The Effect of Xylose on the Growth and Sporulation of an Isolate of *Bipolaris maydis* Race T and its Relation to pH and Ammonium Levels

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ABSTRACT

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Sporulation of an isolate of *Bipolaris maydis* race T (ATCC 36180) was significantly higher and one and a half to two times greater on media containing xylose as a sole carbohydrate source or when used in a 1:1 ratio (w/w) with glucose than on media containing only glucose after 6 days of incubation at 28 C in the dark. The effect of xylose was observed with carbohydrate concentrations of 2, 5, or 10 g/L. Moreover, the magnitude of the effect of xylose on sporulation was the same at 6, 10, or 14 days of incubation. No unique response of mycelial dry weight in the presence of xylose was observed. After 10 days of incubation on media with 5 or 10 g of carbohydrate per liter, mycelial dry weights were significantly higher than after 6 days but comparable to those after 14 days. In the absence of carbohydrate or in the presence of 2 g of carbohydrate per liter there was no change in dry weight after any of the incubation times. Ammonium (as measured by using Nessler's reagent) was produced by the fungus and released in the culture medium. The levels were significantly lower with each increase in concentration of carbohydrate. In addition, ammonium was significantly lower in the presence of xylose than with glucose alone. The differences in ammonium levels due to carbohydrate concentration or xylose were observed at 6, but not at 10 or 14, days of incubation. Also, at the latter times the ammonium levels were comparable at all concentrations of glucose, glucose-xylose, or xylose. The trends in pH values paralleled those seen for ammonium. By 14 days, the final pH and ammonium levels were 8.3 and 16.5 μmoles of NH_4^+, respectively, per milliliter. Furthermore, trends in ammonium and pH levels obtained through titration of the basal medium with ammonium hydroxide were similar to those found in fungal cultures. This suggests that pH and ammonium levels may interact in the culture medium and may affect growth and sporulation of the fungus.

Since abundant sporulation is essential for an epiphytotic of southern corn leaf blight incited by *Bipolaris maydis* Nisikado (Shoemaker) (=*Cochliobolus heterostrophus* Drechsler), factors that affect sporulation are worthy of study. The effect of kind and concentration of carbon sources have been studied with regard to sporulation of *Helminthosporium* spp. pathogenic on gramineous crops (8,11). Many of the carbon sources studied are known to be constituents of cell wall polysaccharides. For example, xylose, a constituent of xylan, which is present in high concentrations in corn cell walls (15), has been observed to especially enhance the sporulation of *Bipolaris maydis* race T (6,7). Studies on the effect of xylose have been given precedence since it has been shown that the fungus has the capacity to produce xylanase which could free xylose from the xylan in the corn cell wall (1,2,9).

While observing the effect of xylose on sporulation, it was noted that the pH of a medium containing xylose increased less rapidly than that of the same medium lacking xylose (6). In one extensive study of nitrogen sources, the effect of xylose on pH was observed not only for L-asparagine but also for several other amino acids (4). Since fungi have the capacity to produce ammonium from L-asparagine, the ammonium generated may cause the increase in pH (10). In view of those findings, the following study examines the effect of xylose on fungal growth and sporulation and their relationship to levels of ammonium and pH in the culture medium.

MATERIALS AND METHODS

Experiments were carried out with a single-spore isolate of *Bipolaris maydis* race T (ATCC 36180) recovered from an ear of corn from a field in Franklin County, OH, in 1970. Routine tests showed that pathogenic and cultural characteristics have remained unchanged for 12 yr. The fungus grew on a basal medium containing: 4.0 g L-asparagine, 1.5 g KH_2PO_4, 0.75 g $MgSO_4 \cdot 7 H_2O$; 1.0 mg each of $CuSO_4 \cdot 5 H_2O$, $Fe_2(SO_4)_3 \cdot 6 H_2O$, $MnSO_4 \cdot H_2O$, $ZnSO_4 \cdot 7 H_2O$; and 20 g of Difco agar per liter of double distilled water. For this set of experiments, a range of carbohydrate concentrations at 0, 2, 5, and 10 g/L was used. The carbohydrates used for this study were D(+)-glucose and D(+) xylose. Treatments consisted of all glucose, all xylose, and glucose and xylose in a 1:1 ratio (w/w).

The medium was prepared as previously described (5). Each compound was individually autoclaved (1.2 kg/cm^2 for 20 min at 121 C) in solution. The pH of the L-asparagine and KH_2PO_4 was adjusted to 5.8 with 1N KOH. An aliquot of each solution was added into a disposable plastic petri dish and melted agar was added to bring the volume to 20 ml. The solution was swirled to insure a homogeneous mixture.

Inoculum for the test media was obtained from 10-day-old cultures grown on a glucose-xylose basal medium. The test media were seeded with a 4-mm-diameter core of mycelium and conidia and incubated for 6, 10, or 14 days at 28 C in the dark. Upon termination of each experiment, four 6-mm-diameter cores taken 1 cm from the growing edge of the thallus were selected and transferred into screw cap vials (15-ml capacity). The fungus was inactivated with a NaOH-ethanol-chlorox perservative (5). The number of conidia per milliliter was determined by microscopic count of 10 random fields for each of five replications. Agar from the mycelium used in the spore count was removed by solubilizing it in an autoclave. The mycelium was placed on a weighed tare, taken to dryness in an oven at 105 C, and weighed after 48 hr.

To determine the dry weight of the mycelium after each incubation period, five cultures from each treatment were placed in 150 ml of distilled water. These were autoclaved (1.2 kg/cm^2 for 20 min at 121 C). The mycelial mat was transferred to a weighed tare, taken to dryness in an oven at 105 C, and later weighed. The values obtained were used as an expression of the growth of the fungus.

Ammonium was assayed by homogenizing three 12-mm-diameter cores of the agar culture in double distilled water, and the

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homogenate was centrifuged at 6,800 *g* at 5 C. The supernatant was analyzed for ammonium with Nessler's reagent (13) and measuring the absorbance at 450 nm (10). This procedure was tested for precision by adding known quantities of $(NH_4)_2 SO_4$ to the basal medium resulting in levels of 0.0, 3.2, 6.3, 12.5 and 25.0 μmoles of NH_4^+ per milliliter. The level of ammonium detected by using Nessler's reagent was compared with known values and shown to be comparable within a 95% confidence interval as determined by Student's *t*-test. The average values detected were 0.0, 2.9, 5.4, 12.3, and 23.2 μmoles of NH_4^+, respectively, per milliliter. This indicated that the use of Nessler's reagent was an effective means of detection of ammonium and that the autoclaved L-asparagine did not interfere with the reagent.

After samples were taken for sporulation and ammonium analysis, the remainder of the fungal culture was macerated in 20 ml of distilled water. After 30 min, pH determinations were made with a pH meter.

The data presented here are representative of three experiments. Values presented are means of five replications and are presented with the 95% confidence intervals. A preliminary report of this study has been given (3).

RESULTS

Sporulation of this isolate of race T increased with increasing carbohydrate concentrations from 0 to 10 g/L. When glucose and xylose were compared as carbohydrate sources, sporulation was significantly higher on media containing xylose than those containing comparable concentrations of glucose. Moreover, the effect of xylose as a sole carbohydrate source on sporulation was comparable to xylose and glucose used in a 1:1 ratio (w/w). Sporulation observed after 6 days was not significantly different from that determined after 10 or 14 days (Fig. 1).

Mycelium dry weight increased as the carbohydrate concentration was increased from 0 to 2, 5, or 10 g/L. After 6 days of incubation, mycelium dry weights were comparable at each concentration of glucose, glucose-xylose, or xylose. After 10 days of incubation on media with 5 or 10 g of carbohydrate per liter, mycelium dry weights were significantly higher than after 6 days but comparable to those after 14 days. In the absence of carbohydrate or in the presence of 2 g of carbohydrate per liter there was no change in dry weight after any of the incubation times (Fig. 2).

Ammonium levels detected were lower as carbohydrate concentration was increased from 2 to 5 or 10 g/L. When glucose and xylose were compared as carbohydrate sources, ammonium levels were significantly lower after 6 days of incubation on media containing xylose than on those containing only glucose. Moreover, the effect of xylose on ammonium was comparable regardless of whether xylose was a sole or partial carbohydrate source. The effect of xylose or carbohydrate concentration on ammonium levels were no longer apparent at 10 or 14 days (Fig. 3).

As with ammonium levels, the pH values detected were lower as carbohydrate concentration was increased from 2 to 5 or 10 g/L. When glucose and xylose were compared as carbohydrate sources, the pH of the culture medium was significantly lower after 6 days of incubation on media containing xylose than on those containing only glucose. This effect of xylose was not seen after 10 or 14 days of incubation, nor was the effect of carbohydrate concentration seen after 6 days. Moreover, there was no significant difference in the

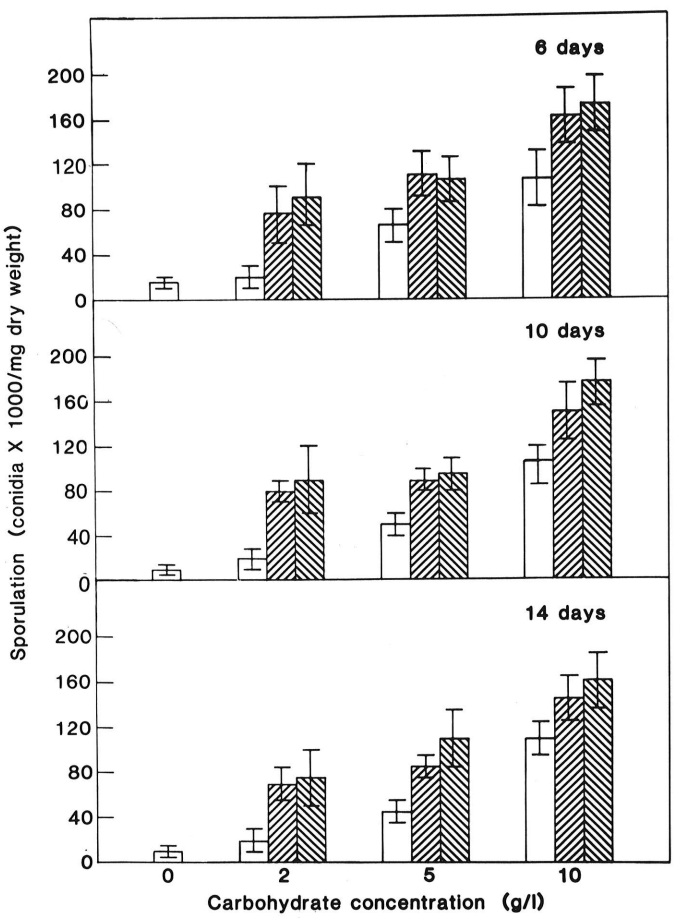

Fig. 1. Sporulation of *Bipolaris maydis* race T isolate 36180 after 6, 10, or 14 days of incubation on a L-asparagine-mineral salts agar medium containing various concentrations of glucose (▭), glucose and xylose added in a 1:1 ratio (w/w) (▨), or xylose (▨). Vertical bars represent means of five replications, and the 95% confidence interval is indicated.

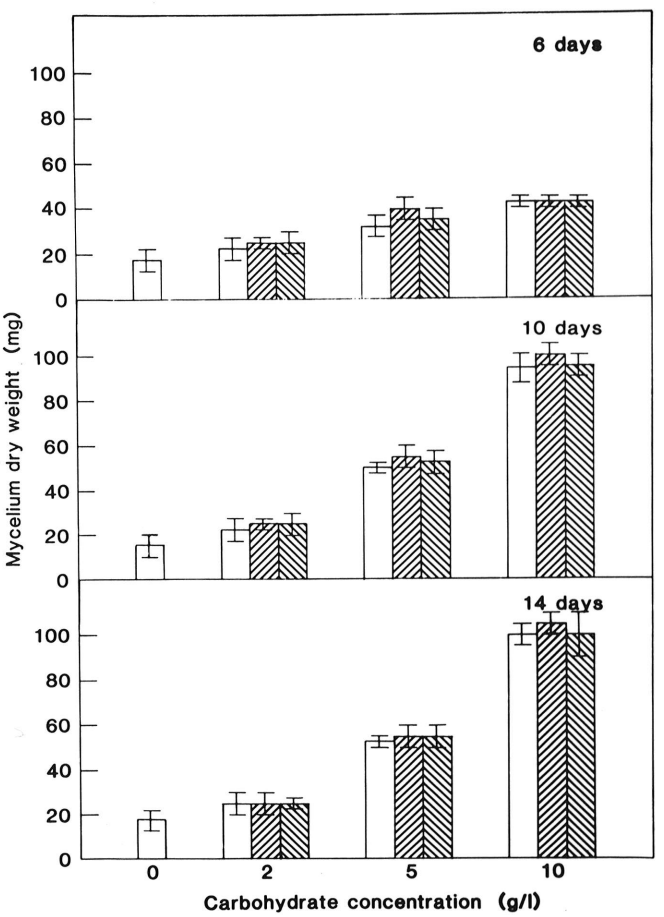

Fig. 2. Mycelium dry weight of *Bipolaris maydis* race T isolate 36180 after 6, 10, or 14 days of incubation on an L-asparagine mineral salts agar medium containing various concentrations of glucose (▭), glucose and xylose added in a 1:1 ratio (w/w) (▨), or xylose (▨). Vertical bars represent means of five replications, and the 95% confidence interval is indicated.

pH of the culture medium with regard to the type or concentration of carbohydrate at 10 or 14 days. (Fig. 4).

Since trends in ammonium levels paralleled the trends in pH of the culture medium, we sought to determine whether the changes in ammonium levels were related to changes in pH. For this study, we titrated the basal medium with ammonium hydroxide and established a titration curve (Fig. 5). Fig. 5 shows that the values for pH and ammonium levels obtained through titration were similar to those found after 6 days of incubation with the isolate of *B. maydis* race T.

DISCUSSION

The data presented here confirm previous reports that the addition of xylose to a culture medium significantly enhances the sporulation of *B. maydis* race T (6,7). This observation has also been recorded with other isolates of *B. maydis* (*unpublished*). The effect of xylose on sporulation was observed at 2, 5, and 10 g/L and after 6, 10, and 14 days of incubation. Moreover, the effects of xylose on sporulation were comparable when xylose was a partial or sole carbohydrate source. However, the response of sporulation to xylose observed after 6 days failed to further increase after 10 or 14 days.

While the addition of xylose significantly enhanced the sporulation of this isolate there was no effect on the mycelial dry weight. Increased concentration of the carbohydrate resulted in increases in the dry weight of the mycelium at 6 days but not after 10 days of incubation in any of the treatments.

Growth of isolate 36180 on the basal medium without a carbohydrate source is an indication that the fungus can metabolize L-asparagine as a carbon source. In order for the fungus to degrade L-asparagine it must produce amino acid oxidases and amidases that release ammonium. The production of amidases and amino acid oxidases has been observed in many ascomycetous fungi (10,12,14,16).

Growth of this isolate on L-asparagine resulted in the production of ammonium in the culture medium. The level of ammonium detected after 6 days of incubation was inversely related to the concentration of carbohydrate added to the culture medium. Ammonium levels in the culture medium were also lower when xylose was present in comparison to glucose alone. After 10 or 14 days of incubation, however, the ammonium levels of all treatments were approximately 16.5 μmoles of NH_4^+ per milliliter.

The production of ammonium in response to carbohydrate levels may be explained by the metabolism of L-asparagine as a carbon source. Sanwal and Lata (16) with *Neurospora crassa* and Kinghorn and Pateman (12) with *Aspergillus nidulans* have reported that the depletion of carbohydrate from the culture medium results in the induction of an NAD-linked glutamate dehydrogenase. This enzyme acts to deaminate glutamate in the production of ammonium and an organic acid. Presumably a similar phenomenon occurs with *B. maydis* race T on L-asparagine.

The response of pH was similar to ammonium levels. In the presence of xylose the final pH was lower in comparison to glucose alone. However, after 10 or 14 days of incubation the pH of all treatments was approximately 8.3. The similarity of the responses of pH and ammonium levels prompted us to titrate the culture medium with ammonium hydroxide. We found that the levels of ammonium and pH observed after titration were similar to those observed after incubation with the fungus. Our observations

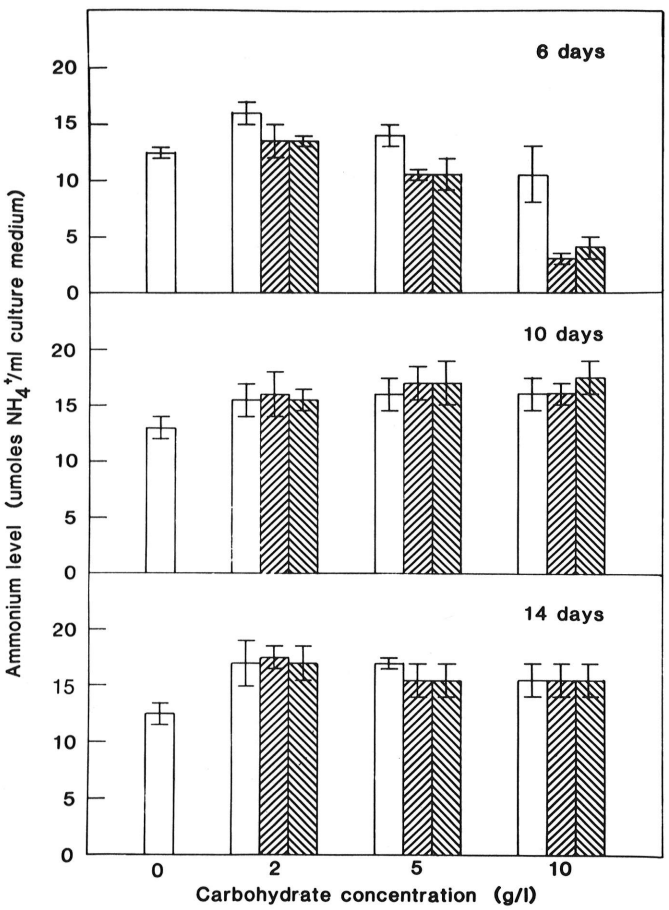

Fig. 3. Ammonium levels produced in cultures of *Bipolaris maydis* race T isolate 36180 after 6, 10, or 14 days of incubation on an L-asparagine-mineral salts agar medium containing various concentrations of glucose (□), glucose and xylose added in a 1:1 ratio (w/w) (▨), or xylose (▥). Vertical bars represent means of five replications and the 95% confidence interval is indicated.

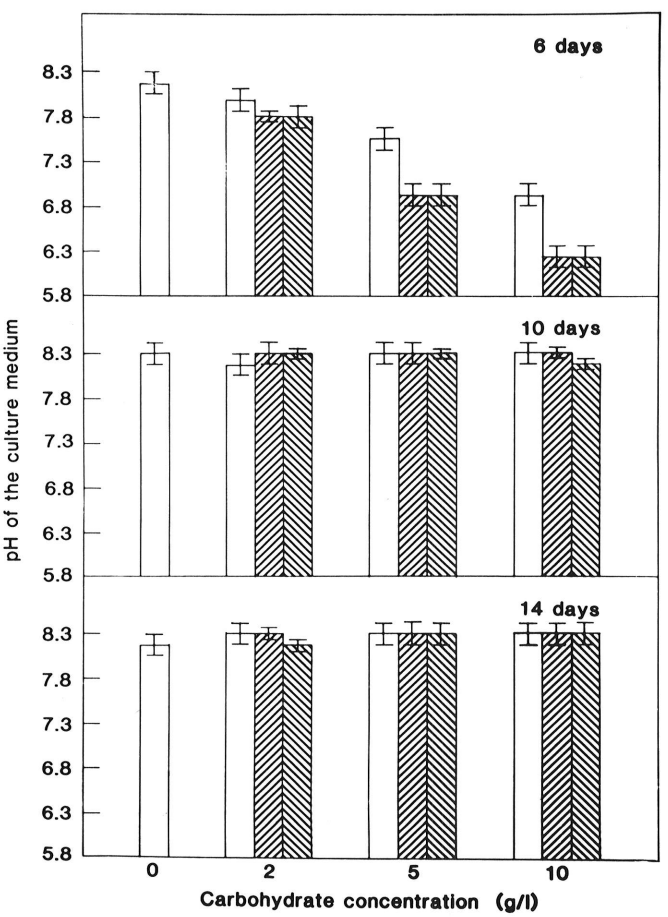

Fig. 4. The pH in cultures of *Bipolaris maydis* race T isolate 36180 after 6, 10, or 14 days of incubation on an L-asparagine-mineral salts agar medium containing various concentrations of glucose (□), glucose and xylose added in a 1:1 ratio (w/w) (▨), or xylose (▥). Initial pH was 5.8. Vertical bars represent means of five replications and the 95% confidence interval is indicated.

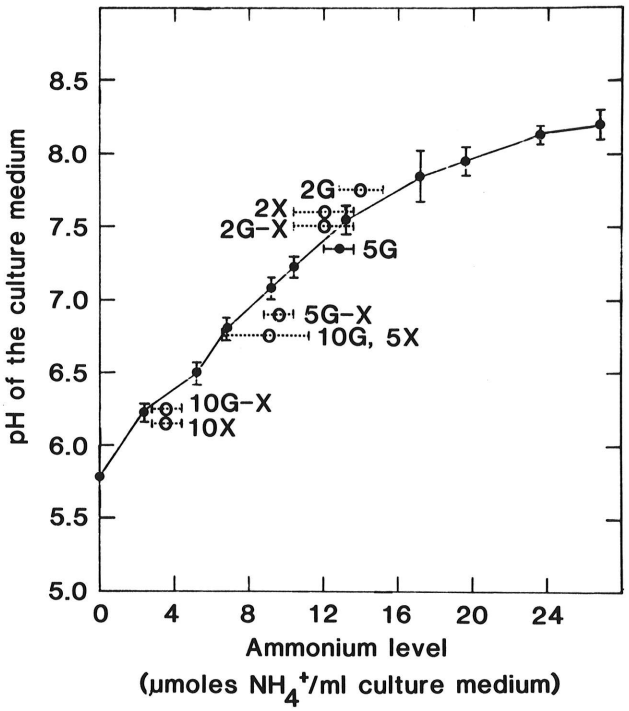

Fig. 5. The response in pH of the unseeded basal medium with increasing concentration of ammonium from ammonium hydroxide is indicated by the titration curve (—●—). Concentrations of ammonium and pH in fungal cultures after 6 days of incubation on an L-asparagine mineral salts medium containing various concentrations (g/L) of glucose (G), xylose (X), or glucose and xylose in a 1:1 ratio (w/w) (G-X) are indicated (--⊙--). Vertical and horizontal bars respectively represent the 95% confidence interval for pH and ammonium.

suggest that the processes involving ammonium production may cause the pH to rise. The high pH of 8.3 and ammonium levels of 16.5 μmoles/ml at 10 days of incubation may be linked with the failure of sporulation and dry weight to further increase.

The observations that sporulation of *B. maydis* race T isolate 36180 in the presence of high carbohydrate concentrations or the presence of xylose was accompanied by lower ammonium levels may give clues to specific physiological processes that mediate fungal sporulation in response to various kinds and amounts of carbon and nitrogen sources. Because xylose is a component of corn cell wall polysaccharides (15), understanding its regulatory role on the sporulation of *B. maydis*, an important corn pathogen, could be of epidemiological importance.

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