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Sequential development of pathogens in the maize tarspot disease complex

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Abstract

The tarspot complex is caused by the interaction of *Phyllachora maydis* and *Monographella maydis*. *Coniothyrium phyllachorae*, possibly a mycoparasite, is found in older ascostromata of *P. maydis*, which always appears first causing tarspot. *M. maydis* follows and is responsible for the damaging "fisheye" symptom. The fisheye symptom is always associated with a tarspot in the center of the lesion, whereas 12 to 20% of the *Phyllachora* ascostromata remained free of *M. maydis*. Inoculations of maize leaves with the *Microdochium* anamorph of the *Monographella* (usually produced in lesions) failed to produce infections. Some infections with *M. maydis* were, however, obtained under unusual conditions in the field. Inoculations onto tarspots in the laboratory were unsuccessful, but in field experiments inoculations with conidia of *M. maydis* enhanced severity of the tarspot complex. Fisheye symptoms of the complex naturally appear 2 to 7 days after the manifestation of *P. maydis*. This is followed a week later by the appearance of *M. maydis* which became predominant in the lesions and is associated with empty perithecia of *P. maydis*. In the early stages of the tarspots pycnidia of the anamorph of *P. maydis*, *Linochora* sp., could occasionally be observed. Ascomata of *M. maydis* were rare in the field. Of the 36 genetic materials of CIMMYT tested, 30 developed the fisheye symptom, 4 tarspots only and 2 remained free of symptoms

Introduction

Tarspot, caused by *Phyllachora maydis* Maubl., was first described in 1904 on maize (*Zea mays* L.) from Mexico [1] and is known to occur only in the Western Hemisphere. The disease is also reported from Bolivia, Colombia, Costa Rica, Dominican Republic, Guatemala, Panama, Peru, Puerto Rico and Venezuela [2–10]. It is also known to occur in Ecuador, El Salvador and Haiti (unpublished, CIMMYT staff). *P. maydis* forms dark erumpent elliptical stromata (tarspots) 0.5–11 mm in size on leaves which may cause decay. In case of severe infection, leaf sheaths and husks also exhibit symptoms. Associated with predominantly young stromata of the fungus is a *Linochora* sp., which we considered to be its anamorph.

Müller and Samuels [11] examined maize leaves collected from Poza Rica, Mexico. They found *Monographella maydis* E. Müller and Samuels and its anamorph *Microdochium maydis*

E. Müller and Samuels associated with the tarspot disease. The association of this facultative parasite with *P. maydis* is very consistent and *M. maydis* appears to be responsible for the excessive leaf necrosis that occurs and the cause of the 'fisheye' symptom of the 'tarspot complex' [12]. In addition, we have often found *Coniothyrium phyllachorae* Maubl. in the tarspot lesions, apparently existing as a mycoparasite. This third fungus of the complex results in five forms of spores associated with the tarspot complex.

Müller and Samuels [11] stated that *M. maydis* is regularly found in green leaves and considered *M. maydis* to be endophytic (Müller, pers. com.) on maize, but turned pathogenic when it came in contact with *P. maydis*. Further information on the *in vitro* behavior of *P. maydis* and *M. maydis* is reported by Dittrich et al. [13]. Hock et al. [12] described the ecological conditions favoring the disease complex and its economic importance in Mexico. We report here on the disease development in relation to the three fungi involved.

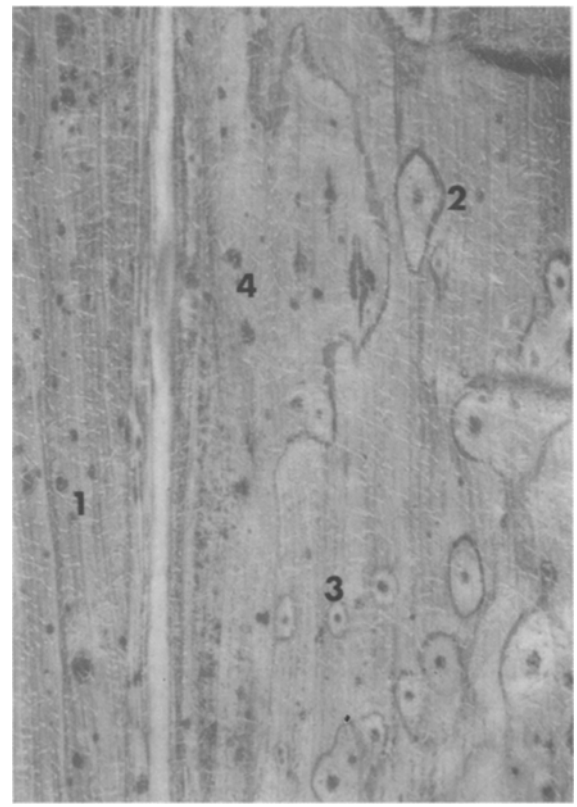

Fig. 1. Symptoms of *Phyllachora maydis* (1), *P. maydis* plus *Monographella maydis* (2), *P. maydis* plus *M. maydis* plus *Coniothyrium phyllachorae* (3), and confluent symptoms (4).

Materials and methods

All field plantings were made during the winter season at the CIMMYT tropical station at Poza Rica, Mexico (20° N latitude, elevation 60 m), and the laboratory examinations were conducted at the CIMMYT El Batan station. Leaf lesions of *P. maydis* on population 22 (resistant) and pool 15 or LG II (susceptibles) were marked when they were about 1 week old and turning from amber to black in color. This experiment was repeated three times at weekly intervals. When the oldest marked lesions were 4 weeks old and the youngest 1 week old the leaves were brought to the laboratory, dissected, stained with lactophenol, and the sporulation in the lesions was determined microscopically at ×100 or ×400. In another experiment, the lesions were marked at 3 and 4 days intervals and the material was examined as above 15 and 16 days later.

Results

Fully developed symptoms of *P. maydis* alone and *P. maydis* plus *M. maydis* are shown in Fig. 1. The early stages of the fisheye symptom are slight depressions around the tarspots. This halo, dark green in beginning, remains almost constant in size and usually turns necrotic within 2 days to 1 week. The lesion size varied with the cultivar and was found to range in diameter between 6.9 (±0.9 SD) × 3.8(±0.5) for pool 15 and 7.6(±0.9) × 4.1(±0.7) mm for LG II and a surface area of 21(±5.2) to 24.5(±0.6) mm^2, respectively [14]. Lesions of the fisheye are 10 to 30 times larger than that of the tarspot (ascostroma) alone. As a consequence, the disease progress curve of the disease complex mounts rather

Table 1. Number of 1 to 4 week old tarspot complex lesions of 20 per age group with spores of *P. maydis* (P), *M. maydis* (M) and *C. phyllachorae* (C) alone, or in combinations

Lesion	Lesions with						
age (week)	P	M	C	PM	PC	MC	PMC
1	11	0	1	8	0	0	0
2	1	11(11)	2	4	1	1	0
3	2	6(6)	5(1)	2	2	2(1)	1
4	0(2)	6(6)	1(1)	2	0	4(3)	5

( ) = empty stromata of *P. maydis*. The mean size of halos was 13.7, 11.5, and 12.7 mm^2 for the 2, 3, and 4 weeks, respectively.

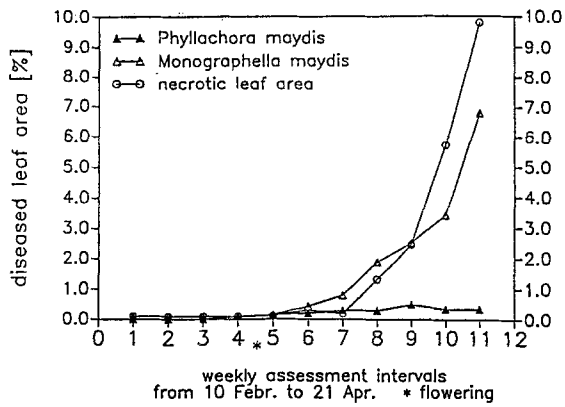

Fig. 2. Disease progress under field conditions of *Phyllachora maydis*, *P. maydis* plus *Monographella maydis*, and necrotic leaf area; all in percentage leaf area measured in weekly intervals from 10 February to 21 April, 1987.

steeply whilst the tarspots, if estimated separately, remain at low values (Fig. 2).

During the early stages of lesion development in the field, the majority of stromata of *Phyllachora maydis* were found to be free of *Monographella maydis*, but later the *Microdochium* anamorph of it and C. phyllachorae predominated (Tables 1 and 2).

Although more than half of the stromata of *P. maydis* (11/20) were found to be free from *M. maydis*, 1 week after the appearance of tarspots, 8 out of 20 marked lesions were already infected by the latter fungus. This finding is supported by another experiment in which 12% of the lesions were found colonized by *M. maydis* on the third day and 20 and 52% after 6 and 8 days (Table 2). From the second week, *M. maydis* became dominant and was associated with empty perithecia of the tarspot pathogen. *C. phyllachorae* was rarely found during the early stage. After

Table 2. Percentage of lesions with *P. maydis* (PM) and *M. maydis* (MM) combined from 25 lesions dissected at 3 to 6 days after the stromata of *P. maydis* were marked

Days after lesions were marked	Tarspot lesions with PM + MM	
	Series 1 (%)	Series 2 (%)
3	–*	12
4	32	–
6		20
8	52	–
9	–	44
12	60	60
15	–	80
16	88	–

* = no data collected.

Table 3. Disease severity of *P. maydis*[a] (PM) and *M. maydis*[b] (MM) 5 weeks after inoculation on tarspots with the *M. maydis* anamorph[c]

Row 1		Row 2		Row 3		Row 4 (control)	
PM	MM	PM	MM	PM	MM	PM	MM
4.3	9.1	4.1	9.2	4.2	7.4	3.5	3.8

[a] Disease severity rated from 1 = no disease to 9 = severe disease.
[b] Based on percentage of leaf area diseased by visual estimate.
[c] Lsd ($p = 0.5$) for PM = 0.84 and MM 2.1.
Rows 1–4 inoculated 4, 3, 2 and 0 times, respectively.

4 weeks only one-third of the lesions contained ascospores of *P. maydis*, 85% were occupied by *M. maydis*, and *C. phyllachorae* was found in 50% of the lesions (Table 1).

M. maydis was detected 3 to 4 days after the incipient stages of *P. maydis* lesions were marked. Our inoculation experiments with *M. maydis* onto the stromata on leaf pieces in Petri dishes failed in the laboratory. So there is no information about the incubation period of *M. maydis*. Early infection by *M. maydis* may occur when the perithecia of *P. maydis* are still closed. At this stage stromata often contained pycnidia of *Linochora* sp. Clearly, the proportion of 'clean' *P. maydis* lesions decreases over time (Tables 1 and 2).

The microscopic examinations showed that nearly all ascospores of *P. maydis* had been released by days 15 and 16. Only 12 to 20% of the tarspots eventually remained free from *M. maydis*. Ascospores of *M. maydis* were rarely found embedded in necrotic lesions. In the laboratory, however, *M. maydis* readily formed perithecia with ascospores on autoclaved maize and wheat straw in about 3 weeks, as Müller and Samuels [11] also found.

Attempts of artificial inoculation with 3–5×10^4/ml conidia of *M. maydis* on the lower leaf surface under protection (plastic cabins) in the field at about 38/18 °C day/night temperatures and 80–100% RH were made. In one of the eight test intervals, lesions of 3–4 mm size were seen after 6 days on 10 plants. In these lesions conidia and sporodochia of *M. maydis*, as well as hyphae penetrating stomata were observed under the microscope. This indicates that under such particular circumstances *M. maydis* can infect maize without previous infection of *P. maydis*. In another field experiment, repeated inoculations of *M. maydis* onto stromata of *P. maydis* yielded significant positive results (Table 3). The inoculation of tarspots with *Microdochium* conidia in the field increased the severity of the MM component of the complex significantly in comparison to the non-inoculated check row (Table 3).

The fisheye symptom of the tarspot complex is common on maize in Mexico. We found it expressed at Poza Rica on 30 of 36 genetic materials (e.g. breeding lines, cultivars used for breeding) with diverse genetic backgrounds. Four only had the pure tarspot symptom (Fig. 1-1). Thus, the degree of stroma development can be an early indicator of the potential damage to be expected from the intervention of *M. maydis*.

Discussion

In all our experiments and observations the typical tarspot symptoms of *P. maydis* appeared first. During 3 years of intensive disease recording in the field we never found fisheye symptoms without tarspot stromata in its center. This pure tarspot form has little effect on the host plant unless dense infection occurs on the leaves [15]. The anamorph *Linochora* sp. apparently does not play a role in the tarspot complex. The disease becomes economically important following infection by *Monographaella maydis* and development of the 'fisheye' symptom (Fig. 1). The typical necrosis around the stromata of *P. maydis*, under favorable climatic conditions, began to manifest itself about 2 days after the tarspots appeared and necrosis usually developed to its final size within 1 week. Inoculation in the laboratory failed, although inoculation with conidia of *M. maydis* without ascostromata succeeded under rather abnormal conditions in the field. Hence, there is no clear evidence that *M. maydis* can express disease symptoms independently in the field. Based on two years of field recordings, we therefore consider lesions of *P. maydis* to facilitate the appearance of *M. maydis*, perhaps by providing a means of entry for this pathogen. The sequential development shown in Table 1 does not argue against *M. maydis* being an endophyte as assumed by Müller and Samuels [11], and has been proved for *Monographella nivalis* in barley [15], although its appearance could also be explained by the prior infection of *P. maydis* and the subsequent superimposed infection by *M. maydis*.

The role of *C. phyllachorae* is not clear. It

usually appears after *M. maydis* is established in the lesions of the tarspot complex. Pycnidia of *C. phyllachorae* are found embedded in the stromata of *P. maydis* which are devoid of ascospores and can be only detected by microscopic examination. The proportion of infected stromata increased towards the end of the season, i.e. early April. Four weeks after first stromata development, *C. phyllachorae* had colonized half of them. Such tarspot lesions had smaller necrotic halos and the tarspots proper appeared to be smaller, more round and more erumpent, which is typical for mycoparasitized Phyllachoraceae [17].

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