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Germination Responses of Seeds of *Vicia angustifolia* L. at Constant, Increased and Decreased Temperature

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Abstract Germination responses of seeds of winter annual *Vicia angustifolia* to the changes in temperature were studied outdoors and in the laboratory. While no seedlings were found outdoors in the summer, many seedlings emerged in mass in the autumn, particularly in October, the season when soil temperature was much lower than that during the summer. Germination of seeds that were incubated at a temperature of constant 4℃, 15℃, 25℃ or 37℃ for 6 months were low. When seeds incubated at low temperature (4℃ or 15℃) for several months were transferred to constant 15℃ or alternating 5/25℃, few seeds germinated. Only seeds that were incubated at high temperature (25℃ or 37℃) for several months germinated at 15℃ or 5/25℃. Germination at 15℃ or 5/25℃ increased with the number of months seeds were incubated at high temperature. More than half of the seeds incubated at 25℃ for 4 months germinated at 15℃. These results indicate that the temperature in the summer is too high to cause germination of *V. angustifolia* seeds, and exposure to high temperature for several months is necessary for germination to occur in the low temperature months of the autumn.

Key words: dormancy, germination, seed, temperature, *Vicia angustifolia*, winter annual.

Vicia angustifolia L. is a winter annual widely distributed in southwest Japan. In the spring, it can be easily found blooming in open grasslands from urban to rural areas. Like many other winter annuals, *V. angustifolia* sets seeds and dies by early summer. Many seeds of *V. angustifolia* spring out from blackened pods and fall onto the ground as the plants wither and desiccate. Although soil water content and soil temperature is high enough for many seeds of perennials or summer annuals to germinate, dispersed seeds of *V. angustifolia* keep dormant in the summer. In the autumn, as if waiting for the winter senescence of summer weeds, seedlings of *V. angustifolia* suddenly emerge and the number of them keeps increasing throughout the early winter.

Dispersal of seeds in the late spring or the summer and germination in the autumn is the general life cycle of winter annual species. As the dispersed seeds do not germinate during summer, there must be some environmental or internal factors that are controlling the timing of the germination. Several workers examined the effect of temperature in the germination of winter annuals and found that the temperature plays an important role in determining the timing of germination (Baskin and Baskin, 1998). For example, 80% of the seeds of *Veronica hederifolia* L. germinated when they were stored wet under high temperature (30℃) for two months, whereas germination scarcely occurred when they were stored at low temperature (4℃) (Roberts and Neilson, 1982a). The same pattern of alleviation from dormancy at high temperature was also reported in other winter annuals: *Aira praecox* L. and *Teesdalia nudicaulis* (L.) R. Br. (Newman, 1963); *Viola rafinesquii* Greene (Baskin and Baskin, 1972); *Torilis Japonica* (Houtt.) DC. (Baskin and Baskin, 1975); *Stellaria media* (L.) Cyrillo, *Valerianella umbilicata* (Sulliv.) Wood f. *intermedia* (Dyal) Eggers and *Phacelia purshii* Buckl. (Baskin and Baskin, 1976) and *Aphanes arvensis* L. (Roberts and Neilson, 1982b).

The object of this study is to elucidate whether exposure to high temperature is

effective in breaking the dormancy of the seeds of *V. angustifolia*. First, seasonal patterns of germination and changes in soil temperature outdoors were monitored to clarify the correlation between decrement in soil temperature and germination pattern of *V. angustifolia* under natural conditions. Next, to elucidate the effects of storage at different temperatures, the germination pattern of seeds which were stored wet at 4 constant temperature regimes (4℃, 15℃, 25℃ and 37℃) were tested at constant and alternating temperature of 15℃ and 5/25℃, respectively.

Materials and Methods

1. Germination and soil temperature—outdoors

In the spring of 1994, 8 quadrats, each 25 cm×25 cm, were established on grasslands or lawns where *V. angustifolia* were growing well. Two quadrats were established on soil in a large, rectangular flowerpot made of plastic (site A). As this pot were left outdoors for several years, many weeds, mostly perennials, were growing in it, naturally. Two quadrats were established on a lawn which had been mowed a few times a year (site B). These total of four quadrats were situated in the Ecology Park of the Natural History Museum and Institute, Chiba in Chiba-shi, Japan. The other four quadrats were situated at the Toho University campus in Funabashi-shi, Japan. Two were established on a grassland where some summer annuals and perennials are growing (site C) and two on a lawn (site D). The grassland and lawn had also been mowed a few times a year.

On June 2nd, 200 seeds of *V. angustifolia* were sown in each quadrat. The seeds were sown evenly and covered with a thin layer of local soil. Seeds sown in the quadrats were collected from naturally growing plants in and around Chiba city in mid-May. The seeds were kept dry at room temperature after they were taken out from blackened pods. Due to the dispersal of naturally ripened seeds of *V. angustifolia* growing inside or around the quadrats, in actuality, the total number of seeds buried in each quadrat most likely exceeded 200.

Soil temperature around the depth where seeds were buried was monitored with a maximum–minimum thermometer. One thermometer was set in each quadrat on the same day when the seeds of *V. angustifolia* were sown. The bulb of the thermometer was buried at a depth of 1 cm immediately outside the quadrat. The upper limit on the thermometer is 60℃.

The number of seedlings of *V. angustifolia* in each quadrat was counted almost once a week. Maximum and minimum temperature were also recorded each time the seedlings were counted. As the thermometers were reset after each recording, the maximum and minimum temperature for the period between countings were recorded.

To determine the strict percentage of germination of newly sown seeds, bags with seeds of *V. angustifolia* inside were buried and the number of germinated seeds inside the bags were later counted. Fifty seeds from the same lot as those sown in quadrats outdoors were placed with some soil in fine-mesh bags (10 cm×7 cm) made of chemical fibers. On June 5th, 10 bags were buried shallowly in site B, and the other 10 in site D. Two bags from each site were exhumed almost once a month and the number of germinated seeds was counted. Even when all the seeds inside the bags were dormant, exhumed seeds and bags were not buried back.

2. Germination test of stored seeds

Seeds of *V. angustifolia* were stored wet on vermiculite moistened with deionized water in 9-cm Petri dishes. Fifty seeds from the same lot as those sown in quadrats outdoors were placed on moistened vermiculite of almost 5 mm thickness in each Petri dish. Seeds in the Petri dishes were incubated under dark conditions at constant temperature of 4℃, 15℃, 25℃ and 37℃, 20 dishes at each temperature. Incubation started on June 30th, 1994. Vermiculite was kept wet by pouring distilled water at intervals of a few days. The number of germinated seeds were counted once a week. Germinated or rotted seeds were removed each time after counting.

After a month from the start of incubation, 4 dishes were chose randomly from each constant temperature regime for germination

testing. After already germinated seeds were counted and removed from the dishes, two dishes were transferred to constant temperature of 15℃ and the other two to alternating temperature of 5/25℃ (12 h/12 h). The number of germinated seeds were counted once a week from transference. Transferences of the dishes from constant temperature to 15℃ or 5/25℃ were repeated 3 more times at 1 month interval. The germination rate at constant temperature was determined with remaining 4 dishes in the constant temperature regimes. Germinated or rotted seeds were removed after every counting.

Results

1. Germination and soil temperature—outdoor

In some quadrats, a few seedlings of *V. angustifolia* were found emerging soon after the sowing (Fig. 1). The emergence of new seedlings, however, stopped abruptly and the seedlings began to die from unknown causes. As all seedlings disappeared in July, no seedlings could be found in any of the quadrats in August. After the emergence of a few seedlings of *V. angustifolia* was observed in September, the number of seedlings sharply increased from early October in all quadrats. This abrupt increment continued until late November or December, when the increase reached a plateau.

Maximum soil temperature in each quadrat at the depth of 1cm was extremely high from July to August. The temperature frequently exceeded 60℃, the upper limit on the thermometer. From late August, maximum temperature decreased gradually or abruptly, depending on the situation the quadrats were established at. The maximum temperature in early October, when the abrupt increment of seedlings was observed, ranged from 25℃ to 35℃.

Minimum soil temperature in each quadrat

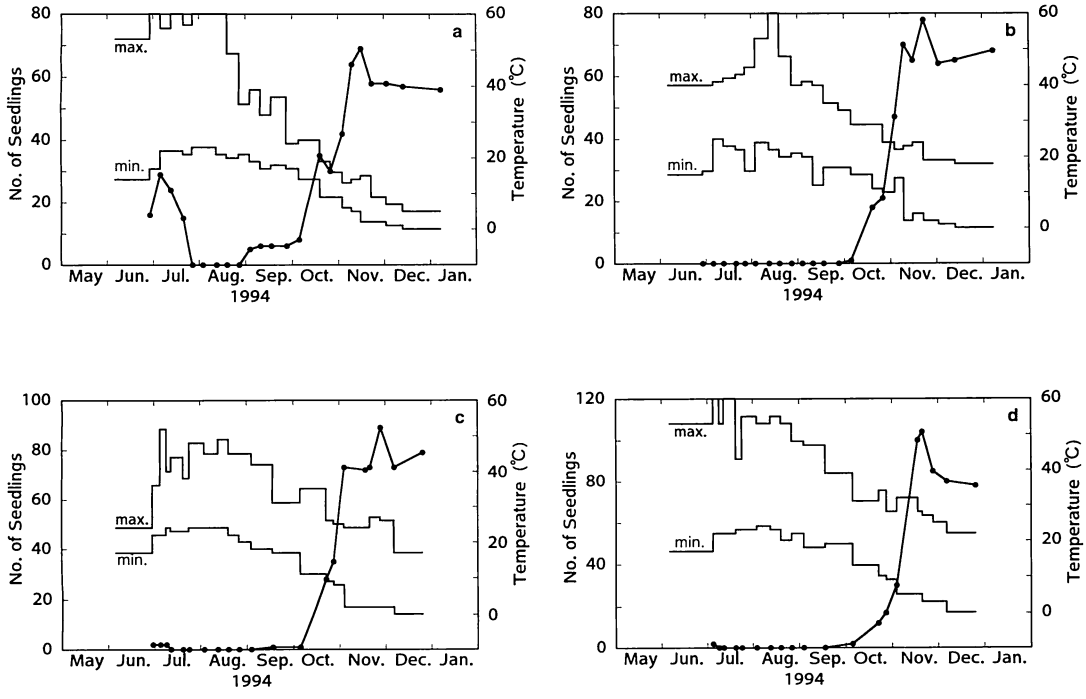

Fig. 1. Seasonal changes in the number of emerging seedlings of *Vicia angustifolia* (closed circles), and maximum and minimum soil temperature (horizontal bars) in one of two quadrats established at each site. Two sites; (a) soil in large flower pots and (b) lawn were situated in the Ecology Park of the Natural History Museum and Institute, Chiba, and the other two sites; (c) grassland and (d) lawn at the Toho University campus. Two hundred seeds of *V. angustifolia* were sown on June 2nd in each quadrat. The time durations of the measurements of maximum and minimum temperatures are represented by horizontal bars. The upper limit on the thermometer is 60℃.

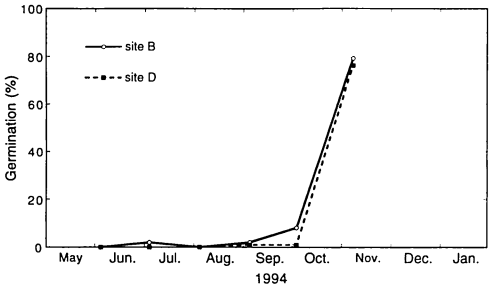

Fig. 2. Seasonal changes in the germination of *Vicia angustifolia* seeds placed in fine-mesh bags buried at two sites; (B) lawn in the Ecology Park of the Natural History Museum and Institute, Chiba and (D) lawn at the Toho University campus. Ten bags with fifty seeds placed inside were buried on June 5th at each site.

at the depth of 1cm seldom exceeded 25℃ and fell below 20℃ from July to August. Minimum temperature decreased gradually in September and rapidly from October to November. In early October, the minimum temperature ranged from 11℃ to 15℃, and in late October from 8℃ to 10℃. Although the minimum temperature in October differed among quadrats as did the maximum temperature, the range of the minimum temperature during the period of abrupt emergence of seedlings was smaller than that of maximum temperature.

The pattern of germination of the seeds inside the buried bags was similar to what was observed in the quadrats (Fig. 2). Although few seeds germinate in the exhumed bags by September, the number of germinated seeds increased markedly in October. Germination percentages exceeded 70% in both sites.

2. Germination test of stored seeds

No seeds germinated when stored wet at constant temperature of 4℃ and 37℃ (Fig. 3). The number of germinated seeds increased gradually from the start of the incubation period at 15℃. Germination at 15℃, however, did not exceed 20% even after 6 months of storage. Seeds incubated at 25℃ germinated abruptly after storage of about 4 months, yet germination soon reached plateau at 20%.

When seeds stored at 4℃ were transferred to 15℃ or 5/25℃, only a few seeds germinated (Fig. 4). The highest germination rate was 5%. As this rate was attained by those seeds that were transferred after one month of storage at 4℃, it most likely was the results of incubation at temperature of 15℃ or 5/25℃ rather than the effect of low temperature of 4℃.

When seeds stored at 25℃ were transferred to 15℃ or 5/25℃, a substantial number of seeds germinated (Fig. 5). Irrespective of the length of storage, germination

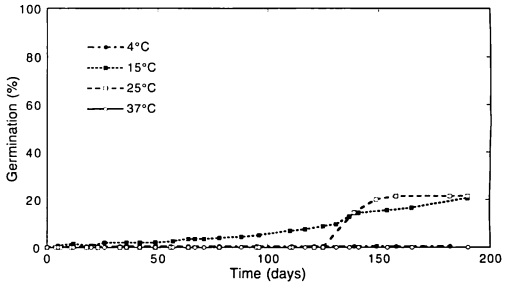

Fig. 3. Germination of *Vicia angustifolia* seeds incubated at constant temperature of 4℃, 15℃, 25℃ and 37℃. Incubation and moistening of seeds began on Day 0.

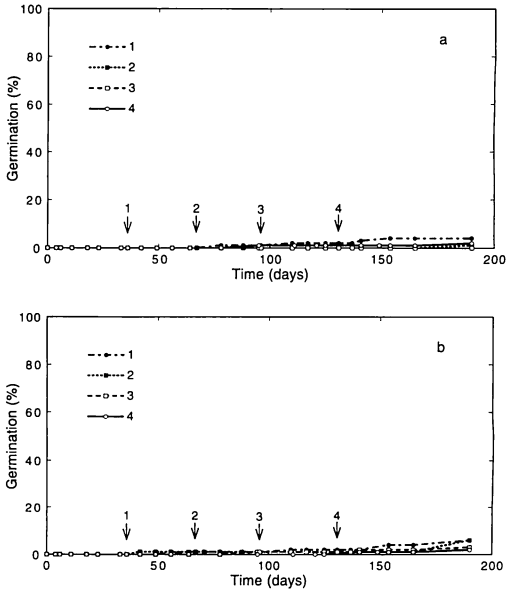

Fig. 4. Germination of *Vicia angustifolia* seeds transferred from 4℃ to (a) constant 15℃ and (b) alternating 5/25℃ (12 h/12 h). Each set (No. 1–4) of 100 seeds was transferred on the day indicated with the arrow of the same number. Incubation and moistening of seeds began on Day 0.

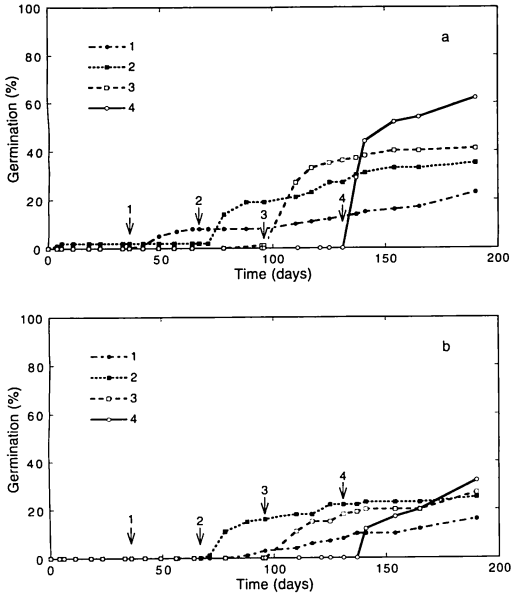

Fig. 5. Germination of *Vicia angustifolia* seeds transferred from 25℃ to (a) constant 15℃ and (b) alternating 5/25℃ (12 h/12 h). Each set (No. 1–4) of 100 seeds was transferred on the day indicated with the arrow of the same number. Incubation and moistening of seeds began on Day 0.

occurred abruptly after the transference, notably when seeds were transferred to 15℃ (Fig. 5a). The degree of the initial sharp increase depended on the number of months the seeds were stored at 25℃, while the inclination of the gradual increase which followed the abrupt initial increment did not. Final percentage of germination increased with the number of months the seeds were stored at 25℃. The highest germination of ca. 60% was attained by seeds transferred after about 4 months storage. Although not as clearly observed as germination at constant 15℃, germination at 5/25℃ showed a similar pattern (Fig. 5b). Germination increased with the number of months the seeds were stored at 25℃.

The germination patterns of seeds stored at 37℃ were similar to those stored at 25℃ (Fig. 6). Germination increased abruptly immediately after the transference to constant 15℃ or alternating 5/25℃. Final percentage increased with the number of days the seeds were stored at 37℃. However, the degree of initial increase and the inclination of gradual increase that followed were not as large as those of the seeds stored at 25℃. The final germination rate of seeds stored at 37℃ was generally lower than those stored for an equal number of days at 25℃.

When the seeds were transferred from constant 15℃ to alternating 5/25℃, no marked effect of transferring was detected (Fig. 7).

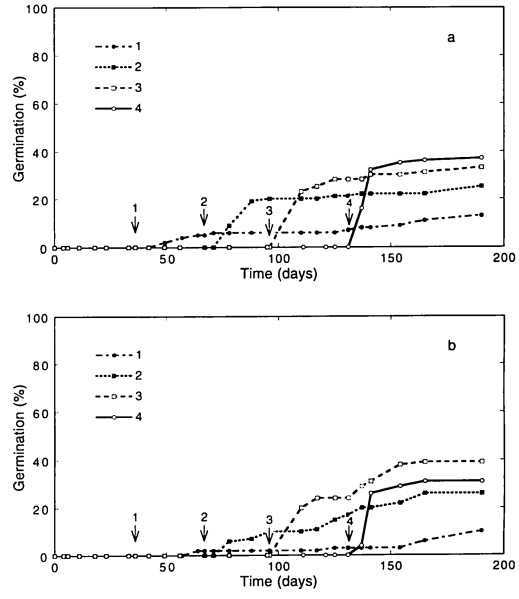

Fig. 6. Germination of *Vicia angustifolia* seeds transferred from 37℃ to (a) constant 15℃ and (b) alternating 5/25℃ (12 h/12 h). Each set (No. 1–4) of 100 seeds was transferred on the day indicated with the arrow of the same number. Incubation and moistening of seeds began on Day 0.

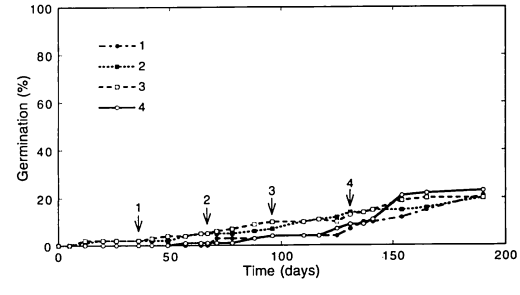

Fig. 7. Germination of *Vicia angustifolia* seeds transferred from constant 15℃ to alternating 5/25℃ (12 h/12 h). Each set (No. 1–4) of 100 seeds was transferred on the day indicated with the arrow of the same number. Incubation and moistening of seeds began on Day 0.

Regardless of the date the seeds were transferred, germination increased gradually in the same pattern as observed among seeds stored at 15℃.

Discussion

The seeds of *V. angustifolia* sown outdoors displayed a germination pattern typical of winter annuals (Fig. 1). Most of the seeds kept dormant until autumn then germinated in mass in October. As more than 70% of the seeds ripened in the current year germinated (Fig. 2), most of the seedlings which emerged in the quadrats must have originated from the seeds sown or dispersed in the current year.

The soil temperature in October, when many of the seedlings emerged, is characteristic in that the maximum and minimum temperature are much lower than the soil temperature in the summer. The decrement in minimum temperature especially indicates that in the autumn, the seeds are exposed to low temperatures never experienced during the summer. As is observed in many other winter annuals, the germination of seeds of *V. angustifolia* started in the season when the soil temperature were on the decrease week by week.

Results of the germination test in the laboratory indicated that although storage at high temperature is necessary, high temperature alone is not sufficient for the seeds to germinate. When seeds were stored wet at one constant low temperature (4 or 15℃) or even when they were transferred to constant temperature of 15℃ or alternating temperature of 5/25℃, few seeds germinated (Figs. 3, 4, 7). When seeds were kept at one constant high temperature (25 or 37℃), a few or no seeds germinated even after storage for many months (Fig. 3). A substantial number of seeds germinated only when they were transferred from high temperature to 15℃ or 5/25℃ (Figs. 5, 6). In addition to high temperature storage, a decrement in temperature is necessary for the actual germination of the seeds of *V. angustifolia* to occur.

Percentage of germination at 15℃ or 5/25℃ increased with the number of months the seeds were stored at high temperature (Figs. 5, 6). The degree of initial sharp increase most notably increased with the length of storage. More than half of the seeds stored at 25℃ for about 4 months germinated immediately after the seeds were transferred to 15℃. Exposure to high temperature for several months is necessary to attain a high percentage of germination after the fall in temperature.

Therefore, in consideration of the germination responses of seeds of *V. angustifolia* to the changes in temperature, the germination pattern of naturally dispersed seeds outdoors can be explained as follows. Soon after the dispersal of seeds in the early summer, most of the seeds of *V. angustifolia* lay deeply dormant. Only a few seeds germinate even when soil temperature falls to near 15℃, a figure occasionally attained in the rainy season of early summer. As the soil temperature rises during the summer, the number of seeds which become potentially able to germinate at low temperature gradually increases. However, as soil temperature never drops to the level suitable for germination, no seedlings emerge during the summer. In October, the soil temperature begins to fall rapidly. When soil temperature drops low enough, seeds ready to germinate at low temperature begin to germinate in mass. As a result, many seedlings erupt above ground from October to November.

The germination pattern of seeds of *V. angustifolia* dispersed outdoors can thus be explained by the responses of seeds to the changes in soil temperature. Like many other winter annuals, soil temperature seems to be one of the most important environmental factors controlling the timing of the germination of seeds of *V. angustifolia*.

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カラスノエンドウの種子発芽に及ぼす温度変動の影響

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越年草であるカラスノエンドウの種子発芽に及ぼす温度変動の影響を屋外と実験室とで調べた．カラスノエンドウの種子発芽と地温の変動を屋外において観測したところ，カラスノエンドウの種子は地温の低下する 10 月頃に一斉に発芽を始めることが観察された．実験室内で，カラスノエンドウの種子の発芽実験を 4℃, 15℃, 25℃, 37℃ の定温条件で行ったところ，6 ヶ月間それぞれの温度の下に置いても，あまり発芽はみられなかった．4℃ もしくは 15℃ の低温条件下に数ヶ月置いた後に，15℃ の定温もしくは 5/25℃ の変温に移しても，ほとんど発芽は見られなかった．数ヶ月間 25℃ もしくは 37℃ の高温に置いてから，15℃ の定温もしくは 5/25℃の変温に移したときのみ多数の種子が発芽した．25℃ もしくは 37℃ の高温下に置く期間が長いほど発芽率は上昇し，25℃ に 4 ヶ月置いた種子を 15℃ に移したときの発芽率は 50% 以上であった．以上の結果から，カラスノエンドウの種子の発芽の適温は，秋に観測される 15℃ 付近の低温であるが，発芽するためには数ヶ月間，夏の高温にさらされることが必要条件であることが示された．