

teristics of the "reflection and transmission spectra" for each of these substances.

6. Solutions of some sulfophthaleins which are yellow in neutral or faintly acid solutions become red in stronger acids and, hence, have been found by Mr. Homer Cloukey to be colored red by such salts as zinc chloride and stannous chloride. But even some neutral salts, such as sodium chloride, change the colors of these solutions and this "salt effect" of acids, bases and salts will be extensively investigated.

7. On account of all of these characteristics of the sulfophthaleins it has been found possible to prepare a series of indicators having a wide range of sensibility of hydrogen ions. By substituting bromo, nitro, methyl, isopropyl, amino and other groups in the benzenesulfonic acid group, and especially in the phenol residue, one is enabled to change the ionization constants of the sulfonic acid and phenol groups greatly and hence prepare indicators covering a wide range of usefulness. The phenol, thymol, and cresolsulfophthaleins, and their bromo derivatives, are highly satisfactory indicators.

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EFFECTS OF LARGE APPLICATIONS OF COMMERCIAL FERTILIZERS ON CARNATIONS.*

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In the investigation of the use of commercial fertilizers in growing carnations by the Illinois Agricultural Experiment Station, it has been found that the lack of appreciation by florists of the relatively high plant food concentrations and often high solubilities of commercial fertilizers, as compared with manure, has often led to a complete loss of a crop of flowers in an effort to produce an extraordinarily large one. On this account, it was considered desirable to study the causes and effects of overfeeding with the more ordinarily used commercial fertilizers.

The fertilizers chosen for the experiment were dried blood, sodium nitrate and ammonium sulfate, acid phosphate and disodium phosphate, and potassium sulfate. For comparison, sodium chloride and sodium sulfate also were used on some sections. Experimental work upon the subject was carried out during the years 1912-15.

Carnations are propagated by means of cuttings, and from these it was

* This paper presents the data contained in the thesis presented by Mr. Muncie in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry in the Graduate School of the University of Illinois.

found impossible to secure a normal growth in either sand or water cultures. Hence, the experimental work was based upon the study of plants grown in soil carefully selected with the view to securing uniformity throughout the benches, watered to give as nearly as possible the same moisture content, and subjected very nearly to identical conditions of heat, ventilation, and illumination. For details regarding the type of soil, its preparation, arrangement of sections, etc., the reader is referred to *Bull.* 176 of the Illinois Agricultural Experiment Station.

The method consisted of weekly applications of the fertilizers at various rates upon isolated sections in the benches, beginning about October 1 and continuing until about May 1 or until injury became serious.

Effects of Overfeeding on Condition of Plants.—The rapidity with which the sections of carnations became affected followed in a general way the solubility of the fertilizer used.* The solubilities** of the pure substances in water per hundred parts at 0° are given in Table I.

TABLE I.—SOLUBILITIES OF PURE SALTS IN WATER AT 0°. (PARTS PER 100.)

NaNO ₃	(NH ₄) ₂ SO ₄	NaCl	KCl	K ₂ SO ₄
72.9	71.0	35.7	28.5	8.5
Na ₂ HPO ₄ .12H ₂ O	CaH ₄ (PO ₄) ₂ .H ₂ O	CaHPO ₄	CaSO ₄ .2H ₂ O	
6.3	4 (15°)	0.028	0.241	

Commercial acid phosphate consists of about equal parts of mono-calcium phosphate and calcium sulfate. Reversion to monohydrogen phosphate in presence of bases in the soil would further decrease the low solubility of the acid phosphate and by double decomposition with calcium, iron and other bases in the soil render the sodium phosphate first applied less soluble, as pointed out by Cameron and Bell.⁷

Dried blood, giving soluble products at a rate depending upon the rapidity with which bacterial decomposition proceeds, could not be rated as having a known solubility without a study of the bacteriological activity of the soil mixture. Tests with litmus paper showed that the surface of the soil, neutral at the beginning of the experiment, became acid seven or eight days after the addition of the dried blood. Soil to which ammonium sulfate was applied became acid as quickly also.

Single applications of ammonium sulfate and sodium chloride at the rate of 12.5 kg. per 100 sq. ft. made on December 3, 1913, produced marked injury within a week's time. Equal amounts of potassium sulfate, at this time, followed by further applications at intervals of one or two weeks, at the rate of 1.25 kg. per 100 sq. ft., produced no signs of injury

* The impurities in the ammonium sulfate, potassium sulfate (in this case 1.26% of chloride as sodium chloride) and disodium phosphate are not sufficient to interfere with the use of the solubilities of the pure substances as a rough measure of the solubilities of the fertilizers themselves.

** Van Nostrand—Chemical Annual, 1910.

until about January 15, when a lack of turgidity became noticeable, followed by a gradual stunting of growth, with the more pronounced signs appearing only after the middle of March. Signs of injury in sections treated in the same manner with sodium phosphate became evident even more slowly, while acid phosphate produced no apparent injury even in the largest applications.

The fertilizers may be grouped into the class, easily soluble and producing almost immediate injury; a second, moderately soluble and producing delayed injury; and a third, difficultly soluble and producing no apparent injury. On days of continuous sunlight a more or less pronounced softness of tissue could be detected by careful observation long before characteristic injuries became apparent.

Effects of Overfeeding with Ammonium Sulfate.—A marked softness of tissue was the earliest sign of overfeeding with ammonium sulfate. A complete plasmolysis took place in that portion of the stem located two and three nodes below the bud and in the portion of the stem just above the node, so that the stem bent completely over. The shoots first affected were those with buds one-half to three-quarters developed. At the same time white spots 0.25 and 1.00 mm. in diameter appeared upon the upper leaves of these and the younger shoots. Microscopic examination of these showed the chlorophyll bearing tissue entirely plasmolyzed.

In contrast to the injury from other fertilizers, practically every flower split.* This splitting was not caused by the pressing outward of the petals as is usually the case, but by a weakening of the tissue at the line joining the sepals to form the calyx cup. Later stages resulted in the drying up of the leaf tips, and the appearance of the white depressions upon the older leaves. The sepal tips very early became brown. Later, pustule-like elevations about 1 mm. across appeared on them, caused by a crystal of ammonium sulfate beneath the epidermis. The injury from excess of ammonium sulfate was more rapid and pronounced in the presence of lime than without it.

Effect of Overfeeding with Sodium Nitrate.—Injury followed heavy applications of sodium nitrate within a few days, the characteristic symptom being an even lightening of color of the foliage over the plant, followed by drying of leaf tips and petals and withering of the plant.

Effects from Large Applications of Sodium Chloride.**—The first appearance of injury from large amounts of sodium chloride was two days after its application, a plasmolysis of the cells of the stem, causing it to lose its rigidity at the crown. When held within supports the plants appeared

* Splits is a trade term denoting flowers with split calyces.

** Sodium chloride, while not strictly a fertilizer, was used in the experiments because of its presence in considerable amounts in kainite and in some grades of commercial potassium sulfate.

normal. Gradually, however, the plants lost their turgidity and the chlorophyll disappeared evenly throughout the entire plant. Tests made in the spring of 1915 with heavy applications of sodium chloride and potassium chloride (12 kg. per 100 sq. ft.) showed the same effect from each of them, while sodium sulfate, like potassium sulfate, showed less injury and that only after a longer period.

Effects of Overfeeding with Potassium Sulfate.—In earlier stages partial wilting occurred on days of sunshine. Drying up of the tips of the leaves and curling of the leaves upward upon their long axis followed, with often, also, a peculiar inhibition of growth on one edge of the leaf, with the same on the opposite edge of another portion, giving the leaf a wavy outline.

A marked stunting of growth was observable. This affected most noticeably the lengthening of the stem, resulting in the later shoots assuming a rosette appearance, due to the leaves of normal length upon a stem with undeveloped internodes less than an inch in length. (The internode in full grown shoots is ordinarily three or four inches long.) The edges of the petals of the flowers after about the middle of January became quite generally withered or crinkled. Those in the center of the flower remained closed quite tightly, while the other two or three rows opened normally. Later, the buds remained closed, although the pistil often pushed its way out and might be seen extending an inch above the top of the bud.

A marked increase in exudation of nectar in the flower was found to have caused the gluing together of the petals, and so prevented their opening. On cloudy days very frequently a calyx cup would be found completely filled with this exudation. The exudation was most plentiful in the flowers from plants receiving a moderately heavy application of potassium sulfate over a long period of time while the heavier applications caused a noticeable but less plentiful increase. A small amount of nectar is found in normal flowers, and somewhat larger amounts in the flowers from plants receiving large applications of sodium phosphate, sodium chloride, ammonium sulfate, or potassium chloride, but not so generally nor in such large amounts as in the sections treated with potassium sulfate. Injury was less marked when ground limestone was added to the soil, in contrast to the effect of liming on the production of injury by ammonium sulfate.

Effects of Overfeeding with Sodium Phosphate.—When moderately large amounts of sodium phosphate were added over a long period (as in 1913-14) no injury was noticeable until about the middle of March, when a retardation of growth was evident from the decrease in height of the plants and abnormally small buds and flowers. These signs of inhibition became steadily more pronounced until the plants were removed from the

benches, about May first. When larger amounts were used (as 12 kg. per 100 sq. ft. in 1914-15) loss of turgidity in the plants, longitudinal rolling of the leaves, death of the leaf tips and softness of the petals of the blossom were evident. These signs of injury appeared, however, only after the middle of January and then only gradually. Injury was less when the soil was limed than when not.

Effects of Overfeeding with Dried Blood.—In none of the experiments with dried blood did injury appear until about the middle of January. At that time a softness of the petals and irregularity of their arrangement, due to the partial opening of the inner and crinkling of the outer ones, became more or less common. The flowers became susceptible to browning when a drop of water from syringing lodged on a petal in a position to be reached by the rays of the sun. The height of the plants was below normal in the spring but rather above in the fall; the color was good. If the applications of dried blood were not continued after signs of injury became apparent, the plants gradually recovered. The same held true for plants overfed with ammonium sulfate in contrast to those which had been injured by potassium sulfate, sodium phosphate, and sodium chloride.

Effects of Overfeeding on the Mineral and Nitrogen Content of Plants.—Effects upon the dry weight and ash are shown in Table II, the samples being the foliage from the shoots gathered January 9, 1915.

TABLE II.—DRY WEIGHT AND ASH IN FOLIAGE.

Section No.	Treatment. Check.	Moist weight.		%	Ash (sulfated) per cent. of dry weight.
		G. 27.6.	Dry weight. 17.8.		
269					13.68.
271	125 P*	32.4	17.6		13.93
273	250 P	32.2	18.3		12.89
275	500 P	30.6	18.9		14.28
277	125 K	26.1	18.4		15.37
279	250 K	36.8	20.4		15.45
281	500 K	32.8	22.6		15.59
283	Check	28.2	19.2		13.19
285	125 NaCl	42.9	22.8		14.45

The increase in both values as the applications of any one fertilizer in a series were increased is shown in the table. The higher values for plants treated with potassium sulfate and sodium chloride over those treated with sodium phosphate correspond to the higher osmotic pressure values obtained from the sap of these plants as well as to the more rapid injury from potassium sulfate.

Determination of the total nitrogen and mineral content of the ash

* N, P and K in the tables are used to indicate ammonium sulfate, disodium phosphate and potassium sulfate, respectively, while NaCl indicates sodium chloride and A. P., commercial acid phosphate. The figures preceding the letters indicate the number of grams applied weekly per 20 sq. ft. of bench space.

from various samples of plants treated with potassium sulfate gave the following values:

TABLE III.—EFFECT OF POTASSIUM SULFATE.

Treatment.	Analyses. Per cent.				
	Na ₂ O.	K ₂ O.	SO ₃ .	N(total).	P ₂ O ₅ .
Check	1.09	5.38	1.07	2.58	0.72
K	1.25	6.62	1.91	2.53	0.70
	0.16	1.24	0.84	—0.05	—0.02

The data show an increased sodium,* potassium and sulfur content, with practically a constant percentage of nitrogen and phosphorus.

A similar study of plants to which ammonium sulfate had been applied gave the results shown in Table IV.

Plants to which sodium phosphate was applied showed a higher phosphorus content, 0.60% P₂O₅ and 1.17% P₂O₅ in a sample of 1915 in which the calcium content was decreased (2.31 and 1.63% CaO, respectively, in the last set of samples); the nitrogen content was increased by applications of sodium phosphate, the values 1.99%, 2.84% and 3.30% being obtained from plants to which had been applied, respectively, none, 250 g. and 500 g. of sodium phosphate per 20 sq. ft. of bench space per week for several weeks.

TABLE IV.—EFFECT OF AMMONIUM SULFATE.

Treatment.	Analyses. Per cent.			
	N(total).	N(by MgO).	SO ₃ .	P ₂ O ₅ .
Check	2.05	0.168	0.75	0.93
N	2.93	0.364	2.10	1.14
	0.88	0.196	1.35	0.21

The ratio 2N/SO₃ in ammonium sulfate is 28/80 = 0.351, that of total nitrogen to sulfur increase is 0.652; and of nitrogen by MgO 0.145. The intake of sulfur when this fertilizer is used is less than is required for the nitrogen then, but in excess of that required to be combined with the nitrogen determined by MgO.** Limestone was found to depress the sulfur intake from ammonium sulfate. Since injury was greater in sections so treated, the injury is not proportional to the intake of sulfur. The intake of phosphorus was increased by the addition of ammonium sulfate, probably due to acidity developed in the soil.

Table V shows the total nitrogen content of some plants from Sections 264 (ammonium sulfate and lime) and 281 (ammonium sulfate). Samples

* Mayer²² states that the addition of soluble potassium salts to a soil causes a partial replacement of the sodium.

** The author would not care to report the presence of ammonium salts in plants not fed with it. It seems, rather, that MgO has caused some decomposition of the organic material; the error due to this is assumed to be the same in both samples.

were collected on April 25, 1914. Section 281 had received but one application at the rate of 12.5 kilos per 100 sq. ft. on December 3, 1913, while applications at the rate of 1250 g. per 100 sq. ft. were made to Section 264 at 15 different intervals of about two weeks after December 20, 1913. Analyses were made of upper and lower portions of the plant separately in order to show any localization of nitrogen in the more vigorously growing portion of the plant.

TABLE V.—TOTAL NITROGEN DETERMINATION ON FOLIAGE.

Sample No.	Plant No.	Section.	Portion.	Condition.	Nitrogen. %.
1	1	264-E	upper	half dead	4.58
2			lower	half dead	4.07
3	4	264-P	upper	half dead	7.78
4			lower	half dead	5.64
5	1	264-P	upper	dead	6.14
6			lower	dead	3.41
7	11	264-E	upper	alive	6.69
8			lower	alive	5.70
9	4	264-E	upper	half dead	7.01
10			lower	half dead	3.34
11	11-15	281-E	upper	dead	4.60
12			lower	dead	3.02
13	16	281-E	upper	partially affected	4.73
14			lower	partially affected	3.78
15	20	281-E	upper	half dead	4.73
16			lower	half dead	2.94
17	7	281-E	upper	slightly affected	4.47
18			lower	slightly affected	3.21

The total nitrogen content of the plants varied from once and a half to more than twice the normal value found in the previous set. Average values for the plants from Section 264 are 6.44% and 4.43%, respectively; for those from Section 281, 4.63% and 3.24%. In each case the more vigorously growing portion contained the larger percentage of nitrogen and the increase over the lower portion is considerably greater in the section to which the smaller applications were made during the entire season. No clear relation is shown between the nitrogen content and the degree of injury. Considerable tolerance for ammonium sulfate is shown when it was applied to the soil in quantities not heavy enough to produce immediate, serious injury. The fact that the dead plants had no higher total nitrogen content than those only injured is evidence that part of the nitrogen when added in small quantities was changed to a nontoxic form, since the dead plants were in this condition as early as March 21, while the living ones though injured undoubtedly continued to take up the salt in solution until samples were taken.

A series of ammonia determinations was made on the sap from "checks" and ammonium sulfate fed plants of the set of 12-9-14. Folin's micro-method for the determination of free¹⁴ ammonia was used, the excess of

sulfuric acid (0.01550) being titrated back with potassium hydroxide 0.02130 with sodium alizarin sulfonate as the indicator. Results are given in Table VI.

TABLE VI.—FREE AMMONIA IN PLANT SAPS.*

Sample No.	Treatment.	Appearance.	Nitrogen. Mg. N per cc.
5	check	normal	none
8	250 N	normal	0.1834
7	500 N	normal	0.1372
2	1000 N	slightly injured	0.6390
1	1000 N	badly injured	1.0560

The white spots on the leaves of plants treated with ammonium sulfate, and of crystals imbedded beneath the epidermis of the sepals were studied by microchemical methods.⁴

1. January 21, 1914. Plant Number 4, Section 281, White Enchantress. Plant apparently normal. A drop of sap from the stem of a shoot was treated with a drop of ammonia-free hydrochloric acid and chloroplatinic acid, and evaporated at room temperature under a loosely covering watch-glass. A few crystal masses, tetrahedral and often aggregated in shape of a cross, appeared. They were yellow in color. Sap from Number 8, somewhat injured, and Number 12, badly affected, gave these characteristic crystals, also.

2. A section of the leaf showing white blotches was immersed in chloroplatinic acid after removal of the epidermis and allowed to remain overnight. Large and perfect crystals appeared, arranged usually around the injured spot, never in it. They were insoluble in 95% alcohol which removed the excess of chloroplatinic acid.

3. A drop of sap from plant Number 4, Section 281 was distilled with a pinch of sodium carbonate over a micro-burner and the distillate caught in a hanging drop of hydrochloric acid in a cover glass placed on a glass ring above it. Treatment as above gave small, yellow tetrahedra insoluble in 95% alcohol.

Ammonium salts were evidently present and apparently caused plasmolysis of certain of the chlorophyll bearing cells. Why injury of this type is caused by ammonium sulfate in contrast to the even lightening of the color of the whole leaf by the other soluble salts, sodium nitrate and sodium chloride, is not known.

Nitrate determinations according to the phenolsulfonic method of Mason²¹ were made upon the sap of a "check" and an ammonium sulfate fed plant from the set of March 9, 1915. The values of 0.01 and 0.40 mg. N as nitrate per cc. of sap, respectively, showed that nitrification was proceeding in the soil although it was quite strongly acid.¹⁷

* In earlier stages of feeding with ammonium sulfate, samples have been taken in which no NH_3 was detected by this method.

Total solids and ash were determined on the sap of the set of 12-9-14. The results, given in Table VII, are calculated to milligrams per cc. of sap.

TABLE VII.—TOTAL SOLIDS AND ASH OF SAP.

Sample No.	Set date.	Section.	Treatment.	Total solids. Mg. per cc.	Ash.* Mg. per cc.	
2	12-9-14	291	1000 N	91.9	...	
3		293	1000 K	104.9	19.2	
5		289	check	63.8	11.8	
6		261	check	62.1	12.1	
7		265	250 N	63.6	13.9	
8		267	500 N	79.9	15.1	
9		277	125 K	64.3	16.1	
10		279	250 K	69.9	17.2	
11		281	500 K	75.7	17.1	
12		283**	check	72.1	15.0	
1		1-9-15	269	check	84.0	7.5
2			271	125 P	81.7	13.2
3	273		250 P	86.7	13.3	
4	275		500 P	93.0	15.1	
5	277		125 K	92.3	13.4	
6	279		250 K	106.3	20.1	
7	281		500 K	133.7	20.0	
8	283**		check	105.1	14.1	

The average total solids content of the sap was 85.1 mg. per cc. and the ash content 14.9 mg. The influence of the fertilizer applications is seen in the increase in both values as the applications of any fertilizer were increased in a series of sections. Sample 3 of the first set and 6 and 7 of the second, all of which were from plants to which large applications of potassium sulfate had been made, showed particularly high values.*** The first set of data was obtained by drying the samples in a Sargent electric oven at 60-70°, the second in a vacuum oven heated to 50° for 12 hours. The actual value for total solids depended on the length of heating but experiments with both sets of data given showed the same relative values after several successive heatings.

* Ash determinations upon the sap were made by careful incineration of the solids in 1 cc. of sap in platinum dishes over a low flame to prevent mechanical loss of particles of the ash. The low chloride content obviates the danger of volatilization of potassium chloride by high temperatures.

** For some reason total solids and ash determinations always ran higher in sap from plants in Section 283 than from those in other "check" sections. The same discrepancy is seen in the osmotic pressure data for these two sets.

*** The determination of total solids with accuracy is not possible on account of the uncrystallizable solutes in the sap, and on this account the mean molecular-weight calculations which often accompany osmotic pressure data were not made. Drying on the water bath was found to cause charring of the sap from plants which had been treated with ammonium or potassium sulfate. The first showed a higher acidity value, the second a higher sugar content.

Determinations of sodium and potassium in the ash from sap obtained on January 9, 1915, from plants treated with potassium sulfate, were made in order to show the increased intake of potassium. Similarly, determinations of phosphorus were made upon the sap from plants fertilized with disodium phosphate. The results, calculated to milligrams per cc. of sap, are given in Table VIII.

TABLE VIII.—MINERAL CONTENT OF SAP.

Sample No.	Section.	Treatment.	Na ₂ O. Mg.	K ₂ O. Mg.	Mg ₂ P ₂ O ₇ . Mg.
9	277	125 K	1.4	9.4	...
10	279	250 K	1.3	10.1	...
11	281	500 K	1.3	10.1	...
12	283	check	1.2	8.4	...
1	269	check	1.5
2	271	125 P	6.1
3	273	250 P	7.5
4	275	500 P	9.6

Effect of Overfeeding on Osmotic Pressure of Sap.—Sap was expressed from the stems of shoots after freezing them with an ice-salt* mixture, and the lowering of the freezing point determined by the method of Harris and Gortner** of allowing supercooling until the solution froze and correcting the value of Δ' obtained by the formula

$$\Delta = \Delta' - 0.0125 u \Delta'$$

where Δ' is the maximum temperature attained in the system and u the difference between this value and the minimum temperature. The relation between Δ and the osmotic pressure given by Lewis¹⁹ in the approximate equation

$$\pi = 12.06 \Delta$$

was used in calculating the value for π .

Description of Experimental Method.—Choosing a time when for two or more hours previous no appreciable draft had been stirring the air in the greenhouse, from four to eight shoots at the same stage of growth were removed from each of the sections of plants and quickly taken to

* Care was taken to select samples from the check and affected plants at the same time of day and shoots in the same stage of growth were taken, to insure freedom from variations in osmotic pressure due to differences in location and illumination, while the fact that the sections studied were usually adjacent obviated the difficulty that differences in temperature change the osmotic pressure of plants. See Dixon and Atkins,¹¹ Atkins,³ Ewart,¹³ Drabble and Drabble,¹² Cavara.⁸

** The method in general was an adaptation of that recommended by Gortner and Harris.^{15,18} André¹ and also Dixon and Atkins¹¹ have shown that successive portions of sap expressed from unfrozen tissue become more concentrated, while the latter have shown that the sap from frozen tissue always has a lower freezing point than that from unfrozen, and that successive portions gave nearly identical lowerings, leading to the conclusion that sap so expressed is representative of that originally within the tissue.

the laboratory. After removal of the foliage from the stems, they were broken at the nodes and placed in hard glass test tubes (25 mm. \times 150 mm.), stoppered with rubber stoppers and sealed with oil paper and rubber bands. Freezing was produced by the use of the ice and salt bath,* giving a temperature of -15° or lower and allowing the tubes to remain in the refrigerator overnight. The tubes were then removed from the bath and after the walls had been cleaned with distilled water and wiped dry, the portions of shoots were removed, thawed gradually, and the sap expressed by pressure from the screw of a tincture press set perpendicular to the wall upon two pieces of $\frac{3}{8}$ inch plate glass. After a first expression, the shoots were rearranged and pressure again applied. The sap was filtered through an S. & S. 589 filter—with a watch glass over the funnel to minimize evaporation—into a small test tube; a drop of xylene was added as a preservative and the tubes placed at once in a refrigerator, at about 10° . The sap after filtration was usually a clear, brown liquid without sediment.

As soon as convenient the freezing-point determinations were made. A thermometer was used having a bulb about 5 mm. by 35 mm., the mercury tube enclosed in a hollow jacket, and graduated to -6.5° in tenths of degrees, upon which, by the aid of a lens, hundredths of a degree could be read without danger from parallax. A stirrer of platinum wire and the thermometer were placed in the 5 cc. of sap contained in a test tube of Bohemian glass (15 \times 120 mm.) and the whole cooled to about $+2^{\circ}$ in an ice and salt bath in a beaker. The tube was wiped free from water and placed within a hard glass test tube (25 mm. \times 150 mm.) set two-thirds way into the ice and salt-freezing mixture. It was found saving of time to place this bath in a Dewar bulb, with inside diameter of 35 \times 130 mm.; the top was closed with a piece of cork; the bath so arranged remaining effective for three hours or more of use. During the entire cooling, the sap was constantly stirred to prevent its freezing about the sides of the tube. The lowest temperature obtained was read to one-tenth, and the maximum, by the aid of a lens, to one-hundredth degree. The tube was removed to a beaker of water, and after the temperature had risen to about 10° , the determination duplicated to within 0.01° , usually without difficulty on the first trial. A typical determination gave the following values:

$$\begin{array}{lll} \Delta' = 1.28 & u = 4.12 & \Delta = 1.214 \\ \Delta' = 1.27 & u = 3.43 & \Delta = 1.216 \\ \text{Average } 1.215 & \text{from which } \pi = 14.64 \text{ atmospheres.} & \end{array}$$

* It was found convenient in case less than a dozen tubes of material were frozen, to place the ice and salt bath in one or two one-liter Jena beakers. In this way the ice can be packed about the upper portions of the test tubes, and the beakers, with five or six test tubes in them, are narrow enough to keep the tops of the test tubes from touching the solution.

TABLE IX.—OSMOTIC PRESSURE DETERMINATIONS.

Date.	Sample No.	Section.	Treatment.	Δ' .	u .	Δ .	π .
11-12-15	1	291	1000 N	1.30	3.21	1.210	14.60
	2	293	1000 K	1.37	4.03	1.261	15.21
	3	295	1000 P	1.32	5.18	1.195	14.41
	4	289	check	1.15	3.90	1.054	12.71
	5	269	check	1.00	1.10	0.946	11.41
11-20-14	1	291	1000 N	1.33	5.67	1.196	14.42
	2	293	1000 K	1.50	3.40	1.396	16.84
	3	295	1000 P	1.10	4.80	0.994	11.99
	4	289	check	1.20	5.80	1.078	13.00
	5	283	check	1.18	2.87	1.098	13.24
12-9-14 (10 A.M.)	7	283	check	1.27	4.43	1.160	13.99
	1	291	1000 N	1.66	5.34	1.513	18.24
	2	291	1000 N	1.43	4.78	1.305	15.73
	3	293	1000 K	1.40	5.30	1.267	15.25
	5	289	check	0.95	4.55	0.856	10.34
	6	261	check	0.99	3.91	0.901	10.86
	7	265	250 N	1.10	5.10	0.990	11.94
	8	267	500 N	1.28	5.40	1.174	14.16
12-9-14 (4 P.M.)	9	277	125 K	1.05	3.75	0.962	11.60
	10	279	250 K	1.18	4.82	0.973	11.73
	11	281	500 K	1.18	4.32	1.076	13.01
	12	283	check	1.06	4.14	0.967	11.68
1-9-15	1	269	check	1.20	4.00	1.100	13.24
	2	271	125 P	1.28	4.62	1.169	14.08
	3	273	250 P	1.32	4.98	1.178	14.20
	4	275	500 P	1.39	3.81	1.284	15.50
	5	277	125 K	1.35	2.65	1.265	15.29
	6	279	250 K	1.58	4.92	1.448	17.49
	7	281	500 K	1.87	4.63	1.722	20.76
	8	283	check	1.28	4.92	1.161	14.04
	9	285	125 NaCl	1.88	2.92	1.771	21.40
	10	287	500 A. P.	1.48	5.52	1.338	16.13

Discussion of Results.—No comparison can be made between the values for the osmotic pressure determined in successive sets on account of variations due to temperature, physiological scarcity of water, etc., but the values obtained from plants in adjacent sections at the one time are regular enough to be comparable.

From the values for osmotic pressure of Samples 7, 8, 2 and 1 of the set of 12-9-14 the conclusion was drawn that the osmotic pressure within the plants increased as the quantity of ammonium sulfate applied to the soil was increased. Samples 2, 3 and 4, and 5, 6 and 7 of the set of 1-9-15 gave similar results with increasing applications of sodium phosphate and potassium sulfate. The values obtained from the application of sodium phosphate were in every case lower than those obtained from application of equal quantities of potassium sulfate or ammonium sulfate. The

samples taken on 11-12-14 and 11-20-14 gave higher values for the sap from plants overfed with potassium sulfate than those treated with ammonium sulfate, but later in the year in the set of 12-9-14 (Samples 1 and 2) the relative values are reversed.

In the set of 12-9-14 plants treated with potassium sulfate at the rate of 1000 g. per section per application were still apparently normal, although the osmotic pressure amounted to 15.25 atmospheres, while plants treated with one-half this weight of ammonium sulfate possessed an osmotic pressure of only 14.16 atmospheres and showed signs of injury. Injury, on the other hand, had not appeared on plants treated with ammonium sulfate (250 g. per section per application) when the osmotic pressure amounted to 12.42 atmospheres as compared to 11.34 atmospheres in the adjacent "check" section (12-9-14—10 A.M.).

The higher value of Sample 1 over Sample 2 (of the set of 12-9-14—10 A.M.) was correlated with a greater degree of injury by the ammonium sulfate. Injury appeared on the plants from sections to which potassium sulfate was applied, only when an osmotic pressure of over twenty atmospheres was reached (1-9-15), and an osmotic pressure value up to 15.50 atmospheres was found in plants on soil treated with sodium phosphate, without injury being apparent. The determination of the value on the sap from plants treated with acid phosphate gave 16.11 atmospheres, yet these plants exceeded in size and vigor those to which no fertilizer was applied (1-9-15). The conclusion to be drawn from these facts is that, with a single fertilizer, injury from overfeeding becomes apparent when a certain osmotic pressure is reached, but that this value is different for different fertilizers.

The injury from applications of sodium chloride at the rate of 125 g. per section per application, occurred at approximately the same time, was very similar to, and was of about the same degree as that from applications of potassium sulfate, in four times these quantities. The relative osmotic pressure values are given in Samples 9 and 10 (1-9-15). The solubilities of these salts, as pointed out on page 2785, at 0° are 35.7 and 8.5, respectively, giving a ratio roughly of 4 to 1.

Effects of Overfeeding on the Total Acidity of the Cell Sap.—Reaction tests with litmus paper showed that the soil receiving no fertilizer or only manure was neutral or slightly alkaline in the fall, and that a gradual change to slight acidity took place during the winter. Commercial acid phosphate, dried blood and ammonium sulfate upon the soil each increased the total acidity,* the first one immediately after application,

* It is not likely that the hydrogen-ion concentration of the soil solution was greatly increased by addition of commercial acid phosphate, since the formula used in its preparation prevents the presence of free sulfuric acid by providing a slight excess of tricalcium phosphate. Salm,²³ using a hydrogen electrode apparatus, found $[H] = 3.3 \times 10^{-5}$ for the di-hydrogen sodium phosphate at 18° in 0.1 N sol.

the latter two within about a week's time. In the case of these fertilizers, the surface of the soil became acid after the lower portions. When disodium phosphate was applied, the surface of the soil became alkaline to litmus, the deeper parts becoming alkaline more slowly. Tests on Section 275 (500 P) on February 18, 1915, and on 291 (1000 P) on March 22, 1915, showed that the soil at each successive inch to the bottom of the bench, was alkaline to litmus. In so far as could be determined by this method, applications of potassium sulfate and of sodium chloride did not change the reaction of the soil.* Hence an opportunity was given to study the effect, upon the acidity of the cell sap, of fertilizers producing increased acidity in the soil, alkalinity, and no change in reaction, and upon the relation the changes bore to injury from overfeeding with the fertilizer.

Determinations were made by titrating at about 15° with CO₂-free KOH, approximately 0.02 N, 1 cc. portions of sap diluted to 6 cc. with CO₂-free water, using phenolphthalein as the indicator. Results are calculated as cc. of normal acid per cc. sap.**

TABLE X.—ACIDITY OF PLANT SAP.***

Date.	Sample No.	Section.	Treatment.	Condition of plants.	Cc. N acid.
12-10-14	1	291	1000 N	affected	0.03068
	2	293	1000 K	normal	0.02492
	3	295	1000 P	normal	0.05490
	4	289	check	normal	0.02048
	5	261	check	normal	0.02090
	6	265	250 N	normal	0.02238
	7	267	500 N	affected	0.02728
	8	277	125 K	normal	0.02088
	9	279	250 K	normal	0.02002
	10	281	500 K	normal	0.02130
	11	283	check	normal	0.02130
1-14-15	12	269	check	normal	0.01977
	13	271	125 P	normal	0.05035
	14	273	250 P	normal	0.06438
	15	275	500 P	normal	0.07415
	16	277	125 K	normal	0.02319
	17	279	250 K	normal	0.02039
	18	281	500 K	affected	0.02422
	19	283	check	normal	0.02252
	20	285	125 NaCl	affected	0.01870
	21	287	500 A. P.	vigorous	0.06981

* See, however, Maschauff.²⁰** For memoir on acidity in plants, see Astruc.²*** Boiling a solution of CO₂ in distilled water under diminished pressure by warming the test tube with the hand was found completely to remove the CO₂. Similar treatment of sap gave identical values for acidity before and after. Hence, the acidity was not due to dissolved CO₂.

Acidity values remained about the same when potassium sulfate was applied, but increased after applications of acid phosphate, ammonium sulfate or disodium phosphate, being proportional in each case to the amount put on the soil. The increased total acidity following applications of disodium phosphate (which is alkaline to phenolphthalein) was unexpected and a more detailed study was made of the sap from these plants. Ether-soluble acids were absent and none of the phosphate was extracted by moisture-free ether. Phosphate was determined in 1 cc. portions of Samples 12-15 and the total acidity of the solution calculated on the assumption of the phosphorus being present (1) as orthophosphoric acid, and (2) mono-alkali phosphate,* the values being given in Table XI.

TABLE XI.—ACIDITY OF SAP BY TITRATION AND CALCULATION.

Sample No.	Treatment.	Mg ₂ P ₂ O ₇ .	Acidity.		
			As H ₃ PO ₄ .	As XH ₂ PO ₄ .	By titration.
12	check	0.0015	0.02692	0.01346	0.01977
13	125 P	0.0061	0.10768	0.05384	0.05035
14	250 P	0.0075	0.13460	0.06730	0.06438
15	500 P	0.0096	0.16348	0.08174	0.07415

The values calculated as XH₂PO₄ agree more closely than those for H₃PO₄, pointing to the presence of the phosphate as mono-alkali phosphates. Subtraction of the "check" value for Mg₂P₂O₇ from each of the other values to obtain the increase in phosphate intake due to applications of disodium phosphate and comparison of the titratable acidity calculated from these results with the excess of acidity of the solutions over that of the "check" gives the following results:

TABLE XII.—ACIDITY AND PHOSPHORUS CONTENT DUE TO OVERFEEDING.

Increase in P ₂ O ₅ .		Titration. (3) As MgmH.	Ratio. (3)/(2).
(1) As Mg ₂ P ₂ O ₇ .	(2) As MgmH.		
0.0046	0.03999	0.03058	0.765
0.0060	0.05388	0.04461	0.827
0.0081	0.07276	0.05438	0.747

The ratio between the value of H determined by titration and by the gravimetric method at 15° was determined to be 0.905, so that the ratios obtained are in the same direction, although the lower values for the sap indicate that some of the phosphate may have been present as the mono-hydrogen phosphate.

This method was applied to the problem of determining the salt in form of which phosphorus enters the plants. In every case increasing applications of disodium phosphate gave higher acidity values. When brown rock phosphate was used (nasturtiums grown in sand culture with Hopkin's nutrient solution omitting phosphorus after the first application) a regular increase up to a maximum in size of plants followed by a

* Two hydrogens of orthophosphoric acid and one of monosodium phosphate when the solution is concentrated at 0° and phenolphthalein is the indicator.²⁸ At higher temperatures, hydrolysis of the salt increases the alkalinity of the solution.

decrease was obtained, without a consistent variation in the acidity of the sap. Rock phosphate fertilizer apparently is not taken into the plant as mono-calcium phosphate.

Reaction of the soil to litmus paper was determined from time to time. After the first applications of sodium phosphate the soil reacted alkaline to litmus on the surface, with decreasing alkalinity or acidity as the distance below the surface increased. On March 22, 1915, Section 295 (to which applications of 1000 g. of sodium phosphate had been made) was found to have an alkaline reaction to litmus paper when tested for each inch of soil down to the bottom of the bench (5 inches). Two shoots each from plant Number 4, badly injured, and plant Number 12, apparently normal, were taken and the sap expressed without previous freezing. The sap reacted acid to phenolphthalein in each case.

The power of soils to absorb bases from salts is well known.⁶ With this in mind, a liter of solution of disodium phosphate was made up with carbon dioxide-free water, and aliquot portions titrated with standard sulfuric acid to a faint rose coloration, using phenolphthalein as the indicator. Six carnation cuttings, rooted in water, were cleansed by repeated washing with distilled water and floated on the surface of 500 cc. of the solution by placing them in holes of a paraffined cork. They were placed in the greenhouse for six days, covered with a large bell jar and shaded during the daytime. The cuttings were taken out, the solution carefully rinsed off and after removal of the roots the remainder of the shoots was frozen, the sap expressed, and 1 cc. portions titrated with standard alkali, using phenolphthalein as the indicator. Comparison was made with the acidity of the sap from cuttings taken from the cutting bench and prepared as in the former case for sap expression.

STRENGTH OF SOLUTION 2 G. $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ PER LITER.			
Titration of 10 cc. portions. H_2SO_4 (0.01550 <i>N</i>).		Titration of plant sap. KOH (0.02130 <i>N</i>).	
(1).	(2).	(1) Check.	(2) Treated.
0.32 cc.	0.31 cc.	0.92 cc.	1.31 cc.
0.0048 cc. <i>N</i> alkali per cc.			

In the absence of soil, the sap had become more acid when the plants were grown in the disodium phosphate solution, hence the increased acidity could not be attributed, at least entirely, to the absorptive power of the soil for bases.

Effect of Large Applications of Potassium Sulfate on Carbohydrate Content of Sap and Foliage.—The increased exudation of nectar and gluing together of the petals in the flowers on plants which had been treated with large amounts of potassium sulfate has been listed among the characteristic signs of overfeeding with this fertilizer (page 2787). An attempt was made to determine the cause of this increased flow.

The amount of nectar present in an affected flower amounted to as much as 1 cc. in the spring of 1912-13, when applications of potassium sulfate,

moderate when compared with those used in 1914-15, were made weekly during the season October to May. In 1914-15 the flow was not so plentiful, although noticeably greater than in the "check" flowers. In the former year, the nectar was a brownish liquid with a sweet and bitter taste, miscible with water, while in the latter year it was a clear, colorless liquid. It had a sweet taste, and was neutral to litmus and phenolphthalein. It charred on ignition on a platinum foil, with the odor of burnt sugar, leaving a small amount of ash which was alkaline to moist litmus paper and to phenolphthalein. Sodium and potassium flame tests were positive, calcium doubtful. No indication of tannin was given by tests with neutral ferric chloride and with potassium ferricyanide and ammonia. A solution made by washing off the nectar with distilled water reduced Fehling's solution. A heavy osazone precipitate of bright yellow color was thrown down upon heating it in a boiling water bath with phenylhydrazine, acetic acid and a crystal of sodium acetate, after three minutes' boiling. Ten minutes' boiling increased the amount. A much heavier osazone precipitate was given after a few minutes' boiling with hydrochloric acid, and a portion of the solution inverted by the Clerget method gave a heavier osazone precipitate than a similar amount before inversion. The rotation in a 1 dm. tube of 1.5° Ventzke was changed to 1.18° V. after the Clerget inversion. Hence, glucose and sucrose were present. The precipitate formed in the hot solution was filtered off and the filtrate again boiled till no further precipitate separated. On cooling the filtrate a further precipitate of sodium acetate and osazone separated. This osazone possessed a roset structure characteristic of maltosazone, and was soluble in the boiling solution and reprecipitated from it on cooling as is maltosazone. Not enough of the precipitate could be obtained after recrystallization for a melting-point determination.* Tests** made with a guaiacol solution and neutral hydrogen peroxide gave a negative test with the exudation, but an equally intensive color with sections of petal, ovary, leaf and stem of both normal and affected plants. Neither of the reagents used alone gave a reaction. Microscopic examination of the lower, plasmolyzed portions of the petals showed the cell walls intact and of normal thickness. It was concluded from this that the increased amount of sugar was not due to breaking down of these cell walls, but was an exudation. Experiments were then undertaken to compare the sugar content of the sap expressed from the stems of the plants not fertilized and of those receiving applications of potassium sulfate. Evidence that a larger amount of sugars was present in the sap of the latter plants was

* Brown and Morris⁵ used 200 g. of leaf tissue in order to obtain enough for preparation of maltosazone.

** Grüss¹⁶ believed gummosis might be caused by an excess of diastatic enzyme, and used this reagent as a means of detecting it.

found during the determination of total solids of the sap (*vide supra*), when the residue from this sap was of greater weight and charred at a lower temperature than that of the check.

The comparative optical rotations* and copper-reducing powers of sap from "check" sections and those which had received applications of potassium sulfate are shown in Table XV.

TABLE XV.—OPTICAL ROTATION AND CU-REDUCING POWER OF SAP SOLUTIONS.

Date.	Treatment.	Rotation circ. degrees.			Reducing power. Mg. CuO.		
		Orig.	Hydrolyzed. ^e	Complete. ^g	Orig.	Clerget.	Complete.
1-9-15 ^a	check	0.73	0.83
	125 K	1.91	1.25
	250 K	1.42	0.97
	500 K	1.49	1.21
2-10-15 ^b	check	3.23	1.20 ^f
	K	3.51	1.35
2-17-15 ^c	check	2.81	1.53	0.67	556	1434	1654
	250-500 K	3.26	2.28	0.79	476.5	1461	1976
3-9-15 ^d	check	2.43	1.43	1.06	521	1276	(1282)
	250-500 K	3.00	1.91	1.34	522	1273	1438

In view of the work of Davis, Daish and Sawyer,⁹ it seems possible, though not proven, that the quantitative relationships of the sugars in expressed sap may not represent the condition within the living tissue. The consistently higher values obtained by both methods of estimation, showed, however, that the application of potash to the soil had resulted in an increased carbohydrate production, in a more rapid hydrolysis of starch, or in a greater permeability of the cell membranes in the mesophyll tissue, so that a larger amount of sugar was found within the conducting and storage tissues.

Leaf tissue (Set 2-10-15) dried at 50-70° was extracted with 80% alcohol (1 g. pptd. CaCO₃ being added to neutralize acids present) and the extracts, after removal of alcohol, cleared with 5 cc. neutral lead acetate, 1 cc. basic lead acetate and alumina cream. The extracts from 7 g.

* A. Schmidt and Häusch half-shadow polariscope, with tubes 4 dm. long, was used. CuO values were obtained by using Defren's¹⁰ solution, the copper being determined by Low's method (Treadwell and Hall, p. 682).

^a 5 cc. sap diluted to 50 cc. cleared with 5 cc. basic lead acetate (sp. gr. 1.115) and an excess of alumina cream.

^b 20 cc. sap diluted to 100 cc. cleared with 10 cc. basic lead acetate and alumina cream.

^c 10 cc. sap diluted to 100 cc. with 5 cc. basic lead acetate and alumina cream.

^d 10 cc. sap diluted to 100 cc. with 2 cc. basic lead acetate and alumina cream.

^e Hydrolyzed 24 hours with 10% 0.5 N HCl at 70°.

^f Clerget inversion.

^g Inversion for 3 hours in boiling water bath of 25 cc. soln. 12½ cc. water and 2.5 cc. HCl sp. gr. 1.19.

made up to 100 cc. gave values shown in Table XVI. A trace only of pentoses was found in the extract.

TABLE XVI.—SUGAR DETERMINATIONS IN EXTRACTS.

Section.	Treatment.	Cupric-reducing power. Mg. CuO.		
		Original.	Clerget.	Complete.
268-270	check	398.0	1656.8	1933.6
277	125 K	652.8	1873.6	1990.4

The results are similar to those in Table XV.

Examination was made for starch in carnation leaves taken from the plant after a day of sunshine by boiling them for some time in alcohol, then in water, and testing leaf sections with an alcoholic solution of iodine; starch was found to be plentiful. Comparative determinations of the starch content* were made upon the residues from sugar extractions, using a diastase solution prepared by extraction of ground malt with monosodium phosphate solution at ice-box temperature, but not dialyzed.** Fifty cubic centimeters of water were added to the residue and the starch gelatinized by boiling for five minutes, with continuous stirring. After cooling to 60°, 5 cc. of the diastase solution were added with a pipet and digestion allowed to proceed for an hour. The mixture was again heated to boiling and 5 cc. of diastase again added and after an hour the mixture was filtered and washed thoroughly. The maltose in the filtrate was hydrolyzed to glucose by the modified Sachsse method and glucose determined with Fehling's solution, correction being made for maltose in the diastase solution. The values obtained for samples from sets of 2-10-15 and 2-17-15 are shown in Table XVII.

TABLE XVII.—STARCH CONTENT OF CARNATION LEAVES.

Treatment.	Starch per cent.	
	2-10-15.	2-17-15.
check	2.72	3.44
K	1.94	3.09

A lower starch content in "check" tissue is indicated by the results. While these analyses were not made over a long enough period to form a basis for a conception of the effect produced by potash upon carbohydrate production and transformations, the higher sugar with lower starch content is interesting in view of the work of Sherman and Thomas²⁴ upon the activating action of potassium sulfate upon diastase.

Summary.

The purpose of the investigation was to determine the effects upon the plants of large applications of certain commercial fertilizers to the soil on which carnations were grown.

* Brown and Morris⁵ state that preliminary washing with cold water as in the O'Sullivan method, is unnecessary in *Tropaeolum majus*.

** Sherman and Schlesinger, THIS JOURNAL, 25, 1619 (1913).

The injuries characteristic of an excess of each fertilizer are recorded from observations made in the greenhouse.

Determinations of dry weight and ash made upon the foliage of the plants, showed an increase in both values with increased applications of the fertilizers.

A sufficient number of determinations of the mineral constituents of the foliage was made to show the increased content of the fertilizing salts in the plants after large applications of them to the soil.

Total nitrogen determinations made upon plants in different stages of injury showed an increased intake of nitrogen when ammonium sulfate was applied but an acquired tolerance by the plant when successive small applications were made. Injury from ammonium sulfate is not proportional to the total nitrogen content.

The sap was expressed from the stems of the plants after freezing to render the plasma membrane permeable to the contents of the cells. Osmotic pressure determinations made upon this sap proved that with each fertilizer used the degree of injury varied with the osmotic pressure, but that not the same degree of injury was caused by different fertilizers at the same osmotic pressure. Injury is not a result of increased osmotic pressure exclusively.

The increase in the osmotic pressure in a series of plants on soil receiving increasing applications of commercial fertilizers was accompanied by an increase in the total solids and ash of the sap and in the amount of the fertilizer taken up by the plant.

Determinations of total acidity showed an increase in the total acidity of the sap of plants fed with ammonium sulfate, disodium phosphate and monocalcium phosphate, when phenolphthalein was used as the indicator.

The relation between the increase in total acidity and in the phosphorus content of the sap when the plants were fed with disodium phosphate proved that the phosphorus was taken in the form of dihydrogen phosphate, due, as was shown, not entirely at least to absorption of the base by the soil but to the selective action of the plant. Applications of potassium sulfate had no effect upon the acidity of the sap.

The sap from the stems of plants grown on soil to which large applications of potassium sulfate had been made showed a higher total sugar content, the same results being obtained with extracts of foliage. The starch content of the foliage of such plants was lower. These data indicate a more rapid hydrolysis of the starch in the foliage in the presence of an excess of potassium sulfate. The increased exudation of nectar in the flowers of these plants probably resulted from this increase in sugar content.

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