タガラシの冬一年草生活史とその発芽特性

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Hirona Okuda and Yasuro Kadono : **Winter annual life cycle of *Ranunculus sceleratus* with special reference to its seed germination characteristics in Japan**

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Abstract

Ranunculus sceleratus L. is a winter annual species that has been common in rice fields and ditches in Japan. Recently, however, its populations have decreased, with extinctions of local populations. To understand the characteristics of winter annual life cycle of this species and the causes of its recent population decline, we studied germination of seeds collected from two populations (Kobe and Tajima). Cold and hot pre-treatments had no significant effect on germination of the Kobe seeds, but the hot treatment seemed to break the dormancy of the Tajima seeds. The optimal temperatures for the germination of seeds from both populations were 20 and 15℃ alternation (day/night), and no germination was observed at higher temperature combinations such as 30 and 25℃. The seeds thus appear to stay in conditional dormancy during the summer and start to germinate at lower temperatures in the autumn. The germination characteristics of the species are adaptive for germination on bare ground after rice harvesting. Recent changes in rice-field environments, such as drying in winter and succession in fallowed rice fields, have caused extinction of local populations of *R. sceleratus*.

Key words : alternating temperature, conditional dormancy, decline of populations, rice field, winter annual.

Introduction

Ranunculus sceleratus L. (Ranunculaceae) is distributed in the temperate regions of the Northern Hemisphere and grows in rice fields and ditches throughout Japan (Kasahara 1981). Although the life cycle of this species has been described as that of a summer or (occasionally) winter annual in Europe (van der Toorn 1980 ; Grime et al. 1988), this is a winter annual in Japan (Kitamura and Murata 1961 ; Kasahara 1981 ; Tamura and Shimizu 1982). That is, the seeds germinate in autumn and the plants bloom and set seeds in the following spring. It is one of the representative weeds found in winter cropping in Japanese agro-ecosystems.

Until recently, this species has been a common weed in Japan. Our field survey, however, has shown that the species is becoming very rare in rice fields, especially in well-drained fields that are left dry from autumn to spring (Kadono and Okuda unpublished data). The species is listed as a threatened plant in some local Red Data Books that have been published recently (e.g., Red Data Book Committee of Miyazaki Prefecture 2000 ; Akita Prefecture 2002).

The germination traits of many agricultural weeds, including winter annuals (e.g., Arai 1961 ; Arai et al. 1961 ; Matsumura 1967 ; Matsuo and Kubota 1993), have most often been studied from the viewpoint of weed control. Recently, however, the loss of biodiversity in agro-ecosystems has become a serious concern (National Institute for Agro-Environmental Sciences 1998), and conservation-oriented ecological studies have become important for future weed management as well as for conservation strategies.

In this study, we investigated the seed germination characteristics of *R. sceleratus* from the viewpoints of two questions : (1) Can the winter annual life cycle observed in Japan be explained by the germination characteristics of the species? (2) Why have populations of the species decreased so rapidly in recent years?

Materials and methods

Collection of seeds

In May 2004, we collected the seeds of *R. sceleratus* before shedding from two rice field populations: Taisanji, Nishi-ku, Kobe City (hereafter described as Kobe), and Genbudo, Toyooka City, Tajima area (hereafter, Tajima), both in Hyogo Prefecture. Kobe is located near the Seto Inland Sea, but Tajima, about 110 km farther north, faces the Japan Sea.

We sampled seeds from more than five plants at each location and mixed them well before dividing the seeds among treatments to minimize the effects of any genetic differences between individuals. Seeds were kept at room temperature in paper envelopes for a few weeks until the experiments started.

Effect of cold and hot treatment

We defined two main temperature treatments: In the cold treatment, seeds were kept in darkness at 4℃ in their envelopes for 0 (control), 2, 4, and 6 weeks. The hot treatment was done at 30℃, but with all other conditions similar to those in the cold treatment.

Thereafter, we placed 50 seeds in each treatment on filter paper moistened with de-ionized water in Petri dishes. We tested germination under combinations of light (12 L 12 D; 33 μmol $m^{-2}s^{-1}$ by fluorescent lamps), dark, aerobic, and anaerobic conditions. Under the dark conditions, the dishes were completely wrapped with aluminum foil. Anaerobic conditions were created by covering the seed bed with a glass plate tightly sealed to exclude air. For all conditions, the experiments were done in triplicate.

The Petri dishes were incubated under two temperature conditions: a 20℃ constant temperature and alternating (20℃ day and 15℃ night) temperatures.

We checked for germination every 3 days until we observed no further germination (30 to 57 days). Dishes in the dark treatment were examined under a safe lamp in a darkened room.

The data were analyzed using one-way ANOVA after arcsine transformation.

Optimal temperature for germination

To determine the optimal temperature for germination, we incubated seeds that had not undergone any pretreatment under 12 h of light and aerobic conditions under five day/night temperature regimes: 30/25, 25/20, 20/15, 15/10, and 10/5℃. Other settings were the same as in the previous experiments. Germination was observed for 48 days.

Results

Effect of cold and hot treatments

Under constant temperature of 20℃, no germination occurred under any treatment and in either population.

Figures 1 and 2 each present three examples of the time course of the cumulative germination rate of seeds from the Kobe and Tajima populations, respectively, under alternating temperature regimes, regarding the four treatment conditions (combinations of light, dark, aerobic and anaerobic conditions).

In the seeds from Kobe (Fig. 1), the germination rate was high under light condition. They germinated well irrespective of oxygen condition. Although pretreatment was not indispensable to induce germination of the seeds under light conditions, a 4- to 6-week hot treatment induced 100% germination. The germination rate was also high in the cold treatment (75% to 81%). We found no statistically significant differences as a function of treatment period in both the cold and the hot treatments ($p > 0.05$). In darkness, no seeds germinated but the seeds showed 30% to 70% germination after a hot treatment that lasted longer than 2 weeks (Fig. 1 C).

In contrast, the seeds from Tajima showed somewhat different responses to the treatments. Increased duration of cold treatment significantly decreased the germination rate ($p<0.001$; Fig. 2). On the other hand, increasing the duration of the hot treatment significantly increased the germination rate ($p<0.001$). These responses were the same both in aerobic and anaerobic conditions as was the case with Kobe seeds. In darkness, the seeds from Tajima showed no germination in any treatment.

Optimal germination temperature

Table 1 shows the cumulative germination rate under different temperature regimes. The

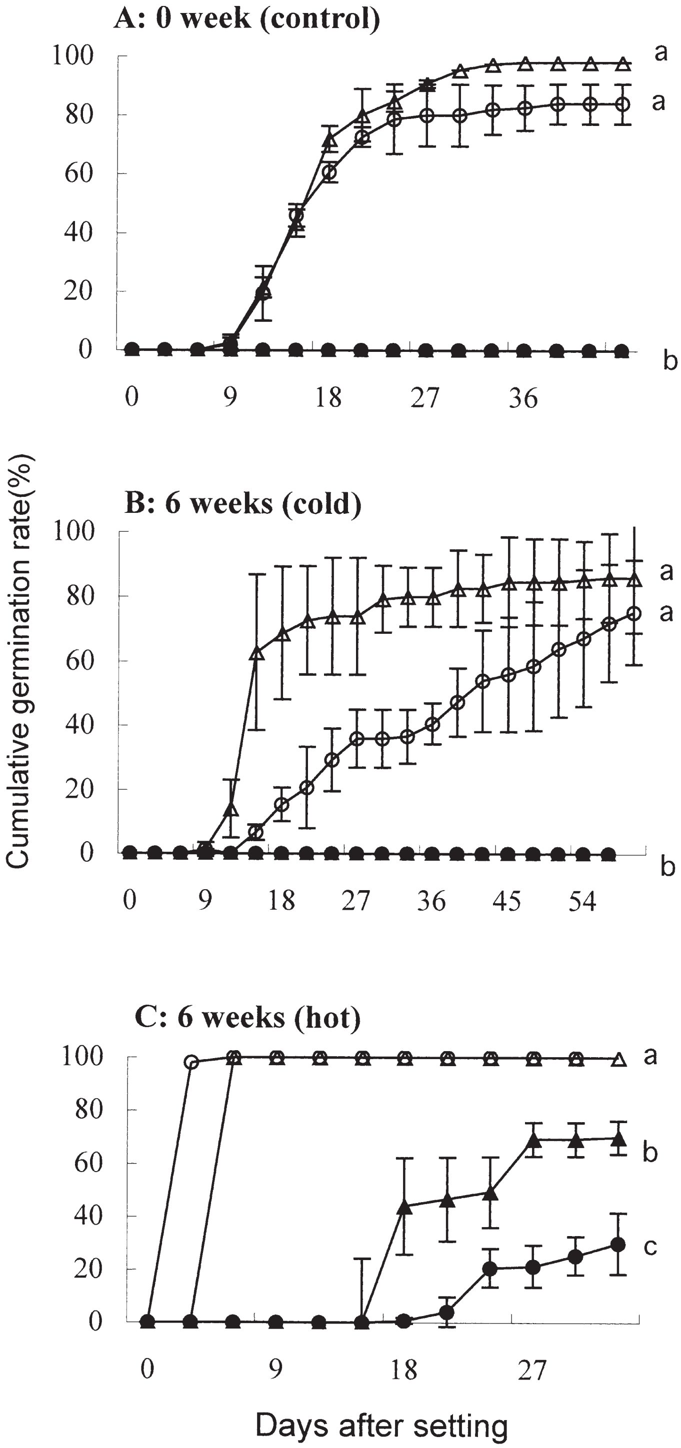

Fig. 1. Time course for the cumulative germination rate of seeds from Kobe under four different treatment conditions at alternating temperatures of 20 and 15℃ (day/night). Values represent means±S.D. and different alphabets indicate significant differences ($p<0.05$). (A) no pretreatment; (B) cold treatment for 6 weeks; (C) hot treatment for 6 weeks. (△) light and aerobic; (○) light and anaerobic; (▲) dark and aerobic; (●) dark and anaerobic.

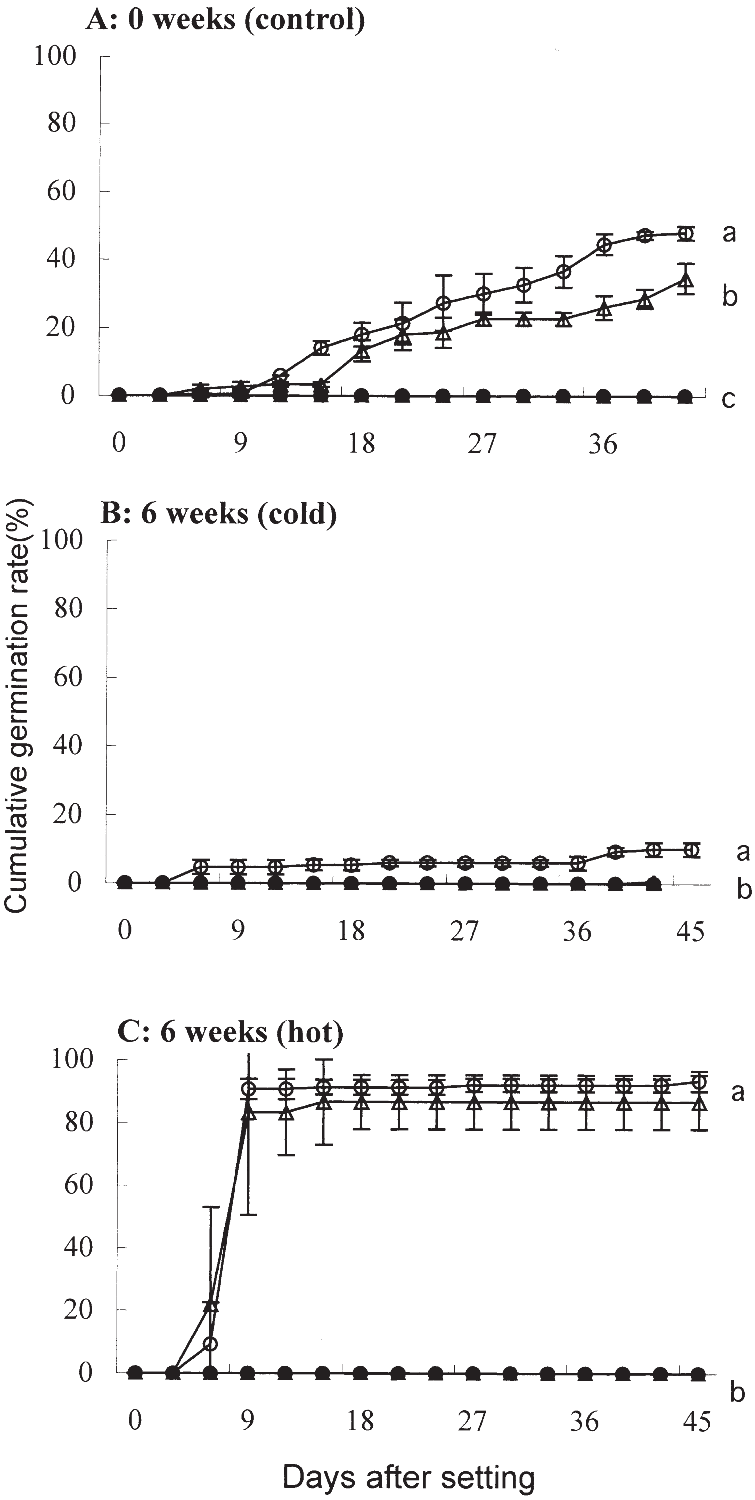

Fig. 2. Time course for the cumulative germination rate of seeds from Tajima under four different treatment conditions at alternating temperatures of 20 and 15℃ (day/night). Values represent means±S.D. (A) no pretreatment ; (B) cold treatment for 6 weeks ; (C) hot treatment for 6 weeks. Others are the same as in Fig. 1.

Table 1. Final germination rate (%) under five fluctuating temperature regimes. The mean and range (in parenthesis) of triplicate experimants are shown for the seeds from Kobe and Tajima

Temperature	Kobe	Tajima
30/25℃	0	0
25/20℃	34.2(27.1–43.9)	12.9(4.4–17.0)
20/15℃	90.6(85.1–94.8)	45.2(44.0–46.0)
15/10℃	75.7(68.6–86.7)	4.1(0–6.3)
10/5℃	13.1(12.3–14.3)	1.3(0–3.8)

seeds from both Kobe and Tajima showed the highest germination rate at 20/15℃ (day/night), although the germination rate was higher for the Kobe seeds (ca. 90%) than for those from Tajima (ca. 45%). At 30/25℃ no germination occurred in seeds from either population.

Discussion

In Japan, the seeds of *R. sceleratus* are shedded in late spring and germination is typically observed in autumn, after harvesting of rice. The seeds of the species of Ranunculaceae, including *R. sceleratus*, require some periods of after-ripening (Tamura and Mizumoto 1972). Although the delay of germination may be partly due to the immature embryos, our results suggest that the seeds are not in a state of innate dormancy, because they can germinate under optimal temperatures without any pretreatment. Actually Takatori (1983) showed that germination rate increased after the storage for one to two weeks under high temperature condition. Thus, the seeds are considered to be in a state of conditional dormancy sensu Baskin and Baskin (1998) after the embryos develop.

The hot treatment promoted germination of the Tajima seeds. This suggests that high summer temperatures break dormancy. This finding contradicts van der Toorn and ten Hove (1982), who reported a requirement for cold stratification for the germination of *R. sceleratus* in Europe. European and Japanese populations of *R. sceleratus* thus seem to have become genetically differentiated, leading to the observed difference in life cycles.

Matsuo and Kubota (1993) showed that stratification at a higher temperature (20℃ in their case) was effective in breaking the dormancy of the seeds of winter annual. However, the seeds from Kobe could germinate without a hot treatment even though they remained dormant during the summer in rice fields. This result can be explained by our observation that no germination occurred at high temperatures such as the 30/25℃ alternation. Takatori (1982) also reported the germination of the seeds of *R. sceleratus* under low temperature (15℃) and no germination under high temperature (25℃). Thus, high summer temperatures appear to inhibit germination during the summer in both populations. Both populations start to germinate only after the temperature decreases. The optimal temperature for germination appears to be the 20/15℃ alternation in our study. These temperatures typically occur in October according to data from meteorological stations near our study sites (data not shown).

Thus, the seeds are in a state of conditional dormancy induced by high temperature during the summer and germinate in autumn. A hypothesis that submergence during the irrigation period inhibits germination of the seeds will not be supported because they germinated irrespective of oxygen condition. Baskin and Baskin (1986) demonstrated that seasonal temperature change controls the timing of germination in seeds of some winter annuals. Relatively low germination rate of the seeds from Tajima may suggest that the period of after-ripening is longer than Kobe populations. Otherwise some pretreatment was necessary to break the dormancy completely.

According to Lehmann (1909) and Takatori (1982), the germination of the seeds of *R. sceleratus* was observed under constant temperature at 15℃ in the light condition. However they did not try the test under alternating temperature. In our study no germination was observed under constant temperature (20℃) and alternating temperatures were required for the germination of seeds. Probert et al. (1987) also reported a stringent requirement for alternating temperatures to permit germination of the seeds of *R. sceleratus*. Although there remains a possibility that lower temperature such as 15℃ may induce the germination even under constant temperatures, alternating temperature seems to promote the germination of the seeds so far as our experiments were concerned. In our study light condition promoted the germination of the seeds as has been already shown by Lehmann (1909) and Takatori (1982).

After harvesting of rice, the ground of rice fields is left bare. Such conditions may promote the germination of seeds because both the presence of light and the alternating temperatures become signals that site conditions are suitable for germination (Grime 1979; Washitani and Takenaka 1987). The germination characteristics of the seeds of *R. sceleratus* appear to be adaptive to starting the life cycle of this species under autumnal conditions in Japanese rice fields.

Growth of *R. sceleratus* indicates wet conditions in the rice fields (Nomiya and Toyohara 1991). However, the recent spread of dried rice fields during the winter has made the habitat unfavorable for this species. Furthermore, the practice of mulching by straws and the increases in the fallowing rice field leading to vegetational succession result in the cover of bare ground. Such conditions are not suitable for germination of the seeds of *R. sceleratus*. The combination of these changes in the rice field environment appears to have led to local extinction of *R. sceleratus* populations in many places.

Variability of seed germination characteristics between populations has been reported in many plant species (for a review, see Baskin and Baskin 1998). For the Tajima seeds, cold treatment seems to delay the after-ripening or induce secondary dormancy and hot treatment seems to promote break that dormancy. On the other hand, the Kobe seeds do not respond significantly to cold and hot treatment. These differences between the two populations may represent adaptations to different environmental conditions at the two sites. However, to generalize about the adaptive significance of individual germination properties, we must analyze seeds from more populations with a wide range of environmental conditions.

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奥田紘奈・角野康郎：タガラシの冬一年草生活史とその発芽特性

タガラシ（キンポウゲ科）は，水田や水路に普通に生育する冬一年草である。しかし，近年，乾田化の進んだ水田を中心に生育地が急減し，地方版レッドデータブックでは絶滅危惧種に挙げる例もみられる。本研究は，ヨーロッパでは夏一年草と報告されるタガラシが，なぜ日本では冬一年草の生活史をとるのか，そして近年の減少の要因は何かを，その発芽特性から解明しようとしたものである。種子は兵庫県神戸市（以下，神戸）と豊岡市（但馬）の2カ所の水田から採集した。神戸の種子は低温処理（4℃）と高温処理（30℃）の有無にかかわらず，明条件の変温条件下（20/15℃）ではよく発芽した。但馬の種子では低温処理は二次休眠を誘導し，逆に高温処理は休眠を打破する傾向が見られた。暗条件では神戸の種子はわずかに発芽したが，但馬の種子は全く発芽しなかった。発芽は好気条件，嫌気条件で両産地とも差はなかった。恒温条件下（20℃）では，低温処理，高温処理を施した場合を含め，いずれの条件下でも全く発芽は見られなかった。また発芽適温を調べた結果，発芽率は20/15℃の変温条件下で最も高く30/25℃のような高温下ではまったく発芽しなかった。

以上の結果より，タガラシの種子は，キンポウゲ科に一般的な後熟を完了した後，夏の高温下では休眠を強いられ，秋になって気温が低下するとともに発芽を開始すると推測された。また変温条件と光が発芽を促進したことから，タガラシの種子は稲刈り後の裸地環境での発芽に適応していると考えられた。近年の乾田化は，湿生植物であるタガラシにとって好ましくないだけでなく，稲刈り後の藁敷きや休耕による植生遷移の進行によって植被率が高まることもタガラシの発芽適地を消失させ，各地の集団が消滅する原因になっていると考えられた。（〒657–8501　神戸市灘区六甲台町1–1　神戸大学理学部生物学科）