Short Research Article

EffectsofDifferentTemperatureTreatmentsonSeedGerminationandSeedlingGrowthof*Rorippa sylvestris*

Abstract:
Temperatureisoneoftheimportantconditionsforplantgrowth,anditisanimportantfactoraffectingtheestablishment and spread of invasive plants. In this study, the northern perennial invasive plant (*Rorippa sylvestris*) was taken as an example. Bycomparing the seed germination rate, germination potential, germination index and vigor index ,the total number of leaves, rootlength, root length and vigor index of Rorippa*sylvestris were determined.* rate, germination potential, germination index and vigorindex ,total number of leaves, root length, lateral root number, seedling biomass and root- shoot ratio under different temperaturetreatments (15, 20, 25, 30, 35, 40℃), the subordinate function was used to compare the temperature tolerance of *R* . *sylvestris in seedgermination. sylvestris* in seed germination and seedling growth. To study the response and tolerance of seed germination andseedling growth of invasive *R. sylvestris to different temperatures, the subordinate function was used to compare the temperaturetolerance of R. sylvestris* in seedgermination and seedling growth. The results showed that high temperature promoted seedgermination of *R. sylvestris*and limitedradicle growth, and it had the strongesttoleranceatdifferent temperatures. Theresultsshowedthathightemperature promotedseedgerminationof R.sylvestrisandlimitedradiclegrowth, andithadthe strongesttolerance at 35℃. Lower temperature was more beneficial to material accumulation of seedlings, and the tolerance of *R. sylvestriswasmore*favorable.

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

Keywords:*Rorippasylvestris;*Temperature;Tolerance;Membershipfunctionmethod

Introduction

Globalwarmingandbiologicalinvasionsareimportantfactorsaffectingbiodiversityandecosystemfunctioning. The spread of invasive species has seriously threatened global biodiversity, ecosystem structure andfunction,socialproductionandhumanhealth[1].Underthegeneralenvironmentofglobalwarming,thetemperature rise in northern China has intensified. Over the past 50 years, there has been an obvious warmingtrend in the average temperature of the northeast region, and the warming rate of the minimum temperature inLiaoning Province is about twice that of the maximum temperature[2]. Seed germination, as the basis for theexpansionandspreadofweeds[3],isakeylinkinthetransformationofpotentialpopulationsintoactual populations,andplaysanextremelyimportantroleinthereproduction,settlement,dispersalofindividualpopulations as well as in the resistance to adverse environments.[4,5], seedling stage is an important period in theprocess of plant development.[6], the adaptability of the seedling to the outside world directly influences the abilityof theplant tocompletethe entirereproductiveperiod successfully, and, in thecase of invasivespecies, it isrelated to the establishment of the entire population. In the case of invasive species, this will be related to theestablishment of the whole population. Temperature is one of the most important factors affecting plant invasion,seedgerminationandseedlinggrowth[7,8],providingopportunitiesforsuccessfulinvasionofexoticspecies.

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, Chinawas initially distributed in Xinjiang Yining, and then gradually spread to Qinghai, Tibet, Gansu and other placeshave distribution[9], in recent years jumped to Dalian, Liaoning, Shenyang, Tieling, etc., is listed as a new recordspecies in Liaoning[10,11,12]. *Rorippa sylvestris* can reproduce through seeds and root tiller buds. Generally, seedreproduction is the mainstay of new invasions, and when a large group has formed at the invasion site, it mainlyrelies on the sprouting of root tillers to spread; the root system is well-developed, and it has a strong ability toinvade the soil, asexual reproduction ability, and the ability to adapt to the environment.[12]. At present, *Rorippasylvestris* has formed several single-optimized communities in the green belts of Liaoning area, and has shownexplosivegrowth, whichis moreharmful.

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

1 Materials andMethods

1.1 Materials

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

1.2 Methods

1.2.1 Seedgerminationexperiments

Theexperimentwasconductedusingthepaper-dishmethod.Seedsof*Rorippasylvestris*withnormalmaturity and full seeds were selected, soaked in distilled water for 12h, and placed in Petri dishes lined withdouble-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 darknessdaily. Each treatment was replicated three times. Seed germination was measured by the radicle breaking throughthe seed coat ≥1 mm, and the number of seed germination was recorded every day during the test, and the filterpaperwaskeptmoist,thenumberofseedgerminationwascountedeveryday,andthetimeofthefirstgermination and the duration of germination were recorded, and the germination was considered to be finished ifno new seeds germinated for 2 consecutive days. The germination rate, germination potential, germination indexandvigorindexof*Rorippasylvestris*seedswerecalculated atdifferenttemperatures.

1.2.2 Seedlinggrowthexperiment

The seeds of *Rorippa sylvestris* were sown evenly on a bed of moist nutrient soil and placed in a lightincubator at 30℃ with 16h of light (intensity 4000lx) and 8h of darkness every day to promote germination. Aftergermination, the seeds were placed at room temperature, and after two months, 50 *Rorippa sylvestris* seedlingseach of similar size and growth were selected and transplanted into small pots with a heightof 8 cm and adiameter of 8 cm, and subjected to different temperature treatments: the parameters of the light incubator were setto be 15, 20, 25, 30, and 35 ℃, and the light-dark ratio to be 16/8h (light 4000lux), and 10 replications were donefor each temperature gradient. 30 After 30 days, the growth conditions were recorded, including: total number ofleaves, root length, number of lateral roots, number of secondary lateral roots, and the root dry weight and crowndry weightoftheseedlingsweredeterminedby usingan electronicbalance.

1.3 Statisticsandcalculations

Germinationrate:G=n/N×100%.

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

Germinationvigor:G_r=m/N×100%,

Where:$G_r$is the germination potential,m is the number of germinated seeds at the peak of germination, and Nisthenumber of testseeds.

Germinationindexes:$G_i=\sum(G_t/D)_t$

Where:$G_i$is the germination index,Gt is the number of germination on day t and Dt is the number of days togermination.

Vigor index (Germination vigor index) *Gv*= *Gi* x seedling root length

(cm)Plantheight:thedistancefromthe baseto the top of theplant;

Totalnumberofleaves(Totalnumberofleaves):allfunctionalleavesofthewholeseedling;Rootlength(

Rootlength):the lengthfromthe root-stemjunction to the roottip;

Numberoflateralrootsper plant:themainrootgrowstoacertainlength,inacertainpartofthelateralfromthe insidetoproduceanumber of branchroots;

Seedlingbiomass(Totaldryweight):dryweightofthewholeseedling;

Biomass root to shoot biomass ratio (Root to shoot biomass ratio) = root dry weight/crown dry

weight.The valueofthe affiliationfunction:R(Xi)=(Xi-Xmin)/(Xmax-Xmin).

Where: X is the measured value of a certain index of the reference plant; Xmax and Xmin are the maximumand minimum values of the index in all the test materials, respectively. The relative germination rate, the relativegermination potential, the relative germination index and the relative root length were obtained by the subordinatefunctionvaluecalculationofthegerminationindicators,andtheaveragevalueofthesubordinatefunctionvalue of each indicator was summed up, which was the total function value. The total function value at each temperaturein theseedlingstagewas calculatedas above.

Overallevaluation:thelargerthevalueofthetotalfunction,themoreresistanttothattemperature.

1.4 dataanalysis

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,andmultiplecomparisons were performedusingthe DUNCANmethod.

Comprehensive evaluation of temperature tolerance of *Rorippa sylvestris* at different temperatures duringseed germination and seedling growth stages, respectively, using the fuzzy mathematical affiliation functionmethod[12].

2 ResultsandAnalysis

2.1 Effectsofdifferenttemperaturesonseedgerminationof*Rorippasylvestris*andevaluationofadaptability

2.1.1 Effectofdifferenttemperaturetreatmentsonthegerminationof*Rorippasylvestris*seeds

Temperature had a significant effect (P<0.05) on germination rate, germination potential, germination indexand vigor index of *Rorippa sylvestris* seeds. The germination rate, germination potential and germination index allshowed an increasing and then decreasing trend with the increase of temperature, and peaked at 35℃. *Rorippasylvestris* did not germinate at 15℃, and the germination rate, germination potential and germination indexachieved larger values at 25-35℃. The vigor index of *Rorippa sylvestris* also had a tendency to increase and thendecrease, withhighvigorat25℃and 35℃and alowpeak at30℃.Itindicated thathighertemperaturespromoted seedgermination andeithertoohighortoolowtemperaturesinhibitedseedradiclegrowth(Table1).

Table1EffectsofdifferenttemperaturesongerminationofR.*sylvestris*Seeds

	Temperature(℃) Temperature	Germinationrate(%) Germinationrate	Germinationpotential (%) Germinationvigor	germinationindex Germinationindexes	vitalityindex Germinationvigorindex
	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
R.sylvestris	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:Differentlettersindicatethatspeciesdiffersignificantly(P<0.05)atdifferenttemperatures.

Note:Differentlettersindicatesignificantdifferencesinspeciesatdifferenttemperatures*(*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 affiliationfunction values of relative germination rate, relative germination potential, relative germination index, and relativeroot length of *Rorippa sylvestris* seeds at different temperatures, and the results showed that the order of thestrength of *Rorippasylvestris*'s tolerance to different temperatures was 35°C>25°C>30°C>40°C>20°C>15°C. Theresultsshowed that*Rorippasylvestris*'stolerancetodifferenttemperatureswashigherathighertemperaturesthan atlowertemperatures(Table2),whichwasnotfavorableforseedgermination.Thetoleranceof*Rorippasylvestris*seedgerminationwashigherathighertemperatures,andthetoleranceofseedgerminationwaslowwhenthetemperature wastoo highortoolow,whichwasunfavorableforseed germination(Table2).

Table2Themembershipfunctionvalueandcomprehensiveevaluationvalueoftoleranceof*R.sylvestris*seedsduringgermination

speciesnameTemperature(°C) Species	Temperature	subordinatefunctionvaluessubordinatefunctionvalues Relative germinationrate Relativeger minationrate	Relative germination potential Relative germinationvigor	relativegermination index(RGI) Relative germinationin dexes	Relativeroot length rootle ngth	collective function value (math.) Total functionv alue	rankings 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
	25	0.5	0.5	0.51	0.41	0.48	2
R.sylvestris	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 EffectsofdifferenttemperaturesonthegrowthofRorippasylvestrisseedlingsandevaluationofacclimatizatio n

2.2.1 EffectofdifferenttemperaturesonthegrowthofRorippasylvestrisseedlings

Differenttemperatureshadsignificanteffectsonplantheight,totalleafnumber,rootlengthandlateralrootnumberof*Rorippasylvestris*seedlings.Plantheight,totalleafnumberandrootlengthallincreasedandthendecreasedwithincreasingtemperature,andthenumberoflateralrootsgraduallyincreasedwithincreasingtemperaturein*Rorippasylvestris*(P<0.05).*Rorippasylvestris*plantheight,totalleafnumber,rootlengthandlateralrootnumberweremaximumat25℃,30℃,20℃and35℃,andminimumat15℃,15℃,30℃and25℃,respectively.Temperaturesweretoohighortoolowforthegrowthof*Rorippasylvestris*(Figures1. A,B,C,andD).

The biomass of *Rorippa sylvestris* seedlings first increased and then decreased with temperature, and theroot-crown ratio decreased with temperature, and *Rorippa sylvestris* slightly increased at 30℃. Biomass wasgreatestat20°Candroot-crownratiowasgreatestat15°C;*Rorippasylvestris*biomassandroot-crownratiowere smallestat15°Cand35°C,respectively.Lowertemperaturesfavored*Rorippasylvestris*biomassaccumulation andbiomass partitioningtorootsatlowertemperatures(Figs. EandF).

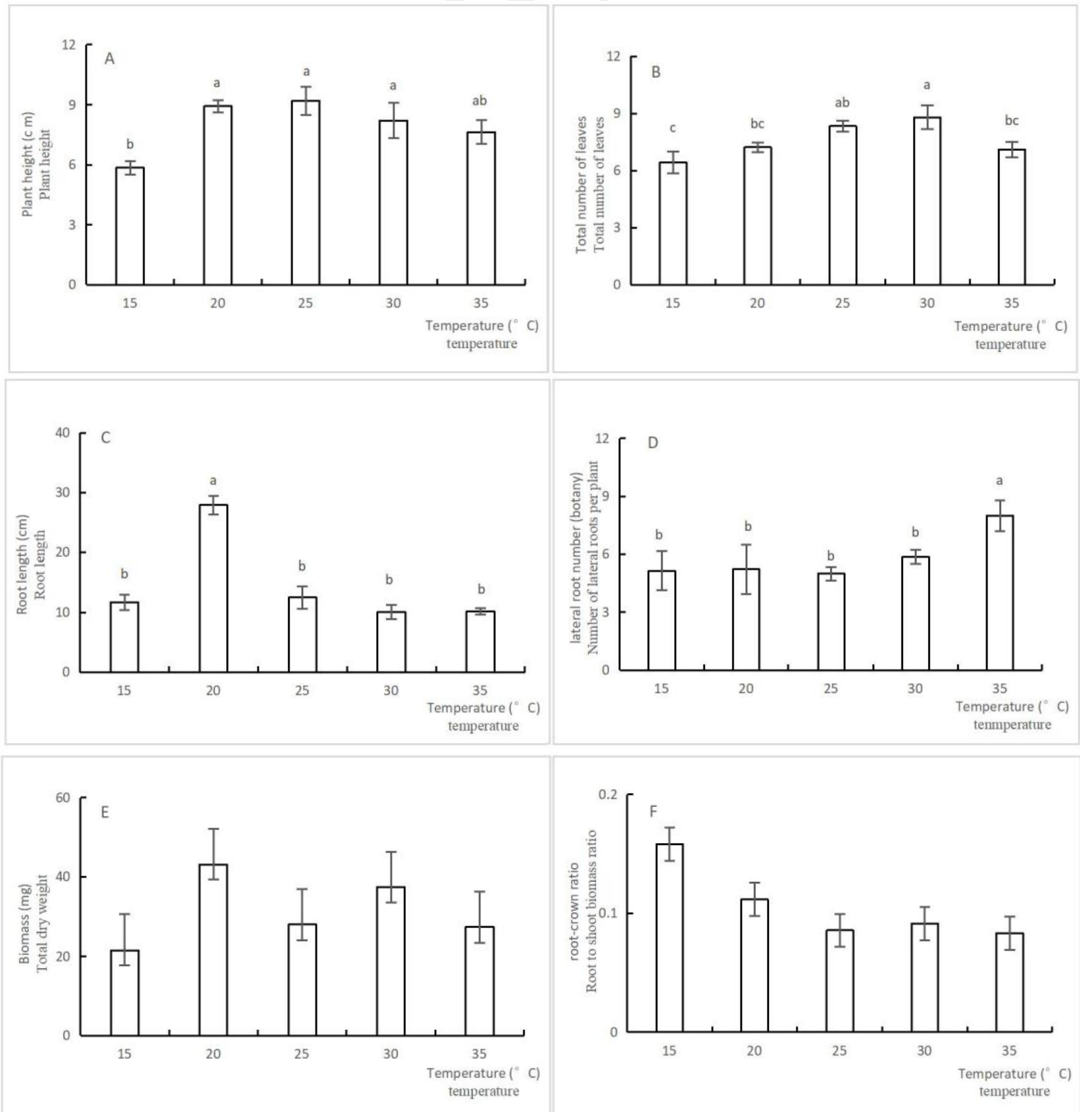

Fig. 1. Effects of different temperatures on plant height (A), total leaf number (B), root length (C), lateral root number (D), biomass (E), and root-crown ratio (F) of Rorippa sylvestris at seedling stage

Note:Differentlettersindicatethatspeciesdiffersignificantly(P<0.05)atdifferenttemperatures.

*Note:Differentlettersindicatesignificantdifferencesinspeciesatdifferenttemperatures(*P<0.05*).*

2.2.2 Evaluation of the adaptability of Rorippa sylvestris to different temperatures at seedlingstage

The above results showed that different temperature treatments had a great influence on the growth of*Rorippa sylvestris* seedlings, and the tolerance of *Rorippa sylvestris* seedlings to different temperatures wasevaluated by the fuzzy mathematical affiliation function through a comprehensive evaluation of the values ofdifferent temperature affiliation functions of four indicators at the seedling stage of *Rorippa sylvestris*. The resultsshowedthat*Rorippasylvestris*seedlingsweretoleranttodifferenttemperaturesintheorderof 30℃>35℃>15℃>20℃>25℃(Table3).*Rorippasylvestris*wastolerantathighertemperatures.

Table3Themembershipfunction valueand comprehensiveevaluationvalueoftheresistanceof*R.sylvestris* seedlingsto differenttemperatures duringthe growthperiod

species nameSpecies	Temperature (°C)Temperature	subordinatefunctionvaluessubordinatefunctionvalues relative plantheightRelativePlant height	Relativetotalnumberofleaves RelativeTotal numberofleaves	Relativeroot lengthRelative root length	Relativenumberoflateral rootsRelativenumberof lateralrootsper plant	collective function value(math.) Totalfunction value	rankings Ranking
	15	0.39	0.49	0.36	0.57	0.45	3
	20	0.48	0.41	0.38	0.31	0.40	4
R.sylvestris	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 DiscussionandConclusion

Plant growth is not only controlled by genetic material, but also by numerous environmental factors, such aslight, temperature, water, soil nutrients and so on. Seed germination is one of the key links in the life history ofplants, and the response of seeds to germination conditions reflects their ecological response to adapt to theenvironment[14], and is also the key for invasive plants to settle into new environments. Temperature, as animportant environmental variable regulating seed germination, is one of the key ecological factors affecting seedgermination[7,8],toohighortoolowwill affectthenormal growthanddevelopmentofplants,WangYuqinet al.[13]found that the seed germination rate of yellow-flowering echinoderms (*Oxytropisochrocephala*) was lower underlowtemperature,ZhangTianxiangetal.[14]foundthatbothlowandhightemperatureswereunfavorableto moringa(*Moringaoleifera*)seedgermination.

Inthisexperiment,itwasfoundthattoohighortoolowtemperatures(15°C,40°C)limitedthegrowthofembryonicrootsafter germinationof*Rorippasylvestris*seedsandaffectedthevigorindexofseedgermination,lowertemperaturesinhibitedthege rminationrate,germination potential,andgerminationindexofseeds,andhighertemperaturespromotedseedgermination.

Germinationrateandgerminationpotentialreflectthegerminationspeedandneatnessofseeds,high germination rate and strong germination potential predict fast and neat germination and strong seedlings; highgerminationrateandlowgerminationpotentialpredictpoorgerminationandweakseedlings[15].*Rorippasylvestris*ger mination rate and germination potential were highestat 35℃,and hightemperaturepromoted itsseed germination and growth. However, the effect of temperature on seed germination is multifaceted, and the useof a single indicator to evaluate its effect is one-sided, and the use of the affiliation function method for thecomprehensive evaluation of plant tolerance has been generally accepted[12], and the use of the affiliation functionmethod can be more comprehensive and more accurate to reflect the tolerance of *Rorippa sylvestris* to differenttemperatures. 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* seedgermination 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℃. Because *Rorippa sylvestris* did not germinate at 15℃, and rootlength was <1mm at 20℃ and 40℃, 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 withglobalwarming.

Temperature plays a very important role in the early growth of plant seedlings. Han Bing et al.[16] showed thatlow-temperaturestresssignificantlyinhibitedthegrowthofseedlings,resultinginadecreaseinthegrowthofplant height, stem thickness, and dry mass compared with the control; Zou Huu[17] showed that high-temperaturestressdecreasedtheaccumulationofdrymatterintheseedlingsofantlerrhododendron(*Rhododendronl* α *toucheae*);andWangJianjunetal.[7]showedthatdifferenttemperatureshaddifferenteffectsonrootlengthand dry matter of Chinese fescue (*Festuca sinensis*) seedlings. The results of this experiment showed that temperaturehad 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 oftemperature, 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℃, and its root length was significantly higher thanother temperatures at 20℃. High temperature promotes the occurrence of leaves and lateral roots of *Rorippasylvestris*, whichhasthe highestnumberof leavesat 30℃and thehighestnumberof lateralrootsat35℃.*Rorippa sylvestris* accumulated the most dry matter at 20℃, but tended to allocate more material to the roots atboth low and high temperatures, probably because the growth of the above-ground part of the plant was limited atlow temperatures, thus allocating more resources to the roots and showing a larger root-crown ratio, As thetemperature increases, it has been shown that warming can increase nitrogen content in the organs of the planttissuesandorgans,whichin turnallowsthesoilenvironmenttobeWithincreasingtemperature,ithasbeenshown thatwarmingcanincreasethenitrogencontentinplanttissuesandorgans,andincreasethesupplyofnutrientstotheplantfrom thesoilenvironment,thuscausing adecreaseinrootpartitioningand anincreaseincrownpartitioningofphotosynthesizedproducts[17,18].Thismaybethereasonforthedecreaseintheroot-crownratioof*Rorippasylvestris*seedlingsatelevatedtemperatures.35℃wasdetrimentaltothedevelopmentof*Rorippasyl vestris*seedlings,indicatingthathighertemperatureshaveaninhibitoryeffecton*Rorippasylvestris*seedlings,whichiscons istentwiththeresultsoftheresearchconductedby WangJJetal.[7],LiuFHetal.[19],andXinFHetal.[20]onthegrowthofChinesefescue,respectively,Thisisconsistentwiththefi ndingsofWangJunetal.,LiuFuhuaetal.,andXinFuhaietal.,whichfoundthathighertemperaturesinhibitedthegrowthofCh inesefescue,*Gleditsiasinensis*,and*Cupressusgigantea*atdifferenttemperatures.Comprehensiveevaluationoftheaffiliat ionfunctionof*Rorippasylvestris*seedlingsintermsofplantheight,totalnumberofleaves,rootlength,andnumberof lateral roots showed that *Rorippa sylvestris* was more tolerant to higher temperatures, with a maximum at 30℃.Theresponseof*Rorippasylvestris*seedsandseedlingstotemperaturewasessentiallysynchronized,withbothhighert emperaturespromotingseedgerminationandinhibitingseedlinggrowth,andlowertemperaturesinhibitingseedgerminati on.Undernaturalconditionshightemperaturesoccurinthesummerandaredetrimentaltoseedlinggrowth,whichmaybeani mportantreasonwhythis clonehasveryfewseededseedlingsundernaturalconditions. Plantspropagated byclonalgrowth tendtohaveaveryhigh mortality rateatthe seedlingstageamongtheprogenyproducedbyseedgermination,andtherateofsuccessfulcolonizationisnotashighasthato ftheclonedprogeny.Inthecaseofinvasiveclones,onceseedsgerminateandpasstheseedlingstage,theycanbesuccessfully colonizedbyasexualpropagation.Therefore,thestudyoftheresponseofseedsandseedlingsof invasive clonestotheenvironmentcanpartlyrevealthemechanismoftheirsuccessfulinvasion.

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