

Article **Seed-Germination Ecology of** *Vicia villosa* **Roth, a Cover Crop in Orchards**

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Abstract: *Vicia villosa* Roth is an annual cover crop that is widely grown in orchards in China. Information on seed ecology is valuable as it helps famers to plant cover crops and manage other weed species in agricultural practice; however, information on *V. villosa* seed-germination ecology is limited. Thus, this study investigated the seed germination and seedling emergence of *V. villosa* under various temperatures, photoperiods, levels of salt stress, pH, levels of osmotic stress, and burial depths. The results showed that the germination values of the *V. villosa* seeds were greater than 93% at the constant temperature range of 5–30 °C and fluctuating temperatures of 5/15 °C–20/30 °C; in particular, the germination of the seeds peaked 20 \degree C and 15/25 \degree C, with germination values of 95% and 94.5%, respectively. Light was not necessary for the *V. villosa* seeds' germination. When the pH was in the range of 5–10, the germination values of the *V. villosa* seeds fluctuated between 85% and 94%, and obvious inhibition of germination was observed at $pH = 4$, with a germination value of 15%. The *Vicia villosa* seeds exhibited obvious salt tolerance, and the seed-germination value was still greater than 50% when the salt concentration reached 280 mM. The seeds were relatively sensitive to osmotic stress, and the germination value was lower than 50% when the osmotic potential was −0.5 MPa. In addition, the germination value of the seeds peaked when the seeds were 1–2 cm underneath the ground; in particular, the seeds still germinated and emerged when the seeds were buried in soli at a depth of 10 cm. These results confirmed that *V. villosa*, as a cover crop, has considerable potential to be planted and grown in orchards in China and, furthermore, that it may contribute to early weed management in fields, supporting the establishment of *V. villosa* populations.

Keywords: temperature; light; pH; salt stress; osmotic stress; burial depth

1. Introduction

Weeds are important biological factors in orchards, with a wide variety of species. They compete successfully with saplings for water, sunlight, space, and nutrients, which seriously inhibits the growth and development of fruit trees and hinders agricultural operations, significantly restricting the efficient production and sustainable development of the fruit-tree industry. According to Zhang and Wang [\[1\]](#page-9-0), weeds, especially tall and winding weed species, consumed soil nutrients, affected peach (*Amygdalus persica* Linn.) tree growth and reduced yields by 10–20%. Weed control in orchards mainly consists of clear tillage weeding, chemical control, and the planting of natural or artificial cover crops. Herbicides (chemical control) play an important role in controlling weeds in orchards. However, studies have found that herbicides have many adverse effects on the growth of fruit trees. Tursun et al. [\[2\]](#page-9-1) found that the use of herbicides in the planting process

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of apricot (*Armeniaca vulgaris* Lam.) trees resulted in a decrease in plant coverage, soil organic matter, and soil moisture, and an increase in soil erosion, runoff, and sand content. Long-term clear-tillage weeding increased management costs and was prone to destroying the soil structure [\[3\]](#page-9-2). Therefore, many integrated weed-management strategies have been encouraged for weed control in orchards, such as interrow cover-crop planting control.

Vicia villosa Roth is an annual species of the legume family, and it is usually grown as a forage or cover crop [\[4\]](#page-9-3). *Vicia villosa* is native to Europe, Central Asia, Iran, and the Eastern United States. In China, *V. villosa* mainly grows along the Yellow River, Huai River, and Hai River Basins at altitudes from 1720–1750 m [\[5\]](#page-9-4). It is mainly used as a cover crop in orchards and can be used as fine pasture or green manure due to its ability to reduce ground-water evaporation, increase organic matter, and enhance microbial diversity in soil [\[6,](#page-9-5)[7\]](#page-9-6). Many studies have also reported that *V. villosa* effectively inhibits the growth of harmful weeds because of its strong ability to compete for light, moisture, nutrients, etc., which is based on the release of the allelochemical, cyanamide [\[8](#page-9-7)[,9\]](#page-9-8).

Usually, *V. villosa* bloom when the air temperature reaches approximately 15 ◦C, but only about 5–15% of the flowers actually form into pods with mature seeds. Under suitable environmental conditions, a single vetch plant can produce approximately 800–1000 seeds. Generally, the rapid establishment of effective populations of *V. villosa* is associated with suitable planting methods, planting density, and seed germination [\[10\]](#page-9-9).

To date, many studies have reported the utilization of *V. villosa* in agricultural practice. Zhou and Everts [\[11\]](#page-9-10) observed that soil mulch with *V. villosa* could not only increase the sugar content of watermelon (*Citrullus lanatus* (Thunb.) Matsum. et Nakai) fruit by 10–15%, but also manage watermelon fusarium wilt cultivar resistance. Spargo et al. [\[12\]](#page-9-11) reported that the yield of maize (*Zea mays* Linn.) can be increased by more than 50% when the field biomass of *V. villosa* reaches a certain value. In addition, *V. villosa* has also been used as an accompanying plant to increase the pollination probability of tomatoes (*Lycopersicon esculentum* Miller) by attracting insects, leading to higher tomato yields [\[4\]](#page-9-3). Moreover, *V. villosa* is capable of providing high amounts of nitrogen (>150 kg ha−¹ of total N) using specific planting times/methods/densities to meet the peak absorption time of cash crops [\[13](#page-9-12)[–15\]](#page-9-13). Pott et al. [\[16\]](#page-9-14) reported that *V. villosa* increased the N content of maize fields, thereby increasing the yield of maize.

The ability of seeds to successfully germinate and establish populations is critical to the success of cover crops in agricultural practices. The germination of seeds is usually affected by many environmental factors, such as light, temperature, soil salinity, soil moisture, soil pH, and seed burial depth [\[17](#page-10-0)[–20\]](#page-10-1). Temperature controls the process of seed germination by affecting the nutrient accumulation of seeds and the activity of protease required for germination [\[21–](#page-10-2)[24\]](#page-10-3). Soil pH affects seed germination through enzymatic reactions [\[25\]](#page-10-4). Both osmotic potential and salt concentration can affect water absorption in seed-germination process [\[26](#page-10-5)[,27\]](#page-10-6). The burial depth of seeds can significantly affect the nonbiological factors required for seed germination. Furthermore, investigating the seedling-emergence response to different burial depths will contribute to the development of agricultural control based on soil covering or tillage [\[28](#page-10-7)[,29\]](#page-10-8).

Vicia villosa is a potential artificial cover species. However, its seed ecology is far from fully known. To the best of our knowledge, limited information is available about the effects of environmental factors on the seed germination and seedling emergence of *V. villosa*, which hinders its large-scale application. Therefore, the purpose of the present study was to investigate the effects of temperature, light, pH, osmotic stress, salt stress, and burial depth on the seed germination and seedling emergence of *V. villosa*.

2. Materials and Methods

2.1. Seed Collection and Preparation

The *V. villosa* seeds were kindly provided by Shandong Institute of Pomology (Tai'an, China). The seeds were cleaned and wrapped with a kraft paper under dry conditions at room temperature (20 \pm 5 °C) until the initiation of experiments. The 1000-seed weight

was 25 ± 3 g. This *V. villosa* cultivar is presently being used as animal feed and as a cover crop in orchards in China.

2.2. General Seed-Germination Test

The following procedures were used for all germination tests, except for specific instructions that are described further below. Seed germination of *V. villosa* was determined by placing 50 seeds evenly in a 9-centimeter-diameter petri dish containing two layers of filter paper (Whatman No. 1, Maidstone, UK) moistened with 8 mL of distilled water (pH = 6.7) or test solution of different pH levels, osmotic potentials, or salt concentrations. All petri dishes were sealed with parafilm to prevent evaporation and then placed in controlledenvironment growth chambers (Model RXZ, Ningbojiangnan Instrument Factory, Ningbo, China) set at a constant temperature of 20 \degree C with a 12-h photoperiod. Fluorescent lamps were used to provide a photosynthetic photon flux density of 200 µmol m $^{-2}$ s $^{-1}$. Seeds with a visible protrusion of the radicle were considered germinated [\[30\]](#page-10-9). The germinated seeds were counted every day for a total of 21 days. The seed-germination values were calculated as the total number of germinated seeds divided by the total number of seeds placed in the petri dish in the initial experiment, multiplied by 100%.

2.3. Effect of Temperature on Seed Germination

Twelve treatment groups were designed according to the temperature conditions commonly found in the fruit-producing regions in China, consisting of 7 constant temperatures (5, 10, 15, 20, 25, 30 and 35 °C) and 5 fluctuating temperatures (15/5, 20/10, 25/15, 30/20 and 35/25 \degree C). These fluctuating temperatures were selected based on temperature variation during the growing season of *V. villosa* in China.

The time to onset of germination (the day when the first germination was observed) was recorded. The days required to reach 50% of germination (t_{50}) were estimated by a linear interpolation [\[31\]](#page-10-10):

$$
t_{50} = (H_p - L_p)^{-1} + L
$$

where *L* is the last day before 50% seed germination was reached, *L^p* is the observed germination percentage on day *L*, and *H^p* is the observed germination percentage on the day when germination reached or exceeded 50%.

2.4. Effect of Photoperiods on Seed Germination

The effect of different photoperiods on seed germination was studied under 7 treatment groups (0/24-, 4/20-, 8/16-, 12/12-, 16/8-, 20/4-, and 24/0-h light/dark). The complete dark treatment was performed immediately by wrapping the seeds in three layers of tin foil to isolate them from light.

2.5. Effect of pH on Seed Germination

Buffer solutions with pH of 4, 5, 6, 7, 8, 9, and 10 were prepared according to Wu et al. [\[32\]](#page-10-11), which included the pH values of surface soil in China (3.7–9.7) [\[33\]](#page-10-12). Distilled water was used as the control ($pH = 6.7$). At the end of test, the pH of buffer solution was measured again and the pH was only slightly changed.

2.6. Effects of Osmotic Stress and Salt Stress on Seed Germination

To determine the effect of drought stress on seed germination of *V. villosa*, 9 treatment groups with different osmotic potentials were prepared $(-0.3, -0.5, -0.7, -0.9, -1.1, -1.3,$ −1.5, and −1.7 MPa) by dissolving 0, 72.5, 112.2, 143.2, 192.6, 233.0, 268.0, 299.3, 327.9, 354.4, and 284.0 g polyethylene glycol 8000 (PEG 8000) in 1 L of distilled water, respectively [\[34\]](#page-10-13).

To determine the effect of salt concentration on seed germination of *V. villosa*, 0, 1.17, 2.34, 4.68, 9.36, 11.70, 14.04, 16.38, and 18.72 g NaCl were dissolved in 1 L of distilled water to obtain 0, 20, 40, 80, 160, 200, 240, 280, and 320 mM NaCl buffer solutions, respectively [\[35\]](#page-10-14).

The seed-germination values (%) of *V. villosa* under different NaCl concentrations and osmotic potentials were both fitted to the following equation [\[30\]](#page-10-9):

$$
G\left(\% \right) = G_{max} / \{1 + \exp[-(x - x_{50})/G_{rate}]\}
$$

where *G* is the total germination (%) at NaCl concentration or osmotic potential *x*, *Gmax* is the maximum germination (%), *x⁵⁰* is the NaCl concentration or osmotic potential required for 50% inhibition of the maximum germination, and *Grate* indicates the slope.

2.7. Effect of Burial Depth on Seedling Emergence

Twenty *V. villosa* seeds were buried at depths of 0, 0.2, 0.5, 0.8, 1, 2, 3, 4, 5, 6, 8, and 10 cm below the soil surface in plastic pots that were 15 cm long, 15 cm wide, and 12.5 cm tall. The experiment was carried out in a controlled-environment growth chamber with a constant temperature of 20 \degree C and a photoperiod of 12/12 h. The soil used in this study (38% clay, 26% silt, 36% sand, pH 7.1, 1.7% organic matter) was autoclaved (killing the original seedbank in soil) before the test and passed through a 3-millimeter sieve. The pots were watered every other day to maintain sufficient soil moisture. When the coleoptile was visible above the soil surface, the seedling was considered to have emerged. The emerged seedlings were counted every day for a total of 21 days. The seedling emergence was calculated as the total number of emerged seedlings divided by the total number of seeds placed in the pots in the initial experiment, multiplied by 100%. At the end of the test, the soil in the pots with the non-emerged plants was removed to check whether the seeds failed to germinate or the coleoptiles failed to emerge.

The seedling emergence values (%) obtained at different burial depths were fitted to a three-parameter sigmoid model [\[36\]](#page-10-15).

$$
E(%) = E_{max} / \{1 + \exp[-(x - x_{50})/E_{rate}]\}
$$

where *E* is the percentage of emerged seedlings at burial depth *x* and *Emax* is the maximum seedling emergence (%), *x⁵⁰* is the depth to reach 50% of maximum seedling emergence, and *Erate* indicates the slope near the *x50*.

2.8. Statistical Analysis

All experiments were arranged in a randomized complete block design with four replications. The whole experiments were repeated twice, and the second run was started within a month of termination of the first run. Each replication was considered as a block and arranged on different shelves in the incubators. Before statistical analyses, the homogeneity (Bartlett's test) and normality (Shapiro–Wilk test) of data were checked. Because the homogeneity of variances was not improved by arcsine square-root transformation on percentage data, the untransformed germination percentage values from repeated experiments were subjected to an ANOVA with the general linear model procedure using SPSS software (v. 19.0, IBM, Armonk, NY, USA). There was no statistically significant ($p > 0.05$) trial-by-treatment interaction between repeat experiments, and the data were therefore pooled across runs and used for subsequent analyses. Regression analysis was conducted using SigmaPlot software (v. 12.5, Systat Software, Point Richmond, CA, USA), and means comparison was performed using Tukey's HSD test at $p \leq 0.05$.

3. Results and Discussion

3.1. Effect of Temperature on Seed Germination

Under a constant temperature, *V. villosa* seeds completed the germination process in a wide temperature range between 5° C and 35° C and the germination values exceeded 90%. The *Vicia villosa* seeds still germinated at 35 ◦C, but the germination value was less than 10% (Table [1\)](#page-4-0). From 5 °C to 30 °C, although the change in final germination values was slight, the time for onset of germination gradually decreased. Li et al. [\[31\]](#page-10-10) also reported that the low temperature did not affect the final germination values of *Bromus* *japonicus* Thunb. ex Murr. Overall, *V. villosa* seeds showed a higher germination value and a faster germination speed at 20 $^{\circ}$ C. When exposed to fluctuating temperatures, the *V. villosa* seeds exhibited over 90% of germination values in all the treatments, except for 25/35 [°]C, and the germination speed was faster under higher temperatures. However, the constant temperature was more suitable for the germination of *V. villosa*. For example, the fluctuating temperature (25/35 °C, mean of 30 °C, Table [1\)](#page-4-0) produced 37% germination, while the constant temperature (30 $^{\circ}$ C, Table [1\)](#page-4-0) produced 94.5% germination.

^a Means followed by the same letter are not significantly different according to Tukey's HSD test at $p \le 0.05$. ^b Calculated as the formula: t₅₀ = (Hp – Lp)⁻¹ + L, where L is the last day before 50% germination was reached, Lp is the observed germination percentage on day L, and Hp is the observed germination percentage on the day when germination reached or exceeded 50%. ^c Abbreviation: NE, not estimated because germination did not reach 50% in all replications.

The results above suggested that planting *V. villosa* seeds at an appropriate temperature range is significant to achieve high seed-germination value at high speed. If other environmental conditions are suitable, sowing seeds at 15–25 ◦C is ideal and may contribute to early seed germination and population establishment. Midsummer in most parts of China is usually accompanied by extremely high temperatures (over $35 \degree C$), which may cause a decrease in the seed germination of *V. villosa*. The sowing in late autumn before the low temperatures in winter, especially in northern China, may create insufficient time for population establishment and plant growth. Thus, early autumn (September to October) and spring (March to April), with average temperatures of over 15 $°C$, may be the best time for sowing *V. villosa* seeds in China [\[37\]](#page-10-16). However, attention should be paid to the low germination speeds at low temperatures when sowing too early in spring.

In this study, the optimal temperature range for *V. villosa* seeds' germination was 15–25 ◦C, which was similar to *Trifolium repens* L. [\[38\]](#page-10-17), a growing cover crop species that is also widely used in the orchards of China. However, it should be mentioned that different varieties of *T. repens* exhibited dissimilar optimal germination-temperature ranges in previous studies [\[39–](#page-10-18)[41\]](#page-10-19). Thus, more *V. villosa* varieties should be used for further investigation in our future studies.

3.2. Effect of Photoperiods on Seed Germination

The effect of photoperiods on *V. villosa* seed germination was not significant, and the seed-germination values were all over 90% when exposed to full light, full darkness, or alternating light-and-dark conditions. The above results indicated that light was not a limiting factor for *V. villosa* seed germination.

One of the decisive factors in the development of interrow-cover-crop species is the need to cover the ground all year to suppress the growth of weeds [\[16\]](#page-9-14). In the orchards of China, the seeds of cover crops are mostly planted between rows, drilled below the soil surface $[42]$; thus, the seeds are inevitably shaded by the soil and tall plants. Therefore, it is very important for seeds to continue to germinate in environments with insufficient light. The results in this study indicated that the seeds of *V. villosa* can still exhibit high germination value under the cover of the plant canopy, which ensures that they can be used as artificially planted cover crops in orchards. The seeds of some other cover crops, such as *T. repens*, *Lolium rigidum* Gaudin, and *Brassica napus* L., also possessed the ability to geminate without light [\[38](#page-10-17)[,43,](#page-10-21)[44\]](#page-10-22).

3.3. Effect of pH on Seed Germination

When treated by solutions with pH ranging from 5 to 10, the *V. villosa* seeds showed high final germination values (of more than 85%); these were significantly higher than those in buffer solution with $pH = 4$ (15%), and the germination values of the seeds peaked (94%) when the seeds were exposed to a solution with $pH = 7.6$ (Figure [1\)](#page-5-0). In most regions of China, the soil is neutral (pH 6.5–7.5) or alkaline (pH 7.5–8.5) [\[45\]](#page-11-0). Therefore, judging only from the influence of soil pH, a conclusion can be drawn that pH is not a limiting factor in the germination of *V. villosa* seeds. Therefore, *V. villosa* can be planted in most areas of China. In addition, the germination of the seeds was always higher than 90% when the pH was from 7 to 9 (Figure [1\)](#page-5-0), which indicated that a neutral or weak alkaline environment may be more suitable for *V. villosa* seed germination and that soil pH can be used as a tool to support the establishment of *V. villosa* population. For example, farmers can promote *V. villosa* germination by maintaining neutral-pH or slightly alkaline soil, helping *V. villosa* establish populations and compete, and restricting harmful weed germination and growth [\[33,](#page-10-12)[46\]](#page-11-1).

Figure 1. Effect of buffered pH on the germination of *Vicia villosa* seeds incubated at 20 °C under a 12/12-h dark/light photoperiod for 21 d. The vertical bars represent the standard error of the means. 12/12-h dark/light photoperiod for 21 d. The vertical bars represent the standard error of the means. Different letters indicate significant differences according to Tukey's HSD test at $p \leq 0.05$. **Figure 1.** Effect of buffered pH on the germination of *Vicia villosa* seeds incubated at 20 °C under a 12/12-h dark/light photoperiod for 21 d. The vertical bars represent the standard error of the means Different lett

3.4. Effects of Osmotic Stress on Seed Germination 3.4. Effects of Osmotic Stress on Seed Germination

As shown in Figure [2,](#page-6-0) the osmotic potential had a significant effect on the germination tion of the *V. villosa* seeds (*p* < 0.0001). When the seeds were exposed to −0.1 MPa osmotic of the *V. villosa* seeds (*p* < 0.0001). When the seeds were exposed to −0.1 MPa osmoticpotential, they maintained a high germination value (92%). When exposed to $\frac{1}{2}$

potential, they maintained a high germination value (92%). When exposed to −0.3 MPa osmotic potential, the germination of the seeds significantly decreased (65%). When the seeds were exposed to osmotic potential of less than −0.9 MPa, seed germination did not occur. The fitting curve showed that the osmotic potential that limited the germination value to 50% was −0.44 MPa. Osmotic potential is an important factor that affects seed germination. In this study, the seed germination of *V. villosa* decreased even under the highest osmotic potential (−0.1 MPa) prepared by dissolving PEG 8000. However, Kalsa and Abebie $[47]$ reported that osmotic priming prepared by dissolving KNO₃ significantly improved the seed germination of *V. villosa*, which might be explained by the enhanced release of ethylene within embryonic tissues in the presence of imbibed NO3⁻¹ ion [\[48](#page-11-3)[,49\]](#page-11-4). In southern China, the annual average precipitation exceeded 1000 mm, causing a humid climate; however, northern China is relatively dry, with an average annual rainfall of only 500 mm [\[50\]](#page-11-5). *Vicia villosa* is more tolerant to osmotic pressure than *T. repens* [\[38\]](#page-10-17), which indicates that *V. villosa* may be more suitable than *T. repens* as a species for cover crops in orchards in northern China.

Figure 2. Effect of osmotic stress on the germination of *Vicia villosa* seeds incubated at 20 °C under a a 12/12-h dark/light photoperiod for 21 d. The vertical bars represent the standard error of the 12/12-h dark/light photoperiod for 21 d. The vertical bars represent the standard error of the means.

aquaticum (L.) Moench, two winter weeds with similar germination and emergence period to *V. villosa*, may prevent *V. villosa* from establishing populations in the early stage due to the competition for light, water, and nutrients with cover crop plants. *Vicia villosa* is much more tolerant of osmotic pressure than *C. bursa-pastoris* and M. aquaticum [\[33](#page-10-12)[,51\]](#page-11-6); thus, we can appropriately reduce the soil moisture by reducing watering to inhibit the seed germination of these two weed species. However, compared with *Avena fatua* L. [\[52\]](#page-11-7), *V. villosa* was more sensitive to osmotic pressure, which requires us to select appropriate herbicides to ensure was of Λ fatus for the ostablishment of better V villege nonviations. the chemical control of *A. fatua* for the establishment of better *V. villosa* populations. In the process of planting *V. villosa*, *Capsella bursa-pastoris* (L.) Medik, and *Myosoton*

3.5. Effect of Salt Stress on Seed Germination

means.

As shown in Figure [3,](#page-7-0) the salt concentration was negatively correlated with the *V. villosa* seed-germination values (*p* < 0.0001), and the seed germination values of *V. villosa*

gradually decreased from 94% to 21% within a salt concentration range from 0 to 320 mM. When the seeds were exposed to the salt concentration range of 0–160 mM, the germination values of the seeds were greater than 80%. When exposed to 240 mM, the germination value was still above 50%; when exposed to high salt concentrations (320 mM), the seeds still maintained a certain degree of germination (21%), while the *T. repens* seed germination was completely inhibited at 160 mM NaCl. In plant-growth stages, seed germination is the most sensitive stage to salt stress, which causes nutritional and ionic imbalances and the excessive accumulation of reactive oxygen species (ROS) [\[53](#page-11-8)[–55\]](#page-11-9). The results above indicated that *V. villosa* has a high tolerance to salt stress and can maintain high adaptability in salt-affected soil. Soil with a NaCl concentration exceeding 20 mM is identified as salt-affected soil [\[56\]](#page-11-10). The ability to germinate under high NaCl concentrations contributes to the promotion of *V. villosa* as a cover crop in a greater diversity of orchard environments, such as orchards of winter jujube (*Elaeagnus macrophylla* Thunb.), which is very suitable for growing in saline alkali land [\[57\]](#page-11-11).

Figure 3. Effect of NaCl concentration on the germination of *Vicia villosa* seeds incubated at 20 °C under a 12/12-h dark/light photoperiod for 21 d. The vertical bars represent the standard error of under a 12/12-h dark/light photoperiod for 21 d. The vertical bars represent the standard error of the means. the means.

3.6. Effect of Burial Depth on Seedling Emergence 3.6. Effect of Burial Depth on Seedling Emergence

The seedling emergence of *V. villosa* gradually decreased with the burial depth in the range of 0 to 10 cm, and a three-parameter logistic model was consistent with the sponding final emergence value under different burial depths (*p* < 0.0001) (Figure 4). corresponding final emergence value under different burial depths (*p* < 0.0001) (Figure [4\)](#page-8-0). When the burial depth was 0–1 cm, the seedling emergence peaked (over 93%); when the When the burial depth was 0–1 cm, the seedling emergence peaked (over 93%); when the burial depth was 2–3 cm, the seedling emergence began to show a slight downward trend burial depth was 2–3 cm, the seedling emergence began to show a slight downward trend (88–89%), which indicated that *V. villosa* seeds should be sown in soil depths ≤3 cm to (88–89%), which indicated that *V. villosa* seeds should be sown in soil depths ≤3 cm to obtain higher seedling emergence values. However, planting *V. villosa* seeds on the soil obtain higher seedling emergence values. However, planting *V. villosa* seeds on the soil surface is not recommended because seeds in soil surfaces tend to be damaged by adverse environmental factors, lack sufficient moisture for seed germination, or are eaten by insects sects or birds. The *V. villosa* seeds had the ability to germinate in deep soil and still main-or birds. The *V. villosa* seeds had the ability to germinate in deep soil and still maintained a tained a certain emergence value (over 22%) at the burial depth of 10 cm (Figure 4), which certain emergence value (over 22%) at the burial depth of 10 cm (Figure [4\)](#page-8-0), which might ϵ where ϵ is the following two reasons. Firstly, light was not an absolute re-explanation of ϵ be explained by the following two reasons. Firstly, light was not an absolute requirement for the germination of the *V. villosa* seeds. Secondly, the *V. villosa* seeds were large and provided sufficient nutrition for the coleoptiles to reach the soil surface. Meanwhile, the non-germinated seeds in the deep soil could still germinate when treated as in the General Seed Germination Test (data not shown) in this study, while fatal germination ocheral beed Schminkhort fest (data hot shown) in this steady, while have germination
was observed in some small weed seeds, such as *Alopecurus japonicus* Steud. [\[16\]](#page-9-14). Thus, the failure of *V. villosa* seedling emergence was due to continued seed dormancy, which might have been caused by the lower gas-diffusion rates in deep soils [\[58\]](#page-11-12). The fitting curve might have been caused by the lower gas-diffusion rates in deep soils [58]. The fitting curve *a* showed that the burial depth required for a 50% inhibition of the seedling emergence was 2.59 cm (Figure [4\)](#page-8-0), much higher than that of *C. bursa-pastoris* (0.57 cm) and *M. aquaticum* (1.08–1.72 cm) [\[33,](#page-10-12)[51\]](#page-11-6). Thus, farmers can appropriately increase the thickness of covering soil to inhibit the seed germination of weeds, while the seedling emergence of *V. villosa* is only slightly affected, which may contribute to the early-stage establishment of *V. villosa* populations. However, this agricultural practice may not be suitable for *T. repens* because its seedling-emergence values were only 40% when the small seeds were buried in soil at a depth of 3 cm [\[38\]](#page-10-17), and similar results were observed in many small-seeded plant species, such as *Meliotus officinalis* L., *Bidens alba* L., and *Medicago lupulina* L. [\[59](#page-11-13)[–61\]](#page-11-14). curve share week seeds, sach to the permane formal stead. [10]. Thus,

Figure 4. Effect of seed-burial depth on the germination of *Vicia villosa* seeds incubated at 20 °C under a 12/12-h dark/light photoperiod for 21 d. The vertical bars represent the standard error of under a 12/12-h dark/light photoperiod for 21 d. The vertical bars represent the standard error of the means. the means.

In summary, the seed germination and seedling emergence of *V. villosa* can be affected by many environmental factors. The seed germination of *V. villosa* can occur in various photoperiods and under a wide range of temperatures, pH values, and salinity levels, which gives this cover crop considerable opportunities to be planted in most orchards in China. The *V. villosa* seeds were relatively sensitive to osmotic potential but highly tolerant of *T. repens* cover crops and *C. bursa-pastoris* and *M. aquaticum* winter weeds, which supports the growth of *V. villosa* in orchards in relatively dry areas and its ability to compete with weeds in the early stages of population establishment. Although the *V. villosa* seedling emergence decreased with increasing burial depth, it could still occur at burial depths of 10 cm. If properly used, this characteristic could be one of the most significant keys to controlling weeds in *V. villosa fi*elds, because many weed seeds are

small and their germination is completely inhibited at burial depths of 2–3 cm, while the germination of *V. villosa* was still over 85%. A neutral-pH or slightly alkaline environment was favorable for the germination of *V. villosa* seeds; thus, sprinkling quicklime powder (when appropriate) combined with a thicker covering soil may significantly increase the advantages of *V. villosa* in seed germination and seedling emergence in the early stages of population establishment. The results above will contribute to the successful planting and large-scale growth of *V. villosa* in orchards if these ecological characteristics are combined with agronomic practices. However, the results of the present study were obtained under laboratory conditions, and their applicability in the planting of *V. villosa* should be verified under field conditions.

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