Growth and Nutrition of an Alternaria Pathogenic to Snapbeans

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ABSTRACT

Alternaria tenuis (Syn. *A. alternata*), the causal organism of Alternaria leaf spot of bean, grew on potato-dextrose agar from 4 to 36 C, the optimum being 28 C. No isolate grew well at 4 or 36 C. Mannose, dextrose, and maltose were the best carbon sources, and fructose supported good growth of isolate H_5. Casein hydrolysate, glutamic acid, asparagine, and tyrosine as amino acid sources supported maximum growth of the fungus. Peptone and sodium nitrate promoted best growth of isolate M_4, whereas peptone and calcium nitrate were superior for isolate H_5. *Alternaria tenuis* grew well over the wide pH range of 4.4 to 7.6, the optimum being 6.5. Phytopathology 60:903-906.

A leaf spot of bean, *Phaseolus vulgaris* L., incited by *Alternaria tenuis* Nees = *A. alternata* (Fries) Keissler, was recently studied in detail by Saad & Hagedorn (8). It causes a troublesome leaf blight under high moisture conditions and relatively cool temp. In view of the importance of snapbeans in Wisconsin and other states and the need to study the diseases involved, it seemed desirable to learn more of the physiology of the organisms that attack this crop. This investigation was conducted to obtain information regarding carbon and N nutrition requirements, and the effects of temp and pH on growth of *A. tenuis*.

MATERIALS AND METHODS.—Three isolates of *A. tenuis* = *A. alternata* (Fries) Keissler were used: A, isolated from diseased bean plants from Antigo, Wisconsin, 1965; H_5, from R. Goth, USDA, Beltsville, Maryland, who isolated it from diseased bean leaves sent from the State of Washington, 1966; and M_4, from T. P. Reiling, Green Giant Co., Dayton, Washington, 1966.

The effect of various carbon sources on the growth of *A. tenuis* was compared in 50 ml of a synthetic liquid medium in still 250-ml Pyrex Erlenmeyer flasks. Individual carbon sources were added to the basal medium before sterilization at the rate equivalent to 10 g dextrose/liter. The basal synthetic medium consisted of $NaNO_3$, 2.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KH_2PO_4, 0.5 g; Na_2HPO_4, 0.65 g; KCl, 0.5 g; distilled water, 1,000 ml. The pH of each media was determined after sterilization.

The various media were sterilized by autoclaving for 15 min at 121 C. Each flask was seeded with a single 3-mm disc of mycelium cut from the advancing margin of a 3-day-old culture of *A. tenuis*, isolate A, growing on potato-dextrose agar (PDA) at 28 C. The cultures were incubated at 28 C for 12 days. Beginning on the 2nd day, four flasks from each treatment were harvested every 2 days. The mycelial growth was collected by filtering through a Büchner funnel, with the aid of suction, on Whatman No. 202 filter paper that had been dried at 80 C for 12 hr and weighed. The mycelium was washed, dried at 80 C for 12 hr, and weighed. The effect of the same carbon sources was compared using the other two isolates of the fungus. Experimental procedures were the same as described above, with the exception that growth was measured 10 days after incubation.

The effect of various N sources on the growth of *A. tenuis* was compared in a synthetic liquid medium with dextrose as the carbon source. In one set of experiments, the N compounds were added in quantities which gave an amount of N equivalent to that present in 3.5 g of KNO_3, except for peptone which was added in the same quantity, i.e., 3.5 g. In the case of urea, it was cold-sterilized by filtering through a Millipore filter and added aseptically. The pH of the medium was adjusted to 6.8 before sterilization. Media were sterilized and the flasks were seeded with isolate H_5 as previously described. The cultures were incubated at 28 C for 10 days, after which the mycelia were harvested, washed, dried, and weighed. In another experiment, the effect of different amino acids on fungus growth was compared. Single amino acids were added to the basal medium at the rate of 0.4 g N/liter. Experimental procedures were the same as described previously. The basal liquid medium consisted of KH_2PO_4, 5.0 g; $MgSO_4$, 2.5 g; $FeCl_3$, 0.02 g; dextrose, 20.0 g; Na_2HPO_4, 12.5 g; citric acid, 5.2 g; distilled water, 1,000 ml.

Temperatures ranging from 4-36 C at 4-degree intervals were used to determine the rate of growth of the three isolates in PDA (pH 6.4). Ryan tubes were used in the study (7). Twenty-five ml of sterilized PDA medium were poured into each of the tubes, and both ends were plugged with cotton. The tubes were seeded with a 3-mm disc of mycelium at one end of the tube and incubated at temp from 4-36 C. Daily growth measurements were taken of the distance from the edge of the growing mycelial frontier to the origin point of seeding.

The influence of pH on growth of *A. tenuis* was investigated at 0.5 pH intervals using the liquid basal synthetic medium as described by Wolpert (12). Pyrex Erlenmeyer flasks of 125-ml capacity were used, each flask containing 35 ml of the medium adjusted to various pH levels. The medium was sterilized by autoclaving for 15 min at 121 C. Each flask was seeded with a single 3-mm disc of mycelium cut from the advancing margin of a 3-day-old culture of *A. tenuis* isolate H_5 growing on PDA at 28 C. The cultures were incubated at 28 C for 10 days, after which the mycelium was harvested, washed, dried, and weighed. The filtrates from the four replicates were collected, combined, and the final pH values determined.

RESULTS.—*Carbon nutrition.*—Dextrose, mannose,

TABLE 1. Effect of various carbon sources on growth of three isolates of *Alternaria tenuis* (*A. alternata*)

Carbon source	Dry wt of mycelium (mg)[a]		
	Isolate A	Isolate M_4	Isolate H_5
Arabinose	90	109	162
Cellobiose	96	86	164
Dextrose	184	197	308
Fructose		114	227
Galactose	107	110	136
Inulin		33	46
Lactose	50	51	67
Maltose	213	212	361
Mannose	222	277	194
Raffinose	51	51	64
Sorbose	93	149	105
Starch		155	166
Sucrose	111	78	103
Xylose		123	102
Control (none)	7	8	10

[a] Growth was measured 10 days after incubation at 28 C; data are avg of two trials of four replicates with isolates M_4 and H_5 and three replicates with isolate A. LSD at 5% = 13.2; at 1% = 18.1 for isolate A. LSD at 5% = 11.2; at 1% = 14.9 for isolate M_4. LSD at 5% = 16.5; at 1% = 22.1 for isolate H_5.

and maltose supported maximum growth of all three isolates (Table 1). Fructose supported good growth of isolate H_5. Figure 1 illustrates the results obtained with selected carbon sources using isolate A. The lag period between the different carbon sources was quite similar, whereas the logarithmic phase differed. The amount of growth of isolate A on the different carbon sources tested increased until the 10th day. Marked differences in yield of mycelium of the different isolates were obtained on the same carbon source and also in the same isolate with different carbon sources. Isolates A and M_4 produced the greatest amount of mycelium on mannose, while isolate H_5 did so on maltose. Of the five hexoses tested, isolate H_5 grew best on dextrose, while isolates A and M_4 grew best on mannose. The two pentoses, arabinose and xylose, supported fair growth of the three isolates. Of the five oligosaccharides tested, maltose supported the most growth of the three isolates, with sucrose and cellobiose producing moderate amounts of mycelial growth. Considering polysaccharides, the greatest amount of mycelium with isolates M_4 and H_5 was produced on starch. Inulin supported very little growth.

Nitrogen nutrition.—Marked significant differences in yield of mycelium were obtained with the different N sources for a given isolate of the fungus (Table 2).

Isolate M_4 produced the maximum dry wt on peptone and sodium nitrate. It produced good growth on the other nitrate compounds tested, limited growth on ammonium sulfate and urea, and poor or no growth on sodium nitrite.

Maximum growth of isolate H_5 developed on peptone and calcium nitrate; it produced good growth on the rest of the nitrate compounds tested and on ammonium sulfate. A limited amount of growth was produced by isolate H_5 on urea and no growth on sodium nitrite.

In a related study, the growth of isolate A was compared on various amino acids in the liquid synthetic medium (Table 2). Differences were highly significant among the various amino acids. Isolate A grew well on most of the amino acids tested, with glutamic acid, asparagine, and tyrosine supporting maximum growth. Casein hydrolysate was an excellent source of N, yielding the highest mycelial dry wt. Growth on aspartic acid, glycine, and proline was similar.

Hydrogen-ion concentration.—The fungus is capable of good growth over a wide pH range (Table 3). Good growth was obtained from pH 4.4 to pH 7.6, the optimum being pH 6.5. Growth was negligible below pH 4.4 and above pH 7.6. At the very low pH levels, the mycelium was exceedingly thin as compared to thicker colonies above pH 5.0. The differences among the treatments were highly significant except for pH 4.4 and 5.0, where the difference was not significant.

Temperature.—Each isolate grew well over a wide

TABLE 2. Effect of various N sources on growth of *Alternaria tenuis* (*A. alternata*) in liquid basal-dextrose medium after 10 days at 28 C

N source	Mycelial dry wt (mg)		Amino acid N source	Mycelial dry wt (mg)
	Isolate M_4	Isolate H_5		Isolate A
Ammonium nitrate	212[a]	254	Alanine	158[b]
Ammonium sulfate	142	228	Arginine	249
Calcium nitrate	284	365	Asparagine	413
Peptone	434	455	Aspartic acid	317
Potassium nitrate	217	273	Casein hydrolysate	447
Sodium nitrate	314	188	Glutamic acid	437
Sodium nitrite	25	10	Glycine	290
Urea	94	97	Leucine	268
Control (none)	31	28	Methionine	139
			Proline	323
			Tryptophan	158
			Tyrosine	406
			Control (none)	17

[a] Data are averages of three trials, four replicates each. Initial pH of medium after autoclaving was 5.6; final pH was 5.6-6.5 for isolate M_4, 5.6-6.8 for isolate H_5. LSD at 5% = 18.8; at 1% = 25.9 for isolate M_4. LSD at 5% = 14.2; at 1% = 19.3 for isolate H_5.

[b] Data are averages of three replicates. Initial pH level was generally 5.7; final levels 5.7-6.2. LSD at 5% = 36.6; at 1% = 49.4.

TABLE 3. Effect of hydrogen-ion concentration on the growth of *Alternaria tenuis* isolate A in a liquid basal synthetic medium after 10 days at 28 C

pH		Ml of M/3 buffer salts			Mycelial dry wt
Initial	Final	H_3PO_4[a]	KH_2PO_4[a]	K_2HPO_4[a]	(mg)[b]
3.0	3.2	1.2	5.8		16
3.5	3.5	0.5	6.5		31
3.9	4.1	0.1	6.9		87
4.4	4.5		7.0		130
5.0	5.2		6.8	0.2	138
5.5	5.7		6.3	0.7	147
6.0	6.2		5.0	2.0	162
6.5	6.8		3.5	3.5	261
7.0	6.8		1.5	5.5	230
7.6	7.8			7.0	121
8.2	8.5			7.0 + 5 g $CaCO_3$	76

[a] 28 ml of basal medium used; total amount of medium per flask-35 ml.
[b] Data are the averages of 4 replicates. LSD at 5% = 8.6. LSD at 1% = 11.7.

range of temp (Fig. 2). Favorable temp for growth of these isolates were between 20 and 32 C, the optimum being 28 C. The extent of growth at temp below the optimum was progressively less down to 4 C, where little growth was observed. At temp above the optimum, the growth rate dropped abruptly.

DISCUSSION.—All hosts supply to their parasites a complex nutritional environment including many organic and inorganic foods. *Alternaria tenuis* grew well on most of the carbon sources tested. Caution should be used in interpreting the results, and particularly in comparing them with studies in which milder or different methods of sterilization were used, because of possible breakdown of sugars during autoclaving. The good growth on the oligosaccharides and polysaccharides may, in part, be a reflection of the partial hydrolysis to utilizable monosaccharides. Taber et al. (11) found that *A. raphani* and *A. brassicae* grew well on most carbon sources tested, with starch supporting the most rapid growth, whereas *A. brassicicola* grew most rapidly on galactose. Reddy et al. (5) in a nutritional study of *A. ricini* found that monosaccharides and disaccharides were better sources of carbon than polysaccharides, sugar alcohols, or organic acids.

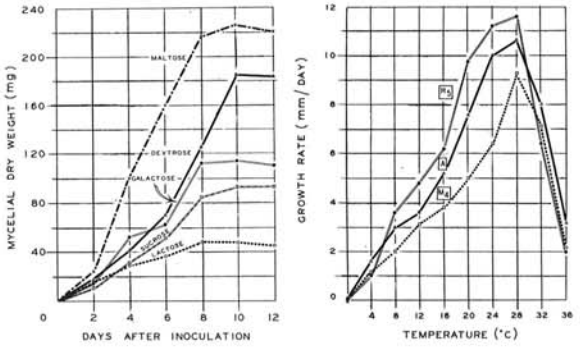

Fig. 1-2. (Left) Growth rate of *Alternaria tenuis* isolate A in still culture on various carbon sources at different time intervals of incubation at 28 C. (Right) Rate of growth of three isolates of *Alternaria tenuis* grown on potato-dextrose agar in Ryan tubes for a period of 8 days at different temp.

Growth of *A. tenuis* on various N sources showed that the isolate grew well on most N sources with the exception of nitrites and urea. Cochrane (1) suggested that poor growth on nitrite was due to free nitrous acid formed in the medium during autoclaving. Rajderkar (4) reported that *A. solani* grew best on peptone, well on calcium nitrate, asparagine, and glycine, and showed no growth in the absence of N. Taber et al. (11) found that two isolates of *A. raphani* grew faster when glutamic acid was supplied as a N source as compared to peptone, ammonium succinate, potassium nitrate, glycine, leucine, or urea. The ability of *A. tenuis* to use ammonium, nitrate, and organic nitrogen justifies its inclusion in the second group of Robbins' classification (6).

The maintenance of an optimum pH for fungal growth is an important factor in nutritional studies, yet according to Cochrane (1) such factors which may change the pH growth curve include temp, time of harvest, and N source. The effects of metabolic activities also complicate pH results, particularly in poorly buffered media. In our pH studies, the use of phosphate buffer of moderately high molarity was very useful because the final pH after 10 days' incubation was almost identical to the initial pH. The present pH studies are in close agreement with the work of Taber et al. (11), who found that *A. raphani* grew well over a wide pH range of 4.8 to 7.2. By using phosphate buffer in our nutritional studies, we found that the differences in the capacity of the different isolates of *A. tenuis* to utilize various N and carbon sources were not due to pH changes of the medium.

Optimum temp results are in agreement with the research results of Stavely & Main (10), who found that the best temp for radial growth of *A. tenuis* was about 27 C. Sobers (9) reported the optimum growth of *A. tenuissima* was 24-28 C; Jackson & Simmons (2) reported that the optimum growth of *A. multirostrata* occurred at 24-28 C. This investigation has shown that the growth rate of the fungus on synthetic media at various temp does not coincide with the rate of pathogenesis. Saad & Hagedorn (8) reported that the best temp for pathogenesis was 16 C. Likewise, Pound (3)

reported that the optimum temp for growth of *A. solani* on synthetic media was 28 C, whereas the optimum for disease development was 16 C.

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