Jpn. J. Crop Sci. 61(1) : 131—135 (1992)

# Maintenance Respiration in Early Growth Stage of Corn (*Zea mays* L.)

Tadachika OOTA*, Katsuhiro WAKABAYASHI** and Katsu IMAI***
(*,***Isotope Center, Tokyo University of Agriculture, Setagaya, Tokyo 156, Japan ;
***Institute of Agriculture and Forestry, University of Tsukuba,
Tsukuba, Ibaraki 305, Japan)
Received July 30, 1991

**Abstract** : The time-course change of dark respiration and the maintenance component of respiration in corn seedlings were studied by the starvation method. In the dark, leaf growth at the 4, 5 and 6th positions from the 14, 21 and 28 day-old plants ceased by 96—120 h. The time-course change of dark respiration was divided into three phases : initial rapid decreasing phase (<10 h), gradual decreasing phase (10—84 h) and non-decreasing phase (84 h<). During the 84—120 h of darkness, shoot respiration attained steady levels (1.06—0.84 mg $g^{-1}$ $h^{-1}$ at 25°C). It was inferred from above two observations that the respiration at 96—120 h measured by IRGA was equivalent to maintenence component for corn seedlings. Younger plants were estimated to have a lower proportion of maintenance component to total respiration than older ones at the initial stage of darkness (10 vs. 18%). The dark efflux of $^{14}CO_2$ from current photosynthates supported these findings.
**Key words** : $^{14}CO_2$, Corn (*Zea mays* L.), Dark respiration, Leaf growth, Maintenance respiration, Starvation method.

**トウモロコシの生育初期における維持呼吸** : 大田忠親・若林克拓・今井　勝*** (東京農業大学アイソトープセンター・***筑波大学農林学系)

**要　旨** : トウモロコシ幼植物における暗呼吸の経時変化及び維持呼吸の割合を飢餓法 (Starvation method) により検討した. 14, 21, 28 日齢の植物の各々第 4, 5, 6 葉の暗黒下での伸長生長は, 96—120 時間で停止した. 地上部の呼吸の経時変化は, 10 時間までの急激な低下, 10—84 時間の漸減, 84 時間以降の定常状態 (1.06—0.84 mg $g^{-1}$ $h^{-1}$, 25°C) の三相に区分された. そこで, 葉の伸長停止を考慮し, 96—120 時間の値をもって維持呼吸と見做した. 暗期初期の呼吸に占める維持呼吸の割合は, 14 日齢の植物で 10%, 28 日齢の植物で 18% と, 齢の進行に伴い上昇した. しかし, これらの値は他で報じられた成熟植物に比べると低い割合であった. また, 光合成により取り込んだ $^{14}CO_2$ の暗黒下での放出の経時変化は, 上記の三相の存在を支持するものであった.
**キーワード** : 暗呼吸, 維持呼吸, 飢餓法, $^{14}CO_2$, トウモロコシ, 葉の生長.

Respiration is required to provide energy and substrates for all biochemical processes in crop plants including the formation of new growth and the turnover of cell components. The concept of distinguishing respiration into growth ($R_g$) and maintenance ($R_m$) components which correspond to the requirements for synthesis of new cell constituents and to the demands of the existing phytomass, has an agronomical significance as proposed by McCree[5] in 1970, where $R_g$ and $R_m$ were expressed as the functions of gross photosynthesis and phytomass. On the other hand, the selection to obtain an increased phytomass by lowering the respiration has been performed in perennial ryegrass without agronomically undesirable consequences[4], though it may not be all the case[8]. Therefore, it is worthwhile to examine the physiological significance of respiration in terms of growth and maintenance.

It was inferred from the $^{14}CO_2$-feeding experiments that when crop plants were kept in the dark, they initially consumed current photosynthates for both $R_g$ and $R_m$ and under prolonged darkness, they exclusively used storage substances for $R_m$[10,11,12,13]. This inference was proved recently in part by using the steady-state $^{13}CO_2$-feeding technique with rice seedlings[19].

The $R_m$ is influenced by temperature[10], day length[2] and plant species[12]. It is also known that $R_m$ shows ontogenetic change in several plant species[1]. In the present study, we aimed at knowing the time-course change of respiration and the $R_m$ in respiration within a narrow range of ontogenesis in corn plants such as 14—28 days-old.

** Present address : Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, Japan.

There are four methods to partition respiration into $R_g$ and $R_m$ ; regression method, starvation method, mature tissue method and theoretical method[1]. Each method has merits and demerits from its own derivation. In the present experiments we adopted the starvation method, though this might have a tendency to estimate $R_m$ lower than that expected from a vigorously-growing plant[1].

## Materials and Methods

### 1. Plant materials

A hybrid corn (*Zea mays* L. cv. Koh 3) was used. Five germinated seeds were sown on each of 1/5000 a Wagner pot packed with vermiculite and were raised in an artificially-lit growth cabinet (12-h photoperiod at 30°C and 12-h dark period at 25°C). Light was supplied by metal halide lamps at 820 $\mu$mol $m^{-2}$ $s^{-1}$ PPFD and the saturation deficit of air was maintained at 1.7/1.3 (light/dark) kPa. Plants were given 1/500 strength liquid fertilizer (HYPONeX®) appropriately throughout the experimental periods. On day 10, plants were thinned to one per pot and at 14, 21 and 28 days after the sowing, they were provided for the growth and respiration measurements.

### 2. Measurement of leaf growth during the prolonged darkness (Expt. 1)

Plants were kept in the dark for 7 days in a growth cabinet at 25°C. The leaf lengths at the 4, 5 or 6th position from the base, respectively, of the 14, 21 or 28 day-old plants were measured once a day by a scale under dim light.

### 3. Measurement of dark respiration rate by IRGA (Expt. 2)

This type of starvation method was demonstrated by McCree[6]. The shoots of the 14, 21 or 28 day-old plants were enclosed with acrylic assimilation chambers (27×15×7 cm) at the end of daytime and the air containing ca. 350 $\mu$mol $mol^{-1}$ $CO_2$ was introduced into each of the chambers at a flow rate of 2.5 L $min^{-1}$ under dark. During the 5 days of the prolonged dark period, respiratory $CO_2$ efflux at 25°C from the shoots was measured by a differential type IRGA (Fuji, Model PHT-IU) and the rate was expressed on the dry weight basis.

### 4. Measurement of dark $^{14}CO_2$ efflux from $^{14}$C-photosynthates (Expt. 3)

Ryle et al[15]. reported this type of starvation method. The shoots of the 14, 21 or 28 day-old plants were enclosed with transparent polyethylene bags (ca. 10 L) and 1.85 MBq $^{14}CO_2$ was generated by adding 10% perchloric acid into $NaH^{14}CO_3$ in the light. After the photosynthetic feeding of $^{14}CO_2$ for 20 min at 25°C and 820 $\mu$mol $m^{-2}$ $s^{-1}$ PPFD, plants were immediately transferred into open-airflow dark chambers (27×15×7 cm). The respiratory $^{14}CO_2$ efflux at 25°C in an air stream (0.4 L $min^{-1}$) from the chamber was absorbed into 1 N NaOH solution for 8 days. The solution was collected at regular intervals and was mixed with the alkaline scintillator (Bray's solution)[3]. Specific radioactivity of the mixture was measured with a liquid scintillation counter (Aloka, Model LSC-900) after the 12 h of darkness and the rate of $^{14}CO_2$ efflux was expressed on the dry weight basis.

Ten plants in Expt. 1 and 4 plants in Expt. 2 and 3 for each age were examined, and the data shown in the figures were expressed as percentages of the initial values taken at the start of the dark period (0h for the leaf length and the $^{14}CO_2$ efflux and 1 h for the IRGA measurement).

## Results

### 1. Growth of leaves in the dark

Figure 1 shows the time-course changes of leaf length at specific positions. Corn plants at 14 and 21—28 days-old, respectively, ceased their leaf growth by 5 and 4 days (120 and 96 h) in the dark. Initial lengths±SE of the 4, 5 and 6th leaves from the 14, 21 and 28 day-old plants were 58.6±2.7, 113.7±4.4 and 125.5±2.8 mm and final ones were 129.4±7.2, 131.4±4.3 and 139.0±3.0 mm, respectively. As the 4th leaves from 14 day-old plants were small, their growth in the dark was the largest among the leaves used. Similar patterns were observed in the case of plant lengths (data not shown).

### 2. Changes of dark respiration rate with time

Respiration rates obtained by IRGA measurements declined exponentially with time in the dark. Just after the transfer of plants into darkness, rapid $CO_2$ efflux occurred and the rates were variable so that we regarded the values after 1 h in the dark as the initial ones. These were well fitted by $Y=81.09-36.74$

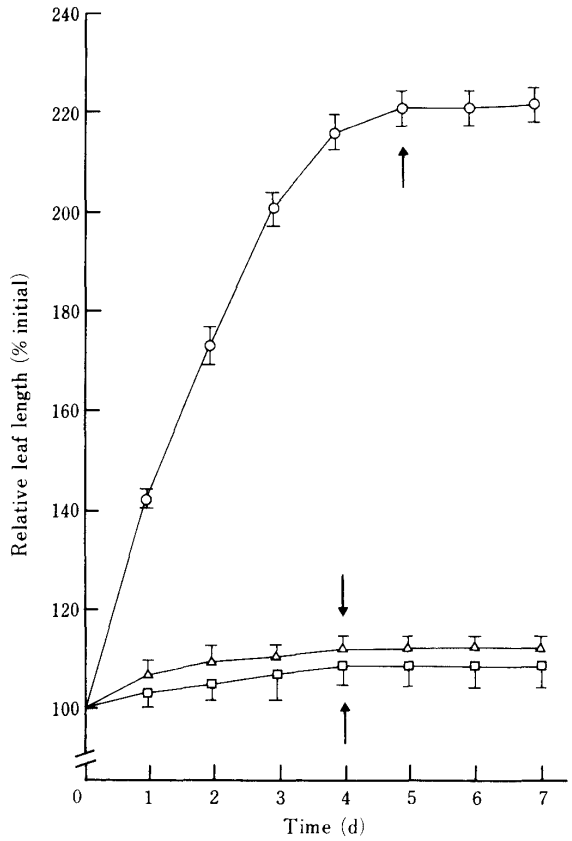


Fig. 1. Growth of leaves in the dark.
○, △, □ : The 4, 5 and 6th leaves of the 2, 3 and 4 week-old plants, respectively. Arrows indicate the cessation of leaf growth and bartical bars, 2 x SEM.

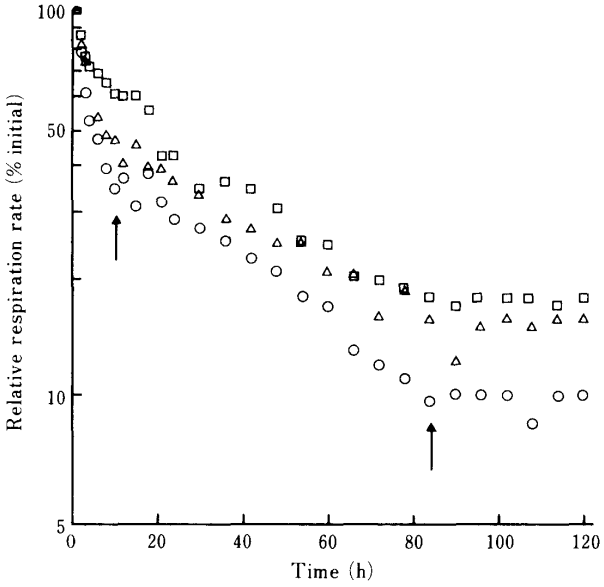


Fig. 2. Changes of respiration rates with time.
Symbols are the same as those in Fig. 1. Arrows indicate turning points of the rates.

logX ($r^2=0.937$), $Y=88.74-37.67$ logX ($r^2=0.968$) and $Y=100.08-41.41$ logX ($r^2=0.977$) for plants at 2, 3 and 4 weeks-old where Y was respiration in % initial and X was time in hours. When plotted on the semilogarithmic graphs (Fig. 2), the relative respiration rates were inversely related to time and the inclination was divided into three phases; initial rapid decrease until 10 h, successive gradual decrease between 10 and 84 h, and steady-state phase after 84 h. Results in Fig. 2 demonstrated that the pattern of relative decline of respiration was different among plant ages. In older plants, the decline of respiration was smaller and the relative level of respiration at prolonged darkness was higher than those in younger ones. Absolute respiration rates of the 14, 21 and 28 day-old plants at 1 h were 10.77, 7.01 and 4.78 mg $g^{-1}$ $h^{-1}$, respectively. These declined to 1.06, 1.06 and 0.84 mg $g^{-1}$ $h^{-1}$ in average during 84—120 h of darkness. At the initial stage of darkness (1 h), the percentages of $R_m$ in total respiration by the 14, 21 and 28 day-old plants were estimated to ca. 10, 15 and 18, respectively, assuming that the respiration during 84—120 h was equivalent to $R_m$.

### 3. Changes of dark $^{14}CO_2$ efflux with time

Since the data plots started from 4.5 h (average of 3—6 h), the initial (0h) $^{14}CO_2$ efflux from shoots was obtained by extraporation. The respiratory efflux of $^{14}CO_2$ from $^{14}$C-photosynthates followed a similar change with that measured by IRGA. However, when relative radioactivities were plotted on the semilogarithmic graphs, three lines were roughly overlapped; initial rapid decrease until 18 h, successive gradual decrease between 18 and 102 h, and steady-state phase after 102 h (Fig. 3). They were fitted to $Y=100 \cdot 0.883X$, $Y=28.84 \cdot 0.947X$ ($r^2=0.904$) and $Y=0.110$, respectively, where Y was relative $^{14}CO_2$ efflux in % initial and X was time in hours. It seemed that the relative level of $^{14}CO_2$ efflux after 102 h was different with age but at that stage, relative values were only $0.110 \pm 0.043$% of the initial ones. Initial radioactivity in the $^{14}CO_2$ efflux was 163.0, 45.0 and 12.5 kBq $g^{-1}$ $h^{-1}$ in the 14, 21 and 28 day-old plants, respectively. These declined to 1.40, 0.59 and 0.26 kBq $g^{-1}$ $h^{-1}$ at 114 h. The

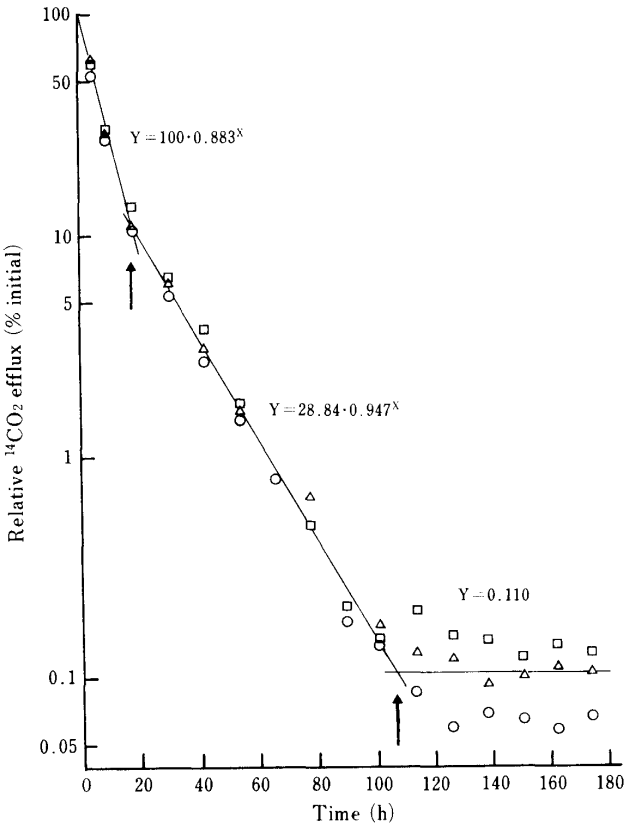


Fig. 3. Changes of dark $^{14}CO_2$ efflux with time.
Symbols are the same as those in Fig. 1. Arrows indicate turning points of the rates.

percentage of $^{14}CO_2$ efflux at 114 h to the initial one was 0.9, 1.3 and 2.1, respectively, at the 14, 21 and 28 day-old plants because $^{14}CO_2$ efflux occupied only part of the respiration under prolonged darkness.

## Discussion

There are several ways of $R_m$ measurement by the starvation method. One is to measure the steady-state $CO_2$ efflux from plants by IRGA under a prolonged darkness[6]. Another is to measure the steady-state $^{14}CO_2$ efflux in the dark in which $^{14}CO_2$ is fed photosynthetically[15]. In the former method, $R_m$ was assumed to be the value obtained at 48 h of darkness and the latter, 24 h of darkness. On the other hand, Yamaguchi and Tanaka[20] regarded corn respiration at 120—192 h (5—8 days) of darkness as $R_m$ when new growth ceased, in which the times of the cessation of leaf number increment and panicle elongation were determined.

To establish an appropriate method of measuring $R_m$ in young corn plant, we compared the above three methods. As shown in Fig. 1, the leaf growth ceased at about 96—120 h (4—5 days) of darkness. The difference (24 h) was probably due to their plant age because the source of energy metabolism from storage materials in older plant was thought to be larger (cf. 120—192 h of Yamaguchi and Tanaka[20]). Figure 2 showed that from about 84 h, respiration rates took a steady-state until 120 h in the dark. Similarly in Fig. 3, $^{14}CO_2$ efflux became the lowest and steady levels were obtained from 102 h of darkness. Our results indicated longer times to obtain $R_m$ than other reported ones but in fact, the measurement of respiration during 96—120 h of darkness by IRGA should be preferred for the estimation of $R_m$. The measurement of $^{14}CO_2$ efflux in a prolonged darkness was not suitable for the estimation of $R_m$ because it showed only a part of respiration.

As to plant age, Silsbury[16] reported that in subterranean clover, $R_m$ decreased slightly with age but the percentage in total respiration increased due to the increase in plant size. Similar small ontogenetic change was observed in sorghum where the decrease of $R_m$ with ontogeny (from 1.6 to 1.2 mg $CO_2$ $g^{-1}$ $h^{-1}$) was ascribed to the decrease in protein content[7]. On the other hand, rather drastic decline of $R_m$ with sorghum ontogeny (from 24 to 6 mg C $gC^{-1}$ $d^{-1}$; vegetative vs. reproductive) was observed in accordance with the decrease in protein content[17]. From the above and our present data (1.06—0.84 mg $CO_2$ $g^{-1}$ $h^{-1}$) by IRGA measurement, it can be said that there is not much difference of $R_m$ in narrow range of ontogeny.

In rice and corn, the percentage of $R_m$ in total respiration was estimated to be 20—30[9,20]. Our results obtained from IRGA measurements were 10—18% at 25°C and the youngest plants showed the lowest percentage and accorded with the tendency observed by Silsbury[16]. This may indicate that at a young seedling stage, the maintenance process is not much significant compared to the construction of new cell components. At the initial stage of darkness, specific respiration rate of the youngest (14 day-old) corn shoots was 10.77 mg $g^{-1}$ $h^{-1}$. This is comparable for young plants, including corn, reported elsewhere[18] and is indicating vigorous construction of new

phytomass.

In the starvation method on corn[20], rice[11] and legumes[10], a rapid decrease of respiration rate at the initial stage of darkness and following gradual decrease at prolonged darkness were reported. Oosaki and Tanaka[12], who applied $^{14}CO_2$ to rice plants under field conditions, assumed from the pattern of $^{14}CO_2$ efflux before and after 48 h of darkness that the characteristics of respiration changed at around 48 h, i.e. substrate from current photosynthates to reserved materials. Our results demonstrated the three phases of respiratory change (Fig. 2) : initial rapid decrease until 10 h, progressive decline during 10—84 h and nonchanging phase after 84 h. Especially in the second phase, both the current photosynthates and the reserved materials were thought to be consumed for $R_g$ and $R_m$ by changing their contribution.

## References


1. Amthor, J.S. 1989. Respiration and Crop Productivity. Springer-Verlag, New York. 1—215.
2. Hansen, G.K. and C.R. Jensen 1977. Growth and maintenance respiration in whole plants, tops, and roots of *Lolium multiflorum*. Physiol. Plant. 39 : 155—164.
3. Ishikawa, H. 1981. Measurement Method for Liquid Scintillation, 2nd ed. Nanzando, Tokyo. 8—23**.
4. Kraus, E., D. Wilson, M.J. Robson and C.L. Pilbeam 1990. Respiration : correlation with growth rate and its quantitative significance for the net assimilation rate and biomass production. In Causes and Consequences of Variation in Growth Rate and Productivity. (Eds.) H. Lambers, M.L. Cambridge, H. Konings and T.L. Pons, SPB Academic Publishing bv, The Hague. 187—198.
5. McCree, K.J. 1970. An equation for the rate of respiration of white clover plants grown under controlled conditions. In Prediction and Measurement of Photosynthetic Productivity. (Ed.) I. Setlik, Pudoc, Wageningen. 221—229.
6. ——— 1974. Equations for the rate of dark respiration of white clover and grain sorghum, as functions of dry weight, photosynthetic rate, and temperature. Crop Sci. 14 : 509—513.
7. ——— 1983. Carbon balance as a function of plant size in sorghum plants. Crop Sci. 23 : 1173—1177.
8. ——— 1988. Sensitivity of sorghum grain yield to ontogenetic changes in respiration coefficients. Crop Sci. 28 : 114—120.
9. Murata, Y. and J. Yamaguchi 1979. Photosynthesis and dry matter production. In Mechanism of Photosynthesis (Protein, Nucreic Acid and Enzyme. Extra 21). (Eds.) H. Fujisige, S. Miyachi and Y. Mukohata, Kyoritu Shuppan, Tokyo. 203—213**.
10. Oota, T., S. Yamaguchi, M. Otani and Y. Murata 1982. Changes in the dark respiratory rate and the use of respiratory substrate after feeding $^{14}CO_2$ in several legume plants. Bull. Radioisotope Res. Inst., Tokyo Univ. Agric. 3 : 13—17*.
11. ——— and Y. Murata 1984. On the use of $^{14}CO_2$ fixed products of photosynthesis for dark respiration in rice seedlings kept in the dark. J. Agric. Sci., Tokyo Nogyo Daigaku 28 : 270—273*.
12. Osaki, M. and A. Tanaka 1979. "Current photosynthates" and "storage substances" as the respiratory substrates in the rice plant. Japan. J. Soil Sci. Plant Nutr. 50 : 540—546**.
13. ——— and ——— 1982. Relationship between "current photosynthate and storage substance respirations" and "growth and maintenance respirations" in the rice plant. Japan. J. Soil Sci. Plant Nutr. 53 : 93—98**.
14. Penning de Vries, F.W.T. 1975. The cost of maintenance processes in plant cells. Ann. Bot. 39 : 77—92.
15. Ryle, G.J.A., J.M. Cobby and C.E. Powell 1976. Synthetic and maintenance respiratory losses of $^{14}CO_2$ in uniculm barley and maize. Ann. Bot. 40 : 571—586.
16. Silsbury, J.H. 1979. Growth, maintenance and nitrogen fixation of nodulated plants of subterranean clover (*Trifolium subterraneum* L.). Aust. J. Plant Physiol. 6 : 165—176.
17. Stahl, R.S. and K.J. McCree 1988. Ontogenetic changes in the respiration coefficients of grain sorghum. Crop Sci. 28 : 111—113.
18. Yamagishi, J., S. Akita and J. Takanashi 1990. Time-course of respiration and its relation to water conditions in various plant species. Jpn. J. Crop Sci. 59 : 169—173.
19. ———, H. Kouchi and T. Yoneyama 1991. Source of respiratory carbon evolved from the shoots of rice seedlings in darkness. Jpn. J. Crop Sci. 60 : 447—452.
20. Yamaguchi, J. and A. Tanaka 1970. Studies on the growth efficiency of crop plants. III. The growth efficiency of the corn plants as affected by growing condition. J. Sci. Soil and Manure, Japan 41 : 509—513**.


* Japanese with English summary.

** Japanese only.