ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLIII, nr 2
1974

Leaf surface microflora of *Hordeum vulgare* L.

R. R. MISHRA and V. B. SRIVASTAVA

Department of Botany, University of Gorkhpur, U.P., India

(Received: September 7, 1973.)

Abstract

The fungal and bacterial population of leaf surface of *Hordeum vulgare* L. has ben described. The phyllosphere and phylloplane regions of green and yellow leaves harboured different dominant species. Bacteria mostly suppressed the fungal growth and possibility of biological control has been suggested. The effect of three amino acids and one organic acid on leaf mycoflora has been studied by foliar spray method. Stimulatory effect of different concentrations of the acids has been noticed. Preferential growth of selected forms of microorganisms may be encouraged by the acid application to act as a mean of biological control.

INTRODUCTION

The leaf surface microbial population of *Triticum aestivum* and *Oryza sativa* has been described by the authors in previous two papers (1970 and 1971). The present paper deals with the microflora of *Hordeum vulgare* L. one of the important crop plants of India. This investigation is important to understand the interaction of the microbial population which may be beneficial or detrimental for the organisms associated with the surface of leaves. The understanding of this complex interaction may lead to suggest the means of biological control of the pathogenic forms and hence this aspect of phytopathological research is of immense importance and great economic value.

The effect of foliar spray of the antibiotics and insecticides has been studied by some workers (Hilborn 1953, Prescott & al. 1955, and Pramer 1958) for the control of the plant pathogens. Growth promoting substances have also been tried by foliar spray method to investigate their effects on rhizosphere flora (Gujarati 1965 and Bhatt 1966). However, no attempts has been made to study the effect of foliar spray of amino acids on the leaf surface microflora and this has been described in the present paper.

MATERIALS AND METHODS

Phyllosphere and phylloplane microflora of *Hordeum vulgare* (barley) were studied from seedling to harvesting stages of the plants. Sampling field was about 2 km south of varsity campus. Two leaves of each type were collected at regular intervals from each plant and 15 plants were sampled every time. Green and yellow leaves were cut into small pieces of 0.5 g of each type and introduced separately into 500 ml conical flasks containing 100 ml of sterilized distilled water. When available, yellow dead leaves were also collected preferably from the same plant for the inoculation purpose. The flasks with leaf pieces were hand shaken for 30 minutes and the suspensions thus obtained were inoculated in Petri-plates containing modified Martin's medium. These leaf pieces were then taken out from distilled water giving 20 changes to remove the loosly adhering spores and hyphal fragments and thereafter were plated out on the plates containing Dox-yeast extract agar medium (pH 4.0) after teasing the pieces into small fragments for the isolation of phyllosphere fungi. Meat-Peptone agar medium (pH 7.0—7.2) was used for the isolation of phyllosphere bacteria. One ml. suspension per plate was used for the assessment of phyllosphere microflora and seven small leaf fragments per plate for phylloplane fungi.

The plates after the inoculation were incubated at 25 ± 1°C for 5—6 days for fungi and 37 ± 1°C for 2 days for bacteria. The observation being cumbersome and tedious only quantitative data was obtained for bacterial population.

The effect of foliar spray of three amino acids, viz., asparagine, glycine and glutamic acid and one organic acid i.e. uric acid was studied by spraying 25, 50 and 75 ppm solution of the acids. All the solutions were prepared in sterilized glass distilled water. Barley plants for this purpose were maintained in earthen pots under identical conditions. Three sprayings were done on the aerial parts of the plants by an automizer in the evening at the interval of 20 days. The phyllosphere microflora of sprayed plants was investigated after three days of spraying. The first spraying was done when plants were 20 days old. Some plants which were simultaneously sprayed with sterilized glass distilled water served as control. The experimental findings of the three sampling periods are consolidated in the Tables 3 and 4.

RESULTS

The dominant fungal forms isolated from the phyllosphere region of green and yellow leaves were nearly the same with varying percentage distribution. *Cladosporium* was dominant throughout the investigation period and was associated with *Alternaria* as a codominant. A number of other forms were infrequent with low percentage occurrence, viz., *Aspergillus niger*, *A. sydowi*, *Penicillium humicola*, *P. notatum*, *P. luteum*, *Paecilomyces fusisporus*, *Memnoniella echinata* and *Myrothecium verrucaria* were restricated to the phyllosphere of green leaves and *Mucor* sp. was confined to yellow leaves. The number of species associated with phyllosphere of green leaves were compartively higher than that of yellow ones (Table 1).

The average fungal colonies per plate exhibited different trends on green and yellow leaves. In former case there was a gradual increase in the colonies reaching the maximum on 29th December, when the plants attained maturity and the population decreased later on. Bacterial population on the other hand exhibited an increasing tendency. In case of yellow leaves, the fungal population was higher in the beginning and decreased in 29th December but again there was increase and maximum populations was recorded in the month of January. Then the fungal population dropped off subsequently. The bacterial population showed two maxima on 29th December and 29th February (Fig. 1). The number of species

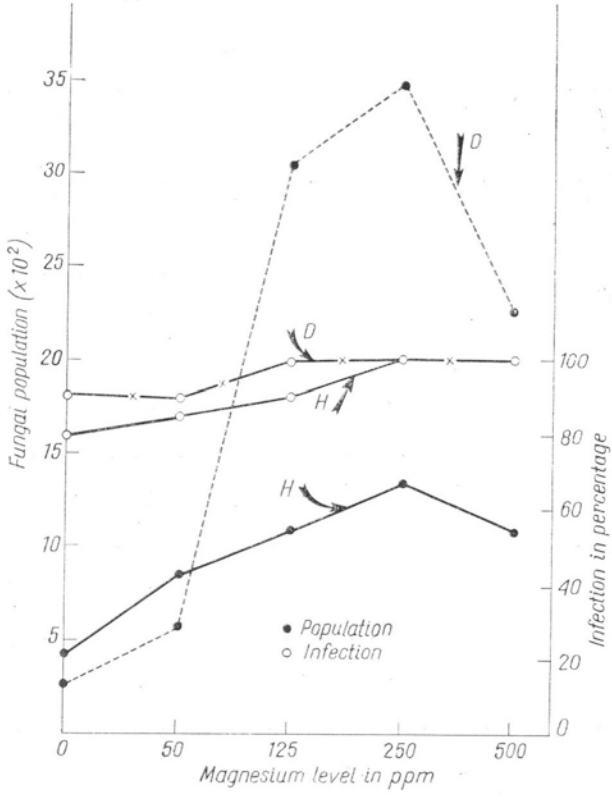

Fig. 1. Bacterial and fungal flora on yellow and green barley leaves

associated with the phyllosphere region of green and yellow leaves at different stages of plant's growth does not show any definite pattern and they fluctuated at different sampling periods (Table 1).

The pattern of distribution of fungal population was different both in quality and quantity in the phylloplane region. None of the forms were continuously present throughout the investigation period. The maximum occurrence was exhibited by *Alternaria* which also showed dominance. *Rhizopus* and *Cladosporium* were infrequent

Table 1

Percentage contribution of phyllosphere fungi in *Hordeum vulgare*

Fungal species	10.11.67	30.11.67	10.1267		29.12.67		13.1.68		27.1.68		10.2.68		29.2.68	
	G	G	G	Y	G	Y	G	Y	G	Y	G	Y	G	Y
Mucor mucedo										2				
Rhizopus nigricans (=*R. stolonifer*)									2	1			1	1
Aspergillus flavus		6							6					1
A. niger		1											1	
A. sydowi											5			
Penicillium humicola		1												
P. notatum			1											
P. oxalicum		5	1	1										
P. luteum														
P. palitans					1									
Paecilomyces sp.	2				1									
Cladosporium spp.	94	85	97	90	75	30	93	86	70	77	85		77	91
Curvularia lunata					1	2								
C. tetramera	2	2			1									
Alternaria spp.			1	9	16	68	7	10	12	8	10	66	15	6
Memnoniella echinata									1					
Fusarium roseum	2				4			4	1	12		4	4	1
Myrothecium verrucaria													3	
Hyaline sterile colonies									8			30		
Black sterile colonies					1									
Number of species	4	6	4	3	8	3	2	3	7	5	3	3	5	5

G — Green leaves; Y — Yellow leaves

Table 2

Percentage contribution of fungal species in phylloplane of *Hordeum vulgare*

Fungal species	30.10.67	10.12.67		29.12.67		13.1.68		27.1.68		10.2.68		29.2.68	
	G	G	Y	G	Y	G	Y	G	Y	G	Y	G	Y
Mucor mucedo			30										
Rhizpus nigricans (= *R. stolonifer*)		60	7	10	25					29		24	35
Aspergillus flavus	45							50				11	
A. niger	5												
A. terreus	5			30									
A. nidulans					15								
A. sydowi						5	17						
Penicillium sp.	5												
P. oxalicum		40	21										
P. palitans				20	15								
P. expansum				10									
Cladosporium spp.	25			30		85	18					20	
Curvularia spp.	5												10
Alternaria spp.	10		42		15	10	45		60	11	27	45	55
Diplococcium sp.					15								
Botrytis cinerea								50		60			
Fusarium sp.					15								
Hyaline sterile colonies							20		40		73		
Number of species	7	2	4	5	6	3	4	2	2	3	2	4	3
Average cols. per plate	7	7	7	5	3	4	10	2	9	8	8	14	8

G — Green leaves ; Y — Yellow leaves

but mostly with higher percentage contribution. Though a number of forms were common in two types of phylloplanes (green and yellow), some species were restricted to one type of leaf. *Aspergillus flavus*, *A. niger*, *A. terreus*, *Penicillium expansum* and *P.* sp. were confined to green phylloplane and *Mucor*, *A. nidulans*, *Diplococcium* and *Fusarium* were isolated only from yellow phylloplane (Table 2).

Number of colonies was higher in the beginning and also in the end and the least on 27th January on the green leaves. In case of yellow leaves, the colony number was lowest on 29th December and for the rest of the period fluctuated between 7 and 10. The number of species associated with the phylloplane of green leaves was maximum in the beginning and decreased subsequently. A somewhat similar trend was noticed in yellow phylloplane as well except that the maximum of species were obtained on 29th December.

Some of the forms were common in phyllosphere and phylloplane regions but some of the species showed restricted occurrence. *Penicillium humicola*, *P. notatum*, *P. luteum*, *Paecilomyces fusisporus*, *Curvularia tetramera*, *Memnoniella echinata*, *Myrothecium roridum* and black sterile colonies were confined to phyllosphere region and *A. terreus*, *P. expansum*, *Diplococcium* and *Botrytis* were isolated only from the phylloplane region (Tables 1 and 2).

The fungal species associated with the leaves sprayed with different acids and water were nearly alike. *Cladosporium*, *Alternaria*, *Rhizopus* and *hyaline* sterile colonies occurred in almost all the sets. The former two species exhibited considerably higher percentage occurrence and the latter two appeared with comparatively low percentage. Other forms were discontinuous in occurrence and with low percentage. Some species showed association only with particular acid-sprayed leaves, viz., *Aspergillus terreus* and *Curvularia* were restricted to the phyllosphere of asparagine sprayed leaves; *Aspergillus* sp. to glycine and *Cephalosporium* (=*Acremonium*) to glutamic acid (Table 3).

The number of species isolated from different types of leaves varied little. However, the average number of colonies per plate showed considerable variation. The different acids and their concentrations affected the phyllosphere mycoflora differently. The effect was also varied on green and yellow leaves. 25 ppm of asparagine proved stimulatory for the phyllosphere of yellow leaves whereas 50 ppm of the same acid was most favourable for the green phyllosphere microfungi. Glycine affected the mycoflora differently. 50 ppm of its spray was stimulatory for the mycoflora of yellow leaves and 75 ppm for green leaves. 50 ppm of glutamic and uric acid was most favourable for green phyllosphere fungi and the same concentration of latter acid proved favourable for yellow leaves as well. The effect of different concentrations of glutamic acid was nearly alike on the yellow leaves.

DISCUSSION

The type and age of the plant, the leaf secretions/excretions and the macro and micro-environmental conditions govern the microbial population of leaf surface. These conditions generally vary from plant to plant and also at different stages

Table 3

Percentage contribution of fungal species from leaf-surface of sprayed leaves of *H. vulgare*

Fungal species	Asparagine						Glycine						Glutamic acid						Uric acid						Water	
ppm.	25		50		75		25		50		75		25		50		75		25		50		75		Control	
leaves	G	Y	G	Y	G	Y	G	Y	G	Y	G	Y	G	Y	G	Y	G	Y	G	Y	G	Y	G	Y	G	Y
Rhizopus oryzae	20				16		20	12	12	2	10	3	10	4	4	6			5	8		10	20	5	12	10
Aspergillus flavus		1							3				15				4			6	5					4
A. terreus			1		2																					
A. niger							4	4	12	3				2	2					2		2			7	
A. sydowi													10	3							4	3	8			
A. ochraclous								2			3			2			2									
A. aculeatus											20						2									
A. sp.												2														
Cephalothecium sp. (= *Trichothecium* sp.)																										
Cephalosporium asperum																		1					4			
(= *Acremonium persicinum*)																		1								
Acremonium vitis	15																									
Botrytis cinerea							12														4					
Cladosporium spp .	15	61	54	77	36	62	52	50	70	95	28	80	15	63	68	68	66	66	74	56	60	67	36	84	66	69
Alternaria spp.	30	34	40	20	36	22	12	30			37	11	45	16	20	20	22	24	8	20	15	8	12	8	15	14
Curvalaria spp.		2																								
Stemphylium spp.								2					5						5							
Fusarium roseum	20	2									2							2				10		3		
Epicoccium. spp.			1			5								2									12			
Hyaline sterile colonies			4	3	10	11			3			4		8	6	6	4	6	8	8	12		8			3

Table 4

Fungal population on the surface of sprayed leaves of *H. vulgare*

Acids	Mycofloral quantity	Acid 25 ppm		Concentration 50 ppm		75 ppm.	
		G	Y	G	Y	G	Y
Asparagine	No. of spp.	5	5	5	3	5	4
	Av. cols./pl.	14	323	35	83	16	32
Glycine	No. os spp.	5	6	5	3	6	5
	Av. cols./pl	13	15	15	277	32	50
Glutamic acid	No. of spp.	6	8	5	4	6	6
	Av. cols./pl.	16	34	26	32	17	34
Uric acid	No. of spp.	5	6	6	6	7	4
	Av. cols./pl.	20	25	23	39	13	19
Water (control)	No. of spp.	4	5				
	Av. cols./pl.	11	42				

G — Green leaves; Y — Yellow leaves

of plant's growth. The microbial population should also correspond to the changing conditions. The various micro-organisms associated with the leaf surface show mutual interaction which proves beneficial to some detrimental for others. The complexity in this region is responsible for various micro-variations in the phyllosphere microflora. Mishra & Srivastava (1970, 1971) while investigating the phyllosphere of *Triticum aestivum* and *Oryza sativa* at different stages of plant growth observed variation in the microflora of two crops. The population further varied at different stages of the same plant species. As evident from the tables 1 and 2, the phyllosphere and phylloplane regions showed variation in the microbial population at different stages of the plant growth. The dominant forms in the phyllosphere and phylloplane regions mostly varied either in type or in percentage occurrence. The phyllosphere forms are mostly those which are typically saprophytes and capable of growing luxuriantly on the leaf surface in the presence of leaf exudate in the form of various organic and inorganic substances (Brown 1922, 1936, Engel 1939 and Hafiz 1952). *Cladosporium* and *Alternaria*, as evident from the table 1 occur with maximum percentage distribution. The former species being saprophyte with ample sporing capacity occupy the maximum leaf surface within a very short time. As evidenced in wheat plant phyllosphere (Mishra & Srivastava, 1970), in this case also once *Cladosporium* got established on the leaf surface and as such it leaves little space for the colonization of other parasitic or saprophytic species. *Alternaria* being parasitic as well survives successfully inside the host tissue and this is quite apparent due to its dominance in phylloplane region. The fungus, therefore, is doubly benefitted and it occupies prominent position in the list of phyllosphere and phylloplane species.

Phyllosphere fungal species are mostly loosely attached with the leaf surface and phylloplane ones have closer association with the leaf surface or may also

invade the leaf tissue. However, as evident from the table 1 and 2, most of the forms were common in the two regions. This is because some of the spores of the so called closely adhering forms might be washed in the solution for inoculation of phyllosphere flora and the vegetative bodies with remaining spores may be present there to appear as phylloplane species. This is apparent with the presence of *Alternaria* in both the regions. As such, with the methods adopted in the present investigation and the results obtained therefrom, it is not convincing to draw a clear cut boundry between the microorganisms of the two regions.

The fungal and bacterial population in the phyllosphere of green and yellow leaves show reversal in their trend. With increase in one, the growth of the other is mostly suppressed and this may be possibly due to the antagonistic effect of the bacterial population on the microfungal flora. A possible mean of biological control is in clue by the results of the present investigation. Such phenomenon has also been observed by some of the previous workers (Voznyakovskaya & Shirokov 1961 and Singh & Sinha 1962).

The effect of foliar spray on the phyllosphere flora is little investigated. Some of the workers have reported increase in the yield of the fruit when sprayed with fungal species to decrease the parasitic forms by antagonistic effect (Voznyakovskaya & Shirokov 1961). Bnatt & Vaughen (1963) suggested the control of *Botrytis cinerea* in varying percentage by the spray of *Cladosporium herbarum*, *Aureobasidium pullulans* or *Penicillium* sp. Some of the concentrations of the acids used in the present investigation proved stimulatory for the phyllosphere fungal population. 50 ppm was suitable in three cases for the phyllopsphere microfungi of the green leaves whereas in the case of yellow on the stimulatory concentration of the acids differed in different cases except glycine and uric acids in which the common stimulatory dose was 50 ppm. The amino acids and uric acid being favourable for microorganisms, if applied in suitable concentration, may stimulate the preferential growth of some species which may become beneficial to the plant. However, the results of the present investigation do not throw any light as to whether the acids affect the microbes by directly entering in their physiological processes or indirectly through host leaf. Possible explanation may be put forth by the results of the further investigation which are in progress in this laboratory.

Acknowledgement

The authors are indebted to Prof. K. S. Bhargava, Head of the Department of Botany, University of Gorakhpur, for providing laboratory facilities.

REFERENCES

Bnatt D. D. & Vaughen E. K., 1963. Investigation-ship among fungi associated with Strawberries in Oregon. Phytopath. 53: 217—220.

Bnatt S., 1966. Age as a factor in the rhizosphere microflora of wild leguminous plants. Ph.D. Thesis, B.H.U., Varanasi, India.

Brown R., 1922. Studies in the physiology of parasitism. VIII. On the exosmosis of nutrient substances from the host tissue into the infection drop. Ann. Bot. Rev. 2: 236—281.

Brown R., 1936. The physiology of host parasite relations. Bot. Hev. Lond. 36: 101—119.

Engel H., 1939. Das Verhalten der Blatter bei Besetzung mit Wasser. Jahrb. Wiss. Bot. 88: 816—861.

Gujahati S., 1965. Investigations into rhizosphere microflora of cultivated legumes. Ph.D. Thesis, B.H.U. Varanasi, India.

Hafiz A., 1952. Basis of resistance in gram to *Mycosphaerella* blight. Phytopath. 42: 422—424.

Hilborn M. T., 1953. Effect of various chemicals on infection by *Rhizoctonia solani* and *Verticillium albo-atrum*. Phytopath. 43: 475.

Mishra R. R. & Srivastava V. B., 1970. Leaf surface mycoflora of *Triticum aestivum* L., Bull. Jour. Bot. club. 97:1.

Mishra R. R. & Srivastava V. B., 1971. Leaf surface fungi of *Oryza sativa* L., Mycopath. Mycol. Appl. 44: 289—294.

Pramer D., 1958. Eradication and therapeutic materials for disease control. Antibiotics. Recent advances in Botany, 452—456.

*Prescott G. C., Emerson H. & Ford J. H., 1955. Jour. Agric. Food Chem. 4: 343—345.

Singh N. P. & Sinha S., 1962. Microorganisms of some vegetable surface and tissues and their antagonisms, 49th Ann. Sess. Proc., Ind. Sci. Congr. Assoc.

Voznyakovskaya Mme Yu. M. & Shirokov Yu. G., 1961. The uses of epiphytic microflora in the control of grey mould of Strawberry. Ereven Acad. Sci. Aremenian, U.S.S.R. p. 170—179.

Present address:
Dr R. R. Mishra and dr V. B. Srivastava
Pilot Plant Laboratories,
Indian Drugs and Pharmaceuticals Ldt.,
Virbhadra, Rishikesh, U.P., India

Microflora powierzchni liści jęczmienia

Streszczenie

Opisano populacje bakterii i grzybów występujących na powierzchni liści *Hordeum vulgare* L. oraz badano współzależności zachodzące między nimi.

Obserwacje prowadzono w ciągu okresu wegetacyjnego na liściach różnego wieku. Zielone i żółte liście jęczmienia charakteryzowały się odrębnymi gatunkami dominującymi. Pewne gatunki grzybów (*Cladosporium i Alternaria*) występują bardzo licznie, inne spotyka się raczej sporadycznie. W trakcie rozwoju rośliny — zarówno na liściach zielonych, jak i żółtych — następuje falowe przenoszenie dominacji grzybów na bakterie. Ponieważ zaobserwowano, że bakterie zwykle ograniczają wzrost grzybów, wobec tego autorzy sugerują możliwość biologicznej kontroli tych zjawisk.

Badano również wpływ trzech aminokwasów (asparaginy, glicyny i kwasu glutaminowego) oraz jednego kwasu organicznego na mikroflorę liściową, przez opryskiwanie roślin roztworami o różnej koncentracji tych związków (25, 50, 75 ppm). Wybiórczy wzrost niektórych mikroorganizmów, osiągnięty przez opryskiwanie kwasami, może być zastosowany jako rodzaj kontroli biologicznej.

---

* Original not consulted.