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RESPONSES OF REPRODUCTIVE TRAITS TO SHORT-TERM ARTIFICIAL WARMING IN A DECIDUOUS ALPINE SHRUB *GEUM PENTAPETALUM* (ROSACEAE)

Naoya WADA, Michiru MIYAMOTO and Satoru KOJIMA

Department of Biosphere Science, Faculty of Science, Toyama University, Gofuku 3190, Toyama 930-8555

Abstract: We examined the effects of short term artificial warming from 4 July to 19 September 1996 (78 days) in an open top chamber (OTC) on fruit production of a deciduous alpine shrub *Geum pentapetalum* (L.) Makino (Rosaceae) in the Tateyama Range, Toyama, Japan. Daily maximum temperature in the growing period significantly increased *ca.* 3.6°C inside the OTC as compared to outside the chamber, while daily minimum temperature significantly decreased *ca.* 1.3°C inside the OTC. Daily mean temperature was not significantly different inside and outside the OTC. There were not any significant differences of shoot characteristics such as the number of leaves, total leaf mass and current stem weight inside and outside the OTC. Dry weight of the main body of the achene (including seed) and individual achene size (IAS) were significantly increased by OTC treatment, while the plume was not. Seed ripening, estimated number of achenes (ENA) and estimated total achene mass per shoot (EAM) slightly increased inside the OTC, but they were not significantly different between the shoots affected by OTC and the unaffected. EAM was significantly positively correlated with current basal stem weight both in OTC-affected and -unaffected shoots. Analysis of covariance showed that EAM in a given stem size was significantly higher in OTC affected shoots than in the -unaffected shoots. These results imply an importance of interaction between fruit production and shoot size when we assess the effects of artificial warming on reproductive traits. Thus, we found that short-term artificial warming first affected the size rather than the number of seeds and fruits, and the reproductive organs were more sensitive to warming than vegetative organs of *Geum pentapetalum*, at least according to the dry weight based analysis.

key words: alpine plant, artificial warming, *Geum pentapetalum*, plant size, seed production

Introduction

In recent years, not only to scientists but also to peoples living in arctic and sub-arctic regions, it is a serious issue how global climatic change caused by human activities will affect terrestrial ecosystems in the Arctic (OECHEL and VOURLITIS, 1994). To understand and predict the dynamics of tundra ecosystems affected by global climatic changes, we need at first to examine how tundra plant species respond to climatic warming. Under these circumstances, in 1990, the International Tundra Experiment

(ITEX) was established by nine countries, Canada, Denmark, Finland, Great Britain, Iceland, Norway, Sweden, United States and Russia (MOLAU and MØLGAARD, 1996). The goal of the ITEX is to understand the response of tundra plant species through simple manipulation and transplant experiments to be conducted at multiple arctic sites (MOLAU and MØLGAARD, 1996). At present, these concerns expand to alpine ecosystems in temperate regions, and there are 4 ITEX active sites in Japan (ITEX Update No. 8, 1996).

In the first step, in 1995, we examined the effects of artificial warming by using the open top chamber (OTC) method (MOLAU, 1993) on the current shoot elongation of two evergreen alpine shrubs (*Empetrum nigrum* L. and *Loiseuleria procumbens* Desv.) in the Tateyama Range, Toyama Prefecture, Japan, and found that current shoot elongation significantly increased in both species by manipulation over a short-term period (KOJIMA *et al.*, 1997). In this paper, we focus on the effect of artificial warming on fruit production of an alpine shrub *Geum pentapetalum* L.

Alpine plants as well as arctic plants struggle against many harsh environmental factors (SAKAI, 1995). Of these, temperature is undoubtedly as a primary factor limiting the reproduction of alpine plants, although pollinator activities and abundance (KEVAN, 1972, 1973; KUDO, 1993), snow-free duration (KUDO, 1991, 1993) and availability of soil nutrients (NADELHOFFER *et al.*, 1991) may also be important. BELL and BLISS (1980) found that seed production of many tundra plants is greatly limited by low temperature in the growing season, which varied from year to year. This also indicates the difficulty of determining which factors are important and how they affect seed production of arctic and alpine plants in a short-term study without any manipulation. Moreover, seed or fruit production is generally related to plant size such as total leaf mass and above-ground biomass. Therefore, we should consider the effects of artificial warming on seed production by taking the plant size into account.

To clarify how strongly temperature in the growing season will affect reproductive traits in short-term manipulation, we examined the effects of artificial warming by using OTC on the number of fruits produced, fruit size, and total fruit mass per shoot of the alpine shrub *Geum pentapetalum*, in relation to shoot size, in the Tateyama Range, Toyama prefecture, Japan.

Materials and Methods

The study site (OTC2) is located at the foot of Mt. Ryûoh of the Tateyama Range about 2700 m above sea level (36°33′42″N, 137°36′29″E: see Fig. 1), in the northern part of the Hida Mountains (Northern Japan Alps). This site is characterized by heavy snow cover from mid-October to mid-June. We established two open top chambers (OTCs, Fig. 2) on the *Geum pentapetalum*, *Empetrum nigrum* and *Loiseuleria procumbens* dominated vegetation (northeast-facing slope) in early July 1996, and automatically measured temperature at *ca.* 5 cm above the ground inside and outside one of the OTCs every one-hour interval from 1 July to 30 September, using KADEC U-II data loggers (Kona System Co. Ltd.). The other OTC was used for observation of flowering phenology and sample collection of *G. pentapetalum* from 4 July to 19 September 1996 (78 days).

The deciduous alpine shrub *Geum pentapetalum* L. (Rosaceae) favors wet habitats,

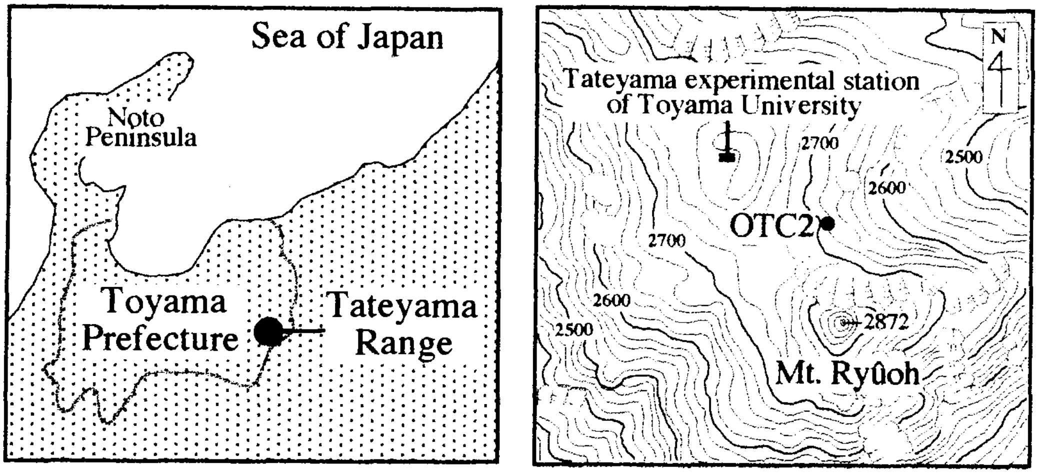

Fig. 1. Study site (OTC2) located at the foot (northeast facing slope) of Mt. Ryûoh (right figure) of the Tateyama Range, Toyama Prefecture (left figure), Japan.

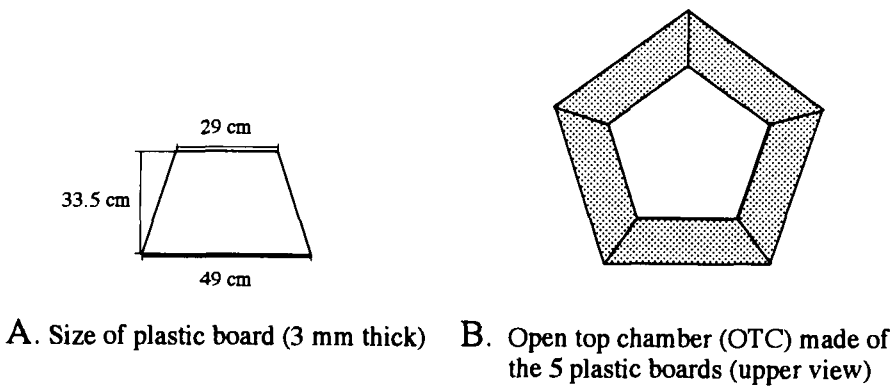

Fig. 2. Size of open top chamber (OTC) made of five plastic boards (A). The basal area of the OTC is 4128 cm^2 (B).

and is distributed in the northern part of Far East Asia, from the central Aleutian Islands to central Japan (HULTÉN, 1968). The flower of this plant has five white petals, and the fruit is a pubescent achene including a nutlet (seed), with a *ca.* 3–5 cm long feather-shaped style (plume: wind dispersal device in maturity).

We collected eleven fruiting shoots, each with leaves and stems, inside and outside the OTC on 19 September 1996. After each shoot was dried (80°C, 48 hours), we measured total leaf mass per shoot and current basal stem weight. Moreover, we analyzed leaf nitrogen content both in OTC-affected shoots and -unaffected shoots by a C-N analyzer (C/N Coder MT-700, Yanaco Co. Ltd.).

Individual achene size (dry weight of achene including dispersal device, hereafter IAS) was calculated by the dry weight of total achenes remaining per shoot divided by the number of achenes, indicating mean achene weight per shoot. After that, we divided the achene into the main body (=seed head, including one seed) and the plume (Fig. 3), and measured each dry weight. Moreover, we dissected each achene main body, and checked the maturity of the seeds.

Because several achenes had already dropped off when we collected samples, we

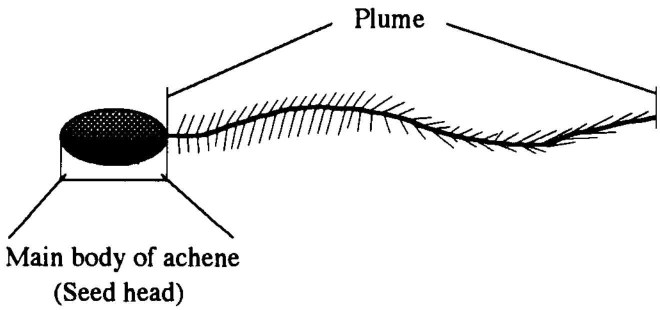

Fig. 3. An achene (fruit) of *Geum pentapetalum*. The main body of the achene includes one seed.

estimated the number of achenes per shoot (hereafter ENA) by counting traces of achenes remaining as hollows on the receptacle of each fruiting shoot. We also estimated total achene mass (dry weight) per shoot (hereafter EAM) as:

$$EAM = IAS \times ENA .$$

Thus, we compared reproductive traits (IAS, ENA and EAM) and shoot sizes (the number of compound leaves, total compound leaf mass and current basal stem) inside and outside the OTC.

Results

Artificial warming by OTC

Differences of temperature (at 5 cm above the ground) inside and outside the OTC are shown in Table 1 and Fig. 4. Daily maximum temperature was significantly higher inside (27.9°C) than outside the OTC (24.3°C) in the growing season (92 days), an increased 3.6°C due to the OTC. Unexpectedly, however, daily minimum temperature was significantly lower inside the OTC (2.9°C) than outside (4.2°C), resulting in no significant difference of daily mean temperature inside (11.5°C) and outside (11.4°C) the OTC (Table 1, Fig. 4). As shown in Fig. 5, we found that daily maximum temperature and Δ Max T (= [daily maximum temperature inside the OTC] − [daily maximum temperature outside the OTC]) were significantly negatively correlated with Δ Min T (= [daily minimum temperature inside the OTC] − [daily minimum temperature outside the OTC]).

Figure 6 shows a typical pattern of temperature change in a sunny day (July 26). The OTC warming effect mainly occurred from 0900 to 1200 when sun-shine radiated into the OTC, while the effect was not so obvious in the other time before 0800 and after 1300. Thus, it was found that the OTC considerably increased maximum temperature only direct sunshine on a sunny day.

Effect of OTC on fruit production, shoot size and leaf quality

Geum pentapetalum mostly flowered from 16 July, 12 days after the OTC was installed, to 24 July. By September, most of the achenes had mature seeds; however, the seed ripening ratio was slightly higher in the OTC-affected shoots than the -unaffected shoots (Table 2). The weight of seed heads was significantly increased by OTC treatment, while the plume weight was not. As a consequence, individual achene size (IAS) differed

Table 1. Effects of OTC on temperature at 5 cm above the ground during the growing season (from July 1 to September 30, 1996). Daily mean temperature, daily maximum temperature, and daily minimum temperature inside and outside the OTC are shown as mean $\pm$ se ($n = 92$). Differences of temperature inside and outside the OTC were tested by the Mann-Whitney U test.

	Inside	Outside	Significance
Mean temperature	11.5 ± 0.5	11.4 ± 0.4	$P = 0.878$
Maximum temperature	27.9 ± 1.2	24.3 ± 1.0	$P = 0.016$
Minimum temperature	2.9 ± 0.4	4.2 ± 0.4	$P = 0.011$

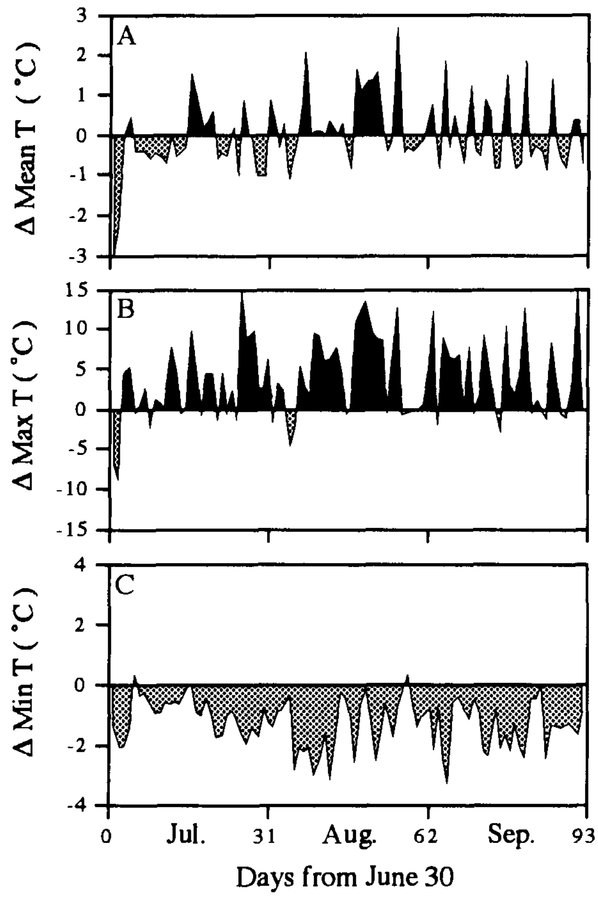

Fig. 4. Differences of temperature (ΔT) inside and outside the OTC. A, daily mean temperature; B, daily maximum temperature; C, daily minimum temperature. Black shaded $= 0°C \geq \Delta T$, light shaded $= 0°C < \Delta T$.

significantly inside and outside the OTC; mean achene weight was heavier inside the OTC than outside.

There is no significant difference of estimated number of achenes (ENA) inside and outside the OTC (Table 2). This is probably because the production of ovules and pistils may be already determined by previous stored resources in the last growing season, so that short-term artificial warming will not influence the number of achenes produced. Estimated total achene mass per shoot (EAM) was slightly higher in fruiting

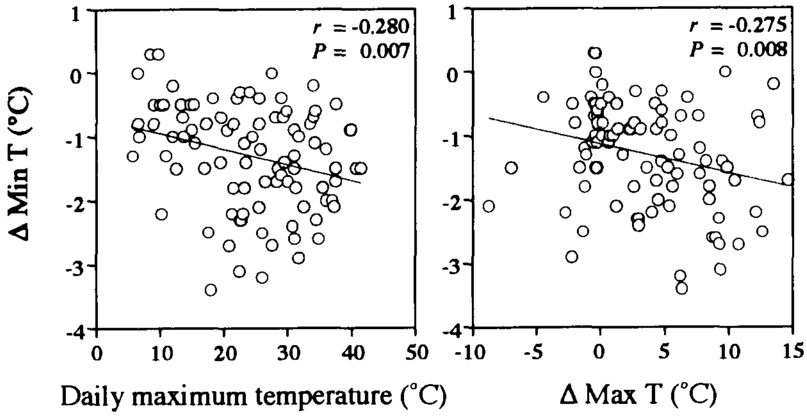

Fig. 5. Relationships between daily maximum temperature, Max ΔT, and Min ΔT from July 1 to September 30 ($n = 92$).

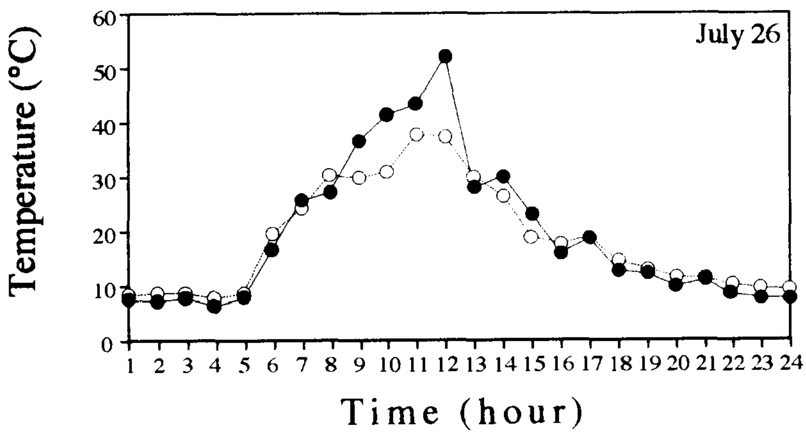

Fig. 6. A typical pattern of temperature change on a sunny day (July 26). Solid circles, inside the OTC; open circles, outside the OTC.

Table 2. Effects of artificial warming by OTC on the reproductive traits per shoot (mean ± sd). Differences of each trait inside and outside the OTC were tested by Student's t-test. Shoot sizes are shown below the reproductive traits. Numerals in parentheses show the number of samples.

Reproductive traits	Inside	Outside	Student's t-test	
			t-value	P
Seed ripening ratio	0.95 ± 0.05 (11)	0.90 ± 0.07 (10)	1.856	0.079
Seed head size (mg)	0.32 ± 0.08 (11)	0.24 ± 0.08 (10)	2.432	0.025
Plume size (mg)	0.26 ± 0.08 (11)	0.23 ± 0.06 (10)	1.221	0.237
Individual achene size (mg)	0.58 ± 0.08 (11)	0.47 ± 0.12 (10)	2.546	0.019
Estimated number of achenes	63.5 ± 16.3 (11)	62.3 ± 8.5 (8)	0.199	0.845
Estimated total achene mass (mg)	37.1 ± 11.3 (11)	32.3 ± 9.5 (8)	0.978	0.342
Shoot sizes				
Number of compound leaves	6.8 ± 1.8 (10)	6.4 ± 3.0 (11)	0.404	0.691
Compound leaf mass (mg)	40.7 ± 14.7 (9)	36.6 ± 19.0 (9)	0.603	0.555
Current basal stem weight (mg)	8.8 ± 5.4 (11)	10.1 ± 4.3 (9)	0.585	0.566

Table 3. Correlation coefficients between reproductive traits and shoot sizes inside (In) and outside (Out) the OTC. ENA, estimated number of achenes; IAS, individual achene size; EAM, estimated total achene mass. Sample size (n) is 9 for inside the OTC, and 8 for outside the one except for the relationship between the reproductive traits and current basal stem weight ($n=7$).

Shoot size	ENA		IAS		EAM	
	In	Out	In	Out	In	Out
Number of compound leaves	0.730*	−0.185	0.419	0.088	0.782*	−0.085
Compound leaf mass	0.682*	0.653	0.353	0.527	0.720*	0.730
Current basal stem weight	0.604	0.728	0.586	0.730	0.772*	0.866*

* $P<0.05$

shoots inside the OTC than outside, although these differences were not significant (Table 2).

There were no significant differences of shoot sizes (the number of compound leaves, compound leaf mass, and current basal stem weight) between inside and outside the OTC (Table 2).

Leaf nitrogen concentrations were $0.77 \pm$ sd 0.18% ($n=10$) for OTC-affected shoots and $0.77 \pm$ sd 0.39% ($n=11$) for the -unaffected shoots, and there was no significant difference ($P=0.503$, by Mann-Whitney U test).

Traits of fruit production in relation to shoot size

The number of fruits and total fruit mass are generally related to plant size. To confirm whether fruit production depends on shoot size or not, we calculated the correlation coefficients between these reproductive traits and the shoot sizes (Table 3). IAS is not significantly correlated with any shoot size, suggesting that the achene weight was increased mostly by the OTC warming effect, rather than by the shoot size effect. Inside the OTC, ENS and EAM were significantly correlated with three shoot characteristics except for the relationship between ENA and basal stem weight (Table 3). Outside the OTC, however, EAM was only correlated significantly with basal stem weight.

To determine which factors (OTC effect or shoot size effect [current basal stem weight]) strongly affect EAM, we analyzed the slope and intercept of the regressions by analysis of covariance (ANCOVA). In the relationship between EAM and current basal stem weight, there is no significant difference of the slope of the regressions inside and outside the OTC ($F_{1,13}=0.255$, $P=0.622$), indicating that increasing ratio of EAM with current basal stem weight is almost the same for shoots growing inside and outside the OTC (see Fig. 7). However, the intercept of the regressions significantly differed inside and outside the OTC ($F_{1,14}=5.411$, $P=0.036$; Fig. 7). This indicates that EAM of a given stem weight is significantly heavier in the OTC-affected shoots than in the -unaffected shoots.

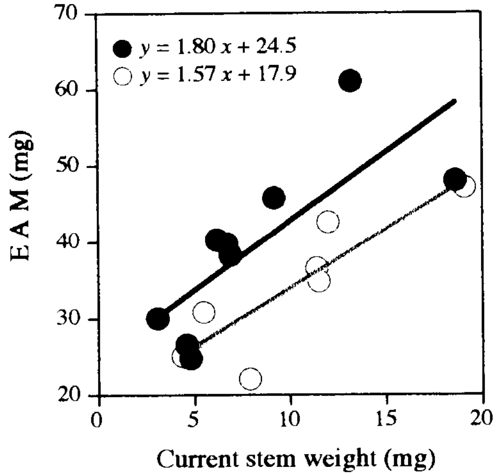

Fig. 7. Relationship between current basal stem weight and EAM (estimated total achene mass per shoot). Black line, inside the OTC; gray line, outside the OTC. Solid circles, inside the OTC; open circles, outside the OTC.

Discussion

Artificial warming by OTC

In this study, the effect of OTC on mean temperature increment was weak as compared to other studies (KOJIMA *et al.*, 1997; NAKASHINDEN *et al.*, 1997; SUZUKI and KUDO, 1997). However, KOJIMA *et al.* (1997) reported that current shoot elongation of two alpine evergreen shrubs *Empetrum nigrum* and *Loiseleuria procumbens* was significantly increased by OTC treatment at this study site (OTC2). Thus, although the OTC warming effect was not strong in the present study, the temperature gain on sunny days might positively affect carbon gain by photosynthesis for alpine plants.

In the present study, we unexpectedly found that daily minimum temperature was significantly decreased by OTC treatment. Thus phenomenon is also observed at other OTC experiment sites (KUDO, per. comm.), suggesting one of the characteristics of OTC-microclimate in a given condition created by microtopography, direction of slope, and so on. We found that daily maximum temperature and Δ Max T were significantly negatively correlated with Δ Min T (Fig. 5). Although we do not know the mechanism causing decrease of daily minimum temperature inside the OTC, the above results imply that there is radiant cooling or a "chimney effect" (difference of temperature inside and outside the OTC causes convective air flow throughout the OTC, resulting in cooling by evaporation of water moisture inside the OTC). Further studies are needed to clarify the mechanism.

Effects of OTC on fruit production and shoot size and quality

In the arctic and alpine plants *Dryas octopetala* (Rosaceae) and *Ranunculus adoneus* (Ranunculaceae), flowers show sun-tracking behavior (flower heliotropism), resulting in higher temperature on the gynoecium than ambient temperature. As a consequence, individual seed or achene weight increased significantly more in intact flowers than in manipulated flowers that were artificially constrained to track the sun (KJELLBERG *et al.*, 1982; STANTON and GALEN, 1989). Thus, for plants inhabiting cold environments, temperature gain on the gynoecium may greatly affect reproductive success. As compared to these studies, artificial warming by the OTC may affect not only the gynoecium of flowers but also photosynthetic organs. Therefore, it is necessary to assess the effects of OTC on the reproductive traits in consideration of interaction between leaf size, leaf quality, stem size and reproductive organs.

In the present study, we found that the individual achene size of *G. pentapetalum* was not significantly correlated with shoot sizes either inside or outside the OTC, but was significantly increased by the OTC treatment in short-term manipulation. Because the difference of seed ripening ratio between OTC-affected and -unaffected shoots was slight, temperature-dependent physiological processes after fertilization, rather than in pollen germination and elongation, probably work well due to the increased temperature, resulting in *ca.* 0.11 mg heavier IAS in OTC-affected shoots.

In contrast, the estimated number of achenes (ENA) and estimated total achene mass (EAM) per shoot were not significantly affected by the OTC treatment. Moreover, leaf nitrogen concentration was not different between the two groups, and showed very low values. Because we sampled the OTC-affected shoots and the -unaffected ones in mid-September when most leaves had already turned red, chlorophyll and some proteins affecting photosynthetic activities in the leaves might have been dissolved and translocated to nonphotosynthetic organs such as stems and roots. If this is the case, our sampled leaves might not reflect the real available resources for seed maturity. Therefore, leaf size and quality at a fruit-developmental stage throughout the time between OTC-affected and -unaffected shoots should be compared, in order to clarify the reason why ENA and EAM were strongly correlated with leaf mass inside the OTC and why not outside the OTC.

In contrast to leaf number and leaf mass, current basal stem size was significantly correlated with EAM both in OTC-affected and -unaffected shoots. From the results of Table 2, EAM was *ca.* 5 mg heavier inside the OTC than outside, but difference was not significant. If we took shoot (stem) size effect into account (by ANCOVA), EAM was *ca.* 6.4 mg (difference of a intercept of the regressions) heavier in the OTC-treated shoots than in -unaffected shoots. These values were nearly the same, although the stem size was not significantly different inside and outside the OTC. Thus, we also need to analyze not only dry weight but also chemical traits of the stem, the same as of leaves throughout the fruit development. It is generally considered that plant (or shoot) size mostly reflects resource availability, and then we expect high correlation between plant size and reproductive biomass. However, our findings in this study imply that the relationship as mentioned above may not be strong if we don't take the quality and phenology of leaves and stems into account. Thus, our studies suggested the importance of interaction between the OTC effects and shoot size effects to clarify how artificial warming by the OTC affects reproductive traits.

In conclusion, we found that artificial warming by the OTC, even over a short-term period, affected the individual achene size (especially in seed head), and EAM of *Geum pentapetalum*, suggesting slightly higher reproductive success. However, the shoot size such as leaf and stem mass did not strongly affected by first-year OTC treatment, at least by the dry weight based analysis, indicating that reproductive organs were more sensitive to warming than vegetative organs. Finally, we stressed temporal responses of leaf and fruiting phenology, and also leaf and stem response to artificial warming, may be very important to understand the detailed mechanism of the OTC effect on reproductive traits.

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