ON-LINE MEASUREMENTS OF CO_2 AND H_2O GAS FLUXES, SAP FLUX AND EXPANSIVE GROWTH IN AN INTACT TOMATO FRUIT

Kitano, Masaharu
Biotron Institute Kyushu University

Araki, Takuya
Biotron Institute Kyushu University

Hamakoga, Michio
Biotron Institute Kyushu University

Eguchi, Hiromi
Biotron Institute Kyushu University

出版情報：BIOTRONICS. 26, pp.85-94, 1997-12. Biotron Institute, Kyushu University
バージョン：
権利関係：

BIOTRONICS **26**, 85–94, 1997

ON-LINE MEASUREMENTS OF CO_2 AND H_2O GAS FLUXES, SAP FLUX AND EXPANSIVE GROWTH IN AN INTACT TOMATO FRUIT

M. KITANO, T. ARAKI, M. HAMAKOGA and H. EGUCHI

Biotron Institute, Kyusyu University 12, Fukuoka 812-8581, Japan

(Received July 28, 1997; accepted August 31, 1997)

KITANO M., ARAKI T., HAMAKOGA M. and EGUCHI H. *On-line measurements of CO_2 and H_2O gas fluxes, sap flux and expansive growth in an intact tomato fruit.* BIOTRONICS **26**, 85–94, 1997. A system for on-line measurements of CO_2 and H_2O gas fluxes, pedicel sap flux and expansive growth in an intact tomato fruit was newly developed, where the gas fluxes in leaflets also can be measured. The system was composed of a fruit chamber, a leaf chamber, CO_2 and H_2O gas analyzing unit and an on-line computer. Furthermore a laser displacement sensors system was equipped in the fruit chamber for evaluating fruit expansive growth, and the pedicel sap flux was evaluated as sum of the H_2O gas flux and the growth rate in the fruit. Diurnal dynamics as affected by light condition were observed in respiration, transpiration, pedicel sap flux and expansive growth in the immature intact fruit, which were clearly enhanced in the light. In fruit water balance, about 30% of the sap flux imported through the pedicel was lost by transpiration from the calyx and the berry, and the residual 70% contributed to fruit expansive growth. These results suggest that the on-line system developed is applicable to quantitative analyses of respiration, sap flux and water balance of an intact tamato fruit in environmental studies on fruit expansive growth and photoassimilate translocation.

Key words: tomato fruit; *Lycopersicon esculentum* Mill.; fruit respiration; fruit water balance; pedicel sap flux; fruit growth.

INTRODUCTION

Expansive growth and sugar accumulation in tomato fruits depend on fruit water balance and translocation of photoassimilates (*1, 3, 6, 8, 15, 16*). Water balance in a tomato fruit has been analyzed by using the detached fruit under steady state (*1, 6*), but dynamics of water balance in the intact fruit has not been analyzed because of difficulties in on-line evaluations of transpirational water loss and imported sap flux in the fruit.

Photoassimilates translocation from leaves to fruits is regulated by the processes of unloading and postphloem transport of sugars in fruits as well as the processes of loading in leaves and long distance phloem transport (*2, 5, 10–13*). In tomato fruits at the stage of rapid sugar accumulation a few weeks after

anthesis, the postphloem transport (i.e. sugar transport from phloem to storage pericarp tissues) has been proved to be apoplastic transport, which involves energy-dependent sugar transport across plasma membranes (*13*). Therefore, it is necessary to investigate effects of fruit respiration on expansive growth and photoassimilate translocation in the intact fruit (*4, 15, 16*).

For dynamic analyses of water balance and fruit respiration with reference to expansive growth and photoassimilate translocation in an intact tomato fruit, the present paper deals with a system for on-line measurements of CO_2 and H_2O gas fluxes, pedicel sap flux and expansive growth in the intact fruit.

MATERIAL AND METHODS

System

A system for on-line measurements of CO_2 and H_2O gas fluxes, pedicel sap flux and expansive growth in an intact tomato fruit was newly developed, where the gas fluxes in intact leaflets also can be measured. Figure 1 shows a schematic diagram of the system. The system was composed of a fruit chamber (FC; $11\times3.5\times4.0$ cm), a leaf chamber (LC; $26\times15\times7.5$ cm), a gas analyzing unit, air ventilating pumps (AP_F and AP_L) and an on-line computer. FC and LC made of transparent acrylic plates were equipped with air mixing micro fans, ventilating openings and those shutters driven by servomotors (SM_F and SM_L). FC was also equipped with a pair of laser displacement sensors (Z4M-W40, OMRON Corporation, Kyoto) to measure fruit expansive growth in FC by applying the on-line system developed in the previous study (*9*). The gas analyzing unit consisted of an infrared CO_2 and H_2O gas analyzer (IRGA; LI6262, LI-COR, inc., Nebraska, U.S.), Teflon tubing and three-way solenoid valves (TSV_1 and TSV_2).

An intact fruit and leaflets were kept in the respective chambers of FC and LC which were ventilated enough by AP_F and AP_L. The sampling path was alternately switched from FC path to LC path or vice versa at intervals of 30s by manipulating TSV_1 and TSV_2 on-line. In the sampling path being operated (FC path or LC path), air sampled from the chamber was circulated into IRGA continuously, and CO_2 and H_2O gas concentrations ($[CO_2]$ and $[H_2O]$) were measured, where signals of $[CO_2]$ and $[H_2O]$ from IRGA were transmitted to the computer at intervals of 1s. For only 15s during this IRGA-analysis, the ventilating openings and air pump of the chamber being sampled were shut down to keep the chamber under the condition of a "closed system", where $[CO_2]$ and $[H_2O]$ varied according to the respective gas fluxes from the fruit or the leaflets. Thereafter the ventilation of the chamber was restarted, and the sampling path was switched to the other chamber. That is, the respective chambers were alternately kept in a "closed system" for 15s every minute. The switching of the sampling path and the on-off action of ventilation were regulated on-line by signals from the computer. Figure 2 shows photographs of fruit and leaf chambers applied in the system.

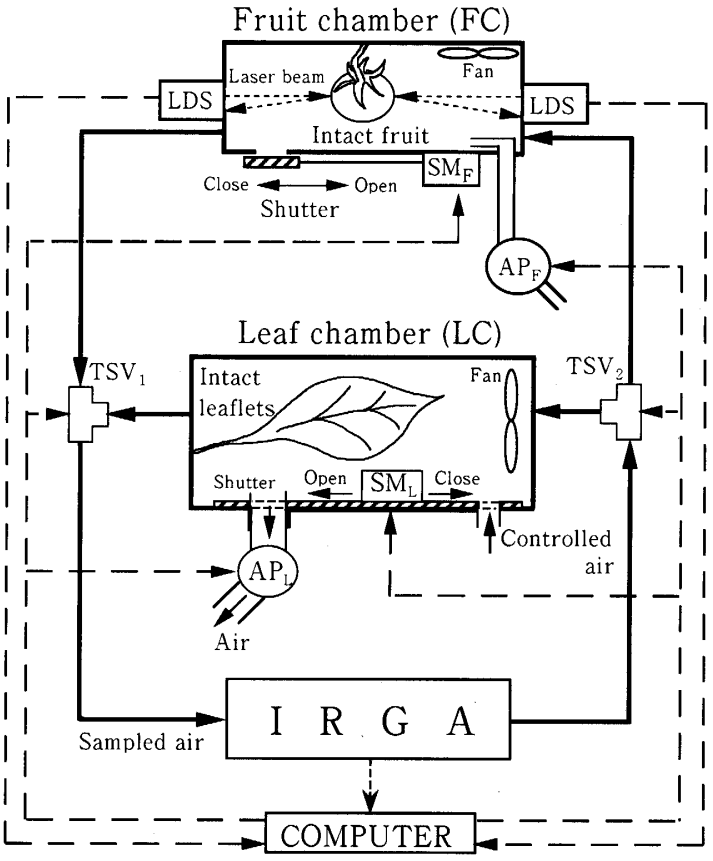

Fig. 1. Schematic diagram of a system for on-line measurements of CO_2 and H_2O gas fluxes, pedicel sap flux and expansive growth in an intact fruit and the gas fluxes in leaflets in a tomato plant: AP_F and AP_L, air pumps for ventilating the fruit chamber (FC) and the leaf chamber (LC), respectively; IRGA, infrared gas analyzer for measuring CO_2 and H_2O gas concentrations; LDS, laser dIsplacement sensors system for measuring fruit expansive growth; SM_F and SM_L, servomotors for driving the shutters of ventilating openings in the respective chambers; TSV_1 and TSV_2, three-way solenoid valves for switching the sampling path between FC path and LC path; dense solid line, air sampling path; broken line, signal line.

Evaluation of gas fluxes

Figure 3 shows typical patterns of time courses of $[CO_2]$ and $[H_2O]$ under the respective closed conditions in FC and LC, where the sampling path was alternately switched between FC and LC with intervals of 30s under light. $[CO_2]$ increased in FC by CO_2 flux (J_C) with fruit net respiration (respiratory CO_2 efflux minus photosynthetic CO_2 influx), but $[CO_2]$ in LC decreased by J_C with leaf net photosysthesis in the leaflets. $[H_2O]$ increased in FC and LC by H_2O flux (J_W) with transpiration from the fruit and the leaflets, respectively. The respective gas fluxes of J_C and J_W can be related to change rates of $[CO_2]$ and $[H_2O]$ in the closed conditions as (*7*)

$$J_C = V_{SYS} \cdot d[CO_2]/dt \quad (1)$$

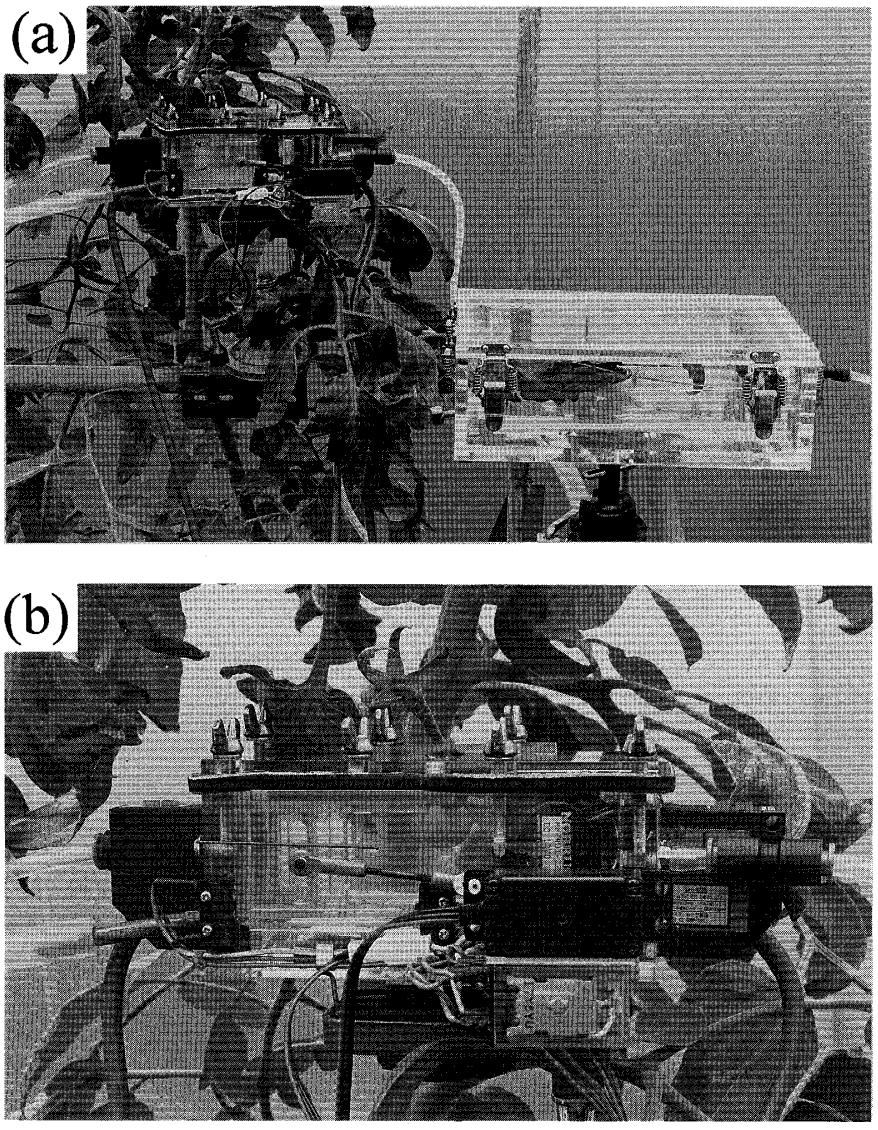

Fig. 2. Photograph (a) of a fruit chamber and a leaf chamber settled on a tomato plant and close up (b) of the fruit chamber.

$$J_W = V_{SYS} \cdot d[H_2O]/dt \qquad (2)$$

where $d[CO_2]/dt$ and $d[H_2O]/dt$ are the change rates of $[CO_2]$ and $[H_2O]$, respectively, and V_{SYS} is the quantity of the air within inner spaces of the closed system, which is different between FC path and LC path. $[CO_2]$ and $[H_2O]$ under the closed condition varied almost linearly as shown in Fig. 3, and therefore $d[CO_2]/dt$ and $d[H_2O]/dt$ were determined on-line by the linear regression analysis with 0.1% significance level. V_{SYS} involved air of inner spaces of the closed chamber (FC or LC), the Teflon tubing, the solenoid valves (TSV_1 and TSV_2) and IRGA (sampling cell, sampling pump, etc.), which were difficult to determine by direct measurement. Therefore, V_{SYS} was determined experimentally for FC path and LC path as follows: A standard gas from a CO_2

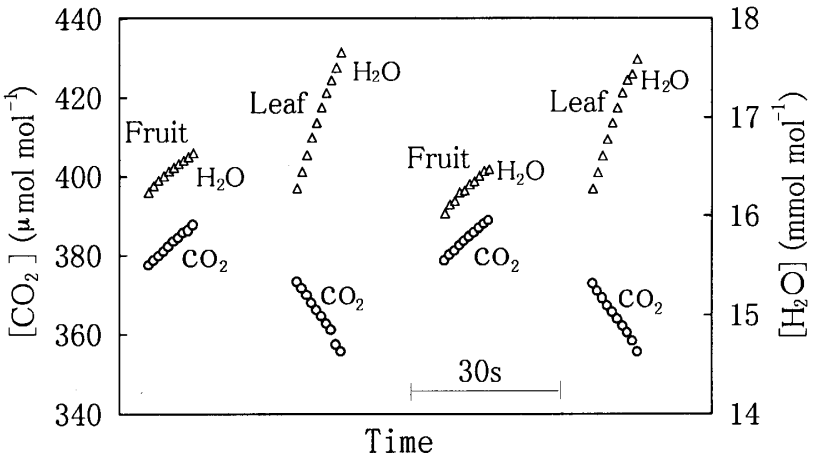

Fig. 3. Linear changes of CO_2 and H_2O gas concentrations in the fruit chamber and the leaf chamber under the closed condition, where the ventilating openings of the respective chambers were alternately shut for 15s every minute.

gas cylinder was supplied into the chamber (FC or LC) at a constant flow rate (q), where air in the chamber was allowed to leak at the same flow rate. Then carbon balance in the chamber can be expressed as

$$q(C_S - [CO_2]) = V_{SYS} \cdot d[CO_2]/dt \tag{3}$$

where C_S is the CO_2 gas concentration of the standard CO_2 gas supplied into the chamber. The standard CO_2 gas cylinder with C_S of 494 μmol mol^{-1} was used in this study, and the flow rate (q) was measured by a mass flow meter (FLOLONE, SEF-21, STEC inc., Kyoto). $[CO_2]$ was measured by IRGA on the sampling path, and $d[CO_2]/dt$ was determined by the linear regression analysis. Then V_{SYS} was evaluated as

$$V_{SYS} = q(C_S - [CO_2])/(d[CO_2]/dt) \tag{4}$$

By adjusting a valve of the CO_2 gas cylinder, q was set at different 16 and 26 rates for the respective chambers of FC and LC, and V_{SYS} in each of the closed condition of FC and LC was evaluated for every q value, where the variance in V_{SYS} values was remarkably small. Therefore, V_{SYS} in volume base was determined by averaging with the small standard deviation as $0.247 \pm 0.009\,l$ in FC path and as $2.61 \pm 0.09\,l$ in LC path. By adopting the respective V_{SYS} values for FC path and LC path, the flux evaluated by $V_{SYS}d[CO_2]/dt$ was almost equal to the flux evaluated by $q(C_S - [CO_2])$ as shown in Fig. 4. This suggests that the gas fluxes can be evaluated accurately from the respective Eqs. (1) and (2) by using V_{SYS} values determined above. J_C and J_W per fruit were represented by J_{CF} and J_{WF}, which involve the fluxes from the berry and the calyx, and J_C and J_W per unit leaf area were represented by J_{CL} and J_{WL}. The outward fluxes were expressed as positive values, and therefore J_{CF} and J_{CL} were positive for respiratory CO_2 efflux and were negative for photosynthetic CO_2 influx, and J_{WF} and J_{WL} were positive for transpirational water loss.

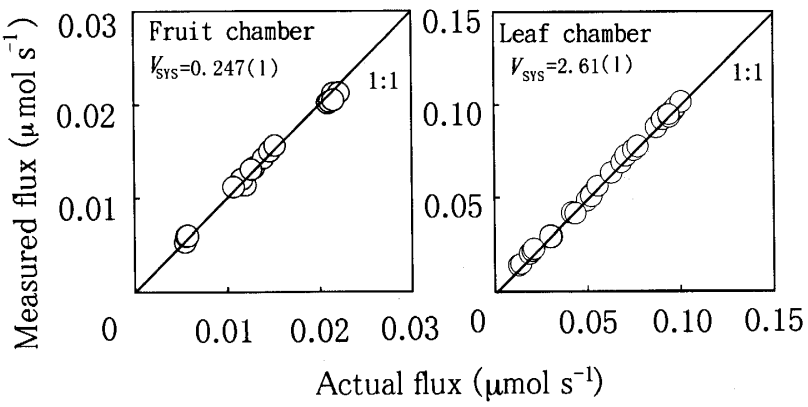

Fig. 4. Relationships between actual CO_2 fluxes and measured CO_2 fluxes in the fruit chamber and the leaf chamber. The actual flux $\{q(C_S-[CO_2])$ of Eq. (3)$\}$ was set at different values by varying rate (q) of gas supply from a CO_2 gas cylinder, and the measured flux was evaluated by $V_{SYS}\cdot d[CO_2]/dt$ of Eq. (3) by using V_{SYS} of 0.247 l for the fruit chamber and V_{SYS} of 2.61 l for the leaf chamber.

Evaluation of fruit expansive growth and pedicel sap flux

For evaluating expansive growth of the fruit kept in FC, fruit diameter (D_F; cm) was measured on-line by the laser displacement sensors (LDS) system equipped in FC (*9*), and the signal of D_F from the LDS system was transmitted to the computer at intervals of 1 min. D_F was converted into fruit volume (V_F; cm^3) on the basis of the predetermined relationship of $V_F=0.4177\cdot D_F^3+1.932$ (*9*), and fruit growth rate (*FGR*) on volume base was evaluated on-line. *FGR* depends on balance among phloem and xylem sap fluxes and transpirational water loss (J_{WF}) in the fruit as

$$FGR=J_P+J_X-J_{WF} \tag{5}$$

where J_P and J_X are the sap fluxes imported into the fruit through phloem and xylem, respectively. Then, the pedicel sap flux (J_P+J_X), which is impossible to measure directly, was evaluated on-line by using measured *FGR* and J_{WF} as

$$J_P+J_X=FGR+J_{WF} \tag{6}$$

Plant materials and experimental conditions

Tomato plants (*Lycopersicon esculentum* Mill. cv. Hausu-Momotaro) were potted in 8 l pots filled with vermiculite and were grown in a phytotron glass room at day/night temperature of 23/18℃ and relative humidity of 70%, where the vermiculite in the pots was kept moistened enough by dripping complete nutrient solution. The plants were pinched at two leaves above the first truss before anthesis of the second truss. A few weeks after anthesis of the second proximal fruit on the first truss, the plant was transferred into a growth chamber with air temperature of 20℃ and relative humidity of 70% under the artificial light of metal halide lamps (YOKO lamp, DR400 TOSHIBA CORPORATION,

Tokyo) through heat absorbing filters (HG, Ohara Optical Grass Mfg. Co. Ltd., Tokyo) with *PPFD* of 300 μmol m^{-2} s^{-1} in a photoperiod of 08:00–20:00. After two days acclimation to the growth chamber condition, the second proximal fruit on the first truss and the matured leaflets in the eighth leaf were kept in the respective chambers of FC and LC: The immature tomato fruit a few weeks after anthesis has been reported to be at the stage of rapid sugar accumulation (*6, 13*), and the eighth leaf in tomato plants has been reported to be one of the major source leaves supplying photoassimilates to the first truss (*14*). J_{CF}, J_{WF}, *FGR* and J_P+J_X in the fruit and J_{CL} and J_{WL} in the leaflets were evaluated in diurnal time course, and each value was represented as mean of three replications.

RESULTS AND DISCUSSION

Figure 5 shows diurnal time courses of J_{CF}, J_{WF}, V_F, *FGR*, J_P+J_X, $-J_{CL}$ and J_{WL}. J_{CF} in the fruit gradually increased in the early morning under the dark, but when the light was turned on, J_{CF} was dropped immediately by the start of CO_2 absorption for photosynthesis in the fruit (calyx and berry): From this drop in J_{CF} by lighting, the photosynthetic CO_2 influx in the fruit was estimated to be about 4 nmol s^{-1}. Thereafter, J_{CF} under light showed gradual increase till the late afternoon. When the light was turned off, CO_2 absorption by photosynthesis in the fruit was stopped, and J_{CF} instantly rose to the maximum, which was followed by two hours decrease under dark. From this time course pattern of J_{CF}, it was estimated that the respiratory CO_2 efflux from the berry gradually increased from the early morning to the late afternoon under light and decreased for two hours after darkening. J_{WF} also responded to the light, where the largely increased J_{WF} was found in the light period. The main part of this response of J_{WF} was considered to be attributed to stomatal response in the calyx, because vapor density difference between the fruit and the ambient air was not increased significantly by lighting.

FGR and J_P+J_X started to increase from the early morning under dark as observed in the respiratory CO_2 efflux from the berry. In the light period, *FGR* and J_P+J_X were kept higher than those under dark but appeared in irregular oscillations: Such oscillation was not found in fruit transpiration (J_{CF}). In the leaflets, $-J_{CL}$ and J_{WL} were also kept at low levels in the dark period: $-J_{CL}$ showed constant negative value by leaf respiration. When the light was turned on, $-J_{CL}$ and J_{WL} rose rapidly by photosynthesis and transpiration activated by the light, respectively, and those were kept at high levels in the light, while slight decreases were found in the late afternoon. Thus, this system performed reliable on-line measurements of diurnal dynamics of CO_2 and H_2O fluxes, pedicel sap flux and expansive growth in the intact tomato fruit as well as CO_2 and H_2O fluxes in the leaflets.

Table 1 shows cumulative respiration (ΣJ_{CF}), transpiration (ΣJ_{WF}), fruit growth (ΣFGR) and pedicel sap influx $\{\Sigma(J_P+J_X)\}$ in the fruit during the light period (08:00–20:00), the dark period (00:00–08:00 and 20:00–24:00) and the whole day (00:00–24:00). ΣJ_{CF} in the light period appeared a little less than

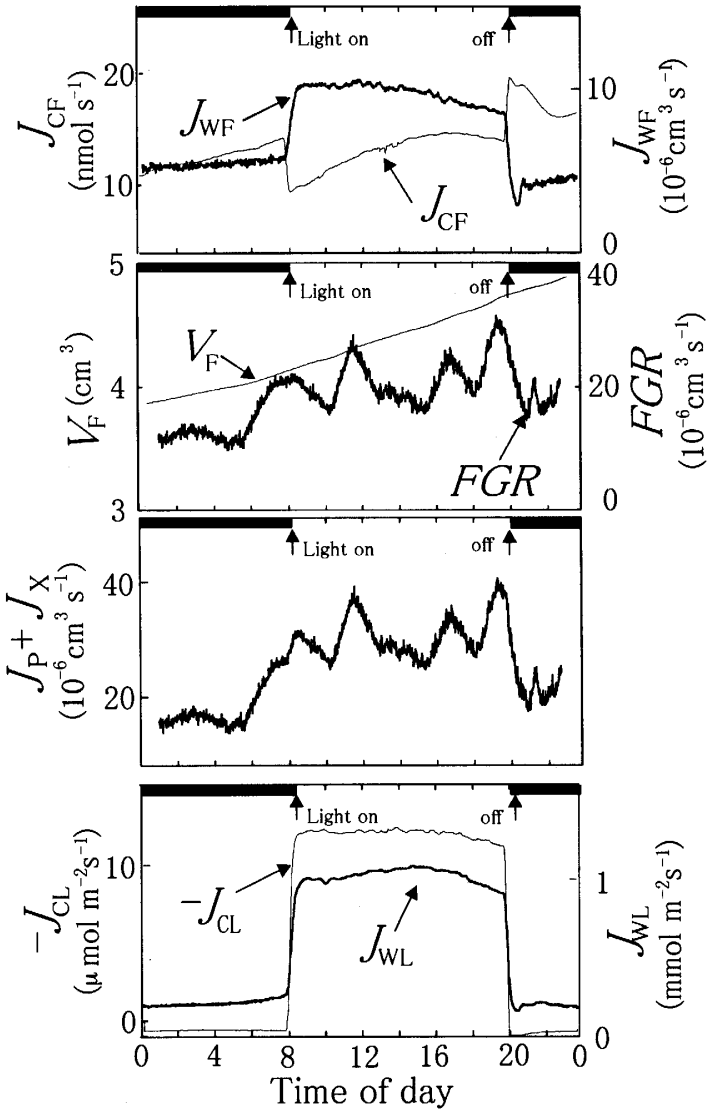

Fig. 5. Diurnal time courses of CO_2 flux ($-J_{CL}$) and H_2O flux (J_{WL}) in leaflets, CO_2 flux (J_{CF}), H_2O flux (J_{WF}), volume (V_F) and fruit growth rate (FGR) and pedicel sap flux (J_P+J_X) in an intact fruit. Values are means of three plants.

that in the dark period, because CO_2 was absorbed under light for photosynthesis in the fruit (calyx and berry). The respiratory CO_2 efflux, however, was estimated to be about 1.3 times larger in the light period than in the dark period: The respiratory CO_2 efflux cumulated during the light period was evaluated as 0.745 mmol by adding CO_2 influx absorbed by the fruit photosynthesis. ΣJ_{WF} in the light period was almost doubled by the enhanced stomatal transpiration in the calyx. Fruit growth and pedicel sap flux were also enhanced by lighting, and ΣFGR and $\Sigma(J_P+J_X)$ in the light period were about 1.7 times larger than those in the dark period. This result suggests that respiration, expansive growth and pedicel sap flux in the intact fruit were enhanced by lighting as well as

Table 1. Cumulative CO_2 flux (ΣJ_{CF}), H_2O flux (ΣJ_{WF}), fruit growth rate (ΣFGR) and pedicel sap flux $\{\Sigma(J_P+J_X)\}$ in an intact tomato fruit during the light period (08:00–20:00), the dark period (00:00–08:00 and 20:00–24:00) and the whole day (00:00–24:00). ΣJ_{CF} values in parentheses represent the cumulative fruit respiration, which were evaluated by adding the cumulative fruit photosynthetic CO_2 influx to ΣJ_{CF} under light. Values are means of three plants.

	ΣJ_{CF} (mmol)	ΣJ_{WF} (cm^3)	ΣFGR (cm^3)	$\Sigma(J_P+J_X)$ (cm^3)
Light	.573 (.745)	.419	.915	1.33
Dark	.595	.213	.547	.760
Light+Dark	1.17 (1.34)	.632	1.46	2.09

Table 2. Proportions of transpirational water loss (ΣJ_{WF}) and fruit growth (ΣFGR) to cumulative pedicel sap flux $\{(\Sigma J_P+J_X)\}$ in an intact tomato fruit during the light period (08:00–20:00), the dark period (00:00–08:00 and 20:00–24:00) and the whole day (00:00–24:00). Values are means of three plants.

	$\Sigma J_{WF}/\Sigma(J_P+J_X)$ (%)	$\Sigma FGR/\Sigma(J_P+J_X)$ (%)
Light	31.4	68.6
Dark	28.1	71.9
Light+Dark	30.2	69.8

photosynthesis and transpiration. Table 2 shows proportions of ΣJ_{WF} and ΣFGR to $\Sigma(J_P+J_X)$ in the fruit, which indicate allocation of the imported sap flux between transpirational water loss and expansive growth in the fruit. About 30% of the sap flux imported through the pedicel was lost by transpiration from the calyx and the berry, and the residual 70% of the imported sap contributed to the fruit expansive growth. Thus, the on-line measurements of J_{WF}, FGR and J_P+J_X made it possible to analyze dynamics of water balance in the intact fruit. The transpiration and the sap allocation observed in the intact fruit were remarkably different from those observed in the detached fruit where the peduncle cut end immersed in a solution bath was allowed to uptake water only through the xylem under complete prohibition of phloem sap influx (*1*). From these results, it is conceivable that the developed system can be useful for quantitative analyses of environmental effects on fruit growth and photoassimilate translocation in a tomato plant with reference to physiological functions in the fruit and leaflets.

REFERENCES

1. Ehret D. L. and Ho L. C. (1986) Effect of osmotic potential in nutrient solution on diurnal growth of tomato fruit. *J. Exp. Bot.* **37**, 1294–1302.
2. Fisher D. B. and Oparka K. J. (1996) Post-phloem transport: principles and problems.

J. Exp. Bot. **37**, 1141–1154.
3. Grange R. I. (1992) Water relations and growth of tomato fruit pericarp tissues. *Plant, Cell and Environ.* **18**, 1311–1318.
4. Grange R. I. and Andrews J. (1995) Respiration and growth in a tomato fruit. *Plant, Cell and Environ.* **18**, 925–930.
5. Ho L. C. and Baker D. A. (1982) Regulation of loading and unloading in the long distance transport systems. *Physiol. Plant.* **56**, 225–230.
6. Ho L. C., Grange R. I. and Picken A. J. (1987) An analysis of accumulation of water and dry matter in tomato fruit. *Plant, Cell and Environ.* **10**, 157–162.
7. Jarvis P. G. and Čatský J. (1971) Gas exchange system. Pages 50–52 *in* Z. Šesták, J. Čatský and P. G. Jarvis (eds) *Plant Photosynthetic Production. Manual of methods.* Dr. W. Junk, The Hague.
8. Johnson R. W., Dixon M. A. and Lee D. R. (1992) Water relation of the tomato during fruit growth. *Plant, Cell and Environ.* **15**, 947–953.
9. Kitano M., Hamakoga M., Yokomakura F. and Eguchi H. (1996) Interactive dynamics of fruit and stem growth in tomato plants as affected by root water condition. I. Expansion and contraction of fruit and stem. *Biotronics* **25**, 67–75.
10. Milburn J. A. (1975) Pressure flow. Pages 328–353 *in* M. H. Zimmermann and J. A. Milburn (eds) *Transport in plants* I. *Phloem transport, Encyclopedia of Plant Physiology, New Series, Vol. 1*, Springer–Verlag, Berlin.
11. Patrick J. K. (1990) Sieve element unloading: cellular pathway, mechanism and control. *Plant physiol.* **78**, 298–308.
12. Patrick J. W. and Offler C. E. (1996) Post–sieve element transport of photoassimilates in sink regions. *J. Exp. Bot.* **47**, 1165–1177.
13. Ruan Y.-L. and Patrick J. W. (1995) The cellular pathway of postphloem sugar–transport in developmental tomato fruit. *Planta.* **196**, 434–444.
14. Tanaka A. and Fujita K. (1974) Nutrio–Physiological studies on the tomato fruit. IV. Source–sink relationship and structure of the source–sink unit. *Soil Sci. Plant Nutr.* **20**, 305–315.
15. Walker A. J. and Ho L. C. (1977) Carbon translocation in the tomato: Carbon import and fruit growth. *Ann. Bot.* **41**, 813–823.
16. Walker A. J. and Thornley J. H. (1977) The tomato fruit: Import, growth, respiration and carbon metabolism at different fruit sizes and temperatures. *Ann. Bot.* **41**, 977–985.