# Marihuana Investigations. III

## The Effect of Region of Growth of Hemp on Response to the Acid and Alkaline Beam Tests

*By B. B. Robinson* and J. R. Matchett†*

### INTRODUCTION

Previous articles (1) of this series have pointed out among other findings that: (1) individual hemp (*Cannabis sativa*) plants, though of the same commercial source of seed and grown side by side, vary widely in response to the acid and alkaline Beam tests; (2) different agronomic varieties of Cannabis vary widely in number and intensity of positive responses; (3) intensity of response increases somewhat with advancing age of plants; (4) sex of plants has probably little bearing on number or intensity of responses; (5) varying fertilizer treatments of the soil have no significant influence on the number or intensity of responses.

It has been shown that neither the alkaline nor the acid Beam tests offer promise as quantitative measures of physiological potency (2). Neither test has been proved adequate as a criterion of the presence or absence of marihuana in the qualitative sense since many specimens respond to neither. Nevertheless the varying responses to the tests definitely indicate variations in the chemical make-up of the resin elaborated by the plant and are valid measures, within obvious limits, of the effects produced by the conditions studied. In the case of the alkaline test the resin constituent measured appears to be cannabidiol recently isolated by Adams (4) and his co-workers. In the case of the acid test the responsive constituent appears to be a naturally occurring combination of an unidentified aldehyde with one or more resin constituents (2).

The purpose of the present research was to study the resin content and Beam test response of plants grown under conditions differing as widely as possible with respect to rainfall, temperature, light and elevation. It will be shown that dry climate is believed to enhance response to the alkaline test but that this influence is small as compared to that of agronomic variety. In the case of the acid test it will be shown that different locations do not produce differences that are significant except when considered with dates of harvest and varieties. Results are presented to show that the resins vary in quantity with age of plant, agronomic variety and regional environment.

### EXPERIMENTAL

#### METHODS OF TESTING

The separate hemp studies were conducted as similarly as possible except that the cultural experiments were carried out at four widely separated locations accessible for the work. The particular locations[1] were: Arlington Experimental Farm, Arlington, Virgina; Delta Experiment Station, Stoneville, Mississippi; Nebraska Experiment Substation, North Platte, Nebraska; and Wisconsin Agricultural Experiment Station, Madison, Wisconsin.

The Nebraska location represented a dry semi-arid region with an elevation of approximately 3000 feet. The rainfall during the growing season at the Nebraska station was equal to the rainfall at the Wisconsin station but the supply of stored moisture in the soil must have been materially less as may be observed by studying Table I. The regional studies in Virgina, Wisconsin and Mississippi were all under so-called humid conditions and below 1000 feet in elevation. The growth of the hemp was probably influenced to some extent by differences in photoperiodism as the Mississippi experiment was located approximately 675 miles south of the Wisconsin experiment. Differences in photoperiodism are known to influence the growth of hemp.

Seed of four single plant selections representing four strains or varieties of hemp were used in the experiments. The selections represented varieties or strains of different origin, Manchurian "A," a selection from seed of Manchurian origin which had been bred and selected for a low alkaline Beam reaction, Manchurian "B," a selection which had been bred and selected similar to "A" but not with an equal result, African"C," a selection from seed received from Tunisia, Africa, and bred and selected for low alkaline Beam response, and Kentucky "D," a single plant selection not selected in any way for the Beam test response. The Kentucky variety from which the selection was made represents a variety which had been grown in the United States for many years but originally was of Eastern Asiatic origin.

* Agronomist of the U. S. Department of Agriculture, Division of Cotton and Other Fiber Crops and Diseases.

† Chemist of the U. S. Treasury Department, Bureau of Narcotics.

[1] The writers wish to express their appreciation to Supt. H. C. McNamara, Stoneville, Mississippi; Supt. L. L. Zook, North Platte, Nebraska; and Prof. A. H. Wright, Madison, Wisconsin, for their coöperation in conducting the cultural tests.

Table I.—Climatological, Cultural and Dates of Sampling Data Pertaining to the Regional Experimental Tests

| Period | Temperature—Mean Degrees, F. | | | | Precipitation—Number, Inches | | | |
|---|---|---|---|---|---|---|---|---|
| | Wisconsin | Nebraska | Virginia | Mississippi | Wisconsin | Nebraska | Virginia | Mississippi |
| Sept. 1938 | 62.0 | 66.0 | 67.4 | 76.2 | 10.29 | 4.56 | 4.27 | 0.26 |
| Oct. 1938 | 55.8 | 58.7 | 58.8 | 66.6 | 0.76 | 0.03 | 1.15 | 1.27 |
| Nov. 1938 | 37.8 | 35.3 | 49.8 | 53.2 | 2.16 | 0.23 | 2.60 | 2.56 |
| Dec. 1938 | 24.2 | 30.0 | 38.4 | 46.6 | 0.89 | 0.16 | 2.69 | 3.38 |
| Jan. 1939 | 26.2 | 32.6 | 37.8 | 49.1 | 1.95 | 0.70 | 3.41 | 12.24 |
| Feb. 1939 | 19.5 | 23.3 | 42.3 | 49.8 | 1.75 | 0.15 | 5.71 | 5.69 |
| Mar. 1939 | 33.0 | 40.5 | 46.4 | 58.2 | 1.25 | 0.88 | 2.89 | 4.17 |
| Apr. 1939 | 42.8 | 49.2 | 53.4 | 61.2 | 3.16 | 1.30 | 3.78 | 5.29 |
| May 1939 | 62.4 | 65.2 | 68.0 | 71.3 | 1.64 | 2.86 | 0.41 | 3.17 |
| June 1939 | 69.8 | 69.6 | 75.4 | 79.7 | 2.33 | 3.85 | 4.55 | 5.61 |
| July 1939 | 74.8 | 79.9 | 76.2 | 82.5 | 1.64 | 0.14 | 2.01 | 1.97 |
| Aug. 1939 | 71.3 | 73.4 | 78.8 | 84.4 | 2.61 | 1.53 | 3.22 | 0.51 |
| Total year | | | | | 30.43 | 16.39 | 36.69 | 46.12 |
| Crop season | 72.3 | 71.1 | 73.5 | 70.6 | 5.54 | 7.97 | 8.30 | 18.47 |
| Elevation—approx. feet | 974 | 3000 | 25 | 127 | | | | |
| Crop season | | | | | | | | |
| No. days long | 86 | 123 | 113 | 134 | | | | |
| Date planted | May 28 | Apr. 24 | Apr. 25 | Mar. 9 | | | | |
| Date 1st sample | June 28 | June 13 | May 29 | Apr. 20 | | | | |
| Date 2nd sample | July 21 | July 10 | July 3 | May 18 | | | | |
| Date 3rd sample | Aug. 10 & 21 | Aug. 9 & 24 | Aug. 15 | July 20 | | | | |

The seed of each selection was divided into four lots, distributed to the four experiment stations and planted in four separate replicated randomized experimental plots. Thus, at each location there were four varieties, each planted in four replicated plots. By using the seed of single plant selections the inheritance or genetic make-up of the plants grown for a single variety at the different stations were as similar as possible. This might not have been necessary if there were available any varieties of hemp which might be considered genetically pure but it is not believed that any hemp breeding has progressed to the point that varieties are available with a genetic purity equal to that found in certain other crops as, for example, cereals.

On three occasions during the growth of the plants, vegetative samples were collected for testing. On the first occasion the plants had not made a large growth so five separate entire plants from each plot were taken. At the second and third sampling the tops were cut from five plants from each plot. At the first two samplings the sex of the plants was indeterminable but at the time of the third sampling the sex was evident. This permitted the third harvest to consist of three female tops and two male tops to represent each plot.

The planting and sampling dates are given in Table I which shows variations due to regional climatic differences. However, in planning the work an effort was made to have similarity of cultural and sampling technique. The first samples were taken 4 to 5 weeks after planting and the second samples collected about 8 weeks after planting. The third sampling was planned to correspond to a definite stage of the plants' growth, *i. e.*, the time the male plants were flowering and shedding pollen. The African "C" and Kentucky "D" were later in maturing than the Manchurian selections and this was so marked that at two of the stations separate days were used in the collection of the third samples.

All of the samples collected were air-dried and then forwarded to Washington, D. C., where weighed samples of 0.5 Gm. were prepared of each plant for testing by the Beam tests. The Beam tests were made in the laboratory of the Internal Revenue Bureau, Philadelphia, Pennsylvania. The methods used and the recording of results were similar to those described in an earlier paper (2). Separate weighed samples of 0.25 Gm. were prepared of each plant. Twenty of these samples representing the same variety at each location and at each period of harvest were bulked together and petroleum ether extractions were made upon them in the laboratory of the Internal Revenue Bureau,[2] Kansas City, Missouri. The following procedure was used:

1. Dry at 65° C. to 70° C. for 12 hours.
2. Weigh a 5-Gm. sample of dry material.
3. Extract with petroleum ether in Soxhlet apparatus for 12 hours.
4. Remove the solvent on a steam bath before a fan.
5. Heat at 100° C. for 15 or 20 minutes to remove moisture.
6. Cool and weigh the residue.
7. Calculate the per cent resin.

The methods of planting, sampling and testing allowed a statistical investigation of the results to determine the significance of differences which were

[2] The writers express their appreciation to Mr. A. J. Mottern and Mr. L. E. Dale, chemists in charge, respectively, of the laboratories at Philadelphia and Kansas City for their assistance in performing these tests.

obtained. The statistical method applied was the "analysis of variance."[3]

## RESULTS

The climatological observations at the four experiment stations are shown in Table I. This table further includes data regarding the dates of planting and dates of sampling. Although the differences in mean temperature are very slight during the growing season at the different stations the growth differences were marked as may be partly shown by the data in Table I which shows the number of days elapsing between planting and the third harvest or time of maturing of the males. This represented only 86 days in Wisconsin, the most northerly station, compared with 134 days in Mississippi, the most southerly station. The same degree of growth required about 123 days in Nebraska which is long, partly due to dry conditions at the time of planting which required nearly two weeks to elaspse before the seedlings emerged.

It is difficult to correlate climatological observations with response to the Beam tests in the present experiments but the regional results lend themselves well for statistical interpretation with other variable factors.

Table II.—Actual Results Obtained in Response to the Alkaline Beam Test. Each Individual Entry in the Table under a State Is the Total Numerical Response of Five Separate Plant Tests and Represents a Single Plot of a Variety at a Location for a Particular Test Period

| Variety | Virginia | | | | Wisconsin | | | | Nebraska | | | | Mississippi | | | | Variety Sub-total | Variety Grand Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *First Sampling* | | | | | | | | | | | | | | | | | | |
| Manchurian "A" | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 2 | 2 | 3 | 0 | 0 | 0 | 0 | 9 | |
| Manchurian "B" | 0 | 0 | 0 | 0 | 0 | 5 | 1 | 0 | 4 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 16 | |
| African "C" | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 6 | |
| Kentucky "D" | 8 | 6 | 3 | 4 | 9 | 4 | 9 | 8 | 8 | 14 | 5 | 10 | 3 | 4 | 4 | 2 | 101 | |
| State subtotals | | 21 | | | | 40 | | | | 58 | | | | 13 | | | 132 | |
| *Second Sampling* | | | | | | | | | | | | | | | | | | |
| Manchurian "A" | 1 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 6 | 1 | 3 | 1 | 0 | 0 | 0 | 0 | 15 | |
| Manchurian "B" | 3 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 3 | 5 | 3 | 4 | 0 | 0 | 0 | 0 | 21 | |
| African "C" | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 2 | 0 | 2 | 1 | 0 | 0 | 1 | 10 | |
| Kentucky "D" | 17 | 8 | 8 | 10 | 7 | 3 | 5 | 6 | 15 | 12 | 9 | 13 | 8 | 9 | 11 | 6 | 147 | |
| State subtotals | | 50 | | | | 25 | | | | 82 | | | | 36 | | | 193 | |
| *Third Sampling* | | | | | | | | | | | | | | | | | | |
| Manchurian "A" | 0 | 0 | 0 | 2 | 2 | 0 | 2 | 3 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 10 | 34 |
| Manchurian "B" | 2 | 0 | 2 | 0 | 1 | 2 | 3 | 0 | 1 | 0 | 0 | 0 | 5 | 0 | 2 | 6 | 24 | 61 |
| African "C" | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 3 | 3 | 3 | 5 | 1 | 0 | 0 | 1 | 1 | 18 | 34 |
| Kentucky "D" | 15 | 12 | 7 | 17 | 9 | 7 | 9 | 9 | 16 | 9 | 14 | 10 | 14 | 11 | 16 | 6 | 181 | 429 |
| State subtotals | | 57 | | | | 51 | | | | 63 | | | | 62 | | | 233 | |
| STATE GRAND TOTALS | | 128 | | | | 116 | | | | 203 | | | | 111 | | | | |

Table III.—Actual Results Obtained in Response to the Acid Beam Test. Each Individual Entry in the Table under a State Is the Total Numerical Response of Five Separate Plant Tests and Represents a Single Plot of a Variety at a Location for a Particular Test Period

| Variety | Virginia | | | | Wisconsin | | | | Nebraska | | | | Mississippi | | | | Variety Sub-total | Variety Grand Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *First Sampling* | | | | | | | | | | | | | | | | | | |
| Manchurian "A" | 0 | 4 | 1 | 5 | 5 | 5 | 5 | 0 | 5 | 1 | 4 | 5 | 0 | 0 | 0 | 0 | 40 | |
| Manchurian "B" | 0 | 0 | 0 | 3 | 1 | 5 | 3 | 1 | 3 | 0 | 2 | 3 | 0 | 0 | 0 | 0 | 21 | |
| African "C" | 2 | 1 | 3 | 3 | 3 | 2 | 1 | 3 | 5 | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 29 | |
| Kentucky "D" | 0 | 1 | 0 | 4 | 1 | 5 | 5 | 4 | 0 | 3 | 3 | 2 | 0 | 0 | 0 | 0 | 28 | |
| State subtotals | | 27 | | | | 49 | | | | 42 | | | | 0 | | | 118 | |
| *Second Sampling* | | | | | | | | | | | | | | | | | | |
| Manchurian "A" | 3 | 1 | 2 | 3 | 6 | 2 | 4 | 4 | 5 | 1 | 3 | 5 | 2 | 1 | 5 | 5 | 52 | |
| Manchurian "B" | 1 | 0 | 2 | 0 | 0 | 1 | 1 | 3 | 4 | 5 | 4 | 4 | 1 | 2 | 3 | 2 | 33 | |
| African "C" | 4 | 3 | 5 | 0 | 10 | 5 | 6 | 8 | 10 | 6 | 7 | 7 | 5 | 8 | 5 | 9 | 98 | |
| Kentucky "D" | 3 | 3 | 5 | 1 | 4 | 3 | 4 | 4 | 2 | 5 | 2 | 4 | 1 | 3 | 1 | 5 | 50 | |
| State subtotals | | 36 | | | | 65 | | | | 74 | | | | 58 | | | 233 | |
| *Third Sampling* | | | | | | | | | | | | | | | | | | |
| Manchurian "A" | 6 | 6 | 8 | 11 | 6 | 7 | 6 | 5 | 7 | 6 | 5 | 5 | 5 | 4 | 9 | 6 | 102 | 194 |
| Manchurian "B" | 5 | 11 | 9 | 5 | 6 | 5 | 6 | 5 | 9 | 5 | 8 | 7 | 6 | 3 | 1 | 1 | 92 | 146 |
| African "C" | 8 | 11 | 13 | 8 | 10 | 10 | 9 | 11 | 14 | 13 | 12 | 13 | 14 | 14 | 18 | 20 | 198 | 325 |
| Kentucky "D" | 6 | 8 | 11 | 2 | 8 | 6 | 4 | 5 | 3 | 2 | 5 | 3 | 7 | 10 | 5 | 17 | 102 | 180 |
| State subtotals | | 128 | | | | 109 | | | | 117 | | | | 140 | | | 494 | |
| STATE GRAND TOTALS | | 191 | | | | 223 | | | | 233 | | | | 198 | | | | |

Tables II and III show the actual results obtained at the four stations upon the four varieties at the three times of sampling. Previous results as mentioned in the introduction have shown the fact that sex plays little part in the response to the Beam tests. However, the results obtained at the third sampling on the two sexes, which are grouped together in Tables II and III, were separated to con-

[3] The writers express their appreciation for suggestions made by Dr. O. A. Pope, U. S. D A., Washington, D. C.

firm or disprove earlier conclusions, and the discussion is presented at another place in this article.

Tables IV and VI contain the results of the analysis of variance of some of the more important relations.

Table IV.—Computations Obtained in Calculating the Analysis of Variance of the Regional Marihuana Studies in 1939

| Variance | D/F | Alkaline Beam Test Mean Squares | Alkaline Beam Test F Found | Alkaline Beam Test F For Odds 99:1 | Acid Beam Test Mean Squares | Acid Beam Test F Found | Acid Beam Test F For Odds 99:1 |
|---|---|---|---|---|---|---|---|
| *Whole Plots* | | | | | | | |
| Varieties | 3 | 779.39 | 160.76 | 4.38 | 128.27 | 29.06 | 4.38 |
| Locations | 3 | 38.39 | 7.92 | 4.38 | 8.31 | 1.88 | 4.38 |
| Var. x location | 9 | 7.36 | 1.52 | 2.94 | 12.98 | 2.94 | 2.94 |
| Block within location | 12 | 5.07 | 1.04 | 2.72 | 4.99 | 1.13 | 2.72 |
| Error (*a*) | 36 | 4.84 | | | 4.41 | | |
| *Split Plots* | | | | | | | |
| Dates | 2 | 40.42 | 19.17 | 4.92 | 580.01 | 217.90 | 4.92 |
| Var. x dates | 6 | 21.44 | 10.17 | 3.07 | 39.79 | 14.95 | 3.07 |
| Location x dates | 6 | 10.14 | 4.81 | 3.07 | 24.46 | 9.19 | 3.07 |
| Var. x loc. x dates | 18 | 6.14 | 2.91 | 2.28 | 9.65 | 3.62 | 2.28 |
| Dates x block within loc. | 24 | 2.51 | 1.19 | 2.07 | 4.68 | 1.76 | 2.07 |
| Error (*b*) | 72 | 2.11 | | | 2.66 | | |
| Total | 191 | 17.64 | | | 14.49 | | |

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

| Variety | Sex | Mean | P. E. | Sex | Mean | P. E. | Difference Male | Difference Female |
|---|---|---|---|---|---|---|---|---|
| | | | | Acid Tests | | | | |
| Manchurian "A" | Male | 1.50 | ± 0.07 | Female | 1.13 | ± 0.05 | 0.37 | ± 0.09 |
| Manchurian "B" | Male | 1.28 | ± 0.07 | Female | 1.06 | ± 0.06 | 0.22 | ± 0.09 |
| African "C" | Male | 2.59 | ± 0.09 | Female | 2.38 | ± 0.08 | 0.21 | ± 0.12 |
| Kentucky "D" | Male | 1.38 | ± 0.12 | Female | 1.19 | ± 0.10 | 0.19 | ± 0.16 |
| | | | | Alkaline Tests | | | | |
| Manchurian "A" | Male | 0.21 | | Female | 0.00 | | 0.21 | |
| Manchurian "B" | Male | 0.31 | | Female | 0.19 | | 0.11 | |
| African "C" | Male | 0.27 | | Female | 0.10 | | 0.17 | |
| Kentucky "D" | Male | 2.44 | ± 0.17 | Female | 2.19 | ± 0.14 | 0.25 | ± 0.22 |

## DISCUSSION OF RESULTS

*Influence of Region.*—Of the four regions studied, three representing humid conditions yielded total influences somewhat similar with the alkaline test. The small differences which exist in regard to region between the "test response totals" for Virginia (128), Wisconsin (116) and Mississippi (111) are not statistically significant but each is significantly different from that obtained in Nebraska (203).

The differences between locations as observed by the acid test are not as great as those by the alkaline test. The greatest difference is between Nebraska (233) and Virginia (191) and the analysis shows that this difference barely gives odds of 19:1 of significance. It is, however, interesting to observe that Nebraska gives the highest value as it did also with the alkaline test.

As fertilizers have been shown to have little or no effect on the response of hemp to either the alkaline or acid Beam tests, the results obtained from the different regions may be considered as having been influenced more by climatic differences than by differences in soil. It is apparent that the soil at all locations could not be similar, and although differences hitherto unaccounted for may have been present in these experiments, it is reasonable to suppose that the greatest variations have been due to climate. Nebraska, the driest and highest in elevation, produced the strongest reactions and Mississippi, with little elevation and the most humid climate, produced the weakest Beam reactions. It would be well if these results could be substantiated by further investigations.

*Influence of Varieties.*—Varieties apparently produce greater differences and influences on the response to the acid or alkaline Beam tests than any other factor which has been studied. This will prove exceptionally fortunate from the breeding point of view if the chemical groups responsible for these tests may later be linked or correlated in some way with the physiologically active substance in Marihuana.

The most striking effect of varieties on the alkaline test results is the low intensities obtained with the varieties Manchurian "A" and "B" and African "C" in comparison with Kentucky "D." The average of the first three varieties is slightly less than 10 per cent of the Kentucky test. While the differences due to location are approximately twofold, they are with varieties tenfold. Mention has been made already that the first three varieties had been bred and selected especially for a low alkaline test, and that the Kentucky had not been selected for that factor.

The differences obtained with the alkaline test between any of the first three varieties in comparison with the Kentucky "D" are highly significant.

The difference between "A" or "C" and "B" are not sufficient to give odds of 19:1.

The acid "test totals" give an entirely different ranking of varieties in respect to their response. These results indicate the slight, if any, positive correlation between the two tests. The lowest ranking variety for the alkaline test, African "C," is by the acid test the highest. Its difference in comparison with any of the other three varieties or the mean of the other three is highly significant. The next greatest difference occurs between the two Manchurian selections, "A" and "B." This difference gives odds of more than 19:1 but not as much as 99:1 of being significant.

The importance of variety results presented above were substantiated for the alkaline test in an earlier investigation in which six varieties, five of which were different from the varieties used in this research, showed the most important difference was due to variety. It is to be noted, however, that varietal effect is markedly less in the acid test than in the alkaline test.

*Influence of Dates of Sampling.*—The alkaline test shows an increase in intensity with the age of the plant. The actual results are: first sampling, 132; second sampling, 193; and third sampling, 233. The differences between the first and third sampling and the difference between the first and second sampling yield odds of significance greater than 99:1 and the difference between the second and third harvest gives odds of at least 19:1.

The acid test yields an increase in intensity with the age of the plant, which is much stronger than the alkaline. The actual results are: first sampling, 118; second sampling, 233; and third sampling, 494. The differences between any two of these samplings are all highly significant, giving odds of more than 99:1.

These results are more striking and significant than those described in an earlier paper (1). In that investigation a significant decrease was obtained in the third harvest by the acid test after the second had increased over the first. It is believed that the response in general increases with age but occasionally factors may depress this reaction.

*Influence of Sex.*—Conclusions were reached in an earlier paper that if differences existed between male and female hemp plants with respect to variety and their response to the Beam tests they were small. In 1939 the sex was recorded only on plants harvested at the last sampling period. Varieties "A," "B" and "C" had such few plants that gave any response to the alkaline test that differences between males and females were not believed to be applicable to the common test of significance as measured by the probable errors. However, the probable errors of the means were determined for the variety "D" for the alkaline test and for the means of all the varieties for the acid test. These means and probable errors are given in Table V. The numbers of the populations involved in the means are small, *i. e.*, females 48 and males 32. It

Table VI.—Results Obtained by Petroleum Ether Extraction of Leaves of Varieties of Hemp Studied in the Regional Experiments, Together with the Variance and Covariance of the Resins and Acid Beam Test Numbers for Mississippi, Nebraska and Wisconsin

| | % Resins Extracted by Petroleum Ether Varieties | | | | Means by | |
|---|---|---|---|---|---|---|
| | Manchurian "A" | Manchurian "B" | African "C" | Kentucky "D" | Dates | Locations |
| Mississippi | | | | | | |
| 1st sampling Apr. 20 | 1.55 | 1.24 | 1.23 | 1.30 | 1.33 | |
| 2nd sampling May 18 | 1.95 | 1.79 | 2.80 | 2.23 | 2.19 | |
| 3rd sampling June 20 | 2.78 | 2.02 | 6.45 | 5.23 | 4.12 | 2.55 |
| Nebraska | | | | | | |
| 1st sampling June 13 | 1.80 | 1.75 | 2.86 | 1.29 | 1.92 | |
| 2nd sampling July 10 | 3.81 | 2.95 | 4.16 | 3.62 | 3.63 | |
| 3rd sampling Aug. 9 & 24 | 4.21 | 4.62 | 6.69 | 5.23 | 5.19 | 3.58 |
| Virginia | | | | | | |
| 1st sampling May 29 | | | | | | |
| 2nd sampling July 3 | 3.35 | 2.91 | 4.37 | 4.29 | 3.73 | |
| 3rd sampling Aug. 15 | 4.86 | 4.27 | 6.54 | 5.07 | 5.18 | |
| Wisconsin | | | | | | |
| 1st sampling June 28 | 2.28 | 2.23 | 2.77 | 2.21 | 2.37 | |
| 2nd sampling July 21 | 2.91 | 2.40 | 3.50 | 2.85 | 2.91 | |
| 3rd sampling Aug. 10 & 21 | 3.89 | 3.45 | 5.19 | 3.99 | 4.13 | 3.14 |
| Variety means | 3.03 | 2.69 | 4.23 | 3.39 | | |

| | Variance and Covariance Mississippi, Nebraska and Wisconsin Mean Squares | | | |
|---|---|---|---|---|
| | D/F | Resins | Acid No. | $r$ |
| Varieties | 3 | 3.5975[a] | 522.1851[a] | 0.959[a] |
| Locations | 2 | 3.2357[a] | 27.0833 | 0.987[a] |
| Varieties x locations | 6 | 0.2085 | 54.8240 | 0.760[b] |
| Harvests | 2 | 20.6094[a] | 1603.8333[a] | 0.999[a] |
| Harvests x varieties | 6 | 1.0125[b] | 180.8240[b] | 0.735[b] |
| Harvests x locations | 4 | 0.7222 | 114.6666 | 0.384 |
| Harvests x locations x varieties (error) | 12 | 0.2942 | 41.1296 | 0.733[a] |
| Total | 35 | 2.0636 | 205.5143 | 0.870[a] |

[a] Significant odds 99:1.
[b] Significant odds 19:1.

is not particularly surprising that small differences might occur but only in one case does the difference in results between males and females appear significant. This is the case of Manchurian "A" where the acid test produces odds of approximately 175:1 of significance, indicating the difference between males and females may be partly varietal. Although Table V shows results in which the males in every case were stronger than the females, previous results in 1938 were not as consistent in this respect. In fact, from six varieties tested only three had male plants stronger than the females. It is interesting to note that the male plants responded as strong if not stronger than the females. The general practice in the past of collecting the resins in Asia has been principally from the female plants.

It is difficult to conclude from these results that sex plays any important role in the response to either the alkaline or the acid test. Possibly small differences may exist and with a greater number of determinations future work may prove the small differences to be significant but from a practical point of view it seems doubtful if the differences which have been found here have any importance.

### ALKALINE VS. ACID TEST

Mention has been made earlier in this article that there existed little or no positive correlation between these two tests. This is true if the sexes are grouped together or the females segregated from the males. Coefficients of correlation as determined for plants between the alkaline and acid tests were obtained on hemp plants grown in 1938. None of these proved large, *i. e.*, $r = 0.17 \pm 0.08$ for female plants of a China variety and $0.26 \pm 0.08$ for male plants of the same variety. Even smaller coefficients were obtained for plants of a Roumanian and Italian varieties.

### QUANTITATIVE DIFFERENCES IN RESINS PRODUCED

Table VI shows the results obtained in extracting samples of leaves with petroleum ether. The results of the first sampling in Virginia are missing as the plants were too small at harvest to furnish material for petroleum ether extraction after the removal of material for the Beam tests. There is easily observed in Table VI the consistent increase in resins that takes place as the plants mature. Of the four varieties of hemp tested, African "C" gave approximately 57% more resins in extraction than Manchurian "B." Of the four regions studied, Nebraska gave approximately 40% more resins than Mississippi. The analysis of variance shows the marked influence that maturity, varieties and environment have on the production of the resins. The analysis of covariance presented in Table VI indicates the strong relationship between the resin results and the acid Beam test numbers which does not differ for different varieties, locations or harvests. No such marked relationship is indicated between the resin content and the alkaline Beam test response.

### CONCLUSIONS

It is concluded that the climatic conditions under which *Cannabis sativa* is grown influence response to the acid and alkaline Beam tests but that this effect is small as compared with that of agronomic variety.

The quantity of resins produced by plants is influenced by plant maturity, variety and environment. The relationship between the resin content and acid Beam test numbers is strong and the same for different varieties, different locations and different harvests.

### REFERENCES

(1) Wollner, *et. al.*, JOUR. A. PH. A., 27 (1938), 29–36.

(2) Matchett, *et. al.*, Scientific Edition, JOUR. A. PH. A., 29 (1940), 399–404.

(3) Unpublished observations, Bureau of Narcotics.

(4) Adams, *et al.*, *J. Am. Chem. Soc.*, 62 (1940), 196.

---

# The Clone in Pharmacognosy

*By William J. Bonisteel**

The concept of the genus (1) was the basis for a symposium at the joint meeting of the Botanical Society of America and the American Society of Plant Taxonomists held at Indianapolis, December 29, 1937. Students of plant life have been working for centuries upon the problems of classification, and while the accomplishment has been great, much remains to be done and in some groups a satisfactory system may never be attained. The problem of species has always been a difficult one. With intergrading characters present, it is possible to have almost as many species as there are individuals. Species splitting can be carried to such an extreme that only a specialist in that field would be able to identify the plant in question.

The sub-species and its various forms are a grouping that is used extensively. In the current U. S. P., this system is followed in many cases. Under the drug, aconite, both sub-species and varieties are used in the defi-

* From the Botanical Laboratory of the Biology Department, Fordham University, New York, N. Y.