Ascospore Survival in Mycosphaerella musicola

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ABSTRACT

Survival of *Mycosphaerella musicola* ascospores in banana leaves was tested at 53, 75, 93, and 100% relative humidities continuously and at 53, 75, and 93% during the day and 100% at night. Germination was greatest with low humidities during the day and high humidities at night. This corresponded to the survival time in tissue exposed to the shade while attached to green banana leaves in the field. A majority of perithecia failed to discharge ascospores after 8 weeks regardless of storage environment except when kept at 5-7 C in a sealed container. In banana fields, survival and infectivity of ascospores are longest in shaded locations with moderate temperatures and with low daytime and high night-time humidities such as commonly occur during dry periods. Even under such conditions, ascospores do not survive in quantity beyond 8 weeks. These results indicate that ambient humidity in the field is a primary factor in the longevity of ascospores. Phytopathology 61:139-141.

Additional key words: leaf spot, bananas, ascospore discharge and germination.

Leach (3) discovered the ascospore stage of banana leaf spot caused by *Mycosphaerella musicola* and studied its role in epidemiology. Ascospores are produced in greatest numbers during the wettest weather (1, 3, 4). The wind-borne ascospores spread more rapidly than water-dispersed conidia from primary infection foci, increasing the rate of disease buildup. The role of conidia in leaf spot epidemiology during a tropical dry season has recently been evaluated (5). During such seasons, when ascospores are not produced, the fate of ascospores already present in diseased leaf tissue is not known. Ascospore survival in different environments was studied to determine what happened to ascospores present in banana leaves during long periods without rain.

MATERIALS AND METHODS.—Banana leaves of the VALERY cultivar, mass-infected naturally with *Mycosphaerella musicola* (Leach), were tested for the presence of viable ascospores. Small samples of infected leaf tissue bearing perithecia were removed, wetted, and placed over slides coated with water agar. Those leaf specimens discharging abundant ascospores with 90% or more germination were pooled, cut into 1-inch squares, thoroughly mixed, and distributed among a set of humidity chambers consisting of desiccator jars with ground glass tops sealed shut with vaseline. Relative humidities (RH) in the chambers were maintained with saturated salt solutions as follows: Mg $(NO_3)_2 \cdot 6H_2O$, 53%; NaCl, 75%; $NH_4H_2PO_4$, 93%; distilled water, 100%. Since the containers were stored at a room temp of 22 to 27 C, RH could have differed slightly from those stated for 25 C. Leaf pieces were removed from four of the chambers at 1- to 2-week intervals to test ascospore discharge and percentage germination on slides coated with water agar. Another set of leaf pieces was removed from chambers at 53, 75, and 93% humidity each afternoon at 4:00 PM and placed in a chamber of 100% RH until 6:30 AM the following day. This was done to simulate alternating low day and high night RH such as occur outdoors. Chambers of the continuous humidity treatment were also opened briefly daily for aeration and to make all treatments comparable. A similar test with fluctuating RH was carried out by placing pieces of ascospore-bearing tissue on green banana leaves in full sun or shade in the field during the day and in a 100% RH moist-chamber at night. Occasionally, when a shower was imminent during the day, the pieces were removed to a sheltered location to avoid ascospore discharge as a result of wetting. Outdoor survival was compared with survival in a sealed Mason jar in a refrigerator at 5-7 C and in a basket on the laboratory shelf.

After 6 weeks' storage at various RH, portions of the leaf tissue were wetted and discharged onto young banana leaves to determine the infectivity of the ascospores still present and viable.

Experiments in humidity chambers were repeated 6 times and outdoor experiments 3 times throughout the year. Because of inherent variability in ascospore germination among different leaf pieces and at different times of the year, the high and low range of germination is shown for the different experiments.

RESULTS.—Low day followed by high night RH preserved germinability for the longest period (Table 1). Germinability decreased fastest at a continuous RH of 75%. Even in the best treatments, germinability decreased rapidly after 6 to 8 weeks. The percentage of samples from the six experiments failing to discharge ascospores is shown in Table 2. Failure to discharge increased to more than 50% in all treatments at 6 to 8 weeks, except with a continuous RH of 53%. Discharge ability was preserved longest at this RH and at 53% during the day and 100% at night. At 100% RH, where discharge failed to occur, tissue had empty perithecia. By placing slides under tissue at 100% RH, it was shown that discharge occurred spontaneously beginning 5 days after storage. At lower RH, perithecia contained ascospores but failed to expel them when wetted.

Data on ascospore survival outdoors, on the laboratory shelf, and in the refrigerator (Table 3) show that survival was still good at 8 weeks in banana shade and 10 weeks in the refrigerator, and poor after 3 weeks on the laboratory shelf or 4 weeks in the sun. Failure to discharge occurred outdoors in the sun at 5 weeks and in the shade at 9 weeks.

The ability of ascospore-bearing leaf tissue stored

TABLE 1. Per cent ascospore germination of *Mycosphaerella musicola* on agar after storage at different relative humidities

	Relative humidity						
	53%		75%		93%		100%
Weeks of storage	Day and night	Day night 100%	Day and night	Day night 100%	Day and night	Day night 100%	Day and night
2	74-100	91-100	80-100	70-100	59-100	93-100	91-100
4	40-90	95-100	0-38	85-100	66-90	93-100	54-100
6	16-53	48-100	0-30	50-100	5-33	0-74	0-23
8	0-26	70-80	0	0-17	0-25	0-30	0
10	0	0-80	0	0	0	0	0

TABLE 2. Per cent leaf samples that failed to discharge ascospores of *Mycosphaerella musicola* after storage at different relative humidities

	Relative humidity						
	53%		75%		93%		100%
Weeks of storage	Day and night	Day night 100%	Day and night	Day night 100%	Day and night	Day night 100%	Day and night
2	0	0	0	0	0	0	0
4	0	25	33	0	0	0	33
6	20	16	50	0	33	66	66
8	25	50	40	60	60	80	60
10	50	50	100	100	100	100	100

for 6 weeks in the various environments to infect banana leaves was tested (Table 4). Material stored at RH of 53 or 75% during the day and 100% at night or in the refrigerator remained the most infective. Material stored in the shade of the banana plant was fairly infective, whereas, storage under all other conditions tested resulted in poor or no infectivity after 6 weeks.

DISCUSSION.—Infectivity of ascospores is dependent on expulsion from the perithecia into moving air, deposition on a susceptible leaf surface, and germination and leaf invasion. At tropical temp, *M. musicola* ascospores did not survive in quantity much beyond 8 weeks because of failure to be released from the perithecium or poor germination after release. The best conditions for survival appear to be those found in tropical dry seasons where high RH occur at night, low RH during the day. High temp such as those occurring on full exposure to sun during the day reduce survival capacity in contrast to shade temp. Under natural conditions, perithecia mature in the shade of the lower leaf canopy in banana plantations. Prolonged periods of 100% RH do not favor survival because ascospores are expelled spontaneously from the perithecium. Continuous low RH favor the capacity for ascospore expulsion over a prolonged period but viability of the expelled spores is reduced.

As observed by Frossard (2), ascospores rapidly lost viability when leaf tissue was stored on the laboratory

TABLE 3. Per cent ascospore germination range of *Mycosphaerella musicola* on agar after storage in different environments

Weeks of storage	Refrigerator, 5-7 C	Shade[a] 15-35 C	Sun[a] 15-38 C	Laboratory shelf, 22-28 C
2	100	90-99	79-98	57-100
3	100	90-100	20-98	55-90
4	95-100	83-96	24-97	0-4
5	95-97	67-86	ND[b]	0-2
6	38-96	56-97	ND	0-4
8	30-97	65-92	ND	0
9	90-95	ND		0
10	85-96			0

[a] Leaf tissue with ascospores attached to banana plant in field during day and placed in chamber of 100% relative humidity during night.

[b] ND = No discharge.

TABLE 4. Ability of ascospores of *Mycosphaerella musicola* to cause leaf spot after storage for 6 weeks in different environments

Storage conditions[a]	Ability to cause leaf spot after storage for 6 weeks[b]
Refrigerator 5-7 C	Very good
75% RH day, 100% night	Very good
53% RH day, 100% night	Good
Shade	Fair
93% RH day, 100% night	Fair to poor
53% RH continuous	Poor
Sun	Nil
Laboratory	Nil
75% RH continuous	Nil
93% RH continuous	Nil
100% RH continuous	Nil

[a] RH = Relative humidity and storage at room temp of 22-28 C.

[b] From 4 to 6 leaf pieces 1-inch square were wetted, and perithecia were allowed to discharge on underside of a recently unfurled banana leaf; very good = 50% or more of leaf area below test pieces necrotic; good = 25% or more; fair = 15% or more; poor = less than 15%.

shelf. This probably results from the absence of saturation for even short periods. Frossard (2) found that ascospores could be preserved for up to 4 months in a refrigerator at 6 C. Similar results were obtained in these studies, and viability was still good at the end of a storage period of 10 weeks in the refrigerator. Thus, for experimental purposes where a large continuous supply of ascospores are required, ascospore-bearing leaf tissue is best stored dry in a sealed container in the refrigerator.

Mycosphaerella musicola ascospores are well adapted for survival, in the absence of rainfall, in lower canopy banana leaves or hanging leaf trash for periods of up to 8 weeks. In Southeast Asia, where most *Musa* spp. originated, and in many other banana-growing areas of the world, rain-free periods seldom exceed 6 to 8 weeks. But in banana zones on the Pacific coast of Central America and in some other irrigated areas of the Western Hemisphere, rain-free periods often exceed 8 weeks. In such areas, few ascospores would survive the dry season, and it is precisely in these areas where control measures are stopped for several months of the year and disease is slow to build up following the resumption of rain.

LITERATURE CITED

1. Brun, J. 1963. La cercosporiose du bananier en Guinée. Étude de la phase ascosporée du Mycosphaerella musicola Leach. Inst. Français de Recherches Fruitiéres Outre-Mer. Paris. 196 p.
2. Frossard, P. 1962. Influence de la température sur la projection et la viabilité de ascospores de Mycosphaerella musicola Leach. Fruits d'Outre Mer 17: 382-385.
3. Leach, R. 1964. Banana leaf spot (Mycosphaerella musicola) on the Gros Michel variety in Jamaica. Government Printer; Kingston. 118 p.
4. Stover, R. H. 1968. Leaf spot of bananas caused by Mycosphaerella musicola: Perithecia and sporodochia production in different climates. Trop. Agr. (Trinidad) 45:1-12.
5. Stover, R. H. 1970. Leaf spot of bananas caused by Mycosphaerella musicola: Role of conidia in epidemiology. Phytopathology 60:856-860.