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Stachybotrys atra, an Effective Aggregator of Peorian Loess

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Stachybotrys atra, an Effective Aggregator of Peorian Loess[1]

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ABSTRACT

Twelve cellulose-decomposing fungi were tested for aggregating ability with Peorian loess containing ground straw at a concentration of 1%. Of the 12 fungi, *Stachybotrys atra* was the most effective soil aggregator. Under laboratory conditions used, it produced from 2 to 30 times more aggregation than any of the other fungi tested.

Studies were made of the influence of various environmental factors on the degree of aggregation effected by *S. atra.* An incubation time of 1 week was sufficient for a relatively high degree of aggregation. In comparison, longer periods resulted in only slightly improved aggregation. Varying the temperature between 20° and 28° C. had no appreciable effect on the aggregation by *S. atra.* Approximately equivalent aggregations were attained at the moisture levels of 20, 25, and 30%, which were definitely superior to the 10 and 15% levels. Alfalfa and straw, either separately or as a mixture, were satisfactory sources of energy material for *S. atra.* The aggregation obtained with alfalfa, however, was somewhat higher than that obtained with straw.

The parent material of many of the better agricultural soils of Nebraska is Peorian loess. This material is almost devoid of organic matter, has a very low aggregate stability, and partly because of the steepness of the slopes where it occurs, it is highly susceptible to erosion by water. As a consequence of the erosion of topsoil in many areas of Nebraska, it is not uncommon to find Peorian loess exposed at the surface. The problem of developing stable aggregates of this parent material thus assumes considerable practical importance.

It is probable that microorganisms, through their actions on crop residues and various other materials, are the most important agents for the development of stable soil aggregates in many soils. (2, 3, 4). Microorganisms, however, vary tremendously in their ability to utilize farm waste materials in the production of stable soil aggregates. Recent studies[3] (4) have been carried out in which 33 soil fungi were compared with respect to their ability to aggregate Peorian loess. With straw as the energy material, they showed wide variability in aggregating effects. The percent aggregation developed by the fungi (wet sieving method, for aggregates which fail to pass 0.2-mm. screen) ranged from 2 to 50.

Since cellulose is without doubt the most abundant constituent in most crop residues, it was thought that a search for effective soil aggregators among cellulose-decomposing microorganisms might be fruitful. Accordingly, studies were undertaken dealing with the effects of 12 cellulose-decomposing fungi on the aggregation of Peorian loess. This paper reports the results of this comparison and of further studies in which the most effective soil aggregator was used.

Experimental Procedure

Fungal cultures.—Twelve cellulose-decomposing fungi (1) were obtained from Dr. W. W. Ray, Chairman of the Botany Department, University of Nebraska. They were: *Myrothecium verrucia, Chaetomium globosum, Monascus purpureus, Helminthosporium, Stachybotrys atra, Curvularia, Cephalothecium roseum, Memnonilla echinata, Aspergillus fumigatus, Aspergillus terreus, Aspergillus flavipes,* and *Thielavia basicala.*

All cultures were maintained on slants of potato-dextrose agar (Difco Co.). Inocula for the aggregation experiments were prepared by suspending the growth from these slants in portions of the salt solution defined below.

Soil material.—Peorian loess was obtained from a road cut near Plattsmouth, Nebr., at a depth of 10 to 20 feet. This material is very low in organic matter (less than 0.5%) and in water-stable aggregates larger than 0.2 mm. (approximately 2%). It is also very low in microbial activity. The percent moisture at 1/3 and 15 atmospheres tension is 21.3 and 8.0% respectively.

Salt solution used.—The following salt solution was used at the rate of 3.5 ml. per 35 gm. of Peorian loess:

NH_4NO_3	8 gm.
K_2HPO_4	4 gm.
$MgSO_4 \cdot 7H_2O$	2 gm.
$FeSO_4$	less than 0.1 gm.
Water (tap)	1000 ml.

Determination of aggregates.—For each test, 35 gm. of Peorian loess was weighed into each of duplicate or triplicate Petri dishes and autoclaved for 30 minutes at 15 pounds pressure. The Peorian loess was brought up to a predetermined moisture level with 3.5 ml. of the above salt solution and inoculum, plus water as needed. At the end of the incubation period, three 10-gm. samples were taken from each Petri dish. One sample was used for determining moisture content and the other two for determining percent aggregation. The aggregates were determined using a wet sieving device in which the samples were placed in cylinders, each 2½ inches tall and 2 inches in diameter with a 0.2-mm. screen on the bottom. The soil samples were allowed to stand in distilled water for 5 minutes and then moved up and down in the same distilled water for 5 minutes at the rate of 28 cycles per minute. The material remaining on the screen was washed into an evaporating dish, oven-dried at 105° C. for 24 hours, and weighed. The percent material aggregated was then calculated.

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[3]Unpublished data, T. M. McCalla, F. A. Haskins, and E. F. Frolik. Univ. of Nebraska.

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Results

Influence of various cellulose-decomposing fungi on aggregation.—The 12 fungi were compared with respect to their ability to promote the aggregation of Peorian loess with ground wheat straw (1% concentration) as the source of energy material. Following inoculation, Petri plates in duplicate were incubated at 24° C. for 14 and 28 days with moisture levels at 15, 25, and 30%. Duplicate aggregate determinations were made on the Peorian loess in each dish. Results are shown in tables 1 and 2.

It is clear from the results that *Stachybotrys atra* is the most effective aggregator of the 12 fungi. The aggregation brought about by *S. atra* ranged from approximately 2 to 30 times that effected by the other fungi. These data also indicate that extending the incubation period from 2 weeks to 4 weeks increased the aggregation appreciably for several of the cultures. The appearance of Peorian loess before inoculation and after inoculation with *S. atra* and incubation for 2 weeks is shown in figure 1. A portion of the inoculated plate of figure 1 is magnified 5 times in figure 2 to illustrate the type of mycelial growth.

Factors influencing the aggregation of Peorian loess by Stachybotrys atra.—Further studies with *S. atra* were designed to furnish information on the environmental conditions under which this organism is most effective in aggregating Peorian loess. Except as noted, the following conditions were used in all experiments: carbon source—ground wheat straw, 1% concentration; initial moisture level—25%; incubation temperature—24° C.; incubation period—2 weeks. Thirty-five gm. of Peorian loess was used in each of triplicate Petri dishes, and duplicate aggregate determinations were made from each dish. The following variables were studied: incubation time, incubation temperature, moisture level, nature and concentration of carbon source.

Table 1.—Aggregation of Peorian loess with fungal cultures incubated for 14 days.

Organism	Percent moisture		
	15	25	30
	Percent aggregation (average)		
None	2.6	2.2	2.1
Monascus purpureus	3.1	2.3	2.2
Aspergillus terreus	3.0	3.5	3.3
Aspergillus flavipes	4.5	3.9	3.4
Aspergillus fumigatus	4.1	6.1	2.8
Thielavia basicala	3.8	3.7	6.9
Myrothecium verrucia	4.0	5.8	8.0
Helminthosporium	4.4	5.4	11.8
Memnonilla echinata	4.5	7.3	10.4
Cephalothecium roseum	3.6	14.7	6.7
Chaetomium globosum	7.0	10.0	10.2
Curvularia	16.6	19.4	10.2
Stachybotrys atra	28.5	54.0	60.6

Table 2.—Aggregation of Peorian loess with fungal cultures incubated for 28 days.

Organism	Percent moisture		
	15	25	30
	Percent aggregation (average)		
None	2.6	10.0	11.8
Monascus purpureus	3.0	15.0	8.8
Aspergillus terreus	3.7	3.9	14.1
Aspergillus flavipes	5.6	5.6	13.2
Aspergillus fumigatus	5.5	5.7	10.2
Thielavia basicala	3.6	8.6	10.0
Myrothecium verrucia	4.2	17.4	19.0
Helminthosporium	5.3	14.6	22.2
Memnonilla echinata	7.0	14.4	26.0
Cephalothecium roseum	5.0	4.8	16.9
Chaetomium globosum	8.4	19.9	25.1
Curvularia	19.6	31.9	22.7
Stachybotrys atra	47.2	62.6	57.8

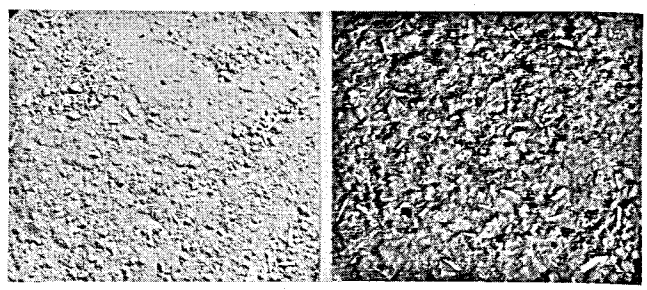

FIG. 1.—The appearance of Peorian loess with ground wheat straw added to make 1% concentration. Left—uninoculated. Right—inoculated with *Stachybotrys atra* and incubated for 2 weeks at 24° C.

FIG. 2.—A portion of the inoculated plate shown in figure 1 magnified 5 times. Note the mycelial growth on the particles of straw and Peorian loess.

Incubation time.—Results of a comparison of incubation periods of 1, 2, 4, and 8 weeks are presented in table 3. The data indicate the attainment in 1 week of a relatively high degree of aggregation. The incubation periods above 1 week and up to 8 weeks increased the degree of aggregation to some extent, but the increase was small.

Incubation temperature.—Different incubation temperatures of 20°, 24°, and 28° C. are all favorable for aggregation by *S. atra,* as shown in table 4. Under the conditions of the experiment, essentially equivalent aggregations were attained at the three temperatures.

Moisture level.—Results of an experiment dealing with the effect of initial moisture level on aggregation are presented in table 5. It is apparent that moisture

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Table 3.—Influence of incubation time on the aggregation of Peorian loess by *Stachybotrys atra.*

Organism	Time in weeks				
	1	2	3	4	8
	Percent aggregation (average)				
None	3.9	5.7	—	—	10.5
S. atra	48.8	50.8	60.9	58.8	66.3

L.S.D. (0.05) = 9.5 for comparison of means of time within *S. atra.*

Table 4.—Influence of incubation temperature on aggregation of Peorian loess by *Stachybotrys atra.*

Organism	Temperature degrees centigrade		
	20	24	28
	Percent aggregation (average)		
None	3.1	2.5	4.6
S. atra	62.3	60.2	59.0

L.S.D. (0.05) N.S. for comparison of means of temperature within *S. atra.*

Table 5.—Influence of initial moisture level on aggregation of Peorian loess by *Stachybotrys atra.*

Organism	Percent moisture				
	10	15	20	25	30
	Percent aggregation (average)				
None	3.8	7.5	2.9	3.3	3.1
S. atra	24.6	36.8	52.8	50.0	52.1

L.S.D. (0.05) = 11.6 for comparing moisture levels within *S. atra.*

Table 6.—Influence of carbon source on the aggregation of Peorian loess by *Stachybotrys atra.*

Carbon source	Percent energy material					
	0.00	0.25	0.50	1.0	1.5	2.0
	Percent aggregation (average)					
Straw	—	32.8	41.7	46.7	56.8	58.9
Alfalfa	3.3	46.8	55.9	58.2	70.9	65.7
Straw-alfalfa*	—	32.8	46.1	62.8	65.6	64.8

L.S.D. (0.05) = 8.4 for comparing concentration of energy material within a treatment or treatments within a concentration.
*Equal weights of both straw and alfalfa added to each Petri dish.

levels of 10% and 15% are suboptimal for aggregation by *S. atra,* and that increasing the initial moisture content from the 20% level to 30% is without effect on the aggregating ability of this organism.

Nature and concentration of carbon source.—In an experiment comparing carbon sources, ground wheat straw, ground alfalfa, and a 1:1 mixture of the two were used at levels of 0.25, 0.5, 1.0, 1.5, and 2.0%. The results presented in table 6 demonstrate that, within this concentration range, there tends to be increased aggregation in response to increased concentration of energy material. It is also apparent that, on a weight basis, alfalfa is superior to straw for the promotion of aggregation by *S. atra.*

Discussion

Of 12 cellulose-decomposing fungi tested, only *Stachybotrys atra* was highly effective in promoting aggregation of Peorian loess under laboratory conditions.

These results indicate that, by the selection of the proper microorganisms, a great deal can be done to change the aggregation percentage of Peorian loess. In addition, when a given microorganism is selected, it is possible to influence the degree to which that microorganism will aggregate the soil by controlling moisture, carbon source and concentration, and time of incubation.

The results also indicate that many of the soil microorganisms which decompose high-cellulose farm residue do so without contributing a great deal to soil aggregation. If the maximum benefit with respect to soil aggregation is to be realized from farm wastes, it would appear that consideration must be given to the selection of effective microorganisms, and as demonstrated in the present study, to the provision of an environment favorable for the functioning of these effective microorganisms.

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