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Longevity and germinability of buried seeds in *Trapa* sp.

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The existence of persistent seed bank of *Trapa* sp. was confirmed by the indoor and outdoor observations. The seeds left under room conditions remained alive for more than five years. Among the ungerminated buried viable seeds, some exhibited germination in the outdoor water tank two years after they were collected from the pond bottom. Germination initiated when water temperature became higher than 10°C and it occurred even under complete darkness. Fluctuation of temperature considerably stimulated germination.

Introduction

The ecological significance of soil buried seed banks has been described by many workers (cf. Harper, 1977; Grime, 1979; Fenner, 1985; Nakagoshi, 1987; Thompson, 1987). In some species, it is normal for a proportion of the seeds to become incorporated into the soil and become part of a store or bank of seeds which can be drawn upon intermittently over a long period (Fenner, 1985). Up to date, many papers have been published concerning the ecology of *Trapa* spp., a floating-leaved aquatic plant. Nevertheless, there have been few reports on longevity and germinability of buried seed of this species. Muenscher (1936) examined the storage and germination of seeds of aquatic plants and carried out the field experiment on *Trapa natans* L. In his report, he describes that the nutlike fruits of *T. natans* do not germinate unless they are first subjected to cold storage in winter, and also suggests by the field tests that none of the chestnuts remain alive for more than one year. On the contrary, Miki (1927) , Uchida (1973) and Hayashi (1983) observed the buried viable seeds in the field, and the latter two authors suggested the existence of persistent seed bank of *Trapa* spp. Uchida (1973) also describes that the seed of *Trapa* spp. can remain alive for 2–9 years or more; though regrettably no data is presented in his paper. The present investigation was thus carried out to evaluate the longevity and germinability of buried seeds of *Trapa* under various environmental conditions. Some of the observations described in the text are still ongoing and the interim results are presented.

Materials and methods

All the fruits and/or seeds used for the present investigation were those heavy and stout enough not to be broken by the strong press of the fingers. Afterwards these heavy and stout seeds were proved to be viable by the following preliminary test.

Materials were sampled from three different ponds; Pond Ojaga-ike in Chiba Pref. (35°33′N, 140°20′E), and Pond Hakucho-ike (35°30′N, 133°02′E) and Pond Hasu-ike (35°18′N, 132°40′E) in Shimane Pref. All the plants collected from these ponds were tentatively identified as *Trapa japonica* Flerov by the two-spined fruit form, but further precise identification should be done in the future.

Germination was determined as the protrusion of the radicle.

Preliminary observation on the germinability of attached seeds

One hundred and twenty-seven mature heavy fruits attached to the parent plants were collected on 31 July 1979 from Pond Ojaga-ike and these were kept at room conditions for two weeks until they were immersed in the outdoor campus concrete pond in Chiba University (c. 0.7 m deep). In spring 1980, germination was observed at three day intervals in the outdoor pond from 12 March. Water temperature of the outdoor pond was monitored by the auto-recording thermograph.

Monthly changes in germinability of buried seeds

During the period from September 1979 to October 1980, buried seeds (including germinating ones) were sampled at least once a month in Pond Ojaga-ike by Ekman-Birge grab. Each sample was taken to cover 0.25 m^2 in bottom area. After counting the numbers of both heavy/stout ungerminated and germinating seeds, ungerminated ones were immersed in the outdoor campus pond as before, and their germination was monitored for each sample. The final germination rate of each sample was determined as relative percentage of seeds which had germinated before 22 June 1980 to the total number of viable seeds collected at each date.

Same observation as mentioned above was done for the buried seeds of Pond Hakucho-ike. Buried seeds per 0.25 m^2 were sampled eight times during May 1986 and July 1986, and ungerminated heavy seeds were immersed in the small water tank settled outdoors of Shimane University. For each sample, germination was monitored at intervals until May 1988 when almost all the seeds germinated.

Long-term observation of the seed longevity

A total of 42 buried viable seeds were collected in May and June 1984 from Pond Hasu-ike, and these seeds were immediately put in the water-filled glass bottle (9 cm in diameter, 20 cm in depth). The bottle was settled at room conditions and germination was observed occasionally. In December 1985, 22 out of 42 seeds were removed to the outdoor small water tank to evaluate the effects of fluctuating temperatures and chilling on the germination.

Germination of seeds buried at different depths

Among the large number of buried seeds collected from Pond Hakucho-ike on 7 March 1986, each of 10 seeds were buried 1, 5 and 10 cm in soil (soft mud of the pond) and settled in the small water tank outdoors, and their germination was monitored at intervals until May 1987 when all the viable seeds had germinated.

Germination of seeds under light, dark and constantly cool conditions

Each of 20 seeds collected as above were put in each three water-filled glass bottles (9 cm in diameter, 20 cm in depth). Of these, one bottle was placed in the cold chamber at 10°C and the other two at room conditions. Of the two bottles at room conditions, one bottle was wholly covered with aluminium foil to observe the germination in continuous darkness. The observation of germination in each bottle has been done occasionally and is still ongoing.

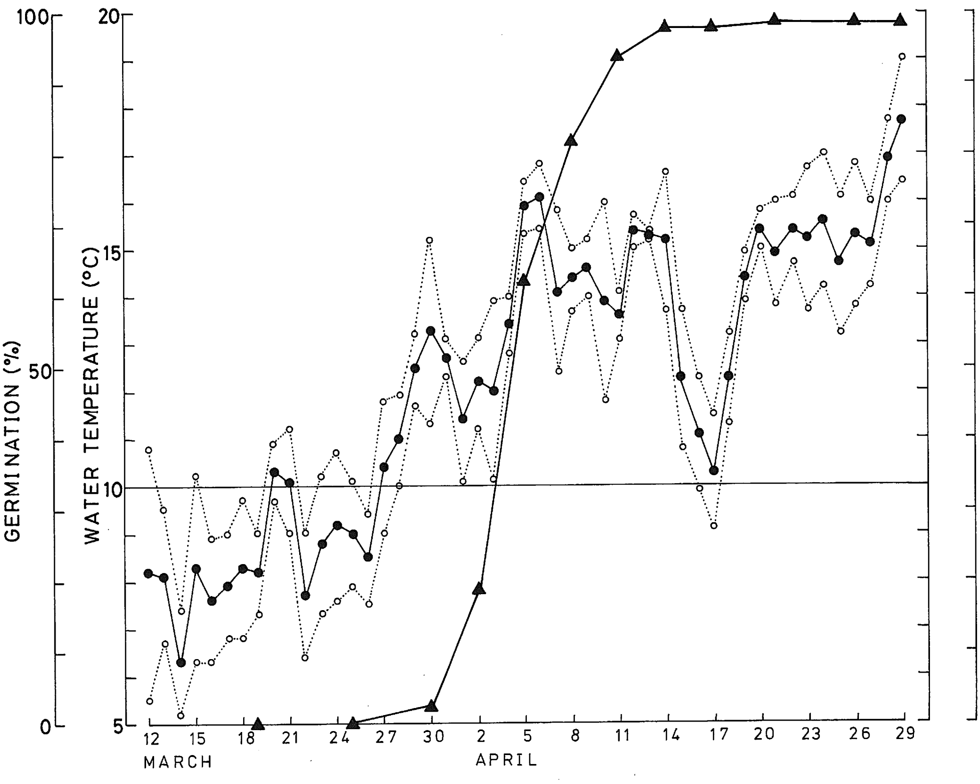

Fig. 1. Temporal changes of germination in the outdoor campus pond in 1980. Triangles show % of germinated seeds relative to total number of mature fruits collected (127 fruits). Filled circles represent daily mean water temperature. Daily maximum and minimum temperatures are also shown by dotted lines. Samples from Pond Ojaga-ike.

Results

Seed germinability of attached fruits

The synchronous germination started from late March when daily mean water temperature of the outdoor pond became higher than 10°C (Fig. 1). During a month the seeds gave more than 98% germination. High percentage of germination proves the present simple non-destructive method for detecting seed germinability to be available.

Monthly changes in germinability of buried seeds

Figure 2 shows the result obtained by the samples collected from Pond Ojaga-ike. Mean number of buried viable seeds per 0.25 m^2 was 49.7±18.2 S.D. (n=23). The first observation of germination in the field was on 31 March when bottom water temperature of the pond became higher than 10°C (cf. Kunii, 1984). Seasonal maximum value of relative percentage of germinated seeds to total viable/germinable seeds was 58.1% observed on 14 May. At that time, 25 out of 43 buried viable seeds had germinated in the field.

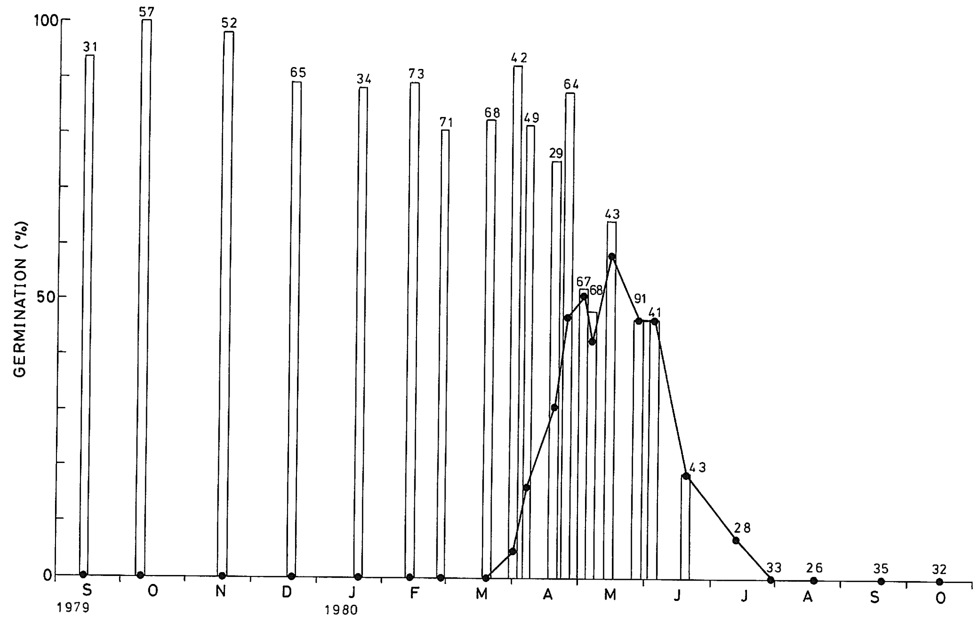

Fig. 2. Seasonal changes in seed germinability and the actual % of germination in the field (Pond Ojaga-ike). Columns show final % of germination determined as the relative number of seeds which had germinated before 22 June 1980 to the total number of viable seeds collected at each date. Filled circles represent the actual % of germination in the field. Figures show total number of viable seeds 0.25 m^{-2} (including both germinated and ungerminated seeds) collected at each date.

By 22 June, ungerminated seeds collected by middle March showed high germination rate (81–100%) and those collected during late March and late April also attained high germination rate (>75%) in the campus outdoor pond. In contrast, those collected after May showed low germination rate (0–64%) and only a few seeds germinated thereafter in the campus pond.

Concerning the monthly changes in germinability of buried seeds, almost the same result was obtained with the sample from Pond Hakucho-ike where 59.4±13.3 S.D. (n =8) buried viable seeds occurred per 0.25 m^2 and seasonal maximum germination rate in the field was 52.9% on 27 May (Fig. 3). The ungerminated seeds collected by 10 May 1986 quickly germinated after they were immersed in the outdoor water tank and, therefore, more than 75% of buried seeds had germinated by late May. In spring of the second year (1987), nearly all the seeds which had not germinated in the first year germinated and attained 97-100% germination in 1987; a total of four seeds out of 252

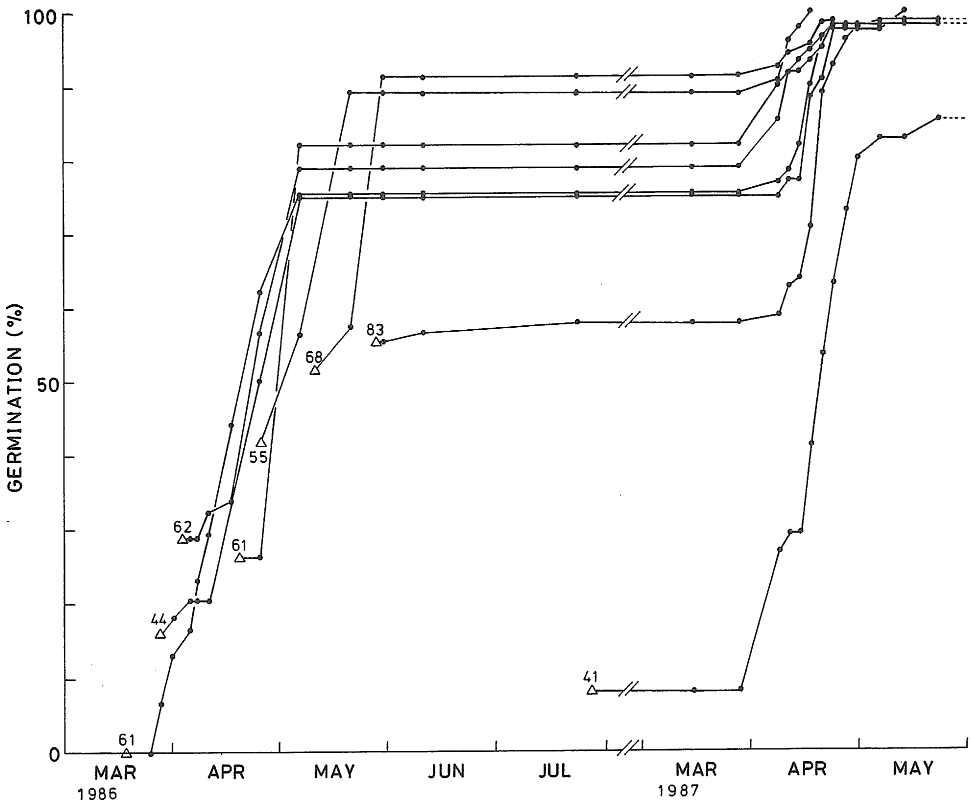

Fig. 3. Temporal changes in seed germination in the outdoor water tank. Triangles represent the actual % of germination in the field (Pond Hakucho-ike) and figures show total number of viable seeds 0.25 m^{-2} collected at each date.

ungerminated seeds collected by 10 May remained ungerminated in 1987, and of these three germinated in the spring of 1988 and only one still continued to be ungerminated.

As for the ungerminated buried seeds collected on 27 May and 26 July, most of them did not show succeeding germination in the water tank in 1986 but germinated in the next spring; 97.3% and 84.2% of the seeds collected, respectively, on 27 May and on 26 July, had germinated by the end of May 1987, and full germination was attained by the end of May 1988.

Seed longevity

Figure 4 shows the result of the observation of seed longevity under room conditions. The buried seeds collected in spring 1984 showed no germination until the end of 1985. After then, the seeds germinated annually in a stepwise fashion and 13 seeds out of 20 germinable seeds had germinated by August 1988. Of the seven ungerminated seeds, six seeds had been dead during the course of the observation and possibly one remains alive. As the buried seeds collected in 1984 were those produced before 1984, it is certain that the viability of seed in *Trapa* can be retained as long as five years or more under stable room conditions. On the contrary, all the seeds removed to the outdoor water tank in December 1985 had germinated by 25 April 1986.

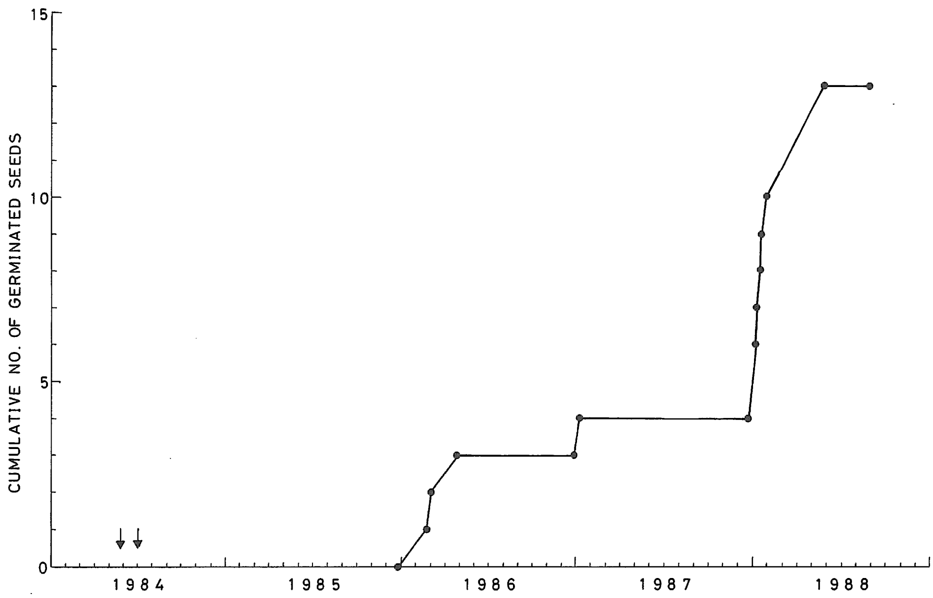

Fig. 4. Temporal changes in the cumulative number of germinated seeds under room conditions. Arrows indicate the sampling dates (25 May and 28 June 1984). Samples from Pond Hasu-ike. See text for further explanation.

Germination of seeds buried at different soil depths

Time-course of germination of seeds buried at different soil depths is shown in Fig. 5. In 1986, seven, four and three seeds germinated at 1, 5 and 10 cm soil depth

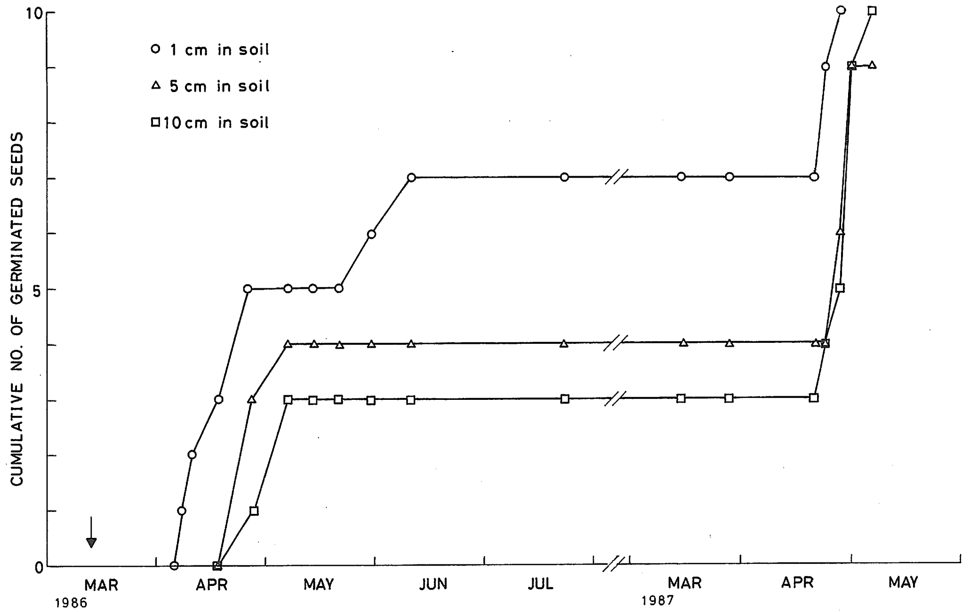

Fig. 5. Temporal changes in the cumulative number of germinated seeds buried at three different soil depths. Arrow indicates the initiation of the experiment. Samples from Pond Hakucho-ike. See text for further explanation.

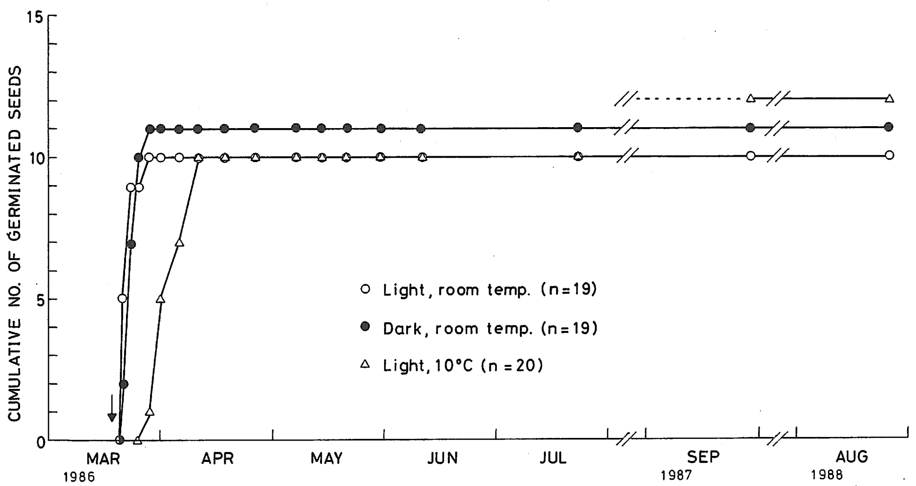

Fig. 6. Temporal changes in the cumulative number of germinated seeds under three different conditions. Arrow indicates the initiation of the experiment. Samples from Pond Hakucho-ike. See text for further explanation.

conditions, respectively. All the rest ungerminated seeds except for a dead seed germinated in the next spring.

Germination of seeds under light, dark and cool conditions

In each condition, about a half of the seeds germinated immediately after the initiation of the experiment (Fig. 6). After that, however, only a slight change in the cool conditions occurred and no further germination was observed in both light and dark conditions in the room. The remaining seeds were all certified as viable in August 1988.

Discussion

The critical water temperature for the spring seed germination was estimated as 10°C by the detailed outdoor experiment and field observations (Fig. 1). This temperature nearly accords with those reported by some authors (Momoshima and Nakamura, 1979; Hayashi, 1983). The seeds could germinate in continuous darkness (Fig. 6), but the germination of seeds buried under both 5 cm and 10 cm of soil delayed in comparison with that under 1 cm of soil (Fig. 5), and more than half of the buried seeds germinated in the next year of the initiation of experiment. This phenomenon may result from the soil atmosphere; Terasawa (1927) using artificial aeration suggests the inhibitory gaseous effect of CO_2 on the germination of *Trapa natans*. However, second years' good germination can not be explained by this gaseous effect. The good germination of seeds being removed from indoor to outdoor conditions and the difference of germination rate shown in Figs. 3 and 6 evidently show considerable effect of chilling and/or temperature fluctuation on germination (Hayashi, 1983). It is probable that the gaseous effect simply acts to reinforce the other inhibitory factors like relatively constant temperatures experienced by buried seeds (Fenner, 1985). In the glass bottle settled under room conditions, seed germination occurred not synchronously but intermittently and not a few seeds remained viable for more than five years (Fig. 4), and even under the outdoor unstable water temperature conditions, some of the seeds remained viable for more than three years (Fig. 3). These results confirm the existence of persistent seed bank of *Trapa* spp. Seasonal changes in seed germinability shown in Figs. 2 and 3 imply the secondary dormancy which must have been acquired during burial. Above all, it is probable that buried viable seeds play an important role in the population dynamics in natural water bodies where catastrophic drawdown occurs. There still remains a large void in the morphology and germination physiology of seed in *Trapa* and it is of considerable interest to examine the mechanisms which prevent (or which initiate) the germination of buried seeds (Grime, 1979; Angevine and Chabot, 1979; Nakagoshi, 1988).

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