

STUDIES ON THE REACTION OF PLANT JUICES¹

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Comparatively little investigation has been done on the actual reaction³ of plant juices upon plant metabolism, notwithstanding its importance. Loeb, in 1919, in a series of five papers on amphoteric colloids in the *Journal of General Physiology*, has shown the importance of the reaction upon the retention and excretion of ions from protein-containing solutions, as well as upon other physical and chemical properties of amphoteric colloids. The factors that may affect the reaction of plant juices are numerous, and as yet are but little understood.

Inasmuch as the soil reaction is one of the important factors affecting the growth of plants, it is quite possible that the degree of acidity of plant juices may be correlated with the reaction of the soil upon which the plants are grown. It has already been shown by Fred and Davenport (4) that the reaction of the medium markedly affects the metabolism of legume bacteria, and that they are rendered inactive at a more or less well-defined critical pH value of the medium. In addition to the reaction of the substrate, meteorological and hereditary factors may be considered as possibly affecting the reaction of plant juices.

The present paper deals with the actual and the total acidities and the total alkalinity of the juices of a number of plants of agricultural importance, together with a study of the influence of liming the soil upon these acidities. The actual acidities of the different parts of plants and their relation to one another are discussed. The effects of age, lack of chlorophyll and changes in illumination, upon the actual and total acidities of plant juices also are considered.

The actual acidity of soil solutions (27) from which plants derive food has been the subject of considerable investigation (25). The studies of Hoagland (12) on the relation of the nutrient solution to the composition and reaction of the cell sap of barley plants, is a step in the right direction.

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² The writer has been obliged to conclude his present studies and finds it advisable to present the data thus far obtained.

³ By actual acidity is meant the amount of dissociated acid. The total acidity includes both the dissociated and undissociated acids.

It has been shown that the actual reaction of the juice of most plants is markedly acid (7, 8), and that only occasionally are species found with cells the juices of which are neutral or slightly alkaline in reaction (5). However, Kappen (13) has recently reported a slight alkalinity in the juice of wheat roots. Rose (20) has also reported the interesting observation that as germination begins, the reaction of the embryos of *Sambucus* changes from alkaline to acid but that the endosperm remains alkaline. The juice of succulent plants has been found by Hempel (11) to be quite acid in reaction, and it was she who first elucidated the nature of the buffer processes existing in the juice of such plants.

For previous work upon reaction as regards both plants and animals, the bibliography compiled by Schmidt and Hoagland (21) is very complete.

METHOD OF DETERMINING THE ACIDITY OF PLANT JUICES

The determination of both the actual and the total acidity (10) of plant juices was carried on by the use of the hydrogen-electrode. It was found that the juice obtained from the plants growing in a single pot was frequently insufficient for carrying on a determination of the actual reaction when a 3-by-10-cm. glass titration tube was used. An effort was made to use the indicator paper method (9) but it was found quite impossible to secure clear or nearly clear plant juice even after filtration by various methods and repeated centrifuging. Unless the juice was very acid, it retained a deep green color. Moreover, it was considered inadvisable to use two methods (the indicator paper method is less accurate than the electrometric) in making comparative determinations, especially in case differences are small.

Accordingly a small hydrogen-electrode vessel was devised as shown in figure 1, in order that determinations of the actual acidity could be made when only three or four drops of plant juice were available. The essential point in the construction of the apparatus is to make the bulb F of such size and shape that a few drops of solution suffice to cover the lower end of the glass tube for the KCl contact and gas-electrode. The small hydrogen-electrode vessel was found to be more convenient than the larger vessel except when the juice was to be titrated, in which case the larger vessel was used. A small quantity of plant juice was found to be preferable to a larger quantity, for with small amounts of juice equilibrium was more rapidly attained. The results obtained with the small apparatus were compared with the results obtained with the larger hydrogen-electrode vessel.

The juice from fresh medium red clover grown out-of-doors was obtained by first crushing the cells in a tinned meat grinder and then pressing out the juice through a clean linen cloth. The extracted juice was not centrifuged. Four drops of the plant juice (0.12 cc.) were introduced with a pipette into the small hydrogen-electrode vessel. The actual reaction of the plant juice was found to be $\text{pH} = 6.02$. The original juice was left standing $\frac{1}{2}$ hour

and a second quantity amounting to 4 drops (0.13 cc.) was used. The actual reaction was found to be $\text{pH} = 6.06$. A 7-cc. sample of the original juice was then run with the larger hydrogen-electrode vessel and the actual reaction of the juice was found to be $\text{pH} = 6.06$. In the determination of the actual

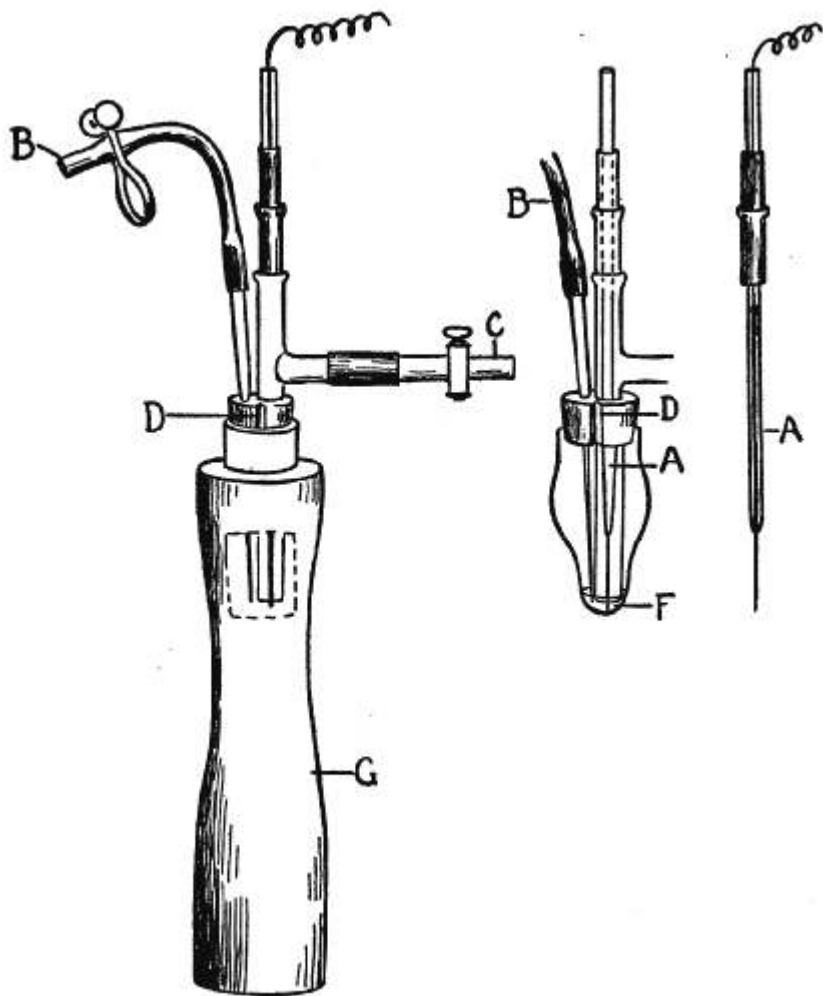


FIG. 1. THE SMALL HYDROGEN ELECTRODE APPARATUS DRAWN TO SCALE

A, Hydrogen-electrode wire at lower end connected with mercury to wire connection at upper end. *B*, Connection with KCl to the calomel electrode. *C*, Hydrogen inlet. *D*, Holes for outlet excess gas. *F*, Plant juice. *G*, Rubber holder for hydrogen electrode vessel with glass tube inserted at lower end for rigidity, and window at upper end for observation.

reaction of some very minute quantities of plant juice, this small type of hydrogen-electrode apparatus served a very useful purpose and facilitated considerably the numerous determinations of the reaction made upon agricultural plants.

Besides determining the actual reaction of plant juice, it was found possible, whenever 5 cc. or more of juice was available, to titrate the total acid by means of the hydrogen-electrode. It is possible to titrate much smaller quantities of juice, but it is not convenient. By titrating the total acidity of the plant juice it is possible to obtain an idea of its buffer action.

The following titrations, taken at random from a large number of determinations, serve to indicate the degree of accuracy that can be secured by the electrometric titration (10) of aliquots of the same sample of expressed plant juice when the readings of E. M. F. are made with a voltmeter. The results are given in table 1.

TABLE 1

Agreement of duplicate determinations of actual acidity upon adding 0.0689 N sodium hydroxide to 10 cc. of juice of medium red clover tops

FIRST TRIAL		SECOND TRIAL	
Actual acidity	NaOH added	Actual acidity	NaOH added
pH	cc.	pH	cc.
6.08	0	6.10	0
6.92	1.0	6.88	1.0
7.53	2.0	7.53	2.0
8.09	3.0	8.07	3.0
8.41	4.0	8.41	4.0
8.69	5.0	8.68	5.0
9.00	6.0	9.02	6.0
9.34	7.0	9.34	7.0
9.63	8.0	9.60	8.0

Without plotting the values, it is obvious that the two successive titrations check very closely. In an electrometric titration, it is frequently possible to observe errors in the reading of the burette or the E. M. F. by the fact that such points ordinarily do not fall close to the trend of the titration curve.

EFFECT OF STANDING ON THE ACTUAL AND TOTAL ACIDITIES

The previous treatment of the plants and the time elapsing between expressing and using the plant juice may influence the actual and total acidities obtained. The total acidity of plant juice will differ according to the pH we select as our end-point. If we select pH = 6.81 we have the turning point for litmus; if we select pH = 8.31 we have the turning point for phenolphthalein. Hence in titrating for total acidity we can tell at once from the curve the amount of alkali required for titration to any given pH and consequently to the color change for any indicator. For example, some medium red clover plants 12 to 14 inches high, growing on a limed plot (pH = 7.53) were cut off just above the surface of the soil at 11.30 a.m. and

TABLE 2

Effect of standing on actual and total acidities of medium red clover juice
 10 cc. of juice titrated with 0.0684 *N* sodium hydroxide

ACTUAL ACIDITY		PLANTS FROM LIMED SOIL		ACTUAL ACIDITY		PLANTS FROM UNLIMED SOIL	
I (1.50 p.m.)				I (8.15 p.m.)			
pH		cc. NaOH added		pH		cc. NaOH added	
6.12		0.0		5.94		0.0	
7.31		1.0		6.45		1.0	
8.05		2.0		6.94		2.0	
8.56		3.0		7.53		3.0	
8.90		4.0		8.07		4.0	
9.20		5.0		8.39		5.0	
9.46		6.0		8.75		6.0	
II (3.05 p.m.)				II (9.30 p.m.)			
6.12		0.0		5.82		0.0	
6.84		1.0		6.45		1.0	
7.87		2.0		6.94		2.0	
8.22		3.0		7.33		3.0	
8.41		4.0		7.97		4.0	
8.98		5.0		8.22		5.0	
9.35		6.0		8.66		6.0	
III (3.55 p.m.)							
6.03		0.0					
6.84		1.0					
7.56		2.0					
8.00		3.0					
8.36		4.0					
8.68		5.0					
9.05		6.0					
IV (5.00 p.m.)							
6.04		0.0					
6.72		1.0					
7.51		2.0					
7.88		3.0					
8.34		4.0					
8.69		5.0					
9.08		6.0					
V (7.10 p.m.)							
Freshly expressed and centrifuged							
6.10		0.0					
6.72		1.0					
7.39		2.0					
7.97		3.0					
8.69		5.0					
9.02		6.0					

placed in beakers containing water. Some medium red clover plants 8 to 10 inches high from an unlimed but otherwise similarly fertilized plot ($\text{pH} = 6.19$) were treated in the same way. Just prior to the first determination in each of the two series of table 2, the lower portion of the plants that had been in the beakers of water were cut off and discarded, and the juice of the upper remaining portion was expressed and centrifuged 15 minutes. The juice of the plants from the limed soil was kept in the dark and 10-cc. aliquots were titrated at intervals throughout the afternoon. The juice of the plants from the unlimed soil was not expressed until in the evening and only two successive titrations were made.

From the table it is evident that on standing in the dark there is a tendency for the medium red clover juice to undergo changes in its actual and total acidities. In the first four determinations on equal aliquots of the same original juice of plants grown on the limed soil there was an increasing total acidity, whereas the fifth determination, made upon freshly expressed and centrifuged juice, behaved more like determinations III and IV than like I and II as regards total acidity and more like determinations I and II than like III and IV as regards initial actual acidity. The juice of the unlimed clover had a greater actual and total acidity, upon allowing the expressed and centrifuged juice to stand in the dark, than when used immediately. When the juice of the limed clover plants was left standing over night for 24 hours and its actual acidity again determined, it was found that the value for the actual acidity had changed from $\text{pH} = 6.12$ to $\text{pH} = 5.91$. This marked increase in actual reaction upon oxidation of the juice is in accord with the observations of Miss Hempel (11) for the juice of succulent plants. The results in table 2 cannot be compared with those in table 1 as it is essential to use fresh plants. Furthermore, as will be indicated, the soil solution and other factors may affect the actual and total acidities of plants.

EFFECT OF LIMING ON THE ACTUAL AND TOTAL ACIDITIES

On comparing determination I of the unlimed clover, in table 2, with determinations I and V of the limed clover, it is found that the unlimed clover has a greater actual and a greater total acidity than the limed clover, regardless of the pH which might be chosen as the end-point.⁴

⁴ In order to compare determination I of the unlimed with determination V of the limed clover as regards total acid, we select any pH as our turning point and note the amount of alkali required to bring the juice to the selected pH end-point. Thus for example, in determination I of the unlimed clover we find that 5 cc. of alkali are required in order to bring the pH of the juice to 8.39, whereas in determination V of the limed clover less than 5 cc. of the alkali is required in order to bring the pH of the juice to 8.39, since 5 cc. carries us 0.30 beyond the end-point $\text{pH} = 8.39$. This shows that the juice of the unlimed clover has a greater total acidity than the juice of the limed clover with reference to $\text{pH} = 8.39$ as our end-point.

It seems important, therefore, to consider the effect of liming the soil on the actual and total acidities of plants grown on such soils. Various crops were grown on Plainfield sand, using pots containing 11,500 gm. of soil. Plant tissue was available from these pots through cooperation with Dr. C. B. Clevenger.

Half of the pots received sufficient ground limestone to neutralize all of the acid as indicated by the Truog test (24), and all the pots received 2 gm. of dipotassium phosphate. The seeds were planted March 1.

The actual and the total acidities of 6 cc. of alsike clover juice were determined for both the limed and the unlimed Plainfield sand. The tops were cut May 13 and the juice was centrifuged for 10 minutes after being expressed. In none of the experiments in this paper was there any water added to the crushed plant tissue or the expressed juice. The values obtained for alsike clover are given in the form of curves in figure 2.

From the curves, it is evident that the actual as well as the total acidities of the juice of the tops of alsike clover plants grown without liming are greater in every case than those with liming. If, for example $\text{pH} = 8.0$ is chosen as the end-point, it is seen at once that the 6-cc. sample of juice from the plants with liming required 2 cc. of alkali, whereas that from plants without liming required 3 cc. to bring the juice to $\text{pH} = 8.0$.

The 7-cc. samples of juice of serradella tops cut on June 2 from limed and unlimed Plainfield sand gave a different relationship (figure 2) from that described for alsike clover. The actual acidity of the plants with liming was greater than that of the plants without liming. As alkali was added, at first the total acidity of the juice of the limed plants is greater, then less, and then greater than that of the juice of the unlimed plants.

When the 8-cc. samples of juice of the tops of garden bean plants, grown on limed and on unlimed Plainfield sand and cut on May 13, were examined, the results represented by the curves in figure 2 were obtained. It is found that the actual as well as the total acidities of the juice of plants with liming was greater than that of the plants without liming. These results might be explained possibly by such a fact as Gile and Ageton (6) have found for beans, namely, that in the ash of bush beans the amount of lime was slightly decreased rather than increased with increasing amounts of calcium carbonate in the soil.

Barley plants, not yet in bloom, were cut off at the surface of the limed and unlimed Plainfield sand on May 12. The expressed plant juices were centrifuged for 15 minutes. The results obtained for the actual and the total acidities of 15-cc. samples of the juices are plotted in figure 3. The initial actual acidity was greater in the plants from unlimed than from limed soil. The total acidity in both cases was practically the same for any given pH as the end-point.

When 15-cc. samples of juice obtained from oat plants cut May 9 on limed and unlimed Plainfield sand were examined, it was found (fig. 3) that

the initial actual acidities of the juices were practically the same. However, as regards total acidity, it was found that no matter which pH was chosen as the end-point, the plants with liming showed a greater total acidity than those without liming.

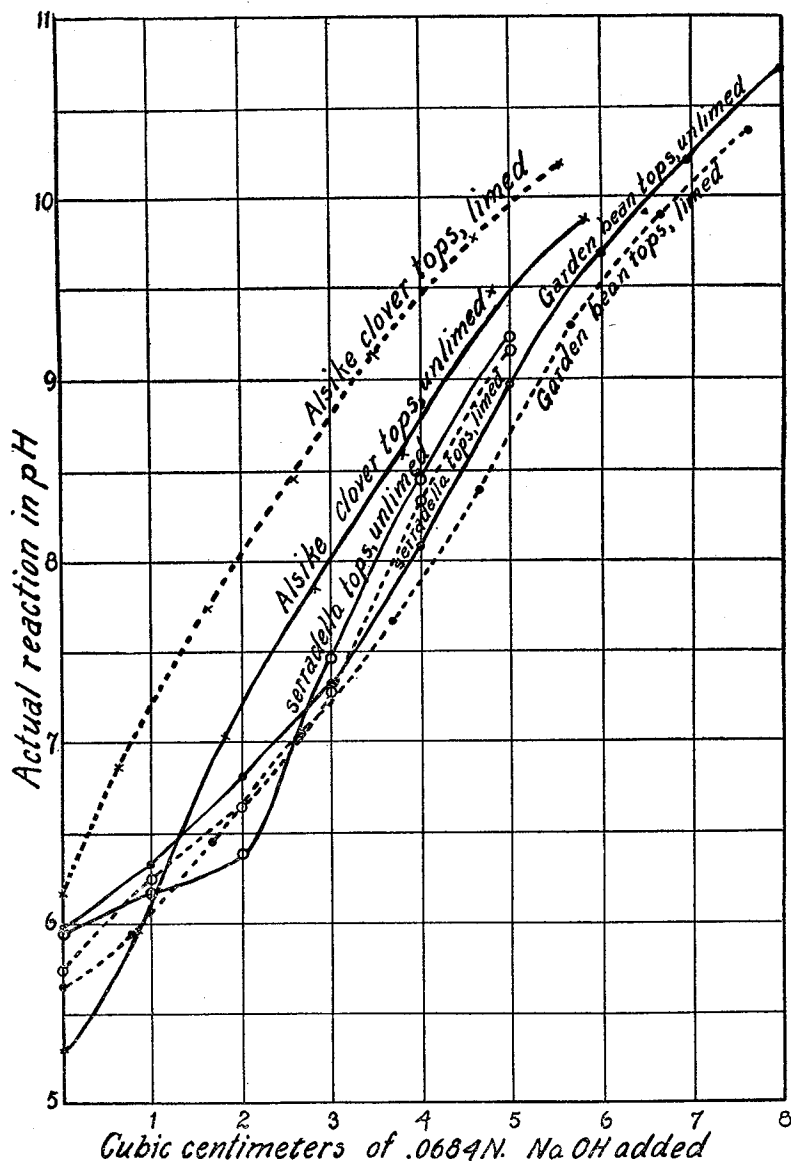


FIG. 2. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES (EXPRESSED IN TERMS OF CUBIC CENTIMETERS OF 0.0684 N SODIUM HYDROXIDE) OF THE JUICES OF PLANTS GROWN ON LIMED AND UNLIMED SOIL

Corn plants growing on soils similar to those of the oat plants were cut May 9. The titration curves of 15-cc. samples of both juices resemble those for oat plants. The initial actual acidities with and without liming were the same. However, for any chosen end-point (pH) the total acidity of the juice of the corn with liming was greater than that without liming.

Truog (25) has suggested that one of the chief functions of calcium, when taken up by the plant in the carbonate or bicarbonate form, is to neutralize and precipitate acids within the plant and thus prevent an injurious degree of acidity. How these irregular results may be harmonized with this suggestion will now be discussed.

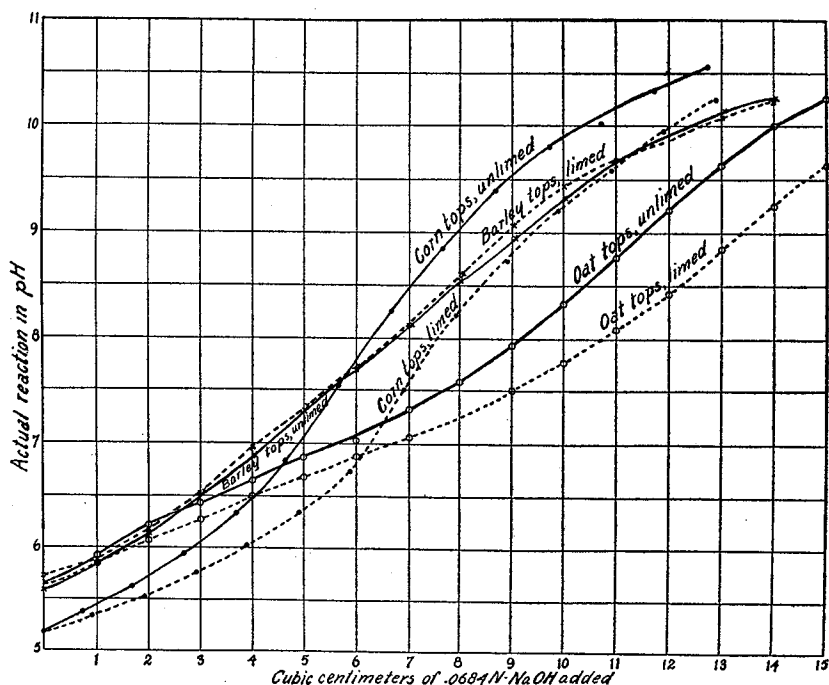


FIG. 3. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF THE JUICE OF BARLEY, OAT, AND CORN PLANTS GROWN ON LIMED AND UNLIMED SOIL

Some of the results from plants grown upon Plainfield sand are most irregular if not abnormal when compared with those from plants grown upon other types of soil. Frequently the addition of lime to Plainfield sand had an unfavorable effect on plant growth even with plants that ordinarily are benefited by liming. This soil thus gave some unexpected results.

In the experiments just described there was not taken into consideration the possibility that differences in actual and in total acidities may exist in different parts of the same plant. With succulent plants in which the leaves are of large size it is relatively a simple matter to separate the leaf, stem, and

root portions of the plants and to compare the actual and the total acidities of corresponding parts. With alfalfa, clover and other plants of a similar nature, it requires considerable time and patience to separate the leaves, stems, and roots from one another in order to obtain sufficient of the plant juice with which to carry on the determinations.

In some of the determinations of the actual acidity of the plant juice of rhubarb leaf-stalk, it was found (8) that the actual reaction varied considerably according to the part of the plant from which the juice was expressed. The observations of Rose (20) upon the reaction of different parts of seed tend to emphasize the fact that it is essential to compare the expressed juices of corresponding parts of plants.

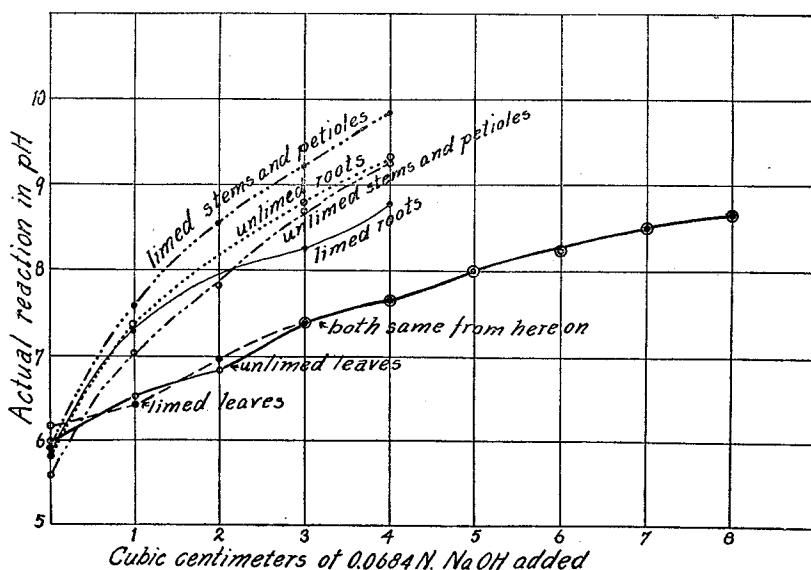


FIG. 4. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF LEAVES, STEMS, PETIOLES, AND ROOTS OF MEDIUM RED CLOVER PLANTS GROWN WITH AND WITHOUT LIMING

The results reported in this paper (fig. 4) point out the fact that leaves, stems, and roots may differ quite considerably in their actual reaction.

In order to control the conditions as much as possible, fresh medium red clover was secured from the adjoining farm plots; one plot had received lime, the other had not. The actual reaction of the limed plot (aqueous extract) was pH = 7.53 and that of the unlimed plot pH = 6.19. The plants from the limed plot were 20 to 24 inches high, whereas those from the unlimed plot were 10 to 18 inches high. When the plants were cut on May 30 those from the unlimed plot appeared a lighter green than the others. In the first determination the leaflets were picked from each plant and their juice expressed

in the usual manner. In order to observe errors that might be introduced as a result of changes in the juice during a 20-minute period of centrifuging, a determination of the actual acidity of a portion of the juice was made immediately after the juice was expressed, whereas the remainder of the juice was centrifuged. The results obtained for the effect of centrifuging on the initial actual acidities of the juices may be stated briefly as follows:

Uncentrifuged

Limed plants	
Leaves.....	pH = 6.19
Stems and petioles.....	pH = 5.95
Unlimed plants	
Leaves.....	pH = 6.02
Stems and petioles.....	pH = 5.63

Centrifuged

Limed plants	
Leaves.....	pH = 6.12
Stems and petioles.....	pH = 5.89
Unlimed plants	
Leaves.....	pH = 5.99
Stems and petioles.....	pH = 5.62

Obviously, only a slight increase in actual reaction of the juice of the above-ground parts occurred during the centrifuging.

The curves (fig. 4) represent the actual and the total acidities (expressed in terms of 0.0684 *N* sodium hydroxide) of the 7-cc. samples of different parts of medium red clover plants. The titration curves make it evident that the initial actual reaction of the leaves, stems and petioles, and roots of plants from limed soil was less than that of the corresponding parts of the plants from unlimed soil. The total acidities of the leaf juices were quite similar in both cases. The juice of the stems and petioles of the plants from limed soil had a smaller total acidity than that of the plants from unlimed soil. The total acidity of the roots without liming was at first greater than that with liming, but became smaller than that with liming as the titration proceeded.

However, the day previous to the above experiment, two lots of medium red clover roots were secured from the same plot as before and determinations made of the actual reactions of the uncentrifuged juice. From table 3 it is evident that the roots with liming had a slightly greater actual acidity than the roots without liming and that the results obtained for the root juice (fig. 4) are reversed.

Timothy and winter wheat plants were cut June 11 at the surface of the soil of the same limed and unlimed plots in which the medium red clover was growing. The actual reactions recorded in table 3 indicate that the effect of liming the soil was to decrease the actual acidity of the juice of timothy and wheat.

White mustard plants were grown on the limed and the unlimed Plainfield sand from March 1 to June 13. When the tops were cut off they were very unequal in size on the limed and unlimed pots. The roots from the limed and unlimed soil with about an inch of the attached stem were the only parts utilized in the determinations of the actual acidities of the juices.

Table 3 makes it clear that the juice of the roots with liming showed a less actual acidity than without liming. Whenever roots were used they were repeatedly washed with pure distilled water and then shaken free of any adhering moisture, after which they were further dried by drawing them

TABLE 3
Actual acidities of juice of different parts of plants grown on limed and unlimed soil

PLANT	PART OF PLANT	TREATMENT	LIMED SOIL	UNLIMED SOIL
			pH	pH
Medium red clover, lot 1	Roots	Uncentrifuged	5.87	5.91
Medium red clover, lot 2	Roots	Uncentrifuged	5.82	5.88
Timothy	Leaves, stems, young spikes	Uncentrifuged	6.17	6.14
Timothy	Leaves, stems, young spikes	Uncentrifuged	6.19	6.12
Winter wheat, lot 1	Tops (pollen stage)	Centrifuged 10 minutes	6.33	5.95
Winter wheat, lot 2	Leaves, stems, no spikes	Centrifuged 10 minutes	6.12	5.77
White mustard	Roots with lower 1 inch of stem	Uncentrifuged	5.91	5.62
White mustard	Entire plants	Uncentrifuged	5.78	5.48
Corn	Tops	Centrifuged	5.48	5.48
Field peas	Tops	Uncentrifuged	6.53	6.80
Buckwheat	Entire plants	Uncentrifuged	5.97	5.48
Alfalfa	Tops	Centrifuged	6.19	5.99
Baltic alfalfa no. 550	Roots	Uncentrifuged	6.12	6.21
Common South Dakota alfalfa, no. 363	Roots	Uncentrifuged	6.12	6.06
Alsike clover	Roots	Uncentrifuged	5.84	5.68

over a good grade of filter paper. These determinations were made possible by using the small hydrogen-electrode vessel. The tops were so unequal in size (plate 1) that it seemed best to utilize the tops of white mustard at an earlier stage, when they were more nearly equal in size and a more fair comparison of the actual acidities of the juices would be possible.

White mustard seed was sown in Colby silt loam in pots containing 11,500 gm. of the soil. To some pots no lime was added; to others 1 per cent sufficient ground limestone was added, to neutralize the acidity as shown by Truog's method. Each pot received 25 cc. of water containing 1 gm. of dipotassium phosphate. The other plants grown on Colby silt loam, which are to be mentioned later, received the same soil conditions as white mustard.

The juice of the white mustard plants was so small in amount that in order to obtain any at all, it was necessary to place the entire plants inside a linen cloth and after crushing the plant cells in a porcelain mortar, the juice was expressed by wringing the cloth. The actual acidities of the juices of the plants under both treatments, as regards liming, are given in table 3. Even at this early stage in the growth of the plants, a distinct decrease in the actual reaction of the juice of plants with liming as compared with that of plants without liming is evident.

When corn plants about 10 to 12 inches high that were grown on limed and on unlimed Colby silt loam were used for determinations of actual acidities,

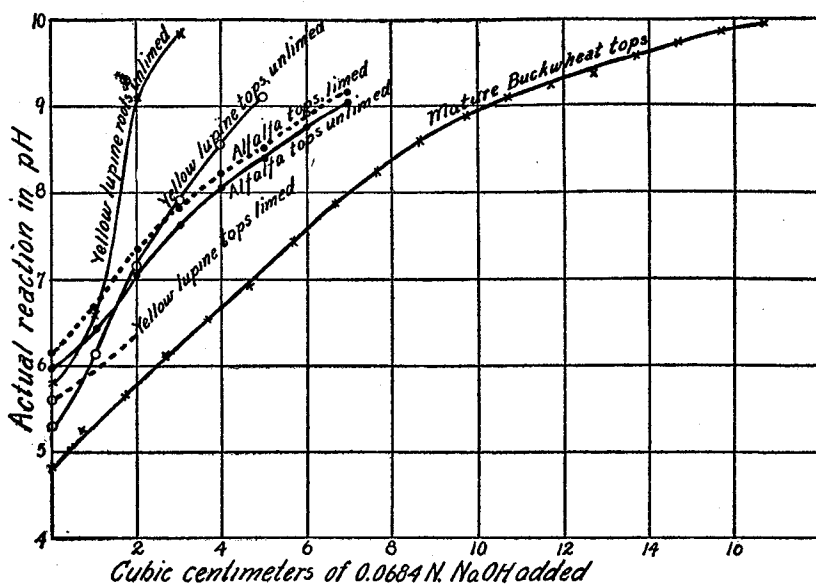


FIG. 5. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF THE JUICE OF BUCKWHEAT TOPS AND OF THE JUICES OF ALFALFA TOPS, LUPINE TOPS, AND LUPINE ROOTS GROWN WITH AND WITHOUT LIMING

the juice in both cases gave a value of $\text{pH} = 5.48$. The small hydrogen-electrode vessel was not available at the time and separate determinations on the leaves and stalks were not made.

The actual acidities of the juices of field pea tops and of entire buckwheat plants grown on limed and unlimed Colby silt loam were determined. The field pea plants in each case were about 8 inches high; the buckwheat plants in each case were about 5 to 6 inches high. The results are given in table 3.

In table 3 it is seen that the acidity of the juice of the pea plants with liming was greater than that of the plants without liming. With buckwheat the converse was true, and the actual reaction with liming was much less than without liming.

Plants from limed and unlimed plots at Monroe, Wisconsin, were brought into the laboratory shortly after being dug up. The roots were well covered with soil and the tops were in excellent condition. Table 3 gives the results for alfalfa tops and several lots of roots, obtained from limed and unlimed plots. Figure 5 gives the titration curves of 9.5-cc. samples of juice of the alfalfa tops from limed and unlimed soil and shows that liming decreased both the actual and total acidities.

Viewing table 3 as a whole, it is found that in one case, liming the soil was followed by no change in the actual acidity of the plant juice. However, in 10 out of 14 cases the addition of lime was followed by a decrease in the actual acidities of the plant juices.

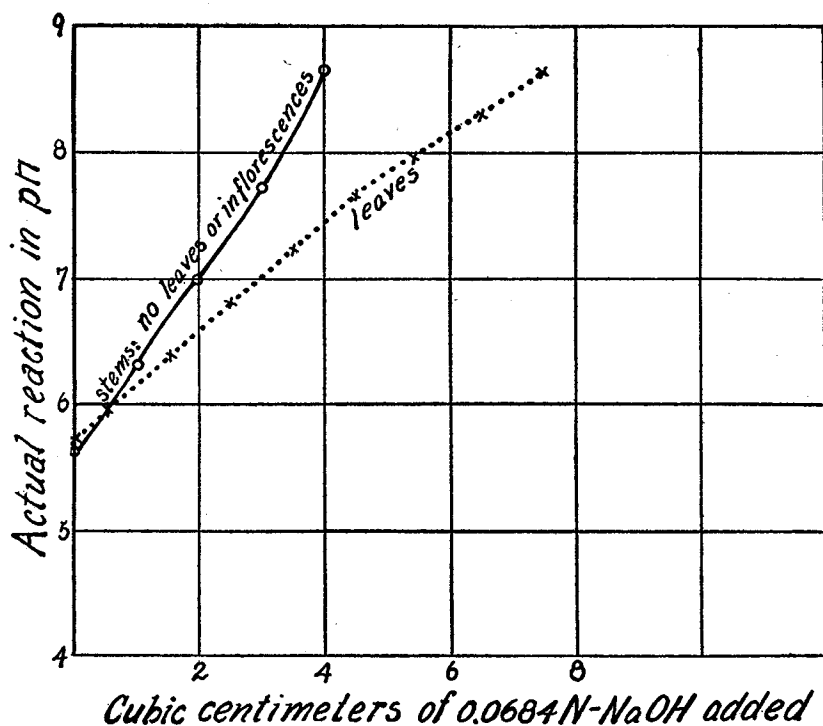


FIG. 6. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF THE JUICE OF THE LEAVES AND STEMS OF ORCHARD GRASS

Although yellow lupines do not grow well on limed Plainfield sand, it was possible to secure sufficient of the plant juice to obtain an idea concerning the effect of liming on the actual acidity of the juice. The plants had grown from March 1 to June 3. The plants without liming were in bloom, whereas the plant with liming was not in bloom and was only about one-half as high as the former. Unfortunately, insufficient juice was obtained from the single plant grown on limed soil to carry on a titration for the total acidity.

In both cases the buds, flowers, and nodules were discarded. A 7-cc. sample of juice was used in each titration. The results obtained for yellow lupine are given in the form of curves in figure 6.

The curves indicate that lime decreased the actual acidity of the juice of yellow lupine tops. Without liming the total acidity of the juice of the tops was greater than that of the roots.

TABLE 4

A summary of the effect of liming the soil upon the actual and total acidities of plant juices

PLANT	ACTUAL ACIDITY	ACTUAL ACIDITY OF JUICE		TOTAL ACIDITY
		Limed	Unlimed	
		pH	pH	
Alfalfa tops.....	Decrease	6.19	5.99	Decrease
Alfalfa roots (Baltic no. 550).....	Increase	6.12	6.21	
Alfalfa roots (Common South Dakota no. 363).....	Decrease	6.12	6.06	
Alsike clover roots.....	Decrease	5.84	5.68	
Alsike clover tops.....	Decrease	6.19	5.28	Decrease
Barley tops.....	Decrease	5.72	5.62	No difference
Buckwheat (entire seedlings).....	Decrease	5.97	5.48	
Corn tops.....	No difference	5.19	5.19	Slight increase
Corn tops.....	No difference	5.48	5.48	
Field peas tops.....	Increase	6.53	6.80	
Garden bean tops.....	Increase	5.65	5.97	Increase
Lupine, yellow tops.....	Decrease	5.63	5.31	
Lupine, yellow roots.....			5.80	
Medium red clover roots.....	Increase	5.87	5.91	
Medium red clover tops.....	Decrease	5.82	5.88	Decrease
Medium red clover leaves.....	Decrease	6.12	5.94	No difference
Medium red clover stems and petioles.....	Decrease	6.19	6.02	
Medium red clover roots.....	Decrease	5.95	5.63	Decrease
Medium red clover roots.....	Decrease	5.92	5.84	Decrease then increase
Mustard, white, roots.....	Decrease	5.91	5.62	
Mustard, white entire plants.....	Decrease	5.78	5.48	
Oat plants.....	Decrease	5.67	5.65	Slight increase
Serradella tops.....	Increase	5.74	5.94	Decrease then increase
Timothy tops.....	Decrease	6.17	6.14	
Timothy tops.....	Decrease	6.19	6.12	
Winter wheat tops.....	Decrease	6.33	5.95	
Winter wheat, leaves and stems, no heads.....	Decrease	6.12	5.77	

Table 4 gives the results of liming the soil upon the reaction of plant juices. Apparently most of the evidence at hand confirms Truog's suggestion² that lime may function to decrease or regulate the actual acidity of the plant protoplasm.

The experiments of Kappen (13) emphasize the fact that as the actual acidity increases in the root juice, there is also a very appreciable increase in the total acidity of the juice. In the titration of the total acidities for limed and unlimed conditions, the writer found that when liming caused a decrease in the actual acidity, frequently there was a decrease in the total acidity; also that when liming produced an increased actual acidity, there was usually an increased total acidity. In this paper, the data for the effect of liming on the total acidities of plant juices are too meager to permit making a conclusive statement regarding it. That the total acidities of plant juices do sometimes fluctuate quite regularly in the same direction as the actual acidities, will be shown in a subsequent paper by Bauer and myself. Miss Irwin (16) has made the interesting observation that under certain conditions the carbon-dioxide production by petals may increase and still the actual reaction of the cell contents may decrease.

AGRICULTURAL PLANTS WITH HIGH ACTUAL AND TOTAL ACIDITIES

As stated the actual reaction of the juice of yellow lupines was found to be $\text{pH} = 5.31$ and $\text{pH} = 5.63$ for unlimed and limed conditions, respectively. Hempel (11) working with 20-day-old white lupine seedlings, found their actual reaction to be $\text{pH} = 5.93$. Of the plants with quite acid juices, buckwheat is of interest. It was mentioned before that the juices of buckwheat (entire seedlings 5 to 6 inches high with but few leaves on each seedling) had actual reactions of $\text{pH} = 5.48$ and $\text{pH} = 5.97$ when the plants were grown on unlimed and limed soil, respectively. Buckwheat tops, partially flowering and in seed, gave a juice with an actual acidity of $\text{pH} = 4.82$. An aqueous extract of the soil on which this buckwheat was grown had an actual reaction of $\text{pH} = 7.68$. The actual acidity of the juice of nearly mature buckwheat plants is markedly greater than during the early stages of growth. The titration values for the actual and the total acidities of the juice of buckwheat plants in their advanced stage are plotted in figure 5.

The curve for buckwheat indicates a marked buffer action of its juice. The 15-cc. sample of the centrifuged juice required the addition of about 4.2 cc. of the alkali to bring the juice to $\text{pH} = 6.81$, which is the color change for litmus, and about 8 cc. of the alkali to bring the juice to $\text{pH} = 8.31$, the color change for phenolphthalein.

VARIATIONS OF REACTION IN DIFFERENT PARTS OF PLANTS

The titration curves, in figure 4, for medium red clover, from limed and unlimed soils, show that the actual acidities and the acid reserves of the juices of the leaves, stems, and roots may differ quite considerably. By acid reserve we mean the amount of dissolved acid or acid salts in the undissociated form that dissociates as we neutralize some of the acid (hydrogen ions). The curves in figure 4 show that for both soils the leaf juice had the

greatest acid reserve. The acid reserve of the root juice, however, was closer to that of the stems and petioles than to that of the leaves, and was somewhat intermediate in position. The results of Kappen (13) indicate that the juice of the above-ground parts of a plant has a greater total acidity than that of the roots.

The importance of determining the actual and total acidities of comparable tissues or organs, or parts of the plants, rather than of the entire tops, is further emphasized in the experiments with sweet clover. It is evident that if plant juice varies in its actual or total reaction according to the part of the plant from which it is expressed, then the number of leaves, stems, roots, etc., on the two lots of plants that are being compared may become a factor of considerable importance.

In order to study the variations of actual reaction in different parts of plants, the results obtained for blue-grass may be examined. The plants were about 20 inches high and in the pollinating stage. The soil reaction was neutral to slightly alkaline. The actual acidities of the uncentrifuged juices of the upper five inches of the tops which included all of the inflorescences, and of the lower 15 inches of the tops, were determined and found to be as follows: upper 5 inches of tops including inflorescences, $\text{pH} = 6.11$; lower 15 inches of tops, $\text{pH} = 5.92$. The results for blue-grass show that plant juice may vary in its actual reaction in different parts of the plant.

The results with orchard grass also show a change of actual reaction of the juice in different parts of the plant. The orchard grass was cut June 9 from a neutral to slightly alkaline soil. Determinations were made of the actual reaction of the different parts of the plants, using 10-cc. samples of juice centrifuged for 10 minutes. The curves in figure 6 plotted from the results obtained, show that the initial actual acidity of the juice of the stems is greater than that of the leaves. Between $\text{pH} = 6$ and 9 the total acidity of the leaf juice is greater than that of the stem juice. Until approximately the point $\text{pH} = 6$ is reached, the conditions of total acidities are reversed from what they are beyond $\text{pH} = 6$.

IS THERE A GRADIENT OF REACTION IN PLANTS?

An extreme case, that of sweet clover, may be taken to emphasize the differences that exist in the reaction of the juice from different parts of the same plant. At 3:45 p.m., May 29, an entire sweet clover plant was dug up. The actual reaction of the soil extract was $\text{pH} = 7.68$. The tops were 24 inches high and the length of thickened root obtained was about 8 to 10 inches. The tops represented the second year's growth. The sunlight was intensely bright and the leaves had lost some of their turgidity. The actual acidity determinations of the juice taken from different portions of the same plant are given in table 5. These results indicate that the juice in this particular plant was acid, neutral, and alkaline, depending upon the portion of

the plant from which the juice was taken. Among the numerous plants examined, the writer has not found another species exhibiting this condition. It is evident from table 5 that in this case a steep gradient existed in the actual reaction from the one end to the other, the upper end being the least acid. The studies of Child (2) on the metabolic and susceptibility gradients in plants and animals are of great interest, and possibly the use of sweet clover in these studies would give further valuable information. At present our results upon acidity gradients are too meager to permit of conclusions regarding their significance.

In connection with acidity gradients in plants it is well also to consider their relation to enzyme activity (3, 17), since the reaction within a plant may range from marked acidity at one end to a considerable alkalinity at the other end. Bunzell (1) has reported the results of a thorough study of the degree of oxidase activity of different plant juices, but has found it necessary to carry on all of the experiments in a solution, the actual acidity of which was greater than that of the neutral point for distilled water. In alkaline solutions, pyrocatechol, as well as most other good oxidase reagents, are oxidized by atmospheric oxygen, so that it is quite impossible to distinguish between the oxidation due to the alkalinity of the solution and that due to the oxidase activity of the plant juice.

In order to demonstrate conclusively that a large plant like this can possess an alkaline reaction of the degree found at one end of the acidity gradient, some second year's growth of sweet clover was cut from various locations about the Soils Building at the Wisconsin station. The entire tops of the various lots gave the following pH values: 7.50, 7.51, 7.54, 7.55, 7.59, 7.80, and 7.90. It is therefore evident that for plants like sweet clover, it is important to compare results from corresponding parts of plants. The different actual acidities of the different parts of sweet clover, together with the different proportions of leaves, stems and petioles in the various lots, undoubtedly have been largely responsible for the above wide range of 7.50-7.90 in the pH value.

THE ACTUAL REACTION OF THE JUICE FROM THE SUCCEEDING YEAR'S
GROWTH OF THE SAME CROP

When the pH values, in table 5 are compared for the first and second year's growth of sweet clover, it is found that the different parts of the first crop of the first year's growth were somewhat more acid than the second year's growth. Further experiments are necessary before a conclusion can be reached in this regard.

Since the second year's growth was much more vigorous than that of the first year, the question arises: do the more vigorously growing plants of a species show a decrease in the actual reaction of their juice compared with that of less actively growing plants of the same species?

TABLE 5

Actual reaction of the juice from different portions of sweet clover plants

ACTUAL ACIDITY	DESCRIPTION OF MATERIAL USED
First crop; First year's growth	
pH	
6.53, 6.65, 6.68, 7.38	Each lot composed of leaves, petioles and buds
6.38, 6.46, 6.48, 6.48	Each lot composed of stems only
First crop; Second year's growth	
8.00	Upper 3 inches of the tops, stem, leaves and buds
7.04	Leaves and petioles of remainder of tops (no stems)
6.68	Stems to about 2 inches above the soil (no leaves)
6.46	2 inches of lower part of stem
	2 inches of upper part of root
5.82	Root: 6-inch portion below the upper 2 inches of root

ACID RESERVE OF THE JUICE OF PLANTS GROWING IN SAME ENVIRONMENT

Since it has been observed that the juice of the second year's growth of sweet clover may be markedly alkaline in reaction, it may be of interest to compare the acid reserve of the juice from the tops of the second year's growth of sweet clover with that of the second year's growth of medium red clover. Both species were growing together in a plot, an aqueous extract of which gave a reaction of $\text{pH} = 7.51$. The plants were cut May 23.

Determinations were also made of the acid reserve of the root juice of the two species of plants which were obtained from the same part of the plot 3 days later. Ten-cubic-centimeter samples of juice centrifuged for 15 minutes were used in every case. The titrations were repeated on a second series of aliquots of the same juice and the results checked very closely. The results are shown in figure 7.

The curves indicate that the juice of the roots in the two cases has a greater acid reserve than the juice of the tops of similar plants cut 3 days previously. They indicate also that the juice of the medium red clover tops and of the roots has a greater acid reserve than that of the sweet clover tops and roots, respectively. Furthermore, the actual acidity of the juice of medium red clover tops is greater than that of sweet clover tops, whereas the actual acidity of the juice of the roots of both species was practically the same.

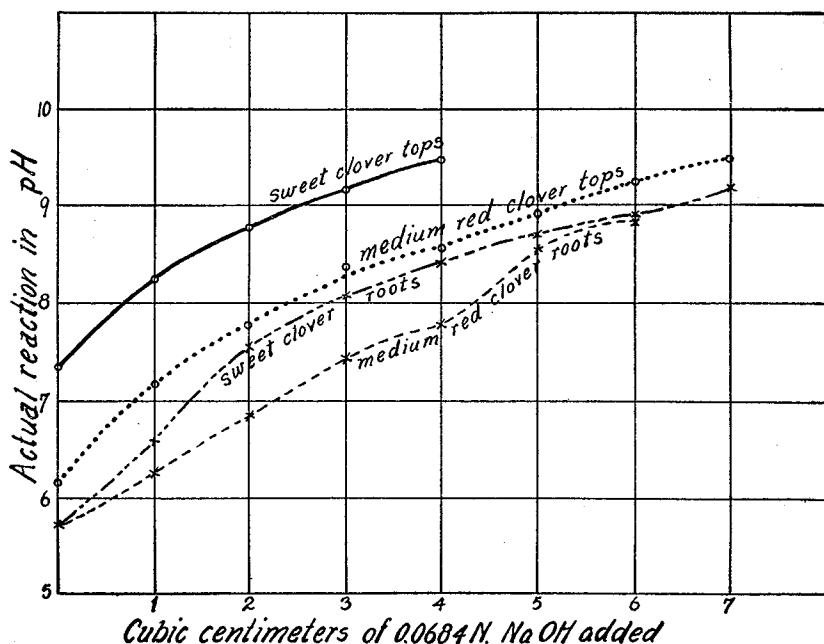


FIG. 7. CURVES SHOWING THE ACID RESERVE OF TOPS OF THE SECOND YEAR'S GROWTH OF SWEET CLOVER AND OF MEDIUM RED CLOVER CUT ON THE SAME DAY, AND THAT OF THE ROOTS OF BOTH SPECIES OBTAINED 3 DAYS LATER

THE ALKALI RESERVE OF PLANT JUICE

It was considered worth while to determine the degree to which the juice of the second year's growth of sweet clover could become alkaline. A group of such plants 24 to 34 inches high was growing luxuriantly near the edge of a burned refuse pile. The reaction of the soil extract was $\text{pH} = 7.68$. The uppermost 2 inches of a large number of the plants were plucked and only the very tip of the axis, together with its unfolding leaves, was retained for the determination of the actual reaction of the juice.

When the tips were cut the sun had been shining several hours, although the previous two days it had been raining. The juice was centrifuged 5 minutes and the actual reaction was found to be $\text{pH} = 8.47$. Since phenolphthalein turns pink at $\text{pH} = 8.31$, the juice of the tips of sweet clover had an actual reaction apparently alkaline to phenolphthalein. The alkali reserve of a 10-cc. portion of centrifuged juice of the tips of sweet clover was then determined by adding different amounts of standard acid to the juice and observing the new pH after each addition of the acid.⁵ The values obtained in this way are given in the form of a curve in figure 8.

⁵ By alkali reserve we mean the change in the actual reaction of the juice upon the addition of acid.

Since sweet clover juice was decidedly alkaline and had a high alkaline reserve, it appeared probable that it might contain more carbonates than the more acid juice of medium red clover tops. The apparatus of Van Slyke (26) was used for the estimation of carbon dioxide in the juices.

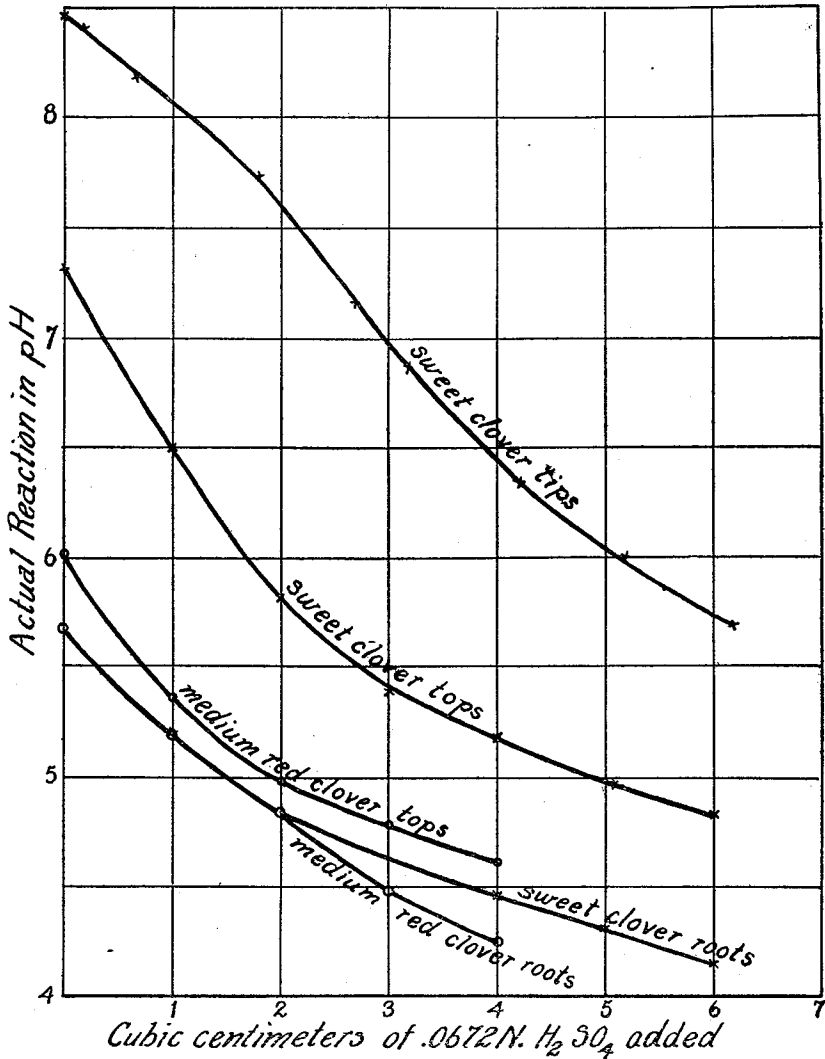


FIG. 8. CURVES SHOWING THE ALKALI RESERVE OF DIFFERENT PARTS OF SWEET CLOVER AND MEDIUM RED CLOVER

The readings of table 6 are not intended for absolute values, and hence corrections have not been made. The juice of sweet clover tops contained approximately 50 per cent more carbon dioxide than did that of medium red

clover. Undoubtedly some of the carbon-dioxide gas existed as dissolved gas in the juice, and hence without further experiments it is impossible to say how much was in the carbonate or bicarbonate form. It is for this reason that the results for medium red clover are given as a control. The results for the two species may therefore serve as a rough comparison of the relative amounts of carbonates and bicarbonates in the juice of the two species.

The juice of the roots of the second year's growth of sweet clover was then examined for its alkali reserve. The juice from the tops of the plants, from which the roots were taken, had an actual acidity of $\text{pH} = 7.90$. Likewise the actual reaction and alkali reserve of the juice of medium red clover roots from the same plot as that from which the sweet clover roots were taken were then determined. The alkali reserve of the juice of the tops of both species from the same plot as before also was then determined. Ten-cubic-centi-

TABLE 6
Total amount of carbon dioxide (uncorrected) in 3-cc. aliquots of the uncentrifuged juice of tops

SWEET CLOVER	MEDIUM RED CLOVER
cc.	cc.
0.142	
0.140	0.090
0.170	0.090
0.140	0.100
0.180	

meter samples of juice were used in the several titrations. The actual acidity and alkali-reserve determinations were carried on at about the same time, since it has been shown especially for some of the succulents that a considerable diurnal variation may take place in the reaction of the juice (14, 15, 18, 19 and 22). From the curves (fig. 8) plotted from the results obtained, it is seen that the juice of the unfolding tips of sweet clover has a much greater alkali reserve than that of the tops, and that of the tops greater than that of the roots. The alkali reserves of the root juices of sweet and of medium red clover are practically identical until $\text{pH} = 4.85$ is reached, at which point the curves begin to diverge. The alkali reserve of the juice of medium red clover tops is greater than that of the roots, though the difference is not as marked as with sweet clover. Much more data are necessary before any far-reaching conclusions can be drawn.

EFFECT OF THE ABSENCE OF CHLOROPHYLL (INHERITABLE) UPON THE REACTION OF PLANT JUICE

In studies upon normal and etiolated lupine seedlings Hempel (11) found that light decreases the total acidity to litmus, but increases the total acidity to phenolphthalein when compared with the total acidity of plants kept

in the dark, although the actual acidity remains nearly constant. The writer was able to secure corn seed that produced seedlings, about 25 per cent of which were pure white.⁶ The juice of both the green and the colorless plants was tested for its actual and total acidity.

A preliminary experiment was carried on, using other corn seedlings. The results of the preliminary experiment are not to be emphasized on account of the fact that it is uncertain whether or not all of the seedlings received similar conditions of illumination.

The seedlings, of which only the tops were used, were 18 days old when the preliminary experiment was carried on. The actual and total acidities of 6-cc. samples of juice that had been centrifuged 15 minutes were determined. If the curves in figure 9 are examined it is found that the juice of

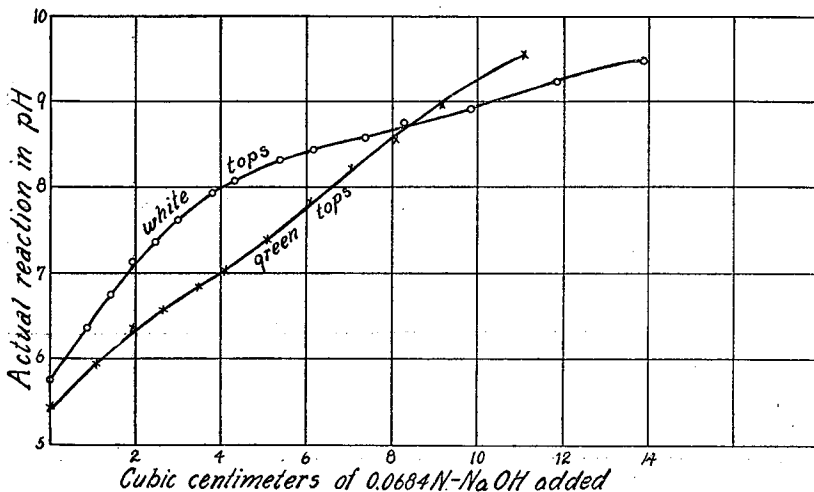


FIG. 9. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF GREEN AND OF WHITE TOPS OF CORN SEEDLINGS

the chlorophylless tops did not behave in a manner similar to that of the chlorophyllous tops as regards total acidity. The total acidity of the juice of the green tops is much greater than that of the white tops until about the point $\text{pH} = 8.7$ is reached, when the curves cross each other. Moreover, the initial actual acidity of the juice of the green tops was greater than that of the white tops. If the chlorophylless tops behaved like etiolated plants, then, according to the interesting observations of Hempel (11) for etiolated lupine seedlings, it should be expected that the chlorophylless tops would have shown a greater total acidity than the chlorophyllous tops to litmus— $\text{pH} = 6.81$, but a smaller total acidity than the chlorophyllous tops to phenolphthalein— $\text{pH} = 8.31$. Neither of these two conditions hold in the curves

⁶ These were furnished through the kindness of E. W. Lindstrom, of the University of Wisconsin.

given; hence, it appears from this preliminary experiment, that as regards total acidity the chlorophyllless plants do not behave as though they were etiolated.

Another similar experiment was carried out in which the different seedlings were grown among one another for 10 days and were given similar environmental conditions. The titrations were made on 10-cc. samples of juice that had been centrifuged 10 minutes.

The results of the determinations are given as curves in figure 10. The curves show much the same results as were observed in a preliminary experiment, and given in figure 9.

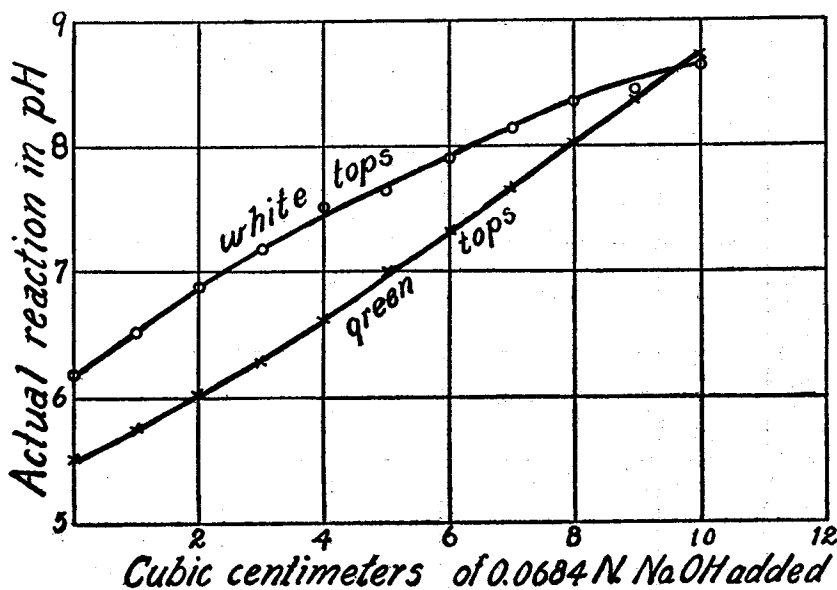


FIG. 10. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF GREEN AND OF WHITE TOPS OF CORN SEEDLINGS

Among the corn seedlings a number of the white tops were tinted somewhat green. As expected, their pH value was intermediate between that obtained for pure white and for green tops. The actual values found were as follows: green tops pH = 5.52, green-tinted white tops pH = 5.85, and white tops pH = 6.16.

EFFECT OF LIGHT AND AGE ON THE REACTION OF THE JUICE OBTAINED FROM PLANTS

Corn seed from a single ear that produced all green seedlings was planted June 3. Half of the seeded area was covered with a large box that was covered with black paper. At the end of 10 days the seedlings with green tops were 5 inches high, whereas the greenish-yellow tops of the darkened

seedlings were 9 inches high. Some of the corn seedlings with green tops were placed in a subcellar in total darkness from June 13 until June 16. Determinations were made of the actual and total acidities of the tops of both the 10- and 13-day-old plants, respectively, using in every case 10-cc. samples of juice that had been centrifuged 10 minutes.

On plotting the data obtained for the 10- and 13-day corn seedling tops (fig. 11), it is found that the total acidity of the juice of the 13-day green tops was greater than that of the 10-day green tops for any pH taken as the end-point. This is in accord with the results of Hempel (11) on white lupine seedlings in which she found that for normal seedlings the total acidity of the juice (taking litmus pH = 6.81 and phenolphthalein pH = 8.31 as end-

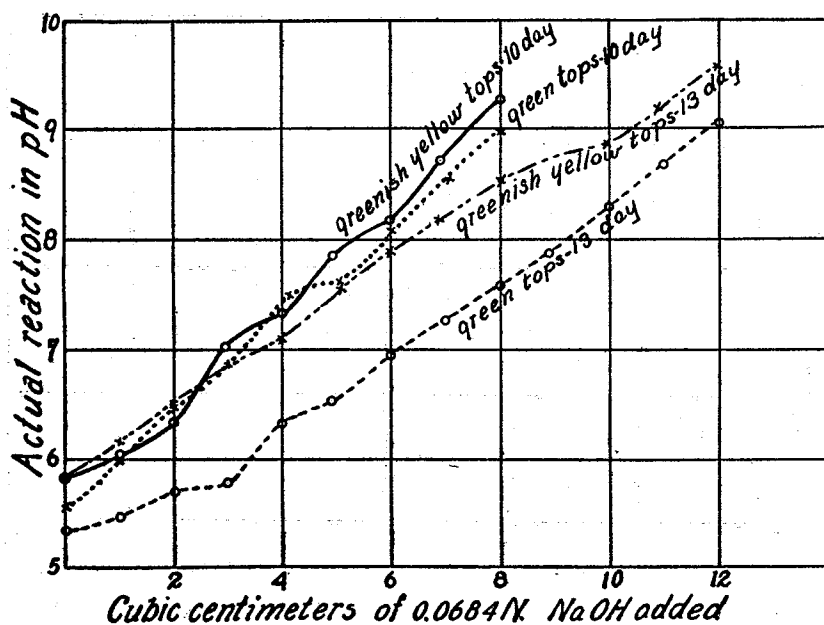


FIG. 11. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF GREEN AND OF GREENISH-YELLOW TOPS OF CORN SEEDLINGS

points) increased with the increasing age of the seedlings. Undoubtedly the 10-day darkened tops must have received very faint illumination, for otherwise the tops would have been more blanched.

For the 10-day seedlings, the initial actual acidity of the darkened tops is less than that of the undarkened tops. The curves for the total acidity of the juices of the two kinds of 10-day-old tops lie very close to one another, but show to a less marked degree the same results as the 13-day-old tops. The close approach to each other of the curves for the 10-day tops may be the result of an insufficient degree of etiolation or may be due to the young age of the seedlings.

When the green seedlings were permitted to remain 10 days in the light and part were then placed in total darkness for 3 days, it is found as before that the initial actual reaction of the juice of the tops exposed 13 days to the light was greater than that of the tops exposed 10 days to the light and 3 days to total darkness. The total acidity of the juice of the tops darkened for 3 days is much less than that of the undarkened tops.

SUMMARY

The actual and total acidities and alkali reserve of a number of agricultural plants have been determined for certain conditions of growth. In order to ascertain the actual reaction of minute quantities of plant juice, a hydrogen-electrode vessel has been used that gave excellent results with only 3 to 4 drops of juice.

The reaction of the juice of a plant appears to be affected by changes in illumination, soil solution, age, and other conditions, indicating that the reaction of plant juice is the resultant of many processes. The results of preliminary experiments bearing upon some of these factors are herein reported.

The juice of buckwheat seedlings was found to possess an actual acidity of $\text{pH} = 5.48$ to 5.97 , whereas in the mature condition a reaction of $\text{pH} = 4.82$ was reached. It appears that during the life cycle of buckwheat plants the juice may undergo a marked change in its actual reaction. The degree of maturity of a plant seems to be an important factor in the degree of actual acidity attained. Table 4 shows that the actual reaction of the juice of many agricultural plants is distinctly acid.

In order to obtain a measure of the buffer action of plant juice, determinations have been made of the acid and alkali reserve of the juice of certain plants. The alkali reserve of the roots of medium red clover and of sweet clover (fig. 8) is less than that of the corresponding tops. The acid reserve of the roots of medium red clover (fig. 4) is less than that of the leaves, even though the actual acidity of the roots is greater than that of the leaves. The unique case of a single plant having juice with a markedly alkaline actual reaction and a steep gradient in actual reaction is reported. The variations in the actual reaction of plant juice in the various portions of the plant have been investigated.

Although the determinations of the total acidity (table 4) have been too few in number, those reported, together with further observations to be presented later, indicate the existence of a close relation between the actual and the total acidity, and appear to show that the total acidity tends to fluctuate in the same direction as the actual acidity, although exceptions may frequently occur. It has been pointed out that the proportion of stems, leaves, petioles, etc., on plants, may influence the values obtained for the actual and the total acidities of the juice. The juice of the younger portions of a plant usually has a lower actual acidity than the older portions.

The total acidity of the juice of chlorophyllless (inheritable) corn seedling tops is less than that of chlorophyllous tops until about pH = 8.6 is passed, after which the reverse is true. The actual and the total acidity of the juice of corn seedling tops increases with the increasing age of the seedlings and decreases with a reduction of the normal illumination.

There is considerable experimental data in favor of the suggestion of Truog that the main specific harmful influence of soil acidity on certain plants is due to its influence in preventing the plants from securing rapidly enough the bases that are needed to neutralize and precipitate acids within the plant. Further experiments under controlled conditions will be necessary before all the factors can be fully understood.

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PLATE 1

EFFECT ON THE GROWTH OF WHITE MUSTARD PLANTS OF THE ADDITION OF LIME
TO PLAINFIELD SAND: 1, LIMED; 2, NOT LIMED

