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INTRODUCTION

Differences in the oxidation potential of tissue fluids of the sexes in dioecious plants have long been known and they appear to be causally related to the contrasts of the staminate and pistillate metabolism (2, 3, 5, 12, 13). Many plant scientists have considered the Manoilov oxidation reaction a valuable test in studies of this type (9, 10, 11). Although its simplicity and wide applicability decommend the Manoilev test, it has not been wholly satisfactory or reliable (8). Color reactions are often unsuitable as quantitative measures of oxidase activity due to the small amount of oxidation necessary to produce marked color change (7).

This investigation describes the use of an iodimetric reaction in a study of press sap from staminate and pistillate plants in several dioecious species of flowering plants. Five different dioecious species were investigated comprising asparagus, hemp, Rumex, spinach and Smilax.

METHODS

Specimens were collected between 5 and 7 A. M. and only plants which were definitely differentiated as staminate and pistillate were selected for analysis. The specimens were dug with care, wrapped in moist paper and taken immediately to the laboratory where they were thoroughly washed, first in tap water and then in distilled water. They were spread upon filter paper until the excess water had been removed.

The desired plant parts were then comminuted with scissors and the pieces well mixed to secure uniformity of sample and placed in 6 x 1 inch test tubes, immediately stoppered with rubber stoppers and frozen with solid carbon dioxide until analyzed. Preliminary to sap extraction the tubes were placed in cool water until thawed. The extraction of the press sap followed immediately.

The sap was extracted by hydraulic press at a pressure of 6000 to 10000 pounds per square inch depending upon the succulence of the tissue. The juice was pressed through canvas that had been washed in alcohol and distilled water respectively. The sap used in the control was boiled over a bunsen flame, immediately removed

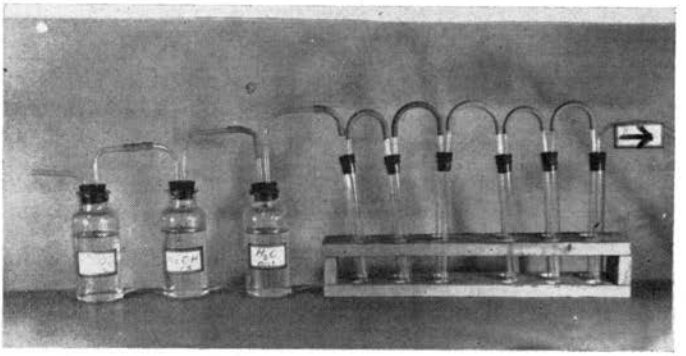

Fig. 1. Aeration apparatus used in determining oxidase activity iodimetrically.

and filtered. Both the boiled juice (control) and the unboiled test sample were added simultaneously to their respective aeration tubes containing the reagent. Aspiration of tubes with air was employed to accelerate completion of oxidation and thus expedite analytical procedures.

The aeration apparatus was composed of 10 x 1 inch test tubes fitted with a two-holed rubber stopper (fig. 1). The test tubes were held in place by means of a specially designed rack. The tubes containing fresh sap were alternated with controls in order to equalize aeration by aspiration. The air was drawn through three 800cc. wide mouth bottles containing 500cc. of a 15% solution of sulphuric acid, 500cc. of a 15% solution of sodium hydroxide, and 500cc. of distilled water respectively.

The reagent used in all determinations was prepared as described by Guthrie (4). A freshly prepared reagent was used for each set of determinations. The pH value of the substrate as determined at 20° C with a quinhydrone electrode varied from 6.4 to 6.7. The desired pH value was obtained by the addition of N/1 sodium hydroxide or N/1 hydrochloric acid. The substrate was then diluted with an equal volume of water and 25cc. portions added to each of the aeration tubes. From 5 to 10 drops of paraffine oil was added as necessary to suppress foaming. The sap (unboiled juice) and the control (boiled juice) were added and aeration started at once. For each determination a water blank (substrate with no plant juice) was used to check the iodine value of the aerated substrate.

When the aeration was completed the contents of each tube were transferred to a 300cc. erlenmeyer flask containing 25cc. of a freshly prepared solution of trichloracetic acid, 25cc. of distilled water were

used in washing the contents from each tube. 50cc. of N/50 iodine in N/10 potassium iodide was then added to each flask and the mixture allowed to stand for thirty minutes. It was then titrated with N/100 sodium thiosulphate. One cc. of a 1% starch paste was used as an indicator. The copper equivalent of the sodium thiosulphate was obtained (6) before each set of determinations was made so each actual volumetric titration could be converted to the corresponding copper equivalent, thus making the results obtained comparable throughout. The difference between the titration of the control and the untreated sap was used as a measure of the oxidase activity of the sample. The oxidizing enzymes in the test sample catalyzed the oxidation of the iodine reducing substance in the substrate. Further details of iodimetric titration procedures as employed in this experiment are given elsewhere (1).

Data and Discussion

Four sets of determinations were made with *Asparagus officinalis,* the first three tests being made on consecutive days on plants of the same sexual maturity. The plants of both sexes were in the early flowering stage and pollen was present in the more mature staminate flowers. The fourth test was made with plants in the post-flowering stage. Though all plants were still green, flowers on staminate plants had dried and the female plants had green berries from 1 to 3 mm. in diameter on them. For each of the four determinations five normal plants of each sex were used. All asparagus plants were gathered at 7 o'clock A. M. and entire tops were used. Two cubic centimeters of sap were used for the test sample and control (boiled sap). Entire tops of staminate plants of asparagus in the flowering stage had a greater rate of oxidase activity than the cor-

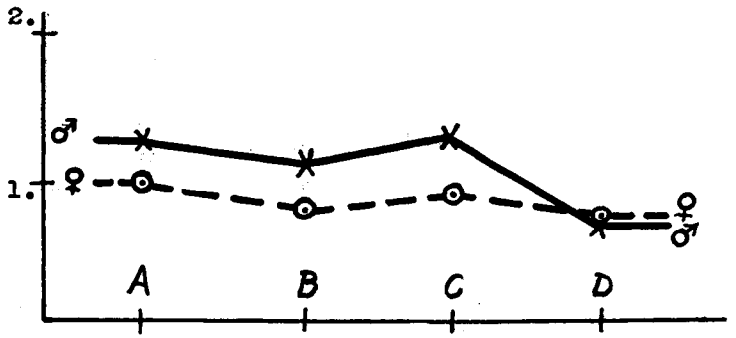

Fig. 2. Rate of oxidase activity in press sap of asparagus plants at two different stages of development. A, B and C are duplicate readings on plants in anthesis; D, plants in post-floral stage. Ordinates (1 & 2), milligrams of copper in two milliliters of sap as titrated with thiosulphate.

responding parts of pistillate plants (table I). Oxidase differences between sexes tended to disappear in the post-floral stage.

The study of *Asparagus officinalis,* thirty-six determinations made on plants from three different localities always showed greater oxidase activity in the male plants in the flowering stage. This condition was reversed after the male flowers had withered and the female plants were in fruit (fig. 2). This latter difference was very small and may have been due to fruits which were left on the plants when comminuted. The sap was thus extracted from the fruits as well as the other plant parts.

Specimens of hemp (*Cannabis sativa*) growing in natural outdoor habitats were also tested, 20 plants of each sex being used for analysis (table II). The flowers of the male plants were largely mature and ripe pollen was much in evidence; flowers of the female plants were in full bloom. Test plants were dug at 5 A. M. Determinations were made of four different plant parts as follows: 1. the tops entire, leaves and inflorescence from the upper one third of the plants; 2. inflorescenses only from the upper third of stems; 3. leaves and stems from the basal one third of the shoot; 4. entire roots, care being taken in washing to include rootlets. One cc. of sap was used and samples were aerated for one hour.

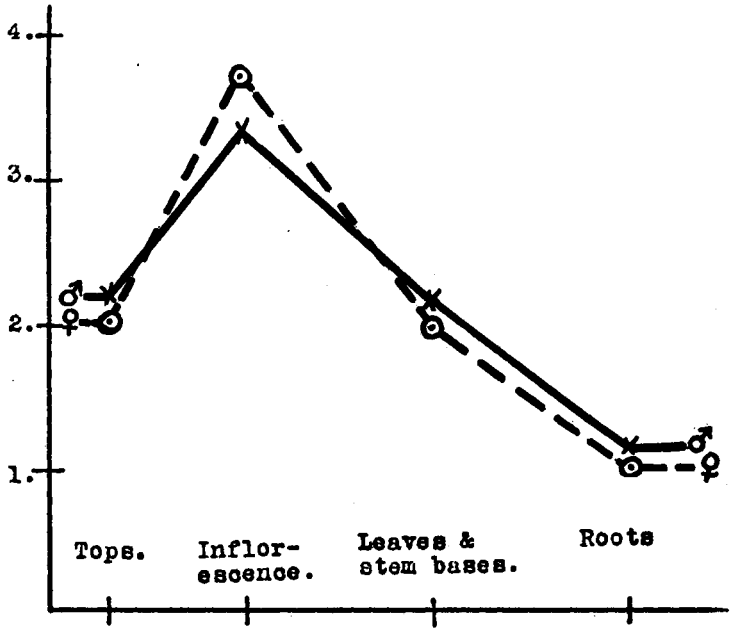

Fig. 3. Rate of oxidase activity in various parts of male and female plants in synthesis. Ordinates as in figure 2.

Table 1, Asparagus

Oxidase activity of asparagus press sap from entire tops of plants in the floral and post-floral stages. Data are recorded as milligrams of copper titrated iodimetrically in two milliliters of sap.

Sex	Test 1	Test 2	Test 3	Average	Difference
A. Plants in flowering stage (full anthesis).					
Female	0.99	0.80	0.91	0.90	.....
Male	1.33	1.06	1.36	1.26	0.36
B. Plants in post-floral stage (early fruiting).					
Female	0.72	.....	.....	.....	—0.01
Male	0.71	.....	.....	.....	.....

Staminate plants and all their parts except inflorescences disclosed higher rates of oxidase activity in hemp plants at the peak of anthesis (fig. 3). With one exception the results of analyses made with other samples of *Cannabis sativa* obtained from three different localities correlated with those of other investigators cited. The results obtained with the entire tops correlate with those obtained by Aitchison (1) and Talley (12) who also found greater oxidase activity in the male hemp plants by means of the colorimetric analyses of press sap.

Study of the enzymatic oxidation rate of *Rumex acetosella* sap is difficult due to the presence of oxalic acid even when neutralized with alkaline calcium or sodium salts. The boiled sap of these plants also showed considerable oxidase activity due to thermostable oxidizing substances. *Rumex acetosella* (Field or Sheep Sorrel) plants in each determination were of different ages, viz: 1. very young plants from 2 to 4 inches in height with no visible evidence of flower parts; 2. plants in flower; 3. mature but green plants in which the flowers had dried on the male individuals and fruits had formed on the female plants. Tests were made on three consecutive days. The specimens used in the first and last determinations, were growing in the same locality, in a very sandy isolated habitat (table III). Since suitable flowering individuals could not be obtained in this locality, plants used in the second determination were obtained from a cut-over lot.

Table 2, Hemp

Oxidase activity of hemp press sap from plants in the flowering stage (anthesis). Data are recorded as milligrams of copper titrated iodimetrically per milliliter of sap.

	Entire Tops	Basal Leaves & Stems	Roots	Inflorescences
Female	2.07	1.96	0.91	3.77
Male	2.19	2.06	1.02	3.37
Difference	0.12	0.10	0.11	—0.40

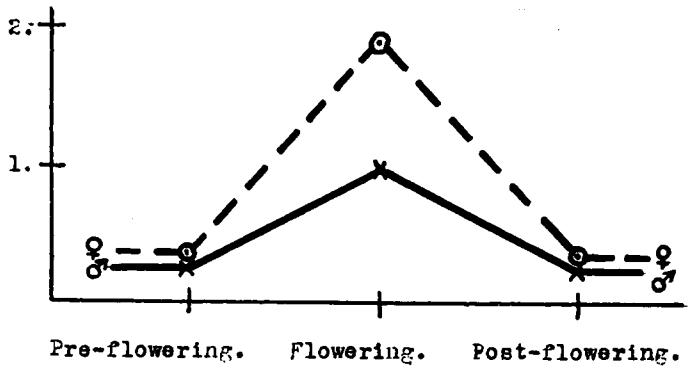

Fig. 4. Rate of oxidase activity in male and female Rumex plants in different stages of maturity. Ordinates as in figure 2.

Plants of Rumex for all three tests were gathered at 6 A. M. on successive days. The entire tops of 10 plants of each sex were analyzed. The oxidase values obtained were all consistently higher in female plants. Similar results were obtained even though the pH of the reagent was varied from 5.3 to 7.6. The results of forty-two determinations made with plants from three different localities nevertheless consistently showed the greater oxidation rate in the sap extracted from the female plants in all the stages of sexual maturity (fig. 4). Rumex thus differs from asparagus, hemp and spinach in having a higher oxidase rate in the tissue fluids of pistillate plants.

Plants of Savoy spinach (*Spinacia oleracea*) comprised the fourth genus tested. Twelve plants of each sex were used which varied in size from 4 to 6 inches in height. They were all in the early flowering stage and no hermaphroditic flowers were found. These determinations showed a slight difference in the rate of oxidase activity, being the more rapid in the male plants (table III).

Mature but green plants of *Smilax herbacea* (Carrion flower) in the post-floral stage were tested (table III). These specimens were taken from a natural moist woodland habitat at 6 A. M. and entire tops of plants were used. Staminate plants had already abscised all flowers and pistillate plants bore young green berries. Plants of *Smilax herbacea* and *Asparagus officinalis* do not pass into a state of senility soon after flowering as do staminate plants of *Cannabis sativa*. The sap of these plants showed a far greater oxidase activity than hemp perhaps due in part to a greater concentration of enzymes in this species or to enzyme concentration associated with desiccation of mature plants. The controls titrated the same as water blanks which suggests the oxidizing substances found

Table 3, Rumex, Spinach, Smilax

Oxidase activity of Rumex, Spinach and Smilax press sap from entire tops of plants in various stages of development. Data are recorded as milligrams of copper titrated iodimetrically in one or two millimeters of sap as indicated.

	Prefloral stage	Floral stage	Post-floral stage
	A. Rumex, 2 cc. of sap		
Female	0.32	1.95	0.30
Male	0.26	0.94	0.24
Difference	—0.06	—1.01	—0.06
	B. Spinach, 2 cc. of sap		
Female	.....	0.91	.....
Male	.....	0.97	.....
Difference	.....	0.6	.....
	C. Smilax, 1 cc. of sap.		
Female	.....	.....	23.36
Male	.....	.....	22.07
Difference	.....	.....	—1.29

in these juices are all thermo-labile. The tissue fluids of pistillate plants of Smilax showed an appreciably higher oxidase rate. Whether this condition prevails throughout the developmental cycle or occurs only at maturity as in asparagus could not be determined due to the inavailability of Smilax plants in earlier phases of growth.

The data indicate that there is a consistent difference in the rate of enzymatic oxidation in the male and female plants of the dioecious species investigated. Examination of the composite results herein show that, while there may be individual differences in plants of the same sex, the greater rate of oxidase activity was consistently in favor of one sex within the same species. Staminate plants did not, however, always exhibit the higher rate of sap oxidase activity in all species. In conclusion, it may be stated that the experimental results show a consistent difference in the oxidase activity of the two sexes within a species. This investigation suggests that the oxidation-reduction potential of tissue fluids probably is an important aspect of sex expression. The iodimetric method of measuring oxidation processes is applicable to such studies and in general it confirms the results of the indophenol oxidase procedure employed in other investigations (1, 5, 12).

Summary

1. In *Asparagus officinalis* the rate of oxidase activity was greater in the male plants during the flowering stages and became some-

what less in the male plants after the flowers had matured and fruit formed on the females.

2. The total oxidase activity was greater in the male plants of *Cannabis sativa* during the early flowering stage in all parts of the plants investigated with the exception of the inflorescence which showed a converse reaction in the majority of plants tested.

3. The oxidase activity was the greater in the female plants of *Rumex acetosella* in all stages of growth investigated.

4. *Smilax herbacea* in the post-flowering stage showed greater oxidase activity in the female plants.

5. *Spinacia oleracea* in the early flowering stage showed the greater oxidase activity in the male plants.

6. The results of this investigation indicate that there is a generally consistent difference in the oxidase activity between the two sexes of the species of dioecious plants investigated.

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