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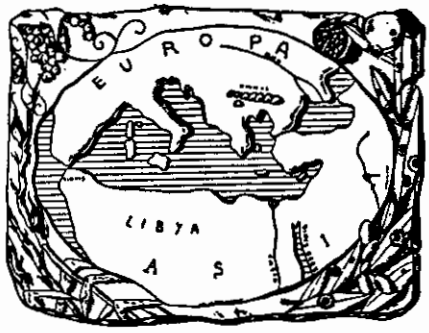
Reprinted from "Phytopathologia Mediterranea"
Vol. XVIII, 1979 - pag. 3-9



UNIONE FITOPATOLOGICA MEDITERRANEA - ITALIA

Phytopath. medit., 1979, *18*, 3-9

Role of ascospores of *Mycosphaerella zeae-maydis* in the epidemics of yellow leaf blight of Maize

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Summary - Corn debris and growing plants sampled from a no-plow plot were examined for the presence of pseudothecia of *Mycosphaerella zeae-maydis*. Pathogenicity to Maize of ascospores produced in them was determined in the greenhouse. Concentration of ascospores in the air was sampled by means of a Burkard spore trap placed in the plot.

Pseudothecia were formed in overwintering leaf debris in May but a few ascospores were observed. Pathogenicity experiments with the pseudothecia in debris were not successful since ascospores did not discharge from the material collected. Pycnidia were numerous in leaf debris and many conidia were found in them.

Pseudothecia were formed in necrotic tissue of lower leaves in growing plants; they were first observed on July 1 and continued to form troughout the groving season. Ascospores developed in these pseudothecia were pathogenic to Corn. Pycnidia were also abundant in necrotic tissue of the lower leaves of Corn plants and could be found in all necrotic leaf tissue throughout the season. Ascospores in the air were trapped continuously from May 31 to August 31, 1974.

Riassunto - RUOLO DELLE ASCOSPORE DI *Mycosphaerella zeae-maydis* NELLE EPIDEMIE DELLA « MACULATURA FOGLIARE GIALLA » DEL GRANTURCO. Residui e piante di Granturco raccolti in inverno da parcelle non coltivate sono stati esaminati per la presenza di pseudoteci di *Mycosphaerella zeae-maydis*. La patogenicità verso Granturco delle ascospore prodotte è stata saggiata in serra mentre la presenza delle ascospore nell'aria è stata rilevata a mezzo di una trappola per spore Burkard installata in campo.

Pseudoteci si sono differenziati in maggio sui residui raccolti ma essi contenevano un modesto numero di ascospore. Le prove di patogenicità non hanno avuto successo a causa della mancata liberazione di ascospore dai residui vegetali utilizzati. Nei residui raccolti erano anche presenti numerosi picnidi maturi.

Pseudoteci sono stati anche osservati nei tessuti necrotici delle foglie basali di piante in attiva crescita; essi erano presenti dai primi di luglio e la loro differenziazione si è avuta durante tutto il ciclo colturale del Granturco.

Le ascospore differenziatesi in tali pseudoteci sono risultate patogene per il Granturco. Sui tessuti necrotici delle foglie basali sono stati abbondantemente presenti per tutto il ciclo colturale delle piante anche picnidi del patogeno.

Le ascospore sono state catturate con continuità dal 31 maggio al 31 agosto.

Introduction

Yellow leaf blight (YLB) of Maize (*Zea mays* L.), which was first observed in the United States in 1965 (McFeely, 1971), is now known to occur in most of the Corn growing areas of this country, and it has also been reported from Argentina (Frezzi, 1972), Canada (Gates and Mortimore, 1969), France (Cassini, 1973), and Kenya (Mukunya, 1975). The agent of the disease was described as a new species of *Phyllosticta, P. maydis* Arny et Nelson (Arny and Nelson, 1971), the sexual stage of which was recently named *Mycosphaerella zeae-maydis* Mukunya et Boothroyd. Mukunya and Boothroyd (1973) reported the discovery of the sexual stage on overwintering corn leaf debris in New York, and suggested that ascospores of *M. zeae-maydis* might act as primary inoculum for the initiation of YLB epidemics. Although ascospores produced

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under artificial conditions from conidial isolates were found by these workers to be pathogenic to corn, non pathogenicity studies were conducted with naturally occurring ascospores as inoculum. Mukunya (1973) showed that ascospores of *M. zeae-maydis* were abundant in the air above corn plants from June 12 to July 10, 1972. Later observations suggested that ascospores were produced throughout the crop season (Boothroyd, 1974). First symptoms of YLB in the field develop in lower leaves, the distal portion of which soon become necrotic under favorable environmental conditions. This necrotic tissue might be a suitable substrate for the development of pseudothecia of *M. zeae-maydis*.

This report presents results of an investigation to search for the sexual stage of *M. zeae-maydis* in corn debris and in necrotic tissue produced in the field during the crop season, and to determine whether or not ascospores that might be produced in such fruiting bodies would be pathogenic to Corn.

Materials and methods

Field experiments and observations. Observations on YLB of Corn in the field were made in no-plow experimental plots at the Cornell Agronomy Farm, Aurora, New York, in 1973, 1974, and 1975. This field has been planted to Corn since 1967 and had a history of YLB since 1968. Corn debris collected in late December 1973, May 3 and 31, and June 13, 1974, and on April 17, 1975 were examined for the presence of pycnidia and pseudothecia of *M. zeae-maydis*. A commercial cultivar of Corn (normal cytoplasm) susceptible to YLB was planted on May 22, 1974. Plant material was sampled and examined at about weekly intervals from June 7 to July 22, and on August 26 and September 2. If fruiting bodies were not present, affected tissue was placed in moist chambers and examined 48 h later. Isolations of pathogens in pure culture were also made. Type and location of symptoms in the leaves and pathogens associated with them were recorded.

The presence of ascospores of *M. zeae-maydis* in the air above corn plants was sampled by means of a Burkard volumetric spore trap (Burkard Manufacturing Co., Ltd., Rickmansworth, Hertfordshire, England) placed in the field. The orifice of the trap was located 1.2 m above the ground level and air flow through it was 9 l/h.

Pathogenicity of ascospores produced from field material. Pieces of necrotic leaves bearing pseudothecia of *M. zeae-maydis* were fastened to the top cover of plates containing water agar. Ascospores were discharged onto the medium. Small agar blocks bearing a number of ascospores were aseptically removed and they were introduced into the whorl of Oh51Atms × B8 seedlings. Plants were then sprayed with sterile distilled water, enclosed in plastic bags, and placed in a mist chamber at 20-22° C. Bags were removed 4 days later and the plants kept in the mist chamber provided with a humid environment for 12 h/day. Plants used as controls were treated similarly except the blocks of agar carried no spores. This experiment was repeated using as plant material the susceptible cultivar of Corn planted in the field. Methods were the same as already outlined except for small pieces of absorbent cotton introduced into the whorl of the plants to cover the agar plugs.

In another experiment, leaf pieces bearing pseudothecia of *M. zeae-maydis* were placed 5-6 cm above the top of seedlings of a susceptible cultivar (normal cytoplasm) grown in pots, or placed on the soil surface of them. Plants thus exposed were enclosed in plastic bags and placed in a growth chamber at 20-22° C for 2 weeks. Plants treated similarly except for the absence of infected leaf pieces served as controls. Bags and leaf pieces were removed from two replicates after 4 days of coverage and others 3 days later.

Pathogenicity and virulence of monoascospore clones. Fifteen conidial clones of *M. zeae-maydis*, labelled Ml through Ml 5, were used to inoculate a susceptible cultivar of Corn. Those clones resulted from monoascospore isolations from necrotic tissue developed on plants growing in the field. A monoconidial isolate of *Phyllosticta zeae* Stout characterized as nonvirulent previously, and a virulent isolate of *P. maydis* (P24) which was isolated from Maize in Cayuga, New York, in 1970, were also included. Seedlings of a commercial cultivar of Corn (normal cytoplasm) susceptible to YLB were inoculated at the fourth-leaf stage (about 14 days old). Inoculum was obtained and prepared as described elsewhere (Jiménez-Díaz and Boothroyd, 1976). Conidial suspensions containing 10^4 spores/ml were applied to the foliage with a De Vilbiss atomizer. Non-inoculated controls were sprayed with sterile distilled water. After inoculation seedlings were enclosed in plastic bags and placed in a growth chamber at 20-22° C and 14 h/day photoperiod of fluorescent light of about 10,000 lux. Bags were removed after 48 h and seedlings were moved to a greenhouse bench. Inoculation was effected in two steps because of space limitation in the growth chamber. Isolates Ml-Mll and P24 were

first used. Isolates MI2-MI5, P24 and *P. zeae* were used 48 h later. Readings on disease severity on a 1-5 scale (1=no symptoms, 5=generalized necrosis) were taken 1 and 2 weeks after inoculation.

Results

Symptomatology. Yellow leaf blight of Corn was severe in the field plots where observations were made. Weather conditions (mean daily temperatures of 13.3-26.1° C in June, 16.1-27.8° C in July and 16.7-23.0° C in August; and total rainfall of 102 mm in June, 55 mm in July and 152 mm in August), and abundant initial inoculum provided by overwintering debris, were apparently conducive for the disease.

The first symptoms of YLB in the field were observed on June 13, when the plants were at the fourth-leaf stage. Symptoms appeared as small chlorotic flecks and round necrotic lesions ranging 1-4 mm in diam. Symptoms were distributed all over the leaf blade in the first and second leaves, but they were mostly located at the tip region in the third and fourth leaves. *Phyllosticta maydis* (the imperfect stage of *M. zeae-maydis*) was consistently isolated from necrotic lesions. In a few cases, a fungus other than *M. zeae-maydis* grew out the plated leaf pieces into the medium. It grew rapidly and produced an aerial mycelium below which abundant pycnidia developed. Those pycnidia extrude a pinkish ooze of small unicelular conidia averaging 2.4×4.8 μm. This fungus was tentatively identified as *P. zeae* Stout.

By June 23, at the fifth-leaf stage, three Corn pathogens were frequently isolated from intermixed necrotic lesions, namely *M. zeae-maydis*, *Colletrotrichum graminicola* (Ces.) Wilson, and *Kabatiella zeae* Narita et Hiratsuka, pathogens of YLB, anthracnose (Arny *et al.*, 1971; Williams and Willis, 1963), and eyespot (Dale, 1963) of Corn, respectively. All sampled plants were affected. Symptoms were either necrosis of the whole leaf, generalized necrosis of the tip of the leaves, or scattered necrotic spots located at the tip region of the leaves. Tip necrosis generally included one third to one fourth of the leaf blade, although distinct necrotic spots were often present in the rest of the tissue. First and second formed leaves were mainly necrotic, and third and fourth leaves showed tip necrosis as the most conspicuous symptom.

On July 1 immature pseudothecia of *M. zeae-maydis* were found in the first and second formed leaves which were already necrotic. By July 8, et the eighth-leaf stage, 90% of the third formed leaf in sampled plants was also completely necrotic. Pseudothecia of *M. zeae-maydis* were found in 40% of the first formed leaves and 35% of the second. Pseudothecia were not observed in the third leaves. The incidence and severity of the symptoms increased with the age of the leave, i.e. decreased with their height in the plant. Generalized necrosis of the leaf tips was the most conspicuous symptom observed in the fourth and fifth sampled leaves; scattered necrotic lesions were present in the distal portion of the sixth and seventh. Pseudothecia of *M. zeae-maydis* were found in necrotic third leaves on July 16, but the asexual stage of the fungus was much more prevalent. By August 26 abundant pseudothecia were present in 95% of the necrotic third leaves sampled (Fig. 1A). By September 2, the fourth and fifth lower leaves were necrotic and the sixth and seventh showed generalized necrosis at the tip of the blade and heavy spotting in the rest of the tissue. Mature and immature pseudothecia of *M. zeae-maydis* were abundant in all tissues presenting a generalized necrosis, up to the seventh leaf. Leaves above this level up to the tassel showed spotting, the intensity of which decreased towards the top of the plant. Pycnidia of *M. zeae-maydis* (Fig. 1B, D) and acervuli of *C. graminicola* were produced in the necrotic spots when they were exposed to a moist environment.

Ascospore content in the air. Ascospores (Fig. 1C) of *M. zeae-maydis* were first caught on May 31, 1974. They were detected everyday throughout the crop season. Figure 2 represents the daily means of the number of ascospores trapped per hour from June 1 to August 31, 1974. Only complete days are represented; days for which some hourly counts were missing have not been considered. A series of peaks occurred in the number of ascospores trapped. The number of ascospores, as represented by the peak counts, increased from June 15 reaching a maximum of about 6,700 ascospores/h in a 9 l flow of air on June 28-29. Counts decreased afterwards to a very low level with a second maximum (4,400 ascospores/h) on July 27, followed by a steady decrease in August. Thus it appeared that a bimodal distribution of the number of ascospores occurred during the season.

Pathogenicity of ascospores. Ascospores of *M. zeae-maydis* produced in growing plants in the field were pathogenic to Corn. Plants inoculated with ascospores in agar blocks showed symptoms by 10 days after inoculation. Symptoms developed in 5 out of 8 inoculated plants; they appeared as small necrotic lesions, ranging 2-4 mm wide and 5-8 mm in length, which were surrounded by yellow halos. Necrotic lesions were located in

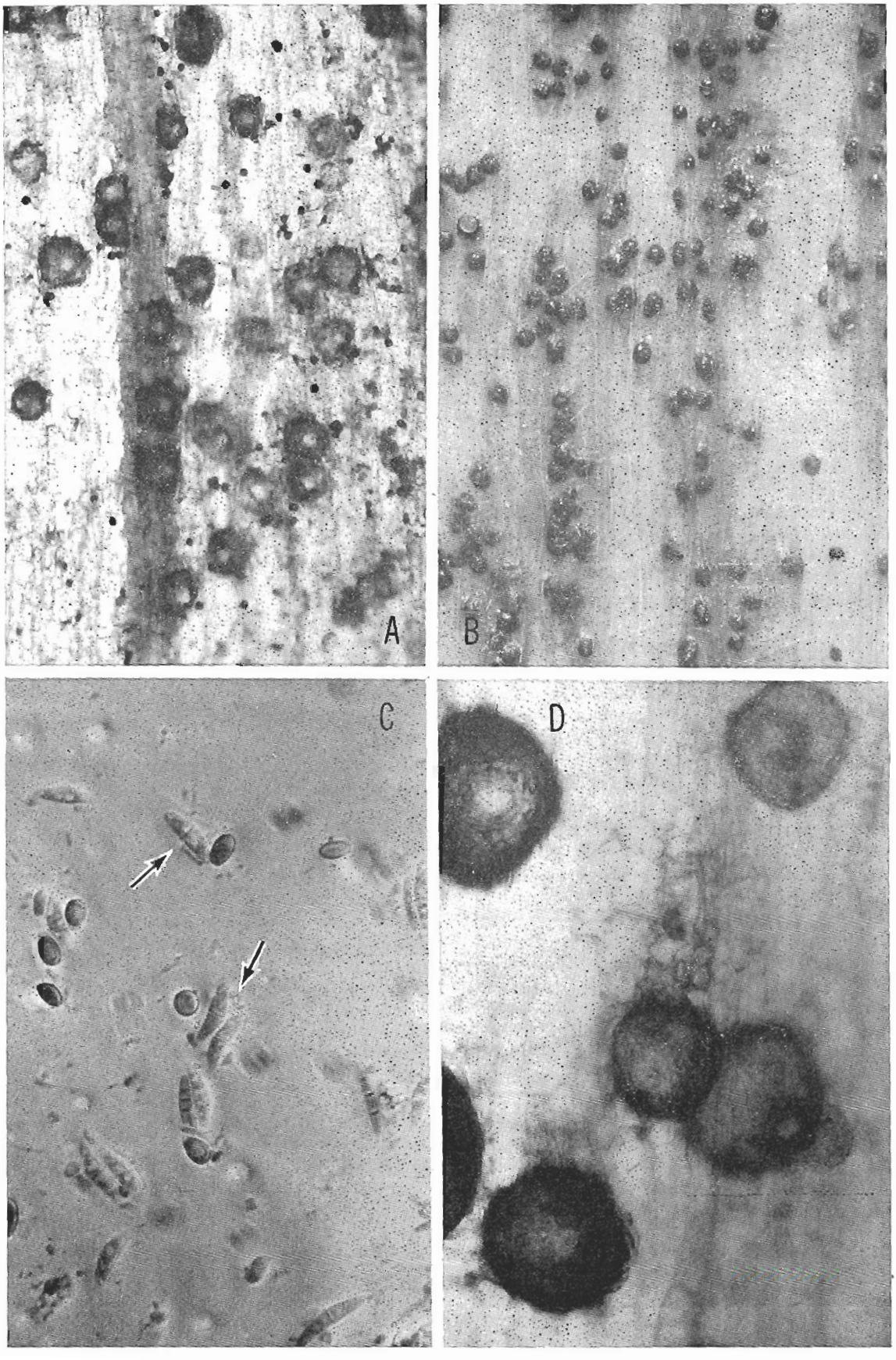
A
B
C
D

TABLE I. - Difference in virulence among fifteen monoascospore isolates of *Mycosphaerella zeae-maydis*. Average disease ratings. (*a*, *b*).

TABELLA I. - *Variazione della virulenza nell'ambito di 15 isolati monoascosporici di* Mycosphaerella zeae-maydis. Valutazione media della malattia.

	Isolates	1st reading (*d*, *e*)	2nd reading
Inoculation 1	M 1	1.2 f (*c*)	1.7 m
	M 2	1.7 fg	3.2 opg
	M 3	2.6 i	3.6 opg
	M 4	2.0 ghi	3.2 opg
	M 5	1.8 fgh	2.9 nopg
	M 6	1.7 fg	2.1 mn
	M 7	2.5 hi	3.7 pg
	M 8	2.5 hi	3.4 opg
	M 9	2.1 ghi	2.8 nop
	M 10	1.9 fghi	2.1 mn
	M 11	1.7 fg	2.7 no
	P 24	2.0 ghi	3.8 g
Inoculation 2	M 12	2.5 l	3.2 tu
	M 13	2.0 k	2.6 t
	M 14	1.6 k	1.7 s
	M 15	2.7 l	3.7 u
	P 24	2.6 l	3.1 tu
	P. zeae	1.0 j	1.0 r

(*a*) Plants of a susceptible cultivar of Corn were inoculated with a suspension of 10^4 conidia/ml in the greenhouse.
(*b*) Disease rating was based on a scale 1-5; 1=no symptoms, 5=generalized necrosis of inoculated leaves.
(*c*) Each figure is average of 12 values.
(*d*) First reading was made 1 week after inoculation and 2nd reading 1 week later.
(*e*) Means having letters in common are not significantly different at 5% level, as established by the Duncan's new multiple range test (Steel and Torrie, 1960).

leaves which were developing in the whorl at the time of inoculation. Symptoms similarly developed in 6 out of 10 plants where agar blocks were covered with absorbent cotton; these symptoms resembled in type and location those which appeared in inoculated plants where absorbent cotton was not used. No symptoms developed in plants used as controls. *Mycosphaerella zeae-maydis* was consistently isolated from necrotic tissue.

Corn seedlings exposed to discharge of ascospores produced in necrotic tissue of growing plants showed symptoms 7 days from the initiation of the exposure. All plants were affected. Symptoms had not developed when plastic bags covering the seedlings were removed from two replicates after 4 days of coverage. However, chlorotic specks appeared by 7 days in plants which has been covered for either 4 or 7 days, followed by the formation of necrotic lesions with yellow halo, the blighting of the leaf tips and the generalized necrosis of the first, second and third lower leaves. Symptoms did not develop in plants used as controls. Pycnidia of *M. zeae-maydis* formed abundantly in necrotic tissue placed in moist chamber.

Pathogenicity and virulence of monoascospore clones. Results of inoculations with monoascospore clones are indicated in Table I. The inoculum concentration and the environmental conditions used in this experiment resulted in a significant variation in degree of virulence among monoascospore clones of *M. zeae-maydis*. As it is indicated, disease reactions were mild 1 week after inoculation, but they increased in severity in 2 weeks. The net increase differed

Fig. 1. - *Mycosphaerella zeae-maydis* in necrotic tissue of Maize. A, pseudothecia; B, pycnidia; C, ascospores (arrows) and unknown fungal spores trapped with a Burkard spore trap at Aurora, New York; D, a close up of pycnidia in necrotic leaf tissue of maize.

Fig. 1. - Mycosphaerella zeae-maydis *in tessuti necrotici di Granturco. A, pseudoteci; B, picnidi; C, ascospore (freccia) e altre spore di funghi non identificati catturate con un captaspore Burkard ad Aurora, New York; D, picnidi a forte ingrandimento entro tessuti fogliari in necrosi.*

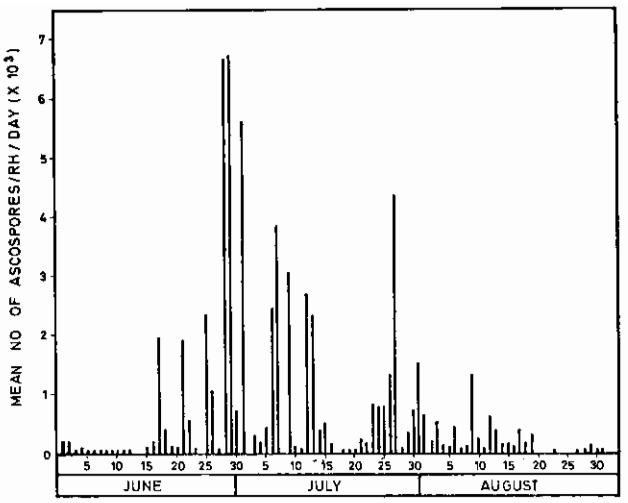

Fig. 2. - Daily means of *Mycosphaerella zeae-maydis* aspore trapped per hour at Aurora, New York, from June 1 to August 31, 1974.

Fig. 2. - Medie giornaliere del numero di ascospore Mycosphaerella zeae-maydis *catturate per ora, ad Aurora, New York, dal 1° giugno al 31 agosto 1974.*

among clones, which also points out the occurrence of variability among them. A continuum in the degree of virulence seemed to occur among clones, with no sharp limits, the more virulent of them being as virulent as isolate P24. The lowest rank in virulence was given to *P. zeae*, which confirmed previous information on its non-pathogenicity to Corn.

Survival of *Mycosphaerella zeae-maydis* in corn debris. Pycnidia were the only fruiting bodies of M. *zeae-maydis* found in leaf debris taken from the soil surface on December 1973. By early May 1974, a few pseudothecia were observed in ground leaf debris but pycnidia were fairly abundant. Conidia were abundant in the pycnidia but only a few ascospores had formed in the pseudothecia. On May 31 and June 13 samples included leaf pieces and ear husks removed from the ground surface and from the stalks above ground. Fruiting bodies of *M. zeae-maydis* were not present in ear husks, and they did not develop when pieces of tissue were placed in moist chamber. Pycnidia were abundant in leaf sheaths and leaf blades from above ground and in leaf sheaths from the soil surface, but pseudothecia, either mature or immature, were less numerous. Many fruiting bodies resembling pseudothecia were found lacking spore content. Leaf blades sampled from the soil surface were mostly decomposed.

On April 17, 1975 leaf debris from above plant parts harbored many fruiting bodies resembling pycnidia and pseudothecia of *M. zeae-maydis*, however those fruiting bodies were desiccated and were found devoid of spore content. Abundant conidia were produced in desiccated fruiting bodies, and new pycnidia but no pseudothecia developed, when pieces of leaf debris were placed in moist chamber.

Mycosphaerella zeae-maydis was recovered in pure culture from material collected on December 1973, April 1975 and May 1974. Recovery was not attempted on June 1974.

Discussion

The display of symptoms of YLB on the plants in the field suggest that the first infections took place when the developing leaves were still within the whorl of the young plant. Initially necrotic spots developed in the distal portions of the lower leaves. These portions appeared blighted later on and they usually harbored pseudothecia of *M. zeae-maydis*. Pseudothecia were formed in necrotic leaf tissue of growing plants throughout the crop season. Pseudothecia with mature ascospores were first found in necrotic first and second formed leaves, but later on they were formed in necrotic tissue of upper leaves, including blighted apices of the seventh leaves.

Pseudothecia of *M. zeae-maydis* were also produced in overwintering leaf debris. In 1974 mature pseudothecia in debris were observed in May, but not before, and they were more abundant by June 13, although in both cases the asexual fruiting bodies were more prevalent. Leaf debris from the 1974 crop, sampled on April 1975, had fruiting bodies resembling pseudothecia but they were devoid of spore content. Mukunya (1973) suggested that the differentation of pseudothecia of *M. zeae-maydis* takes place during the winter and early spring. He also reported that abundant pseudothecia were mature by May. Our observations appear to be in keeping with his findings. The ascospore concentration in the air above corn plants (Fig. 2) points out the continuous availability of pseudothecia to form throughout the crop season. Pseudothecia formed in debris may account for the early peaks of ascospore trapping in middle and late June, since by that time first symptoms were developing in growing plants and pseudothecia were not found in them. The heavy ascospore catches by the end of June and the fluctuations of the trappings during late season may be explained by the production of new pseudothecia in growing plants and possibly in standing debris, since debris on the soil were decomposed by late June.

Our results showed that ascospores of *M. zeae-maydis* produced in growing plants are pathogenic to Corn. The concentration of airborne ascospores and their ability to infect Corn suggest that they may play an important role in the epidemics of YLB. The possibility that ascospores formed in overwintering corn debris might act as primary inoculum is only indicated by the availability of ascospores in the air from the beginning of June. However, whether ascospores from overwintering debris are able to infect corn plants still needs to be proved. Our attempts to test the pathogenicity of ascospores naturally occurring in overwintering debris were not successful, possibly because of the unsuitability of the material chosen, as it is sug gested by the fact that during the routine observations many fruiting bodies resembling pseudothecia lacked spore content. Our failures, however, do not rule out the possibility that pseudothecia developed in overwintering debris may provide primary inoculum for the development of YLB. The abundance of pycnidia observed in overwintering debris and their apparent viability suggest that they, as well as pseudothecia, may provide inoculum for YLB. Conidia are disseminated by water splash but they are not wind borne (Mukunya, 1973), which suggests that they may provide a local spread of the pathogen. Ascospores are airborne and if they are carried by wind to long distances, as other species of *Mycosphaerella* do (Hare and Walker, 1944), they may provide a way for long range dissemination of *M. zeae-maydis*.

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