Interaction of pH and Temperature with Exogenous Carbon and Nitrogen Nutrition in Conidial Germination by Aspergillus flavus

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ABSTRACT

Maximum germination by *Aspergillus flavus* conidia occurred at 35 C in two axenic nutritional systems. However, the rate of germination in glucose plus amino acid nitrogen, supplied as peptone, was much more rapid than in glucose plus ammonium nitrogen. Percentage germination at reduced temp (25 C) in glucose plus peptone was more than double that in glucose plus ammonium. Using both citrate-phosphate and phosphate buffers, a broad pH optimum range (pH 3.0 - 7.5) for germination was observed in glucose plus peptone, while a narrower optimum range (pH 4.5 - 6.0) was observed in glucose plus ammonium. Low soil temp and high soil pH may restrict A. *flavus* conidial germination less in soil environments where amino acid nitrogen is the principal nitrogen source.

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The toxigenic fungus, *Aspergillus flavus* Link ex Fries, may colonize peanut (*Arachis hypogaea* L.) fruits in soil. Conidial germination, the initial event of this activity, may be inhibited in soil by inadequate exogenous carbon and nitrogen substrates (1, 2, 5) and by volatile soil fungistatic substance (3). This study was undertaken to investigate the effects of temperature and pH on conidial germination by *A. flavus*.

The clone of *A. flavus* used and methods used to prepare standardized washed conidial suspensions, glassware, and chemical solutions were the same as used previously (5). B solution (5) was used as a basal inorganic medium (pH 5.7) in all germination experiments other than pH studies. Nutritional systems of glucose plus NH_4Cl, and glucose plus peptone were employed. All data are based on two counts of 100 conidia/count in each of two 9-cm diam glass petri plates (10 ml/plate), and 1×10^4 conidia/ml was used throughout. All experiments were conducted three or more times in whole or in part.

Effect of temperature on conidial germination.—In B solution containing glucose plus ammonium (4.0 mg C plus 0.26 mg N/ml), maximum germination (83.4%) was observed at 35 C after 16 h. Lower percentage germination occurred at 30 C (74.6%), 25 C (30.6%) and 20 C (2.8%). Germination decreased sharply above 35 C as low percentage germination (0.6%) was observed at 40 C. Similar trends were also observed in B solution containing glucose plus peptone, except that germination was equally high at 30 C and 35 C (98.3%). Even though the incubation period (12 h) was shorter and total carbon supplied lower (2.0 mg C plus 0.5 mg peptone/ml) in this nutritional system, germination was much greater at 25 C (64.3%), 30 C and 35 C than in glucose plus ammonium. Germination at 20 C was 1.0% and neither germination nor swelling of conidia was observed at 5 C and 45 C in either medium.

Rate of conidial germination in glucose plus ammonium and glucose plus peptone.—At 30 C, germination was complete (98.5%) at 12 h in glucose plus peptone, while complete conidial germination (97.5%) required 28 h in glucose plus ammonium. In the latter medium, germination at 4, 8, 12, 16, 20 and 24 h was 1.0, 18.0, 54.5, 81.0, 88.5 and 95.6%, respectively. In contrast, germination in glucose plus peptone at 4 and 8 h was 2.5 and 82.5%, respectively. On the basis of these data, germination was examined at 8 h in B solution containing glucose plus peptone at 30 C and 35 C; percentage germination was typically higher at 35 C than at 30 C in these tests.

Griffin (2) also observed highest conidial germination by *A. flavus* in peanut geocarposphere soil at 35 C.

TABLE 1. Influence of pH on percentage germination of conidia of *Aspergillus flavus* at 30 C in glucose plus NH_4Cl or glucose plus peptone and in two buffers at 1×10^4 conidia/ml

Initial pH	Citrate-phosphate[a] buffer, % germination				Phosphate buffer,[b] % germination			
	Glucose[c] +NH_4Cl[d]	Final pH	Glucose[e] +Peptone[f]	Final pH	Glucose[c] NH_4Cl[d]	Final pH	Glucose[e] +Peptone[f]	Final pH
3.0	38.5[g]	2.95	94.5[h]	2.95	--	--	--	--
3.5	44.0	3.50	97.5	3.62	--	--	--	--
4.0	55.5	3.90	97.7	4.05	--	--	--	--
4.5	80.0	4.60	98.2	4.52	--	--	--	--
5.0	87.2	4.75	98.0	5.12	--	--	--	--
5.5	83.2	5.20	98.9	5.60	--	--	--	--
5.7	--	--	--	--	84.2[g]	4.65	99.2[h]	5.55
6.0	84.0	5.70	95.2	6.00	73.5	5.45	97.0	5.95
6.5	50.5	6.00	94.0	6.45	57.2	5.90	97.2	6.45
7.0	47.2	6.40	93.2	6.80	52.5	6.35	95.5	6.75
7.5	--	--	--	--	53.5	6.75	90.0	7.20
8.0	--	--	--	--	56.2	6.60	83.7	7.30

[a] 0.005 M citric acid, 0.01M sodium phosphate, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl.
[b] 0.01M sodium phosphate, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl.
[c] Glucose supplied at 4.0 mg C/ml.
[d] NH_4Cl supplied at 0.26 mg N/ml.
[e] Glucose supplied at 2.0 mg C/ml.
[f] Peptone supplied at 0.5 mg/ml.
[g] After 16 h.
[h] After 12 h.

Nitrate and ammonium are both poorer sources of nitrogen for *A. flavus* germination than amino acid nitrogen on an equivalent nitrogen basis (5). This suggests that in an environment rich in amino acid nitrogen (possibly the peanut fruit geocarposphere), *A. flavus* may be more competitive during germination and colonization at temp below the optimum than in one containing only inorganic nitrogen. We have observed *A. flavus* conidial germination in peanut geocarposphere soil as low as 20 C (2). In nonsterile peanut field soil at 30 C, glucose plus peptone supported much higher germination of *A. flavus* conidia than glucose plus ammonium (1).

The effect of pH on conidial germination.—Citrate-phosphate buffer and phosphate buffer were used in order to have adequate buffering capacity over a wide pH range. In glucose plus peptone a very broad pH optimum was observed (Table 1); percentage germination was greater than 90% after 12 h from pH 3.0 to 7.5. In contrast, the pH-optimum range was narrower in the glucose plus ammonium system; germination after 16 h was 80% or greater from pH 4.5 to 6.0. The drop in pH in glucose plus ammonium medium above initial pH 5 most likely accompanied the use of ammonium. Citrate probably had little direct nutritional effect on the fungus, as judged by percentage germination in the overlapping pH range for the two buffer systems.

In an acidic medium, it is possible that conidia may take up less ammonium than amino acids (from peptone) (4). At soil pH greater than 6.0, *A. flavus* may be much less competitive during germination in an environment containing only inorganic nitrogen than in one containing amino acid nitrogen as well. A survey of five Virginia peanut fields by us indicated that pH of soil samples (six per field) ranged from 4.9 to 6.7 by the saturation extract method and from 5.1 to 7.3 by the 1:1 soil extract method. As the pH of some peanut soil samples exceeded 6.5, inhibitory soil volatiles may be more important to *A. flavus* conidial germination in these soils than in lower pH soils, as suggested by the data of Hora and Baker (3). Also, it appears from our data that germination in the peanut field soils of pH 5.0 - 6.0 should not be inhibited *directly* by pH.

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