

Marihuana Investigations. II

The Effect of Variety, Maturity, Fertilizer Treatment and Sex on the Intensity of Response to the Beam Tests

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INTRODUCTION

In a previous communication (1) it has been pointed out that (1) individual Cannabis plants, though of the same agronomic variety and grown side by side, vary widely in capacity to respond to the acid and alkaline Beam tests; (2) the substance responsive to the tests, when detectable at all, is always found in the tops and is generally found in all parts of the plant except pith, lower stalk and roots; (3) many plants respond to the tests when as small as three inches tall; (4) response continued throughout the life of the plant; and (5) male and female plants respond alike to the tests.

With a view to continuing the study of these and related phenomena the research here reported was initiated. Its objectives were to study (1) variation in response to the tests among different agronomic varieties; (2) the effect of fertilizer treatments on response to the tests; (3) variation in response with age of plants; and (4) difference in responses on the part of male and female plants.

Since the completion of the present research a pure substance, known as cannabidiol, has been isolated from Cannabis resin (2). This compound has been found to respond intensely to the alkaline Beam test. Cannabidiol accounted for a considerable percentage of the resin from which it was prepared and it is not unreasonable to assume that it is the chemical individual to which the test is principally due. It would appear that intensity of response to the alkaline Beam test constitutes a valid criterion of differences in chemical makeup of resins of different origin despite the fact that the cannabidiol has been found physiologically inactive in dogs.

The acid Beam test, while by no means so firmly founded, chemically, must also be considered a measure of some quality of the resin and the test results described below are accordingly considered worthy of record though of lesser interest than those obtained employing the alkaline Beam test. There is evidence that intensity of response to the acid test is conditioned by the method of testing. The results recorded were obtained, of course, following a strictly uniform procedure.

EXPERIMENTAL

Material and Methods Employed.—On April 30, 1938, a plot of Cannabis was planted on the lowlands of the Department of Agriculture Experimental Farm in Arlington, Virginia, which was adjacent to the plot planted for the 1937 investigations. The plot consisted of twelve rows, three and one-quarter feet apart and one hundred and thirty feet long, the inner ten rows being divided into ten parts each, thus forming one hundred sections, ninety-six of which were utilized for planting the six agronomic varieties studied. Each variety was planted in sixteen randomized plots.

The outer rows and four remaining sections were not concerned in this report except that they assured identical conditions of sunshine and crowding to the remainder of the plot.

Fertilizer was applied to the various experimental plots. Nitrogen was applied in the form of sodium nitrate at the rate of 100 pounds per acre, 18% superphosphate at the rate of 200 pounds per acre and muriate of potash at the rate of 100 pounds per acre. This was applied directly in the row at the time of planting. By using each of these three fertilizers by itself, by combining them in couplets, by using them all together, there were even different treatments provided for each of the six varieties. Adding to this a control plot for each of the six varieties, there results a total of eight different treatments, or 48 combinations of treatment *versus* variety. In view of the fact that each of these plots was planted in duplicate, 96 supervised plots were set out.

The varieties planted were as follows: Seed from Spantov province, Roumania, called Roumanian No. 1; seed from Canad province, Roumania, called Roumanian No. 2; seed from Cluj province, Roumania, called Roumanian No. 3; seed from Manchuria, called Manchurian; seed from China, called Chinese; and seed from Italy, called Italian.

Early growth was slow, presumably due to drought. Some male plants began flowering when only eighteen inches tall, but all continued to grow, as the season progressed, to an average height of nine feet.

By June 10th male flowers began to appear among the Roumanian varieties and by July 28th the sex

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of all plants was apparent. By September the males were fast being denuded.

The growth types were as follows:

A. Plants short in height and very early in maturing. The internodes were shorter than the other types and the lateral branches shorter and gave the appearance of being much more compact. Roumanian No. 1 and No. 3 represented this type.

B. Plants medium tall, open branching heads and medium in time of maturing. Represented by Roumanian No. 2.

C. Plants tallest in growth type, retaining very few branches but of open branching growth type and medium in time of maturing. The most favorable type for the production of fiber. Represented by the Italian variety.

D. Plants medium tall with open branching type of growth but late in maturing. The *D* type is similar in many ways to the *B* type, except that the *D* is later in maturing than the *B* type represented by the Manchurian and Chinese varieties. The Chinese variety had very broad leaflets which could be seen and distinguished at a distance from the other varieties. The Manchurian differed from the Chinese in this respect, that its leaflets were narrower.

On three occasions during the growth of the plants, portions of the tops were cut from each of three plants in each of the ninety-six plots, making a total of two hundred and eighty-eight samples tested on each occasion. An effort was made to sample one male and two females on each occasion, but in the two earlier tests it was frequently not possible to distinguish the sex. Each plant, however, was tagged and its sex ultimately determined. Only a half dozen plants, thus tagged, died during the testing period. The samples gathered were dried in the laboratory air.

Date	Days Old	Average Height, cm.	Stage Growth	Gathered Males	Gathered Females	Drying Period	Temp. of Drying, °C.
6-18-38	49	60-100	Few flowers	133	155	4 days	30
6-30-38	61	120-200	Flowering	121	167	11 days	25
7-29-38	90	240	Full flower	96	192	7 days	25

Above are tabulated pertinent data regarding the plants on each of the three test occasions:

The test procedure employed was essentially that described in a previous report (1). The uniformity of the samples permitted the procedure to be standardized as follows:

1. Cover $\frac{1}{2}$ Gm. of crushed sample with 15 cc. of pure ethyl acetate and allow to extract 1 minute or more.
2. Add $\frac{1}{2}$ Gm. of Norit (activated carbon) and stir.
3. Filter through a dense paper and place 5 cc. of filtrate in each of two white porcelain dishes.
4. Evaporate the solvent on a steam bath before a fan.

5. *Alkaline Test.*—To the residue in one dish add 5 drops of Beam's alkaline reagent (2% alcoholic potassium hydroxide) and observe any purple or violet color which appears within thirty minutes.

6. *Acid Test.*—To the residue in the second porcelain dish add 1 cc. of Beam's acid reagent (saturated alcoholic HCl) and observe any cherry-red color which develops at once.

The plants were classified in six categories based on intensity of response to the tests. Those referred to as "0" were negative; those referred to as "1" showed a trace of color; and the other classes showed color intensity increasing in the indicated order. It may be stated that tests listed as "2," "3," "4" or "5" were clearly positive. Those listed as "1" could scarcely be considered adequate for identification, although a definite trace of color was produced in each case.

Tests were made in the numerical order of test plots, which numbers were random with respect to the various varieties and fertilizer treatments. In this way those making the tests were not aware at any time which variety or treatment was being tested. The data recorded were assembled after all chemical work had been completed.

Table III presents the results of an "Analysis of Variance" of all the alkaline and acid test data showing the effect thereon of each of a number of variables and combinations of variables. Under the heading "required" appear the numbers which must be derivable from the data variations if the probability that the indicated effect of the corresponding variable is not due to chance is to be 19 to 1 or 99 to 1 as the case may be. In the column headed "Found" are recorded, opposite the pertinent variable, the numbers actually derived where these were found to be larger than the required numbers. Where no number is recorded the number derived was numerically smaller than the required value and the effect of the variable in question is not to be considered statistically significant.

RESULTS

ALKALINE TEST

The data obtained from the testing are assembled in Table I. Each of the individual numbers recorded is the sum or total of the "test intensity numbers" of the three individual plants tested from each of the 96 plots. The data for each of the three test occasions are separately grouped. The two numbers under each "Variety—Fertilizer Treatment" heading record the totals from duplicate plots. The sub-totals are the sums of all the individual totals occurring in the columns. The other totals are self-explanatory.

Effect of Variety.—Attention is invited to the striking difference in test response among the various varieties as reflected in the columns headed "Variety Sub-total" and "Variety Grand Total." The three Roumanian varieties are thus shown to re-

spond intensely to the test while the Chinese and Manchurian varieties respond very feebly. These differences are statistically significant. All of the plants which tested negatively occurred in the latter two varieties and the great majority of their positive responses were categorized "1" and "2." The Italian variety is intermediate in intensity of test response but more nearly resembles the Roumanians than the others. Of chief concern is the demonstration that samples of Manchurian or Chinese Cannabis may be encountered which cannot be identified by the alkaline Beam test as at present carried out. A considerable amount of the hemp now growing in this country is originally of Manchurian or Chinese origin.

Effect of Age.—The sum of the variety sub-totals for each of the three samplings as recorded in Table I indicate the differences that appeared in test response with advancing age of the plants. The most intense colors were obtained on the second occasion when most of the plants were flowering. The difference between these figures and those obtained on the first occasion are statistically significant, but the small diminution in test totals recorded between the second and third test occasions may be due to chance variation. It is noteworthy that the number of negative ("0") tests did not vary considerably among any of the three test occasions. The

increasing intensity was accompanied by a corresponding decrease in the feebly positive categories "1" and "2." Since the period covered by the tests began before inflorescence and continued through the beginning of decadence of the males it would appear representative of the whole growth period. It is indicated that intensity of response to the test increases as the plants mature though previous tests have elicited strong responses in very young plants.

Effect of Fertilizer.—The sub-totals and grand totals of the vertical columns in Table I illustrate the differences in test response determined by the fertilizer treatments of the various plots. The figures do not vary considerably and it is inferred from the statistical analysis that under the conditions studied the fertilizer treatments used do not demonstrably affect the capacity of the plants to elaborate the substance, or substances, responsive to the test.

Effect of Sex.—Variation in test response due to sexual characteristics of the plants is shown in the first half of Table IV where the figures given in the several columns indicate the percentage of the total plants of each variety and sex which were categorized under each of the "test intensity number" classifications. If all plants of each sex be considered, irrespective of variety, no difference is to be found in test response between male and female plants. It is of interest to note, however, that if

Table I.—Tabulation of Alkaline "Test Intensity Totals." The Individual Entries Are "Test Intensity Totals" for the Three Plants Sampled from Each of the 96 Plots on the Date Indicated. The Totals in the Column Headed "Variety Grand Totals" Are the Sums of the Individual Variety Totals for the Three Test Occasions. The Indicated Fertilizers Are "N"—Nitrogen, "P"—Phosphorus, "K"—Potassium

Variety	Fertilizer										Variety Sub-totals	Variety Grand Totals					
	None	N	P	K	NP	NK	PK	NPK									
<i>First Sampling—June 18, 1938</i>																	
Chinese	0	2	1	1	3	2	0	2	4	3	1	1	4	3	0	2	29
Manchurian	3	1	0	3	1	6	3	3	2	2	9	2	2	2	5	4	48
Italian	7	7	6	9	6	9	8	5	3	7	8	7	5	9	7	7	110
Roumanian No. 1	9	9	9	10	5	9	9	9	8	10	4	12	9	9	7	9	137
Roumanian No. 2	10	9	12	10	6	9	9	10	9	9	8	9	9	8	9	9	145
Roumanian No. 3	6	9	12	9	9	9	9	9	9	9	8	9	8	9	8	9	141
Fertilizer Sub-totals	72		82		74		76		75		78		77		76		
Age Total																	610
<i>Second Sampling—June 30, 1938</i>																	
Chinese	3	6	4	3	2	5	6	1	3	2	3	1	3	1	4	1	48
Manchurian	2	1	4	0	5	1	5	1	4	1	3	3	4	2	3	4	43
Italian	10	9	10	9	10	10	11	8	9	9	10	10	9	10	10	9	153
Roumanian No. 1	12	9	12	9	7	9	9	10	9	8	9	9	12	10	12	10	156
Roumanian No. 2	10	12	8	11	9	10	12	10	10	8	11	10	10	10	8	9	158
Roumanian No. 3	9	7	9	9	8	7	9	9	9	9	11	10	9	9	10	9	143
Fertilizer Sub-totals	90		88		83		91		81		90		89		89		
Age Total																	701
<i>Third Sampling—July 29, 1938</i>																	
Chinese	3	2	2	1	2	1	7	1	2	4	4	1	0	2	1	0	33
Manchurian	2	2	5	4	1	2	3	3	1	3	3	0	3	3	1	3	39
Italian	8	9	9	9	9	9	10	8	9	8	9	9	7	9	9	9	140
Roumanian No. 1	9	10	10	9	10	10	10	9	10	8	10	12	8	10	8	7	150
Roumanian No. 2	13	9	9	8	11	10	11	9	11	9	11	9	11	10	12	12	165
Roumanian No. 3	10	12	11	9	10	10	10	10	13	10	11	9	9	9	11	12	166
Fertilizer Sub-totals	89		86		85		91		88		88		81		85		
Age Total																	693
GRAND TOTALS	251		256		242		258		244		256		247		250		

each variety be considered separately, three show slightly greater "test response" means among the males, and the other three show slightly greater "test response" means among the females. It must be concluded that difference in test response between male and female plants, if it exists at all, is of no considerable consequence. No inference may be drawn from these results regarding the relative physiological potencies of male and female plants.

ACID TEST

Table II and the second halves of Tables III and IV record the acid test data. The significance of the various numbers in the tables is similar to that referred to above for the alkaline test data.

It is plain that no very striking differences in capacity to respond were disclosed among the several varieties. Statistical analysis shows that the widest difference in "test response totals," 87 to 125

Table II.—Tabulation of Acid "Test Intensity Totals." The Individual Entries Are "Test Intensity Totals" for the Three Plants Sampled from Each of the 96 Plots on the Date Indicated. The Totals in the Column Headed "Variety Grand Totals" Are the Sums of the Individual Variety Totals for the Three Test Occasions. The Indicated Fertilizers are "N"—Nitrogen, "P"—Phosphorus, "K"—Potassium

Variety	Fertilizer								Variety Sub-totals	Variety Grand Totals
	None	N	P	K	NP	NK	PK	NPK		
<i>First Sampling—June 18, 1938</i>										
Chinese	0 3	2 3	3 3	1 3	2 1	3 3	4 1	0 4	36	
Manchurian	6 6	3 3	6 1	0 3	3 3	1 2	1 0	5 7	50	
Italian	2 2	1 0	2 3	5 5	2 2	1 2	2 1	3 3	36	
Roumanian No. 1	3 3	2 0	6 2	3 1	0 1	2 0	0 4	1 2	30	
Roumanian No. 2	0 2	3 3	1 1	0 2	1 2	0 3	1 1	2 3	25	
Roumanian No. 3	3 0	4 2	3 2	2 5	4 3	0 1	3 0	0 0	32	
Fertilizer Sub-totals	30	26	33	30	24	18	18	30		
Age Total										209
<i>Second Sampling—June 30, 1938</i>										
Chinese	4 3	3 1	4 3	4 3	2 5	0 2	4 5	6 3	52	
Manchurian	3 4	3 2	5 3	2 6	3 2	1 5	1 1	3 2	46	
Italian	3 0	3 1	2 4	2 0	4 2	3 2	2 3	3 3	37	
Roumanian No. 1	2 4	2 3	4 5	3 4	1 4	3 1	3 4	3 5	51	
Roumanian No. 2	2 3	4 3	3 2	1 2	4 2	3 3	3 3	1 4	43	
Roumanian No. 3	3 2	4 0	3 3	0 2	0 4	1 2	2 2	3 2	33	
Fertilizer Sub-totals	33	29	41	29	33	26	33	38		
Age Total										262
<i>Third Sampling—July 29, 1938</i>										
Chinese	1 2	2 1	1 0	4 1	0 4	3 2	1 1	0 4	27	115
Manchurian	1 0	7 3	2 0	0 3	2 1	0 1	2 3	0 4	29	125
Italian	0 1	3 0	1 1	0 4	0 1	0 0	0 2	0 1	14	87
Roumanian No. 1	0 3	1 3	2 3	0 2	1 3	5 2	2 2	0 4	33	114
Roumanian No. 2	2 0	1 3	7 0	2 2	4 5	1 2	3 4	1 3	40	108
Roumanian No. 3	0 3	3 3	3 2	0 1	4 1	1 2	3 2	3 5	36	101
Fertilizer Sub-totals	13	30	22	19	26	19	25	25		
Age Total										179
GRAND TOTALS	76	85	96	78	83	63	76	93		

Table III.—Computations Obtained by Analysis of Variance. The Designations of Contributors Are: "V"—Varieties, "D"—Dates Harvested, "N"—Nitrogen, "P"—Phosphorus, "K"—Potassium

Variance	D/F	Alkaline Beam Test			Acid Beam Test		
		Mean Squares	Found	Required	Mean Squares	Found	Required
<i>Whole Plots</i>							
Block	1	4.0138			3.5556		
Variety	5	582.6917	256.78	3.45	3.5972	2.42	
N	1	0.2222			0.0139		
P	1	5.0138			7.3473		
K	1	1.1250			3.1250		
NP	1	0.0140			0.3472		
NK	1	0.1250			0.1250		
PK	1	0.0556			0.3472		
NPK	1	0.2221			10.1250		
VN	5	2.6472			2.3639		
VP	5	0.7722			0.9805		
VK	5	1.8833			2.9083		
VNP	5	1.4389			1.6139		
VNK	5	1.3833			2.2083		
VPK	5	1.6472			1.2805		
VNPK	5	1.1472			1.1583		
Error (a)	47	2.2692			2.8959		

Table III (Continued)

Split Plots										
Dates	2	26.4479	13.69	4.85	3.10	18.3993	10.13	4.83	3.10	
D Block	2	15.3368	7.94	4.85	3.10	0.8367				
DV	10	7.1771	3.71	2.72	2.04	4.8201	2.65	2.72	2.04	
DN	2	0.7743				3.6909	2.03	2.72	2.03	
DP	2	0.3577				1.9201				
DK	2	1.0729				0.5104				
DNP	2	1.6284				2.6701				
DNK	2	0.5104				4.1354				
DPK	2	1.3159				0.1701				
DNPK	2	0.0868				0.9479				
VDN	10	0.6613				2.6534				
VDP	10	3.3784				2.6159				
VDK	10	1.5937				0.8812				
VDNP	10	1.5909				3.1243				
VDNK	10	3.7062				4.1312	2.28	2.72	2.04	
VDPK	10	3.1951				3.4159				
VDNPK	10	1.0243				0.7687				
Error (b)	94	1.9325				1.8155				
Total	287	12.4930				2.4494				

(Italian-Roumanian No. 2), is probably not due to chance variation, the odds being greater than 19 to 1 but less than 99 to 1.

Far greater were the differences found with respect to age. The test response total was greatest on the second occasion and, rather surprisingly, declined on the third to a value smaller than that of the first. The analysis presented in Table III shows that the variations were statistically significant.

Fertilizer treatment was found, as in the alkaline test, essentially without effect on the results.

Sex variations (Table IV) proved to be similar

to those found for the alkaline test, that is, without significance when all varieties are considered as a whole. In the case, however, of the individual varieties, Roumanian No. 2 and Chinese, differences between responses of males and females were, respectively, 3.8 and 3.7 times the probable error. This difference is probably not due to chance variation, the odds being approximately 95 to 1. Since these rather striking differences are balanced by opposite differences in the other varieties the question of sex effect can scarcely be considered settled but it is plain that any real difference must be small.

Table IV.—Results Showing the Male and Female Plants Separated for Their Response to the Beam Tests

Variety	Number Plants	Test Intensity in Per Cent					Mean	P. E.	Difference P. E.	
		0	1	2	3	4			Male	Female
Alkaline Test										
<i>Males</i>										
Roumanian No. 1	55		1.8	10.9	74.5	12.7	2.98 ± 0.05			
Roumanian No. 2	50			10.0	68.0	22.0	3.12 ± 0.05			
Roumanian No. 3	47		2.1	6.4	80.8	10.6	3.00 ± 0.05			
Italian	66		3.0	18.2	63.6	15.2	2.91 ± 0.06			
Manchurian	71	35.2	36.6	25.4	2.8		0.96 ± 0.07			
Chinese	61	34.4	52.5	9.8	1.6	1.6	0.84 ± 0.07			
<i>Females</i>										
Roumanian No. 1	89		3.4	6.7	64.0	24.7	1.1	3.13 ± 0.05	-0.15 ± 0.07	
Roumanian No. 2	94			8.5	55.3	31.9	4.3	3.32 ± 0.05	-0.20 ± 0.07	
Roumanian No. 3	97			8.2	67.0	22.7	2.1	3.19 ± 0.04	-0.19 ± 0.06	
Italian	78		3.8	23.1	71.8	1.3		2.71 ± 0.04	0.20 ± 0.07	
Manchurian	73	32.9	53.4	9.6	4.1			0.85 ± 0.06	0.11 ± 0.09	
Chinese	83	45.8	41.0	9.6	3.6			0.71 ± 0.07	0.13 ± 0.10	
Acid Test										
<i>Males</i>										
Roumanian No. 1	55	43.6	49.1	7.3				0.64 ± 0.06		
Roumanian No. 2	50	44.0	52.0	4.0				0.60 ± 0.05		
Roumanian No. 3	47	42.6	51.1	6.4				0.64 ± 0.06		
Italian	66	37.9	57.6	3.0	1.5			0.68 ± 0.05		
Manchurian	71	32.4	43.7	23.9				0.92 ± 0.06		
Chinese	61	23.0	59.0	18.0				0.95 ± 0.06		
<i>Females</i>										
Roumanian No. 1	89	30.3	50.6	19.1				0.89 ± 0.05	-0.25 ± 0.08	
Roumanian No. 2	94	29.8	58.5	10.6	1.1			0.83 ± 0.04	-0.23 ± 0.06	
Roumanian No. 3	97	36.1	54.6	9.3				0.74 ± 0.04	-0.10 ± 0.07	
Italian	78	50.0	46.2	3.8				0.54 ± 0.04	0.14 ± 0.06	
Manchurian	73	35.6	49.3	12.3	2.7			0.82 ± 0.06	0.10 ± 0.08	
Chinese	83	38.6	54.2	7.2				0.69 ± 0.04	0.26 ± 0.07	

CONCLUSIONS

The experiments herein described lead to the following conclusions:

1. Different agronomic varieties of Cannabis vary markedly in chemical makeup of resin as evidenced by wide differences in response to the alkaline Beam test.
2. Treatment of the soil with various fertilizers alone and in combination is without effect on alkaline Beam test response under the conditions studied.
3. Male and female plants respond essentially alike to the alkaline Beam test.
4. Intensity of response to the alkaline Beam test tends to increase with age of plants at least until the time of flowering.
5. Intensity of response to the acid Beam test was not definitely influenced by varying variety, fertilizer, sex or age of plants except that there was observed a statistically significant diminution in intensity after the plants had flowered.

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Polyploidy in Relation to Chemical Analysis

By Wm. J. Bonisteel*

Polyploidy is present in nearly one-half of the flowering plants. Economic plants that are polyloid in nature include apples, wheat, oats, corn, tomatoes, tobacco (1) and many other plants that are of horticultural interest. Polyploids are organisms that have more than two sets of homologous chromosomes. Cytological and genetical studies show that interspecific hybridization, chromosome duplication and meiotic aberrations have played an important part in the development of new and improved forms of plant life. The function that polyploidy exerts in the chemical constituents in the realm of drug plants seems to have been largely overlooked up to the present time.

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By the use of chemical substances it is possible to induce polyploidy in certain plants (2). Sterile triploids may have their chromosomes doubled and thus become fertile plants. Polyploid plants are generally characterized by larger size, vigor, hardiness and larger cells.

Polyploidy and Plant Constituents.—Plant breeding means the production and maintenance of new types through the direction and control of the natural processes of fertilization (3). Digitalis may be cited as a type that is carefully selected and bred for uniform constituents. That polyploidy induces chemical changes in the cellular constituents of plants has been shown by Crane and Zilva (1933) in their studies on the vitamin C content of tetraploid tomatoes which is approximately double that in the diploid plant from which they were derived. In the apple considerable variation is found in the vitamin C content between diploid and triploid varieties. Mangelsdorf and Fraps (4) have shown the effectiveness of genic changes in the production of the nutritional biochemical vitamin A when suitable pollinations are made in corn. Each gene for yellow induced approximately 2.5 units of vitamin A per Gm. of seed and they were able to increase the vitamin A content from 0.05 unit to as high as 5.00 units per Gm. Beasley (5) found that in tetraploid cottons doubling the chromosome number gave octoploids and the fiber length was increased. Such results show that a definite quantitative relationship exists between the genetic factors in a plant and the chemical substance produced.

Polyploidy and Toxicity in the Genus Aconitum.—In a previous paper (6) Sparks Aconite was investigated cytologically and this clone was found to have two somatic chromosome numbers. The majority of the root tips have the triploid ($3n = 24$) chromosome number but sectors were found in a few root tips which had the hexaploid ($6n = 48$) chromosome number. Thus Sparks Aconite is a member of the polyloid series of aconites. Preliminary tests indicate that this clone was toxic. It is to be noted that if a sector with 48 chromosomes reached a growing point a new type of plant would result. Since Sparks Aconite is a sterile clone and has never been known to produce seeds in nature or under adequately controlled hybridization pollinations such a 48 chromosomed plant would be fertile and produce viable seeds. Whether such a plant would be toxic or not is undetermined at the present time. Experiments now being conducted show that aconites can be induced to double their chromosomes by suitable treatment with colchicine. Treated plants show the characteristic stunted growth, thick heavy leaves and swellings indicative of chromosome doubling as a result of colchicine treatment.

A study was made of the chromosome numbers of several types of aconites and this was compared with the published chemical analysis and the assumed