

Short Research Article

Effect of Different Temperature Treatments on Seed Germination and Seedling Growth of *Rorippa sylvestris*

Abstract:

Temperature is one of the important conditions for plant growth, and it is an important factor affecting the establishment and spread of invasive plants. In this study, the northern perennial invasive plant (*Rorippa sylvestris*) was taken as an example. By comparing the seed germination rate, germination potential, germination index and vigor index, the total number of leaves, root length, root length and vigor index of *Rorippa sylvestris* were determined. rate, germination potential, germination index and vigor index, total number of leaves, root length, lateral root number, seedling biomass and root-shoot ratio under different temperature treatments (15, 20, 25, 30, 35, 40°C), the subordinate function was used to compare the temperature tolerance of *R. sylvestris* in seed germination and seedling growth. To study the response and tolerance of seed germination and seedling growth of invasive *R. sylvestris* to different temperatures, the subordinate function was used to compare the temperature tolerance of *R. sylvestris* in seed germination and seedling growth. The results showed that high temperature promoted seed germination of *R. sylvestris* and limited radicle growth, and it had the strongest tolerance at different temperatures. The results showed that high temperature promoted seed germination of *R. sylvestris* and limited radicle growth, and it had the strongest tolerance at 35°C. Lower temperature was more beneficial to material accumulation of seedlings, and the tolerance of *R. sylvestris* was more favorable.

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The results provide theoretical basis for revealing the diffusion and invasion mechanism of *R. sylvestris*. The results provide theoretical basis for revealing the diffusion and invasion mechanism of *R. sylvestris*.

Keywords: *Rorippa sylvestris*; Temperature; Tolerance; Membership function method

Introduction

Global warming and biological invasions are important factors affecting biodiversity and ecosystem functioning. The spread of invasive species has seriously threatened global biodiversity, ecosystem structure and function, social production and human health^[1]. Under the general environment of global warming, the temperature rise in northern China has intensified. Over the past 50 years, there has been an obvious warming trend in the average temperature of the northeast region, and the warming rate of the minimum temperature in Liaoning Province is about twice that of the maximum temperature^[2]. Seed germination, as the basis for the expansion and spread of weeds^[3], is a key link in the transformation of potential populations into actual

populations, and plays an extremely important role in the reproduction, settlement, dispersal of individual populations as well as in the resistance to adverse environments.^[4,5] seedling stage is an important period in the process of plant development.^[6] the adaptability of the seedling to the outside world directly influences the ability of the plant to complete the entire reproductive period successfully, and, in the case of invasive species, it is related to the establishment of the entire population. In the case of invasive species, this will be related to the establishment of the whole population. Temperature is one of the most important factors affecting plant invasion, seed germination and seedling growth^[7,8], providing opportunities for successful invasion of exotic species.

Rorippa sylvestris are cruciferous (Brassicaceae) nasturtium genus (*Rorippa*) perennial herbaceous plants, early spring sprouting earlier, more sensitive to temperature. It is native to Europe and southwestern Asia, China was initially distributed in Xinjiang Yining, and then gradually spread to Qinghai, Tibet, Gansu and other places have distribution^[9], in recent years jumped to Dalian, Liaoning, Shenyang, Tieling, etc., is listed as a new record species in Liaoning^[10,11,12]. *Rorippa sylvestris* can reproduce through seeds and root tiller buds. Generally, seed reproduction is the mainstay of new invasions, and when a large group has formed at the invasion site, it mainly relies on the sprouting of root tillers to spread; the root system is well-developed, and it has a strong ability to invade the soil, asexual reproduction ability, and the ability to adapt to the environment.^[12] At present, *Rorippa sylvestris* has formed several single-optimized communities in the green belts of Liaoning area, and has shown explosive growth, which is more harmful.

Given that *Rorippa sylvestris* is able to reproduce rapidly and establish a single-optimal community after invading a new ecosystem, this experiment was conducted to investigate the response and tolerance of *Rorippa sylvestris* seed germination and seedling growth to different temperatures by using the seeds and seedlings at the early stage of invasion, with a view to providing theoretical basis for the study of *Rorippa sylvestris*'s invasion mechanism.

1 Materials and Methods

1.1 Materials

Rorippa sylvestris seeds were collected from the north side of the College of Plant Protection, Shenyang Agricultural University, and the weight of 1,000 grains was 0.0524 g. The seeds were kept in a dry environment after shade-drying.

1.2 Methods

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1.2.1 Seed germination experiments

The experiment was conducted using the paper-dish method. Seeds of *Rorippa sylvestris* with normal maturity and full seeds were selected, soaked in distilled water for 12h, and placed in Petri dishes lined with double-layer filter paper (50 seeds per dish). They were placed in a light incubator at 15°C, 20°C, 25°C, 30°C, 35°C and 40°C, respectively, and the incubator was treated with 16h of light (intensity 4000lx) and 8h of darkness daily. Each treatment was replicated three times. Seed germination was measured by the radicle breaking through the seed coat ≥ 1 mm, and the number of seed germination was recorded every day during the test, and the filter paper was kept moist, the number of seed germination was counted every day, and the time of the first germination and the duration of germination were recorded, and the germination was considered to be finished if no new seeds germinated for 2 consecutive days. The germination rate, germination potential, germination index and vigor index of *Rorippa sylvestris* seeds were calculated at different temperatures.

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1.2.2 Seedling growth experiment

The seeds of *Rorippa sylvestris* were sown evenly on a bed of moist nutrient soil and placed in a light incubator at 30°C with 16h of light (intensity 4000lx) and 8h of darkness every day to promote germination. After germination, the seeds were placed at room temperature, and after two months, 50 *Rorippa sylvestris* seedlings each of similar size and growth were selected and transplanted into small pots with a height of 8 cm and a diameter of 8 cm, and subjected to different temperature treatments: the parameters of the light incubator were set to be 15, 20, 25, 30, and 35 °C, and the light-dark ratio to be 16/8h (light 4000lux), and 10 replications were done for each temperature gradient. After 30 days, the growth conditions were recorded, including: total number of leaves, root length, number of lateral roots, number of secondary lateral roots, and the root dry weight and crown dry weight of these seedlings were determined by using an electronic balance.

1.3 Statistics and calculations

Germination rate: $G = n/N \times 100\%$.

Where: G is the germination rate, n is the number of normal seedling seeds generated at the end of germination test; N is the number of seeds for test.

Germination vigor: $G_r = m/N \times 100\%$,

Where: G_r is the germination potential, m is the number of germinated seeds at the peak of germination, and N is the number of test seeds.

Germination indexes: $G_i = \sum (G_r/D)_i$

Where: G_t is the germination index, G_t is the number of germination on day t and D_t is the number of days to germination.

Vigor index (Germination vigor index) $G_v = G_t \times$ seedling root length

(cm) Plant height: the distance from the base to the top of the plant;

Total number of leaves (Total number of leaves): all functional leaves of the whole seedling; Root length (

Root length): the length from the root-stem junction to the root tip;

Number of lateral roots per plant: the main root grows to a certain length, in a certain part of the lateral from the inside to produce a number of branch roots;

Seedling biomass (Total dry weight): dry weight of the whole seedling;

Biomass root to shoot biomass ratio (Root to shoot biomass ratio) = root dry weight/crown dry weight. The value of the affiliation function: $R(X_i) = (X_i - X_{\min}) / (X_{\max} - X_{\min})$.

Where: X is the measured value of a certain index of the reference plant; X_{\max} and X_{\min} are the maximum and minimum values of the index in all the test materials, respectively. The relative germination rate, the relative germination potential, the relative germination index and the relative root length were obtained by the subordinate function value calculation of the germination indicators, and the average value of the subordinate function value of each indicator was summed up, which was the total function value. The total function value at each temperature in the seedling stage was calculated as above.

Overall evaluation: the larger the value of the total function, the more resistant to that temperature.

1.4 data analysis

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Data were statistically analyzed using SPSS 22.0 software, plotted in Excel, one-way analysis of variance (ANOVA) was used to compare the significance of differences between different treatments of the same indicator, and multiple comparisons were performed using the DUNCAN method.

Comprehensive evaluation of temperature tolerance of *Rorippa sylvestris* at different temperatures during seed germination and seedling growth stages, respectively, using the fuzzy mathematical affiliation function method^[12].

2 Results and Analysis

2.1 Effects of different temperatures on seed germination of *Rorippa sylvestris* and evaluation of adaptability

2.1.1 Effect of different temperature treatments on the germination of *Rorippa sylvestris* seeds

Temperature had a significant effect ($P < 0.05$) on germination rate, germination potential, germination

index and vigor index of *Rorippa sylvestris* seeds. The germination rate, germination potential and germination index all showed an increasing and then decreasing trend with the increase of temperature, and peaked at 35°C. *Rorippa sylvestris* did not germinate at 15°C, and the germination rate, germination potential and germination index achieved larger values at 25-35°C. The vigor index of *Rorippa sylvestris* also had a tendency to increase and then decrease, with high vigor at 25°C and 35°C and a low peak at 30°C. It indicated that high temperatures promoted seed germination and either too high or too low temperatures inhibited seed radicle growth (Table 1).

Table 1 Effect of different temperatures on germination of *R. sylvestris* seeds

Temperature (°C)	Germination rate (%)	Germination potential	germination index	vitality index
		(%)	Germination indexes	Germination vigor index
15	0.00 ± 0.00c	0.00 ± 0.00d	0.00 ± 0.00c	0.00 ± 0.00c
20	5.33 ± 3.33bc	2.67 ± 0.67cd	0.39 ± 0.25c	0.00 ± 0.00c
25	18.00 ± 7.57ab	6.00 ± 2.31c	1.58 ± 0.67ab	3.28 ± 1.38a
30	18.67 ± 4.67ab	12.00 ± 2.31b	1.73 ± 0.42ab	1.18 ± 0.28bc
35	30.00 ± 3.06a	17.33 ± 1.76a	2.66 ± 0.30a	2.18 ± 0.25ab
40	6.00 ± 1.15bc	4.00 ± 1.15cd	0.88 ± 0.15bc	0.00 ± 0.00c

Note: Different letters indicate that species differs significantly ($P < 0.05$) at different temperatures.

Note: Different letters indicate significant differences in species at different temperatures ($P < 0.05$).

2.1.2 Evaluation of the adaptation of *Rorippa sylvestris* seed germination to temperature

The fuzzy mathematical affiliation function method was used to comprehensively evaluate the affiliation function values of relative germination rate, relative germination potential, relative germination index, and relative root length of *Rorippa sylvestris* seeds at different temperatures, and the results showed that the order of the strength of *Rorippa sylvestris*'s tolerance to different temperatures was $35^{\circ}\text{C} > 25^{\circ}\text{C} > 30^{\circ}\text{C} > 40^{\circ}\text{C} > 20^{\circ}\text{C} > 15^{\circ}\text{C}$. The result showed that *Rorippa sylvestris*'s tolerance to different temperatures was higher at high temperatures than at low temperatures (Table 2), which was not favorable for seed germination. The tolerance of *Rorippa sylvestris* seed germination was higher at high temperatures, and the tolerance of seed germination was low when the temperature was too high or too low, which was unfavorable for seed germination (Table 2).

Table 2 The membership function value and comprehensive evaluation value of tolerance of *R. sylvestris* seeds during germination

subordinate function values subordinate function values

speciesname	Temperature(°C)	Relative germination rate	Relative germination potential	relativegermination index(RGI)	Relativeroot length	collective function value (math.)	rankings
Species	Temperature	Relativegerminationrate	Relativegerminationpotential	Relativegerminationindex	rootlength	Total functionvalue	Ranking
	15	0.00	0.00	0.00	0.00	0.00	6
	20	0.33	0.33	0.36	0.00	0.26	5
<i>R.sylvestris</i>	25	0.5	0.5	0.51	0.41	0.48	2
	30	0.54	0.5	0.53	0.29	0.47	3
	35	0.6	0.56	0.63	0.26	0.50	1
	40	0.5	0.5	0.64	0.00	0.41	4

2.2 Effects of different temperatures on the growth of *Rorippa sylvestris* seedlings and evaluation of acclimatization

2.2.1 Effect of different temperatures on the growth of *Rorippa sylvestris* seedlings

Different temperatures had significant effects on plant height, total leaf number, root length and lateral root number of *Rorippa sylvestris* seedlings. Plant height, total leaf number and root length all increased and then decreased with increasing temperature, and the number of lateral roots gradually increased with increasing temperature in *Rorippa sylvestris* ($P < 0.05$). *Rorippa sylvestris* plant height, total leaf number, root length and lateral root number were maximum at 25°C, 30°C, 20°C and 35°C and minimum at 15°C, 15°C, 30°C and 25°C, respectively. Temperatures were too high or too low for the growth of *Rorippa sylvestris* (Figures 1. A, B, C, and D).

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The biomass of *Rorippa sylvestris* seedlings first increased and then decreased with temperature, and the root-crown ratio decreased with temperature, and *Rorippa sylvestris* slightly increased at 30°C. Biomass was greatest at 20°C and root-crown ratio was greatest at 15°C; *Rorippa sylvestris* biomass and root-crown ratio were smallest at 15°C and 35°C, respectively. Lower temperatures favored *Rorippa sylvestris* biomass accumulation and biomass partitioning to roots at lower temperatures (Figs. E and F).

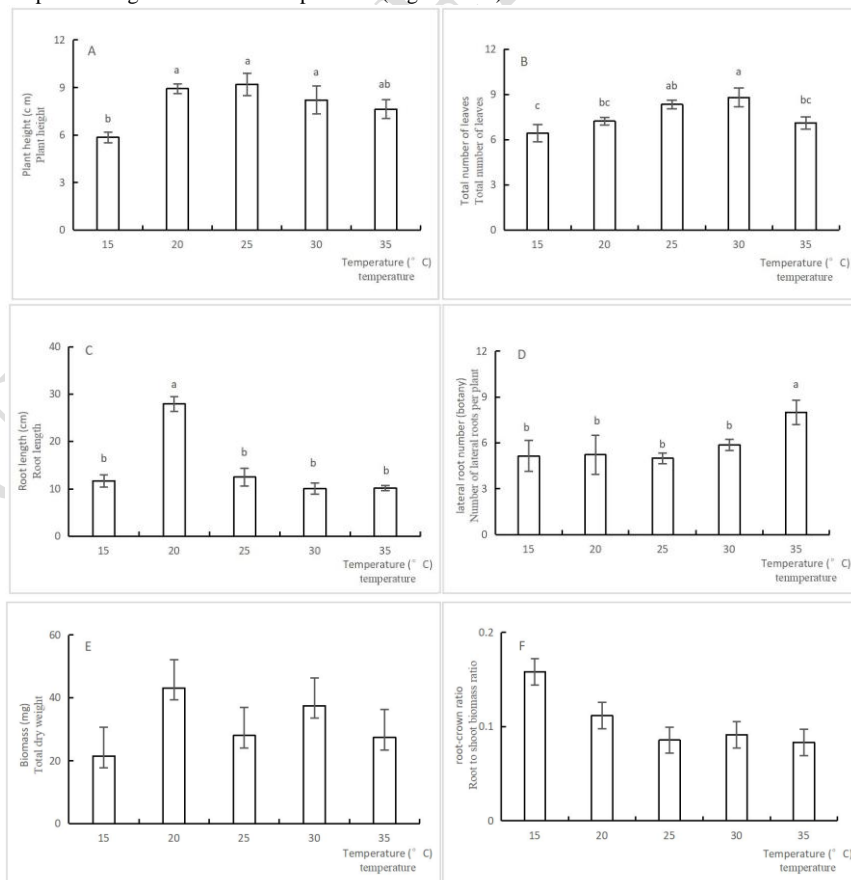


Fig. 1. Effects of different temperatures on plant height (A), total leaf number (B), root length (C), lateral root number (D), biomass (E), and root-crown ratio (F) of *Rorippasylvestris* at seedling stage

Note: Different letters indicate that species differs significantly ($P < 0.05$) at different temperatures.

Note: Different letters indicate significant differences in species at different temperatures ($P < 0.05$).

2.2.2 Evaluation of the adaptability of *Rorippasylvestris* to different temperatures at seedling stage

The above results showed that different temperature treatments had a great influence on the growth of *Rorippasylvestris* seedlings, and the tolerance of *Rorippasylvestris* seedlings to different temperatures was evaluated by the fuzzy mathematical affiliation function through a comprehensive evaluation of the values of different temperature affiliation functions of four indicators at the seedling stage of *Rorippasylvestris*. The results showed that *Rorippasylvestris* seedlings were tolerant to different temperatures in the order of $30^{\circ}\text{C} > 35^{\circ}\text{C} > 15^{\circ}\text{C} > 20^{\circ}\text{C} > 25^{\circ}\text{C}$ (Table 3). *Rorippasylvestris* was tolerant at high temperatures.

Table 3. The membership function value and comprehensive evaluation value of the resistance of *R. sylvestris* seedlings to different temperatures during the growth period

species	Temperature (°C)	subordinate function values		subordinate function values		collective function value (math.)	rankings
		relative plant height	Relative Total number of leaves	Relative root length	Relative number of lateral roots per plant		
<i>R. sylvestris</i>	15	0.39	0.49	0.36	0.57	0.45	3
	20	0.48	0.41	0.38	0.31	0.40	4
	25	0.35	0.33	0.43	0.40	0.38	5
	30	0.46	0.47	0.40	0.63	0.49	1
	35	0.51	0.53	0.32	0.50	0.47	2

3 Discussion and Conclusion

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Plant growth is not only controlled by genetic material, but also by numerous environmental factors, such as light, temperature, water, soil nutrients and so on. Seed germination is one of the key links in the life history of plants, and the response of seeds to germination conditions reflects their ecological response to adapt to the environment^[14], and is also the key for invasive plants to settle into new environments. Temperature, as an important environmental variable regulating seed germination, is one of the key ecological factors affecting seed germination^[7,8], too high or too low will affect the normal growth and development of plants, Wang Yuqin et al.^[13] found that the seed germination rate of yellow-flowering echinoderms (*Oxytropis ochrocephala*) was lower under low temperature, Zhang Tianxian et al.^[14] found that both low and high temperatures were unfavorable to moringa (*Moringa oleifera*) seed germination.

In this experiment, it was found that too high or too low temperatures (15°C, 40°C) limited the growth of embryonic roots after germination of *Rorippa sylvestris* seeds and affected the vigor index of seed germination, lower temperatures inhibited the germination rate, germination potential, and germination index of seeds, and higher temperatures promoted seed germination.

Germination rate and germination potential reflect the germination speed and neatness of seeds, high germination rate and strong germination potential predict fast and neat germination and strong seedlings; high germination rate and low germination potential predict poor germination and weak seedlings^[15]. *Rorippa sylvestris* germination rate and germination potential were highest at 35°C, and high temperature promoted its seed germination and growth. However, the effect of temperature on seed germination is multifaceted, and the use of a single indicator to evaluate its effect is one-sided, and the use of the affiliation function method for the comprehensive evaluation of plant tolerance has been generally accepted^[12], and the use of the affiliation function method can be more comprehensive and more accurate to reflect the tolerance of *Rorippa sylvestris* to different temperatures. In this experiment, the relative value of traits was used to analyze the temperature tolerance of *Rorippa sylvestris* seed germination at different temperatures, and the results showed that *Rorippa sylvestris* seed germination was less tolerant at too high or too low a temperature, but more tolerant at higher temperatures, and *Rorippa sylvestris* was the most tolerant at 35°C. Because *Rorippa sylvestris* did not germinate at 15°C, and root length was <1mm at 20°C and 40°C, it is known that *Rorippa sylvestris* requires high germination temperature; higher temperature promotes *Rorippa sylvestris* seed germination, which may be its adaptive strategy to cope with global warming.

Temperature plays a very important role in the early growth of plant seedlings. Han Bing et al.^[16] showed that low-temperature stress significantly inhibited the growth of seedlings, resulting in a decrease in the growth of plant height, stem thickness, and dry mass compared with the control; Zou Huu^[17] showed that high-temperature stress decreased the accumulation of dry matter in the seedlings of antler rhododendron (*Rhododendron l*

touchaeae); and Wang Jianjun et al.^[7] showed that different temperatures had different effects on root length and dry matter of Chinese fescue (*Festuca sinensis*) seedlings. The results of this experiment showed that temperature had a significant effect on plant height, leaf number, root length and lateral root number of *Rorippa sylvestris* seedlings. Both plant height and root length had a tendency to increase and then decrease with the increase of temperature, and the effect of low temperature on the growth of plant height and root length of *Rorippa sylvestris* was more obvious, and its plant height was maximized at 25°C, and its root length was significantly higher than other temperatures at 20°C. High temperature promotes the occurrence of leaves and lateral roots of *Rorippa sylvestris*, which has the highest number of leaves at 30°C and the highest number of lateral roots at 35°C. *Rorippa sylvestris* accumulated the most dry matter at 20°C, but tended to allocate more material to the roots at both low and high temperatures, probably because the growth of the above-ground part of the plant was limited at low temperatures, thus allocating more resources to the roots and showing a larger root-crown ratio. As the temperature increases, it has been shown that warming can increase nitrogen content in the organs of the plant tissues and organs, which in turn allows the soil environment to be. With increasing temperature, it has been shown that warming can increase the nitrogen content in plant tissues and organs, and increase the supply of nutrients to the plant from the soil environment, thus causing a decrease in root partitioning and an increase in crown partitioning of photosynthesized products^[17,18]. This may be the reason for the decrease in the root-crown ratio of *Rorippa sylvestris* seedlings at elevated temperatures. 35°C was detrimental to the development of *Rorippa sylvestris* seedlings, indicating that high temperatures have an inhibitory effect on *Rorippa sylvestris* seedlings, which is consistent with the results of the research conducted by Wang J. et al.^[7], Liu F. H. et al.^[19], and Xin F. H. et al.^[20] on the growth of Chinese fescue, respectively. This is consistent with the findings of Wang Jun et al., Liu Fuhua et al., and Xin Fuhai et al., which found that high temperatures inhibited the growth of Chinese fescue, *Gleditsia sinensis*, and *Cupressus gigantea* at different temperatures. Comprehensive evaluation of the affiliation function of *Rorippa sylvestris* seedlings in terms of plant height, total number of leaves, root length, and number of lateral roots showed that *Rorippa sylvestris* was more tolerant to higher temperatures, with a maximum at 30°C. The response of *Rorippa sylvestris* seeds and seedlings to temperature was essentially synchronized, with both high temperatures promoting seed germination and inhibiting seedling growth, and lower temperatures inhibiting seed germination. Under natural conditions, high temperatures occur in the summer and are detrimental to seedling growth, which may be an important reason why this clone has very few seeded seedlings under natural conditions. Plants propagated by clonal growth tend to have a very high mortality rate at the seedling stage among the progeny produced by seed germination, and the rate of successful colonization is not as high as that of the cloned progeny. In the case of invasive clones, once seeds germinate and pass the seedling stage, they can be successfully colonized by asexual propagation. Therefore, the study of the response of seeds and seedlings of

invasive clones to the environment can partly reveal the mechanism of their successful invasion.

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