CARBOHYDRATE–NITROGEN RATIOS WITH RESPECT TO THE SEXUAL EXPRESSION OF HEMP

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(WITH TWO FIGURES)

Introduction

Most of the recent work on the determination of sex in the higher plants has been done by cytologists and geneticists. These investigations have resulted in the chromosome theory of sex inheritance and sex determination, which is supported by much evidence in the dioecious forms of the higher plants.

Any adequate theory of sex inheritance and determination in the angiosperms must do more than account for the usual approximate 1:1 ratio of the sexes in dioecious plants. It must also explain the reversal of sexes within the so-called dioecious plants and the determination and development of both types of sporophylls on the same plant, as in the monoecious types. It should even explain the development of both types of sporophylls within one flower, as is the case with the majority of the angiosperms. The segregation of the microsporophylls and the macrosporophylls to individual plants should be considered the exception and not the general case. Any theory of sex determination based only on dioecious types is subject to many exceptions and criticisms.

The absence, or apparent absence, of allosomes in many dioecious plants and the frequency of reversal from the staminate to the pistillate condition, or *vice versa,* in forms in which allosomes have been reported, have led to differences of opinion as to the final importance of the "sex chromosomes" in sex determination in the dioecious angiosperms. The reversal of the sexes in dioecious plants indicates that sex determination and inheritance may result from the action of two different, but closely related, sets of factors in the development of these plants. The prevailing 1:1 ratio of the sexes indicates that a genetical tendency to produce one or the other type of flower is probably inherited in a strictly Mendelian manner. The evidence from the development of intersexes under certain environmental conditions shows clearly that the final display of the staminate or of the pistillate condition is determined by physiological as well as by genetic factors, even in dioecious plants. Probably the development of staminate and pistillate flowers is more dependent on physiological factors in monoecious than in dioecious plants. In plants with perfect flowers the development of stamens and pistils is probably chiefly dependent on physiological factors. Indeed it would be difficult to explain the determination of the

sex of the floral organs in monoecious plants and in plants with bisexual flowers on only a Mendelian basis, although genetic factors undoubtedly play an important part in determining the order of appearance and the arrangement of stamens and pistils in bisexual flowers and of pistillate and staminate flowers in monoecious species.

If the chromosome theory of sex inheritance is accepted for dioecious plants, it need not imply that sex determination is finally accomplished by means of the chromatin content alone. The observations in nature indicate that the potentialities for both types of spores are present at all times in all heterosporous angiosperm sporophytes, whether dioecious, monoecious, or perfect with respect to flower types. This has frequently been assumed. In that case dioecism would be considered as the suppression of one type of sporophyll on a given plant and monoecism would imply the suppression of one type of sporophyll only within a given region of the floral structures. The inherent nature to behave in this manner, under the usual conditions of growth, is probably controlled by genetic factors. Whether or not this is the case, there remains the need for an explanation of the controlling internal physiological mechanism that is functioning at the time of determination of staminate and pistillate structures. This internal physiological mechanism is at present the unknown factor. Regardless of our lack of knowledge concerning the nature of this physiological mechanism, it has been assumed to be the fluctuating factor which makes possible the reversal of the sexes in dioecious plants. This same concept can be applied to the development of only one type of fertile sporophylls in one region and of the other type of fertile sporophylls in another region of the same heterosporous plant. The physiological mechanism in question need not be fixed; perhaps it may be changed by an altered environment or by the metabolism of the plant.

Geneticists in general do not attempt to explain the mechanism by which a gene, or set of genes, brings about the manifestation of the particular morphological and physiological characters with which they are connected, but this manifestation is thought to be the result of a series of processes which begins with the interaction between the chromosome substance and the particular type and state of the cytoplasm within which the chromosomes are contained. Many genes are recognized which are influenced, or limited, in their actions by the conditions of the plant or of the tissue in which the character is expressed. If this is the case for genes influencing sexual expression, then it is obviously necessary to postulate a difference in the physiological conditions varying with time or location, or both, which makes possible the expression of the staminate or pistillate conditions in the ordinary monoecious plant or in a plant with perfect flowers. The genetic constitution is not known to be different in the stami-

nate and pistillate organs of these plants, with the possible exception of *Begonia* (**20**). Thus there is need for a study of the physiological differences associated with the development of stamens and pistils to explain the determination of sex in the higher plants. It is obviously difficult to obtain data which are conclusive in this respect. It will ultimately be necessary to determine whether or not the physiological differences represent the changes which accompany or follow the real determining physiological factors.

The problem of the physiological factors associated with the formation of staminate and pistillate structures respectively has not been directly attacked. Obviously the difficulties in making such a study on plants with bisporangiate flowers are very great. The problem should be less difficult in such monoecious plants as corn, and least difficult in a dioecious plant like hemp.

Review of literature

KLEBS (**11**) emphasized the complexity of the factors influencing the types of structures formed by plants. He considered the living cell as being influenced by three sets of factors: (1) the specific structure, (2) the internal conditions, and (3) the external conditions. His use of the term specific structure includes what might be termed the inherent composition of the cell. By varying the environmental factors in his studies on *Saprolegnia mixta* (**11**) and *Sempervivum funkii* (**10**), KLEBS showed clearly that the behavior of these organisms is not controlled by a fixed inner rhythm but is subject to control (within limits) by external factors.

Since the work of KLEBS, the most important advances in the mechanics of development and in our knowdge of the factors determining differentiation in the seed plants have fallen along two general lines. KRAUS and KRAYBILL (**12**) report a correlation between the carbohydrate and nitrogen content of the tomato plant and fruitfulness. The literature on carbohydrate-nitrogen ratios has become so extensive that no attempt will be made to review it here. The writer knows of no work pertaining to carbohydrate-nitrogen ratios in the two sexes of a dioecious angiosperm.

Another line of advance in our knowledge of the factors determining growth and reproduction in the angiosperms was initiated by GARNER and ALLARD (**6**) in their work on photoperiodicity. Probably the reaction of the plant to the proper photoperiod is only one means of producing an internal condition favorable to the formation of flowers and of influencing sexual reproduction. It should be possible to produce this same end result by altering one or more factors in the environment of the plant other than the photoperiod.

Some photoperiodic studies have been conducted on dioecious plants.

SCHAFFNER (**23, 25**), MCPHEE (**15**), and ADAMS (**1**) have varied the daily period of illumination on hemp. SCHAFFNER found that a decreased length of day is accompanied by an earlier flowering and an increase in the relative number of sex reversals. He reported that almost 100 per cent. of the staminate plants show some pistillate structures when flowering occurs in the middle of the winter. MCPHEE failed to obtain as high a percentage of reversals or intersexes as SCHAFFNER reported. ADAMS gave no data on this phase of the subject.

WESTER (**29**), in an account of the work of CIESIELSKY on sex determination in hemp, referred to the latter's conclusion that sex is normally determined by the age of the pollen at the time of pollination, but BESSEY (**2**) and BOSE (**3**) failed to find any support for this theory from their experiments on pollen age and sex.

The cytological aspect of sex inheritance in hemp has been studied by several workers. STRASBURGER (**28**) and MCPHEE (**14**) failed to find any apparent sex chromosomes, but SINOTÔ (**26**) and HIRATA (**9**) reported allosomes from studies on meiosis in staminate plants.

The genetical aspect of sex inheritance in hemp has been studied in breeding experiments by MCPHEE (**16**) and HIRATA (**8**). MCPHEE suggested that the genetical mechanism is of the XY type, the staminate plants being heterozygous for the factor or factors involved. The conclusions of MCPHEE are supported by the work of HIRATA.

It is generally agreed that the sexual expression of hemp can be modified in certain strains by environmental factors. MCPHEE (**16**), HIRATA (**7**), and BOSE (**3**) have discussed the frequency of sex reversals in different strains of hemp. CORRENS (**5**) has classified hemp as trioecious because the appearance of monoecious plants is so frequent in this species.

SCHAFFNER (**24**) has caused hemp plants to pass from a vegetative condition into a flowering condition and then back to a vegetative state for a prolonged period of time by changing the photoperiod. When flowering recurs the staminate plants frequently produce some intersexes. BOSE'S (**3**) observations show that the reversals of sex are more common in the staminate plants. He found that the occurrence of intergrades in the field is limited almost completely to the flowers formed at the beginning or near the end of the period of flowering. These two observations support the view that conditions existing within the plant largely influence the types of flowers found at a given time. If the determining factors are physiological, one might expect that the conditions within the plant determining the formation of staminate flowers, or inhibiting the formation of pistillate flowers, on a genetically staminate plant would be gradually developed. One need not assume that these internal physiological factors remain fixed from the time of initiation of the first flower primordia until the cessation of flower formation.

Pritchard (**21**) obtained sex reversals in hemp by mutilating the plants, that is, by removing the floral branches or leaves. Covering the tips of the plants with manila bags was found to be equally effective in this respect. The injection of solutions of carbohydrates and nitrogenous compounds into the stem after mutilation seems to be of further help in causing the production of intersexes in some cases. Bose (**3**) attempted to repeat this work, using the transpiration stream as a means of distributing the liquids through the plant instead of injecting them mechanically. Both investigators found that decapitation and covering the tips with bags are the best means of forcing sex reversals in the field. Bose doubted the significance of his results since some intersexes appeared in his controls.

The metabolic differences between the sexes in dioecious angiosperms have not been investigated to any extent. Satina and Blakeslee (**22**) applied several biochemical tests to certain Mucorales in an effort to distinguish metabolic differences between the heterothallic strains of this group of fungi. They applied similar tests to the leaves of some of the dioecious angiosperms, hemp being among those tested. They found that the Manoilov reaction, which is based on the reduction of potassium permanganate, is of a different order for the leaves of staminate and pistillate plants of hemp. They also reported a difference between the sexes in the natural color of the alcoholic extracts and in the oxygenase and the peroxidase activities, while the pH of the two types of plants is practically the same. They used only the leaves from staminate and pistillate plants. Camp (**4**) reported that the catalase activity of reproductive organs, or structures closely associated with them, is higher in the case of staminate tissues than in the corresponding tissues of pistillate plants of the same species. The same relationship was reported by him for the catalase activity of the staminate structures or flowers in contrast to activity of the pistillate structures or flowers of monoecious plants or plants with bisexual flowers.

Many indications of physiological differences between staminate and pistillate angiosperms exist. Such differences are frequently assumed to be important in sex determination, but there is a noticeable paucity of data on the subject. The results here presented are the outcome of an attempt to ascertain some of the differences and similarities in staminate and pistillate plants of hemp.

Methods

Hemp seeds obtained from a commercial seed company were planted in soil trays on February 7 and transplanted into a deep soil bed in the greenhouse on February 22. The length of the photoperiod was increased to 15 hours by means of a battery of electric lights. A few male plants were beginning to flower on March 19. On April 2 typical male and

typical female plants were selected for analyses. The most mature flowers in the inflorescences of the staminate plants were beginning to shed pollen, but most of the flowers on each plant were immature at this time. A few of the flowers on the pistillate plants were fully developed, but no seed had been set. The average heights were 40 cm. for the pistillate plants and 52 cm. for the staminate plants. On April 5 another collection was made. These plants were in essentially the same stages of development as those collected three days earlier.

The plants were cut at the cotyledonary node at 4 P. M. and placed in separate weighed flasks for the determination of their fresh weights. Each plant was then cut into small pieces. The macerated tissue was mixed thoroughly and divided into two portions, each of which was weighed.

One of the portions was used for the determination of the percentage of dry matter in the sample and subsequently employed for the estimation of nitrogen. The other portion of each plant was used for the determination of carbohydrates. It was placed in 80 per cent. alcohol with a slight excess of calcium carbonate and refluxed for one hour to complete the extraction of the soluble carbohydrates. The procedure given by LOOMIS (**13**) was followed for the separation of the soluble and insoluble carbohydrates. All extracts were cleared by the use of natural lead acetate and deleaded by the use of potassium oxalate. Tests for reducing sugars were made on aliquots of the extracts obtained in this manner. Total sugars were determined on aliquots of these extracts after they had been hydrolyzed with 2 per cent. hydrochloric acid at 70° C. for 10 minutes and then neutralized with sodium carbonate.

That portion of the sample of each plant which was insoluble in 80 per cent. alcohol was hydrolyzed with 2 per cent. hydrochloric acid at the temperature of boiling water for two hours. The solution was then neutralized, cleared, and deleaded. Aliquots of this liquid were used for reducing sugar tests to estimate the acid-hydrolyzable polysaccharides present in each plant.

The carbohydrate fractions were determined by the use of Fehling's solution. The cuprous oxide was estimated by the volumetric iodide method given by MAHIN and CARR (**17**). Determinations were made on duplicate aliquots of each fraction of the carbohydrates. Each class of carbohydrates was expressed in terms of its glucose equivalent.

The nitrogen content of that portion of each plant used for dry weight determinations was estimated by the Kjeldahl method. Digestion was accomplished by the use of 20 cc. of concentrated sulphuric acid and 0.5 gm. of cupric sulphate. The distillate was received in normal acid and titrated with normal sodium hydroxide using methyl red as an indicator.

Results

The determinations of the dry weights show no differences between the percentages of moisture present in the aerial portions of the staminate and pistillate plants. There is a marked difference in the content of reducing sugars in the plants of the two sexes. The average content of reducing sugars expressed in terms of the fresh weight of the plants is 0.56 per cent. for the male plants and 0.23 per cent. for the female plants in the collection made on April 2. The same values for the collection made on April 5 are 0.81 for the males and 0.27 for the females. This striking difference still exists for the collections of both dates if the values are computed in terms of the dry matter. The differences in the contents of total sugars, expressed as glucose, also show the males to be higher than the females in the total quantity of the more soluble carbohydrates present on both dates. The results of these determinations are given in columns 2–6 inclusive of tables I and II.

The acid-hydrolyzable polysaccharides, expressed in terms of glucose, are more abundant on the average in the males than in the females, but the content of polysaccharides varies so much within the staminate plants that the average value for the group loses its possible significance (see columns 8 and 9 of tables I and II). The contents of the various classes of carbohydrates in the staminate and pistillate plants collected on April 5 are presented graphically in figure 1.

The average content of nitrogen is higher in the females than in the males, whether the values are computed on the basis of fresh weight or on the basis of dry matter. The results of the determinations of nitrogen are given in columns 12 and 13 of tables I and II.

The differences in the contents of the various carbohydrate fractions and of the nitrogen present in the staminate and pistillate plants are accentuated when the results are expressed in terms of the ratios of the carbohydrates to the nitrogen. The ratios of the reducing sugars to the nitrogen are higher for the staminate than for the pistillate plants. The same relationship holds for the ratios of the total sugars to the nitrogen. The average values of the ratios of the polysaccharides to the nitrogen are higher for the males than for the females; however, a few males have lower values than some few of the females for this ratio. The ratios of the various groups of carbohydrates and of the total carbohydrates to the nitrogen for the plants of both sexes are given in table III. The ratios for the plants collected on April 5 are presented graphically in figure 2.

Discussion

The determinations of the content of dry matter fail to show any differences between the sexes. If differences between the compositions of the

TABLE I

Fresh weights and percentages of dry matter and moisture and amounts of reducing sugars, total sugars, polysaccharides, total carbohydrates, and nitrogen as percentages of fresh and dry weights of staminate and pistillate hemp plants harvested April 2

Plant no.	1	2	3	4	5	6	7	8	9	10	11	12	13
	Total fresh weight	Dry matter	Moisture content	Reducing sugars		Total sugars		Polysaccharides		Total carbohydrates		Nitrogen	
				Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis
	gm.	%	%	%	%	%	%	%	%	%	%	%	%
						Staminate plants							
1	13.1	21.03	78.97	0.79	3.78	1.38	6.58	3.01	14.3	4.39	20.9	0.81	3.88
2	11.3	20.81	79.19	0.48	2.34	1.43	6.90	3.01	14.5	4.44	21.4	0.80	3.84
3	15.8	20.86	79.14	0.43	2.07	1.24	5.94	2.78	13.3	4.02	19.2	0.90	4.29
4	5.7	20.59	79.41	0.47	2.25	2.13	10.27	2.81	13.6	4.94	23.9	0.85	4.12
5	3.4	24.49	75.51	0.40	1.62	3.47	14.05	5.70	23.1	9.17	37.2	0.77	3.11
6	17.6	21.12	78.88	0.78	3.68	1.45	6.88	2.75	13.0	4.20	19.9	0.67	3.16
Average	11.2	21.48	78.52	0.56	2.62	1.85	8.44	3.34	15.3	5.19	23.7	0.80	3.73
						Pistillate plants							
1	14.0	21.43	78.57	0.20	0.94	1.15	5.33	2.96	13.8	4.11	19.1	0.99	4.63
2	15.0	20.55	79.45	0.20	0.98	1.05	5.12	2.27	11.0	3.32	16.1	0.90	4.36
3	17.6	20.57	79.43	0.22	1.09	1.15	5.60	2.94	14.3	4.09	19.9	0.95	4.63
4	12.8	21.16	78.84	0.22	1.02	1.09	5.14	2.98	14.1	4.07	19.2	0.95	4.48
5	20.5	21.28	78.72	0.25	1.18	0.99	4.64	2.71	12.7	3.70	17.3	0.90	4.25
6	13.5	18.77	81.23	0.27	1.46	0.90	4.83	2.44	13.0	3.34	17.8	0.88	4.71
Average	15.5	20.63	79.37	0.23	1.11	1.06	5.11	2.71	13.2	3.77	18.3	0.93	4.51

TABLE II

Fresh weights and percentages of dry matter and moisture and amounts of reducing sugars, total sugars, polysaccharides, total carbohydrates, and nitrogen as percentages of fresh and dry weights of staminate and pistillate hemp plants harvested April 5

Plant no.	1	2	3	4	5	6	7	8	9	10	11	12	13
	Total fresh weight	Dry matter	Moisture content	Reducing sugars		Total sugars		Polysaccharides		Total carbohydrates		Nitrogen	
				Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis
	gm.	%	%	%	%	%	%	%	%	%	%	%	%
						Staminate plants							
1	9.4	23.27	76.73	0.78	3.36	1.94	8.34	2.62	11.2	4.56	19.5	1.04	4.47
2	8.8	22.42	77.58	0.76	3.39	1.49	6.64	2.68	12.0	4.17	18.6	0.88	3.92
3	8.5	23.11	76.89	0.80	3.45	1.81	7.83	3.81	16.5	5.62	24.3	1.04	4.52
4	6.0	24.17	75.83	1.02	4.19	2.66	10.94	3.89	16.0	6.55	26.9	1.02	4.22
5	28.2	20.13	79.87	0.36	1.76	1.28	6.33	2.22	11.0	3.50	17.3	0.93	4.64
6	7.1	23.78	76.22	1.12	4.68	1.95	8.18	3.39	14.3	5.34	22.5	0.84	3.52
Average	11.4	22.81	77.19	0.81	3.47	1.86	8.04	3.10	13.5	4.96	21.5	0.96	4.22
						Pistillate plants							
1	16.7	23.98	76.02	0.26	1.08	1.17	4.88	2.63	11.0	3.80	15.9	1.12	4.68
2	21.7	17.18	82.82	0.20	1.16	0.99	5.78	1.88	10.9	2.87	16.7	0.92	5.33
3	9.2	25.31	74.69	0.31	1.21	1.21	4.77	3.09	12.2	4.30	17.0	1.22	4.82
4	18.5	20.52	79.48	0.26	1.28	0.97	4.74	2.10	10.3	3.07	15.0	1.00	4.87
5	19.0	20.97	79.03	0.34	1.63	1.09	5.19	2.37	11.3	3.46	16.5	0.98	4.70
6	25.5	23.11	76.89	0.23	1.01	1.13	4.90	2.68	11.5	3.80	16.4	1.12	4.82
Average	18.4	21.85	78.15	0.27	1.23	1.09	5.04	2.46	11.2	3.55	16.3	1.06	4.87

sexes exist, they are to be found in the compositions of the dry matter and not in the percentages of dry matter.

The analyses show several striking differences in the compositions of the dry matter of the sexes. The average content of carbohydrates is higher in the males than in the females. The significance of the average values is decreased by the fact that the lowest values for individual staminate plants

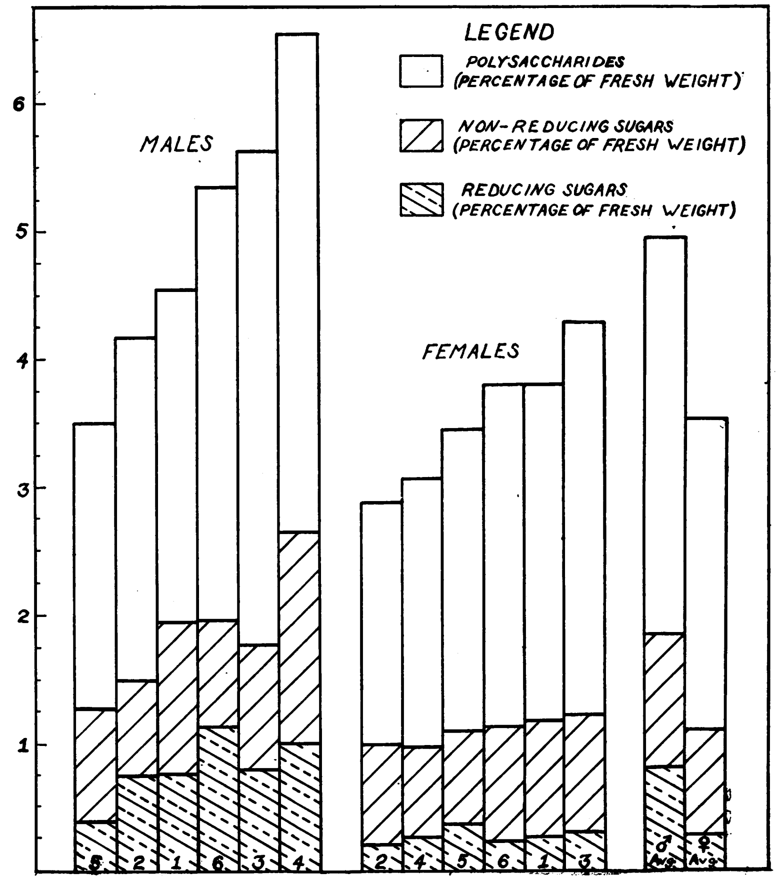

FIG. 1. Polygons showing carbohydrate contents of staminate and pistillate plants of hemp harvested April 5. The number in the base of each polygon refers to the experimental number for the plant.

are less than the highest values for a few of the pistillate plants. Tables I and II show that in this case one is not only comparing staminate and pistillate plants but is also contrasting the largest staminate plants and the smallest pistillate plants. Usually the pistillate plants have a much greater total fresh weight than the staminate plants at the time of flowering. There are

TABLE III

RATIOS OF CARBOHYDRATE FRACTIONS TO THE NITROGEN

PLANT NO.	REDUCING SUGARS/ NITROGEN	TOTAL SUGARS/ NITROGEN	POLYSACCHARIDES/ NITROGEN	TOTAL CARBOHYDRATES/ NITROGEN
Staminate (April 2)				
1	0.98	1.70	3.70	5.40
2	0.60	1.79	3.77	5.56
3	0.48	1.38	3.10	4.48
4	0.55	2.50	3.31	5.81
5	0.52	4.51	7.42	11.93
6	1.16	2.17	4.11	6.28
Average......	0.71	2.34	4.24	6.58
Pistillate (April 2)				
1	0.20	1.16	2.99	4.15
2	0.22	1.17	2.52	3.69
3	0.23	1.21	3.09	4.30
4	0.23	1.15	3.14	4.29
5	0.28	1.09	2.99	4.08
6	0.31	1.02	2.76	3.78
Average......	0.25	1.14	2.91	4.05
Staminate (April 5)				
1	0.75	1.86	2.51	4.37
2	0.86	1.69	3.05	4.74
3	0.77	1.74	3.66	5.40
4	1.00	2.60	3.80	6.40
5	0.38	1.37	2.38	3.75
6	1.33	2.32	4.05	6.37
Average......	0.85	1.93	3.24	5.17
Pistillate (April 5)				
1	0.23	1.04	2.35	3.39
2	0.22	1.09	2.05	3.14
3	0.25	0.99	2.53	3.52
4	0.26	0.97	2.10	3.07
5	0.35	1.11	2.41	3.52
6	0.21	1.02	2.39	3.41
Average.....	0.25	1.04	2.31	3.34

individual exceptions to this rule which probably can be accounted for in part by the lack of genetical uniformity in the seeds.

In contrast to the percentages of total carbohydrates, the percentages of total sugars not only show a higher average value for the staminate

plants in contrast to the average value for the pistillate plants, but there is no overlapping in the quantities of carbohydrates soluble in 80 per cent. alcohol present in the individual plants of the two sexes. The lowest value for any of the males is 1.24 per cent. of the fresh weight, while 1.21 is the highest percentage for any one female plant. This difference in the quantities of total sugars in the plants of the opposite sexes is found to be due almost entirely to the quantities of reducing sugars. This class of carbohydrates

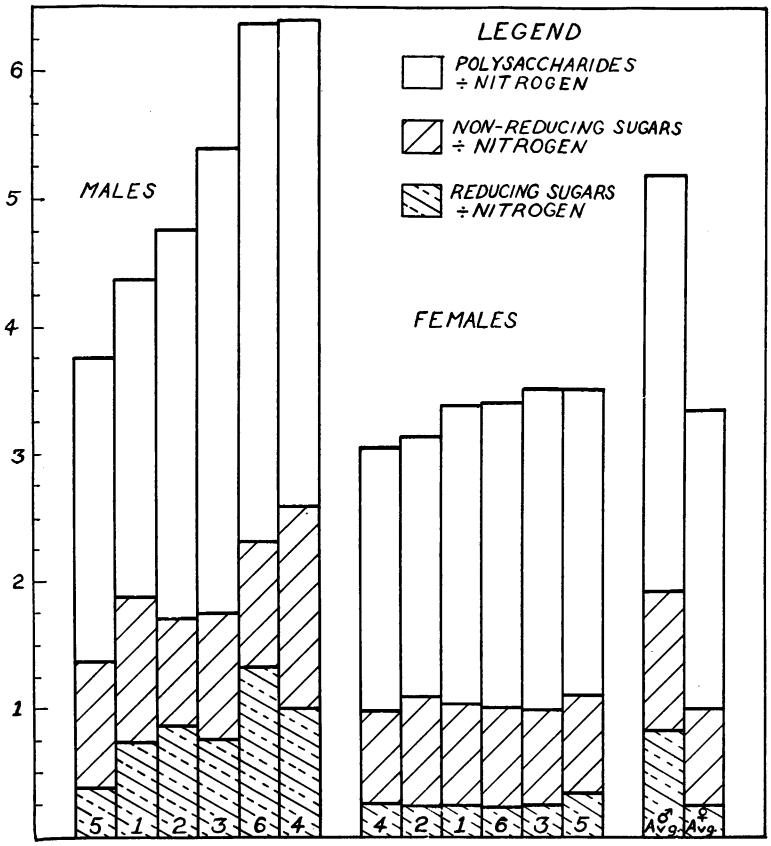

FIG. 2. Polygons showing carbohydrate-nitrogen ratios of staminate and pistillate plants of hemp harvested April 5. The number in the base of each polygon refers to the experimental number for the plant.

presents the most contrasting difference between the chemical compositions of the sexes of hemp under the conditions in which these plants were grown. The average values for the reducing sugars, expressed as percentages of the fresh weight, are from two to three times as high for the males as for the females. The ranges of variation within the two sexes cover two separate

regions. Although there is a difference in the quantities of total sugars in the two sexes, it is based chiefly on the differences in the reducing sugars and not on the quantities of non-reducing sugars. Figure 1 presents these facts graphically and also indicates the ranges of variability in the contents of carbohydrates of the two sexes.

The pistillate plants are relatively constant; that is, the analyses show little variation in the percentages of reducing and total sugars in these plants. The situation existing within the staminate plants is different. The quantities of reducing and total sugars fluctuate from values slightly greater than those for the highest pistillate plants up to values clearly of a different order.

The higher content of carbohydrates and the wider range of fluctuation in all classes of carbohydrates in the staminate plants, in contrast to the condition in the pistillate plants, are very suggestive. One is inclined to consider that the presence of the greater quantities of reducing and total sugars in the males is not a result of the inability to synthesize polysaccharides, since they too are usually more abundant in the staminate plants. Nor is one justified by these analyses alone in assuming that the polysaccharides have been digested more rapidly in the males than in the females. Regardless of the causes of the differences in the quantities of the various fractions of carbohydrates in the sexes, the constancy of the females is in keeping with the later behavior of the plants. The females flower more gradually and apparently retain their vegetative vigor for a longer period of time than do the males. The staminate plants usually turn yellow and die rapidly after flowering. The greater variability in the content of carbohydrates in the males and their early senescence stand in contrast to the small amount of variability in the carbohydrates and the slow loss of vegetative vigor in the females, when both sexes are growing under early spring conditions in the greenhouse.

The results of the nitrogen analyses are not so significant. The pistillate plants have a larger average percentage of their fresh weights composed of nitrogen than have the staminate plants. However, the values for the individual staminate and pistillate plants do not fall into two groups as in the case of the reducing sugars. It is not advisable to attempt any detailed evaluation of the nitrogen determinations, since they include all of the reduced forms of nitrogen within the plants. It is probable that most of this nitrogen is present in the plant in complex organic molecules.

The ratios of the various classes of carbohydrates to the nitrogen present some interesting differences between the sexes (table III and fig. 2). The ratios of the reducing sugars to the nitrogen again show the females to be less variable than the males. The ratios are much greater for the staminate plants than for the pistillate plants; the average values for the males

are about three times as great as are those for the females. The greater ratios of the males are due chiefly to their higher content of reducing sugars but are partly the result of their lower content of nitrogen. This same situation exists in the ratios of the total sugars to the nitrogen, but the differences between these ratios for the sexes are not so great. It is evident that the main differences in the ratios of the total sugars to the nitrogen are chiefly the result of the differences in the ratios of the reducing sugars to the nitrogen. The constancy of the females is again evident in these ratios, while the males continue to show a greater variability.

The ratios of the polysaccharides to the nitrogen show that the males have a higher average ratio than the females, but the ranges of variability in these two groups overlap. These ratios obviously are not so significant as the ratios of the sugars to the nitrogen in differentiating between the sexes of hemp grown under the conditions of these experiments. The combinations of these ratios for the different plants, that is, the ratios of the total carbohydrates to the nitrogen, give higher average values for the staminate plants than for the pistillate plants. The lowest ratio for a staminate plant collected on April 2 is higher than the highest value for any pistillate plant collected on that date. The same relationship exists for the ratios of the staminate and pistillate plants collected on April 5. The ratios are greater for the plants collected on April 2 than they are for the collection of April 5. This is partly the result of a lower nitrogen content in the plants of both sexes collected on April 2 and a lower content of polysaccharides in the plants collected on April 5.

From the preceding discussion it is evident that the contents of carbohydrates and nitrogen are different in staminate and pistillate hemp plants at the time of flowering, when they are grown under the conditions of these experiments. These differences are seen to be the result of a relatively high content of sugars and a relatively low content of nitrogen in the staminate plants. These facts can be interpreted to explain the early senescence of the staminate plants and the retained vegetative vigor of the pistillate plants of greenhouse hemp. KRAUS and KRAYBILL (**12**) and others have shown that the relationships between the carbohydrates and nitrogenous materials are largely the determining factors in changing from the vegetative to the fruiting state in several bisporangiate angiosperms. The results obtained in these analyses of hemp plants in the early stages of flowering do not necessarily show any relationship to the quantities of these substances which were present before the plants began flowering, but it is evident that these staminate and pistillate plants have different carbohydrate-nitrogen ratios at the time of flowering. It remains to be determined whether the differences between the sexes in these ratios are causal physiological factors connected with the formation of stamens and pistils, or

whether they are merely accompanying factors. It does not necessarily follow that the conditions in the meristematic regions of the staminate and pistillate plants at the time of flower formation are properly reflected by the analyses of the whole aerial portions of the plants.

MURNEEK (**18**) has shown that tomato plants grown with a shortage of nitrogen turn yellow and decline vegetatively. This condition can soon be overcome by furnishing the plant available nitrogenous compounds. The staminate hemp plants, soon after they flower, act similarly to the nitrogen-starved tomato plants. A difference exists chiefly in the fact that the staminate hemp plants continue to decline even though they have the same source of nitrogen compounds as have the pistillate plants. They are apparently exhausted by the flowering process to the extent that they are unable to continue the assimilation of nitrogen rapidly enough to regain their lost vegetative vigor, as does the tomato plant.

The spider flower (*Cleome spinosa*) yields some interesting information with respect to types of flowers formed and the quantity of nutritive materials present. STOUT (**27**) has described the development of alternating whorls of fertile and sterile flowers in this bisporangiate angiosperm. MURNEEK (**19**) has altered the nitrogen available to this plant and finds intermittent sterility in plants with an abundance of or with a shortage of available nitrogen in the soil. MURNEEK found a difference in the growth of the plants and in the frequency of the alternating whorls of sterile and fertile flowers, but he was unable to cause the plant to overcome the tendency to form alternately fertile and sterile flowers by changing the quantity of nitrogen available to its roots. The removal of flowers containing fertile pistils has a direct effect on the type of flowers produced in the meristematic region of the spike. The percentage of the flowers able to set seed was increased greatly by this procedure. The intermittent sterility in the spider flower results from the alternation of whorls of flowers with aborted stamens and large functional pistils with whorls of flowers containing aborted pistils and large functional stamens. Thus the functional sex of the flowers of *Cleome spinosa* varies with some internal regulating condition determining the development of the essential floral parts. Since the removal of fertile flowers and fruits increases the width of the zones of fertile flowers being formed nearer the apex of the inflorescence, it seems that the formation of functionally pistillate flowers and the maturation of fruits both require similar, or the same, substances from the plant. Either these substances are not present in sufficient quantities or else they are not conducted to all parts of the inflorescence rapidly enough to permit a continuous formation of fertile flowers and the simultaneous maturation of fruits.

Since the removal of fruits is known to allow the nitrogenous compounds

to accumulate in certain plants (**18**), it is probable that the fertility of the flowers produced by *Cleome spinosa* is partly dependent upon the nitrogen supply available in the tissues in close proximity to the flower primordia. If this is true for the spider flower, one might consider that the functionally staminate flowers of *Cleome spinosa* were formed from meristematic regions lower in certain nitrogenous elements, or with a higher carbohydrate-nitrogen ratio, than the regions forming functionally pistillate flowers. The condition in hemp plants might be essentially the same except that the usual presence of aborted stamens or pistils within the flowers is not encountered. Since these analyses include the whole aerial portions, it is not safe to assume positively that the formation of stamens takes place in tissue with a higher carbohydrate-nitrogen ratio, while pistils are formed in a corresponding tissue with a lower carbohydrate-nitrogen ratio. If this were definitely proved, it would not necessarily follow that the sexual nature of the flower depended entirely on the quantities of these metabolic materials; but it is interesting to note this possible relationship between the sexual nature of the plant and the quantities of sugars and nitrogenous compounds present.

A further investigation into other aspects of the physiological differences and similarities between the sexes in hemp and in other plants is needed before one can properly evaluate the true significance of the carbohydrate-nitrogen ratios with respect to sex determination.

Summary

1. The aerial portions of staminate and pistillate plants of hemp were analyzed at the time they were coming into flower. Determinations were made of the total fresh weight of the individual plants and of the moisture, reducing sugars, total sugars, polysaccharides, and reduced forms of nitrogen.

2. Little if any difference in the percentages of moisture and dry matter exists between the sexes of hemp.

3. Staminate plants have higher average percentages of total carbohydrates, polysaccharides, and sugars than pistillate plants under the conditions of these experiments.

4. The reducing sugars are much more abundant in the staminate plants than in the pistillate plants.

5. Nitrogen is relatively more abundant in the pistillate plants than in the staminate plants.

6. The carbohydrate-nitrogen ratios of the sexes are discussed with respect to the differences in the sexual expressions and the habits of growth of the plants.

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