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The aerobiological significance of smut spores in Tulsa, Oklahoma

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Abstract

Few aerobiological studies have focused on smut spores, teliospores of fungi within the order Ustilaginales, but the scientific literature provides evidence of the potential aerobiological significance of these plant pathogens. The atmosphere in Tulsa, Oklahoma was monitored for the presence of smut teliospores using a Burkard Volumetric Spore Trap. Smut spores were identified in the atmospheric samples every day from May to October during 1991 and 1992 at concentrations that were normally below 1000 spores/m^3. The peak concentration observed during this study was almost 6000 spores/m^3. Daily concentrations fluctuate due to a variety of factors such as precipitation, relative humidity, percent sunshine, and the phenology of fungi in relation to their hosts. In northeastern Oklahoma, the most prevalent species of smuts in the atmosphere during the spring include *Sphacelotheca occidentalis*, *Ustilago tritici*, and *U. kolleri*. In the fall, spores of *U. brumivora*, *U. bullata*, and *U. maydis* are more common.

Keywords: Aerobiology; Smut spores; Teliospores; Ustilaginales

1. Introduction

Members of the order Ustilaginales, smuts, create black, dusty spore masses similar to soot on the plants they infect (Alexopoulos and Mims, 1979). There are approximately 1200 species of smuts within 50 genera (Vanky, 1987). The majority of species are classified into two large genera, *Tilletia* and *Ustilago*. Smuts are serious plant pathogens on cereal crops, causing millions of dollars in crop damage each year (Alexopoulos and Mims, 1979). Many native grasses and other plants are also affected, and the number of hosts for smuts approaches nearly 4000 species. Teliospores are the characteristic asexual spores of members of Ustilaginales. Typically, teliospores are globose with smooth, echinate, or reticulate sculpting patterns; they have yellow to brown pigmented walls and are dispersed by wind (Alexopoulos and Mims, 1979).

The season for teliospore production varies for the different smuts. Hamilton (1959) reported that airborne spores of *Tilletia* peaked in late August and early September while *Ustilago* spores climaxed between mid-June and the end of July. As typical of most fungi, the airborne spore concentrations of these two genera depend on a number of meteorological factors. Levels decrease when conditions are rainy or extremely humid, and they increase during periods of brilliant sun, strong winds, and high barometric pressure (Hamilton, 1959). As members of the 'dry air spora', conditions such as low humidity levels and gusty winds enhance the spore dispersal mechanism employed by the smuts (Hirst, 1953; Levetin, 1995).

In addition to the aerobiological significance of teliospores as plant pathogens, it has been suggested that these spores could serve as potential aeroallergens. The atmosphere is often saturated with these fungal spores for an extended period of time. Also, individuals have skin-tested positive for these fungal allergens and have IgE antibodies to the antigens in their blood (Burge, 1985; Santilli et al., 1985). Although some smut

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extracts are routinely used for diagnosis and desensitization, further research in this area must be conducted in order to determine the full extent of the allergenicity and clinical significance of various smut species.

Several aerobiological studies have shown the presence of smut spores in the atmosphere of ecologically diverse locations. Halwagy (1989) reported that *Ustilago* species were the second most common fungal spore type in the Kuwait atmosphere. Shaheen (1992) identified *Tilletia* and *Ustilago* spores in the atmosphere of Jordan with the peak concentrations occurring during the summer. Mishra (1987) showed smut spores peaked during dry conditions of December and January in India, while Rubulis (1984) reported that smut spores peaked in late spring and fall in two cities in Sweden. In addition, the presence of airborne smut spores are routinely reported by members of the Aeroallergen Monitoring Network of the American Academy of Allergy and Immunology (AAAI, 1995). These previous studies generally lumped the smuts into one or two categories with no attempt to identify the species involved. The present study examined the airborne concentration of smut teliospores in the Tulsa atmosphere during 1991 and 1992 in order to identify the most abundant smut spores.

2. Materials and methods

2.1. Oklahoma smut species

Reference slides of the smut species native to Oklahoma were prepared from specimens housed in the herbarium of the Department of Plant Pathology at Oklahoma State University. Spores were scraped off the infected plant material and mounted in a drop of Lacto-phenol with polyvinyl alcohol (Levetin, 1989). The slides were allowed to dry at room temperature for 4–5 days until the mounting medium thoroughly hardened. They were then microscopically examined, and spores measured with a Nikon Labophot at 1000 ×. The average size of the spores of each reference sample was determined based on the measurements of six spores of each particular species.

2.2. Aerobiology

Atmospheric sampling in Tulsa, Oklahoma, was conducted using a Burkard Volumetric Spore Trap, located on the roof of a building on the campus of The University of Tulsa. The intake orifice of the sampler was approximately 12 m above ground. The samples were collected onto Melinex tape, which had been previously greased with a thin film of Lubriseal (Thomas Scientific, Swedesboro, NJ). The tape was changed every 7 days and then divided into 1-day segments, which were mounted on microscope slides. The slides were stained with a glycerin-jelly stain containing basic fuchsin and then analyzed using light microscopy (Levetin, 1991). All teliospores were enumerated on one sweep down the length of the slide, and counts were converted into atmospheric concentrations. Average daily concentrations of teliospores were determined for all days during the months of May through October of 1991 and 1992.

Meteorological data (average temperature, relative humidity, precipitation, wind speed, and percent sunshine) were obtained from the NOAA (National Oceanic and Atmospheric Administration) weather station in Tulsa which is located approximately 8 km northeast of the sampling station at the university. Spore concentrations during the peak month each year were correlated with meteorological conditions using a Pearson correlation with Statistix (version 3.5) Analytic Software.

Table 1
Morphological characteristics of the teliospores of *Ustilago* species identified on Oklahoma crops

Species size	Mean spore size (μm)	Spore morphology
U. avenae	6.3 × 5.4	Subglobose, light brown minutely punctate on thin wall area
U. boutelouae	9.5 × 8.3	Globose to subglobose, golden, asperulate
U. brumivora	8.9 × 7.9	Globose to subglobose, brown, rugose
U. bullata	10.6 × 9.5	Globose to subglobose, brown, rugose to verrucose
U. cynodontis	6.7 × 6.5	Globose to subglobose, golden, smooth
U. hordei	7.2 × 6.5	Globose to subglobose, golden brown, smooth
U. kolleri	7.0 × 6.7	Globose to subglobose, brown, smooth with thin wall area prominent
U. maydis	8.4 × 7.7	Globose to subglobose, golden, echinate
U. minima	4.1 × 3.7	Globose to subglobose, golden brown, smooth
U. nuda	6.5 × 5.8	Globose to subglobose, golden brown, minutely punctate especially on thin wall area
U. rabenhorstiana	11.6 × 9.6	Globose to subglobose, brown, asperulate
U. striaeformis	10.5 × 9.8	Globose to subglobose, golden, asperulate
U. syntherismae	11.0 × 9.1	Globose to subglobose, brown, punctate to verrucose
U. tritici	6.3 × 5.1	Subglobose, golden to brown, punctate on thin wall area

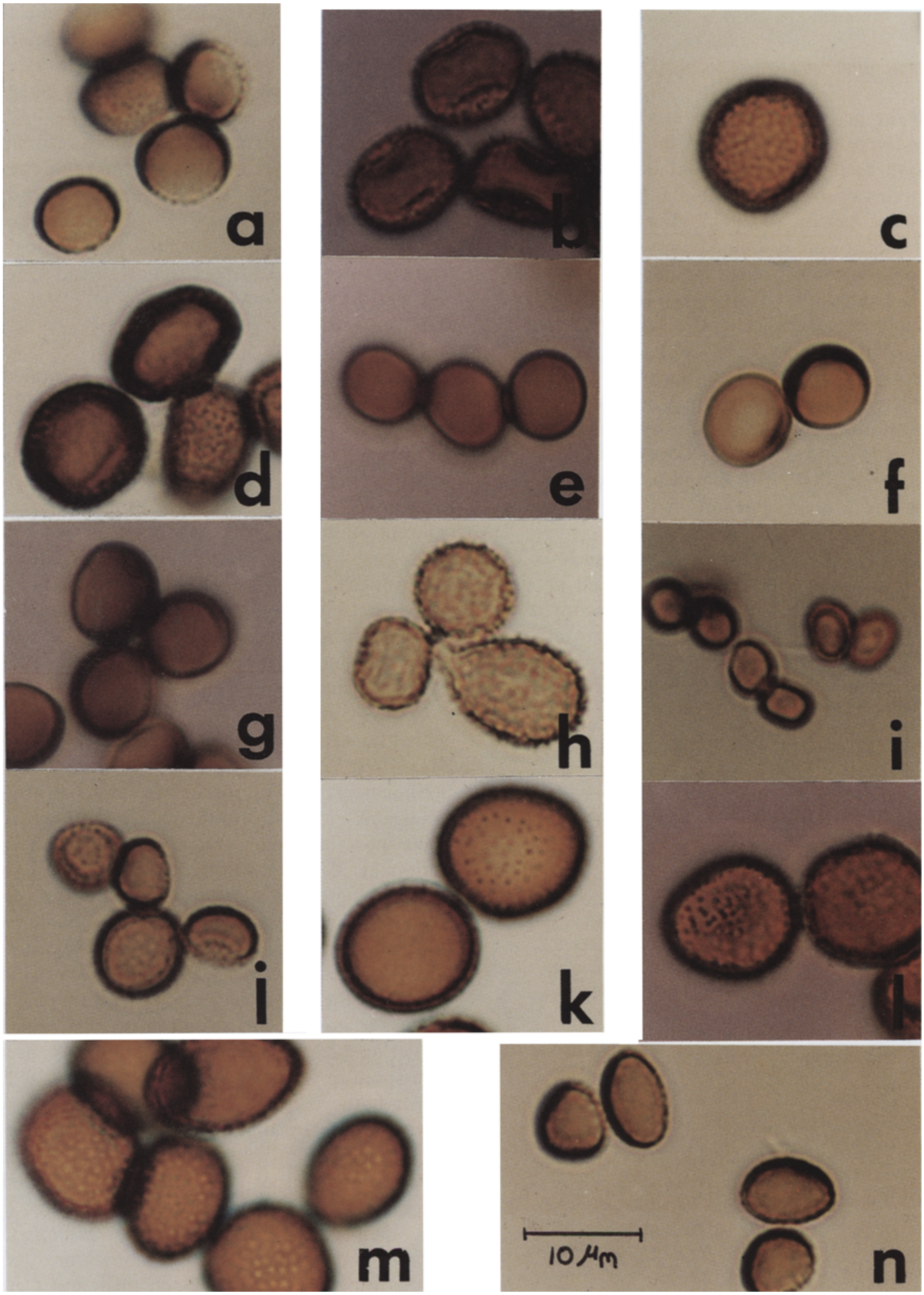

Fig. 1. Teliospores of *Ustilago* species identified on Oklahoma crops. (a) *U. avenae*, (b) *U. boutelouae*, (c) *U. brumivora*, (d) *U. bullata*, (e) *U. cynodontis*, (f) *U. hordei*, (g) *U. kolleri*, (h) *U. maydis*, (i) *U. minima*, (j) *U. nuda*, (k) *U. rabenhorstiana*, (l) *U. striaeformis*, (m) *U. syntherismae*, (n) *U. tritici* (Original magnification: × 500).

3. Results

3.1. Oklahoma smut species

Based on the herbarium records at Oklahoma State University's Department of Plant Pathology, 28 species of smuts have been recorded in Oklahoma. These include 14 species of *Ustilago* (Table 1, Fig. 1) and 12 species in other genera (Table 2, Fig. 2). Reference slides of all spores were examined, and spore sizes and morphology were recorded. *Ustilago* spores ranged in size from 3–4 μm up to 10–11 μm. In contrast, the spores of the other genera were often larger, ranging in size from 5–6 μm up to 22–24 μm. For all of the species studied, the distinguishing morphological characteristics recorded included spore shape, color, and wall ornamentation. The morphological features described in Tables 1 and 2 were based on examination at a magnification of 1000 ×. It should be noted that for some spores the wall ornamentation is not visible at lower magnifications.

The spore walls of a number of species, such as *U. avenae* and *U. kolleri*, have wall areas which appear thinner and less pigmented using light microscopy. For some species the surface morphology of the thin area also appears different than the rest of the spore. It was noted that many spores showed signs of collapse around these thinner areas changing the shape of the spore from globose to subglobose.

Although the herbarium specimens examined had confirmed identities, the spore morphology of the *Tilletia caries* does not match descriptions in the literature (Duran and Fischer, 1961; Alexopoulos and Mims, 1979). Based on spore morphology and host species, this smut appeared to be *Ustilago tritici*.

Table 2
Morphological characteristics of the teliospores of non-*Ustilago* species identified on Oklahoma crops

Species	Mean spore size (μm)	Spore morphology
Cintraetria taubertiana	12.6 × 10.3	Globose to subglobose, brown, smooth
Melanopsichium pennsylvanica	9.7 × 8.6	Globose to subglobose, golden brown, punctate
Sphacelotheca cruenta	8.3 × 7.9	Globose to subglobose, golden brown, smooth
Sphacelotheca occidentalis	13.9 × 12.6	Globose to subglobose, golden brown, asperulate
Sphacelotheca sorghi	8.9 × 7.8	Globose to subglobose, golden brown, minutely roughened
Sorosporium ellisi	15.2 × 14.1	Globose to subglobose, golden brown, punctate
Sorosporium sorghi	5.9 × 5.4	Globose to subglobose, golden brown, smooth
Sorosporium sytherismae	12.4 × 10.6	Globose to subglobose, golden brown, minutely roughened
Tilletia caries[a]	6.3 × 5.4	Subglobose, brown, punctate on thin wall area[a]
Tilletia foetida	16.3 × 15.0	Globose to subglobose, pale golden yellow, smooth
Tilletia laevis	17.0 × 15.2	Globose to subglobose, golden, smooth
Tilletia pulcherrima	24.3 × 22.0	Globose to subglobose, dark brown, rugose to warty

[a]Although the identification of this specimen had been verified as *Tilletia caries*, the spore characteristics do not match those in the literature. The authors believed that it is actually *Ustilago tritici*.

3.2. Aerobiology

Teliospores of Ustilaginales were identified on all slides from the Burkard sampler from May through October of 1991 and 1992 (Figs. 3 and 4). The omission of data on 2 July 1991 did not indicate the absence of teliospores in the atmosphere but rather an error in data collection. For 1991, the atmospheric teliospore concentration generally fell between 100 and 1000 spores/m^3 with the mean daily concentration for the season at 287 spores/m^3 (Fig. 3). Table 3 indicates that the average daily concentrations of teliospores were greatest during September and October while the lowest concentrations were noted in May and June. However, the single highest peak in the atmospheric concentration (1874 spores/m^3) occurred on 5 July 1991; the lowest concentration (6 spores/m^3) was reported on 29 May 1991.

Similarly, for 1992, the average range of atmospheric teliospore concentration remained between 100 and 1000 spores/m^3 with the daily mean for the 6-month period at 356 spores/m^3. Contrary to the data from 1991, the greatest atmospheric concentrations of teliospores occurred in May and October while the lowest concentrations appeared in June and August (Table 3, Fig. 4). These observations coincide with the occurrences of the single highest and lowest peaks during 1992. The highest concentration of 5906 spores/m^3 occurred on 12 May 1992 while the lowest concentration of 6 spores/m^3 was reported on 4 August 1992.

For both years, different species of smuts were observed at different times of the year. During the months of May and June, a significant portion of the smut spore component of the atmosphere belonged to the species which infect Bermuda grass (*Ustilago cynodontis*), Johnson grass (*Sphacelotheca occidentalis*), oat (*U. kolleri*), and wheat (*U. tritici*). Smut spores recognized in the atmosphere during September and October included those which attack corn, (*U. maydis*) and several grasses native to Oklahoma (*U. brumivora* and *U. bullata*).

Meteorological variables and smut spore concentrations were correlated during October each year, the month with the peak concentrations (Table 4). The

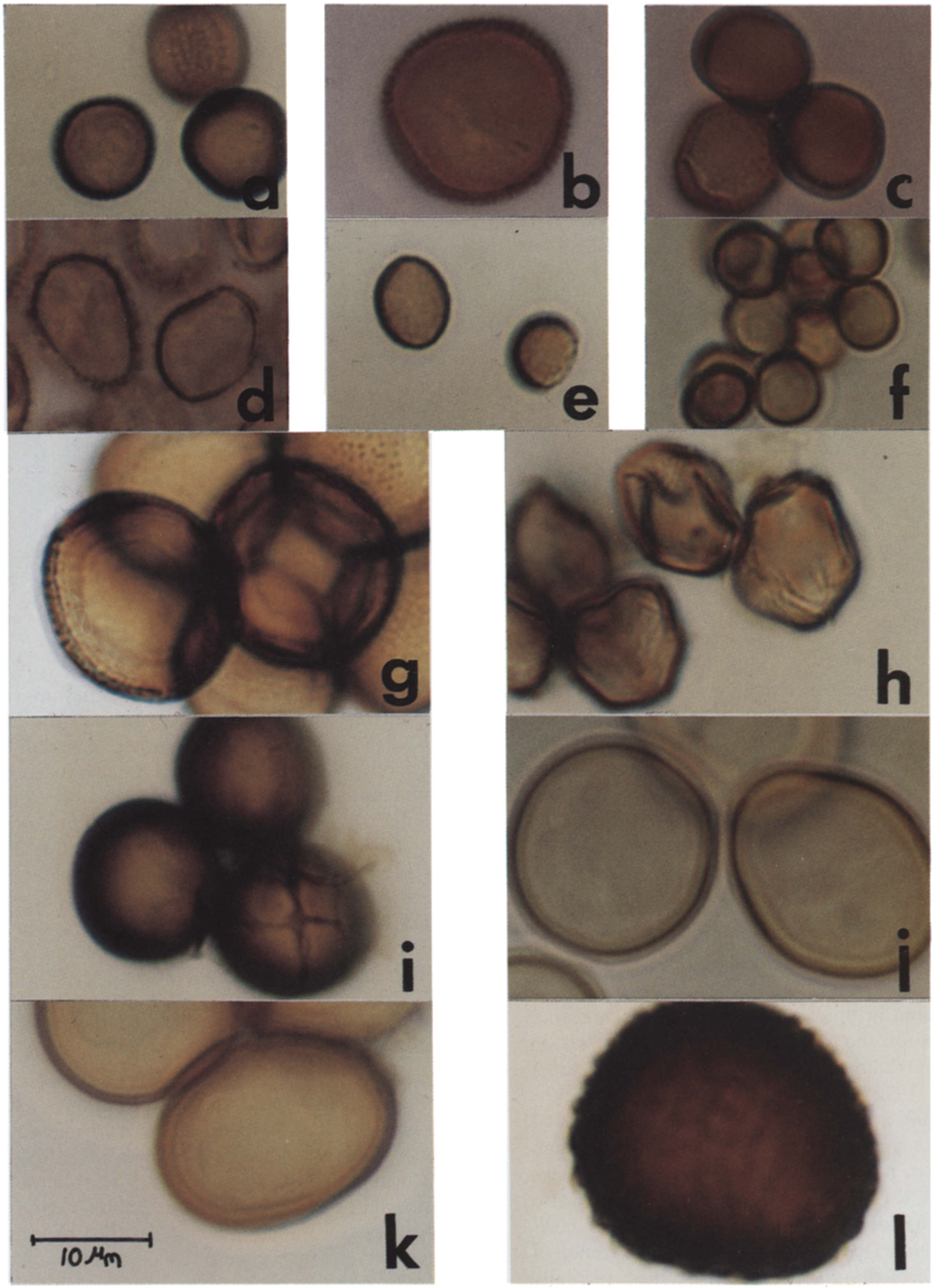

Fig. 2. Teliospores of non-*Ustilago* smut species identified on Oklahoma crops. (a) *Sphacelotheca cruenta*, (b) *Sphacelotheca occidentalis*, (c) *Sphacelotheca sorghi*, (d) *Melanopsichium pennsylvanica*, (e) *Tilletia caries* (Note: Although the identification of this specimen had been verified as *Tilletia caries*, the spore characteristics do not match those in the literature. The authors believed that it is actually *Ustilago tritici*.), (f) *Sorosporium sorghi*, (g) *Sorosporium ellisi*, (h) *Sorosporium syntherismae*, (i) *Cintraetria taubertiana*, (j) *Tilletia foetida*, (k) *Tilletia laevis*, (l) *Tilletia pulcherrima* (Original magnification: × 500).

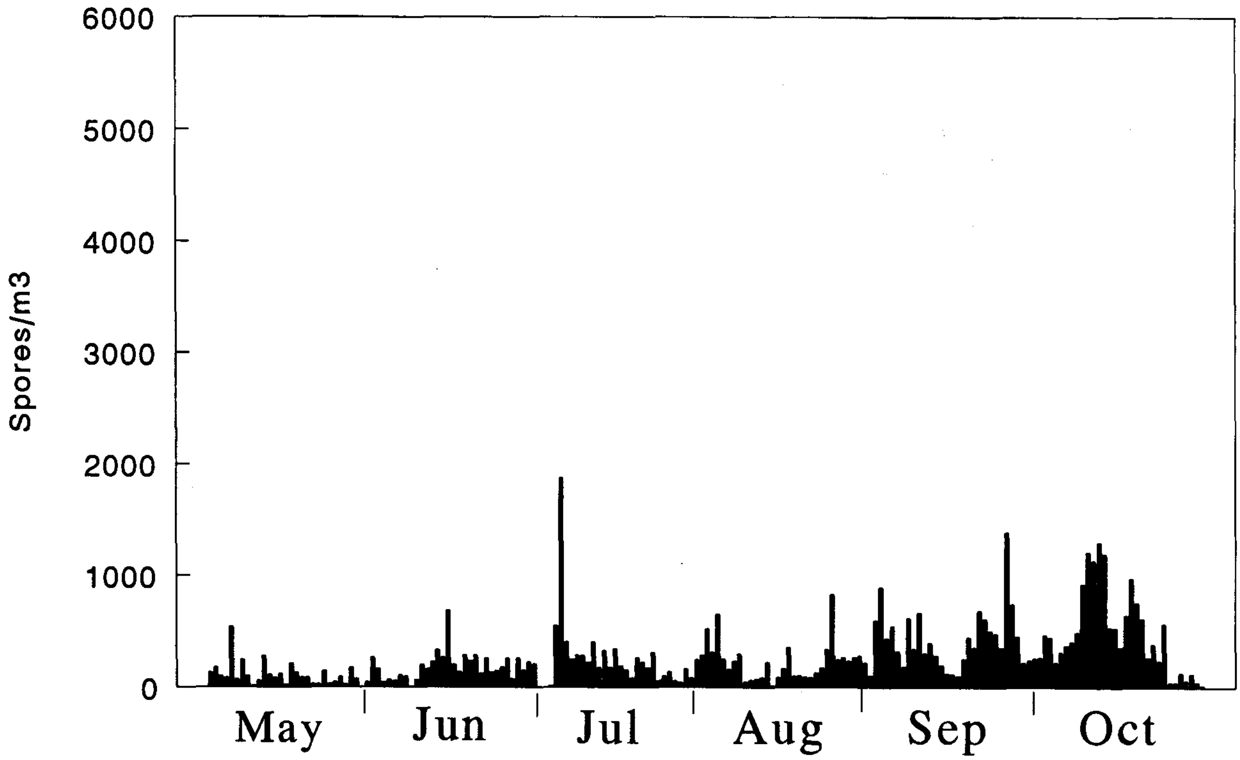

Fig. 3. Average daily concentration of smut teliospores in Tulsa, Oklahoma from May to October 1991.

mean daily teliospore concentration showed significant negative correlations with the average daily relative humidity and with precipitation. The correlations between concentration and percent sunshine and between concentration and average daily temperature were positive; however, the correlation with temperature was not significant in 1992 ($P > 0.05$). The correlation between teliospore concentration and average wind speed was slightly negative, but not significant.

4. Discussion

Data collected in this study indicate the significance of teliospores of the Ustilaginales as bioaerosols in northeastern Oklahoma. From May through October of 1991 and 1992, these spores were present in the atmosphere 100% of the days. Since different species of smuts produce peak concentrations at different times during the year, these spores are prevalent in the atmosphere for at least 6 months. Hamilton (1959) also showed seasonal occurrence of the teliospores of *Ustilago* and *Tilletia*. Despite the consistent occurrence of smut spores throughout the 6 months studied, the daily and monthly atmospheric concentrations of these spores experienced many fluctuations from May through October. The concentrations reported in this study are daily averages (Figs. 3 and 4); however, hourly concentrations may differ significantly throughout the day.

Many factors contribute to the variations in the teliospore concentrations. As members of the ‘dry air spora’, daily fluctuations in weather, especially wind speed, precipitation, and relative humidity are known to influence the atmospheric concentration of smut spores (Hirst, 1953). As anticipated, the data obtained during this study showed significant negative correlations between concentration and relative humidity and between concentration and precipitation. A significant positive correlation was seen between concentration and percent sunshine; however, there was not a significant correlation with average wind speed. Although spore dispersal of members of the ‘dry air spora’ is increased by increasing wind speeds, it is difficult to demonstrate this relationship during routine air sampling. Hasnain (1993) could find no positive correlations between wind speed and concentrations of various spore types; in fact, he found negative correlations with several spore types including *Cladosporium*, also considered a member of the ‘dry air spora’. Hart et al. (1994) also showed negative correlations between wind speed and both Poaceae and *Urtica* pollen. The absence of a positive correlation between concentration and wind speed can possibly be attributed to the variability of wind coupled with the lack of isokinetic sampling. While wind speeds often change minute to minute, the sampling flow rate of the Burkard sampler remains fixed and can result in overestimates or underestimates of atmospheric concentrations (Hirst, 1953; Hasnain, 1993).

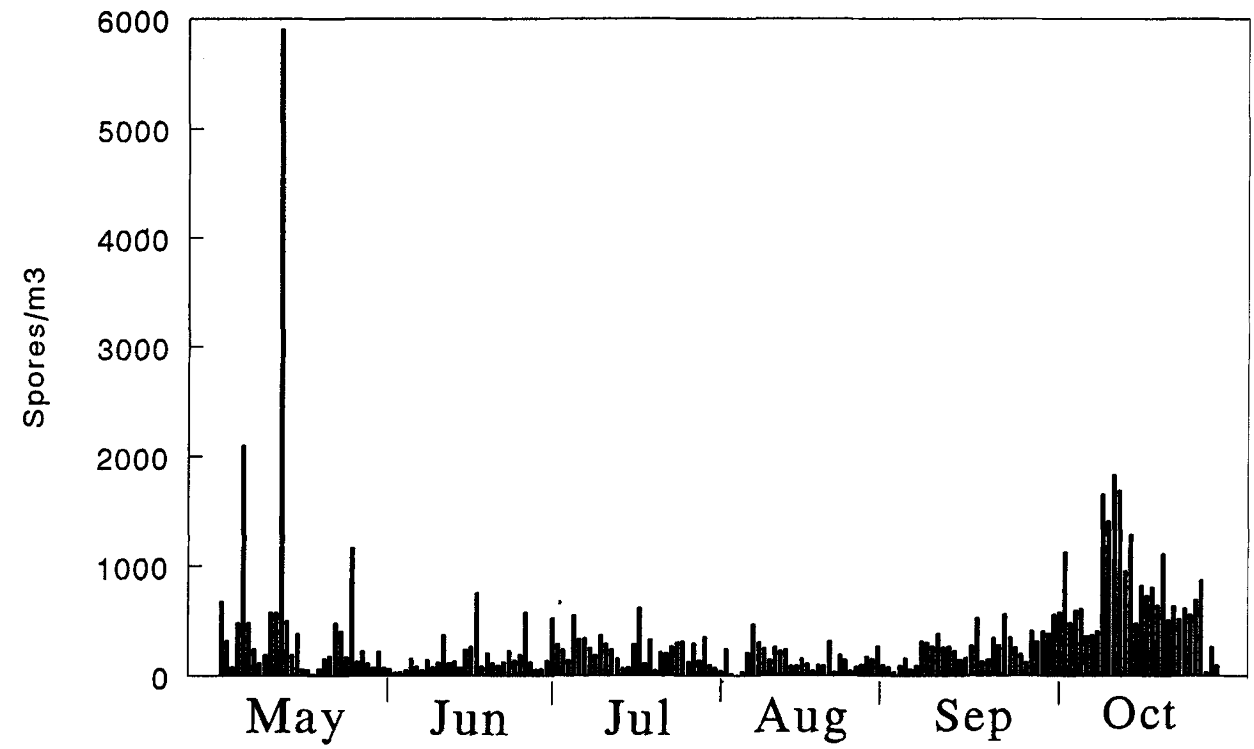

Fig. 4. Average daily concentration of smut teliospores in Tulsa, Oklahoma from May to October 1992.

Of primary importance to those smuts classified as 'loose' is the phenology of their host plants. For loose smuts, spore production and dispersal are often dependent on the flowering cycle of the infected plants. Teliospores are released when the plant flowers in preparation for pollination (Walker, 1957). Loose smuts commonly infect a wide variety of grasses, such as Johnson and Bermuda as well as various cereal crops.

In northeastern Oklahoma, both Johnson and Bermuda grasses heavily populate the extensive pasturelands of this area (Oklahoma State University Extension, personal communication, 1993). The teliospores of the two different types of smuts which infect these grasses (*Sphacelotheca occidentalis* and *Ustilago cynodontis*, respectively) were seen in remarkably high concentrations during the spring and summer months in correspondence with their host's flowering season. *Ustilago tritici* which causes loose smut of wheat was also abundant during the spring. In addition, during the months of September and October, other teliospores were observed in high concentrations in the atmosphere. Based on morphological characteristics, these teliospores are believed to belong to *U. brumivora* and *U. bullata*, which infect grasses native to the northeastern Oklahoma area.

Table 3
Average teliospore concentration in the Tulsa atmosphere, May–October 1991 and 1992

Month	1991 average daily concentration spores/m^3	1992 average daily concentration spores/m^3
May	117	533
June	188	168
July	256	257
August	229	158
September	437	255
October	487	755
May–October 6-month mean	287	356
Cumulative 6-month total	52 521	65 467

Table 4
Correlations for meteorological variables influencing the average daily smut teliospore concentrations during October 1991 and October 1992

Meteorological factors	1991	1992
Average relative humidity	−0.5905	−0.5093
	$P<0.001$	$P<0.005$
Precipitation	−0.4451	−0.4321
	$P<0.05$	$P<0.05$
Average wind speed	−0.2822	−0.0029
	$P>0.05$	$P>0.05$
Percent sunshine	0.5484	0.5264
	$P<0.005$	$P<0.005$
Average temperature	0.4018	0.2893
	$P<0.05$	$P>0.05$

For those smuts classified as 'covered', the phenology of the host plant is less important. Instead, many of the covered smuts depend on human intervention for the dispersal of their teliospores. Smuts that produce spores in a covered structure such as a gall or a glume often infect economically important cereal crops such corn, wheat, oat, and barley. During harvesting, teliospores are released from this protective body (Walker, 1957). Since various grains are planted and harvested at different times during the year, the atmospheric concentrations of the teliospores may also vary. For example, in Oklahoma, farmers harvest wheat and oat crops beginning in April and continuing through June; they harvest corn in September and October. Thus, during the spring months, a significant number of the teliospores in the atmosphere were identified as *U. kolleri* which infects oats, while during September and October, the primary teliospore components in the atmosphere were those of corn smut, *U. maydis*.

In conclusion, this study indicates that in addition to their role as serious pathogens of several economically important plant species, the smut fungi are also significant components of the atmosphere in Tulsa, Oklahoma from May through October. Due to a variety of factors, different species of smuts occur in higher atmospheric concentrations at certain times of the year. Although this study was limited to northeast Oklahoma, many of the smut species as well as many of the host plants have a cosmopolitan distribution. Similar aerobiological patterns can be expected in many areas. Further investigation into these often overlooked fungi is urgently needed to completely define the aerobiological importance of their spores in diverse geographic locations. Likewise, since the allergenicity of smut spores remains to be fully defined, additional research on the clinical significance of teliospores as potential aeroallergens is essential.

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