Satoko Kawarasaki¹,²,³, Yoshiko Abe¹ and Yoshimichi Hori¹: Effect of temperature on achene germination in five Mutisieae understory herbaceous species (Asteraceae)

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Abstract

We measured the final germination rates of achenes, the number of days needed to accomplish 50% of the final germination (T₅₀), and mortalities under various temperatures in the range 5 to 25°C for five understory perennial herbs (four species with large achenes of ca. 10 mg weight : Pertya robusta, P. triloba, P. rigidula and Ainsliaea acerifolia var. subapoda, and one species with small achenes (1 mg) : A. apiculata) to investigate variation of these traits. Achenes were collected in Ogawa and Mito in Ibaraki Prefecture, and in Iwakuni in Fukui Prefecture. T₅₀ ranged from 35 to 76 days at 5°C and became shorter with increasing temperature. The final germination rates of most samples were over 95%. Ainsliaea acerifolia had the longest T₅₀ (76 days) and the lowest final germination rate (86.1%) at 5°C, showing a dormant ability to some extent. Moreover, A. apiculata possessing small achenes showed a lower final germination rate (83%) at 25°C, and dormancy at higher temperatures. Fungal attack was thought to cause the death of achenes during the germination period, and mortalities increased with increasing temperature. However, there were no significant differences in final germination rates and T₅₀s between two populations of P. robusta from Ogawa and Mito, though the Ogawa population had a higher mortality than the Mito population (27% vs. 18% at 25°C). Mortalities were higher in P. triloba and P. rigidula than in the other species. There were intra- and interspecific variations of temperature dependency of achene mortality during germination, and these variations may be one of the factors limiting the distribution of each species.

Key words: achene germination response to temperature, final germination rate, mortality, T₅₀, understory herbaceous species.

Introduction

Germination success is an important factor determining the fitness of plants, especially in plants that regenerate only by seeds. Pertya robusta (Maxim.) Beauverd, P. triloba (Makino) Makino, P. rigidula (Miq.) Makino, Ainsliaea acerifolia var. subapoda Nakai (hereafter, A. acerifolia) and A. apiculata Sch.Bip. are understory perennial herb species belonging to the tribe Mutisieae (Asteraceae) and regenerate only by achenes. These five species have specific distribution areas (see Fig. 1). As plant physiological responses are thought to be closely correlated with climatic conditions of habitats, we therefore expect temperature effects on germination traits and the existence of intra-and interspecific variation in germination traits among these species.

The achenes of the five species are relatively large weighting 7–13 mg, except for A. apiculata (1 mg). In general, understory plants produce larger seeds than plants growing in open habitats (Salisbury 1975 ; Luffmansteiner 1979 ; Foster and Janson 1985 ; Mazer 1989). Plants with large seeds have large seedlings and can tolerate shaded environments (Salisbury 1975 ; Moles and Westoby 2004), or they may possess the ability to germinate below the litter (Leishman and Westoby 1994). On the other hand, plant mortality is higher in the seedling stage than in other life stages (Harper 1977 ; Solbrig 1980). Mortality may also be high during seed germination because many physiological processes occur
in seed germination, and plants may be sensitive to external factors during the transition from seeds to seedlings. Although large seeds are considered advantageous for survival because of their larger resource under shaded conditions, some seeds do die during germination. What factors cause the death of large achenes and how many seeds die in actual populations of the five Mutisieae species? Are there temperature dependencies for factors contributing to the death of achenes, and is there intra- and interspecific variation of achene mortality?

A theoretical study proposed that large seeds tend to lack an ability to lie dormant because seedlings of large-seeded plants have a larger resource and can establish themselves in relatively unfavorable environments, such as shaded conditions (Rees 1994). On the other hand, non-dormant seeds during germination would be under strong selective pressure by unexpected events such as a sudden coldness, a late frost, a severe drought and so on, since they cannot avoid unexpected unfavorable conditions through the utilization of a dormant period. Therefore, germination traits of non-dormant seeds may be coupled with the climatic conditions of their habitats. We wanted to determine whether these five species have a dormant ability and, especially, whether the germination traits of *A. apiculata* were different to those of the other species since *A. apiculata* has conspicuously smaller achenes than members of the other species. Moreover, if dormancy is detected, does the dormant ability depend on temperature and are there intra- and interspecific differences in dormancy?

We examined temperature effects on germination responses, including final germination rates, the number of days needed for 50% of the final germination rates ($T_{50}$), mortalities, and whether there exist variations in germination responses among the five species and among intraspecific regional populations.

**Materials and methods**

The five Mutisieae species are understory perennial herbs that reproduce only by achenes. They flower in the autumn and then disperse their achenes. Achenes commence germination in the next spring. All of the species, with the exception of *A. apiculata*, are summergreen herbs and have chasmogamous flowers. *Ainsliaea apiculata* is an evergreen herb and bears cleistogamous flowers. The distribution ranges of these species are shown in Fig. 1. In the autumn of 1995, we collected achenes before dispersal at three sites. Achenes of *P. robusta*, *P. triloba* and *A. acerifolia* were collected from Ogawa Forest Reserve, Ibaraki, where their distributions
are sympatric (Kawarasaki and Hori 2001). Achenes of *P. robusta* and *A. apiculata* were collected from Mito, Ibaraki, where their distributions are sympatric. Achenes of *P. rigidula* were collected from Iwakuni National Forest, Fukui. The achenes of each species were collected from more than one-hundred individuals. Their localities, elevation, environmental characteristics and achene weights are shown in Table 1. The achenes were air-dried at room temperature for ca. one month and then kept at almost the same air temperature as that of the outdoors, in Ibaraki University in Mito, Ibaraki.

Fifty achenes from each species/population were placed in a petri dish (φ 8.5 cm) with soil that had been sieved through a 5 mm mesh on May 8, 1996. To mimic the natural habitat of achenes during germination, we put the petri dishes into soil. The soil was derived from the achene collection sites, except for *P. rigidula*, whose soil was from Mito. Pappuses were removed from achenes just before the experiment. Achenes were incubated at five constant temperatures (5, 10, 15, 20 and 25°C) using a regime of 12-h light and 12-h dark with four replications in temperature-gradient growth chambers (TG-200-ADCT, Nippon Medical & Chemical Instrument, Osaka). In order to keep achenes from drying, we put distilled water into the 20 and 25°C petri dishes almost daily, and almost every three days for the other petri dishes. We recorded the number of germinated and dead achenes everyday, and removed those that had either germinated or died. Germination was defined as protrusion of a radicle, and the death of an achene was confirmed from its softness when pinched with a pair of tweezers. The duration of the experiment was 120 days, except for *A. acerifolia* achenes incubated at 5°C, for which 150 days were needed.

Final germination rates, mortalities and Tₐ (the number of days to reach 50% germination of the final germination) estimates for each dish were calculated. Final germination rates are the number of germinated achenes per number of all live achenes during the incubation time. The following logistic equation is a good representation of the relationship between the cumulative per-
From the fitted equations, T₅₀ can be calculated as follows:

\[ T_{50} = \frac{\ln A}{R} \]

where \( P \) is the relative percentage to the final germination rate, \( A \) and \( R \) are fitted parameters, and \( T \) is the incubation time (expressed in days). From the fitted equations, \( T_{50} \) can be calculated as follows:

\[ T_{50} = \frac{\ln A}{R} \]

The effects of temperature and species on final germination rates, \( T_{50s} \) and mortalities were examined using two-way analysis of variance (ANOVA) and a post-hoc test by the least squares method. The final germination rates and mortalities were arcsine transformed for these tests.

**Results**

Final germination rates of most treatments were over 95% (Fig. 2 A). Temperature had a significant effect on germination rate (Table 2 A). Final germination rates at 5°C were significantly lower than those at other temperatures (p<0.01 ; least squares test). The final germination of *A. apiculata* showed significant differences from that of the other species (p<0.05 ; least squares test), and unlike the other species, *A. apiculata* had a low germination rate at a lower temperature (83.0% at 25°C). There was no intraspecific variation of final germination between the two populations of *P. robusta*.

Temperature also had a significant effect on \( T_{50} \) (Table 2 B). \( T_{50} \) decreased exponentially with increased temperature (Fig. 2 B) and there were significant differences between all temperature combinations, except for temperatures between 20°C and 25°C (p<0.05 ; least squares test). In all populations, \( T_{50} \) at 20°C and 25°C were shorter than 12 days, and \( T_{50} \) at 5°C was 35–76 days. The longest \( T_{50} \) was observed in *A. acerifolia* at 5°C (76 days), and there were significant differences between *A. acerifolia* and *P. robusta* collected in Ogawa (p<0.05 ; least squares test), *A. acerifolia* and *P. triloba* (p<0.01 ; least squares test), and *A. acerifolia* and *P. rigidula* (p<0.05 ; least squares test). The \( T_{50} \) of *A. apiculata* showed significant differences from that of the other species (p<0.05 ; least squares test), and *A. apiculata* had the smallest \( T_{50} \) at lower temperatures (34.8 days at 5°C). *Pertya robusta* did not display intraspecific variation in \( T_{50} \) between the two sampled populations.

Temperature had a significant effect on \( T_{50} \) (Table 2 C). The mortality of achenes tended to increase with increasing temperature (Fig. 2 C), and there were significant differences between \( T_{50} \) estimates at 25°C and other temperatures, between those at 20°C and 10°C and those at 20°C and 5°C (p<0.05 ; least squares test). *Pertya triloba* and *P. rigidula* showed relatively high mortalities (20–40%) in all temperature re-
gimes, and there were significant differences in mortality between *P. triloba* and the other species and between *P. rigidula* and the other species (*p < 0.01; least squares test*). The two species in the genus *Ainsliaea* showed relatively low mortalities (<10%) throughout the experiment. The mortality was significantly different between the Mito and Ogawa populations of *P. robusta* (*p < 0.01; least squares test*), and between the two *Ainsliaea* species (*p < 0.01; least squares test*).

### Discussion

Conspicuous dormant ability was observed both in large-achened *A. acerifolia* at 5°C and small-achened *A. apiculata* at higher temperatures. *Ainsliaea acerifolia* had a lower final germination rate and a longer T₅₀ at 5°C than the sympatric species *P. robusta* and *P. triloba* in Ogawa. No difference was observed in final germination rates or T₅₀ between the two *Pertya* species. In a study of *Plantago asiatica* from several sites over the Japanese archipelago, populations from higher latitudes or higher altitudes had a narrower range of temperature for germination and tended to be dormant at lower temperatures (Sawada et al. 1994). The authors suggested that seedlings of these populations have adapted to survive the low temperatures that frequently occur in the early growing season at these locations. On the other hand, on Mt. Fuji, seeds of *Reynoutria japonica* from higher elevations had higher final germination rates and higher germination speeds (being the reciprocal of T₅₀) at lower temperatures than seeds from lower elevations (Mariko et al. 1993). The authors concluded that the greater germination ability of upland plants at low temperature compensates for the shortened growing season at upland locations. The interspecific variation of germination traits among *A. acerifolia*, *P. robusta* and *P. triloba* from Ogawa may be due to the same factors controlling germination traits in *Plantago asiatica*. The distribution of *A. acerifolia* extends far north to China (Fig. 1B). The climate at the center of the distribution of *A. acerifolia* is expected to be cooler than that associated with *P. robusta* and *P. triloba*. *Ainsliaea acerifolia* shows dormancy to some extent at 5°C, and this appears to be an adaptation to low tem-
temperature as a means of avoiding damage to seedlings by unexpected coldness or late frost in the early growing season. Furthermore, leaf expansion was earlier and optimal temperature for photosynthesis lower in A. acerifolia than in the other two sympatric species (Kawarasaki and Hori in preparation). These facts may also suggest that A. acerifolia has better adapted to low temperature than the other two species.

The achenes of A. apiculata are much smaller than those of the other species, and they become dormant at high temperatures. The possession of small achenes in this species contradicts the notion that plants inhabiting unfavorable environments, such as shaded environments like understory, have large seeds with a larger reserve in order to tolerate poor growth conditions (Venable and Brown 1988; Rees 1994). Ainsliaea apiculata is normally considered an understory plant. Although it bears only 2–4 leaves within one year after germination on the forest floor, it expands many leaves successively and develops an elongated flower stem within three months in the laboratory under high light conditions. As a consequence, Hori and Kawarasaki (1996) considered it a "pseudo-understory" plant. This species is also unlike typical understory plants in view of its smaller achene size, dormancy at higher temperature and leaf phenology. Dormancy at higher temperature, which is found in many summer annual plants, is considered an adaptation to avoid hot and/or dry conditions during summer (Baskin and Baskin 1988). Small-achened A. apiculata differs from the large-achened species in germination traits. Further study is needed to clarify how A. apiculata acquired small-shaped achenes and an ability to become dormant at higher temperatures.

Pertya triloba and P. rigidula showed slight dormancy at 5°C. On the other hand, geographical variation was not observed in final germination rates or T₅₀s between the two populations of P. robusta, even though the average air temperature in Mito is ca. 3°C warmer than it is in Ogawa (Table 1). A larger difference in temperature may be needed to generate sufficient selective pressure essential for the development of intraspecific variation in final germination rate and T₅₀.

Achene mortalities depended on temperature throughout the experiment, and dead achenes were always covered by fungal hyphae, suggesting that the main cause of achene death is pathogenic fungi. The seeds had a structural injury caused by fungi (Tillman-Sutela et al. 2004), and the fungi were responsible for the death of the seeds (O’Hanlon-Manners and Kotanen 2004). There were interspecific variations in mortality. Furthermore, our study also revealed the existence of intraspecific variation of mortality of P. robusta achenes: the Ogawa population had a higher mortality than the Mito population (Fig. 2 C). Two causes of intraspecific variation in mortality may be considered. First, there are variations in resistant ability of achenes to fungal injury between the two populations. Second, soil from Ogawa contained more and/or stronger fungi. Through one way or another, achene mortality would be one of factors that limit the distribution areas of the species. In spite of the possession of large seeds, P. triloba and P. rigidula showed higher mortalities. In contrast, small-ached A. apiculata unexpectedly had a low mortality. These mortalities would strongly affect the distribution areas of each species.

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References


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