IDENTIFICATION AND VIABILITY OF AIRBORNE HYPHAL FRAGMENTS

by 6408

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TABLE OF CONTENTS

INTRODUCTION

Much work has been done in the study of airborne fungi since the early studies of Miquel (1883). Investigations have been concerned primarily with the fungus spore content of the atmosphere. The presence of numerous small hyphal fragments in the air was largely overlooked until the recent studies of Pady and Kramer (1960), Pady and Gregory (1963), and Harvey (1970). However, little work has been done in an attempt to determine the relative importance of these hyphal fragments as airborne propagative units. Therefore, the present investigations were mainly concerned with the concentration of hyphal fragments in the air under varying environmental conditions, their isolation, germination, culture of colonies developed from single isolated hyphal fragments, and identification. Some attempt has also been made to interpret the question of viability of these hyphal fragments in the atmosphere.

REVIEW OF LITERATURE

The studies of airborne fungi were begun by Miquel (1883) in the city of Paris. These and other early studies were primarily concerned with the spore content of the atmosphere. Although hyphal fragments were mentioned from time to time, comparatively little attention has been paid to them as an important component of the air-spora until recently.

Hyphal fragments have been reported from the upper atmosphere by a number of workers. Perhaps, the first was that of Meier (1935), who found them on slides exposed by Lindbergh on his trip over the arctic in 1933. Newman (1948), reported the presence of a few hyphae, probably of sooty mold fungi, collected during his journey on an aeroplane from New Zealand to the United Kingdom in 1947. Pady and co-workers (Pady and Kapica, 1953; Kelly and Pady, 1953), reported the presence of hyphal fragments over various arctic and non-arctic parts of Canada. The high numbers encountered, up to $46/M^3$ of air, were surprising. Many of the hyphae were actually conidiophores of the Alternaria-type, occurring in small clumps. Later, Pady and Kapica (1955), again reported that hyphal fragments were present in the air during cross Atlantic flights from Montreal, Canada to London, England. They were especially abundant in tropical air in concentrations up to $48/M^3$, but were almost completely absent in the polar air. In a recent study, Kramer et al (unpublished), reported them to comprise an important part of the air biota of the upper atmosphere over the Pacific and the continental United States.

Sreeramulu (1958), recorded the presence of the hyphal fragments in a series of exposures over the Mediterranian sea from the deck of a ship enroute from London to India. The average number of hyphae on the 14 slides

was 3.7/M^3 as compared to 55.8 fungus spores/M^3. The highest number of hyphae was 10.2/M^3 obtained near Gibralter as compared to 106/M^3 fungal spore concentration.

Kramer et al (unpublished), during surface level trapping of air biota on the Pacific ocean, found hyphal fragments to comprise one of the principal forms of biota in marine air. The numbers of hyphal fragments collected throughout all three cruises were comparatively high. They comprised 3% of the total biota collected during a 44 day cruise from San Diego, California to Manzanillo, Mexico; 9% of the total biota during 46 days on the seas of Japan and Okhotsk; and they occurred on 14 of 26 days at sea between Tahiti and Balboa, Panama Canal zone.

There have been numerous reports of hyphal fragments in the air at surface level over terrestrial areas. Last (1956), collected them at Rothamsted, England. Pady (1957), found them to constitute one of the major components of the air-spora in Kansas ranging from 2-15/M^3 of air. In a continuation of this study, Pady and Kramer (1960), exposed silicone slides for 30 minutes each day over a two year period on the roof of a campus building at Manhattan, Kansas. As a result of this survey, hyphal fragments were found to be present in the air throughout the year with highest numbers occurring during the growing season. Numbers in winter varied from 35 to 212/M^3, and in summer from 177-1800/M^3. Fragments varied in size and length, mostly from 5-12u, occasionally to 100u. The majority were brown, septate, and thick walled, but hyaline ones were also present. Viability ranged from 29-80%. In a preliminary study, single hyphal isolates produced colonies of Cladosporium, Alternaria, and Penicillium.

Hamilton (1959), reported the occurrence of fungus conidiophores and hyphae in the air throughout a 5 month survey in London. There was a well defined daily periodicity in the number of mycelial fragments in the air with maxima occurring between 1500-1700 hours. A similar pattern was also reported by Pady and Gregory (1963) in a study at Rothamsted, England. Kramer et al (1964) reported the occurrence of abundant numbers of hyphal fragments during prolonged dry periods which allowed them to become fragile and more easily detached and carried into the air. In recent studies at Cardiff, Harvey (1970), showed that these hyphal fragments are common constituents of the air-spora over both land and sea. The recorded concentrations of hyphal fragments were generally low. He mentioned the importance of climatic factors, temperature, rainfall and wind, in determining fluctuations in concentration. Estimation of viability was still problematic. He further emphasized that release of hyphal fragments into the air is entirely passive and wind is principally responsible for their erosion from plant and soil surface.

Lacey (1962), sampled the air-spora of two adjacent rural sites, half a meter above ground. One of the sites was in a valley near a stream and the other on an exposed hill top in Berkshire. The mycelial fragments usually consisted of broken pieces of conidiophores of Cladosporium. The difference between the two sites was striking for fungal spores, pollen etc., but the average percentage of mycelial fragments caught in the two areas was 0.1% and 0.3% respectively. From the differences between the two sites (450 meter apart), it was evident that local ecology of an area has a major influence on its air-spora through local flora and microclimate.

There have been several reports of hyphal fragments germinating on

exposed slides that were incubated (Pady and Kramer, 1960). Pady and Gregory (1963), in their Rothamsted survey, studied the number and viability of airborne hyphal fragments. According to them, concentration of hyphal fragments ranged from 10-599/M^3. Germination averaged 16.2% on silicone slides, whereas, on glycerine jelly, germination was irregular but averaged 8.6%.

With the exception of the study made by Lacey (1962), little information is available on the air-spora, including hyphal fragments of two contrasting ecological sites located near one another. The present work was designed to study the differences in concentration and viability of hyphal fragments at a grassland and woodland site located near one another. Single fragment isolations were made in order that colonies could be developed and identifications made.

MATERIALS AND METHODS

Sampling Sites. Two sampling sites were selected for this study. They were situated 6.6 miles north of Manhattan, Kansas, on the east side of Tuttle Creek Reservoir on the premises of the Elks Club private recreation area. This reservoir is approximately one mile across in this area. The first site was located at the base of a wooded ravine with canopy of deciduous trees, primarily oaks and elms. This site was approximately 1090 ft above sea level and 2500 ft east and downwind from the direction of prevailing winds and from the main body of Tuttle Creek Reservoir. This location is designated as the woodland site. The second site was located on the crest of a hill approximately 1240 ft above sea level, in a typical tall grass prairie 1500 ft southeast and overlooking the woodland site. This location is designated as the prairie site.

Sampling Techniques. Samples were taken regularly during a 4 month period from July to October 1970, usually in a series of 4-5 consecutive days each week. For collecting samples, two Kramer-Collins spore samplers (Kramer and Pady, 1960) were used throughout this study (Figs. 1 & 2). The Kramer-Collins spore sampler is an automatically operated impinging type sampler that deposits spores and hyphal fragments in hourly bands on silicone-coated microscope slides.

At both locations, Kramer-Collins samplers were placed on a box with the intake tube 2 ft above ground. Each sampler was equipped with a directional intake tube so that the orifice always pointed into the wind. Slides were changed daily between 10-10:30 in the morning. Exposed slides were kept dry in a plastic slide box until isolation of hyphal fragments was completed. This was done during the afternoon of the day on which the slides were changed.

Fig. 1. Kramer-Collins Sampler set up at the woodland site.

Fig. 1

Fig. 2. Kramer-Collins Sampler set up at the prairie site.

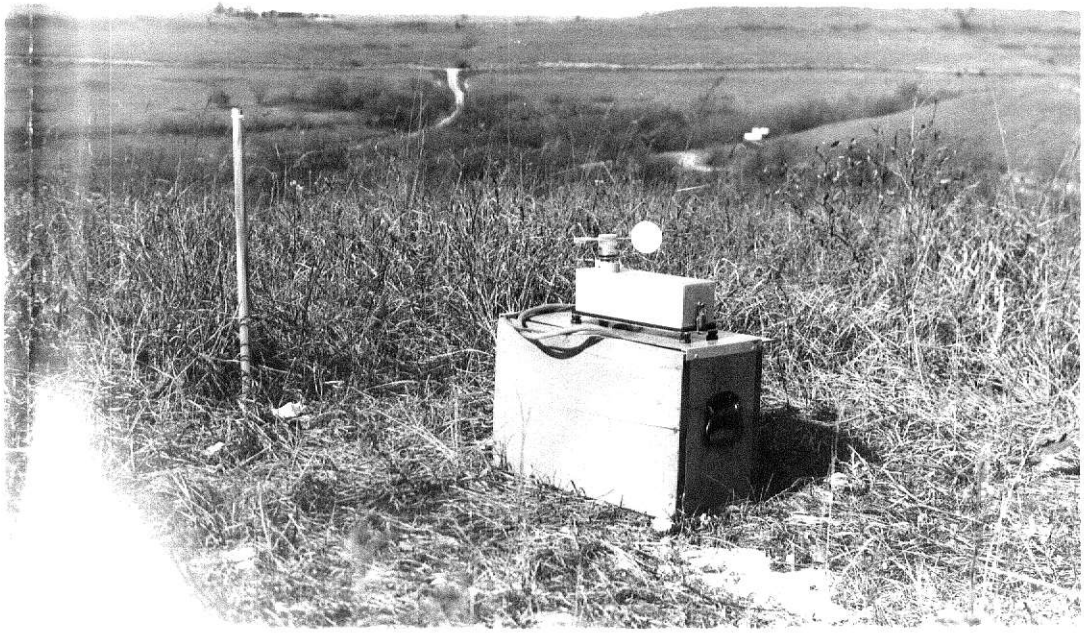

Fig. 2

Maximum and minimum temperatures and relative humidity were recorded daily.

Isolation and Germination of Hyphal Fragments. In the laboratory, each slide was examined under a Wild dissecting microscope. Hyphal fragments, particularly dematiaceous forms, which were easier to find with the dissecting microscope, were picked up with a microneedle and transferred to a petri dish of freshly poured water agar. Care was taken to pick up fragments from each band on the slide. Each fragment was moved repeatedly over the surface of the agar until it appeared clean of contaminating spores. Each fragment was then transferred to another clean petri dish containing Difco Potato-Dextrose medium. The position where each hyphal fragment was placed on the medium, was marked on the underside of the petri dish in order that it could easily be detected to check for germination. Usually 12-24 hyphal fragments were isolated from each slide every day. At least 6 fragments were placed equidistant from each other on each plate which was then incubated at room temperature. After 48 hours, each fragment was examined under the microscope to determine if germination had occurred and to check for contamination. These were then allowed to develop colonies.

Colonies developed from germinating fragments were examined daily after the formation of mycelium to the sporulating stage. The measurements of the colony, size, shape and color were studied. Each colony was transferred to a new petri dish in order to maintain pure culture. Transfers were then made from these plates to PDA slants in order that specific identifications could be made at a later time.

After sufficient fragments had been isolated from the exposed slides, these slides were sprayed with water and placed in a moist chamber at room temperature for 24 hours to allow the remaining fragments to germinate. After

incubation, the slides were dried at room temperature. They were later mounted in lactophenol-cotton blue for reading. Counts of germinated and non-germinated hyphal fragments were made for both dematiaceous and hyaline forms.

In an attempt to determine the potential maximum percentage viability of hyphal fragments, colonies of Cladosporium, Alternaria, Helminthosporium etc., were grown on cellophane over PDA. The cellophane pieces with sporulating colonies were then allowed to dry at room temperature for varying lengths of time before testing. Periodically, portions of these colonies were scraped off with the help of an inoculating needle and streaked on fresh PDA plates. The plates were then incubated for 24 hours at room temperature. Counts of germinated and non-germinated fragments were also made of a few young colonies prior to the sporulating stage. Thus, the percentage germination and viability were estimated for the fragments dried in the laboratory.

RESULTS

Five genera of fungi were identified from isolated hyphal fragments. Of these, Cladosporium comprised 62.9% of the total. These isolates were either identified as C. cladosporioides (Fres.) DeVries or C. herbarum (Pers.) Link (Table I). Other genera included Alternaria (30.6%), sterile mycelial colonies (2.2%), Helminthosporium (1.7%), Penicillium (1.7%), and Curvularia (0.9%). There were no significant differences in the occurrence of any of the genera isolated at the two sites.

Hyphal fragments ranged in length from 5-20u, however, a few of them were as long as 100u or more. They were often branched and either septate or non-septate. The majority of them were dematiaceous but hyaline ones were also present. Most of them were thought to be portions of conidiophores.

Germination of hyphal fragments occurred at one or both ends by means of germ tubes emerging through the septal walls (Figs. 3 & 4). Lateral germination with germ tubes emerging through the side walls of the fragments was not observed. Germination on slides often produced germ tubes that gave rise directly to conidiophores producing clusters of spores.

Hyphal fragments which were caught on all 43 sampling days from July to October 1970, ranged from the lowest numbers in August with averages of $7.5/M^3$ at the woodland site and $7.1/M^3$ at the prairie site to the highest numbers in September of $14.1/M^3$ and $15.2/M^3$ respectively. However, there was considerable day to day variation despite their consistent occurrence. In order to obtain information on the germination of hyphal fragments collected at the two sites, counts were made from exposed slides that had been incubated in a moist chamber (Table II). There were no significant differences in the germination of hyphal fragments collected at the woodland

Fig. 3. Photomicrograph of germinating hyphal fragment. Note the germ tube emerging from one end only. (Magnification - 1647X)

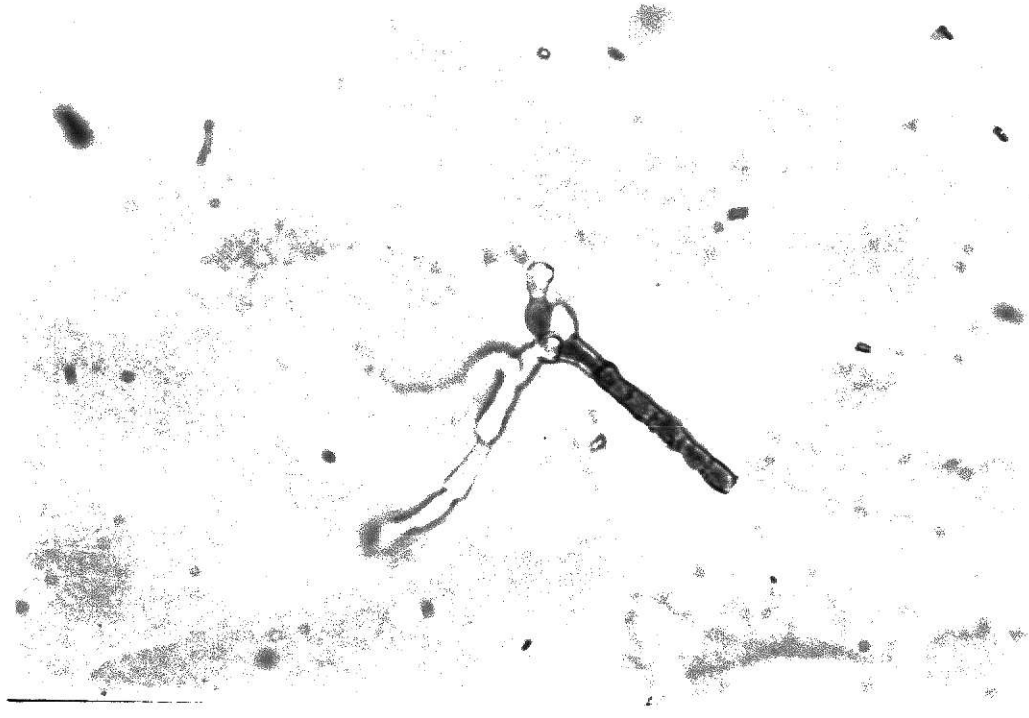

Fig. 3

Fig. 4. Photomicrograph of germinating hyphal fragment. Note the germ tubes emerging from both the ends. (Magnification - 1647X)

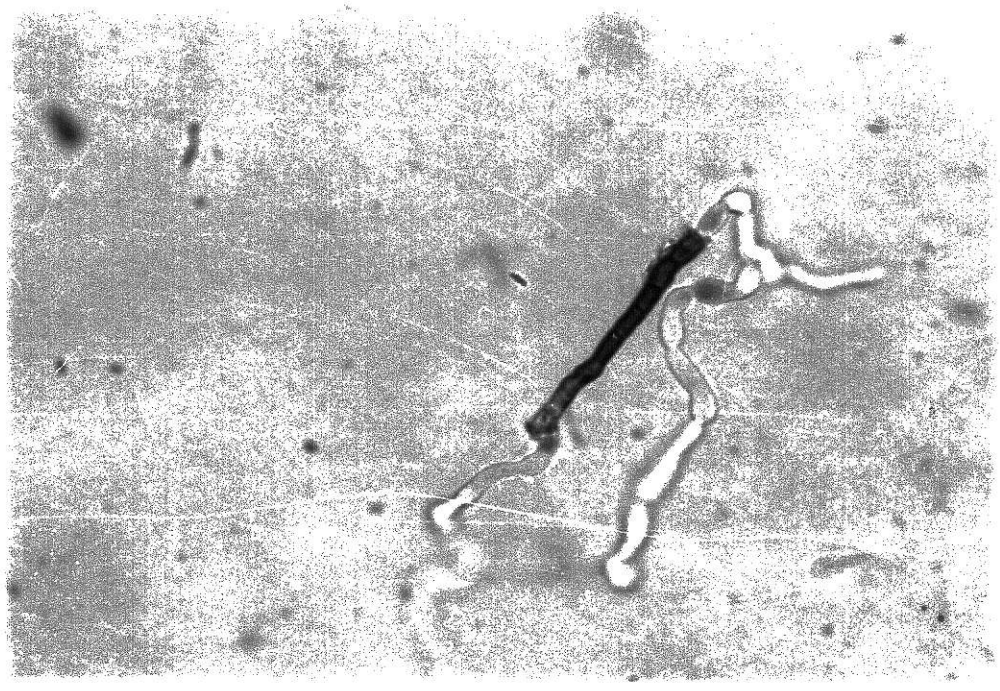

Fig. 4

TABLE I

Identification of Fungal Colonies Developed from Isolated Hyphal Fragments in Woodland (W) and Prairie (P) Sites. July - October, 1970.

	July		August		September		October		Total Colonies	Percentage of Total Colonies
	W	P	W	P	W	P	W	P	Isolated	Identified
Cladosporium	10	16	2	1	35	39	19	22	144	
C. clodosporioides	(8)	(13)	(2)	(1)	(30)	(35)	(17)	(19)		62.9%
C. herbarum	(2)	(3)	-	-	(5)	(4)	(2)	(3)		
Alternaria	6	-	-	3	21	10	15	15	70	30.6%
Helminthosporium	1	2	1	-	-	-	-	-	4	1.7%
Penicillium	2	-	-	-	1	1	-	-	4	1.7%
Curvularia	1	-	-	-	1	-	-	-	2	0.9%
Sterile colonies	2	1	-	-	-	2	-	-	5	2.2%
	Total Number of Colonies Identified								229	100
	W	P	W	P	W	P	W	P		
Total Numbers of Hyphal Fragments Isolated During 43 Sampling Days	140	120	96	96	160	152	137	139	1040	

and prairie sites. There was also no significant difference in the germination of pigmented and hyaline hyphal fragments. There were differences, however, in the germination of fragments at different times of the growing season. During August which was extremely hot and dry, the percentage of germinated hyphal fragments was low as compared to other months, especially September. This was a month when temperature and available moisture were suitable for the growth and development of fungi in the surrounding areas. It was during this time that the highest rate, 50.3% of germination was obtained. The average percentage germination of pigmented fragments for 43 sampling days at both the locations was 21.4%. From 3847 fragments counted, 826 germinated (Table II). Likewise, the average percentage germination of hyaline fragments at both the locations was 26.7%. From 1253 hyaline fragments counted on the slides, 335 germinated. The average percentage germination of both pigmented and hyaline hyphal fragments at both the locations was 22.7%.

Potential percentage viability of hyphal fragments has been recorded in Table III. Of 1390 hyphal fragments counted, over approximately 48 consecutive days, 566 germinated and formed colonies. Thus the potential percentage viability estimated for this period averaged approximately 41%.

TABLE II

Germination of Pigmented and Hyaline Hyphal Fragments Incubated Directly on Silicone-coated Slides. July - October, 1970.

			PIGMENTED			HYALINE			
Month	Number Days Sampled	Location	Number Counted	Number Germinated	Percentage Germination	Number Counted	Number Germinated	Percentage Germination	Total Percentage Germination
July	13	Woodland	387	80	20.6%	270	69	25.5%	22.8%
		Prairie	591	108	18.2%	298	77	25.8%	20.8%
August	8	Woodland	273	41	15.0%	117	16	13.6%	14.6%
		Prairie	268	50	18.6%	98	10	10.2%	16.3%
September	10	Woodland	668	178	26.6%	135	68	50.3%	30.6%
		Prairie	746	144	19.3%	142	52	36.6%	22.1%
October	12	Woodland	411	109	26.5%	107	24	22.4%	25.7%
		Prairie	503	116	23.0%	86	19	22.0%	23.0%
	Total 43		Total Counted 3847	Total Germinated 826	Average 21.4%	Total Counted 1253	Total Germinated 335	Average 26.7%	Average of Total 22.7%

TABLE III

Potential Maximum Percentage Viability of Hyphal Fragments Dried and Germinated at Room Temperature from January 11 - February 27, 1971.

Date	Number Counted	Number Germinated	Percentage Germination
January 12	134	61	44.5%
January 13	142	57	40.1%
January 15	108	35	32.4%
January 18	167	60	35.9%
January 26	120	44	36.6%
February 3	142	59	41.5%
February 13	184	74	40.2%
February 20	194	83	42.7%
February 27	199	93	46.7%
	Total Number Counted 1390	Total Number Germinated 566	Average Percentage Germination 40.1%

DISCUSSION

Hyphal fragments are the broken pieces of fungal mycelium, primarily conidiophores, which occur in the atmosphere. They occur wherever fungal spores are present. They are most common during the growing season when climatic factors are favourable for the development of fungi with aerial sporulating structures. During hot summer months (July-August), and cold winter months (November-January), the concentration of these hyphal fragments decreases considerably. In recent studies at Cardiff, Harvey (1970), mentioned the importance of climatic factors, such as, temperature, rainfall and wind in determining fluctuations in concentration. He emphasized that release of hyphal fragments into the air is entirely passive and wind is principally responsible for their erosion from plant and soil surfaces.

The frequency of all the genera identified from both the locations was similar (Table I). From the differences between the two sites, it is evident that local ecology of an area has a major influence on the distribution and concentration of hyphal fragments through local flora and microclimate as pointed out by Lacey (1962).

From the single hyphal fragment isolations, Cladosporium was the most frequently isolated fungus. This is consistent with studies of air-spora in the northern parts of the world done at ground level (Kelly and Pady, 1953; Last, 1956; Pady and Kramer, 1960; Lacey, 1962; and Harvey, 1970), at high altitudes (Kelly and Pady, 1953; Pady and Kapica, 1953, 1955), or at surface level over the oceans (Sreeramulu, 1958; and Kramer et al, unpublished). Two species, C. cladosporioides (Fres.) DeVries and C. herbarum (Pers.) Link were consistently identified from the isolates. Alternaria,

Helminthosporium, Penicillium and Curvularia, which were identified from the single fragment isolations, also have been found to be common constituents of the air-spora by numerous workers throughout the world (Pady and Kramer, 1960; Hirst, 1953; Pady and Gregory, 1963; Harvey, 1970; Kramer et al, unpublished).

The percentage germination of both pigmented and hyaline hyphal fragments on exposed slides varied according to the climatic conditions (Table II). The highest percentage was in September with 30.6% at the woodland and 22.1% at the prairie site. The greater percentage germination in September at both the locations is attributed to the favourable climatic conditions (optimum temperature 65-85°C, and moderate rainfall), during which more hyphal fragments are produced and released into the air. The lowest percentage germination was in August with 14.6% at the woodland and 16.3% at the prairie site. This is probably because of the high temperature range (89-105°C), with practically little or no precipitation. These conditions would induce desiccation of the protoplasm, hence retarding germination. Although, there was some difference in percentage germination of pigmented and hyaline hyphal fragments, this difference was not considered to be significant.

The question of how long these hyphal fragments remain viable in the atmosphere and function as propagative units, still remains a problem. An attempt has been made to obtain some information on this problem by artificially obtaining fragments from viable cultures. Assuming that the maximum potential viability of hyphal fragments would be represented by the percentages obtained in the laboratory, only 41% of these hyphal fragments are potentially viable (Table III). Under the less favourable field conditions,

it is assumed that the percentage would be lower. Since the average percentage germination of both the pigmented and hyaline fragments under natural conditions at both the locations was 22.7% (Table II), it suggests that there is an approximate 44% loss in viability of these hyphal fragments in the general population of the air-spora.

SUMMARY

Hyphal fragments were collected with a Kramer-Collins spore sampler at a prairie and a woodland site located near one another from July to October, 1970. Colonies developed from isolated hyphal fragments were: Cladosporium, Alternaria, Helminthosporium, Penicillium, Curvularia, and a few sterile mycelial types. Cladosporium was the most common fungus. Two species of Cladosporium were identified as C. cladosporioides (Fres.) DeVries and C. herbarum (Pers.) Link.

Concentrations ranged from $7.4/M^3$ at the woodland and $7.1/M^3$ at the prairie site in August to $14.1/M^3$ at the woodland and $15.2/M^3$ at the prairie site in September. August was an exceptionally hot and dry month while September was more favourable for the growth and development of fungi.

There was little difference in composition of hyphal fragments at both the locations. The percentage germination of both hyaline and pigmented fragments on exposed slides was 26.7% for hyaline and 21.4% for pigmented at both locations. The average percentage germination of pigmented and hyaline hyphal fragments at both locations was 22.7%. Thus, there was also no significant difference in percentage germination of pigmented and hyaline fragments at both locations.

The potential percentage germination, as studied at room temperature in the laboratory, averaged 41%. This suggested an approximate 44% loss in the viability of hyphal fragments in the atmosphere.

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ABSTRACT

Studies of the concentration, identification and viability of hyphal fragments in the air were made at two ecologically different sites; one on an exposed prairie hill top, the other in a wooded ravine at the base of the hill. Samples were taken daily with a Kramer-Collins spore sampler. Hyphal fragments were caught on all 43 sampling days from July to October, 1970. These ranged from the lowest numbers in August with averages of $7.4/M^3$ at the woodland site and $7.1/M^3$ at the prairie site, to the highest numbers in September of $14.1/M^3$ and $15.2/M^3$ respectively.

Hyphal fragments were isolated each day from slides exposed at both locations by picking off with a microneedle and transferring to a plate of Potato-Dextrose agar. Fragments were cleaned of contamination by repeatedly moving them across the surface of the agar and finally to a new position. From 12-24, such fragments were isolated each day from each location. Only pigmented hyphal fragments that ranged in length from 5-2 u (occasionally 100u or more), were isolated in this manner. Germination occurred either at one or both ends. Colonies developed from these were: Cladosporium, Alternaria, Helminthosporium, Penicillium, Curvularia and a few sterile mycelial forms. Two species of Cladosporium were identified as C. cladosporioides (Fres.) DeVries, and C. herbarum (Pers.) Link. The most common fungi isolated from both locations were Cladosporium (62.9%) and Alternaria (30.5%).

Exposed slides were also incubated to allow the hyphal fragments to germinate directly on the slides. With these preparations, it was possible to obtain percentages of germination of a large number of both hyaline

and pigmented hyphal fragments. These were 21.4% for pigmented fragments at both locations and 26.7% for hyaline fragments at both locations.

In order to compare these figures with a potential maximum percentage viability, hyphal fragments from freshly grown colonies were incubated on nutrient plates at room temperature. An average of 41% of these germinated, indicating an approximate 44% loss in viability of hyphal fragments in the atmosphere.

With the exception of the study made by Lacey (1962), little information is available on the air-spora, including hyphal fragments of two contrasting ecological sites, located near one another. The present work was designed to study the difference in composition, concentration and viability of hyphal fragments in two such sites under varying environmental conditions. There were no striking differences in either the composition, concentration, or viability of hyphal fragments between the two sites.