[FROM THE DIVISION OF CHEMICAL RESEARCH OF THE KENTUCKY AGRICULTURAL EXPERIMENT STATION.]

ON THE COMPOSITION OF THE ASH OF THE SAP, LEAVES AND YOUNG STEMS OF THE WILD GRAPE VINE (VITIS CORDIFOLIA).

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During the early spring of this year we had the opportunity to observe a very large and rapid flow of sap from the cut ends of a wild grape vine. It therefore occurred to one of us (Kastle) that it might be of interest to compare the mineral components of this sap with those of the leaves and new stems of this plant.

The relation existing between the composition of the sap of a growing plant and that of the new growth thereof has always been a matter of interest to the agricultural chemist and the plant physiologist. It is curious to note, however, that despite the well recognized importance of the sap to the requirements of the plant, especially in so far as the mineral constituents are concerned, very little work has been done on this subject; and at present nothing much is known as to the relation, if any, existing between the mineral components of the sap and those of the growing parts of the plant.

On looking over the literature of this subject for the past several years it has been found that while a large number of analyses have been made of a great variety of plants both as to amount and kind of mineral and organic matter that they contain, but few analyses have been made of the sap. It is true that a great deal of work has been done on the sap of the sugar maple tree and also on the juice of the sugar cane, but only so far as the sugar content is concerned, and that from a commercial standpoint. Moreover, the juice of the sugar cane, as this is obtained in practice, is not a true sap of the plant but contains besides the sap, a considerable quantity of the cellular contents pressed out in the juice, in the process of manufacture.

As a matter of fact, there are very few plants indigenous to the middle and northern states which will, at any period of the year, exude sufficient sap in a reasonable length of time to furnish the requisit amount of material for chemical analysis and this is perhaps one reason why no greater amount of work has been done on the sap itself. There are plants, however, that are exceptions to the above rule, chief among which are the grape vine and the sugar maple tree, both of which, in the early spring, as is well known, yield abundant sap in a very short time.

There are recorded in the literature (accessible to us at present) a few analyses of the sap and different parts of the cultivated grape vine.

ASH OF THE SAP (REBTHRÄNENASCHE, NOS. 4 AND 5, PAGE 62).

Crude ash.	Crude ash. Sand and carbon.	Carbonic acid.	Pure ash.	In 100 parts of the pure ash. K_2O.	Na_2O.	CaO.	MgO.	Fe_2O_3.	P_2O_5.	SO_3.	SiO_2.	Cl.
No. 4[1].	..	..	...	16.20	...	63.73	8.54	0.29	4.35	2.22	1.25	4.41
No. 5, 0.119[2].	..	..	0.084	36.14	16.76	20.83	2.87	Trace	7.66	9.99		4.61

ASH OF THE UPPER AND LOWER LEAVES. WOLFF, PAGE 63.

Crude ash.	Crude ash. Sand and carbon.	Carbonic acid.	Pure ash.	In 100 parts of the pure ash. K_2O.	Na_2O.	CaO.	MgO.	Fe_2O_3.	P_2O_5.	SO_3.	SiO_2.	Cl.
No. 10,[3] upper leaves 9.72	.	..	6.96	10.73	..	48.17	7.35	10.20	3.51	6.27	15.50	0.82
No. 11, lower leaves 11.40	..	..	8.47	7.55	..	50.89	7.00	9.28	3.18	3.21	12.41	1.62

ASH OF THE YOUNG STEMS AND LEAVES, WOLFF, PAGE 64.

	Crude ash.	Crude ash. Sand and carbon.	Carbonic acid.	Pure ash.	In 100 parts of pure ash. K_2O.	Na_2O.	CaO.	MgO.	Fe_2O_3.	P_2O_5.	SO_3.	SiO_2.	Cl.
No. 24. A. Barbera stems[4]	..	..	..	2.66	34.54	0.88	36.83	6.64	2.32	7.69	3.42	6.36	0.74
No. 24. A. Grignolino stems	..	..	..	2.41	33.47	1.77	35.18	6.50	1.90	10.01	4.67	6.01	0.91
No. 27. A. Pinot stems	..	..	..	2.91	32.84	1.78	32.76	5.38	2.47	10.56	4.07	8.60	1.26
No. 30. B. Barbera stems	..	..	.	3.37	34.98	2.00	33.64	11.89	0.77	8.43	2.87	4.67	1.05
No. 32. B. Grignolino stems	..	..	..	2.51	36.58	3.81	30.55	12.67	1.35	7.67	2.98	3.57	0.67
No. 34. B. Fresca stems	..	..	..	2.63	36.09	2.59	35.58	8.50	1.10	7.82	2.96	4.28	1.25
No. 22. A. Barbera leaves	..	..	..	8.34	6.53	0.64	45.06	8.04	1.04	0.92	2.82	34.22	0.62
No. 25. A. Grignolin leaves	..	..	..	9.52	6.36	0.64	41.61	8.36	0.74	0.66	1.41	39.44	0.50
No. 28. A. Pinot leaves	..	..	..	8.30	6.65	0.58	46.32	8.78	1.28	0.90	1.41	33.11	0.46

[1] No. 4. C. Neubauer, *Ann. Oenologie*, **4**, 102–116.

[2] No. 5. J. Nessler and Muth, "*Weinlaube*," **1871**, 51. This last sap was collected from a four year old vine (Black Burgundy) and 2 liters contained 3.998 grams solid matter consisting of 1.618 grams organic matter and 2.380 grams mineral matter including CO_2. In the organic matter there was found 0.176% N equivalent to 1.10% protein bodies. It required two days to collect the two liters of sap.

[3] Nos. 10 and 11. T. Peneau, *Centr. Agrik.-chem.*, **1878**, 366; *Ann. agron.*, **3**, 131–140 (1877).

[4] Nos. 21–34. E. Rotondi, *Relazione della r. stanzione enologica d.' Asti*, **1878**, S. 111–117 (also in *Centr. Agrik.-chemie.*, **1879**, 531).

Whether these materials were from the same vine or different vines, we have no means of determining.

Thus Dr. Emil Wolff,[1] in his book on ash analysis, gives the preceding analyses of the different parts of the cultivated grape vine and also of the sap.

In the *Fourth Report of the Kentucky Geological Survey*, **1861**, page 314, Dr. Robert Peter gives the following data on this subject:

"Bleedings of the grape vine. In the spring of 1860, the writer (Dr. Robert Peter) caused to be collected a quantity of the 'bleedings' of a Lenoir grape vine, from a branch which had been cut off for the purpose, with a view to ascertain by analysis, whether any notable quantity of the essential materials of the soil, etc., was contained in it. The result was as follows:

"One hundred parts of the clear, colorless fluid, evaporated to dryness at 212° F., left 0.286% of extract which was of a light chestnut color, and contained a vegetable substance resembling gum. This, when incinerated, left 0.084% of ashes, so that the amount of the organic matters was 0.202%. The composition of the ashes was found to be as follows:

Carbonate of lime	0.0419
Magnesia	0.0057
Phosphoric acid	trace
Sulfuric acid	0.0027
Potash	0.0240
Soda	0.0004
Silica	0.0048
Loss	0.0045
	0.0840

"So that, as might have been expected, this sap of the vine contains a portion of those mineral materials which are essential to vegetable nourishment."

"The stalks and branches of the vine contain considerable proportions of potash, lime, and phosphoric acid, yielding from above two to more than three per cent of their weight of ashes. The following is about an average account of the composition of the ash, according to Bruchauer:

Potash	34.1
Soda	7.6
Lime	32.2
Magnesia	4.7
Oxide of iron	0.2
Phosphoric acid	16.4
Sulfuric acid	2.7
Chloride of sodium	0.8
Silica	1.5

[1] Aschen Analysen, Wolff, **Berlin, 1871**.

"The leaves contain a large percentage of mineral matters, derived from the soil. But both leaves and prunings should be left to decay on the soil, for which, according to experience in Europe, they are the best manure in the cultivation of the vine."

In 1879, E. Rotondi and A. Ghizzoni,[1] in their article entitled "Researches on the Bleeding of the Vine," after giving the results of analysis of the sap of vines cut in April and May, the first author remarks that on the average, a liter of vine sap contains 0.147 gram of solids and 0.052 gram of ash. The red varieties yield more than the white. The time of cutting does not vary the phosphoric acid and potash contained in the sap.

Ghizzoni's work leads him to the conclusion that sap taken from a lower part of the plant contains more mineral and less organic matter than that taken from the upper parts.

In 1892, L. Roos and E. Thomas,[2] in an article entitled "Contribution to the Study of the Growth of the Vine," have determined at regular intervals of two weeks from May 15 to September 20, the proportion of dry matter, nitrogen, potash, phosphoric acid, sugar and acid in the year's growth (berries, leaves, and vine) of a vine of the Aramon variety. The vineyard from which the vine for examination was chosen, was located on a clay lime soil. It had been planted in 1884 and grafted with Aramon in 1886. The vine selected had been manured with farm manure in 1890. For each analysis a sufficient quantity of material was taken from the new growth in different parts of the vine, and the berries, leaves and stems were separated from each other.

The results of the work except that obtained on the sugar and acidity which have been omitted, are given in the following table.

Roos and Thomas conclude, therefore, that the phosphoric acid decreased from the time of flowering to maturity in the leaves and berries. It remained very constant, however, in the stems, except at the period just before blooming (June 14), when only traces were found. It is suggested that this may be the result of an error in analysis. The dry matter increased steadily in the leaves and stems. In the fruit it remained stationary at between 14 and 16% until the berries began to form, when it decreased to 8.82%. It then remained between 8 and 10% for several weeks, after which it increased to 16.20% by accumulation of sugar in the berries.

These authors also sum up, as part of their conclusions, applicable to this variety of grape, and to the conditions of the experiment, as follows:

1. That the dry matter increases steadily from the beginning of growth to time of harvesting.

[1] *Biedermann's Centr.*, **1879**, 527–530. See also *J. Chem. Soc.*, **1880**, II, 133.

[2] *Ann. Agron.*, **18**, 238–260 (1892). See also *Exper. Sta. Rec.*, **4**, 217.

COMPOSITION (FRESH MATERIAL) OF THE VINE AT DIFFERENT STAGES OF GROWTH.

Date. 1891.	Stage of growth.	Dry matter.			Nitrogen.			Ash.			Potash.			Phosphoric acid.		
		Fruit. Per cent.	Leaves. Per cent.	Stems. Per cent.	Fruit. Per cent.	Leaves. Per cent.	Stems. Per cent.	Fruit. Per cent.	Leaves. Per cent.	Stems. Per cent.	Fruit. Per cent.	Leaves. Per cent.	Stems. Per cent.	Fruit. Per cent.	Leaves. Per cent.	Stems. Per cent.
May 17	Bunches formed but very small....	14.27	21.85	13.20	0.49	0.74	0.21	1.29	1.58	1.12	0.47	0.33	0.37	0.14	0.22	0.06
May 31	Berries more distinct.............	15.15	23.03	17.45	0.52	0.94	0.31	1.32	1.82	1.18	0.43	0.44	0.38	0.17	0.21	0.08
June 14	Two or three days before flowering	16.50	22.25	27.68	0.49	0.74	0.36	1.17	1.62	1.00	0.30	0.38	0.35	0.12	0.15	trace
June 28	Flowering past..................	14.31	26.10	23.27	0.31	0.67	0.23	0.94	2.40	1.28	0.35	0.52	0.45	0.09	0.14	0.06
July 12	Berries the size of an ordinary pea	8.82	30.30	27.10	0.26	0.78	0.27	0.38	2.51	1.34	0.15	0.38	0.42	0.04	0.11	0.06
July 26	Berries very rich in juice (60%)...	9.87	29.78	30.92	0.21	0.65	0.24	0.46	2.67	1.47	0.16	0.42	0.44	0.04	0.12	0.08
Aug. 9	Berries translucid, seeds visible....	9.47	31.25	28.65	0.16	0.52	0.23	0.37	3.45	1.55	0.16	0.33	0.42	0.03	0.13	0.09
Aug. 23	Bunches beginning to ripen, berries purple......................	10.52	33.30	30.60	0.11	0.60	0.20	0.40	3.66	1.53	0.16	0.31	0.25	0.03	0.12	0.06
Sept. 6	As above, but color deeper	15.15	36.80	43.86	0.11	0.56	0.22	0.47	3.68	1.73	0.22	0.29	0.39	0.04	0.12	0.07
Sept. 20	Little change in appearance	16.20	37.00	43.80	0.09	0.50	0.20	0.38	4.58	1.82	0.16	0.27	0.18	0.03	0.04	0.07

2. That the assimilation of fertilizing materials is continuous up to maturity.

3. That it cannot be admitted, as various authors have maintained, that the fruit is formed at the expense of the substance accumulated in the leaves and stems at the time of flowering or a few days after. These organs do not act as intermediate agents in the formation of the fruit.

Several analyses of the berries, leaves and new wood of different varieties of grapes have also been made in the New York State Agricultural Experiment Station,[1] at Geneva, N. Y. The results obtained on the leaves and new wood are given in the following table:

Variety of grape.	Moisture. Per cent.	Nitrogen. Per cent.	Phosphoric acid. Per cent.	Potash. Per cent.
Catawba:				
Leaves	72.63	0.449	0.090	0.301
New wood	56.69	0.269	0.087	0.282
Concord:				
Leaves	71.49	0.533	0.088	0.462
New wood	53.60	0.288	0.079	0.306
Delaware:				
Leaves	72.58	0.422	0.088	0.269
New wood	52.94	0.301	0.099	0.278
Diamond:				
Leaves	78.44	0.330	0.080	0.230
New wood	72.27	0.304	0.086	0.242
Niagara:				
Leaves	69.00	0.691	0.130	0.536
New wood	54.33	0.324	0.096	0.429
Worden:				
Leaves	69.71	0.385	0.055	0.221
New wood	46.50	0.326	0.080	0.241

No discussion was made of the above results and these were given among several other analyses of fruits and vegetables, as matters of general interest and for reference.

G. Chippaz in an article[2] on the fertilizer requirement of grapes gives the results of analytical studies made with grape vines and the fruit at six different periods during the growing season, with a view of determining a suitable fertilizer for grapes as indicated by the amount of nitrogen, potash, phosphoric acid and lime required from the soil.

The data obtained show that about three-fourths of the total amount of nitrogen and five-eighths of the total amount of potash are taken up in the interval between the time growth starts and the beginning of flowering season. The absorption of these elements practically ceased when the fruits began to show color. The phosphoric acid appears to

[1] *Bull.*, **265**, 225 (1905).

[2] *Preg. Agr. et Vit.* (*Ed. l'est.-Centre*), **29**, 517–522 (1908). See also *Exper. Sta. Rec.*, **20**, 753 (1908–'09).

be taken up almost entirely before the beginning of the flowering season. Lime is taken up quite regularly throughout the growing season, the amount increasing as growth advances. It is estimated that a hectare (2.47 acres) of grapes removes from the soil annually from 40 to 80 kg. of nitrogen, 8 to 20 kg. of phosphoric acid, and 30 to 80 kg. of potash.

So far as we have been able to ascertain from an examination of the literature at present accessible, this about represents the present status of our knowledge of this subject. As has been said in the foregoing, our attention was directed to this matter by observing a very rapid and continuous flow of sap from the cut ends of the main trunks and branches of a wild grape vine (*Vitis cordifolia*, Michx).

Accordingly, on April 16th, about 400 cc. of this sap were collected and on June 14th samples of the succulent stems and young leaves were gathered from the same vine for analysis. The specific gravity of the sap, together with the chlorine, nitrate and percentage of ash therein were determined by Kastle. The analyses of the ash of the sap, stems and leaves were made by Shedd. We are also indebted to Dr. Buckner of this laboratory for the preparation of the ash of the leaves and stems for analysis and for the determination of the total moisture and ash in these products.

A few days previous to the collection of the sap, the main trunks and branches of the vine, which was a very large one, had been cut off. The cut ends of the main trunks and branches had been bleeding very freely ever since and from some of them a continuous drip of sap amounting almost to a continuous stream issued from the wounds. In some places on the main trunk of the vine, where the drops of sap had run down to the root, there accumulated a somewhat viscid, semi-solid mass, which on close examination appeared pulpy, very much like filter-paper pulp, and when pressed between the thumb and finger it left a residue having the appearance of cellulose. On closer examination this substance proved to be a mold, which had developed on the sap.

The sap was found to have a faintly acid taste and reacted faintly acid to blue litmus paper. Fifty cc. on titration, required 1.4 cc. 0.1 *N* NaOH, using phenolphthalein as indicator. Hence 100 cc. sap would require 2.80 cc. 0.1 *N* NaOH for neutralization.

The results of the analysis of the sap, leaves and stems of this vine are given in the following table. The results given under head of fresh sample have been calculated from the corresponding ash analysis. The sulfuric acid as given in the columns, under original sample, is undoubtedly low in all cases, since sulfur is lost on ashing and to obtain the actual amounts present in the green samples, the Osborn sodium peroxide method should have been used on the original material:

PERCENTAGE COMPOSITION OF THE ASH OF THE SAP, LEAVES AND YOUNG STEMS OF THE WILD GRAPE VINE (VITIS CORDIFOLIA, MICHX.).

Determined.	Sap.[1] I. Ash of sap.	II. Fresh sap.	Leaf. III. Ash of leaf.	IV. Green leaf.	Stem. V. Ash of stem.	VI. Green stem.
Water at 100°	..	99.6340[2]	..	75.4700	..	79.2500
Organic matter	..	0.2782	..	22.8500	..	20.0437
Silica, SiO_2	0.405	0.0005	5.890	0.1372	0.400	0.0041
Ferric and aluminic oxides, $Fe_2O_3 + Al_2O_3$	0.540	0.0006	0.920	0.0214	0.030	0.0003
Calcium oxide, CaO	19.490	0.0220	30.900	0.7200	10.920	0.1114
Magnesium oxide, MgO	3.900	0.0044	5.740	0.1337	3.390	0.0346
Soda, Na_2O	1.500	0.0017	1.530	0.0356	1.680	0.0171
Potash, K_2O	41.380	0.0468	14.710	0.3427	38.070	0.3883
Phosphorus pentoxide, P_2O_5	5.090	0.0058	9.700	0.2260	12.520	0.1277
Sulfur trioxide, SO_3	4.590	0.0052	2.720	0.0634	2.240	0.0228
Carbon dioxide, not determined	..	....	..	....	..	....
Chlorine, determined only in the sap	..	0.0008	..	....	..	....
	76.895	100.0000	72.110	100.0000	69.250	100.0000
d 25°/25°	..	1.0035	..	....	..	....
Total solids	..	0.3660	..	...	..	....
Nitrogen as nitrates	..	0.0075	..	0.0045	..	0.0056
Crude ash	..	0.1130	..	2.3300	..	1.0200

It will be seen from columns II, IV, and VI of the above table that, as might be anticipated, there is a great concentration of ash constituents in the stem and leaf as compared with the sap; and further that in the leaf the total ash is more than double the amount found in the stem, despite the fact that the water is about the same in each. So far as the several constituents of the ash are concerned, it will be seen from columns I, III and V of the above table that the silica in the sap and stem are practically the same, whereas in the ash of the leaf it is greatly increased. It will be seen further that the oxides of iron and aluminium are greatly decreased in the stem as compared with the sap, whereas in the leaf they are somewhat increased in amount. The numbers for lime are also of interest. In the sap, we observe about twice as much as in the stem, and in the leaf about half as much more as in the sap, or three times the amount found in the ash of the stem. In the ash of the sap

[1] By weight.

[2] On evaporation of the sap at 100° and subsequent ignition of the dry residue at low red heat the following numbers were obtained:

Water and volatil matter	99.634%
Loss on ignition	0.253%
Crude ash	0.113%
Total	100.000%

and stem, the magnesia is about the same, whereas in the ash of the leaf it is somewhat increased. The soda in all of these ashes is about the same, whereas while the potash in the ash of the sap and stem is high and about the same in each, it is greatly diminished in the ash of the leaf, while it is still present in considerable amount. The quantity of phosphoric acid in the ash of the leaf and stem is considerably increased as compared with that present in the ash of the sap, being greatest in the stem. The sulfuric acid in the ash of the stem and leaf both show a decrease as compared with that present in the ash of the sap. It should be borne in mind, however, that as pointed out in the foregoing, our determinations failed to reach all of the sulfur contained in these materials. In all cases the lime, potash and phosphoric acid are the largest ash constituents, the former showing an accumulation in the leaf, the potash a diminution in the leaf and the phosphoric acid an accumulation in both the leaf and stem. The large amount of nitrates in the sap is also a matter of interest.

A comparison of the amount of nitrate found in the sap, with that contained in the leaf and stem, shows that about one-third thereof has been consumed in the leaf and growing stem. This is in harmony with the idea that in the chlorophyllous plants the nitrates furnish the principal source of nitrogen for the elaboration of protein and other nitrogenous compounds.

Whether these relations, as indicated above, will hold throughout the entire growing season in a matter which is open to conjecture and can only be determined by further analyses.

A still better comparison, as throwing light on the possible significance of the several ash constituents for photosynthesis and growth, is that based on the actual amounts of the several ash constituents contained in the fresh material. It is evident from columns II, IV, and VI of the above able that the following relations hold for the several ash constituents, reckoned on the basis of the several amounts of these substances contained in the sap as unity in every case:

	Sap.	Stem.	Leaf.
Organic matter	1	72	82
Total ash	1	9	20
Silica	1	8	274
Iron and alumina	1	1	35
Lime	1	5	33
Magnesia	1	8	30
Soda	1	10	20
Potash	1	8	7
Phosphoric acid	1	22	39
Sulfuric acid	1	4	12

It is evident from this comparison that there is a marked accumulation of the ash constituents of the sap in the growing leaf. That such is the case serves to indicate a close correlation between the mineral elements of the ash and the chemical changes occurring in the leaf. As is well known, the principal seat of the changes taking place in the life and growth of the plant is in the leaf, and here we find a great concentration of the ash constituents, as compared with the sap. As compared with the leaf, the woody stem of the plant is of slower growth and in a sense at least secondary to it, in order of development. In the stem, therefore, while we find considerable concentration of the ash constituents of the sap, it is by no means as high, in most cases at least, as in the leaf. It will be observed, of course, that the organic matter contained in the stem is nearly as great as that found in the leaf. It should be borne in mind, however, that the greater amount of this represents woody fiber and reserve material which has been translocated from the leaf and hence there has been less concentration of the ash constituents of the sap in the stem than in the leaf, where the organic matters are largely the product of photosynthesis.

It is a matter of common experience that if pruned just before budding and after the sap has begun to flow freely, and especially if much bleeding or loss of sap occur, the grape vine will suffer considerable injury, due to loss of reserve materials, mineral matter and water required for the development of the new growth. Hence the common practice of pruning such vines in the late fall or early spring, before the rise of the sap. All of these considerations, therefore, serve to emphasize the importance of the sap in the life and growth of the plant. The fact that a soil solution is being continually imbibed by the plant roots, while pure water is being driven off in such large amounts from the foliage, with corresponding concentration of the several mineral constituents in the various tissues of the plant, renders a study of the sap, such as we have described above, of great interest to students of plant physiology. We propose therefore to considerably extend the scope of this investigation as soon as the necessary materials can be obtained so as to include in our comparisons, the soil solution, the sap from various portions of the plant and several plant tissues which we have not as yet been able to examin.

[CONTRIBUTION FROM THE HARRIMAN RESEARCH LABORATORY (ROOSEVELT HOSPITAL), NEW YORK CITY.]

PHENOLPHTHALEIN AND ITS COLORLESS SALTS.[1]

[THIRD PAPER.]

PREPARATION OF MONOBASIC PHENOLPHTHALATES.

BY P. A. KOBER AND J. THEODORE MARSHALL, with the assistance of E. N. ROSENFELD.

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Introduction.—For many years, until it was disproven by Meyer and

[1] Read in part before the Section on Organic Chemistry, Eighth International Congress of Applied Chemistry, Sept. 10, 1912, New York City.