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The Destruction of Vanillin in the Soil  
by the Action of Soil Bacteria

By

WILLIAM J. ROBBINS

Assisted by A. E. Elizondo

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# THE DESTRUCTION OF VANILLIN IN THE SOIL BY THE ACTION OF SOIL BACTERIA

By

WILLIAM J. ROBBINS

Assisted by A. E. ELIZANDO

In an earlier publication (6)\* it was concluded that the addition of a given toxic organic compound may produce no harmful effects in one soil and decidedly harmful effects in another, depending on the presence and action of suitable microorganisms which destroy the toxic compound. In the present paper further evidence is offered to justify the application of this conclusion to a number of soils in which the effect of vanillin on the growth of higher plants has been tested. Vanillin is an aldehyde which is harmful in water culture at a concentration of 1 part per million (7) to wheat plants, and which has been isolated from unproductive soil (8). The writer has shown (6) that in certain Alabama soils it is rapidly destroyed by the action of bacteria.

## THE PRESENCE OF VANILLIN-DESTROYING BACTERIA IN SOILS OTHER THAN ALABAMA SOILS

Four investigations in addition to the one carried on at the Alabama Experiment Station (5) have been made on the effect of the addition of vanillin to the soil on the growth of plants. Davidson (2) working at the New York State College of Agriculture found that vanillin had little bad effect on the growth of wheat. Skinner (9) in field tests at the experiment farm of the Agricultural Department at Arlington, Virginia, found that vanillin stunted the growth of cow peas, garden peas and string beans. He found vanillin present in the soil of these plots six months after its application. The same investigator in pot experiments found vanillin to be harmful to wheat plants grown in infertile Florida sandy loam but to have no effect in fertile Hagerstown loam. Fraps (4) at College Station, Texas, found in general little harmful effects from the application of vanillin to potted soil. He also found that the vanillin rapidly disappeared during the course of the experiment. Upson and Powell (10) at Lincoln, Nebraska, report that vanillin shows very little

(\* Reference is made by number to Literature cited p.)

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and 8/28/10

Robbins

harmful effect in the soil on the growth of wheat.

From the above brief review it can be noted that Davidson, Fraps and Upson and Powell found little harmful effect from the addition of vanillin to the soil. Skinner also found vanillin to have little or no effect when added to the fertile Hagerstown loam. He obtained, however, marked bad effects in the Arlington soil, in the infertile Florida sandy loam and in the infertile Susquehanna sandy loam.

From our own work on Alabama soil we assume that the results first cited where little harmful effect was observed were due to the fact that bacteria which fed on vanillin were present and the soil conditions were suitable for their growth and their destructive action on vanillin. On the addition of vanillin to these soils the bacteria destroyed the vanillin and little or no bad effect resulted. The results in the Arlington soil, in the Florida sandy loam and the Susquehanna sandy loam would be explained as due either to the absence of suitable bacteria or to conditions in those soils which prevented the growth and the action on vanillin of the bacteria. Under either of these conditions the added vanillin would persist and evidence its toxicity. To demonstrate the correctness of this view samples of all the above soils except the Hagerstown loam, Florida sandy loam and Susquehanna sandy loam have been examined for the presence of vanillin-destroying bacteria. In addition the effect of vanillin on the numbers of microorganisms in the Arlington soil has been studied.

Through the courtesy of Dr. T. L. Lyon of the New York State College of Agriculture a sample of soil was received from the same field and as nearly as could be determined from the same spot as that used by Davidson in the experiments noted above. This soil was collected with a sterile spoon and shipped by express in a sterile can. Upon its receipt, using all precautions to avoid contamination, a small quantity was added to a sterile nutrient solution containing vanillin.<sup>2</sup>

<sup>2</sup>This solution contained:

Na NO <sub>3</sub> .....	0.1 gm.
K <sub>2</sub> HPO <sub>4</sub> .....	0.1
Mg SO <sub>4</sub> .....	0.05
NHy Cl .....	0.1
KCl .....	0.05
Vanillin .....	0.1
Water Dist. ....	200 cc.

The solution soon became cloudy and within a few days a test for vanillin using the acid nitrate of mercury reagent described by Estes (3) showed that the vanillin had disappeared. The cloudy solution was plated out and from the isolations made, bacteria were obtained which, in pure culture, destroyed vanillin.

By the kindness of Dr. G. L. Fraps four of the soils used by him in his work with vanillin were collected and shipped in sterile cans. The four soils were those referred to in his publication (4) as Nos. 876, 870, 1956 and 114. All four of the soils contained vanillin destroying bacteria.

From Dr. F. W. Upson the Black Meadow soil and Lancaster fine sandy loam referred to in the work by Upson and Powell cited above were received. These soils were also collected and shipped under sterile conditions. The presence of vanillin-destroying bacteria in these two soils was also demonstrated and pure cultures of vanillin-destroying bacteria isolated from them. With these soils there was also forwarded a can of what appeared to be quartz sand in regard to which Dr. Upson stated.

"We get a very marked difference between the sand and the two soils in regard to their ability to destroy vanillin and cumarin."

In this sand no organisms destroying vanillin could be demonstrated.

Through the kindness of Dr. Oswald Schreiner and Dr. J. J. Skinner, a sample of the Arlington soil was received. This sample was collected and shipped under sterile conditions. Vanillin-destroying bacteria were found to be present in this soil and were isolated in pure culture.

We have, therefore, demonstrated the presence of vanillin-destroying bacteria in those soils to which the addition of vanillin has been found to have little bad effect on the growth of plants. The sand is of much interest as here we apparently have a case in which the vanillin persists and evidences its toxicity because of the absence of vanillin-destroying bacteria. The Arlington soil is also of much interest because in this case the vanillin persists and is toxic even though vanillin-destroying bacteria are present in the soil. We, therefore, assume that conditions are not suitable in this soil for the growth and the action of the vanillin-destroying bacteria present. To test the correctness of

this assumption the effect of the addition of vanillin to this soil on the number of microorganisms in it was determined.

EFFECT OF THE ADDITION OF VANILLIN TO THE ARLINGTON  
SOIL ON THE NUMBER OF MICROORGANISMS  
DEVELOPING IN IT

As was pointed out in a previous publication (6) the addition of vanillin in suitable concentration to a soil in which the vanillin-destroying bacteria are present and are under conditions which allow them to act produces an initial decrease in the number of microorganisms present which will develop on Brown's albumen agar (1). This decrease is followed by a marked temporary increase in the number as the vanillin-destroying bacteria feed on the vanillin and multiply. With the exhaustion of the vanillin and its decomposition products the number of microorganisms returns to normal. By studying, therefore, the effect of vanillin on the number of microorganisms in the Arlington soil it was hoped that an indication might be found as to whether the conditions in that soil are suitable for their action on vanillin.

Dr. Oswald Schreiner was kind enough to furnish us with a quantity of the Arlington soil. The soil as received was very acid having a lime requirement by the Veitch method of 4740 pounds per acre.

Nine kilograms of the air dry soil were placed in two-gallon pots and the pots were brought to the optimum water content with distilled water. At the same time nine kilograms of air dry Norfolk sandy loam from the station farm were placed in two-gallon pots. This soil was practically neutral, having a lime requirement of 600 pounds per acre. It was also brought to optimum water content with distilled water. After standing 30 days the soil from two pots of the Arlington soil was removed, mixed with a sterile spatula on sterile paper and repotted. These served as checks. The soil from two other pots was similarly treated but 9 gms. of vanillin was added to each pot. The soil from two additional pots was removed, 9 gms. of vanillin added to each pot and the soil well inoculated with a suspension of a pure culture of a vanillin-destroying bacterium isolated from Alabama soil. Two pots of the Norfolk sandy loam were prepared as checks and two pots of the same soil were prepared to each of which 9 gms. of

vanillin were added. From time to time the number of microorganisms developing in the pots was determined by the methods described by Brown (1) using his albumen agar. Each soil was plated in duplicate using dilutions of 1-20,000 and 1-200,000 as described by Brown. The number of microorganisms developing in the pots is given in Table 1.

TABLE 1  
*Micoorganisms in Millions per gm. of Air Dry Soil.*  
*Soil Potted June 5th, Vanillin Added July 5.*

Treatment	June 15	June 26	July 9, 4 days after treatment	July 18, 13 days after treatment	July 27, 22 days after treatment	Aug. 4, 30 days after treatment	Aug. 17, 43 days after treatment	Aug. 31, 57 days after treatment
None—Arlington Soil ----	4.74	7.32	1.52	.92	2.52	4.76	5.49	2.66
Vanillin—Arlington Soil ---	14.92	10.26	0.12	0.18	0.05	0.14	0.14	0.96
Vanillin & Van.-destroying bacteria—Arlington soil	8.12	4.46	0.11	0.06	0.00	0.14	0.21	0.32
None—Alabama soil -----	9.76	8.83	7.88	4.98	8.26	4.45	5.45	3.76
Vanillin—Alabama soil ---	11.48	8.16	1.28	5.76	22.22	22.44	14.68	18.12

The data in Table 1 indicate that the persistence of vanillin in the Arlington soil is due to some condition or conditions which prevent the destructive action on vanillin of the vanillin-destroying bacteria. As was found before (6) the addition of vanillin to the Alabama soil first produces a decrease in the number of microorganisms which will develop on Brown's albumen agar. This decrease is followed by an increase in which the numbers far exceed those present in the normal soil. There is then a return to normal. In the Arlington soil, however, no such phenomenon occurs. The addition of vanillin produces a marked decrease in the numbers but this is not followed by any increase. In fact, as far as bacteria are concerned, the upper layers of soil remain practically sterile as the majority of microorganisms indicated in the table as developing in the vanillin treated Arlington soil were molds. Only occasional bacterial colonies appeared on the plates from these pots. Even the addition of a pure culture of a bacterium known to destroy vanillin does not im-

prove conditions as can be noted from the table. There can be no question regarding the persistence of vanillin in this soil. It rose to the surface of the soil where it was observed in crystals more than 40 days after its addition to the pots. It is, therefore, believed that the persistence of vanillin in the Arlington soil is due to conditions in that soil which prevent the action of the bacteria on the vanillin.

What these conditions are can not be definitely stated at the present stage of the investigation. The soil, as was indicated above, is very acid. It was found, however, that in an acid Alabama sandy loam, having a lime requirement of 3400 pounds per acre, vanillin, at the same concentration as was used in the Arlington soil, was entirely destroyed in less than 57 days (6).

Soil extracts of Alabama soil and Arlington soil to which vanillin was added showed no difference in the rate at which vanillin was destroyed by a pure culture of a vanillin-destroying bacterium.

It is possible that the persistence of vanillin in this soil is due to poor oxygenation conditions. It has been found that oxygen seems to influence the rate at which vanillin is destroyed by a pure culture of a vanillin-destroying bacterium and the destruction of vanillin by this organism is an oxidative process, at least in its early stage. The Arlington soil is a heavy silty clay loam which compacts easily, probably excluding oxygen to a large measure.

Some condition which may be poor oxygenation is certainly unfavorable for bacterial growth in this soil. This can be seen from the fact that, as is indicated in Table 1 in the data for the check pots, the mere removal, mixing and repotting caused a marked decrease in the numbers of bacteria found 4 days later. This decrease is perhaps best explained as a dilution effect. If we assume that the upper layer of soil contained numerous bacteria, while in the lower reaches of the soil the bacteria which will develop on plates under aerobic conditions were few or absent, then in mixing the soil as was done at the time of treatment there would be a decrease in the numbers because those bacteria in the upper layers were spread through the contents of the entire pot. Not only is this decrease evident 4 days later, but it continues to be evident for something over 22 days as it is not until 30 days after



treatment that the numbers of bacteria in the untreated Arlington soil approach normality. This seems, perhaps, to be slower than would be expected if oxygen were the limiting factor in the multiplication of the bacteria. In the untreated Alabama soil which is a porous sandy loam no decrease in numbers is produced by the mixing and repotting.

A further investigation of soils in which vanillin has been found to persist, to determine whether bacteria capable of destroying vanillin are present and if they are, to determine why they do not act on the compound is advisable.

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