DIURNAL VARIATION IN CAROTENE CONTENT IN PLANTS

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IN CAROTENE CONTENT OF PLANTS

By

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PREFACE

Periodicity seems to be a universal biological phenomenon, under the control of extrinsis or intrinsic factors. In the plant kingdom such specific factors include climate, ecological associations, and host-parasite or symbiotic relationships. A qualitative diurnal variation in the carotene content of several species of plants has recently been reported.

The economic importance of the amount of carotene contained in food plants is due to the fact that certain of the carotenoid pigments are precursors of vitamin A. The possibility that by selecting a suitable time of harvesting one might obtain a crop containing larger amounts of carotene invests such an observation with great agronomic importance.

The actual role of the carotenoids in photosynthesis has not been completely established, although there is some experimental evidence that the carotenoids act as receptors for light energy, converting it, as does chlorophyll, into energy suitable for the reduction of carbon dioxide.

These theoretical and practical considerations prompted a more quantitative survey of several species of food and forage plants and it is with such a survey that this study is concerned.

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INTRODUCTION

In 1946 Roberts (1) reported a series of experiments planned to evaluate the possible relation between pigment content of various plants during the daily dark period and blossom induction. A prominant feature of his data is the pronounced diurnal cycle of carotene content in many species. Roberts also indicated that the pigment content is affected by such cultural conditions as photoperiod and temperature. These interesting observations are somewhat open to question because of the small size (0.5 gram) of the samples used, the failure to correct for varying moisture content of the tissue, and the qualitative nature of his analytical procedure which consisted of development of the pigment bands on a chromatographic column and measuring the band widths with a vernier caliper.

Zafren (2), in connection with the problem of preserving the maximum carotene content in hay, made a study of the content of that pigment in the leaves at different times of day. He found that during times of most intense illumination the carotene content was lower than at early morning and late evening. Growing leaves shielded from the sun for a few hours showed little change in carotene content. Cut leaves kept in the dark for the same length of time retained a greater portion than similar leaves kept in the light. The significance of Zafren's observations is not as great as might otherwise be the case because of the extremely limited amount of data presented.

Neither of these workers was able to attribute this variation to any one cultural or environmental factor, although Roberts (1) suggested that production of floral primordia was involved.

Bernstein, et al., (9) noted that samples of turnip greens collected in the afternoon had a lower carotene content than samples collected in the morning.

An extended search of the literature has failed to disclose any further reference to this particular phenomenon. Periodicity in other phases of plant physiology, however, has been observed by several workers: photosynthesis, (3) and (4); respiration, (5); dry-matter, (6); and transpiration, (7).

The series of experiments discussed herein constitutes an attempt to confirm the phenomenon observed by Roberts (1), Zafren (2), and Bernstein (9) and to place it on a quantitative basis. It was hoped that some information could be acquired which would point to the controlling factor or factors in the cylic variation in the carotene content of the plants studied.

EXPERIMENTAL

I. Materials

Leaves from both monocotyledenous and dicotyledenous plants were used. The monocotyledenous plants included Triticum aestivum, L., var. Tenmarq (winter wheat) seedlings, grown under normal greenhouse conditions in mid-winter of 1948-49; Triticum aestivum, L., var. Clarkan (spring wheat) and Avena sativa, L., var. Wintok (oat) seedlings, grown under normal greenhouse conditions in the spring of 1949; and Holcus sudanensis, L., (Sudan grass), grown under field conditions in well-fertilized Kirkland silty clay loam in the summers of 1947 and 1948, and sampled while still in a vegetative state.

The dicotyledenous plants consisted of Brassica Rapa, L., var. Seventop (turnip greens) and Beta vulgaris, L., var. Cicla (Swiss chard) both at midgrowth and grown under field conditions on well-fertilized Kirkland silty clay loam in the late spring and early summers of 1947 and 1948; and Ipomoea Batatas, Lam., (sweet-potato) cuttings, grown in sand in open cold-frames in the summer of 1948. The plots and greenhouse were at Stillwater, Oklahoma.

II. Methods

Sampling. Except in the case of the greenhouse experiments, leaves were selected from the entire plot, care being taken to obtain insofar as possible leaves of uniform size and stage of development. In the greenhouse experiments, a

swath of grass large enough to provide sufficient tissue for the sample was cut. The tissue after harvesting was either extracted at once or quick-frozen. In the latter case the samples were left in frozen storage at -14 degrees centigrade until all samples had been collected. The procedure was uniform within each experiment.

The turnip green sample consisted of medium sized, fully developed leaves in which the midrib had been removed before chopping. The Swiss chard was sampled by taking the smallest fully developed leaves and dissecting out the midrib. The third or fourth leaf from the apex of the sweet-potato plant was selected and the petiole removed. The entire leaf blade was used in all the cereal grasses. The Sudan grass leaves sampled were the third from the apex of the plant. The trimmed tissue was finely chopped with shears, mixed well, and 5 to 10 gram representative samples taken in duplicate for carotene determination and a 10 to 25 gram sample taken for dry-matter.

Carotene. The method of Wall and Kelley (8) modified as follows was used to determine the carotene content of the tissue:

The samples were extracted with about 100 ml. of a mixture of two parts 95 percent ethanol and one part petroleum ether (Skellysolve B) until completely homogenized in a Waring blendor. The mixture was filtered and the residue washed with extraction reagent until the washings were free of color. The filtered extract was frequently stored under refrigeration until several samples had been extracted. Phasic separation was achieved by adding water; the aqueous layer was then removed and extracted

three times with 25 ml. portions of petroleum ether. The combined ether layers were well washed with water. The ether solutions were then reduced to small volume on a steam plate and run through a chromatographic adsorption column containing a mixture of 75 percent diatomaceous earth (Johns-Manville "Hyflo-Supercel") and 25 percent activated magnesium oxide (Westvaco Chlorine Products Co.). The chromatogram was developed and the alpha-, beta-, and gamma-carotene eluted with a three percent solution of acetone in petroleum ether. The carotene contained in the eluate was determined photo-colorimetrically using the Evelyn instrument at a wave-length of 440 millimicrons. The values were obtained by comparison with a standard carotene solution containing 90 percent beta- and 10 percent alpha-carotene.

Dry-matter. The samples were placed in evaporating dishes and dried to constant weight in a thermostatically controlled electric oven at 100 degrees centigrade.

Climatic Data. The climatic data used in experiment III was collected by the author with a Julien P. Friez recording hygro-thermograph located adjacent to the experimental plot and from an Eppley pyrheliometer connected to a Leeds and Northrup Micromax automatic recorder located on the roof of a three-story building about three blocks north of the plot. Unfortunately, the means for collecting this type of data did not become available until the spring of 1949.

Shading. The sweet-potatoes used for the shading experiments were growing in cold-frames whose sides were of sufficient

height to support coverings. The partial shading was accomplished by spreading a double thickness of cheesecloth over the required area. Full shading was provided by a khaki-colored wool blanket spread in a like manner. The plants were exposed only long enough to allow sampling.

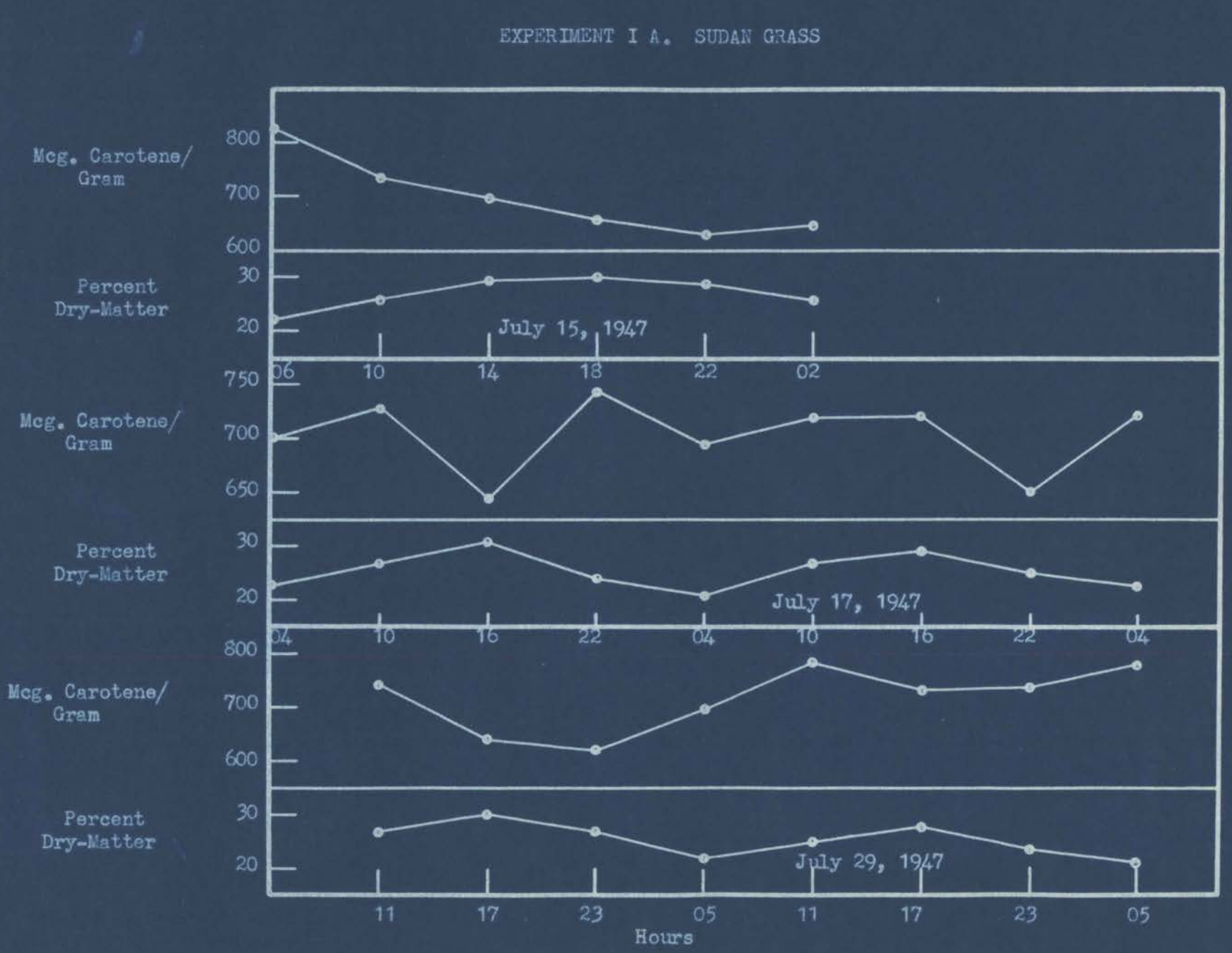
EXPERIMENT I A. SUDAN GRASS
Mcg. Carotene/ Gram
800
700
600
Percent Dry-Matter
30
20
July 15, 1947
06
10
14
18
22
02
Mcg. Carotene/ Gram
750
700
650
Percent Dry-Matter
30
20
July 17, 1947
04
10
16
22
04
10
16
22
04
Mcg. Carotene/ Gram
800
700
600
Percent Dry-Matter
30
20
July 29, 1947
11
17
23
05
11
17
23
05
Hours

Figure 1

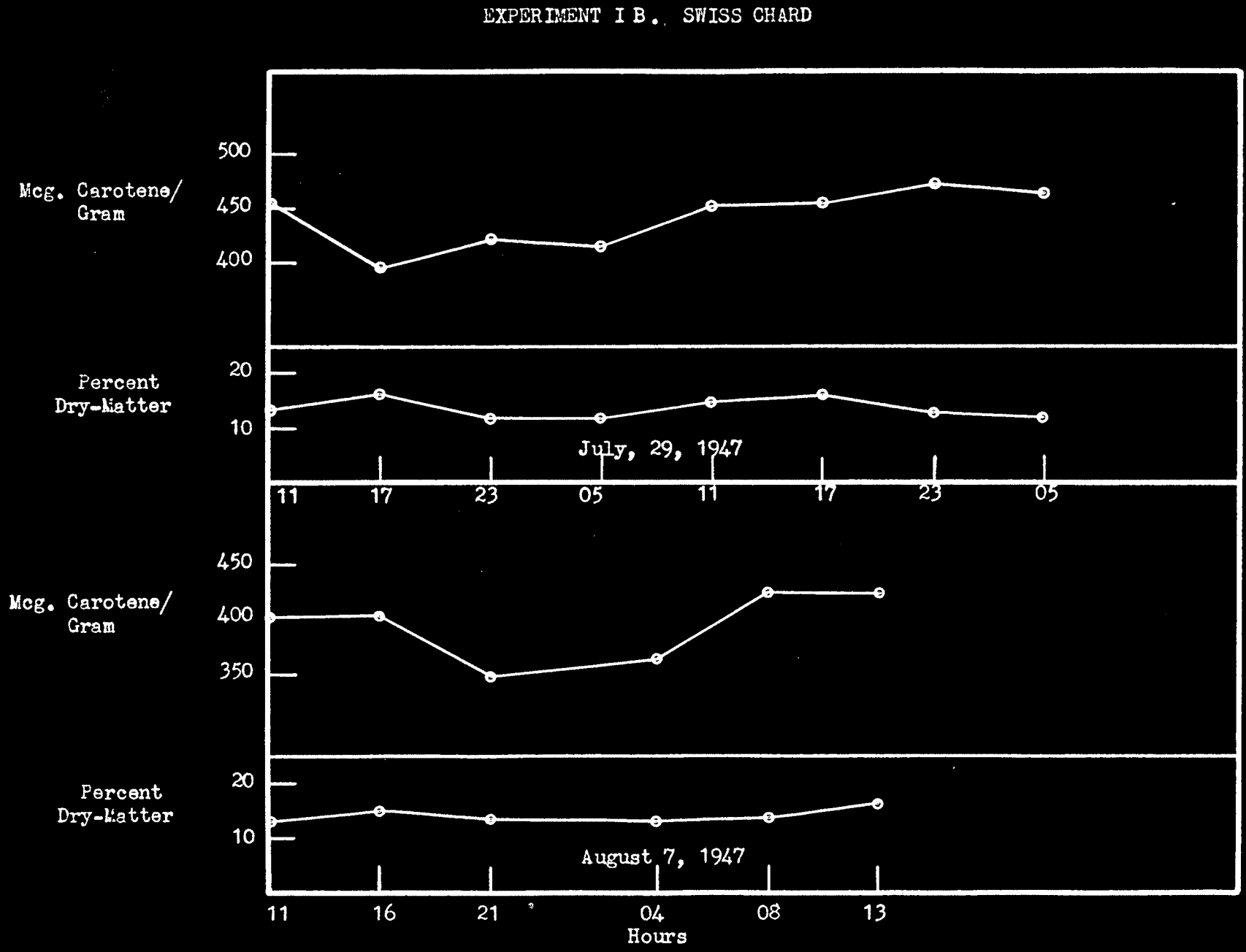
EXPERIMENT I B. SWISS CHARD
Mcg. Carotene/ Gram
500
450
400
Percent Dry-Matter
20
10
July, 29, 1947
11
17
23
05
11
17
23
05
Mcg. Carotene/ Gram
450
400
350
Percent Dry-Matter
20
10
August 7, 1947
11
16
21
04
08
13
Hours

Figure 2

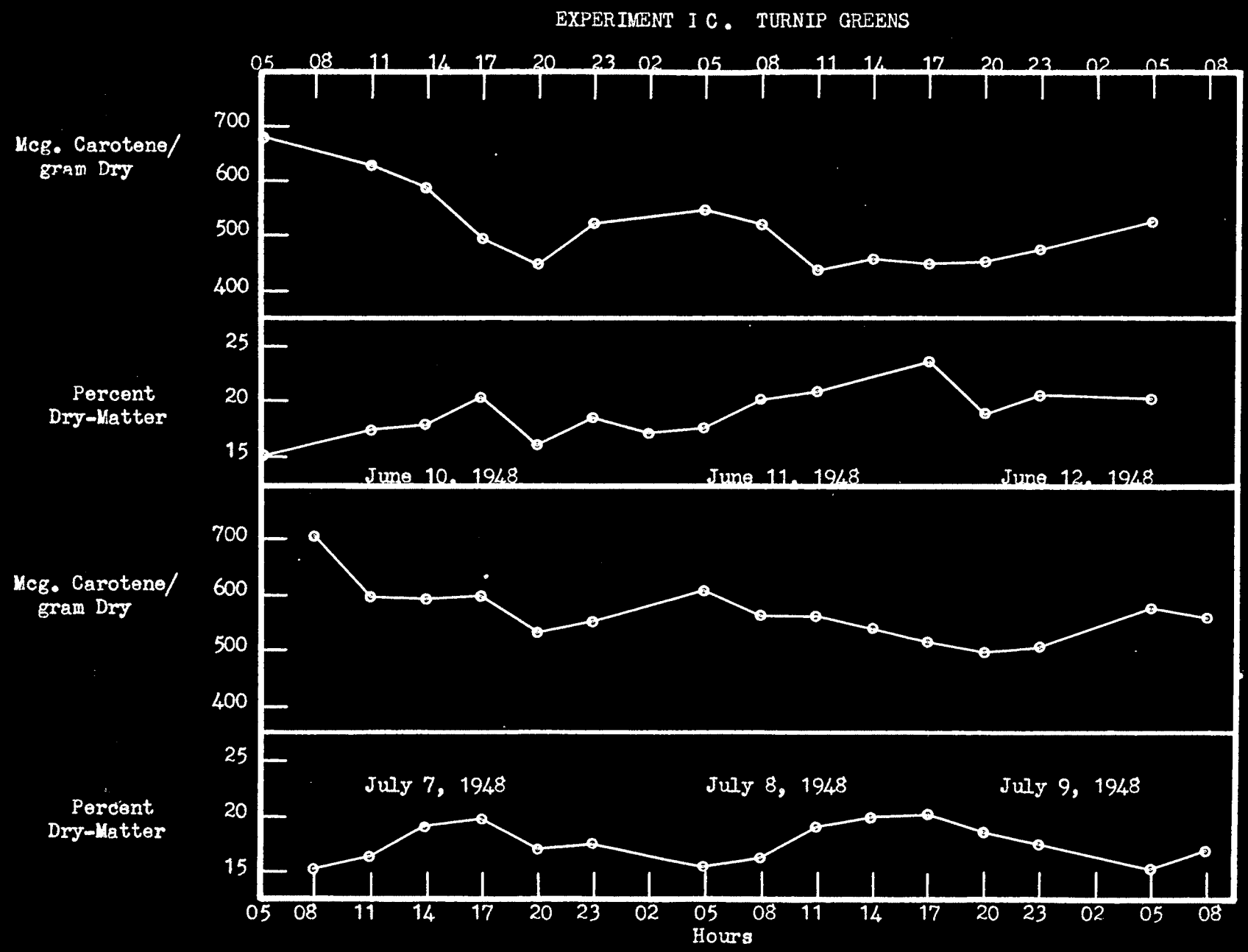
EXPERIMENT I C. TURNIP GREENS
05 08 11 14 17 20 23 02 05 08 11 14 17 20 23 02 05 08
Mcg. Carotene/ gram Dry
700
600
500
400
Percent Dry-Matter
25
20
15
June 10, 1948
June 11, 1948
June 12, 1948
Mcg. Carotene/ gram Dry
700
600
500
400
Percent Dry-Matter
25
20
15
July 7, 1948
July 8, 1948
July 9, 1948
05 08 11 14 17 20 23 02 05 08 11 14 17 20 23 02 05 08
Hours

Figure 3

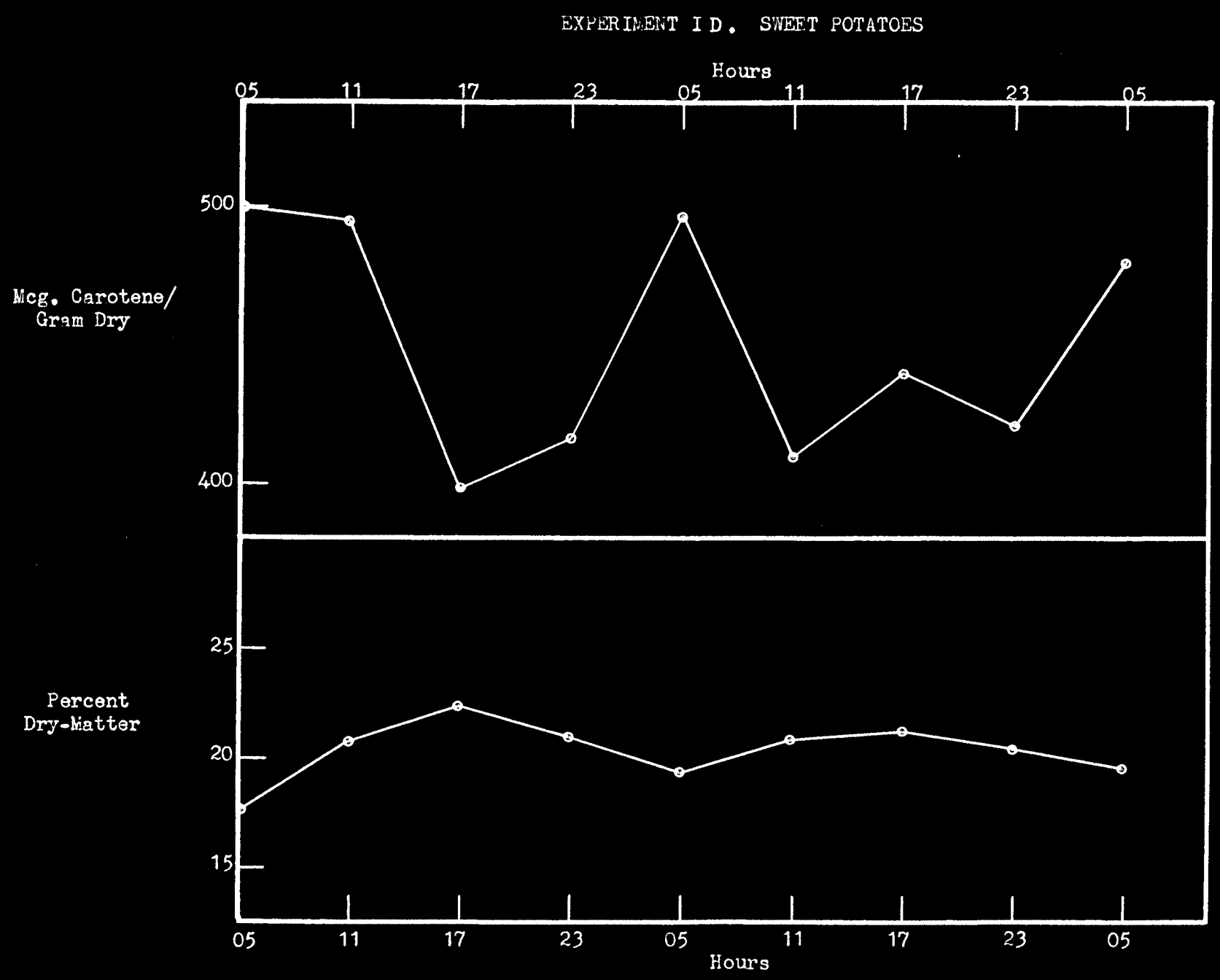
EXPERIMENT I D. SWEET POTATOES
Hours
05
11
17
23
05
11
17
23
05
500
400
Mcg. Carotene/
Gram Dry
25
20
15
Percent
Dry-Matter
05
11
17
23
05
11
17
23
05
Hours

Figure 4

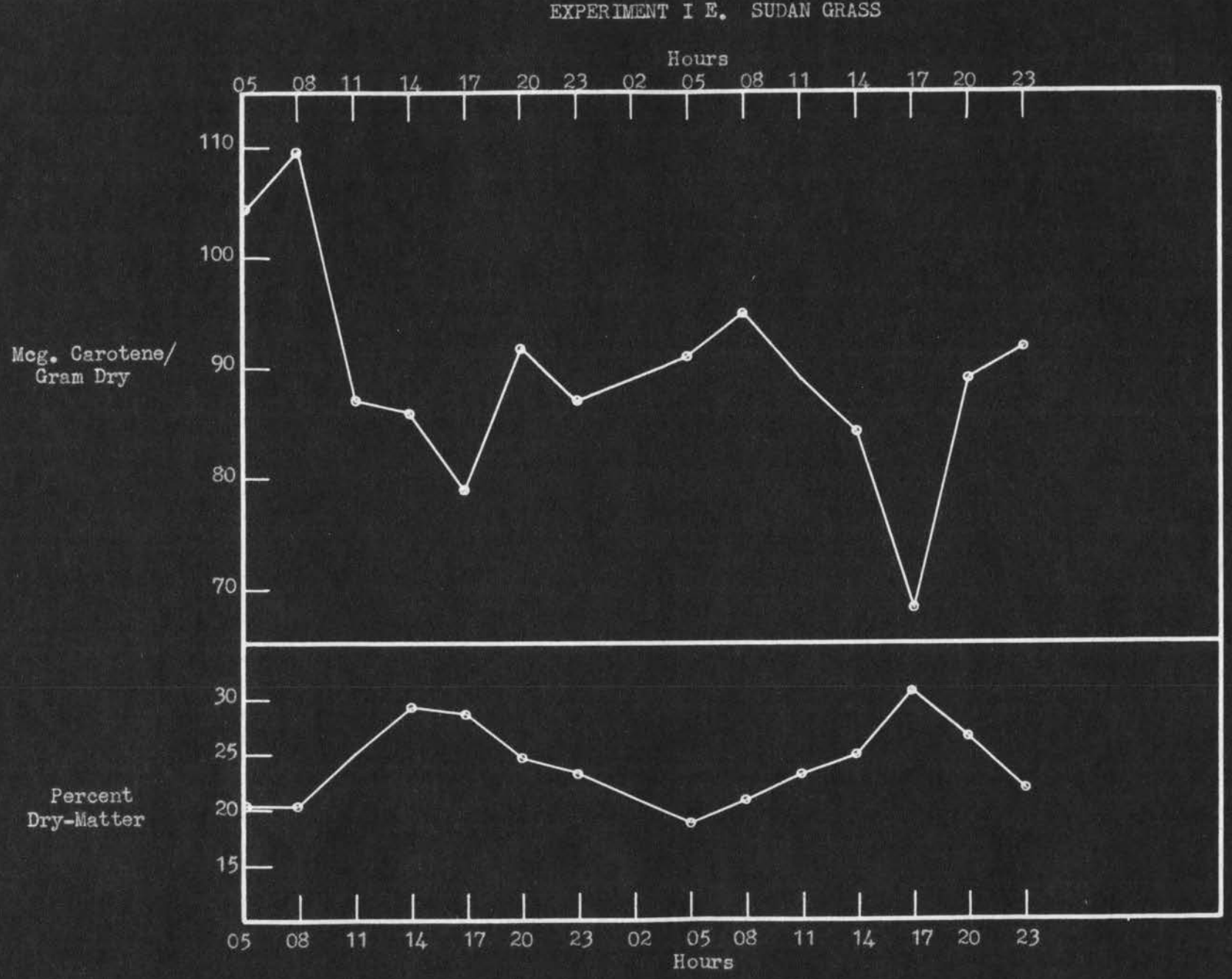
EXPERIMENT I E. SUDAN GRASS
Hours
05 08 11 14 17 20 23 02 05 08 11 14 17 20 23
110
100
90
80
70
Mcg. Carotene/
Gram Dry
30
25
20
15
Percent
Dry-Matter
05 08 11 14 17 20 23 02 05 08 11 14 17 20 23
Hours

Figure 5

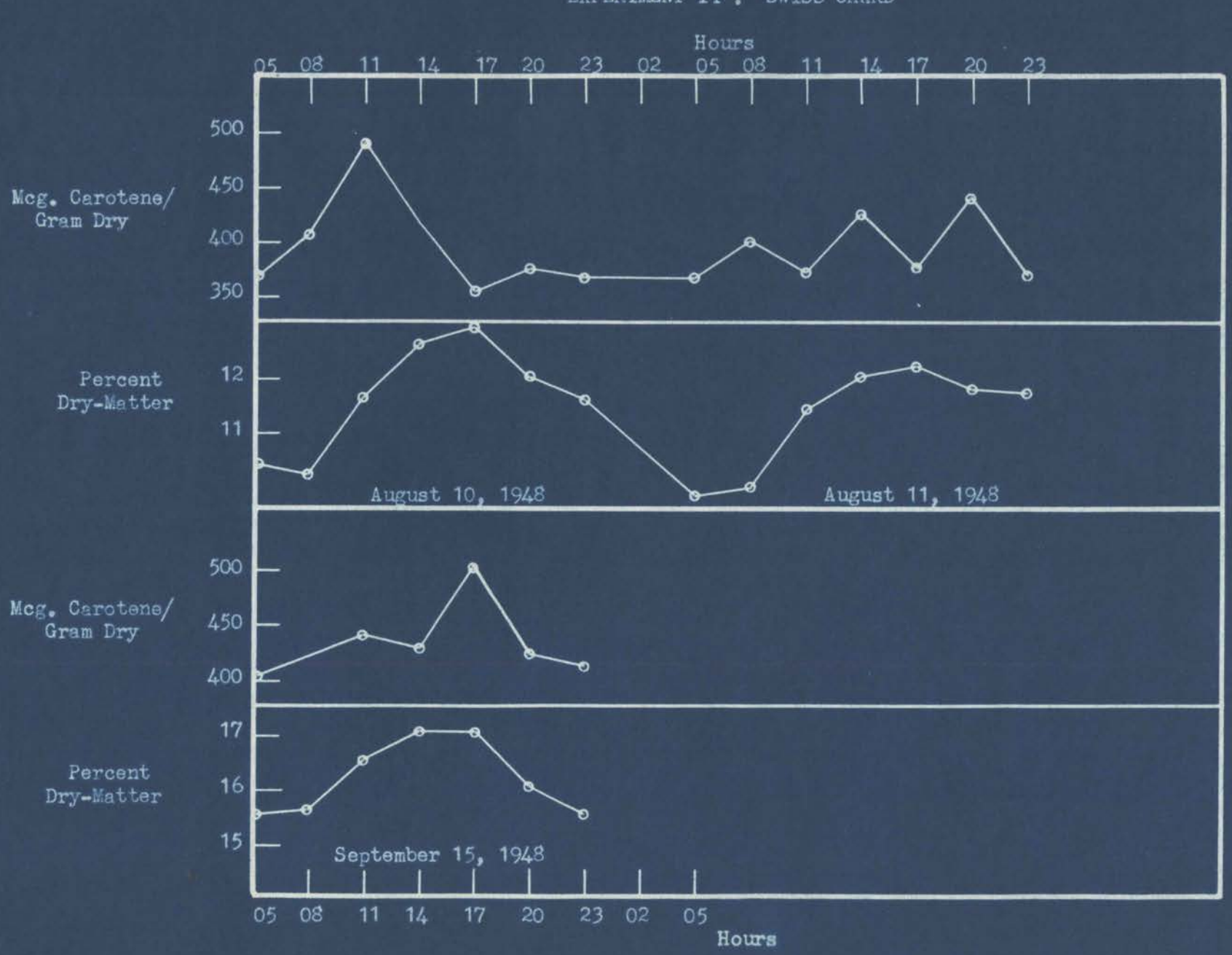
EXPERIMENT IF . SWISS CHARD
Hours
05 08 11 14 17 20 23 02 05 08 11 14 17 20 23
Mcg. Carotene/ Gram Dry
500
450
400
350
Percent Dry-Matter
12
11
August 10, 1948
August 11, 1948
Mcg. Carotene/ Gram Dry
500
450
400
Percent Dry-Matter
17
16
15
September 15, 1948
05 08 11 14 17 20 23 02 05
Hours

Figure 6

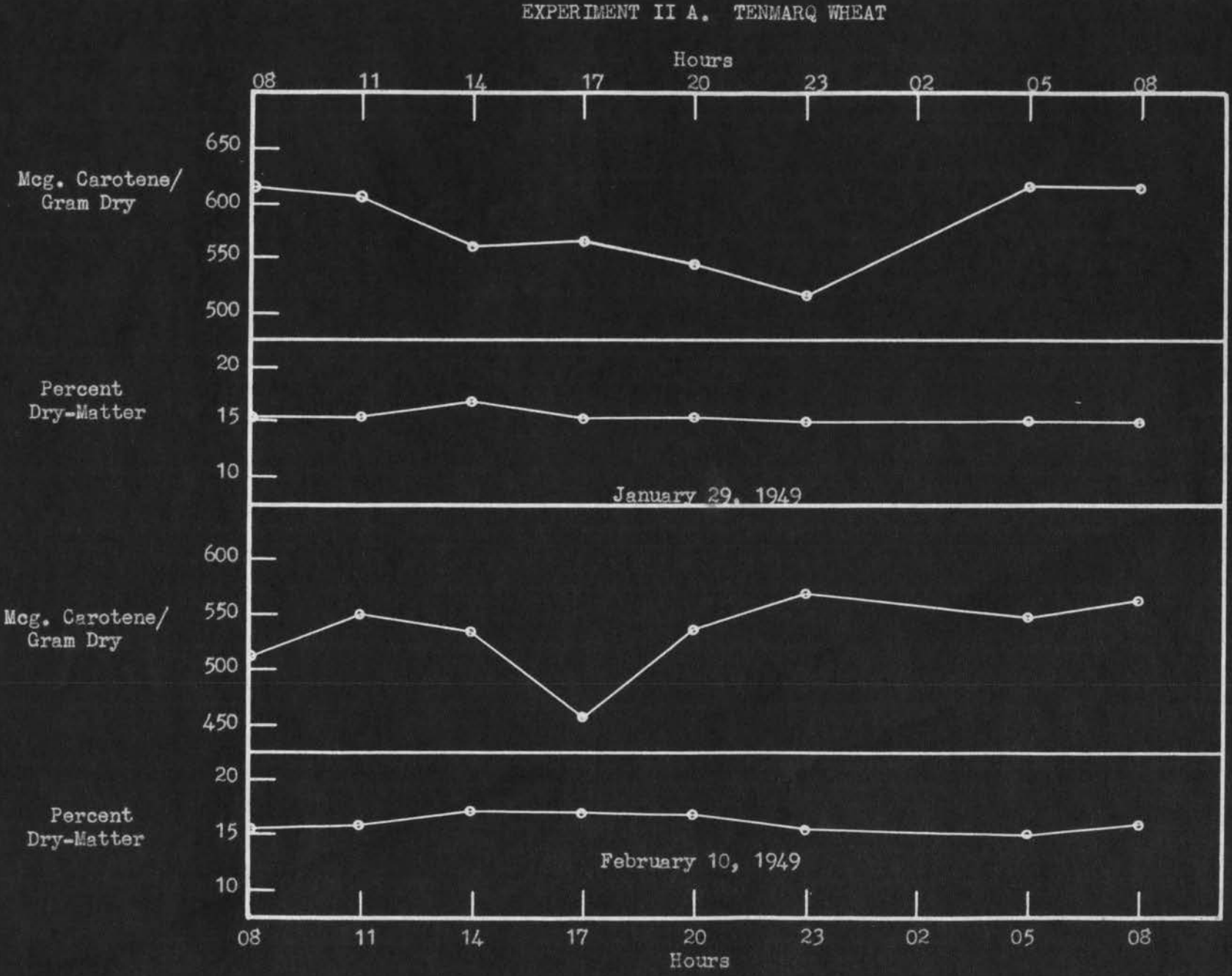
EXPERIMENT II A. TENMARQ WHEAT
Hours
08
11
14
17
20
23
02
05
08
650
600
550
500
Mcg. Carotene/
Gram Dry
20
15
10
Percent
Dry-Matter
January 29, 1949
600
550
500
450
Mcg. Carotene/
Gram Dry
20
15
10
Percent
Dry-Matter
February 10, 1949
08
11
14
17
20
23
02
05
08
Hours

Figure 7

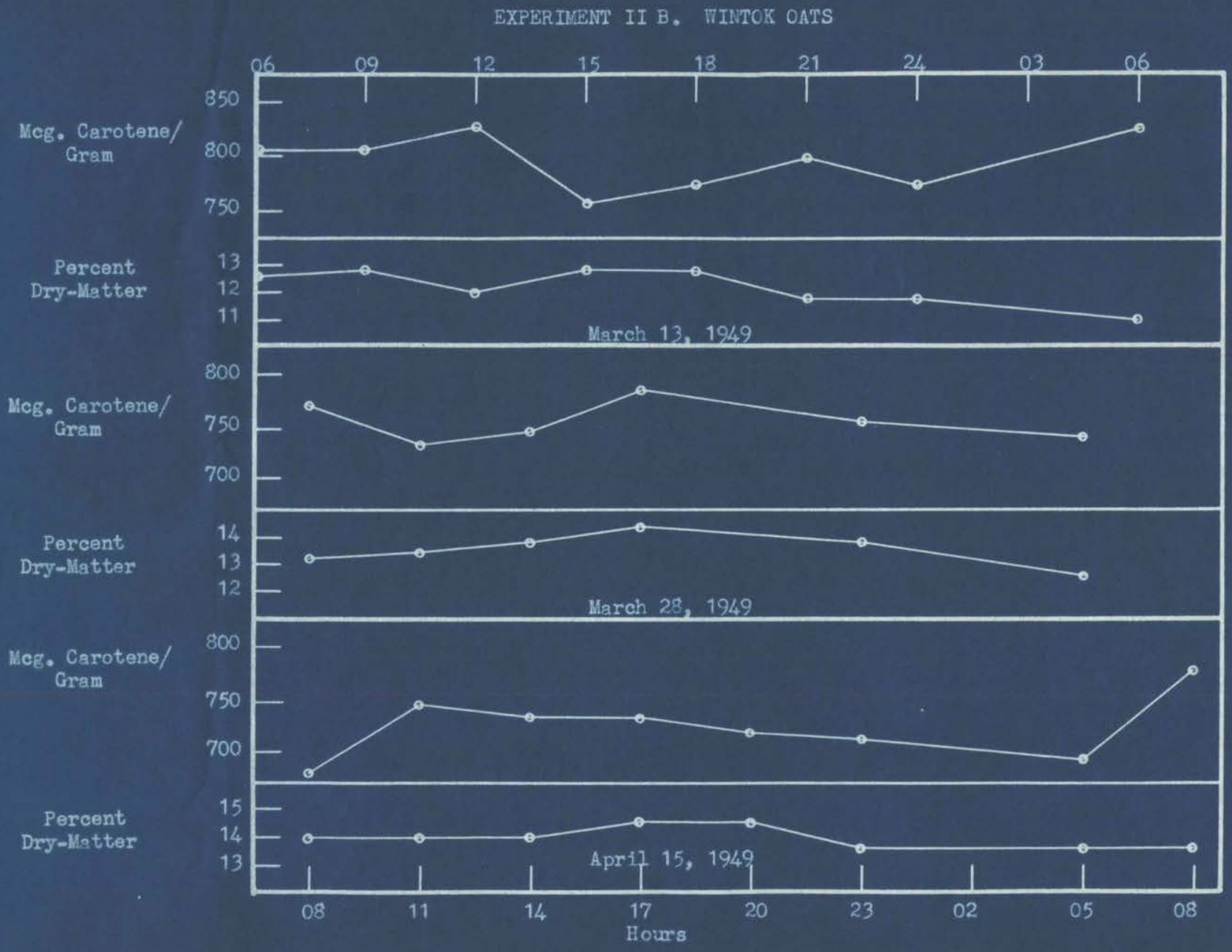
EXPERIMENT II B. WINTOK OATS
06
09
12
15
18
21
24
03
06
Mcg. Carotene/ Gram
850
800
750
Percent Dry-Matter
13
12
11
March 13, 1949
Mcg. Carotene/ Gram
800
750
700
Percent Dry-Matter
14
13
12
March 28, 1949
Mcg. Carotene/ Gram
800
750
700
Percent Dry-Matter
15
14
13
April 15, 1949
08
11
14
17
20
23
02
05
08
Hours

Figure 8

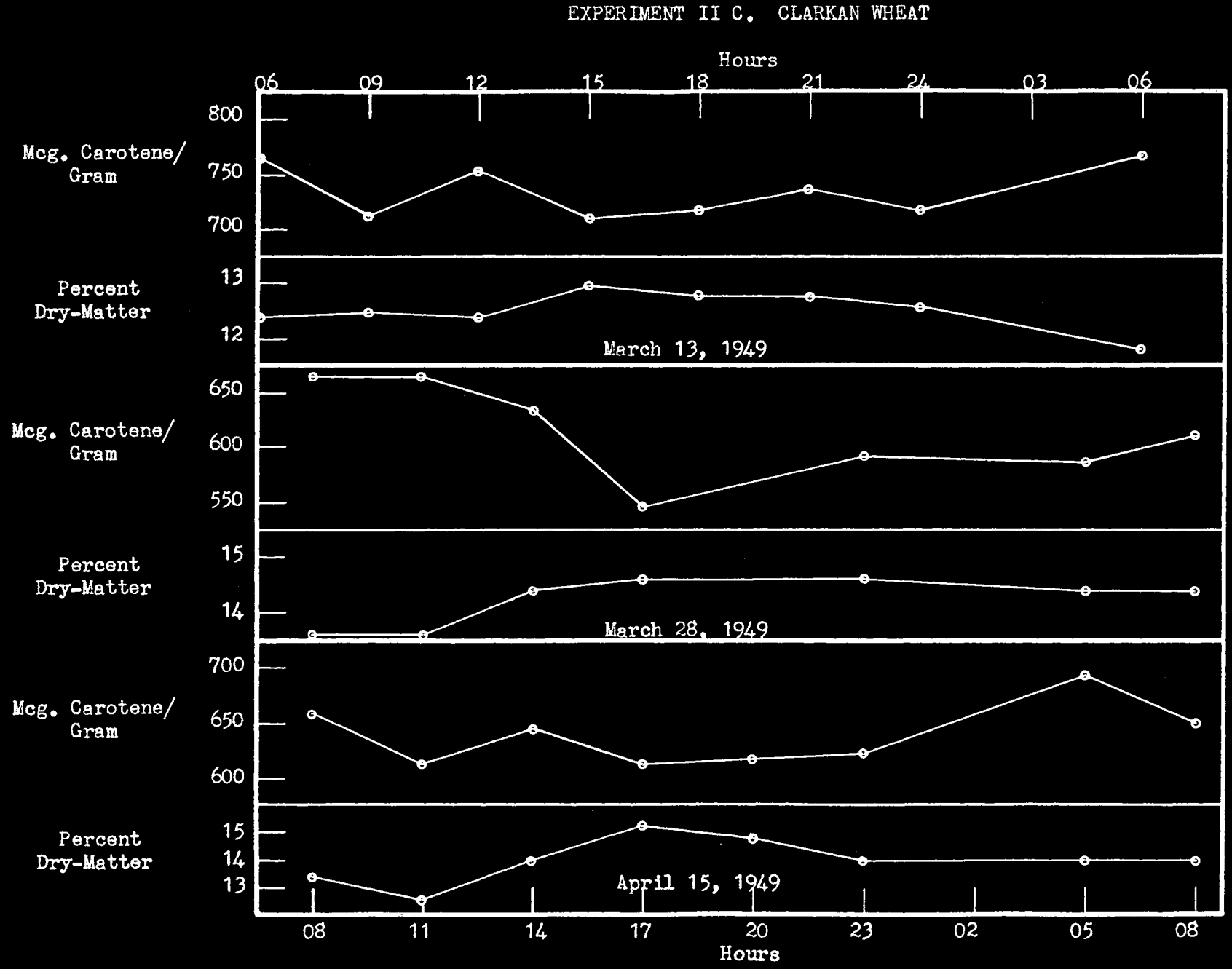
EXPERIMENT II C. CLARKAN WHEAT
Hours
06
09
12
15
18
21
24
03
06
800
750
700
Mcg. Carotene/ Gram
13
12
Percent Dry-Matter
March 13, 1949
650
600
550
Mcg. Carotene/ Gram
15
14
Percent Dry-Matter
March 28, 1949
700
650
600
Mcg. Carotene/ Gram
15
14
13
Percent Dry-Matter
April 15, 1949
08
11
14
17
20
23
02
05
08
Hours

Figure 9

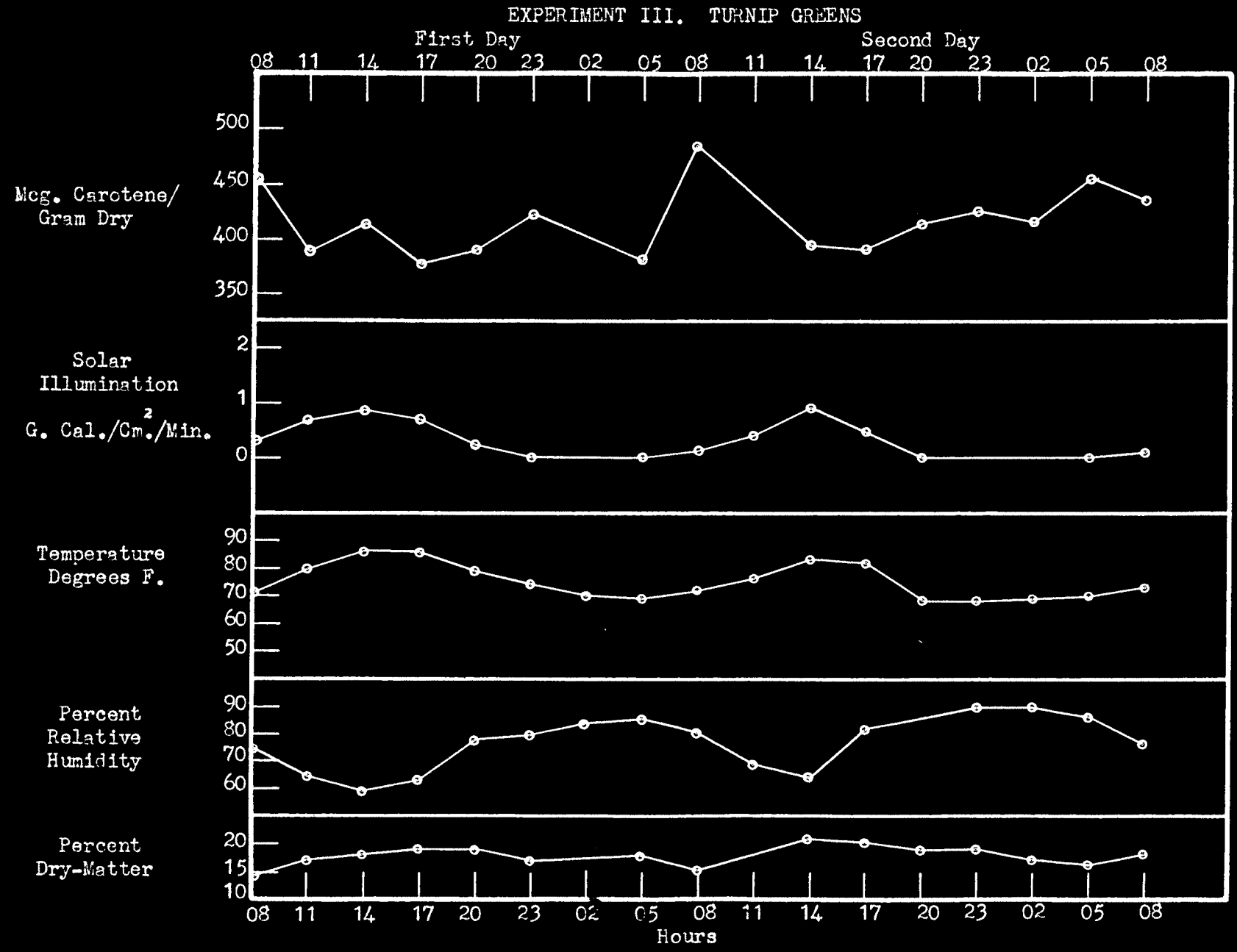
EXPERIMENT III. TURNIP GREENS
First Day
Second Day
08 11 14 17 20 23 02 05 08 11 14 17 20 23 02 05 08
Mcg. Carotene/ Gram Dry
500
450
400
350
Solar Illumination
G. Cal./Cm.²/Min.
2
1
0
Temperature Degrees F.
90
80
70
60
50
Percent Relative Humidity
90
80
70
60
Percent Dry-Matter
20
15
10
08 11 14 17 20 23 02 05 08 11 14 17 20 23 02 05 08
Hours

Figure 10 A

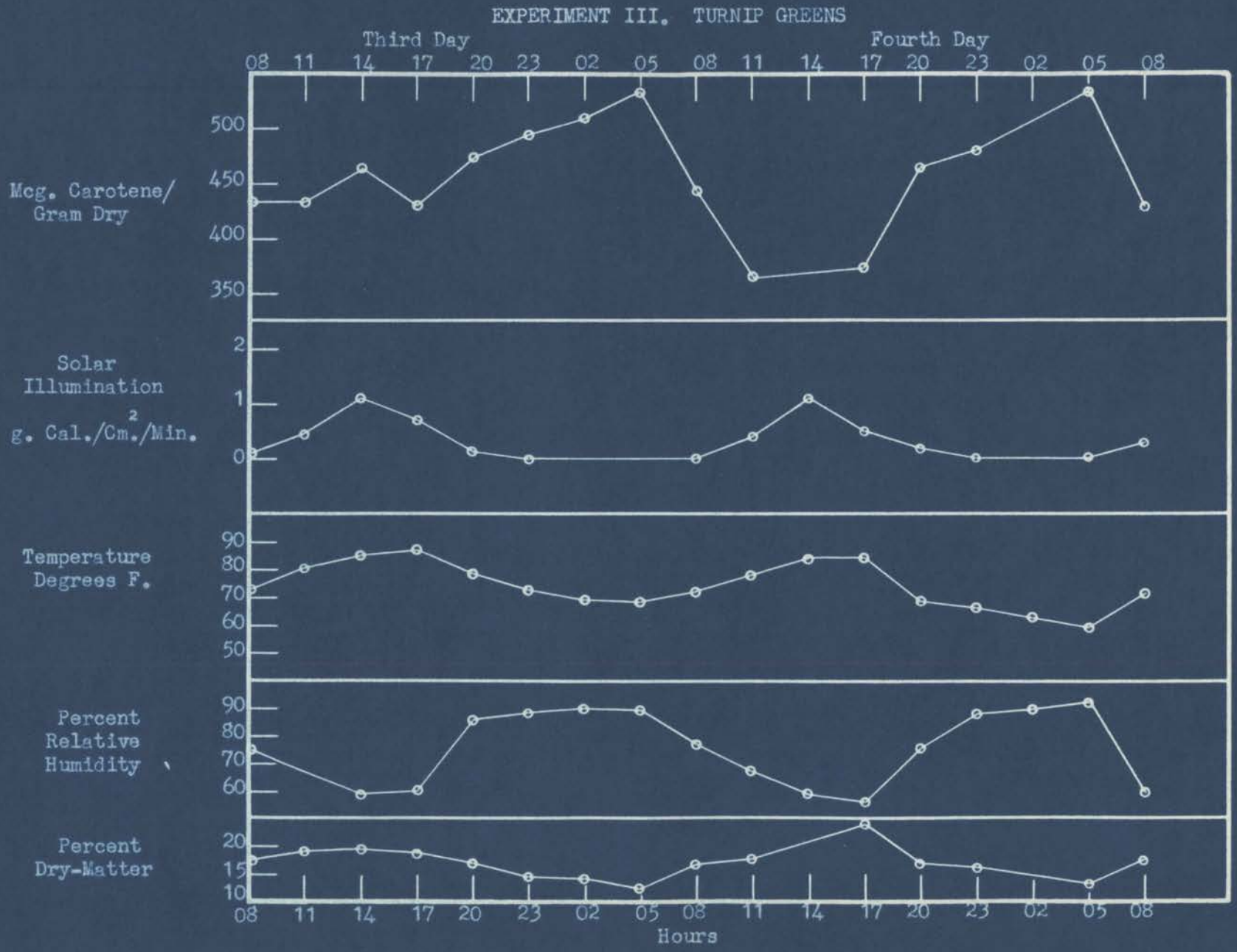
EXPERIMENT III. TURNIP GREENS
Third Day
Fourth Day
08 11 14 17 20 23 02 05 08 11 14 17 20 23 02 05 08
Mcg. Carotene/ Gram Dry
500
450
400
350
Solar Illumination
g. Cal./Cm.²/Min.
2
1
0
Temperature Degrees F.
90
80
70
60
50
Percent Relative Humidity
90
80
70
60
Percent Dry-Matter
20
15
10
08 11 14 17 20 23 02 05 08 11 14 17 20 23 02 05 08
Hours

Figure 10 B

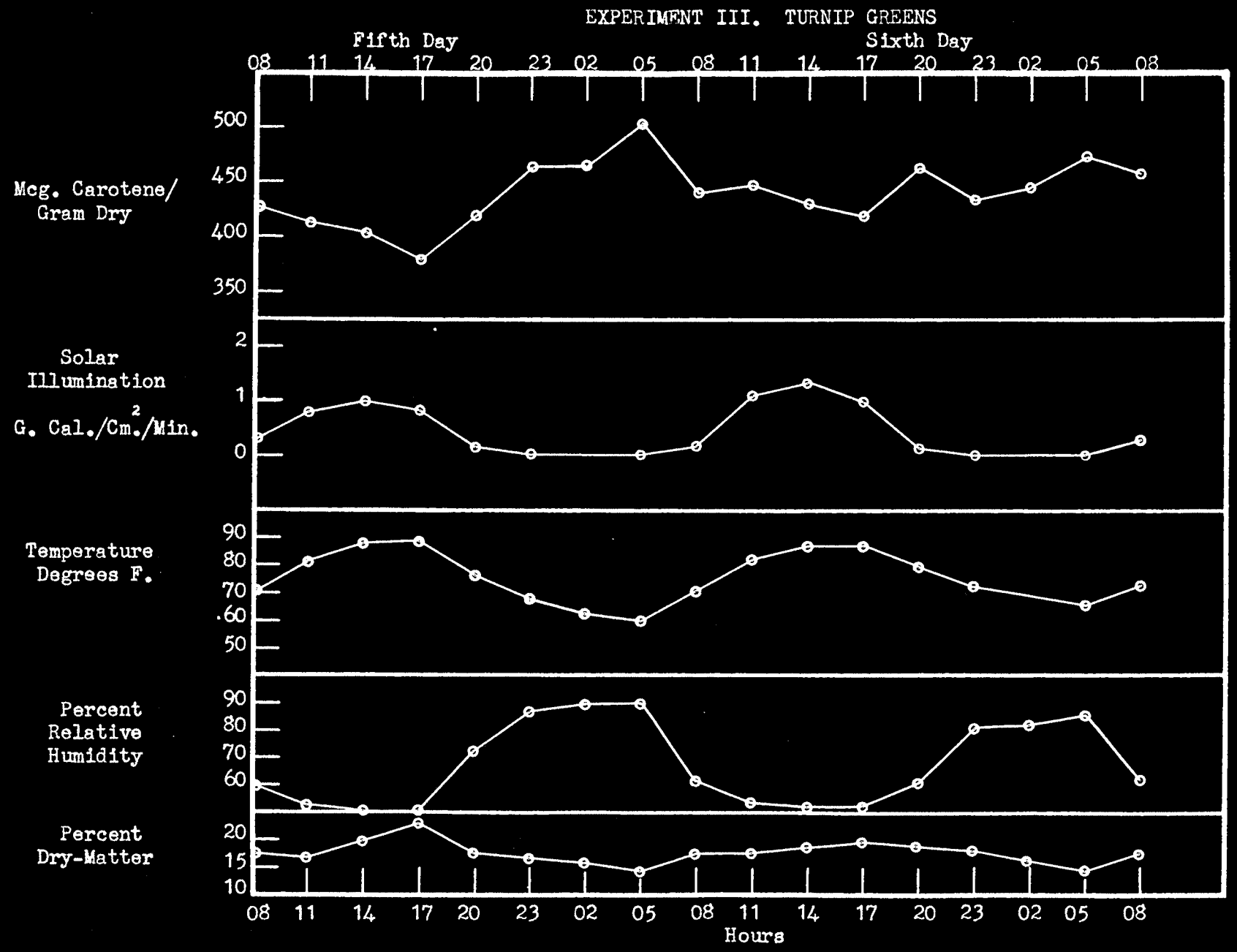
EXPERIMENT III. TURNIP GREENS
Fifth Day
Sixth Day
08 11 14 17 20 23 02 05 08 11 14 17 20 23 02 05 08
Mcg. Carotene/ Gram Dry
500
450
400
350
Solar Illumination
G. Cal./Cm.²/Min.
2
1
0
Temperature Degrees F.
90
80
70
60
50
Percent Relative Humidity
90
80
70
60
Percent Dry-Matter
20
15
10
08 11 14 17 20 23 02 05 08 11 14 17 20 23 02 05 08
Hours

Figure 10 C

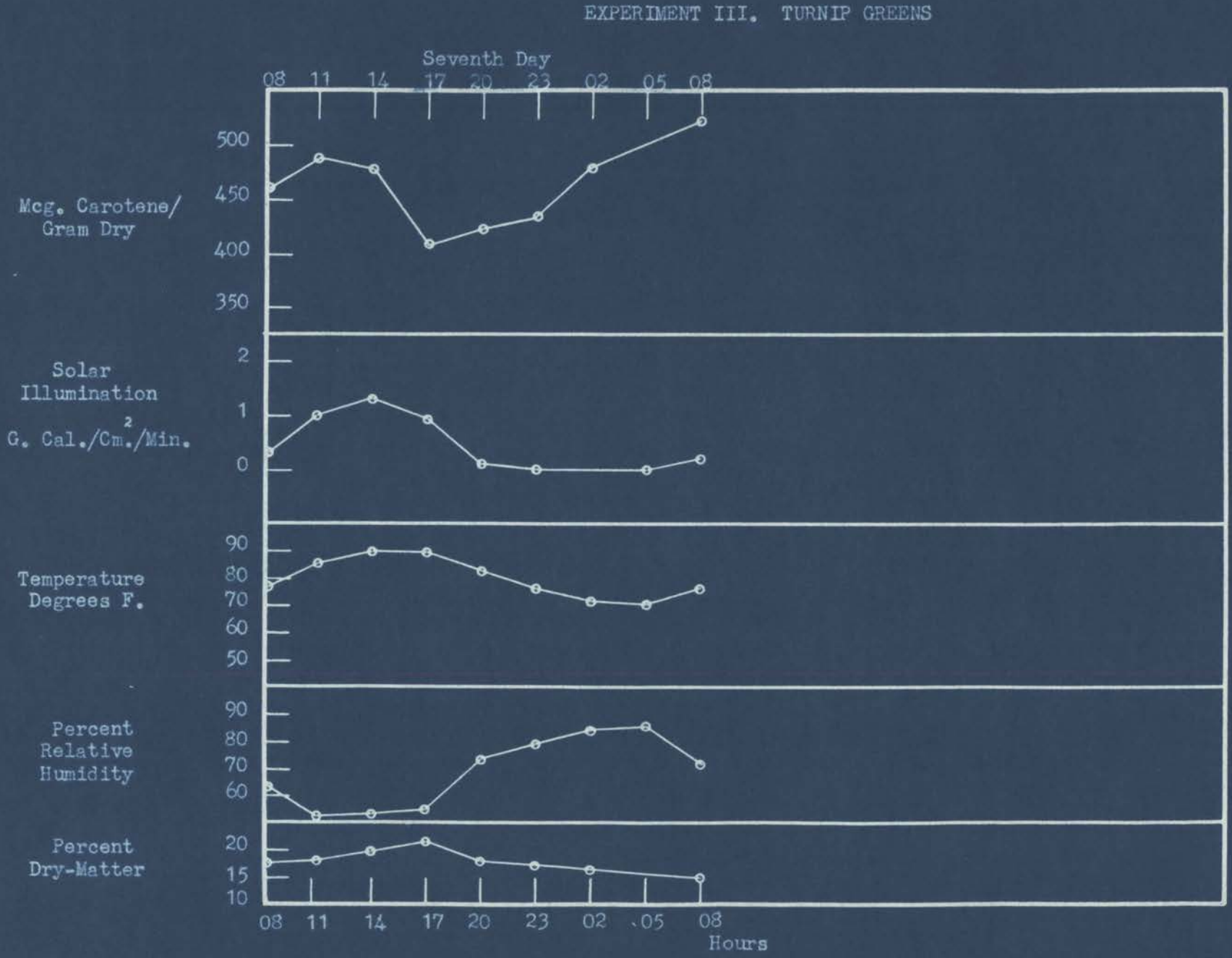
EXPERIMENT III. TURNIP GREENS
Seventh Day
08 11 14 17 20 23 02 05 08
Mcg. Carotene/ Gram Dry
500
450
400
350
Solar Illumination
G. Cal./Cm.²/Min.
2
1
0
Temperature Degrees F.
90
80
70
60
50
Percent Relative Humidity
90
80
70
60
Percent Dry-Matter
20
15
10
08 11 14 17 20 23 02 05 08
Hours

Figure 10 D

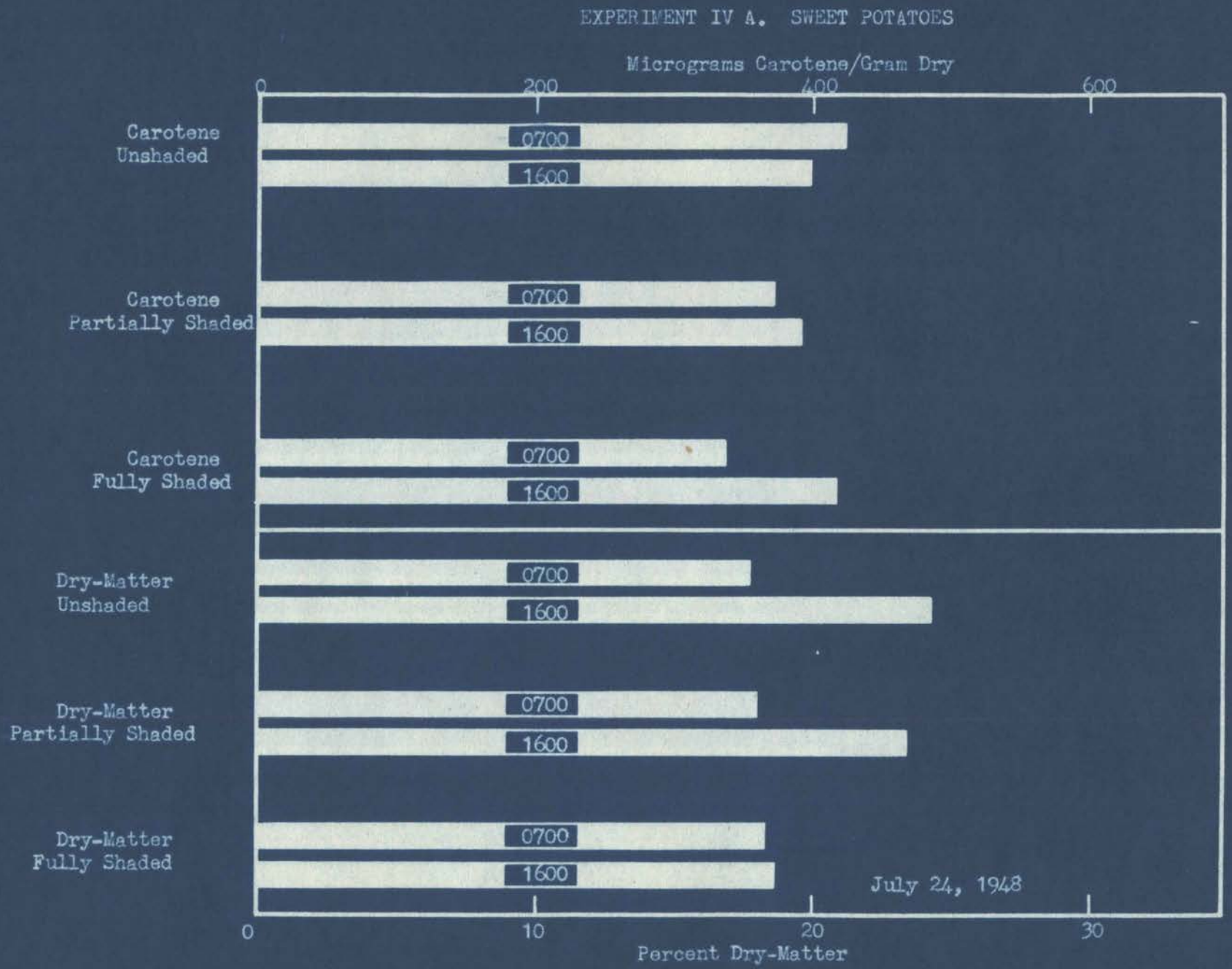
EXPERIMENT IV A. SWEET POTATOES
Micrograms Carotene/Gram Dry
0
200
400
600
Carotene Unshaded
0700
1600
Carotene Partially Shaded
0700
1600
Carotene Fully Shaded
0700
1600
Dry-Matter Unshaded
0700
1600
Dry-Matter Partially Shaded
0700
1600
Dry-Matter Fully Shaded
0700
1600
July 24, 1948
0
10
20
30
Percent Dry-Matter

Figure 11

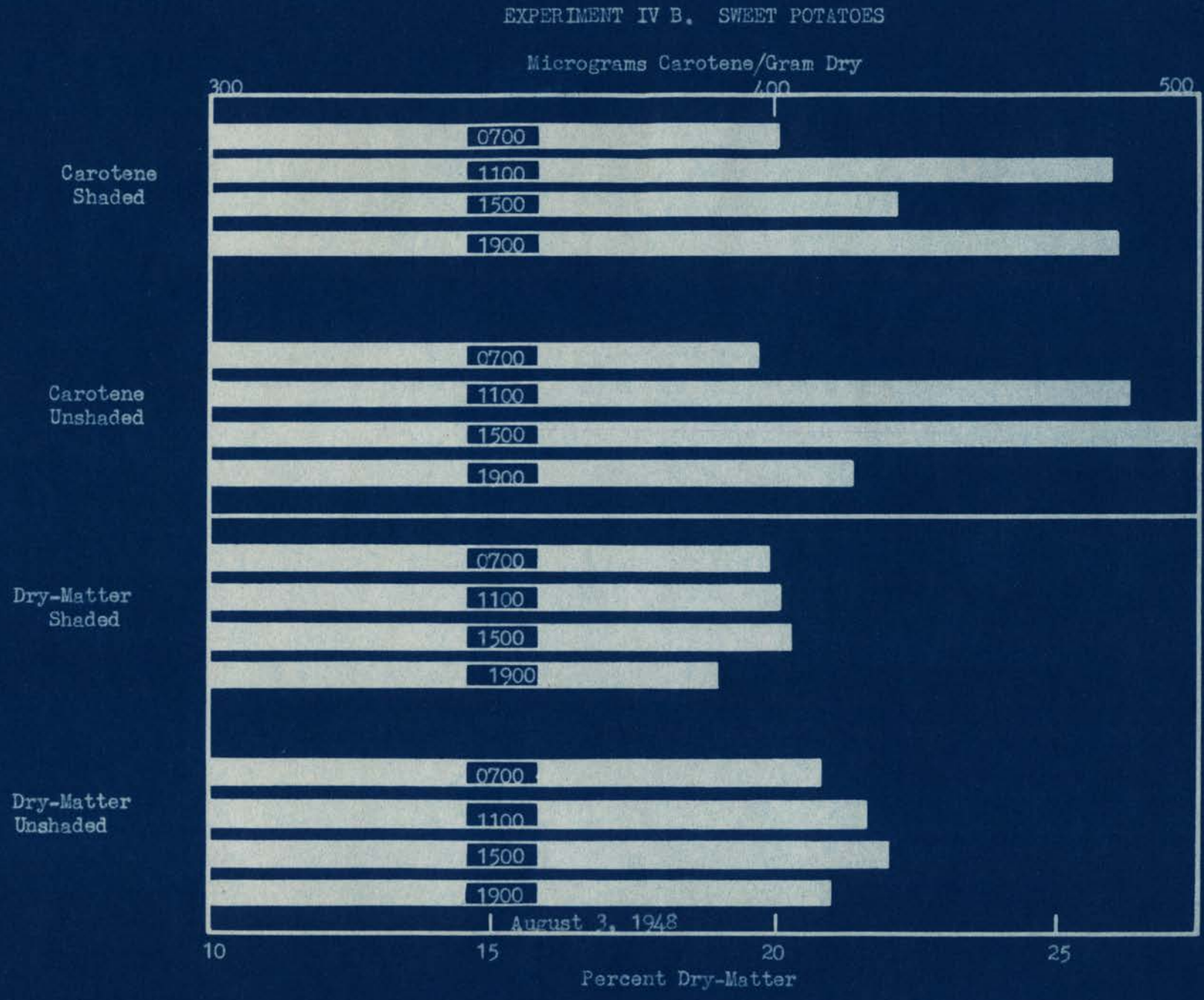
EXPERIMENT IV B. SWEET POTATOES
Micrograms Carotene/Gram Dry
300
400
500
Carotene Shaded
0700
1100
1500
1900
Carotene Unshaded
0700
1100
1500
1900
Dry-Matter Shaded
0700
1100
1500
1900
Dry-Matter Unshaded
0700
1100
1500
1900
August 3, 1948
10
15
20
25
Percent Dry-Matter

Figure 12

RESULTS

Initial experiments (I and II) were carried out to determine whether or not the periodic phenomenon observed by Roberts (1), Zafren (2), and Bernstein, et al. (9) could be demonstrated. If demonstrable, more extensive examination would indicate the quantitative magnitude of such changes. Figures 1 through 9 present the data obtained from these experiments. In general there was a quantitative decline in the carotene content in the late afternoon with a rise during the hours of darkness reaching a maximum in the early morning. The characteristic cycle was repeated with some slight variations on subsequent days of experiments lasting more than one day. Comparison of the data from these two series of experiments indicates that greenhouse versus field environment had little or no effect upon the observed phenomenon. Although the different plants studied did not contain the same amounts of carotene and although their periods of maximum and minimum carotene contents fell at somewhat different times of the day, the general pattern of diurnal fluctuation was present in most of the cases studied. Inspection of the data as a whole shows that while in general the pattern of maximum carotene values in the early morning and minimum values in the late afternoon was followed, there were occasions when the maxima and minima occurred at unexpected times. Unfortunately, no adequate explanation of this phase of the phenomenon can be advanced at this time.

In an attempt to relate certain available climatic data, namely, solar radiation, air temperature, and relative humidity,

to the carotene content variation, experiment III was carried out in which samples were taken every three hours for seven consecutive days. The data obtained in this experiment are presented in Figure 10. Throughout the experiment the general pattern of diurnal variation is evident. The difference between the daily maximum and the daily minimum for the successive days of this experiment varied from 5 to 35 percent. The maximum most often occurred at 5 a.m. and the minimum at 5 p.m.

It is generally accepted that rate of photosynthesis is positively correlated to light intensity. This led to an investigation of the possible effects of shading on the carotene content, the data from which investigation are presented in Figures 11 and 12. The values, though limited in number, did not show any correlation between shading and the amount of carotene in the plant (sweet-potato).

In most of the experiments, although the times at which the maximum and minimum values occurred were repeated on successive days and generally were in the neighborhood of 5 a.m. and 5 p.m., respectively, the maximum-to-minimum decline in values varied between days. The declines were: turnip greens, 20 and 16 percent (Expt. I), 28, 23, 5, 35, 35, 16, and 17.5 percent (Expt. III); Swiss chard, 18 and 31 percent; sweet-potato, 22 and 16 percent; Sudan grass, 16, 31, and 31 percent; Tenmarq wheat, 18 and 31 percent; Wintok oat, 8 percent; and Clarkan wheat, 8 percent.

DISCUSSION

A study of the carotene content of several species of the higher plants has demonstrated a quantitatively measurable periodic variation. In general there was found to be a constant decline during the day, followed by a restoration during the night. What factor or factors (light intensity, relative humidity, temperature, and perhaps other environmental conditions) are responsible for this periodic variation is still to be established.

The initial series of experiments amply confirmed the periodic phenomenon observed by Roberts (1), Zafren (2), and Bernstein, et al. (9). A notable feature of the data from these experiments is the fact that at no time did the daily decline from maximum to minimum carotene values observed reach the magnitude of those reported by Roberts (1), whose data indicated declines of 50 to 70 percent. This disparity may be partially explained by differences in technique employed and in plants observed. Due to his technique, as has been previously noted, the data reported by Roberts (1) was essentially qualitative in nature, as contrasted to quantitative data presented in this study. Roberts (1) used, almost exclusively, mature flowering plants in his experiments, while, for this study, plants in the vegetative state were used.

An attempt was made to correlate available climatic data (solar radiation, air temperature, and relative humidity) with carotene content variation. Although the carotene values seem

to vary inversely with solar radiation, air temperature, and percent dry-matter and directly with relative humidity, the data in general agrees with the statement of Shirley (3) in regard to photosynthesis in which he says:

> "Attempts to correlate rate of photosynthesis with measurement of climatic factors have not met with particular success. Since photosynthesis is dependent upon a number of factors, first one then another may be limiting."

While this experiment adds support to the observation that there is a diurnal variation in the carotene content, it indicates even more strongly that closely controlled climatic conditions will be needed to demonstrate which environmental factor is effecting the change. It is interesting to note that on the third and fourth days of Experiment III, the decline from maximum to minimum were 5 and 35 percent, respectively. The climatic data indicate that as far as weather was concerned, the days were very closely similar.

The shading experiments did not show any affect of reduction in light intensity on the carotene content of the plant under observation (sweet-potato).

Shirley (3) has observed that under conditions favorable to rapid photosynthesis, the rate of photosynthesis tends to decrease with increasing time, due to an accumulation of carbohydrate in the leaves. Weintraub (5) confirmed this finding and listed as possible internal causes: stomatal closure affecting carbon dioxide diffusion, photic or thermal fatigue of the assimilation mechanism, photodestruction of chlorophyll, accumulation of assimilates, and reduction of the assimilation

surface through phototaxis of the chloroplasts. Some light has been thrown on the possible relation of carotene to photosynthesis by Dutton and Manning (10) in their studies of the diatom, Nitzchia closterium. These workers found that in the organism studied light absorbed by some or all of the carotenoid pigments could be utilized in photosynthesis. Carotenoid photosynthesis probably utilized the same enzyme system as chlorophyll photosynthesis.

In view of the preceding observations, certain deductions may be tentatively made regarding the reasons for the variations in carotene content found in the experiments herein described. Since carotene is relatively easily oxidized by the oxygen of the air, it is possible that during times of high photosynthetic activity, the presence of relatively high amounts of oxygen in the chlorophyllous tissue as a product of carbon dioxide assimilation might cause an increased rate of oxidation of carotene. Further, carotene is itself somewhat labile to high light intensities, particularly in the lower wavelengths. Its photodestruction at such times may contribute, along with that of chlorophyll, to the slowing down of the photosynthetic processes noted by Shirley (3) and Weintraub (5).

It is known that the enzyme, lipoxidase, which is present in most chlorophyllous tissue, catalyzes the destruction of carotene and it may be that the higher temperatures of the latter part of the day, both immediately and as a cumulative effect, enhance the activity of this enzyme and further contribute to the lowered carotene contents observed at those

times. The immediate availability of oxygen produced by the photosynthetic process has previously been pointed out. The converse of the foregoing would then follow as a contributing factor to the maximum usually found in the cooler early morning hours.

An increase of 20 percent in the carotene content of a food or forage crop from late afternoon to early morning is of no small agronomic importance. If, as is the case with some crops produced for dehydration, the selling price were based on carotene content at time of delivery to the processor, the difference in carotene content might well make the difference between success and failure of a farming operation. If it can be sufficiently established that an increase in the amount of carotene in a crop plant is to be realized by harvesting in the early morning hours, there is no apparent reason why farmers, with the modern equipment at their disposal, would not prefer to harvest his crop at those times. Dehydrating plants could easily accommodate themselves to such a schedule.

These observations should also be considered by scientific investigators who intend to determine the carotene content of a food or feed or use it as an index of the effect of some other variable. Unless the characteristic diurnal variation in the carotene content of the plant in use were appreciated and taken into consideration, the efforts of his investigation could well be vitiated.

SUMMARY

1. It has been demonstrated that there is a quantitatively measurable diurnal variation in the carotene content of certain species of plants. A pattern in which the maximum values occur in the early morning and the minimum values in the late afternoon is usually followed. The periodicity under observation is not the same for all species, and the same plant does not at all times follow the same pattern.

2. Although the characteristic pattern of the species is followed in most cases, the range between maximum and minimum carotene values on different days may vary. In a few instances little or no variation was demonstrable.

3. Shading had no significant effect upon the carotene content of intact sweet-potato leaves.

4. Attempts to correlate certain climatic data, namely solar radiation, air temperature, and relative humidity, with carotene content showed that an inverse relationship existed between the carotene values and solar radiation and air temperature and a direct relationship between carotene and relative humidity. Amount of carotene varied inversely with the percent dry-matter in the tissue.

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