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Regulatory Effects of Nursery Mode and Canopy Closure on the Establishment Survival Rate of *Tetrastigma hemsleyanum* and Delineation of the Optimal Closure Range

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Abstract To clarify the regulatory factors affecting the establishment survival rate of *Tetrastigma hemsleyanum*, cutting propagation experiments and field establishment trials were conducted to compare survival performance under different nursery modes and canopy closure conditions. The results demonstrated significant differences in survival rates among the three nursery modes, with container substrate cultivation showing significantly higher survival than direct field cuttings. Establishment survival also differed significantly across canopy closure levels, exhibiting a unimodal response pattern along the closure gradient. Quadratic regression analysis indicated that the predicted maximum survival rate occurred at a canopy closure of approximately 0.67. Based on comprehensive statistical analyses and trend fitting, the optimal canopy closure range for the establishment of *T. hemsleyanum* was determined to be 0.6–0.7. These findings provide quantitative support for understory cultivation management of *T. hemsleyanum*.

Keywords *Tetrastigma hemsleyanum*; Establishment; Nursery mode; Canopy closure; *Survival rate*

1 Introduction

Tetrastigma hemsleyanum Diels et Gilg is a perennial climbing vine belonging to the Vitaceae family and is one of the important medicinal resource plants in southern China. Its tuberous roots are rich in polysaccharides, flavonoids, and various bioactive compounds, exhibiting considerable pharmacological potential in anti-inflammatory, antitumor, and immunomodulatory applications. With increasing market demand and continuous depletion of wild populations, artificial cultivation has become an essential approach for ensuring sustainable resource utilization. Within the cultivation system, establishment represents the critical transition from nursery production to subsequent field management. The survival rate during this stage directly affects planting costs, population structural stability, and future yield potential. Forest ecological studies have demonstrated that the seedling establishment phase often constitutes a demographic bottleneck in regeneration processes, where high mortality restricts the transition of individuals to stable populations (Chang-Yang et al., 2021; Stone et al., 2025). Under closed canopy conditions, seedlings may persist in the understory for extended periods, yet only a small proportion successfully overcome early survival constraints, thereby influencing community structure and regeneration trajectories (Lin et al., 2014). Therefore, improving establishment survival is a prerequisite for large-scale cultivation and stable production.

T. hemsleyanum is commonly distributed along forest edges or in open woodland environments and is considered a typical understory-adapted species. Canopy closure, as a structural indicator of canopy coverage, directly regulates understory light intensity, light quality, and microclimatic conditions. Under closed canopies, light availability may decline to 10%~15% of full sunlight, substantially affecting seedling photosynthetic performance and carbon balance (Zhou et al., 2023). Shade-tolerant or semi-shade-tolerant species generally exhibit higher light-use efficiency under moderate diffuse light, whereas excessive irradiance may induce photoinhibition and severe shading may reduce net photosynthetic rates due to insufficient light availability (George and Bazzaz, 1999; De Lombaerde et al., 2020). Variations in canopy gap size and openness can markedly alter early seedling survival conditions; however, increased light availability may simultaneously promote rapid expansion of understory vegetation, generating an ecological filtering effect (Lu et al., 2021; Liu et al., 2022). Thus, canopy closure not only determines the light environment but also indirectly influences water balance and neighborhood competition intensity, thereby jointly affecting establishment survival. Nevertheless, quantitative studies examining the gradient response of *T. hemsleyanum* establishment survival to canopy closure and delineating an optimal closure range remain limited.

In addition to light conditions, nursery mode constitutes a key technical factor influencing establishment success. Global-scale restoration studies have shown that seedling size and root development quality significantly affect post-transplant survival (Andivia et al., 2021). Larger seedlings or those with well-developed root systems typically exhibit enhanced water uptake capacity and carbon reserves, thereby improving tolerance to drought or low-light stress (Wu et al., 2024). Container cultivation can improve substrate structure and rhizosphere aeration, contributing to root integrity and facilitating recovery after transplanting, whereas direct field cuttings are more susceptible to soil compaction and pathogen pressure. The degree of matching between seedling quality and stand conditions is therefore critical for successful establishment. However, in the cultivation practice of *T. hemsleyanum*, the combined regulatory effects of nursery mode and canopy closure have not yet been systematically compared or ecologically interpreted.

The present study focuses on the establishment survival rate of *T. hemsleyanum*, systematically comparing responses under different nursery modes and canopy closure conditions. By analyzing their regulatory effects and delineating the optimal canopy closure range, this study aims to provide a scientific basis for large-scale understory cultivation of *T. hemsleyanum* and offer reference insights for ecological suitability studies of other understory medicinal plants.

2 Materials and Methods

2.1 Study area

The experimental site was located in the Lühetang forest region of Shouchang Forest Farm, Zhejiang Province, China. The area is characterized by a subtropical humid monsoon climate, with a mean annual temperature of 16 °C~18 °C and abundant annual precipitation. The terrain consists primarily of low mountains and hills, with elevations ranging from 200 to 400 m above sea level. The soil type is red soil with slightly acidic properties and a soil depth generally exceeding 30 cm. The dominant forest types include *Cunninghamia lanceolata* plantations, *Phyllostachys edulis* (Moso bamboo) forests, natural broadleaf forests, and *Metasequoia glyptostroboides* stands, providing suitable ecological conditions for understory cultivation experiments.

2.2 Cutting propagation experiment design

2.2.1 Nursery mode settings

Cutting propagation experiments were conducted in spring 2021. Cuttings were collected from healthy 1-3-year-old mother plants. Each cutting contained 2-3 nodes, with a length of 10-15 cm and at least two retained leaves.

Three nursery modes were established:

(1) Mode I: Container cultivation in a greenhouse using a self-formulated substrate composed of peat (20%), organic fertilizer (20%), rice husk powder (10%), yellow subsoil (48%), and calcium–magnesium phosphate fertilizer (2%). Container size was 6.5 cm × 6.5 cm.

(2) Mode II: Non-woven fabric container cultivation. The substrate consisted of peat (40%), rice husk powder (10%), organic fertilizer (10%), vermiculite (10%), and perlite (25%). Container size was 5 cm × 8 cm.

(3) Mode III: Direct cutting insertion in prepared field beds in the forest, with trenches 30 cm wide and 30 cm deep.

Each nursery mode included 50 randomly assigned cuttings per replicate, with three replicates per treatment, totaling 450 cuttings.

2.2.2 Operational procedures and variable control

Cutting size, timing (mid-March to before the plum rain season in June), and routine management practices were standardized across treatments. Container seedlings were maintained under shaded conditions with substrates kept moist but well-drained. Field cuttings received equivalent irrigation management. No additional growth regulators were applied, ensuring that treatment differences primarily reflected nursery mode effects.

2.3 Field establishment experiment design

2.3.1 Plot arrangement

From April to June 2022, container-grown seedlings produced in 2021 were transplanted into four forest stand types. The site conditions were as follows:

Plot 1: Moso bamboo forest, canopy closure 0.5, quasi-wild establishment;

Plot 2: Chinese fir forest (*Cunninghamia lanceolata*), canopy closure 0.6, quasi-wild establishment;

Plot 3: *Metasequoia glyptostroboides* stand, canopy closure 0.7, cultivation in biodegradable planting bags;

Plot 4: Natural broadleaf forest, canopy closure 0.8, quasi-wild establishment.

For quasi-wild establishment, plant spacing was 0.3 m × 0.5 m. Each plot included 100 seedlings per replicate, with three replicates, totaling 1 200 seedlings.

2.3.2 Measurement of canopy closure

Canopy closure was determined by averaging measurements from multiple sampling points within each plot. At least five evenly distributed points were established per plot. Canopy cover proportion was visually estimated at each point, and the mean value was calculated as the canopy closure index for that plot.

2.4 Survival assessment and indicator definition

Survival in the cutting experiment was defined as successful root formation accompanied by resumption of new shoot growth. Establishment survival in the field was defined as seedlings maintaining normal aboveground growth without mortality until October of the same year.

Survival rate was calculated as: Survival rate (%) = (Number of surviving individuals / Total number of individuals surveyed) × 100%.

2.5 Data analysis and statistical models

2.5.1 Comparison of survival rates

Differences in survival rates among nursery modes and forest stand treatments were analyzed using contingency table chi-square (χ^2) tests. When overall differences were significant, pairwise comparisons were performed using Fisher's exact test, with Bonferroni correction applied for multiple comparisons.

2.5.2 Effect size estimation

Effect size of survival differences between treatments was quantified using Relative Risk (RR), and 95% confidence intervals were calculated to evaluate the magnitude of treatment effects.

2.5.3 Analysis of canopy closure response trends

To analyze the response pattern of survival rate to canopy closure, a quadratic polynomial model was constructed for descriptive fitting between canopy closure and survival rate. Curve visualization was used to evaluate the presence of a unimodal response pattern and to delineate the canopy closure range associated with higher survival. All statistical analyses were conducted using proportional data, with a significance level set at $\alpha = 0.05$.

3 Results and Analysis

3.1 Differences in cutting survival among nursery modes

The survival performance of *Tetrastigma hemsleyanum* cuttings under the three nursery modes is presented in Figure 1. Each mode included 50 cuttings. Mode I had an average of 47 surviving individuals, corresponding to a survival rate of 94%; Mode II had an average of 45 survivors, with a survival rate of 90%; and Mode III had an average of 35 survivors, with a survival rate of 70%.

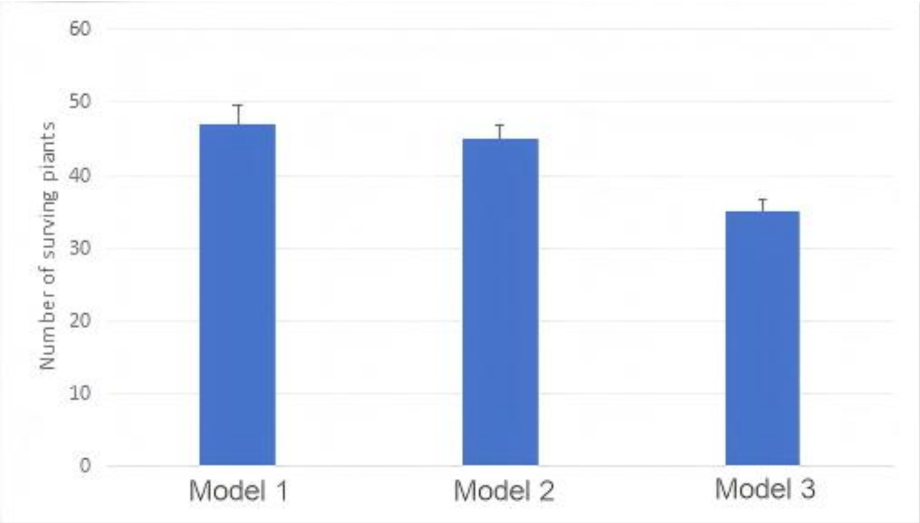

Figure 1 Survival rate under different nursery modes

The contingency table chi-square test indicated significant differences in survival rates among nursery modes ($\chi^2=12.74$, df=2, $p=0.0017$). Pairwise comparisons showed that the difference between Mode I and Mode III was significant (Fisher's exact test, $p<0.01$). The difference between Mode II and Mode III reached significance before correction ($p\approx0.02$) but was not significant after Bonferroni adjustment. No significant difference was detected between Mode I and Mode II ($p>0.05$).

Relative risk (RR) analysis showed that, compared with Mode III, both Mode I and Mode II had RR values greater than 1, with 95% confidence intervals not crossing 1. Overall, container-based nursery modes exhibited approximately a 20-percentage-point higher survival rate than direct field cutting.

3.2 Effects of Canopy Closure Gradient on Establishment Survival

The establishment survival of *T. hemsleyanum* under different forest stand conditions is shown in Figure 2. Each plot included 100 transplanted individuals. In the canopy closure 0.6 stand (*Cunninghamia lanceolata* plantation), the average number of surviving plants was 85 (85% survival); in the 0.5 closure stand (Moso bamboo forest), 60 plants survived (60%); in the 0.8 closure stand (natural broadleaf forest), 75 plants survived (75%); and in the 0.7 closure stand (*Metasequoia glyptostroboides*, biodegradable planting bags), 95 plants survived (95%).

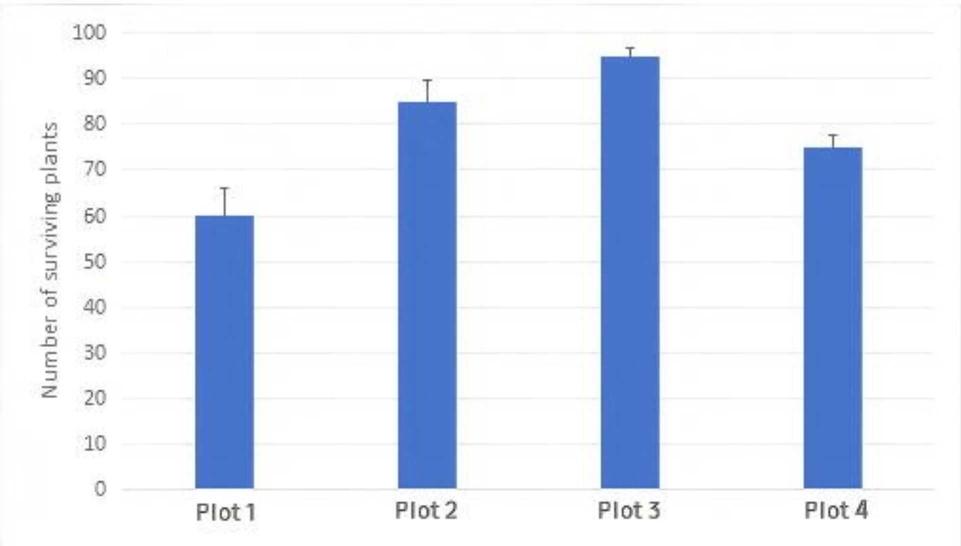

Figure 2 Establishment survival rate under different canopy closure levels

The contingency table chi-square test revealed significant differences in survival rates among the four stand conditions (χ^2=39.96, df=3, p<0.001). Survival rates ranked as follows: 0.7>0.6>0.8>0.5. Pairwise comparisons indicated that survival under canopy closure 0.7 was significantly higher than under 0.5 and 0.8 (p<0.001), and survival under 0.6 was significantly higher than under 0.5 (p<0.001). No statistically significant difference was detected between 0.6 and 0.8.

Relative risk analysis showed that the establishment survival rate under canopy closure 0.7 was approximately 1.58 times that under closure 0.5, with the 95% confidence interval not crossing 1. Overall, survival increased as canopy closure rose from 0.5 to 0.7 and declined at 0.8.

3.3 Integrated response characteristics of nursery mode and stand conditions

The stage-wise results showed that container substrate nursery modes exhibited higher survival during the cutting phase. In the field establishment experiment, survival under canopy closure levels of 0.6~0.7 was generally higher than under 0.5 or 0.8, with the highest survival rate (95%) observed at canopy closure 0.7. In contrast, survival declined under both lower and higher closure levels. These results reflect differences in establishment survival across nursery modes and stand conditions. As the experimental design did not constitute a fully factorial combination, the integrated response characteristics are based on stage-wise comparisons.

3.4 Summary of the response pattern between canopy closure and survival

To examine the relationship between canopy closure and establishment survival rate, scatter fitting was performed between canopy closure values and corresponding survival rates (Figure 3). The results showed a near-unimodal response pattern along the canopy closure gradient. Survival increased progressively as canopy closure rose from 0.5 to 0.7 and declined at 0.8.

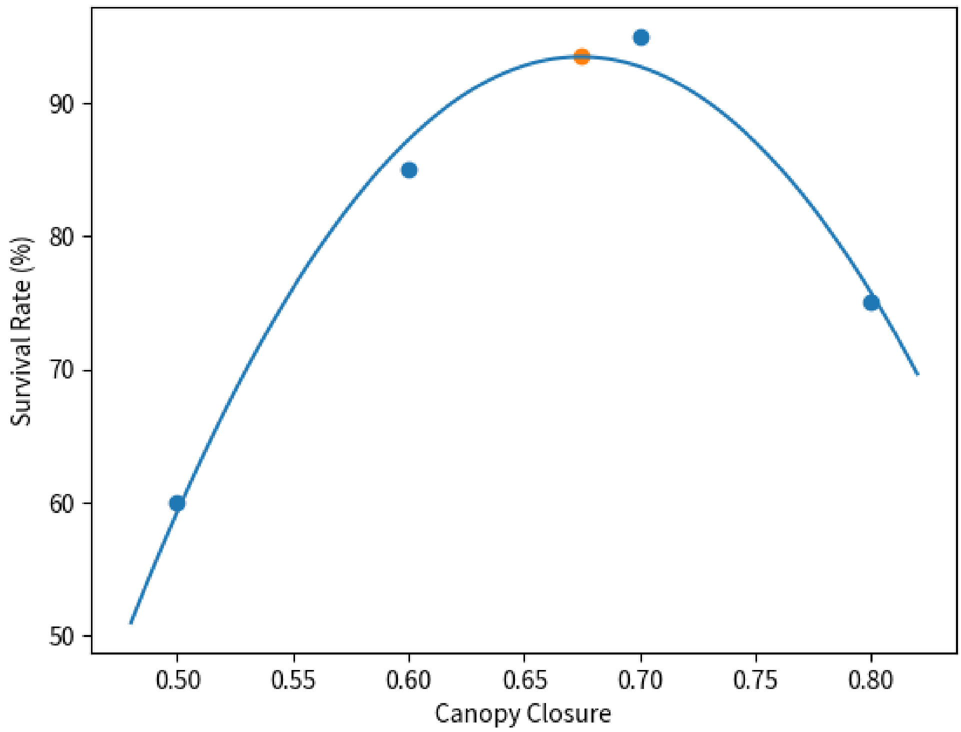

Figure 3 Response of survival rate to canopy closure

A quadratic polynomial model was applied for descriptive fitting, and the vertex of the fitted curve corresponded to a canopy closure of approximately 0.67, with a predicted survival rate of approximately 93%~94%. The fitted trend was consistent with the observed data pattern. Given that the canopy closure gradient consisted of only four discrete levels and that stand type and cultivation method differed among plots, the fitted model is primarily descriptive and used to delineate the range associated with higher survival. Based on both fitted results and observed data, the optimal canopy closure range for *T. hemsleyanum* establishment was defined as 0.6~0.7.

4 Discussion

The principal findings of this study regarding the establishment survival rate of *Tetrastigma hemsleyanum* can be interpreted within an ecological regulation framework integrating individual attributes, resource availability, and neighborhood pressure. Studies on forest regeneration have demonstrated that the transition from establishment to stable growth often constitutes a demographic bottleneck, where minor differences in individual traits and microhabitat conditions may be amplified into substantial variation in survival outcomes (Chang-Yang et al., 2021). In the present study, survival rate exhibited a pronounced nonlinear response to canopy closure, indicating that *T. hemsleyanum* is sensitive during the establishment stage to both excessive environmental stress and excessive resource limitation. This pattern is consistent with conclusions that niche differentiation and negative density dependence jointly shape seedling survival dynamics (Johnson et al., 2017). Therefore, the optimal canopy closure should be understood as a manageable operational range that reduces desiccation risk and temperature fluctuations while maintaining adequate photosynthetic returns and keeping understory competition at a moderate level. Long-term plot studies have further shown that habitat heterogeneity and density dependence exert persistent influences on seedling survival (Magee et al., 2020), providing theoretical support for the canopy closure range identified in this study.

From a mechanistic perspective, the unimodal response pattern observed here is consistent with classical interpretations of understory plant responses to light environments. Excessive shading may reduce net photosynthetic capacity and disrupt carbon balance, whereas excessive irradiance may increase transpiration and leaf temperature, leading to water deficit and photoinhibition risk. Moreover, changes in canopy structure influence ventilation and humidity conditions, thereby altering pathogen incidence. Research on understory vegetation as an ecological filter has indicated that shading and understory structural characteristics jointly regulate seedling growth and survival (George and Bazzaz, 1999). Multifactorial experiments have further demonstrated significant interactions among light, temperature, and understory cover, with combined effects often exhibiting nonlinear characteristics (De Lombaerde et al., 2020). Consequently, differences in survival under varying canopy closure levels observed in this study likely reflect the combined effects of light availability, water balance, and microclimatic stability rather than changes in light intensity alone.

Differences among nursery modes further highlight the importance of individual attributes during the establishment stage. A global meta-analysis has shown that initial seedling size and stress-resistance traits significantly affect post-transplant survival, with larger individuals generally exhibiting higher survival probabilities across most site conditions (Andivia et al., 2021). In restoration experiments conducted under extreme heat stress, initial seedling height explained survival variation more effectively than functional trait indicators (Gardiner et al., 2019). Additionally, life-history stage modulates the relative importance of neighborhood effects, with individual attributes and microhabitat conditions often playing a dominant role during early stages (Pu et al., 2020). These findings are consistent with the present results, indicating that root system quality and individual robustness developed during the nursery stage determine the capacity of seedlings to overcome the establishment bottleneck. Container-based nursery cultivation enhances rhizosphere conditions and preserves root integrity, thereby increasing tolerance thresholds to water fluctuations and shading stress and improving establishment stability.

In a broader comparative context, the conclusion that moderate canopy closure favors survival is largely consistent with observations from forest regeneration and restoration studies across multiple regions, although its applicability remains context-dependent. Research in northern coniferous forests has shown that overstory density and ground vegetation cover jointly determine seedling emergence and survival, and management must balance seedbed improvement with competition control (Kyrö et al., 2021). For *T. hemsleyanum*, an understory medicinal vine, the objective of establishment emphasizes stable survival and subsequent tuber development. Its sensitivity to strong irradiance and excessive moisture conditions may differ from that of typical tree regeneration. Therefore, under similar canopy closure levels, variation in soil moisture, ventilation, and understory vegetation structure among forest types may lead to different survival outcomes, representing an important source of inter-plot variability.

It should be noted that the canopy closure gradient in this study was relatively limited and did not constitute a fully factorial design. Accordingly, inferences regarding potential interactions between nursery mode and canopy closure should be made with caution. Future studies should refine canopy closure gradients under controlled forest-type conditions and incorporate high-resolution monitoring of microclimate and soil moisture to further disentangle the relative contributions of light, water availability, and competition. Long-term follow-up experiments are also necessary to verify whether the identified optimal establishment range can be consistently translated into subsequent growth performance and population structural advantages, thereby enhancing both theoretical interpretation and practical management guidance.

5 Conclusion

This study systematically analyzed differences in establishment survival of *Tetrastigma hemsleyanum* under different nursery modes and canopy closure conditions. The results demonstrated that nursery mode significantly affected establishment survival. Container-based substrate cultivation resulted in higher survival rates, indicating that initial seedling quality and root system development are fundamental factors determining establishment stability. Optimization of nursery practices enhances seedling adaptability to understory environments.

Canopy closure exhibited a significant gradient effect on establishment survival, with survival rates displaying a unimodal response pattern along the closure gradient. Based on statistical testing and response curve analysis, the optimal canopy closure range for *T. hemsleyanum* establishment was determined to be 0.6–0.7. Within this range, understory light and microclimatic conditions were relatively balanced, and establishment survival remained comparatively stable.

Overall, establishment success of *T. hemsleyanum* is jointly regulated by seedling quality and stand structure. Developing establishment strategies that align nursery practices with appropriate canopy closure conditions represents an effective technical pathway for improving the stability of large-scale understory cultivation. By delineating the optimal canopy closure range from an ecological regulation perspective, this study provides quantitative support for understory cultivation management of *T. hemsleyanum*.

Author Contributions

Li Jianhui and Xu Yonghong designed and conducted the experiments. Li Jianhui, Zhang Yehua, and Xu Yonghong performed the data analysis and drafted the initial manuscript. Fang Yumin and Fan Jianzhong contributed to the experimental design and analysis of the experimental results. Xu Yonghong conceived and led the project and supervised the experimental design, data analysis, manuscript writing, and revision. All authors have read and approved the final version of the manuscript.

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