

Seasonal fluctuations of phyllosphere and phylloplane fungi of Egyptian wheat

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Mazen M. B., S. I. I. Abdel-Hafez, G. M. Shaban: *Seasonal fluctuations of phyllosphere and phylloplane fungi of Egyptian wheat*. Acta Mycol. 21(1) : 109 - 116, 1985.

Forty-nine species and 20 genera were collected from the phyllosphere and phylloplane of wheat plants on 1% glucose and 50% sucrose-Czapek's agar at 28°C. The total counts of phyllosphere and phylloplane fungi displayed seasonal periodicities and the highest counts were found in April and May 1977, 1978. In the case of phyllosphere the most frequent species were *Aspergillus niger*, *Penicillium corylophilum* and *Alternaria alternata*; in the phylloplane *Aspergillus niger* and *A. flavus*.

INTRODUCTION

The term phyllosphere fungi was coined by Last (1955) and Ruinen (1956) to describe organisms found on the leaf surface. Kerling (1958) suggested that the phylloplane should be used when referring to the fungi on the leaf surface. Phylloplane as described by Last (1971) is the outer skin of the leaf surface and is thus an excellent site for both saprophytic and parasitic fungi. Several investigations were made on the activity of micro-organisms on the leaf surface (Di-Menna 1959, 1971; Clark, Paul 1970; Hudson 1971; Statt 1971; Diem 1973; Dickinson, Donnel 1977; Abdel-Hafez 1981 and oth.).

In Egypt, few investigations on leaf surface microflora had been conducted (Moubasher et al. 1971; Abdel-Wahab 1975; Zaki 1975; Abdel-Fattah et al. 1977; Abdel-Gawad 1978; El-Maghraby 1980). The aim of the present investigation was to study the seasonal periodicities of the phyllosphere and the phylloplane fungi of wheat cultivated in El-Minya Governorate, Egypt.

MATERIALS AND METHODS

Samples of wheat (*Triticum vulgare*) leaves were collected from wheat fields in El-Minya Governorate every fortnight during the period December 1976 - May 1977 and December 1977 - May 1978. Samples were placed in polyethylene bags and transferred immediately to the laboratory.

Ten g of wheat leaf segments (1 cm²) were placed in a sterile conical flask (250 ml) which contained 100 ml sterile distilled water and shaken by hand with a rotating motion for 10 minutes. Twenty ml of the suspension were transferred to another flask which contained 80 ml water. One ml of the water suspension was transferred to a sterile petri-dish, and poured with melted but cooled agar medium.

The cut pieces (1 cm²; 20 segments in each sample) of wheat leaves (of 12 samples of plant) after shaking for 10 minutes in sterile distilled water were removed and thoroughly dried between sterilized filter papers. Four pieces were placed on the surface of the agar medium in each plate. Five plates were used for each sample of the phyllosphere and the phylloplane and for each type of media (1% glucose 50% sucrose-Czapek's agar + rose bengal (1/15000) as bacteriostatic agent (Smith, Dawson 1944). The plates were incubated at 28°C and the developing fungi were identified and counted.

RESULTS AND DISCUSSION

Forty-nine species which belong to 20 genera were collected from the phyllosphere (20 genera and 42 species; and 16 genera and 39 species on 1% glucose and 50% sucrose-Czapek's agar respectively) and phylloplane (9 genera and 18 species; and 8 genera and 17 species respectively) of wheat plant on the two media at 28°C (Table 1). All these species were isolated previously from the phyllosphere and phylloplane of some Egyptian plants (Moubasher et al. 1971; Zaki 1975; Abdel-Wahab 1975; Abdel-Fattah et al. 1977; Abdel-Gawad 1978; El-Maghaby 1980) as well as from leaves of wheat plant cultivated in Saudi Arabia (Abdel-Hafez 1981).

The results of Table 1, show clearly that the total counts of phyllosphere fungi displayed seasonal periodicities and the highest counts on the two media were estimated in May and April 1977; and the poorest in December and February 1976 - 78. This means that the phyllosphere cells are too weak to defend themselves against the phyllosphere fungi which are mainly saprophytes. Thus, the phyllosphere fungi at the later stages of the plant could live actively, as saprophytes or weak parasites on the senescent or the dying cells of the leaves and increased their

Table 1
Isolation of fungi from phyllosphere /per g fresh weight/ and phylloplane

Species	Phyllosphere				Phylloplane			
	1 % glucose		50% sucrose		1 % glucose		50% sucrose	
	TC	NCI	TC	NCI	TC	NCI	TC	NCI
Total count	15970		15225		431.5		500.5	
<i>Aspergillus</i>	7270	12	8065	12	289.5	12	305.5	11
<i>A. niger</i> Van Tieghem	4085	12	5905	12	145.5	12	163.5	11
<i>A. flavus</i> Link	330	8	225	7	38.0	9	34.0	8
<i>A. fumigatus</i> Presenius	2260	8	1305	7	87.0	7	85.5	8
<i>A. terreus</i> Thom	155	7	265	9	5.0	3	10.5	5
<i>A. ochraceus</i> Wilhelm	160	5	105	3	1.0	1	0.0	0
<i>A. versicolor</i> /Vuill./ Tirab.	135	3	25	2	9.5	3	4.0	2
<i>A. candidus</i> Link ex Fr.	40	2	0	0	0.0	0	0.0	0
<i>A. carneus</i> /Van Tiegh./ Bloch.	35	2	5	1	0.0	0	0.0	0
<i>A. nidulans</i> /Eidam/ Winter	35	2	10	1	3.5	3	0.0	0
<i>A. sydowii</i> /Bain. et Sort./Thom et Church	35	2	20	3	0.0	0	0.0	0
<i>A. amstelodani</i> /Mangin/Thom et Church	0	0	15	1	0.0	0	3.5	3
<i>A. chevalieri</i> /Mangin/Thom et Church	0	0	475	4	0.0	0	4.5	2
<i>Penicillium</i>	480	11	485	9	29.5	8	12.5	6
<i>P. corylophilum</i> Dierckx	130	5	30	3	0.0	0	0.0	0
<i>P. funiculosum</i> Thom	95	5	0	0	0.0	0	0.0	0
<i>P. citrinum</i> Thom	75	4	90	4	0.0	0	0.0	0
<i>P. chrysogenum</i> Thom	50	3	215	7	1.5	2	7.0	5
<i>P. oxalicum</i> Thom	35	3	25	3	19.5	3	0.0	0
<i>P. nigricans</i> /Bain./ Thom	80	2	20	1	0.0	0	0.0	0
<i>P. waksmani</i> Zaleski	15	1	0	0	0.0	0	0.0	0
<i>P. brevicompactum</i> Dierckx	0	0	95	2	0.0	0	5.5	4
<i>P. jenseni</i> Zaleski	0	0	10	1	0.0	0	0.0	0
<i>P. roqueforti</i> Thom	0	0	0	0	8.5	2	0.0	0
<i>Alternaria</i>	4420	10	3690	10	89.5	10	100.0	8
<i>A. alternata</i> /Fr./ Keissler	300	8	1150	6	89.5	0	92.0	8
<i>A. tenuissima</i> /Kunze ex Pers/Wiltshire	4120	4	2435	4	0.0	0	8.0	3
<i>A. geophila</i> Deaze Waka	0	0	105	3	0.0	0	0.0	0

<i>Fusarium</i>	1000	8	575	6	1.0	2	0.0	0
<i>F. solani</i> /Mart./ Appi. et Willems.	370	6	575	6	1.0	2	0.0	0
<i>F. moniliforme</i> Sheldon	115	3	0	0	0.0	0	0.0	0
<i>F. oxysporum</i> Schlecht.	155	3	0	0	0.0	0	0.0	0
<i>Curvularia</i>	330	7	60	5	0.0	0	0.0	0
<i>C. lunata</i> /Wakker/ Boedijn	125	5	45	4	0.0	0	0.0	0
<i>C. spicifera</i> /Bain/ Boedijn	130	4	15	2	0.0	0	0.0	0
<i>C. tuberculata</i> Jain	75	3	0	0	0.0	0	0.0	0
<i>Stachybotrys chartarum</i> /Ehr. ex Link/ Hughes	220	9	55	5	0.0	0	0.0	0
<i>Mucor</i>	300	7	405	5	12.0	3	28.0	3
<i>M. hiemalis</i> Wehner	160	4	0	0	0.0	0	0.0	0
<i>M. racemosus</i> Presenius	60	3	155	4	11.0	2	0.0	0
<i>M. spinescens</i> Lendner	80	2	0	0	10.0	1	0.0	0
<i>M. pusillus</i> Lindt	0	0	250	4	0.0	0	28.0	3
<i>Cladosporium herbarum</i> /Pers/Link ex S.F. Gray	1520	6	1365	6	1.5	2	22.5	7
<i>Epicoecium purpurascens</i> Ehrenb. ex Schlecht.	100	5	260	7	0.0	0	7.0	5
<i>Scopulariopsis brevicaulis</i> /Sacc./Bain	65	3	40	2	1.0	2	0.0	0
<i>Paecilomyces variotii</i> Bainier	30	2	60	3	0.0	0	0.0	0
<i>Hemicola grisea</i> Traaen	35	2	45	1	0.0	0	0.0	0
<i>Nigrospora oryzae</i> /Berk. et Br./ Petch	45	2	25	2	0.0	0	0.0	0
<i>Rhizopus stolonifer</i> /Ehrenb. ex Fr./Lindt	25	2	10	1	6.0	4	13.5	2
<i>Sporotrichum roseolum</i> Oudem. et Bijen.	15	2	0	0	0.0	0	0.0	0
<i>Trissmatostroma salicis</i> Corda	50	2	5	1	0.0	0	0.0	0
<i>Cephalosporium roseum</i> Oudem.	15	1	0	0	0.0	0	0.0	0
<i>Doratomyces purpureofuscus</i> /Fr./Morton et Smith	20	0	0	0	0.0	0	0.0	0
<i>Drechlera sativus</i> /Ito et Kurib./ Drechsler	15	1	0	0	0.0	0	0.0	0
<i>Ulocladium botrytis</i> Preuss	10	1	0	0	1.5	3	11.5	3
<i>Botryotrichum piluliferum</i> Sacc. et March	0	0	30	2	0.0	0	0.0	0
Sterile mycelium	40	2	50	3	0.0	0	0.0	0

TC - total counts in every sample; NCI - number of isolation; High occurrence - more than 5 cases /out of 12/; Moderate occurrence - between 3-5 cases; Low occurrence - 2 cases; Rare occurrence - 1 case

populations dramatically as reported by several workers (Last 1955; Kerling 1958; Dickinson 1967; Diem 1967; Di-Menna, Parle 1970; Abdel-Gawad 1978).

Aspergillus was the most frequent genus in the phyllosphere of wheat on the two media. It emerged from 100% of the samples comprising 45.5 and 53% of the fungi on glucose and 50% sucrose-Czapek's agar respectively. It was also the most common genus in the phyllosphere of some Egyptian summer and winter plants namely, *Lathyrus odoratus*, *Hordeum vulgare*, *Triticum vulgare*, *Lupinus termis*, *Carthamus tinctorius*, *Gossypium barbadense*, *Hibiscus subdariffa*, *Hibiscus esculentus*, *Corchorus olitorius* and *Zea mays* (Abdel-Gawad 1978); *Prunus*, *Citrus*, *Gossypium*, *Punica* and *Saccharum* (Abdel-Wahab 1958); and broad-bean (Abdel-Fattah et al. 1977). Its count displayed seasonal periodicities and the highest counts were recorded in March and May 1977; and March and May 1977 and 1978 on 1% glucose and 50% sucrose-Czapek's agar respectively. Ten and 12 species of the genus were identified on the two media respectively, from which *Aspergillus niger*, *A. flavus*, *A. fumigatus* and *A. terreus* were isolated with high seasonal frequencies. In the phyllosphere of wheat plant cultivated in Assiut Governorate, Abdel-Gawad (1978) found that *A. niger*, *A. fumigatus*, *A. terreus*, and *A. flavus* were encountered 5, 7, 3 and 1 months (out of 8) contributing 6.5, 9.7, 2.2 and 0.5% of the total fungi respectively. Their counts showed seasonal fluctuations almost parallel to those of the genus counts. They were also recovered from the phyllosphere of some Egyptian plants (Moubasher et al. 1971; Abdel-Wahab, 1975; Abdel-Fattah et al. 1977 and Abdel-Gawad 1978) and wheat plant cultivated in Saudi Arabia (Abdel-Hafez 1981). *A. chevalieri* and *A. amstelodami* were encountered only in the phyllosphere on 50% sucrose-Czapek's agar. These two species are well known as osmophilic (Raper, Fennel 1965; Moustafa, Al-Mussalam 1975; Abdel-Hafez et al. 1977; Moubasher et al. 1980; Abdel-Hafez 1981; Moubasher et al. in press).

Penicillium ranked second on 1% glucose agar, but it retreated to third place on 50% sucrose agar and the highest counts were recorded in January 1977 and March 1978; and February 1978 respectively. The most frequent species were *P. corylophilum*, *P. funiculosum* and *P. citrinum* on 1% glucose agar and *P. chrysogenum*, *P. citrinum* and *P. corylophilum* on 50% sucrose agar which occurred in 33.3-41.7%; and 25-50% of the samples; and accounting for 0.5-0.8%; and 0.2-1.0% of the total fungi on the two media respectively. Abdel-Gawad (1978) isolated 7 species of *Penicillium* from the phyllosphere of wheat culti-

vated in Assiut Governorate and these were *P. citrinum*, *P. corylophilum*, *P. verruculosum*, *P. chrysogenum*, *P. jensenii*, *P. funiculosum* and *P. oxalicum* which contributed 0.03 - 1.4% of the total fungi.

Alternaria occupied the third place on 1% glucose agar, but was promoted to the second place on 50% sucrose agar. Three species were identified of which *A. alternata* and *A. tenuissima* were the most common on the two media, Abdel-Gawad (1978) found *A. alternata* in the phyllosphere of all samples of wheat comprising 8.6% the total fungi. Abdel-Hafez (1981) isolated 4 species of *Alternaria* from the phyllosphere of wheat plant cultivated in Saudi Arabia and these were *A. alternata*, *A. tenuissima*, *A. phragmospora* and *A. raphani*.

Curvularia, *Stachybotrys*, *Fusarium*, *Epicoccum* and *Cladosporium* were less frequent than the previously genera in the phyllosphere of wheat plant on the two media. They emerged from 41.7 - 75.0% and 41.7 - 58.3% of the samples comprising 0.6 - 9.5% and 0.4 - 9.0% of the total fungi on the two media respectively. From these genera *C. lunata*, *C. spicifera*, *S. chartarum*, *F. solani*, *E. pururascens* and *C. herbarum* were the most common. All these species were isolated from the leaf surface of some Egyptian plants (Abdel-Wahab 1975; Abdel-Fattah et al. 1977; Abdel-Gawad 1978). It is worth mentioning that *Cladosporium* represented mainly by *C. herbarum* was one of the basic constituents of phyllosphere fungi accounting for 50.4 and 24.4% of the total fungi of wheat plant cultivated in Assiut (Abdel-Gawad 1978) and Saudi Arabia (Abdel-Hafez 1981) respectively. In El-Minya, it comprised 9.5 and 9.0% of the total fungi recovered on 1% glucose and 50% sucrose Czapek's agar respectively. *C. herbarum* was also one of the dominant fungi on leaf surface of numerous plants (Hogg, Hudson 1966; Di-Menna, Parle 1970; Moubasher et al., 1971; Abdel-Wahab 1975; Abdel-Fattah et al. 1977; Abdel-Gawad 1978).

The results presented in Table 1 show clearly that the total counts of phylloplane fungi showed regular seasonal periodicities and the highest counts were estimated in March - May 1977 and 1978 when the plant was almost mature.

The most frequent species were *Aspergillus niger*, *A. flavus*, *A. fumigatus* and *Alternaria alternata*. Abdel-Gawad (1978) found that *Aspergillus fumigatus*, *A. niger*, *A. terreus*, *A. flavus* and *Alternaria alternata* accounted for 21.5, 6.4, 5.5, 0.9 and 19.3% of the total fungi of phylloplane of wheat plant cultivated in Assiut respectively. Abdel-Hafez (1981) reported that *Cladosporium herbarum*, *Aspergillus niger*, *A. ochraceus*, *Alternaria alternata* and *A. phragmospora* were the most prevalent in the phylloplane of wheat plant cultivated in Saudi

Arabia and emerged from 87.5, 62.5, 54.2, 91.7 and 66.7% of the samples constituting 24.9, 4.5, 2.2, 19.9 and 4.6% of the total fungi respectively. *Cladosporium* which was isolated in low counts from the phylloplane (0.34 and 0.45% on 1% glucose and 50% sucrose agar respectively) accounted for 26.7 and 35.7% of the total fungi of phylloplane of wheat plant cultivated in Assiut (Abdel-Gawad 1978) and Saudi Arabia (Abdel-Hafez 1981) respectively, *A. amstelodami* and *A. chevalieri* were encountered only on 50% sucrose-Czapek's agar.

We would like to thank Prof. A. H. Moubasher, Dean of the Faculty of Science, Qatar University, for kindly reading the manuscript.

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