Physiological Studies on Tobacco Plants I.

Relationship between Photosynthetic Activity and Growth Stage,
with Special Reference to the Effect of Topping-Treatment*

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On the photosynthetic activity of higher plants, there have been many investigations in which the infra-red gas analyzer was used as the detecting apparatus1-7, but comparatively little is known as to the relationship between the photosynthetic activity and the growth of higher plants. Moreover, almost of them have adopted an air flow method, and there still remained much room for considering the effect of the velocity of air flow on photosynthesis rate, and this introduced noticeable discrepancy in those studies7' 8>

An attempt has been made in this paper to develop a closed system not only avoiding such a difficulty, but also giving reliable measurements of small changes in photosynthetic and respiratory activities of the test plants throughout the whole course of their growth and maturation. In the present study intact tobacco plants were used in place of detached leaves or punches of leaves, and the photosynthetic activity was studied throughout the full course of the growth stage of each individual plant. Special attention was also paid to the effect of topping-treatment**** on the photosynthetic activity.

In the following paper of this series, attention will be drawn to the marked changes in the cell constituents during the growth, and a full discussion of the close relationship among the photosynthetic activity, the composition of cell constituents and the growth stage will be presented.

Materials and Methods

(1) Materials: The following three cultivated varieties of Nicotiana tabacum were used: (1) Bright Yellow, (2) White Burley and (3) Bitchâ. The former two

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**** Topping-treatment is a special and important cultivation technique in tobacco cultivation, which is the cutting-off of flower buds at an early growth stage (about 60-70 days after transplantation) for the purpose of concentrating nutrients into the development of leaves. The way in which this treatment is carried out affects seriously the yield and quality of tobacco leaves.
varieties are sensitive to the topping-treatment, while Bitchû is a variety native to Chûgoku District which is rather insensitive to this treatment. This is the reason for the choice of these three varieties. Test material of each variety consisted of 100 individuals, whose seedlings were transplanted from the Okayama Tobacco Experiment Station (Tamashima City, Okayama) to the experimental field of our laboratory. Observations reported here started 20 days after transplantation and finished 120 days after transplantation.

The cultivation of plant materials was carried out under nearly natural conditions in Wagner-pots (1/50,000) containing fine sand and field soil (1: 2). A culture solution* was given to the plants regularly once a day.

(2) Measurement of photosynthetic activity: Photosynthetic activity was measured by the system as shown in Fig. 1 which consisted of an assimilation chamber, an infra-red gas analyzer (Beckeman L/B Model 15A coupled to a Varian recording potentiometer) and the under-mentioned accessory parts in closed series.

(i) A block diagram of the system used in this experiment is shown in Fig. 1. The test plant was placed in the assimilation chamber (A) and illuminated at 25°. Illumination was provided by a bank of 29 fluorescent lamps (Mitsubishi, de Luxe cold-white, 40 watt) placed above and all around the chamber. Light intensity was about 25,000 lux at the level of the surface of the test plant. The gas stream passing through the assimilation chamber (A) was circulated through the whole system by a circulating pump (P1). Besides the main path of the gas stream (A—C1—S1—C2—C6—P1—A), there was also a branch path, namely, C2—K—N—F—C3—R—S2—SC—C4—C5—C6. The gas flux both in the main path and in the branch path was regulated at 300 ml/min. The components used in the system had the following purposes: Kel-F filter (K), for the removal of fine particles contaminated in the gas stream; needle valves (N), for the regulation of gas flux; pressure pulsation reducer (R), for reducing the pulse in the gas stream arising from the action of circulating pumps; desiccator tubes (S1—S3), for keeping the gas stream dry; accessory circu-

* The composition of the culture solution in ppm: N, 100; P2O5, 50; K2O, 200; CaO, 200; MgO, 25; Fe2O3, 5; MnO2, 1.5; B, 0.1; Zn, 0.2; Cu, 0.05.
lating pump \((P_2)\), for circulating and uniformizing the gas in the large assimilation chamber. The range of the infra-red gas analyzer was 0~600 ppm CO2.

(ii) Assimilation chamber: Three different chambers, i.e., a two pile glass cloche, a three pile glass cloche (shown in Fig. 2A) and a vinyl chamber (shown in Fig. 2B), were used variously according to the size of the test material. Out of the three parts of the glass cloche (top, middle and bottom), the middle part could be removed when a two pile cloche was enough to hold the young material of smaller size. A vinyl chamber was made out of a gastight ‘vinyl sheet’ and kept in a cylindrical shape (45 cm in diameter; 120 cm in height) with fine bamboo hoops*. When in use, the assimilation chamber was soaped slightly to keep out water drops.

(iii) Determination of the total volume of the whole system: As mentioned above, three different kinds of assimilation chamber were used in the measurement of photosynthetic activity. Due to the disparity in the total volume of the whole system in the three chambers, the reading of CO2 concentration on the recorder \((\Delta \text{ppm})\) will be different according to the choice of assimilation chamber. The total volume, which had to be determined in order to compare the \(\Delta \text{ppm}\) values with each another, was calculated from the change in CO2 concentration, when a definite amount of CO2, which was produced by the addition of the given concentration of HCl to the known concentration of NaHCO3, was evolved in the system. In Table 1 are collected the results of these measurements. The observed values of the change in CO2 concentration \((\Delta \text{ppm})\) were recalculated in terms of the \(\Delta \text{ppm}\) in the case of a two pile glass cloche by multiplying by the coefficient given in the 4th column of Table 1.

* Detailed explanation in text.

![Fig. 2. A whole view of the assimilation chamber used.](image)

A. Three pile glass cloche; B. Vinyl chamber.

<table>
<thead>
<tr>
<th>Assimilation chamber used</th>
<th>Total volume (L)</th>
<th>Required amount of CO2 for a change of 1 ppm in the system used ((\mu g))</th>
<th>Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two pile glass cloche</td>
<td>43.0</td>
<td>84.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Three pile glass cloche</td>
<td>65.5</td>
<td>128.7</td>
<td>1.53</td>
</tr>
<tr>
<td>Vinyl chamber</td>
<td>136.0</td>
<td>267.2</td>
<td>3.17</td>
</tr>
</tbody>
</table>

* The writers knew afterwards that Nichiporovich et al.14) have designed almost the same kind of assimilation chamber as ours.
(iv) Measurement and calculation: After adjusting the gas analyzer against zero gas (100% pure N₂) and upscale gas (600 ppm CO₂ in pure N₂), a suitable assimilation chamber was chosen in which the test material was placed, and the whole measuring system was constructed. After establishment of the equilibrium of gas flux in the whole system, light was turned on for 15 minutes, and then turned off for 15 minutes, and this procedure was repeated three times. From the average reading of the indicator of the recorder (given in mV value), the ΔmV-value per 3 minutes was calculated both under light and dark conditions. The change in CO₂ concentration (Δppm) was determined by a calibration curve in which the reading of the indicator (mV) was plotted against the concentration of CO₂ (ppm). From the values of ppm, the increment or decrement in CO₂ concentration (µg) was determined. By this procedure, the total activity of photosynthesis per individual plant, namely photosynthetic rate expressed on a whole plant basis, was obtained, and photosynthetic activity per unit leaf area was also computed. The former is tentatively designated as “PS_w” and the latter “PS_LA” in this paper.

(3) Measurement of leaf area: The trait of the present investigation was an accurate measurement of the minute changes in photosynthetic activity of the same intact individual plant in the whole course of its growth, and consequently it was necessary to measure the leaf area without damaging the test plant. The following method was proved to be most stable: leaf margins of the test plant were brushed over with a dilute solution of dye (e.g., 50 mg Brilliant green in 200 ml water with 2~3 drops of neutral soap solution*). A sheet of paper was put on each leaf blade of the test plant, and the leaf patterns printed with dye were cut out. After drying these patterns overnight and weighing, the leaf area was calculated from the calibration curve of the area of paper pattern against its weight. The total sum of the leaf area of tips**, smoking leaf**, cutter** and lug** was used in the calculation of photosynthetic activity per unit leaf area (“PS_LA”).

Results

(1) Progress of growth stage: (a) Plant height—Typical examples of growth curves of the varieties used are given in Fig. 3 in which solid lines and broken lines represent those of the untopped and topped plants, respectively. Those of White Burley are not shown in the figure, but are strikingly similar to those of Bright Yellow. As can be seen from this figure, there was little if any difference in the growth curves of the three varieties used, and in all three an inconspicuous increase in plant height until about 55 days after transplantation was followed by a rapid growth stage (60~80 days after transplantation), finally coming to a standstill period.

(b) Budding—The commencement of the budding stage differed in the three varieties used as follows: Bright Yellow, 68th day after transplantation; Bitché, 74th day; White Burley, 76th day. The topping-treatment was carried out at the time of the opening of the first top-flower, and the dates of topping-treatment for three varieties differed consequently, as follows: Bright Yellow, 78th day after transplantation; Bitché, 85th day; White Burley, 92nd day.

* After the measurement of leaf area, the dye solution was washed away.
** These are the special technical terms of tobacco cultivation, and correspond to the following Japanese technical terms: Ten-ha, Hon-pa, Chô-ha and Do-ha, respectively.
(c) Total leaf area—Fig. 3 also shows the variation in the total leaf area of the test plants. In the three varieties, the development of leaves was completed not later than the 70th day after transplantation (Bitchû), the 75th day (Bright Yellow) and the 80th day (White Burley), respectively. The shape of increment curve of total leaf area was similar to that of plant height, except for a gradual decrease in leaf area observed at the latest stage, which was attributable to the withering-up beginning from about 90 days after transplantation.

(2) Variation in photosynthetic activity: (a) Total activity of photosynthesis per individual plant ("PS\(_w\)")—From the topped plots and untopped plots of each of the three varieties, 5~8 individual plants of relatively similar size were chosen, and with them the measurements of the total activity of photosynthesis per individual

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**Fig. 3.** Typical growth curves of the varieties used.

A. Total leaf area; B. Plant height.

- **Bright Yellow** (untopped plant)
- **"** (topped plant)
- **Bitchû** (untopped plant)
- **"** (topped plant)
plant ("PSW") (represented in mg CO$_2$ per minute) were carried out. Fig. 4 summarizes the results for each individual plant of the three varieties used. The variation in activity from one individual plant to another was practically negligible.

As shown in Fig. 4, there were two distinguishable types in the curves of "PSW"
against growth stage in the tested three varieties. Among them, *Bright Yellow* and *White Burley* belong to the first type which has two peaks in its curve. The maximal "PSw"-value observed at the budding period was followed by a gradual decrease, which in its turn was followed by a small retrieval observed at the end of the flowering period. To these two varieties, the topping-treatment brought a temporary decrease immediately after the treatment and again a small retrieval of the activity was observed about 10 days later than in the untopped plant.

The difference in cultivational properties—i.e., *Bright Yellow* and *White Burley* belong to the air-cured type and *Bitchu* to the sun-cured type in the technical terms of tobacco cultivation—seemed to be reflected in the "PSw"-growth stage curves as shown in Fig. 4. In the case of *Bitchu*, there were three peaks in the curve: before the budding period (60 days after transplantation), at the flowering period (80 days after transplantation) and at the late maturing period (100 days), respectively, and the second peak was followed by a marked decrease until the third peak appeared. Besides this peculiar feature of *Bitchu* variety in the photosynthetic activity per individual plant in relation to the progress of growth stage, the experiments with *Bitchu* variety also showed that the topping-treatment had no effect on the photosynthetic activity, and there was no significant difference in the curves of photosynthetic activity between the topped plants and untopped ones.

(b) Photosynthetic activity per unit leaf area ("PSLA"): The photosynthetic activity per unit leaf area ("PSLA") can be calculated from the quotient: total activity of photosynthesis per individual plant ("PSw")/total leaf area of the same plant. While there was a remarkable difference between the "PSw"-growth stage curves of the three varieties, this was not the case in the photosynthetic activity per unit leaf area ("PSLA").

As can be seen from Fig. 5, the time course of "PSLA" showed a peak at about 50 days after transplantation irrespective of the varieties used, while, in the case of "PSw" of the three varieties used, their maximal peaks ranged from 60 to 70 days after transplantation. The maximal value of photosynthetic activity per unit leaf area was 24 mg CO₂/100 cm²/hr., and this is in agreement with the values reported by other investigators.

The first peak was followed by a gradual decrease, which in its turn was followed by a small retrieval observed at the late flowering period (about 100 days after transplantation), which finally fell off. Whereas the differences among the three varieties used were almost negligible in the time course of photosynthetic activity per unit leaf area in the case of the untopped plants, a noticeable difference can be observed in the topped plants in the following three points: (a) First, with both *Bright Yellow* and *White Burley* topping-treatment brought a remarkable decrease in "PSLA", while *Bitchu* was almost insensitive to this treatment. (b) Secondly, in connection with the small retrieval in "PSLA" observed at the late flowering period, that of the topped plant fell behind that of the untopped plant by 10 days in the cases of *Bright Yellow* and *White Burley*, while there was little time lag between untopped and topped plants in the case of *Bitchu*. (c) Thirdly, both the drop in photosynthetic activity observed at the early stage of maturation and the retrieval at the late stage of maturation were more remarkable in *Bitchu* than in *Bright Yellow* and *White Burley*.

These physiological characteristics of *Bitchu* variety may reflect its cultivational properties, namely that the time for picking the leaves of *Bitchu* comes late in comparison with *Bright Yellow* and *White Burley*, and the topping-treatment is not
absolutely necessary for a good harvest of Bitchû leaves.

(3) Variation in respiratory activity: The total activity of respiration per individual plant showed a similar rise and fall to that of photosynthetic activity, though the extent of variation was small. The first peak was observed at about 60 days after transplantation in the case of Bitchû, and 80 days in the cases of Bright Yellow and White Burley, and after 90 days or more a small, second peak was observed in every case.

Fig. 5. Relation between photosynthetic activity per unit leaf area and progress of growth stage.

A, ★—★, White Burley (untopped plant)
   ○—○, " (topped plant)
B, ▲—▲, Bright Yellow (untopped plant)
   △—△, " (topped plant)
C, ×—×, Bitchû (untopped plant)
   ×—×, " (topped plant)
* Explanation in text.
Discussion

The investigation reported in this paper is of interest in the following two points. In the first place, the ups and downs in the curves of photosynthetic activity during the whole stage of growth could be traced while keeping the test plant intact. Secondly, the effect of topping-treatment was judged by a comparison among three varieties of tobacco plant of different cultivational characteristics. It may be worth while to discuss these points.

(1) Relation between photosynthetic activity and progress of growth stage: From the comparison of Figs. 3, 4 and 5, it will be seen that both the total leaf area and “PS_w” reached the maximal value at the early budding stage (i.e., 60~70 days after transplantation), while the maximum of “PS_LA” was observed at a still earlier period, i.e., 50 days or so after transplantation, at a stage in which tobacco plants had not reached the climax of their growth. The physiological significance of these observations is that the photosynthetic activity of the whole leaf system was very active till about 50 days after transplantation, and after that the increase in “PS_w” was small as compared with that in total leaf area, because old leaves having poor activity increased in number and contributed only to the increase in leaf area.

Taken together with the changes in cell constituents during the whole stage of growth which will be reported in detail in our next paper, the observations reported in this paper become more interesting. The starch contents of leaves reached the maximum about 50 days after transplantation, whereas that of protein-N lagged far behind it, reaching its maximum 10 days later. From these two findings it can be suggested that the active photosynthesis till about 50 days after transplantation results in a good reservation of starch, and this is followed by protein synthesis in which the substances derived from starch serve as a carbon source for protein, and protein synthesis is most active at the period of the climax of leaf area development, i.e., 60~70 days after transplantation.

Taken together with the data obtained with rice plants, the results presented here are of interest, as the rather complex ups and downs in “PS_w” during the growth have been confirmed and two or three peaks have been observed in the curves of photosynthesis rate against its time course. Shimizu and Tsuno reported that the photosynthetic activity of rice plants during the whole of their growth stages had also three peaks, namely, the first peak at the maximal tillering period, the second at the early heading stage and the last at the late heading period.

The results presented in this paper are incompatible with Miyazaki's results. He reported that the photosynthetic activity, “PS_LA”, decreased gradually with the progress of growth stage, and that the photosynthetic activity per mg protein-N was, however, almost constant irrespective of the growth stage. On the contrary, in our experiment, there can be seen a rather complicated ups and downs in “PS_LA”, and the maximal value was observed at about 50 days after transplantation and the second, small retrieval peak was seen at the late stage of flowering. Examining Miyazaki's data carefully, it seems that his conclusion that there was only a simple decrement in the curve of “PS_LA” was rather overhasty, because there were many observed values which deviated from a monotonous decrement curve. Moreover, his experiment was carried out using detached tobacco leaves and was limited to the early stage of growth, while in our investigation intact and individual plants as a whole were used as test materials and the activities were examined throughout
the whole stage of growth. It is likely that there are considerable differences between the results obtained with detached leaves and those obtained with the whole individual plant. The investigation of this possibility must be a subject for future research.

(2) Effect of topping-treatment: Among our observations, the following was of interest from the view point of the cultivation of tobacco plants. The topping-treatment is effective in both Bright Yellow and White Burley for producing tobacco leaves of good quality, while Bitchû, a native variety, is rather insensitive to it. These cultiginal characteristics may be closely related to the physiological behaviour that the photosynthetic activities of the former two varieties were sensitive to the topping-treatment, while those of the latter variety were insensitive to it. This point is being investigated further.

The authors wish to thank the members of the Okayama Tobacco Experiment Station of the Japan Monopoly Corporation (Messrs. K. Ishidoya, E. Masuda, Y. Kawakami and T. Miyazaki) for supplying the tobacco seedlings for this study. Thanks are also due to Miss Mary F. McCrimmon for her kindness in reading the original manuscript.

Summary

1) Using a measuring system, in which a Liston-Becker infra-red gas analyzer (Model 15A) was used as the detecting apparatus, the relationship between the progress in growth stage and the changes in photosynthetic activity of tobacco plants was investigated, and the effect of the topping-treatment was also studied here. The following three cultivated varieties of tobacco plant were used as the test materials: White Burley, Bright Yellow and Bitchû.

2) The principal results obtained in this study were as follows:
(a) The changes in the total activity of photosynthesis per individual plant with the progress of growth stage was not simple, but there were two or three maxima which were found at the maximal growth stage and at the periods of maturity.
(b) The maximum of the photosynthetic activity per unit leaf area was observed at an earlier stage than the maximum of total activity of photosynthesis per individual plant, i.e., about 50 days after transplantation, and it was followed by a decrease, which in its turn was followed by a small increase at the climax of maturity.
(c) A marked effect of topping-treatment was observed in the cases of White Burley and Bright Yellow, but this was not the case with Bitchû.

3) The relationship among the photosynthetic activity, the progress of growth stage and the effect of topping-treatment was discussed.

References

藤茂 宏・和田喜徳・砂口博志・大森 譲：タバコ植物の生理学的研究 I.光合成活性と生育段階との関連および摘芯処理の光合成活性におよぼす影響

1) Infra-red gas analyzer (Liston-Becker Model 15 A) を検出器として用いた測定装置を組み立て、タバコ植物の光合成活性と生育段階との関連および摘芯処理の光合成活性におよぼす影響をしらべた。実験材料としては次の 3 株培品種のタバコを用いた：ホワイトバレー種、ブライトイェロー種および偽中種。

2) 得られた知見の主なものは次の如くである。
   (a) 生育段階の進行に伴なう個体当たりの全光合成活性の消長は単調な経過ではなく、最大成長期から成熟期にかけて 2 乃至 3 の山を示した。
   (b) 単位葉面積当たりの光合成活性は個体当たりの全光合成活性が最高値を示す時期よりももっと早く、移植後 50 日ごろに最大であった。その後、次第に低下し、成熟期に再び若干の回復を示すことが認められた。
   (c) 摘芯処理の影響はホワイトバレー種とブライトイェロー種の場合は顕著に認められたが、在来種の偽中種ではほとんど認められなかった。

3) 生育段階の進行に伴なう光合成活性の消長および摘芯処理の影響について論議した。（岡山大学理学部生物学教室）