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PHOTOPERIODIC RESPONSES OF HEMP

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Introduction

Photoperiodic investigations of hemp ***(Cannabis sativa*** L.) were undertaken to find procedures that might facilitate the breeding and perhaps suggest improved methods of field production of the crop. Environmental variables have long been known to influence the flowering behavior of hemp. Following the discovery of photoperiodism, a number of investigations indicated that the occurrence of certain abnormalities of flowering could be largely attributed to effects of that factor. Some of these abnormalities are of such a nature that several improvements in methods of breeding and production could possibly be based upon them if they could be produced at will. In this study the photoperiodic responses of the plant were re-examined in conjunction with variations in other environmental factors to find combinations of conditions that might lead to this end.

Hemp is dioecious and normally produces male and female plants in about a **1 : 1** ratio. Significant deviations, however, sometimes occur, and in many such cases the females exceed the males in number. Also, sexual intergrades occur in which plants that are predominantly either male or female produce floral structures of the opposite sex. In some varieties these irregularities are reported to be of more frequent occurrence than in others. Within a single strain, however,

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the extent to which these intersexual forms occur varies with respect to both the percentage of the population concerned and the degree to which the character is exhibited by the individual plant; it is apparent that both conditions are in some way related to variations in the environment.

Review of literature

The influence of environmental factors on the production of sexual irregularities in hemp has been under investigation for more than 100 years. BRESLAVEC (2) stated that MOLLIARD (10) in 1897 was the first to find a method of inducing sex reversals in hemp, but BRESLAVEC cited references to work conducted as early as 1827 in which attempts were made to alter the sex ratios MOLLIARD grew hemp in the greenhouse in winter and attributed the sex reversals to the low light intensities prevailing at that season.

According to MCPHEE (**8**), TOURNOIS (20) also observed hermaphroditic flowers on hemp plants grown in the greenhouse in winter. In 1899 HALSTEAD (5) started experiments designed to alter the sex ratios of hemp by manipulation of environmental factors. PRITCHARD (13) reported definite cases of sex reversals in hemp and was of the opinion that the condition could be influenced by removal of flowers.

SCHAFFNER published a series of papers (**15–18**), most of them between 1920 and 1930, dealing with the effects of various environmental factors on the ratios of male to female hemp plants and on the occurrence of intersexual flowers on plants of each sex. He early became convinced that differences in light, presumably in intensity, were responsible for differences in the degree to which intersexual flowers were formed in winter and spring plantings of hemp. Upon learning of the discovery of photoperiodism by GARNER and ALLARD (**3**), SCHAFFNER (16) concluded that his results with hemp planted in winter and in spring were to be explained on the basis of seasonal change in photoperiod. Since SCHAFFNER was able to obtain, from a single source of seed, populations in which no intersexual plants occurred under one set of conditions and populations in which all the plants produced intersexual flowers under another, he held that sex in hemp was not inherited but that its expression was dependent upon environmental conditions.

MCPHEE (**9**) did not accept this viewpoint because he was able to produce seed all of which gave rise to female plants if he used pollen from male flowers developed on female plants. HIRATA (6) confirmed MCPHEE's findings and demonstrated an unlike pair of chromosomes in male hemp plants. He was unable to find such a pair in female plants; hence he concluded that the determining mechanism of sex inheritance was of the XY type. He held, however, that the production of intersexual flowers on hemp was undoubtedly influenced by environmental factors.

Several workers have studied effects of various nutrient conditions on sex expression in hemp. SCHAFFNER (**17**) found that the incidence of intersexual flowers, even during the short days of winter, was slightly greater if the plants received high concentrations of nitrogen. TIBEAU (**19**) obtained only female plants from a lot of seedlings supplied with a nutrient solution having a high nitrogen concentration and only male plants from a similar lot supplied with a low level of nitrogen. In Iowa, BLACK (1) conducted fertilizer tests with hemp in which he varied nitrogen, phosphorus, and potassium. He reported small and, in most cases, insignificant differences in sex ratios among treatments and did not mention any intersexual plants.

Contradictory conclusions concerning effects of environment on hemp have resulted, in part, from differing usage of terms. SCHAFFNER (**15**), for example, applied the term "sex reversal" to any plant that produced structures of both sexes on the same plant. Thus a female plant that bore a single male flower or even part of a male flower was said to have undergone sex reversal. MCPHEE (**8**), on the other hand, reserved the term for plants that had expressed their sex and that had subsequently produced flowers exclusively of the opposite sex. Various other workers did not clearly define their terms; hence it is uncertain which usage they have adopted.

Some of the apparent lack of agreement came about through use of different varieties of hemp. In occasional experiments in which several named varieties were tested, outstanding varietal differences in flowering behavior have occurred (8). Relatively few workers, however, clearly indicated the variety with which they worked.

Briefly, past work on hemp shows that photoperiod exerts a strong influence on time of flowering. This effect is modified to varying degrees by the interaction of other environmental factors with photoperiod. The incidence of intersexual flowers or of monoecious plants is also influenced by photoperiod, but here, also, other environmental variables interact with photoperiod and modify the effect. There is some indication in published work that strains or varieties of hemp differ markedly in the degree and type of their sexual abnormalities. A survey of the literature suggests that combinations of environmental conditions might be found that would insure the production of male flowers on female plants when they are desired.

Material and methods

Hemp seed, variety Kentucky, was used exclusively in all but one experiment, in which half the plants were of the Chile variety and the rest were Kentucky.

The early experiments were performed with Kentucky seed from a commercial source. This seed produced plants of both sexes in about equal numbers and is referred to hereafter as "normal" seed. For later experiments strains were produced by the methods of MCPHEE (8) that gave rise to female plants only. Seed from these strains is called "female" seed. All plants from this seed had the typical female growth habit and produced abundant female flowers, but some developed variable numbers of male flowers in addition. Plants of the latter type are probably the same as some of the monoecious kinds of hemp described by other workers (4, 7).

Male and female hemp plants respond to photoperiod somewhat differently. It is convenient, therefore, to be able to restrict a particular experiment to plants of one sex or the other. A source of "male" seed yielding only male plants would have been very useful, but its production would have depended initially on the use of female flowers formed by male plants, as was done by RICK and HANNA for *Asparagus officinalis* L. (**14**). Relatively few of these flowers were observed in our material, and in nearly all cases they failed to produce seed. The female type of seed, however, was readily produced in quantities of many thousands and was then used in most experiments.

Experiments were performed in the greenhouse, in outdoor photoperiod houses, and in lighted growth chambers. Special details of culture and procedure are described for the individual experiments as they are discussed.

Experimentation and results

FLOWERING RESPONSE AT VARIOUS CONSTANT PHOTOPERIODS.-Hemp flowers promptly if subjected to photoperiods of 14 or fewer hours daily and with considerable delay, or not at all, on photoperiods of 16 or more hours. In an experiment conducted at Beltsville in the greenhouse in March and April, lots of 72 hemp plants were placed for 53 days on photoperiods of 8, 11, 14, 17, and 20 hours. The treatments were started as soon as the seedlings came up. All lots were exposed to natural light, beginning at 8: 00 A.M.; those that required photoperiods longer than the prevailing duration of daylight received supplemental light of 20-40 ft-c. from 100-watt incandescent-filament lamps, beginning at sundown each day. During the 53-day period all plants on the 8-, ll-, and 14-hour photoperiods flowered. The male plants shed their pollen and died, and the female plants produced a crop of seed that nearly reached maturity. Plants on the 17- and 20-hour photoperiods, on the other hand, produced no visible flowers and were still green and growing when the experiment was terminated.

In another experiment conducted outside during the summer, plants of Kentucky and Chilean varieties of hemp were subjected to 10-, 13-, 16-, and 19-hour photoperiods. All received 10 hours of natural light daily, from 7 :00 A.M. to 5 :00 P.M. Those on longer photoperiods received incandescent-filament light at intensities of 20-40 ft-c. for the required number of hours beginning at 5:00 P.M. The 50 plants on lo-hour photoperiod and the 56 plants on 13-hour photoperiod all flowered, and the females all matured seed. On the 16-hour photcperiod 28 of the 58 plants produced flowers; only 4 were female, and of these only 1 produced seed. On 19-hour photoperiod 19 plants flowered, all male, and 40 remained vegetative. The plants on 10- and 13-hour photoperiods attained average heights of about 12 and 18 inches, respectively, while those on 16- and 19-hour photoperiods exceeded 6 feet. There was no difference between varieties in the response to photoperiod. The excess of males over females on the longer photoperiods is attributed to the fact that flowering is delayed less in male than in female plants by long photoperiods.

These results from plants grown with various fixed photoperiods are in reasonably good agreement with field observations. SCHAFFNER (18) planted hemp on natural photoperiod at Columbus, Ohio, at 2-week intervals from July 15 to the following May 15. He did not indicate the dates of blooming, but flowering plants were obtained from every planting. The longest days in the latitude are approximately 15 hours.

FLOWERING RESPONSE FOLLOWING SHIFT FROM LONG TO SHORT PHOTOPERIODS.—The photoperiodic conditions and the time required to induce flowering of plants that had been grown with long photoperiods were determined. Such procedures are useful in synchronizing the blooming dates of plants that are to be crossed. Lots of 16 plants that had attained ages of 3, 4, and 5 weeks on 18-hour photoperiods were transferred to 8-hour photoperiods for various numbers of days and were then returned to 18-hour photoperiods. Short-day treatment for 14 days resulted in flowering of all plants of the two oldest lots and nearly all of the youngest one (table 1). Lots of different ages differed in the number of 8-hour photoperiods required for the production of equal effects, older lots requiring fewer days than younger ones. Flower buds on some lots were visible within about 10 days after transfer to short photoperiods. Hemp plants can thus be held for periods of several weeks in a flowerless condition by subjecting them to long photoperiods and then can be brought into full bloom in little over 2 weeks by appropriate shortening of the photoperiods to which they are exposed.

TABLE 1

EFFECT OF AGE OF PLANT AND NUMBER OF 8-HOUR PHOTOPERIODS ON FLOWERING OF KENTUCKY HEMP

AGE OF PLANTS AT START OF TREATMENT (WEEKS)	PLANTS FLOWERING IN RESPONSE TO VARIOUS NUMBERS OF 8-HOUR PHOTOPERIODS*								
	0	1	2	4	6	8	10	12	14
3	0	0	0	0	0	2	2	6	14
4	0	0	0	0	4	9	8	8	16
5	0	0	0	7	9	16	16	16	16

* Sixteen plants per lot.

LOCALIZATION OF FLOWERING RESPONSE IN TWO-BRANCHED PLANTS.—The flower-inducing stimulus resulting from treatment of hemp plants with short photoperiods in nearly all cases remained rather definitely localized in the parts of the plant exposed to such treatment. This result was obtained from experiments with two-branched plants, one branch of each of which received 8-hour photoperiods in a temperature-controlled box, while the rest of the plant, including the other branch, was given 18-hour photoperiods outside the box. Branching was induced by pinching out the tops of seedlings and forcing the two opposite buds at a node near the base. The plants remained on 18-hour photoperiods from planting time until the branches were subjected to different photoperiodic treatments.

In each of two experiments the short-day branches of 20 plants were subjected to 50° F. and of 20 others to 70". The rest of the plant in every case was exposed to the fluctuating temperatures of the greenhouse, which averaged about 70". The experimental treatments were continued for 10 days, after which the plants were placed on 18-hour photoperiods at greenhouse temperatures.

Flowers were formed on the short-day branch of every plant in both experiments, but they became evident considerably earlier on branches held at higher temperatures. Ten days after the short-day treatments were started, buds on the branches of male plants held at 70° F. were about 2 mm. in diameter. At this time buds were present also on branches held at 50" but were not yet of macroscopic size. After 6 more days at greenhouse temperature and 18-hour photoperiods, however, even these buds were large enough for identification on all plants.

No flower buds could be found on the long-day branch of any of the 80 plants in the two experiments at the end of the 10-day treatments. Observations were continued for several weeks, during which time both branches received

18-hour photoperiods. The long-day branches of two plants in the first experiment and five in the second ultimately produced a few flowers. Whether these resulted from cross transfer of the flowering stimulus from the short-day to the long-day branches or from conditions occurring subsequent to the treatment is not known. It seems evident, however, that cross transfer of such a stimulus is not extensive in hemp.

Light energy required to inhibit flowering.-A study was made to determine the minimum amount of light, in extension of the natural photoperiod, that would prevent the flowering of hemp. The experiments were performed in midsummer with greenhouse-grown female plants which had produced several pairs of leaves at the time the experiments were started. Representative plants dissected at that time had no floral primordia in their terminal buds. All leaves except three, one from each of the three uppermost fully expanded pairs, were removed. These leaves were left, as nearly as possible, on one side of the stem, so that they would be equally irradiated when the plants were irradiated with a point source.

During the 14-day experimental treatments the plants received 8 hours of natural light outdoors and 16 hours of artificial light from incandescent-filament lamps indoors each 24 hours. During the 16-hour intervals twelve lots of six plants each were subjected to different intensities, which ranged from 0.001 to 60 ft-c. by. approximately threefold steps. For the five highest intensities, plants were placed at appropriate distances from a 300-watt incandescent-filament lamp, the only source of light in the room, and for the seven lowest ones at proper distances from a 6-watt incandescent-filament lamp in a different room. The plants were dissected 2 weeks after the treatments were concluded. Flowering occurred in all plants that received 0.02 ft-c., or less and failed in all that received 0.12 ft-c. or more. At 0.05 ft-c., five flowered in a lot of six.

Another experiment with twelve plants per lot was then designed to cover by approximately twofold steps a range of intensities from 0.002 to 0.25 ft-c. The photoperiodic treatments were continued 14 days for half these plants and 21 days for the rest. This time the division point between flowering and nonflowering lots occurred at 0.03 ft-c. This value was in approximate agreement with results obtained in the first experiment and was about the same as was found for Biloxi soybean and for cocklebur in similar experiments (11).

These results indicate that the intensities of 20-40 ft-c. used in the greenhouse to extend the natural photoperiods to 18 or more hours may be one hundred times as much as needed. ‘Plants growing close together shade one another, however, so that it is necessary to use excess radiation to insure that enough energy reaches the shaded leaves to prevent them from producing an effective flower-forming stimulus.

Production of female seed. - Among the plants of the preceding experiments, numerous females bearing a few normal male flowers were observed (fig. 1). Certain of these plants were isolated and self-pollinated. Other female plants on which no male flowers were present were also isolated and pollinated with some of this same pollen. All seeds from these controlled pollinations gave rise to female plants, thus confirming McPhee’s (8) results.

A large quantity of seed of this type was produced for use in subsequent experiments by pollinating female plants with a mixture of pollen from all the male flowers found on a large number of female plants. Many of the plants were harvested separately, so that in some of the subsequent experiments it was possible to compare the progenies of some of these different female parents as to their tendencies to produce male flowers on female plants. In all, about 40,000 seeds of this type, of which approximately 2,500 were used in photoperiod experiments, were produced. All these plants that were permitted to flower proved to be females.

FIG. 1.-Monoecious hemp plant showing awnlike styles and stigmas of numerous female flowers. Note one or more male flowers near base of each female inflorescence.

EFFECT OF NUTRITION AND LIGHT INTENSITY ON OCCURRENCE OF MALE FLOWERS ON FEMALE PLANTS.—Two experiments tested the interaction of two levels of nitrogen and two levels of light intensity on the production of male flowers on female plants. In the first experiment plants from normal hemp seed were grown in sand. The seeds received tap water while germinating, but as soon as the seedlings could be transplanted to sand in larger glazed pots, they were given a complete nutrient solution containing 20 p.p.m. of nitrate nitrogen for a period of 10 days. Then, on December 28, the plants were divided into four lots of 90 each. Two of these were shifted to a solution devoid of nitrogen but otherwise complete and the other two to a solution containing 211 p.p.m. of nitrate nitrogen. One lot on each nitrogen treatment was shaded with tobacco cloth that transmitted about 63% of the incident light. From the time of planting until the shading and nitrogen treatments were started on December 28, the plants received 18-hour photoperiods daily. After that time they were exposed to the natural short photoperiods then prevailing. When the experiment was terminated in February, all plants had formed macroscopic flower buds.

In the entire experiment there were 169 males and 191 females. Slightly more females than males occurred in each of the four treatments, but in no treatment was the difference greater than might be expected for a 1: 1 ratio.

The extreme difference in amounts of nitrogen given to various lots resulted in large differences in height at the time of flowering (fig. 2). The treatments resulted in an even greater difference in the number of female plants that produced visible male flowers. In the nitrogen-deficient lots male flowers were developed by only 11 of the 98 female plants, whereas in the high-nitrogen lots they were formed by 64 plants in a population of 98 females. This difference in number of female plants with visible male flowers probably resulted mainly from the slow

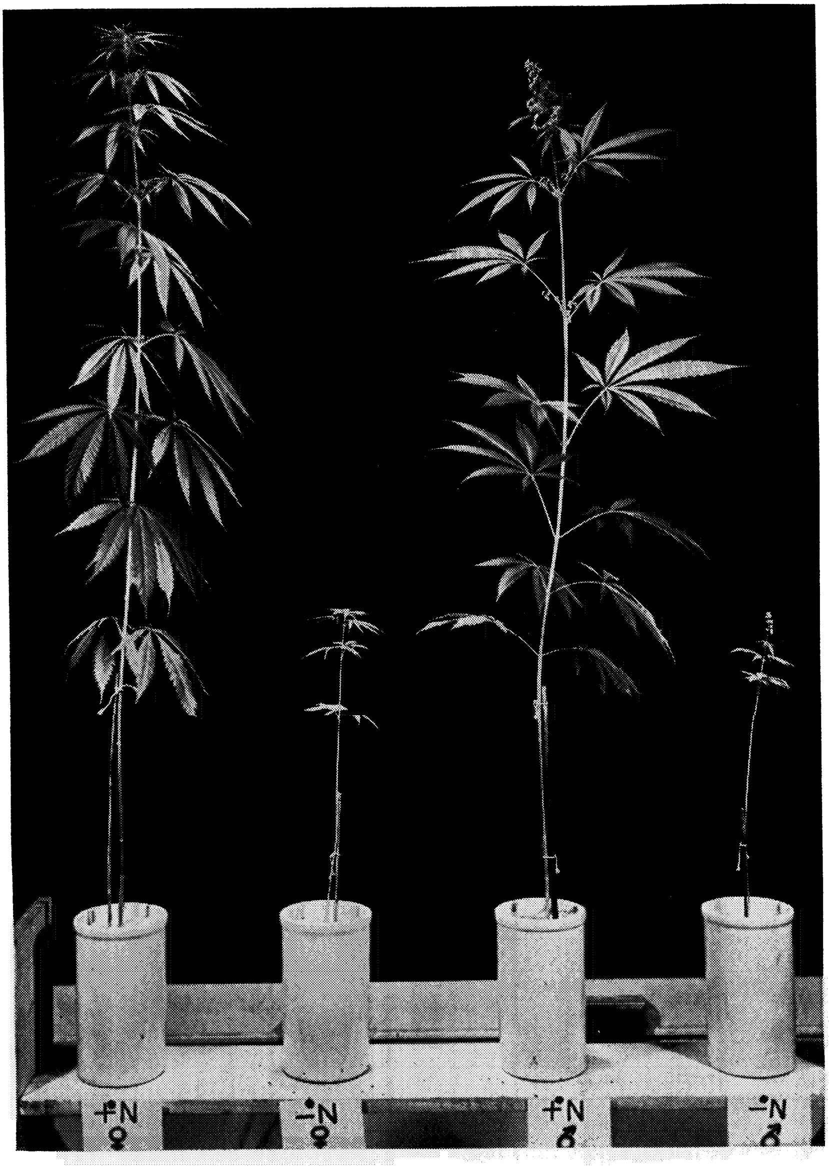

FIG. 2 .-Flowering of male and female hemp plants grown on two levels of nitrogen. Two plants on left are female; other two are male. Large plants received a high level of nitrogen and small ones a low level.

development of male flower buds on the low-nitrogen plants.

Light intensity had a significant effect on the number of female plants producing male flowers only in the high-nitrogen lots; high light intensity resulted in a greater number of such plants than did low light intensity.

Another experiment dealing with effects of light intensity and nitrogen supply was performed outdoors from mid-May to mid-July. The plants were grown in soil in 6-inch pots, and twice a week half of them were given 0.5 l. of sodium nitrate solution containing 70 p.p.m. of nitrogen. The other half received no additional nitrogen. Half of each of these two lots was shaded, so that the plants received only 30% of the natural light intensity; the other half received full intensity. All were grown on natural photoperiods under these conditions from June 14, when they were 1 month old, until June 28. Then the four different lots were subjected to 8-hour photoperiods for 2 weeks, but with light intensity and nitrogen treatments continued without change. At the end of this time heights were measured, and female flowers were visible on all plants.

TABLE 2

EFFECTS OF NITROGEN NUTRITION AND LIGHT INTENSITY ON GROWTH AND FLOWERING OF FEMALE HEMP PLANTS

Supplemental nitrogen (p.p.m.)	Relative light intensity (%)	Average height (inches)	Plants with male flowers*
0........	100	25.5±1.9	19
	30	33.3±1.17	22
70.........	100	28.8±0.80	19
	30	40.4±1.03	23

* Thirty-six plants per treatment.

Significant differences in height resulted from both nitrogen-nutrition and light-intensity treatments (table 2), but the differences among treatments in numbers of female plants that formed male flowers were not significant. The average percentage of female plants that produced male flowers in this entire experiment was 57, while for the high-nitrogen lots of the preceding experiment this percentage was about 65.

EFFECT OF DAYLENGTH ON OCCURRENCE OF MALE FLOWERS ON FEMALE PLANTS.—SCHAFFNER (18) showed very clearly that high percentages of hemp plants grown during the short days of midwinter produced intersexual flowers and that no such flowers were formed on plants grown in midsummer. Whether this was a result of the difference in length of photoperiod or of some other variable light factor, such as total energy, was not shown, however.

To clarify this point, hemp plants grown during winter, spring, and early summer were subjected to several daily durations of photoperiod, and the occurrence of male flowers on female plants was observed. Plantings were made on December 16, March 7, and May 19 in soil in flats. The seedlings were grown for 30 days with 18-hour photoperiods, which consisted of all the natural light available at the particular season, with supplemental light of an intensity of 20-40 ft-c. from incandescent-filament lamps added at sundown to complete the photoperiod. Early in the 30-day period the plants were transplan.ted to 4-inch pots, and at the end of the period, when they were about a foot high, four different treatments were started. These consisted of 8-, 11-, and 14-hour photoperiods and the natural photoperiods that prevailed at the time the respective experiments were in progress. The 8-, 11-, and 14-hour photoperiods consisted, in each case, of natural radiation in the greenhouse from 8 : 00 A.M. until 4: 00 P.M. At 4: 00 P.M. all lots were moved into adjoining dark-rooms, where the 8-hour lots received no light and the 11- and 14-hour lots received 3 and 6 hours of incandescent-filament radiation. At 8: 00 A.M., electric time switches turned on lights in all sections, and the trucks of plants were then promptly returned to the greenhouse.

The lots on natural photoperiods were left in the greenhouse continuously. The periods from sunrise to sunset when photoperiod treatments were started were 9.7, 12.9, and 14.9 hours, respectively, for the December, March, and May plantings. All natural-day lots thus received more hours of natural radiation per day and greater total radiant energy than did any of the lots on controlled photoperiods. All plants in the experiment flowered, and all were female.

TABLE 3

EFFECTS OF VARIOUS PHOTOPERIODS APPLIED AT DIFFERENT TIMES OF YEAR ON THE PRODUCTION OF MALE FLOWERS BY HEMP PLANTS DERIVED FROM FEMALE SEED

PLANTING DATE	AV. NO. OF PLANTS AT EACH PHOTOPERIOD	PERCENTAGE OF PLANTS BEARING ONE OR MORE MALE FLOWERS*			
		8 hours	11 hours	14 hours	Natural photoperiod
December 16, 1944. .	126	24.6	27.8	0.0	23.0
March i', 1945.	168	36.1	44.3	.0	11.6
May 19, 1945.	179	30.5	28.9	0.0	0.0

* All plants flowered, and all were female.

The production of male flowers by these female plants was prevented by photoperiods of 14 hours, regardless of the time of year they were applied (table 3). At 8- and 1 l-hour photoperiods plants bearing male flowers varied from about 25 to 45%, with no significant difference between the two photoperiods. In the lots that received natural photoperiods male flowers were abundant on plants of the December planting, reduced in number on those of the March planting, and absent from those of the May planting. This experiment and the previously described experiments in which light intensity was a variable thus confirm SCHAFFNER'S conclusion (18) that the greater production of intersexual flowers by hemp in winter than in summer is induced by the short photoperiods rather than by the low total radiation prevailing at that time of year.

EFFECTS OF LOW TEMPERATURE PRECEDING OR DURING PHOTOPERIODIC INDUCTION ON DEVELOPMENT OF MALE FLOWERS ON FEMALE PLANTS.—The effects of low temperature, applied just before or at the time of treatment with short photoperiods, upon the subsequent production of male flowers by female plants were studied in two separate experiments.

Female seed was used in both experiments, and the seedlings were grown in a controlled-environment room, where they received daily 16-hour photoperiods with light of about 2000-ft-c. intensity for the first 10 days. For the first experiment two lots of 48 plants each were then subjected for 2 weeks to two different temperature treatments. During this 2-week period the photoperiod was still maintained at 16 hours, but the intensity was held at 2000 ft-c. for only 8 hours and was then dropped to 150 ft-c. for the second 8 hours. One of the two lots was continued at 70" F. constant temperature, while the other lot received 70° during the first 8 hours of each photoperiod and 55° during the second 8 hours and during the following 8-hour dark period.

After the temperature treatments were finished on August 21, the plants were transplanted to large boxes of soil outdoors. The natural photoperiod at that time of year was short enough to induce prompt flowering, and minimum night temperatures for the first 3 weeks after the plants were moved outside averaged 58" F. First flowers were observed 21 days later on a few of the plants. By October 4 all plants were in advanced stages of flowering, and, although all were female, many of them produced so many male flowers that abundant seed formation throughout the group resulted from natural pollination.

Male flowers were produced by 35 of the 48 plants that received 55° F. and by 21 of the 45 plants that received 70°. The difference between treatments was significant at the 1% level. The percentage of plants that formed male flowers was high in both lots, a possible consequence of the relatively low night temperatures after they were transplanted. The significant difference between the two lots, however, must have been a result of the temperature treatments applied before the plants were subjected to short photoperiods and, therefore, before they had begun to develop their flowers.

The second experiment was concerned with the effects of temperature treatments at the time flowers were being formed. Plants of this experiment were from the same source as those of the preceding one. Until the seedlings were about 2 weeks old, they were given the same treatment as were those of the first experiment. When the temperature treatments were started, the photoperiod was reduced to 8 hours. One of two lots of 24 plants was subjected to 55° F. during the 16-hour dark periods, while the other was continued at 70". At the end of 2 weeks of treatment both lots were moved to the greenhouse to 16-hour photoperiods and temperatures of about 75°. All the plants of this experiment flowered, and all were female. In the 55" lot 71% of the plants formed a few male flowers, while in the 70" lot only about 12% formed male flowers. The difference is significant at the 1% level.

The data of both these experiments thus indicate that formation of male flowers on female plants is promoted by the occurrence of low temperature just before or during the time of flower-bud formation.

POSSIBLE HERITABLE VARIATIONS IN TENDENCY OF FEMALE PLANTS TO FORM MALE FLOWERS.—During these experiments female seed was produced from three successive generations of female plants. The pollen was in all cases derived from the male flowers produced on female plants and was usually a mixture from several such plants. Only a few plants were self-pollinated; at the time the work was done, a study of possible hereditary relations had not been considered. The seed from the various seed parents was saved separately, however, and records were kept as to whether the seed parents themselves had produced any male flowers.

Since the amount of seed from a single plant was rarely sufficient for an entire experiment, the practice of planting seed from several separate lines and distributing the seedlings of each line equally throughout the various lots of an experiment was adopted. In certain experiments it was therefore possible to make comparisons of lines derived from female parents that had produced male flowers with others in which the female parent had not produced such flowers.

One such experiment involved 588 female plants derived from seed parents that had produced male flowers and 582 from seed parents that had produced female flowers only. The plants were grown simultaneously and were equally subjected to the various environmental variables. In the former lot, 201 plants, or 34%, produced male flowers, and, in the latter, 136 plants, or 23%, were of that type. The difference is significant at the 1% level.

Another experiment included 468 female plants from female parents that had formed male flowers and 409 from female parents that had not. The percentages of these plants that formed male flowers were 13.7 and 4.9, respectively. Although the percentages were small, the difference is significant at the 1% level. The two experiments indicate that the occurrence of monoecism is to some extent hereditary, and the results suggest that, had more appropriate procedures and careful selection been employed, even greater differences could have been demonstrated.

OCCURRENCE OF FLORAL PRIMORDIA ON PLANTS PRIOR TO SHORT-DAY TREATMENT.—Representative plants were dissected before each experiment to determine whether they had already initiated floral primordia. Final data were not usually taken until the flower buds were visible to the unaided eye, but plants of lots which, because of the treatment they had received, were still not in bloom at that time were then dissected. Special attention was given to the terminal bud. Floral primordia were invariably absent from these when the experiments were begun and were still absent at the close of the experiment in most plants that had not produced visible flowers by that time.

The results of certain later experiments, however, indicated that floral primordia might have been present in axillary buds at nodes some distance below the terminal. Careful dissections of the terminal and all axillary buds of representative plants from one of the threshold experiments were, therefore, made at the time the experiment was started, and of others after it was finished. Floral primordia were found at the beginning of the experiment in buds located in the axils of a few of the fully expanded leaves several nodes below the terminal, but they were not present in such buds at the lowermost six to eight nodes of the plants or in those of the younger nodes nearer the top of the plant. These buds were in reality short lateral axes consisting of several nodes and internodes. At the base of each such axis was a pair of opposite prophylls, and the floral primordia occurred in the axil of one or both of these. Any male flowers present on the plant were usually at the lowermost of these buds that contained floral primordia.. Male flowers sometimes occurred in the axils of both prophylls and sometimes in only one. They were completely absent from some plants but usually occurred at two or three nodes. At the lower nodes at which flower buds were formed, a female flower was frequently found at one prophyll and a male flower at the other. At higher nodes, however, female flowers were usually present in the axils of both.

The threshold experiment mentioned was performed with these plants in the usual way, and certain lots that received treatments unfavorable to flowering remained in an apparently vegetative condition. These plants were dissected some weeks later. The primordia of male flowers, which were known to have been present when the experiment was started, had developed no further and in most cases had begun to deteriorate. The female flowers had grown little but had not degenerated, and new ones had been formed at a few of the nodes farther up the main axis,

The data clearly show that floral initiation can take place in hemp under photoperiodic conditions not conducive to further development of the flowers. Further studies were not made to determine how extensively this condition occurs in hemp or how universally female hemp plants produce male flower primordia of this type. In this particular experiment, however, 18-hour photoperiods were not long enough to inhibit floral initiation completely at these nodes.

Discussion

Photoperiodic control can be useful for the hemp breeder. By use of short photoperiods several generations of plants can be produced each year. Under such conditions hemp plants will flower when only a few inches tall, and, if yield is not important, as is often the case in the early stages of a breeding program, the time required for the life-cycle can thus be appreciably reduced.

Photoperiodic control also makes it possible to synchronize the flowering dates of plants that differ in earliness and thus makes possible the crossing of such plants. Probably the greatest use to which it can be put, however, is to stimulate the production of male flowers on female plants, for only by means of these can self-pollination be effected.

Short photoperiod has been shown in these experiments and in those of SCHAFFNER and others to be especially conducive to the formation of male flowers on female plants, but the photoperiodic conditions under which field-grown hemp usually develops might operate against the formation of such flowers. This could result from the fact that plants started in the spring begin their development in long days that inhibit flowering. They begin to initiate floral primordia as soon as the days begin to shorten, initiation thus occurring on the longest days at which flowering can occur. These relatively long days have been shown to be inhibitory to the development of intersexual flowers and of male flowers on female plants. Greatest numbers of male flowers have been observed to occur on female plants that either were started in short days of 8-11 hours as seedlings or were transferred abruptly from long days to short days at a later stage of development. These facts would therefore suggest that, if such flowers are desired in the field, planting should be delayed as late as possible, so that the seedlings will be subjected very early in their development to daylengths considerably shorter than the maximum on which flowering can occur. Since the plants would possibly be dwarfed by such a procedure, an increased rate of seeding might be necessary.

This procedure was tested by L. E. HESSLER[3] in a small field plot at the Kentucky Experiment Station with female seed produced in these Beltsville experiments. All the plants were female, and large numbers of them produced variable numbers of male flowers. The plants were open-pollinated and pro-

[3] "Investigations with Hemp Breeding and Hemp Fiber Quality" (Project Annual Report, Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, 1946 [a typewritten report filed in the Division]).

duced a fair yield of seed. Samples of this seed were planted in the greenhouse at Beltsville, and 94.5% of the resulting seedlings proved to be female. The 5.5% of plants that were male evidently were derived from pollen produced by normal male plants in adjacent fields or by wild hemp plants. Presumably an equal percentage of female plants was derived from the same pollen source, making the total approximately 11%.

A more elaborate study was made by FEASTER[4] at Western Kentucky Substation, Princeton, Kentucky, with seed derived from female lines developed in these photoperiodic experiments. FEASTER made plantings on April 18, May 11, June 2 and 18, and July 16 in plots 20 × 20 feet. Estimated percentages of female plants bearing male flowers in September in these respective plantings were 15, 50, 80, 90, and 100. The male flowers in the April 18 planting appeared about 2 weeks later than the female flowers. This interval became progressively less in the succeeding plantings, and in the final planting of July 16 male and female flowers were developed simultaneously.

It may be noted that plants of a May planting grown with natural photoperiods in pots in the greenhouse (table 3) produced no male flowers, whereas about 50% of the plants of FEASTER's May planting in the field produced them abundantly. These divergent results could have been due to different cultural conditions or to inherited differences in the seed. FEASTER'S seed was derived from the same original stock, but during several generations selection had been made for high expression of monoecism.

These results of HESSLER and FEASTER clearly establish the feasibility of producing female seed in commercial plantings by methods suggested in these photoperiodic experiments. Field conditions even more favorable to the production of female seed could possibly be found.

For small breeding plots it should be practical to use artificial light to extend the daylength at the time when the natural photoperiod is beginning to decrease, and thus to prevent flowering. Discontinuance of the supplemental light after the natural days had become appreciably shorter would then serve to transfer the plants abruptly from long days inhibitory to flowering to days short enough to promote the formation of both male and female flowers on the female plants. Such a procedure should have the further advantage of bringing the plants into bloom after most of the normal males or wild males in the vicinity had shed their pollen, thus increasing the probability of obtaining a high percentage of female seed.

Sex ratios that did not deviate significantly from unity were obtained in all populations of hemp produced from the commercial Kentucky seed used in these experiments. There are numerous reports in the literature, however, of significant deviations (6-9, 12), and in many such cases the female plants outnumber the males. Explanations for such deviations have been based on the possible higher mortality of male-producing pollen grains or of the male seedlings (7) and the possible production of apomictic seed.[5] Another explanation would obviously be based on the production of male flowers

[4] "Hemp Investigations" (Project Annual Report, Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, 1951 [a typewritten report of 42 pages filed in the Division]).

[5] K. P. BUCKHOLTZ, "Hemp Investigations" (Project Annual Report, Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, 1942 and 1943 [typewritten reports of 13 and 27 pages, respectively, filed in the Division]).

on female plants. Such flowers produce pollen of only one type, the kind that results in the production of female seeds, whereas that from pure male plants is of two kinds occurring in equal amount. The occurrence of appreciable numbers of male flowers on female plants would increase the percentage of the female-producing type of pollen in the field and should thereby increase the percentage of female seed. This explanation would account for variations in the sex ratios of seed produced under different environmental conditions because environmental factors influence the numbers of male flowers formed on female plants. This probably is not the only cause of a high percentage of female plants, but it is one that has been demonstrated to operate under some field conditions.

Summary

1. Flowering of hemp, ***Cannabis sativa*** L., variety Kentucky, occurred in all plants at photoperiods of 8-14 hours, inclusive. At photoperiods of 16-20 hours flowering was incomplete and greatly delayed.

2. Seedling plants 3-5 weeks old flowered within about 2 weeks when shifted from 16-hour to 8-hour photoperiods. Five-week-old plants required fewer 8-hour photoperiods than did 3-week-old plants for equal promotion of flowering.

3. When one branch of a two-branched plant was subjected to short photoperiods, the production of flowers was restricted to that branch. Temperatures as low as 55” F. applied during the dark periods did not inhibit flowering.

4. Intensities of light from an incandescent-filament lamp greater than 0.03 ft-c. inhibited flower-bud initiation of hemp when applied daily during the 16-hour periods between successive 8-hour periods of natural light outdoors.

5. Pollination of female plants with pollen from male flowers produced on female plants resulted in seed that produced only female plants. This “female” seed was produced in such abundance that subsequent experiments were done with plants from this source.

6. The relative abundance of nitrogen in the nutrient solution did not greatly modify the extent to which male flowers were formed on female plants, but it influenced their rate of development.

7. Full intensity of natural light resulted in production of male flowers on a greater percentage of female plants than did lower intensities of natural light.

8. Transfer of plants from long photoperiods to photoperiods appreciably shorter than that critical for flowering resulted in production of male flowers on a greater percentage of female plants than occurred when they were transferred to photoperiods only slightly shorter than the critical one.

9. A low temperature during or immediately prior to photoperiodic induction resulted in more extensive production of male flowers on female plants than did a higher temperature.

10. The tendency of plants of certain female lines to form male flowers was significantly greater than that of plants of other lines, indicating that selection for abundant production of male flowers on female plants might be successful.

11. Detailed dissection of all the buds on plants that had failed to flower when grown on long photoperiods revealed the presence of a few floral primordia in the axils of certain fully expanded leaves. Both male and female flower buds were present in these positions, but they failed to continue development except when subjected to short photoperiods.

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SOME EFFECTS OF CERTAIN PLANT GROWTH SUBSTANCES ON MAIZE[1]

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Introduction

The primary objective of investigations summarized in this paper was to determine the effect of application of certain growth substances on development of the tassel and ear of maize plants. DUNGAN and GAUSMAN (3) found that clipping corn plants delayed anthesis and silking. This was found to offer a practical means of synchronizing inbreds and single crosses which do not normally tassel and silk at the same time. Delay in reproductive development, however, was usually accompanied by a statistically significant reduction in yield. Since it was suspected that applications of growth

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