Survival of *Colletotrichum graminicola*: Importance of the Spore Matrix

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ABSTRACT

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Spores of *Colletotrichum graminicola* are produced in association with a water-soluble matrix material composed of polysaccharide(s) and protein. Spore masses (spores embedded in spore matrix) placed at relative humidities of 90, 80, 70, 60, and 45% lost moisture rapidly and at low relative humidities (70% or less) became dry and powdery within 48 hr. Spores in masses stored at these relative humidities retained viability (measured by germination bioassay) throughout a 4-wk period. However, when the spore matrix was removed prior to storage, viability of the population was significantly reduced within 24 hr (at each relative humidity treatment) and viability was completely lost within 48 hr. Addition of concentrated crude matrix or partially purified matrix components to washed (matrix-free) spores prior to storage at low relative humidity maintained viability of the spore population. The data suggest that in the field spores may survive and be dispersed in dry particulate matter. Hydrolase and invertase enzymes are present in the matrix. Desiccation of the matrix at low relative humidity did not result in a complete loss of enzyme activity. Two roles for the spore matrix in survival of the pathogen are suggested: protection of spores against desiccation and increase in efficiency of germination and penetration through invertase and hydrolase activities.

Additional key words: corn anthracnose, corn, *Zea mays* L., cuticle.

Maize anthracnose has become a significant disease of corn in the United States (7,8,11,13,14,16,20,22,26,27,29,30). The pathogen, *Colletotrichum graminicola* (Ces.) Wils., causes leaf blight, stalk rot, and root rot of seedlings as well as ear and kernel infections (10,18,19,26,28). Although the disease may occur at any time throughout the growing season, crop damage generally has been attributed to leaf blight and stalk rot of plants at stages of maturity either at or past anthesis.

If the infection occurs at the seedling stage, crop loss is sometimes sufficient to require replanting or crop rotation (7). As seedlings mature, further development of leaf lesions is restricted, with lesions often being present only as chlorotic flecks. When plants reach a later stage of maturity (at anthesis and later) lesions enlarge and the fungus sporulates (10,18,19). Thus, lesions formed early in the growing season serve as inoculum for subsequent infections.

Because the fungus overwinters in crop residue on the soil surface (25) the disease often occurs when minimum tillage is practiced (7,10,18). Conventional tillage or crop rotation is often recommended to control the disease in fields where anthracnose has been a problem during the previous growing season (7). Where corn follows corn, conventional tillage reduces inoculum and early disease development thereby reducing or eliminating "secondary"

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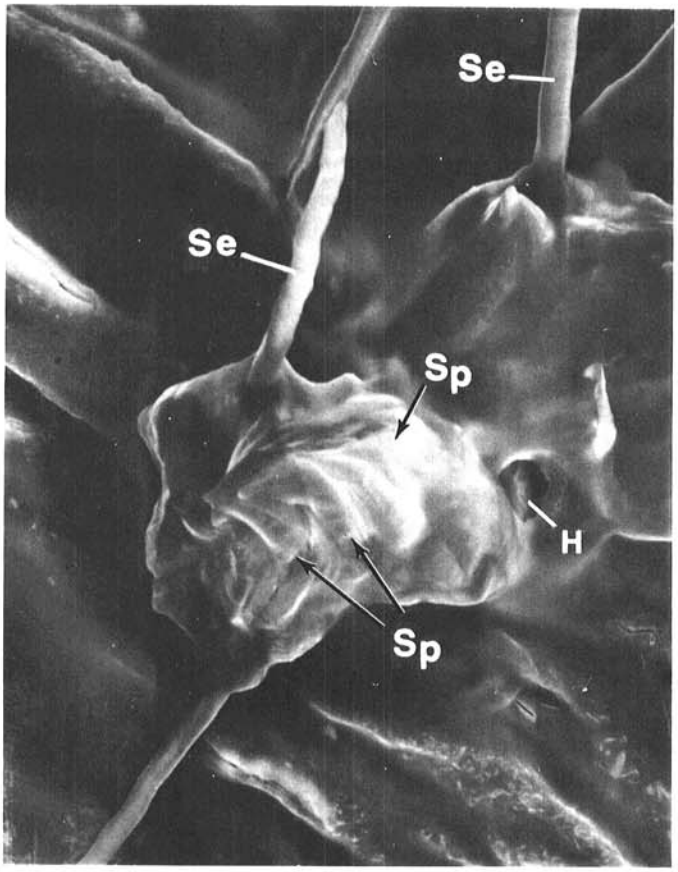

Fig. 1. An acervulus of *Colletotrichum graminicola* on the surface of an infected corn leaf. The outlines of several spores (Sp) embedded in the mucilaginous matrix of the spore mass are visible. A hole-like area (H) is visible where the matrix appears to have flowed around rather than covered the leaf surface. Setae (Se) protrude from the spore mass. Photomicrograph taken by scanning electron microscopy of unfixed, uncoated tissue (magnification × 1,000).

spread of the pathogen when the crop matures.

Free water is necessary for successful penetration of the corn leaf by *C. graminicola* spore germlings and disease severity is most often associated with periods of wet weather. It is generally assumed that spread of the fungus is dependent upon wet, windy weather (10). It is also assumed that this requirement for free water restricts the spread of the pathogen to relatively short distances. Such assumptions do not adequately account for the rapid spread of maize anthracnose across the corn belt (7,10,11,13,16,22,27), the random appearance of epiphytotics (7,27), or the occurrence of late-season stalk infections in the absence of infested residue from previous crops (10). These incidences of disease might be explained if spores could be dispersed without water as wind-blown "dry" particulate matter.

Spores of *C. graminicola* are embedded in a mucilaginous matrix (spore matrix) in acervuli on infected tissue (Fig. 1) and when the fungus sporulates on agar media. In the field as well as in culture the wet spore masses eventually become dry. If viability is retained by some proportion of spores after desiccation of the mucilaginous matrix, the resulting "dry" particulate matter could represent a source of wind-blown inoculum.

Previous studies in this laboratory have demonstrated that the spore matrix contains invertase (5,6) and a nonspecific hydrolase (2,3). It was suggested that the invertase functions in the acquisition of a carbon source for the germinating spore (5) and that hydrolase activity accounts for the partial degradation of the corn leaf cuticle which occurred when leaves were incubated in the presence of hydrolase-active matrix preparations (3). Preliminary assays of matrix material also revealed the presence of substantial levels of polysaccharide and protein.

In the present investigation we report the ability of *C. graminicola* spores to survive extended periods of desiccation at low relative humidities and demonstrate the involvement of the spore matrix and partially purified matrix components in that survival.

MATERIALS AND METHODS

Influence of relative humidity on spore survival. To obtain sufficient spores in natural association with matrix material, the

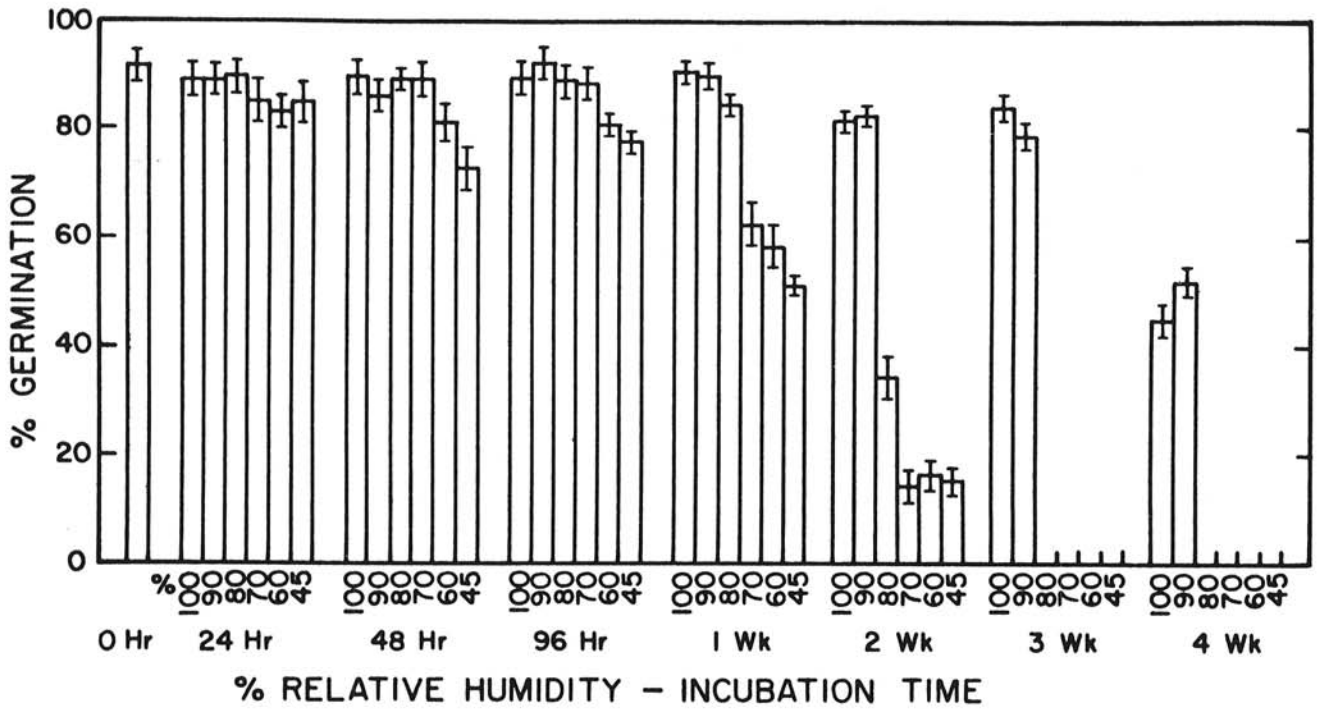

Fig. 2. Germination of spore populations of *Colletotrichum graminicola* after storage of spore masses (spores embedded in spore matrix) at various relative humidities throughout a 4-wk period. Spore masses were not rehydrated. Variance in the data is expressed as the 95% confidence interval.

fungus was cultured on sterile, 0.22-μm, MF type Millipore filters (Millipore Corp., Bedford, MA 01730) placed on the surface of oatmeal agar in petri dishes. The culture plates were then inoculated with spores of *C. graminicola* by flooding with 0.2 ml of a spore suspension containing ~6×10^6 spores per milliliter prepared from a 10-day-old culture. The culture plates were placed under constant fluorescent light (3,500 lux) at 24 C (9). After 10 days, the Millipore filters were covered with the pink spore masses which are typical of *C. graminicola*. The filters were removed from the agar surface and adhering agar was removed.

The spore masses were then stored on the filters at 25 C at relative humidities (RH) of 100, 90, 80, 70, 60, and 45% for the desired times. Relative humidity chambers consisted of airtight plastic boxes ($10.5 \times 10.5 \times 9$ cm) containing aqueous glycerine solutions at concentrations (specific refractive indices) to provide the designated relative humidities at 25 C (1). Refractive indices as measured with a Bausch & Lomb refractometer were 1.3773 (90% RH), 1.4015 (80% RH), 1.4191 (70% RH), 1.4329 (60% RH), and 1.4486 (45% RH). Sterile distilled water (refractive index 1.3329 at 25 C) was used in the 100% RH chamber. The glycerine solutions contained 1% (w/v) $CuSO_4 \cdot 5\ H_2O$ to prevent the growth of microorganisms (24). Filters with their associated spore masses were uniformly positioned 1.5 cm above the surface of the glycerine solution in each relative humidity chamber.

Spore survival after storage at various relative humidities over a 4-wk interval was determined by measuring the ability of spores to germinate. Spore suspensions were prepared by transferring individual filters with associated spore masses from the relative humidity chambers to sterile distilled water. Spore masses were soaked for 5 min after which the spores were brought into suspension by vigorous agitation on a vortex mixer. The spore concentration was adjusted to 10^6 spores per milliliter and 0.1 ml of the suspension was plated on 0.5% sucrose agar. Spores were incubated for 12 hr at 25 C, after which they were killed and stained with lactophenol-cotton blue and observed for germination. Spores were considered to have germinated if a germ tube was protruding from the spore wall. Each treatment was replicated three times. A minimum of 750 spores per replicate per time treatment were counted for determination of percentage germination.

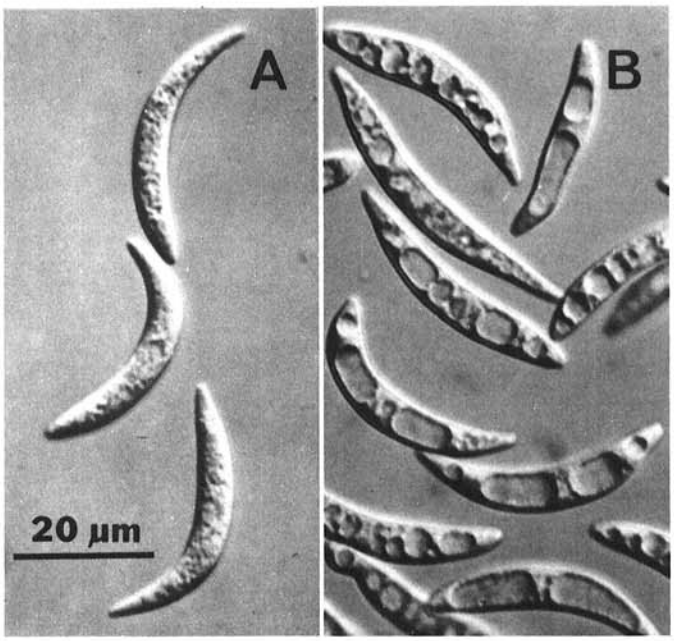

Fig. 3. Spores of *Colletotrichum graminicola* from spore masses (spores embedded in spore matrix) which had been stored at 45% relative humidity (RH) for 4 wk. **A,** Spores from spore masses which were not rehydrated prior to preparation of suspensions and **B,** spores from spore masses rehydrated at 100% RH for 24 hr prior to preparation of suspensions. Note the disruption of cellular integrity in A but not B. Interference-contrast optics.

Rehydration of spore masses and influence on germination. In preliminary studies it was found that spore masses became progressively drier and sometimes brittle as the relative humidity decreased. Thus, the preparation of spore suspensions by submersion of spore masses taken from low relative humidity directly into water could have resulted in lethal osmotic shock. To test this possibility, spore masses which had been stored for 4 wk at 90, 80, 70, 60, and 45% RH were transferred to 100% RH for rehydration. Spore masses were rehydrated for 24 and 72 hr prior to preparation of spore suspensions. Spore viability was determined by the spore germination assay as described above. The experiment was repeated three times with each treatment carried out in triplicate.

Determination of hydrolase and invertase activities in spore matrix preparations. It has been suggested that invertase and hydrolase enzyme activities in the spore matrix of *C. graminicola* may play a role in anthracnose disease development (2,3,5,6). Therefore, it was important to determine the extent to which enzyme activity was retained when the spore matrix was subjected to drying at various relative humidities over time.

Enzyme activity was assayed in matrix which had been stored at various relative humidities over a 3-wk period. The source of matrix was the spore preparations used to test survival of spores after storage at different relative humidities. Spore suspensions (10^6 spores per milliliter) prepared as for germination assays were centrifuged (2,000 *g*, 5 min) and the supernatants were filtered through 0.22-μm (pore size) Millipore filters to ensure the complete

TABLE 1. Influence of rehydration on germination of *Colletotrichum graminicola* spores stored at various relative humidities (RH)

Storage RH[a]	Germination (%) without rehydration[b]	Germination (%) after rehydration at 100% RH	
		24 hr	72 hr
90%	51.0 ± 3.4[c]	70.0 ± 4.0	67.1 ± 4.8
80	0	0	19.9 ± 3.9
70	0	0	8.5 ± 2.3
60	0	3.6 ± 1.7	28.6 ± 3.9
45	0	50.7 ± 8.4	51.9 ± 6.8

[a] Spore masses maintained at specified relative humidity at 25 C for 4 wk.
[b] Spore suspensions (10^6 spores per milliliter) were prepared in sterile distilled water and percent germination was determined 12 hr after plating spores on 0.5% sucrose agar.
[c] Variance expressed as the confidence interval, $P = 0.05$.

TABLE 2. Germination of *Colletotrichum graminicola* spores after removal of the spore matrix and storage at various relative humidities (RH)

Germination (%) of washed spores[a] with matrix removed	64.7 ± 2.9[b]
Germination (%) of unwashed spores with matrix intact	68.8 ± 3.1
Germination (%) of unwashed spores stored at 100% RH for 24 hr	66.0 ± 2.5

Storage RH(%)	Germination (%) of washed spores stored at various RH	
	24 hr	48 hr
100	28.7 ± 3.8	11.9 ± 2.2
90	2.1 ± 0.9	0
80	1.4 ± 0.8	0
70	0.6 ± 0.6	0
60	1.5 ± 1.2	0
45	1.4 ± 0.9	0

[a] Washed spores were prepared by repeated (5 ×) centrifugation (2,000 *g*, 5 min) and resuspension in sterile distilled water.
[b] Spore suspensions (10^6 spores per milliliter) were prepared in sterile distilled water and percent germination determined 12 hr after plating spores on 0.5% sucrose agar. Variance is expressed as the confidence interval, $P = 0.05$.

exclusion of spores from the filtrate. Previous studies (5) had shown that the filtrate from the first aqueous spore wash contained 98% of the water-soluble protein from the matrix material. The spore filtrates were frozen (−20 C) until assayed for enzyme activity.

Invertase (EC 3.2.1.26) activity was measured by determination of reducing sugars by the method of Somogyi (23) and by using Nelson's (17) arsenomolybdate reagent as a color developer. Incubation mixtures consisted of 0.25 ml of 0.73 M sucrose, 0.50 ml of 0.1 M sodium acetate buffer (pH 4.7), and 0.25 ml of filtrate. Reaction mixtures were incubated at 30 C for 10 min after which the enzymatic reaction was stopped by addition of 1.0 ml of Somogyi's alkaline copper reagent (23). After the addition of 1.0 ml arsenomolybdate reagent (17), absorbance was determined at 520 nm. Autoclaved filtrate preparations served as controls and each assay was run in triplicate. A unit of invertase was defined as the amount of enzyme which catalyzed the release of reducing sugars equivalent to 1 μg of glucose per hour under conditions of the assay. Specific activity was given as invertase units per milligram of protein. Protein was determined by the method of Lowry et al (15).

Hydrolase activity (nonspecific esterase, hydrolyzing carboxylic acid esters, EC 3.1.1) was determined on the basis of hydrolysis of *p*-nitrophenol from *p*-nitrophenylpropionate by a modification of the method of Huggins and Lapides (12). Reaction mixtures consisted of 1.25 ml of 0.05 M phosphate buffer (pH 7.5), 0.25 ml of 6×10^{-4} M *p*-nitrophenylpropionate in the same buffer, and 1.0 ml of spore filtrate preparation. The release of *p*-nitrophenol from *p*-nitrophenylpropionate was measured spectrophotometrically at 400 nm at 25 C. One unit of activity was defined as the amount of enzyme which catalyzes the hydrolysis of 1 μg *p*-nitrophenol from the ester per hour under conditions of the assay.

Influence of spore matrix on spore survival. The importance of the spore matrix of *C. graminicola* to spore survival was investigated by incubating spores in the absence of matrix at various relative humidities followed by measurement of their ability to germinate. Spores with associated water-soluble spore matrix were scraped from the surface of 7-day-old cultures and suspended in sterile distilled water (approximately 20 ml per 9-cm-diameter culture plate). Spores were washed free of matrix material by repeated centrifugation (2,000 *g*, 5 min) and resuspension in sterile distilled water. Spores were washed a total of five times but only two washings were required to remove the matrix material. The removal of matrix material was monitored by assaying each supernatant for protein (15), for absorbance at 280 nm, for invertase and hydrolase activities, and for the presence of carbohydrate by the anthrone test (4).

Washed spores were resuspended in distilled water to a concentration of 10^6 spores per milliliter. The spores from 20-ml aliquots of the suspension were collected on Millipore filters (8 μm pore size) and the filters were placed in relative humidity chambers and incubated for 24 and 48 hr. The diameter of the layer of spores collected on the filter was 15 mm. Spore suspensions (10^6 spores per milliliter) were prepared after the incubation period and spore survival was measured by determining germination on sucrose agar as described above. The experiment was repeated three times and each treatment was replicated in triplicate. A minimum of 750 spores were counted for each replication of each treatment. The germination of washed (matrix removed) and unwashed spores also was determined for each spore preparation at the time the spores were harvested from culture plates.

Fractionation of the spore matrix. Matrix material was separated from spores (8-day-old cultures) by the spore-washing procedure using 20 ml of sterile distilled water per culture plate. Following centrifugation (6,000 *g*, 20 min, 4 C) the supernatant containing the matrix material was filtered through a Millipore filter (0.22 μm pore size) and the filtrate was concentrated approximately fifty-fold in dialysis tubing against PEG 6000 at 4 C. The resulting concentrated matrix preparation was centrifuged again (6,000 *g*, 20 min, 4 C) and the clear supernatant was either stored (−20 C), used immediately for fractionation by gel chromatography, or dialyzed, reconcentrated and used in gel chromatography.

Five milliliters of concentrated matrix preparation (approximately 900 μg protein per milliliter) were applied to a column (2.5 × 34 cm) of Sephadex G200 that had previously been equilibrated to pH 7.0 with 0.05 M phosphate buffer. The column was eluted with phosphate buffer (0.05 M, pH 7.0) and 3-ml fractions were collected. The flow rate was maintained at approximately 4.7 ml/hr/cm^2.

Each fraction was monitored for absorbance at 280 nm, for protein content (15), for hydrolase and invertase activities, and for the presence of carbohydrate (4). Fractions representing principal components of the matrix were pooled and concentrated to approximately 5 ml at 4 C against PEG 6000.

The ability of matrix and partially purified matrix components to sustain spore viability was estimated using the bioassay for spore germination. Suspensions of washed spores (10^6 spores per milliliter) were prepared and the spores from 20-ml aliquots were

TABLE 3. Retention of hydrolase activity in spore matrix preparations from *Colletotrichum graminicola* spore masses stored at various relative humidities

Storage period	Relative humidity					
	100	90	80	70	60	45
24 hr	342 ± 66[a]	237 ± 24	254 ± 30	263 ± 21	336 ± 62	276 ± 37
48 hr	386 ± 9	388 ± 45	291 ± 35	257 ± 14	182 ± 23	156 ± 8
1 wk	309 ± 58	211 ± 16	263 ± 7	157 ± 38	124 ± 21	142 ± 9
2 wk	594 ± 51	115 ± 34	74 ± 17	26 ± 10	29 ± 3	46 ± 14
3 wk	192 ± 10	76 ± 24	37 ± 6	43 ± 8	36 ± 5	26 ± 10

[a]Values represent specific activity (units per milligram protein, one unit of hydrolase is equivalent to that which catalyzes the hydrolysis of 1 μg *p*-nitrophenol from *p*-nitrophenyl propionate per hour under conditions of the assay). Each value is the mean of nine observations from three, three-replicate experiments. Variance is given as the standard error of the mean.

TABLE 4. Retention of invertase activity in spore matrix preparations from *Colletotrichum graminicola* spore masses stored at various relative humidities

Storage period	Invertase activity after storage at relative humidity (%)					
	100	90	80	70	60	45
24 hr	4,699 ± 551	5,111 ± 276	4,017 ± 1,232	6,183 ± 839	6,605 ± 1,616	8,266 ± 1,952
48 hr	5,698 ± 326	6,120 ± 631	6,391 ± 117	7,313 ± 348	6,886 ± 736	7,042 ± 594
1 wk	4,668 ± 149	5,740 ± 504	6,950 ± 937	3,360 ± 674	4,231 ± 202	3,288 ± 542
2 wk	4,032 ± 597	3,478 ± 521	4,103 ± 300	4,921 ± 628	3,180 ± 123	3,314 ± 650
3 wk	3,322 ± 315	3,304 ± 1,072	2,061 ± 712	2,061 ± 712	3,290 ± 814	2,042 ± 525

[a]Values represent specific activity (units per milligram protein, one unit of invertase is equivalent to that which catalyzes the release from sucrose of 1 μg glucose per hour under conditions of the assay). Each value is the mean of nine observations from three, three-replicate experiments. Variance is given as the standard error of the mean.

collected on Millipore filters (8 μm pore size). Fifty microliters of test solutions were uniformly applied to the spores and the filters were placed in 80% RH chambers at 25 C for 24 hr. Test solutions included unconcentrated crude spore matrix, partially purified matrix components, and water as a control. After the storage interval, spore suspensions were prepared and germination assays were performed as previously described. Each treatment was carried out in triplicate for each of three experiments.

RESULTS

Retention of spore viability at various relative humidities. When spore masses were incubated at 80% RH or less, their surface texture appeared dry rather than moist and mucilaginous within 24 hr from the start of incubation. Within 48 hr, drying was so extensive that when spore masses stored at 70% RH or less were scraped from their filter supports the material was dry and powdery. Generally, spore masses stored at 90% RH exhibited dryness of surface texture after 48 hr of incubation, but never appeared as dry as those stored at lower relative humidities.

At the initiation of the experiment (0 hr) the percent germination of "fresh" unstored spores was 91.5 ±2.9% (Fig. 2). Spores stored at 60 and 45% RH for 48 hr exhibited a significant decrease in germination (81.2 ± 3 and 72.5 ± 4%, respectively). As shown in Fig. 2, spore germination decreased progressively with time so that by 2 wk germination of spores stored at 80, 70, 60, and 45% RH was reduced to 33.9 ± 4.6, 14.3 ± 2.7, 16.5 ± 2.9, and 14.9 ± 2.5%, respectively. And after 3 wk no germination was observed for spores stored at 80% or less RH. Germination of spores stored at 100 and 90% RH also was reduced with storage time. At high relative humidity a significant loss of germination (as compared to 0 hr germination) occurred only after 2 wk of incubation when germination dropped to 81.2 ± 2.2% at 100% RH and 82.3 ± 2.4% at 90% RH. Even after 4 wk a level of 44.4 ± 2.9% and 51.0 ± 3.4% germination was observed for spores stored at 100 and 90% RH. Thus, under the conditions of the germination bioassay, viability apparently was lost when spores were stored at 80% RH or less for 3 wk.

Spore germination after rehydration (100% RH) of spore masses. When spore masses which had been stored for 4 wk at various relative humidities were rehydrated at 100% RH for 24 and 72 hr prior to preparing suspensions, germination again was observed (Table 1). Thus, spore populations that apparently had lost viability (Fig. 2) had, in fact, maintained a considerable level of spore viability. When spores were observed through the microscope it was found that those which had not received the rehydration treatment exhibited disruption of the cytoplasm (Fig. 3A) and lacked the vacuolation and cytoplasmic integrity characteristic of living *C. graminicola* spores (Fig. 3B). Spores that

TABLE 5. Protection of washed *Colletotrichum graminicola* spores from desiccation at 80% RH for 24 hr by treatment with spore matrix and partially purified matrix components prior to storage

Material added to washed spores	Spore germination (%)		
	Exp. 1	Exp. 2	Exp. 3
Crude matrix[a]	30.4 ± 5.5[b]	10.2 ± 2.4	22.2 ± 2.5
Band 1	47.1 ± 5.1	81.5 ± 3.1	···
Band 2	41.2 ± 5.1	71.4 ± 8.2	60.8 ± 4.2
Band 3	47.3 ± 4.5	90.6 ± 2.5	16.9 ± 1.9
Water	0.1 ± 0.1	2.9 ± 1.5	0

[a]Bands 1, 2, and 3 represent pooled effluents from Sephadex G200 fractionation of crude matrix material. Different matrix and band fractions were used in each of the three experiments.

[b]Each value is the average of no fewer than 750 spores observed for each of three replicates for each experiment. Variance given is at $P = 0.05$.

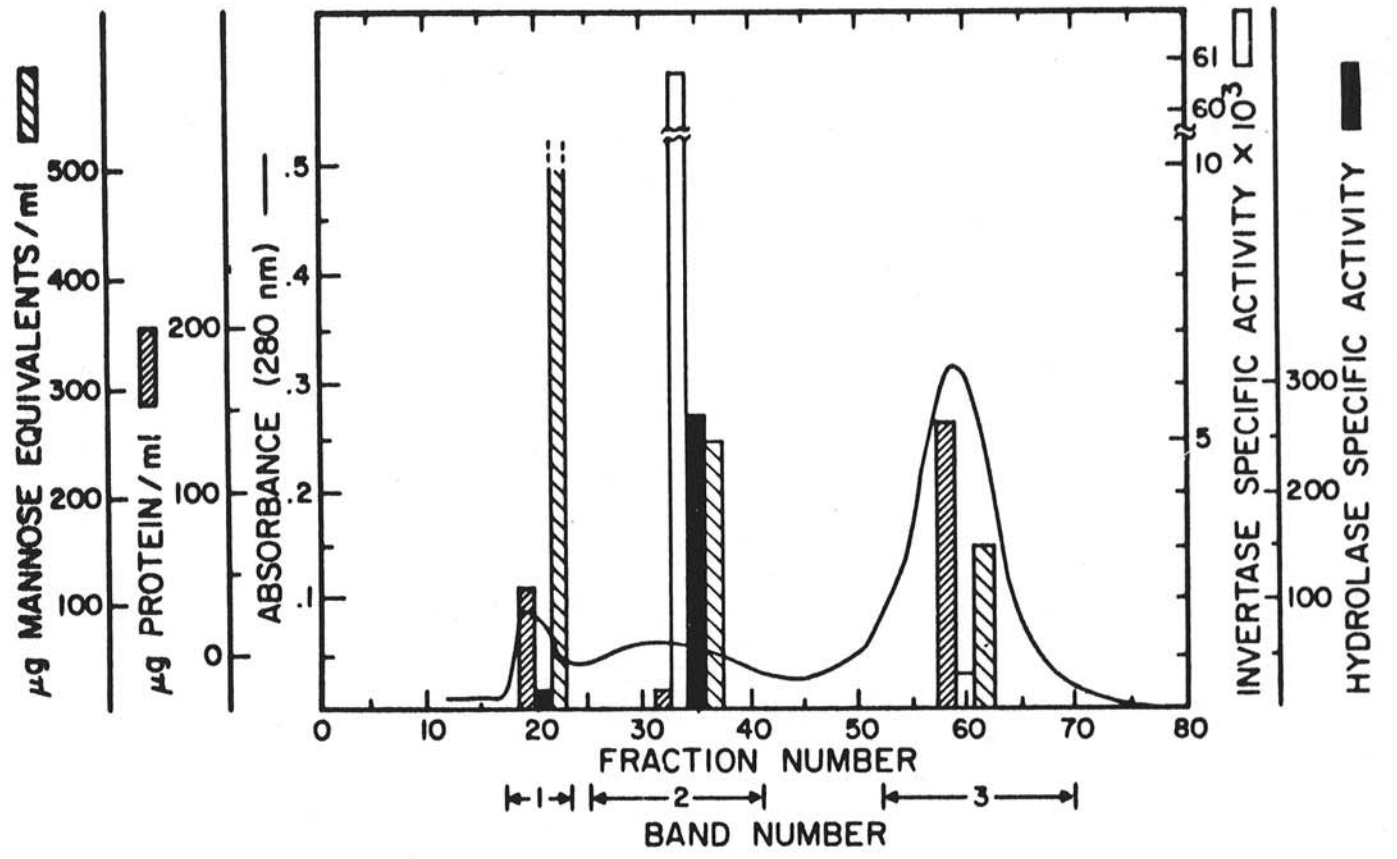

Fig. 4. Sephadex G-200 gel filtration of the water soluble spore matrix of *Colletotrichum graminicola*. Crude dialyzed matrix (900 μg protein per milliliter) was eluted through the gel with 0.05 M phosphate buffer (pH 7.0). Fractions (3-ml) were collected and assayed for hydrolase, invertase, absorbance at 280 nm, protein content, and carbohydrate content as described in the text. Fractions representing three peaks of absorbance at 280 nm were pooled and designated as Bands 1, 2, and 3.

had undergone the rehydration treatment (Fig. 3B) could not be distinguished from spores taken directly from 7-day-old cultures. These observations suggest that the apparent loss of viability of spores stored at RH of 80% or less probably was caused by osmotic shock.

Storage at low relative humidity (45 and 60%, compared to 70 and 80% RH) also appears to have protected spores from loss of viability (Table 1). Spores stored at 60 and 45% RH exhibited significantly better germination (28.6 ± 3.9 and 51.9 ± 6.8%, respectively) than spores stored at 80 and 70% RH (19.9 ± 3.9 and 8.5 ± 2.3%, respectively) following the 72-hr rehydration period (Table 1). The reason for this apparent protection is unknown. It is possible that drying of the spore mass at low relative humidity reduced spore metabolism to the extent that intracellular nutrients essential for germination were not depleted.

Viability in the absence of the spore matrix. Removal of the spore matrix by the washing procedure had no influence on the abiltiy of spores to germinate if the germination assay was carried out immediately (Table 2). However, germination was reduced significantly (maximum of 2.1 ± 0.9% for spores stored at 90% RH) when matrix-free spores were maintained at 90% RH or less for 24 hr. After 48 hr of storage, no germination was observed in spores maintained at 90% RH or less. Thus, even brief (24-hr) storage without the spore matrix material resulted in a loss of viability at each relative humidity level (Table 2), a loss that would not have occurred in the presence of matrix material over the same storage period (Fig. 2, Table 1).

Microscopic observation of spores stored at 90% RH or less revealed extensive disruption of the cytoplasm. The protective influence of the matrix appears then to be associated in part with preventing spore desiccation.

As shown in Table 2, significantly reduced spore germination also occurred when matrix-free spores were maintained at 100% RH for 24 and 48 hr (28.7 ± 3.8% and 11.9 ± 2.2% germination, respectively). Because desiccation does not occur at 100% RH, these data suggest that the matrix has an effect on spores in addition to preventing desiccation. Such an effect could be to suppress metabolism and prevent the utilization of nutritional reserves in an environment not conducive to germination.

Retention of invertase and hydrolase activities in the spore matrix. The matrix from spore masses stored at various relative humidities (Fig. 2) was collected and assayed for invertase and hydrolase activities (Tables 3 and 4). Enzyme activity was present in matrix preparations from spores maintained at each relative humidity throughout a 3-wk storage period. Considerable variation in specific activity of each enzyme at each storage time and relative humidity treatment was observed. This variation may result from the differential rates of drying of the matrix material which occurs at each relative humidity level. The data show that there was a general loss of enzyme activity with time and that the loss was most pronounced for spore masses that had been stored at lower relative humidity. Regardless of the observed variation in enzyme activity, it is important to note that activity was present at all relative humidity treatments throughout the 3-wk storage period. This suggests that the natural drying of the spore matrix (which occurs in the field) would have relatively little effect on enzyme activity.

Matrix components and their role in maintaining spore viability. Molecular sieve gel chromatography resulted in separation of the matrix into three major peak areas based on absorption at 280 nm (Fig. 4). Fractions representing these peak areas were pooled and designated as bands 1, 2, and 3. All fractions included in band 1 contained hydrolase but not invertase activity; those representing band 2 contained both hydrolase and invertase activities; and those fractions representing band 3 contained unknown protein(s) with trace levels of invertase. Carbohydrate was associated with all fractions. The calculated range of molecular weight for each band was 4.0×10^5 and larger (band 1), $2.0–2.5 \times 10^5$ (band 2), and $0.3–0.4 \times 10^5$ (band 3).

Two methods of further purification of whole matrix and bands 1, 2, and 3 were attempted: DEAE cellulose chromatography with a linear NaCl gradient (0 to 0.25 M) in phosphate buffer (0.01 M, pH 7.0); and passage through Sephadex QAE-A25 gel eluted with 0.1 M Tris buffer at pH 9.0, 8.0, and 7.2 in that order. These procedures neither improved the separation of protein components beyond that observed with Sephadex G200 nor did they dissociate carbohydrate material from the major protein components of the matrix.

Treatment of washed spores with crude matrix prior to storage at 80% RH for 24 hr resulted in a significant increase in spore germination compared to water-treated controls (Table 5). Spores also were protected from a loss of germinability by treatment with the partially separated matrix components designated as bands 1, 2, and 3 (Table 5, Fig. 4). However, the data did not demonstrate that protection was associated with a specific elution band.

DISCUSSION

The results of this study clearly demonstrate that under laboratory conditions, spore populations of *C. graminicola* retained substantial levels of viability even after storage of the spore mass at relative humidities as low as 45%. The apparent loss of viability of spores stored at 80% RH or less for 3–4 wk (Fig. 2) was probably the result of too-rapid a change in osmotic potential during preparation of spore suspensions from dry spore masses. This hypothesis is supported by the data which show that spore viability was again evident after rehydration of spore masses at 100% RH (Table 1) and by the observation that spores retained cytoplasmic integrity only if rehydrated prior to preparation of suspensions (Fig. 3A and 3B).

Removal of the mucilaginous, water-soluble spore matrix from spores prior to storage at any relative humidity for as little as 24 hr resulted in a significant reduction in viability of the spore population (Table 2). However, washed spores were protected from a loss of germinability by treatment either with concentrated crude matrix material or with components of the matrix which had been partially purified by Sephadex gel chromatography (Table 5, Fig. 4). Therefore, we propose that the water-soluble matrix protects spores of *C. graminicola* from periodic desiccation and rehydration due to changing relative humidity in the field.

Because spore viability is retained in the dry spore mass, it is reasonable to assume that spore masses produced on leaf tissue and subjected to a low relative humidity could serve as sources of secondary inoculum after even a brief period of rain. Furthermore, we suggest that desiccation of the spore mass allows for the wind dissemination of spores in the form of dry particulate matter. It would be difficult to determine the distance over which spore masses could be wind disseminated without loss of viability. However, it is evident that spread of *C. graminicola* is not dependent upon wet, windy weather as previously assumed (10).

Anthenill and Nicholson (3) demonstrated the presence of a heat-labile cuticle-degrading factor in the *C. graminicola* spore matrix and suggested that cuticle degradation, and therefore efficient penetration, was associated with hydrolase(s) in the matrix. Bergstrom (5) demonstrated that germination of *C. graminicola* spores was enchanced by sucrose and suggested that invertase in the spore matrix may aid in the germination process. Invertase and hydrolase enzyme activities in the spore matrix were not eliminated by desiccation of the matrix at low relative humidity (Tables 3 and 4). These enzymes probably would not be inactivated by drying of the matrix under field conditions.

Efficient germination and penetration are necessary for survival of this pathogen in the field. Liquid water is required for spores to germinate. Periods during which liquid water is available in the field are likely to be of short duration. Germination and penetration may take up to 12 hr (21). The protection provided by the matrix is lost when water breaks up the spore mass. Thus, the spore matrix not only protects spores from desiccation, but also contains invertase and hydrolase enzymes which aid germination and penetration.

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