

THE INFLUENCE OF THE SUBSTRATUM ON THE  
PERCENTAGE OF SEX REVERSAL IN WINTER-  
GROWN HEMP.\*

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In my paper on "The Influence of Relative Length of Daylight on the Reversal of Sex in Hemp" attention was called to the fact that apparently the substratum acts in inducing sex reversal along with relative length of the daily illumination period, by the following statement:† "A few experiments so far conducted seem to indicate that the percentage of reversal for any given length of daylight will be greater in a rich soil with abundant nitrogen than in a poor substratum low in nitrogen." Opportunity has not been available to test out completely the exact status of this observation. But it is apparently important to have a suitable substratum along with the proper length of daily illumination in order to obtain the expected sex reversal. The evidence so far obtained is, therefore, presented at the present time for the benefit of those who wish to carry on sex reversal investigations with the hemp (*Cannabis sativa* L.).

The highest percentages of reversal have been obtained when the hemp was planted in rich sandy loam with a rich supply of well-rotted cow manure, as stated in former papers. When hemp of the ordinary types is planted in such soil on shallow greenhouse benches with the ordinary greenhouse temperature and normal water supply, the percentage of plants showing sex reversal is roughly, inversely proportional to the relative length of daylight, ranging from 0% about the 15th of August, to 90% or over in December, to 0% again by the first of May. The reversal takes place in both directions about equally, although so far the staminate plants have shown a little higher reversal than the carpellate plants.

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\* Papers from the Department of Botany, The Ohio State University, No. 153.

† Ecology 4:330. 1923.

The very highest reversal for carpellate plants was from two beds planted on December 19, 1919, as follows:

1st bed—Total carpellate individuals	18.	Showing reversal,	16
2nd bed— “ “ “	36	“ “	32
Totals.....	54		48

Thus nearly 89% showed sex reversal. In a bed planted December 15, 1920, with 29 carpellate plants 25, or 86+%, showed reversal.

The highest reversal for staminate plants was obtained in two beds planted respectively on December 1 and December 15, 1920.

Planted Dec. 1—Total staminate individuals	66.	Showing reversal	62
Planted Dec. 15— “ “ “	42	“ “	39
Totals.....	108		101

Approximately 93½% showed sex reversal.

On January 2, 1922, duplicate plantings were made by Mr. Donald B. Anderson, graduate student, in pure quartz sand and in pure well-rotted manure. This experiment was carried on for a different purpose but the writer had the opportunity of studying these plants for sex reversal. These plantings were in deep tin trays which were immersed in water kept at a temperature of 30–35° C. The plants in the sand were provided from time to time with the following nutrient solution which was decidedly deficient in nitrogen:

- Purified calcium chloride (Ca Cl<sub>2</sub>)—4 grams in 1000 cc. water.
- C. P. potassium chloride (KCL) —1 gram in 1000 cc. water.
- C. P. magnesium sulfate,  
(Mg. SO<sub>4</sub>+7H<sub>2</sub>O) —1 gram in 1000 cc. water.
- C. P. potassium nitrate (KNO<sub>3</sub>) —.5 gram in 1000 cc. water.

On account of the nature of Mr. Anderson's experiment, it was possible to study only the staminate plants.

1. Planted in pure manure with high moisture—total staminate plants 13; showing reversal 12, pure 1.
2. Planted in pure manure with low moisture—total staminate plants 19, showing reversal 14, pure 5.
3. Planted in quartz sand with low nutrition and high moisture—total staminate plants 20, showing reversal 10, pure 10.
4. Planted in quartz sand with low nutrition and low moisture—total staminate plants 18; showing reversal 9, pure 9.

There was some mortality among these plants before blooming, but apparently not much. The original seeds and seedlings had not been counted. In these experiments, therefore, the staminate plants which survived showed the following percentages of sex reversal:

Planted in manure—

Total staminate plants 32, of which  $81\frac{1}{4}\%$  showed reversal to femaleness and only  $18\frac{3}{4}\%$  remained pure.

Planted in sand—

Total staminate plants 38, of which only 50% showed reversal to femaleness and 50% remained pure.

In the autumn and winter of 1922, the writer continued the experiments, but used the ordinary, variable greenhouse temperature, the trays being kept on the benches. The same nutrient solution was used for the quartz sand cultures, and distilled water was used for watering. The manure used was apparently not rotted enough at the first planting date, which caused the carpellate plants to have poor root systems and to die before they came into bloom. For this reason the first planting of November 10 was discarded. The other plantings were as follows:

1. Planted December 2 in rich cow manure. Total staminate plants 15—showing reversal 12, or 80%; pure staminate 3, or 20%.

Carpellate plants not studied.

2. Planted December 2 in quartz sand. Total staminate plants 4—showing reversal 1, or 25%; pure staminate 3, or 75%. Total carpellate plants 9—showing reversal 0, or 0%; pure carpellate 9, or 100%.

There was a high mortality among these plants before they came into bloom.

3. Planted December 20 in rich cow manure. Total staminate plants 21—showing reversal 19, or 90+%; pure staminate 2, or 9+%.

Carpellate plants not studied.

4. Planted December 20 in quartz sand. Total staminate plants 12—showing reversal 3, or 25%; pure staminate 9, or 75%. Total carpellate plants 17—showing reversal 0, or 0%; pure carpellate plants 17, or 100%.

In all of these sand experiments there was considerable mortality; so if the plants that are capable of reversal under the given environment have a greater mortality than those which have a more persistent sexual state, the ratios would be somewhat modified. But this is not probable.

Taking into account the time of the year in which the planting was done the average reversal for the staminate plants should have been about 90% and for the carpellate plants about 83% if planted in rich, well manured soil. The plants in the pure manure approached these figures in spite of a rather high mortality in this substratum.

But the average reversal for the 34 staminate plants in the sand was only 33% and among the total of 26 carpellate plants in the sand cultures studied, there was no sex reversal whatever. Part of this result was probably due to the high mortality among the carpellate plants after they began to bloom and before the individuals had time to change their sex in any flower buds produced. It must be remembered that usually the great majority of carpellate plants are much more tardy in showing sex reversal than the staminate plants, and are, therefore, less favorable objects for study, when grown in very sterile soils, than the staminate plants.

As stated above, the 68 staminate plants grown to maturity in the pure manure cultures showed nearly 84% reversal to femaleness, approximating the 90% or more usually obtained when the plants are grown on the greenhouse benches in well manured sandy loam at the same season of the year.

It is thus evident that although a primary cause of sex reversal in hemp is the relative length of daylight and darkness during the 24 hours, the percentage of reversal and even the very possibility of reversal depends to a considerable extent on whether the plant is drawing the proper nutrients, especially nitrogen, from the substratum at the time.

The reversal of sex is probably brought about by a physiological condition dependent in some way on the carbohydrate-nitrogen ratio in the cells. Gardner\* in his studies in the nutrition of bisporangiate strawberries (Senator Dunlap) as related to yield, came to the following conclusions in regard to the sex development and reversal which appeared in his cultures: "Low carbohydrate and low starch content at the time of fruit bud differentiation led to the production of female flowers in a variety that normally is hermaphroditic." "It is suggested that variations in the relative carbohydrate content are responsible for changes in sex development, low carbohydrate content

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\* Gardner, V. R. Studies in the Nutrition of the Strawberry. Univ. of Missouri Agr. Sta. Research Bull. 57:1-32, March, 1923.

being associated with the female condition, high carbohydrate content with hermaphroditism." This of course might be true for the bisporangiate-flowered strawberries and not necessarily for other types of plants.

Apparently the Senator Dunlap Strawberry, *Arisaema triphyllum* (L.) Torr., and *Cannabis sativa* L. are each good species for studying the relation that may exist between the substratum, the carbohydrate-nitrogen ratio of the protoplast, and the sex determination and sex reversal that occur so readily with the proper environment in these plants. Physiological ecology must soon take its place as a fertile field of research among its sister divisions in the botanical field if we are to obtain a better understanding of the great and fundamental problems presented by living things.