

### Conclusions.

(1) The yellow color of flours is due to a very minute quantity of a colored substance which is contained in the fat. When the fat is removed, high-grade flours become white. Exposure to the sunlight or treatment with nitrogen peroxide changes the colored compound into one or more colorless compounds. Both the fat and solutions of the fat from thoroughly bleached flours are practically colorless. Overtreated (so-called "overbleached") flours have a yellow to brownish-yellow color, and the fat, as well as the solutions of the fat, from overtreated flours are also colored.

(2) Bleaching with nitrogen peroxide does not increase the acidity of flours, while overtreating them with the same agent does.

(3) Neither the absorption of a flour nor the expansion of its gluten is affected by bleaching.

(4) Bread made from bleached flours does not differ in weight, lightness, texture, odor or taste from that made from unbleached flours; it is, however, in all cases whiter, where high-grade flours are used. Low-grade flours, when bleached, produce bread with an uninviting color.

(5) Bleached flours sometimes yield bread containing nitrites and at other times bread free of nitrites. In all cases the amount of nitrites in the bread is much smaller than that in the flour.

(6) The quantity of peroxide may be so increased as to seriously injure the quality of the flour, but such a quantity at the same time unfavorably affects the color.

(7) Low-grade flours when bleached do not resemble patent flours in appearance.

(8) Many of the conflicting opinions in regard to the effect nitrogen peroxide has on wheat flour are to be attributed to the investigation of flours that had been "overtreated."

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## THE POWER OF SODIUM NITRATE AND CALCIUM CARBONATE TO DECREASE TOXICITY IN CONJUNCTION WITH PLANTS GROWING IN SOLUTION CULTURES.<sup>1</sup>

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Investigations upon the nature and action of toxic agents upon organisms have shown that there is not always a simple relation between them. Although the harmful effect of the toxic agent upon the organism is the main factor in the problem, it is no less true that the organism exerts an

<sup>1</sup> Published by permission of the Secretary of Agriculture.

influence upon the toxic agent which may modify its action to a greater or less extent. The present paper reports the results of a study of the action of living plants upon solutions of toxic organic compounds with and without the addition of sodium nitrate, calcium carbonate and other substances.

It has been known for some time that the addition of a second solute to a toxic solution often decreases the toxicity of the solution. Krönig and Paul<sup>1</sup> found that the addition of hydrochloric acid and of halogen salts to solutions of mercuric bichloride decreased their toxicity. Kearney and Cameron<sup>2</sup> showed that the toxicity of sodium carbonate and of magnesium salts is greatly lowered or overcome by the addition of calcium salts to the solution. True and Gies<sup>3</sup> have demonstrated the same thing for mixtures of the salts of heavy metals with salts of the light metals. Pigorini<sup>4</sup> has shown that the toxicity of silver nitrate may be remarkably lowered by the addition of the poisonous sodium thiosulphate, although in this case there would be an actual chemical interaction between the toxic agents.

The question arises whether the presence and activity of the organism does not play a part in ameliorating the toxic conditions. Reed has pointed out in a recent article<sup>5</sup> that such an action appears to play some part in the observed antagonism between calcium and magnesium. In the present study particular attention was paid to the effect of the plant upon the toxicity of organic compounds with and without certain inorganic salts.

Wheat seedlings of uniform age and size were employed in all the experiments described in this paper. The seeds were germinated on perforated cork plates, which floated on the surface of a pan of water.<sup>6</sup> The water cultures were made by placing the solution to be used into salt mouth glass bottles having a capacity of 250 cc. Ten seedlings were inserted in the same number of notches cut in the edge of each cork in the manner described by Whitney and Cameron<sup>7</sup> and by Livingston.<sup>8</sup>

The water used in making solutions was taken from the laboratory still and shaken with washed carbon black. At the end of thirty minutes it was filtered and was then ready for use. This method of treating ordinary distilled water with an insoluble absorbent agent as described

<sup>1</sup> Z. Hyg., 25, 1 (1897).

<sup>2</sup> Report 71, U. S. Dept. Agr. (1902).

<sup>3</sup> Bull. Torr. Bot. Club, 30, 390 (1903).

<sup>4</sup> Atti. r. accad. Lin., Classe sci. fis. mat. nat. (5) 16 (1), 359 (1907).

<sup>5</sup> Ann. Bot., 21, 565 (1907).

<sup>6</sup> For details of the method by which the seedlings were grown, the reader should see Plant World, 9, 13 (1906), and Bull. 40, Bureau of Soils, U. S. Dept. Agr. (1907).

<sup>7</sup> Bull. 23, U. S. Dept. Agr. (1904).

<sup>8</sup> Plant World, 9, 13 (1906).

by Livingston<sup>1</sup> is found to give physiologically pure water, and appears to be generally applicable to physiological work.

In estimating the growth of the plants in different solutions, records were kept of the weight of the green tops and of the amount of water transpired. It was found, however, that for measuring the effects of the different compounds employed in these experiments the transpiration record was more useful than the green weight. This is undoubtedly due to the fact that in many cases the root growth was more affected than the top growth. This would seem to be a necessary consequence of the more intimate contact with the toxic agent and doubtless for this reason, the root has been a standard indicator of toxicity studies in plant physiology. It has been shown that transpiration is more nearly proportional to the growth of both roots and tops of wheat and is therefore a better indicator of the effect on the plant than the weight of the green tops. In Table IV both green weight and transpiration are given together with a photograph of the plants themselves, and a comparison of the three records will serve to illustrate this point.

The experiments which are to be described were designed to study the effect of various treatments in overcoming the action of some organic substances which the writers have shown to be toxic to plant growth.

*The Effect of Root Oxidation and of Adding Pyrogallol.*—It was shown that tyrosine lost its toxic properties as a result of oxidation incident to continued exposure to the air. The aqueous solution of tyrosine, which was perfectly colorless when first prepared, became dark brown upon standing in contact with the air for several months. The exact nature of the products of oxidation is somewhat in doubt, although it is probable that homogentisinic acid and other oxidation products of tyrosine are present. These oxidation products were favorable to plant growth. The same relation was shown by the action of the compounds neurine, choline, and betaine. Increased state of oxidation was accompanied by decreased toxicity. It is not to be presumed that increased oxidation of an organic compound always produces a decrease in toxic effects, but such a result undoubtedly follows in certain cases.

Experiments were performed in which certain organic compounds possessing known toxic properties were subjected to mild oxidation. It seems possible that beneficial changes might be brought about by oxidation. It has been shown by the work of Raciborski<sup>2</sup> that the roots of plants possess very definite powers of oxidation. The writers<sup>3</sup> have shown, furthermore, that the roots of wheat plants grown in soil extracts

<sup>1</sup> Bull. 36, Bureau of Soils, U. S. Dept. Agr. (1907).

<sup>2</sup> Bull. Acad. Sci. Cracovie. Math-nat. Cl., 1905, 338; *Ibid.*, 668.

<sup>3</sup> J. Biol. Chem., 3, Proc., 24 (1907).

and synthetic nutrient solutions are capable of quite energetic oxidation.

Experiments were accordingly planned to show whether the solutions of organic compounds would be less toxic to a second set of plants by reason of the oxidation performed by the first set. Solutions of five different compounds were prepared in concentrations of 1,000, 500, 250, 100, 50, 25 and 1 part per million. Wheat seedlings were installed in these solutions and allowed to grow twelve to fourteen days and the toxic effects noted. The first set of plants were then removed, the water lost by transpiration restored, and second sets of wheat seedlings installed.

Table I shows that for three of the five substances the lowest concentrations causing injury to plants had been altered during the growth of the plants. The concentrations causing the death of the plants were the same for both the first and second sets of plants.

TABLE I.—SHOWING THE EFFECT OF PLANTS IN ALTERING THE CONCENTRATIONS AT WHICH INJURY WAS FIRST SHOWN IN VARIOUS TOXIC SOLUTIONS.

(p.p.m. = parts per million).

Solutions.	Lowest concentrations causing injury.	
	First crop.	Second crop.
Arbutin.....	25 p.p.m.	Originally 500 p.p.m.
Cumarin.....	1 p.p.m.	" 100 p.p.m.
Cinnamic acid.....	25 p.p.m.	" 25 p.p.m.
Sodium cinnamate.....	100 p.p.m.	" 100 p.p.m.
Vanillin.....	50 p.p.m.	" 500 p.p.m.

It will be seen that the arbutin solution which originally contained 500 p.p.m. was so reduced in toxicity by the growth of the first set of plants that it was no more toxic than a fresh solution containing 25 p.p.m. of arbutin. When cumarin solutions were replanted, the solution originally containing 100 p.p.m. was no more toxic than a freshly prepared solution of 1 p.p.m. The cinnamic acid solution showed no apparent improvement when used the second time; it was thought that this might have been due to the acid properties of the compound. The experiment was accordingly repeated with sodium cinnamate, which was much less toxic to seedlings than was cinnamic acid, as was shown by True,<sup>1</sup> but the growth of the first set of wheat plants did not perceptibly alter the point of injury for the second crop. It would seem from this that the activities of the roots were not able to alter sufficiently the properties of cinnamic acid to change its toxicity to seedlings. The question whether the activities of the roots were not modified by the properties of the cinnamic acid in such a way that the ameliorating powers were lost, seems worthy of more careful study than we have been able to give it.

The toxicity of the vanillin solutions was likewise greatly reduced by the growth of one set of plants. A solution which had originally con-

<sup>1</sup> Am. J. Sci. (IV), 9, 183 (1900).

tained 500 p.p.m. of vanillin appeared to be no more toxic to the second set of plants than a freshly prepared solution containing 50 p.p.m. had been to the first set of plants.

The roots growing in the stronger solutions of vanillin oxidized some of the vanillin to a dark purple dye, which was deposited on the root, similar to the oxidation effects noticed when roots grow in solutions of  $\alpha$ -naphthylamine, benzidine, etc. The point may be raised, and with some propriety, that the first set of plants absorbed and removed some of the toxic substances from the solutions. While this may be true, it is not probable that the amounts absorbed were sufficient to account for such differences as were noted in the case of arbutin, the concentration of which was so altered that the action of a solution originally containing 500 p.p.m. was no more injurious than that of a freshly prepared solution containing 25 p.p.m. That the concentration at which injury was manifested was altered, while that at which death occurred was not, is in accord with the assumption upon which the experiment was made. The roots which grew in the stronger solutions were soon seriously injured, hence any power they may have possessed to oxidize, or otherwise to ameliorate toxic conditions had a very limited time in which to act.

Without speculating too much upon the nature of the changes produced by the action of the growing plants, it may be pointed out that they appeared to alter materially the toxicity of three of the five solutions used. Although the plants undoubtedly absorbed some of the dissolved matter from the solutions through their roots, they accomplished a still greater reduction in toxicity by their activities in the solution. It would seem that the oxidizing powers of the roots are able to change some of the organic compounds into other compounds less toxic to plants.

The beneficial action of certain organic substances has been pointed out in various experiments described by Livingston<sup>1</sup> and by the writers<sup>2</sup> which showed, for example, that the addition of 500 p.p.m. of pyrogallol to an unproductive soil increased the growth of wheat plants 101 per cent. The addition of small amounts (2 to 10 p.p.m.) of pyrogallol or  $\alpha$ -naphthylamine to the extract of an unproductive soil also has a very beneficial effect. Organic substances, like the ones mentioned, cannot be considered as plant nutrients; even if they were, the small amounts added would be insufficient to account for the results produced. Their action may be upon the plant itself, causing it to resist the toxic action of the soil extract, or else these substances, being quite active chemically, act directly upon the toxic substances in the solution.

It became of interest, therefore, to test the action of pyrogallol upon

<sup>1</sup> Bulls. 28 and 36, Bureau of Soils, U. S. Dept. Agr.

<sup>2</sup> Bull. 40, Bureau of Soils, U. S. Dept. Agr.

some substance which was known to have a decidedly toxic action upon plants. Cumarin was selected as the substance to be tried because it had a nearly neutral reaction in aqueous solution. Four cultures of wheat plants were set up in each of four solutions containing 75, 50, 25 and 5 p.p.m. of cumarin respectively, together with four cultures in distilled water. Half of the cultures received pyrogallol at the rate of 2 p.p.m. and the other cultures served as controls for comparison. The experiment ran ten days, and growth was measured by transpiration.

The plants grown in solutions of cumarin containing 75 p.p.m. were killed alike whether they contained pyrogallol or not. In 50 p.p.m. of cumarin the growth was only 36 per cent. of the control plants, but the addition of pyrogallol allowed a growth which was 82 per cent. of the controls. The solutions containing 5 p.p.m. of cumarin supported a growth which was 93 per cent. of the controls, and when pyrogallol was added the plants made a growth which exceeded the controls by 16 per cent. These results make it almost certain that pyrogallol had affected the toxic action of the cumarin.

Pyrogallol has no direct value as a nutrient substance for the higher plants, so far as known. Nevertheless, the growth of plants in the cumarin solutions was benefited in each case, and in the solution containing 5 p.p.m. of cumarin the growth of plants was distinctly better than in distilled water. This result would seem to indicate that the presence of the pyrogallol had not only been able to overcome the deleterious effect of the cumarin but also to bring about a slightly greater growth. From this it would appear that the presence of an organic compound possessing no value as a nutrient may aid the plants in overcoming toxic conditions.

*The Effect of Adding Sodium Nitrate and Calcium Carbonate.*—To study this matter further, experiments were made in which substances commonly used as fertilizers were added to solutions of organic substances which were known to have a marked toxic action upon wheat plants. Forty plants in four cultures were used for each concentration of the toxic agent employed. The fertilizer salts were added to two of the cultures and comparisons were made with the two which received no fertilizer material. Sodium nitrate and calcium carbonate were the two substances experimented with. The former is especially efficient in producing improved growth when added to toxic soil extracts, in fact it almost invariably produces increased plant growth when applied to soils or water cultures. Calcium carbonate is likewise efficient in improving growth and is found to be quite generally beneficial when applied with green manures which are known to contain a variety of organic compounds.

Solutions were prepared containing arbutin, cumarin and vanillin, making them up in the concentrations shown in Tables II and III. One

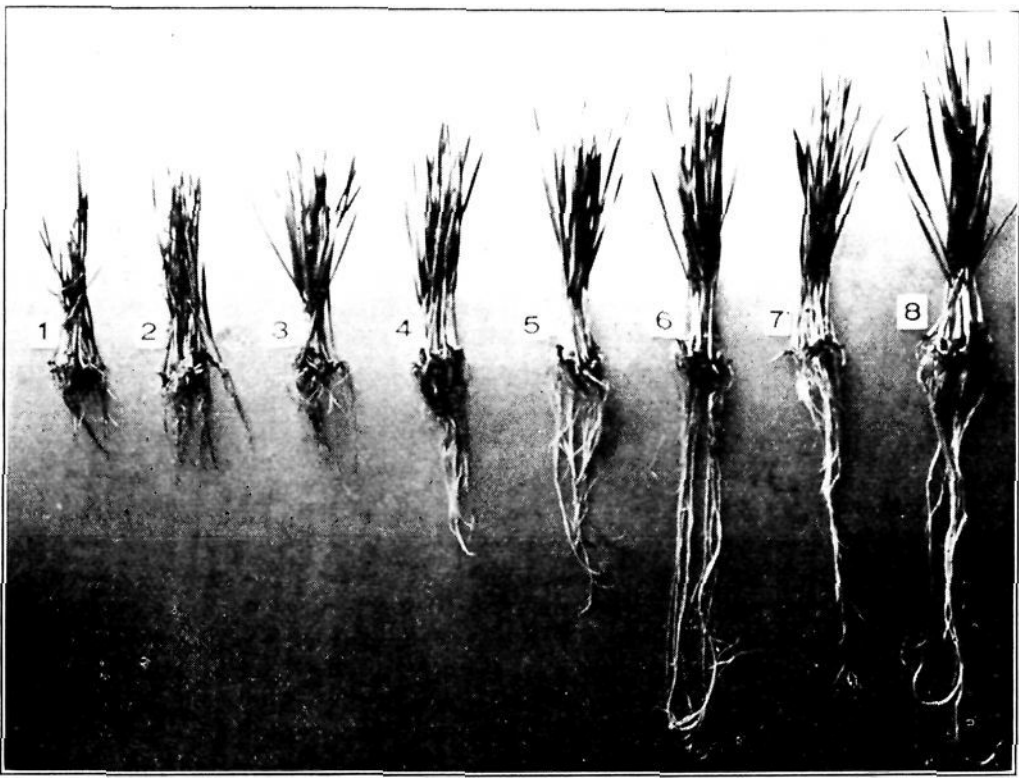


Fig. 1. Effect of calcium carbonate in overcoming the toxicity of cumarin. Plants grown in: (1) Cumarin solution 25 p.p.m.; (2) Cumarin solution plus 2000 p.p.m.  $\text{CaCO}_3$ ; (3) Cumarin solution 10 p.p.m.; (4) Cumarin solution plus 2000 p.p.m.  $\text{CaCO}_3$ ; (5) Cumarin solution 1 p.p.m.; (6) Cumarin solution plus 2000 p.p.m.  $\text{CaCO}_3$ ; (7) Distilled water; (8) Distilled water plus 2000 p.p.m.  $\text{CaCO}_3$ .

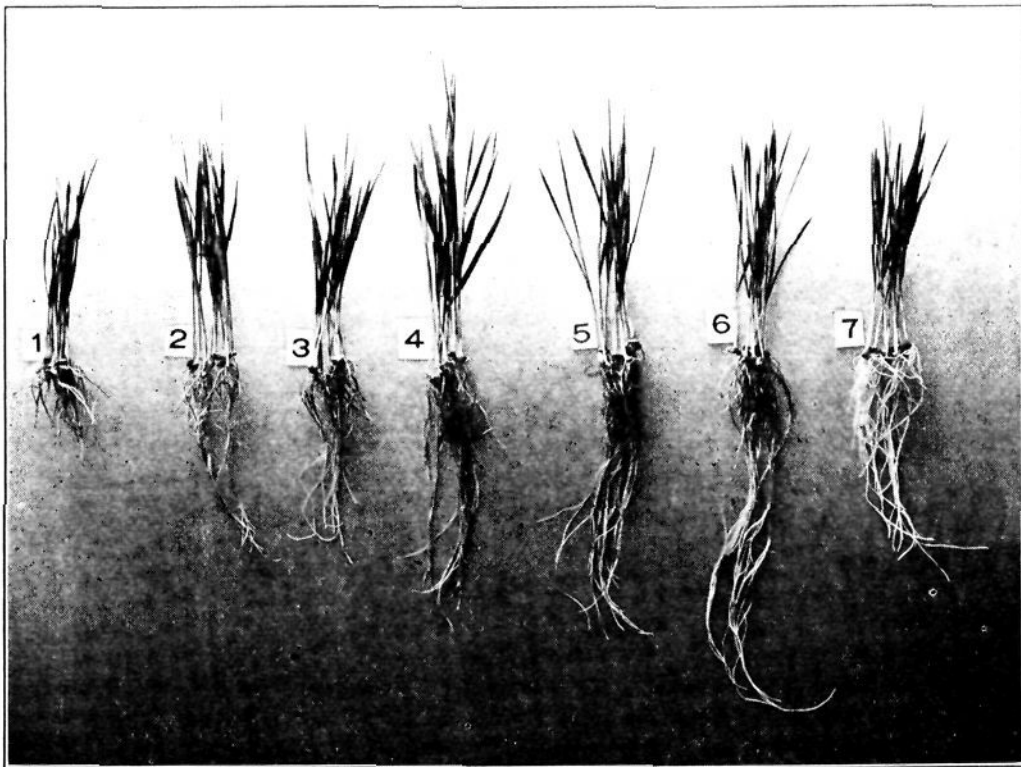


Fig. 2. Effect of sodium nitrate in overcoming the toxicity of vanillin for the first and second crop of wheat plants. (1) First crop in vanillin solution; (2) Second crop in vanillin solution; (3) First crop in vanillin solution plus 100 p.p.m.  $\text{NaNO}_3$ ; (4) Second crop in vanillin solution plus 100 p.p.m.  $\text{NaNO}_3$ ; (5) First crop in vanillin solution plus 2000 p.p.m.  $\text{CaCO}_3$ ; (6) Second crop in vanillin solution plus 2000 p.p.m.  $\text{CaCO}_3$ ; (7) Control in distilled water.





series of solutions served as controls, to another sodium nitrate was added at the rate of 100 p.p.m. of  $\text{NO}_3$ , to another series calcium carbonate was added at the rate of 2000 p.p.m., except that arbutin was omitted from this series. The arbutin cultures were allowed to grow for nine days, the cumarin cultures for eleven days, and the vanillin cultures for ten days.

Tables II and III show the comparative growth of wheat plants in various concentrations of the toxic substances and the effect of the inorganic salts upon them. The growth of control plants in pure distilled water is used as a basis for the comparison and represented as 100 in each case.

TABLE II.—EFFECT OF ADDING SODIUM NITRATE TO TOXIC SOLUTIONS. RELATIVE GROWTH MEASURED BY TRANSPIRATION.

(p.p.m. = parts per million).

No.	Solutions.	Relative growth.	
		Without nitrate.	100 p.p.m. $\text{NO}_3$ added.
1	Control in distilled water.....	100	289
2	Vanillin, 500 p.p.m.....	25	34
3	“ 100 “ .....	53	184
4	“ 25 “ .....	80	238
5	“ 10 “ .....	126	246
6	“ 1 “ .....	132	271
1	Control in distilled water.....	100	...
2	Arbutin, 500 p.p.m.....	23	27
3	“ 100 “ .....	41	78
1	Control in distilled water.....	100	...
2	Cumarin, 100 p.p.m.....	dead	dead
3	“ 50 “ .....	47	53
4	“ 10 “ .....	66	105

TABLE III.—EFFECT OF ADDING CALCIUM CARBONATE TO TOXIC SOLUTIONS. RELATIVE GROWTH MEASURED BY TRANSPIRATION.

(p.p.m. = parts per million).

No.	Solutions.	Relative growth.	
		Without carbonate.	With 2000 p.p.m. $\text{CaCO}_3$ added.
1	Controls in distilled water.....	100	209
2	Vanillin, 500 p.p.m.....	25	107
3	“ 100 “ .....	53	127
4	“ 25 “ .....	80	183
5	“ 10 “ .....	126	184
6	“ 1 “ .....	132	201
1	Controls in distilled water.....	100	...
2	Cumarin, 100 p.p.m.....	dead	dead
3	“ 50 “ .....	dead	dead
4	“ 25 “ .....	36	58
5	“ 10 “ .....	74	127
6	“ 1 “ .....	97	166

It is quite evident from a survey of the results that these inorganic salts had a beneficial action when added to the toxic solutions, the effect being more marked in the case of the weaker solutions. The plants which grew in the cumarin solutions containing 25 p.p.m. and less are represented in Fig. 1.

It may be noted that one of the toxic substances (vanillin) produced, in the lower concentrations, what is ordinarily interpreted as stimulation. The phenomenon of increased growth in the presence of weak poisons is quite general and has been worked out in much detail by Raulin,<sup>1</sup> Richards,<sup>2</sup> Ono,<sup>3</sup> and others. There can be little question but that the increased growth of the plants in the vanillin solutions was caused by some stimulating action of the vanillin upon the functions of the plants. Turning now to the column expressing the effect of the inorganic salts upon the vanillin, no stimulating effect will be noticed. None of the solutions containing vanillin plus an inorganic salt produced an effect upon growth greater than did distilled water containing only the inorganic salts. In other words, the stimulating effect of the toxic agent totally disappeared.

It would appear that the fertilizer salts either had an action upon the toxic organic substances ameliorating the conditions for the growth of plants, or that they acted upon the plants in such a way that increased growth was possible in spite of the presence of the toxic compounds. Further evidence on these points was gained from the results of replanting certain of the solutions. The vanillin solutions enumerated in Tables II and III received a second set of wheat seedlings after the first had been removed. The water lost by transpiration was restored by adding distilled water, and the plants were allowed to grow nine days. The behavior of the second set of plants indicated that the conditions for growth were on the whole, even better than those existing during the growth of the first set, except in the solution originally containing 500 p.p.m. vanillin, in which the plants were again killed. In the first crop the injurious effect of vanillin itself was primarily on the root development and this condition was largely ameliorated by the calcium carbonate and sodium nitrate when added to the vanillin solutions. In the second crop the root system was, generally speaking, much improved in all three series, including those without fertilizer salts, again showing that activities of the living roots were able in part to overcome the toxic agents.

In order to ascertain whether the growth of the plants was a correct indication of the presence of vanillin, the solution was submitted to a

<sup>1</sup> Ann. Sci. Nat. Bot. [V] 11, 91 (1869).

<sup>2</sup> Jahrb. wiss. Bot., 30, 665 (1897).

<sup>3</sup> Jour. Coll. Sci. Tōkyō, 13, 141 (1900).

chemical examination.<sup>1</sup> Fairly large amounts of vanillin were shown by this test to be present in the solutions originally containing 500 p.p.m. vanillin, while only traces could be found in the solutions originally containing 25 or 10 p.p.m. No vanillin could be demonstrated in any of the solutions in which calcium carbonate or sodium nitrate had been present. There had been a diminution and even a total disappearance of the toxic substance.

An additional experiment will be described for the purpose of giving a direct comparison between the growth of the first and the second set of plants in toxic solutions containing inorganic salts. Three different solutions were employed in this experiment: the first contained 100 p.p.m. vanillin; the second, 100 p.p.m. vanillin plus 100 p.p.m. sodium nitrate; the third, 100 p.p.m. vanillin plus 2000 p.p.m. calcium carbonate. A set of wheat plants was allowed to grow in each of these solutions for eight days. The growth of the plants was of the same general character as represented in corresponding treatments in Tables II and III. After removing the plants from these solutions the original volumes were restored by the addition of distilled water to replenish that transpired by the first set of plants, and a second set of seedlings was installed. Nothing was added to the solutions except the distilled water. At the same time a set of plants was installed in a new set of solutions, precisely similar in composition to the original set. Accordingly, the plants in the new solution represented a first crop in toxic solutions containing fertilizers, and the replanted set represented a second crop in the originally similar solutions. The plants in these solutions together with controls in pure distilled water were allowed to grow ten days. The relative growth of the plants in the various solutions is given in Table IV and the plants are shown in Fig. 2.

The numbers and order of the solutions in the table correspond to those of the plants shown in the figure.

It will be seen that the results of this experiment confirm those of preceding experiments in showing that the toxic properties were ameliorated both by the action of plant roots and by the presence of inorganic salts. Where the two agencies worked in conjunction, the growth of the plants was the best. The second crop showed the better growth in each

<sup>1</sup> The method used seems to have been first described by Moerk, *Amer. J. Phar.*, 63, 572 (1892), and cited in *Z. anal. Chem.*, 32, 242 (1893). It consists in decolorizing the vanillin solution (if necessary) with freshly precipitated lead hydroxide, then adding bromine water, drop by drop, until a slight excess is present. Ferrous sulphate is finally added until the maximum blue-green color is reached. The test was slightly impaired in solutions to which calcium carbonate had been added, by the yellow color formed with the reagents, but the presence of sodium nitrate does not interfere with the test. It was not found to be strictly quantitative although the amount of color produced was indicative of the approximate amount of vanillin present.

case, owing to the action of the plant roots and the inorganic salts during the growth of the first crop.

TABLE IV.—RELATIVE GROWTH OF THE FIRST AND SECOND SETS OF PLANTS IN SOLUTIONS CONTAINING 100 PARTS PER MILLION VANILLIN WITH AND WITHOUT CERTAIN INORGANIC SALTS.

(p.p.m. = parts per million).

No.	Solutions.	Relative transpiration.	Relative green weight of tops.
1	First crop in vanillin solution.....	45	100
2	Second crop in vanillin solution.....	93	103
3	First crop in vanillin solution + NaNO <sub>3</sub> 100 p.p.m.....	114	99
4	Second crop in vanillin solution + NaNO <sub>3</sub> 100 p.p.m.....	190	135
5	First crop in vanillin solution + CaCO <sub>3</sub> 2000 p.p.m.....	141	111
6	Second crop in vanillin solution + CaCO <sub>3</sub> 2000 p.p.m.....	166	100
7	Controls in pure distilled water.....	100	100

Another experiment upon the action of these fertilizers was performed, using arbutin as the toxic substance and growing two sets of plants in the solution. Two cultures, each containing 10 wheat plants, were made for each concentration of solution employed. Arbutin was shown to be decidedly toxic to wheat plants, killing at a concentration of 500 p.p.m. and injuring at 25 p.p.m. As before, sodium nitrate and calcium carbonate were added to the solutions. The set of solutions receiving sodium nitrate was accidentally lost before it was chemically examined for arbutin and, hence, does not appear in the records of the second crop. The growth of the plants in these solutions is shown in Table V where the figures represent the relative transpiration. The first crop grew eleven days, the second crop ten days.

TABLE V.—RELATIVE GROWTH OF WHEAT PLANTS IN ARBUTIN SOLUTIONS WITH AND WITHOUT CERTAIN INORGANIC SALTS. GROWTH MEASURED BY TRANSPIRATION.

(p.p.m. = parts per million.)

No.	Solutions.	Relative transpiration.	
		First crop.	Second crop.
1	Controls in distilled water.....	100	100
2	Arbutin, 1000 p.p.m.....	23	28
3	“ 500 “.....	27	36
4	“ 200 “.....	41	71
5	“ 100 “.....	45	94
6	“ 50 “.....	80	125
7	“ 1000 “ + calcium carbonate 2000 p.p.m....	31	37
8	“ 500 “ + “ “ 2000 “... ..	51	84
9	“ 200 “ + “ “ 2000 “... ..	56	74
10	“ 100 “ + “ “ 2000 “... ..	70	148
11	“ 50 “ + “ “ 2000 “... ..	92	154
12	Distilled water + “ “ 2000 “... ..	109	147
13	Arbutin, 1000 p.p.m. + sodium nitrate 100 p.p.m.....	33	..
14	“ 500 “ + “ “ 100 “.....	53	..
15	“ 200 “ + “ “ 100 “.....	72	..
16	“ 100 “ + “ “ 100 “.....	83	..
17	“ 50 “ + “ “ 100 “.....	91	..
18	Distilled water + “ “ 100 “.....	146	..

It will be seen from these figures that the general order of results was the same here as in the preceding experiment where a second set of plants was grown in toxic solutions. In making the comparison it is of course necessary to use the growth in "replanted" distilled water as the basis of the comparison and not fresh distilled water, since all solutions used for second crops contained the waste products of the first crop. The second crop was in all cases better than the first. Where the calcium carbonate had been added to the lower concentrations of arbutin, the second crop was remarkably good. These results bear out the results of chemical tests to determine the presence of arbutin.

It was found that Pauly's<sup>1</sup> diazobenzene-sulphanilic acid reagent could be used as an indicator for arbutin. While it is only approximately quantitative it gave good indications. Pure standard solutions of arbutin are colored bright crimson by the addition of a few drops of diazobenzene-sulphanilic acid reagent. Weak solutions of arbutin, especially after the growth of plants, are strongly tinged with yellow. The results of the chemical tests made after the growth of the first and second crops are given in Table VI.

TABLE VI.—ARBUTIN REMAINING IN SOLUTION AFTER GROWTH OF FIRST AND SECOND CROP OF WHEAT.

(p.p.m. = parts per million.)

No.	Solutions.			Results of tests to indicate arbutin.	
				After first crop.	After second crop.
1	Originally containing arbutin	1000	p.p.m.	abundant	moderate
2	" "	500	"	abundant	moderate
3	" "	200	"	moderate	weak
4	" "	100	"	weak	trace
5	" "	50	"	none	none
6	" "	1000	" + CaCO <sub>3</sub> , 2000	abundant	weak
7	" "	500	" + " 2000 "	moderate	weak
8	" "	200	" + " 2000 "	weak	trace
9	" "	100	" + " 2000 "	trace	none
10	" "	50	" + " 2000 "	none	none

An inspection of these results shows that the calcium carbonate had the same action upon arbutin as the inorganic salts used in the previous experiment had upon vanillin. There had been a disappearance of arbutin from the solutions in which plants grew, but much more had disappeared from solutions containing the inorganic salt.

In regard to the question as to how the toxic substance was caused to disappear, three possibilities seem to present themselves: (1) The plants themselves absorbed part of the toxic substance and oxidized some of it; (2) the inorganic salts had a direct action upon the toxic substance; (3) the plants and inorganic salts working together had a direct or in-

<sup>1</sup> Z. physiol. Chem., 42, 508 (1904); *Ibid.*, 44, 159 (1905).

direct action upon the toxic substance. The first possibility was tested by the experiment already described in which a second set of plants was grown in the toxic solutions. It was there shown that the plants did have an ameliorating action, although they were only able to overcome the toxic substances in the lowest concentrations.

In connection with the second possibility, *viz.*, that the fertilizer substances had a direct action upon the toxic substance, the following experiment will be of interest. A solution containing 100 p.p.m. of vanillin was prepared; a portion of it received sodium nitrate equivalent to 100 p.p.m. of  $\text{NO}_3$ , another portion received calcium carbonate at the rate of 2000 p.p.m., and still another portion received both sodium nitrate and calcium carbonate. These solutions were then allowed to stand ten days. At the end of that time another set of solutions, exactly similar to the first, was prepared. They were compared by growing plants in them and using the growth of the plant as an indicator of the ameliorating action of the salts added. Four cultures comprising forty plants were used for testing each solution. It was thought that any action the inorganic salts might have had, would be shown by the growth of the plants in the solutions.

The results of the experiment are presented in Table VII.

TABLE VII.—EFFECT OF TEN-DAY ACTION OF SODIUM NITRATE AND CALCIUM CARBONATE ON THE TOXICITY OF VANILLIN SOLUTIONS. GROWTH MEASURED BY RELATIVE TRANSPIRATION. (p.p.m. = parts per million.)

No.	Solutions.	Solutions prepared at time of planting.	Solutions prepared ten days before planting.
1	Controls in distilled water.....	100	100
2	Vanillin, 100 p.p.m.....	63	56
3	“ 100 “ + $\text{NaNO}_3$ , 100 p.p.m.....	127	100
4	“ 100 “ + $\text{CaCO}_3$ , 2000 “.....	125	166
5	“ 100 “ + $\text{NaNO}_3$ , 100 “ + $\text{CaCO}_3$ , 2000 p.p.m.....	225	215

From these figures it will be seen that the action of sodium nitrate was not the same as that of calcium carbonate. The latter produced beneficial effects which were appreciably increased when it stood in contact with the toxic solution for ten days. Sodium nitrate, on the contrary, did not show any increase in its ameliorating powers upon standing, in fact the solutions appeared to become somewhat poorer in both the cases in which sodium nitrate was allowed to stand. The results seem to suggest that calcium carbonate has the power to act independently in ameliorating the toxicity of vanillin, and the lack of ameliorating action of the sodium nitrate may be due to the formation of intermediate compounds having as great or greater toxicity than the original. These points seem worthy of further study.

Regarding the third possibility it might be said that the experimental data thus far obtained, go to show that the physiological activities of the plants were able to ameliorate the toxic conditions and also that the action of certain inorganic compounds in the solution is able to bring about some improvement due probably to direct action on the toxic substances, but far greater improvement is effected by the combined action of plants and inorganic salts. In other words, the plants and inorganic compounds working together are able to accomplish more in the way of destroying a toxic substance than either can do working alone. That such a substance as vanillin is actually destroyed, does not admit of doubt, if any conclusion is to be drawn from the experiments previously recorded. Whether the destruction of vanillin took place in the solution or within the cells of the wheat plants was not ascertained, but it probably took place in the solution. This may be safely inferred from the behavior of the plants. Vanillin itself has a very inhibitive action upon the growth of the roots of wheat seedlings in water cultures. All plants grown in vanillin solutions showed more harmful effects in the growth of roots than in the growth of tops. When, however, fertilizer ingredients were added in sufficient amounts to ameliorate distinctly the growth of plants, the roots made as good or better relative growth than the tops. (Fig. 2.)

### Summary.

The activities of the plant roots are able to decrease the toxicity of organic compounds to a certain extent, provided the original concentration of the solution is below that able to cause death of the plants. It is probable that the oxidizing power of the root plays a greater or less part in the process of amelioration. The first set of plants may have absorbed directly some of the toxic material from the solutions, but the greatly diminished toxicity of the solutions as well as the formation of dyestuffs indicated that other changes had taken place.

The addition of certain inorganic salts to solutions of toxic organic compounds was distinctly beneficial to plant growth. That the inorganic salts and the physiological activities of the plant working together, had accomplished the destruction of toxic substances was shown by both plant growth and chemical tests.