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SEED DORMANCY IN *TRILLIUM CAMSCHATCENSE* (MELANTHIACEAE) AND THE POSSIBLE ROLES OF LIGHT AND TEMPERATURE REQUIREMENTS FOR SEED GERMINATION IN FORESTS[1]

TETSUYA KONDO[2,4], MAKOTO MIKUBO[2], KEISUKE YAMADA[2], JEFFREY L. WALCK[3], AND SITI N. HIDAYATI[3]

[2]Graduate School of Agriculture, Hokkaido University, Kita 9, Nishi 9, Kita-ku, Sapporo, 060-8589, Japan; and [3]Department of Biology, Middle Tennessee State University, Murfreesboro, Tennessee 37132 USA

- *Premise of the study*: Seeds of some temperate plants require multiple treatments to break complex forms of dormancy, such as deep simple double morphophysiological dormancy (MPD), but embryo growth and radicle and cotyledon emergence have not been studied in depth for this level of MPD. Here we studied *Trillium camschatcense*, a species that is purported to have this MPD and that is sensitive to habitat fragmentation with decreased recruitment at forest edges.
- *Methods*: *Trillium camschatcense* seeds were collected and experiments conducted in Hokkaido, Japan over 5 years. Growth of embryos and emergence of radicles and cotyledons were tracked in relation to field temperature to determine specific temperature and light requirements for these stages under laboratory conditions.
- *Key results:* Low (winter) temperatures overcame dormancy in the underdeveloped embryos, and embryo growth and radicle emergence occurred between July and September, ca. 1 year after seed dispersal. Radicles emerged optimally over a narrow temperature range (20–25°C), in darkness, and at constant temperatures. Roots developed during the second autumn. Cotyledons needed a second low temperature (second winter) to emerge from seeds with roots, doing so in April, slightly over 1.5 years after dispersal.
- *Conclusions:* Seeds of *T. camschatcense* have deep simple double MPD and requirements for radicle emergence: darkness and constant temperatures. Ecologically, edges of forests may be deleterious for germination of the species due to increased light and to higher temperature fluctuations as compared to the interiors of forests. Thus, these specific requirements may play an important role in reducing seed germination of this plant at forest edges.

Key words: alternating temperature; double dormancy; edge effect; habitat; morphophysiological dormancy; seedling; underdeveloped embryo

Seeds of many temperate plants around the world are dormant at the time of dispersal, and they require warm or cold temperatures in conjunction with dry or moist conditions to overcome dormancy; these treatments are known as after-ripening, warm stratification, or cold stratification (Baskin and Baskin, 1998). Following one period of treatment, their seeds are nondormant and germinate when temperatures and moisture conditions are favorable. However, seeds of other temperate species have very complex forms of dormancy that need multiple treatments for dormancy release. For example, some seeds require a period of warm stratification followed by cold stratification (Kondo et al., 2005), while others need two periods of cold stratification interrupted by warm stratification (Barton and Schroeder, 1942) for complete germination (i.e., emergence of both roots and shoots). Moreover, emergence of the root and the shoot from seeds with multiple dormancy-break periods may occur at the same time (Hidayati et al., 2000b) or at different times (Kondo et al., 2004).

With rare exceptions (Baskin and Baskin, 1995), seeds of temperate species with these complex dormancy break mechanisms have underdeveloped embryos that must grow before the radicle emerges and are classified as having some level of morphophysiological dormancy (MPD) (Baskin and Baskin, 1998). For example, the seed dormancy of plants such as *Viburnum* species, that require warm stratification for root emergence and then cold stratification for shoot emergence is referred to as "deep simple epicotyl MPD" or generally as "epicotyl dormancy" (Baskin et al., 2009a). Temperature conditions for embryo growth followed by radicle emergence and cotyledon emergence for epicotyl dormancy have been relatively well studied (Baskin and Baskin, 1998; Adams et al., 2003; Hidayati et al., 2005; Kondo et al., 2004, 2005). Recently, the concept of epicotyl dormancy has expanded with the findings that moderate temperatures may overcome root dormancy and shoot dormancy separately; this situation has been called "non-deep simple epicotyl MPD" (Baskin et al., 2008, 2009b; Jayasuriya et al., 2010). In contrast, the precise requirements for embryo growth and for root and shoot emergence rarely have been examined for seeds that need cold + warm + cold stratifications for germination. This kind of dormancy is named "deep simple double MPD," or generally "double dormancy" or "2-year seeds". Roots emerge and grow during relatively warm temperatures following the first winter, and shoots emerge following a second winter. Barton and Schroeder (1942) were the first researchers to document double dormancy. However, little research has been conducted on this kind of dormancy since that time (Barton, 1944; Whigham, 1974; Takagi, 2001a, b).

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[4] Author for correspondence (e-mail: kondo@res.agr.hokudai.ac.jp)

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The genus *Trillium* (Liliaceae, Melanthiaceae, or Trilliaceae) includes approximately eight species in Asia and ca. 42 species in North America (Freeman, 1975). These species are an important component of the herbaceous flora in temperate forests of Asia and North America, particularly the southern Appalachians (Mabberley, 2008). Of the ca. 50 species worldwide, the type of seed dormancy has been reported for only five of them, all of which occur in North America. *Trillium erectum* L., *T. grandiflorum* Salisb., and *T. ovatum* Pursh have double dormancy (Barton, 1944; Jules and Rathcke, 1999), whereas *T. flexipes* Raf. and *T. sessile* L. have epicotyl dormancy (Baskin and Baskin, 1998). Underdeveloped embryos in *Trillium* seeds have been reported (Martin, 1946), but specific light and temperature conditions related to embryo growth and to root and shoot emergences have not been documented. We selected the Asian species *T. camschatcense* Ker-Gawl. because it purportedly has double dormancy. This species belongs to the *Erectum* group of the genus along with the North American *T. erectum* (Ohara and Kawano, 2005), whose seeds have double dormancy (Barton, 1944).

Samejima and Samejima (1962) performed tests on dormancy and germination of seeds of *T. camschatcense* (as *T. kamtschaticum*), and they suggested that seeds of this species have double dormancy. However, in their experiments, only one pot with 50 seeds was used in each treatment, they observed only 6% radicle emergence and 4% (green) seedling emergence. Therefore, their results were inconclusive. Further, they did not monitor the growth of embryos, which is an important criterion to determine the level of MPD, nor did they examine the effects of light and temperature on root emergence and cotyledon emergence. We also hypothesized that seeds of *T. camschatcense* have double dormancy, and we monitored the embryo growth and radicle emergence and cotyledon emergence in the field and investigated the light and temperature requirements for radicle emergence and cotyledon emergence in detail.

Trillium camschatcense is found from northern Honshu and Hokkaido (Japan) to the Kamchatka Peninsula (Russia) and throughout the Kurile Islands (Tatewaki, 1957; Ohara and Kawano, 2005). This white-flowered species is perennial and grows in mesic deciduous forests taking more than 10 years to initiate flowering, which occurs from late April to early May (Ohara and Kawano, 2005). This species was once abundant in Hokkaido. Now, it is being considered for inclusion on the endangered list because its habitats have been severely disturbed by urbanization and agricultural activity (Ohara and Kawano, 2005). Large, beautiful populations are still often found in and around urban districts in Hokkaido. These remnant populations are valuable for recreation, environmental education, and tourism. Therefore, information on this species is also vital from the fields of nature conservation, ecology, horticulture, and landscape architecture for the establishment or maintenance of populations.

Many aspects of the biology of *T. camschatcense* have been studied: phenology, seasonal growth pattern (Ohara and Kawano, 2005), genetic diversity, breeding system (Ohara et al., 1996, 2006; Ohara and Kawano, 2005; Kubota et al., 2008), resource allocation (Tomimatsu and Ohara, 2006b), and seed dispersal by myrmecochory (Ohara and Kawano, 2005). The seedling stage of *T. camschatcense* is highly affected by edge effects (Tomimatsu and Ohara 2004, 2006a) as is that of other *Trillium* species (Jules and Rathcke, 1999; Schmucki and de Blois, 2009). Tomimatsu and Ohara (2004) found that the density of *T. camschatcense* seedlings was considerably lower at the edge than in the interior of the forest, and they suggested that microclimatic conditions associated with the edge environment might be deleterious to seed germination. However, knowledge pertaining to its seed germination, which is fundamental for propagation of the species and for understanding edge effects, is lacking.

In the present study, we document the phenology of embryo growth, radicle emergence, and cotyledon emergence in field experiments. These studies were then combined with laboratory experiments on light and temperature conditions for radicle emergence and cotyledon emergence. The information from the field and laboratory investigations enabled us to confirm whether seeds of this species have double dormancy. This study is the first on embryo growth and specific light and temperature requirements for root and shoot emergences from seeds having double dormancy, including *T. camschatcense*. On the basis of our results, we also discuss why germination of this species may be compromised at the edges of forests.

MATERIALS AND METHODS

Preparation of seeds—Slightly brown and soft fruits were collected from a natural population of *T. camschatcense* in a remnant secondary deciduous forest on the campus of Hokkaido University, Sapporo, Japan on 25 July 2002, 29 July 2003, 3 August 2004, 1 August 2005, and 28 July 2007. These fruits were placed on moistened newspapers that lined the bottom of stainless steel trays in a laboratory at ca. 20°C. Following 1–7 d on the newspapers, elaiosomes attached to seeds were removed by hand scrubbing the seeds with gauze under running water. The seeds were then placed in a mortar with a small amount of water to keep them moist, similar to conditions in the fruit, for a few days until the initiation of experiments (hereafter, sowing).

Definition of radicle emergence and cotyledon emergence—We defined radicle emergence as when the radicle tip emerged more than 1 mm from the seed coat and cotyledon emergence as when the whole cotyledon appeared aboveground.

Field experiments for phenology of embryo growth, radicle emergence, and cotyledon emergence—A nontemperature-controlled metal framehouse, located outdoors on the campus of Hokkaido University, was used to monitor phenology. This framehouse was covered with shade cloth (shading rate 70%) from mid-June until the last week of October to simulate conditions on the natural forest floor, where seeds would reside after dispersal. Snowfall covered the ground of the framehouse from 6 December 2002 to 1 April 2003 and from 8 December 2003 to 25 March 2004. Temperature at the soil surface was measured in three places every 15 min throughout the study using thermo data loggers (RT-30S, Espec Mic, Aichi, Japan). Daily mean, maximum, and minimum temperatures were determined from these data.

Phenology of embryo growth—On 25 July 2002, 10 fresh seeds were cut into thin sections using an automicrotome (Plant Microtome Automatic MT-2, Nippon Medical & Chemical Instruments, Osaka, Japan), and initial embryo length in each seed measured using an optical microscope equipped with a micrometer. A plastic nursery tray was filled with red clay granules 1–3 mm in diameter, which was used as soil to create uniform conditions throughout all our experiments and to avoid the growth of fungi. On 29 July 2002, five fine-mesh polyester bags containing ca. 500 fresh seeds each were buried at a depth of 3 cm in the tray, which was then placed on the floor in the framehouse.

Ten seeds were removed at random from the bags on 25 October 2002 and on 8 February 2003, and their embryo lengths measured as described already. Embryo lengths in 10 seeds were measured approximately every month from 10 April 2003 to 11 July 2003 and about every week from 11 July 2003 to 5 September 2003. On 5 September 2003, lengths of fully elongated embryos, i.e., embryo length just before radicle emergence, were recorded. Embryo length during the study period was calculated as a percentage of that of fully elongated embryos.

Phenology of radicle emergence—On 29 July 2002, 50 fresh seeds were placed in each of four fine-mesh polyester bags and were buried 3 cm deep in a

tray filled with soil and placed on the floor in the framehouse. In preliminary experiments, radicles began to emerge from seeds 1 year after seed dispersal during the summer. Therefore, radicle emergence was monitored about every month between 10 May 2003 and 11 July 2003 and about every week between 11 July 2003 and 26 September 2003. At each scoring, seeds with an emerged radicle were removed from the bags, and the remaining seeds were reburied.

Phenology of cotyledon emergence—On 29 July 2002, ca. 500 fresh seeds were placed in each of five fine-mesh polyester bags and buried 3 cm deep in a tray filled with soil and placed on the floor in the framehouse. On 18 September 2003, bags were exhumed, and seeds with a 5–10 mm long radicle were selected. Fifty seeds were planted into each of four pots filled with soil and placed on the floor of the framehouse. The seeds were covered with ca. 1 cm of sieved soil. Because, in preliminary experiments, cotyledons emerged from seeds in the spring after dispersed seeds had passed through two winters, emergence of cotyledons was checked at 4–5 d intervals between 27 March 2004 and 5 May 2004.

Laboratory experiments on light and temperature requirements for embryo growth, radicle emergence, and cotyledon emergence—Seeds were placed in 90-mm-diameter glass or plastic Petri dishes on three sheets of filter paper moistened with distilled water. Petri dishes were sealed with parafilm (Pechiney Plastic Packaging, Menasha, Wisconsin, USA) to retard water loss during incubation. Experiments were conducted in light- and temperature-controlled incubators set at 12 h/12 h alternating temperature regimes or at constant temperature regimes. In alternating temperature treatments, 30°/20°, 25°/15°, or 20°/10°C were used to simulate natural temperatures during summer, 15°/5°C for autumn or spring, and 0°C or 5°C for winter. In constant temperature treatments, 25°C (or 20°C) was used for summer, 10°C for autumn or spring, and 0°C for winter. In light treatments, the daily photoperiod was 12 h in both alternating and constant temperature regimes. In the alternating temperature regimes, the high temperature period corresponded to the 12 h of light and the low temperature period corresponded to 12 h of darkness. However, seeds incubated at 0°C were kept in constant darkness. Keeping the photoperiod constant in all experiments allowed us to directly reveal the effects of light and temperature on germination. The light source was cool white fluorescent tubes, and photon irradiance (400–700 nm) at seed level was 15–20 $\mu mol \cdot m^{-2} \cdot s^{-1}$. In darkness, Petri dishes at both alternating and constant temperature regimes were sealed with parafilm and wrapped with two layers of aluminum foil. Seeds in dark were exposed to fluorescent light for about 1 min in the laboratory when radicle emergence was monitored at about 1-wk, 2-wk, or 30-d intervals. At each observation period, seeds with an emerged radicle were removed from the dishes, parafilm was exchanged, aluminum foil replaced if needed, and filter

TABLE 1. Temperature treatments for experiments testing the effects of various alternating temperature sequences in light on embryo growth and on radicle emergence for *Trillium camschatcense* seeds. Percentages of seeds with and without an emerged radicle and of rotten seeds are based on four replications of 50 seeds each 415 d after the initiation of the experiment. Embryo lengths are expressed as a percentage of the fully elongated embryo ($N = 10$). The asterisk indicates that the embryo length differed significantly from the initial embryo length (17.8%).

Treatment no.	Temperature treatments based on transition of seasons (Experiment was terminated 415 d after initiation)	Seeds with emerged radicle (%)	Seeds without emerged radicle (%)	Rotten seeds (%)	Embryo length (%)
All seasons (first summer → autumn → winter → spring → second summer): summer temperature: 25°/15°C or 20°/10°C; winter temperature 5°C or 0°C					
1	25°/15°C (60 d) → 15°/5°C (60 d) → 0°C (120 d) → 15°/5°C (90 d) → 25°/15°C	0.0	100.0	0.0	29.9
2	25°/15°C (60 d) → 15°/5°C (60 d) → 5°C (120 d) → 15°/5°C (90 d) → 25°/15°C	0.0	91.0	9.0	–
3	25°/15°C (60 d) → 15°/5°C (60 d) → 0°C (120 d) → 15°/5°C (90 d) → 20°/10°C	0.0	93.0	7.0	25.1
4	25°/15°C (60 d) → 15°/5°C (60 d) → 5°C (120 d) → 15°/5°C (90 d) → 20°/10°C	0.0	97.0	3.0	–
Without first summer (autumn → winter → spring → second summer)					
5	15°/5°C (60 d) → 0°C (120 d) → 15°/5°C (90 d) → 25°/15°C	2.0	97.5	0.5	37.4*
6	15°/5°C (60 d) → 5°C (120 d) → 15°/5°C (90 d) → 25°/15°C	0.0	100.0	0.0	–
Without first summer and autumn (winter → spring → second summer)					
7	0°C (90 d) → 15°/5°C (90 d) → 25°/15°C	0.5	99.5	0.0	22.6
8	5°C (90 d) → 15°/5°C (90 d) → 25°/15°C	0.5	99.5	0.0	–
Without autumn (first summer → winter → spring → second summer)					
9	25°/15°C (60 d) → 0°C (120 d) → 15°/5°C (90 d) → 25°/15°C	0.5	99.0	0.5	30.4
10	25°/15°C (60 d) → 5°C (120 d) → 15°/5°C (90 d) → 25°/15°C	0.0	99.5	0.5	–
11	20°/10°C (60 d) → 0°C (120 d) → 15°/5°C (90 d) → 25°/15°C	0.5	99.5	0.0	–
12	20°/10°C (60 d) → 5°C (120 d) → 15°/5°C (90 d) → 25°/15°C	0.0	99.5	0.5	–
Without autumn and spring (first summer → winter → second summer)					
13	25°/15°C (60 d) → 0°C (120 d) → 25°/15°C	0.0	100.0	0.0	20.4
14	25°/15°C (60 d) → 5°C (120 d) → 25°/15°C	0.0	92.5	7.5	–
15	25°/15°C (60 d) → 0°C (120 d) → 20°/10°C	0.0	99.0	1.0	20.8
16	25°/15°C (60 d) → 5°C (120 d) → 20°/10°C	0.0	96.5	1.0	–
17	20°/10°C (60 d) → 0°C (120 d) → 25°/15°C	0.0	100.0	0.0	–
18	20°/10°C (60 d) → 5°C (120 d) → 25°/15°C	0.0	99.5	0.5	–
19	20°/10°C (60 d) → 0°C (120 d) → 20°/10°C	0.0	100.0	0.0	–
20	20°/10°C (60 d) → 5°C (120 d) → 20°/10°C	0.0	94.0	6.0	–
Without spring (first summer → autumn → winter → second summer)					
21	25°/15°C (60 d) →15°/5°C (60 d) → 0°C (120 d) → 25°/15°C	0.5	98.0	1.5	22.8
22	25°/15°C (60 d) →15°/5°C (60 d) → 5°C (120 d) → 25°/15°C	0.0	97.5	2.5	–
Without first summer and spring (autumn → winter → second summer)					
23	15°/5°C (60 d) → 0°C (120 d) → 25°/15°C	0.0	99.5	0.5	26.5
24	15°/5°C (60 d) → 5°C (120 d) → 25°/15°C	0.0	100.0	0.0	–
25	15°/5°C (60 d) → 0°C (120 d) → 20°/10°C	0.0	97.5	2.5	23.8
26	15°/5°C (60 d) → 5°C (120 d) → 20°/10°C	0.0	96.5	3.5	–
Without first summer, autumn, and spring (winter → second summer)					
27	0°C (90 d) → 25°/15°C	0.5	99.5	0.0	24.4
28	5°C (90 d) → 25°/15°C	0.0	100.0	0.0	–
29	0°C (90 d) → 20°/10°C	1.5	98.5	0.0	25.6
30	5°C (90 d) → 20°/10°C	0.0	100.0	0.0	–
31	5°C (180 d) → 20°/10°C	0.5	99.5	0.0	–
32	5°C (180 d) → 25°/15°C	1.0	99.0	0.0	–

TABLE 2. Temperature treatments to test the effects of light and of summer temperature regime during a sequence of constant temperature regimes, simulating a transition of seasons (first summer → fall → winter → spring → second summer) on radicle emergence from seeds of *Trillium camschatcense*. Light and dark conditions were set up for each temperature sequence.

Treatments	Temperature regimes for transition of seasons (Experiment was terminated 450 d after initiation)
Summer temperature at 20°C	
All seasons	20°C (60 d) → 10°C (60 d) → 0°C (120 d) → 10°C (60 d) → 20°C (150 d)
Without first summer	10°C (60 d) → 0°C (120 d) → 10°C (60 d) → 20°C (210 d)
Without fall	20°C (60 d) → 0°C (120 d) → 10°C (60 d) → 20°C (210 d)
Without winter	20°C (60 d) → 10°C (60 d) → 10°C (60 d) → 20°C (270 d)
Without spring	20°C (60 d) → 10°C (60 d) → 0°C (120 d) → 20°C (210 d)
Without first summer and fall	0°C (120 d) → 10°C (60 d) → 20°C (270 d)
Summer temperature at 25°C	
All seasons	25°C (60 d) → 10°C (60 d) → 0°C (120 d) → 10°C (60 d) → 25°C (150 d)
Without first summer	10°C (60 d) → 0°C (120 d) → 10°C (60 d) → 25°C (210 d)
Without fall	25°C (60 d) → 0°C (120 d) → 10°C(60 d) → 25°C (210 d)
Without winter	25°C (60 d) → 10°C (60 d) →10°C (60 d) → 25°C (270 d)
Without spring	25°C (60 d) → 10°C (60 d) → 0°C (120 d) → 25°C (210 d)
Without first summer and fall	0°C (120 d) → 10°C (60 d) → 25°C (270 d)

papers were watered. If we noticed fungi on the seed surface, then seeds were washed with running water using a tea strainer with fine mesh and filter papers and Petri dish were exchanged.

Effects of alternating temperatures in light on embryo growth and on radicle emergence—On 1 August 2003, initial embryo length was measured in 10 fresh seeds. Then, to obtain a baseline measurement of embryo growth under natural conditions, about 200 seeds were placed in fine-mesh polyester bags, buried in soil in a plastic nursery tray, and stored on the floor of the framehouse. Seeds were removed from the bags on 20 August 2004, and the length of fully elongated embryos was measured (N = 10). To reveal which temperatures are effective for radicle emergence, on 3 August 2003, four replicates of 50 fresh seeds were incubated at 32 treatments in light with alternating temperature sequences (Table 1). Radicle emergence was checked about every month until the final temperature regime, then checked about every week. During scoring, soft and black seeds that collapsed when pinched with forceps were counted as rotten. This experiment was terminated 415 d after sowing. In 12 of 32 temperature treatments, embryo length was measured at the end of the experiment and calculated as a percentage of that of fully elongated embryos.

TABLE 3. Treatments used to test the effects of temperatures in the sequence of 15°/5°C → 0°C →15°/5°C on cotyledon emergence from seeds of *Trillium camschatcense* with an emerged radicle.

Treatments (Experiment was terminated 450 d after initiation.)	No. of seeds in each of four replications
Effects of first temperature regime of 15°/5°C	
15°/5°C (90 d) → 0°C (120 d) → 15°/5°C (30 d)	25
0°C (120 d) → 15°/5°C (120 d)	25
Effects of the length of 0°C period	
15°/5°C (90 d) → 0°C (0 d) → 15°/5°C (150 d)	30
15°/5°C (90 d) → 0°C (30 d) → 15°/5°C (120 d)	30
15°/5°C (90 d) → 0°C (90 d) → 15°/5°C (60 d)	30
15°/5°C (90 d) → 0°C (120 d) → 15°/5°C (30 d)	30
Effects of the final temperature regime	
15°/5°C (90 d) → 0°C (120 d) → 15°/5°C (30 d)	30
15°/5°C (90 d) → 0°C (120 d) → 20°/10°C (30 d)	30
15°/5°C (90 d) → 0°C (120 d) → 25°/15°C (30 d)	30

Effects of light and summer temperature regime during a sequence of constant temperatures on radicle emergence—In preliminary experiments using seeds collected in 2005, fresh seeds were buried under field conditions. When they were exhumed in May 2006 and moved to the incubator, radicles emerged from a high percentage of seeds in darkness at constant temperatures, but they failed to emerged or emerged from a low percentage of seeds in light at alternating temperatures. Therefore, the effects of light vs. darkness at constant temperatures on radicle emergence were investigated in more detail in this experiment. On 1 August 2007, four replications each of 30 fresh seeds were subjected to temperature sequences based on the transition of seasons in light and in darkness (Table 2). Seeds with an emerged radicle were counted at about 30-d intervals until 240 d after sowing, and thereafter at ca. 2-wk intervals until 450 d after sowing.

Effects of light and of temperatures on the final stage of radicle emergence—On 1 August 2007, four replications each of 30 fresh seeds were subjected to 18 treatments. During a temperature sequence of 20°C (60 d) → 10°C (60 d) → 0°C (120 d) → 10°C (60 d) → final temperature (150 d), the final temperature was varied among constant temperatures of 10°, 15°, 20°, 25°, and 30°C and among alternating temperatures of 15°/5°, 20°/10°, 25°/15°, and 30°/20°C. In addition, seeds were incubated in both light and darkness at each temperature treatment. Radicle emergence was scored about every 30 d until the final temperature, and then every 2 wk. The experiment was terminated 450 d after sowing.

Effects of a range of diurnal fluctuating temperatures on the final stage of radicle emergence—On 1 August 2007, four replications of 30 fresh seeds each were incubated in the dark at a temperature sequence of 20°C (60 d) →10°C (60 d) → 0°C (120 d) → 10°C (60 d) → final temperature (150 d). The three final temperature regimes, represented a range of diurnal fluctuations: 20° (range = 0), 22.5°/17.5° (range = 5°), and 25°/15°C (range = 10°). Radicle emergence was monitored at ca. 30 d intervals until 240 d after sowing, and then at ca. 2-wk intervals until 450 d after sowing.

Effects of low temperature on growth of seedlings—On 3 August 2005, five fine-mesh polyester bags each containing ca. 500 fresh seeds were buried at a soil depth of 3 cm in a plastic nursery tray filled with soil, and placed on the floor in the framehouse. On 7 September 2006, bags were removed from the soil, and seeds with an emerged radicle (about 5 mm long) were selected. Two replications of 20 seeds each were incubated either at 15°/5°C (250 d) or at 15°/5°C (90 d) → 0°C (120 d) →15°/5°C (40 d). Seeds were kept in light when at 15°/5°C and in darkness when at 0°C. Because the radicle, rhizome, hypocotyl, and cotyledon are difficult to distinguish during the initial stage of seedling growth, the length of the whole seedling was measured with a digital vernier caliper at 10–60 d intervals for 250 d. Mean seedling length was calculated by measuring 40 seeds in each temperature treatment, and seedlings were photographed to record development.

Effects of temperatures on cotyledon emergence from seeds with an emerged radicle—On 3 August 2005, five fine-mesh polyester bags each containing ca. 500 fresh seeds were buried at a depth of 3 cm in a plastic nursery tray filled with soil and placed on the floor in the framehouse. On 22 September 2006, bags were removed from the soil, and seeds with a radicle ca. 2 cm long were selected. For observations on cotyledon emergence, polyethylene containers (7 × 7 × 3.5 cm deep), each with six 5-mm-diameter drainage holes in the bottom, were filled with soil sterilized at 160°C for 2 h. Four replications each of 25 or 30 seeds with an emerged radicle were planted in soil at a depth of 1 cm. Containers were watered from the bottom and covered with a transparent vinyl bag with small puncture holes to reduce evaporation of water but to allow exchange of oxygen and carbon dioxide. These containers were transferred to nine temperature treatments based on the sequence of 15°/5°C → 0°C → 15°/5°C (or 20°/10°C, 25°/15°C) to test the effects of the first temperature of 15°/5°C, length of 0°C period, and final temperature (Table 3). Seeds were kept in darkness at 0°C and in light at other temperatures. Cotyledon emergence was observed every 2–4 d until 240 d after seeds with an emerged radicle were planted.

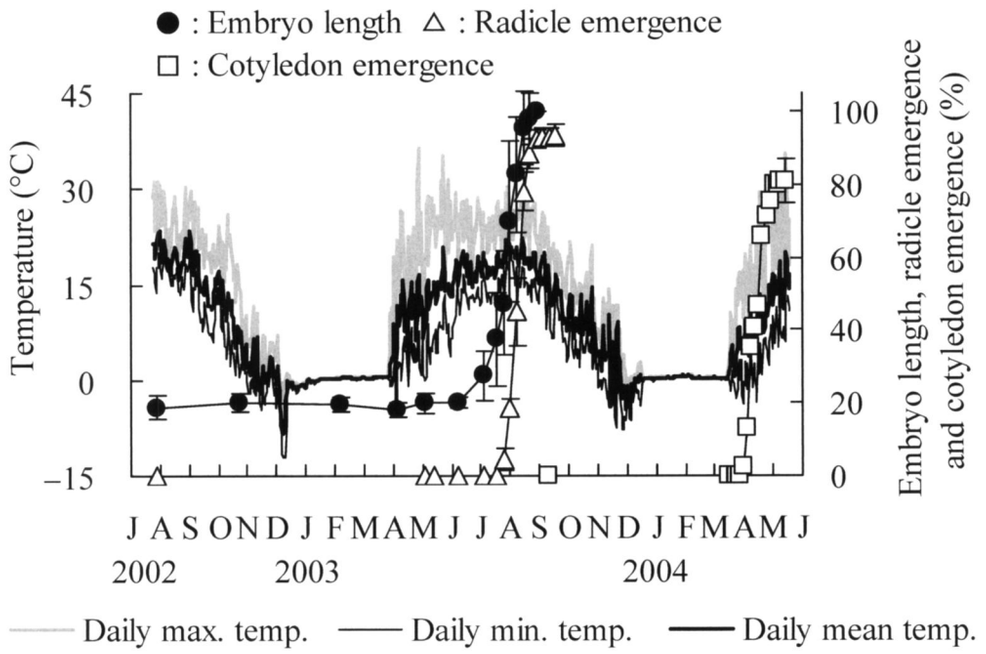

Fig. 1. Daily maximum, daily minimum, and daily mean temperatures and the phenology of embryo growth (mean ± 1 SD), radicle emergence, and cotyledon emergence from seeds of *Trillium camschatcense* outdoors in a framehouse in Hokkaido, Japan. Embryo length is expressed as a percentage of the fully elongated embryo.

Statistical analyses—The following analyses were carried out using the program SPSS Statistics 17.0 (IBM, Somers, New York, USA) to compare percentages of radicle or of cotyledon emergence: (1) *t* tests when two treatments were involved ($P < 0.05$), (2) one-way ANOVAs followed by Scheffé's tests ($P < 0.05$) when three or more treatments were tested with one factor, or (3) two- or three-way ANOVAs in experiments with two or more factors ($P < 0.05$). Treatments in which no radicles or cotyledons emerged were excluded from the analyses. Percentage values were arcsine square-root transformed for analyses, but nontransformed data are shown in figures.

RESULTS

Field experiments for phenology of embryo growth, radicle emergence, and cotyledon emergence—The initial embryo length in fresh seeds in July 2002 was 0.3 ± 0.1 mm (mean ± SD), which was 18.8% of that of the fully elongated embryo measured in September 2003 (1.6 ± 0.3 mm) (Figs. 1, 2). Embryos hardly grew until mid-June 2003, when they started to elongate. They grew rapidly to 82.8% of the length of fully elongated embryos by 15 August 2003 and to 100% by 5 September 2003. Between 13 June 2003 and 5 September 2003, when embryos grew rapidly, the average maximum and minimum temperatures and the mean daily temperature were 24.4°, 14.7°, and 18.5°C, respectively (Fig. 1).

Two percent of seeds already had an emerged radicle (about 2 cm) when examined on 10 May 2003 after the first winter following dispersal (Fig. 1). Radicles had emerged from 4.7% of the seeds on 1 August 2003 and from 93.0% by 12 September 2003. The average maximum and minimum temperatures and the mean daily temperature between 1 August 2003 and 12 September 2003, when radicles emerged rapidly, were 24.7°, 16.0°, and 19.3°C, respectively (Fig. 1).

On 12 April 2004, following two winters since sowing and 18 d after snowmelt, cotyledon emergence from 2.0% of the seeds was observed and continued to 80.0% by 14 May 2004 (Fig. 1). The average maximum and minimum temperatures and the mean daily temperature between 12 April 2004 and 14 May 2004, when cotyledons emerged rapidly, were 24.7°, 16.0°, and 19.3°C, respectively.

Laboratory experiments on light and temperature requirements for embryo growth, radicle emergence, and cotyledon emergence—*Effects of alternating temperatures in light on embryo growth and on radicle emergence*—In all treatments in light and with alternating temperature sequences, radicles emerged from ≤2.0% of seeds (Table 1). The maximum percentage of rotten seeds was 9.0. Intact seeds without an emerged radicle remained viable for the duration of the experiment. Initial embryo length was 17.8% of the fully elongated embryo, and embryos grew to only 20.4–37.4% by the end of the experiment. Only embryo length in treatment no. 5 was significantly longer as compared to initial embryo length ($t = 3.48$, df = 18, $P = 0.03$).

Effects of light and of summer temperature regime during a sequence of constant temperatures on radicle emergence—Emergence of radicles was strongly dependent on light regimes and on summer temperature regime during the temperature sequences, but it was similar between light regimes regardless of the summer temperature (Table 4; Fig. 3). Higher percentages of radicles emerged in darkness, and particularly at a summer temperature of 25°C over all temperature sequences, than in light or at a summer temperature of 20°C. Among all treatments, radicle emergence was highest for sequences that contained 0°C (120 d) → 10°C (60 d) in darkness, particularly 0°C (120 d) → 10°C (60 d) → 25°C in darkness.

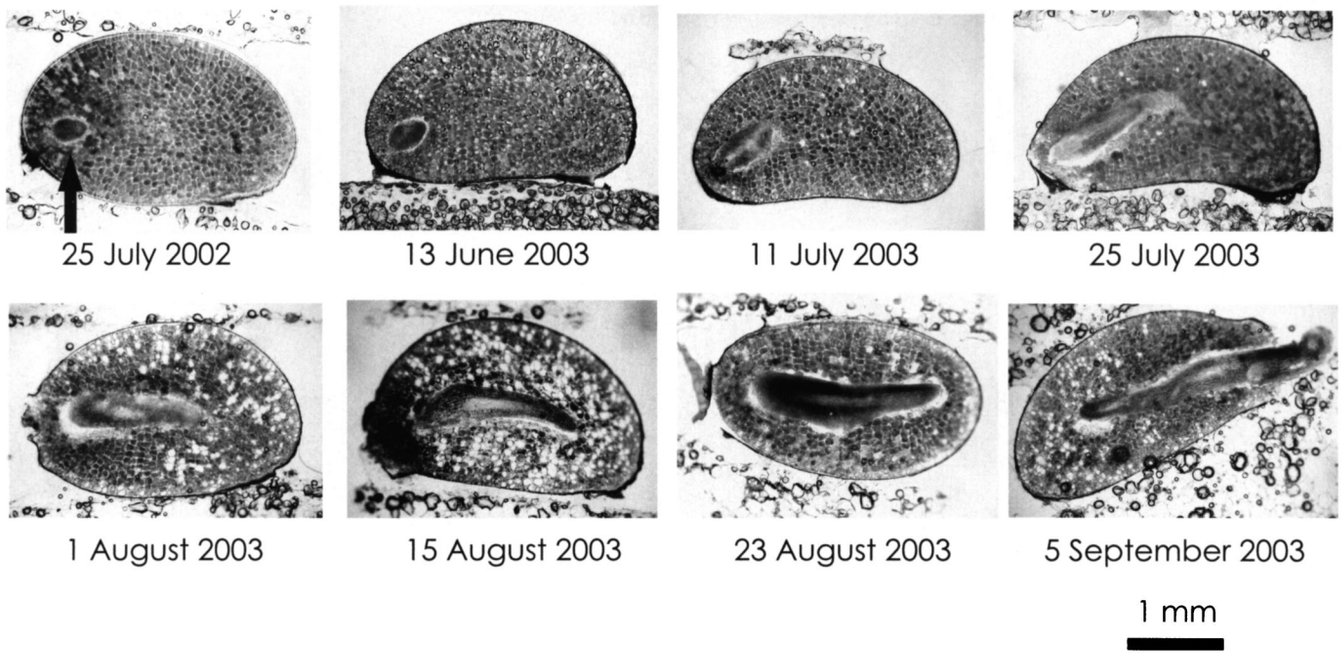

Fig. 2. Embryo (arrow) growth in seeds of *Trillium camschatcense* outdoors in a framehouse in Hokkaido, Japan.

Effects of light and of temperatures on the final stage of radicle emergence—Emergence of radicles was highly dependent on light regime and on final temperature (Table 5; Fig. 4). Highest percentages of radicles emerged at final constant temperatures of 20° and 25°C in darkness, and little radicle emergence occurred at final alternating temperatures both in light and in darkness. In addition, radicle emergence occurred over a broader range of final temperatures in darkness than in light.

Effects of a range of diurnal fluctuating temperatures on the final stage of radicle emergence—Radicles emerged from 82–89% of seeds when incubated at a final temperature of 20° and 22.5°/17.5°C representing daily fluctuations of temperatures ≤5°, whereas they emerged from only 11% at 25°/15°C with a daily fluctuation of 10° (Fig. 5).

Effect of low temperature on growth of seedlings—Seedlings incubated at continuous 15°/5°C and at the sequence 15°/5°C (90 d) → 0°C (120 d) → 15°/5°C (40 d) grew rapidly and developed radicles, rhizomes, hypocotyls, and cotyledons during the first 90 d, but then growth mostly ceased during the next 120 d (Fig. 6). Hypocotyls and cotyledons grew rapidly during the second 15°/5°C period following 0°C in seedlings exposed to the temperature sequence (Figs. 6, 7A). In contrast, hypocotyls and cotyledons of seedlings kept at 15°/5°C for the duration of the experiment stopped growing and failed to elongate (Figs. 6, 7B).

Effects of temperatures on cotyledon emergence from seeds with an emerged radicle—Cotyledons emerged from 92% of seeds with an emerged radicle incubated at 15°/5°C (90 d) → 0°C (120 d) → 15°/5°C (30 d), whereas they emerged from only 63% of seeds given 0°C (120 d) → 15°/5°C (120 d) (i.e., without the first 15°/5°C period) and variation among replicates was large (Fig. 8A). No cotyledons emerged from seeds when incubated at a temperature sequence without a period of 0°C (Fig. 8B). However, with an increase in the length of 0°C period, cotyledon emergence increased from 85 to 96%. Cotyledons emerged from 84–96% of the seeds, regardless of the final temperature in the sequence 15°/5°C (90 d) → 0°C (120 d) → final temperature (30 d) (Fig. 8C).

DISCUSSION

Classification of dormancy—Seeds of *T. camschatcense* are dispersed in summer (between late July and early August), and at the time of dispersal, they contain underdeveloped embryos

TABLE 4. Results of a three-way ANOVA showing the effects of light, summer temperature regime, and temperature sequence on radicle emergence from seeds of *Trillium camschatcense*.

Source	Sum of squares	df	Mean square	*F*	*P*
Light regime	3.818	1, 72	3.818	186.429	<0.001
Summer temperature regime	2.460	1, 72	2.460	120.138	<0.001
Temperature sequence regime	5.882	5, 72	1.176	57.452	<0.001
Light × summer temperature	0.450	1, 72	0.045	2.209	0.142
Light × temperature sequence	0.265	5, 72	0.126	6.169	<0.001
Summer temperature × temperature sequence	0.632	5, 72	0.053	2.585	0.033
Light × summer temperature × temperature sequence	0.137	5, 72	0.027	1.340	0.257

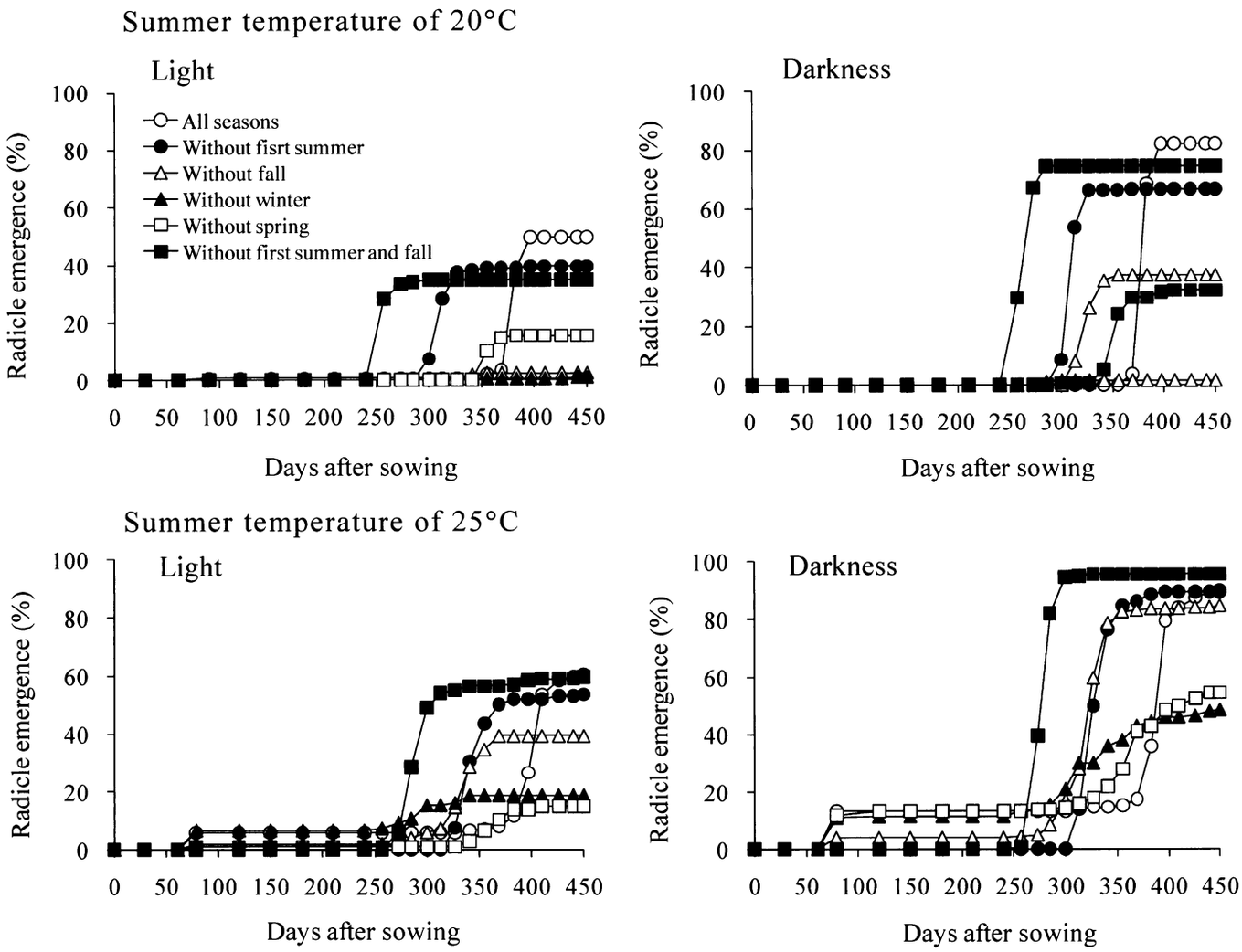

Fig. 3. Effects of light and of summer temperature regime during a sequence of temperatures (see Table 2) on radicle emergence from *Trillium camschatcense* seeds.

(Figs. 1, 2). Embryos grew the next summer (from mid-June to early September) following the first winter in the field. Radicles emerged from the seeds immediately after the full elongation of embryos mostly in early September. Cotyledons emerged the following spring (from mid-April to mid-May) after the second winter following dispersal (Fig. 1). In the laboratory experiments, optimal radicle emergence occurred in dark conditions and at a temperature sequence that included low temperatures: 0°C (120d) → 10°C (60d) (Fig. 3). Further, seeds with an emerged radicle needed a low temperature of 0°C for cotyledon emergence (Figs. 6, 7, 8B). Thus, seeds of *T. camschatcense* have deep simple double MPD. Seeds with this kind of dormancy have underdeveloped embryos at seed dispersal, need the first low (winter) temperature period to overcome embryo dormancy, allow embryo growth followed by radicle emergence, and require the second low (winter) temperature period for cotyledon emergence from seeds with an emerged radicle (Baskin and Baskin, 1998).

TABLE 5. Results of a two-way ANOVA showing the effects of light and of final temperature on radicle emergence from seeds of *Trillium camschatcense* in the temperature sequence of 20°C (60 d) → 10°C (60 d) → 0°C (120 d) → 10°C (60 d) → final temperature.

Source	Sum of squares	df	Mean square	*F*	*P*
Light regime	1.604	1, 54	1.604	116.952	<0.001
Final temperature regime	11.562	8, 54	1.445	105.351	<0.001
Light × final temperature	0.516	8, 54	0.065	4.706	<0.001

Evolutionary considerations—Relatively strong selective pressures must have occurred to produce the germination pattern seen in *T. camschatcense* and other species with double dormancy. Roots and shoots emerge simultaneously in spring in many species, and thus, the seedlings are readily able to photosynthesize and grow. In contrast, root emergence and shoot emergence from *T. camschatcense* seeds are separated by about 8 mo. Radicles emerge from *T. camschatcense* seeds in early September, ca. 1 yr following seed dispersal. In early September, light intensities on the floor of mesic deciduous forests of Hokkaido, Japan are low due to a dense tree canopy. Further, the severe environment of winter follows in a few months.

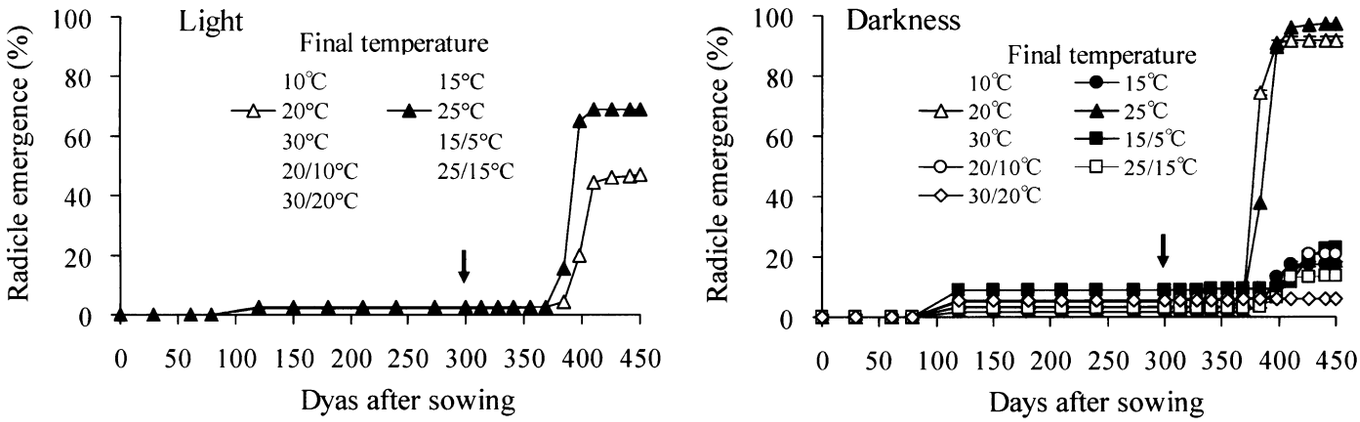

Fig. 4. Effects of light and of final temperatures in the sequence 20°C (60 d) → 10°C (60 d) → 0°C (120 d) → 10°C (60 d) → final temperature (shown in each graph) on radicle emergence from *Trillium camschatcense* seeds. The arrow indicates when incubation at the final temperature started. Data at 10°, 15°, 30°, 15°/5°, 20°/10°, 25°/15°, and 30°/20°C in light, and those at 10° and 30°C in darkness, are not shown because final germination was ≤2%.

Therefore, we suggest that it would be advantageous for seeds with an emerged radicle to develop a root system during autumn and for their cotyledons to be not exposed to winter conditions. Otherwise, the cotyledons may not have enough time to grow before winter, and the plant would die. In spring immediately after snow melt, cotyledons uniformly emerge from seeds with a developed root system, and the seedlings can photosynthesize sufficiently before leaves on the tree canopy produce shade.

With the results of our study, we now know how to manipulate root and shoot emergences from seeds of *T. camchatcense*. Therefore, we can test the hypothesis that radicle emergence in early autumn and low temperature requirement for cotyledon emergence from seeds with an emerged radicle is an adaptation of seedlings to the forest light environment and severe winter conditions. First, we will produce seeds with an emerged radicle in autumn and spring in the laboratory, then plant them in autumn and spring, respectively, in the field. We predict that seeds with an emerged radicle planted in autumn will develop a root during autumn and sprout cotyledons in spring; however, seeds with an emerged radicle planted in spring will not sprout cotyledons, and they will die or will sprout cotyledons the following spring. Second, we will produce seeds having cotyledons in autumn and spring in the laboratory, then plant them in autumn and spring, respectively, in the field. We predict that seedlings (with cotyledons) planted in autumn will die during winter, but seedlings planted in spring will grow and become established.

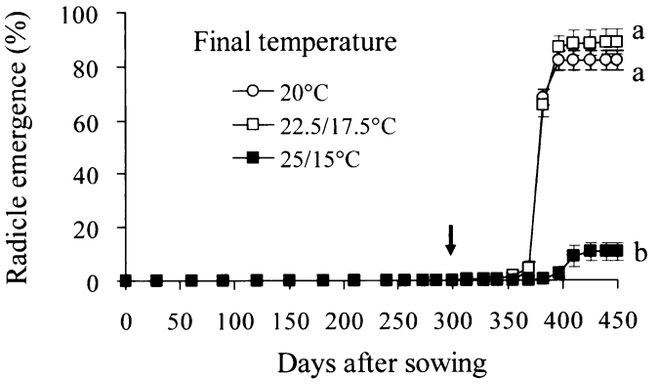

Fig. 5. Effects of a range of final temperatures given at the end of the sequence 20°C (60 d) →10°C (60 d) → 0°C (120 d) →10°C (60 d) → final temperature in darkness on radicle emergence (mean ± 1 SD) from *Trillium camschatcense* seeds. The arrow indicates when incubation at the final temperature started. Final emergence percentages with different letters are significantly different (Scheffé's test, $P < 0.05$).

Specific requirements for radicle emergence—Seeds of many species germinate equally well or better in light than in darkness (Grime et al., 1981; Baskin and Baskin, 1988). Relatively few species have higher germination in darkness than in light (Baskin and Baskin, 1998). Studies on seeds with MPD that do not have double dormancy have found high percentages of germination in light (Hidayati et al., 2000a, b, 2001; Baskin et al., 2002; Kondo et al., 2002, 2004, 2005, 2006; Walck and Hidayati, 2004b; Karlsson and Milberg, 2007; Kondo and Sato, 2007; Vandelook et al., 2007, 2009; Phartyal et al., 2009a, b; Vandelook and Assche, 2008, 2009). In seeds with double dormancy, studies on six plant species were conducted either in pots or (presumably) outdoors (Barton and Schroeder, 1942; Barton, 1944; Whigham, 1974), and we assume that seeds were in darkness while buried in soil. For seeds of *T. grandiflorum* (Solt, 1998), *Polygonatum odoratum* (Mill.) Druce var. *pluriflorum* Ohwi (Takagi, 2001a), and *P. macranthum* (Maxim.) Koidz. (Takagi, 2001b), which have double dormancy, radicle emergence did not differ in light and darkness. However, a description of the light intensity used in these experiments is lacking. In our study, we examined light linked with temperature and found that radicle emergence from *T. camschatcense* seeds was significantly higher in darkness than in light (Figs. 3, 4; Tables 4, 5). Light quality, light intensity, duration of light exposure, and timing of light exposure during seasons affect germination, but we were unable to conduct experiments on these aspects.

Furthermore, seed germination in many species is promoted by alternating temperatures and rarely by constant temperatures (Baskin and Baskin, 1998). Seeds with MPD that do not have double dormancy germinate to high percentages at alternating temperatures in experiments using incubators (Hidayati et al.,

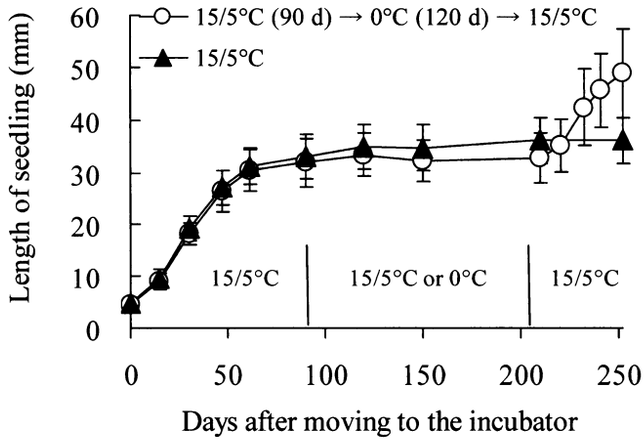

Fig. 6. Effects of temperature on growth of *Trillium camschatcense* seedlings. Length (mean ± 1 SD) of the whole seedling was measured because it is difficult to distinguish radicles, rhizomes, hypocotyls, and cotyledons in the initial stage of growth.

2000a, b, 2001; Baskin et al., 2002; Kondo et al., 2002, 2004, 2005, 2006; Walck and Hidayati, 2004b; Karlsson and Milberg, 2007; Kondo and Sato, 2007; Vandelook et al., 2007, 2009; Phartyal et al., 2009a, b). Seeds with double dormancy germinate to high percentages at alternating temperatures in experiments either conducted in a cold frame or outdoors (Barton, 1944; Barton and Schroeder, 1942; Whigham, 1974) as well as at constant temperatures in experiments using incubators (Solt, 1998; Takagi, 2001a, b). However, none of these studies compared the effects of constant vs. alternating temperatures as we did for seeds of *T. camschatcense*.

In our laboratory experiments, radicle emergence was low from *T. camschatcense* seeds at alternating temperatures (Table 1; Fig. 4). Radicle emergence was high at sequences of constant temperatures (Figs. 3–5) or at sequences of constant + alternating temperatures when the maximum and minimum temperatures differed by less than 5°C during the final stage of emergence (Fig. 5). These results indicate that radicle emergence from seeds of *T. camschatcense* is promoted by constant temperature and inhibited by alternating temperature. On the other hand, under the framehouse covered with shade cloth in the field, embryos began to grow in mid-June, and radicles emerged from 93% of the seeds by mid-September (Fig. 1). The average daily maximum and minimum temperature on the soil surface during this period was 24–25° and 15–16°C, respectively. Our framehouse underwent alternating temperatures, and a large percentage of radicles emerged from the seeds. Thus, our laboratory and field experiments with regards to alternating vs. constant temperature requirements for radicle emergence seem contradictory.

However, two caveats for the difference in radicle emergence between our laboratory and field experiments need to be considered. First, the daily maximum and minimum temperatures on the soil surface in the field represent the average of daily instantaneous values. In addition, seeds were buried 3 cm depth in the soil, and temperature fluctuations under the soil are usually buffered compared with air temperatures. Therefore, diurnal temperature under the soil in the field where seeds were placed probably fluctuated less than on the soil surface (which is where we measured it) and in the laboratory experiments. This difference could be one of the causes for high radicle percentage in the field experiments and low radicle emergence at alternating temperatures in the laboratory experiments. Second, optimum temperatures for radicle emergence were within the narrow range of 20–25°C, and radicle emergence was strongly inhibited at

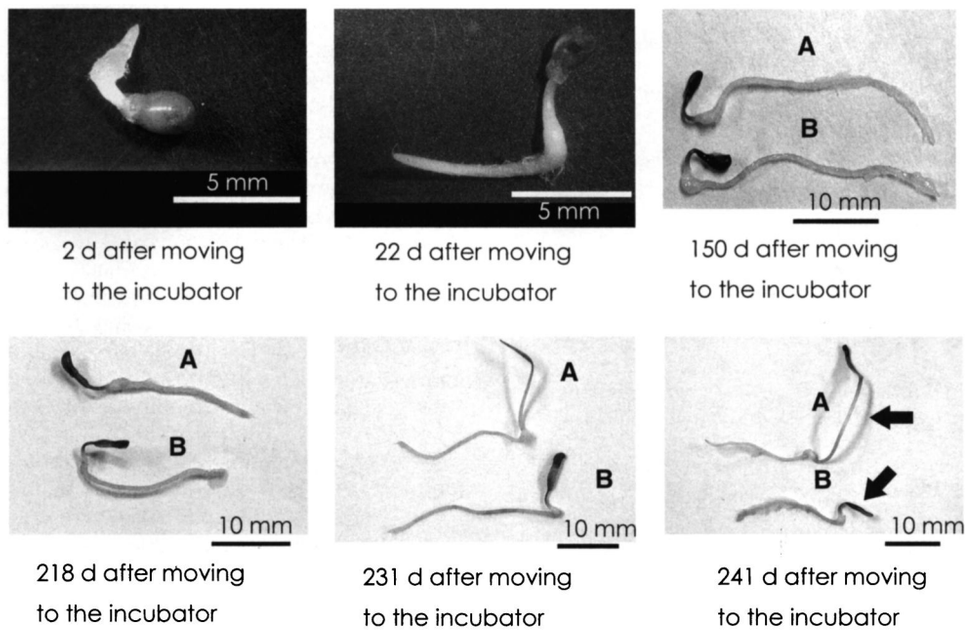

Fig. 7. Growth of *Trillium camschatcense* seedlings. Seeds with an emerged radicle, developed under field conditions, were exposed to two temperature regimes in incubators for 250 d: (A) 15°/5°C (90 d) → 0°C (120 d) → 15°/5°C (40 d) or (B) constant 15°/5°C. At 2 d and 22 d, seedlings were at the same temperature regime and looked identical between treatments. In the initial stage of growth, it is difficult to distinguish radicles, rhizomes, hypocotyls, and cotyledons. Arrows indicate growth of hypocotyl and cotyledon.

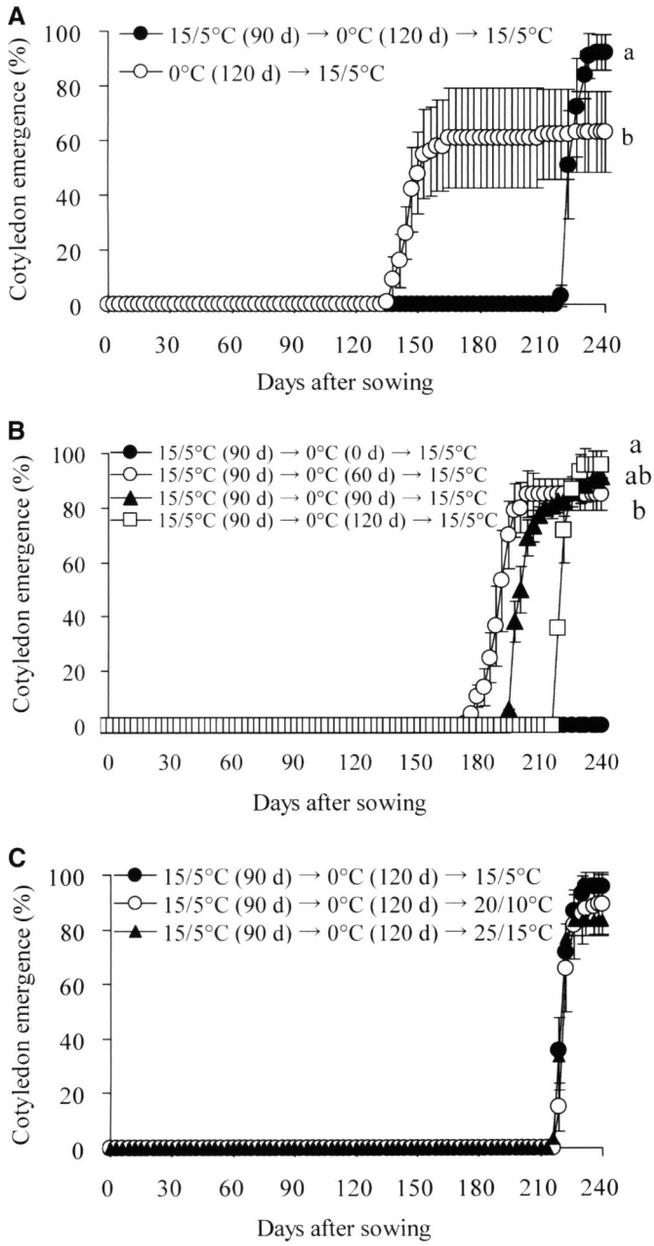

Fig. 8. (A) Effects of temperature sequences either with a first exposure to 15°/5°C or without such exposure, (B) effects of various durations of 0°C in the sequence 15°/5°C → 0°C → 15°/5°C and (C) effects of various final temperatures at the sequence 15°/5°C (90 d) → 0°C (120 d) → final temperature (30 d) on cotyledon emergence (mean ± 1 SD) from seeds with an emerged radicle. Final percentages with dissimilar letters indicate significant differences among treatments in panel A (t =3.44, df = 6, P = 0.01) and in panel B (Scheffé's test, $P < 0.05$); final percentages in panel C did not differ significantly among treatments (Scheffé's test, $P > 0.05$).

15°C (Fig. 4), suggesting that a period of 15°C for 12 h at 25°/15°C in the incubator may reduce radicle emergence.

Radicles emerged from 2% of seeds in the phenology study in May following seed dispersal, and the radicles were already about 2 cm long, though radicles emerged from most seeds in August (Fig. 1). Further, in the laboratory experiments, radicles emerged from 10–15% of seeds at high or moderate temperature (summer or autumn temperature in the year of seed dispersal) before the first low temperature period (Figs. 3, 4). These results suggest that radicles may emerge from some seeds without being first exposed to low temperatures of winter (i.e., within the year of seed dispersal). Thus, temperature requirements of *T. camschatcense* for radicle emergence vary somewhat, albeit relatively little. In contrast, radicle emergence in *Convallaria majalis* L., a species with double dormancy, occurred at a constant temperature of 25°C or alternating 30/20°C, but higher emergence took place if seeds were first pretreated at 5°C (Barton and Schroeder, 1942).

Specific requirements for cotyledon emergence—In seeds of *Smilacina racemosa* (L.) Desf. (Barton and Schroeder, 1942), *C. majalis* (Barton and Schroeder, 1942), *P. odoratum* var. *pluriflorum* (Takagi, 2001a), and *P. macranthum* (Takagi, 2001b), all reported to have double dormancy, shoot emergence was high when seeds with an emerged radicle were subjected to high temperatures for several months before the second low temperature period. But when seeds were not subjected to warm temperature before that second low temperature period, shoot emergence was lower. In experiments on *T. erectum* and *T. grandiflorum* (Barton, 1944), cotyledons emerged from many seeds subjected to warm temperature for several months before the second low temperature period. Similarly, cotyledon emergence in seeds of *T. camschatcense* was low and varied considerably when seeds with an emerged radicle were incubated at 0°C → 15°/15°C, but it was high when they were incubated at 15°/5°C → 0°C → 15°/5°C (Fig. 8A). Thus, our study supports previous reports (Barton, 1944; Barton and Schroeder, 1942; Takagi, 2001a, b) on seeds with double dormancy. Seeds with an emerged radicle needed low temperature at 0°C for 60 d or more for cotyledon emergence (Fig. 8B), then cotyledons emerged over a wide range of temperatures (Fig. 8C).

In the field, radicle emergence from *T. camschatcense* seeds started in summer (early August) following the year of seed dispersal and finished by early autumn (mid-September) (Fig. 1). Seeds with an emerged radicle developed roots, rhizomes, and hypocotyls at a simulated autumn temperature (15°/5°C) within about 90 d, but they stopped development until subjected to a spring temperature (15°/5°C) after winter (0°C) (Figs. 6, 7). Therefore, if radicles emerged in late autumn or at the beginning of winter, the period required for developing roots, rhizomes, and hypocotyls would be insufficient and cotyledon emergence in spring would be compromised. Thus, the reason why radicles did not emerge at 30°C and 15°C and only at 20–25°C (Fig. 4) may be an adaptation to ensure that they emerge at the beginning of autumn and have sufficient time to grow before winter. Ecologically, it may be critical for seedlings to have a sufficiently large root system by the second spring with which to supply adequate water and nutrients for the growing shoot.

Ecological implications of germination requirements in relation to edges of forests—Light and temperature conditions in habitats are very important for determining germination, and hence, the ecological distribution of species. Higher germination in darkness than in light has been interpreted as a mechanism to prevent seedling establishment on the soil surface in a harsh environment, particularly with regards to soil moisture (Walck and Hidayati, 2004a). Germination in darkness also would promote establishment of a species under dense leaf canopies and restrict it from growing in gaps or open areas, such as the case for several forest geophytes like species in *Muscari* (Doussi and

Thanos, 2002). On the other hand, responses to alternating temperatures have been interpreted as a mechanism to detect gaps (Washitani and Takenaka, 1987) or to sense burial depth (Teketay, 2002). Thus, the interaction between light and temperatures plays an important role as a cue for germination. For example, seed germination of *Arthropodium cirratum* (Forst. F.) R. Br., which grows in a narrow habitat range close to the high tide mark on coastal sea cliffs, was promoted in low light intensity or continuous darkness at constant temperatures compared to high light intensity and alternating temperatures. Germination of this species would be avoided in stressful sites with high light or extreme alternating temperature (Conner and Conner, 1988).

Trillium camschatcense primarily grows in the interior of mesic deciduous forests, and recruitment of the species is reduced at the edges of forests. In the interior of forests, light intensity is lower (Kapos, 1989; Chen et al., 1993; Matlack, 1993; Young and Mitchell, 1994; Davies-Colley et al., 2000), leaf litter is higher (Meekins and McCarthy, 2001), soil is moister (Kapos, 1989; Chen et al., 1993; Matlack, 1993), and air and soil temperatures are lower (Kapos, 1989; Chen et al., 1993; Matlack, 1993; Young and Mitchell, 1994; Davies-Colley et al., 2000) than at the forest edge. Moreover, the daily amplitude of air and soil temperatures is reduced inside the forest (Chen et al., 1993). With regards to *T. camschatcense*, the number of seedlings was significantly negatively correlated with air and soil temperatures (Tomimatsu and Ohara, 2004).

Radicle emergence in *T. camschatcense* is enhanced with darkness and with constant temperatures, which are more characteristic of forest interiors with reduced sunlight penetration through the tree canopy and by increased leaf litter. Hence, increased light and increased fluctuation of temperatures may reduce radicle emergence at the forest edge, where soil moisture deficit also may occur in summer when radicles emerge rapidly. Ecologically, seeds of *T. camschatcense* may encounter favorable conditions in the forest interior for the exacting light and temperature requirements of radicle emergence. In contrast, the microclimatic conditions at the edges of forests would not be favorable for germination. Thus, our results may explain the mechanism behind the decreased recruitment of the species at edges of forests as found by Tomimatsu and Ohara (2004).

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