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Timing of bud flush and bud set of seedlings of *Abies sachalinensis*, *Picea. glehnii* and *P. jezoensis* transplanted to warm sites

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温暖地に移植したトドマツ・アカエゾマツ・エゾマツ実生の開芽期と冬芽形成期

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1. Introduction

Many studies provide evidence that the phenological events of animals and plants are responding to climate change (Walther *et al*., 2002). Phenology has received much research focus because many organisms are changing the timing of certain activities within their life cycles in response to ongoing global warming (Root *et al*., 2003). The growing season is often lengthened, and plant growth may be enhanced by global warming (Gordo and Sanz, 2010). Furthermore, changes in the timing of flowering and fruit set may affect plant-animal interactions (Walther *et al*., 2002; Gordo and Sanz, 2010).

The phenology of certain plant characteristics during spring, such as bud flush and flowering, tends to be occurring earlier in general (Doi and Katano, 2008). In comparison, the phenology of certain plant characteristics during fall, such as leaf coloration or defoliation, tends to be occurring later (Matsumoto *et al*., 2003). Thus, the growing period of plants has been lengthening in recent decades (Menzel and Fabian, 1999). However, the responses of plants to the warming climate vary depending on species and location (Doi and Katano, 2008; Gordo and Sanz, 2010). Temperate and boreal forests in the Northern Hemisphere cover a wide geographical area and act as substantial carbon sinks (Magnani *et al*., 2007). Therefore, it is

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important to establish the potential impacts of global warming on these forests.

In this study, we transplanted the seedlings of three conifer species, *Abies sachalinensis*, *Picea glehnii*, and *P. jezoensis* to a single native site (Furano) and two warm sites (Chichibu and Chiba) in May 2016. We compared the timing of bud flush and bud set of these seedlings in the warm sites compared to the native site. Our results are expected to provide clear insights on how climate warming is affecting the phenology of the seedlings of three boreal conifers.

2. Materials and Methods

The seeds of the three conifers were collected from the University of Tokyo Hokkaido Forest (UTHF), Graduate School of Agricultural and Life Sciences, the University of Tokyo in Furano, central Hokkaido, Japan (hereafter, referred to as Furano). Two-year-old seedlings of each species were prepared in Yamabe nursery (43° 13′ 10"N, 142° 22′ 55"E) of the UTHF. Warm sites were Kagemori nursery (35° 59'02"N, 139° 04'34"E) of the University of Tokyo Chichibu Forest (UTCF) in Chichibu, Saitama Prefecture (hereafter, Chichibu) and Goudai nursery (35° 11'33"N 140° 06'33"E) of the University of Tokyo Chiba Forest (UTCBF) in Kamogawa, Chiba Prefecture (hereafter, Chiba). The average of mean annual temperature from 2001 to 2010 for Furano, Chichibu, and Chiba was 6.4℃ 13.2℃ 13.9℃ respectively (The University of Tokyo Forests Graduate School of Agricultural and Life Sciences, The University of Tokyo, 2003; 2005a; 2005b; 2006; 2007; 2008; 2009; 2010; 2011; and 2012). We planted 48 seedlings of each species at the native site (Furano) and at the two warm sites (Chichibu and Chiba) in May 2016. Seedlings were planted using a single-plot design with approximately 40 cm interval and were fully irrigated after planting with no fertilization during the experiment.

Photographs of terminal buds for 12 seedlings (4 seedlings × 3 species) per site were taken with a digital camera (Fig. 1) from the first week in two warm sites (the third week in Furano only) in April to the second week in August (Table 1). Overall, 19 digital photographs were taken at Chichibu and Chiba, and 17 times at Furano (Table 1). Day of Year (DOY) of bud flush date and bud set date was determined based on the first day of bud flush and the presence of newly formed winter buds, according to the method of Goto (2018). Furthermore, the period from bud flush date to bud set date was calculated for each seedling.

Digital loggers (Data Collector RTR-500DC) were installed near the planting sites in November 2016 to record air temperature. Effective cumulative temperature (above 5℃) was calculated from the mean annual day temperature by the initial date as 1th January.

3. Results and Discussion

In total, 36 seedlings (4 seedlings × 3 species × 3 sites) were observed in this study. However, we could

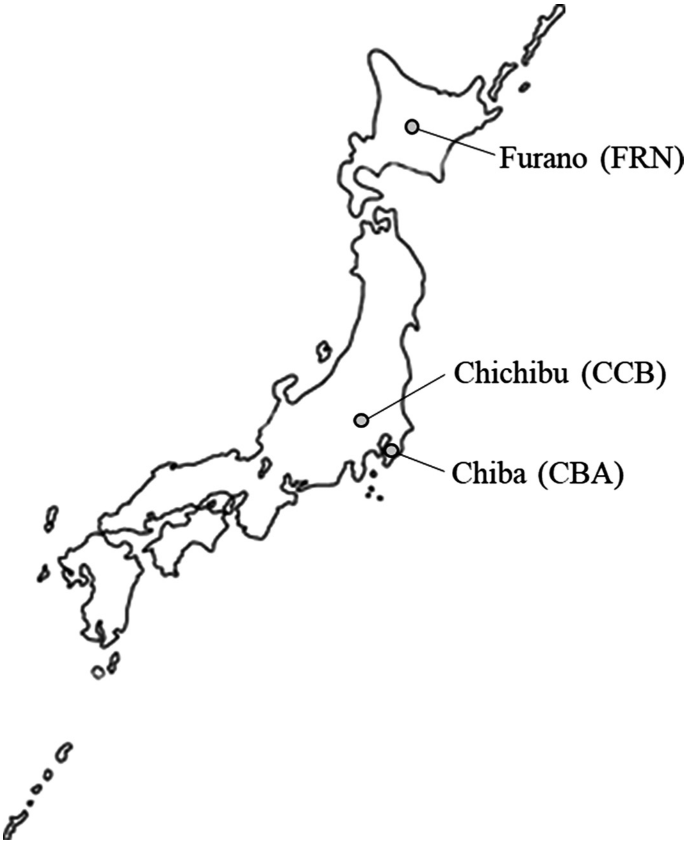


Fig. 1. Location of the three planting sites.

not identify the bud set date of four *P. glehnii* seedlings (No. 348 and 364 in Furano and No. 196 and 235 in Chichibu), because winter buds were tightly hidden in needles. In addition, four *P. jezoensis* seedlings (No. 114 and 127 in Chiba and No. 243 and 268 in Chichibu) did not exhibit normal bud flush or bud set. Overall, bud phenology data of 12, 8, and 8 seedlings of *A. sachalinensis*, *P. glehnii*, and *P. jezoensis*, respectively were used in the analyses of this study (Table 2). Although the bud flush and bud set dates for all species were 30 to 45 days earlier at the warm sites (Chichibu and Chiba), as compared to the native site (Furano), the length of the shoot elongation period was not affected (Table 3).

Effective cumulative temperature was higher in Chichibu and Chiba compared to Furano (Fig. 2). The DOY that effective cumulative temperature exceeded 200 was 144 in Furano, 116 in Chichibu, and 108 in Chiba (Fig. 2). The relationship between bud phenology and effective cumulative temperature was shown in Figure 3. Based on the average cumulative effective temperature at the three sites, the effective cumulative temperature of bud flush for *A. sachalinensis*, *P. glehnii*, *P. jezoensis* was 186, 250, and 112, respectively, while that of bud set was 629, 1002, and 594, respectively.

Table 1. Timing of the observation of bud phenology at three sites.

		Furano		Chichibu		Chiba	
Month	Week	Date	DOY	Date	DOY	Date	DOY
April	1st	-	-	5th	95	6th	96
	2nd	-	-	11th	101	13th	103
	3rd	18th	108	18th	108	20th	110
	4th	25th	115	25th	115	27th	117
May	1st	2nd	122	2nd	122	2nd	122
	2nd	9th	129	9th	129	11th	131
	3rd	16th	136	16th	136	18th	138
	4th	23th	143	23th	143	25th	145
	5th	30th	150	30th	150	31th	151
June	1st	6th	157	6th	157	7th	158
	2nd	13th	164	13th	164	14th	165
	3rd	20th	171	20th	171	22th	173
	4th	27th	180	27th	180	28th	179
July	1st	7th	188	6th	187	6th	187
	2nd	14th	195	12th	193	13th	194
	3rd	18th	199	19th	200	19th	200
	4th	25th	206	26th	207	26th	207
August	1st	3rd	215	3rd	215	1st	213
	2nd	8th	220	10th	222	10th	222

In general, the growing season is expected to be lengthened by global warming. For instance, over a 30-year observation period from the 1960s in Europe, the average growing season lengthened by 10.8 days due to global warming (Menzel and Fabian, 1999). However, the current study showed that the period from bud flush to bud set was similar between the native site and warm sites for all three species, because both bud flush and bud set dates were 30-45 days earlier at the warm sites compared to the native site (Table 2, 3). The effective cumulative temperature of bud flush was higher in Chiba for all three species than in Chichibu and in Furano (Fig. 2, 3). The effective cumulative temperature of bud set was highest in Chiba for *A.sachalinensis* and *P. jezoensis* and in Chichibu for *P. glehnii* (Fig. 2, 3). The effective cumulative temperature of bud set of *A. sachalinensis* and *P. jezoensis* was approximately 600, while that of *P. glehnii* was approximately 1000, regardless of planting site location (Fig. 3). This phenomenon might partly explain the reason why shoot elongation period was similar among sites (Table 3).

Table 2. Phenological data for *As* (*A. sachalinensis*), *Pg* (*P. glehnii*), and *Pj* (*P. jezoensis*), 0: winter bud, 1: bud flushing, 2: bud set, - : not observed. *ID represents individuals for bud flush or bud set date could not be determined. "na" represents data for bud flush or bud set is not available (see Materials and Methods).

Species	Site	ID	April				May					June				July				August	
			1st	2nd	3rd	4th	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd
As	Furano	297	-	-	0	0	0	0	0	1	1	1	1	1	1	2	2	2	2	2	2
	Furano	303	-	-	0	0	0	0	0	1	1	1	1	1	1	2	2	2	2	2	2
	Furano	314	-	-	0	0	0	0	1	1	1	1	1	1	2	2	2	2	2	2	2
	Furano	329	-	-	0	0	0	0	0	1	1	1	1	1	2	2	2	2	2	2	2
	Chichibu	149	0	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2
	Chichibu	162	0	0	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2
	Chichibu	172	0	0	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2
	Chichibu	188	0	0	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2
	Chiba	5	0	0	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2
	Chiba	17	0	0	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2
	Chiba	30	0	0	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2
	Chiba	46	0	0	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2
Pg	Furano	348*	-	-	0	0	0	0	0	1	na	na	na	na	na	na	na	na	na	na	na
	Furano	351	-	-	0	0	0	0	0	0	1	1	1	1	1	1	1	1	2	2	2
	Furano	364*	-	-	0	0	0	0	0	0	1	na	na	na	na	na	na	na	na	na	na
	Furano	377	-	-	0	0	0	0	0	0	1	1	1	1	1	1	1	1	2	2	2
	Chichibu	196*	0	0	1	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na

Table 2. (Continued)

Pg	Chichibu	206	0	0	0	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2
	Chichibu	221	0	0	0	0	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2
	Chichibu	235*	0	0	1	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
	Chiba	54	0	0	0	0	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2
	Chiba	67	0	0	0	0	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2
	Chiba	80	0	0	0	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2
	Chiba	90	0	0	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2
Pj	Furano	390	–	–	0	0	0	1	1	1	1	1	1	1	2	2	2	2	2	2	2
	Furano	404	–	–	0	0	0	0	1	1	1	1	1	1	1	2	2	2	2	2	2
	Furano	416	–	–	0	0	0	1	1	1	1	1	1	1	2	2	2	2	2	2	2
	Furano	427	–	–	0	0	0	1	1	1	1	1	1	1	1	2	2	2	2	2	2
	Chichibu	243*	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
	Chichibu	255	0	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2
	Chichibu	285	0	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2
	Chichibu	268*	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
	Chiba	101	0	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2
	Chiba	114*	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
	Chiba	127*	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
	Chiba	137	0	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2

Table 3. Median and range (min.-max.) of day of year (DOY) for bud flush, bud set, and shoot elongation period.

Species	Site	Bud flush		Bud set		Shoot elongation period	
		Median	Range	Median	Range	Median	Range
A. sachalinensis	Furano	143.0	(13–143)	183.0	(178–188)	43.5	(35–45)
	Chichibu	108.0	(101–108)	150.0	(150–157)	42.0	(42–56)
	Chiba	110.0	(110–110)	151.0	(151–158)	41.0	(41–48)
P. glehnii	Furano	146.5	(143–150)	206.0	(206–206)	59.5	(56–63)
	Chichibu	118.5	(115–122)	178.0	(178–178)	59.5	(56–63)
	Chiba	119.5	(110–122)	173.0	(173–179)	53.5	(51–69)
P. jezoensis	Furano	129.0	(129–136)	182.0	(176–188)	49.5	(47–59)
	Chichibu	103.0	(103–103)	146.5	(143–150)	43.5	(40–47)
	Chiba	101.0	(101–101)	148.0	(145–151)	47.0	(44–50)

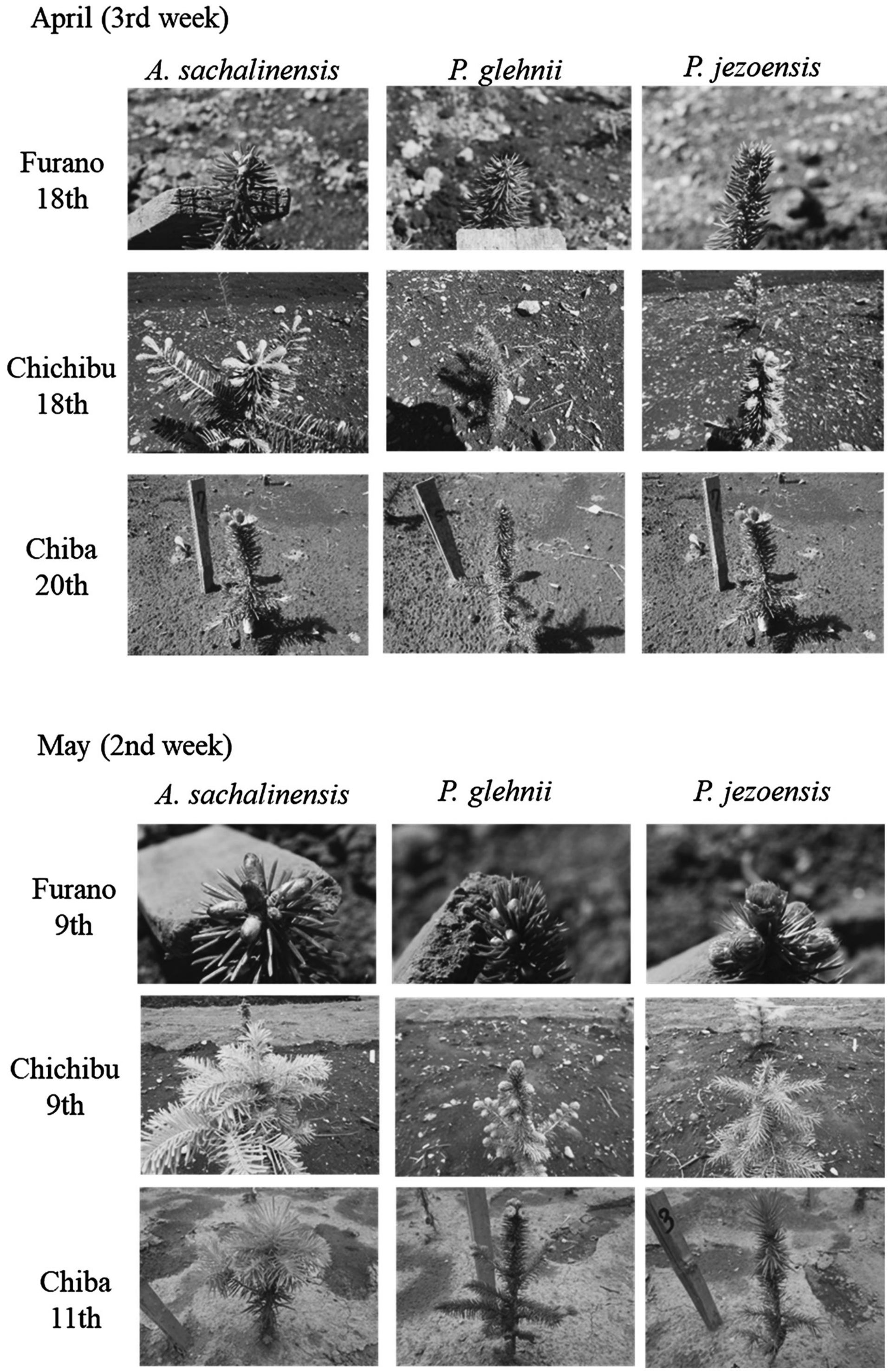
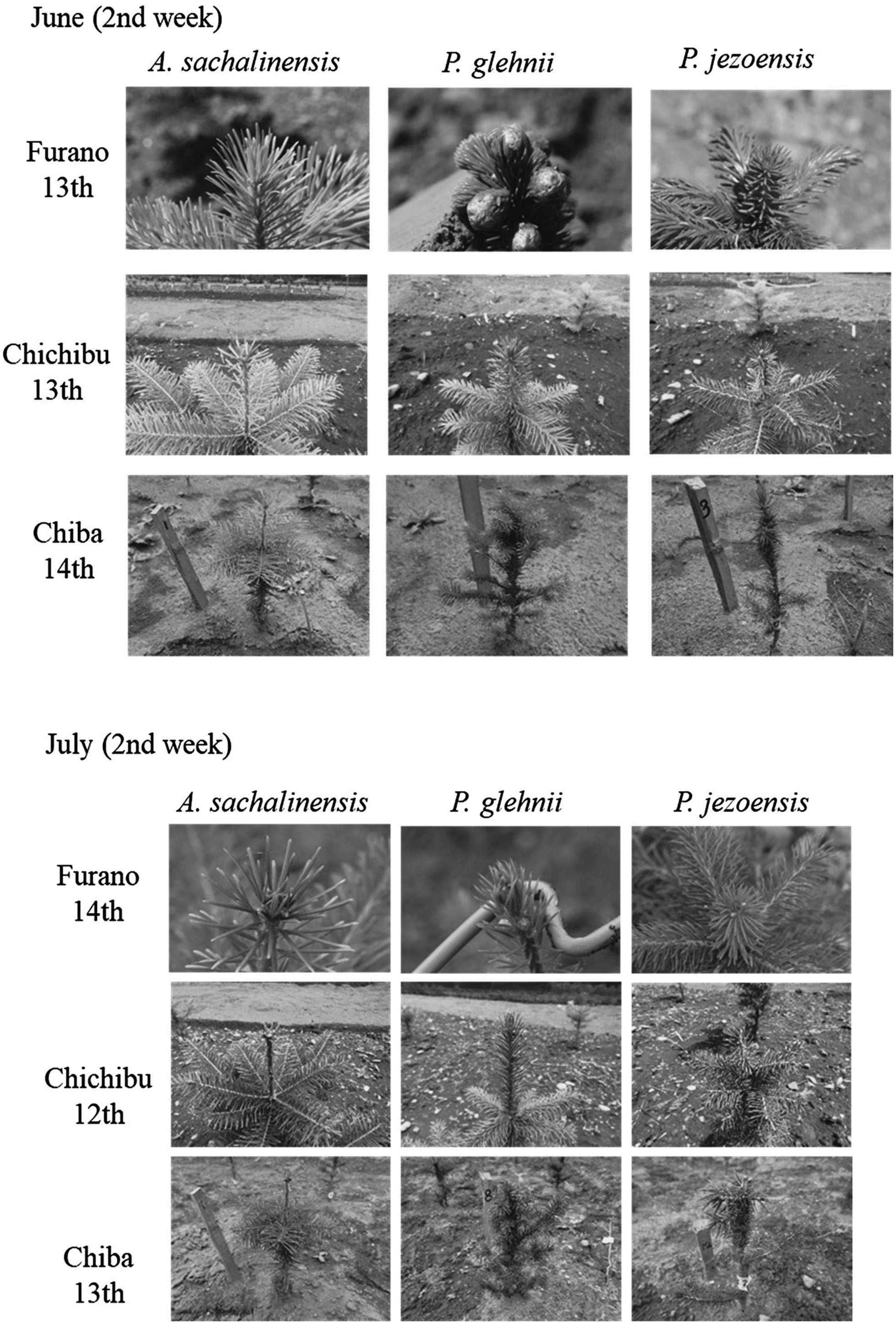


Fig. 2. Serial photographs of bud phenology for three boreal conifers: *A. sachalinensis*, *P. glehnii*, and *P. jezoensis*



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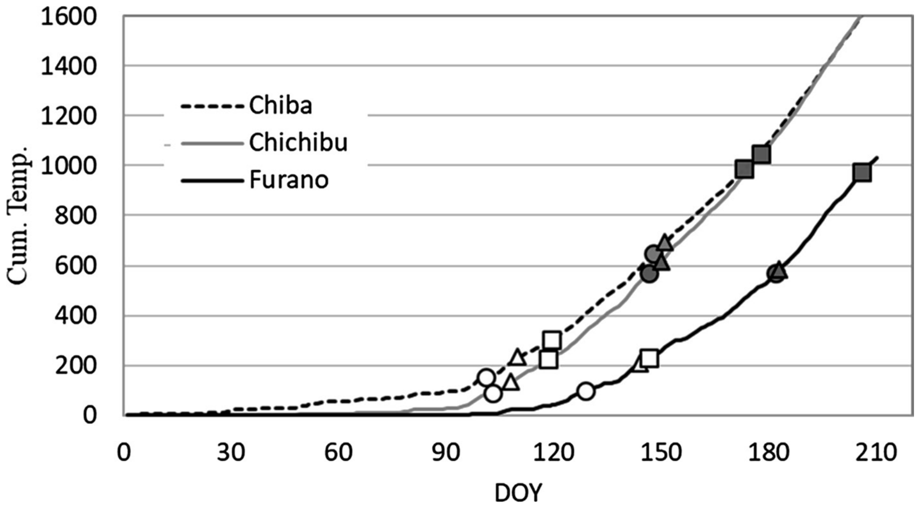

Fig. 3. Effective cumulative temperature (Cum. Temp.), along with day of year (DOY) of bud flush and bud set for three boreal conifers. ○ : *P. jezoensis*, △ : *A. sachalinensis*, □ : *P. glehnii*, open symbol: bud flush, closed symbol: bud set

In this study, digital photos were used to evaluate the status of bud phenology (Fig. 1). However, it was difficult to determine the timing of bud set, especially for *P. glehnii*. Goto (2018) investigated the timing of bud flush and bud set of seedlings of the same three boreal conifers in Furano, and found that the *P. jezoensis* exhibited the earliest bud phenology among three conifer species. The author also found that *P. glehnii* was characterized by mid-term bud flush, and that it had the latest bud set, which facilitated a long growing period. Furthermore, the author found that *A. sachalinensis* was intermediate between the two other *Picea* species. The relationship of bud phenology for the three species monitored in this study was consistent with that obtained by Goto (2018).

In conclusion, we found that the timing of both the bud flush and bud set was earlier in the warm sites than in the native site for all conifer species examined, *A. sachalinensis*, *P. jezoensis,* and *P. glehnii*. Therefore, the period from bud flush to bud set for these species may not largely change owing to global warming.

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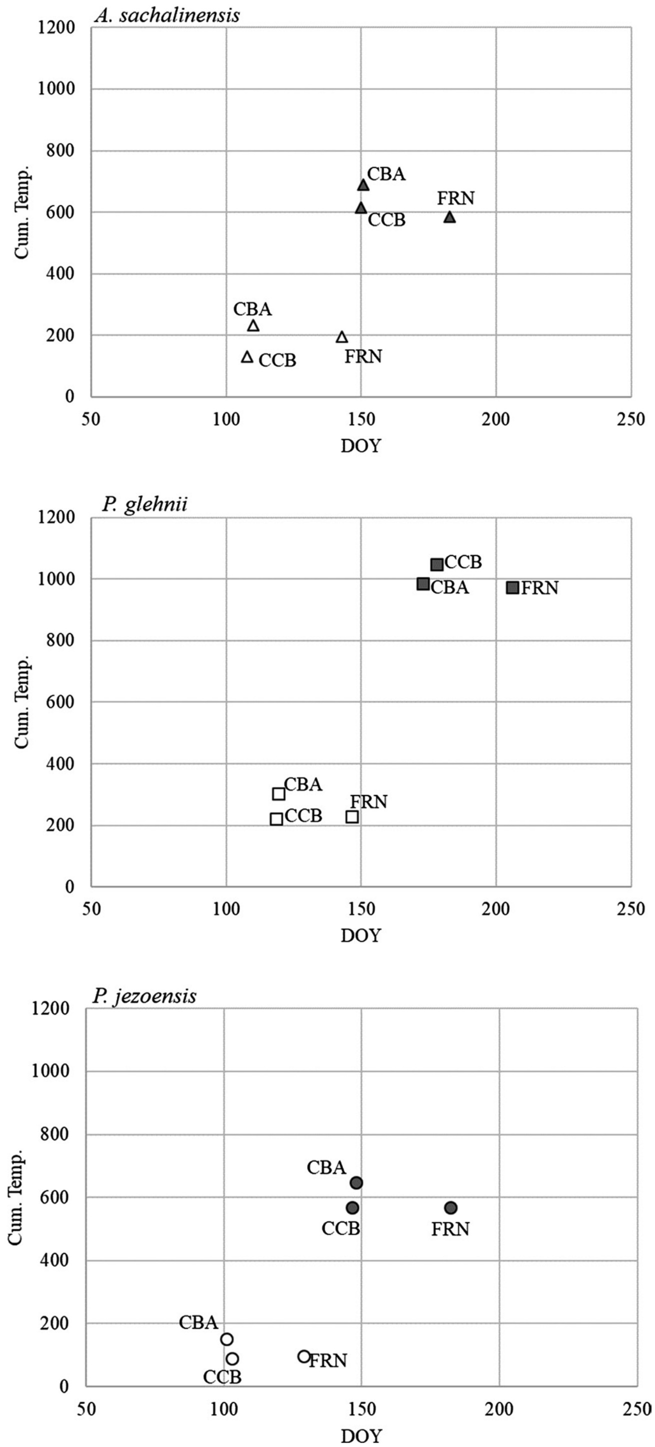

Fig. 4. Relationship between day of year (DOY) and effective cumulative temperature (Cum. Temp.) of bud phenology (bud flush and bud set) for three boreal conifers. ○ : *P. jezoensis*, △ : *A. sachalinensis*, □ : *P. glehnii*, open symbol: bud flush, closed symbol: bud set, CBA: Chiba, CCB: Chichibu, FRN: Furano

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