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Ascospore Release of *Mycosphaerella caryigena* and Downy Spot Disease Occurrence on Pecan Groves in Central Nuevo Leon, Mexico[1]

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ABSTRACT

Downy spot by *Mycosphaerella caryigena* Demaree and Cole [anomorph=*Cercosporella caryigena* (Ell. and Ev.) Hoehn] is a common foliar disease on pecan (*Carya illinoensis* Koch.) groves in Central Nuevo Leon, Mexico. In extreme situations, severe defoliation may affect nut yields in several subsequent crops. Fungicide spray programs rarely are successful because of an apparent inconsistent infection period from one year to another. Two different pecan groves were studied for ascospore release of *M. caryigena* and disease occurrence over a period of three subsequent years. Mature ascospores of *M. caryigena* were consistently present in dead leaves on the ground in April and May every year. Ascospores in the air were coincident with the first spring rains, were intermittent throughout the spring and summer, and decreased in late summer. Downy spot symptoms appeared within a period of 6 to 8 weeks after detection of ascospores in the air. Disease severity was different in every grove and in different years.

RESUMEN

La mancha vellosa causada por *Mycosphaerella caryigena* (fase asexual = *Cercosporella carygena*) en una enfermedad foliar común en plantaciones de nogal pecanero (*Carya illinoensis* Koch.) en la región central del estado de Nuevo León, México. En casos extremos, la defoliación puede reducir notablemente la producción de nuez en varias cosechas subsequentes. Los programas de aplicación de fungicidas raramente proporcionan un combate efectivo de la enfermedad, debido aparentemente a que el período de infección ocurre en fechas distintas entre un año y otro. Se efectuó un estudio en dos diferentes plantaciones de nogal para determinar la liberación de ascosporas de *M. caryigena* y la incidencia de la enfermedad durante un período de tres años subsecuentes. Se encontraron en forma consistente ascosporas maduras de *M. caryigena* en las hojas secas presentes en el suelo durante los meses de abril y mayo de cada año. En el aire, las ascosporas fueron coincidentes con la presencia de las primeras lluvias de la primavera, fueron intermitentes por toda la primavera y el verano y decrecieron al final de éste. Los síntomas de mancha vellosa aparecieron durante un período de 6 a 8 semanas después de detectar las ascosporas en el aire. La severidad de la enfermedad fue diferente en cada plantación y en años diferentes.

Additional index words: epidemiology, ecology, Cercosporella caryigena.

Downy spot caused by *Mycosphaerella caryigena* Demaree and Cole is a common foliar disease of pecan (*Carya illinoensis* Koch.) in the United States and Mexico (Cortés-Ortega et at, 1989; Drye, 1976; Goff, 1980; Littrell and Bertrand, 1981; Sánchez, 1976). The disease produces 2 to 5 mm diameter whitish spots which later turn yellow or brown. Chlorophyll is destroyed and photosynthesis and transpiration are reduced in the affected tissue (Demaree and Cole, 1932; Loustalot and Hamilton, 1941). The disease produces early defoliation (Demaree and Cole, 1932) which reduces tree carbohydrate reserves and subsequently the crop of the following year (Worley 1979a, 1979b). The fungus overwinters in fallen leaves and develops pseudothecia during autumn and winter (Fig. 1A). Ascospores mature in the following spring and constitute the primary inoculum for the next growing season (Demaree and Cole, 1932; Drye, 1976; Goff, 1980; Goff et al, 1987; Littrell and Bertrand 1981).

In the state of Nuevo Leon, Mexico, pecan groves occupy ca. 7,700 hectares, with an estimated annual production of 2,000 t of nuts (Cortés-Ortega, 1998). Downy spot is a common foliar disease in groves with a high population of 'Western' and 'Mahan' cultivars that is more severe particularly in those areas near the irrigation channels of the grove (Cortés-Ortega et al, 1989). Attempts for chemical control over the years, even with different fungicides and spray programs, have produced inconsistent results (González-Garza and Cortés-Ortega, 1982), apparently because of an irregular infection period from one year to the other (Cortés-Ortega et al, 1989). The objectives of this study were to determine ascospore release of *M. caryigena* and downy spot disease incidence over a period of three subsequent years. Part of this work has been previously published (Solís-Gutiérrez, et al., 1985; Solís-Gracia et al., 1996).

MATERIALS AND METHODS

The study was conducted in two pecan groves with history of moderate and severe symptoms of downy spot disease. One grove was located in Montemorelos, Nuevo Leon. The experiment was established on trees of the 'Western' cultivar planted at a spacing of 15 X 15 m. The second grove was located in General Teran, Nuevo Leon. This grove was planted with 'Western', 'Wichita' and 'Mahan' cultivars planted at a spacing of 20 X 20 m. In both cases, the trees were 12 years old.

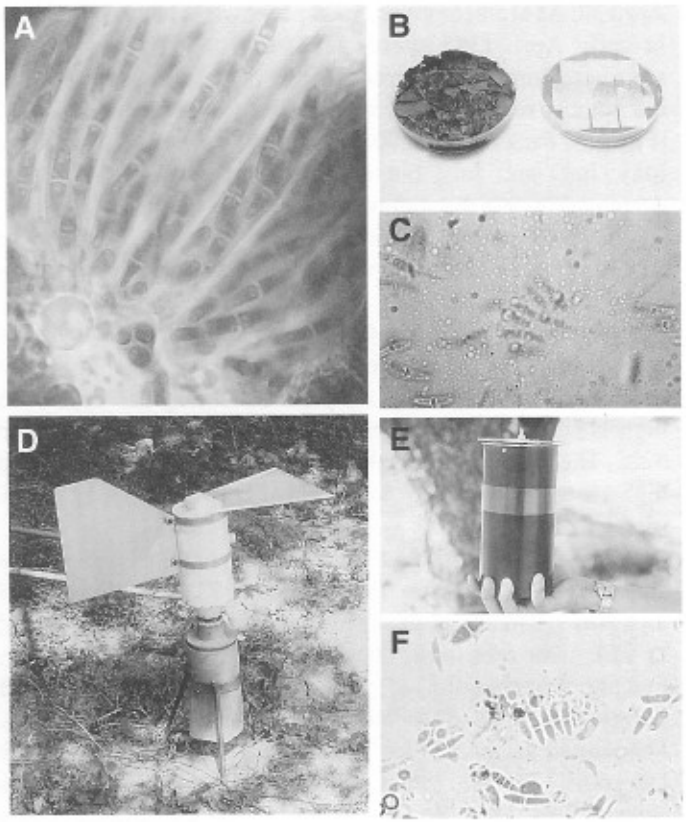

Fig. 1. *M. caryigena* ascospore sampling. **A**, *M. caryigena* pseudothecia observed in overwintered pecan leaves. **B**, overwintered pecan leaves arranged in a moist chamber for determination of ascospore maturity. **C**, ascospores released from pseudothecia present in overwintered pecan leaves after their incubation in moist chambers. **D**, volumetric spore trap used to collect ascospores in the air. **E**, hygrothermograph drum and collecting tape used for ascospore air sampling. **F**, ascopores of *M. caryigena* collected in the air.

Pseudothecia maturity. Fallen pecan leaves were collected in each grove in November of 1983, 1984, and 1985, confined in cages made with chicken wire, and left on the ground exposed to the weather during the winter every year. Overwintered leaves were sampled from the cages during the spring of 1984, 1985, and 1986, brought to the laboratory, and placed in moist chambers to stimulate ascospore release (Goff, 1980). The chambers were assembled as previously described (Goff, 1980; Goff et al., 1987) with some modifications. Moist paper towel discs were placed in two bottom halves of 10 cm diameter petri dishes. Leaf fragments were placed on the total area of the moist disc with the stroma side up. Three pieces of adhesive laminating tape measuring 2 X 6.5 cm were coated with vaseline petroleum jelly and placed on the second petri dish bottom to collect the ascospores. A glass slide was placed between the tape pieces and the moist paper to avoid wetting the tape. The petri dish with the leaf pieces was inverted and placed on the top of the half containing the pieces of tape (Fig. IB). The chambers were sealed using adhesive tape and were incubated for 48 hours at 28ºC. After incubation, the chambers were disassembled and the tape pieces were removed and mounted with lactophenol-cotton blue. The tapes were examined under the microscope and the spores with the morphology and size of *M. carygena* were counted on 20 fields at 40X (Fig. 1 C). Eighteen slides from six moist chambers were evaluated every week for each grove.

Ascospore air sampling. Two seven-day volumetric spore traps (Fig. 1D) were built with PVC material, according to the model described by Gadoury and MacHardy (1983). One spore trap was placed in each grove to sample the ascospores of *M. caryigena* in the air. The traps were situated next to the cages containing the overwintered pecan leaves. The opening of the trap was situated at 70 cm above the ground. The spore trap suctioned 40.7 cubic meter of air daily by a fan generated by a 12-volt motor. The spores were collected on a strip of laminating tape coated with Vaseline petroleum jelly. The tape was placed around a hygrothermograph drum that was installed inside the PVC volumetric spore trap (Fig. 1E). The strip was changed every week and cut into seven pieces. Each piece corresponded to each day of sampling. The tape pieces were mounted on glass slides with a drop of lactophenol-cotton blue to stain the spores and facilitate their counting. A transect of 0.4 X 40 mm along the center of the tape was examined and the ascospores of *M. caryigena* were counted (Fig. 1F). The air was sampled daily from April throughout September of 1984, 1985, and 1986. A hygrothermograph and a pluviometer were installed in each grove to monitor environmental conditions.

Disease occurrence. Five 20-year-old 'Western' pecan

Table 1. Rainfall occurrence during days in which more than 2000 ascospores of *Mycosphaerella caryigena* were detected in the air.

Years	Days with > 2000 ascospores	Rain on same day	Rain 1-3 days prior	Rain 0-3 days prior
1984	25	8	10	18
1985	27	8	11	19
1986	24	9	9	18
1984-1986	76	25	30	55

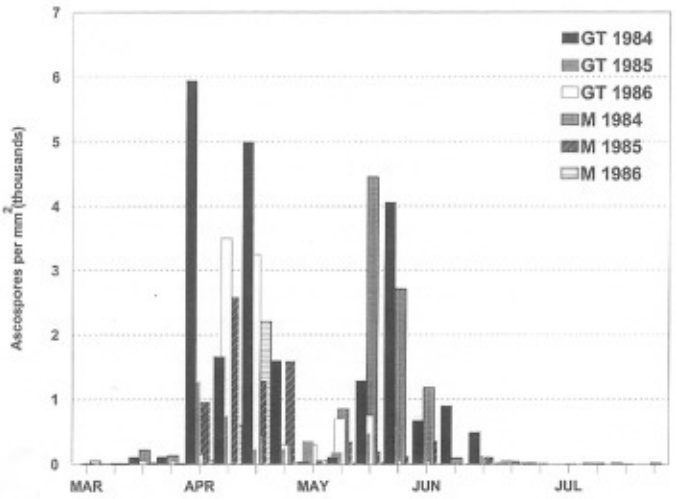

Fig. 2. Weekly sampling of ascospores of Mysocphaerella caryigena released from pseudothecia present in overwintered pecan leaves form General Teran (GT) and Montemorelos (M) after being incubated in a moist chamber for 48 hours from 1984 to 1986.

trees were selected in each of the groves on April of 1984, 1985 and 1986. Four new flushes were selected per tree, one for each point of the compass. Three compound leaves were marked in each fluch at three different levels: basal, medium and apical. The number of leaflets were counted in each marked compound leaf. The leaves were examined weekly and the time of symptom appearance, the number of spots, an the number of leaflets were recorded.

RESULTS

Pseudothecia maturity. Ascospores of *M. caryigena,* ejected from pseudothecia present in overwintering leaves placed in moist chambers, were detected from March to July (Fig. 1C and 2). The results were similar in both groves during the three years. Values of less than 250 spores per mm² of collecting tape were observed during March. Largest numbers of ascospores (up to 5944, 2561, and 3420 per mm² of collecting tape) were observed during April and May in 1984, 1985 and 1986, respectively. The number of spores observed decreased to very low numbers by June of every year (Fig 2).

Ascospore air sampling. Low numbers of ascospores of *M. caryigena* (26 ascospores on April 7) were detected in the air in early April of 1984 in General Teran grove (Fig. 3A). Larger ascospore numbers were detected later that month (1085 ascospores on April 26). The highest amount of ascospores detected was 52,290 on May 18 (it is important to mention that the spore trap fell during a storm in this day and the opening slot was at ground level). Other peaks in spore collection occurred at the end of May, early June and in August, 1984. In Montemorelos, ascospores were detected in the air on April 20, 1984 (18 spores). Higher amounts of spores were detected in late May, late June, and during July. The highest peak in ascospore numbers observed was 6090 on May 29 (Fig. 3B).

In 1985, ascospores were detected in the air in early April (70 ascospores on April 8) in General Teran. The highest amount, 6965 ascospores, was seen on April 15. Other peaks (3200-4500 ascospores) occurred on April 20, 30 and June 9, and 29 (Fig. 3C). In Montemorelos, 35 ascospores were detected on April 1. Very high amounts were detected by April 12 (7945 ascospores). There were several peaks in ascospore production from April to September. The highest peak observed was 19,180 ascospores on May 30 (Fig. 3D).

In 1986, ascospores were detected in the air in early April (35 ascospores on April 3) at General Teran. Larger numbers (2100-44,380 ascospores) were seen during late April and early May (Fig. 3E). The highest numbers of ascospores occurred on April 23. At Montemorelos, ascospores were detected in the air in early April (385 spores on April 1). Several peaks in ascospore numbers occurred during April. The highest peak in the number of ascospores was 10,535 ascospores on April 16 (Fig. 3F). Peaks above 2000 ascospores were observed during May, June and July, but in general, the number of spores decreased during the summer.

In both groves, the occurrence of rain accompanied or preceded most of the days with the highest amounts of spores in the air in all three years (Table 1). However, high numbers of ascospores in the air on days with no rain were recorded, but some of these readings were coincident with irrigation on the groves. The average day temperature during the days with highest numbers of ascospores in the air varied between 19 and 32ºC. The average relative humidity on the days that presented higher numbers of ascospores varied between 55% and 87% (data not shown).

Disease progress. In 1984, disease symptoms appeared on July 9 at General Teran. By September 21, the affected leaf area was 7% (data not shown). After this date, the spots started to fuse with each other and the leaf areas turned necrotic, making the counting of the spots difficult. Five percent defoliation was observed by September 27 (Fig. 4). Defoliation progressed to 51% by October 16 and to 89% by December 15. At Montemorelos, downy spot symptoms appeared by June 29. The affected leaf area was 72% by August 31. Defoliation was 13% by September 21, 61% by October 19, and 98% by November 15 (Fig. 4).

In 1985, downy spot symptoms appeared on May 31 in both groves. At General Teran, the affected leaf area was 4% by September 6 and 11% by October 10. Defoliation was 23% on July 26, 62% on October 24, 84% on November 21, and 100% by December 13 (Fig. 4). At Montemorelos, 23% of affected leaf area was seen by September 6. Defoliation was 40% by late August and 70% by early November (Fig. 4).

In 1986, disease symptoms appeared on June 9 in both groves. At General Teran, defoliation was 6% by August 4, 58% by September 15 and reached 85% by early November (Fig. 4). At Montemorelos, defoliation was 70% by July 28 and 100% by September 22 (Fig. 4).

DISCUSSION

Ascospores of *Mycosphaerella caryigena* were released from pseudothecia present in overwintering leaves when they were placed in moist chambers. The seasonal pattern of

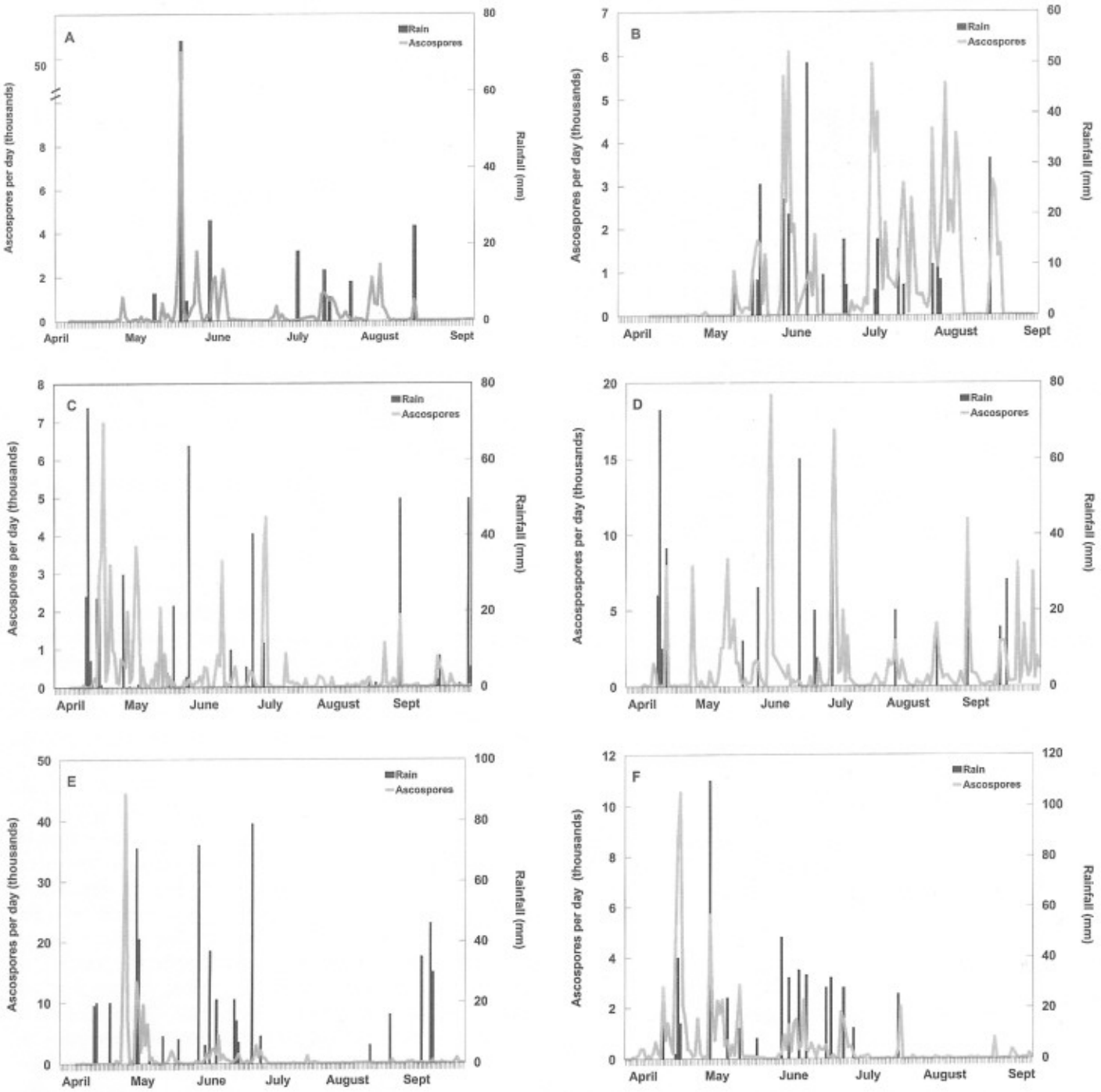

Fig. 3. Ascospores of *M. caryigena* collected daily in the air and daily rainfall records in General Teran during 1984 (A), 1985 (C), and 1986 (E), and in Montemorelos during 1984 (B), 1985 (D), and 1986 (F).

ascospore ejection from the pseudothecia was very consistent in both groves during the three years. Ascospores were collected during spring and summer but the maximum release occurred in April and May. Occurrence of mature pseudothecia and ascospores during spring has been observed for *M. caryigena* in pecan groves in the eastern states of the United States (Demaree and Cole, 1932; Goff et al, 1987) and for other Mycosphaerella species in other areas (Kessler, 1981; Ostry et al, 1982; Patton and Spear, 1983; Quintero and Romero, 1970; Sanchez et al, 1979). Results of this three-year study in the central part of Nuevo Leon, Mexico, indicated that *M. caryigena* ascospores were mature and ready for infection at the time of the new flush production every year. However, some differences were observed in the abundance of mature ascospores among the three years. There was a relative higher amount of mature ascospores of *M. caryigena* in 1984 than in 1985 and 1986. The winter temperatures of 1983-84 were notably lower (freezes of -12ºC) than those of 1984-85 and 1985-86. The extremely low temperatures in the winter of 1983-84 may have led to favorable conditions for ascospores development, as reported for *M. caryigena* and other related ascomycetes (Drye, 1976; Gadoury et al., 1984).

Ascospores were detected in the air in April during the three years at both Montemorelos and General Teran (Fig. 3). In 1984, however, the number of ascospores detected in the air reached large amounts until the middle of May. In the same year, disease symptoms appeared on June 29 and July 9, in Montemorelos and General Teran, respectively. It has been reported that downy spot symptoms in pecan groves appear between 6 to 8 weeks after the initial infection (Goff 1980; Goff et at, 1987; Littrell and Bertrand, 1981). Therefore, the primary infection in 1984 in both groves likely occurred at the middle or the end of May. This time coincided with the period when high amounts of spores were observed in the air of the groves (Fig. 3A and B). Contrasting with the observations from 1984, very large numbers of ascospores were detected in the groves air during April in both 1985 and 1986 (Fig. 3C, D, E, and F). These years, the symptoms appeared at the end of May and the beginning of June. This indicates that the primary infection apparently occurred in April.

Rainfall seemed to have a major influence on ascospore release. Large amounts of ascospores appeared in the air always after the first spring rains (Fig. 3). This was remarkable during 1984 when very few ascospores were collected in the air during April and high peaks were observed when the rains occurred during May. In 1985 and 1986, rainfall occurred during April and very large numbers of spores were collected during that month (Fig. 3C, D, E, and F). Furthermore, the highest peaks in spore collection were generally accompanied or preceded by rainfall. However, some peaks in spore numbers were observed in days with no precipitation. Other factors such as dew, relative humidity or irrigation could have had an effect on spore release. These findings are similar to those reported in previous studies (Goff et al., 1987) where rain was found to increase the release of *M. caryigena* ascospores but appreciable amounts of spores were detected in the air during some days with no precipitation. The average temperature registered during the days with higher numbers of ascospores collected was in the same range as the average temperatures registered during the time of the sampling and no particular pattern in relation to ascospores release was detected.

First appearance of disease symptoms in both groves varied from late May through early July during the three years of the study. There was a close relationship between the number of downy spots lesions on the leaves and the intensity of tree defoliation. Downy spot symptoms and defoliation were more severe at Montemorelos. This grove had a tree spacing 15 x 15 m in contrast to 20 x 20 m at General Teran. This observation is in agreement with some reports where severity of downy spot disease has been mostly associated with groves of high density and poor air circulation (Demaree and Cole, 1932; Goff, 1980; Littrell and Bertrand, 1981; McGlohon, 1975; Payne, 1979). Defoliation was also more intense when symptoms appeared earlier in the season. This happened in 1985 and 1986 in both groves when disease symptoms appeared at the end of May or the beginning of June (Fig. 4).

Results indicate that there is inoculum for downy spot disease in the area of Montemorelos and General Teran during spring and summer since the growers do not eliminate the

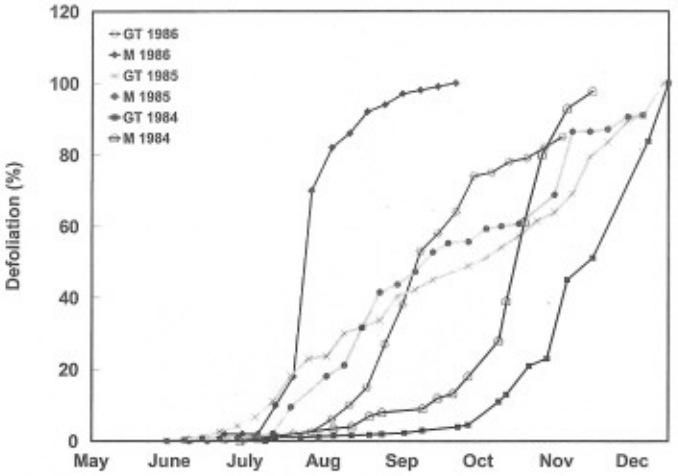

Fig. 4. Defoliation in pecan trees affected with downy spot disease during 1984, 1985 and 1986 in General Teran (GT) and Montemorelos (M).

fallen leaves from previous season. According to these results, although there are mature ascospores present on the ground and ready to be released in April, the primary infection apparently occurs shortly after the occurrence of the first rains of the year. The irregular occurrence of the first rains every year may be the explanation of the wide period in which the symptoms of downy spot disease normally occur in the area. The time of the symptoms appearance may vary with the pattern of the rain timing. Therefore, in years with rain in early spring, there would be early infections and a severe defoliation can be anticipated, especially in those groves of higher density. In contrast, the occurrence of disease symptoms may be delayed and the effect of the disease on tree defoliation can be mild or with no apparent effect during dry spring seasons. However, late infections may also produce early defoliation in groves of high density and poor aeration. It has been documented that early defoliation of pecan trees will cause a severe depletion of tree reserves and a reduction of yield the following year (Worley, 1979a, b); therefore, any premature leaf loss will have a detrimental economic effect. It is clear that the occurrence of rain has an effect on the development of the infection and disease symptom occurrence. This statement is supported by the observations that during the three years of sampling, large numbers of ascospores in the air coincided with the first rains of the spring season. In addition, the appearance of disease symptoms occurred 6-8 weeks from the time when large numbers of spores were detected in the air.

In summary, our results indicate that ascospores are mature in April but their release is regulated by rain. So, irregular pattern of spring rains in the central area of Nuevo Leon may be the reason for the inconsistent pattern of symptom appearance. A successful chemical control program has been established in the Montemorelos and General Teran area (Cortés-Ortega, 1998 unpublished; Manzano-Flores et al, 1985) which it is timed to protect the foliage during the more likely time for presence of inoculum and initial infection occurrence. The program consists of three fungicide applications (Mancozeb has shown excellent results) at 15-day intervals after the new flush appearance and a fourth

application in June, one month after the third application. The program has been used in the area during recent years and has provided a remarkable reduction of disease incidence and defoliation.

There is some information that the anomorph *Cercosporella caryigena* may also play a role as an additional source of inoculum for disease development in pecan groves in South Carolina (Drye, 1976; Goff et al., 1987). In our study, we observed both conidia and conidiophora of *C. caryigena* in sections from downy spot lesions examined under the microscope. The presence of *C. caryigena* in stem cankers and other parts of affected trees and its potential role as additional inoculum source in the central part of Nuevo Leon remains to be determined.

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