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The Cause of the Disappearance of Cumarin, Vanillin, Pyridine and Quinoline in the Soil

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THE CAUSE OF THE DISAPPEARANCE OF CUMARIN, VANILLIN, PYRIDINE AND QUINOLINE IN THE SOIL[1]

By
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Since the proposal of the soil toxin theory of soil fertility by DeCandolle (4) [2] in 1832, and its further elaboration in recent years by the Bureau of Soils of the U. S. Department of Agriculture, evidence has been offered to support or discredit it along several lines. One of these has been the demonstration that organic substances, either found in the soil or reasonably assumed to be there, are toxic to crop plants in water culture. As a further step in this same direction, the effect on plants of these substances, when added to the soil, has been studied.

The results obtained when the compounds are added to the soil have been conflicting. Some investigators have found that the compounds which are toxic to plants in water culture are decidedly harmful when added to the soil. They have also found that the compound and its toxic effects persist for a considerable space of time. Others have found that the same compounds have little or no toxic action in the soil or even prove decidedly beneficial to the growth of the plants. They have also found that the compounds disappear rapidly in the soil.

The work recorded in the following pages was undertaken to determine the cause of the disappearance of these compounds in the soil. With a clear understanding of why they disappear in one soil, it would appear possible to explain why they persist in other soils and to examine intelligently methods for eliminating them from soils in which they are known to exist and in which they may be a contributing cause to infertility.

[1] The writer wishes to express his indebtedness to Mr. A. E. Elizondo for careful and conscientious assistance.

[2] Reference is made by number to "Literature Cited," p. 63.

LITERATURE [3]

Duggar (5) states that "wheat in paraffined pots containing rich garden loam is practically unaffected by pyridine at the enormous rate of 8,000 parts per million, this solution being used to moisten the soil to 60 percent of its water holding capacity."

Davidson (3) studied the effect on the growth of wheat of cumarin and vanillin when added to Dunkirk clay loam. It was found that 180 parts per million [4] of cumarin, or 600 parts per million of vanillin depressed the yield somewhat. Davidson concludes that even this effect is on the soil and not on the plant.

Skinner (28) in pot experiments, found that vanillin added to the soil before potting, at a concentration of 500 parts per million, was harmful to wheat plants grown in infertile Florida sandy loam soil and infertile Susquehanna sandy loam, but had no effect on wheat grown in fertile Hagerstown loam. Vanillin added to plots of the experiment farm of the Agricultural Department at Arlington at the rate of 285 pounds per acre stunted the growth of cowpeas, garden peas and string beans. The same investigator found vanillin present in the soil of these plots six months after its application, and demonstrated by pot experiments that the compound still injuriously affected the growth of plants.

Fraps (6) has found that vanillin at a concentration of 100 parts per million is injurious to corn or oats in but one of eight soils, but is injurious in all cases at a concentration of 200 parts per million. Cumarin was injurious at 100 parts per million in six out of nine experiments; at 200 parts per million in five out of seven, and at 300 parts per million in one of two. He also found that the vanillin and cumarin rapidly disappeared during the course of the experiment.

Funchess (7) found that pyridine and quinoline in Norfolk sandy loam or Cecil clay in pots had little harmful effects or proved decidedly beneficial to the growth of corn or oats. Vanillin and cumarin had

[3] Only the literature dealing with the effect on plant growth of vanillin, cumarin, pyridine and quinoline when added to the soil is cited here. In water culture, according to Schreiner, Reed and Skinner (22), vanillin is harmful to wheat at a concentration of 1 part per million; cumarin, at 1 part per million; pyridine, at 50 parts per million and quinoline at 5 parts per million.

[4] Expressed in parts per million of air-dry soil.

little or no toxic effect. In all cases the beneficial effect was intensified, or the toxic effect entirely eliminated when the pots were allowed to stand for a period of about six months after the application of the compounds.

Upson and Powell (30) report that vanillin in the soil, even at a concentration of 1000 parts per million, shows very little harmful effect on the growth of wheat. Cumarin acted quite differently in the soil from what it did in water culture.

EXPERIMENTAL

Soils Used

The soils used were a Norfolk sandy loam from what is known as the "Culler's rotation plot" and a sandy loam from plots on the Experiment Station Farm which have received annual applications of ammonium sulfate. The former soil was practically neutral in reaction, and is of fair fertility. The latter was decidedly acid. Clover grows poorly upon it. The first soil is similar to one used by Funchess in which the results noted above were obtained.

Chemicals Used

The vanillin, cumarin, pyridine and quinoline were Merck's C. P. chemicals.

The Effect of Vanillin, Cumarin, Pyridine and Quinoline on the Microorganisms of the Soil

Nine kilograms of air dry soil (Norfolk sandy loam) were placed in two gallon pots. The pots were thoroughly watered with tap water and allowed to stand for thirty days. At the end of that time the soil was removed from the pots and spread on sterile paper. The compounds were added, thoroughly mixed with the soil by means of a sterile spatula and the soil repotted. The vanillin and cumarin were added at the rate of 9 gms. per pot; the pyridine and quinoline at the rate of 14 cc. per pot. Each treatment was made in duplicate. The soil in two pots was removed, mixed and repotted without treatment. These two pots served as checks. An attempt was made to keep the water content uniform throughout the series by weighing about once a week and making up the loss with distilled water. The number of microorganisms devel-

oping in the pots was determined by the methods described by Brown (2), using his albumen agar. Each soil was plated in duplicate, using dilutions of 1 to 20,000 and 1 to 200,000, as described by Brown.

The Number of Microorganisms. The number of microorganisms developing in the pots are given in Table I.

TABLE I—*Microorganisms in Millions per 0.25 gm. of Air Dry Soil. Soil Potted June 30th. Compounds Added to Pots July 29th.*

Treatment	July 13	July 26	Aug. 3 5 days after treatment	Aug. 15 17 days after treatment	Aug. 30 32 days after treatment	Sept. 22 55 days after treatment	Oct. 22 85 days after treatment
None	1.25	1.29	1.64	1.44	1.37	1.15	.89
Vanillin	1.35	1.39	.42	10.63	11.84	2.74	1.05
Cumarin	1.18	1.72	3.08	44.55	5.42	2.91	1.52
Pyridine	1.27	1.25	1.97	31.13	5.73	2.22	1.18
Quinoline	1.11	1.33	.60	1.12	3.05	6.34	10.21

From Table I it can be noted that in all cases the compounds have caused a marked increase in the numbers of microorganisms. In the case of vanillin and quinoline this occurs after an initial depression. In the case of pyridine and cumarin no such depression is evident. It should be remembered, however, that if a determination had been made sooner after the addition of the compound to the pots, a depression in numbers might also have been found in the case of the latter two compounds.

The maximum number of organisms developing in four days at room temperature on the medium used is 178.20 millions per gm. of air dry soil in the cumarin treated pots 17 days after treatment. This is almost 40 times as many organisms as were present in the untreated pots. The maximum number of organisms observed was 218.24 millions in one of the cumarin treated pots at the end of 17 days. The actual number in any of the treatments may have been larger than is indicated in the table as the greatest increase may have occurred in the case of some of the treatments at a time which fell between determinations.

The maximum number of organisms occurs at different times depending on the treatment. In the cumarin and pyridine treated soils the organisms reach their

maximum numbers first, followed by those in the vanillin and quinoline treated soils. Quinoline depresses the number of microorganisms for the longest period, a period of 17 days. Its odor also persisted longest, being still evident 55 days after the treatment.

The Flora of the Plates. No complete data on the flora of the plates was recorded. It was noted, however, that the increase in the numbers of microorganisms was due chiefly to the development of bacteria and not to an increase in the numbers of Actinomyces. As far as the medium would allow differentiation, the number of Actinomyces colonies previous to treating the soil was 30-40 per plate at a dilution of 1 to 20,000 or about 20-30 percent of the total number of microorganisms. The number of Actinomyces colonies per plate at the 1-20,000 dilution after the soil had been treated with the compounds is given in Table II. Each figure is the average of a count of two plates from each of two duplicate pots.

TABLE II. *Number of Actinomyces Colonies Appearing on the 1 to 20,000 Dilution Plates.*

Treatment	August 3rd—5 days after treatment		August 15th—17 days after treatment		August 30th—32 days after treatment	
	No. of Actinomyces colonies	Percentage of total organisms	No. of Actinomyces colonies	Percentage of total organisms	No. of Actinomyces colonies	Percentage of total organisms
None	46	30	72	36	38	29
Vanillin	4	11	0	0	0	0
Cumarin	21	7	17	1.3	87	17
Pyridine	66	35	179	23	207	41
Quinoline	11	21	17	10	6	0.8

From the data given in Table II it is evident that the number of Actinomyces, both actual and relative to the total number of organisms, decreased markedly in the pots treated with vanillin, cumarin and quinoline. In the pyridine treated pots the actual number of Actinomyces increased. The bacteria, however, in the pyridine treated pots increased in proportion, as is indicated by the fact that the percent of Actinomyces colonies did not increase decidedly.

By comparing the figures in Table I and Table II we find a more or less close correlation between the lowest percentage of Actinomyces and the greatest number of microorganisms. In fact, at the time of

maximum increase in numbers of microorganisms in the cumarin, vanillin, and quinoline treated soils the Actinomyces have practically disappeared, reappearing later with the decrease in numbers as is shown for cumarin in Table II, and as was observed for quinoline and vanillin at a date later than August 30. The significance and cause of this is not clear.

Discussion. The increase in numbers of microorganisms observed in the treated pots appears to be much similar to that found in "partial sterilization" with steam, carbon disulfide, toluol, etc., by Hiltner, Russel and Hutchinson and others. They have found in general that treatment of the soil by sterilizing agents, in quantity or degree insufficient to cause complete sterility, frequently produces an enormous temporary increase in the microscopic flora of the soil. The increase is often preceded by a depression. Buddin (1) has studied the effects of pyridine. He found a marked effect, obtaining a maximum number of 3,500 millions of organisms in the pyridine treated pots when the check gave but 13 millions.

The explanations offered for this phenomenon have been various. Russell and Hutchinson (18) have stated that the increase in the number of bacteria is due to the fact that the antiseptic destroys the bacteria consuming protozoa. Other explanations offered by various investigators are summarized by Lipman (12) as "(1) increase in availiable food for bacteria; (2) rendering soil toxins insoluble; (3) destroying bacteriotoxins; (4) acceleration of biological processes." Buddin (1) suggests for pyridine that pyridine affords a magnificient diet for bacteria, and provides the simple case of two or three species feeding directly on the substance itself or its decomposition products. Davidson (3) observed a growth of molds and fungi in a solution containing 200 parts per million of cumarin which had stood for some time and suggests that microorganisms may destroy cumarin and vanillin in the soil. (Funchess (8, 17) has found that pyridine and quinoline are apparently nitrified in the soil. These facts and suggestions, as well as others not mentioned, appeared to indicate that Buddin's explanation might also hold for vanillin, cumarin and quinoline. In order to determine whether microorganisms acted on these compounds in the soil, the following method was used:

Comparative Effect on Plant Growth of Toxins in Sterile and Inoculated Soil.

In each of 30 two-liter bottles 500 grams of air dry soil were placed. This soil came from the ammonium sulfate manured plots, referred to above. Ninety cc. of tap water were added to each of the bottles. To six of the bottles 0.5 gm. of vanillin was added and to six others 0.5 gm. of cumarin. The 30 bottles were plugged with cotton and sterilized in an autoclave for 3 hours at 15 lbs. pressure. After sterilization 0.5 cc. of pyridine was added to 6 of the bottles and 0.5 cc. of quinoline to 6 others. These compounds were added by means of sterile pipettes. The remaining six bottles received no treatment. Half, 3, of each set was inoculated by adding about 2 cc. of a suspension of normal soil in sterile water and all were incubated in a dark cupboard at room temperature for 57 days. At the end of that time ten wheat grains, sterilized by Wilson's method (31) were dropped into each bottle. Sixteen days after planting the wheat the sterile bottles were tested for sterility by plating some of the soil in Brown's albumen agar. All were sterile save one of the quinoline series which contained fungi. At the same time the wheat plants were removed from the bottles and the tops and roots measured. Due to the difficulty in removing the plants from the narrow necked bottles considerable of the roots was lost in all cases in which they had penetrated the soil. The results are given in Table III. The method used in growing the plants and the results with the set treated with pyridine are shown in Plate I, figure 1.

Table III. *Growth of Wheat in Sterile and Inoculated Soil Which Had Been Incubated for 57 Days.*

Treatment	Number of plants from 30 seeds		Average length of tops in centimeters		Average length of roots in centimeters	
	Sterile	Inoculated	Sterile	Inoculated	Sterile	Inoculated
Cumarin	0	22	0	12.3	0	10.5
Pyridine	18	24	5.3	24.1	3.7	8.1
Vanillin	24	25	2.6	17.3	1.2	10.4
Quinoline	0	5	0	20.2	0	7.0
None	25	20	6.9	19.2	3.3	15.4

From the data it is evident that inoculating the untreated steamed soil with an infusion from normal soil

produces a decided increase in the growth of wheat. Explanations for this effect based on increased plant food produced by bacterial action might be offered. The appearance of the roots of the plants, however, make it evident that the steam heating of the soil had produced toxic material and that the inoculation had caused its disappearance, or nullified its effect.

Inoculation has also markedly improved the growth of the plants in pyridine, quinoline, vanillin, and cumarin treated soils. The production of toxic conditions in the steamed soil makes it more difficult to determine whether the improved growth in these cases is caused by the action of the microorganisms on the toxic material produced by steaming or by their action on the compounds. The fact, however, that the toxic effect of the compounds is evident on the germination (cumarin and pyridine) and on the growth (cumarin, pyridine and vanillin) in the sterile soil while in the inoculated soils this effect has disappeared almost completely in the case of pyridine and vanillin, and very largely in the case of cumarin would lead us to believe that microorganisms had acted on the cumarin, vanillin and pyridine. This is also substantiated by the fact that no odor of pyridine, cumarin nor vanillin remained in the soil removed from those bottles which were inoculated while it was still present strongly in the sterile bottles. The case of quinoline may be considered doubtful. The odor of quinoline still clung to the soil in both sterile and inoculated bottles.

That microorganisms have neutralized the toxicity of the vanillin, cumarin and pyridine and also acted on the quinoline is shown more clearly by the following:

The soil removed from the bottles was dried for four days. It was then placed in tumblers and brought to a uniform water content. The soil from each bottle filled two tumblers. Ten wheat seeds were planted in each tumbler and allowed to grow for 11 days. Of course, the soil from all the bottles, both sterile and inoculated, was inoculated by the handling. The results are given in table IV; in Plate I, figures 2 and 3, and in Plate II, figures 4, 5 and 6.

TABLE IV. *Growth of Wheat in Soil From Sterile Bottles and Inoculated Bottles.*

Treatment	Number of plants from 60 seeds		Average length of tops in centimeter		Green weight of tops per 10 plants in grams		Dry weight of tops per 10 plants in grams	
	Soil from sterile bottles	Soil from inoculated bottles	Soil from sterile bottles	Soil from inoculated bottles	Soil from sterile bottles	Soil from inoculated bottles	Soil from sterile bottles	Soil from inoculated bottles
Cumarin	10	54	4.0	17.0	0.250	1.049	0.035	0.126
Pyridine	43	34	15.8	15.9	1.005	1.120	0.115	0.115
Vanillin	46	47	10.0	17.2	0.525	1.087	0.077	0.131
Quinoline	39	34	7.4	16.4	0.42	1.002	0.045	0.109
None	40	46	14.5	14.6	0.806	0.950	0.099	0.113

From the growth of the wheat plants in the untreated soil from the sterile and inoculated bottles it is evident that the difference between the two has largely disappeared. This is perhaps due to bacterial action in the soil which came from the sterile bottle. Less marked differences than are given in Table III are also noted between the soil from the sterile and inoculated bottles in the case of all the compounds. This is particularly true of pyridine. Comparing the growth of the wheat in the treated and untreated soils it is evident, however, that the toxic effect of cumarin, vanillin and quinoline is still present in the soil from the sterile bottles. In the soil from the inoculated bottles the growth in the vanillin and cumarin treated soils is as great if not greater than that in the untreated. The toxic effect of the quinoline has also largely disappeared. It would seem clear then that the microorganisms have in some way neutralized the toxicity of vanillin, cumarin, pyridine and quinoline.

Discussion. The development of material toxic to higher plants in soil which has been steam heated has been observed by others. Pickering (13, 14, 15, 16) observed that the germination and the growth of plants is retarded in heated soils. He believes that the toxic substances formed are organic in nature. Pickering also found that the toxic qualities of heated soils are reduced on storing them under moist aerated conditions. This he believes is not due to bacterial action but to chemical changes in which the action of water is particularly concerned. Russel and Petherbridge (19) state that there is no evidence that the active substances in steam heated soils are necessarily

organic, but suggest that ammonia and other inorganic substances may be responsible for the toxicity. They also state that they could obtain no definite proof that the harmful effect on germinating seeds passes off after a time. Seaver and Clark (20, 21) emphasize the fact that the decomposition products found in heated soil may be toxic to higher plants but favorable for the development of Pyronema or other moulds. Schreiner and Lathrop (27) found that in steam heated soils there was an increase in water soluble constituents and in acidity. They seem inclined to believe that the toxicity of the heated soils to plant growth is due to the development of organic harmful material. Johnson (11) states that ammonium compounds develop in heated soils and are responsible for the harmfulness of the soil to green plants. The writer's results do not indicate whether the toxic material formed in the soil used in the experiment noted above is organic or inorganic. They do show, however, that the toxicity has been largely destroyed by the action of microorganisms.

The results also show that under the conditions of the experiment and in the soil used microorganisms have largely neutralized or destroyed the toxicity of vanillin, cumarin, pyridine and quinoline. The probable method by which this neutralization is accomplished is indicated in the following section.

Bacteria Feeding on Vanillin, Cumarin, Pyridine and Quinoline.

As there seemed no doubt but that microorganisms were instrumental in destroying the toxicity of the four compounds used attempts were made to isolate the organisms responsible.

Vanillin. A nutrient solution was prepared containing vanillin as the only source of carbon.[5]

To 50 cc. of this solution, after sterilization, were added a few grains of soil from a vanillin treated pot used in the experiments reported above. In a few days the cloudy appearance of the medium showed a heavy

[5] This solution contained:

$NaNO_3$	0.1	gm.
K_2HPO_2	0.1	gm.
$MgSO_4$	0.05	gm.
NH_4Cl	0.1	gm.
KCl	0.05	gm.
Vanillin	0.1	gm.
H_2O distilled	200	cc.

multiplication of bacteria. In ten days the odor of vanillin had entirely disappeared though it was still present in the check flasks. At the end of two weeks an ether extract, evaporated to dryness, showed vanillin to have disappeared from the inoculated flasks, but to be present in the checks.

After the solution had become cloudy, as is described above, it was plated out and five isolations of bacteria were made. These all seemed to be the same organism. Using the medium given above it was found that this organism could rapidly cause the disappearance of the vanillin. Since the vanillin was the only source of carbon in the solution it is evident that the organism used it as a food.

Cumarin. The same procedure was followed in the search for organisms responsible for the disappearance of cumarin. The solution used was the same as that given for vanillin and contained cumarin at a concentration of about 500 parts per million substituted for vanillin. Again the cloudy appearance of the medium showed a rapid multiplication of organisms and the cumarin disappeared. The solution was plated out and six isolations were made, three of which used cumarin as a source of carbon. These three appeared to be identical organisms as far as could be judged from their growth on agar. It is understood, however, that this simple criterion is not sufficient to prove their identity. An ether extract made four days after inoculating 50 cc. of the nutrient solution containing approximately 500 parts per million of cumarin with one of these organisms, showed that the cumarin had disappeared. The temperature of incubation was about 25 to 30 degrees C.

Pyridine. For the isolation of organisms acting on pyridine a nutrient solution was prepared containing pyridine as the only source of nitrogen[6]. This solution was sterilized and, by means of a sterile pipette, sufficient pyridine was added to make a concentration of 1000 parts per million. The solution was inoculated with a small amount of soil from one of the pyridine treated bottles. The contents of the flask became

[6] This solution contained:

K_2HPO_4	0.1	gm.
$MgSO_4$	0.05	gm.
$FeSO_4$	0.001	gm.
KCL	0.05	gm.
C. P. dextrose	1.0	gm.
Dist. H_2O	100	cc.

cloudy and the odor of pyridine disappeared. The solution was plated out and three isolations were made, one of which proved to be an organism which destroyed pyridine. With this bacterium present the odor of pyridine disappeared in five days and was replaced by a characteristic sour smell. As the pyridine was the only source of nitrogen in this solution it is evident that pyridine is a very favorable source of nitrogen for this organism. No attempt was made to determine whether the pyridine might not also serve as an energy source for this organism.

Quinoline. Attempts were also made by the same methods to secure an organism acting on quinoline, but thus far none such has been found.

These three organisms, one acting on cumarin, one on vanillin and one on pyridine are different species. They also seem to be specific inasmuch as, with the solutions and concentrations used, the organism acting on vanillin will not act on cumarin or pyridine and vice versa.

It has also been demonstrated by water culture and soil experiments that the bacterium feeding on vanillin will in pure culture entirely destroy the toxicity of vanillin for wheat plants. It has also been found that the bacterium feeding on cumarin will in pure culture destroy the toxicity of that compound for wheat plants. Further work on the physiology of these organisms is being undertaken.

Discussion. The enormous increase of bacteria in the vanillin, cumarin or pyridine treated soils, the disappearance of the toxic effects of the compounds in inoculated soil, but not in sterile soil, and the isolation of specific microorganisms using them as food would seem to indicate that their disappearance in the soils used in this investigation is due to the fact that they serve as very favorable food sources to definite species of bacteria. While no specific organism using quinoline was isolated, the effect of quinoline on the microorganisms in the pots and the results secured with the sterile and inoculated soils would seem to indicate that quinoline, in the soils used, suffers the same fate.

The relation of this data to the phenomena found in "partial sterilization" may be pointed out. The initial decrease in numbers noted in the vanillin and quinoline treated soils is probably due to the toxic action of the compound on some species of the bacteria present.

The later increase in numbers would seem to be due to the fact that specific organisms find the compound a very favorable food source. With the exhaustion of the compound and perhaps its decomposition products the organisms which fed upon them decrease in numbers. In view of these results it would seem advisable to re-investigate the effect of steam, carbon bisulfide and other agents which have been found to produce large increases in the number of microorganisms in the soil, bearing in mind the possibility that the increase may be due to the fact that the compounds may serve as food sources to bacteria or the treatment of the soil may make food supplies available. (Compare with Greig Smith's (9, 10) suggestions).

These results would also seem to be of considerable significance to those who are considering the soil toxin theory of soil fertility. They show that the disappearance in the soil of organic material toxic to higher plants may in some cases be accomplished by microorganisms and apparently by specific microorganisms. This view assigns the important role of destruction in the soil of such compounds as vanillin and cumarin to bacteria and not to the oxidizing action of the plant roots as might be inferred to be the case from the work of Schreiner, Reed and Skinner (23, 24, 25, 26, 29.) The persistence of the compounds on the other hand, such as occurred with vanillin in some of the soils used by Skinner (28), would appear to be due to the absence of suitable organisms or to the presence of conditions which inhibit their acting on the compounds in question.

Since the disappearance of these four compounds is due to biological factors it can be inferred that the addition of a given organic compound may produce no harmful effects in one soil and decidedly harmful effects in another depending on the presence and action of suitable organisms. This is probably the explanation for the varying and in some cases apparently conflicting results obtained with the same compound by different workers as summarized in the early part of this paper. Not only may this be inferred but it can be conceived that the same soil under some conditions of temperature, oxygen, soluble salt and water supply, etc., may allow the persistence of a harmful compound and under other conditions may eliminate it rapidly. What those conditions are can probably be discovered by a study of the physiology of the organisms involved.

SUMMARY

1. Vanillin, cumarin, pyridine and quinoline when added separately to the soil used in these experiments at a concentration of approximately 1000 parts per million of air dry soil produce a great temporary increase in the number of bacteria which will develop on Brown's albumen agar.

2. In the case of vanillin and quinoline it is shown that this increase in numbers is preceded by a decrease.

3. The number of Actinomyces colonies in the soils treated with cumarin, vanillin and quinoline decreases, reaching a minimum roughly corresponding with the maximum in bacterial numbers.

4. Steam sterilizing of the soil used in these experiments produces material toxic to the growth of wheat plants. Soil microorganisms destroy the toxicity of the steamed soil under the conditions of the experiment reported.

5. The effect on the growth of wheat of vanillin, cumarin, pyridine and quinoline in sterile soil and in soil which had been sterilized, reinoculated and incubated was compared. In the inoculated soil the toxicity of the four compounds largely disappears. It persists in the sterile soil.

6. Specific bacteria were isolated from the soils used which utilize cumarin, vanillin and pyridine as food sources.

7. The bacterium feeding on vanillin will in pure culture destroy the toxicity of vanillin to wheat.

8. The bacterium feeding on cumarin will in pure culture destroy the toxicity of cumarin to wheat.

9. The increase in the numbers of bacteria in the soils treated with the four compounds mentioned and the disappearance of the toxicity of these substances in inoculated soil is therefore believed to be due to the fact that they serve as favorable food sources to definite species of bacteria.

LITERATURE CITED

1. Buddin, W.
1914. Partial sterilization of soil by volatile and non-volatile antiseptics. In Jour. Agr. Sci. V. 6, pt. 4, p. 416-451, 4 figs.

2. Brown, P. E.
1913. Methods for bacteriological investigations of soils. Iowa Agr. Exp. Sta. Research Bul. 11, p. 381-407.

3. Davidson, J.
1915. A comparative study of the effect of cumarin

and vanillin on wheat grown in soil, sand and water cultures. In Jour. Am. Soc. Agr. V. 7, No. 4, p. 145-158, No. 5, p. 221-238.

4. De Candolle, A. P.
1832, Physiologie vegetale 3 vols., 1579 p. Paris.

5. Duggar, B. M.
1911. Plant Physiology 516 p. Illus. New York.

6. Fraps, G. S.
1915. The effect of organic compounds in pot experiments. Tex. Agr. Exp. Sta., Bul. 174, p. 1-13.

7. Funchess, M. J.
1916. The effects of certain organic compounds on plant growth. Ala. Agr. Exp. Sta., Bul. 191, p. 103-132, 8 pls.

8. Funchess, M. J.
1917. The nitrification of pyridine, quinoline, guanidine carbonate, etc., in soils. Ala. Agr. Exp. Sta., Bul. 196, p. 65-84.

9. Greig Smith.
1911. The bacterio toxins and the agricere soils. In Centrbl. Bakt, (etc.), Abt. 2, Bd. 30, No. 7-12, p. 154-156.

10. Greig-Smith.
1912. The agricere and the bacterio-toxins of the soil. In Centrbl. Bakt. (etc.), Abt. 2, Bd. 34, No. 8-9, p. 224-226.

11. Johnson, J.
1916. Preliminary studies on heated soils. In Science, N. S. V. 43, No. 1108, p. 434-435.

12. Lipman, J. G.
1916. Microorganisms as a factor in soil fertility. In Marshall, C. E. Microbiology 2nd Ed. Div. 3, ch. 1, p. 289-314.

13. Pikering, S. U.
1908. Studies on germination and plant growth. In Jour. Agr. Sci. V. 2, pt. 4, p. 411-434, fig. 1.

14. ———
1908. The action of heat and antiseptics on soils. In Jour. Agr. Sci. V. 3, pt. 1, p. 32-34.

15. ———
1910. Studies in the changes occurring in heated soils. In Jour. Agr. Sci. V. 3, pt. 3, p. 258-276.

16. ———
1910. Plant growth in heated soils. In Jour. Agr. Sci. V. 3, pt. 3, p. 277-284, pl. 16-18.

17. Robbins, W. J.
1916. The cause of the disappearance of cumarin, vanillin, pyridine and quinoline in the soil. Preliminary note.In Science N. S., V. 44, p. 894-895.

18. Russel, E. J. and Hutchinson, H. B.
1909. The effect of partial sterilization of soil on the production of plant food. In Jour. Agr. Sci. V. 3, pt. 2, p. 111-144, 4 figs. pl. 8-9.

19. Russel, E. J., and Petherbridge, F. R.
1913. On the growth of plants in partially sterilized soils. In Jour. Agr. Sci. V. 5, pt. 3, p. 248-287, 1 fig. pls. 8-11.

20. Seaver, F. J., and Clark, E. D.
1910. Studies in pyrophilous fungi. II Changes brought about by the heating of soils and their relation to the growth of Pyronema and other fungi. In

Mycologia V. 2, No. 3, p. 109-124, pl. 24-26.

21. ———
1912. Biochemical studies on soils subjected to dry heat. In Biochem. Bul. V. 1, No. 3, p. 413-427, pl. 7.

22. Schreiner, O., and Reed, H. S., assisted by Skinner, J. J.
1907. Certain organic constituents of soils in relation to soil fertility. U. S. Dept. Agr. Bu. Soils, Bul. 47, p. 1-52, 5 pls.

23. Schreiner, O., and Reed, H. S.
1908. The power of sodium nitrate and calcium carbonate to decrease toxicity in conjunction with plants growing in culture solutions. In Jour. Am. Chem. Soc. V. 30, No. 1, p. 85-97, fig. 2.

24. ———
1909. The role of oxidation in soil fertility. U. S. Dept. Agr., Bu. Soils, Bul. 56, p. 1-52.

25. Schreiner, O., and Skinner, J. J.
1910. Some effects of a harmful organic soil constituent. U. S. Dept. Agr., Bu. Soils, Bul. 70, p. 1-98, fig. 31.

26. ———
1911. Organic compounds and fertilizer action. U. S. Dept. Agr., Bu. Soils, Bul. 70, p. 1-31, plates 2 and fig. 5.

27. Schreiner, O., and Lathrop, E.
1912. The chemistry of steam heated soils. U. S. Dept. Agr. Bu. Soils, Bul. 89, p. 1-37.

28. Skinner, J. J.
1915. Field test with a toxic soil constituent: vanillin. U. S. Dept. Agr., Bul. 164, p. 1-9. 4 pls.

29. Skinner, J. J.
1916. The effect of vanillin and salycilic aldehyde in culture solution and the action of chemicals in altering their influence. In Plant World, V. 19, No. 12, p. 371-378, fig. 3.

30. Upson, F. W., and Powell, A. R.
1915. The effect of certain organic compounds on wheat plants in the soil. Preliminary paper. In Jour. Ind. and Eng. Chem. V. 7, No. 5, p. 420-422. pl. 1.

31. Wilson, J. K.
1915. Calcium hypochlorite as a seed sterilizer. In Am. Jour. Bot. V. 2, No. 8. Bibliography, p. 426-427.

PLATE 1

Fig. 1. Wheat in soil treated with pyridine. Three bottles on left are sterile; three bottles on right are inoculated.
Fig. 2. Wheat in soil untreated. Three tumblers on left contain soil from inoculated bottles; three tumblers on right contain soil from sterile bottles.
Fig. 3. Wheat in soil treated with pyridine. Three tumblers on left contain soil from inoculated bottles; three tumblers on right contain soil from sterile bottles. Compare with Fig. 1.

PLATE 2

Fig. 4. Wheat in soil treated with vanillin. Three tumblers on left contain soil from inoculated bottles; three tumblers on right contain soil from sterile bottles.
Fig. 5. Wheat in soil treated with cumarin. Three tumblers on left contain soil from inoculated bottles; three tumblers on right contain soil from sterile bottles.
Fig. 6. Wheat in soil treated with quinoline. Three tumblers on left contain soil from inoculated bottles; three tumblers on right contain soil from sterile bottles.

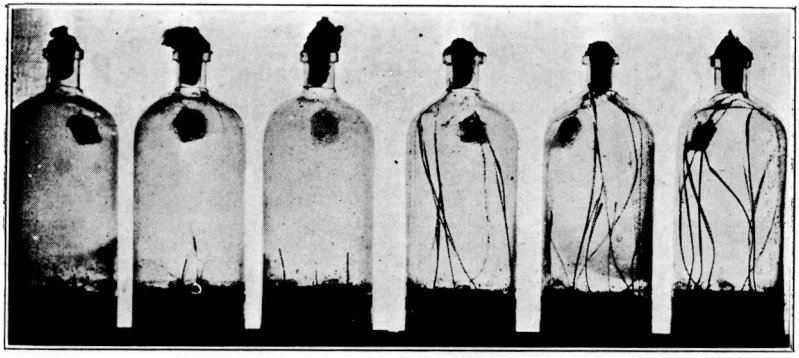

Fig. 1.

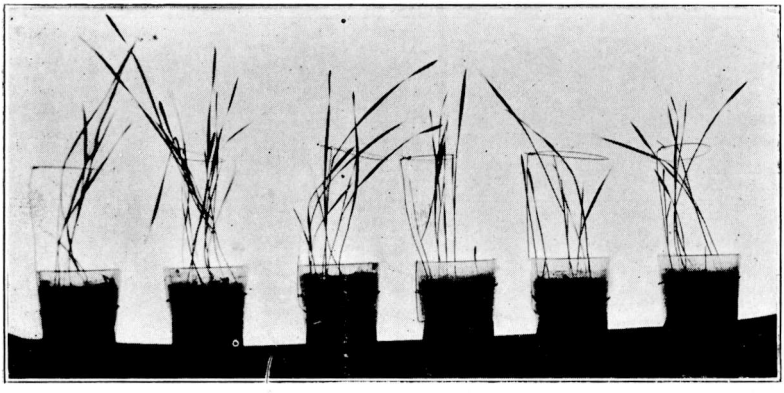

Fig. 2.

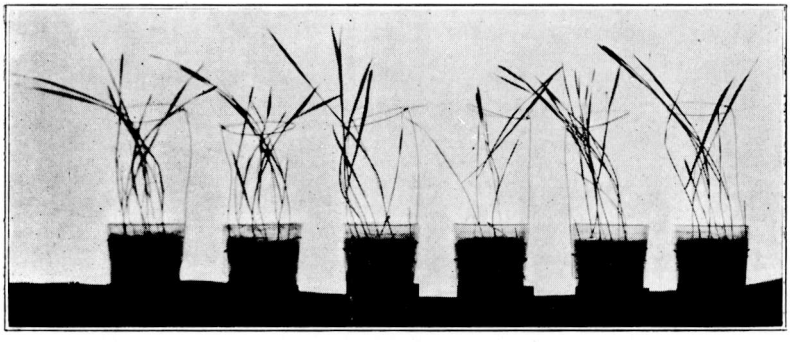

Fig. 3.

PLATE II.

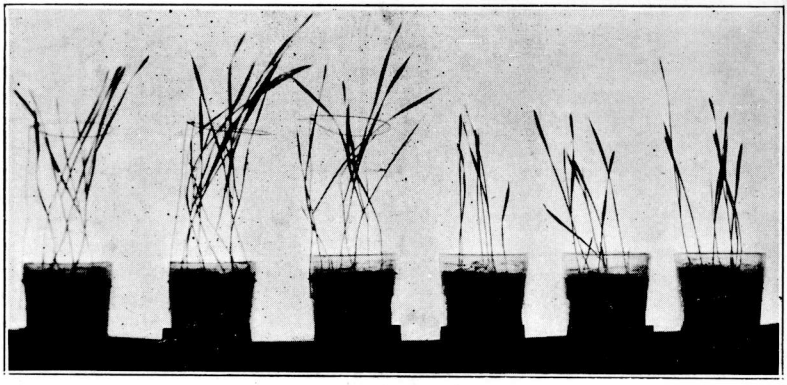

Fig. 4.

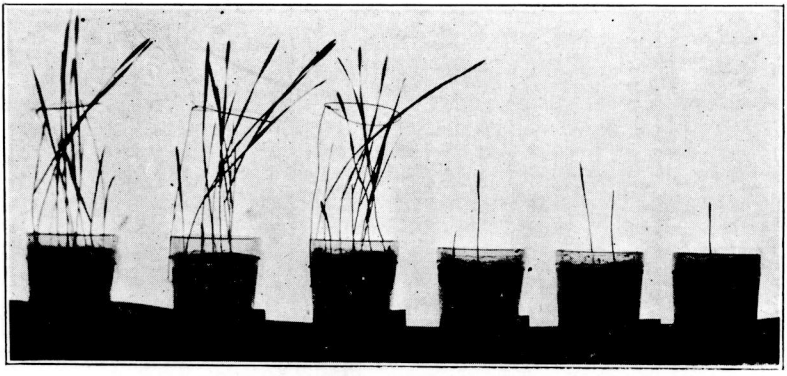

Fig. 5.

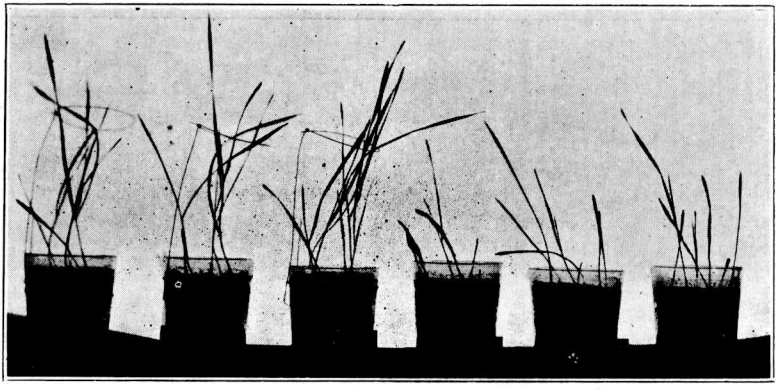

Fig. 6.