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Effects of range, seasons and storage under different temperatures on germination of *Senecio vulgaris* (Asteraceae) seeds

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Abstract

Invasive plants colonize new environments, become pests and cause biodiversity loss, economic loss and health damage. *Senecio vulgaris* (Common groundsel, Asteraceae), a cosmopolitan weed widely distributes in the temperate area, is reported with large populations in the north-eastern and south-western parts, but not in southern, central, northern or north-western part of China. We studied the germination behavior of *S. vulgaris* to explain the distribution and the biological invasion of this species in China. We used seeds originated from 12 populations in native and invasive range (six populations in each range) to conduct germination experiments in a climate chamber and ambient condition. When incubated in climate chamber (15°C) seeds from the majority of population showed >90% germination percentage (GP) and the GP was equal for both ranges. The mean germination time (MGT) was significant different among the populations. Under ambient conditions, significant effect of range, storage conditions (stored at 4 or 27°C) and seasons (in summer or autumn) were observed on the GP while the MGT was only affected by the season. In autumn, the GP was higher (38.6%) and the MGT was slightly longer than that in summer (4.5%). In autumn, seeds stored at 4°C showed higher GP than those stored at 27°C, and seeds from invasive population revealed higher GP than those from native populations. High GP and short time for seed emergence demonstrated that in *S. vulgaris* seeds

checked in this study were from *Senecio vulgaris* ssp *vulgaris*, the non – dormancy subspecies. The results also implied that the high temperature exceeds the threshold for *S. vulgaris* to germinate and storage at high temperature cause *S. vulgaris* seeds lost the viability greatly. This demonstrates the reason why in Wuhan *S.vulgaris* can't establish natural and viable populations, and also explain why *S.vulgaris* is scattered in China.

Key word: Seed dormancy, seed viability, *Senecio vulgaris* ssp *vulgaris*, invasive plant, distribution

Introduction

Invasive plants colonize new areas, become pests and cause biodiversity loss, economic loss and health damage (Keller *et al.* 2011). An invasive species is a non-native species whose introduction does or is likely to cause economic or environmental harm or harm to human, animal, or plant health (Horan & Lupi 2010). One of such invasive species is *Senecio vulgaris* (Common groundsel, Asteraceae) that most probably originated from southern Europe and widely distributes in the temperate area all over the world (Robinson *et al.* 2003). Despite the wide distribution of *S. vulgaris* in China, its occurrence is scattered, with large populations reported in the north–eastern and south–western parts, but not in southern, central, northern or north-western part of China (doi.org/10.7287/peerj.preprints.1612v1).

Germination is an important stage in the life cycle of plants and germination behavior limits the distribution. We had germinated the seeds in autumn and spring in Wuhan, Central China, where no natural *S. vulgaris* populations are established. We observed that the plants from seeds germinated in spring ended their life cycle in late spring or early summer, with seed dispersion; and plants and from seeds germinated in autumn grew in winter and ended in late spring as well. From this observation, we had a preliminary hypothesis that the *S. vulgaris* seeds cannot germinate, or survive the hot summer in Wuhan. This is the reason why no observable natural *S. vulgaris* populations established in Wuhan and other area in the north–eastern and south–western where it is extremely hot in summer.

To test the hypothesis, we collected seeds from various populations from the native (Europe) and invasive range (China), stored the seeds in different condition (4 and 27 °C), and germinated them in a controlled condition (15 °C) and ambient conditions in summer and autumn in Wuhan. Particularly, we addressed the following questions: (1) Do the *S. vulgaris* seeds have dormancy or not? (2) Do the storage condition and seasons have an effect on seed germination? (3) Does the germination behavior vary depending on the origin of range and the populations?

Materials and Methods

Species description

Senecio vulgaris is an erect herbaceous annual plant growing up to 45 cm tall (Stace 1997), has a thick taproot, and possesses an ephemeral strategy typical of many weedy species (Weiner *et al.* 2009). *S. vulgaris* is ubiquitous weed found in the temperate zones of Europe, North and south America, North Africa and Asia (Senesac 1991). In warmer climates such as California, however, it is a winter annual that appears soon after fall irrigation. Its optimal growing temperature is estimated to be 22°C from meristem tips grown in static tube culture (Walkey & Cooper 1976b). Plants of *S. vulgaris* develop from seeds annually, and each plant can produce an average of 830 seeds (Kadereit 1984). But, large plants of *S. vulgaris* can produce over 1700 seeds (Royer & Dickinson 1999).

Seeds source

Seeds from 9 populations of *S. vulgaris* in their native area (Europe) and 7 populations in invasive areas (China) were sampled in different sites in 2012 and 2013, respectively (Table 1). Seeds of each population were grown for one generation in a climate room (20 °C, 18/6 hours, light/dark) in October and November 2013. And seeds collected from these plants grown in climate room were used for the germination experiment in a climate chamber in December 2013 and January 2014, 3-4 weeks later after the seeds harvesting.

Another set of seeds from the plants grown in the climate room were germinated and then the plants from them cultivated in a greenhouse in spring 2014 and harvested on June of the same year. The resulting seeds of the first flower heads per each plant were harvested and used in the second germination experiments. From each population, three plants from different maternal families that contained a large number of good seeds were selected. The germination experiment was done one week after seeds collection from mother plants.

Germination experiment design

Germination in a climate chamber

From the 16 populations, we used 6 native and 6 invasive populations for the germination experiment in a climate chamber. Three maternal families per population, 10 seeds per family, and in total 360 seeds were used for this experiment. The seeds used in this experiment did not benefit any special treatment, only to be air dried in paper bags and kept in room temperature in winter (below 20°C).

The single layer of Whatman No.1 filter paper was placed inside Petri dish (9 cm diameter) and moistened with distilled water. After the filter papers were wet, 10 seeds from the same maternal family were placed on the top of the filter paper and spaced as evenly as possible. The Petri dishes containing seeds were then placed in a climate chamber (at 15 °C, 12/12 hour, light /darkness). Germinated seeds were recorded daily. Germination test criterion was the protrusion

of the radicle and the data collection continued until germination had ceased. The duration of the experiment was 19 days.

Germination in ambient conditions

For this experiment, three families from each of 6 native and 6 invasive populations were selected, and 40 seeds were chosen per family. These seeds were divided into two lots and each lot had the seeds collected from 36 plants representing the 12 populations. The lots were then stored under two different temperature conditions: (i) Room temperature: seeds in the paper bag were placed in plastic bag containing a bag of silica gel to absorb moisture thereby abating humidity inside the plastic bag and placed in the box at ambient conditions in room temperature. (ii) At low temperature: Another lot of seed was put in plastic bag and tightly sealed and stored in a refrigerator (cold and dry at 4°C). In total 1440 seeds were used in this experiment. The germination experiment was carried out twice, in July and in October. The experiment done in July used seeds stored for one month (seeds harvested in June) while the experiment conducted in October used seeds stored for 4 months.

In July (summer) 10 seeds per family from lots stored at different conditions were planted on a filter paper soaked with tap water. After sowing, Petri dishes were placed in plastic bags to prevent evaporation and placed in a large container and then put it in a place where seeds received enough sunlight. The Petri dishes were left for 12h in the plastic bags and opened for (30) minutes allowing seeds or seedlings to be oxygenated. A few drops of tap water were added to keep constant moisture level in the Petri dish. Every morning, the Petri dishes were observed to monitor the number of sprouted seeds. Germination was considered to be the incidence of radicle protrusion. Records of daily temperature through the relevant experimental period were obtained from the meteorological office of Wuhan City.

In October (autumn), after 4 months of seeds storage, another germination experiment was done with the seeds from both groups stored in different conditions. The germination experiment and data recording was carried out following the same procedure as in July with a minor modification where the Petri dishes were not wrapped in plastic bags, because the temperature was not very high during that period.

Germination parameters

Two germination characteristics which are germination percentage (GP) and mean germination time (MGT) were estimated. The germination percentage is an estimate of the viability of a population of seed and the mean germination time is a reciprocal of germination rate that indicate the seeds emergence speed, and its characteristics can be determined in a few days. GP is expressed as percentage according to the following equation described by Ellis & Roberts (Ellis & Roberts 1981) : $GP = \text{Number of germinated seeds} / \text{Total number of sowed seeds} \times 100\%$. MGT was determined according to the equation of Ellis & Roberts (Ellis & Roberts 1980): $MGT = \sum dn / \sum n$, where n is the number of seeds newly germinated on days d, d refers as days counted from the beginning of germination test, and $\sum n$ is the total seeds germinated.

Data analysis

In analysis of the data from the germination experiment in the climate chamber, the germination parameters (GP and MGT) were log-transformed before further statistical analysis. A two level nested-ANOVA was performed for the first experiment to assess the difference in GP and MGT between range and populations within the range.

In analysis of the data from the germination experiment under ambient condition, one - way ANOVA tests were conducted to check the difference of GP and MGT among the populations within the range. The results of the One - way ANOVA tests indicated that there were generally no significant difference of GP and MGT among the populations within the same range, storage under the same condition and germinated in the same season (data not shown). Hence, three way-ANOVA was used for the second experiment to observe any significant differences in GP and MGT due to ranges, storage conditions and seasons on seed germination.

All statistical methods were performed using R software, version 3.2.1(R Team 2015).

Results

Germination experiment in climate chamber

The *S. vulgaris* seeds started to germinate at the 4th and 5th day after sowing for invasive and native populations, respectively. A high germination took place between the 4th and the 16th day (Fig.1). At the end of the germination period (the 19th day), all populations had > 80 % GP and 8 of the 12 populations had $\geq 90\%$ GP (Fig.2). The final GP (91.1%) was the same for invasive and native populations (Figure 1-2). In addition, there was no significant difference in GP between the ranges and the populations within the ranges (two – level nested ANOVA, df=1 and 10, $P > 0.05$).

The mean germination time (MGT) was not statistically different between the ranges, however, within the ranges, the populations were significantly different (two – level nested ANOVA, df=1 and 10, for range: $F=0.631$, for populations within range: $F=2.398$, $P=0.039$). The highest value of the MGT (13.51 days) was found in population from Tongjiang which belongs to the invasive range while the lowest value (7 days) was recorded in population from Oestgeest that belongs to native range (Fig. 2).

Germination experiment under natural conditions

Compared to the seeds germinated in summer, the GP of the seeds germinated in autumn was much higher, no matter which range the seeds from or under what kind of conditions the seeds were stored (Fig.3, Fig.4a). GP of *S.vulgaris* seeds were significantly different between the seasons (S), storage conditions (SC) and ranges (R). The interactions of $SC \times S$ and $R \times S$ were significant. However, the interactions of $R \times SC$ and $R \times SC \times S$ were not statistically significant (Table. 2). Seasons had a strong effect. But the influence of range and storage condition on the GP depends on seasons.

In autumn, GP of the seeds from invasive range was significantly higher than that from native range, no matter under what kind of conditions the seeds were stored. In summer, there was no difference between invasive and native seeds in relation to GP (Fig.4b). In autumn, final GP of seeds stored under 4°C was 54.17 %, and final GP of those stored under 27°C was 22.78 %, while the GP of the seeds germinated in summer was no more than 5% and was not different between the seeds stored under different condition (Fig.4c).

The *S. vulgaris* seeds started to germinate at the 2nd day after sowing in autumn, and in summer they started germination at the 5th day. Most germination in autumn took place between the 2nd and 8th day after sowing. MGT for the two seasons (summer and autumn) was statistically different and no interaction was revealed to be significant (Table 2). Higher MGT was recorded in autumn for both ranges and both storage conditions than in summer.

Discussion

Do the *S. vulgaris* seeds germinate and survive in the hot summer?

Plant seeds germinate over a wide range of temperatures, but the maximum and minimum temperatures vary with the species. The optimum growing temperature for *S. vulgaris* was estimated to be 22°C from meristem tips grown in static tube culture (Walkey & Cooper, 1976). Robert and Popay (1970a) recorded a maximum seed emergence between 5 and 20°C during the germination tests of *S. vulgaris* seeds at constant temperatures ranging between 4 and 35°C, and found a decreased GP with rising temperature when it was over 25°C and practically no germination at 35°C. Ren & Abbott (1991) also found that seed of *S. vulgaris* germinated at almost all temperature ranging from 4 to 35°C, but showed 0 % GP at 35°C. It is clear that *S. vulgaris* have threshold temperature beyond which it cannot thrive.

In our study, the results showed a high GP (91.51%) at 15°C and a significant low GP in summer, compared to that in autumn. This difference is explained by the climate in Wuhan. In summer, the max temperature ranged from 37.2°C to 40°C, while it is generally below 30°C in autumn (Figure S1, Qian et al., 2007). The high temperature exceeds the threshold for *S. vulgaris* to germinate. Additionally, seeds storage in room temperature and subjected to a variation of high temperature failed to get good germination in autumn, compared to the seeds storage at 4°C. This indicates that subjection to high temperature cause *S. vulgaris* seeds lost the viability greatly. To summarize, the hot summer in Wuhan inhibited germination and damaged seeds of *S. vulgaris*.

Do GP and MGT of *S. vulgaris* seeds vary depending on the range and populations?

Senecio vulgaris species has two subspecies: *Senecio vulgaris* ssp *vulgaris* and *Senecio vulgaris* ssp *denticulatus* (Kadereit, 1984). The cosmopolitan weed *S. vulgaris* var. *vulgaris* is likely to

have originated from the non-weedy *S. vulgaris* ssp. *denticulatus* from which it differs by showing no seed dormancy, completing its life cycle from germination to seed formation much faster, and lacking ray florets (Comes et al., 1997; Moritz & Kadereit, 2001).

In the germination test conducted by Abbott et al (1988) with seeds of non-radiate *S. vulgaris* at 19/15°C (day/night), the first germination appeared 2 days after sowing and the GP was about 90 % at the end of the germination period, which was 28 days. Ren and Abbott (1991) carried out a germination test over a range of temperature (4-35°C) with seeds of *S. vulgaris* from Mediterranean and British origin, and found high GP of 62.7% and 82% at 16°C and 20°C respectively, for the fresh seed of *S. vulgaris* from British populations; however, in the same experiment, fresh seeds of *S. vulgaris* from the Mediterranean populations showed low germination (8%) only at 9°C. Moreover, the experiment with *S. vulgaris* ssp. *denticulatus* sown on 8 different dates, revealed that the rapid germination happened 20 days after sowing, while the shortest germination period was 50 days (Kadereit, 1984).

To make a summary, the majority of populations of *S. vulgaris* var. *vulgaris* shown no dormancy, while the populations of ssp. *denticulatus* from the Mediterranean area showed strong dormancy. The ecological consequence of seed dormancy is the fact that germination is prevented at a time of the year when the environment does not remain favorable long enough for seedlings to become established and thus survive. The strong dormancy of some *S. vulgaris* population from the Mediterranean area showed was regard as adaption trait for the long, dry and hot summer in this area (Ren & Abbott 1991).

Moreover, germination of seeds from *S. vulgaris* ssp *vulgaris* can be influenced by geographical variation. Seeds of *S. vulgaris* collected from six sites along a 700-km transect between Lexington (Kentucky) and Essexville (Michigan) in May 2000, were exposed to constant temperatures (5-25°C), and two patterns appeared in GP for seeds incubated at higher and lower temperature: (1) seeds from the southern locations averaged 80 to 90% germination across the range of 5 to 25°C; (2) seeds from northern locations had reduced germination as incubation temperatures were close to 5 or 25°C (Figuroa et al., 2010).

Germination of *S. vulgaris* seeds influenced by genetic variation as well. Richards (1975) reported that seed of *S. vulgaris* collected from a Mediterranean population in south Yugoslavia (YY, as genotype) was slower to germinate than British seeds (RR, rr as genotypes); particularly, the homozygotes, rr showed a quick germination in 5 days with 80% GP, and the germination was slower and similar in RR and YY genotypes.

This study showed that at an optimal condition (15 °C, in the climate chamber) GP was not different among the ranges and the population within the range. Most population showed > 90% GP and the first germination took place the 4th day after sowing. In germination experiment conducted in autumn, the well-kept seeds gained > 50% average final GP at the end of the 20 - days period. These results agreed with those from the germination experiment using ssp *vulgaris*

and different from those using *ssp denticulatus*. Hence, we conclude that the seed used in the first experiment were not dormant and they were from populations of *ssp vulgaris*.

We found at the 15 °C climate chamber, the MGT was different between populations. The MGT of seeds from Oestgeest, The Netherlands was 7.0 days and MGT of Tongjiang, China was 13.5 days. This implies that the germination speeds of the former might be two times fast as that of the later. We also found in autumn, the well-kept seeds from invasive plants gained about 65% GP, those from native plants gained about 45 %; and the different of GP between the range was significant (Table 4, Figure 3b). Higher GP of invasive populations indicated that the invasive *S. vulgaris* might more adapt to the environment in China.

Conclusion

Our study investigated the dormancy potential in freshly harvested seeds of *S. vulgaris*; the effect of storage conditions, seasons and ranges on the germination of the same species. It concludes convincingly that seeds of *S. vulgaris* could germinated in short time after sowing and had high percentage of germination at 15 °C or 25 °C in a climate chamber or ambient condition. Thus, the *S. vulgairs* seeds collect from the populations of *Senecio vulgaris ssp vulgaris*, the non – dormancy subspecies. Low GP for seeds germinated in summer (above 30 °C) and seeds kept at room temperature (about 27 °C) implied that the high temperature exceeds the threshold for *S. vulgaris* to germinate and storage at high temperature cause *S. vulgaris* seeds lost the viability greatly. This demonstrates the reason why in Wuhan *S.vulgaris* can't establish natural and viable populations, and also explain why *S.vulgaris* is scattered in China.

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Table1. Origin of the populations of *Senecio vulgaris* used in this study

Range	Country	Location	Collected		
			Year	Latitude	Longitude
Native	Spain	Barcelona ²	2012	41.67	2.73
Native	Switzerland	Fribourg ²	2012	46.79	7.15
Native	The Netherlands	Leiden ^{1,2}	2013	52.17	4.48
Native	The Netherlands	Lisse ¹	2012	52.25	4.55
Native	The Netherlands	Oestgeest ^{1,2}	2013	39.36	-9.16
Native	Germany	Potsdam ¹	2012	52.39	13.06
Native	Poland	Pulawy ²	2012	51.39	21.96
Native	United Kingdom	St Andrew ^{1,2}	2012	56.33	-2.78
Native	The Netherlands	Teylingen ¹	2013	52.21	4.49
Invasive	China	Fuyuan ^{1,2}	2013	48.37	134.29
Invasive	China	Hegang ^{1,2}	2013	47.33	130.29
Invasive	China	Lijiang ^{1,2}	2013	26.87	100.24
Invasive	China	Luobei ¹	2013	47.57	130.82
Invasive	China	Siping ^{1,2}	2013	43.17	124.38
Invasive	China	Tongjiang ^{1,2}	2013	47.98	133.17
Invasive	China	Yichun ^{1,2}	2013	47.72	128.79

Table2. Analysis of variance of final germination percentage (GP, %) and mean germination time (MGT, days) for *Senecio vulgaris* seeds from 6 native and 6invasive populations stored different conditions (4 °C and 27 °C) and germinated in different seasons (summer and autumn).

Source of variation	df	GP	MGT
		F	F
Range (R)	1	10.0804**	3.1723
Storage condition (SC)	1	6.7871*	0.5177
Season (S)	1	117.2067***	38.0943***
R×SC	1	0.18	0.005
R×S	1	3.9866*	0.0523
SC×S	1	6.3788*	0.1391
R×SC×S	1	0.0033	0.0071

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure 1 Cumulative germination percentage of *Senecio vulgaris* seeds from 6 native and 6 invasive populations in a climate chamber (15 °C, 12h/12h, dark/light) during 19 days (■ seeds from the native populations, ◆ seeds from the invasive populations). 10 seed per 3 maternal family per population.

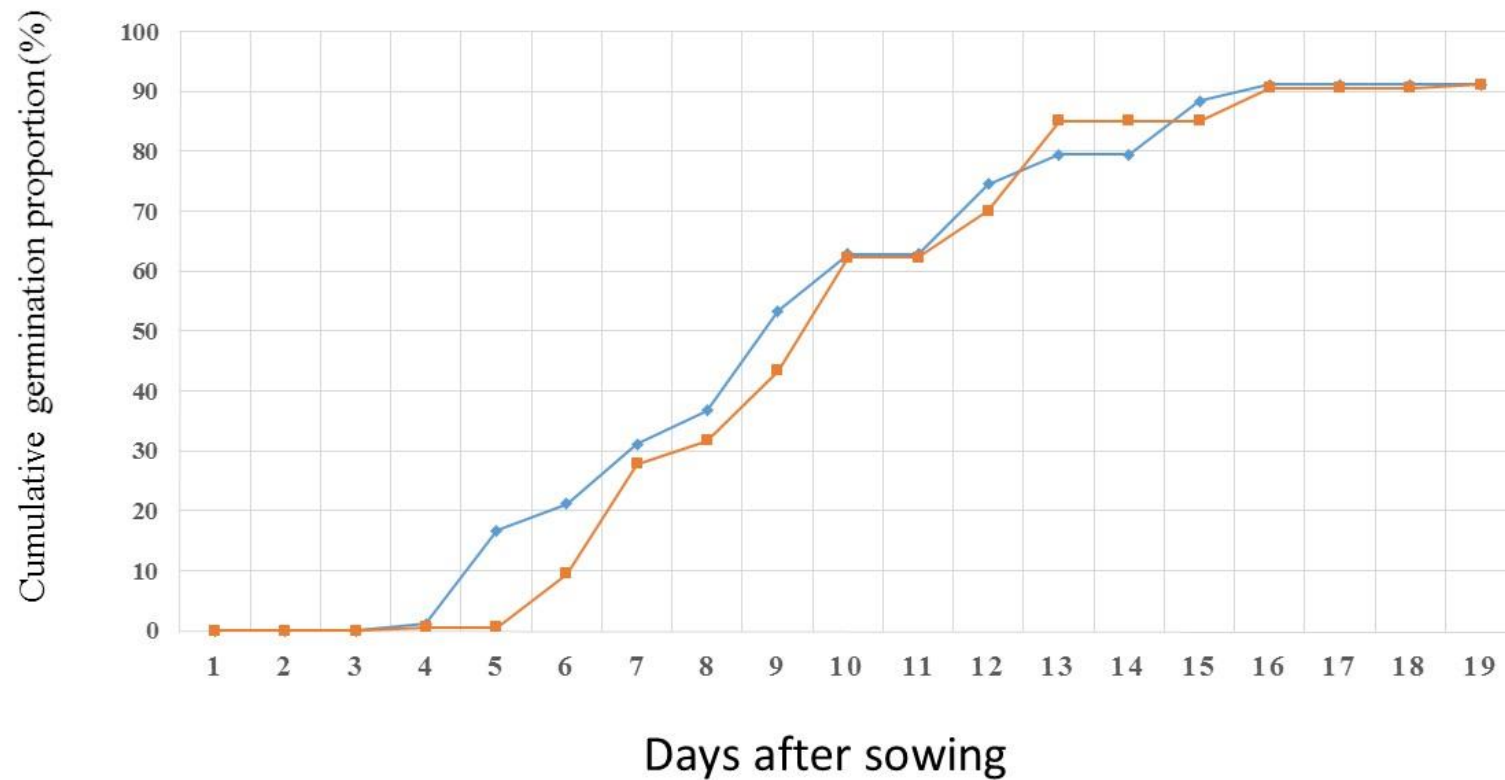


Figure 2 Mean final germination percentage (GP, %, bars) and mean germination time (MGT, days, dots) for *Senecio vulgaris* seeds from 6 native and 6 invasive in a climate chamber (15 °C, 12h/12h, dark/light) during 19 days. Error bars : standard error. 10 seed per 3 maternal family per population.

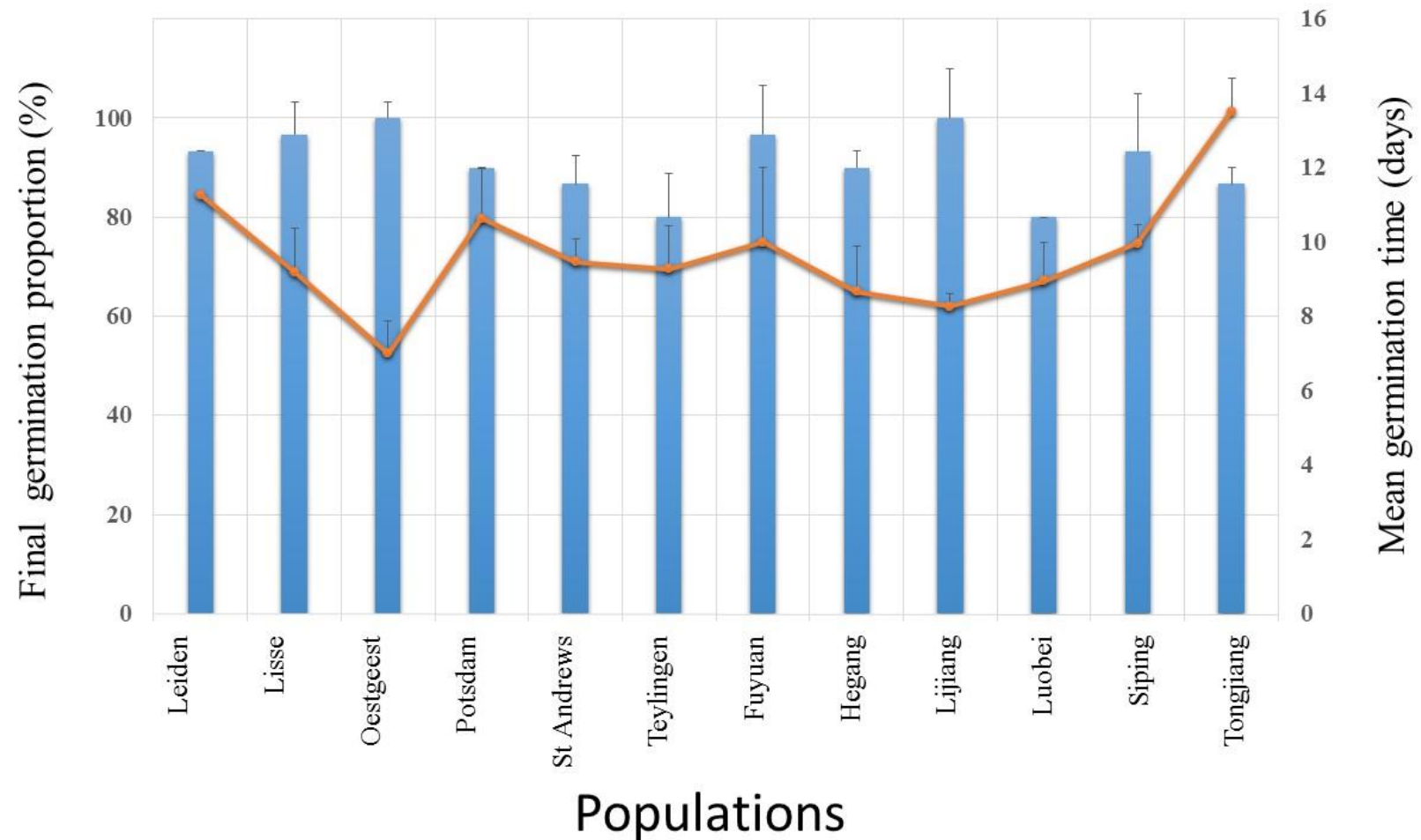


Figure 3 a: Cumulative germination percentage (GP) of *Senecio vulgaris* seeds from 6 native and 6 invasive populations stored in different conditions and germinated during summer and autumn (◆: Germination in summer, seeds stored at room temperature (ca.27°C) for one month, ■: Germination in summer, seeds stored at 4°C for one month, ▲: Germination autumn, for seeds stored at room temperature (ca. 27°C) for three month, ×: Germination in autumn, for seeds stored at 4°C for three month). b: Daily average temperature during the experiment in summer (◆)and in autumn (■). Daily average temperature throughout the relevant experimental period were obtained from the meteorological office of Wuhan City.

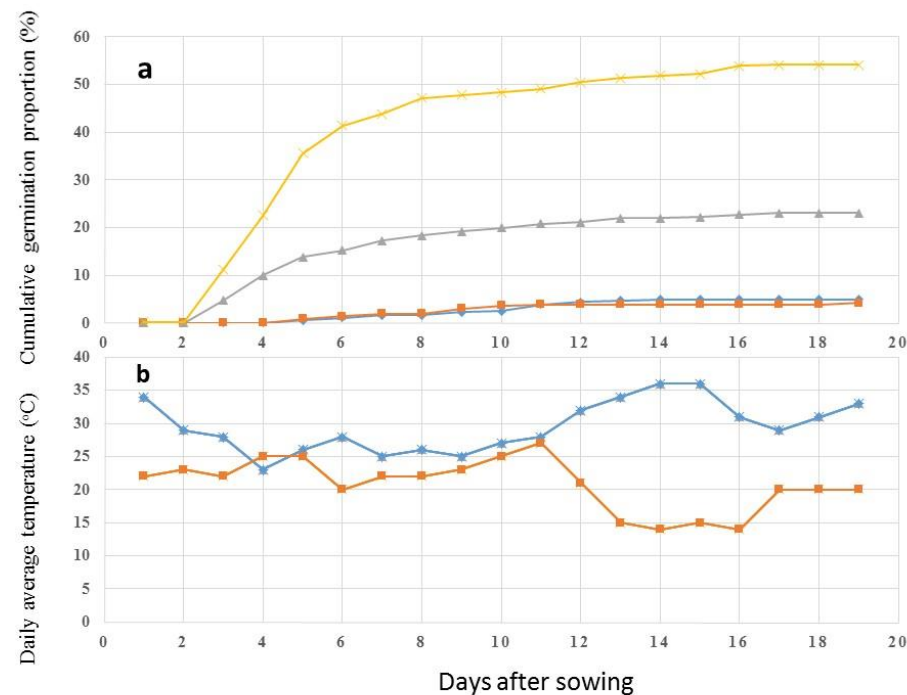


Fig.4 Comparison of the final germination proportion (GP) of *Senecio vulgaris* seeds germinated in different seasons, from different ranges and stored under different conditions and. a: Comparison of GP of seeds germinated in autumn and summer in 4 different groups divided by the range and storage conditions (at 4 °C and 27 °C) ; b: Comparison of GP of seeds from native and invasive range in 4 different groups divided by the different storage conditions (at 4 °C and 27 °C) and germinating seasons (summer and autumn); c: Comparison of GP of seeds stored under different condition in 4 different groups divided by the ranges and germinating seasons.

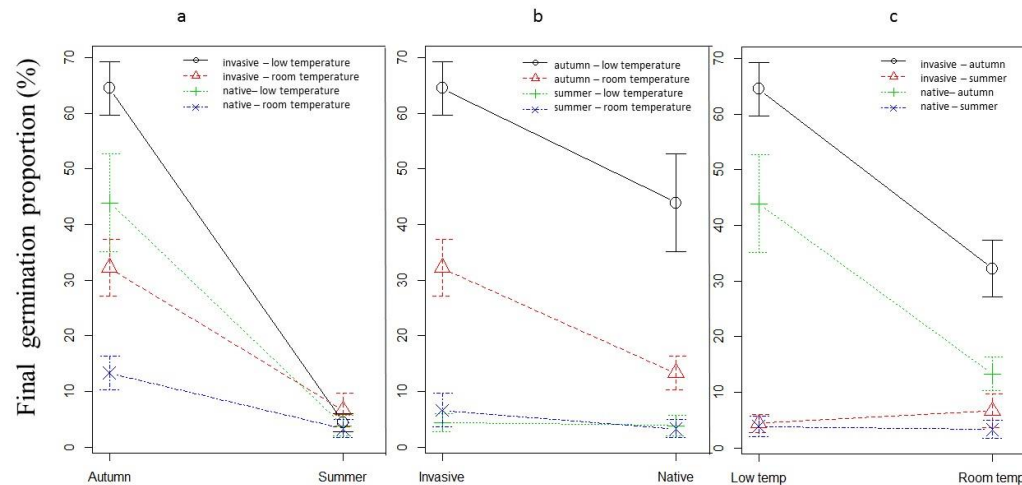


Fig.s1. Monthly temperature at the course of the year in Wuhan (Hubei): (◆) maximum monthly temperature, (■) average monthly temperature and (▲) minimum monthly temperature.

