

Germination Traits and Adaptive Regeneration Strategies of the Three *Carpinus* Species

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Germination tests in the three sympatric *Carpinus* species, *C. laxiflora*, *C. tschonoskii*, and *C. cordata*, were carried out to clarify their germination traits in relation to the regeneration strategies. Seeds did not germinate just after ripening under any conditions in the three species. After one or two rounds of cold stratification, however, *C. laxiflora* and *C. tschonoskii* showed high germination percentages ($> 80\%$) at alternating temperature in light while *C. cordata* did not. After a long-term (10 months) cold stratification, about 100% of *C. cordata* seeds germinated, although about 70% of these seeds were in "conditional dormancy" and cannot germinate in the dark. Seed germination in the three *Carpinus* species was enhanced by alternating temperature, but not by light exposure. These results suggest that the seeds of the three species are dormant just after ripening and the dormancy is broken by cold stratification, with intra-generic variations in the breakage. Germination traits in each species may be closely related to the regeneration strategy; *C. cordata* maintains a persistent seed bank with seed dormancy and regenerates after disturbances as a seed bank strategist, whereas the other two species germinate in the next spring after seedfall and regenerate depending either on existing disturbed-sites or sites of recent disturbances after seedling establishment as a seed rain strategist (*C. tschonoskii*) or seedling bank strategist (*C. laxiflora*).

Key words: *Carpinus* species, regeneration strategy, seed dormancy, seed germination

Germination traits, such as responses to light and temperature regimes and seed dormancy, have important implications for the life history strategy of plant species, because they affect the early stage of the regeneration process (Harper, 1977; Grime, 1979; Cook, 1980; Baskin and Baskin, 1998). Plant seeds are, in general, classified into two types based on germination strategies; selective and non-selective to variable environmental conditions (Washitani, 1989). Non-selective seeds are non-dormant and can germinate under minimum water and temperature conditions, while selective seeds which show dormancy to some extent, can only germinate under favorable growing conditions (Washitani, 1989; Baskin and Baskin, 1998). Non-selective seeds are attributable to both small-seeded species, such as light demanding pioneers, and to large-seeded species with shade-tolerance (Baskin and Baskin, 1998). The former type have adopted a seed rain strategy in which seed dispersal plays a role in increasing the chance of arriving at safe sites, while the latter have a seedling bank strategy in which seedlings are able to persist under the closed canopy and to wait for a gap that provides favorable growth conditions (Garwood, 1989; Alvarez-Buylla and Martinez-Ramos, 1990). On the other hand, plants producing selective seeds with dormancy adopt a seed bank strategy represented by the maintaining of a seed bank under unfavorable conditions for seedling survival. Once a disturbance occurs, the seeds detect changes in light- and temperature-regimes and germinate (Washitani, 1989). These germination traits are diversified even in same and similar taxa, and thus may explain the co-existence of congeneric species (Baskin *et al.*, 1994).

The three Japanese *Carpinus* species (*C. laxiflora*, *C. tschonoskii*, and *C. cordata*) are widely distributed in the temperate zone in Japan (Kitamura and Murata, 1965), and are main components of secondary growth forests (Suzuki, 1986). They co-occur sympatrically sometimes accompanied by

another *Carpinus* species, *C. japonica*. Shibata and Nakashizuka (1995) clarified the differences in regeneration strategies of these four *Carpinus* species in a temperate deciduous forest. They partly explained the co-occurrence based on seed and seedling demography. On the other hand, the differentiation of germination traits may contribute to co-existence through different responses to the spatio-temporal variations of the environment. However, this possibility was not examined in the previous study (Shibata and Nakashizuka, 1995).

In this paper, I compared the germination traits of the three *Carpinus* species, *C. laxiflora*, *C. tschonoskii*, and *C. cordata*, based on the results of germination tests, and discuss the difference in their germination traits in relation to the life history strategies.

Materials and Methods

1 Materials

Fruits of the three *Carpinus* species were collected in October 1995 from trees in the Ogawa Forest Reserve, Ibaraki Prefecture, central Japan ($36^{\circ}56' N$, $140^{\circ}35' E$, altitude 610–660 m). The seeds removed from the fruits were identified as sound or empty by flotation. These seeds which sank in water were sound and assumed to be viable. They were subjected to tests immediately. Some morphological features of *Carpinus* seeds were evaluated in relation to the seed germination traits. Kernel seed masses and seed coat masses were estimated from sound and empty seed weights; Seed coat mass was regarded to be equivalent to dry weight of empty seed, while the kernel mass was computed by subtracting seed coat mass from the total dry weight of a seed (seed coat + kernel). "Seed coat toughness" values, which are defined here as press intensity to break seed coat, were measured by a load-sell (Maruhishi SV-950).

2 Germination test

Five replicates of 50 seeds of each *Carpinus* species were placed on sheets of filter paper on 0.8% agar in 8.5-cm diameter plastic Petri dishes. Germination tests were carried out for 30 days under the following four conditions in temperature-controlled incubators; (i) alternating temperature in light; (ii) alternating temperature in darkness; (iii) constant temperature in light; (iv) constant temperature in darkness. In the germination tests, the seeds were placed under diurnal conditions of alternating temperature of 20°C (16h)/30°C (8h), or constant temperature of 23°C. Under light conditions, the seeds were exposed to cool white fluorescent light for 8 h a day during the higher temperature cycle of the temperature alternation. The photosynthetic photon flux density (at 700 nm) at seed level was adjusted to $36 \mu\text{mol m}^{-2}\text{s}^{-1}$. To achieve dark conditions, the Petri dishes were put in black plastic bags.

The emergence of radicles from the endocarp was used as a criterion for determining seed germination. The first trial of the germination test (S0) was conducted just after ripening without any stratification. If the cumulative germination percentages under all conditions were less than 80% after incubation for 30 days, the seeds were placed in a refrigerator at 4°C for 30 days as a cold stratification in the darkness (without light), and subjected to the next trial (S1). If the cumulative germination percentages did not reach 80% after the third trial (S2), the seeds were stored in a refrigerator at 4°C for 10 months as a long cold stratification, and subjected to the final trial (S3). After the germination test, the seeds which did not germinate were cut and their status (alive or dead) was checked. Germination percentages were calculated to the number of viable seeds. The effects of two factors (light and temperature) and their interactions on seed germination were tested by a two-way ANOVA using arcsine-transformed data of the germination percentages.

3 Definition of dormancy

Seed dormancy is classified into three states; "non-dormancy," "dormancy" and "conditional dormancy" by required conditions for seed germination such as temperature or light (Vegis, 1964; Baskin and Baskin, 1989). Seeds population in many species with seed dormancy consist of sub-populations in the three different states of dormancy during the process of dormancy breakage (Baskin and Baskin, 1989; Washitani, 1989; Suzuki, 1997). In this paper, "dormant" are defined as seeds which were unable to germinate at alternating temperature in light, "non-dormant" seeds as those which were able to germinate at constant temperature in darkness, and "conditional dormant" seeds as those which

were able to germinate only at alternating temperature in light (Suzuki, 1997).

Results

1 Seed morphology

Carpinus tschonoskii produced the largest seed (12.8 mg in dry weight), 1.2 times as heavy as the seed of *C. laxiflora*, the smallest among the three *Carpinus* species (Table 1). Kernel weight was greatest in *C. cordata* (5.7 mg), 2.4 times of the *C. laxiflora* and 1.8 times of the *C. tschonoskii* value. The ratio of seed coat to total seed weight was largest in *C. tschonoskii* (75%), and smallest in *C. cordata* (41%). *C. tschonoskii* had toughest seed coat (3.4 kg cm^{-2}), more than twice the value for *C. laxiflora* (1.6 kg cm^{-2}).

2 Germination traits

The seeds of *C. laxiflora* just after ripening did not germinate under any of the conditions (Fig. 1a). After one month of cold stratification, more than 80% of seeds germinated under all conditions, and there was no significant difference between alternating and constant temperature (ANOVA: $F = 1.80$, $d.f. = 1$, $p > 0.05$). On the other hand, the germination percentages were significantly lower in darkness than in light (ANOVA: $F = 18.60$, $d.f. = 1$, $p < 0.01$) (Fig. 1a).

In *Carpinus tschonoskii*, without any stratification after ripening, the seeds did not germinate under any conditions (Fig. 1b). Although they germinated after one months cold stratification, the germination percentages varied widely depending on the conditions; the percentage was significantly larger at alternating temperature than at constant temperature (ANOVA: $F = 20.65$, $d.f. = 1$, $p < 0.001$) and was greater in light than that in darkness (ANOVA: $F = 245.88$, $d.f. = 1$, $p < 0.001$). Germination percentage after the first cold stratification was 70% at alternating temperature in light, but only 15% at constant temperature in darkness (Fig. 1b). After the second cold stratification, the cumulative germination percentages increased under all conditions, especially in darkness from 10 % to 62 % at alternating temperature and from 5% to 50% at constant temperature. Alternating temperature (ANOVA: $F = 8.34$, $d.f. = 1$, $p < 0.05$) and light (ANOVA: $F = 113.67$, $d.f. = 1$, $p < 0.01$) also affected the seed germination positively in this species (Fig. 1b).

In the germination test, even after the second cold stratification, *C. cordata* seeds did not germinate under any conditions (Fig. 1c). However, about 40% of seeds which had been subjected to at alternating temperature in light germinated, and 5–10% under the other conditions, during 10 months of cold stratification (third stratification). In the final germination test, almost all seeds germinated in light under

Table 1 Morphology of the three *Carpinus* species.

Species name	No. of sample seeds	Seed weight ^a (mg)	Empty seed weight ^b (mg)	Kernel weight ^c (mg)	Seed cost ratio (%)	Seed cost toughness parameter
<i>C. laxiflora</i>	30	4.9 ± 0.5	2.5 ± 0.4	2.4	51	1.6 ± 0.2
<i>C. tschonoskii</i>	30	12.8 ± 1.5	9.6 ± 1.7	3.2	75	3.4 ± 0.5
<i>C. cordata</i>	30	9.6 ± 1.2	3.9 ± 0.6	5.7	41	2.2 ± 0.4

^a obtained by (a–b). Mean ± SD was shown for each parameter.

both temperature conditions. In darkness, the final germination was 30% at alternating and 20% at constant temperature. Alternating the temperature did not affect the seed germination, but the presence of light did (ANOVA: $F = 645.10$, $d.f. = 1$, $p < 0.01$) (Fig. 1c).

3 Changes in seed dormancy

Although the seeds in the three *Carpinus* species were dormant just after ripening, their dormancy was broken by the

warm and cold stratification. However, the proportion of seeds in each state of dormancy and the process to break the dormancy differed from species to species (Fig. 2). The dormancy of most *C. laxiflora* seeds was broken by the first cold stratification, and the percentage of conditional dormant seeds was only 12%. In contrast, the seed dormancy of *C. cordata* was not broken until the third stratification (a long cold stratification). Conditional dormant seeds occupied about 70% of the seed population. For *Carpinus tschonoskii*, about 70% of the seeds were released from dormancy by the first stratification. However, 90% of germinated seeds originated from the conditional dormant seeds. After the second stratification, 83% of the seeds were released from their dormancy, and the proportion of conditional dormant seeds decreased to 36%.

Discussion

1 Intragenetic variation in germination traits

Germination percentages for the Japanese *Carpinus* species

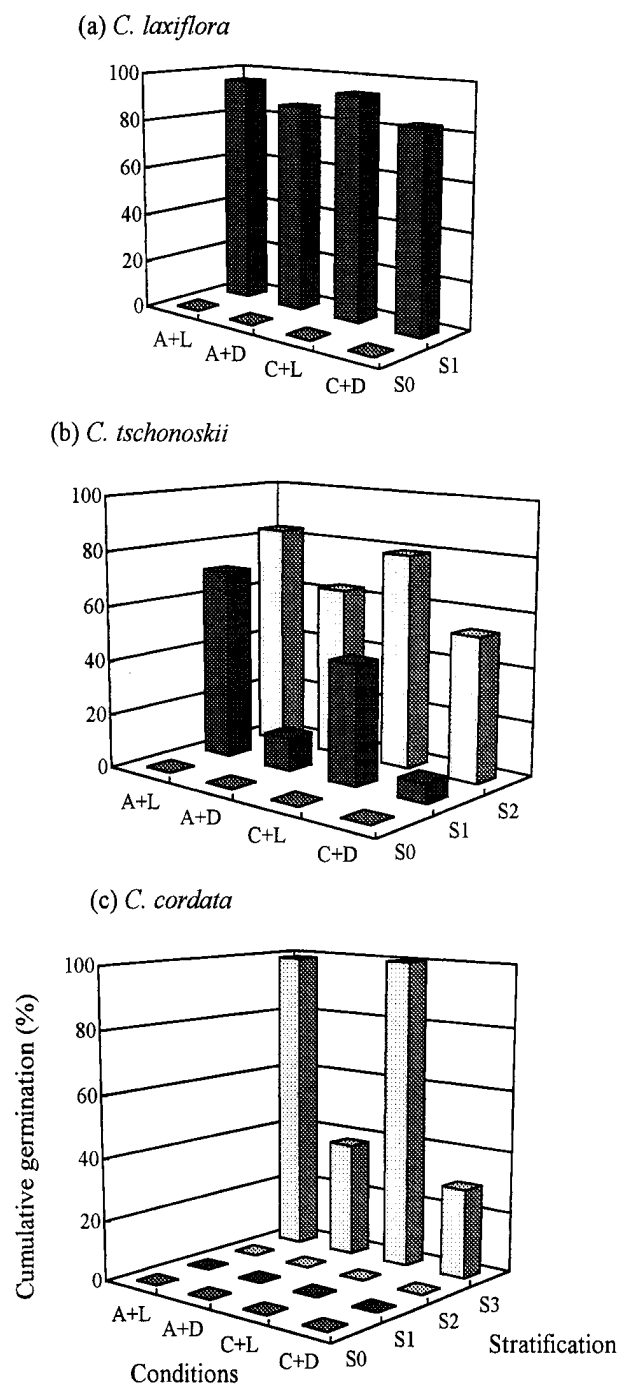


Fig. 1 Effects of stratification on seed germination percentages after one month of incubation in the three *Carpinus* species. S0, without stratification; S1, after the first stratification for a month; S2, after the second stratification for another one month (two months in total); S3, after third (final) stratification for six months.

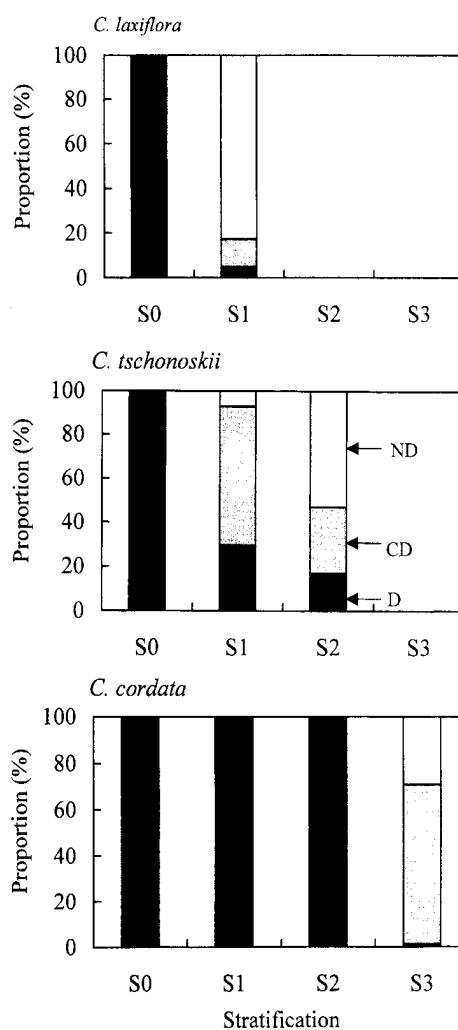


Fig. 2 Changes in the proportion of seeds in each state of dormancy in the three *Carpinus* species following stratification. D, dormant seed; CD, conditional dormant seed; ND, non-dormant seed. Notations for S0, S1, S2 and S3 are same as in Fig. 1.

in the present study (80–90%) were high compared to the values reported in some earlier studies; 48% in *C. japonica* (Kamiyama *et al.*, 1978), 25% in *C. laxiflora* (Yanagisawa, 1985), and 17% in *C. tschonoskii* (Tsutsui *et al.*, 1976). One reason for the lower germination rates in these earlier studies may be a failure to break the dormancy caused by the methodology and stratification regimes (Katsuta and Yokoyama, 1998).

Seed dormancy in the three *Carpinus* species was recognized in that their seeds just after ripening did not germinate at all (Fig. 1). After one month of cold stratification, more than 90% in *C. laxiflora* and 70% in *C. tschonoskii* of seeds germinated at alternating temperature in light which was near optimal for germination. Physiological dormancy of other *Carpinus* seeds just after ripening was also reported (Baskin and Baskin, 1998; Suszka, 1968; Rudolf and Phipps, 1974). The embryo excised from the seed coat easily germinated without any stratification in *C. tschonoskii*, but not in the other two species (Suzuki, unpublished data). This result suggests that *C. tschonoskii* seeds are combinationally dormant: the germination is physiologically inhibited by the impermeable seed coat (Baskin and Baskin, 1989). The greater allocation of dry matter (75%) to, and therefore stronger, seed coat in this species would support this (Table 1).

The seed dormancy of *C. cordata* was not broken until third stratification (a long-term cold stratification). Warm and cold stratification, but not cold stratification alone, may affect the seed dormancy in *Carpinus* species (Suszka, 1968; Rudolf and Phipps, 1974). Each germination trial and subsequent period of storage in a refrigerator appeared to correspond to a warm and cold stratification, respectively, and may also affect the breakage of seed dormancy in spite of different responses to stratification among the species.

Light exposure enhanced the seed germination in the three *Carpinus* species, but to what extent varied among the species (Fig. 1). On the other hand, alternating temperature did not affect positively the germination in *C. laxiflora* nor *C. cordata*, while the germination percentage in *C. tschonoskii* was higher at alternating than constant temperature (Fig. 1). However, with further stratification, there would be no difference in germination responses to the temperature regime.

Even after a long cold stratification, *C. cordata* was the most conservative of the species in terms of ending its seed dormancy (about 70% of conditional dormant seeds) (Fig. 2). This result suggests that the seeds of *C. cordata* may have strategy to be buried in soil. In contrast, *C. laxiflora* seeds, more than 80% of which germinated on the first stratification, seem to prefer not to be buried in soil. *C. tschonoskii* may also have difficulty in maintaining a persistent seed bank, because the proportion of dormant and conditional dormant seeds rapidly decreased following stratification (Fig. 2).

2 Linkage to regeneration strategy

The variation in germination traits of the three *Carpinus* species may have important implications for life history

strategies of these species. These species ripen and disperse their seeds in autumn (October–November) when it is warm enough for germination. They commonly exhibit seed dormancy and may suppress germination in autumn to avoid seedling mortality in winter. During the winter, the seed dormancy will be broken by natural stratification, and the seeds will be ready for germination in spring. On the other hand, the differences in germination traits among the three *Carpinus* species suggest specialization into: (1) “seed bank strategists” having a persistent seed bank with seed dormancy and using only safe sites for regeneration, (2) “seedling bank strategists” which maintains a seedling bank at stable sites until a gap in the canopy appears, or (3) “seed rain strategists” which disperse their seeds to find safe sites for regeneration (Garwood, 1989; Alvarez-Buylla and Martinez-Ramos, 1990).

C. laxiflora and *C. tschonoskii* seedlings emerge the spring after seed dispersal, due to the early breakage of the seed dormancy by a short warm and cold stratification. However, there is a difference in seedling survival under the closed canopy; *C. laxiflora* had higher rate (7.5%) than *C. tschonoskii* (2.7%) (Shibata and Nakashizuka, 1995). These findings suggest that *C. tschonoskii* is, relatively, a seed rain strategist utilizing only existing disturbed-sites for regeneration, whereas *C. laxiflora* is a seedling bank strategist (compared to *C. tschonoskii*), and uses both stable and recently disturbed sites in the old-growth forests (Shibata and Nakashizuka, 1995). However, the difference of these strategies may not be definitive, because the three *Carpinus* species are commonly characterized by the relatively light seeds loaded on the wing, all of which can disperse their seeds widely by wind (Shibata and Nakashizuka, 1995). The high germination percentages of *C. laxiflora* under all conditions (Fig. 1) strongly suggests this species to be a seedling bank strategist. In contrast, *C. tschonoskii*, which needs more stratification for the breakage of seed dormancy and requires much light and alternating temperature on germination, may have adapted to germinate only at recently-disturbed sites exposing mineral soil without litters.

To end the seed dormancy in *C. cordata*, a relatively long-term stratification is needed, and light exposure and alternating temperature enhanced the seed germination (Fig. 1). In the field, the new seedlings of this species appear approximately 1.5 years after seed dispersal in contrast to the other two species which emerge the next spring. Seedlings of this species emerge every year in spite of extreme year-to-year variation in seed production. Moreover, the average emergence rate of *C. cordata* was 5.5%, lower than the values for the other two species (10.7–11.2%) (Shibata and Nakashizuka, 1995). These results of field observation and the germination test support a persistent seed bank in *C. cordata*. This *Carpinus* species may adopt a seed bank strategy, while waiting for disturbances it forms a seed bank and responds quickly to the formation of safe sites with exposed mineral soil which accompanies some improvements of light and temperature regimes (Washitani, 1989).

I thank T. Kanazashi, K. Osumi, and K. Hoshizaki for their critical reading of the manuscript. I also thank M. Shibata and H. Tanaka for support and helpful suggestions during the course of this study. This study was supported by grants from the Ministry of Agriculture, Forestry and Fisheries (BCP-97-III-A).

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(Accepted May 25, 2000)