# III. PLANT GROWTH EXPERIMENT

## Effects of elevated temperature and $CO_2$ on the growth of C3 plant and C4 plant populations

## III-1. MATERIALS AND METHODS

### *III-1-1. Plant growth conditions; temperature and $CO_2$ condition in both chambers and integrated solar radiation*

Five plots were prepared in the TGC and CTGC: ambient conditions (Control plot), 2℃ higher condition than ambient with ambient $CO_2$ (T2 plot), 4℃ higher condition than ambient with ambient $CO_2$ (T4 plot), 2℃ higher condition than ambient with 1.4 fold concentration of ambient $CO_2$ (CT2 plot), and 4℃ higher condition than ambient with 1.8 fold concentration of ambient $CO_2$ (CT4 plot).

The simulated global warming conditions in all plots were maintained throughout an experimental period (cf. Figs. 7, 9 and 15). The lowest and highest weekly mean air temperatures in the Control plot were 1.5℃ in late-January and 26.5℃ in early July, respectively. For the other plots, the temperatures were 5.9 and 31.1℃ in the T4, and 5.1 and 30.8℃ in the CT4 plot, respectively. The daily mean air temperature in the Control plot exceeded 23.0℃ from July to September, except for the last week of July when it abruptly fell down to 20.9℃. The air temperature gradually fell down from September 1998 onward, and then entered winter season with the accompanying lower air temperatures. The mean air temperatures of the Control, T2, CT2, T4, and CT4, throughout total experimental period were 14.3, 16.3 16.4, 18.5, and 18.4℃, respectively. The half-monthly integrated solar radiation greatly fluctuated, as shown in Fig. 16. The highest monthly integrated solar radiation was 385.4 MJ $m^{-2}$ in May.

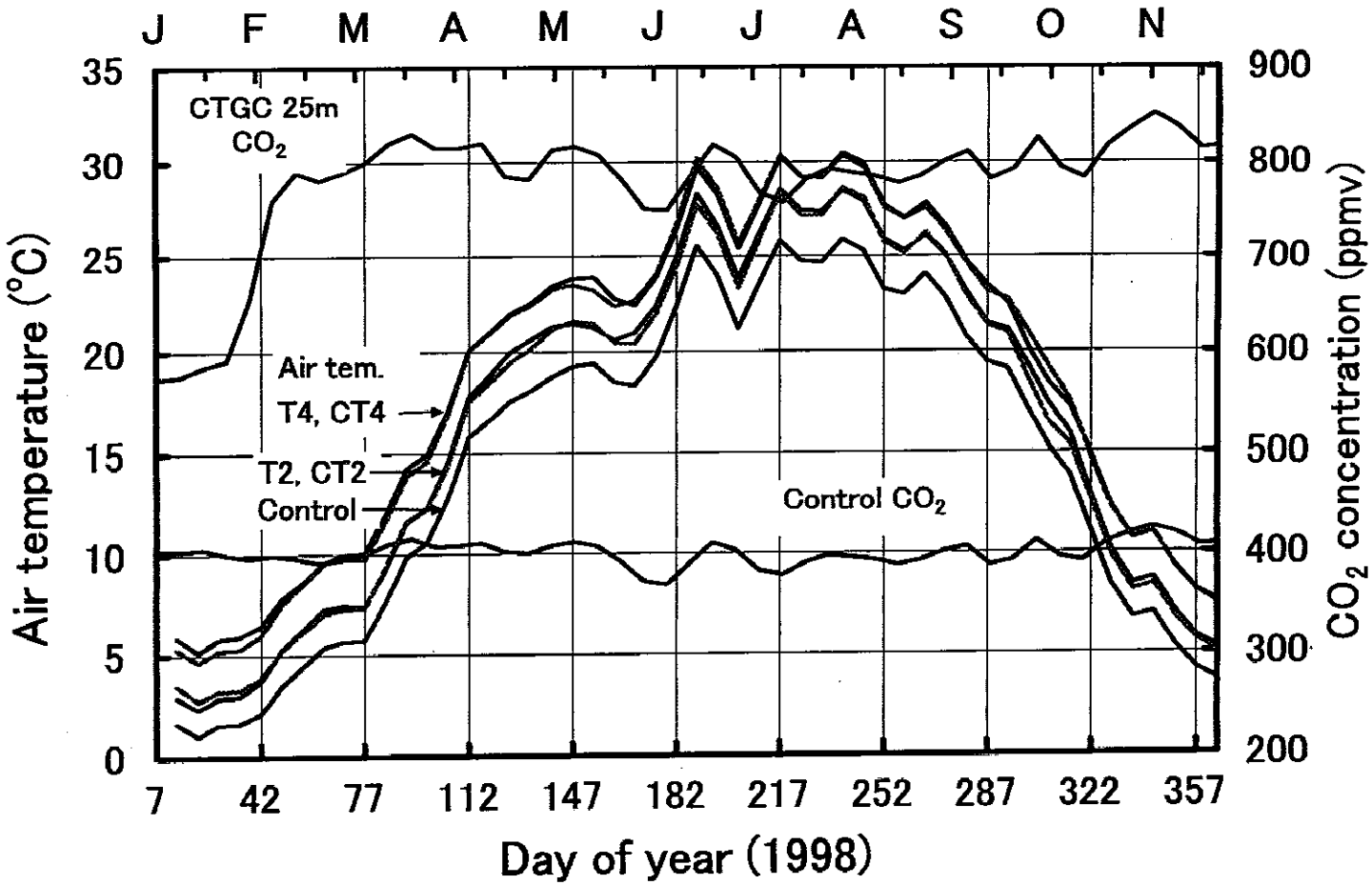



**Figure 15.** Running mean air temperature during the two weeks in each plot and $CO_2$ concentration of ambient and 25 m away from air inlet in the $CO_2$-Temperature Gradient Chamber.

The simulated global warming conditions were sustained in all plots in accordance with the short- and long-term irregular fluctuations, including the diurnal and seasonal change in meteorological factors (e.g. extreme diurnal and annual changes of temperature and light, moisture, $CO_2$ concentration and so on). Since these irregular fluctuations are generally seen in the field, only results obtained with similar fluctuations in an artificial environment can be used to predict global warming effects. However, many previous studies using facilities such as the growth chamber have been examined under constant temperature conditions. Those classical facilities made it difficult to predict how the plant growth (e.g. plant phenology) would be altered under global warming conditions, since they could not simulate natural habitat conditions which are composed of many meteorologically complex factors.

### *III-1-2. Experimental species and culture*

Three annual weed species, *C. album* (C3), *S. viridis* (C4), and *E. crus-galli* (C4), were selected from the vegetation table of an agricultural field in a Japanese temperate zone (Acker-Unkraut gesellschaften, Miyawaki, 1981). They were classified as summer annual strong weeds at an early stage in early secondary succession in Japan (Hayashi, 1977) with a $NADP^+$-ME subtype of C4 photosynthesis. The seeds of the three species were obtained from at least 50 different plants between September and October, 1997, in an agricultural field near University of Tsukuba, Ibaraki, Japan.

Each plot contained 18 containers, sized by 60 (L) X, 40 (W) X 24 (D) ($cm^3$). The containers were filled to the top with a 3:2:1 mixture of

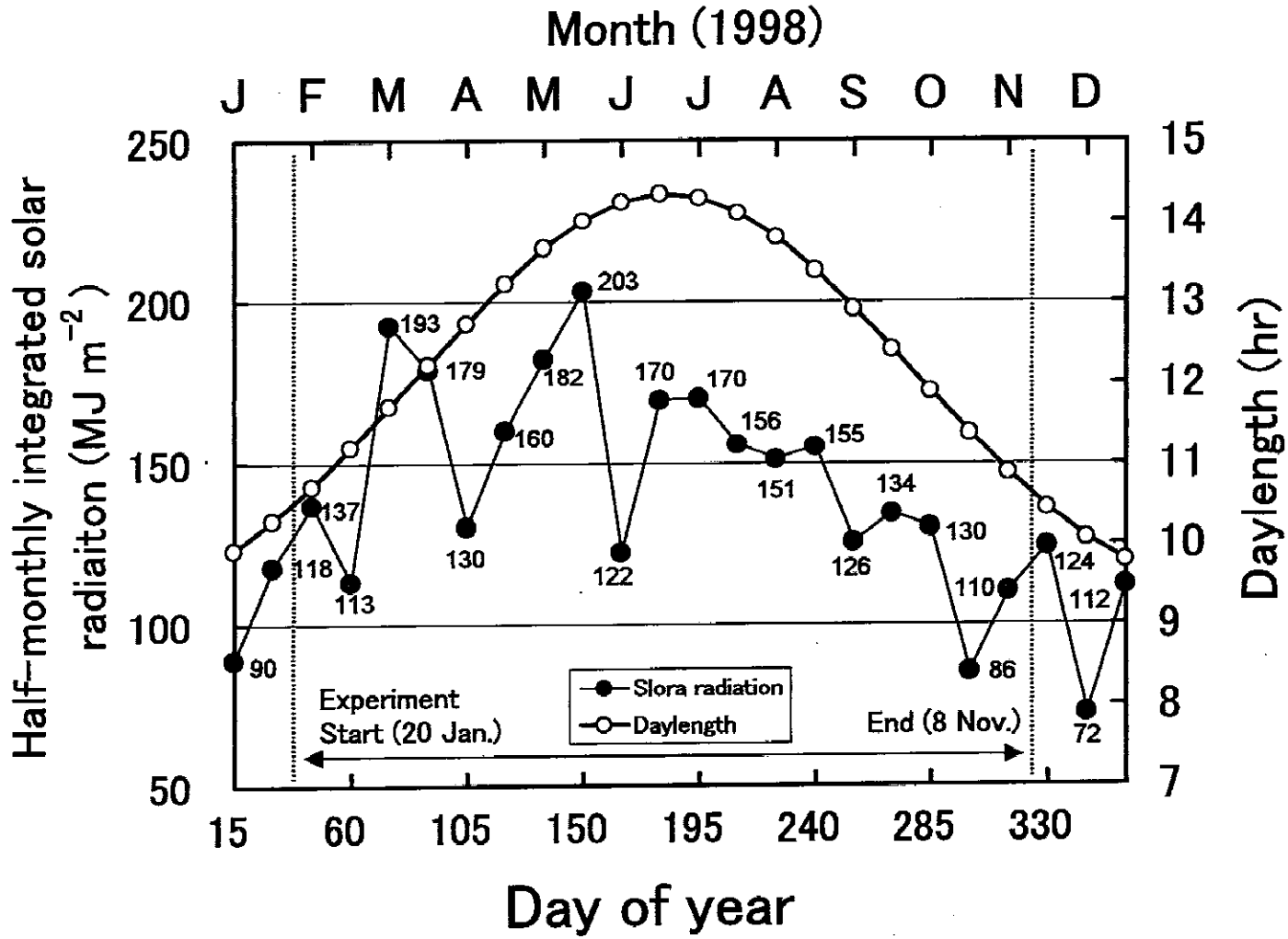



**Figure 16.** The fluctuation of half-monthly integrated solar radiation in 1998.

sand-clay-loam (66%, 19%, and 15%, respectively), leaf mold, vermiculite and 1g nutrient (8:15:7, NPK) $L^{-1}$ soil. Six containers for each species and each plot were placed just prior to sowing the seeds. In order to get sound seedlings, on Jan. 23, 1998, ten seeds were sowed at a 0.5 cm depth with a spacing of 8 cm. After the emergence of the seedlings, a planting density of 100 plants per $m^2$ was achieved by thinning the plants and leaving only the most normal one at each point. These containers were rotated within the same plot every 3 d in order to minimize local environmental differences such as light, air temperature, and $CO_2$ concentration. Sufficient water was irrigated every night throughout the growing season using a microjet sprinkler system suspended from the ceiling. Each container was fertilized with 40 g of nutrient mixtures (8:15:7, NPK) applied in two equal doses of 20 g from the vegetative stage through the emergence stage to the flowering stage.

### *III-1-3. Plant development and seed collecting*

In order to compare phonological response to increased temperature and $CO_2$ among the plots, seedling emergence and flowering number were checked. The emerged seedlings in each container were counted after a shoot extended about 0.2 cm long. The flowers per container were also determined every 2 days throughout the flowering period.

The ears of 12 shoots per container for *S. viridis* and *E. crus-galli* populations were wrapped with coarse cloth in order to collect seed. They were collected when the above-ground parts of plant were completely dried, and then those seeds were counted. For *C. album* seeds were obtained by manual

picking because the seeds do not shed for some time.

### *III-1-4. Plant growth and biomass allocation*

Non-destructive growth measurements were made at about three-week intervals. The linear regression was calculated from the relationships between shoot length and diameter(Mousseau, 1993). In order to calculate the regression equation, the plants were harvested two times during the vegetative stage and one time during the flowering stage. The plants were then separated into shoot, leaf, root, and reproductive parts. The areas of all leaves for 12 individuals from each container were measured by passing each leaf through a leaf area meter (AAM-7, Hayashi Denkou, Tokyo, Japan). Total leaf area for the population was calculated from the specific leaf area (*SLA*; unit leaf area relation to unit weight). Dry weight of the components were taken after drying them in a forced air draft oven at 80℃ to constant weights.

Applying the linear regression to the non-destructive measurements, the time-progressions of total standing biomass and leaf area were estimated for each population, and were plotted as a function of time. Then, the functional growth analysis was used (Radford 1967; Hunt 1982) to calculate the leaf area index (*LAI*) and the net assimilation rate (*NAR*) at approximately three-week intervals. Total dry weights included all dead leaves collected at each measurement.

Three containers were picked for measuring total final dry weight per unit ground area. All shed leaves of *C. album* population were collected to calculate total dry-matter production. The last harvest was performed when the

above-ground part of the plant was completely dried.

### *III-1-5. Data analysis*

Two-way analyses of variance (ANOVA) were used to test for the main effects of the plots. Multiple comparisons of means were calculated using the Tukey test (HSD) to determine whether the means of the dependent variable were significantly different at the 0.05 probability level.

## III-2. RESULTS

### *III-2-1. Phenological response to elevated temperature and $CO_2$ conditions*

Seedling emergence and flowering times for the three experimental plants were significantly advanced by elevated temperature while elevated $CO_2$ had little effect (Table 2). The seedling emergence DOY (day of year) of the *C. album* (C3) population was 81, 64, 63, 54, and 57 in the Control, T2, CT2, T4, and CT4 plots, respectively, and thus the seedlings emerged 27, 24, 17, and 18 d earlier in the T4, CT4, T2, and CT2 plots, respectively, compared with the Control plot. The seedling emergence times of *C. album* population were approximately 26 d earlier in the 4℃ warmed plots (T4 and CT4) and 18 d earlier in the 2℃warmed plots (T2 and CT2) than in the Control plot. The flowering times of *C. album* population were approximately DOY 151, 157, 172, and 174 in the T4, CT4, T2, and CT2 plots, respectively, while the mean

Table 2. Effects of elevated temperature and $CO_2$ on phenology of the three annual species grown in plant population level. Seedling emergence time and flowering time as day of year are, respectively, defined as the emerged time of 50% of finally emerged seedlings and the flowering time of 50% of total plants in each container. Values are expressed as the mean days with ±standard error, and different superscript letters within a row indicate significant difference at $P<0.05$. Periods of vegetative stage are defined as the duration from the emergence time to the flowering time. Diff. indicates differnce with the Control plot

| | Experimental regime | | | | |
|---|---|---|---|---|---|
| | Control | T2 / CT2 | Diff. | T4 / CT4 | Diff. |
| **Seedling emergence time (day of year, n=6)** | | | | | |
| *Chenopodium album* (C3) | 81.0±2.7$^{a}$ | 63.7±2.1$^{b}$ | -17.3 | 54.0±2.7$^{c}$ | -27.0 |
| | | 63.0±1.0$^{b}$ | -18.0 | 56.7±1.2$^{c}$ | -24.3 |
| *Echinochloa crus-galli* (C4) | 102.0±2.0$^{a}$ | 67.7±1.5$^{b}$ | -34.3 | 56.3±2.1$^{c}$ | -45.7 |
| | | 68.3±2.1$^{b}$ | -33.7 | 54.0±1.7$^{c}$ | -48.0 |
| *Setaria viridis* (C4) | 106.3±1.2$^{a}$ | 80.7±1.2$^{b}$ | -25.7 | 71.3±2.3$^{c}$ | -35.0 |
| | | 81.3±1.2$^{b}$ | -25.0 | 71.3±1.2$^{c}$ | -35.0 |
| **Flowering time (day of year, n=3)** | | | | | |
| *Chenopodium album* (C3) | 204.3±2.3$^{a}$ | 171.7±2.3$^{b}$ | -32.6 | 151.0±5.3$^{c}$ | -53.3 |
| | | 173.7±3.1$^{b}$ | -30.7 | 157.0±5.3$^{c}$ | -47.3 |
| *Echinochloa crus-galli* (C4) | 182.0±0.0$^{a}$ | 155.7±3.1$^{b}$ | -26.3 | 143.0±1.7$^{c}$ | -39.0 |
| | | 154.3±3.1$^{b}$ | -27.7 | 141.0±0.0$^{c}$ | -41.0 |
| *Setaria viridis* (C4) | 178.3±1.2$^{a}$ | 164.3±1.2$^{b}$ | -14.0 | 147.7±1.2$^{d}$ | -30.7 |
| | | 159.7±1.2$^{c}$ | -18.7 | 146.3±1.2$^{d}$ | -32.0 |
| **Period of vegetative stage (days, n=3)** | | | | | |
| *Chenopodium album* (C3) | 123.3±2.1$^{a}$ | 108.0±4.4$^{bc}$ | -15.3 | 97.0±4.6$^{c}$ | -26.3 |
| | | 110.7±2.5$^{b}$ | -12.7 | 100.3±6.1$^{bc}$ | -23.0 |
| *Echinochloa crus-galli* (C4) | 73.0±2.0$^{a}$ | 88.0±4.4$^{b}$ | -15.0 | 86.7±3.1$^{b}$ | -13.7 |
| | | 86.0±1.7$^{b}$ | -13.0 | 87.0±1.7$^{b}$ | -14.0 |
| *Setaria viridis* (C4) | 72.0±2.0$^{a}$ | 83.0±1.2$^{b}$ | -11.7 | 76.3±1.2$^{c}$ | -4.3 |
| | | 78.3±1.2$^{c}$ | -6.3 | 75.0±0.0$^{ac}$ | -3.0 |

flowering times for the plants in the Control was DOY 204. The emergence of the two C4 plant species, the *E. crus-galli* and *S. viridis* populations, was more sensitive to elevated temperature than that of the C3 plant; that is, the *E. crus-galli* and *S. viridis* populations emerged approximately 46 and 35 d earlier in the T4 plot, 48 and 35 d earlier in the CT4 plot, 34 and 26 d earlier in the T2 plot, and 34 and 25 d earlier in the CT2 plot, than those of the Control plot. The flowering times of *E. crus-galli* and *S. viridis* populations were DOY 143 and 148 in the T4 plot, DOY 141 and 146 in the CT4 plot, DOY 156 and 164 in the T2 plot, DOY 154 and 160 in the CT2 plot, and DOY 182 and 178 in the Control plot, respectively. The flowering time was more sensitive in the C3 plant than the C4 plants, while seedling emergence time was more sensitive in the C4 plants.

As a result, the temperature-induced changes in the vegetative periods of the C3 and C4 plants were species-specific. The vegetative period of the *C. album* population was 123, 108, 111, 97, and 100 in the Control, T2, CT2, T4, and CT4 plots, respectively, showing a significant decrease with increasing temperature. By contrast, the two C4 species showed a prolonged vegetative period in elevated temperature and $CO_2$ conditions, in comparison with the Control plot. However, a significant difference was not observed between the elevated-temperature and $CO_2$ plot and the elevated-temperature plot. The vegetative periods of the *E. crus-galli* population were 73, 88, 86, 87, and 87 in the Control, T2, CT2, T4, and CT4 plots, respectively. Also, those of the *S. viridis* population were 72, 83, 78, 76, and 75 in the Control, T2, CT2, T4, and CT4 plots, respectively.

***III-2-2. Dry-matter accumulation of the C3 and C4 plant populations to***

***elevated temperature and $CO_2$***

There was a great variance in the developmental stages of each plot because the elevated temperature caused a significant advance in the plant phonologies. Thus, the total dry weights at particular time points, which included the above- and below-ground parts and shed leaves, differed greatly among the plots. For example, in all species, the plants grown in the T4 and CT4 plots were at a vigorous vegetative growth stage on DOY 132, but ones in the Control plot were at the seedling stage (cf. Figs. 17). The *S. viridis* populations in the T4 and CT4 plots also showed the same trend with the *C. album* and *E. crus-galli* populations. This difference in developmental growth stage between plots resulted in a remarkable difference in the total dry weight at the same time. On DOY 148, the total dry weight of the *C. album* populations grown in the T4 and CT4 plots were 2.5 and 3.1 times greater than those in the Control plot, respectively. Also, the total dry weight of the *E. crus-galli* population was approximately 2.7 times greater in the T4 plot, and 3.1 times greater in the CT4 plot, compared with those in the Control plot (cf. Figs. 17b). The *S. viridis* populations also showed a similar trend: 4.1 times and 4.9 times greater in the T4 and CT4 plots than the Control plot, respectively (cf. Fig. 17c).

The total final dry weight per ground area (*TFDW*) of the *C. album* population showed a significant decrease of 19.6% and 33.9% in the T2 and T4 plots, respectively, compared with the Control plot (cf. Fig. 18). By contrast, in the CT4 plot the *TFDW* increased significantly by 33.9% ($P < 0.05$). There was no significant difference in the *TFDW* between the CT2 and Control plots.

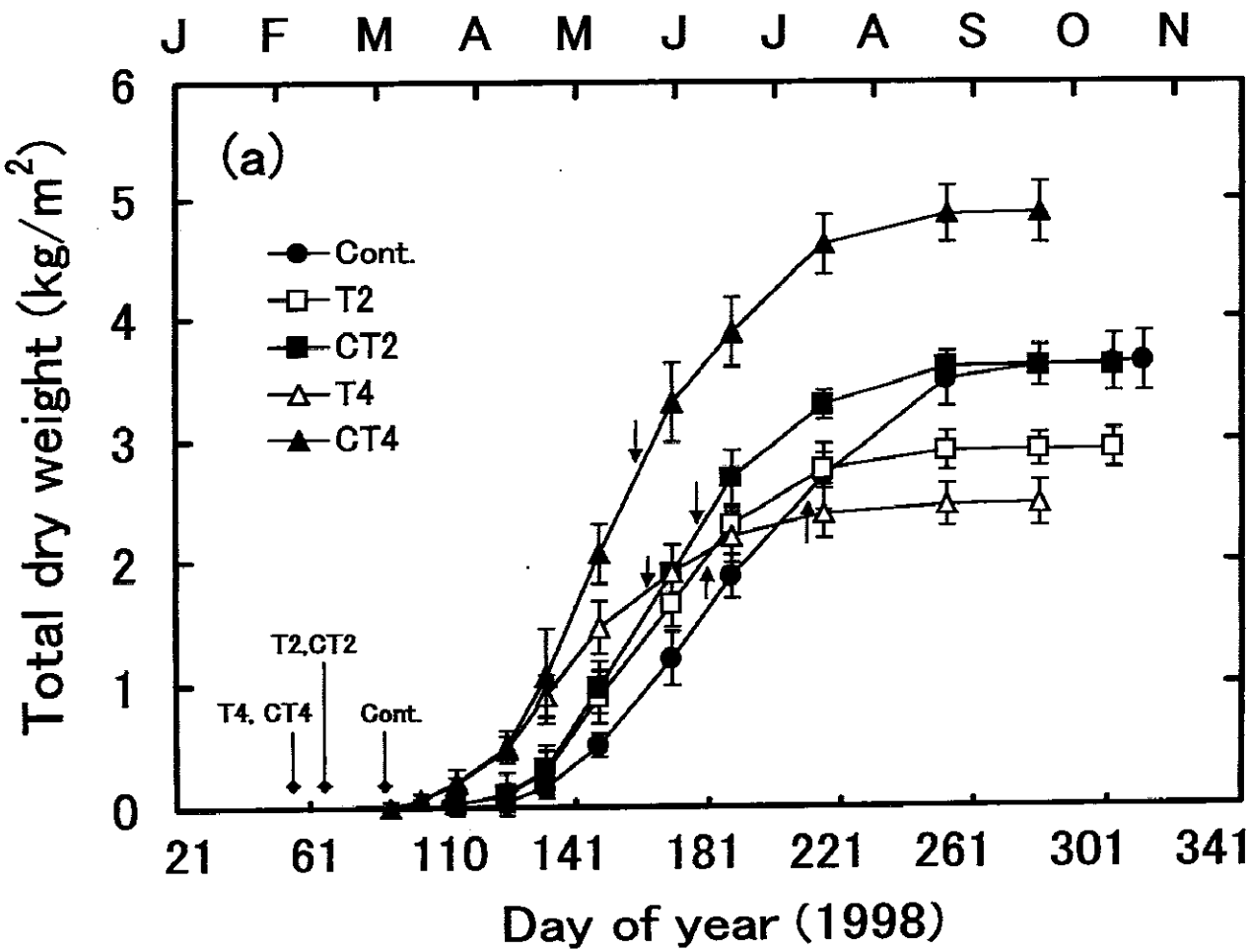
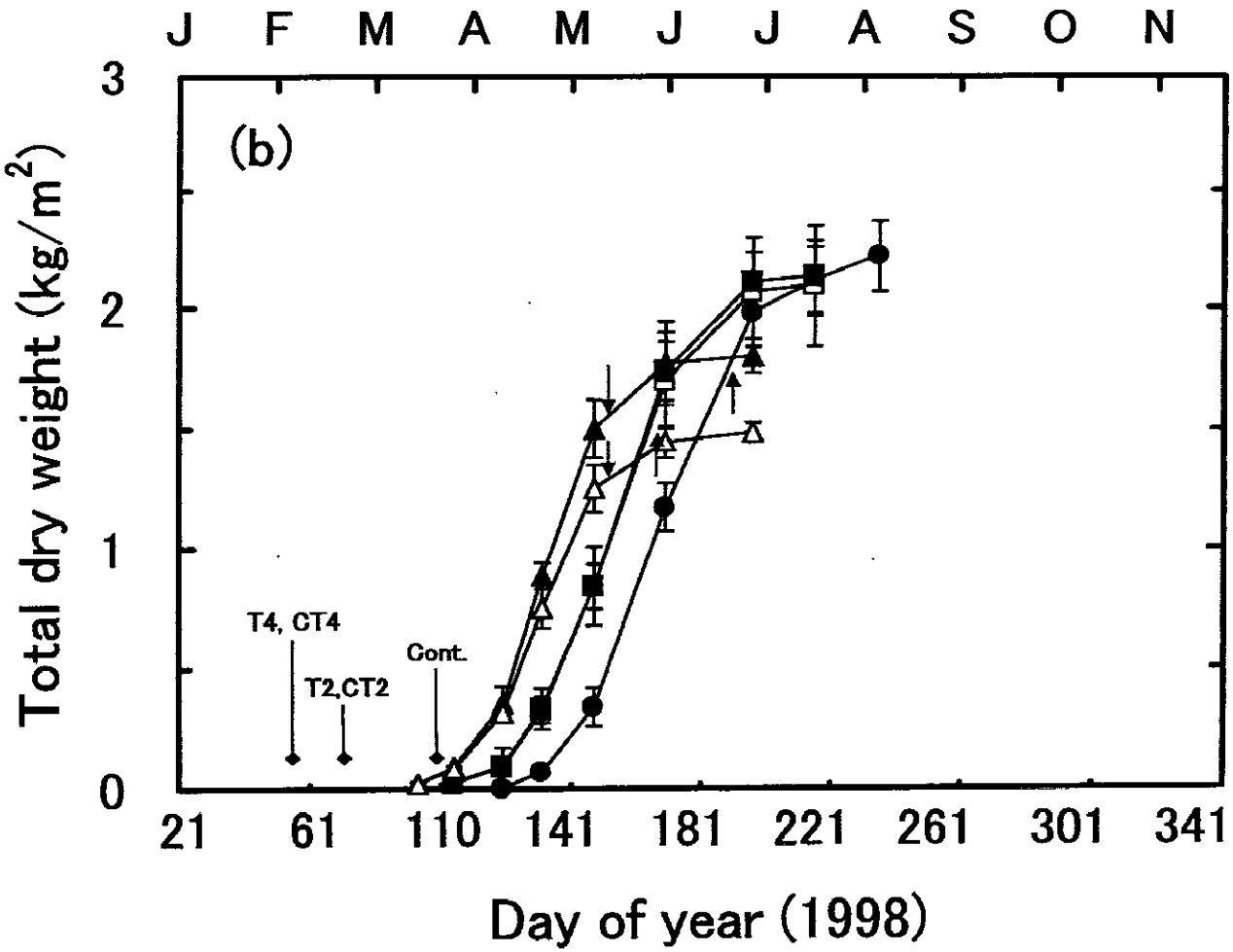



**Figure 17.** Dry matter weight as a function of plant age grown in enriched $CO_2$ and increased temperature of *C. album* (a), *E. crus-galli* (b), and *S. viridis* (c) populations. Symbols represent means and error bars represent±1 SE (note difference in scale between graphs).The arrows represent the approximate time at emergence (diamond head) and flowering time (triangle head).

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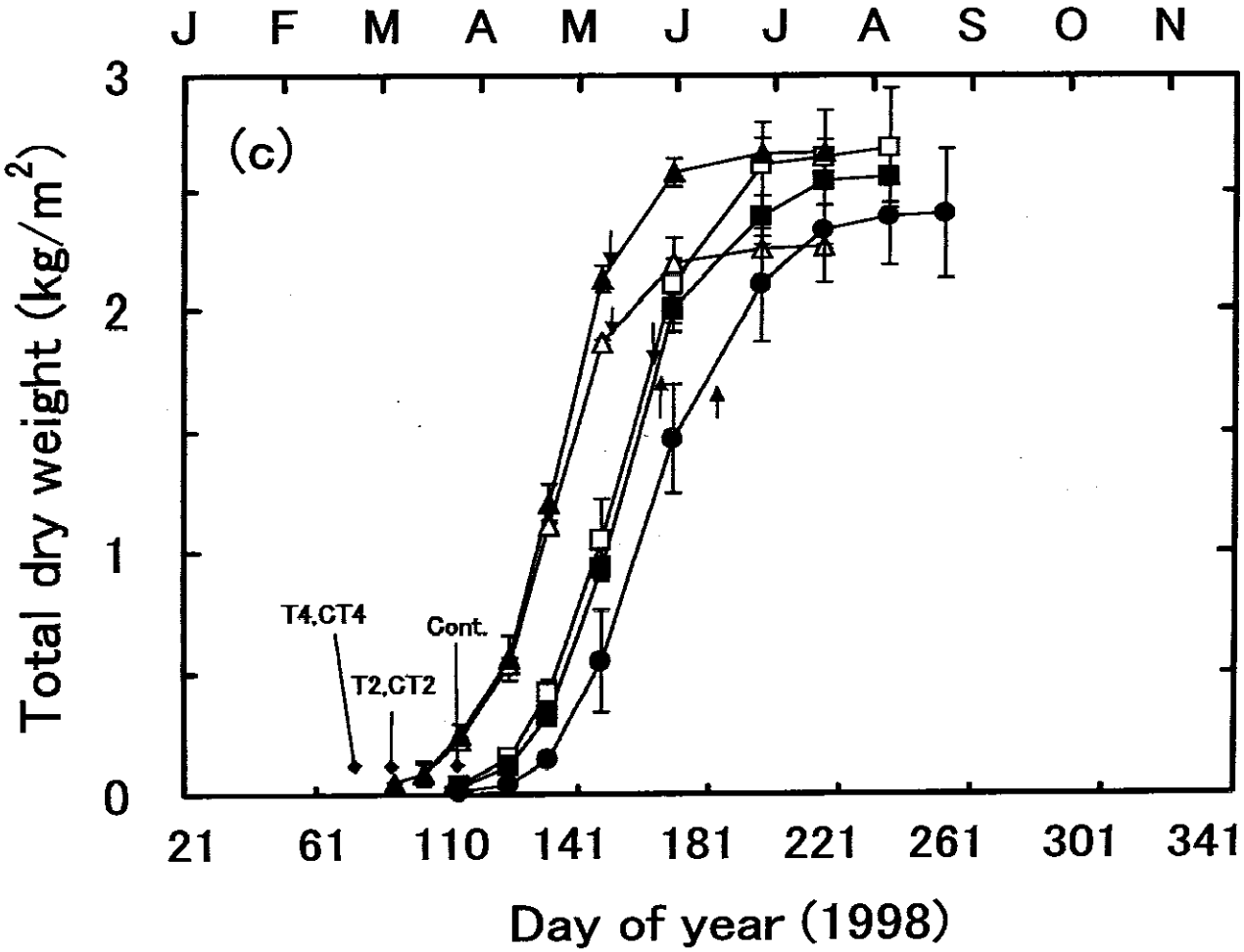


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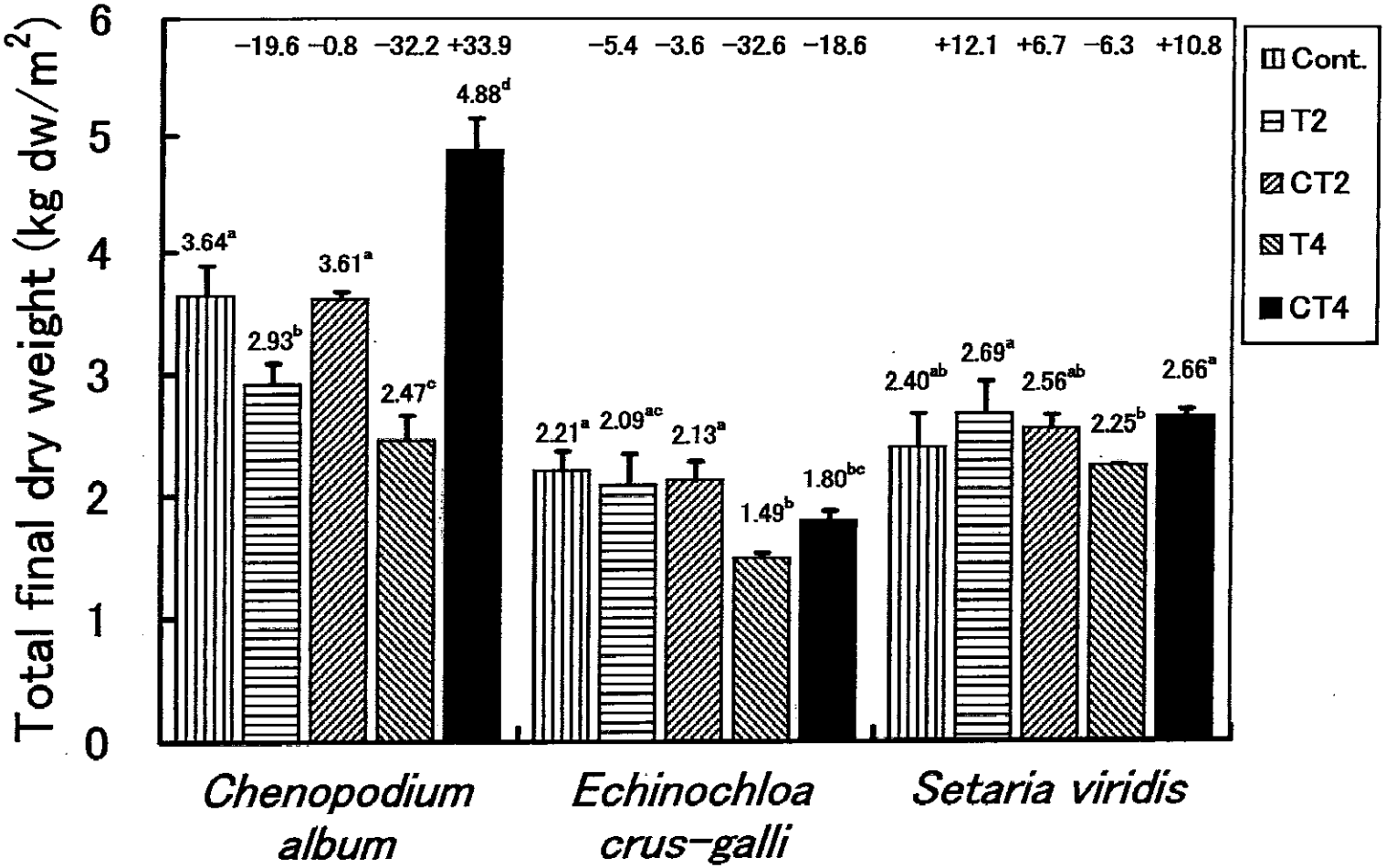



**Figure 18.** Total final dry weight in each plant grown in elevated temperature and $CO_2$ of *C. album*, *E. crus-galli*, and *S. viridis* populations. The ratios to the Control plot are displayed on the top of the graph. Different *superscript letters* indicate significant difference at $P < 0.05$ (n=3). Error bars represent standard error.

Within the same temperature plot, elevated $CO_2$ conferred a significant increase in the *TFDW* of *C. album* population. The *TFDW* of the *C. album* population was 24.2% higher in the CT2 plot compared with the T2 plot, and it showed an increase of 97.6% in the CT4 plot compared with the T4 plot.

The two C4 plant species populations showed different responses in the *TFDW*. The *TFDW* of the *E. crus-galli* population showed a significant decrease of 32.6% in the T4 plot and also a significant decrease of 18.6% in the CT4 compared with the Control plot, whereas there were no significant differences in the T2 and CT2 plots (Fig. 18). The *S. viridis* populations had no significant differences in the *TFDW* in all plots compared with the Control plot, but it was 18.2% higher in the CT4 plot than in the T4 plot. The *TFDWs* of the C3 plants were 1.6, 1.4, and 1.3 times greater in the Control, T2, and T4 plots, respectively, than those of the C4 plants. There was an increase of 2.2 times in the CT4 plot in comparison with the T4 plot, but it was 1.5 times higher in the CT2 plot than in the T2 plot.

### *III-2-3. Growth analysis*

As was the case in dry-matter production, in particular for the *C. album* population, the relative effects of elevated $CO_2$ on *NAR, RGR,* and *LAI* were temperature dependent (Figs. 19, 20, and 21). The relative *NAR, RGR,* and *LAI* of the *C. album* in elevated $CO_2$ plots to the non-elevated $CO_2$ plots populations were high in high temperature season. The positive effects of elevated $CO_2$ on *NAR* resulted in direct enhancement of *RGR*. As a result, relatively high *RGR* in the elevated $CO_2$ plots showed in late-growth season in which plants was

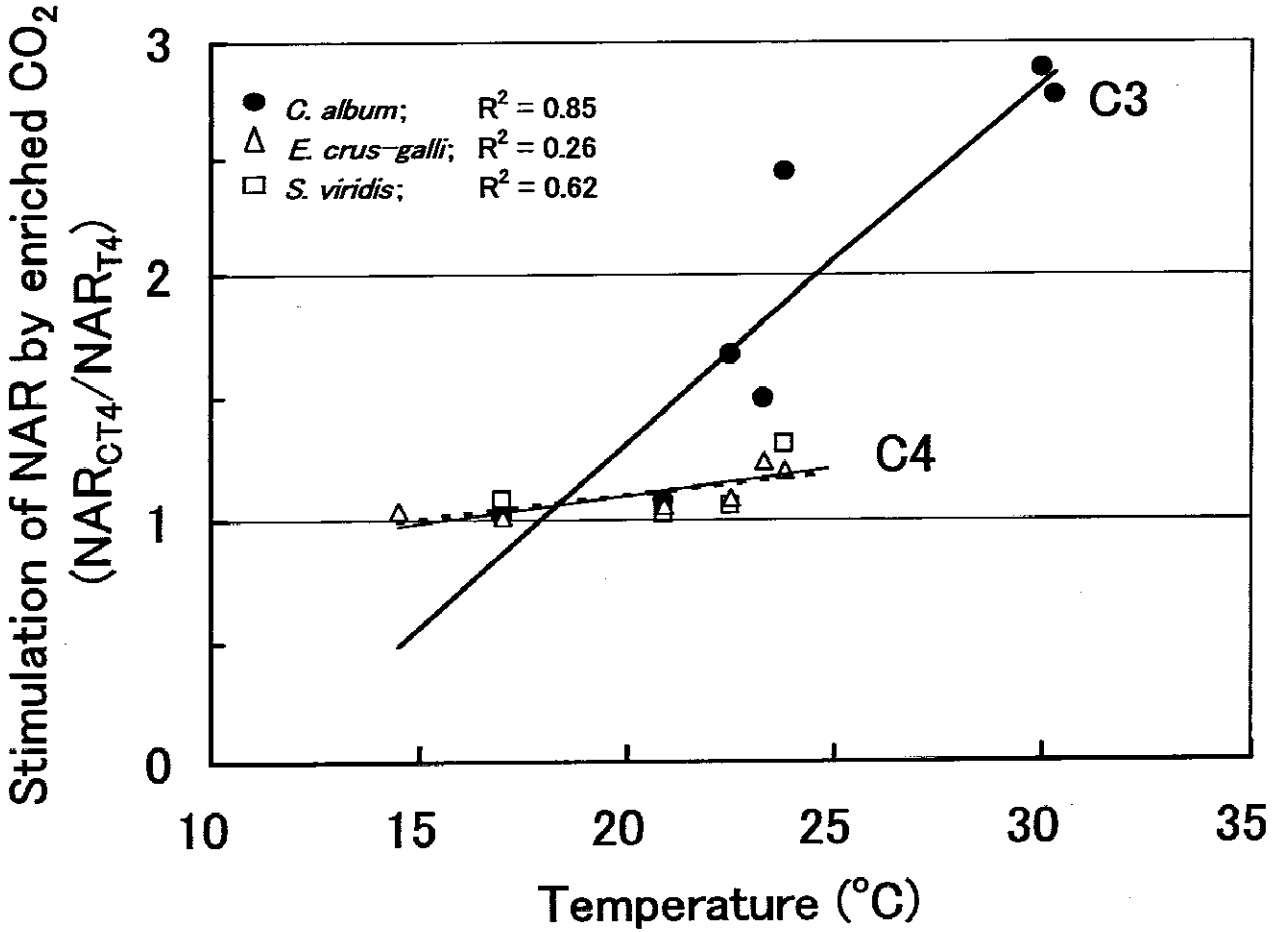



**Figure 19.** The dependence of $CO_2$ stimulation of *NAR* on temperature in the *C. album* (C3), *E. crus-galli* (C4), and *S. viridis* (C4) populations.

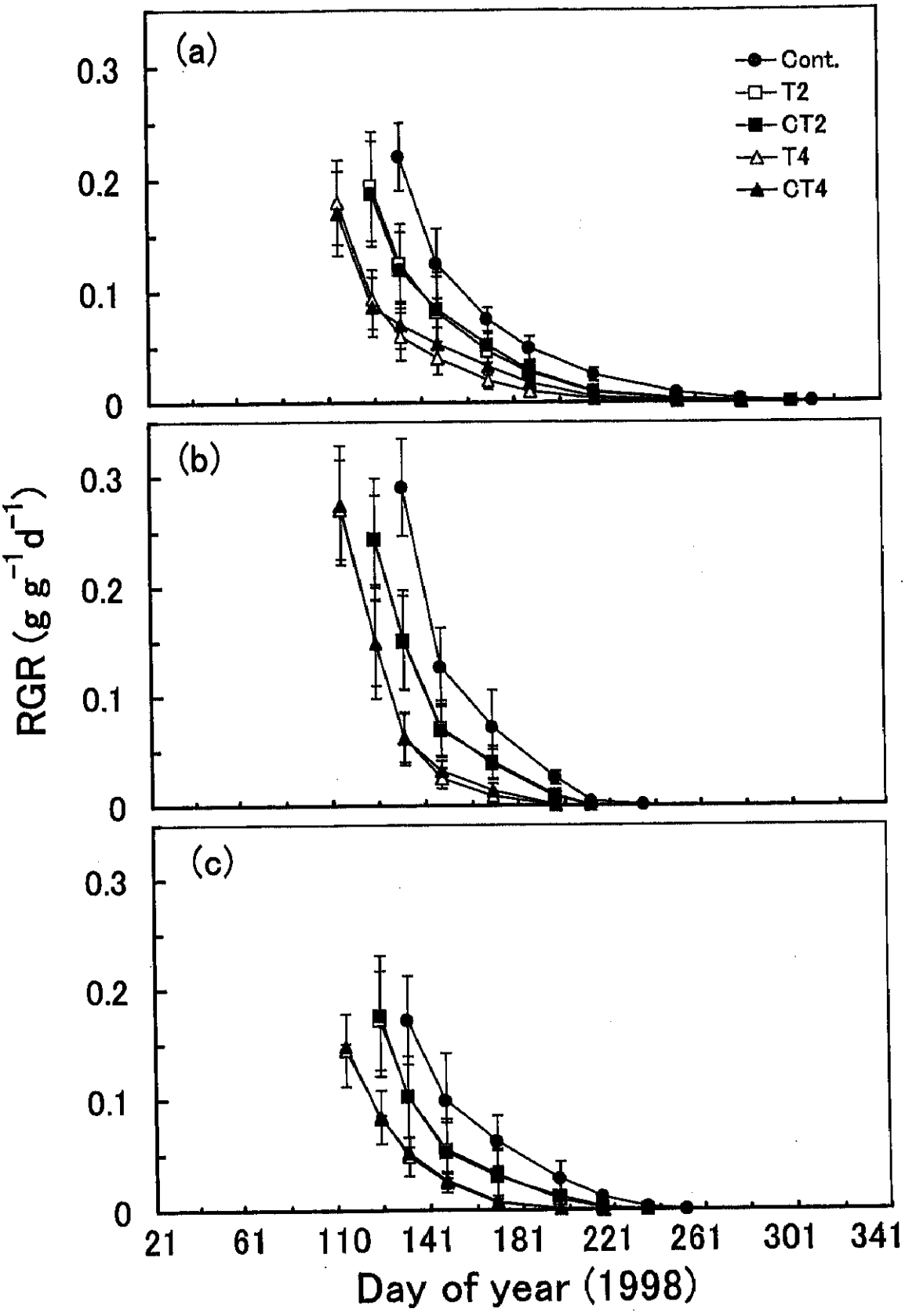



**Figure 20**. Seasonal change of *RGR* of *C. album* (a), *E. crus-galli* (b), and *S. viridis* (c) populations under the elevated temperature and $CO_2$ concentration.

reproductive stage.

Figure 19 shows the relationships between the stimulation of the *NAR* and the elevated $CO_2$ and temperature. The stimulation of the *NAR* to elevated $CO_2$ was computed as ($NAR_{CT4}/NAR_{T4}$), where the subscripts CT4 and T4 indicate elevated $CO_2$ and ambient conditions with 4℃ warmed condition. The values were plotted against the mean air temperature between the two measurement times. The stimulation of elevated $CO_2$ on the *NAR* of the C3 plants showed linear dependence for air temperature (cf. Fig. 19, $r^2$=0.85). The stimulation of the *NAR* to elevated $CO_2$ was clearly a function of temperature. However, the two C4 plant populations showed a clearly lower effect on the *NAR* from the elevated $CO_2$, compared with the *C. album* population (cf. Fig. 19). On the other hand, there was very little effect on the *NAR* from elevated $CO_2$ for the two C4 plant populations, which increased slightly in the high temperature season. Owing to the temperature-dependence of the *NAR*, the increased growth temperature significantly enhanced dry-matter production in the elevated-temperature plots (Figs. 17 and 18).

Elevated $CO_2$ also significantly enhanced the *RGR* in the CT2 and CT4 plots during the high temperature season (Fig. 20), compared with the warmed plots without $CO_2$ enrichment. The elevated $CO_2$ in the CT4 plot almost completely compensated for the depression of the *RGR* observed in the T4 plot in the summer (Fig. 20). The increase in *RGR* under the high temperature condition was due to the increased *NAR* and relatively high *LAI*.

The *LAI* increased with increasing temperature (Fig. 21). The *LAI*s of the three species rapidly increased from emergence to late-flowering and sharply decreased as the seeds were ripening after flowering. Also, these seasonal

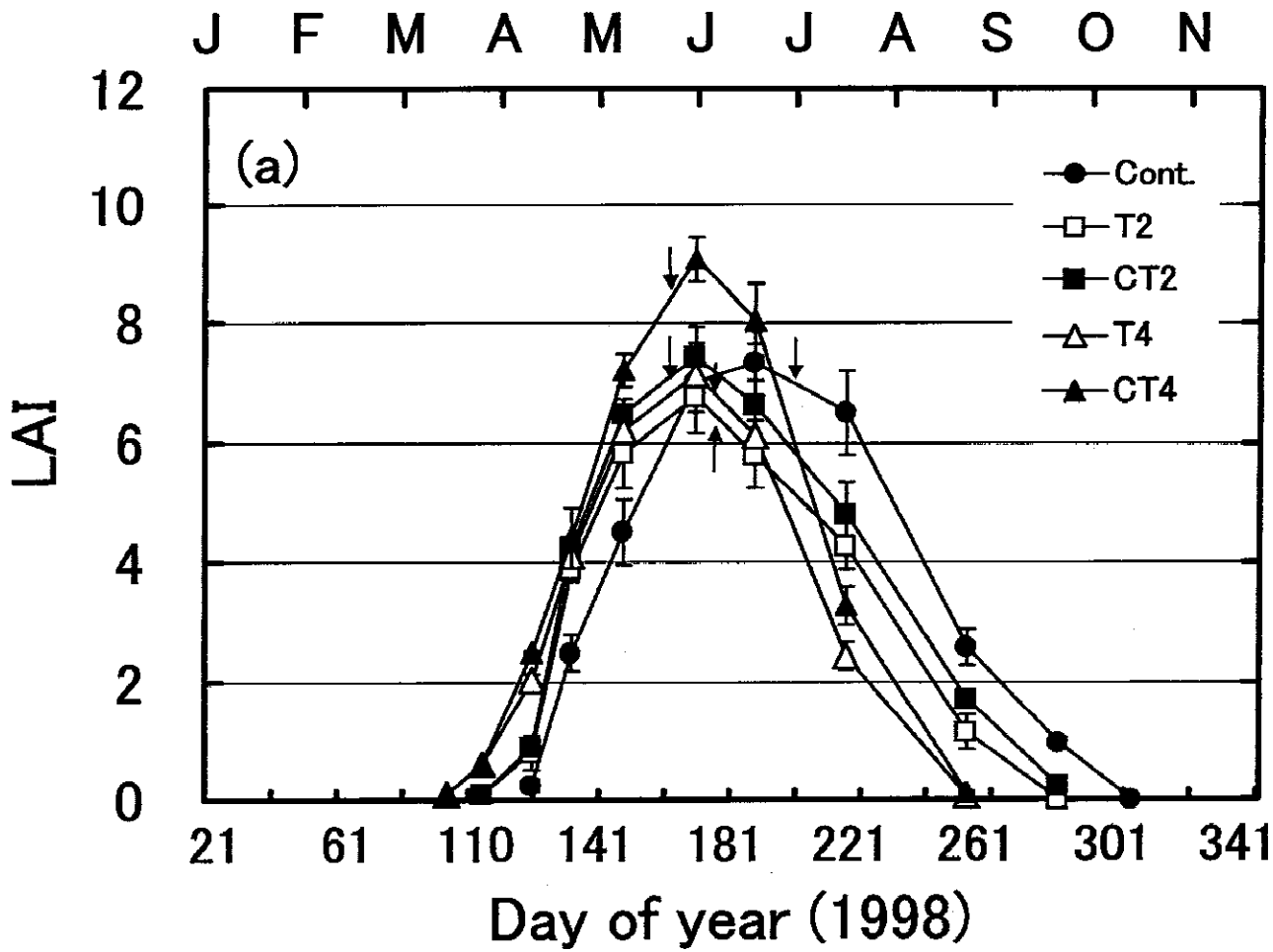
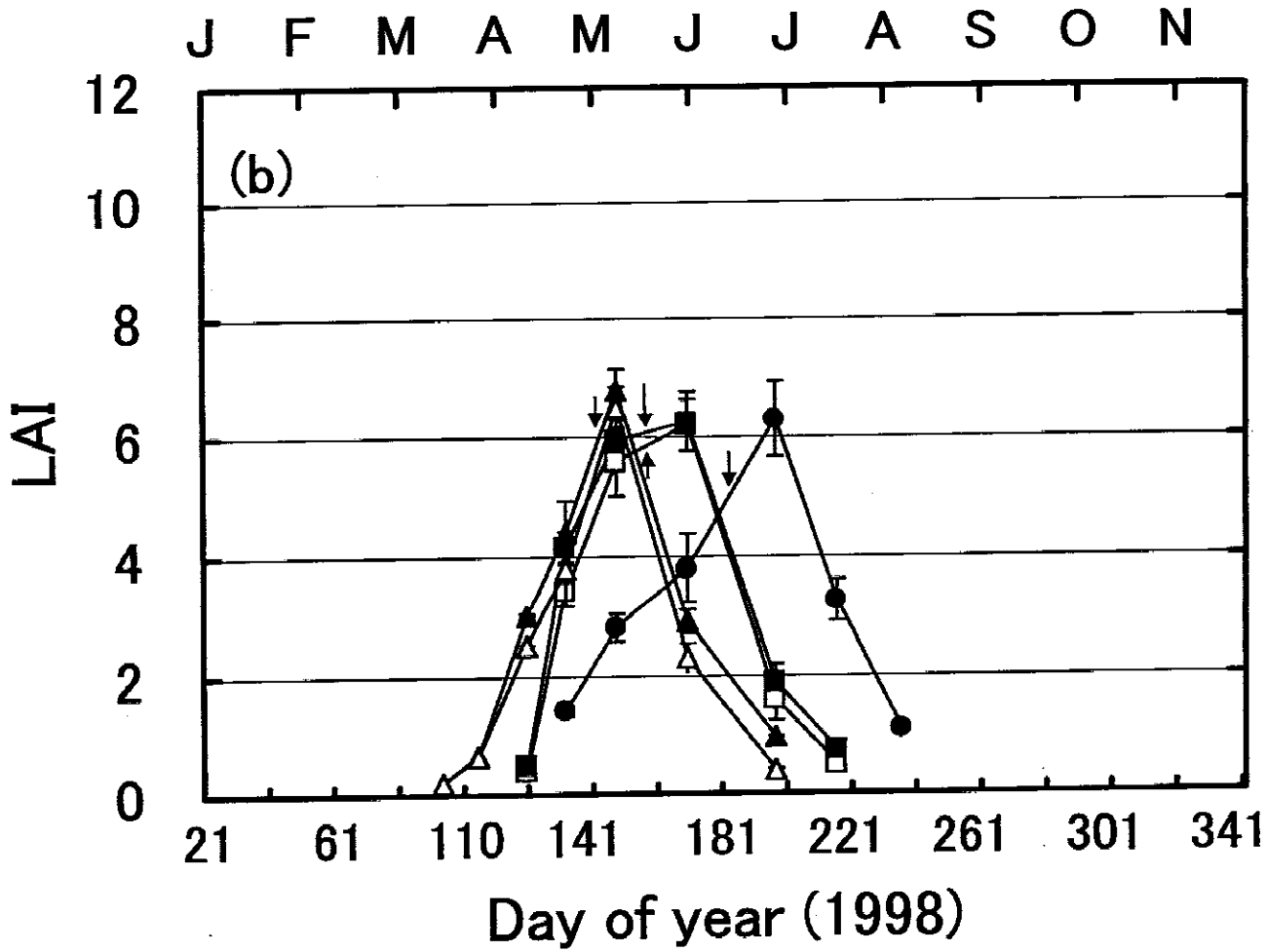



**Figure 21.** Seasonal change of LAI of *C. album* (a), *E. crus-galli* (b), and *S. viridis* (c) populations under increased temperature and $CO_2$ concentration. Arrows indicate flowering time.

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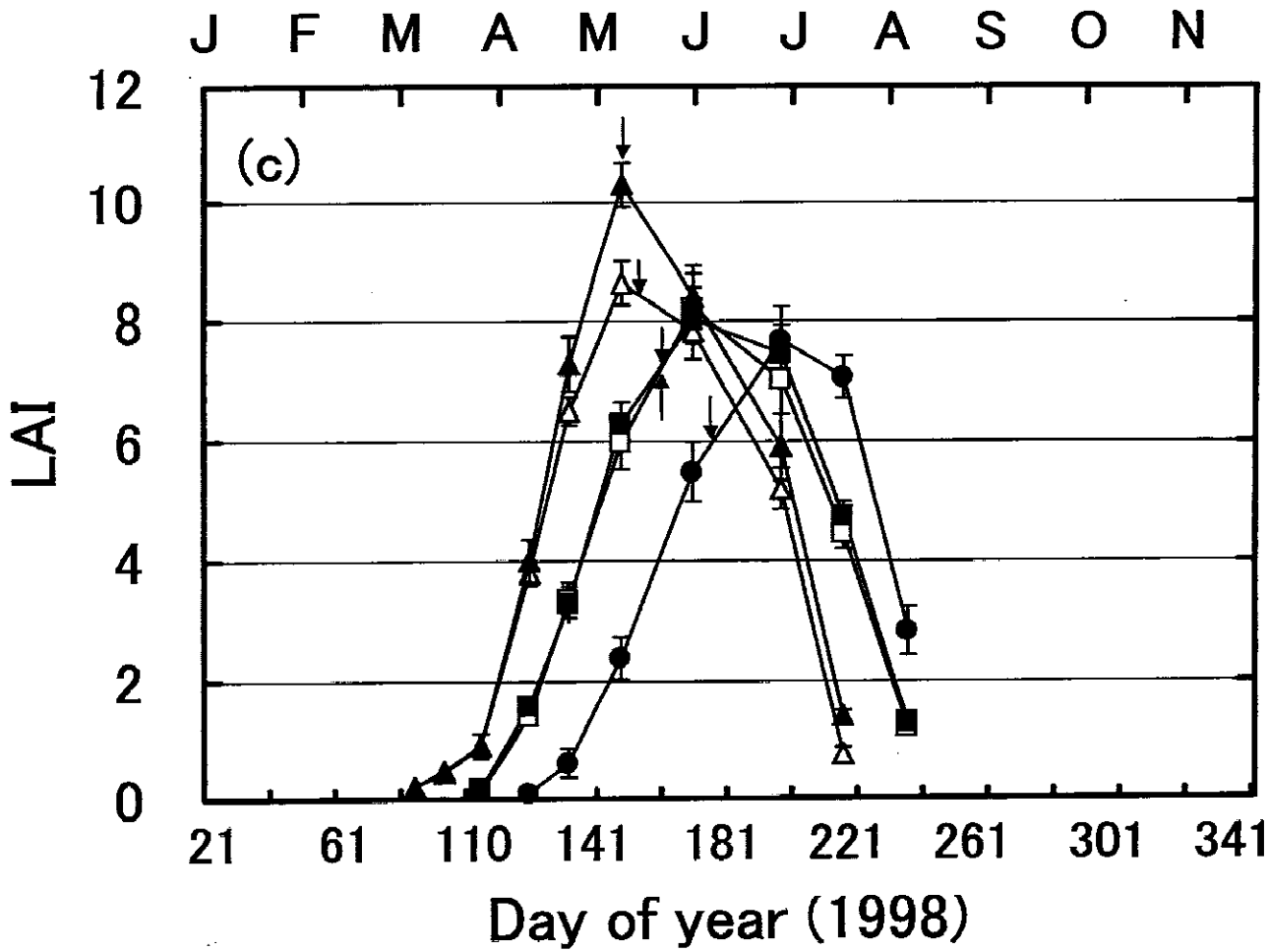


**Figure 21 (continued).**

changes of the *LAI* happened earlier in the temperature-elevated plots than in the Control plot (cf. Fig. 21). The duration of the canopy composed of photosynthetic leaves was significantly longer for the *C. album* population than for the two C4 plant populations, and the Control plot canopy had a longer duration than the canopies of the temperature-elevated plots.

The maximum *LAI* of the *C. album* population was reached earlier in the elevated-temperature plots than in the Control plot. The *LAI* of the *C. album* population grown in the CT plots was slightly higher than in the T plots during the growth season (cf. Figs. 21a). The maximum *LAI* of the *C. album* population was 8.9 in the CT4 plot, but 7.1 in the T4 plot in mid-June. For the two C4 plant populations, the maximum *LAI*s (in late-May) in the T4 and CT4 plots were 6.5 and 6.8 for the *E. crus-galli* population and 8.6 and 10.3 for the *S. viridis* populations, respectively. By contrast, the maximum *LAI*s of the two C4 plants were significantly lower in the Control plot than those of the T4 and CT4 plots. The maximum *LAI* of the *S. viridis* population was significantly higher in the CT4 plot than in the T4 plot (cf. Fig. 21b), whereas the *E. crus-galli* populations showed no significant difference (cf. Fig. 21c).

### *III-2-4. Reproductive output in population level*

There was no significant difference in vegetative tiller numbers among the *C. album* populations (cf. Table 2). The *E. crus-galli* populations grown in the T4 and CT4 plots increased the vegetative tiller number by 18.8% and 14.2%, respectively, compared with those in the Control plot, and no significant differences were observed between them. In the *S. viridis* populations, the

Table 3. Reproductive output of plant populations of the three annual weeds grown under elevated temperature and $CO_2$. Values are expressed as the average with ±standard error. Reproductive index is defined as the ratio of total seed weight to total final dry weight. Different *superscript letters* within a row indicate significant difference at $P<0.05$. Diff. indicates differnce with the Control plot

| | Control | T2 / CT2 | Diff. | T4 / CT4 | Diff. |
|---|---|---|---|---|---|
| Vegetative tiller number (No./$m^2$) | | | | | |
| *Chenopodium album* (C3) | 100.0±0.0$^a$ | 90.3±9.6$^a$ | -9.7 | 97.2±2.4$^a$ | -2.8 |
| | | 97.2±2.4$^a$ | -2.8 | 97.2±2.4$^a$ | -2.8 |
| *Echinochloa crus-galli* (C4) | 273.6±12.0$^a$ | 265.3±22.9$^a$ | -3.0 | 325.0±22.0$^b$ | 18.8 |
| | | 263.9±10.5$^a$ | -3.6 | 312.5±18.2$^b$ | 14.2 |
| *Setaria viridis* (C4) | 402.8±12.7$^a$ | 520.8±25.3$^b$ | 29.3 | 504.2±11.0$^b$ | 25.2 |
| | | 522.2±44.6$^b$ | 29.7 | 487.5±19.1$^b$ | 21.0 |
| Total seed dry weight (kg/$m^2$) | | | | | |
| *Chenopodium album* (C3) | 0.588±0.054$^a$ | 0.413±0.046$^b$ | -29.7 | 0.502±0.145$^{ab}$ | -14.6 |
| | | 0.682±0.074$^a$ | 15.9 | 1.260±0.204$^c$ | 114.4 |
| *Echinochloa crus-galli* (C4) | 0.544±0.042$^{ab}$ | 0.652±0.071$^a$ | 20.0 | 0.464±0.029$^b$ | -14.7 |
| | | 0.663±0.080$^a$ | 22.0 | 0.622±0.014$^a$ | 14.4 |
| *Setaria viridis* (C4) | 0.346±0.021$^a$ | 0.397±0.021$^{abc}$ | 14.8 | 0.386±0.031$^{ab}$ | 11.5 |
| | | 0.430±0.021$^{bc}$ | 24.4 | 0.457±0.034$^c$ | 32.1 |
| Reproductive index | | | | | |
| *Chenopodium album* (C3) | 0.205±0.006$^a$ | 0.190±0.013$^a$ | -7.3 | 0.349±0.018$^b$ | 70.4 |
| | | 0.189±0.052$^a$ | -7.9 | 0.326±0.043$^b$ | 59.2 |
| *Echinochloa crus-galli* (C4) | 0.345±0.014$^a$ | 0.399±0.011$^{bc}$ | 15.7 | 0.382±0.020$^{ab}$ | 10.8 |
| | | 0.394±0.019$^{bc}$ | 14.4 | 0.425±0.008$^c$ | 23.5 |
| *Setaria viridis* (C4) | 0.192±0.015$^a$ | 0.190±0.013$^a$ | -1.1 | 0.208±0.008$^a$ | 8.5 |
| | | 0.204±0.009$^a$ | 6.4 | 0.208±0.012$^a$ | 8.3 |
| Seed weight ($10^{-3}$g, n = 7200) | | | | | |
| *Chenopodium album* (C3) | 0.353±0.029$^a$ | 0.398±0.048$^a$ | 12.8 | 0.346±0.029$^a$ | -2.1 |
| | | 0.367±0.049$^a$ | 3.9 | 0.400±0.056$^a$ | 13.3 |
| *Echinochloa crus-galli* (C4) | 1.210±0.137$^a$ | 1.417±0.103$^b$ | 17.1 | 1.380±0.087$^b$ | 14.1 |
| | | 1.256±0.088$^a$ | 3.9 | 1.317±0.130$^a$ | 8.8 |
| *Setaria viridis* (C4) | 2.369±0.178$^a$ | 1.894±0.162$^a$ | -20.0 | 1.733±0.153$^a$ | -26.8 |
| | | 1.728±0.260$^a$ | -27.1 | 2.383±0.172$^a$ | 0.6 |

vegetative tiller number of the T2, CT2, T4, and CT4 plots increased by an average of 26.3%, compared with the Control plot, but it was not affected by the $CO_2$ enrichment under the same temperature regime.

In the *C. album* populations, the total seed weight per unit ground area (*TSW*) greatly increased from the $CO_2$ enrichment, but in the corresponding temperature plot, it was greatly reduced by the elevated temperature (cf. Table 3). While the *TSW* of the *C. album* populations decreased by 29.7% in the T2 and 14.6% in the T4 plot, compared with the Control plot, it showed an increase of 114.4% in the CT4 plot. The *TSW* in the CT4 plot was 2.5 times greater than that in the T4 plot. Similarly, the *TSW* in the CT2 plot was 1.7 times greater than that in the T2 plot, though it was not significant. In the *C. album* populations, the *TSW* decreased to 29.7% and 14.5% in the T2 and T4 plots, respectively, compared with that of the Control plot. In the *E. crus-galli* populations, the *TSWs* were significantly reduced in the T4 plot, but no significant difference was detected among the Control, T2, and CT4 plots. In the *S. viridis* populations, the *TSW* was 32.1% greater in the CT4 plot than in the Control plot, and was 18.5% greater than in the T4 plot.

The reproductive index of the *C. album* population increased 1.7 times in the T4 plot and 1.6 times in the CT4 plot compared with that in the Control plot (cf. Table 3). In the *E. crus-galli* populations, the reproductive index increased in the CT4 plot compared with the Control, but no significant difference was observed among other plots. In the *S. viridis* populations, the reproductive index showed no difference among the plots.

### *III-2-5. Temperature and light conditions during vegetative and reproductive stages*

The mean air temperatures during the vegetative and the reproductive stages showed different tendencies among the three species. These temperature differences resulted from species-specific responses of plant phenology to elevated temperatures. The mean air temperatures during the vegetative stage of the *C. album* populations were 1.5, 1.1, 0.7, and 0.5℃ lower in the T2, CT2, T4, and CT4 plots, respectively, than in the Control plot (cf. Table 4). However, the mean air temperatures during the reproductive stage were 3.1, 3.5, 4.0, and 4.5℃ higher in the T2, CT2, T4, and CT4 plots, respectively, than in the Control plot.

The mean air temperatures during vegetative stage of the *E. crus-galli* populations were 1.6℃ lower in the T2 and CT2 plot and 2.5℃ lower in the T4 and CT4 plots, respectively, than in the Control plot. Also, the mean air temperatures during reproductive stage of the *E. crus-galli* and *S. viridis* populations showed a significantly higher value in the elevated temperature plots than in the Control plot (Table 4). Integrated solar radiation (*ISR*) during the vegetative stage for the *C. album* population in the Control was significantly higher than those of the other plots but it was lower during the reproductive stage in the Control plot (cf. Table 4). These differences mainly resulted from the difference in the period of vegetative stage.

The mean air temperatures during the vegetative stage of the *E. crus-galli* populations were 1.6℃ lower in the T2 and CT2 plots and 2.5℃ lower in the T4 and CT4 plots, respectively, than in the Control plot. Also, the mean air

Table 4. Mean air temperature, accumulated temperature, and integrated solar radiation during vegetative stage and reproductive stage for *Chenopodium album*, *Echinochloa crus-galli*, and *Setaria viridis* populations. Vegetative stage is defined as period from seedling emergence time to flowering time. Reproductive stage is defined as period from flowering time to two months after flowering time. Values are expressed as the mean days±standard errors for four to three containers per species per treatment. Different *superscript letters* within a row indicate significant difference at $P<0.05$. Diff. indicates differnce with the Control plot

| | Vegetative stage | | | | | Reproductive stage | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | T2 / CT2 | Diff. | T4 / CT4 | Diff. | Control | T2 / CT2 | Diff. | T4 / CT4 | Diff. |
| Mean air temperature (°C) | | | | | | | | | | |
| *Chenopodium album* (C3) | 18.4±0.1$^{a}$ | 16.9±0.0$^{b}$ | -1.5 | 17.7±0.1$^{cd}$ | -0.7 | 23.2±0.0$^{a}$ | 26.3±0.0$^{b}$ | 3.1 | 27.2±0.3$^{c}$ | 4 |
| | | 17.3±0.1$^{bc}$ | -1.1 | 17.9±0.0$^{ad}$ | -0.5 | | 26.7±0.2$^{b}$ | 3.5 | 27.7±0.3$^{c}$ | 4.5 |
| *Echinochloa crus-galli* (C4) | 19.2±0.1$^{a}$ | 17.5±0.1$^{b}$ | -1.7 | 16.6±0.2$^{d}$ | -2.6 | 24.6±0.0$^{a}$ | 24.7±0.1$^{a}$ | 0.1 | 25.4±0.3$^{a}$ | 0.8 |
| | | 17.7±0.3$^{b}$ | -1.5 | 16.9±0.2$^{c}$ | -2.4 | | 24.9±0.6$^{a}$ | 0.3 | 25.3±0.7$^{a}$ | 0.7 |
| *Setaria viridis* (C4) | 18.8±0.0$^{a}$ | 18.2±0.1$^{b}$ | -0.6 | 19.1±0.1$^{c}$ | 0.3 | 24.6±0.0$^{a}$ | 25.6±0.5$^{b}$ | 1.0 | 25.7±0.4$^{b}$ | 1.1 |
| | | 18.2±0.1$^{b}$ | -0.6 | 18.7±0.0$^{a}$ | -0.1 | | 25.6±0.1$^{b}$ | 1.0 | 25.7±0.0$^{b}$ | 1.1 |
| Accumulated temperature (°C·days) | | | | | | | | | | |
| *Chenopodium album* (C3) | 1641.3±23.6$^{a}$ | 1293.4±18.6$^{b}$ | -347.9 | 1250.6±19.3$^{b}$ | -390.7 | 1645.5±0.0$^{a}$ | 1949.4±8.5$^{b}$ | 303.9 | 2032.8±27.1$^{c}$ | 387.3 |
| | | 1334.7±23.6$^{b}$ | -306.6 | 1284.9±19.6$^{b}$ | -356.4 | | 1973.0±13.8$^{b}$ | 327.5 | 2074.2±26.7$^{c}$ | 428.7 |
| *Echinochloa crus-galli* (C4) | 1124.7±11.1$^{a}$ | 1057.6±33.8$^{ab}$ | -67.1 | 1098.7±21.9$^{ab}$ | -26.0 | 1195.4±0.0$^{a}$ | 1211.9±11.3$^{ab}$ | 16.5 | 1248.7±18.9$^{b}$ | 53.3 |
| | | 1023.4±90.2$^{b}$ | -101.3 | 1037.4±32.4$^{ab}$ | -87.3 | | 1218.0±38.6$^{ab}$ | 22.6 | 1237.9±39.3$^{ab}$ | 42.5 |
| *Setaria viridis* (C4) | 1024.1±0.0$^{a}$ | 1115.1±57.6$^{b}$ | 9.1 | 1094.7±43.6$^{bc}$ | 70.6 | 1195.2±0.0$^{a}$ | 1268.3±31.1$^{b}$ | 73.1 | 1274.0±27.0$^{b}$ | 78.8 |
| | | 1052.9±26.6$^{ac}$ | 28.8 | 1061.8±0.0$^{ac}$ | 37.7 | | 1260.0±9.4$^{b}$ | 64.8 | 1268.9±0.0$^{b}$ | 73.7 |
| Integrated solar radiation (MJ/m$^2$) | | | | | | | | | | |
| *Chenopodium album* (C3) | 1711.2±18.5$^{a}$ | 1518.0±9.8$^{b}$ | -193.2 | 1345.8±14.1$^{c}$ | -365.4 | 1046.0±28.7$^{a}$ | 1181.3±0.9$^{b}$ | 135.3 | 1207.8±25.4$^{b}$ | 161.8 |
| | | 1544.2±14.7$^{b}$ | -167.0 | 1427.1±16.9$^{b}$ | -284.1 | | 1178.5±5.6$^{b}$ | 132.5 | 1171.4±13.2$^{b}$ | 125.4 |
| *Echinochloa crus-galli* (C4) | 1114.2±33.6$^{a}$ | 1293.1±29.9$^{b}$ | 178.9 | 1228.5±32.7$^{c}$ | 114.3 | 828.3±20.7$^{a}$ | 814.2±2.7$^{a}$ | -14.1 | 808.8±16.9$^{a}$ | 19.5 |
| | | 1246.2±82.2$^{c}$ | 132.0 | 1226.6±55.8$^{c}$ | 112.4 | | 813.0±15.0$^{a}$ | -15.3 | 822.6±13.3$^{a}$ | -5.7 |
| *Setaria viridis* (C4) | 1296.2±0.0$^{a}$ | 1148.1±21.5$^{b}$ | -148.1 | 1094.1±6.8$^{c}$ | -202.1 | 839.8±0.0$^{a}$ | 842.5±11.5$^{a}$ | 2.7 | 827.1±9.6$^{ab}$ | -12.7 |
| | | 1104.0±22.3$^{b}$ | -192.2 | 1080.1±0.0$^{b}$ | -216.1 | | 831.4±12.0$^{ab}$ | -8.4 | 817.0±0.0$^{b}$ | -22.8 |

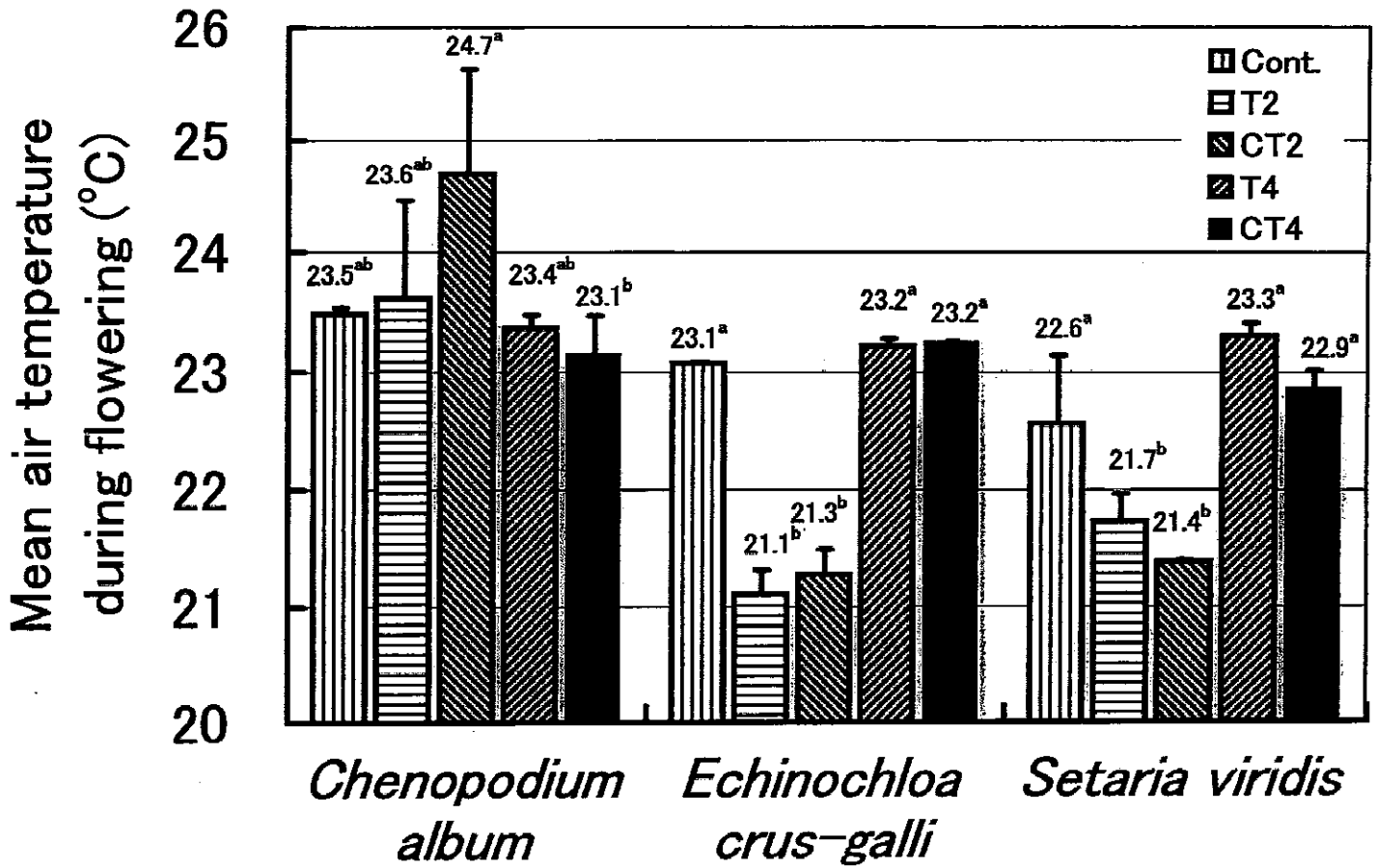


**Figure 22.** Daily mean air temperature around flowering time for *C. album*, *E. crus-galli*, and *S. viridis* populations.

temperatures during the reproductive stage of the *E. crus-galli* and *S. viridis* populations showed a significantly higher value in the elevated-temperature plots than in the Control plot (Table 4).

The integrated solar radiation (*ISR)* during the vegetative stage of the *E. crus-galli* population in the Control plot was significantly lower than that in the other plots, but there was no significant difference between the plots during the reproductive stage. While the *ISR* during the vegetative stage of the *S. viridis* population in the Control plot was significantly higher than that of all the other plots, during the reproductive stage it was only significantly higher in the CT4 plot. The mean *ISR* during the vegetative and the reproductive stages of the *S. viridis* populations in all plots was 1221.8MJ $m^{-2}$ and 817MJ $m^{-2}$, respectively.

Because the temperature most strongly influences pollination during the flowering time, the mean air temperatures in the four weeks surrounding the flowering time for the three species were calculated on the basis of the daily mean air temperature (cf. Fig. 22). In general, the mean air temperatures did not show great differences among the plots because the flowering time shifted with elevated temperature. The mean air temperatures around the flowering time of the *C. album* populations were 23.5, 23.5, 24.7, 23.4, and 23.1℃ respectively in the Control, T2, CT2, T4, and CT4 plots. Those of the two C4 species showed similar trends. While the mean air temperatures around the flowering time of the *E. crus-galli* population in the Control plot were 1.8℃ and 2.0℃ higher than those in the T2 and CT2 plots, respectively, there was little difference (about 0.1℃) compared with the T4 and CT4 plots.

The mean air temperatures around the flowering time of the *S. viridis* population in the Control plot were 0.9℃ and 1.2℃ higher than in the CT2 and

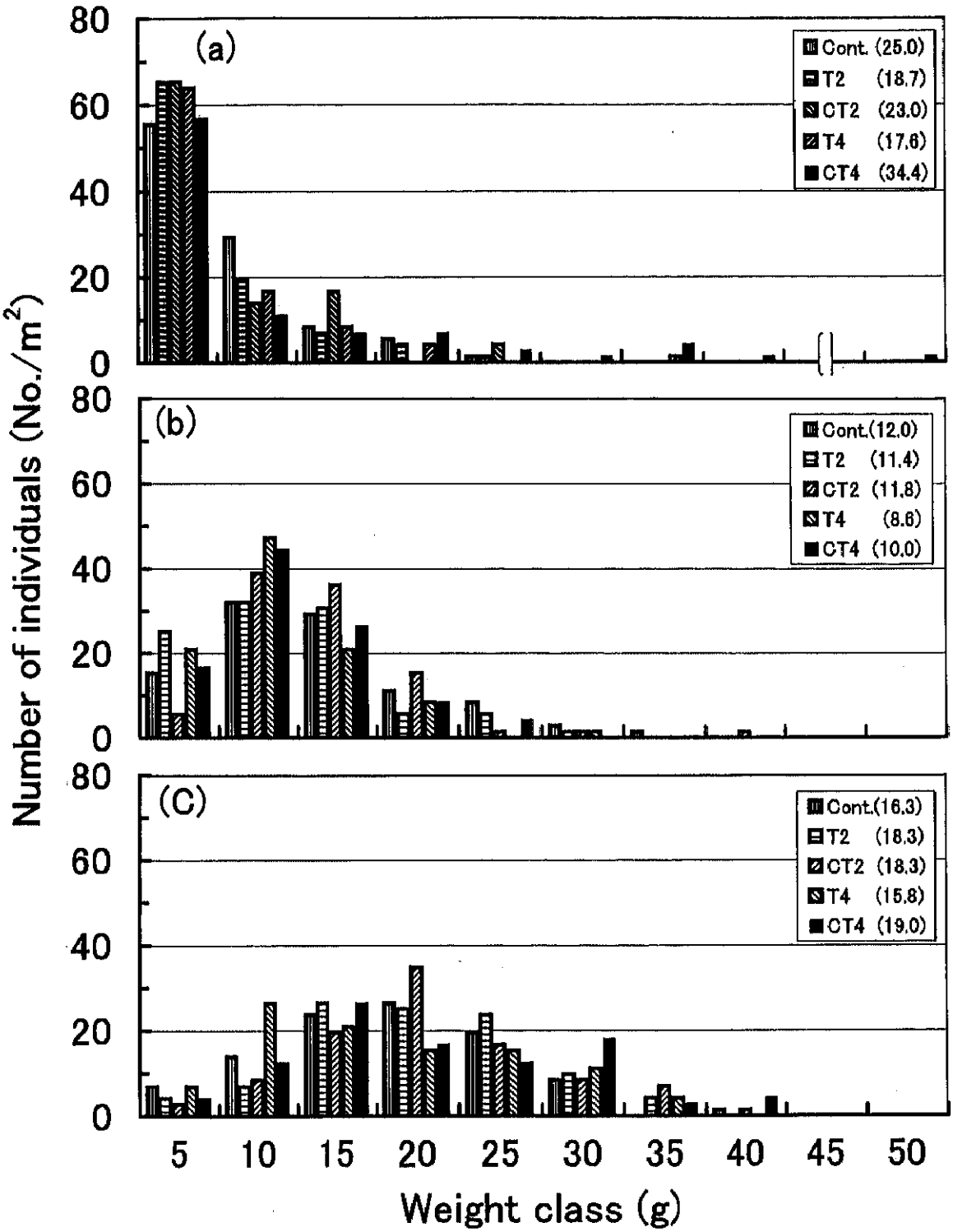


**Figure 23**. The distribution of the individual weights of the *C. album* (a), *E. crus-galli* (b), and *S. viridis* (c) populations grown in elevated temperature and/or $CO_2$. Individual size (weight) for two C4 plants included in all shoots divided from one seed. Values in the index box indicate mean individual weight.

T2 plots, respectively, but there was no significant difference between the T4 and CT4 plots.

### *III-2-6. Effects of elevated temperature and $CO_2$ on size hierarchies*

Most of the three experimental plant species developed inflorescences at the top of the main and/or divided shoot during the experimental period. The mean plant weights of the *C. album* populations significantly decreased with the elevated temperature. However, the mean plant weight in the Control plot showed no significant difference from that in the CT2 plot, while it was significantly higher than that in the CT4 plot. The mean plant weights of the *C. album* populations were 25.0, 18.7, 23.0, 17.6, and 34.4 g in the Control, T2, CT2, T4, and CT4 plots, respectively (cf. Fig. 23a). The weight range distribution of the *C. album* population grown in the elevated temperature and $CO_2$ plot ranged widely, particularly in the CT4 plot.

The mean plant weights of the two C4 plant populations in the Control, T2, CT2, T4, and CT4 plots were 12.0, 11.4, 11.8, 8.6, and 10.0 g in the *E. crus-galli* populations, respectively, and 16.3, 18.3, 18.3, 15.8, and 19.0 g in the *S. viridis* populations, respectively, (cf. Figs. 23b and c).