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Introduction

The earliest published record of diurnal changes in sap acidity appears to be the report of Heyne (8) in 1813 who detected it by the sense of taste. He found that *Bryophyllum calycinum* was more sour early in the morning than in late afternoon. According to Richards (19), these observations were corroborated by Link who tested the juice from the same species and other crassulaceous forms with litmus paper. Other investigators also verified these observations and various explanations were offered for such diurnal changes in acidity (6, 21). Succulents were used in most of the early investigations on diurnal changes in sap acidity and the acidity there of was measured as measured was total titratable acidity. Gustafson (6) recorded diurnal changes in total and actual acidity of the juice of *Bryophyllum calycinum,* and found the lowest pH at 4 P. M. on a sunny day. The hydrogen-ion concentration underwent a similar series of changes on cloudy days, the chief difference being that changes were more pronounced on sunny days. Gustafson reported pH 3.90 for 10:00 A. M. and pH 5.37 for 4 P. M. for whole tops of *Bryophyllum calycinum.* Ingalls and Shive (13) recorded pH values 3.44 and 4.93 at 9 A. M. and 5 P. M., respectively, for stems, and pH 3.34 and 4.92 at 7 A. M. and 5 P. M. for leaves of *Bryophyllum.*

More recent investigations have disclosed diurnal periodicity of total and free acidity in non-succulents. Truog and Meacham (24) reported diurnal changes in actual acidity of the juice of alfalfa tops. They give pH values of 5.97 and 6.00 for plants cut at 6 A. M. and 2 P. M. respectively on a clear, warm day. Garner, Bacon and Allard (4) observed diurnal fluctuations in biloxi soy bean in the vegetative stage. They recorded pH values of 6.22, 6.19 and 6.14 respectively for samples collected in the morning, at midday and in the afternoon. Hurd (9, 11) found that wheat plants cut at 9 A. M. almost invariably had a higher hydrogen-ion concentration than those cut from the same plot at 1 P. M. According to her data the difference in pH values for these times was never less than 0.14. On the other hand, Hurd (10) reported that there was very little change in pH of corn between 8:30 A. M. and 12:30 P. M.

Clevenger (2) followed the diurnal changes in actual acidity of

leaves, stems and roots of cow pea during a 24 hour period, making determinations every two hours. He found the hydrion concentration of leaves and stems to be highest in the morning and lowest during the night; the greatest actual acidity for leaves and stems respectively, was reached at 10:37 A. M. and 7:38 A. M. and the lowest at 12:32 A. M. and 9:41 P. M. Garner, Bacon and Allard (4) observed that in the thin-leaved species the general tendency was toward an increase in average active acidity with increase in duration of the daily light period. Haas (7) reported greater hydrion concentration for the juice of tops of corn seedings exposed 10 days to the light and 3 days to total darkness. Loehwing (16) found that prolonged periods of strong illumination were accompanied by lower acidity levels and smaller diurnal fluctuations in the juice of wheat plants. In practically all cases maximum acidity was observed during morning hours and minimum acidity in afternoon or evening (15).

In an attempt to verify and extend some of the foregoing data an experiment was undertaken to determine changes in pH of tissue fluids that may occur in common non-succulents at short intervals during consecutive 24 hour periods. Plants chosen for these experiments were Reid's Yellow Dent corn (*Zea Mays*), hemp (*Cannabis sativa*), Ito San soy bean (*Glycine max* Merr.) and tobacco (*Nicotiana tobacum*).

Procedure

Reid's Yellow Dent corn was planted in pots June 17 and kept in the greenhouse until July 29 when the pots were moved outside where the plants had direct sunlight. Determinations were made on August 11 and 12 and the samples for analysis included all of the tissues 4 inches above the prop roots. Seeds of Little Turkish tobacco were planted in January and seedlings transplanted in June. Plants remained outside from July 29 until August 11 and 12 when the determinations were made. They had not yet reached the flowering stage. Only the upper leaves were used. Hemp used for the final determinations were taken from the drug garden of the State University of Iowa and was in the flowering stage when determinations were made. Separate determinations were made for male and female plants. Tops of main stem and branches of the same plant were used, and everything in the terminal 6 inches was included. Leaves as far as 12 inches below tips of main stems or branches were included.

Ito San soy beans taken from the University drug garden were approximately 2½ months old and just approaching the anthesis

stage when analyzed. The parts used were collected on two consecutive days. In the case of hemp and corn, before the determinations were begun plants were selected for uniformity and marked with the hour at which they were to be cut. Tobacco plants were similarly marked, and for every determination leaves were selected from corresponding levels of each plant.

A few preliminary experiments with frozen and unfrozen tissues indicated that larger quantities of juice can be obtained from frozen material than from unfrozen samples (18). These results are in agreement with those of Dixon and Atkins (3), Gortner and Harris (5) and Meyer (17). Dixon and Atkins also found that successively expressed samples of sap from frozen tissues were more uniform than those from unfrozen material. In order to begin the freezing of the tissues as quickly as possible after cutting a plan similar to that described by Meyer (17), was employed, using small pieces of solid CO_2. A thermos jug packed with dry ice was taken to field or plant house where the plants were cut. In every case the plant tissues were immediately chopped finely and mixed well. A sample was placed in a tared weighing bottle for moisture content determination and the remainder of the tissue (approximately 10 grams) was placed in a precooled stoppered pyrex tube taken from the jug; the rubber stopper was then replaced in the tube, the tube labeled and returned to the thermos jug. In this way there was little opportunity for pH or other physiological changes to take place in the plant tissues. After returning to the laboratory, more solid CO_2 was packed around the tubes in the jug and the temperature brought to —70° C.

A few preliminary experiments were undertaken with frozen tissues of soy bean to determine the influence of pressure employed on amounts of sap obtained and on the hydrion concentration of the sap. In all cases a pressure of 10,000 pounds yielded more sap than 5,000 pounds, but pressures up to 15,000 pounds produced almost identical yields. Knudson and Ginsberg (14) found that in the case of pre-frozen leaves of Iresine a pressure of 50,000 pounds yielded a more concentrated sap than a pressure of 10,000 pounds and concluded from this and other data that the amount of pressure applied is an important factor for certain types of sap analysis.

In order to insure uniformity for the data reported herein, a pressure of 15,000 pounds was employed in all cases. A Carver press was used (17). Canvas filter pads previously boiled and rinsed in distilled water were placed below and above the tissue in the cylinder and the tissue evenly packed. The expressed sap was collected

in a graduated tube and immediately brought to 25° C., the temperature at which all pH determinations were made. The measurements were made as quickly as possible to prevent or minimize changes which might occur in the acidity of the expressed juice. That changes do occur in acidity of plant juices upon standing has been shown by Clevenger (2), Haas (7), Hurd (9) and others who emphasize the importance of making determinations of acidity as quickly as possible. Since the expressed juices contained very little solid material and because of the suggestion made by Loehwing (16) concerning changes which might take place while centrifuging expressed juices, none of the sap was centrifuged.

The pH determinations were made potentiometrically (1), using a saturated calomel half-cell and quinhydrone electrode. Before a series of determinations was begun and several times during the progress of the series, the apparatus was carefully checked against a known buffer solution. Tissue samples were taken at consecutive 4 hour intervals. Each daily series of determinations was started at 2 P. M. and continued until 10 A. M. the following day.

Data and Discussion

The results of this investigation are in general agreement with those of other investigators (2, 6, 13, 16, 20) in which observations were made of press sap at regular intervals during a 24 hour period. The most obvious difference between the data of the present and of earlier investigators appears to be in the magnitude of fluctuations.

The pH value for corn was 5.18 at 6 A. M. and 5.20 at 10 A. M. (table 1) Hurd found that the pH of the juice of corn did not change significantly between 8 A. M. and 12:30 P. M. Hurd's results are in a general way corroborated by the present investigation. In tobacco (table 2), the lowest pH reading of 5.28 occurred at 6 and 10 A. M.

A greater hydrion concentration occurs in male than in female hemp plants throughout the series of determinations (table 3.). This is in agreement with results recorded by Talley (23) who found the average pH value of the sap of male plants to be 0.37 lower than that of females. In the present investigation the average difference in pH between the sexes was 0.40. In every case the juice of male plants was more acid than that of female plants.

The diurnal acidity data for soy bean (table 4.) show approximately similar levels (6.12 and 6.13) at 2 A. M. and 2 P. M. This tendency toward low pH levels in early morning and in early after-

Table 1. Corn

pH values of expressed juice (at 25° C.) of tops of Reid's Yellow Dent corn and moisture content (percentage of wet weight) of tissues used.

Hour Cut	Moisture Content	pH
2 P. M.	83.88	5.36
6 P. M.	82.08	5.47
10 P. M.	83.69	5.32
2 A. M.	84.55	5.17
6 A. M.	85.17	5.18
10 A. M.	85.48	5.20

Table 2. Tobacco

pH values of expressed juice (at 25° C.) of leaves of tobacco and moisture content (percentage of wet weight) of tissues used.

Hour Cut	Moisture Content	pH
2 P. M.	85.70	5.57
6 P. M.	85.07	5.57
10 P. M.	86.26	5.42
2 A. M.	86.28	5.37
6 A. M.	87.03	5.28
10 A. M.	84.84	5.28

noon in soy bean was also found in preliminary experiments by the writer. The pH values given by Garner, Bacon and Allard (4) for Biloxi soy bean for early morning, noon and late afternoon were 6.22, 6.19, and 6.14 respectively. These results appear to substantiate the hypothesis that active acidity increases during daylight hours in soy bean. These workers contrast the increase in sap hydrion concentration of soy bean, a legume, from morning to afternoon with the corresponding pH decrease in non-leguminous, non-succulents and in succulents from morning to afternoon. Rogers and Shive (20) have recorded diurnal changes in expressed sap of whole soy bean plants, determinations being made at the same hours as in the present study. An increase in acidity at 6 P. M. was followed by a decrease at 10 P. M. after which the acidity again increased, reaching its highest level at 6 A. M. No consistent correlation was observed between moisture content of tissues and hydrion concentration of juices of plants studied (tables 1 to 4).

An attempt was made to determine whether variation in sap pH is correlated with a change in temperature. The temperature was recorded at the time of each cutting for determinations of hemp and soy bean. No consistent correlation between sap pH and

temperature was discovered in the plants studied (tables 3 and 4). Gustafson (6), however, found that actual and total acidity of *Bryophyllum calycinum* decreased at approximately the same rate in plants kept in a warm greenhouse and plants kept at a lower temperature outside when the day was bright. On cloudy days plants exposed to the low temperature showed a lower rate of deacidification than those in the warm greenhouse. He concluded from this and other experiments that light is a much more important factor than temperature in the decomposition of tissue acids. When the light was intense, temperature did not seem to play any part. Hurd (12) found that hydrion concentration varied with the temperature at which wheat plants were grown, but in this study the pH values reflected the degree of vigor of the plants and seemed to be concomitant to unbalanced metabolism brought about by unfavorable temperatures.

Spoehr (22) probably gave the first definite account of the deacidification of malic acid on exposure to light. He stated that substances formed by photolysis of plant acids could be used in sugar synthesis in non-succulents as well as succulents. According to Richards (19), Astruc reported that much less acid was formed

Table 3. Hemp

pH values of expressed juice (at 25° C.) of tops of male and female hemp plants. Temperature (C.) of air near plants is given for each time of cutting.

		Moisture Content		pH	
Hour Cut	Temperature	Male	Female	Male	Female
2 P. M.	28	75.22	71.63	6.18	6.68
6 P. M.	23	75.66	69.97	6.57	7.32
10 P. M.	16	74.78	67.13	6.48	6.98
2 A. M.	12	75.87	70.16	6.41	6.57
6 A. M.	16	75.03	68.95	6.42	6.77
10 A. M.	27	74.32	68.36	6.40	6.57

Table 4. Soy Bean

pH values of expressed juice (at 25° C.) of tops of soy bean and temperature of air near plants at time of each cutting.

Hour Cut	Temperature	Moisture Content	pH
2 P. M.	28	63.00	6.12
6 P. M.	23	65.11	6.21
10 P. M.	16	68.43	6.37
2 A. M.	12	68.60	6.13
6 A. M.	16	76.13	6.23
10 A. M.	27	64.55	6.20

in succulents at night when they had been exposed during the previous day in an atmosphere devoid of CO_2.

When the foregoing data for non-succulent herbaceous species are viewed as a whole, some but by no means all species are found to show a decline in sap acidity during periods of daylight. The cyclic diurnal drop in sap pH of certain non-succulents suggests analogy with the photolysis of organic acids encountered in succulents such as Bryophyllum. The exceptional behavior of soy bean sap in this respect found in the present experiment confirms the earlier work of other investigators, and suggests that the diurnal cycle of sap acidity in legumes as a class may differ from succulents and other non-succulent herbs. Taken in their entirety, the data for non-succulents seem to warrant the inference that several as yet unidentified factors play as great and perhaps even a larger role than acid photolysis in the diurnal regulation of tissue fluid reaction.

Summary

1. Active acidity of expressed tissue fluids from leaves and tender tops of stems of corn, tobacco, hemp and soy bean showed definite diurnal fluctuations of varying magnitude.
2. Maximum sap acidity was reached in the early morning in all except soy bean and male hemp.
3. In soy bean corresponding sap acidity levels were attained in early morning and early afternoon.
4. The temperature at which freezing of tissues takes place apparently does not appreciably affect the pH values of the press sap obtained from ordinary non-succulents.
5. In the plants studied, small differences in magnitude of pressure at which sap is expressed from tissues appear to exert little influence on the pH of the sap sample obtained.
6. In the case of pH of press sap obtained from a variety of herbaceous non-succulent species at regular intervals during a 24 hour period, the lack of correlation thereof with time of day, atmospheric temperature, and moisture content of tissues suggests that the observed diurnal changes in pH are by no means as definitely controlled by photolysis of tissue acids as has been found in succulents.

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