

house without fogging and a greenhouse without ventilation but with fogging gave the best rooting, root growth, and survival, whereas greenhouse environments with ventilation had poorer performance and in one case higher temperatures. Heated mini-tunnels likewise improved winter rooting and physiological performance in *Eucalyptus dunnii* by protecting plants from low temperatures and maintaining adequate humidity, while supplemental lighting served mainly to overcome seasonal constraints rather than replace environmental conditioning (Schilisting et al., 2025). Practical regulation should therefore use shade nets or protected structures to buffer radiation and temperature, maintain gentle ventilation sufficient to renew air, and gradually increase light exposure and air movement only after rooted cuttings begin active leaf expansion (Xue et al., 2023; Shen et al., 2025).

5.3 Nutrient supply and pest and disease control during the seedling stage

After initial callus and root formation, nutrient supply becomes necessary to sustain the transition from root induction to seedling growth, but fertilization should remain balanced and stage-specific because excessive or poorly matched nutrition can impair seedlings rather than improve them (Pascual et al., 2018). In mulberry rapid propagation, only clean water was used during the first four days after insertion, after which Hoagland nutrient solution was introduced and replaced every five days in summer, illustrating the principle that nutrient supply should follow rather than precede early rooting stabilization (Shen et al., 2025). Fertilization can substantially improve cutting and seedling growth when correctly managed: NPK at 20~30 g/L increased rooting and sprouting of apical forest-tree cuttings, moderate N and P improved cocoa cutting growth, and balanced mineral nutrition is repeatedly identified as a determinant of adventitious rooting and survival (De Almeida et al., 2017). Substrate-linked nutrient strategies also matter. Cocopeat improved aeration and water-holding capacity over sterilized soil in clonal forestry cuttings, cattle-manure-containing substrates improved seedling growth and nutrient accumulation in African mahogany, and bio-inoculants such as AMF or PGPR enhanced P, K, Ca, and chlorophyll in rooted hazelnut cuttings, although nitrogen remained limiting when N supply was inadequate (Kamara et al., 2025).

Pest and disease control during the seedling stage should emphasize prevention, sanitation, and environmental regulation, because contaminated cuttings, substrates, irrigation water, and poorly ventilated wet nursery conditions allow pathogens to spread early and persist into field planting. Integrated management begins with healthy propagules and clean infrastructure: cuttings can carry surface microorganisms, so disinfection of plant material, tools, hands, containers, and substrates is essential, and contaminated media may require steam or other disinfestation measures (Shen et al., 2025). Disease suppression can also be supported biologically and culturally, since compost-based substrates may provide pathogen suppressiveness, beneficial microorganisms can improve nutrition and resistance, and irrigation, humidity, storage, and nursery hygiene all directly affect seedling health (Pascual et al., 2018). Nursery production should therefore include routine removal of diseased seedlings and residues, maintenance of irrigation-water quality, controlled access and operation records, and balanced nutrition that avoids weakening seedlings or predisposing them to infection (Ventura et al., 2019).

6 Construction of a Standardized Seedling Production System for *Tetrastigma hemsleyanum*

6.1 Hardening, transplanting, and establishment management of rooted cuttings

Hardening of rooted cuttings should proceed by gradual acclimatization, not abrupt transfer, because newly rooted propagules often suffer transplant shock before they become fully coupled to the field environment (Grossnickle and Macdonald, 2018). In carob, rooted cuttings were transplanted into sterilized sand:soil substrate and kept under rooting-phase conditions for four weeks before relative humidity was progressively reduced to normal greenhouse conditions, showing a practical model for staged acclimation (Essahibi et al., 2017). Comparable medicinal-plant studies also show that post-rooting hardening can determine final survival, with rooted *Aphanamixis polystachya* reaching 72% survival after hardening and micropropagated *Alangium salviifolium* achieving 60% transfer to soil after greenhouse acclimatization (Pandey et al., 2022; Gurav et al., 2025). By contrast, *Ruta chalepensis* plantlets survived at 95.2% after effective hardening, indicating that acclimatization quality can sharply change establishment outcomes even when rooting has already occurred (Qahtan et al., 2021).