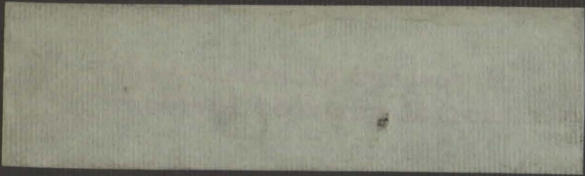
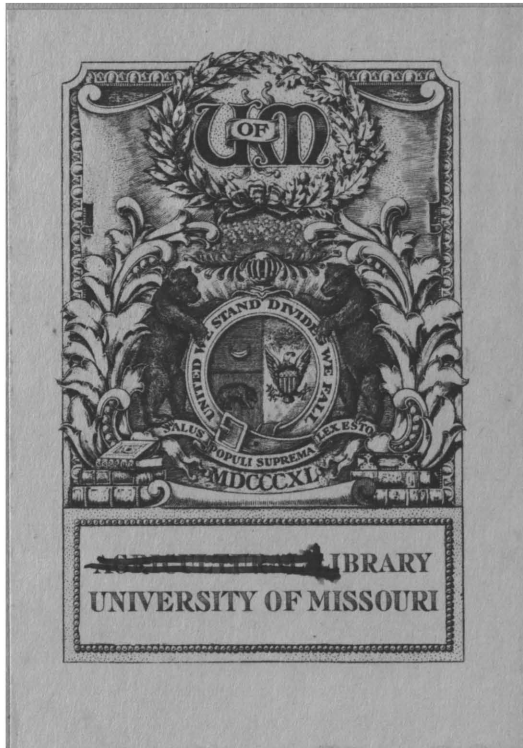


UM Libraries Depository



103284905002





This Thesis Has Been

**MICROFILMED**

Negative No. T- **1074**

Form 26















PHYSIOLOGICAL STUDIES ON MONASCUS

by

Emma Bee Mundy, A.B., B.S. in Ed.



SUBMITTED IN PARTIAL FULFILLMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF  
MASTER OF ARTS

in the

GRADUATE SCHOOL

of the

UNIVERSITY OF MISSOURI

*Approved May 14, 1914*  
*George M. Reed*

1914



378.7M71

XM92

## PHYSIOLOGICAL STUDIES ON MONASCUS.

### Introduction.

Moldy silage, because of its economic importance, has attracted considerable attention in the last few years. Certain molds, because of their general and frequent occurrence and peculiar characteristics, have become of considerable interest from both a practical and a scientific standpoint. It is thought by many farmers that moldy silage has been responsible for the deaths of stock. If this be true either all the moldy silage, which in some cases is a considerable amount, must be thrown away, or the risk of poisoning stock must be taken. From a scientific point of view, the interests are more diverse. The molds may be of interest because of their own characteristics, either morphological or physiological, or they may be studied because of the changes which they bring about in the substratum on which they grow. These changes may be either desirable or undesirable. It is now generally accepted that the fermentations of silage are, in part at least, due to the action of bacteria, yeasts, and molds, and that the quality of



the silage produced depends to some extent upon their presence and activities. On the other hand, frequently conditions are such that the changes these organisms effect are undesirable from the standpoint of good silage. In order, however, to understand any change brought about by the mold in the substratum or medium, it is necessary to study the physiological characteristics of the mold.

For the past five years a red mold has persistently occurred in the silos located on the Dairy Farm of the Agricultural College, of the State University of Missouri. Some years this mold appeared only in small quantities; other years it has been found occurring throughout the silage to a depth of fifteen feet, sometimes infecting areas two to three feet square, and often adhering to, and growing upon, the sides of the concrete silo. From time to time reports have been received from various points over the state that this red mold is quite common in silos. It has even been suggested that stock have been killed as a result of feeding upon the infected silage.

In looking over the literature, which is very meagre on this subject, the red fungus is reported from various points in the United States, being mentioned frequently in farm magazines and papers. Buchanan (2), in 1909, mentions it as occurring in Iowa, but says, "no (previous) record has been found of its occurrence in America and no record of its occurrence in silage." However, reports of its occurrence now seem to be



rather common. Recently, in Wallace's Farmer, published in Iowa, several accounts of moldy silage appear. One account from Kansas mentions the red mold specifically. Nevertheless, the only previous work upon the physiology of this fungus consists in rather brief physiological studies made in connection with more elaborate work on its morphology.

A few years ago the Dairy Department of the University of Missouri collected some of this red moldy silage from the silo and brought it into the laboratory for identification. At first it was thought that the red color was due to some bacterium which was associated with a white mold. However, it soon became evident that the mold was responsible for the red color. Because of its peculiar characteristics a sample was turned over to the Botany Department for identification and study.

In the late fall after the silos on the Dairy Farm have been opened, fresh samples of silage infected with this red mold may be obtained almost any time. The fungus occurs scattered throughout the silage to a depth of ten or fifteen feet. Samples were collected and brought to the laboratory for study. Wherever the mold was present the silage was matted together into tight masses by a white web-like growth. Upon pulling the masses apart the pieces of silage in the center were stained a deep carmine red. This color was especially evident when grains of corn or portions of an old ear were ~~collected~~ found near the center





of the mass. From these moldy samples inoculations were made upon dextrose potato agar sticks which were then plated. By repeated effort a pure culture of the mold was obtained, a microscopical examination of which showed the mold to correspond to that which Buchanan (2) tentatively called Monascus purpureus Went.

Went's identification of Monascus purpureus came as a result of study made upon the red fungus which the Chinese use in the manufacture of "Ang-quac" (red rice), a substance used as a food coloring and a beverage. The preparation of "Ang-quac," Went says, was kept secret a long time by the Chinese but was found out and described to him by a friend. The process of making it is as follows: Rice is cooked and spread out upon trays. When cool, some of the "Ang-quac" powder is sprinkled over it, and the rice set away in a cave for six days. At the end of this time the whole amount is colored a deep red. This is then dried and later pulverized. Went obtained his original culture from some infected rice grains which he first washed in hydrochloric acid and rinsed in sterile water to kill all possible foreign organisms. Then he placed the rice grain in a nutrient solution where the fungus developed. He studied the morphology of the organism in detail and did some work on the physiology.

This fungus is at once interesting because of the striking red color that appears in connection with its development.



As has been previously mentioned, the color diffuses out into the substratum, staining it a deep red. In examining the fungus some of the mycelial threads are observed to be stained a deep red while others are colorless. Since little work has been done upon the physiology of the mold and very little upon the pigment, this phase has been given emphasis in the work at hand.

#### Morphology.

The morphology of this mold has been studied and described by earlier workers, as Went (7), Barker (1), Olive (6), and others. Therefore a brief description of its characteristics will suffice here.

The mycelium is much branched, septate, and granular. The older hyphae are very vacuolate and often times are stained red. Two kinds of spores are formed, those that are borne asexually, known as conidia, and those which develop within the fruiting body known as ascospores. The conidia are borne in chains or singly at the tips of hyphae. They are spherical or nearly so and vary greatly in size. They are, however, considerably larger than, though not so numerous as, the spores which are developed in the fruiting structure. In the cultures grown in the laboratory the conidia never appeared to be colored or tinged with red.

The fruiting structure, which Went refers to as a sporangium, has led recent writers, as Barker and Olive, to class the



mold with the Ascomycetes, since the fruiting body appears to be a typical perithecium containing, in its early development, several asci which contain many spores. The asci appear to disintegrate before maturity of the perithecium and the spores are then set free in the perithecium. The young perithecium oftentimes is red, but with age the structure usually assumes a brown color. At this time it is surrounded by a mass of short hyphae which have grown out around it as a protective layer. The perithecia occur in great abundance throughout the growth and are often imbedded within the medium. At maturity the wall ruptures and the numerous, small, elliptical ascospores are set free.

#### Isolation and Gross Cultural Studies.

Samples of moldy silage were brought into the laboratory and from these, series of transfers were made. For all this preliminary work two per cent dextrose potato agar plates were used. During this phase of the study many other molds were isolated in addition to many bacteria. When a pure culture of *Monascus* was obtained by the plate method, the mold was transferred and kept growing on two per cent dextrose potato agar slopes, from which all subsequent inoculations were made. While isolating *Monascus*, it was noticed that one bacterium, in particular, frequently appeared with *Monascus*, and that, when it was present, the medium immediately surrounding the mold became a deep carmine red. Furthermore, the mold in pure cul-



ture on dextrose potato agar was not able to produce the red color. From seventy-five to one hundred dextrose potato agar slopes were inoculated with *Monascus* for comparison in color variations. The mold became a brownish gray and the medium a dark copper color, but the characteristic red color never appeared. These facts seemed to indicate that the production of the red color was made possible by the presence of the bacteria. Consequently, a pure culture of the bacterium was obtained, and dextrose potato agar slopes were inoculated both with *Monascus* and the bacteria. In every case the color was present after a few days. The bacteria grew rapidly and soon covered the surface of the slope. Although the mold grew more slowly it developed rather tall aerial hyphae. About five days after inoculation, the bacteria surrounding the mold colony gradually turned a deep carmine color, which finally spread entirely over the surface of the slope and later throughout the medium. Certain known bacteria, *B. coli*, *B. vulgatus*, *B. megatherium*, and *B. mycoides* were then used in the same way to determine whether only the one specific organism behaved in this manner, or whether any bacterium would bring out the color. The results showed that *B. vulgatus* of the known organisms brought out the color just as the unidentified form. Since this form did not show any particularly peculiar characteristics it was not identified, although in appearance it much resembled *B. vulgatus*. From these experiments it is evident that a pure culture of *Monascus* on





dextrose potato agar will not produce the carmine red color, but if contaminated with certain bacteria the color will develop.

The cultural characteristics of *Monascus* on other media were determined to see if the fungus persistently behaved <sup>as was indicated</sup> by the above experiments on dextrose potato agar. About twenty-six different kinds of media were prepared, and *Monascus* grown upon them to ascertain the effect upon growth and color production. Since the mold was taken originally from silage it seemed natural to suppose that a silage decoction would prove a most suitable medium. Consequently, silage agar was made by using 100 grams of fresh silage to 500 cc. of water. This was autoclaved at ten pounds pressure for twenty minutes, then filtered and the amount lost through evaporation restored by addition of water. To this solution, 20 grams of agar, melted in 500 cc. of water, were added. Then the medium was again put in the autoclav at ten pounds pressure for twenty-five minutes. It was then titrated to determine the acidity, which was 1.4 cc. acid, per 100 cc., and filtered into test tubes which were sterilized at ten pounds pressure for thirty minutes. When removed, the medium was cooled in slopes. Inoculations were made upon the silage agar slopes, but only a very small amount of white growth appeared. No color, whatever, developed.

It seemed peculiar that an abundant growth did not occur on silage agar since the mold develops so vigorously on silage. Consequently several methods of making this medium were tried



out. Finally a medium was made by using approximately 50 grams of the dry weight of fresh silage, to which 1000 cc. water were added. This was allowed to stand for twenty-four hours, at the end of which time the solution was filtered off. A portion retained as a liquid medium was placed in flasks and test tubes, and sterilized by the discontinuous method. Another portion of the solution was made into agar media as follows: two per cent agar was melted in a small amount of the solution and then added to the whole. This was filtered, tubed, and sterilized in the autoclav at a very low temperature and cooled in slopes. The mycelial growth which developed, although it was not as abundant as on other media, and produced no color, was very much better than any observed on silage agar previously made. However, these results did not seem consistent or conclusive in themselves. Consequently, other media were tried out to see what ones, if any, would allow growth and color production. In all cases agar and liquid media were prepared according to Duggar.(3) The results are given in tabulated form in Table I.



TABLE I

Character of Growth of *Monascus* on Different Media.

Media	Amount of Growth	Character of Growth	Presence of Perithecia	Coloring Matter	
				In fungus	In media
Beans (string)	Fair	Mycl. thinly covering surface of beans	+	Pink to white	None
Bean (dry) agar	none	-----	-----	---	----
Bread	Good	Mycl. over entire surface	+	Gray to red	Carmine red
Bean agar +2% dextrose	Fair	Very little surface growth. Mycl. throughout medium	+	Yellow Brownish	same
Clabber	Very good	Aerial hyphae well developed. Mycl. throughout medium	+	Brownish-red	Carmine red throughout
Corn grains crushed	Very good	Aerial hyphae fairly well developed. Mycl. through medium	+	Brownish-red	Carmine red throughout
Corn agar	poor	Very little aerial growth but growth all through medium	+	Reddish	Red
Corn sol.	poor	No surface growth. Mass of mycelium in solution	+ ?		Red
Full nutri.sol.	Very good	Dense mat of felt over surface	+	White to gray to pink	Solution colored blood orange
Green corn	Very good	Mycl. developed throughout mass of corn	+	Gray to red	Parts of corn colored deep red.



Table I (contd.)

Media	Amount of Growth	Character of Growth	Presence of Perithecia	Coloring Matter	
				In fungus	In media.
Milk (sweet)	Very good	Dense thick mat of felt over surface.	+	Red	Solution colored deep red throughout
Milk (sour)	Very good	Not quite as dense as sweet milk	+	Red	Solution pink to deep red
Potato agar+2% dext.	Very good	Mycl. growing abundantly over surface and in media	+	Grayish-brown	Copper color
Potato sol.+2% dext.	Fair	Dense but not thick mat of mycl. over surface of sol.	+	White to brown	Slightly brownish
Potato agar+5% dext.	Fair	Not as abundant as 2% dext.	+	Yellowish brown	Copper color.
Potato slope	Fair	Covered surface of potato	+	White	None
Prune Sol.	Fair	Rather dense mat over surface	+	White to gray	Brownish
Rice (whole, moistened)	Very good	Mycl. grows rapidly over surface and throughout medium	+	Brownish red	Rice grains stained carmine red
Rice agar	Poor	Very little surface growth. Mycelium developed through medium.	+	Reddish	Red





Table I (contd.)

Media	Amount of Growth	Character of Growth	Presence of Perithecia	Coloring Matter	
				In fungus	In media
Rice solution	poor	No surface growth. Mass of mycelium in solution	+?	reddish	red
Silage, sterile	very good	Most abundant mycelial growth throughout mass	+	white	Turning red in region of corn grain or cob
Silage agar	poor	Sparse mycelial growth over surface	+	white	----
Silage agar +2% dext	very good	Mycelial growth well developed over surface and in media	+	reddish-gray	Dark red
Silage solution	fair	Mat over surface of solution	+	gray	----
Silage solution +2% dext	better than above	Mat over surface of solution	+	reddish gray	reddish brown



In all the media used perhaps the most striking results were obtained on bread, clabber, crushed corn, green corn, milk (sweet and sour), whole rice, sterile silage, and silage agar plus two per cent dextrose. On each of these, abundant growth occurred accompanied by the production of the carmine red color. In the milk cultures a dense, thick, dark red mat of mycelium developed on the surface of the liquid. The red color gradually diffused into the liquid until the milk became carmine red in color throughout. In the case of the crushed corn medium which was prepared by filling small culture dishes half full of crushed corn thoroughly moistened, then sterilized, a fairly good surface growth developed over each bit of crushed corn. Simultaneously with the development of the mycelium, the color appeared and diffused throughout the crushed corn until every particle was colored a deep, dark red. The growth obtained on the whole rice was much the same. In twenty-four to thirty-six hours' time after inoculation small tufts of white aerial hyphae could be seen. The substratum immediately surrounding the tufts likewise showed the red color. As growth proceeded, the color diffused throughout the medium, which, at the end of two weeks, was completely red. The rice medium was prepared by placing 15 grams of rice to 25 cc. of water in a 150 cc. Erlenmeyer flask and sterilizing, during which the moisture was all taken up by the grains. The green corn medium was made by chopping up the green corn plants on which the young ears were formed,



yet undeveloped. This material was then placed in flasks of various sizes and a small amount of water added, then sterilized by the discontinuous method. Silage was prepared in the same way. After inoculation both showed a fairly abundant growth of a delicate, white, cob-web-like mass of mycelium throughout the mass of material. In the green corn the characteristic red color developed along with the mycelium and diffused rapidly, especially on pieces of the young ears. On the silage, however, although the white mycelial growth was much more abundant than on the green corn the red color did not appear until about two weeks later, when it was first observed in a grain of corn and a piece of cob to which a few imperfect grains were still attached. From these parts the color diffused slowly to other surrounding fragments of the silage. On the silage agar plus dextrose as on dextrose potato agar, abundant surface growth appeared and upon microscopic examination of a bit of the agar it was found to be invaded throughout by the mycelium which produced perithecia within the agar. But in the case of silage agar plus sugar a dark red color developed, whereas in dextrose potato agar only a copper color appeared. The standard full nutrient solution mentioned was prepared in the following proportions, which are essentially like those outlined by Duggar:

1.0 gram	$\text{KNO}_3$	in 20 cc.	redistilled $\text{H}_2\text{O}$
.5 "	$\text{KH}_2\text{PO}_4$	" 20 cc.	" "



.25 gram $\text{MgSO}_4$	in 20 cc. redistilled $\text{H}_2\text{O}$		
2.00 mg. $\text{FeCl}_3$	" 20 cc.	"	"
5. grams ( $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ )	in 20 cc."	"	"

Fifty cc. of the above solution were placed in 150 cc. Erlenmeyer flasks, sterilized at ten pounds pressure for twenty-five minutes and inoculated when cool. The value of this solution as a source of food for *Monascus* was first tested out; so that, if it proved a good medium, it might be used as a standard for future work where it is desirable to know all the constituents in the medium. As indicated in the table, a fair amount of growth developed.

However, after this work, the facts still remained that the color had not been produced on the plain silage agar, on silage solution, or on dextrose potato agar unless contaminated, but that on certain <sup>other</sup> very complex compounds color did appear. It was thought that the acidity of the medium might have some influence upon growth and color production, and that in the case of dextrose potato agar, which was neutral, the bacteria produced acid. Consequently, the bacteria were tested out for acid formation by using dextrose litmus potato agar. Several tests were made, but no signs of acid production were apparent. Then a series of full nutrient solutions as outlined above were made acid and alkaline in varying proportions described in the following experiment to determine the effect of varying amounts of acid and alkali on growth and color production.





Effect of Acid and Alkali on Growth and Color Production  
of Monascus.

A standard full nutrient solution was made neutral. Fifty cc. of the solution were poured into each of twenty-six flasks of 150 cc. capacity. All glassware used was thoroughly cleaned in cleaning solution, rinsed first in tap water, then in distilled water, and finally twice in redistilled water. The cultures were all made in duplicate. To twelve of the cultures varying amounts of standardized normal hydrochloric acid were added; to the other twelve the same amounts of standardized normal sodium hydroxide were added. The remaining two cultures were left neutral for comparison.

The amounts of acid and alkali added were as follows:

1.	2	cultures(50 cc. each),	.05 cc. NaOH	per 50 cc.
2.	2	" " "	.1 " "	" " "
3.	2	" " "	.5 " "	" " "
4.	2	" " "	1.0 " "	" " "
5.	2	" " "	2.5 " "	" " "
6.	2	" " "	5. " "	" " "
7.	2	" " "	Neutral	
8.	2	" " "	.05 " HCl	" " "
9.	2	" " "	.1 " "	" " "
10.	2	" " "	.5 " "	" " "
11.	2	" " "	1.0 " "	" " "
12.	2	" " "	2.5 " "	" " "
13.	2	" " "	5. " "	" " "





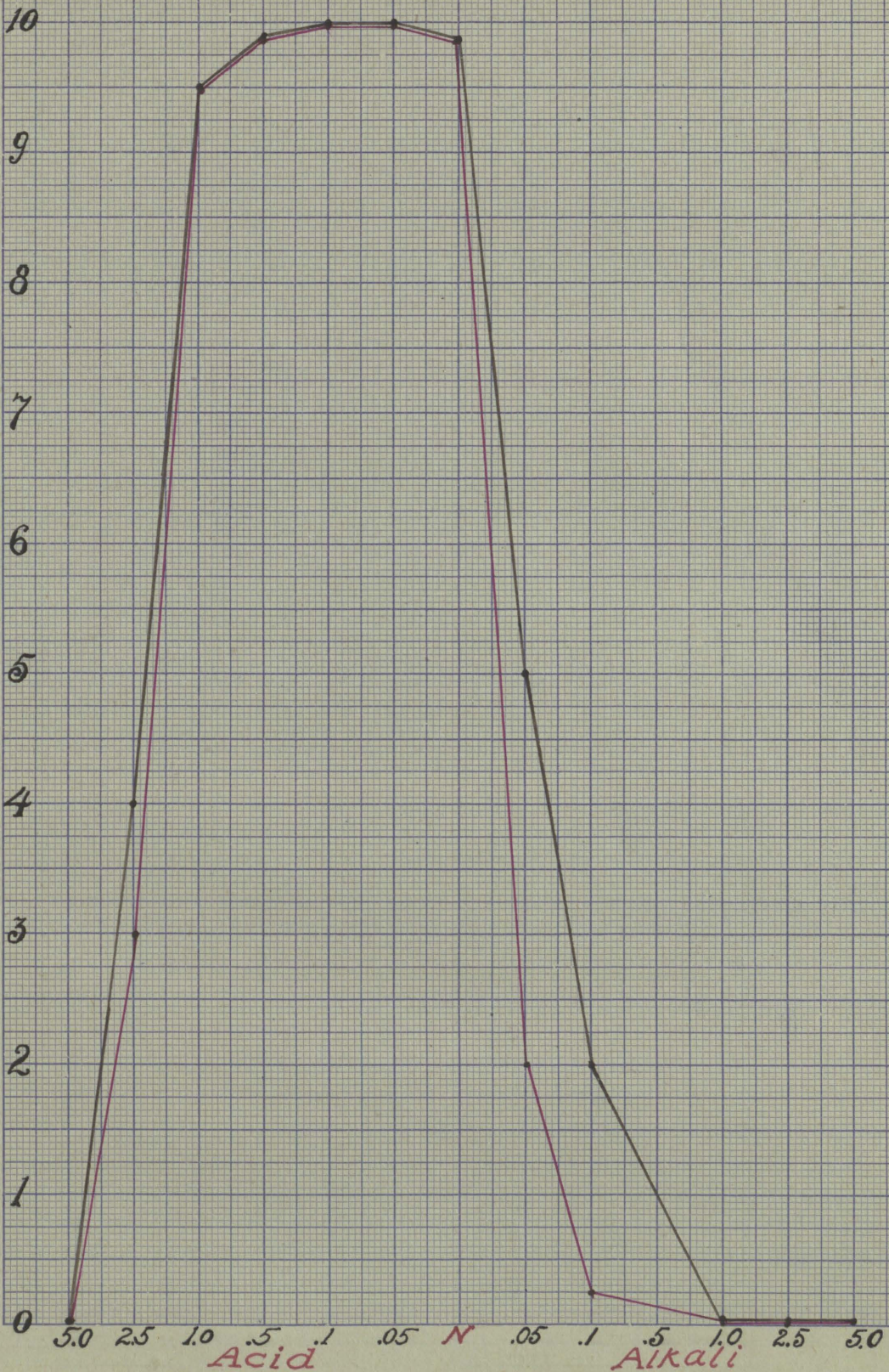
## GRAPH I.

Graph I shows in a comparative way the effect of different amounts of acid and alkali on growth and color production of *Monascus*.

The blue line represents the growth curve; the red, color production. The figures at the base to the left of N (neutral) represent the number of cc. of acid used; to the right of N, the number of cc. of alkali used.

Ten is taken as an arbitrary standard representing the maximum growth and color production in the experiment.

Graph No. 1.





These flasks were then inoculated and incubated at 28° C. for three weeks. At the end of this time the following results were obtained:

5.	cc. acid	shows no growth;	no color.
2.5	" "	"	very little growth; slight color.
1.0	" "	"	abundant growth; solution, deep orange.
.5	" "	"	about same as 1.0 cc.
.1	" "	"	maximum amount, though not much better than 1. and .5 cc. Color of solution about same.
.05	" "	"	growth not quite as good as .1 cc.
Neutral		"	growth about like 1. cc acid.
.05 cc.	alkali	"	growth not nearly as dense as on 1. cc. acid. Very marked decrease.
.1	" "	"	still less growth. Solution a very pale straw color. Mycelium a delicate pink.
.5	" "	"	very slight growth. Mycelium white.
1.	" "	"	a few scattered white colonies.
2.5	" "	"	no growth
5.	" "	"	no growth

A comparison of the different amounts of growth and the color produced in the different solutions is given in Graph I.

From these data it is evident that an acid medium is more favorable to growth and color production in *Monascus* than an alkaline medium. But the growth in the neutral medium would seem to indicate that acid is not essential. The essential point seems





to be that the medium must be acid or neutral for growth and color production.

A Comparison of the Effect of Different Acids in the Same Varying Amounts on Growth and Color Production.

Since an acid medium seemed to be favorable for growth, it was thought worth while to test out the effect of different amounts of several acids, and make a comparison of them. Besides hydrochloric acid, lactic and acetic were used because of their occurrence in silage. The acids were standardized and the full nutrient solution made neutral. To the latter the following amounts of acid were used in duplicates of each series.

.1 cc. acid in 50 cc. full nutrient						
.5 "	"	"	"	"	"	"
1. "	"	"	"	"	"	"
1.5 "	"	"	"	"	"	"
2. "	"	"	"	"	"	"
2.5 "	"	"	"	"	"	"
3. "	"	"	"	"	"	"
3.5 "	"	"	"	"	"	"
4. "	"	"	"	"	"	"

Two neutral cultures were used as checks.

After incubation at 28° for three weeks the final observations were made.

In neutralizing the standard nutrient solution a precipitate formed during sterilization which had an inhibiting effect upon



the growth in the first three amounts of all acids used. Consequently, the results on these are not included.

Of the three acids used, hydrochloric seemed the least favorable. In this no growth occurred in the solutions beyond 3 cc. of the acid. The color of the solution was a deep burnt straw.

The cultures containing lactic acid showed a much better development of the fungus. A good mat of felt was formed in the cultures of greatest acidity. The color produced was much like that in the hydrochloric cultures except that there was more red present. The colors of the mold in each case were about the same, varying from a deep pink to gray.

In the case of acetic acid by far the best results were obtained from the standpoint of color. As far as the amount of growth was concerned it did not vary greatly although, as mentioned before, both lactic and acetic showed good growth in solutions of highest acid content. But the color of the fungus in acetic acid was a light grayish red and the solution a deep cherry red — the deepest red obtained in any liquid medium used, with the exception of milk.

While these results are not conclusive in any degree, they do show, first, that such organic acids as lactic and acetic are more favorable for the development of *Monascus* than hydrochloric acid. Second, that in acetic acid the characteristic color is produced. The fact that the solution in the acetic acid



cultures were a deep red should not be considered very significant for that may have been due to the fact that acetic acid was a better solvent than the others, and was thereby able to dissolve out the pigment. But the fact that the fungus itself is red in the case of acetic <sup>acid</sup> cultures is of some significance.



Compounds Used by Monascus for Carbon Assimilation.

It seemed evident from the preliminary culture studies that *Monascus* behaved very differently on different media. Consequently, an experiment was set up to determine what organic compounds seemed best suited for the development of the mold.

A full nutrient solution was made up as outlined before except that no sugar was included. In place of the sugar other organic compounds were substituted in varying amounts. The different compounds and amounts used per 50 cc. of solution were as follows:

1. Cornstarch	.5 g.	6. Lead acetate	.05 g.
"	2.5 g.	" "	.15 g.
2. Peptone	.5 g.	7. Gallic acid	1. g.
"	2.5 g.	" "	2. g.
3. Maltose	.5 g.	8. Ethyl alcohol	.05 g.
"	2.5 g.	" "	.25 g.
4. Glycerine	.5 g.	9. Citric acid	.1 g.
"	1.5 g.	" "	.3 g.
"	2.5 g.	10. Cane sugar	.5 g.
5. Sodium tartrate	.5 g.	" "	2.5 g.
" "	2.5 g.	11. Minus any organic compound.	

Fifty cc. of each solution were placed in clean 150 cc. flasks, using, in all, forty-four flasks. They were sterilized in the autoclav at ten pounds pressure for thirty minutes. After





cooling they were inoculated and incubated at 26° C.

After two weeks' growth final observations were made which are recorded in Table II.

TABLE II.

Effect of Different Organic Compounds on *Monascus*.

Organic Compounds	Character of Growth	Coloring Matter	
		In fungus	In solution.
Corn starch .5 g.	No growth on surface. Small amount of slimy growth in solution	white	----
Corn starch . 2.5 g.	as above	white	----
Peptone .5 g.	Very thin mat of felt over surface	yellowish pink	yellowish-pink
Peptone 2.5 g	Fairly dense mat of felt over surface	pinkish	straw-color
Maltose .5 g.	Fairly dense mat over surface	brownish	brownish
Maltose 2.5g.	Not as dense as above	brownish	brownish
Glycerine . .5 g.	Very sparse, thin growth on surface	white	----
Glycerine . 1.5 g.	Sparse, thin growth on surface	white	----
Glycerine . 2.5 g.	Best of three. A very thin mat over surface and extending upon sides of flask	white	----
Sodium tartrate .5 g	no growth	----	----
Sodium tartrate 2.5 g.	no growth	----	----



Table II (contd.)

Organic Compounds	Character of Growth	Coloring Matter	
		In fungus	In solution
Lead acetate .05 g.	Few, small masses scattered over surface	white	----
Lead acetate .15 g.	Few, small masses scattered over surface. (Little better than above).	white	----
Full nutrient minus any organic compound	none	-----	----
Gallic acid 1. g.	no growth	-----	----
Gallic acid 2. g.	no growth	-----	----
Ethyl alc. .05 g.	Few masses of mycelium over surface.	white	----
Ethyl alc. .25 g.	Small masses of mycelium over entire surface.	white	----
Citric acid .1 g.	no growth	-----	----
Citric acid .3 g.	no growth	-----	----
Cane sugar .5 g.	Dense, thick mat of felt over entire surface	pinkish-white to gray	blood-orange
Cane sugar 2.5 g.	As above, perhaps a little denser	same as above	deep blood orange

Lafar (5) mentions that *Monascus* was observed growing on glycerine vats in a soap factory. Hence, the six flasks of full



nutrient solution plus glycerine were allowed to develop about four weeks longer. At the end of this time a rather dense mat of mycelium had developed over the surface. The mycelium showed a variety of colors. The upper surface of the mat was colored from brownish to gray to white. Looking at the underside of the mat from beneath, dark red areas were apparent. However, the solution remained colorless or nearly so, and the amount of growth was by no means as abundant as in the case of the sugars.

Buchanan (2) set up an experiment to determine the ability of *Monascus* to use glycerine, in which he used tap water containing 5, 10, 20, and 40 per cent of glycerine. His results were as follows: "Growth occurred in the 5 and 10 per cent solutions. In the 5 per cent solution small masses of mycelium could be observed within a few days, floating in the liquid. These continued to enlarge slowly for two months, at the end of which time they formed a semi-transparent mass of a quarter of the volume of the medium. The interior of such masses was found to be densely matted, and of a deep carmine color. In a 10 per cent solution of glycerine growth was slower. The colonies or mycelial balls remaining smaller and more compacted."

This experiment was repeated in connection with this particular phase of the work and practically the same results, as those outlined by Buchanan, obtained.

It was mentioned in the first part of this paper that, in the masses of silage infected with *Monascus*, the red color was es-



pecially vivid if a grain of corn or a portion of an old ear was found in the mass. Consequently, the following experiment was set up which gave some very interesting results: Five culture dishes were lined with filter paper thoroughly moistened. Into one dish some leaves of the corn silage were placed, into a second dish, some pieces of corn stalk of silage, into a third, portions of old cobs from silage, into a fourth, pieces of pith of corn stalk <sup>of</sup> silage with epidermis removed, into a fifth, corn grains and pieces of ears from the silage. The dishes were then covered and sterilized. When cool the material within was inoculated. Forty-eight hours after inoculation a dense, white, mycelial growth developed over each material. Growth continued for a month. At the end of this time all appeared practically the same as they were at the end of the first forty-eight <sup>hours</sup> except in the case of the corn grains and pieces of ears. These showed the carmine red color in addition to the white mycelial growth, part of which had become a dark reddish gray.

This experiment was repeated both with the silage material and also with fresh green corn in which no matured grains were yet developed. As a substitute, portions of young developing ears were used. In these last two experiments the results were identical with those of the first.

From the results recorded in table II it is evident that not only some organic compound is absolutely essential but that





the sugars are by far the best compound for the growth of *Monascus*. Certain foods as Gallic acid, citric acid, and sodium tartrate which are used by other molds do not apparently serve as a carbon supply for *Monascus*.

The experiments with the different portions of silage and of the green corn, point to the fact that some compound or compounds within the seed and ears are especially favorable to growth, and essential for color production, and that color production is not always proportional to the amount of growth, as is suggested by Went (7).



Effect of Certain Chemical Elements on Growth  
and Color Production.

After testing out what organic compounds were used by the mold, and since the standard full nutrient solution was used rather extensively as a culture medium, it seemed of interest to determine just which of the elements were necessary for growth and color production, or, conversely, to see the effect upon growth and color production of the absence of any one of the elements used in the standard nutrient solution. This was done by substituting a non-essential element, thereby maintaining a balanced solution.

The following solutions were therefore prepared in duplicates of 50 cc. each:

1. Standard nutrient solution (check).

2. Minus nitrogen

1. gram KCl

.5 "  $\text{KH}_2\text{PO}_4$

.25 "  $\text{MgSO}_4$

2. mg.  $\text{FeCl}_3$

5 grams  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$

100 cc. redistilled  $\text{H}_2\text{O}$

3. Minus potassium

1. gram  $\text{NaNO}_3$

.5 "  $\text{NaH}_2\text{PO}_4$

.25 "  $\text{MgSO}_4$



2. mg.  $\text{FeCl}_3$   
 5. grams  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$   
 100 cc. redistilled  $\text{H}_2\text{O}$

## 4. Minus phosphorus

1.0 gram  $\text{KNO}_3$   
 .5 "  $\text{K}_2\text{SO}_4$   
 .25 "  $\text{MgSO}_4$   
 2.00 mg.  $\text{FeCl}_3$   
 5. grams  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$   
 100 cc. redistilled  $\text{H}_2\text{O}$

## 5. Minus magnesium

1.0 gram  $\text{KNO}_3$   
 .5 "  $\text{KH}_2\text{PO}_4$   
 .25 "  $\text{Na}_2\text{SO}_4$   
 2.00 mg.  $\text{FeCl}_3$   
 5. grams  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$   
 100 cc. redistilled  $\text{H}_2\text{O}$

## 6. Minus sulphur

1.0 gram  $\text{KNO}_3$   
 .5 "  $\text{KH}_2\text{PO}_4$   
 .25 "  $\text{MgCl}_2$   
 2.00 mg.  $\text{FeCl}_3$   
 5. grams  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$   
 100 cc. redistilled  $\text{H}_2\text{O}$



## 7. Minus iron

1.0 gram  $\text{KNO}_3$ .5 "  $\text{KH}_2\text{PO}_4$ .25 "  $\text{MgSO}_4$ 2.00 mg.  $\text{NaCl}$ 5. grams  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ 100 cc. redistilled  $\text{H}_2\text{O}$ 

## 8. Minus all mineral constituents

5. grams  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ 100 cc. redistilled  $\text{H}_2\text{O}$ 

The cultures were sterilized at low pressure and, when cooled, inoculated. After incubation at  $28^\circ \text{C}$ . for three weeks the following results were obtained:

## 1. Standard Nutrient Solution:

Fairly heavy pink to gray wrinkled mat over surface of solution. Solution colored blood orange.

## 2. Full Nutrient Solution minus Nitrogen:

Very slight amount of germination.

No surface growth whatever.

## 3. Full Nutrient Solution minus Potassium.

Rounded, isolated colonies extending over entire surface tending to grow together. Colonies pink, carmine on under side. Solution deep blood orange color, not clear.







## GRAPH II

A graphical representation of the effect upon growth and color production of the absence of different mineral elements. The blue line represents the growth curve; the red, color production.

Ten was taken as an arbitrary standard for comparing the different solutions. It represents the maximum amount of growth and color production in the whole set. The spaces below ten, approaching zero, represent a gradual decrease.

# Graph No 2.

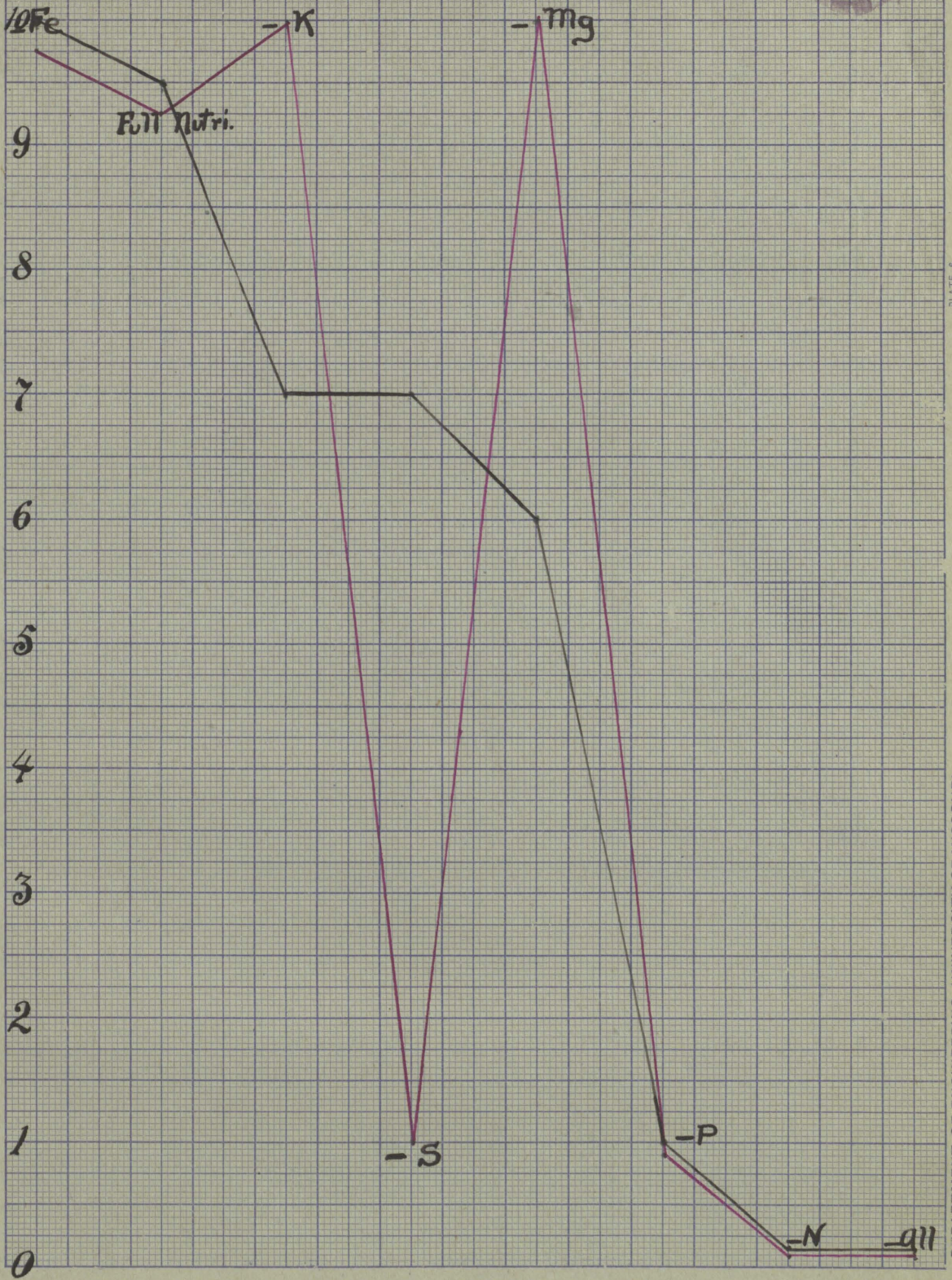
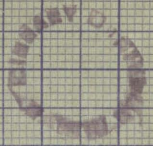


PLATE 6

DEPARTMENT OF ENGINEERING, UNIVERSITY OF MISSOURI



4. Full Nutrient Solution minus Phosphorus:

Very thin delicate web-like mass of mycelium in solution. No surface growth whatever. Solution pale transparent straw color.

5. Full Nutrient Solution minus Magnesium:

Rounded isolated colonies over surface, not quite as thick as - K. Colonies white with minute deep pink spots over surface of colonies, making them appear irregularly spotted. Solution deep straw color.

6. Full Nutrient Solution minus Sulphur:

Fairly heavy wrinkled dark gray mat over surface. In places the mat shows a pinkish color. Solution first a milky, pale green but turning to straw color.

7. Full Nutrient Solution minus Iron:

Very good growth; like, and perhaps a little better than, standard nutrient.

8. Minus all elements except Sugar:

Slight amount of germination, but no growth beyond that.

For a comparison of the amounts of growth and color production, see Graph II.

Although these results are only preliminary, it is evident that certain of the elements in the nutrient solution influence growth and color production directly, while others act more indirectly. The presence of phosphorous and nitrogen seem essen-



tial for growth. In fact, with the possible exception of iron, all the elements are necessary for a fair development of the mold. The amount of iron used in the nutrient solution is very small and a slight trace from some outside source might enter in the minus iron solution, which amount would be sufficient for the development of the mold.

In the case of color production, however, a somewhat different condition obtains. It is obvious that color would not be produced in solutions minus nitrogen and minus phosphorous when little growth occurs in these. But in the case of the solution minus sulphur a fair amount of growth develops but not the characteristic color. This seems to suggest that sulphur is partly essential for color production. In a solution minus potassium, and one minus magnesium, although growth is only medium, color production is very good. These facts indicate that the production of the pigment does not depend entirely upon, or develop in proportion to, the amount of growth of the fungus, as is suggested by Went (7).

#### Temperature Relations of Monascus.

Four flasks containing 15 grams of whole rice and 25 cc. of water were sterilized, and, when cool, inoculated with *Monascus*. One was incubated at 12° C., another at room temperature, about 21° C., another at 28° C., and the fourth at 36° C. In less than thirty-six hours the medium at 28° showed evidences of germination. The culture at 36° also showed signs of germination.





But those at 21° and 12° showed no signs of germination at this time. In about three days, growth was evident on the medium at 21°. In ten days time the rice in the flask at 28° was red throughout, due to the development of the mold through the medium. The flask at 21° showed the fungus to have developed all over the surface of rice and <sup>in</sup> over half its depth. In some places the red color had reached the bottom of the medium. The fungus at 38° had grown almost as much as that at 21° but instead of the carmine color, it was a dark brownish-gray red. No visible growth occurred at 12°, but after remaining at 12° for nearly two weeks, the flask was transferred to room temperature whereupon the fungus became active and soon developed throughout the medium producing the characteristic color.

These results are quite in accordance with the results of other workers, the optimum temperature being somewhere around 26°-28°. Furthermore, the maximum temperature for growth of the mold corresponds quite nearly to the temperature in silos, according to Esten and Mason, <sup>(4)</sup> in 1912.

#### Oxygen Relation.

The oxygen relation of *Monascus* seems to be somewhat uncertain. Growth may occur in the silo at a depth of 15 feet as well as near the surface. However, it is never found much below this depth unless near the sides of the silo. Last fall an experimental silo on the Dairy Farm was filled about one-third full of silage; then later filled to the top. In order to



mark the place between the two, a gunny-sack was thrown on top of the first deposit. Later the remainder was filled in. This spring when the gunny-sack was exposed it was practically covered with *Monascus*. Circular areas red at the center and shaded off into the white mycelium at the edges of the colonies, five to ten inches in diameter, were developed directly on the gunny-sack. Here and there pieces of silage were matted to the sack by the mycelium. Transfers were made directly from the gunny-sack, and, by isolation, pure cultures were obtained. No trace of *Monascus* could be found in the silage at this depth other than that which occurred on the gunny-sack and immediately surrounding it.

In cultural studies made in the laboratory it has been observed that with two exceptions *Monascus* behaves like an aerobë. With the exceptions of corn and rice liquid extract aerial hyphae always developed, and if the flask containing the inoculated solution be slightly tipped from side to side the mold will later develop on the thin film of solution that remained on the sides. Furthermore, if the mat of felt growing on the surface of the liquid be shaken down to the bottom of the vessel, new surface growth develops immediately. Several flasks of silage solution were prepared by adding to half of them a pad of absorbent cotton just thick enough so that the saturated cotton came to the surface of the liquid. The other flasks contained the same amount of silage solution without the cotton. All the flasks were inoculat-



ed. In twenty-four hours, the mold on the cotton pad had germinated. Growth continued very rapidly until the whole surface of the cotton was covered with the mold at about the same time that the first aerial hyphae appeared on the solution alone. The solution minus the cotton finally, however, developed a mat of felt over the surface.

In the corn and rice extracts, as mentioned above, surface mycelium, if any, develops very tardily. Before any signs of aerial hyphae appear, there is a fairly well developed slimy mass of mycelium and, in some, fruiting bodies down in the liquid. Later, in most instances, small aerial clusters of hyphae develop.

Although some growth may occur under anaerobic conditions, it seems doubtful that the mold will develop to any extent without the presence of free oxygen. Its occurrence in the lower depths of silos, as suggested by farmers themselves, is in part most probably due to the fact that the silage when packed was not sufficiently tramped, thereby leaving spaces of air through the silage. The gunny-sack, mentioned above, undoubtedly allowed some oxygen to be held at that depth, and as the mold occurred nowhere else in that region of silage except in close association with the sack, the presence of oxygen there was undoubtedly partly responsible for the growth of the mold.

Another factor which certainly has some influence upon the presence of molds in silage, and which may be correlated with



the oxygen factor, is that of moisture. Very often silage is put away too dry. Molds develop in much greater abundance where the silage is just very slightly moist than where it is sufficiently moist. If corn already dry, because of lack of rains at the time it is maturing, be put away with insufficient wetting, not only is this condition of dryness favorable to the molds, but aeration is included, so that the molds also have a fairly good supply of oxygen for growth. Farmers are beginning to realize that the absence of air and presence of sufficient moisture are of great importance in preventing the development of molds in silage.





Studies on the Pigment.

As suggested by Went (7), although the carmine red pigment produced by *Monascus* offers opportunity for study, it has not been thoroughly worked out because its physiological relations to the fungus were never understood. In this work, studies made upon the pigment as such, have been brief and incomplete. Went, however, worked with the pigment rather extensively, and most of his results will therefore be included in this account.

A microscopic examination of some of the mycelial threads shows at once that certain hyphae are red, while others remain uncolored. Upon close inspection it appears that the more vacuolate hyphae are usually stained, while the more dense ones exhibit no color. This would indicate age to be a factor in pigment production. Yet this does not always hold, for on certain media the hyphae never become red, while on others they are red even in very young cultures. According to Went, the granular protoplasm, not the cell sap, within the hypha is stained red. This, he says, can easily be determined by plasmolysis. However, this point does not seem conclusive, for in plasmolysis the cell sap may diffuse out leaving the pigment in the cell. When the state of complete plasmolysis is reached the contents appear red, but this may be due to the fact that the stain which was once in the cell sap did not pass out during exosmosis. It would be difficult to prove that the protoplasm



was stained red in the first place. Furthermore, this would be very unusual, for it is usually the cell sap which is stained.

Went says that the absence of oxygen is often the cause of the mycelium remaining uncolored; the fungus does not develop when oxygen is absolutely lacking. Yet he maintains that the color is not brought about by simple oxidation, but is the result of a vital process. This fact he demonstrated by the following experiment: He took an uncolored submerged mat of mycelium and divided it into two parts. After killing one half, both portions were exposed to the air. The portion which was alive became red, while the other remained uncolored. The presence of oxygen may have some effect, but it is not the controlling factor in color production, for the fungus has been grown under conditions such that oxygen was present, yet no color developed. For example, in the experiment where different parts of silage were inoculated, the conditions there were aerobic, yet only in the case of the corn grains did color develop.

The solubility of the pigment was tested by the use of ethyl alcohol, ether, xylol, and water. A beautiful deep red solution was dissolved out by alcohol and ether; but no effect was produced by xylol. The pigment is slightly soluble in water but fades out rapidly. This corroborates the work of Went who finds that the pigment is dissolved easily in ethyl alcohol, methyl alcohol, ether, chloroform, glacial acetic acid, acetone, acetic ether; and insoluble in water, dilute acids, benzolé,



ether of petrolium, turpentine, carbon bisulphide, and glycerine.

An extract of the pigment for study was obtained by first breaking to pieces a whole rice culture which was stained throughout. About twenty-five cc. of absolute alcohol were added to this and allowed to stand for twenty-four hours. At the end of this time, the alcohol in solution, now a deep red, was filtered off. The solution obtained was, in transmitted light, a deep transparent red, and in reflected light, greenish. The rice still remained a deep red and more alcohol was added, but it never seemed able to extract all the color from the rice, i.e., the rice seemed to be permanently stained. The paper used as a filter was also stained a deep red.

Tests were then made upon the alcoholic solution. A small amount of concentrated hydrochloric acid was added to a few cc. of the solution. A deep yellow color resulted which did not come back to the original when sodium hydroxide was added. To another portion of the extract, sodium hydroxide was first added, with a yellow color resulting. Hydrochloric acid was added but no marked change occurred. About ten cc. of the extract were placed in an evaporating dish and set in an open window. When all the alcohol had evaporated, a deep red, sticky residue, was left. A microscopic examination revealed no crystal formation whatever. Upon re-adding alcohol the residue was dissolved. Another small quantity was kept in direct sunlight for six hours without showing any effect. The effect of continued exposure to light was



brought about by placing an amount of the solution in a flask and sealing with paraffin. The flask was placed in a window so that direct sunlight fell upon it for about four hours per day, and reflected light for two hours. At the end of a month the solution had faded considerably, becoming a pale burnt straw color. In diffuse light, however, the color is very lasting. Went says that the color of the Ang-quac is almost indestructible. Lafar (5), however, says that in durability the color cannot compare with the aniline dyes. Hence, it will never be a product of much value.

The pigment was tested for presence of acid by adding litmus solution to a small quantity of the extraction. The litmus, it is true, turned slightly reddish, but it was thought to be wholly due to the red in the pigment. Went says the pigment is composed of C, H, O, but N is lacking. Further than that little has been determined of its exact chemical nature.

#### SUMMARY.

It is evident from the results of experiments and tests outlined in this paper that growth and pigmentation in Monascus purpureus offer many problems. Although the work done in this connection is only preliminary, some striking results of the peculiar behavior of the fungus have been brought out.

In the first place, as might be supposed, Monascus does not produce acids or alkalis. This was demonstrated in the following manner: The mold was grown on sterile litmus sweet milk





and sterile litmus sour milk with checks of both kinds of milk without litmus. Abundant growth, accompanied by brilliant color production, occurred on all, without any suggestion of production of acid or alkali. Moreover, growth and the production of the red color is not altogether dependent upon the presence of acid. An alkaline medium is unfavorable for growth, but on a neutral or very slightly acid medium, growth and pigment production are abundant. This fact is brought out not only by the full nutrient cultures, but also by the corn and rice cultures on which the fungus develops so abundantly and produces color. When tested these cultures were found to be neutral. Yet such organic acids as lactic and acetic seem to render a given medium much more suitable for development of *Monascus* than hydrochloric or a neutral medium. This fact corresponds, moreover, to conditions in silage. During the fermentations which go on in silage the two principal acids formed are lactic and acetic.

The problem dealing with the effect of certain chemical elements upon growth and color production, shows clearly that the amount of color produced does not always depend upon the amount of growth present. That is, the factors which cause the color to appear may not always be the same as those which bring about abundant growth.

Unlike the pigments of certain bacteria, as *B. prodigiosus*, that of *Monascus* is not dependent upon temperature conditions that do not otherwise influence the development of the fungus.



On a given medium the color production is always constant with the possible exception of the influence of the absence of oxygen. External conditions, with that exception, seem to have no direct effect upon the pigment.

Oxygen probably plays a very important role in the life of the fungus. Few molds are able to live as anaerobes, and from all evidence *Monascus* is an aerobe. The presence of molds throughout silage points to the fact that either the silage has not been properly tramped in or has been insufficiently moistened.

The most puzzling and contradictory results are those obtained from the complex organic media on which *Monascus* was grown for cultural study. The facts on the one side are these: On milk, bread, sterile silage, sterile green corn, rice, and crushed corn there occurred abundant growth of the fungus, and abundant production of the characteristic carmine red pigment. On the other side, abundant growth occurs on dextrose potato agar, but very little color is apparent unless bacteria are added. Again, although the original supply of the fungus was collected from silage, no silage medium, other than sterile silage, could be made which would yield the characteristic amount of growth and color. And although growth was abundant on sterile silage color was never produced except where the fungus was in contact or association with grains of corn or pieces of ears.

In testing out the foods which serve for carbon supply, the sugars, particularly cane sugar, are by far the most favorable for the fungus. Other organic compounds are used but



slightly by the mold. A peculiar condition was revealed in this experiment, i. e., that *Monascus* developed very slightly in the cultures where starch was used for the carbon food, yet it grows very abundantly on rice which is composed almost entirely of starch. On the other hand, in dextrose potato agar where both sugar and starch are supplied, abundant growth occurs, but no color appears in the medium unless bacteria are introduced.

The explanation of some of these facts is obvious, while of others it is mere conjecture.

In the case of dextrose potato agar it is likely that the color is formed in the fungus, but does not dissolve out in the medium to any great extent. The bacteria when introduced form a slimy mass over the surface of the agar, and as the color is produced they take it up readily. It may be, however, that the bacteria really bring about some change in the medium thereby rendering the fungus capable of producing the color.

One fact which seems absolutely clear is that complex organic compounds are necessary for complete development of the mold and color. Of these compounds, the sugars or sugar-like substances are the ones used. However, the fact that rice is such a favorable medium led to the carrying out of several experiments, the results of which may throw some light on this particular study.

Two liquid cultures of rice solution were used. One culture was inoculated with *Monascus*; the other was left as a



check. At the end of four weeks a slimy mass of mycelium had developed within the inoculated culture. The following tests were made:

(a) For starch - (iodine)

The reaction in the check was blue.

The reaction in the inoculated culture was a reddish purple.

(b) For sugar - (Fehling's)

The check showed no sugar.

The inoculated culture showed the presence of sugar.

From these tests but one conclusion can be drawn, that is, that *Monascus* by secreting the enzyme diastase changed the starch into sugar, which, from a previous experiment, seems to be the most usable compound for the fungus. A series of media were treated with commercial diastase to see if it rendered the medium more favorable for the fungus. But it was impossible to introduce the diastase without also introducing bacteria. Hence, in most cases, contamination by molds and bacteria prevented a normal development of *Monascus*. Went suggests the possibility that *Monascus* contains a diastatic ferment but he does not offer much proof for his theory.

It may be that the products formed during the change from starch to maltose are used by the fungus to a better advantage than the end product of the reaction. Two kinds of corn agar





were made. One was made from ungerminated corn while the other was made from corn which had germinated and produced radicles one to two cm. long.

After inoculation and growth of *Monascus* on the two kinds of media, the ungerminated corn agar became a deep rose pink throughout while the germinated, in which enzyme activity had gone on, showed more of a brown color. The aerial growth in either case was very small.

If it be true, therefore, that some product formed during the change is more favorable than the end product, this would explain why a medium could not be made which would best serve the fungus for a food supply, since in this, either starch or sugar must be used.

In the case of the silage it is possible that by sterilization two changes are brought about which have a great deal to do with the development of the fungus. First, probably some of the compounds are broken down by heat, and rendered unfit as food for the mold. Second, since most of the sugars which were originally present in the corn before fermentation are now changed into other compounds which may not be of value to the mold, the fungus may depend to a large extent upon the activities of other organisms in the silage, for its food supply, instead of upon the silage as such. When they are killed their by-products are no longer available. When sugar is added to the silage as was done with silage agar, the mold grows and produces color abundantly.



The fungus is, however, able to use the organic foods stored up in the corn grains, and, as is found to be true, it attacks the grains, invading the tissues throughout. When it is once established it is able to continue its development. In the case of green corn abundant sugars are present so that *Monascus* grows readily upon it and produces the characteristic pigment.

The color production, as is usually the case in the formation of pigments, is probably an end- or by-product of metabolism of no particular use to the mold but an inevitable product when the proper foods are supplied to the developing fungus.

As has been previously mentioned the pigment is able to be dissolved out by certain substances and kept in solution. Although the eastern peoples use the pigment from *Monascus* as a dye, and an industry has been built up because of its use, the aniline dyes are by far more satisfactory and lasting.

The facts that the natives of China, according to Lafar (5), use the pigment in preparing a beverage, and, as is mentioned by Went (7), that it is used in coloring small fish, known as fish of *Mancascar*, which are served as a food delicacy in Java, tend toward proving that the fungus is not poisonous. It is possible, however, that growing on silage, *Monascus* produces some compounds which are poisonous. However, the stock on the State Farm have fed upon moldy silage for years and no deaths have been known to occur.

From these facts a variety of conclusions may be drawn, some of which are fairly well established facts, while others are



only hypotheses.

1. The fungus grows vigorously on a neutral or slightly acid medium, but feebly on an alkaline medium. Such organic acids as lactic and acetic are more favorable than hydrochloric.

2. Color production does not necessarily accompany abundant growth.

3. The fungus does not produce acids or alkalies.

4. Oxygen is necessary for the development of the fungus.

5. Temperature affects the color production only as it affects the growth of the mold.

6. Liquid and agar media do not seem to be the most favorable for development of *Monascus*.

7. The fungus grows best on complex organic compounds.

8. The most suitable organic compounds for foods are the sugars or sugar-like substances.

9. On starchy media the fungus probably secretes the enzyme diastase which converts the starch into sugars which are available as food.

10. The fullest development of the mold in silage is probably dependent upon the activities of other organisms.

11. The carmine red pigment is not due to simple oxidation only. It is probably one of the end- or by-products of metabolism within the cells and depends upon the best food conditions for its development.



12. The pigment is soluble in certain solutions as ethyl alcohol, methyl alcohol, ether, chloroform, glacial acetic acid, acetone, and acetic ether; but insoluble in water, dilute acids, benzole, ether of petrolium, turpentine, sulphur of carbon, glycerine, and xylol.

13. Facts seem to indicate that molds in silage are due to inadequate tramping and insufficient moisture, yet their occurrence probably does not render the silage poisoning.





## BIBLIOGRAPHY.

1. Barker, B. T. P.       The Morphology of the *Ascocarp* in  
                              *Monascus*.  
                              *Ann. Bot.* 17 : 167        1903
2. Buchanan, R. E.       *Monascus purpureus* in Silage.  
                              *Mycologia*, Vol. II, No. 3     May 1910.
3. Duggar, B. M.         Fungous Diseases of Plants.
4. Esten and Mason       Silage Fermentation.  
                              *Storrs Agric. Exp. Sta. Bult.* No. 70     Jan. 1912.
5. Lafar                  *Monascus purpureus* und der chinesische  
                              *Ang-Khak (Ang-Quac)*  
                              *Handbuch der tech. Mykologie*, Vol. IV, p. 265.
6. Olive, E. W.         The Morphology of *Monascus purpureus*.  
                              *Bot. Gaz.* 39 : 56        1905
7. Went, C.               Le Champignon De L'*Ang-Quac*.  
                              *Ann. des sciences nat., Botan.*, 1895, 8 ser. Bd. 1,  
                              S. 1.





## PLATE I

This photograph was taken of the gunny sack which had been placed between the two layers of silage in the experimental silo on the Dairy Farm. Note the rounded colonies of *Monascus*, the red centers, showing dark in the photograph, with a white periphery of uncolored mycelium. Note also the masses of silage attached to the sack.

*Plate 1.*



DRIV. 1911

















328.7M71  
XME2

University of Missouri - Columbia



010-100920489

~~This thesis is never to leave this room.~~  
~~Neither is it to be checked out overnight.~~

