Alteration of Germination Patterns of Sclerotia of Macrophomina phaseolina on Soil Surfaces

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ABSTRACT

Sclerotia of *Macrophomina phaseolina* on the surface of nonsterile soil exhibited an abnormal germination pattern (AG). An intermediate germination (IG) pattern was induced with sclerotia undergoing AG by amendment with appropriate nutrients. Sclerotia on nonsterile soil amended with 5×10^{-2} M concns of several sugars, amino acids and organic acids exhibited mainly normal germination (NG), and their germ tubes colonized the soil surface. Autoclaved soil induced NG (98%), but there was little colonization of the soil. The following sugars, amino acids, and organic acids effectively (80-98%) stimulated NG: maltose, D-ribose, DL-aspartic acid, glutamic acid, L-leucine, DL-phenylalanine, L-serine, citric, malonic, and tartaric acids. Lactose, L-cystine, urea, and all tested mineral salts were ineffective; DL-methionine was inhibitory at 5×10^{-2} M. Continuous leaching of sclerotia in vitro was fungistatic. The NG of sclerotia of *M. phaseolina* in soil is apparently nutrient-dependent. Also, a nutrient-dependent mechanism is suggested which limits germ tube emergence and conserves the viability of these propagules.

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The influence of soil fungistasis on heterogeneous fungal structures, as sclerotia, has received limited attention. Of 116 species of fungi surveyed for sensitivity to fungistasis, Lockwood (6) lists the

sclerotia of only one species. However, sclerotia and similar propagules are important in the long-term survival of many fungi in soil, presumably because of their durable structure and greater endogenous nutrient reserves than spores or mycelium. It has not been established, in most cases, whether persistence in soil by these propagules is the result of inherent dormancy, or quiescence induced by the soil environment. However, Emmatty and Green (4) found that failure of microsclerotia of *Verticillium albo-atrum* to germinate in soil was due to nutrient-dependent fungistasis.

The importance of exogenous nutrients for germination of sclerotia of *Macrophomina phaseolina* (Tassi) Goid. in soil was suggested by Ashworth (1) who found that susceptibility of Persian melon seedlings may be related to their sugar content. Smith (8) showed that root exudates from sugar pine seedlings, especially the amino acid fraction, enhanced germination of these sclerotia in soil.

We report here an abnormal germination pattern of sclerotia of *M. phaseolina* on the surface of nonsterile soil, and the effects of soil sterilization and soil amendment on germination.

MATERIALS AND METHODS.—An isolate of *M. phaseolina* from snap bean was used. Sclerotia were from 4-day-old cultures grown on cellophane disks (cellulose xanthate) over potato dextrose agar (PDA)

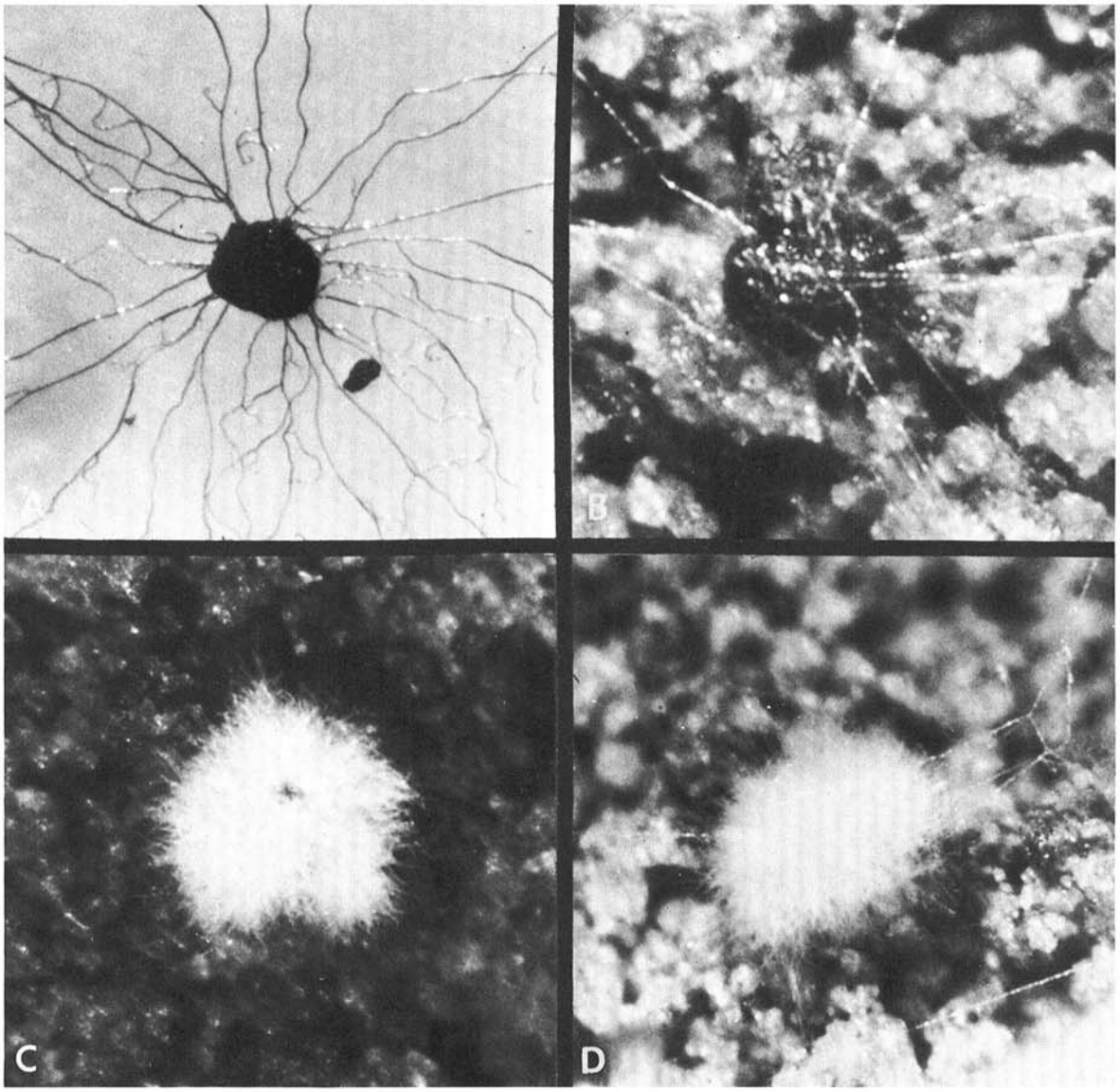

Fig. 1-(A to D). Germination patterns of sclerotia of *Macrophomina phaseolina* on soil surfaces: **A)** normal germination on PDA; **B)** normal germination on sterile soil; **C)** abnormal germination on nonsterile soil; and **D)** intermediate germination on nonsterile soil nutrient-amended after initial germination.

at 32 C. The sclerotia were dried 3 h at room temp, ground in a mortar to break up aggregates of sclerotia and washed through a series of screens of 100-325 mesh. Sclerotia retained on a 160-mesh screen (96 μm), after sieving through a 140-mesh screen, were used. The sclerotia were washed several times with sterile phosphate buffer (pH 6.5) by repeated centrifugation (1,475 *g* for 3 min) and decantation to remove exogenous nutrients.

The soil was of the Warsaw sandy loam series (sand-64%, clay-29%, silt-7%, and organic matter-3.5%). It was rendered free of *Macrophomina* sp. by sifting through a 20-mesh screen and autoclaving for 1 h at 1.05 kg-force/cm^2 (15 psi). The water-holding capacity (WHC) was adjusted to 30% (w/w) and a small portion of nonsterile soil was incorporated to reestablish the natural microflora. The soil was stored at least 4 wk at 23 C before use.

To determine the sensitivity of sclerotia to fungistasis induced by intermittent leaching in vitro, the Millipore filter apparatus described by Emmatty and Green (4) was used. Sclerotia were leached for 4 days with sterile, deionized water at 4, 23, and 32 C. In this method, at intervals of 1 min, 1.5 ml of water flow over the sclerotia placed between two filter disks. Sclerotia were removed at 6-h intervals and classified for germination, using the following rating system: no germination (A); one to three germ tubes (B); and four+ germ tubes (C). Germ tubes shorter than the diam of the sclerotium were not counted. Viability of the leached sclerotia was determined during and at the conclusion of the tests by transferring them to cellophane disks over PDA and incubating for 15-20 h at 23 C.

Germination of sclerotia on the surface of nonsterile, sterile, and amended soils was determined as follows. The soil, adjusted to 50% WHC, was placed in 60 X 15 mm petri dishes, compressed, and leveled to a smooth surface. After dilution, washed sclerotia were sprayed on the soil surface to give 400-500 sclerotia/petri dish. After incubation for 48 h at 23 C, germination was determined in situ (5) using a Stereozoom 7 microscope (Bausch and Lomb) at X140 magnification. In a series of parallel treatments, sclerotia were sprayed on the surface of soil previously sterilized by autoclaving [1 h at 1.05 kg-force/cm^2 (15 psi)] and on nonsterile soil amended with various sugars, amino acids, organic acids, and other sources of carbon, nitrogen, and mineral salts. The soil amendments included DL-arabinose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-ribose, sucrose, DL-alanine, arginine, asparagine, DL-aspartic acid, L-cystine, glutamic acid, glutamine, glycine, L-leucine, L-lysine,

TABLE 1. Comparative effects of sugars, organic acids and alcohols on germination patterns of sclerotia and soil colonization by *Macrophomina phaseolina*

Treatments	Concentrations											
	5×10^{-2}M				5×10^{-3}M				5×10^{-4}M			
	Germination types (%)[a]			Soil invasion[b]	Germination types (%)			Soil invasion	Germination types (%)			Soil invasion
	AG	IG	NG		AG	IG	NG		AG	IG	NG	
Maltose	4	8	88ABC[c]	+++	43	17	40BC	+	98	0	2B	–
D-Ribose	7	8	83ABC	+++	56	24	20BCDE	+	84	10	6B	–
Sucrose	16	17	67BCD	++	60	20	20BCDE	+	86	10	4B	–
D-Fructose	15	23	62CD	++	50	30	20BCDE	+	70	20	10B	–
D-Glucose	14	28	58CD	++	80	12	8DE	–	96	4	2B	–
DL-Arabinose	32	24	44DE	+	64	28	8DE	–	70	22	8B	–
D-Galactose	32	28	40DEF	+	80	10	10CDE	–	92	2	4B	–
Lactose	75	8	12FG	+	74	14	12CDE	–	94	4	2B	–
Citric Acid	0	4	96A	+++	42	24	34BCD	–	92	6	2B	–
Tartaric Acid	2	3	95A	+++	39	28	33BCD	–	91	6	3B	–
Malonic Acid	3	5	92AB	+++	59	16	25BCDE	–	97	1	2B	–
Oxalic Acid	34	5	61CD	–	70	12	18CDE	–	89	5	6B	–
Ethanol	23	14	63CD	+	26	24	50B	–	72	16	12B	–
Methanol	75	4	16EFG	–	92	5	3E	–	93	2	5B	–
H_2O Control (Autoclaved Soil)	0	2	98A	–	3	0	97A	–	3	4	93A	–
H_2O Control (Nonautoclaved Soil)	92	3	5G	–	94	2	4E	–	89	4	7B	–

[a]NG, AG, and IG are normal, abnormal, and intermediate types of germination, respectively.

[b]+++, ++, +, and – represent vigorous, moderate, sparse, and no soil surface colonization, respectively.

[c]Values are actual means of three replications from 200 sclerotia/replication. For statistical analysis, arc sin-transformed data were used. Mean values not followed by the same letter within a column differ significantly as determined by Duncan's multiple range test ($P = 0.05$).

TABLE 2. Comparative effects of amino acids on germination of sclerotia and soil colonization by *Macrophomina phaseolina*

Treatments	Concentrations											
	5×10^{-2}M				5×10^{-3}M				5×10^{-4}M			
	Germination types (%)[a]			Soil invasion[b]	Germination types (%)			Soil invasion	Germination types (%)			Soil invasion
	AG	IG	NG		AG	IG	NG		AG	IG	NG	
L-Glutamic Acid	0	2	98A[c]	+++	50	14	36AB	+	76	12	12A	–
L-Leucine	4	2	92A	+++	64	12	24ABC	–	86	6	8A	–
DL-Aspartic Acid	2	8	90AB	+++	40	20	40A	+	70	16	18A	–
DL-Phenylalanine	4	10	86AB	+++	64	18	18ABC	–	70	16	14A	–
DL-Serine	6	12	82AB	+++	62	14	24ABC	+	66	16	18A	–
DL-Valine	18	20	62BC	++	36	8	36AB	–	62	24	14A	–
Asparagine	20	19	61BC	++	38	46	16ABC	–	54	34	12A	–
L-Proline	34	20	46CD	+	68	8	24ABC	–	90	6	4A	–
Glycine	45	20	35CD	+	72	8	24ABC	–	78	18	4A	–
DL-Alanine	40	28	32CD	+	60	24	16ABC	–	68	16	16A	–
L-Lysine	53	17	30CD	+	66	22	12ABC	–	84	6	10A	–
Glutamine	43	28	29CD	+	82	6	12ABC	+	92	4	4A	–
Arginine	52	21	27CD	+	90	6	4C	–	82	8	10A	–
DL-Methionine	0	0	19D	–	88	2	10BC	–	62	26	12A	–
L-Cystine	70	16	14D	–	74	12	14ABC	0	60	30	10A	–

[a]NG, AG, and IG are normal, abnormal, and intermediate types of germination, respectively.

[b]+++, ++, +, and – represent vigorous, moderate, sparse, and no soil surface colonization, respectively.

[c]Values are actual means of three replications from 200 sclerotia/replication. For statistical analysis, arc sin-transformed data were used. Mean values not followed by the same letter within a column differ significantly as determined by Duncan's multiple range test ($P = 0.05$).

TABLE 3. Comparative effects of mixtures of amino acids and/or sugars on germination patterns of sclerotia and soil colonization by *Macrophomina phaseolina*

Treatments	types (%)[b]			Soil invasion[c]	types (%)			Soil invasion	types (%)			Soil invasion
	AG	IG	NG		AG	IG	NG		AG	IG	NG	
ALPSV[d]	0	2	98A[f]	++	28	16	56A	–	70	16	14A	–
FGMRS + ALPSV[e]	2	4	94AB	++	64	14	22BCDEF	+	94	4	2A	–
DL-Aspartic Acid + D-Ribose	4	6	90ABC	++	28	26	46ABC	+	86	6	8A	–
DL-Aspartic Acid + Maltose	4	6	90ABC	++	70	12	18BCDEF	–	90	4	6A	–
DL-Phenylalanine + D-Ribose	6	6	88ABCD	++	54	20	26ABCDE	+	88	4	8A	–
DL-Phenylalanine + Sucrose	6	10	84ABCD	+	70	12	18BCDEF	–	86	6	8A	–
DL-Phenylalanine + Maltose	8	8	84ABCD	++	88	6	6EF	–	92	8	6A	–
L-Leucine + Maltose	12	8	80ABCD	+	27	23	50AB	–	94	4	2A	–
DL-Aspartic Acid + Sucrose	6	14	80ABCD	+	60	20	20BCDEF	–	78	16	6A	–
L-Leucine + D-Ribose	7	15	78ABCD	+	82	8	10DEF	–	84	10	6A	–
L-Leucine + Sucrose	14	10	76CDE	+	38	24	38ABCD	–	92	2	6A	–
FGMRS[g]	7	21	72CDEF	+	68	16	16CDEF	–	92	6	2A	–
Glycine + D-Galactose	26	12	62DEFG	+	32	28	40ABC	–	80	12	8A	–
DL-Alanine + D-Galactose	28	14	58EFGH	–	56	16	28ABCDE	–	70	16	14A	–
DL-Methionine + D-Galactose	43	14	43FGH	+	60	14	26ABCDE	–	90	4	6A	–
Glycine + Lactose	40	20	40FGH	+	88	10	2F	–	90	8	2A	–
DL-Methionine + Lactose	14	4	28GH	–	94	4	2F	–	88	6	6A	–
DL-Alanine + Lactose	61	12	27H	–	88	8	4EF	–				

[a]Concns of individual amino acids or sugars in each mixture.

[b]AG, IG, and NG are abnormal, intermediate, and normal patterns of germination, respectively.

[c]+++, ++, + and – are vigorous, moderate, sparse, and no soil surface colonization, respectively.

[d]Combination of DL-aspartic acid, L-leucine, DL-phenylalanine, DL-serine, and DL-valine, each 1×10^{-2}M.

[e]Each amino acid and sugar was applied at 5×10^{-3}M.

[f]Data are actual means of three replications, from 200 sclerotia/replication. For analytical purposes, arc sin-transformed data were used. Means having the same letter within a column are not significantly different from one another as determined by Duncan's multiple range test ($P = 0.05$).

[g]Combination of D-fructose, D-glucose, maltose, D-ribose, and sucrose, each at 1×10^{-2} M concn.

DL-methionine, DL-phenylalanine, L-proline, DL-serine, tyrosine, DL-valine, citric acid, malonic acid, oxalic acid, tartaric acid, K_2HPO_4, K_2SO_4, KNO_3, KNO_2, $(NH_4)_2HPO_4$, $(NH_4)_2SO_4$, NH_4NO_3, $MgSO_4$, ethanol, methanol, and urea. These amendments were added singly and in various combinations to the soil at 5×10^{-2}, 5×10^{-3}, and 5×10^{-5} M (w/w) soil at 30% WHC and the soil was then adjusted to 50% WHC. Soil amendments were made both before the sclerotia were sprayed on the soil surface, and after the initial incubation period.

Preliminary observations of germination of sclerotia on sterile, nonsterile, and nutrient-amended soils indicated three distinct germination patterns. These included: *normal germination* (NG) - a limited number of germ tubes formed with colonization of the soil surface; *abnormal germination* (AG) - an uncountable number of germ tubes formed with no colonization of the soil; and *intermediate germination* (IG) - soil colonization by germ tubes from sclerotia with AG, following soil amendment with specific nutrients.

Colonization of the soil surface following germination of sclerotia was determined as follows. The soil dishes were prepared as described and, prior to spraying the sclerotia on the soil surface, one-half of the surface was covered with aluminum foil. After 48 h at 23 C, sclerotial germination and colonization of the nonsprayed, covered half of the soil surface was rated as vigorous (+++), moderate (++), sparce (+), or none (-).

The persistence of the various amendments in soil, and the duration of their effect on the germination pattern, was determined by spraying washed sclerotia on the exposed half of amended soil from day 0 to the 4th day after amendment. Germination and colonization was determined using the procedures described above. Controls consisted of sclerotia stored dry at 6 C in closed vials. Incubation was for 20 h at 23 C, after which the soil surface was sprayed with lactophenol aniline blue to prevent further germination, and the plates were stored at 4 C until examined.

All tests were replicated at least three times, done

TABLE 4. Comparative effects of sugars, amino acids, and their mixtures on germination patterns of sclerotia and soil colonization by *Macrophomina phaseolina* 4 days after soil amendment

Sugars and amino acids[a]	Germination types (%)[c]				Mixtures of sugars and/or amino acids[b]	Germination types (%)			
	AG	IG	NG	Soil invasion		AG	IG	NG	Soil invasion
Sucrose	28	19	59B[d]	+	FGMRS + ALPSV[e]	34	16	50A[g]	++
Maltose	34	26	41BCDEF	+	DL-Aspartic acid + D-Ribose	12	40	48A	+
D-Ribose	30	30	40BCDEF	+	DL-Phenylalanine + D-Ribose	20	40	40AB	+
D-Glucose	32	36	32BCDEFG	–	DL-Phenylalanine + Sucrose	16	44	40AB	+
D-Fructose	41	30	29BCDEFGH	–	DL-Glutamic acid + D-Ribose	25	37	38AB	+
Lactose	64	26	12FGH	–	L-Leucine + Sucrose	42	20	38AB	+
					ALPSV[f]	30	40	32AB	+
DL-Phenylalanine	18	30	52BC	+	L-Leucine + D-Ribose	50	18	32AB	+
L-Leucine	30	21	49BCD	+	Glycine + D-Galactose	30	40	32AB	+
DL-Aspartic Acid	29	32	39BCDEF	+	DL-Aspartic Acid + Maltose	42	28	30AB	+
DL-Serine	8	60	32BCDEFG	–	FGMRS[g]	44	30	26ABC	+
Glycine	47	33	20CDEFGH	–	DL-Phenylalanine + Maltose	40	38	22ABC	+
DL-Methionine	33	0	16DEFGH	–	DL-Methionine + D-Galactose	34	2	22ABC	–
L-Glutamic Acid	56	30	14EFGH	+	DL-Aspartic acid + Sucrose	40	40	20ABC	+
DL-Alanine	65	23	12FGH	–	DL-Alanine + D-Galactose	60	20	20ABC	–
DL-Valine	44	44	12FGH	+	DL-Alanine + Lactose	66	14	20ABC	–
L-Cystine	65	27	8GH	–	Glycine + Lactose	62	20	18ABC	–
H_2O Control (Autoclaved Soil)	0	3	97A	–	L-Leucine + Maltose	36	48	16BC	+
H_2O Control (Nonautoclaved Soil)	86	8	6H	–	DL-Methionine + Lactose	88	8	4C	–

[a]Individual sugars or amino acids were applied at 5×10^{-2} M concns.

[b]With the exceptions noted, individual sugar(s) and/or amino acid(s) in each mixture were applied at 2.5×10^{-2} M concns.

[c]AG, IG, and NG are abnormal, intermediate, and normal patterns of germination, respectively.

[d]Values are actual means of three replications, from 200 sclerotia/replication. Arc sin-transformed data were statistically analyzed. Means having the same letter(s) within a column do not differ significantly as determined by Duncan's multiple range test ($P = 0.05$).

[e]Mixture of the sugars D-fructose, D-glucose, maltose, D-ribose, and sucrose, plus the amino acids. DL-aspartic acid, L-leucine, DL-phenylalanine, DL-serine, and DL-valine, each at 1×10^{-3} concn.

[f]Mixture of the five amino acids (see d) each at 1×10^{-2} M concn.

[g]Mixture of the five sugars (see d) each at 1×10^{-2} M concn.

in duplicate and repeated three times.

RESULTS.—When sclerotia were leached continuously in the Millipore apparatus at 4, 23, and 32 C, no germination occurred until after 24 h, when 0, 5, and 8% of the sclerotia germinated at the respective germination temp. All produced fewer than four germ tubes per sclerotium. Germination of washed, nonleached sclerotia on cellophane disks over PDA at 23 C for 15 h was 72%, with 41% with four+ germ tubes per sclerotium. The viability of sclerotia leached at 4 C continued to decline and, after 96 h, less than 15% germinated.

Sclerotia on nonsterile, nonamended soil exhibited a distinct, abnormal germination (AG), compared to the normal germination (NG) pattern on sterile soil, or on nonsterile, amended soil. An intermediate germination (IG) pattern was observed with sclerotia with AG subjected to certain amendments in situ or upon transfer to an amended soil (Fig. 1).

Depending on the amendment and its concn, different percentages of sclerotia with the AG, NG, and IG patterns were observed. Of the eight sugars tested, maltose and D-ribose (5×10^{-2} M) were the most effective in inducing the NG pattern (Table 1). The stimulatory influence of D-fructose, D-glucose, and sucrose was generally high (50-70% NG) at this concn. Lactose was practically nonstimulatory (12%), while DL-arabinose and D-galactose had only moderate influence (44-40%). Of sclerotia on nonsterile, unamended soil surfaces only 5% had the NG pattern, while most (92%) exhibited the AG type.

More than 90% of the sclerotia on soil amended with citric, malonic, and tartaric acids showed the NG pattern (Table 1). The stimulatory influence of oxalic acid and ethanol was similar to that of D-glucose, D-fructose, and several other sugars. As with lactose, NG of sclerotia on soil amended with methanol was less than 20%. Amendments that were nonstimulatory at 5×10^{-2} M and lower concns were urea, K_2HPO_4, K_2SO_4, KNO_3, KNO_2, $(NH_4)_2HPO_4$, NH_4NO_3, $(NH_4)_2SO_4$, and $MgSO_4$.

Of the 16 amino acids (5×10^{-2} M) with which soil was amended, DL-aspartic acid, glutamic acid, L-leucine, DL-phenylalanine, and DL-serine vigorously stimulated NG (82-98%) (Table 2). Good stimulation of NG (46-62%) was observed on soil amended with asparagine, L-proline, and DL-valine, while only modest stimulation (27-35%) occurred on soil with DL-alanine, arginine, glutamine, glycine, and L-lysine. L-cystine and DL-methionine were nonstimulatory at 5×10^{-2} M and lower concns. DL-methionine showed an inhibitory effect at 5×10^{-2} M. Germination of sclerotia was 100% (the sum of the three types of germination) on soil amended with all other amino acids, but over 80% did not germinate within 48 h on soil amended with DL-methionine. Soil amended with all amino acids at 5×10^{-3} M and lower concns showed only moderate stimulation of the NG pattern.

Soils amended with mixtures of sugars and amino acids were equally effective in stimulating the NG pattern (Table 3). Sugars or amino acids which by themselves were poor stimulators; e.g., lactose or alanine, were also poor stimulators in mixtures. For statistical analysis, all data were arc sin-transformed. A mixture of five sugars (D-fructose, D-glucose, maltose, D-ribose, and sucrose–each 10^{-2} M) was significantly less effective in inducing NG than a mixture of five amino acids (DL-aspartic acid, L-leucine, DL-phenylalanine, DL-serine, and DL-valine) at similar concns. When the five sugars and five amino acids were mixed, each at a lower concn (5×10^{-3} M), the stimulation of NG was high (94%). Mixtures of sugars and/or amino acids at 2.5×10^{-3} M and lower concn stimulated NG only moderately.

Individual sugars or amino acids, and their combinations were significantly more effective in stimulation of NG of sclerotia on soil on the 1st day (Tables 1, 2 and 3) than on the 4th day after amendment (Table 4). For example, overall means of 60, 50, and 71% of the sclerotia with NG were observed on the 1st day following soil amendment with all eight individual sugars, 16 amino acids, and their 18 combinations, respectively. Overall means of only 32, 21, and 30% of sclerotia with NG were obtained from sclerotia placed on soil the 4th day following soil amendment with all individual sugars, amino acids, and their combinations, respectively.

Vigorous soil invasion by the mycelium occurred on soil amended with maltose, D-ribose, citric acid, malonic acid, tartaric acid (Table 1), and valine (Table 2) at 5×10^{-2} M. On soil amended with DL-arabinose, D-fructose, D-glucose, sucrose, L-lysine, and L-proline at 5×10^{-2} M, soil invasion by the fungus was moderate, while only sparse invasion occurred with ethyl alcohol, DL-alanine, arginine, asparagine, glutamine, and glycine at 5×10^{-2} M. No soil invasion by the fungus was observed with lactose, oxalic acid, methyl alcohol, L-cystine, and D-methionine at 5×10^{-2} M. Urea, and all inorganic salts at 5×10^{-2} M and lower concns failed to stimulate soil invasion. Although 98% of all sclerotia on autoclaved soil exhibited NG, no soil invasion was observed with either the autoclaved or nonsterile soils (control).

Even though several combinations of sugars and/or amino acids stimulated high germination of sclerotia with NG patterns at 2.5×10^{-2} M, they were less effective in inducing soil invasion (Table 3). Only few of the mixtures; e.g., DL-phenylalanine + D-ribose, DL-aspartic acid + D-ribose, DL-phenylalanine + maltose, etc., stimulated moderate soil invasion at this concn. For most other mixtures of sugars and/or amino acids at 2.5×10^{-2} M and lower concns, soil invasion was either sparse or nonexistent. This trend was also observed on soil on which sclerotia were placed 4 days following soil amendment with individual sugars, amino acids, and their mixtures at 5×10^{-2} M (Table 4).

Concentrations of all amendments to soil significantly affected the proportions of sclerotia with NG and AG patterns. For all eight sugars at 5×10^{-2}, 5×10^{-3} and 5×10^{-4} M, the overall means

of sclerotia with NG were 60, 17, and 5%, respectively, while those with AG were 22, 63, and 85%, respectively. Overall means of sclerotia with NG for all the individual amino acids at 5×10^{-2}, 5×10^{-3} and 5×10^{-4}M were 51, 19, and 10%, respectively, while those of sclerotia with AG were 28, 64, and 75%, respectively.

DISCUSSION.—The alterations in the germination pattern of sclerotia of *M. phaseolina* on the soil surface following soil sterilization or soil amendment with appropriate nutrients, compared to nonsterile soil, suggests nutrient-dependence for germination in soil. It also suggests a mechanism which limits the number of germ tubes produced, which may also be nutrient-dependent.

Sclerotia on the surface of nonsterile soil produced an uncountable number of aerial germ tubes that were restricted in development and failed to colonize the soil. No germ tubes were produced from that portion of the sclerotium in direct contact with the soil surface. Smith (8) found that sclerotia buried in soil failed to germinate unless suitable nutrients were added. In our studies, the availability of nutrients, either by amendment of nonsterile soil or by autoclaving before sclerotia were added to the soil surface, altered the germination pattern so that germ tubes either grew over the soil surface (sterile soil) or invaded the soil surface (amended soil) to produce typical mycelial elements. The availability of nutrients to support colonization also restricted the number of germ tubes produced and this mechanism may be important in maintaining the viability of the sclerotium for repeated flushes of new growth.

That the germination pattern of sclerotia could be altered by soil amendment after one germination pattern (AG) had begun also suggests a nutrient-dependent mechanism for germination.

The more highly stimulatory influence of several of the individual amino acids or their combinations (Table 1, 2, and 3) over that of any sugar or mixtures of sugars agrees with Smith (8), who reported higher sclerotial germination in soil amended with the amino acid fraction of root exudates from sugar pine seedlings, than with the carbohydrate fraction. However, the stimulation by citric, malonic, and tartaric acids is at variance with the results of Smith (8) who found the organic acid fraction of root exudates to be nonstimulatory. Differences in the nature and concns of the organic acids may account for the different results. Differences in NG stimulation among individual amendments may be due to their comparative suitability as sources of carbon or nitrogen for germination and subsequent growth, which in turn is dependent on enzymes of the fungus that are either adaptive or constituitive. Differences in the effective concns of individual amendments on NG (Table 1 and 2), and their differential persistence in soil (Table 4) may be due, in part, to the degree of competition for the substrate in the soil. Thus, a simple and universally usable sugar, e.g. glucose, at 5×10^{-2} and 5×10^{-3}M, stimulated only 57 and 32% NG, respectively, compared to 70 and 50%, respectively, for ethanol (Table 1), a poor source of carbon for many microorganisms. The longer-persisting influence of sucrose, over its individual monosaccharide constituents D-fructose and D-glucose (Table 4), may also be due to the simplicity of use and microbial competition for the monosaccharides.

The poor utilization of amendments such as lactose (Table 1) and L-cystine (Table 2) apparently reflects the inability of this fungus to utilize these specific compounds due to lack of appropriate enzyme systems. The inhibition of germination on soil surfaces amended with DL-methionine (Table 2) suggests a toxic effect similar to that described for other fungi with this amino acid (2). The ineffectiveness of organic salts to alter germination patterns is not unusual. Mineral salts and inorganic nitrogen compounds have generally been ineffective in stimulating spore germination in soil (3).

Greater stimulation of NG by mixtures of sugars and amino acids (Table 3), even at lower concns, over the individual amendments suggest a favorable carbon-nitrogen ratio effect. Similar effects have been shown for other fungi (7), and probably explains the response to several of the individual amino acids or their combinations that provide carbon as well as nitrogen, compared to the NG stimulation of all sugars and their combinations.

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