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The effects of large applications of

commercial fertilizers on

carnations

THE EFFECTS OF LARGE APPLICATIONS OF COMMERCIAL FERTILIZERS ON CARNATIONS

BY

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I INTRODUCTION

The use of commercial fertilizers in growing carnations has been a subject of investigation by the Illinois Agricultural Experiment Station since the fall of 1908 and some results of this study have been recently published[a]. A lack of appreciation of the relatively high plant food concentrations and often great solubilities of commercial fertilizers as compared with manure have often lead to a complete loss of a crop of flowers by florists in an effort to produce an extraordinarily large one. On this account, it was considered desirable to study the cause and effects of overfeeding with the more ordinarily used commercial fertilizers.

II EXPERIMENTS IN GREENHOUSE

The fertilizers chosen for the experiment were dried blood and later, ammonium sulfate; acid phosphate, for which commercial di-sodium phosphate was later substituted, and potassium sulfate. 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 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 thruout 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 and its preparation, etc. the reader is referred to Bul. 176 of the Illinois Agricultural Experiment Station.

Preliminary experiments during the years 1910-12 had shown that eight times the "normal" amounts of dried blood, of commercial acid phosphate, or of

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[a] "The Use of Commercial Fertilizers in Growing Carnations." H. B. Dorner, F. W. Muncie, and A. H. Nehrling. Bul. 176, Illinois Agr. Exp. Sta. (1914).

potassium sulfate used in the experimental work of 1910-13[a] could be applied without danger of overfeeding, if the dried blood was added in four installments thruout the season of August 1 to May 1. With these results in mind, preliminary experiments were made, during 1911-12, of applying these three fertilizers singly as top dressings lightly worked into the soil, at intervals of one week during the period of November 7 to April 12. Two pounds per 100 square feet of the one used was increased to one and a half times this amount in the latter part of the year. In 1912-13, applications were made in a similar manner at the rate of 1250 gms. per 100 square feet at weekly intervals during the period October 4 to April 25. The varieties Enchantress, a carnation of the "bush" type, and White Perfection, resembling the "tree" type, with thinner and narrower leaves and wiry stems, were used[b]. Comparison was made of the production and quality of flowers from these sections with those from sections fertilized "normally" and observations taken upon the specific effects of each of the fertilizers used.

In 1913-14, di-sodium orthophosphate on account of its greater solubility in water, was substituted for acid phosphate and experiments were begun with ammonium sulfate. An initial application of the fertilizer used, at the rate of 12.5 kilos per 100 square feet, was worked into the top inch of soil on December 3. For purposes of comparison a similar large application of sodium chloride was made to one section. Further applications at the rate of 1250 gms. per 100 square feet were made, at approximately weekly intervals during the period January 10 to April 4, 1914, to those sections to which sodium phosphate, dried blood, or potassium sulfate was applied. The injuries from the heavy application of ammonium sulfate and sodium chloride were so serious that no further applications of these

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[a] The experimental work referred to consisted of a comparison of the value of manure and a complete fertilizer composed of 4 pounds of dried blood, 2 pounds of acid phosphate, and 2 pounds of potassium sulfate per 100 square feet of bench space.

[b] See sections F, G, H, I Tables 2 and 3.

fertilizers were made to the sections, but ammonium sulfate at the rate of 1250 gms. per 100 square feet was applied to other sections on the remaining dates. To one set of sections finely ground limestone at the rate of 20 pounds per 100 square feet was worked into the soil with hand trowels before setting in the plants. The other set received no application of limestone. Thruout the year applications of potassium sulfate and of ammonium sulfate were made to other sections as fresh material was needed for examination or to substantiate, by duplication, the symptons noted as a characteristic result of large applications of a fertilizer. The varieties White Perfection and White Enchantress (a sport from Enchantress and practically identical in its habit) were grown in the experiments of this year.

During the season of 1914-15 the experimental sections were arranged in series of three to which was applied, after October 31 at intervals varying from one to three weeks, one of the fertilizers, ammonium sulfate, di-sodium phosphate, or potassium sulfate in amounts equivalent to 1.5 Kg., 3 Kg., and 6 Kg. respectively per 100 square feet of bench space. Also, one section received applications of ammonium sulfate at the rate of 24 pounds per 100 square feet (10,886.4 gms.); a second received applications of potassium sulfate, and a third, of di-sodium phosphate at the rate of 12 Kg. per 100 square feet. Acid phosphate was used on one section at the rate of 6 Kg. per 100 square feet and sodium chloride on another at one fourth this rate. During the season, as the need arose for further material, applications of some one of these fertilizers were made to other sections. Between the different series "check" sections, receiving no fertilizer, were placed for comparison. A space of two inches without soil separated each two of the sections.

Observations on the effects of overfeeding upon the condition of the plants, together with a study of the effects upon the production of flowers and their quality, formed the basis for the laboratory experiments described in the latter part of this paper.

1. 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[a]. The solubilities of the pure substances in water per hundred parts at 0°C. are given in Table 1. (Van Nostrand- Chemical Annual 1910).

Table 1. - Solubilities of Pure Salts in Water at 0° C.

Salt	Parts per 100	Formula
Ammonium sulfate	71.0	$(NH_4)_2SO_4$
Sodium chloride	35.7	$NaCl$
Potassium chloride	28.5	KCl
Potassium sulfate	8.5	K_2SO_4
Sodium phosphate (di)	6.3	$Na_2HPO_4 \cdot H_2O$
Calcium phosphate (mono)	4. (15°)	$CaH_4[PO_4]_2 \cdot H_2O$
Calcium sulfate	.241	$CaSO_4 \cdot 2H_2O$

Commercial acid phosphate consists of about equal parts of monocalcium phosphate and calcium sulfate. As pointed out by Cameron and Bell[14], the action of soluble phosphates "is primarily on the plant and not on the soil" so long as "the basic matter in the soil is sufficient to combine with the phosphoric acid" for "the least soluble phosphates are the ones which will be formed and will control the concentration of soil moisture." Further, "this fact, together with the well-known phenomena of absorption, gives a satisfactory explanation of the observation that the concentration of the soil moisture" (in phosphate) "is low and varies but little for different soils and with the total amount of phosphoric acid in the soils." So far, then, as increasing the concentration of the soil solution is concerned, no effect would be looked for until the addition of soluble phosphate was more than sufficient to combine with all the basic matter in the soil.

[a] The impurities in the ammonium sulfate, potassium sulfate (2.12 percent of sodium as sodium chloride, by analysis) 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.

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.

Single applications of ammonium sulfate and sodium chloride at the rate of 12.5 Kg. per 100 square feet 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 square feet, produced no signs of injury 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, then, 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

The marks of injury from overfeeding with ammonium sulfate were as follows:

(1) A marked softness of tissue was the earliest sign of overfeeding.

(2) 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 the white spots .25 to 1.00 millimeter in diameter appeared upon the upper leaves of these and the younger shoots. Microscopic examination showed the chlorophyll bearing tissue entirely plasmolyzed.

(3) In contrast to the injury from other fertilizers, practically every flower split[a]. 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.

(4) Later stages resulted in the drying up of the leaf tips, and the appearance of the white depressions upon the older leaves.

(5) The sepal tips very early became brown. Later, pustule-like elevations about 1 millimeter across appeared on them, caused by a crystal of ammonium sulfate beneath the epidermis.

(6) The injury from excess of ammonium sulfate was more rapid and pronounced in the presence of lime than without it.

Effects from Large Applications of Sodium Chloride[b]

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

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[a] Splits is a trade term denoting flowers with split calyces.
[b] 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.

Effects of Overfeeding with Potassium Sulfate

The characteristics of injury from potassium sulfate appeared in the following order:-

(1) In earlier stages a softness of tissue on days of sunshine.

(2) Drying up of the tips of the leaves and curling of the leaves upward upon their long axis. 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.

(3) A marked stunting of growth. 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.)

(4) 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 outer two or three rows opened normally. Later, the buds remained closed, altho the pistil often pushed its way out and might be seen extending an inch above the top of the bud.

(5) A marked increase in exudation of nectar in the flower was found to have caused the glueing together of the petals, and so prevented their opening. On cloudy days very frequently a calyx cup would be found completely filled with this secretion. The secretion was most plentiful in the flowers from plants receiving a moderately heavy application of potassium sulfate over a long period of time (as in the experiments of 1912-13, while the heavier applications of the succeeding years caused a noticeable but less plentiful increase). A small amount of nectar was 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.

(6) 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

(1) 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 square feet 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.

(2) 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.

2. Effects of Overfeeding on the Production and Quality of Flowers

The effect of the injuries pointed out in the preceding section is shown in the record given in Tables 2 and 3 of the production and quality of flowers grown on the overfed plants during the season of 1912-13. The daily production of flowers was totaled weekly and their quality determined by the average size and length of stem, number and percentage of flowers with perfect calyces and the number and percentage of normally strong stems (designated in the table as "firsts"). The total or average of these values for the first eleven weeks is listed under the first season, for the second eleven weeks under the second and the remaining eight under the third season in Table 2 which contains the records for the variety Enchantress and Table 3, for White Perfection.

Table 2. - Effects of Overfeeding on Production and Quality

of Carnations

Enchantress

Sec	Treatment	Flowers	Perfect		Size inches	Length of stem inches	Firsts	
			no.	percent			no.	percent
Season 1								
F	Dried blood	242	228	94.2	2.78	10.00	187	77.2
G	Acid phosphate	239	230	96.2	2.84	10.67	207	86.6
H	Potassium sul- fate	209	195	93.3	2.76	10.94	146	69.8
I	Check	218	200	91.7	2.74	11.37	161	73.8
Season 2								
F	Dried blood	269	239	88.8	3.28	16.12	264	98.1
G	Acid phosphate	246	196	79.6	3.38	16.49	242	98.3
H	Potassium sul- fate	216	163	75.4	3.29	14.69	191	88.4
I	Check	219	165	75.4	3.40	15.94	212	96.8

Enchantress (Table 2 continued.)

Sec	Treatment	Flowers	Perfect no.	Perfect percent	Size inches	Length of stem inches	Firsts no.	Firsts percent
				Season 3				
F	Dried blood	274	260	94.8	3.12	15.28	266	97.0
G	Acid phosphate	283	241	85.1	3.38	17.23	259	91.5
H	Potassium sulfate	67	55	82.0	3.13	14.88	67	100.0
I	Check	241	192	79.6	3.38	16.54	220	91.2

Table 3.- Effects of Overfeeding on Production and Quality of Carnations

White Perfection

Sec	Treatment	Flowers	Perfect no.	Perfect percent	Size inches	Length of stem inches	Firsts no.	Firsts percent
				Season 1				
F	Dried blood	264	237	89.7	2.65	10.29	236	89.3
G	Acid phosphate	256	225	87.8	2.65	10.25	227	88.6
H	Potassium sulfate	238	223	93.6	2.64	9.66	221	92.8
I	Check	219	184	84.0	2.67	9.88	200	91.3
				Season 2				
F	Dried blood	233	212	90.9	3.04	16.32	217	93.1
G	Acid phosphate	217	197	90.7	3.08	16.17	184	84.7
H	Potassium sulfate	214	201	93.9	3.30	15.02	206	96.2
I	Check	226	201	88.9	3.10	15.58	216	95.5
				Season 3				
F	Dried blood	145	136	93.7	2.92	16.40	142	97.9
G	Acid phosphate	155	137	88.3	3.05	17.65	153	98.7
H	Potassium sulfate	57	56	98.2	2.90	12.81	57	100.0
I	Check	152	132	86.8	3.11	16.66	150	98.6
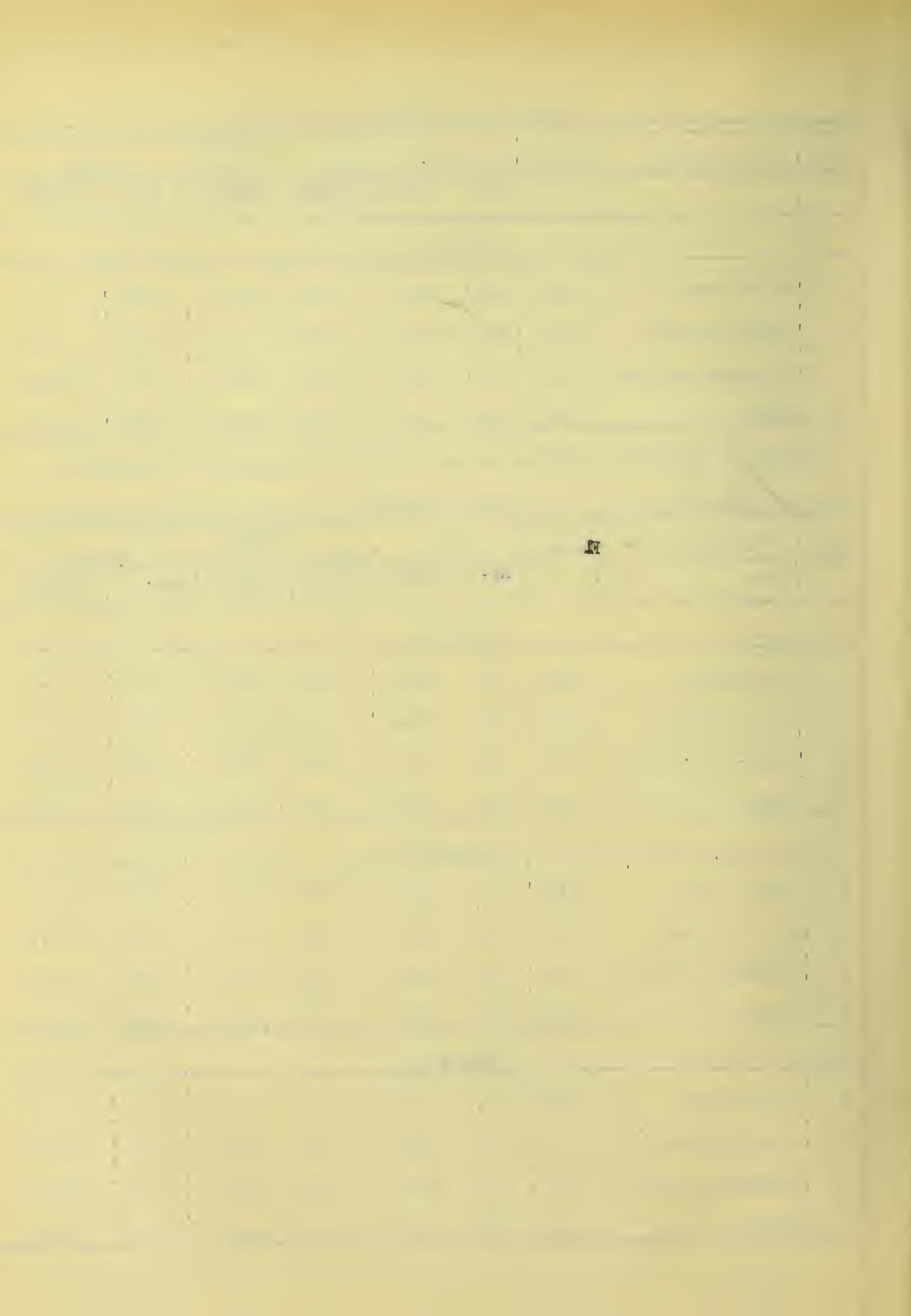
The following points are brought out in a study of these tables:

(1) Thruout the first and second seasons the number of flowers produced on plants overfed with dried blood exceeded that from any of the other treatments, while in the third season, the number was larger than that produced from the "check" plot and was exceeded only by the production from the plot to which acid phosphate was applied. When potassium sulfate was applied, Enchantress showed the injury by a lower production in each season, the difference being particularly noticeable in the last season. The variety White Perfection gave similar results with the exception of the first season, where injury does not show in the difference in production. This difference in varieties corresponds with the observation that White Perfection is less sensitive to overfeeding than the variety Enchantress. The noticeable increase in production by the plants to which large amounts of acid phosphate were applied accords with the observation that not only no apparent injury followed the use of very large amounts of this fertilizer, but the plants were more vigorous even than those of the "check" sections.

(2) In regard to the production of flowers with perfect calyces, no discussion need be made excepting to point out that even in the later stages of overfeeding with these fertilizers, no decrease in percentage of flowers with perfect calyces was found, the variation being, if anything, in the opposite direction[a]. Since the flowers on the overfed plants were not fully turgid, it might be expected that no pressure sufficient to break apart the calyx would be exerted by the growing flower.

(3) The decrease, during the later seasons in the average size of flower from those plants overfed with dried blood and with potassium sulfate, and the full size of those flowers from plants to which large amounts of acid phosphate were applied, is noticeable in the data. Similarly, a decrease in length of stem of the flowers overfed with dried blood and with potassium sulfate, and an increase quite noticeable over the "check", in the sections where acid phosphate was used in large amounts,

[a] Ammonium sulfate affected the flowers differently, however.

is apparent.

(4) The observation that Enchantress plants in the earlier stages of overfeeding with potassium sulfate showed the effect by a partially wilted condition, is borne out in the percentage of normally strong-stemmed flowers (designated "firsts") during the first two seasons. The stems of White Perfection, being more wiry on account of the heavier ring of schlerenchyma tissue, did not give signs of this loss of turgidity altho in either variety the difference, in comparison with "checks", was evident on days of bright sunshine.

III EXPERIMENTS IN THE LABORATORY

Experiments were undertaken in the laboratory to make clear some of the effects of the large applications of commercial fertilizers on the metabolism of the plants, and to show whether or not a relation existed between them and the effects upon the condition of the plants and the number and quality of flowers produced as recorded in the preceding pages. In every case the effect was studied by a comparison of the values obtained from overfed plants with those from "checks" which had received either no, or only moderate, applications of commercial fertilizer. The foliage of the plants was used for the determination of dry weight, ash, mineral and total nitrogen content. In the later work sugars and the relative starch content were estimated in the foliage of "check" plants and those which had received heavy applications of potassium sulfate. The sap from the stems of carnation shoots was expressed and the following determinations were made upon these samples:

(1) Total solids and ash.

(2) Content of the salt used in overfeeding.

(3) Osmotic pressure.

(4) Total acidity.

(5) Carbohydrate content.

(6) Free ammonia and nitrates.

1. Effects of Overfeeding on the Mineral and Nitrogen

Content of Plants

The effect of applying fertilizers to the soil upon the consequent mineral content of plants has been a subject of frequent investigation with a large number of agricultural crops, altho, to the author's knowledge, not with carnations. Analyses are recorded by Andre [2] and in Wolff's Aschen-Analysen [59] of plants of this species grown under ordinary conditions. The percentages of minerals in the pure[a] ash of the foliage are given in Table 4.

Table 4. - Mineral Content of Ash of Foliage of Dianthus caryophyllus (from Wolff)

	percent			percent
K_2O	36.88	P_2O_5		10.94
Na_2O	.38	SO_3		4.59
CaO	27.69	SiO_2		3.71
MgO	8.27	Cl		1.46
Fe_2O_3	6.42			

Pure ash 4.41 percent of dry weight.

These analyses were made upon the perpetual carnation, two to eight year stock, and so are not strictly comparable to results from first year stock used in this experimental work. However, the relatively high calcium and potassium content and the small content of chloride are noticeable. The determination of mineral content of the ash was undertaken in order to establish with these plants the actual intake of the salts applied to the soil. In the investigation of sap, the amount obtainable from material available at the time of a collection of samples usually was from 5 to 10 cc. and the accuracy of results is limited by the extent to which the small samples could be accurately measured; in some cases the results in a series are fragmentary from lack of sufficient material. The method used for ex-

[a] Wiley, Ag. Anal. III (1914) 46.

pression of sap is given under the discussion of effects upon osmotic pressure
values, since it was for this purpose primarily that the expressions were made.

The effect of applications of the fertilizers upon the dry weight and
ash is shown in Table 5, the samples being the foliage from the shoots gathered
January 9, 1915.

Table 5.-Dry Weight and Ash in Foliage

Section no.	Treatment	Moist Weight gms.	Dry Weight percent	Ash[a] percent of dry weight
269	check	27.6	17.8	13.68
271	125 P[b]	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 corres-
ponds to the higher osmotic pressure values obtained from the sap of these

[a] The ash determinations were made by careful incineration of 1 gm. of air
dry material till volatile matter was driven off, followed by treatment with con-
centrated sulfuric acid, careful heating to drive off the excess, and ignition
to white heat in a muffle.

[b] N, P, and K in the tables are used to indicate ammonium sulfate, di-sodium
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 used in each application.

plants (Table 19).

Determination of the amounts of sodium and potassium in the plants when potassium sulfate was applied gave an increase in the potassium content, and also an increase in the amount of sodium. On the other hand, when sodium phosphate was added to the soil, the sodium content increased with a decrease in the content of potassium. These results are shown in determinations from the set of samples(foliage) taken on March 13, 1913 and April 25, 1914 given in Table 6[a].

Table 6.-Sodium and Potassium Content of Plants

Sample date	Treatment	Na_2O percent	K_2O percent
3-13-13	check	1.09	5.38
3-13-13	K	1.25	6.62
4-25-14	check	.55	4.34
4-25-14	P	1.75	3.59

These results agree with the statement of Mayer[45] that in replacement of bases in the soil, potassium is able to release sodium and be itself retained by the soil.

In connection with the study of the influence of liming upon the rapidity with which injury from overfeeding became apparent, determinations[b] were made of the sulfate, phosphate, chloride, and calcium content of the foliage of plants grown during 1913-14, samples being collected April 25, 1914. The results are

[a] Alkalies were determined by driving off the volatile matter at low temperature, leaching the charred mass with hot water, igniting the residue to whiteness, then evaporating the leachings to dryness and igniting at low red heat. The rest of the procedure is as given in section (h) Bul. 107, U. S. Dept. Agr. B. Chem. p.17.

[b] The methods used were as outlined in Bul. 107, U. S. Dept. Agr., B. Chem. p. 15 et. seq. excepting that the ash was removed from the platinum dishes as well as possible with hot water, and the remainder with a little dilute nitric acid. Correction is made for calcium added, using a 50 percent alcoholic solution which wet the dry sample much better than an aqueous one.

given in Table 7, where in addition to the letters previously used to explain the treatments, the "Ca" indicates the addition of lime to soil.

Table 7.-Calcium and Acid Content of Foliage

Section no.	Treatment	CaO percent	P₂O₅ percent	SO₃ percent	Cl
275	check	6.70	.93	.85	trace
291	P	4.28	1.24	.95	trace
292	P + Ca	7.56	.91	.91	"
281	N	4.84	1.14	5.73	trace
264	N + Ca	5.00	.73	1.23	"
261	K	5.30	1.02	4.11	opalescent
262	K + Ca	6.86	1.01	3.36	"

The small amount of chloride found in spite of the care used during ignition to prevent the volatization of potassium chloride is perhaps explained by the low chloride content of the water used in the greenhouses[a]. The potassium sulfate used contained about 2 percent of sodium as sodium chloride, which accounts for the somewhat larger amount obtained when this salt was used as a fertilizer. The depressing effect of the application of sulfates and phosphate upon the content is seen in the values obtained lower than in the "check" in every case where lime was not added. The increase of phosphate content when sodium phosphate but no lime was used and when ammonium sulfate was added alone is evident. The soil in the latter case was quite acid. Where lime was used with the soluble phosphate no increase is found in the phosphate content. The values are higher for sulphate in every case where a sulfate was applied, particularly without lime, the content rising in the case of ammonium sulfate fed plants to nearly 6 percent. Since ob-

[a] Analyses of water from drift wells of this section show about 2.5 mg. of chloride per 1000 cc. U. of Ill. Bul. 4, p. 180.

servation showed that those sections which received ammonium sulfate alone did not show so serious injury as those which received limestone also, the injury must be attributed to the ammonia rather than to the sulfate.

For the determination of the total nitrogen content of the foliage of the plants, samples were prepared by grinding the dried foliage fine enough that 75 percent or more would pass a 60 mesh sieve, and nitrogen determined by the Kjeldahl method, 25 cc. of concentrated sulfuric acid with a crystal of copper sulfate and 10 gms. of potassium sulfate being employed for digestion of one gram samples. Bumping of the solution during distillation was prevented by the addition of a few pieces of granulated zinc. The receiving flasks contained from 10 to 30 cc. of standard sulfuric acid (N .1651) according to the nitrogen content of the sample and the excess was titrated back with standard potassium hydroxide (N .2105) using Congo red or sodium alizarin sulfonate as the indicator. Duplicates were run on all samples. A set of results on "check" and on potassium fed plants grown during 1912-13(samples taken 3-13-13) are given (Table 8) because they indicate the degree of variation among the individual plants.

Table 8.-Total Nitrogen Determination on Foliage

Plant no.	Treatment	Nitrogen percent	Plant No.	Treatment	Nitrogen percent
1	check	2.57	1	K	2.53
2	"	2.61	2	"	2.46
3	"	2.45	3	"	2.45
4	"	2.64	4	"	2.56
5	"	2.53	6	"	2.65
6	"	2.69	7	"	2.60
7	"	2.55	8	"	2.38
8	"	2.53	11	"	2.55
9	"	2.53	15	"	2.60
10	"	2.67	18	"	2.55
			20	"	2.55

The average from the first treatment is 2.57 percent and the maximum variation .12 percent; from the second treatment, it is 2.53 percent and the maximum variation .12 percent. These results show also practically the same percentage of total nitrogen in plants overfed with potassium sulfate as in the "checks". The average total nitrogen content of the plants is 2.55 percent.

Table 9 shows the total nitrogen content of some plants from sections 264 (ammonium sulfate and lime) and 281 (ammonium sulfate). Samples were collected on April 25, 1914. Section 281 had received but one application at the rate of 12.5 kilos. per 100 square feet on December 3, 1913, while applications at the rate of 1250 gms. per 100 square feet 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 if a localization of the salt in the more vigorously growing portion of the plant, occurred. The variety White Enchantress or White Perfection is indicated by the letter E or P after the section number.

Table 9.-Total Nitrogen Determination on Foliage

Sample no.	Plant No.	Section	Portion	Condition	Nitrogen percent
1	1	264-E	upper	half dead	4.58
2			lower	" "	4.07
3	4	264-P	upper	" "	7.78
4			lower	" "	5.64
5	1	264-P	upper	dead	6.14
6			lower	"	3.41
7	11	264-E	upper	alive	6.69
8			lower	"	5.70
9	4	264-E	upper	half dead	7.01
10			lower	" "	3.34
11	11-15	281-E	upper	dead	4.60
12			lower	"	3.02

Table 9 (continued)

13	16	281-E	upper	partially affected	4.73
14			lower	" "	3.78
15	20	281-E	upper	half dead	4.73
16			lower	" "	3.44
17	7	281-E	upper	slightly affected	4.47
18			lower	" "	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 percent and 4.43 percent respectively; for those from section 281, 4.63 and 3.38 percent. 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 direct relation is shown between the nitrogen content and the degree of injury. The higher content of the plants of the first section points out the ability of the plant to accumulate a much larger amount of nitrogen when ammonium sulfate is added to the soil in small quantities than when enough is applied to produce immediate serious injury. Either the plants became tolerant to larger quantities of the ammonium sulfate itself when the concentration in the soil solution was increased gradually, or some of the salt was transformed into non-toxic forms. The fact that the dead plants had no higher total nitrogen content than those only injured is evidence of this also, since the dead plants were in this condition as early as March 21, while the injured ones continued to transpire and undoubtedly to draw up more of the salt in solution until samples were taken. The unevenness of the bench and washing of the fertilizer during watering which was observed to take place, would account for the unequal extent of injury among the plants of the section.

Comparative determinations of ammonia were made on plant number 20,

section 281-E (N) and plant number 9, section 265-E ("check") by aspirating 2 gm.

samples in 500 c.c. Kjeldahl flasks with 20 c.c. of Greenbank alkali for 48 hours.

The receivers contained 25 c. c. of standard sulfuric acid (N .1651). The excess

of acid was titrated back with standard alkali (N .2105) using methyl orange as

the indicator. The results, calculated as mg. of nitrogen per gm. of material

were:

	No. 20, 281-E	No. 9, 265-E
Upper	5.5657	3.7548
Lower	5.2283	3.6512

The excess of nitrogen as free ammonia over the "checks" was 1.8109 and

1.5771 mg. respectively for upper and lower portions. The results do not mean that

free ammonia existed in plants unfertilized with ammonium salts, for the decomposing

action of sodium hydroxide on protein even in the cold would account for the am-

monia obtained from the "checks"; they do not even indicate the presence of am-

monium salts in the plants fed with ammonium sulfate, but simply that treatment

in the cold with concentrated alkali produced a larger amount of ammonia in these

samples than in the "checks". 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[29]

micro-method for the determination of free ammonia in urine was used on the as-

sumption that a method applicable to the estimation of ammonia in the presence of

such an easily decomposible substance as urea would be suitable for the plant sap.

The excess of sulfuric acid (N .01550) was titrated back with potassium hydroxide

(N .02130) using sodium alizarin sulfonate as the indicator. The pipettes, whose

calibration is given in the description of methods for determining acidity, were

used, so that an accuracy of 1 percent is probable. Results are given in Table 10.

Table 10.-Free Ammonia in Plant Saps

Sample no.	Treatment	Condition	Nitrogen mg. N per c.c.
5	check	normal	none

Table 10(continued)

8	250 N	normal	.1834
7	500 N	"	.1372
2	1000 N	slightly injured	.639
1	1000 N	badly "	1.056

The presence of white spots on the leaves of plants treated heavily with ammonium sulfate, and of crystals imbedded beneath the epidermis of the sepals has been mentioned in the description of the symptons of overfeeding with this salt. Proof for the presence of ammonium salts was sought for by microchemical means.[6]

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 chlorplatinic acid after removal of the epidermis and allowed to remain over night. Large and perfect crystals appeared, arranged usually around the injured spot, never in it. They were insoluble in 95 percent alcohol which removed the excess of chlorplatinic 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 on a cover glass placed on a glass ring above it. Treatment as above gave small, yellow tetrahedra insoluble in 95 percent alcohol.

Nitrate determinations were made upon the sap of a "check" and an ammonium sulfate fed plant from the set of March 9, 1915. The phenol-sulfonic acid method recommended by Mason[43] was used since the determinations given in Table 7 had

shown that only a trace of chloride was present in the plants. The values of .01
and .40 mg. N as nitrate per c.c. of sap respectively showed that nitrification was
proceeding in the soil altho it was quite strongly acid.

Total solids and ash were determined on the sap of 12-9-14, 1 to 4 c.c.
being used. Similar determinations were made on sap taken from plants on 1-9-15.
The results, given in Table 11 are calculated to mgs. per c.c. of sap.

Table 11.-Total Solids and Ash of Sap

Sample no.	Set date	Section	Treatment	Total solids mgs.	Ash[a] mgs.
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[b]	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

[a] Ash determinations upon the sap were made by careful incineration of the
solids in 1 c.c. of sap in platinum dishes over a low flame to prevent mechanical
loss of particles of the ash. The low chloride content does away with the danger
of volatilzation of potassium chloride by high temperature.
[b] 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.

Table 11 (continued)

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[b]	check	105.1	14.1

The average total solids content of the sap was 85.1 mg. per c.c. 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 determination of total solids with
accuracy is not possible on account of the colloidal and uncrystallizable solutes
in the sap. 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[a]. The first set of data was obtained by drying 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 suc-
cessive heatings.

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 di-sodium phosphate. The
results, calculated to mgs. per c.c. of sap are given in Table 12.

[a] See pages 34 and 45.

Table 12.-Mineral Content of Sap

Sample no.	Section	Treatment	Na_2O mg.	K_2O mg.	$Mg_2P_2O_7$ 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

The data given have shown that when ammonium sulfate, potassium sulfate or sodium phosphate is used in heavy applications, there is an increase in the acid and base content of the plant; that potassium partially displaces sodium from the soil but is not itself displaced by the sodium; that calcium restricts the intake of phosphate and sulfate into the plant; that when ammonium sulfate is applied the total nitrogen content of the plant increases, and there are ammonium salts in the sap. The results were obtained both upon the foliage and the sap of the plants.

2. Effects of Overfeeding on the Osmotic Pressure

of the Cell Sap

Determinations of the osmotic pressure of the cell sap of overfed plants in comparison with "check" plants were undertaken with the expectation of establishing a relationship between the different values for osmotic pressure obtained after different fertilizers or one fertilizer in different amounts had been applied, and the rapidity and degree of injury from overfeeding. The method consisted in the expression of the sap and determination of the osmotic pressure by

the relation between its value and the lowering of the freezing point. Dixon and Atkins[24] in their investigation of the variations in the osmotic pressure of the cell sap of _Hedera helix_, and Atkins[4] in the study of the osmotic pressure of plant organs showed that the greatest variations in the osmotic pressure occurred in the leaves, which elaborate material and store little or none, while similar plant organs in the same species, excepting the leaves, had similar osmotic pressures. A variation was found in the osmotic pressure due to different ages of leaves on the plants and to different locations with respect to the sun. For instance, the ultimate, penult, and antepenult leaves of _Hedera helix_ were found to give in this order higher values, and leaves located on the south side of the plant to have a higher osmotic pressure than those on the north. Ewart[27], also, in the study of the ascent of sap in trees proved that a progressive lowering of the osmotic pressure occurred as the distance above the ground increased. Drabble and Drabble[25], in their study of the effect of physical environment on the osmotic strength of cell sap of plants concluded "(1) that in any area the osmotic strength varies with the physiological scarcity of water; (2) in all plants growing under the same conditions, the osmotic strength of the cell sap is generally the same; (3) the effect of increased temperature on the osmotic strength of the sap is quite appreciable." Cavara's[16] conclusions after a series of determinations of osmotic pressure values of cell sap had been obtained, were that (1) each species of plant had a characteristic osmotic pressure; (2) that variations due to ecological conditions ranked before those due to the plant structure and the elaboration of material capable of exerting an osmotic pressure when in solution in the sap. He pointed out that in halophytic plants, which are subjected to conditions similar to overfed ones, variation of the saltiness of the substratum was capable of causing a large variation in the osmotic pressure. It was also proven that the osmotic pressure varied with the progress of the assimilative processes, and was higher in the evening than morning.

These conclusions of previous workers determined the precautions necessary

in the selection of samples from the plants. On account of the variability in the
osmotic pressure of the sap from leaves, it was decided to obtain the sap from the
stems. Another reason which influenced this choice was the fact that the stems
contain the main conducting tissues, and the sap from them would, more nearly than
that from the leaves, represent the actual absorption of the salts from the soil.
Obtaining the sap from the roots for a series of determinations thruout the year
was, of course, not possible. In any organ, however, the osmotic pressure values
would be changed by any variation in the amounts of organic acids, carbohydrates,
etc. elaborated by the plant under influence of the salts absorbed from the soil.
Samples for a series of determinations were always collected from the several
sections with as little delay as possible. Care was exercised that no draft was
stirring the air in the greenhouse for some time before the samples were taken and
the differences due to variations in the temperature in different parts of the
house (at the times amounting to as much as five degrees) obviated as nearly as
possible by having the sections from which the samples to be compared were secured,
small (3 x 4 feet), and adjacent. Shoots with buds one-half to two-thirds fully
developed were chosen, and the several shorter internodes at the bottom and the
two just below the bud discarded. On account of the long period of cloudy weather
during the late fall of 1914, it was not possible in the successive sets to secure
samples taken under the same conditions of sunlight and temperature. Differences
existed also in the percentage of moisture in the soil when the different sets of
samples were secured. Hence the values from the same section at different times
were not comparable as were the values from samples taken from different sections
at the same time.

Attempts were made in the preliminary work to determine the osmotic
pressure values by the plasmolytic method, that is, to measure the osmotic pressure
in terms of the concentration of a salt solution isotonic with the cell sap. The
method used by DeVries,[21] of observing the separation of the plasma membrane from

the cell wall, was discarded because the absence of colored cell sap in the carnation made the observations too inaccurate to give a practicable method. That of determining the concentration of solution at which portions of leaf with the epidermal tissue removed from one side showed no change in curvature[7] after immersion in solution, also was found too approximate to be satisfactory. A third method is based upon the loss or gain of weight of a portion of the material after successive immersions in solutions of different concentrations, removal of the solution from the surface and weighing.[8,9,10] This method, tried with portions of stem and root one inch long split into four quarters, and of leaf with the epidermis in part removed, gave fairly satisfactory results, but was discarded because of its tediousness.

The researches of Osterhout and other workers[a] have shown that the permeability of the plasma membrane is subject to periodic variations and is changed under the influence of the ions in contact with it; and that "contraction may take place, due to chemical action and not osmotic pressure." The large amount of experimental work based upon the plasmolytic method makes it questionable whether such variations would affect the results based upon any but very slight changes in the osmotic pressure. Yet, in this study, the use of such methods would necessitate the additional investigation into the effect of the increased amounts of the salt, taken into the plants under the conditions of the experiment, upon the permeability of the plasma membrane[b]. While a study of the injurious effects of overfeeding might be approached in this way, it was not desirable so to complicate the determination of relative osmotic pressure values. The method based on the determination of the lowering of the freezing point does away with the necessity for considering these factors in the osmotic pressure determinations.

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[a] See references (57), (50), (46), (41), (42), (56).
[b] Lewis[39] found that leaves of a number of non-halophytes after immersion in sea water possessed a higher chloride content and osmotic pressure than before.

In 1913, Dixon and Atkins[24] published evidence confirming the observations of Andre[1] that when sap is expressed from fresh tissue, the successive portions become more concentrated. They further showed that when tissue is first frozen by means of liquid air, thawed, and then the sap expressed, the values for freezing point lowerings are always greater than those obtained on sap expressed from untreated tissue.   Successive pressings, also, gave nearly identical lowerings. The natural conclusion is that the sap expressed from tissue so frozen has the same concentration as that originally within the tissue. Gortner and Harris[30] worked out a method using an ice and salt bath and this was followed in preparing the tissue of the carnations for expression. Gortner and Harris do not publish data on the concentrations of successive portions of sap secured by expression of tissue so frozen, and Maximow[44] in his researches on the protective action of salt solutions against freezing of cells of red cabbage and Tradescantia has proven that some salts do exert such a protection against freezing. The death of these cells is usually brought about, however, after exposure to a temperature of around -17° C. for four to five hours, even after immersion in the salt solutions. An exposure for some twelve hours to around -15° C. as obtained in the method used in the following experimental work would without doubt be sufficient to produce death. The ease with which the sap could be expressed and its clearness are evidence, also, that the plasma membrane did not retain its semipermeability.

The method ordinarily used for the determination of freezing point lowering is that of Beckmann. The use of a Beckmann thermometer necessitates obtaining 12 to 15 c.c. of sap, (enough completely to cover the bulb). Dixon and Atkins[23] and Ohlweiler[49] have made use of a thermo-couple in place of the Beckmann, the method requiring as little as 2.5 c.c. of sap, according to the first authors. The number of carnation shoots of approximately the same stage of growth available at any one time was usually less than eight, and yielded less than 10 c.c. of sap. It was found in preliminary experiments, however, that the differences in the freezing

points of sap from the differently treated plants was large enough to give ample
accuracy for the purposes of the investigation when a thermometer, reading to hun-
dredths of degrees only, and requiring only 5 c.c. for covering the bulb, was used.

Lewis[40] has developed the relation between the lowering of the freezing
point and the osmotic pressure of a solution and expressed it by the equation:

$$\pi = 12.06\,\Delta - .021\Delta^2$$

where π is the osmotic pressure in atmospheres, and Δ the lowering of the freezing
point in centigrade degrees. In order for the second term on the right of the
equation to produce a variation of one tenth atmosphere, the value of Δ must be
larger than 2.4. Since the differences in the values of the osmotic pressure con-
siderably exceeded one tenth atmosphere, the second term was omitted in the calcu-
lations and use made of the more approximate relationship:

$$\pi = 12.06\,\Delta$$

Dixon and Atkins determined the value for Δ by slightly supercooling the
solution and causing crystallization by the addition of a minute crystal of ice.
In this experimental work it was found more convenient to follow again the method
recommended by Harris and Gortner,[33] that of allowing supercooling until the solution
freezes, then correcting the value of Δ' obtained, according to the formula:

$$\Delta = \Delta' - .0125\,u\,\Delta'$$

where Δ' is the maximum temperature attained by the system and u the difference
between this value and the minimum temperature.

Description of Experimental Method

A description of the procedure follows. 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 were removed from each of the sections of
plants and quickly taken to 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. x 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[a], giving a temperature of about - 15° C. and allowing the tubes to remain in the refriger- ator over night. 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 re- moved, thawed gradually, and the sap expressed. For this purpose the shoots were placed between pieces of 3/8 inch plate glass, and pressure exerted by the screw from a tincture press set perpendicular to the wall. After a first expression, the shoots were rearranged and pressure again applied. The sap was filtered thru an S. & S. 589 filter-with a watch glass over the funnel to minimize evaporation - into a small test tube; a drop of xylol was added as a preservative and the tubes placed at once in a refrigerator, the temperature in which was about 10° C. The sap after filtration was usually a clear brown liquid without sediment.

As soon as convenient the freezing point determinations were made. A thermometer (E. H. Sargent & Co. make) with a bulb about 5 mm. by 35 mm., and the mercury tube enclosed in a hollow jacket, was used for the determinations. It was graduated to -6.5° in tenths of degrees, and, by the aid of a magnifying lens, hundreths of a degree could be read without danger from parallax. A stirrer of platinum wire and the thermometer were placed in the 5 c.c. of sap contained in a test tube of Bohemian glass (15 x 120 mm.) and the whole cooled to about +2° C. 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. x 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

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[a] 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.

Dewar bulb, with inside dimensions of 35 x 130 mm.; the top was closed with a

piece of cork. This bath so arranged remained 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 hundreth degree. The

tube was removed to a beaker of water, and after the temperature had risen to about

10° C., the determination duplicated to within one hundredth of a degree, usually

without difficulty on the first trial. A typical determination gave the following

values:

$$\Delta' = 1.28 \qquad U = 4.12 \qquad \Delta = 1.214$$

$$\Delta' = 1.27 \qquad U = 3.43 \qquad \Delta = 1.216$$

Average 1.215 from which

$$\pi = 14.64 \text{ atmospheres}$$

Experimental Data

Set 1. 10-28-14. Before the first applications of fertilizer were

made to the sections, material was taken from sections 261, 262, 281, and 282.

10 a. m. No sunshine.

Table 13.-Osmotic Pressure Determinations[a]

Date	Sample no.	Section	Treatment	Δ'	U	Δ	π
11-3-14	1	261	check	1.32	4.70	1.203	14.47
"	2	262	"	1.34	4.18	1.23	14.83
"	3	281	"	1.34	3.88	1.24	14.92
"	4	282	"	1.38	3.81	1.27	15.31

The mean of the determinations was 14.88 and the maximum variation from

it .43 atmosphere pressure. The values in this set for "check" plants were higher

[a] Calibration of the thermometer by freezing distilled water gave a freezing
point of +.04, constant before and after a set of determinations were made and on
successive days. Corrections are made in the values given for Δ and π .

than any obtained later from "check" plants. As it was impossible to count on
having the same moisture content in the soils and similar temperature conditions at
times when the sets were taken, it is not surprising that considerable variation
should be found in the values from time to time.

Set 2. Samples taken 11-3-14. 10 a. m. Clear. Soil moist. One
application of fertilizer on 10-31-14.

Table 14.-Osmotic Pressure Determinations

Date	Sample no.	Section	Treatment	Δ'	U	Δ	π
11-4-14	5	289	check	1.12	4.03	1.024	12.33
"	7	293	1000 K	1.23	3.77	1.130	13.60
"	8	295	1000 P	1.08	3.12	.960	11.57

The osmotic pressure in the plants to which potassium sulfate had been
applied was higher than the "check", while that for plants to which sodium phos-
phate was added was even somewhat lower than the "check".

Set 3. 11-12-14. 10 a. m. Clear. Applications of fertilizer made
on 10-31-14 and 11-10-14.

Table 15.-Osmotic Pressure Determinations

Date	Sample no.	Section	Treatment	Δ'	U	Δ	π
11-17-15	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	.946	11.41

The plants in section 291 already showed the characteristic white
spots from overfeeding with ammonium sulfate, while the plants from other sections
were apparently normal. The osmotic pressure of the plants receiving potassium

sulfate was somewhat higher than that of those receiving ammonium sulfate, altho
injury was apparent only on the latter.

Set 4. 11-20-14. 10 a. m. Cloudy. No draft. Applications of
fertilizer 10-28-14; 11-10-14; 11-16-14.

Table 16.-Osmotic Pressure Determinations

Date	Sample no.	Section	Treatment	Δ'	U	Δ	π
11-25-14	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	.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
	7	283	check	1.27	4.43	1.160	13.99

In this set also, the plants fed with potassium sulfate had a higher osmotic
pressure than those treated with ammonium sulfate, yet did not show signs of in-
jury. The osmotic pressure of the plants treated with sodium phosphate was lower
than that of the "checks", as in set 2. The mean of the values from "checks" is
13.41 atmospheres and the maximum variation is .58 atmosphere.

Set 5. 12-9-14. After eight days of cloudy weather. 10 a. m. No draft.
Applications of fertilizers on 10-31-14; 11-10-14; 11-16-14; 11-23-14; 11-30-14
on sections 293, 265, and 267.

Table 17.-Osmotic Pressure Determinations

Date	Sample no.	Section	Treatment	Δ'	U	Δ	π
12-16-14	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	.95	4.55	.856	10.34

Table 17 (continued)

12-16-14	6	261	check	.99	3.91	.901	10.86
"	7	265	250 N	1.10	5.10	.990	11.94
"	8	267	500 N	1.28	5.40	1.174	14.16

Sample number 2 was taken from plants only slightly injured by the ammonium sulfate, number 1 from those badly affected, the greatest osmotic pressure being correlated with the more serious injury. In all sections treated with fertilizer the osmotic pressure in the plants exceeded that in the "checks". In samples 6, 7, and 8 the effect upon the osmotic pressure of increasing amounts of ammonium sulfate is brought out.

Set 6. 12-9-14. 4 p. m. Cloudy. Applications of fertilizer to these sections on 10-31-14; 11-6-14; 11-16-14; 11-23-14; 11-30-14.

Table 18.-Osmotic Pressure Determinations

Date	Sample no.	Section	Treatment	Δ'	U	Δ	π
12-14-14	1	277	125 K	1.05	3.75	.962	11.60
"	2	279	250 K	1.18	4.82	.973	11.73
"	3	281	500 K	1.18	4.32	1.076	13.01
"	4	283	check	1.06	4.14	.967	11.68

The effect on the osmotic pressure of increasing amounts of potassium sulfate is shown in this table. The total solids and ash determinations on samples 2 and 3 have values corresponding to the slight differences found in the osmotic pressures of these two samples[a].

Set 7. 1-9-15. 4 p. m. No ventilation nor draft in greenhouse. Cool, sunny day. All plants turgid. Applications of fertilizer in addition to those

[a] Page 22. The abnormally high values of sap from this "check" section are seen in the values for total solids and ash given there.

listed in set 6, on 12-16-14; 1-4-15.

Table 19.-Osmotic Pressure Determinations

Date	Sample no.	Section	Treatment	Δ'	U	Δ	π
1-18-15	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	21.36
"	10	287	500 A.P.	1.48	5.52	1.338	16.13

Samples 1 to 4 showed the effect on the osmotic pressure of applications of sodium phosphate in increasing amounts. Similarly the effect of increasing amounts of potassium sulfate was shown by results on samples 5 to 8. A section treated with sodium chloride for the sake of comparison with the fertilizers used gave a very high value for the osmotic pressure altho the application (125 gms. per application) was comparatively moderate. Of the plants from which samples were taken only those from sections 281 and 285 showed signs of injury. These two sections gave osmotic pressures of 20.76 and 21.40 atmospheres respectively. On the other hand the plants in section 287 to which acid phosphate was applied were the largest and most vigorous in the house, and yet gave an osmotic pressure value of 16.11 atmospheres, considerably above that of the "check" plants.

Set. 8. 2-10-15. 4 p.m. After 3 days of sunshine. A slight draft. Applications of fertilizer in addition to those listed in set 7 were made on 1-15-15;

2-6-15. Three separate samples were taken from each treatment. Shoots taken from the plants for securing these samples were in all stages of growth, from those having buds less than 1/3 developed to those with full grown flowers. The "checks" were made up of 37 shoots of which 6 had flowers, 15 fully grown buds and 16 buds 1/2 or less than 1/2 developed. Of the 35 shoots from plants treated with potassium sulfate, 5 bore flowers, 15 fully grown buds, and 15 buds 1/2 or less than 1/2 developed, hence, the averages of samples were comparable in this respect.

Table 20.-Osmotic Pressure Determinations

Date	Sample no.	Section	Treatment	Δ'	U	Δ	π
2-12-15	1	270	check	1.14	6.06	1.014	12.24
"	2	270	"	1.06	4.84	.956	11.56
"	3	268	"	1.20	4.10	1.099	13.24
"	4	277	125 K	1.38	4.82	1.257	15.16
"	5	277	125 K	1.42	4.78	1.295	15.65
"	6	277	125 K	1.36	3.64	1.258	15.19

The mean of the determinations from "check" plants was 12.35 atmospheres and maximum variation .89 atmosphere. That of the plants treated with potassium sulfate was 15.33 atmospheres and the maximum variation .32 atmosphere. The difference between the means of the two sets was 2.98 atmospheres.

Discussion of Results

As pointed out before, no comparison can be made between the values for the osmotic pressure determined in successive sets. The mean of all "check" determinations is 12.95 atmospheres and the maximum variation more than 2 atmospheres. 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 (Table 17) 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 (Table 19) 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 (Tables 14, 15, 16, and 19). The samples taken on 11-12-14 and 11-20-14 (Table 15 and 16) 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 3, Table 17) the relative values are reversed.

In the set of 12-9-14 (Table 17) plants treated with potassium sulfate at the rate of 1000 gms. per section per application were still apparently normal, altho 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 gms. per section per application) when the osmotic pressure amounted to 11.94 atmospheres as compared to 10.86 atmospheres in the adjacent "check" section (Table 17).

The values of samples 1 and 2, Table 17, showed that the greater degree of injury with ammonium sulfate was accompanied by a larger value for the osmotic pressure. Injury appeared on the plants from sections to which potassium sulfate was applied, only when an osmotic pressure of over twenty atmospheres was reached (Table 19), 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 (Table 19). The conclusion to be drawn from these facts is that, with a single fertilizer, injury from overfeeding becomes apparent when a cer-

tain osmotic pressure is reached, but that this value is different for the different fertilizers. If it could be shown that the increase in osmotic pressure was due solely to increased intake of the salt used in overfeeding, this would amount to saying that injury became apparent when the concentration within the plant of the salt used in overfeeding reached a certain value. The accuracy of the analytical methods used on the small quantities available was not sufficient to attempt to determine whether this was true; the larger amounts of sugars found in the sap from potassium fed plants (page 52) renders this unlikely.

The injury from applications of sodium chloride at the rate of 125 gms. 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 Table 19, samples 7 and 9. The solubilities of these salts, as pointed out on page 4 , at 0° C. are 35.7 and 8.5 respectively, giving a ratio roughly of 4 to 1.

In two sets, 2 and 4, the osmotic pressure in plants grown previous to January 9 on soil treated with sodium phosphate was slightly less than that of the "check" plants. The values in the set 7, with determinations of the phosphorus content, showed that, at this date at any rate, the phosphate content of the sap was increased. The earlier data are not corroborated by determinations of the amount of phosphate present, unfortunately, so that it could be decided whether or not the soluble phosphate in the soil was fixed by the bases present until a considerable excess had been applied, and so prevented from entering the plant. This result would be expected from the quotations already made (page 4) from Cameron and Bell.

3. 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 acidity, the first one immediately after application, 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 di-sodium 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. Tests were made upon the acidity of the sap of the plants, and quantitative determinations of the relative acidity of the plants in the differently fertilized sections.

Astruc [3] in a memoir on the subject of vegetable acids reviews the methods for determining "free and combined acids" in plants and discusses the theories of other workers in regard to the effects of acids within the plant upon its activities.

The method for determining total acidity of a sample as given by Astruc is as follows: "The organ to be studied is weighed and triturated quickly in a glass mortar with a little distilled water; there is obtained thereby a sort of broth which is transferred to a Bohemian beaker, care being taken that in the washings of the mortar the last traces of the mixture which adhere to it are removed. The vessel is then heated on a water bath to $80°$-$90°$ C., and maintained at this temperature for about 20 minutes; the mixture is filtered. The filtrate is washed with a known volume of boiling distilled water and to the clear liquid is added a certain number of drops of an alcoholic solution of phenolphtalein. Little by little, then, by means of a Mohr burette, a solution of N/50 KOH is added just to a bright rose coloration; the number of cubic centimeters of solution employed is noted and the quantity corresponding to 1 gm. of the organ calculated."

As pointed out by Detmer[20] in the discussion of methods, where it is
possible to secure enough for the purpose, the sap obtained by expression from
the organ may be titrated. Freedom is secured in this way from possible error in
transferring the material from the mortar and of insufficient washing after fil-
tration. Assuming the conclusions of Dixon and Atkins that sap expressed from
frozen tissue is an accurate representative of that within the organ, titration of
the sap so obtained from plants to be compared gives an accurate measure of their
relative total acidity. The method outlined above gives the value not only for
the acidity as measured by the concentration of hydrogen ion in the sap, but for
the reserve acidity due to the presence of slightly ionized acids or their acid
salts, which continuously ionise as titration proceeds, without changing greatly
the hydrogen ion concentration of the solution[a]. The acidity of the sap of plants
may be due to mineral acids from the soil solution, carbonic acid, acid phosphates,
and organic acids and their acid salts.

The organic acids of the sap are formed for the most part as by-products
in the process of respiration[20,36] by partial oxidation of carbohydrates. The fur-
ther oxidation to carbon dioxide and water is hastened by an increase in light
intensity and in temperature, and vice versa. The precautions taken in securing
samples for expression of sap (page 29) should prevent error arising from variations
in these factors. Carbonic acid and bicarbonates are removed in the method out-
lined. Phosphoric acid and the mono-alkali phosphates are acid to phenolphthalein
and may be titrated by the use of this indicator[58] while di-alkali phosphate is
slightly alkaline to phenolphthalein.

Methods of Determination of Acidity

Sap obtained as previously described and kept with one drop of xylol for
preservative, at icebox temperature, was used in the titrations, which were made as

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[a] Salm (52) using the gas chain method (35) found $H = 3.3 \times 10^{-5}$ in a N/10 sol.
of NaH_2PO_4 at 18°C. while the total titrable hydrogen in such a solution is around
.1 mole if phenolphthalein is the indicator.

soon as possible after expression. It was not heated since trial showed that a
coagulation of proteid occurred and it was feared that other changes tending to
vary the acidity of the solution would result. Experimental data are given lat-
er showing the absence of titratable quantities of carbon dioxide in the sap.
Portions of one cubic centimeter each were pipetted into test tubes (25 mm. by
130 mm.) and the pipettes washed successively with three portions of about 1 c. c.
each of carbon dioxide-free water. The sap was further diluted with carbon
dioxide-free water to about 6 c.c., the water being previously cooled so that
the titration was carried out at the temperature of about $15°$ C. Three drops of
a 1 percent solution of phenolphthalein in 50 percent alcohol were added, and
standard carbon dioxide-free KOH, approximating N/50, added from a Mohr burette,
drop by drop, to faint rose coloration. Results were calculated as cubic cen-
timeters of normal acid per c.c. of sap.

Experimental

Pipettes of the ordinary type were used, their accuracy being esti-
mated by the following calibration - the weight of distilled water flowing from
each when drained 15 seconds after emptying and then touched to the side of the
weighing bottle used to contain the water.

Table 21.-Calibration of Pipettes

Pipette No.	Weight Distilled Water	Error percent
1	1.0009	+.09
2	1.0078	+.78
3	1.0004	+.04
4	0.9986	-.14
5	1.0018	+.18
6	1.0037	+.37
7	1.0086	+.86
8	0.9955	-.45

Table 21.-(Continued)

9	1.0020	+.20
10	0.9985	-.15

Seven of the ten delivered somewhat more than the weight of one cubic centimeter of water at this temperature, hence the results as given by the method are in these cases slightly higher than would be given by 1 c.c. of sap, but they are comparable to about one percent, and the differences in the values obtained were greatly in excess of this.

The standard alkali used in titration was prepared by diluting ten times a carbonate-free KOH solution (N .2105) prepared by the method of Küster[a] and standardized against oxalic acid. The standard acid was made by a similar dilution of sulfuric acid (N .1651) and the normality of the two determined by titration with one another and against the stronger acid and alkali respectively.

Normality of KOH , N.02126

Normality of H_2SO_4, N.01626

Set 1. Data are given in Table 13 in terms of c.c. of normal acid per c.c. of sap on samples from the set of 12-9-14. Applications of fertilizer had been made on 10-31-14; 11-7-14; 11-23-14; and on 11-30-14 to all sections excepting 291 and 295.

Table 22.- Acidity Determinations

Date	Sample	Section	Treatment	Condition of plants	c.c. N acid
12-10-14	1	291	1000 N	affected	.03068
"	2	293	1000 K	normal	.02492
"	3	295	1000 P	"	.05490
"	4	289	check	"	.02048
"	5	261	check	"	.02090
"	6	265	250 N	"	.02238

[a] Treadwell and Hall, II, p. 555.

Table 22. (Continued)

12-10-14	7	267	500 N	affected	.02728
"	3	277	125 K	normal	.02088
"	9	279	250 K	normal	.02002
"	10	281	500 K	"	.02130
"	11	283	check	"	.02130

The mean value of the "check" determinations is .02089 and the maximum variation .00041. The results show an increase in acidity when ammonium sulfate was used as the fertilizer, and a still larger value when di-sodium phosphate was applied. The latter result is interesting in view of the fact that di-sodium phosphate is somewhat alkaline to phenolphthalein. Plants from sections to which potassium sulfate was applied had a normal acidity value.

That this acidity is not due to carbon dioxide in the sap is shown by the following experiments. Distilled water was approximately saturated with CO_2 prepared from precipitated calcium carbonate and dilute sulfuric acid, and washed with a solution of sodium carbonate. One cubic centimeter was pipetted out and, after dilution to 6 c.c., titrated with approximately N/50 KOH. An acidity value equal to .0145 c.c. normal acid per c. c. of this solution, was obtained. A few drops of kerosene (tested for neutrality) to prevent excessive frothing and a small piece of clay plate were added to a second portion, and the solution boiled for one minute under diminished pressure by warming with the hand. One drop of KOH turned the solution to a deep red, showing the complete removal of the carbon dioxide by this method. One c.c. of sample number 3 of the next set was so treated and a similar portion titrated without suction. The number of c.c. of alkali required before suction was 3.03; to the portion after suction, 3.00. Hence no titratable quantity of carbon dioxide was present. Nevertheless, the second set were subjected to this precaution before titration. A similar treatment of sample

number 10 of the same set gave the following values, in number of c. c. of alkali required per 1 c.c. of sap: before suction, 3.32 c.c.; after suction, 3.30 c.c. These titrations were made on 1-15-15, 20 hours after the values given in Table 14 were obtained. The number of cubic centimeters of alkali required for 1 c.c. of number 10 in the previous titration was 3.29, showing no change to have taken place in the acidity when kept at icebox temperature for 20 hours, with xylol as a preservative.

Set 2. Samples of 1-9-15. Further applications of fertilizer 12-16-14; 1-4-15.

Table 23.-Acidity Determinations

Date	Sample no.	Section	Treatment	Condition of plants	c.c. N acid
1-14-15	1	269	check	normal	.01977
"	2	271	125 P	"	.05035
"	3	273	250 P	"	.06438
"	4	275	500 P	"	.07415
"	5	277	125 K	"	.02319
"	6	279	250 K	"	.02039
"	7	281	500 K	affected	.02422
"	8	283	check	normal	.02252
"	9	285	125 NaCl	affected	.01870
"	10	287	500 A.P.	vigorous	.06981

The high acidity value was found in plants from soil to which either commercial acid phosphate acid or di-sodium phosphate had been added, and increased quantities of the latter salt produced higher acidity values. The "check" values were about the same as in Set 1. Potassium sulfate produced no change in the acidity but sodium chloride caused a slight decrease.

A more detailed study was made of the sap from plants grown on soil to

which di-sodium phosphate had been applied (numbers 2, 3 and 4, Table 23). One cubic centimeter of sample number 3, diluted to 6 c.c. was extracted with three successive portions of ether, which had been distilled over sodium and kept free from moisture with sodium ribbon until use. The ether was evaporated on the water bath, and 6 c.c. of carbon dioxide-free water added. Titrations were made (1) of the original, giving 3.01 c.c. of KOH (N.02126) required; and (2) of the ether extract, one drop of alkali producing a deep red. Hence ether soluble acids were absent. The addition of ammonium molybdate and ammonium nitrate to the ether extract after slightly acidifying and warming the solution gave only a faint yellow color, hence not more than a trace of phosphate was present in it.

Phosphate[a] was determined in the portion of sap remaining after ether extraction after addition of a few drops of dilute nitric acid and one gram of ammonium nitrate. In the original solution the phosphate in one cubic centimeter was precipitated with barium hydroxide and barium chloride, the precipitate digested till flocculent, and filtered. After being washed free from chloride, the precipitate was ignited, taken up with nitric acid, made just ammoniacal, then just acid and the phosphorus determined as in the preceding sample. $Mg_2P_2O_7$ in solution after ether extracting = .0078 gm.; in original solution = .0084. The results of phosphorus determinations, made by the latter method on 1 c.c. of samples 1 to 5 of the set of 1-9-15 are given in Table 24.

Table 24.- Phosphorus Determinations

Date	Sample	Section	Treatment	$Mg_2P_2O_7$ gms.
2-2-15	1	269	check[b]	.0015
"	2	271	125 P	.0061

[a] The double precipitation method (U.S. Dept. Agr. Bur. Chem., Bul. 107 p.8) was used in all phosphate determinations.
[b] Determination of the phosphate content of the sap from section 283, also a "check", gave .0036 mg. $Mg_2P_2O_7$. Osmotic pressure, total solids and ash determinations on this sample gave abnormally high values, however, so that the value obtained with the sample from the section 269, adjacent to the "P" sections, is used. The increase in acidity with increased applications of di-sodium phosphate holds with either value.

Table 24 (Continued)

2-2-15	3	273	250 P	.0075
"	4	275	500 P	.0096
"	5	277	125 K	.0022

The value for acidity of the samples given in Table 23 may be termed also milligrams of hydrogen per c. c. of sap titratable with phenolphthalein. Assuming the phosphorus to be present in the sap as ortho-phosphoric acid or as mono-alkali phosphate, the number of milligrams of titratable hydrogen present as such may be calculated in terms of milligrams per c.c. of sap. These values are given in Table 25.

Table 25.-Content of Titratable Hydrogen per c.c.Sap

Sample no.	Treatment	By titration mg. H.	By phosphate as H_3PO_4 mg. H	Determination as XH_2PO_4 mg. H.
1	check	.01977	.02692	.01346
2	125 P	.05035	.10768	.05384
3	250 P	.06438	.13460	.06730
4	500 P	.07415	.16348	.08174
5	125 K	.02319	.03628	.01814

The values for milligrams of hydrogen per c.c. obtained by titration and by calculation from the weights of $Mg_2P_2O_7$, assuming the phosphate to be present as the di-hydrogen salt, agree fairly well. The reaction during titration proceeds according to the equation:

$$NaH_2PO_4 + NaOH \leftrightarrow Na_2HPO_4 + H_2O.$$
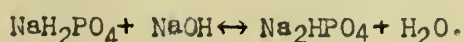
The reaction is not quite complete toward the right, however, owing to slight hydrolysis of the di-sodium phosphate with the production of alkali and the degree of this hydrolysis is dependent on the concentration of the salt in solution and the temperature. The ratio of the values for acid hydrogen obtained by the two methods

at 15° C. was determined in a solution of mono-sodium phosphate in approximately the strength found in the solution of the sap, made up with carbon dioxide-free water.

Approximately 1.2 gm. $NaH_2PO_4H_2O$ (Kahlbaum) were dissolved in 100 c.c. solution in carbon dioxide-free water at room temperature. 5 c.c. portions were made slightly acid with dil. HNO_3, 2 gm. NH_4NO_3 added, and phosphorus determined.

$Mg_2P_2O_7$ obtained : (1) .0601 gm. (2) .0596 gm. Average .0598 gm.

Mol. Wt. $Mg_2P_2O_7$ = 222.64

$$\frac{.0598 \times 2}{222.64 \times 5} = .1074 \text{ mg. H per c.c.}$$

Titrations were carried out on 10 c.c. portions after cooling to 15° using standard KOH (N.02126).

c.c. KOH required : (1) 45.72 (2) 45.67 Average 45.70

45.70 X .02126 X .1 .0972 mg. H per c.c.

Ratio $\frac{\text{titration}}{\text{precipitation}} = \frac{.0972}{.1074} = .905$

By subtraction of the acidity value of the "check" (Table 23) from that of samples 2,3, and 4, the acidity value (by titration) in each of the samples due to applications of sodium phosphate, was obtained. Similarly, subtraction of the weight of $Mg_2P_2O_7$ obtained in the "check" from the other three gave the weights of phosphorus (as $Mg_2P_2O_7$) due to the same cause.

The values are as follows:

Table 26.-Acidity and Phosphorus Content Due to Overfeeding

Sample 2:	.0036 Gm. $Mg_2P_2O_7$ ⇌ .03228 Mg. H per c.c. sap					
Sample 3:	.0060 "	"	⇌ .05388 "	"	"	"
Sample 4:	.0081 "	"	⇌ .07276 "	"	"	"
Sample 2:	By titration		.03058 "	"	"	"
Sample 3:	"	"	.04461 "	"	"	"
Sample 4:	"	"	.05438 "	"	"	"

The ratios of values in milligrams of hydrogen calculated from titration to those obtained by precipitations of phosphorus are:

Sample 1: $\dfrac{.03058}{.03228} = .947$

Sample 2: $\dfrac{.04461}{.05388} = .827$

Sample 3: $\dfrac{.05438}{.07276} = .747$

The ratios are in the same direction as those obtained with the pure salt[a]. It was concluded that phosphate from di-sodium phosphate was taken into the plant in form of the di-hydrogen salt. The decreasing value of the ratio as the applications of the di-sodium salt increased, points to the probability that some of the phosphate was taken in, in the latter cases, as the di-sodium salt.

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 gms. of sodium phosphate had been made) was found to give 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[b]. With this in mind, a liter of solution of di-sodium 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 c.c. of solution by placing them in holes of a paraffined

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[a] No account is taken in these calculations of the influence of other salts present on the value obtained by titration with phenolpthalein.

[b] See discussion by Cameron and Bell (13)

cork. They were placed on the greenhouse - covered with a large bell jar and shaded during the day-time- and allowed to remain for 6 days. 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 c.c. 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 gms. $Na_2HPO_4 \cdot 12H_2O$ per liter.

Titration of 10 c.c. portions, using 3 drops phenolphthalein.

$$H_2SO_4 \text{ (N .01550)}$$

(1)	(2)
.32 c.c.	.31 c.c.

.0048 c.c. N alkali per 10 c.c.

Titration of Plant Sap.

KOH (N .02130)

| (1) Check	(2) Treated
.92 c.c.	1.31 c.c.

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

Discussion of Results

The sap from the stems of plants grown on a soil slightly acid to litmus paper had a total acidity value of about .022 c.c. of normal acid per c.c. of sap. Applications of potassium sulfate did not change this value, but ammonium sulfate, commercial acid phosphate and di-sodium phosphate caused an increased total acidity. Di-sodium phosphate was taken into the plant in form of di-hydrogen salt. The increase in acidity was not associated with injury from overfeeding, excepting as an indication in the case of the sodium phosphate of the amount of phosphate taken into
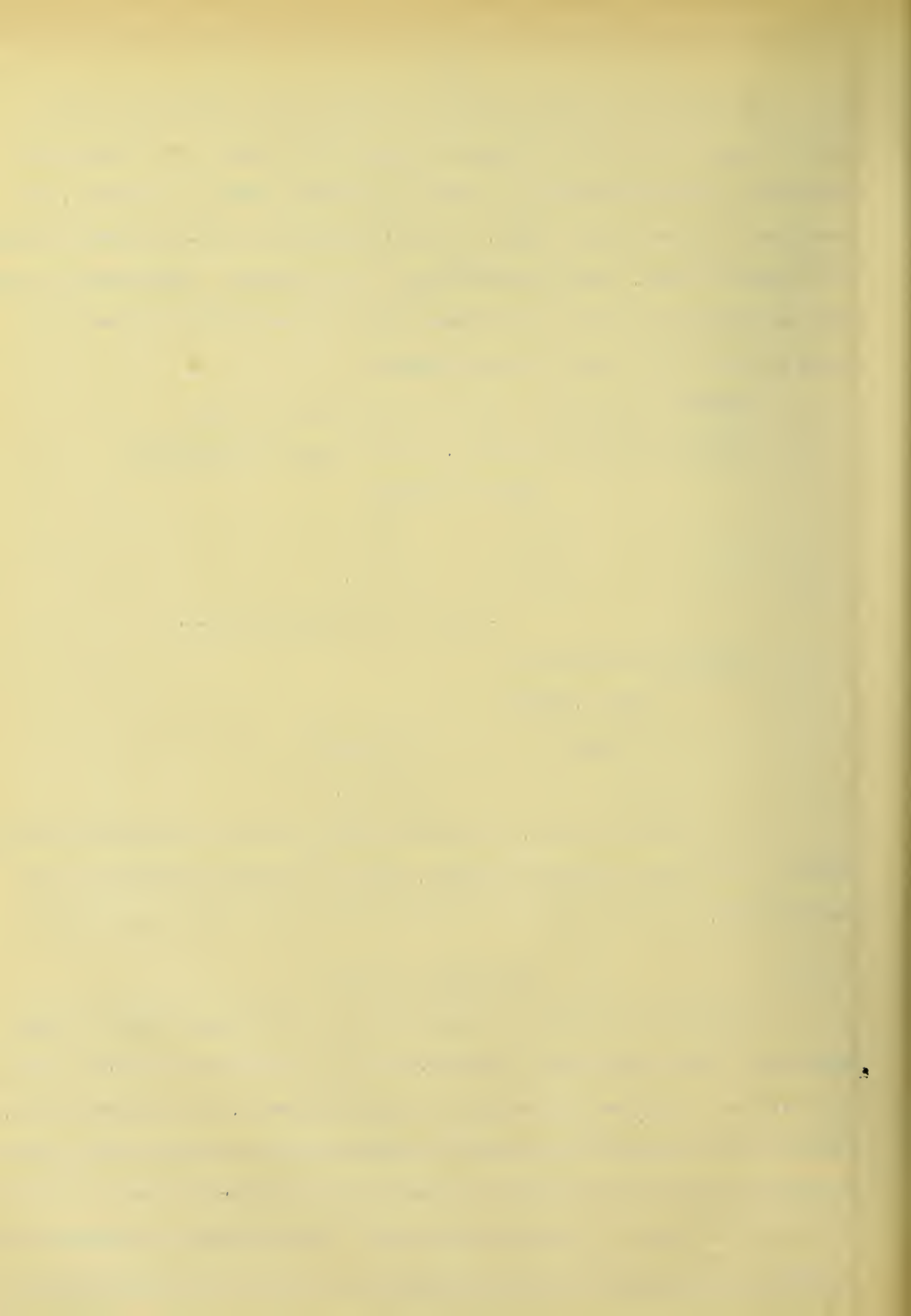
the plant. On account of the nitrification of ammonium sulfate even in soils which react acid[a] and the removal of the ammonia by microfungi[32] and the consequent presence of nitric and sulfuric acids, the increase in acidity is not an indication of the amount of ammonium sulfate taken into the plant. The ash analyses[b] made on plants from limed and unlimed soil showed the high sulfate content that could be present with less injury to the plant than where the presence of lime inhibited its intake. According to an abstract of an article by Comes[18], nitrogenous fertilizers, especially manure, decrease the acidity of the cell sap of wheat. In cultures not sterile, however, the acidity undoubtedly is increased, altho, it is true, probably owing rather to the side reactions than to the presence of the salt itself. The evidence that di-sodium phosphate is taken into the plant in form of di-hydrogen phosphate and that the increase in acidity is due to the increased amount of this salt present is interesting in view of the proposed use of phosphates for the purpose of securing for the plant immunity from disease. The hydrogen ion concentration would increase but comparatively little[c]; the ability of the plant to retain this ion concentration would, however, be considerably greater.

4. Effect of Large Applications of Potassium Sulfate
### on the Carbohydrate Content of Sap and Foliage

The increased exudation of nectar 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 7). An attempt was made to determine the cause of this increased flow. Braconnot as early as 1843[8] investigated the nectar of the flowers of a large number of species of plants and gave as the average composition:

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a Page 21.
b Page 16.
c A N/10 solution of mono-sodium phosphate has a hydrogen ion concentration of 3.3 X 10^{-5}.
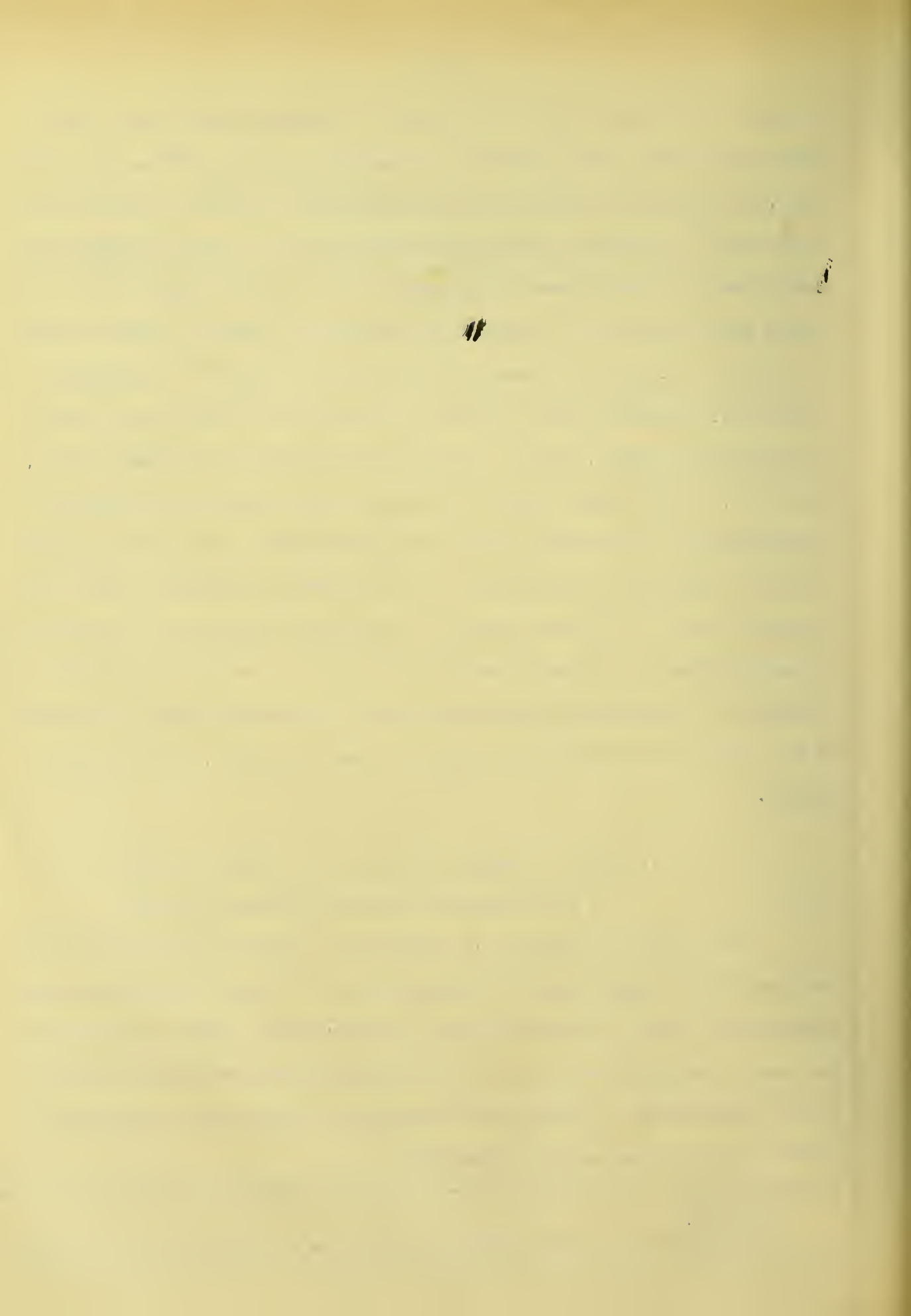
Sucrose	13 percent
Uncrystallizable sugar	10 percent
Water	77 percent

Biot[7] described the honey dew from the linden as containing dextrose and a large proportion of sucrose, while Langlois[28] listed among the components of the mixture: dextrose, uncrystallizable sugar, mannite, mucilage, vegetable albumenoids, a little tannin, inorganic salts, potassium acetate, calcium sulfate, lime, and potassium and calcium chlorides.

The amount of nectar present in an affected flower amounted to as much as 1 c.c. 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, altho 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. In the latter year it was a clear, colorless liquid with a sweet taste, 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]. 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[b], 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

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[a] Haas and Hill - The Chemistry of Plant Products (1913) p.191.

[b] Sherman, Org. Anal. (1912) p. 69. 2 gms. of phenylhydrazine in 8 c.c. 50% acetic acid was used with 10 c.c. of the solution.

inverted by the Clerget[a] 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 Clerget inversion. Hence dextrose and sucrose were present. The

precipitate formed in the hot solution was filtered off and the filtrate again

boiled till no fertilizer precipitate separated. On cooling the filtrate a further

precipitate of sodium acetate and osazone separated. This osazone was character-

istic of maltose in that the crystals possessed the rosette structure characteristic

of maltose, and were soluble in the boiling solution and reprecipitated on cooling

it[b]. Not enough of the precipitate could be obtained after recrystallization for

a melting point determination. Tests[c] made with 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. 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

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 found during the determination of total solids of the sap (page 22), when the

residue from this sap was of greater weight and charred at a lower temperature than

that of the check. The results, with the small amount of material available, are

necessarily not complete but are given as offering a possible explanation for the

increased exudation.

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[a] Sherman - Org. Anal. (1912) p.94.
[b] Mulliken - Identification of Pure Organic Compounds (1911) p.32. Hawk,
Practical Physiol. Chem. (1914) p. 29.
[c] Grüss (31) believed gummosis might be caused by an excess of diastatic
enzyme, and used this reagent as a means of detecting it.

Studies of the sugar content of plant saps were made as early as 1882-1883 by Müller - Thurgau[48] and Kayser[37] who investigated the reducing power of plant saps upon Fehling's solution. The method consisted in determining this before and after boiling with hydrochloric acid; the former results were expressed as reducing sugars and the increase after boiling with acid as cane sugar. Filtered sap expressed from the organ under investigation was used by Kayser. It was "neutralized with soda", and clarified with lead acetate. After filtration and washing of the precipitate the excess of lead was removed with hydrogen sulfide; after filtering off the lead sulfide, the filtrate and washings were boiled to remove the excess of hydrogen sulfide. Soxhlet's[a] modification of Fehling's solution was used in determination of the cupric-reducing power. Meyer[47] in 1885 working with plant sap also, deemed clarification necessary only in the sap from plants in which starch is a reserve material, these darkening on boiling. Sap from plants not elaborating starch was not clarified.

Brown and Morris[10], in 1893, making use of more accurate methods in their investigation of the carbohydrates in the foliage leaves of Tropaeolium majus and Helianthus annuus proved the presence of maltose in the extract of the dried leaves, both by analysis of the maltosazone and by the increase in cupric-reducing power and decrease in rotation of the clarified solution following boiling with hydrochloric acid, after cane sugar had been hydrolyzed with invertase. Their method is given in some detail:

1. In securing the solution for analysis, leaves, dried in a steam heated oven, were ground to a fine powder. After ether extraction to remove fat and chlorophyll, the residue was dried and the sugar extracted from a weighed quantity with two successive portions of 80-85 percent alcohol at 45-50 . Ammonia was added to prevent inversion by the acids also extracted and the alcohol removed by distillation. The solution was then clarified with basic lead acetate and made up to volume for analysis.

[a] Allen - Com. Org. Anal. (1912) I p. 318.

2. Cupric-reducing power and optical rotation of the solution were determined.

3. A second portion, hydrolyzed with invertase, was used for the determination of sucrose.

4. A third portion was inverted by digestion with 6 percent of concentrated hydrochloric acid for three hours, and cupric-reducing power and optical rotation again determined.

The advantage of this procedure lies in the distinction made between cane sugar and the maltose, which in earlier work was hydrolyzed with the former, the change in rotation and reducing power being attributed to sucrose alone. The combined results from the use of Fehling's solution and the polariscope furnished the data necessary for calculation of dextrose, levulose, maltose. and sucrose. Also, the polariscope readings served as a check on the correctness of assuming the cupric-reducing power to be due to reducing sugars. Campbell[15] in his study of the daily fluctuations in the content and proportions of sugars in mangold leaves calculated as maltose the increase in cupric-reducing power after boiling with 10 percent hydrochloric acid for an hour, the sucrose having first been hydrolyzed by boiling the solution with 2 percent citric acid for 7 minutes. Alumina cream or kieselguhr was used as the clarifying agent. Campbell's work is interesting from the standpoint of this investigation because he found that the dextrose and levulose content of mangold leaves from plants treated with potassium sulfate at the rate of 500 pounds per acre, was only one half that of leaves from plants receiving none of this fertilizer; the maltose content, on the other hand, was considerably increased.

Bryan[12] using 3.5 and 7 c.c. of basic lead acetate (sp. g. 1.25) as a precipitant for a 100 c.c. solution containing 5 gms. of dextrose and levulose, and 1 gm. each of ammonium tartrate and magnesium sulfate, showed that 3.35 and 8.34 percent of glucose and 8.03 and 19.91 percent of fructose were removed by the

precipitate . Little glucose and no fructose were removed by neutral lead acetate, which is recommended by Bryan, Given and Straughn[11] as the clarifying agent for plant extracts. Parkin[51] has made a study of basic lead acetate and charcoal as clarifying agents previous to the determination of sugars in the extracts of leaves of the Snow-drop. Charcoal was found unsuitable on account of the absorption of sugars. Basic lead acetate (5 c.c. sp. g. 1.25) lowered the rotation and, to a slight extent, the cupric-reducing power of one percent solutions of glucose and levulose. However, when tannin was first added and the greater part of the lead removed in precipitating it, no change took place in either property with one percent solutions of dextrose, levulose and sucrose, either singly or in a mixture, even tho the excess of lead was not removed. The apparent contradiction in these results is probably due to the greater concentration of sugar used by Bryan.

Basic lead acetate was chosen as the clarifying agent in these experiments for the following reasons:

(1) As a rule the use of alumina cream alone left the solution too turbid to permit polariscopic measurements being made.

(2) After neutral lead acetate had been added to complete precipitation, basic lead acetate produced a further precipitate.

(3) Tannin was detected in some of the samples of sap by the use of potassium ferricyanide and ammonia. No test could be obtained for it after clarification[a].

(4) The sap from the set of 3-9-15 upon clarification with alumina cream alone was found clear enough to polarize. Portions of 5 c.c. of sap were made up to 50 c.c. (1) with 5 c.c. of alumina cream and (2) with 1 c.c. of basic lead acetate (sp. g. 1.115) and 5 c.c. alumina cream, and filtered thru dry filters. The rotations are given in Table 26. The reading sample of number 1 was not quite distinct on account of a slight turbidity.

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[a] Saponines[26] optically active glucosides, are found in several genera of Caryophyllaceae but not in the carnation to the writer's knowledge. They precipitated by basic lead acetate, according to Haas and Hill. (l.c. p. 181).

Table 27.-Rotation of Solutions with Different Clarifying Agents

Sample no.	Treatment	Clarifying agent	Rotation circ. degrees
I	Check	Alumina cream	2.359
II	"	Basic lead acetate Alumina cream	2.428
III	K	Alumina cream	3.000
IV	K	Basic lead acetate Alumina cream	3.035

The increase in rotation when basic lead acetate was used amounted to one percent in the latter case, where both solutions were clear, a difference that could be accounted for by the increased volume of precipitate made by the addition of the lead salt.

The lead still in solution was removed by the addition of alumina cream in excess before making the solution up to volume.

Sucrose was determined by hydrolysis with hydrochloric acid according to the Clerget[17] method. Parkin[a], checking the amount of sucrose by fermentation with yeast, has shown that reliable results are obtained by this method. No maltose was present in the extract used by Parkin, however. Sigmond[55] has determined the constant for hydrolysis at 74°C. of an approximately 2 percent maltose solution with hydrochloric acid approximately half normal, to be .0024. The fraction hydrolyzed in 7 minutes at this temperature would amount to less than 1 percent. Hence, the use of the Clerget method was considered safe. The drop in rotation and increase in cupric-reducing power by hydrolysis upon the water bath for 3 hours over those values obtained after Clerget inversion were calculated as maltose. The concentration of hydrochloric acid used was 2.5 c.c. (sp. g. 1.19) with 25 c.c. of clarified solution and 12.5 c. c. of water, the solution being made up to 50 c.c. after hydrolysis, a little alumina cream being added if the color was too dark for polariscopic readings. For determination of reducing sugars the Defren[19] method, applicable to

[a] l.c. p. 11.

mixtures of dextrose, sucrose and maltose, was used, the cuprous oxide being determined by Low's[a] method for the determination of copper.

For optical measurements, a Schmidt and Hänsch half shadow polariscope, reading accurately when the solution was clear to .05° Ventzke was employed; 4 dm. tubes were used to secure the highest reading possible.

Observations upon the optical rotation of the clarified sap from the set of 1-9-15 gave the values shown in Table 28. The solutions were made with 5 c.c. of sap, 5 c.c. basic lead aceate (sp. g. 1.115) and an excess of alumina cream diluted to 50 c.c. and filtered thru dry filters.

Table 28.-Optical Rotation of Sap Solutions

Section no.	Treatment	Rotation circ. degrees.	
		Orig.	Inverted [b]
269	Check	.73	.83
277	125 K	1.91	1.25
279	250 K	1.42	.97
281	500 K	1.49	1.21

Similarly 20 c.c. of the sap of the set of 2-10-15 were made up to 100 c.c. with 10 c. c. of basic lead acetate (sp. g. 1.115) and an excess of alumina cream. The rotations were: (1) "Check", 3.23 ; (2) "K", 3.51 . After a Clerget inversion the rotation was 2.03 and 2.11 respectively.

In the sap from the set of 2-17-15, 10 c.c. from "check" and "K" samples were made up to 100 c.c. with 5 c.c. of the basic lead acetate and 12 c.c. alumina cream and filtered thru dry filters. The results are given in Table 29, calculated to the basis of 100 c.c. of the original clarified solution.

[a] Treadwell and Hall, Anal. Chem. (1912) II p.682.

[b] In this case after inversion with 10 percent N/2 HCl for 24 hrs. at 70°.

Table 29.-Sugar Determinations on Sap

Section no.	Treatment	Rotation circ. degrees			Reducing Power mg. CuO		
		orig.	Clerget	complete	orig.	Clerget	complete
272-280	Check	2.81	1.53	.67	556	1434	1654
279-281	250-500 K	3.26	2.28	.79	776.5	1461	1976

Using the values given by Defren[a] for the reducing power of dextrose and maltose, and the formula

$$[\alpha]_D = \frac{100\ a}{lc} \qquad \text{or } a = \frac{lc\ [\alpha]}{100}$$

for expressing the rotation when the values of $[\alpha]_D$ are:

Sucrose, 66.5

Dextrose, 53

Levulose, -90.8 at 20° C.

Maltose, 138

the calculations are as follows, I indicating "check" and II, "K" samples:

	I	II
Mg. CuO after Clerget inversion	1434	1461
" " before " "	556	776.5
" " due to invert sugar	878	684.5
Mg. invert sugar (as dextrose)	387.2	303.2
x $\frac{342}{360}$ mg. sucrose	367.8	288.0
Mg. CuO after complete inversion	1654	1976
" " after Clerget inversion	1434	1461
" " due to hydrolysis of maltose	220	515

102.1 mg. maltose reduces 140 mg. Cu O

102.1 "   " $\frac{360}{342}$ x 102.1 mg. = 107.4 mg. dextrose

a Sherman, Org. Anal. (1912) p.76
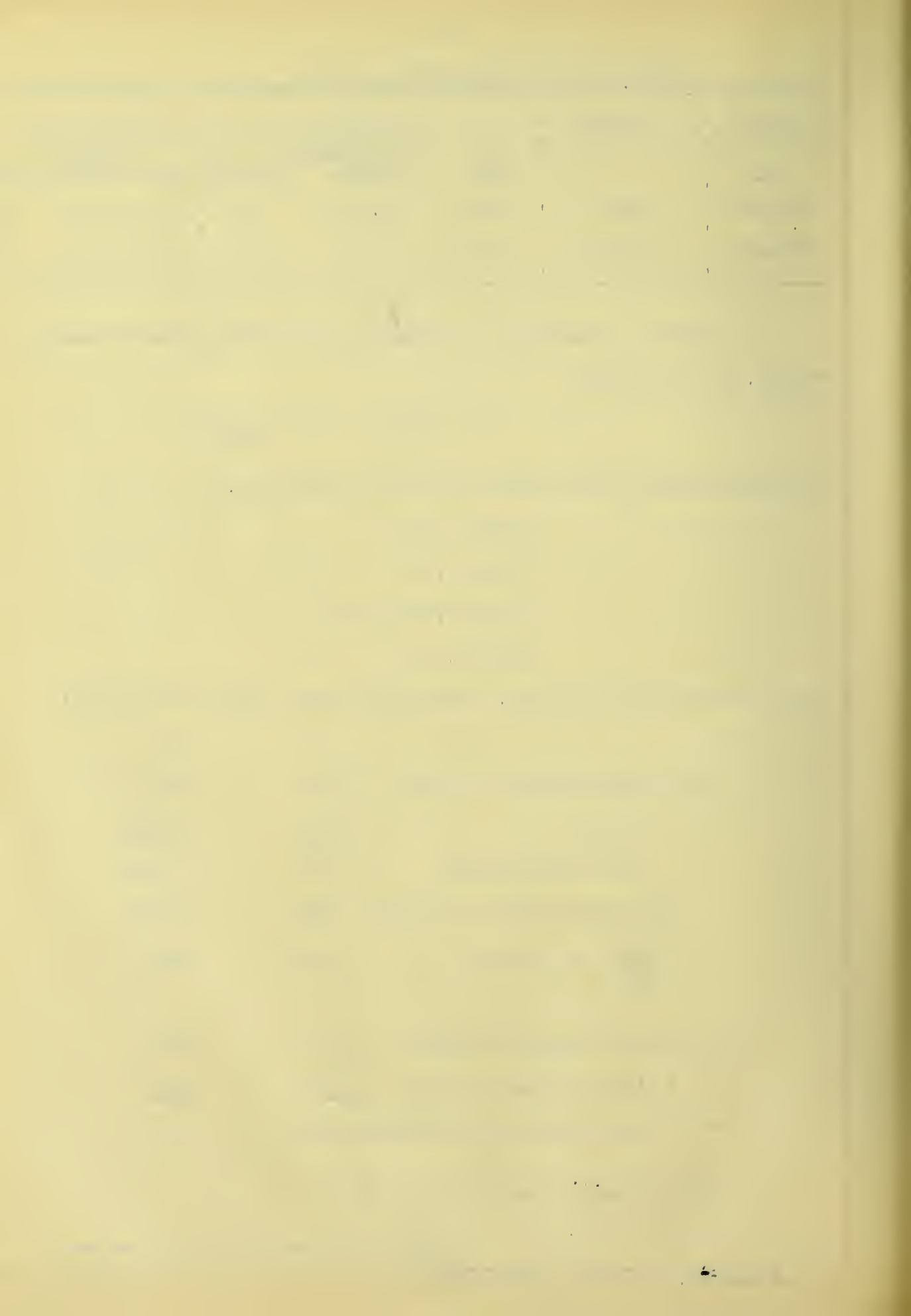
107.4 mg. dextrose reduces 240 mg. CuO

. . 100 mg. increase in reducing power (as CuO) is equivalent to inversion of 102.1 mg. of maltose.

(2)   Mg. maltose present   224.6   525.8

Total reduction (orig. sol.)   556.0   776.5

Mg. CuO reduced (in orig. sol.)

by maltose $\frac{303}{253}$ $\frac{725}{51.0}$

Mg. CuO reduced by other sugars

Calculated as dextrose   113.8   22.8

The drop in rotation[a] after Clerget inversion was respectively 1.28 (I) and .98 (II). These figures would correspond to 371 mg. and 284 mg. of sucrose, which gives a satisfactory agreement with the values 367.8 and 288.0 mg. obtained by the copper reduction method. Upon complete inversion a drop in rotation of .86 and 1.49 respectively was found. These figures correspond[b] to 261 mg. and 453 mg. of maltose respectively, which can be considered a fair but no better agreement with the values 224.6 mg. and 525.3 mg. obtained by the copper reduction method. The results in terms of mgs. of sugar per 100 c.c. of clarified sap solution are compared in Table 30, I indicating the "check" and II the "K" sections.

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[a] 100 mg. sucrose⇌105.2 mg. invert sugar or 52.6 mg. of dextrose and levulose. If $a = \frac{lc\;[\alpha]}{100\,a}$, then a for 100 mg. sucrose in a 4 dm. tube, $a = .266°$; for the dextrose, $a = .112°$; for levulose, $a = -.191$, giving a final rotation of -.079. The drop in rotation per 100 mg. of sucrose $= .345$, and the milligrams of sucrose present equal the observed drop times 100 divided by .345.

[b] 100 mg. maltose ⇌105.2 mg. dextrose. For 100 mg. maltose, $a = .552$; for 105.2 mg. dextrose, $a = .223$. The drop in rotation times 100 divided by $(.552-.223)$ $=$ mg. of maltose.

Table 30.-Sugars in Clarified Sap

Sugars	Amount mg. per 100 c.c.			
	By Cu-reduction		By rotation	
	I	II	I	II
Sucrose	367.8	288	371	284
(Dextrose)	113.8	22.8		
Maltose	224.6	525.8	261	453

Reducing sugars other than maltose present in the original solution are calculated as dextrose.

The data from the second set (3-9-15) were obtained with a solution of 10 c.c. of sap made up with 2 c.c. of basic lead acetate (sp. g. 1.115) and 5 c.c. of alumina cream, to 100 c.c.

Table 31.-Sugar Determinations of Sap

Section no.	Treatment	orig.	Rotation circ. degrees		Reducing power mg. CuO		
			Clerget	complete	orig.	Clerget	complete
286-288	check	2.43	1.43	1.06	521	1276	(1282)
279-281	250-500 K	3.00	1.91	1.34	522	1273	1438

The amounts of sugar, calculated from increase in reducing power and from the drop in rotation are given in Table 32, expressed as milligrams of sugar per 100 c.c. of clarified sap.

Table 32.-Sugars Present in Clarified Sap

Sugar	Amount			
	By Cu-reduction		By rotation	
	I	II	I	II
Sucrose	318	316	295	322
(Dextrose)	⎯	132	⎯	⎯
Maltose	(61)	168.5	112.6	173.2

The figures in the preceding two tables in parentheses are probably due to an error in manipulation, judging from the value for maltose obtained from the drop in rotation. On this account no calculation is made of the amount of reducing sugars, other than the maltose in the original solution. It is justifiable to include the data only because of the consistently larger rotation in sap from plants fed with potassium sulfate is again seen, and because the larger drop in rotation and increase in cupric-reducing power was found after hydrolysis with hydrochloric acid on the water bath. The value for maltose calculated by these two methods in the case of sample II checks satisfactorily.

As no more material was available for sap expression, an attempt was made to study the sugars obtained by extraction of the dried foliage. Seven grams of the air-dry foliage of the set of 2-10-15, dried at a temperature of 50 -70° in order not to cause charring, were mixed with 1 gm. of precipitated calcium carbonate[51], extracted with two successive 100 c.c. portions of 80 percent alcohol at about 70° for 24 hours, and the residue washed thoroly with warm alcohol. Water was added and the alcohol evaporated off after addition of a little ammonia. The solutions were clarified with 5 c.c. of neutral lead acetate, 1 c.c. of basic lead acetate (sp. g 1.115) and 10 c.c. of alumina cream, and made up to 100 c.c. The filtrates were too dark for polariscopic readings. An attempt was made to decolorize them by filtering thru a little (.3 gm.) animal charcoal but without success. The extracts were tested for pentoses or pentosans by boiling with an equal volume of concentrated hydrochloric acid and exposing anilin acetate paper to the vapor[a] but only a trace of pink color was given. Iodine and potassium iodide gave no test for dextrine[b], and potassium ferricyanide and ammonia, none for tannin. Determination of the cupric-reducing power was made upon the solutions. The results in mg. CuO per 100 c.c. of the solution are given in Table 33.

[a] Sherman, Org. Anal. (1912) p. 57.

[b] Allen, Com. Org. Anal. (1912) I p. 419.

Table 33.-Sugar Determinations in Extracts

Section no.	Treatment	Cupric-reducing power mg.CuO		
		original	Clerget	complete
268-270	check	398	1656.8	1933.6
277	125 K	652.8	1873.6	1990.4

Calculations after the plan followed in the preceding data give the following values:

	I (chk)	II (K)
(Dextrose)	5.33	216.4
Sucrose	531.5	515.1
Maltose	282.6	119.3

The "check" extract is noticeable for its lower content of reducing sugars other than maltose, and its higher content of maltose. The cupric-reducing values obtained from the sap of this set have not been included because no trustworthy results were obtained excepting those for the reducing sugars present in the solution before inversion. It was interesting at this point, however, to find that 20 c.c. of these saps, made up with 10 c.c. basic lead acetate (sp. g. 1.115) and 20 c.c.alumina cream to 100 c.c. had reducing balues before inversion of 1718.4 mg. CuO and 1101.6 mg. CuO per 100 c.c. for the "check" and "K" samples respectively.

Examination was made for starch in carnation leaves taken from the plant after a day of sunshine. After boiling them for sometime in alcohol, then in water, leaf sections were tested with an alcoholic solution of iodine; starch was found to be plentiful.

Comparative determinations of the starch content[a] were made upon the residues from sugar extractions, using a diastase solution prepared by extraction

[a] Brown and Morris (1. c. p. 629) state that preliminary washing with cold water as in the O'Sulllivan emthod, is unnecessary.

of ground malt with acid phosphate solution at icebox temperature, but not dialyzed.[a]
Fifty cubic centimeters of water were added to the residue and the starch gelatin-
ized by boiling for five minutes, with continuous stirring. After cooling to 60°,
5 c.c. of the diastase solution[b] were added with a pipette and digestion allowed
to proceed for an hour. The mixture was again heated to boiling and 5 c.c. of
diastase again added and after an hour the mixture was filtered and washed thoroly.
The maltose in the filtrate was hydrolyzed to dextrose by the modified Sachsse
method[c] and dextrose determined with Fehling's solution. The values obtained were
27.2 mg. and 19.4 mg. respectively for the "check" and "K" samples of this set
(2-10-15), the results being calculated as mg. starch per gm. of air dry foliage.
A similar determination was made upon the foliage from the set of 2-17-15. For
"check" and "K" samples of this foliage the values, 34.4 mg. and 30.9 mg. per gm.
foliage were obtained.

Discussion of Results

The results of these experiments show a higher total sugar content of
sugars in the sap from plants over-fed with potassium sulfate. A consistently
larger value was found for substances hydrolyzed by three hours heating with hy-
drochloric acid upon the water bath, and the correspondence of results by optical
and cupric-reducing methods of determination was close enough to lead to the con-
clusion that the excess sugar was maltose. The maltose content of the foliage
extract of plants so fed was less than that of the "check", while the starch con-
tent also was lower. The data are too incomplete to be used as a basis for a

[a] Sherman & Schlesinger - J. Am. Ch. Soc. 25 (1913) 1619.
[b] Reducing sugar blanks were made upon diastase solution prepared as for this
purpose; i.e., 20 gms. of this sample of malt digested for 1.5 hrs. with 50 c.c. of
mono-sodium phosphate solution (containing .0276 gm. of the phosphate), and filtered.
The original reducing power of the solution was 44.44 mg. CuO per c.c.; after hy-
drolysis by the Sachsse method, 74.8. If the original reduction were caused by mal-
tose alone, the solution after hydrolysis should give a value of 73.5 mg. CuO per
c.c.
[c] U. S. Dpt. Agr. Bur. Chem., Bul. 107, p. 53.

theory regarding the increased hydrolysis of starch in the foliage of plants so fed, or of increased permeability of the cell membrane under the influence of the potassium sulfate. In this connection, however, the recent article of Sherman and Thomas[53] showing the stimulation of diastatic activity by potassium sulfate is suggestive.

IV 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.

The experiments in the greenhouse gave a means of observing the injuries characteristic of each fertilizer and of measuring the effects of large applications upon the condition of the plants and the production and quality of flowers.

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. The effect of liming upon the intake of the fertilizers was also studied in a series of ash determinations.

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.

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 there is not the same degree of injury caused by different fertilizers at the same osmotic pressure.

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, di-sodium phosphate and mono-calcium phosphate, when phenolphthalein was used as the indicator.

The relation between the increase in total acidity and in the phosphorus content of the ash when the plants were fed with di-sodium phosphate proved that the phosphorus was taken into the plant in the form of di-hydrogen phosphate, due, as was shown, not entirely at least to absorption of the base by the soil but partially 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 due to the presence of a larger amount of maltose. The starch content of the foliage of such plants was shown to be 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 secretion of nectar in the flowers of these plants probably resulted from this increase in maltose content.

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