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The Influence of Photoperiodicity on the Sexual Index in Hemp (*Cannabis sativa* L.)

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Souhrn

1. Vysévali jsme konopí (*Cannabis sativa* L.) postupně v měsíčních intervalech od listopadu do srpna v letech 1952/53 a 1953/54. U jednotlivých výsevů byla u samčích a samičích rostlin sledována doba do květu. Samčí rostliny kvetou zpravidla dříve než rostliny samičí. Při prodlužujícím se dnu (nad 14 hod.) přestanou samičí rostliny kvést (pokud nekvetly v tuto dobu, nerozkvetou) a pokračují jen ve vzrůstu. Při silně ubývajícím dnu (den kratší než 15 hod.) počnou kvést a jejich doba do květu se tak prodlužuje až na 265 dnů. Jsou to vždy rostliny samičí. Rostliny samčí kvetou i při dnu delším než 14 hod. Jsou tedy fotoperiodicky rostlinami neutrálními s tendencí být rostlinami dlouhého dne. Rostliny samičí jsou rostlinami krátkého, lépe řečeno ubývajícího dne. Nejmenší podíl samčích rostlin je ve výsevech nejkratšího dne.

2. V polních podmínkách jsme v letech 1953/54 provedli zastiňování mladých rostlin konopí v různých fázích růstu. Ukázalo se, že snižování světelné intensity zvyšuje podíl samičích rostlin a to zejména, trvalo-li zastiňování 14 až 21 dnů a začalo-li v roce 1953 do 14 dnů a v roce 1954 do 7 dnů od vzcházení. Různé výsledky z obou let vysvětlujeme různými klimatickými podmínkami.

3. Za určitých podmínek se vyskytují u konopí též rostliny intersexuální. V našich pokusech byl jejich výskyt vázán na velmi krátký den (11 až 13 hod.) a vyskytovaly se v množství 22—30 %. Považujeme intersexuální rostliny za rostliny samčí, u nichž vliv krátkého dne nedovolil čistou pohlavní diferenciaci. Ačkoliv se tedy tyto rostliny vyskytují pouze při extrémně krátkých fotoperiodách (optimálních pro rostliny samičí), je jejich počet dnů do květu přibližně střední mezi počtem dnů potřebných pro rostliny samčí a samičí. Po trojnásobně opakované dekapitaci intersexuálních rostlin se při dosažení potřebné délky dne vyvinuly tyto rostliny v rostliny samčí.

4. Roubováním různě pohlavních rostlin nebylo dosaženo žádné změny pohlaví, pravděpodobně pro krátké trvání vegetativního sblížení.

Summary

Hemp (*Cannabis sativa* L.) was sown at intervals from November to August. For each sowing the time of flowering was recorded for male and female plants separately. Male plants usually flowered earlier than female. When the period of daylight exceeds 14 hours, that is when the days are lengthening in April, the female plants stop flowering and continue their vegetative growth. They start flowering again when the days are shortening considerably, in August, when the daylight period is about 15 hours. The male plants flower even when the day is longer than 14 hours.

In experiments during which the plants were shaded it was found that reduction of light intensity leads to an increase in the number of female plants, particularly if shading is carried out in the early stages of the plant's development.

The occurrence of intersexual plants was observed only during the short day period (11 to 13 hours), when they composed 22—30% of the total. When the day exceeds 13 hours intersexual plants no longer develop.

The grafting of plants of varying sex did not produce any sexual change—probably due to the fact that the period of vegetative approximation was short.

Introduction

Hemp is a dioecious plant with a sharp sexual dimorphism. A very small proportion of monoecious plants occur (these are in fact female plants with a small number of male flowers). Their monoecious character is, however, very unstable and in order to maintain it constant selection is necessary (ARINSHTEYN and SENCHENKO 1956). Under quite exceptional conditions intersexual plants can occur, in which case they are abundant.

Hemp has for a long time been the classical material for experiments on the sexuality of dioecious plants.

The question of whether or not male and female sex of dioecious plants is already determined in the embryo has been on the whole settled by SCHAFFNER'S (1921) extensive work, which gives a clearly negative result.

Similar conclusions from work with hemp (LYUBICH 1950) and European hop (*Humulus lupulus* L.) indicate that both sexes are already established in the seed (OSVALD and BLATTNÝ 1954). The determination of sex in young plants is still in the experimental stage (KULIK 1953).

The fact that the sexual index of hemp changes under the influence of environmental conditions has been well known and fully documented. It has not, however, been sufficiently clear which components of the environment are the most decisive in determining the sexual index, how they act in the formation of sexual polarity and at which period of ontogenesis their intervention is necessary for them to be effective (BORTHWICK and SCULLY 1954) MATUSIEWICZ (1953) has found that when the day was artificially shortened (10 hours) hemp flowered after 67 days, while the control plants flowered after

127 days. He does not mention whether the plants were male or female. He followed the growth of male and female plants with short and long days. When the day was shortened to 10 to 8 hours intersexual plants also occurred.

In the present experiments the influence of light intensity and day length has been the chief subject of study.

Results and Discussion

Ia. The flowering period of male and female plants in relation to photoperiodism

In 1952 and 1954 sowing was carried out at monthly intervals from November to August. The seed was sown in boxes in the glasshouse. The total number of plants in the experiment was 6,152 with 1,333 and 4,819 in the different series. The variety of hemp used was "Jihoslovenská krajová". The soil and watering was the same for all plants. They sprouted evenly, on an average 8 days after sowing.

The flowering period was counted when about 30% of the plants began to flower, the remainder flowered within two further days. Results are given in graphs 1 and 2.

For ♀ plants the period of vegetation up to the start of flowering was often very different for the different sowings. For ♂ plants the length of the vegetative period up to the time of flowering was less influenced by the external environment. As a rule ♂ plants flower earlier than ♀. In some sowings ♂ plants flowered later than ♀.

It is clear from the experiments that for the formation of flowers and flowering of ♀ plants rapidly lengthening days are not favourable. When in April the day exceeds 14 hours ♀ plants cease flowering. They only continue to grow, without regard to age. Only when the day is considerably shortening in August, with a day length of about 15 hours, the temporarily non-flowering ♀ plants start to flower again.

The ♂ plants of hemp sown in November start to flower on 21 March, i. e. after 126 days, and flowering is 100%. Female plants start to flower on March 3, that is after 108 days. Some of them stop growing until in June the lower nodes send out new shoots. The foundations of flowers are formed on them, but flowering starts on August 8, i. e. 265 days after sowing. Flowering was "postponed" in this way in 22·1% of the female plants.

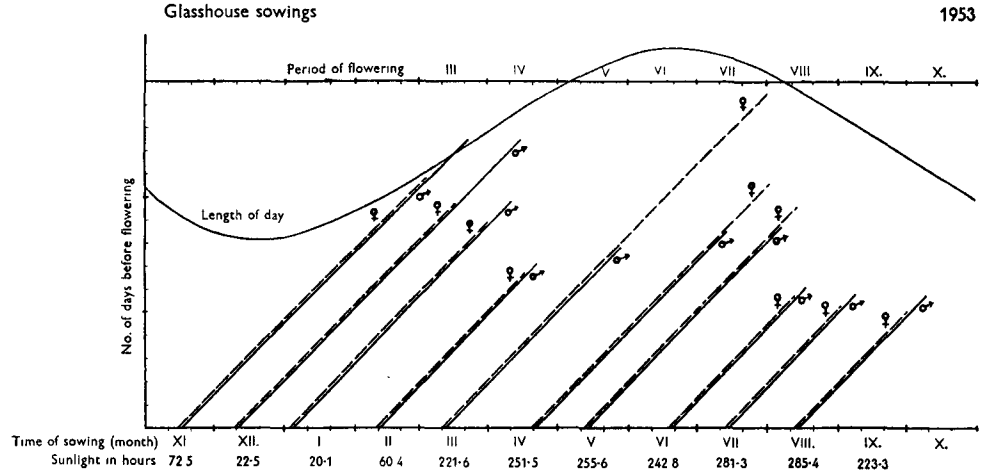
"Postponement" of flowering was repeated in further sowings up to the February sowing. This is only a quantitative difference. Plants from these sowings, insofar as they did not flower, continued to grow, did not send out auxiliary shoots, but flowered in August on their main axes. The majority of the female plants flowered within 67 days, later plants in 186 days.

In the case of the March and April sowings, plants from which should have flowered in 80 days in May to June, the female plants developed only "vegetatively" and began to flower—as did some of the February-sown plants—after 145 days, i. e. in August when the day was shortening to 15 hours.

The male plants from the February sowing flowered in April after 72 days and from the March sowing in May after 79 days.

Thus the female plants in the glasshouse did not flower from the middle of April to the end of July, i. e. when the day was longer than 14 hours. They are, therefore, s h o r t - d a y photoperiodic plants.

The male plants flowered without almost any delay even when the day was longer than 14 hours, i. e. in May, June and July as well. They are, therefore, n e u t r a l photoperiodic plants, w i t h a t e n d e n c y t o w a r d s

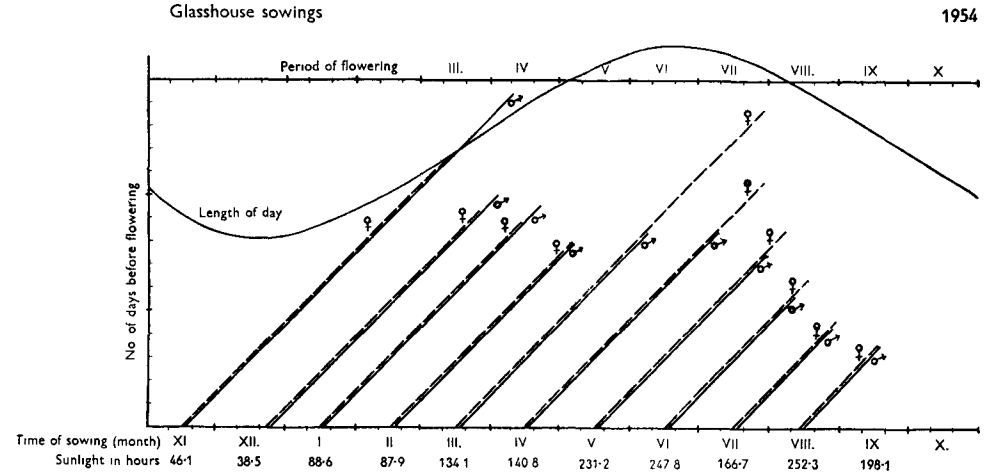


Explanation of graphs

The ordinate represents the period before flowering, 1 section = 10 days. The lower abscissa shows the month and day of sowing. 1 section = 10 days.

The second line shows the period of sunlight in hours for each month. The upper axis shows the day and month of flowering. (The curve shows day length in hours.)

Graphs 1 a 2 show clearly that male plants do not always flower earlier than female.



being long-day plants (the smallest number of male plants was to be found in the November to January sowings, when there are 8—10 hour days).

In the case of the May, June and July sowings female plants flowered later than the male, but as compared with earlier sowings they considerably shortened the time required before coming to flower. Female plants from the August sowing needed 50 days before flowering in 1953 and only 36 days in 1954. In 1954 there was less sunshine than during the same period in 1953.

The influence of a too-short day and definitely also the reduction in daylight intensity started to be effective in plants sown in November, December, January and February. Female plants from the September sowing flowered on November 4, after 57 days. Intersexual plants began to appear in this sowing. The occurrence of intersexual plants is particularly typical for plants from the November, December, January and February sowings. Hemp sown in September and October always perished in the winter. The sexual index of plants sown in November, December, January and February changes in favour of female plants: there is a greater number of female plants than male; with high light intensity the index is nearly 1 : 1. This corresponds to the results of experiments involving the reduction of illumination, which caused an increase in the number of female plants.

Ib. Sowing of Hemp at time intervals in the open

In addition to sowing in the glasshouse hemp was sown on field plots in April, May, June, July and August of 1953 and 1954.

The experiment was repeated over two consecutive years — 1953 and 1954. The plots measured 100 × 150 cm. with twofold repetition.

The sexual index and period from sowing to flowering was followed for male and female plants.

Further the size of the plants in correlation to the period required before flowering was followed.

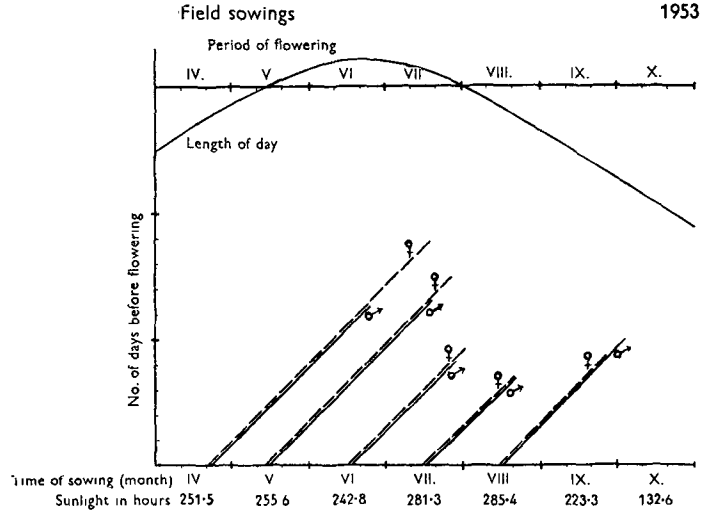
The height of the plants decreased with the shortening of the time needed for flowering: in 1953 the average height of plants from the first sowing was 175 cm., from the last sowing 9 cm.; in 1954, 190 cm. and 35 cm. The experimental results are set out in tables and graphs 3 and 4.

With regard to the period required from sowing to flowering, in 1953 the difference between male and female plants of the April sowing was very considerable; for male plants the time required was 15 days shorter than for female plants. This difference was less for later sowings: for the May sowing by 9 days, for the June sowing by 5 days. For the July sowing the period required from sowing to flowering is the same for male and female plants and for the August sowing it is 7 days longer in male plants than in female.

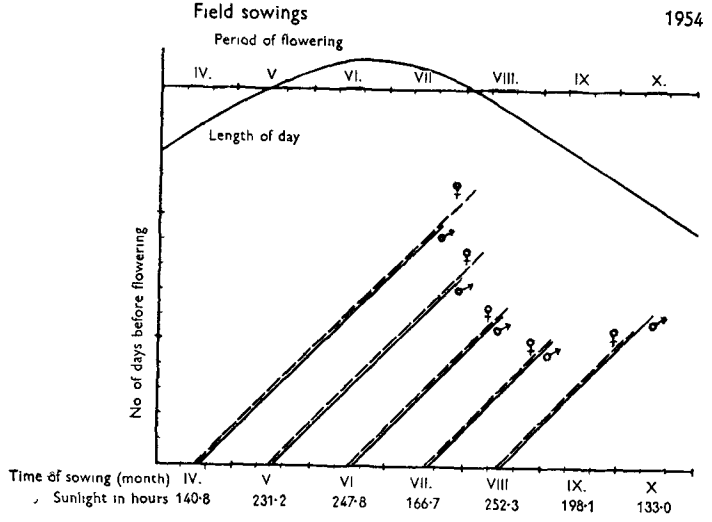
In 1954 the time required before flowering was 14 days shorter for male plants of the April sowing than for female, for those of the May sowing 10 days and for the June sowing 3 days. For the July sowing this period was the same for male and female plants and for the August sowing it was 3 days longer for male plants than for female.

There were differences between results for the two years, but they were

quantitative and not qualitative. The general rule to be deduced from experiments in the open seems to be the same as that deduced from the glasshouse experiments: female plants are short-day (i. e. shortening day) photoperiodic plants, male plants are neutral with a tendency to be long-day plants.



Graph 3.



Graph 4.

A further experiment was carried out in order to demonstrate that sex is unstable and influenced by photoperiodism.

Hemp was sown in the glasshouse on November 18 and left to grow until

Table 1

1953

Date of sowing	Number ♀ plants	%	Start of flowering		Number of days before flowering	
			♂ plants	♀ plants	♂ plants	♀ plants
21. 4.	276	57·5	23. 6.	18. 7.	64	89
14. 5.	15	60·0	18. 7.	27. 7.	66	75
15. 6.	301	58·3	27. 7.	1. 8.	42	47
15. 7.	125	54·3	20. 8.	20. 8.	35	35
14. 8.	25	50·0	3. 10.	26. 9.	51	44

Table 2

1954

Date of sowing	Number ♀ plants	%	Start of flowering		Number of days before flowering	
			♂ plants	♀ plants	♂ plants	♀ plants
16. 4.	148	58·3	21. 7.	4. 8.	97	111
15. 5.	140	50·5	29. 7.	7. 8.	76	86
15. 6.	170	55·6	14. 8.	17. 8.	61	64
15. 7.	385	51·8	3. 9.	3. 9.	51	51
13. 8.	99	48·5	13. 10.	6. 9.	61	54

it flowered. The plants flowered on March 10 and were intersexual. On March 15 they were decapitated above the third node. They sent out new shoots, which flowered again on April 16 and were again intersexual. Decapitation was repeated. The plants again came into flower and flowered from May 25 as male plants—in the period of longer days.

II. Pilot experiments were carried out on the effect of the artificial reduction of illumination on the sexual index of hemp plants.

Illumination has a certain effect on the formation of the male or female sex in dioecious plants both of hemp (BORTHWICK and SCULLY 1954, HESLOP-HARRISON 1957) and of hop (VALÁŠKOVÁ 1952). This is also supported by the second series of the present author's experiments. An attempt was made to ascertain the phase of ontogenesis at which this effect is exercised. Hemp was sown on plots measuring 1·5 m². Reed screens fixed in pairs on 150 cm.-high constructions were used to reduce light intensity. These screens were set up for varying numbers of days at varying periods following the sprouting of the plants. Control plots were not screened. In 1953 the hemp was sown on May 22, in 1954 on May 12, and nearly all plants had come up within a week. Plants shaded for 21 days were partially etiolated after shading and were shorter than those on the control plot, later, however, they nearly equalled the control plants in growth.

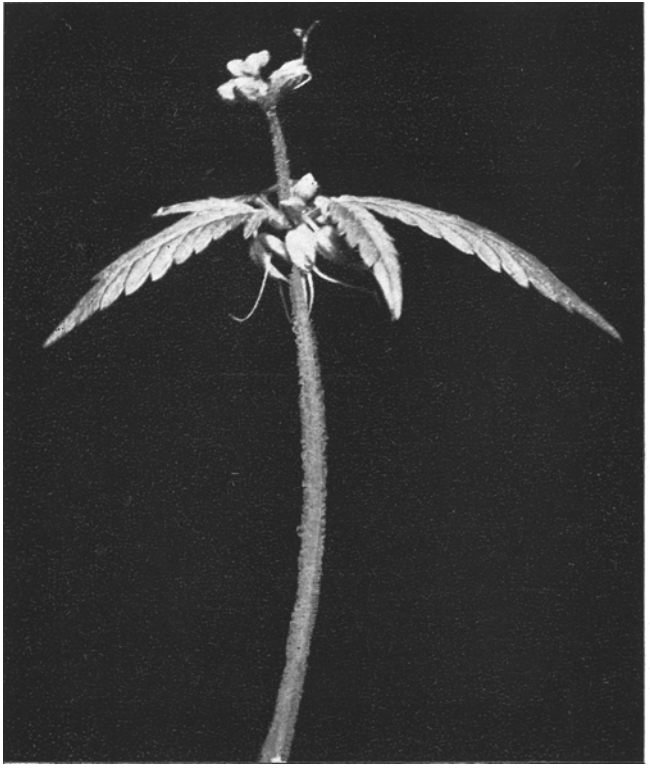


Fig. 1.

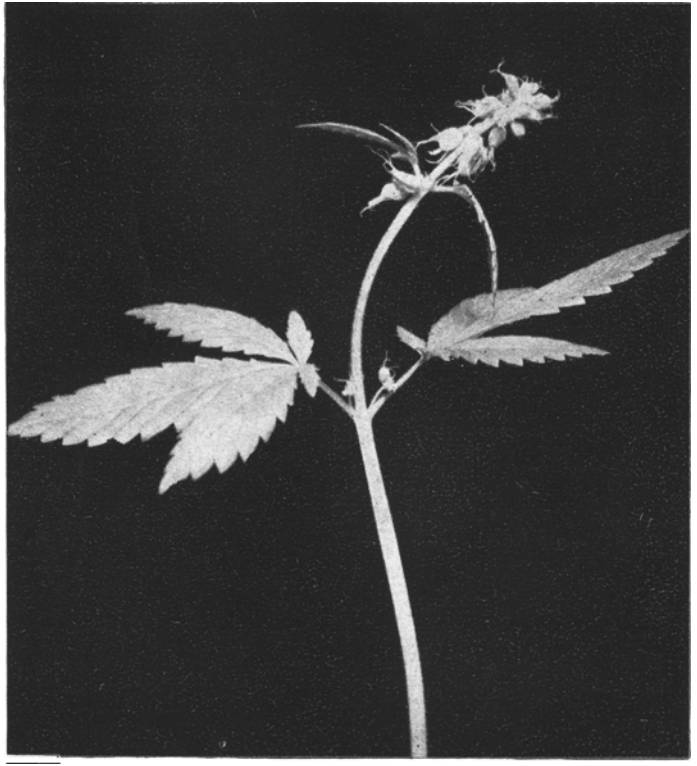


Fig. 2.

Fig. 1 and 2. Intersexual flowers in hemp.

In both 1953 and 1954 screening was carried out: a) immediately after the plants came up, b) 7 days after coming up, c) 14 days after coming up—in every case for 7, 14 and 21 days.

The increase in the proportion of female plants obtained by shading was, as compared with the unshaded control, in 1953 5·1—6·1%, in 1954 1·6 to 4·5%, average for both years 4·32%. Differences in these pilot experiments between the variants were small and probably insignificant.

Nevertheless, concurrent results for the two years show that changes in the sexual index caused by decreased light intensity depend on the ontogenetical age of plants and the duration of the decrease in light intensity. The most effective was shading which started immediately after the plants came up, or that starting a week after coming up and lasting 14 to 21 days (1954 results), or shading starting 7 or 14 days after the plants came up and lasting 14 to 21 days (1953 results).

According to these preliminary experiments it would seem that some kind of preformation of sexual polarity takes place at least partially in the period up to 14 days after the plants come up if the experimental conditions last for 14 to 21 days. In this case it would be necessary to determine the time-limits for the influencing of sex formation in the periods mentioned. These experiments will be continued.

III. The development of intersexes in hemp

In scarcely any other plant species do intersexual plants occur so plentifully and regularly—only under certain environmental conditions, however—as they do in hemp. In their habit the plants are male, but with the difference that in addition to male sex organs, stamens, they also bear female organs, pistils and ovaries with stunted seeds. This is the case only in some flowers (photo).

The cause of the development of intersexual plants and the time of their occurrence had not been sufficiently elucidated. On uncovered plots in our latitudes and climatic conditions they had not been observed by the author nor recorded in the literature.

In the glasshouse experiments already described (v. I) intersexual plants were found in 1952—1954 only from autumn to spring, or in individual cases among plants sown in September to October, and then abundantly among plants sown from November to February (22% to 30%). Among plants sown in March they were not to be found.

It was noticed that the smallest number of male plants occurred among those sown in November, December and January, i. e. when the days were at their shortest (10—8 hours). With the decrease in male plants their place was partially taken by intersexual plants. Even if the intersexual plants were added to the number of male plants, the decrease in the proportion of male plants would be greatest for the December sowing. In the January sowing their proportion would exceed that of the female plants and in the March sowing the proportion of male plants would go down to normal. Intersexual plants did not occur in later sowing. In the September sowing intersexual

plants again began to appear and they flowered after the same period from the date of sowing as the female plants.

The time required by intersexual plants to come to flower varied according to the month of sowing. Intersexual plants sown in December required 10 days less before flowering than the female plants. In later sowings they flowered at the same time as the female flowers. Male plants, however, sown in January and February required in all cases a longer period than female and intersexual plants. Intersexual plants of the November sowing started to flower on March 10. Those of the February sowing started to flower at the latest in April 9. Thus new intersexual plants did not appear once the day length had reached $13^1/_2$ hours. Experiments with reduced light intensity, which showed that this influence is effective on young plants, also lead to the conclusion that here, too, the influence of the short days in November to February, when the plants were still young, had a decisive effect on the formation of intersexes. Of all the methods of changing sex which have been tested on hemp, photoperiodism has been the only one to prove successful (BRESLAVEC 1937).

On the basis of his experiments SCHAFFNER (1931) came to the conclusion that the fluctuation of sex in male hemp plants is primarily caused by photoperiodic influences.

In the light of the present experiments it can be assumed that intersexual plants are actually male in the first place and that the influence of the short day has made a pure sexual differentiation impossible in them. It is further considered that the occurrence of these plants is limited to the shortest-day period, although in view of the presence of both sexual components they should represent some kind of mean between plants requiring long and short days for flowering, i. e. between male and female plants. In the opinion of the author intersexual plants are plants which have been diverted from the normal development of sexual polarity by their environment (very short days with low light intensity).

This instability and the very existence of intersexual plants are, in the opinion of the author, a clear indication of the fact that the elements of both sexes are present in the hemp seed and that one of them is more or less suppressed during development.

IV. Grafting of plants of opposite sex

With a view to the possibility of changing sex in hemp, plants of opposite sex were grafted on to each other. Grafting was carried out at a stage of growth in which the sex of the plant was usually recognizable. Males were grafted on to female stocks (10 cases), and females on to male stocks (10 cases). No change of sex occurred in any of these either in the graft or the stock.

Similar results are given by KUHN (1941). He found that grafting had no effect on sex and no such change could take place owing to the short period of approximation. Undoubtedly photoperiodic influences also played their part, which was unknown to Kuhn—female plants do not flower when the days are long. In his sowings only male plants flowered during the long days.

He states that he knows of no cases in which grafting has caused a sex change. LIMBERK (1954) showed that in the European hop a sex change can take place, although only to a slight extent, following a long symbiosis of the graft and stock when a special method of grafting was used (a root onto young wood). The change occurred in buds growing on the root.

It seems most likely that a sex change in hemp could be brought about by grafting at a very early stage of the plant's development, before complete sexual differentiation has taken place. So far, however, no reliable method of determining this stage is known.

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Влияние фотопериода на половой индекс конопли

ЯРОСЛАВ ЛИМБЕРК

Резюме

От ноября до августа нами поочередно высевалась конопля (*Cannabis sativa* L.) и, сравнивая разные сроки посева, мы следили за периодом цветения мужских и женских растений. Мужские растения цветут обычно раньше женских. Если в апреле длительность освещения выше 14 часов, женские растения перестанут цвести и только продолжают расти. К цветению переходят при сокращении дня в августе, когда длина дня около 15 часов. Мужские растения цветут и в условиях более чем 14-часового освещения. В опытах по затенению растений нами установлено, что снижением интенсивности света повышается число женских растений. Нами установлены интерсексуальные растения в условиях короткого дня (11—13 часов) в количестве 22—30%. При удлинении дня на больше, чем 13 часов, развития интерсексуальных растений не наблюдается. Прививками растений разного пола — вероятно из-за короткого вегетативного сближения — не достигнуто никаких изменений пола.