Proceedings of the Iowa Academy of Science

Volume 60 | Annual Issue Article 23

1953

Buffer Capacity and pH of Press Sap in Relation to Dioecism of Phanerogams

Mary Claire Hoxmeier
Briar Cliff College

Buffer Capacity and pH of Press Sap in Relation to Dioecism of Phanerogams

By Sister Mary Claire Hoxmeier

Numerous experiments have been performed in the endeavor to identify the factors which underlie the sexual dimorphism in dioecious species of flowering plants (2, 7, 8, 9, 11). The discovery in 1920 (5) that the photoperiod has a fundamental relationship to inception of reproductive processes has given renewed impetus to studies of sex expression in dioecious species of plants in the belief that it also may be conditioned by a similarly specific stimulus. Studies of physico-chemical attributes such as respiration, tissue fluid reaction, and the role of specific cell components have disclosed certain functional processes which are correlated with sex differences. Though none of these has as yet been shown to be causative in its effect on sex expression, considerable evidence discloses that redox systems of tissue fluids are intimately correlated with sex (1, 3, 6). This investigation supplies data on the acid reserves on buffering action and pH of tissue fluids of staminate and pistillate plants of hemp and spinach. This study endeavors to correlate existing information on hydrion concentrations of tissue fluids in the two sexes of dioecious plants with new data on buffer capacity. Such data may in turn permit correlations with studies on oxidase activities which distinguish the sexes of dioecious species (1, 3, 4, 6).

Methods

A preliminary series of hemp (*Cannabis sativa*) were grown in a greenhouse in two gallon jars containing ordinary garden soil. Plants of this first series were exposed to extremes of photoperiod in order to determine the effect of daylength on dates of flower formation and anthesis. Daily temperature averaged 68° F. and humidity was kept at 75% or above by sprinkling. Plants from seed sown on December 1 were grown under a continuous 24 hour photoperiod by extending daylight by electric light from Mazda lamps until February 10 in order to prevent premature flowering of small plants under the otherwise naturally short day conditions of winter months. After February 10, plants were exposed to a ten hour photoperiod. Initial analyses of press sap were made on February 10 on entire tops of vegetative hemp plants. Similar subsequent determinations were made one and three weeks later (February 17

and March 10) on potted hemp plants in early stages of anthesis and fruiting, respectively. Pollen had been almost completely shed by staminate plants in the final series and young fruit capsules were present on pistillate plants (table I).

A second series of hemp plants were started on December 31 from seedlings transplanted to soil on greenhouse benches. By use of a ten hour photoperiod plants of the second series were brought into flower at the age of 75 days (table II).

A California variety of prickly seeded spinach (*Spinacia oleracea*) was also planted in soil of greenhouse benches on January 19 and grown under temperature and humidity conditions similar to those employed for hemp (table III). Plants in this first spinach series were grown in short day (10 hour photoperiod) for 58 days and then transferred to long day (24 hour photoperiod). A second planting of prickly seeded spinach was similarly made on February 19 except that plants were grown in a 24 hour photoperiod for 99 days and then exposed to a 10 hour photoperiod until final analyses at 106 days of age (table V.).

Plants or parts thereof as needed were harvested between 8 and 9 A.M. comminuted, weighed, packed in pyrex tubes and sealed for immediate freezing in dry ice until analysis. Six to seven gram fresh weight samples of young tissues provided adequate yields of press sap but larger samples of mature hemp tissues were needed. To maintain consistent results hydraulic pressure of 12,000 lbs. was applied in all cases. The expressed sap was adjusted to a temperature of 25° C. by means of a water bath and the initial pH read immediately by potentiometer (10). After the initial pH was recorded, buffer curves were tabulated for staminate and pistillate plant parts by titrating a total of 5 ml. of N/40 HCl or KOH into the original sap, at successive additions of 0.5 ml. each. In the interest of brevity only buffer capacity of press sap of hemp and spinach plants is reported herein except in figure 1 but further details on procedure and complete buffer curves are reported elsewhere (6). Complete representative buffer curves are given for a few plants to illustrate the general buffing action of the press saps studied (Fig. 1).

Data and Discussion

Hemp

Initial pH of press sap from 2 to 3 inches of male tops of hemp showed a higher hydrion concentration than from female tops (table 1). In long day the average initial pH for six male tops was 6.67 and for the females was 6.73 for plants at 72 days of age.

These results are in accord with previous determinations made on staminate and pistillate hemp plants (4, 11). After the photoperiod had been experimentally shortened to 10 hours for 1 week, pH determinations were repeated. Changes in photoperiod obviously influenced the free acidity of plant tissues.

When floral shoots had appeared buffer determinations were made on the staminate tops just before anthers opened at 93 days of age. A week later pistillate flower tops were analyzed. Since hemp females lag behind the males in floral development the one week lapse of time permitted study of developmentally comparable material (table I). The last determinations were made 17 days later, age 110 days. Pollen had been almost completely shed before the fresh material was tubed and young fruits were well developed. Both sexes reached their maximum acidity at this stage with lower acidity in the sap of male plants. The data on entire tops of hemp plants indicate only small and perhaps insignificant differences in pH of press sap except in the late flowering stage at the 93 to 100 day age period.

In analyses of the second hemp crop described below, shoot tissues were divided into flowers, stem tops and stem bases to determine pH values and buffer capacity of the different parts. The in-

Table 1

Hydrion concentration (pH) of press sap from terminal 2-3 inches of hemp tops. Plants in 24 hour photoperiod until 72 days of age; in 10 hour day thereafter.

Age in Days	72	80	93	100	110
Males	6.67	6.93	6.76	.....	6.00
Females	6.73	6.99	.....	6.61	6.02

itial analyses of press sap from the second series of hemp (started December 31) were made at 75 days of age when plants were about 36 inches tall and in full anthesis. Measurements of the original pH and buffer capacity were made of flowers, and of lower and upper stems plus leaves. The data of four determinations were averaged (table II). The data on the second hemp crop can be quickly summarized as follows: In short photoperiods (10 hour day) the original pH of sap from pistillate flowers (A-table II) was significantly higher (0.45 pH units) than in males. Staminate flowers were better buffered than females against HCl by 0.80 pH units but pH of KOH end points were about the same in flowers of both sexes. Original sap of pH of stem tops and bases were about the same in the two sexes of hemp in short day. No data were obtained on buffer action of stem sap of short day plants.

Table 2

Hydrion concentration (pH) and buffer action of press sap of hemp grown in short and long photoperiods.

Part	Initial pH	HCl E.P.*	Difference	KOH E.P.	Difference
A. Ten hour photoperiod					
Staminate plants, age 75 days					
Flowers	6.13	3.59	2.54	9.80	3.67
Stem Tops	6.47	.....	.....	.....	.....
Stem Bases	6.43	.....	.....	.....	.....
Pistillate plants, age 75 days					
Flowers	6.58	3.24	3.34	10.23	3.65
Stem Tops	6.48	.....	.....	.....	.....
Stem Bases	6.44	.....	.....	.....	.....
B. Sixteen hour photoperiod					
Staminate plants, age 95 days					
Flowers	6.09	3.94	2.15	9.46	3.37
Stem Tops	6.45	3.50	2.95	9.70	3.25
Stem Bases	6.61	2.80	3.81	10.40	3.79
Pistillate plants, age 95 days					
Flowers	6.62	3.70	2.92	9.80	2.88
Stem Tops	6.31	3.20	3.11	10.10	3.79
Stem Bases	6.09	2.17	3.92	10.99	4.90

*E. P.—End point pH reading after addition of 5 ml. N/40 HCl or KOH to 1 ml. of press sap.

In more mature plants of hemp in the early fruiting stage (age 95 days) in 16 hour photoperiod (B-table II), the initial pH of pistillate floral sap continued to be significantly greater than in males (0.53 pH units). The sap of long day male flowers was, however, better buffered than females both against HCl but not against KOH. In stem bases, sap pH of males rises from 6.43 to 6.61 on the shift from short to long day but falls in female plants from 6.44 to 6.09. Sap of stem tops and bases of male hemp plants are better buffered than females against HCl and KOH at 95 days of age While the photoperiod was increased from 10 to 16 hours per day after hemp plants had become reproductive at 75 days of age in an attempt to insure general luxuriance growth, the difference between short and long day plants described above are probably the attributes of the more advanced stage of development at 95 days over 75 day old plants rather than due to increase day length.

Data herein on buffer capacity and the general shape of buffer curves (6) suggests that the primary buffering agents in plant cells are weakly dissociated organic acids and their salts. Hempel (10) has suggested the role of malic acid and its salts in maintaining a range of sap pH *in vivo* compatible with normal metabolism. By

analogy, the known diurnal cycle of tissue fluid reaction of succulents due to photolysis of organic acids gives support to Hempel's explanation. The precise quantitative levels of tissue fluid reactions as reported herein seem to be correlated with sex differences in hemp, both in respect to sap pH and buffer capacity. The shape of buffer curves (fig. 1) for hemp sap also indicate the probable buffering role of soluble phosphates (6, 10).

Spinach

Tests on prickly seeded spinach similar to those employed for hemp were made for purposes of comparison between a short day species (hemp) and a long day species (spinach). Spinach plants were initially grown in a 10 hour photoperiod until the first sampling of plants in anthesis at 58 days of age. Remaining plants were shifted to a 24 hour day with natural daylight extended by use of Mazda lamps (table III).

The data on press sap of spinach show less variation of pH among plant parts than in hemp plants. Transition from 10 to 24 hour photoperiod causes relatively little change in original pH of press sap in either sex even though the plants of the latter group were almost twice the age (106 days) of those in short day (58 days of

Table 3

Hydrion concentration (pH) and buffer action of press sap of spinach plants grown in short and long photoperiods.

Parts	Initial pH	HCl E.P.*	Difference	KOH E.P.	Difference
A. Plants in anthesis in 10 hour photoperiod					
Staminate plants, age 58 days					
Entire Tops	6.10	2.75	3.35	11.00	4.90
Stem Tops	6.20	.....	.....	.....	.....
Stem Bases	6.38	.....	.....	.....	.....
Pistillate plants, age 58 days					
Entire Tops	5.90	2.25	3.65	11.70	5.80
Stem Tops	6.14	.....	.....	.....	.....
Stem Bases	6.25	.....	.....	.....	.....
B. Early fruiting stage in 24 hour photoperiod					
Staminate plants, age 106 days					
Entire Tops	6.10	3.00	3.10	11.00	4.90
Stem Tops	5.70	.....	.....	.....	.....
Stem Bases	6.13	.....	.....	.....	.....
Pistillate plants, age 106 days					
Entire Tops	6.19	2.40	3.79	11.50	5.31
Stem Tops	6.19	.....	.....	.....	.....
Stem Bases	5.94	.....	.....	.....	.....

* See footnote Table 2.

age). Sap from stem tops of male plants showed a moderate decrease in pH in the shift from short to long day. Staminate plants were somewhat better buffered than females against both HCl and KOH in short and long day. The press sap of flowers in long day was only slightly more acid in male than in female spinach plants (table IV). Abundant pollen had formed on the 78 day staminate flowers. When the last pH value was made pollen had been completely shed and seeds had developed (table IV).

Table 4

pH Value of Press Sap from Spinach Flowers During Long Day Conditions (24 Hour Illumination)

Age	Male pH	Age	Female pH
78 days	6.01	80 days	6.17
106 days	5.80	106 days	5.95

In an attempt to determine the interplay between the length of photoperiod and stage of development, a final series of spinach plants was grown under conditions similar to those just described except that they were at first grown in long day (24 hour) photoperiod for 99 days and then transferred to short day (10 hour day). Flower primordia appeared in six weeks and 57 day old plants were in anthesis (table V).

It is to be noted (table V) that at the outset the female plants surpassed the males in free acidity but after three weeks this condition was reversed. Likewise the shift from a more acid top and alkaline base to a more alkaline top and acid base took place in both sexes. The fact that the initial pH became more basic after the photoperiod is decreased is consistent with the findings through-

Table 5

Sap Hydrion Concentration (pH) of Tissue Fluid of the Upper and Lower Portions of Flowering Spinach in Long Day to Short Day Conditions.

A. Long day—24 hour photoperiod				
Age of Plants	pH Value			
	Male		Female	
	Tops	Bases	Tops	Bases
57 days	6.20	6.35	6.04	6.15
86 days	6.03	5.83	5.94	5.76
92 days	6.32	6.11	6.59	6.40
99 days	5.96	5.75	6.07	5.67
B. Short day—10 hour photoperiod				
100 days	6.19	6.03	6.51	6.05

out the study. The inversion in pH gradients in stems might well have been a developmental effect as it has been found possible to rejuvenate or reconvert plants in the reproductive stage to renewed vegetative activity by alteration of the photoperiod. The data of other authors on sex reversal by switch in photoperiod have been contradictory and where sex reversal has been reported it probably has been a purely phenotypic rather than a genotypic response

Differences in buffer action of press sap of hemp (a short day species) and spinach (a long day species) are demonstrated in the form of their respective buffer curves (fig. 1). The curves in this figure were obtained from flowering tops in a similar stage of development. Spinach buffer curves, although similar in form to hemp, offered less effective buffering in the initial phase. Both male and female spinach bases were the poorest buffers. Maximum buffering was obtained in the male hemp against HCl and the poorest buffering in the female spinach against KOH. All plants were better buffered against acid than base and the best buffering was manifested in the center third of the buffer curves. Buffering was more effective in the male than in the female plants and better buffer action against acid than base was observed in both hemp and spinach.

The data of the two plants further disclosed that maximum acid buffering appeared in the flowers and seeds. Throughout the study buffer curves suggestive of phosphate buffers were found. The more acid the press sap the better was the buffer effect against acid and the more alkaline the initial pH the better the buffering against alkali.

Summary

1. The original press sap of staminate plants of hemp and spinach plants tends on the whole to be more acid than that of pistillate plants.
2. Press sap of staminate hemp and spinach plants shows a better buffer capacity than that of females, especially toward acid.
3. The greatest difference between the sexes in hemp and spinach was found in flowers, both in respect to initial sap pH and buffer capacity.
4. During a lengthened photoperiod buffer action was most pronounced in the ripened blossoms and least effective in the older tissues of the plants.
5. Maximum acidity occurred in fruits, minimum acidity in stem bases.

6. Press sap was more alkaline in short than in long photoperiods.
7. The greatest alkalinity occurred just before flower primordia were formed.

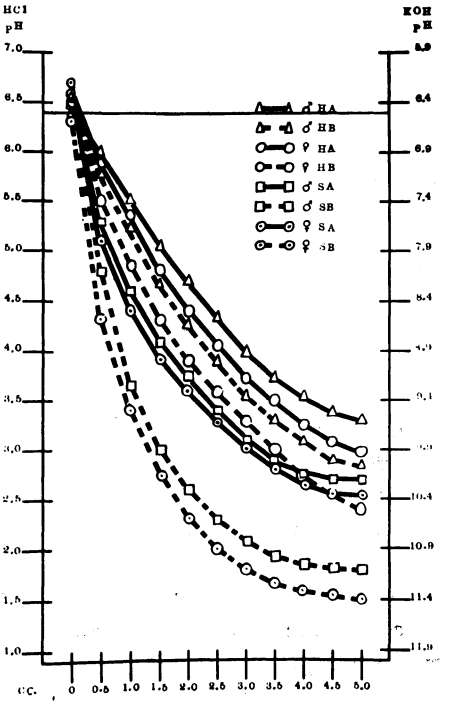

Figure 1. Comparison of buffer curves of press sap from entire tops of staminate (♂) and pistillate (♀) plants in the flowering phase, grown in a 24 hour photoperiod. HA, hemp titration curve of one ml press sap against five ml. N/40 HCl; HB, hemp titration against N/40 KOH; SA, spinach curve against HCl; SB, spinach curve against KOH. HCl titration against successive 0.5 ml. additions of HCl on ordinates at left; KOH titration on ordinates at right. Amounts of N/40 HCl and KOH added to one ml press sap shown on abscissa.

ACKNOWLEDGMENT

The writer is indebted to Dr. W. F. Loehwing, Head of the Department of Botany, The State University of Iowa, Iowa City, Iowa for his valuable assistance and suggestions.

Bibliography

1. Aitchison, J. A. Oxidase activity in certain dioecious plants. Thesis, State University of Iowa, Iowa City, Iowa. pp. 40. 1933.
2. Bressman, E. H. Inheritance of sex in certain plants. Am. Jour. Bot. 21:328-349. 1934.
3. Bunzell, H. H. The relationship existing between the oxidase activity of plant juices and their hydrogen ion concentrations, with a note on the cause of oxidase activity in plant tissues. Jour. Biol. Chem. 28:315-333. 1916.
4. Cromer, H. Diurnal changes of sap acidity of certain plants. Thesis, State University of Iowa. 1932.

5. Garner, W. W. and Allard, H. A. Effect of relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Ag. Res. 18:553-606. 1920.
6. Hoxmeier, Sister Mary Claire. Sap buffer capacity in relation to sex of dioecious plants. Thesis, State University of Iowa, Iowa City, Iowa, 55 pp. 1933.
7. Joyet-Lavergne Ph. La physico-chemie de la sexualité. pp. 457. Borntraeger, Berlin. 1931.
8. Loehwing, W. F. Physiological aspects of sex in angiosperms. Bot. Rev. 4:581-625. 1938. Also: Plant Physiol. 5:293-307. 1930.
9. Riede, W. Die Abhängigkeit des Geschlectes von den Auszenbedingungen. Flora 115:259-272. 1922.
10. Small, J. Hydrogen ion concentration in plant cells and tissues. Protoplasma Monograghien. Borntraeger Bros. Berlin. 1929.
11. Talley, P. J. Physiological differences in the sexes of Cannabis sativa. Thesis State University of Iowa. 1930. Also Plant Physiol. 9:731-748. 1934.

Briar Cliff College
Sioux City, Iowa