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STUDIES ON THE OCCURRENCE OF FUNGI IN A WHEAT–FIELD. II. FUNGI ON THE PHYLLOPLANE AND FUNGAL AIR–SPORA

Giuseppe Caretta, Giuseppe Del Frate, Paola Della Franca, Maria Guglielminetti, Anna Maria Mangiarotti and Elena Savino
Istituto di Micologia Medica "R. Ciferri & P. Redaelli"
Università degli Studi di Pavia. Italy
Via S. Epifanio 14, 27100 Pavia (Italy)

ABSTRACT

Fungi recorded from northern Italy on the phylloplane of wheat during its life cycle and the ambient fungal air-spora were compared. On the phylloplane 68 species, belonging to 35 genera, were identified: ***Alternaria alternata, Aureobasidium pullulans var. pullulans, Cladosporium cladosporioides*** *and* ***Epicoccum purpurascens*** *were present all months. A few pathogens were occasionally recorded such as* ***Dreschslera*** *anamorph of* ***Cochliobolus sativus, Erysiphe graminis*** *and* ***Septoria tritici.***

In general, a gradual transition in species was noticed from the initial development to leaf senescence with an apparent change in fungal population in April.

In the air ***Cladosporium, Alternaria, Epicoccum*** *and* ***Fusarium*** *were the dominant genera showing high peaks of concentration between June and late August.*

We noticed the greatest abundance of fungi on the phylloplane in April with a relatively low concentration in the air; in June and July the situation was reversed.

RESUMEN

[Estudio sobre la presencia de hongos en campo de trigo. II Hongos del filoplano y esporas fúngicas aéreas]

Se compararon las especies fúngicas detectadas en el filoplano del trigo durante su ciclo de vida con el registro de esporas aéreas ambientales en la zona norte de Italia. Del filoplano se aislaron 68 especies pertenecientes a 35 géneros, que incluyeron: ***Alternaria alternata, Aureobasidium pullulans var. pullulans, Cladosporium cladosporioides*** *y* ***Epicoccum purpurascens*** *(presentes todos los meses). Se registraron ocasionalmente unos pocos patógenos tales como* ***Drechslera*** *anamorfo de* ***Cochliobolus sativus, Erysiphe graminis*** *y* ***Septoria tritici.***

En general, se aprecia una transición gradual en las especies desde el inicio del desarrollo de la senescencia de la hoja con un aparente cambio de la población fúngica en abril.

Los géneros dominantes en el aire que mostraron un alto peak de concentración entre junio y agosto corresponden a ***Cladosporium, Alternaria, Epicoccum y Fusarium.***

Destacamos la gran abundancia de hongos en abril, con una relativa baja concentración en el aire; en junio y julio, la situación fue a la inversa.

INTRODUCTION

The first part of this research described the succession of mesophilic, thermophilic and keratinophilic micro-fungi in wheatfield soil (5). This second part compares the fungi on the wheat phylloplane with fungal spores in the surrounding air.

MATERIALS AND METHODS

Sampling procedures and cultural methods for isolation of phylloplane fungi.

Wheat leaves were collected monthly from January to July 1983 from plants selected along the diagonal of a wheatfield plot. A description of the site, chemical data of the soil and climatic data are in the first part of this study (5). At each sampling 2 leaves from each of 10 wheat seedlings were collected; the leaves were transported within an hour to the laboratory in separate plastic bags, taking care to avoid contamination, and all mycological processing was carried out on the day of collection.

Each leaf was aseptically cut into three segments and subjected to the following three methods:

1) leaf impression: the leaf surface of the first segment was temporarily pressed against malt extract agar (MEA), amended with CAF (0.5o/oo) and streptomycin sulphate (1 o/oo), in Petri dishes. The dishes were incubated at 25° C for 7 days;
2) leaf washing: the second segment of each leaf was shaken for 20 min. in 50 cm sterile distilled water. The suspension was diluted at 1/10 and 1/100 and, in duplicate, 0.1 cm of each dilution was spread on Petri dishes containing MEA. The dishes were incubated at 25° C for 15 days;
3) damp chamber: each third segment was placed individually in 16 cm diameter plastic dishes containing a 12 cm diameter sterile filter paper moistened with 5 ml of sterile distilled water containing streptomycin sulphate (40 μg/ml). The dishes were incubated at 25° C for 14-20 days.

All resulting fungal colonies were directly identified or transferred to an appropriate medium for identification. Each fungal species was counted only once per dish, even if more than one colony of the same species was present.

Sampling of air—spora

Spore concentration was determined by a Hirst automatic volumetric spore trap (10) installed over the wheat field. Its orifice was at 2 m above ground level. Air was sampled weekly from 1st October 1982 to 30th September 1983.

Slides, coated with silicone, were exposed for 24 hours by the trap and then mounted with glycerin jelly containing basic fuchsin as a stain. The suction rate of the trap was kept at 10 l/min throughout the trapping period. The slides moved at 2 mm per hour. We counted the fungal spores along four traverses parallel to the direction of travel at intervals of 500 μ in width. The fungal spores were examined by microscopy using 250 magnification. A total of 51 air sampling were made.

RESULTS

Phylloplane fungi

A total of 1557 fungal isolations representative of 68 species belonging to 35 genera were identified. All fungi recorded by the three methods are listed in Table 1.

Acremoniella atra, Gibberella acuminata, Gliomastix cerealis, Erysiphe graminis, Septoria tritici and synnematous Hyphomycetes **Cephalotrichum stemonitis** and **Trichurus spiralis** were recorded mainly by the damp chamber method. The leaf washing method encouraged isolation of **Aureobasidium pullulans** var. **pullulans, Brettanomyces claussenii, Fusarium dimerum, Metschnikowia pulcherrima**, and **Torulopsis magnoliae**. The filamentous fungi **Botrytis, Drechslera, Epicoccum, Gliocladium, Nigrospora, Trichocladium, Trichoderma, Rhizopus** and **Ulocladium** were isolated more frequently by the leaf impression method. Certain genera were always present, others occurred only occasionally. The genera constantly present were: **Alternaria, Aureobasidium, Aspegillus, Cladosporium, Epicoccum** and **Penicillium**. **Alternaria, Aspergillus** and **Cladosporium** were each represented by five species with **A. alternata, A. fumigatus** and **C. cladosporioides** dominant. **Penicillium** was represented by twelve species with **P. aurantio-candidum** dominant.

The air—spora concentration

Results for the air-spora are expressed as monthly averages of spore concentration (Table 2). The mean spore concentration over the period from October 1982 through September 1983 was 4976 spores per cubic metre. Spore concentration showed pronounced seasonal variation.

The highest values were found in late spring (June, 14872 spores/m^3), summer (July, 10293/m^3) and early autumn. In winter, as in early spring, spore concentration was lower (January 519/m^3 and April 798/m^3). The most common sporetype was **Cladosporium**, which accounted for 71.39% of the total of counted spores. The second most common was **Fusarium** which amounted to 13.7% **Alternaria** and **Epicoccum** totalled 2.05% and 1.97% respectively.

All other particles recognizable as fungal spores amounted to 10.8%; most were spores of genera **Aspergillus** or **Penicillium, Botrytis, Drechslera, Ganoderma, Oidium, Polythrincium, Stemphylium, Torula, Ulocladium** and **Ustilago**. **Cladosporium** spores were regularly trapped from the wheat field air throughout the year, but mainly from June to August with a peak in June (11797 spores/m^3).

Fungal spores of **Fusarium** were present all year round but with relatively high incidence in late spring and in summer with a peak in June (2496 spores/m^3). **Alternaria** and **Epicoccum** showed a similar incidence and concentration: they were found in moderate amounts all year, with the greatest concentrations in October and from July to September, except that **Alternaria** was absent in January.

DISCUSSION

In general the fungi of the wheat phylloplane

was composed of ascomycetes and fungi imperfecti.

The transitory appearance of some genera and species on phylloplane seems signifcant. **Botrytis, Cephalotrichum, Oidiodendron, Trichoderma, Septoria, Metschnikowia** and **Torulopsis** were present until April; **Acremonium, Trichocladium, Verticillium** did not appear before April, and **Acremoniella, Drechslera, Gliomastix** were isolated only in April.

Similarly some species of the dominant genera occurred until April, e.g. **A. flavus, C. tenuissimum, P. brevi-compactum, P. variabile, S. roseus, Rh. stolonifer,** while others were present only from April, e.g. **A. chlamydospora** and **C. herbarum.** An important change in the fungal composition of leaf surfaces thus seems to occur in April.

Flannigan and Campbell (8) in a study of the mycota of the flag leaf, bracts and caryopsis of wheat growing in eastern Scotland found the **A. pullulans, C. herbarum,** and **C. cladosporioides** were the earliest established filamentous fungi in all organs of wheat; **A. alternata** and **V. lecanii** followed and E. **purpurascens** appeared later on the dying leaf and bracts and on the ripening cariopsis.

The naturally-occurring yeasts **Sporobolomyces** and **Cryptococcus** and the filamentous fungi **Cladosporium, Alternaria, Epicoccum** and **Aureobasidium** are the common leaf saprobes.

Their incidence on Gramineae, and their role in controlling plant pathogens, is reported in many investigations (1, 7, 9).

Following the schematic classification of epiphytic fungi on leaves proposed by Dickinson (6), the fungal population on wheat leaves during this investigation consisted mainly of non-pathogenic epiphytes, but some species of phytopathological interest were occasionally recorded: **Erysiphe graminis, Drechslera** anamorph of **Cochliobolus sativus** and **Septoria tritici.**

The comparative data between the genera found on wheat phylloplane and the concentration of the fungal air-spora showed some discrepancies. The phylloplane appeared to be colonized by different genera very abundant in January, less in February, but increasing in March and April. The fungi decreased with the increasing of the plant growth and in the senescent leaves the number was lower. On the contrary we had the highest spore concentration in the air in June and the lowest in January and March.

Cladosporium. Epicoccum and **Fusarium** behaved in the same manner: they showed the greatest abundance on the phylloplane in late winter and in early spring while, at the same time, they had a relatively low concentration in the air.

On the other hand these fungi showed the highest concentration in the air in June, July and August when on the phylloplane they were descreasing. **Alternaria** had the highest occurrence on the leaves in June and the maximum concentration in the air in September.

The **Fusaria** isolated from wheat phylloplane include soil-borne species **(F. oxysporum),** air-borne species **(F. lateritium)** and those that Burgess (2) included in the category of **Fusaria** which are common in soil but have efficient mechanisms for air dispersal **(F. moniliforme).** Although **F. oxysporum** has been isolated from the air (11, 13) it is not well adapted to air dispersal. Its presence in the atmosphere can probably be attributed to wind-blown soil or organic debris (11,12) and crop residues stubbles. Dispersal by rain and water-splash or wind-blown soil would account for it ability to colonize aerial plant parts. The high concentration of air-borne macroconidia of **Fusarium** at Pavia could be correlated with stages in the development of local vegetation and crops.

It is interesting to note that some of the dominant genera occurring on the wheat leaves **Alternaria, Aureobasidium** and **Epicoccum** were rarely isolated from the soil of the same weath field (5). On the other hand very similar patterns on wheat leaves and in wheat soil were observed for **Aspergillus, Fusarium** and **Penicillium.**

While we could note some differences among fungi isolated from wheat, maize and rice soils (3, 4, 5), the fungi recorded from the phylloplane of these three cereals are almost the same.

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TABLE 1: Fungi isolated from phyllophane by three different methods: leaf impression (1), leaf washing (2), damp chamber (3).

	JAN			FEB			MARCH			APRIL			MAY			JUNE			JULY			TOTAL		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Acremoniella atra Saoc.										–	–	10										–	–	10
Acremonium kiliense Grutz													1	–	–	2	–	5				3	–	5
A, murorum (Corda) W. Gams																1	-	-				1	-	-
Alternaria alternata (Fr.) Kessler	10	1	3	5	2	10	6	-	6	3	-	20	8	-	3	20	1	3	-	-	20	52	4	65
A. chlamydospora Mouchacca										1	-	1	10	-	-							11	-	1
A. longipes (Ellis & Everth.) Mason				2	-	10	2	-	-	-	1	-	-	1	-	21	3	-				25	5	10
A. tenuissima (Kunze: Fr.) Wilta										2	3	2										2	3	2
A. triticina Prasada & Prabhu										1	-	-										1	-	-
Alternaria sp.													4	-	-				14	-	-	18	-	-
Aspergillus alutaceus Berk. & Curt.							3	-	-													3	-	-
A. Clavatus Desm.													1	-	-							1	-	-
A. flavus Link	1	-	-	-	1	-	1	-	-													2	1	-
A. fumigatus Fres.	-	11	-							-	1	-	1	-	-	1	-	-	1	-	-	3	12	-
A. niger van Tieghem	2	-	-							1	-	-										3	-	-
Aspergillus sp.				1	3	-																1	3	-
Aureobasidium pullulans (de Bary) Arn. var. **pullulans**	5	20	-	5	20	-	3	20	-	-	20	-	2	10	-	3	4	-	4	-	-	22	94	-
Brettanomyces claussenii Custera													-	2	-							-	2	-
Botrytis cinerea Pers: Fr.	2	-	-				3	-	-													5	-	-
Cephalotrichum stemonites (Pers.) Link	3	-	20																			3	-	20
Chaetomium globosum Kunze ex Stend.							1	-	5	-	-	5				-	5	-				1	5	10
Cladosporium cladosporioides (Fresen.) de Vries	5	-	-	5	1	10	1	10	-	3	-	2	12	-	-	-	2	-	-	-	20	25	13	32

	JAN			FEB			MARCH			APRIL			MAY			JUNE			JULY			TOTAL		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
C. elatum (Harz.) Nannf.	1	2	-																			1	2	-
C. herbarum (Pers.) Link.										-	-	2	5	-	-	6	3	-				11	3	2
C. oxysporum Berk. & Curt.				2	2	10				14	20	10	2	-	-	5	-	-				23	22	20
C. tenuissimum Cooke				4	1	-	8	-	20	10	4	-										22	5	20
Cladosporium spp.	1	-	-	1	-	-	20	20	1	13	-	-	-	-	2	2	6	-				37	26	3
Cryptococcus albidus (Saito) Skinner							3	3	-				-	2	-							3	5	-
C. laurentii (Kufferath) Skinner	3	9	-	-	2	-	5	4	1				4	6	-	6	3	-				18	24	1
Drechslera anamorph of **Cochliobolus sativus** (Ito & Kuribayashi) Drechsler ex Dastur										1	-	-										1	-	-
Epicoccum purpurascens Ehrenb. ex Schlecht	15	-	-	10	-	10	20	-	13	4	1	15	13	-	3	20	1	-	3	-	-	86	2	41
Erysiphe graminis DC.	-	-	1				-	-	14				-	-	10	-	-	10				-	-	35
Fusarium dimerum Penzing										-	5	-										-	5	-
F. lateritium (Nees.) Sny. & Hans							2	1	-													2	1	-
F. moniliforme Sheld							-	-	7	5	2	10	1	-	-							6	2	17
F. oxysporum Schlecht. emend. Sny. & Hans	2	-	-	6	-	5	4	-	-				2	-	-				1	-	-	15	-	5
Gibberella acuminata Wollenw.							-	-	5	-	-	10										-	-	15
Gliocladium roseum Bain							16	-	-	1	-	-	5	-	-	3	-	-				25	-	-
Gliomastix cerealis (Karst.) Dickinson										-	-	2										-	-	2
Metschnikowia pulcherrima Pitt & Miller							-	3	-													-	3	-
Mucor hiemalis Wehmer	-	20	-																11	20	20	11	40	20
Mucor racemosus Fres.	-	10	-										-	10	-							-	20	-
Nigrospora anamorph of **Khuskia cryzae** Hudson																5	-	-				5	-	-

	JAN			FEB			MARCH			APRIL			MAY			JUNE			JULY			TOTAL		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Oidiodendron cerealis (Thum.) Barron	-	4	-				-	-	5	-	3	-										-	7	5
Penicillium aurantio-candidum Dierckx	-	10	-	-	10	-	3	20	1	10	-	-							1	-	-	14	40	1
P. brevi-compactum Dierckx	5	1	-	16	-	-				-	3	-										21	4	-
P. charlesii Smith	3	-	-																10	-	-	13	-	-
P. chrysogenum Thom	-	1	-	-	1	-										1	-	-				1	2	-
P. decumbens Thom	-	5	-	1	-	-																1	5	-
P. digitatum Saccardo							-	-	5	-	3	-							1	-	-	1	3	5
P. expansum Link	-	1	-																			-	1	-
P. frequentans Westling	1	5	-	6	-	-							5	-	-				1	-	-	13	5	-
P. funiculosum Thom																1	-	-				1	-	-
P. purpurogenum Stoll										-	-	5										-	-	5
P. variabile Sopp	10	-	-	-	2	-	-	4	1													10	6	1
P. velutinum van Beyma	2	-	-							1	-	-	1	-	-							4	-	-
Penicillium sp.	3	-	-	2	-	-	1	1	-	1	4	-				4	-	-	4	4	-	15	9	-
Phoma herbarum Westd.							-	1	-	-	1	-	1	-	-	4	10	-				5	12	-
Rhizopus oryzae Went & Prinsen Geerling	10	-	-	10	-	-							10	-	-	2	-	-				32	-	-
Rh. stolonifer (Ehrenb.: Fr.) Lind.	10	-	-	2	-	-	10	-	-	3	-	10										25	-	10
Rhodotorula graminis Di Menna							3	2	-	1	-	-	15	-	-	-	10	-	1	-	-	20	12	-
R. pallida Lodder																6	1	-				6	1	-

	JAN			FEB			MARCH			APRIL			MAY			JUNE			JULY			TOTAL		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
R. rubra (Demme) Lodder	1	3	-																			1	3	-
Septoria tritici Rob.	-	-	10																			-	-	10
Sporobolomyces roseus Kluyver & Van Niel	-	2	-				8	1	-	-	4	-										8	7	-
Torula graminis Desm.							-	-	10	3	-	-				-	10	-				3	10	10
T. herbarum Pers. ex Gray																			2	-	-	2	-	-
Torulopsis magnoliae Lodder & Kreger-van Rij	-	10	-							-	2	-										-	12	-
Trichocladium asperum Harz										1	-	-	10	-	-							11	-	-
Trichoderma viride Pers. ex Gray	1	-	-				2	-	-	10	-	-										13	-	-
Trichurus spiralis Hasselbr.							-	-	10	-	-	1	-	-	10							-	-	21
Ulocladium alternariae (Cooke) Simmons	1	-	-																2	-	-	3	-	-
Verticillium tenerum (Nees ex Pers.) Link.										-	-	1	-	-	3							-	-	4
Sterile cultures										5	-	-	7	-	-	1	-	-				13	-	-

TABLE 2

AVERAGE SPORE CONCENTRATION PER m^3

	All genera	Alternaria	Cladosporium	Epicoccum	Fusarium
October	6242	210	3342	183	541
November	3767	53	2801	105	140
December	1101	11	814	17	92
January	519	0	404	4	1
February	576	4	211	7	38
March	517	2	256	3	64
April	798	7	244	3	304
May	4253	16	2960	9	710
June	14872	25	11797	106	2496
July	10293	232	7441	308	1870
August	8370	214	5908	212	1120
September	8295	399	4213	166	639

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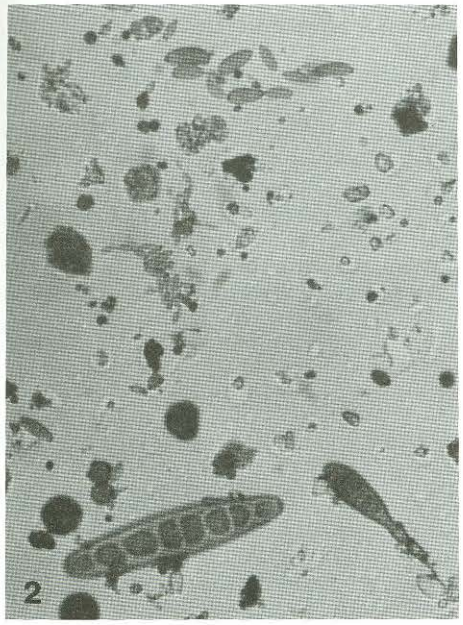

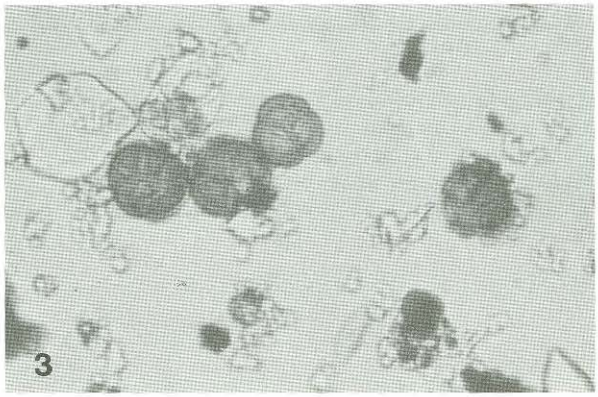

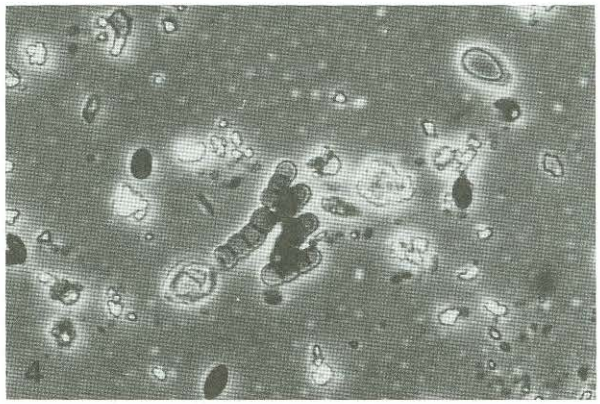

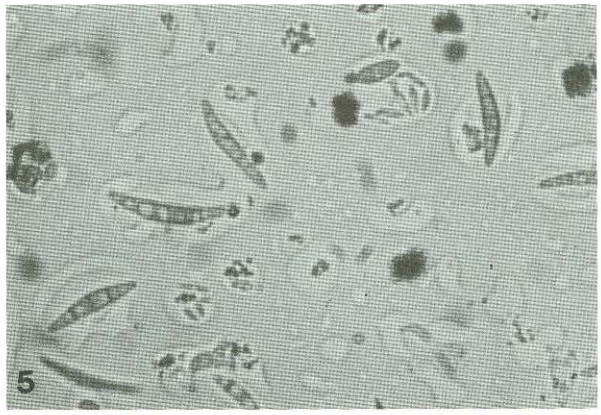

Fungal spores of the Form Genera recognized in deposits by impaction. Fig. 1: **Alternaria** 400X; Fig. 2: **Drechslera** and **Cladosporium** 400X; Fig. 3: **Epicoccum** 400X; Fig. 4: **Torula** and **Ganoderma** 400X; Fig. 5 **Fusarium** 250X.