Effects of Plant Gender, Cutting Age, and Propagation Medium on Rooting and Growth of *Juniperus communis* Cuttings

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Abstract

Juniperus communis L. is a slow-growing, dioecious conifer of increasing ecological and commercial value, but large-scale production is limited by slow, erratic adventitious rooting. The present study examined the influence of cutting age, plant gender and propagation substrate on survival, and growth of *J. communis* stem cuttings under greenhouse conditions. Shoots were taken from male and female stock plants and divided into one-year-old and two-year-old cutting material. Two-year-old cuttings were propagated in rockwool, pumice, peat: perlite (1:1, v/v), and vermiculite: perlite (1:1, v/v) mix, while one-year-old cuttings were propagated in pumice and vermiculite: perlite (1:1, v/v) mix. Survival, root number, root length, and shoot length were assessed over a nine-month period.

Plant gender, propagation substrate and their interaction had significant effects on survival at three, five and nine months after planting in two-year-old cuttings. Overall, well-aerated substrates, particularly pumice and rockwool, consistently promoted better rooting performance than vermiculite:perlite. Male cuttings propagated in pumice and rockwool achieved the highest survival rates (80% and 84%, respectively), whereas female cuttings grown in vermiculite:perlite had consistently low survival (12%). Root number was the most responsive growth parameter, with male cuttings in rockwool and female cuttings in pumice producing the highest root numbers.

Cutting age influenced the response to both plant gender and substrate. In two-year-old cuttings, survival and root number were significantly affected by gender, substrate, and their interaction. In contrast, one-year-old cuttings showed more uniform rooting responses, with few significant effects of gender or substrate. Root length in one-year-old cuttings was greater in pumice (82.82 mm) than in vermiculite:perlite (64.96 mm) after five months of propagation.

Overall, plant gender and propagation substrate had greater effects on rooting performance in two-year-old than in one-year-old cuttings. Well-aerated substrates, particularly pumice and rockwool, promoted more favourable rooting than vermiculite:perlite, while one-year-old cuttings exhibited more consistent propagation outcomes. These findings demonstrate that cutting age, plant gender, and substrate selection should be considered jointly when developing vegetative propagation protocols for *J. communis*. Developing reliable vegetative propagation methods will support its ecological restoration, conservation, and commercial cultivation.

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CHAPTER 1 – INTRODUCTION

1.1 Introduction

Juniperus communis L. is a slow-growing, long-lived, dioecious coniferous species, which is widely distributed in temperate and boreal regions of the Northern Hemisphere (Nowak-Dyjeta et al., 2017; Markó et al., 2021). The species is important for natural ecosystems and is also becoming economically significant in pharmaceuticals, cosmetics and, most importantly, the global gin industry, where juniper cones (berries) are an essential ingredient (Judžentienė, 2019). The wild population of *J. communis* has been under pressure due to the increasing demand for juniper berries, and concerns have been raised about the sustainability of this resource, genetic diversity and conservation (Markó et al., 2021).

Seed germination *in J. communis* is frequently slow and inconsistent. The seeds can exhibit deep physiological dormancy, a long stratification period, and poor germination rates (Roşca, 2009). Seedling establishment can be slow, and seedling mortality can be high, particularly when environmental conditions change (Houle & Babeux, 1994). Mycorrhizal associations may also be important for the establishment of conifer seedlings, as they enhance nutrient and water uptake. These factors restrict the use of seed propagation for commercial-scale production, restoration and cultivation systems.

Consequently, vegetative propagation has become an important alternative propagation method for *J. communis*. Propagation by stem cuttings can result in clonal plants, preserve desirable characteristics, and prevent problems associated with seed dormancy and seedling variability (Hazubska-Przybył, 2019). Large-scale clonal propagation can, however, reduce genetic diversity if only a small number of mother plants are used; this risk can be minimised by collecting cuttings from genetically diverse stock plants (Hartmann et al., 1990). *J. communis* is regarded as difficult to propagate, although it has potential. In previous research, delayed adventitious root formation and extended rooting periods, as well as low and variable rooting responses, have been reported in *J. communis* cuttings, even under greenhouse or nursery conditions and with the application of exogenous auxins, such as indole-3-butyric acid (IBA) (Hartmann et al., 1990; Ragonezi et al., 2010; Hazubska-Przybył, 2019).

The success of rooting in *J. communis* depends on the interaction of various physiological and environmental factors, such as cutting maturity, collection time, endogenous hormone status, auxin treatment, and the physical and chemical properties of the propagation medium (Hazubska-Przybył, 2019). Although previous studies have examined factors such as auxin application, cutting type, and propagation substrate, limited information is available on the combined effects of plant gender, shoot age, and propagation substrate on the rooting performance of *J. communis* cuttings. Given these constraints, it is important to explore how cutting age, plant gender, and propagation substrate interact in the vegetative propagation of *J. communis* in a controlled greenhouse environment. Knowledge of these factors is crucial for enhancing rooting success, early growth, and survival, as well as for developing more reliable propagation methods for commercial production and conservation.

1.2 Physiology of Rooting in Conifers

The formation of adventitious roots in woody plants such as *J. communis* is a complex and energy-intensive process. It includes wound response, callus formation, meristematic activity, initiation of root primordia and root elongation. The processes can take a long time before the conifer cutting produces functional roots, during which it utilises stored carbohydrates (Villar-Salvador et al., 2015). Thus, the availability, distribution and metabolic activity of carbohydrates are significant for rooting success.

The relationships among growth, storage, and stress responses in *Juniperus species* are closely linked to long-term carbon balance (Villar-Salvador et al., 2015; Druege et al., 2019). This is especially crucial in vegetative propagation, where cuttings are abruptly removed from the mother plant, and thus have less water available and may be less efficient at photosynthesis and metabolism. In such instances, rooting will be induced not only by hormones but also by the cutting's capacity to sustain respiration and cell differentiation over time (Druege et al., 2019).

1.3 Constraints in Conifer Rooting

Environmental factors and intrinsic biological factors related to gymnosperm physiology limit adventitious rooting in conifers. Conifers are also known to have reduced rooting competence due to early tissue maturation, limited capacity for dedifferentiation, and developmentally regulated cell reprogramming (Steffens & Rasmussen, 2016; Díaz-Sala, 2020).

These biological limitations contribute to the slow and often variable rooting responses reported in *J. communis*. As a result, successful propagation frequently requires extended rooting periods and carefully controlled environmental conditions to support root initiation and development (Schoonmaker et al., 2021).

1.4 Key Context and Rationale

In *J. communis,* vegetative propagation is a viable alternative to seed propagation, but rooting success is influenced by multiple interacting biological and environmental factors. Rooting response may be affected by the following factors: cutting age, plant gender, and propagation substrate, especially in slow-rooting conifers, where cuttings need to remain viable for longer before functional roots form (Hazubska-Przybył, 2019).

Short-term assessments may underestimate the rooting potential of *J. communis*, as delayed rooting and callus development have been reported in this species (Abshahi et al., 2022). Thus, assessment over a period of months is necessary to determine survival, root development and early shoot growth. Substrate conditions are also important, as moisture-holding capacity, aeration, and physical structure can influence root initiation and elongation.

The vegetative propagation of *J. communis* is an important area of research to reduce pressure on wild populations and to ensure a reliable supply of plant material for commercial and conservation use. Therefore, this study investigated the effects of cutting age, plant gender and propagation substrate on survival, adventitious rooting and early shoot growth of *J. communis* cuttings under controlled greenhouse conditions.

1.5 Research Aim

This study aimed to investigate the effects of cutting age, plant gender and propagation substrate on rooting success, root development and early shoot growth of *J. communis* stem cuttings under greenhouse conditions.

1.6 Research Objectives

1. Evaluate the effect of cutting age (one-year and two-year-old shoots) on rooting success and root development of *J. communis cuttings*, considering that the maturity of the propagating tissue affects adventitious rooting.
2. Assess the effect of plant gender (male and female) on rooting success, root development, and early plant growth.
3. Evaluate the effects of different propagation substrates, having distinct physical and chemical characteristics, on rooting and early vegetative growth of *J. communis* cuttings, especially under prolonged rooting periods.
4. Explore potential interaction effects between cutting age, plant gender and propagation substrate.

1.7 Hypothesis

It was hypothesised that cutting age, plant gender, and propagation substrate would influence adventitious rooting, survival and early shoot growth of *J. communis* cuttings, both as individual factors and through their interactions. The younger physiological condition and lower tissue lignification of one-year-old cuttings were expected to result in higher rooting capacity and more uniform root development than in two-year-old cuttings. Differences in resource allocation, stored reserves, and physiological condition between male and female cuttings were also expected to lead to differences in rooting and early growth responses. Furthermore, propagation substrates with a favourable moisture retention-aeration balance were expected to result in higher survival, root initiation, and root elongation than substrates with a less favourable air–water balance. Thus, the general hypothesis was that the main and interaction effects of cutting age, plant gender and propagation substrate would significantly affect the rooting and early growth performance of *J. communis* cuttings.

1.8 Research questions

1. 1. What are the effects of cutting age, plant gender and propagation substrate on survival, rooting, root development and early shoot growth of *J. communis* cuttings?
2. Which propagation substrate improves rooting response in male and female *J. communis* cuttings?
3. How does cutting age affect the survival, rooting and early growth of *J. communis* cuttings?

CHAPTER 2 – LITERATURE REVIEW

2.1 Challenges of Seed Propagation in *Juniperus communis* L.

Juniperus seed propagation is known to be inefficient and unpredictable due to physiological, ecological and environmental factors (Gruwez et al., 2014; Thomas et al., 2007). *J. communis* seeds are usually deeply physiologically dormant, requiring a long cold stratification period to break dormancy and promote germination. Even with stratification, germination percentages tend to be low and quite variable within and among populations (Roșca, 2009). Beyond dormancy-related issues, *J. communis* seed viability is often diminished by immature embryo development, predation and fungal infection, which can result in reduced germination (Houle & Babeux, 1994). Consequently, a large number of seeds are required to produce a limited number of seedlings, making seed propagation inefficient and potentially expensive for commercial or restoration applications.

Another constraint in the life cycle of *J. communis* is seedling establishment. Although germination is often successful, seedlings have poor early growth and are highly vulnerable to environmental pressures, such as drought, extreme temperatures, and competition (Ward, 1982; Verheyen et al., 2009). Seeding mortality has been reported at high rates in the wild and in nurseries, especially in the early years of growth (Gruwez et al., 2016). This greatly limits the feasibility of using seed propagation systems for commercial production. Seed-based propagation also presents problems for conservation and industry practices regarding genetic uniformity and predictability (Thomas et al., 2007; Gruwez et al., 2014). Plants derived from seed exhibit a high degree of genetic variability, which may be undesirable when uniform growth rates, chemical profiles, and/or fruit quality are required, such as in managed cultivation systems feeding into the gin industry. Additionally, collecting more seeds from wild populations may put further pressure on already limited populations.

These challenges demonstrate that seed propagation cannot be the sole approach for *J. communis* planting stock production. The limitations of dormancy, germination, seedling survival and genetic variability suggest the need for alternative propagation methods such as vegetative propagation.

2.2 Stem Cuttings Propagation

Stem cutting propagation has been extensively studied as an alternative to seed propagation in *J. communis* due to problems with sexual reproduction (Jesus et al., 2020). Cutting propagation is a promising method for producing genetically identical plants and maintaining desirable characters such as growth forms and chemical profiles, thereby reducing reliance on wild seed sources (Edson et al., 1996). Thus, stem-cutting propagation is a potential method for the conservation, restoration, and commercial production of *J. communis*.

However, *J. communis* is often reported as difficult to root (Hazubska-Przybył, 2019). Research on *Juniperus* species reports delayed adventitious root formation, lengthy rooting periods (often more than several months), and low or inconsistent rooting percentages, even under nursery or greenhouse conditions (Abshahi et al., 2022). These traits set *J. communis* apart from many woody species and pose challenges for its vegetative propagation. Several factors interact to affect the rooting of *Juniperus* cuttings. The type of cuttings and the physiological status of the mother plant are key factors, as tissue maturity influences the capacity for root formation (Ragonezi et al., 2010). *Juniperus* cuttings are often semi-hardwood type because they are less susceptible to desiccation and damage than softwood cuttings, while still being able to initiate adventitious roots (Goncharenko et al., 2020). However, even within semi-hardwood material, considerable variation in rooting can occur depending on the age of the shoot and the position from which the cutting is taken.

The time of year when cuttings are collected also influences rooting in *Juniperus*. Seasonal changes affect carbohydrate and hormone levels and metabolic processes, which in turn affect rooting ability. Higher survival rates for cuttings taken in cooler seasons have been observed, but root initiation can still be slow and delayed (Denaxa et al., 2021). The higher survival observed during cooler conditions may be associated with seasonal temperature effects and winter chilling, which have been reported to influence the rooting potential of some Juniperus cuttings (Basham, 1981). This underscores the need to assess rooting responses over a longer period and not just in the short term. The use of exogenous auxins, such as indole-3-butyric acid (IBA), is a common practice to improve the rooting of *Juniperus* cuttings. Although auxin application may enhance rooting percentage and root growth, responses are variable and can be influenced by interactions with cutting maturity, substrate properties and environmental factors (Hazubska-Przybył, 2019; Abshahi et al., 2022). As a result, exogenous auxins are insufficient to overcome rooting problems in *J. communis*.

Substrate is also an important factor in rooting. The physical properties of the substrate, including aeration, water-holding capacity and oxygen levels at the stem base, play a critical role in rooting (Bunt, 1988; Raviv et al., 2019). In *Juniperus* species, highly porous, well-drained substrates are reported to be more suitable for root development over time. Conversely, substrates with low air content can limit rooting, even with the addition of auxins (Cope et al., 2013).

In summary, previous studies show that stem cuttings of *J. communis* can be used for vegetative propagation, but are variable and responsive to several factors (Blythe et al., 2007; Druege et al., 2019). This highlights the need for comprehensive research into the interactions between cutting age, plant gender and substrate under controlled conditions, rather than isolating variables.

2.3 Effects of Plant Gender on Growth and Reproduction

Juniperus communis is a dioecious species, with male and female reproductive organs found on different plants. Dioecy in woody plants is frequently accompanied by plant gender-specific growth, physiology and allocation traits. Many ecological and physiological studies have reported gender-specific variation in plants of *Juniperus species*, including photosynthetic capacity, carbon (C) allocation, secondary metabolism, and responses to environmental stressors (Nowak-Dyjeta et al., 2017; Markó et al., 2021; Tian et al., 2022).

Juniperus females typically experience greater reproductive demands for producing conifer cones and seeds, which can affect vegetative performance and non-structural carbohydrate allocation. In *J. communis* and other species, females have been found to allocate more resources to reproduction and storage, while males sometimes show increased growth under resource-poor or stressful environments (Iszkuło et al., 2011; DeSoto et al., 2016). Such allocation patterns can affect subsequent growth of cuttings after collection for propagation.

The storage of carbohydrates and secondary growth also vary between male and female *Juniperus*. Studies show that plant gender-related differences in non-structural carbohydrate concentrations may influence regrowth and stress tolerance, and could affect the survival of cuttings during the rooting process (Vizcaíno-Palomar et al., 2014). Adventitious root production is costly, and variation in internal resource supply between male and female cuttings may contribute to differences in rooting and initial growth. Although considerable evidence exists for physiological differences between plant genders in *Juniperus species*, the impact of plant gender on plant propagation remains to be clarified. Most propagation studies do not consider plant gender, and those that have found mixed results.

Crucially, plant gender-related differences in *Juniperus* are not always expressed, and may depend on environmental conditions and physiological stage (Nowak-Dyjeta et al., 2017; Markó et al., 2021). Plant gender differences in growth and physiology may be diminished or obscured under controlled greenhouse or nursery conditions, but may be more pronounced in variable or stressful environments (Marion & Houle, 1996). This context dependency suggests that it is essential to explicitly test the effects of plant gender in combination with other critical factors on propagation success. The inclusion of plant gender as a factor in the current study provides an opportunity to evaluate potential differences between male and female *J. communis* cuttings in rooting success and early growth under equally favourable propagation conditions.

This will enable objective assessment of both direct effects of plant gender and interactions with cutting age and substrate used in propagation, thereby filling a gap in the available propagation literature.

2.4 Propagation Substrate

The composition of the substrate is one of the most important factors influencing the development of adventitious roots, as it shapes the physical and chemical environment around the cutting base. Substrate properties such as air space, water-holding capacity, drainage, oxygen availability, and chemical stability (Sun et al., 2008) may affect rooting in *Juniperus* species. The slow, prolonged rooting process of *J. communis* requires the substrate to maintain an appropriate moisture-to-air balance for an extended period to ensure the survival of cuttings and the initiation of root formation (Güney et al., 2021).

Good drainage and high porosity are preferred in substrates used for rooting woody and conifer cuttings, as they help minimise anoxia at the base of the cuttings (Soffer & Burger, 1988; Raviv et al., 2019; Hartmann et al., 2011). Even with the application of auxins, low substrate aeration can limit oxygen supply, reduce respiration and limit root initiation (Abshahi et al., 2022). High water-holding-capacity substrates, on the other hand, can help keep cuttings hydrated during early propagation but can limit root growth if oxygen diffusion is restricted during long-term mist propagation (Jackson, 1985; Drew, 1997). The substrates used in the present study were chosen to represent contrasting physical conditions relevant to cutting propagation. Rockwool was added as a stable inert substrate with good porosity. Pumice was added as a mineral substrate to improve drainage, aeration, and structural stability. Peat: perlite (1:1, v/v) was used as one of the most frequently used organic–inorganic propagation media, a mixture of moisture-holding peat and aerating perlite. Vermiculite: perlite (1:1, v/v) was added to provide higher moisture retention with added aeration so that comparisons could be made between the wetter and more aerated rooting environments.

Propagation substrates for *Juniperus* species can be inert, mineral, organic or mixed media, including rockwool, pumice, peat-based, and vermiculite-based mixes (Hartmann et al., 2011; Bunt, 1988). Rockwool and pumice can offer high air-filled porosity and structural stability, which can help to keep oxygen available for extended periods of rooting (Gruda, 2012). Peat- and vermiculite-based substrates can hold water, potentially promoting cutting hydration, but their water-holding capacity needs to be carefully managed to prevent reduced aeration (Yafuso et al., 2019).

Cole and Dunn (2002) evaluated expanded polystyrene beads as a substitute for perlite in peat-based rooting media for woody ornamentals, such as *Juniperus*. They determined that expanded polystyrene could be used to replace perlite in some media. However, rooting responses varied among species and were lower in Juniperus than in similar formulations containing perlite, with fewer, shorter roots. This indicates that the choice of rooting media should not be based solely on physical substitution, as particle structure, stability, and contact with the cutting base may influence air and water relations during rooting. In addition to the physical structure, the chemical characteristics of the substrate, including salinity, pH and nutrient buffering capacity, can also affect cutting survival during long rooting periods (Abad et al., 2001; Gruda, 2019). Nutrient uptake is restricted during early root development, but chemically stable, low-salinity media can minimise stress and prevent damage to cutting tissues (Gupta et al., 2023).

Substrate effects are well known, but only a few studies have compared multiple substrates under the same greenhouse conditions for *J. communis* (Sanz Gallego et al., 2025). Furthermore, the effects of the substrate are often treated independently of other biological factors, such as cutting age and plant gender. This restricts the knowledge of the interaction between the rooting medium and the physiological state of the cutting (Ragonezi et al., 2010). Thus, the present study enabled comparison of substrates with different physical properties to determine which conditions would promote survival, root development, and early growth of *J. communis*, and whether these responses interacted with stock plant age and plant gender.

2.5 Interactions between Biological and Environmental Factors

Although the effects of various factors on adventitious root development in *Juniperus* spp. have been well described, little is known about the combined effects of biological factors and rooting substrates during long-term rooting periods. Biological factors are also important in determining rooting potential, particularly in woody conifers, where shoot lignification is age-related (Hazubska-Przybył, 2019). Differences between shoot tissues of one-year-old and two-year-old plants may therefore affect root initiation. The propagation substrate can also interact with these biological factors, thereby affecting gas diffusion, water content, and mechanical stability around the cutting base (Soffer & Burger, 1988; Abshahi et al., 2022). Substrates with high air-filled porosity can provide aerobic conditions for root initiation during long rooting periods. On the other hand, substrates with high water-holding capacity can cause anaerobic stress when oxygen levels are reduced, thereby reducing respiration and root initiation (Abshahi et al., 2022). The effect of the substrate may differ between cutting types because physiological differences associated with cutting age may affect sensitivity to oxygen and water availability.

Plant gender is another source of biological variability that has received little attention in propagation studies of *J. communis*. Male and female plants may differ in growth, carbohydrate reserves and stress responses, suggesting different resource allocation strategies with respect to reproduction (DeSoto et al., 2016; Nowak-Dyjeta et al., 2017; Markó et al., 2021). These differences may affect vegetative propagation, particularly if cuttings take a long time to root and must rely on their own reserves for metabolism until roots are established. However, the effect of plant gender is rarely discussed in propagation research, and when it is, the results can vary or be context-dependent (Khoshhal Sarmast et al., 2019; Sanz Gallego et al., 2025). There is some evidence that gender differences in vegetative performance may be less evident under controlled greenhouse conditions, while other evidence suggests that gender differences may be expressed in interaction with environmental or substrate conditions (Khoshhal Sarmast et al., 2019; Sanz Gallego et al., 2025). This context specificity is a reason to test plant gender alongside other propagation factors in a combined experimental design.

Taken together, these findings indicate that the rooting of *J. communis* plants cannot be explained by a single factor alone. Instead, propagation responses can be influenced by interactions among cutting age, internal resource state, plant gender, and the physical properties of the rooting medium, particularly during the extended rooting period characteristic of this species (Hazubska-Przybył, 2019; Sanz Gallego et al., 2025). Multifactor experiments are therefore important for improving the understanding and application of vegetative propagation in *J. communis*.

2.6 Aeration-Moisture Balance in Rooting Medium

The physical environment of the basal zone of the woody cutting plays an important role in adventitious rooting, especially the interaction between medium aeration and moisture (Soffer & Burger, 1988). Under intermittent mist propagation, the surface of the cutting foliage is kept wet to minimise transpiration; however, the rooting medium needs to be well aerated to prevent anoxia at the base of the cutting (Leakey et al., 1990; Ofori et al., 1996). Reinl et al. (1991) found that high moisture content in the rooting medium increased survival and percentage of rooted cuttings of the woody species *Juniperus* because of improved water status and xylem water potential of the cuttings. However, they also concluded that increased moisture levels can lead to decreased oxygen levels because water and air compete for substrate pores.

This research demonstrates that the suitability of a substrate for conifer rooting depends not only on the components present but also on the medium's ability to maintain steady-state contact and water potential at the base of the cutting while maintaining air passages. Reinl et al. (1991) also illustrated the importance of controlled and properly documented moisture regimes when assessing rooting performance in propagation substrates. This makes a clear case for reporting substrate preparation, wetting and moisture regimes when assessing rooting performance in propagation substrates.

2.7 The Biology of Adventitious Rooting in Conifers

Internal physiological and external propagation factors influence adventitious rooting in conifers. Adventitious root formation in woody plants is typically considered a multi-phase process, including induction, initiation, and expression (Bellini et al., 2014; Druege et al., 2016; Ragonezi et al., 2010). The induction phase involves molecular and biochemical reprogramming before the appearance of visible roots. This is followed by the initiation phase, during which cell division and root primordia organisation take place, and the expression phase, when root primordia elongate and emerge as adventitious roots (Bellini et al., 2014; Druege et al., 2016; Ragonezi et al., 2010).

The transition from one phase to another may be slow and incomplete in conifers, leading to delayed root emergence, reduced root number, and early cessation of root growth. Gymnosperm physiology, such as tissue maturation, decreased cellular plasticity and reliance on stored carbohydrates during the early rooting period, can limit conifer adventitious rooting (Ragonezi et al., 2010; Druege et al., 2016; Díaz-Sala, 2019). These limitations account for the lower and more variable rooting potential of many conifer species compared with many angiosperm species. Zavattieri et al. (2016) reported that endogenous hormones, carbohydrate status, explant maturation, temperature, rooting environment, and biological factors, such as microbial partners, interact to influence conifer rooting. Thus, rooting may be slow, variable or incomplete even if cuttings are treated with auxin and propagated under controlled conditions. Conifer propagation studies also show that poor root development and early termination of root growth are important factors limiting the success of vegetative propagation in conifers (Zavattieri et al., 2016).

These biological limitations are directly applicable to *J. communis*, which is known for slow and variable rooting. Rooting takes a long time, and early root emergence is not a good indicator of successful rooting. Instead, root number, root length, survival, and shoot growth should be assessed over time to determine whether cuttings are progressing through root induction, initiation, and expression. This is also why longer assessment times (three, five and nine months after planting) are used to assess rooting performance in slow-rooting conifers.

The internal constraints of conifer rooting also indicate the need to take into account cutting-related factors such as stock plant age, cutting health and plant gender, as well as external propagation factors. These variables may affect the physiological activity of cuttings and their ability to initiate and express adventitious roots. Thus, the present study investigated the rooting of *Juniperus communis* with respect to both biological and propagation substrate factors under controlled greenhouse conditions.

2.8 Seasonal Collection and Multiple Parameter Rooting

The time of year when cuttings are collected is a known factor affecting adventitious rooting in woody plants. McCrary (1980) evaluated seasonal rooting in 10 woody ornamental species, including shore juniper, ‘Bar Harbour’ juniper and red cedar. Rooting was assessed using various parameters such as rooting percentage, root quality grade, root ball diameter and root dry weight. This indicates that rooting performance can be better understood by measuring multiple rooting parameters. McCrary (1980) found that species varied in their seasonal rooting response. Shore juniper rooted best from spring cuttings, whereas ‘Bar Harbour’ juniper rooted best from winter hardwood cuttings. Rooting of red cedar was poor. These results suggest that even among coniferous species, rooting response may differ depending on species and season of cutting collection.

More recent juniper propagation studies also indicate the need for multiple rooting parameters. Abshahi et al. (2022) investigated stem cuttings of *Juniperus sabina* L. Across different seasons, IBA treatments and substrates were assessed, and rooting percentage, root biomass, and specific root length were measured. This shows that the success of rooting should be judged not solely by the cuttings' ability to root, but also by the quality and development of the root system. Thus, the timing of the season and the type of rooting measurements are critical when assessing slow-rooting conifers such as *J. communis*. A multiparameter approach and a longer observation period can give a better understanding of rooting performance than a single short-term rooting percentage.

2.9 Medium, Plant Gender and Auxin Interactions

Rooting medium, plant gender, and auxin treatment may interact to affect *J. communis* propagation. Sanz Gallego et al. (2025) conducted a recent greenhouse study to assess the impact of rooting medium, plant gender and indole-3-butyric acid (IBA) on vegetative propagation in *J. communis*. They studied male and female cuttings with either 0 ppm IBA or 4000 ppm IBA. Two rooting media were tested: rooting medium I (RM_I), a 50:50 mixture of professional substrate and perlite, and rooting medium II (RM_II), a 65:25:10 mixture of unfertilised blonde Peat, professional substrate, and Perlite.

Sanz Gallego et al. (2025) found that rooting percentage and number of primary roots were affected by rooting medium, plant gender and IBA treatment. The average rooting percentage of female cuttings was higher than that of male cuttings, 29.69% and 19.90%, respectively. Rooting medium also affected rooting success, with RM_II having a higher average rooting percentage than RM_I (28.89% vs. 20.70%). The number of primary roots was also greater in RM_II than in RM_I, with mean values of 7.46 and 6.04 roots per cutting, respectively. There was a significant interaction between rooting medium and IBA treatment for rooting percentage. The highest rooting percentage was achieved with female cuttings treated with 4000 ppm IBA and propagated in RM_II, at 45.57%. This is significant because it suggests that auxin action in *J. communis* is not independent of the rooting environment; rather, the rooting medium influences cuttings' response to auxin.

Other *Juniper* species have also been reported to have interaction-based responses. Abshahi et al. (2022) investigated the effect of IBA concentration, substrate and season on the rooting of stem cuttings of *Juniperus sabina L.* They used five IBA concentrations (0, 1000, 2000, 4000, and 8000 ppm), four substrates (perlite, perlite–cocopeat, pumice, and mixed substrate), and four seasons (winter, spring, summer, and autumn) in their experiment. The highest rooting percentage was achieved with spring cuttings treated with 1000 ppm IBA and propagated in perlite–cocopeat (1:1), at about 62%. Güney et al. (2021) also showed that the rooting success of juniper cuttings was influenced by auxin treatment and greenhouse temperature. They investigated the propagation of *Juniperus communis* 'Hibernica', *Juniperus chinensis* 'Stricta', and *Juniperus chinensis* 'Stricta Variegata' from cuttings under two greenhouse temperature regimes and various auxin treatments. They determined the rooting percentage, callus percentage, root length, and number of roots, and found that rooting responses varied with the propagation environment and auxin treatment.

These studies indicate that rooting in *junipers* is not affected by a single factor alone, but by a combination of plant material, auxin treatment, rooting medium, season and greenhouse environment. Thus, propagation research on *J. communis* should take into account biological and environmental interactions. Previous juniper propagation studies helped justify the design of the present study, which examined plant gender, cutting age, and propagation substrate in the greenhouse. This is important because the interaction between the physiological state of the cutting and the physical characteristics of the rooting medium over a prolonged propagation period may affect rooting in slow-rooting conifers.

2.10 Inherent Rooting Difficulty in *Juniperus*

Juniperus species are generally considered difficult to propagate by stem cuttings because adventitious rooting is often slow and variable, and depends on both the physiological state of the cutting and the propagation conditions. Rooting in conifers can be constrained by tissue maturity, decreased rooting competence, carbohydrate supply, plant growth regulators, temperature, light conditions and rooting substrate (Ragonezi et al., 2010; Zavattieri et al., 2016). This challenge is also apparent in *J. communis*. Sanz Gallego et al. (2025) reported that, even under greenhouse conditions with IBA treatment, the highest rooting percentage in common juniper was 45.57% in female cuttings treated with 4000 ppm IBA and propagated in RM_II. This indicates that auxin treatment and a suitable rooting medium can improve rooting, but they do not completely remove the inherent difficulty of rooting in this species.

Thus, slow and variable rooting in *J. communis* should be interpreted as a combination of inherent biological constraints and external propagation factors. Propagation strategies for this species should take into account the long rooting period, variation in cutting material and the effect of the rooting environment.

2.11 Role of Temperature in Adventitious Rooting in Conifers

Temperature is an important factor affecting adventitious root induction and root growth in woody plants. Temperature may affect rooting responses in *junipers,* since root initiation and root elongation require active metabolic processes and appropriate propagation conditions. Güney et al. (2021) studied the propagation of *Juniperus communis* 'Hibernica', *Juniperus chinensis* 'Stricta' and *Juniperus chinensis* 'Stricta Variegata' from hardwood cuttings under different greenhouse temperatures and exogenous auxins. They compared two greenhouse conditions: Greenhouse 1, with an air temperature of 20 ± 2 °C, rooting table temperature of 25 ± 2 °C and relative humidity of 70 ± 2%, and Greenhouse 2, with an air temperature of 20 ± 2 °C, rooting table temperature of 20 ± 2 °C and relative humidity of 70 ± 2%.

Three auxins, indole-3-butyric acid (IBA), indole-3-acetic acid (IAA) and α-naphthalene acetic acid (NAA), were also tested at 3000 ppm and 5000 ppm. Rooting percentage, callus percentage, root length and number of roots were measured. Generally, the highest rooting percentages were achieved in Greenhouse 1, with a rooting table temperature of 25 ± 2 °C. The best rooting results were 93.33% for *J. communis* 'Hibernica' with 5000 ppm IAA, 66.67% for *J. chinensis* 'Stricta' with 5000 ppm IAA, and 60.00% for *J. chinensis* '*Stricta variegata'* with 3000 ppm IBA (Güney et al., 2021). These results indicate that the rooting response of junipers may vary with propagation temperature and auxin treatment. Thus, rooting-zone temperature should be considered when evaluating adventitious rooting in slow-rooting conifers like *J. communis.* In the current study, this justifies interpreting the rooting and early growth responses in the context of the controlled greenhouse environment and the prolonged rooting period.

2.12 Seasonal, Plant Gender and Biological Regulation of Rooting in *Juniperus communis*

Seasonal timing, plant gender and biological treatments may affect rooting success in *J. communis*. Khoshhal Sarmast et al. (2019) investigated the effects of season, plant gender and *Agrobacterium rhizogenes* strains on adventitious root induction in male and female *J. communis* cuttings. They found that the season of collection affected rooting response, with the highest rooting percentage in female plants from cuttings collected in September. However, no significant seasonal differences in rooting percentage were found for male plants. Similarly, Khoshhal Sarmast et al. (2019) demonstrated that biological treatment with *A. rhizogenes* strains had an effect on adventitious root induction in *J. communis*. This suggests that rooting in common juniper is not only dependent on the time of cutting collection and plant gender, but also on biological factors that may affect root induction. The results support the idea that rooting responses in *J. communis* are complex and can be influenced by the physiological state of the cutting and the propagation treatment applied.

Therefore, season, plant gender and biological regulation should be considered when interpreting rooting behaviour in *J. communis*. This is relevant to the present study because plant gender was one of the experimental factors, and the results were evaluated over a longer propagation period.

2.13 In Vitro *Juniperus* Research Providing Evidence for Intrinsic Difficulty of Adventitious Rooting

Rooting is a limiting stage in the in vitro *propagation of Juniperus*. Koçer et al. (2011) investigated indirect organogenesis from bud explants of *J. communis*. They found that genotype, plant gender, sampling time, and combinations of growth regulators affected callus induction and adventitious root development. This suggests that morphogenic responses of common juniper are influenced by both biological and cultural conditions. A review of *Juniperus* tissue culture also shows that in vitro propagation has been studied in several juniper species, and that successful regeneration and rooting are species-dependent and depend on the type of explant and culture conditions (Hazubska-Przybył, 2019). Thus, even under controlled in vitro conditions, *Juniperus* tissues can exhibit different responses during regeneration and root formation.

These findings suggest that poor or inconsistent rooting of *Juniperus* is not only due to external propagation conditions but may also result from intrinsic biological limitations of the plant material. Therefore, propagation studies of slow-rooting species like *J. communis* should allow for delayed rooting and evaluate rooting over longer periods (e.g., three, five and nine months after planting), as in the present study, rather than relying on short-term observations.

2.14 Gap and Justification

From the literature reviewed above, there are several gaps in the vegetative propagation of *Juniperus communis*. While several studies have investigated vegetative propagation in *Juniperus* species, many have focused on individual factors such as auxin treatment, cutting collection time or propagation substrate (Hazubska-Przybył, 2019; Ragonezi et al., 2010). Thus, the interaction between biological and physical aspects of rooting is poorly understood, particularly in the slow-growing, hard-to-root *J. communis*. Physiological age and maturity of the cutting affect the rooting ability of woody cuttings. Tissue maturity, lignification and hormone status can affect adventitious rooting capacity (Ragonezi et al., 2010; Díaz-Sala, 2020). Little is known, however, about the response of different cutting age classes under the same greenhouse conditions, especially in slow-rooting conifers such as *J. communis*.

Another factor that could affect propagation response in this dioecious species is plant gender. Male and female plants may differ in growth, resource allocation, carbohydrate status and stress tolerance in ecological and physiological studies (Nowak-Dyjeta et al., 2017; Markó et al., 2021). However, the role of plant gender in the propagation of *J. communis* has not been extensively investigated. Where it has been studied, the effects appear to be variable or context-dependent, suggesting that plant gender may interact with other factors such as cutting age and propagation substrate.

The physical properties of the substrate also play an important role in the rooting success of woody cuttings, as aeration, moisture-holding capacity and oxygen diffusion are important for root initiation and root growth (Soffer & Burger, 1988; Dirr & Heuser, 2006). However, few studies have compared different propagation substrates under the same environmental conditions for *J. communis*. In addition, the interaction between the substrate and biological factors, such as cutting age and plant gender, is rarely investigated.

Another limitation is that many propagation studies have been conducted over relatively short periods and have focused mainly on rooting. Early evaluations may underestimate the rooting potential and subsequent growth of *J. communis* because this species can exhibit slow and delayed rooting responses (Ragonezi et al., 2010; Sanz Gallego et al., 2025). Longer assessment periods are therefore needed to evaluate early and later rooting responses and growth of rooted cuttings. In the present study, rooting and growth responses were assessed at three, five and nine months after planting. Thus, the present study was conducted to address these gaps by investigating the effects of cutting age, plant gender, and propagation substrate on survival, rooting success, root growth, and early shoot growth of *J. communis* stem cuttings in a greenhouse environment. The purpose of this study was to investigate the influence of biological and substrate-related factors on rooting performance in this slow-rooting conifer over an extended propagation period.

CHAPTER 3 - MATERIALS AND METHODS

3.1 Location and Propagation Setup

The study was carried out at the Massey University Plant Growth Unit (PGU), Manawatū Campus, Palmerston North, New Zealand (40.3750° S, 175.6130° E). The experiment, including cutting preparation, planting, maintenance and data recording, was carried out in the PGU greenhouse facilities. Propagation was carried out in a greenhouse conducive to rooting slow-growing conifer cuttings. Cuttings were exposed to natural daylight, and green shade netting was used to minimise excess light and moisture loss. Propagation tables were bottom-heated, and the rooting media were kept at a temperature range of 18–25 °C, which is suitable for adventitious rooting in *junipers* and other conifers (Güney et al., 2021).

The relative humidity around the cuttings was maintained by an automatic misting system throughout the experiment, operating for 15 seconds every hour from dawn to dusk to maintain high humidity and reduce moisture loss from the cuttings during the trial.

3.2 Plant Material

The stem cuttings of *Juniperus communis* L. were collected from mature parent plants cultivated under the same management conditions at Massey University, Manawatū Campus, Palmerston North, New Zealand (40.3852° S, 175.6179° E). Male and female parent plants were distinguished based on their reproductive structures, with male plants identified by the presence of pollen-producing structures and female plants identified by the presence of berries. Cuttings were then collected separately from the identified male and female parent plants. Cutting age was calculated from the age of the shoot tissue, not the age of the entire plant. One-year-old cuttings were taken from sub-terminal shoots of the current season, and two-year-old cuttings were taken from mid-shoot areas of the previous season. The collection was done in winter, June 2025, in New Zealand. Cuttings were transported to the greenhouse for treatment and planting as soon as possible to prevent dehydration and stress.

All cuttings were standardised at 12 cm in length to maintain uniform cutting material and to provide sufficient stem length for handling, basal IBA treatment and planting in the propagation substrate. This length was also consistent with cutting lengths used in previous Juniperus propagation research, where cuttings of 10–12 cm were used (Guney et al., 2021). All cuttings were dipped at the basal end for about 3 seconds in rooting hormone gel containing indole-3-butyric acid (IBA) at 3 g/L.

3.3 Propagation Substrates

Different propagation substrates were tested to cover a range of physical and chemical properties found in propagated plants. These were rockwool cubes, pumice, peat: perlite and vermiculite: perlite. Rockwool cubes were soaked before planting to ensure the substrate was saturated and to prevent initial drought stress in the cuttings. Pumice, with a particle size of around 2–4 mm, had high air-filled porosity and drainage. Peat: perlite and vermiculite: perlite mixes were prepared fresh at a 1:1 (v/v) ratio to ensure adequate moisture retention and aeration. Substrates were carefully filled into the growing container to provide good contact between the substrate and the cutting base without excessive compaction that would reduce aeration. All cuttings were planted to a depth of 3-5 cm.

The aim was to use substrates with different physical properties (water retention, aeration, stability, etc.) to evaluate their effects on rooting and root development of *J. communis* cuttings under uniform greenhouse conditions (Dirr & Heuser, 2006).

3.4 Experimental Design

The study evaluated the effects of plant gender, shoot age, and propagation substrate on the survival, rooting, and early growth of *Juniperus communis* cuttings. The experimental structure consisted of two plant genders (male and female), two shoot ages (one-year-old and two-year-old shoots), and propagation substrates that differed by shoot age group. Two-year-old cuttings were evaluated in four propagation substrates, whereas one-year-old cuttings were evaluated in two propagation substrates. Therefore, the substrate treatments were not identical between the two cutting-edge groups, and the two age groups were analysed separately rather than making direct cutting-age × substrate comparisons. For two-year-old cuttings, four propagation substrates were evaluated: rockwool, pumice, peat: perlite (1:1, v/v), and vermiculite: perlite (1:1, v/v). This resulted in eight treatment combinations (2 genders × 4 substrates). For one-year-old cuttings, two propagation substrates were evaluated: pumice and vermiculite: perlite (1:1, v/v), resulting in four treatment combinations (2 genders × 2 substrates). Therefore, a total of 12 treatment combinations were included in the study.

The difference in propagation substrates between the two cutting-age groups was due to the availability of suitable one-year-old cutting material at the time the experiment was established. Sufficient one-year-old cuttings were available to establish treatments using pumice and vermiculite: perlite (1:1, v/v), but there was insufficient suitable one-year-old material to establish additional rockwool and peat: perlite treatments. Therefore, the one-year-old cutting group had an incomplete substrate treatment structure compared with the two-year-old group and was treated as a separate experimental group for statistical analysis. The two-year-old cuttings had sufficient material to be evaluated across all four substrates: rockwool, pumice, peat: perlite, and vermiculite: perlite.

Twenty-five cuttings were used for each treatment combination. The individual cutting was considered the experimental and observational unit because treatments were applied to individual cuttings, and measurements were recorded separately for each cutting. All cuttings in each treatment combination were used for survival/mortality and root number. Survival was recorded as the percentage of cuttings remaining alive at each assessment time. Root number was analysed, with unrooted or dead cuttings assigned a root number of zero. Root length and shoot length were analysed on the surviving rooted cuttings. Cuttings were collected from male and female stock plants maintained under the same environmental conditions. Treatments were placed in 50-cell propagation trays with an 8 cm cell depth, and all trays were maintained on the same mist bench. Because treatments were applied to individual cuttings and were not independently replicated across separate benches, trays or experimental runs, treatment effects should be interpreted as responses of propagated cuttings under the conditions of this study.

3.5 Rooting and Growth Measurements

Rooting was assessed at three, five and nine months after planting to evaluate rooting success, root growth, and early shoot growth, which are typical traits of slow-rooting conifers such as *J. communis*. Rooting success was measured as a binary variable, with cuttings classified as rooted or non-rooted based on the presence of adventitious roots. A cutting was classified as rooted if it formed visible adventitious roots, and cuttings that were dead or without visible roots were recorded but not used for further measurements of root growth. Root growth of rooted cuttings was assessed by measuring the length of the longest adventitious root and counting the number of adventitious roots. Root lengths were measured with a stainless-steel ruler (only visible roots) and handled with care to avoid damage. No additional nutrients or fertiliser were supplied to the propagation media during the experimental period, including between the five and nine-month assessment periods.

Vegetative growth was assessed by measuring the length of the longest shoot on each rooted cutting at three, five and nine months after planting, where applicable. Shoot length was measured only on rooted cuttings because unrooted cuttings did not provide meaningful growth measurements. Data were collected on a per-cutting basis at each assessment time. Because propagation substrate treatments differed between one-year-old and two-year-old cuttings, data from each cutting-age group were analysed separately. Root number, root length and shoot length were evaluated to determine the effects of plant gender and propagation substrate on vegetative propagation success.

Data on root number included all cuttings, both rooted and non-rooted (or dead), to evaluate rooting performance. However, root length and shoot length were measured only in rooted cuttings, as unrooted cuttings did not have meaningful measures of growth. This ensured uniformity in data collection and statistical analysis.

3.6 Statistical Analysis

Data were analysed separately for each assessment time point at three, five and nine months after planting. Survival/mortality, root number, root length and shoot length were the response variables.

The data were analysed separately for two-year-old and one-year-old cuttings because the propagation substrate treatments differed between cutting age groups. For each cutting-age group and assessment time, the effects of plant gender, propagation substrate and the plant gender × substrate interaction were evaluated.

The binary survival data were used to analyse survival, with surviving cuttings coded as 1 and dead cuttings as 0. Two-way analysis of variance (ANOVA) with replication was used to analyse survival separately for each cutting-age group and assessment time. Survival and mortality percentages were also calculated and reported in the Results.

All cuttings in each treatment combination were used for root number, and unrooted or dead cuttings were assigned a root number of zero. Two-way ANOVA with replication was used to analyse root number and test the effects of plant gender, propagation substrate and their interaction. Root length and shoot length were measured on surviving rooted cuttings, as these measurements were not meaningful for dead or unrooted cuttings. Where analyses were conducted using treatment means, two-way ANOVA was used to examine the effects of plant gender and propagation substrate within each cutting-age group. The analyses were interpreted with caution due to limited independent replication.

Standard errors (SE) were calculated for treatment means using the formula $SE = SD/\sqrt{n}$, where SD is the standard deviation, and n is the number of observations contributing to the mean. SE values were used to express variability around the treatment means and are presented as mean ± SE in the relevant tables and figures. For root-number measurements, n was 25 cuttings per treatment.

Statistical significance was determined from the ANOVA results using the corresponding F-statistics and p-values, with $p \leq 0.05$ considered statistically significant. Standard errors were used only to express variability around treatment means and were not used to determine statistical significance. No separate t-tests were used for pairwise comparison of treatment means.

Data were analysed in Microsoft Excel and R statistical software (version 4.3.1; R Foundation for Statistical Computing, Vienna, Austria) and statistical significance was set at $p \leq 0.05$.

CHAPTER 4 – RESULTS

4.1 Survival and Mortality

4.1.1 Survival and mortality of two-year-old cuttings

In two-year-old *Juniperus communis* L. cuttings, survival varied among plant gender and propagation substrate combinations across the three assessment times (Table 4.1). At three months after planting, survival was 100% in both rockwool and pumice for male and female cuttings. In contrast, the lowest survival was observed in female cuttings propagated in vermiculite: perlite (1:1, v/v), with only 12% survival and 88% mortality recorded at three, five and nine months after planting. Overall, survival was greater in rockwool and pumice than in the peat: perlite (1:1, v/v) and vermiculite: perlite (1:1, v/v) treatments throughout the assessment period.

Table 4.1: Survival and mortality of two-year-old *Juniperus communis* L. cuttings at three, five and nine months after planting.

Plant gender	Substrate	Survival/Mortality (%)		
		Three months	Five months	Nine months
Female	Rockwool	100 / 0	56 / 44	44 / 56
Female	Pumice	100 / 0	76 / 24	68 / 32
Female	Peat: Perlite (1:1, v/v)	52 / 48	48 / 52	40 / 60
Female	Vermiculite: Perlite (1:1, v/v)	12 / 88	12 / 88	12 / 88
Male	Rockwool	100 / 0	92 / 8	84 / 16
Male	Pumice	100 / 0	80 / 20	80 / 20
Male	Peat: Perlite (1:1, v/v)	56 / 44	48 / 52	48 / 52
Male	Vermiculite: Perlite (1:1, v/v)	64 / 36	64 / 36	64 / 36

4.1.2 Survival and mortality of one-year-old cuttings

In one-year-old cuttings, survival was highest in pumice at three months after planting, with 100% survival recorded for both female and male cuttings (Table 4.2). Survival in vermiculite: perlite (1:1, v/v) remained 72% for both plant genders across all assessment times. By nine months, survival in pumice was 72% for female cuttings and 60% for male cuttings.

Table 4.2: Survival and mortality of one-year-old *Juniperus communis* L. cuttings at three, five and nine months after planting in different substrates.

Plant gender	Substrate	Survival/Mortality (%)		
		Three months	Five months	Nine months
Female	Pumice	100 / 0	76 / 24	72 / 28
Female	Vermiculite: Perlite (1:1, v/v)	72 / 28	72 / 28	72 / 28
Male	Pumice	100 / 0	80 / 20	60 / 40
Male	Vermiculite: Perlite (1:1, v/v)	72 / 28	72 / 28	72 / 28

Survival analysis showed that plant gender, propagation substrate, and the plant gender × substrate interaction significantly affected the survival of two-year-old cuttings at three, five and nine months after planting (Table 4.3). In one-year-old cuttings, propagation substrate significantly affected survival at three months after planting, but plant gender and the plant gender × substrate interaction were not significant at any assessment time. The propagation substrate did not significantly affect the survival of one-year-old cuttings at five or nine months after planting (Table 4.3).

Table 4.3: *P*-values for the effects of plant gender, propagation substrate and their interaction on survival* of one-year-old and two-year-old *Juniperus communis* L. cuttings at three, five and nine months after planting.

Cutting age	Source of variation	Three months	Five months	Nine months
Two-year-old	Plant gender	0.0030	0.0003	<0.001
Two-year-old	Substrate	<0.001	<0.001	0.0002
Two-year-old	Plant gender × substrate	<0.001	0.0080	0.0423
One-year-old	Plant gender	1.000	0.821	0.524
One-year-old	Substrate	<0.001	0.498	0.524
One-year-old	Plant gender × substrate	1.000	0.821	0.524

*Survival was analysed using binary survival data, where surviving cuttings were coded as 1 and dead cuttings as 0. *P*-values were obtained from two-way ANOVA with replication within each cutting-age group and assessment time.

4.2 Root number

4.2.1 Effects of plant gender, substrate and plant gender × substrate interaction in two-year-old cuttings

At three months after planting, the number of roots per cutting in two-year-old *Juniperus communis* cuttings was significantly affected by plant gender ($p = 0.0026$) (Table 4.4). When averaged across propagation substrates, male cuttings produced a greater mean number of roots per cutting (6.00) than female cuttings (4.08). Propagation substrate also significantly affected root number ($p = 1.20 \times 10^{-6}$) (Table 4.4). When averaged across plant genders, cuttings propagated in rockwool produced the highest mean number of roots (7.12), followed by pumice (6.10), peat: perlite (1:1, v/v) (4.60), and vermiculite: perlite (1:1, v/v) (2.34).

A significant plant gender × substrate interaction was observed ($p = 1.04 \times 10^{-7}$) (Table 4.4; Figure 4.1). The significant interaction indicates that the effect of propagation substrate on root number differed between male and female cuttings. Male cuttings produced the highest root numbers in rockwool (9.28 ± 0.92) and peat: perlite (1:1, v/v) (6.80 ± 1.62), whereas female cuttings produced the highest root numbers in pumice (8.52 ± 1.08). In contrast, vermiculite: perlite (1:1, v/v) resulted in the lowest root number for female cuttings (0.44 ± 0.30).

Table 4.4: Two-way analysis of variance (ANOVA) showing the effects of plant gender, substrate, and their interaction on the number of roots per cutting in two-year-old *Juniperus communis* L. cuttings at 3 months after planting. *

Source of variation*	Df	SS	MS	F	*p*-value
Plant gender	1	184.32	184.32	9.33	0.0026
Substrate	3	646.68	215.56	10.91	1.20×10^{-6}
Plant gender × substrate	3	764.28	254.76	12.89	1.04×10^{-7}
Error	192	3794.40	19.76	-	-
Total	199	5389.68	-	-	-

*Data represent the number of roots per cutting of two-year-old *Juniperus communis* L. stem cuttings evaluated three months after planting. Each treatment combination consisted of 25 cuttings (n = 25). Propagation substrates included rockwool, pumice, peat: perlite (1:1, v/v), and vermiculite: perlite (1:1, v/v). Df = degrees of freedom; F = F-statistic from the analysis of variance (ANOVA); ANOVA = analysis of variance; *p* = probability value; v/v = volume/volume.

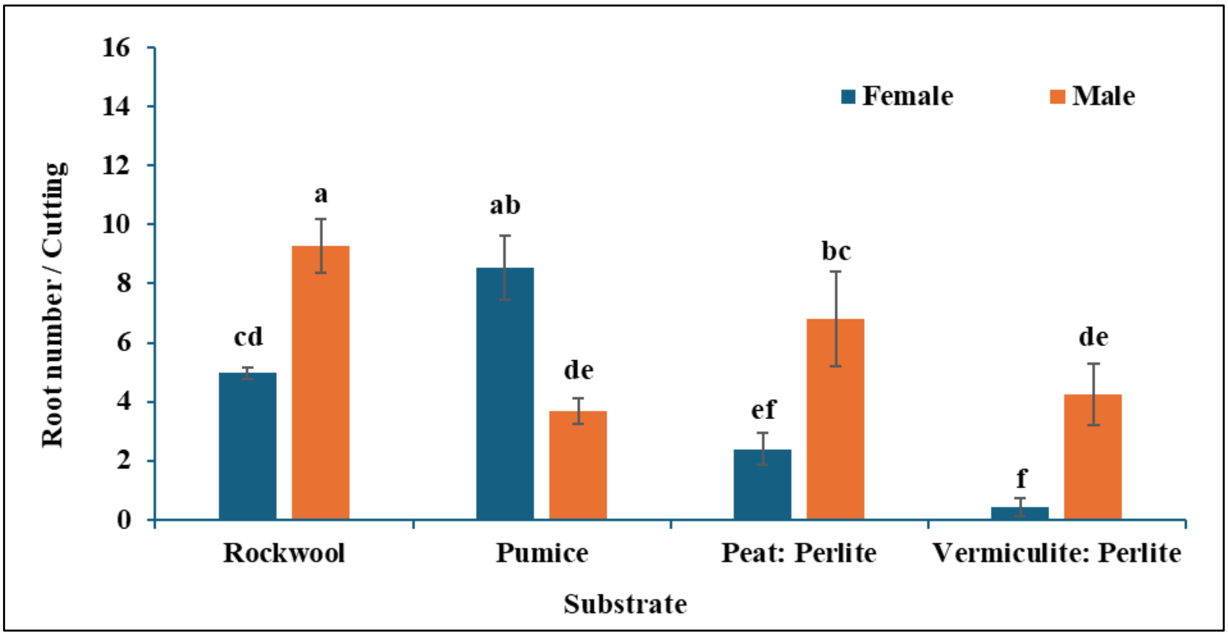

Figure 4.1: Mean number of roots per cutting (± SE) of two-year-old *Juniperus communis* L. stem cuttings grown in four propagation substrates as affected by plant gender at three months after planting. Each treatment consisted of 25 cuttings (n = 25). Error bars represent standard error.

At five months after planting, plant gender significantly affected root number ($p = 0.0102$), propagation substrate significantly affected root number ($p = 1.74 \times 10^{-4}$), and the plant gender × substrate interaction was significant ($p = 5.01 \times 10^{-6}$) (Table 4.5; Figure 4.2).

The significant plant gender × substrate interaction indicates that the effect of propagation substrate on root number differed between male and female cuttings. Female cuttings produced the greatest number of roots when propagated in pumice, whereas male cuttings showed comparatively high root production in rockwool and peat: perlite (1:1, v/v). In contrast, vermiculite: perlite (1:1, v/v) resulted in lower root numbers in female cuttings. These results indicate that root number in two-year-old *Juniperus communis* cuttings was influenced by the combined effects of plant gender and propagation substrate.

Table 4.5: Two-way ANOVA results for the effects of plant gender, substrate, and their interaction on root number per cutting in two-year-old *Juniperus communis* L. cuttings at five months after planting. *

Source	Df	F	*p*-value
Plant gender	1	6.73	0.0102
Substrate	3	6.99	1.74×10^{-4}
Plant gender × substrate	3	9.77	5.01×10^{-6}
Error	192	-	-
Total	199	-	-

*Data represent the number of roots per cutting of two-year-old *Juniperus communis L*. stem cuttings evaluated five months after planting. Each treatment combination consisted of 25 cuttings (n = 25). Df = degrees of freedom; F = F-statistic; ANOVA = analysis of variance; *p* = probability value.

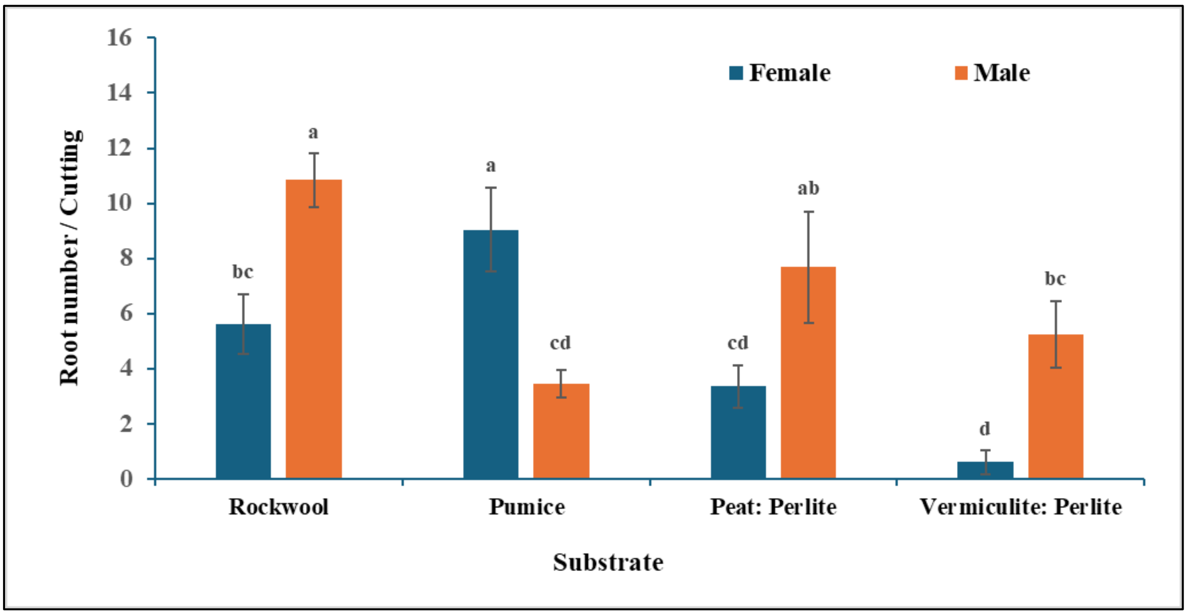

Figure 4.2: Mean number of roots per cutting (± SE) for male and female two-year-old *Juniperus communis* L. cuttings grown in four propagation substrates at five months after planting. Propagation substrates included rockwool cubes, pumice (2–4 mm particle size), peat: perlite (1:1, v/v), and vermiculite: perlite (1:1, v/v). Each treatment consisted of 25 cuttings (n = 25). Error bars represent standard error.

At nine months after planting, plant gender significantly affected root number ($p = 0.0118$), whereas propagation substrate did not significantly affect root number ($p = 0.0523$). However, the plant gender × substrate interaction was significant ($p = 1.04 \times 10^{-6}$) (Table 4.6; Figure 4.3). The significant plant gender × substrate interaction indicates that the effect of propagation substrate on root number differed between male and female cuttings. Female cuttings produced the highest mean root number when propagated in pumice (13.08 ± 2.43 roots per cutting) (Figure 4.3), whereas male cuttings produced the highest mean root number in rockwool (12.00 ± 1.52 roots per cutting). Male cuttings also produced greater root numbers than female cuttings in peat: perlite (1:1, v/v) and vermiculite: perlite (1:1, v/v). The lowest root number was observed in female cuttings propagated in vermiculite: perlite (1:1, v/v). These results indicate that root number in two-year-old *Juniperus communis* cuttings was influenced by the combined effects of plant gender and propagation substrate.

Table 4.6: Two-way analysis of variance (ANOVA) showing the effects of plant gender, propagation substrate, and their interaction on the number of roots per cutting of two-year-old stem cuttings of *Juniperus communis* L. at nine months after planting. *

Source	Df	F	p-value
Plant gender	1	6.46	0.0118
Substrate	3	2.62	0.0523
Plant gender × substrate	3	11.02	1.04×10^{-6}
Error	192	-	-
Total	199	-	-

*Values represent the number of adventitious roots per cutting of two-year-old *Juniperus communis* L. stem cuttings evaluated nine months after planting. Df = degrees of freedom; F = F-statistic; ANOVA = analysis of variance; p = probability value.

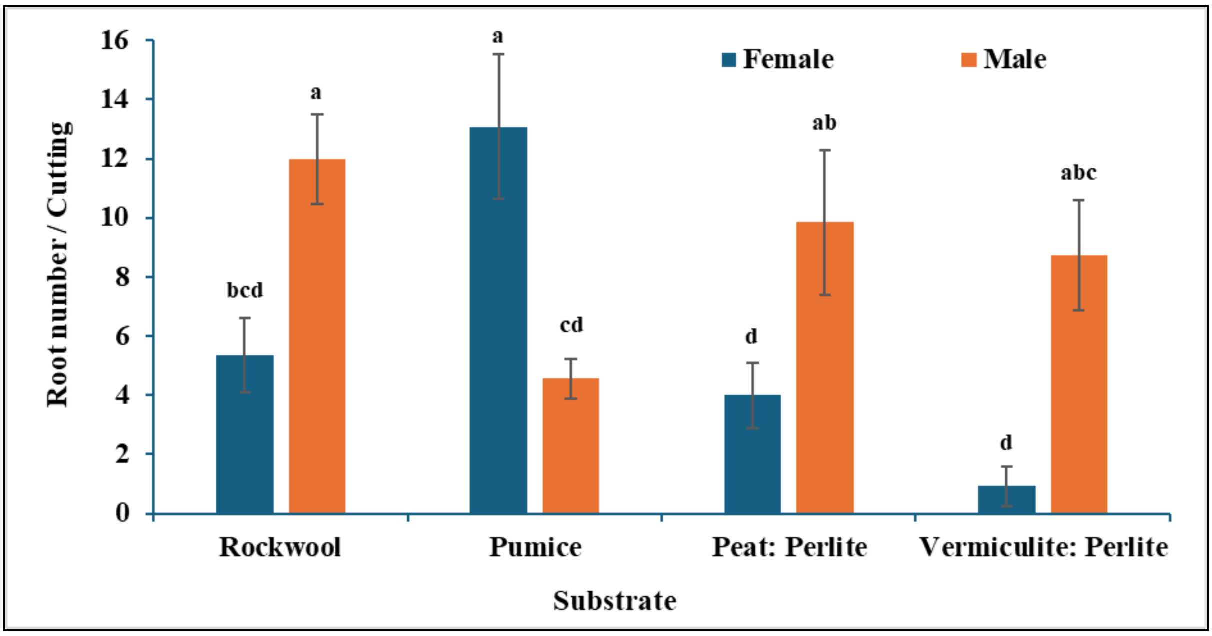

Figure 4.3: Mean number of roots per cutting (± SE) of two-year-old stem cuttings of *Juniperus communis* L. as influenced by plant gender (female and male) and propagation substrate at 9nine months after planting. Propagation substrates included rockwool cubes, pumice (2–4 mm particle size), peat: perlite (1:1, v/v), and vermiculite: perlite (1:1, v/v). Error bars represent standard error.

4.2.2 Effects of plant gender, substrate and plant gender × substrate interaction in one-year-old cuttings

Root number in one-year-old *Juniperus communis* cuttings was not significantly affected by plant gender, propagation substrate or the plant gender × propagation substrate interaction three, five and nine months after planting ($p > 0.05$). Female cuttings produced slightly higher mean root numbers than male cuttings at three and five months, but the differences were not significant. Root numbers were also similar between pumice and vermiculite: perlite (1:1, v/v). At nine months, mean root production was slightly higher in vermiculite: perlite (1:1, v/v) (12.12 roots per cutting) than in pumice (10.00 roots per cutting), but this difference was not significant. These results show that the number of roots was not significantly affected by plant gender, propagation substrate and their interaction during the evaluation period in one-year-old cuttings.

4.3 Root length

Root length was measured only on surviving rooted cuttings; therefore, sample sizes varied among treatments, and the results were interpreted cautiously.

4.3.1 Effects of plant gender and substrate on root length in two-year-old cuttings

At three months after planting, plant gender did not significantly affect root length ($p = 0.579$), and propagation substrate did not significantly affect root length ($p = 0.815$) in two-year-old cuttings. No significant differences in root length were observed between male and female cuttings or among the propagation substrates evaluated.

At five months after planting, plant gender did not significantly affect root length ($p = 0.851$), and propagation substrate did not significantly affect root length ($p = 0.637$). Root length remained similar across plant genders and propagation substrates.

At nine months after planting, plant gender did not significantly affect root length ($p = 0.880$), and propagation substrate did not significantly affect root length ($p = 0.671$). No significant variation in root length was detected among plant genders or propagation substrates at the final assessment.

Only rooted cuttings that survived were measured for root length, and treatment means were used for analysis. Therefore, the plant gender × substrate interaction was not reported as the interaction effect could not be separated from the residual variation in this analysis.

Overall, these results indicate that root length in two-year-old *Juniperus communis* L. cuttings was not significantly influenced by plant gender or propagation substrate throughout the experimental period. The detailed root-length data are presented in the Appendix (Figures A1 and A2).

4.3.2 Effects of plant gender and substrate on root length in one-year-old cuttings

At three months after planting, root length was not significantly affected by plant gender (p = 0.799) or propagation substrate (p = 0.601) in one-year-old cuttings. Although female cuttings propagated in pumice produced slightly longer roots than the other treatment combinations, the observed differences were not statistically significant.

At five months after planting, plant gender did not significantly influence root length (p = 0.077). In contrast, the propagation substrate had a significant effect on root length (p = 0.0096). Across both plant genders, cuttings propagated in pumice developed longer roots (82.82 mm) than those propagated in vermiculite: perlite (1:1, v/v) (64.96 mm). This result indicates that pumice provided more favourable conditions for root elongation than vermiculite: perlite (1:1, v/v) at this stage of development.

At nine months after planting, neither plant gender (p = 0.822) nor propagation substrate (p = 0.385) significantly affected root length. Root growth remained comparable between male and female cuttings and between the two propagation substrates evaluated.

Only rooted cuttings that survived were measured for root length, and treatment means were used for analysis. Therefore, the plant gender × substrate interaction was not reported because the interaction effect could not be separated from the residual variation in this analysis.

Overall, root length in one-year-old *Juniperus communis* L. cuttings was generally unaffected by plant gender throughout the study period. Propagation substrate influenced root length only at five months after planting, when cuttings grown in pumice produced significantly longer roots than those grown in vermiculite: perlite (1:1, v/v). The detailed root-length data are presented in the Appendix (Figures A5 and A6).

4.4 Shoot length

Shoot length was measured only on surviving rooted cuttings; therefore, sample sizes varied among treatments, and the results were interpreted cautiously.

4.4.1 Effects of plant gender and substrate on shoot length in two-year-old cuttings

Two-year-old *Juniperus communis* L. cuttings showed no measurable increase in shoot length at three months after planting. There was no difference in mean shoot length among plant genders and propagation substrate combinations.

Plant gender had no significant effect on shoot length at five months after planting ($p = 0.546$), and propagation substrate had no significant effect on shoot length ($p = 0.577$). The mean shoot length of female cuttings propagated in pumice was the highest (58.11 mm), whereas the mean shoot length of female cuttings propagated in peat:perlite (1:1, v/v) was the lowest (22.75 mm), but these differences were not significant.

Although the treatment means differed numerically, the variation among the surviving rooted cuttings meant that these differences were not statistically significant.

At nine months after planting, shoot length was not significantly affected by plant gender ($p = 0.883$) or propagation substrate ($p = 0.996$). There was no difference in mean shoot length between propagation substrates or between male and female cuttings. Female cuttings propagated in pumice had the longest mean shoot length (60.35 mm), while male cuttings propagated in pumice had the shortest mean shoot length (32.33 mm). These differences, however, were not statistically significant.

Only rooted cuttings that survived were measured for shoot length, and treatment means were used for analysis. Therefore, the plant gender × substrate interaction was not reported because the interaction effect could not be separated from residual variation in this analysis.

Overall, shoot length of two-year-old *Juniperus communis* L. cuttings did not differ significantly between genders of plants or propagation substrates during the study period. The detailed shoot-length data are presented in the Appendix (Figures A3 and A4).

4.4.2 Effects of plant gender and substrate on shoot length in one-year-old cuttings

One-year-old cuttings of *Juniperus communis* L. showed no measurable increase in shoot length at three months after planting. Mean shoot length was 15 mm across all treatment combinations.

At five months after planting, shoot length was not significantly affected by plant gender ($p = 0.116$) or propagation substrate ($p = 0.544$). The mean shoot length varied from 27.50 mm for female cuttings in vermiculite: perlite (1:1, v/v) to 32.22 mm for male cuttings in vermiculite: perlite (1:1, v/v). However, these differences were not significant.

Plant gender had no significant effect on shoot length at nine months after planting ($p = 0.233$), and propagation substrate had no significant effect on shoot length ($p = 0.115$). The female cuttings propagated in pumice had the highest mean shoot length (49.47 mm), whereas the male cuttings propagated in vermiculite: perlite (1:1, v/v) had the lowest mean shoot length (31.22 mm). However, the differences between treatments were not significant. Only rooted cuttings that survived were measured for shoot length, and treatment means were used for analysis. Therefore, the plant gender × substrate interaction was not reported because the interaction effect could not be separated from the residual variation in this analysis. Overall, the absence of significant differences indicates that neither plant gender nor propagation substrate produced statistically detectable differences in shoot growth of one-year-old Juniperus communis L. cuttings during the study period. The detailed shoot-length data are presented in the Appendix (Figures A7 and A8).

4.5 Overall Summary of Results

Survival and mortality of *Juniperus communis* L. cuttings varied among cutting-age groups, plant genders, and propagation substrates. In two-year-old cuttings, the highest survival percentages were generally observed in rockwool and pumice, whereas the lowest survival occurred in female cuttings propagated in vermiculite: perlite (1:1, v/v). In one-year-old cuttings, survival remained relatively high across propagation substrates throughout the study period.

Root number was the response variable most strongly influenced by treatment effects. In two-year-old cuttings, root number was significantly affected by plant gender at three, five and nine months after planting. The propagation substrate also significantly affected root number at three and five months, and significant plant gender × substrate interactions were detected at all assessment times. In contrast, the number of roots in one-year-old cuttings was not significantly affected by plant gender, propagation substrate, or their interaction.

Root length was generally less responsive to treatment effects than root number. In two-year-old cuttings, root length was not significantly affected by plant gender or propagation substrate at any assessment time. In one-year-old cuttings, propagation substrate significantly affected root length at five months after planting, whereas no significant effects were observed at third or ninth months.

Shoot length, as an indicator of shoot growth, did not differ significantly between treatments in either cutting-edge group during the study period. Plant gender and propagation substrate did not significantly affect shoot length in either one-year-old or two-year-old cuttings. Survival and root number were the most responsive measures of cutting performance, while root length and shoot length were generally less responsive to the treatments tested, except for the significant effect of propagation substrate on root length of one-year-old cuttings at five months after planting.

CHAPTER 5 – DISCUSSION

5.1 Overview of Treatment Responses

One-year-old and two-year-old *Juniperus communis* L. cuttings were propagated under greenhouse conditions to determine survival, adventitious rooting, and early shoot growth, and to examine the influence of plant gender and propagation substrate. Survival/mortality of cuttings and root number showed clearer treatment-related responses than root length or shoot length. Treatment effects were also greater for two-year-old cuttings than for one-year-old cuttings, as indicated by survival analysis. This pattern is important because cutting survival and adventitious root initiation are key early stages in successful vegetative propagation, followed by root elongation and shoot growth (Hartmann et al., 2011).

The results support the general concept that adventitious rooting in conifers is a complex process influenced by both biological and environmental factors. Tissue maturity, decreased cellular plasticity, carbohydrate utilisation, environmental conditions, and the physical characteristics of the rooting medium can limit conifer rooting (Ragonezi et al., 2010; Druege et al., 2016; Díaz-Sala, 2020). This was shown in the present study by clearer treatment effects on survival/mortality and root number, while root length and shoot length were less responsive to treatment factors.

The results also suggest that the rooting of *J. communis* cuttings should be evaluated over months, rather than relying solely on short-term observations. Low rooting percentages and long rooting periods have been reported previously in *J. communis* and other *Juniperus* species (Hazubska-Przybył, 2019; Abshahi et al., 2022). Thus, consistent with the prolonged rooting periods reported previously, the three, five and nine-month evaluations in this study provided a more comprehensive assessment of survival, root initiation, root elongation and early shoot growth over time.

5.2 Survival and Mortality Responses

The combinations of plant gender and propagation substrate had different survival and mortality rates, particularly in two-year-old cuttings. Survival analysis showed that plant gender, propagation substrate, and the plant gender × substrate interaction were significant at three, five and nine months after planting. Survival is an important criterion for propagation success because Juniperus communis is a slow-rooting conifer, and cuttings may require several months to establish a sufficiently developed root system capable of supporting continued water and nutrient uptake and subsequent shoot growth.

In the present study, root development was assessed using root number and root length. By five months after planting, surviving two-year-old cuttings had developed adventitious roots, although root number differed significantly according to plant gender, propagation substrate, and their interaction. At nine months, root number remained significantly affected by plant gender and the plant gender × substrate interaction, whereas root length did not differ significantly among plant genders or substrates. Thus, the differences in survival between treatments should be interpreted together with the root development measurements rather than survival alone.

The highest survival for two-year-old cuttings was in rockwool and pumice for both female and male cuttings at three months. This suggests that these substrates provided favourable conditions for maintaining cutting viability during the early stages of propagation. Rockwool and pumice are both highly porous and well-aerated, supplying oxygen at the cutting base while allowing excess water to drain and maintaining a more favourable air–water balance (Soffer & Burger, 1988; Raviv et al., 2019). Root initiation and elongation are dependent on active respiration (Reinl et al., 1991; Druege et al., 2016; Soffer & Burger, 1988; Raviv et al., 2019), and adequate oxygen supply is required for respiration and for initiation of adventitious roots, particularly in slow-rooting conifer species.

For several treatments, survival was highest at the three-month assessment and lower at subsequent assessment times. This indicates that some cuttings were initially viable but were unable to sustain physiological function during the extended rooting period. Slow-rooting conifers may gradually decline if cuttings fail to establish a functional root system, as stored carbohydrate reserves are depleted, water uptake is reduced, and physiological stress increases. This loss of survival over time may be due to some cuttings failing to establish a sufficiently developed root system to support continued water and nutrient uptake under the propagation conditions employed.

A detailed comparison of root structure between treatments was not possible because root architecture was not specifically assessed in this study. Root development was evaluated using root number and root length, and these measurements were used to describe differences in rooting responses among treatments.

The lowest survival rate was observed for two-year-old female cuttings propagated in vermiculite: perlite (1:1, v/v), with 12% survival across all three assessment periods. Vermiculite-based media can retain water, but if the pores are filled with water, aeration may be reduced, limiting the oxygen available around the cutting base (Soffer & Burger, 1988; Reinl et al., 1991; Raviv et al., 2019). In mist propagation, excessive moisture around the cutting base can cause physiological stress. The moisture retention and aeration of the rooting medium are critical for successful rooting of woody plant cuttings (Hartmann et al., 2011; Raviv et al., 2019). The lower survival in vermiculite: perlite (1:1, v/v) may therefore suggest that the air–water balance of this substrate was less favourable for long-term survival than that of rockwool or pumice.

This result is in line with the literature reviewed in Chapter 2, which indicates that the physical characteristics of the rooting medium have a strong influence on the survival and rooting of juniper cuttings. Rockwool and pumice have high air-filled porosity and structural stability, which can help maintain oxygen availability around the cutting base during extended rooting periods (Gruda, 2012). Peat and vermiculite-based substrates, on the other hand, can retain water, but their water-holding capacity must be managed to prevent reduced aeration (Yafuso et al., 2019). Reinl et al. (1991) also found that the moisture level in the rooting medium affected the survival and rooting of juniper cuttings, as water and air compete for substrate pore space. Therefore, the poor survival of two-year-old female cuttings in vermiculite: perlite in the present study may be related to a less favourable air–water balance during the extended propagation period.

The survival of one-year-old cuttings was more uniform than that of two-year-old cuttings. For vermiculite: perlite (1:1, v/v), survival was 72% for both plant genders during the assessment period. Survival analysis showed that propagation substrate had a strong influence on the survival of one-year-old cuttings at three months after planting, but not at five and nine months. Plant gender and plant gender × substrate interaction were not significant at any assessment time. This indicates that the survival response was more uniform in the younger cutting material than in the two-year-old cuttings.

In woody plants and conifers, the ability to form adventitious roots can be influenced by tissue maturation and lignification, with younger tissues tending to have higher rooting potential (Ragonezi et al., 2010; Díaz-Sala, 2020). This more uniform and greater survival of one-year-old cuttings may therefore be associated with their more juvenile physiological state, such as increased cellular plasticity and ability to initiate roots. The lower survival of two-year-old cuttings, on the other hand, could be attributed to an age-related reduction in rooting ability and increased tissue maturity, which could decrease the ability of cuttings to root and survive during longer propagation periods (Ragonezi et al., 2010; Díaz-Sala, 2020).

The survival responses in the present study are also consistent with previous juniper propagation studies, which showed that rooting success was influenced by substrate, plant gender, and the physiological state of the cutting material. Sanz Gallego et al. (2025) found that rooting percentage in *J. communis* was influenced by rooting medium, plant gender and IBA treatment, and that the average rooting percentage of female cuttings was higher than that of male cuttings. The present study, however, showed very low survival in two-year-old female cuttings in vermiculite:perlite, suggesting that plant gender effects may depend on the rooting medium and cutting age. Likewise, Khoshhal Sarmast et al. (2019) reported that the rooting response of *J. communis* was influenced by season and plant gender, and Abshahi et al. (2022) reported that the rooting of *Juniperus sabina* was influenced by substrate, season and IBA treatment. These comparisons suggest that survival in juniper propagation is not determined by a single factor but by a combination of biological and substrate-related factors.

5.3 Root Number Responses

Root number was the most responsive rooting measure in this study, as it reflected adventitious root initiation more clearly than root length or shoot length. In slow-rooting conifers, root initiation is often slow and variable; therefore, the number of roots per cutting is a useful indicator of propagation success (Ragonezi et al., 2010; Hazubska-Przybył, 2019). Root length, in contrast, reflects later root elongation after root initiation has occurred.

Root number was significantly affected by plant gender at three, five and nine months in two-year-old cuttings. The mean number of roots per cutting was greater for male than for female cuttings when averaged over substrates. This supports the inclusion of plant gender in propagation research on dioecious plants. Male and female plants of *J. communis* can differ in growth, carbohydrate allocation, photochemistry and stress responses (Nowak-Dyjeta et al., 2017; Markó et al., 2021; Rabska, 2020). Female plants can also invest more in cone and seed production, which can impact internal resource allocation and vegetative performance (Iszkuło et al., 2011; DeSoto et al., 2016). These differences could potentially contribute to the greater mean root number observed in male cuttings if male cuttings had greater availability of resources for adventitious root initiation and development. However, the present study did not measure carbohydrate allocation or physiological activity directly, so these factors cannot be confirmed as the cause of the higher root number in male cuttings. The number of stock plants and the uniformity of their physiology may have influenced the results. Cuttings were taken from similar positions where possible. However, differences among cuttings could have been due to variations in carbohydrate reserves, prior reproductive effort, shoot maturity, and parent plant condition. This is particularly true for *J. communis*, where plant gender may be linked to resource allocation and physiological state (Nowak-Dyjeta et al., 2017; Markó et al., 2021; Rabska, 2020).

Plant gender effects were not uniform across all cutting categories. Plant gender differences were more evident in two-year-old cuttings because root number did not differ significantly between plant genders in one-year-old cuttings at three, five and nine months. This trend is similar to the survival results, which also indicated higher treatment effects in two-year-old cuttings. It also suggests that the impact of plant gender in dioecious species can vary with tissue stage, growing environment and resource availability (Stark & Martz, 2018; Markó et al., 2021).

Propagation substrate significantly affected root number at three and fifth months, and the plant gender × propagation substrate interaction was significant at three, five and nine months in two-year-old cuttings. This indicates that substrate performance differed between male and female cuttings. Male cuttings had more roots in rockwool and peat: perlite (1:1, v/v), while female cuttings had more roots in pumice in some assessments. The results agree with earlier research indicating that rooting in *Juniperus* may be influenced by interactions among the rooting medium, cutting physiology, and other propagation factors (Hazubska-Przybył, 2019; Abshahi et al., 2022; Sanz Gallego et al., 2025).

The number of primary roots in *J. communis* was affected by rooting medium, plant gender and IBA treatment, as reported by Sanz Gallego et al. (2025). The more favourable rooting medium produced more primary roots than the less favourable medium, suggesting that the rooting medium environment can influence root initiation and early root development. Similarly, the present study revealed that the propagation substrate influenced the root numbers in two-year-old cuttings, particularly in rockwool, pumice, and peat: perlite mixtures. However, in contrast to Sanz Gallego et al. (2025), where female cuttings had a higher average rooting percentage than male cuttings, the present study found that the mean number of roots was higher in male two-year-old cuttings than in female two-year-old cuttings when averaged across substrates. This suggests that plant gender effects in the propagation of *J. communis* may be influenced by the age of the cutting, the rooting medium and the type of rooting parameter measured.

The favourable performance of pumice and rockwool in some root number comparisons may be related to their drainage, structural stability, and aeration. Good drainage and high air-filled porosity of the rooting medium can help to facilitate oxygen diffusion around the basal end of the cutting, particularly for slow-rooting cuttings that are retained in the propagation medium for several months (Soffer & Burger, 1988; Reinl et al., 1991; Raviv et al., 2019; Hartmann et al., 2011).

Other juniper studies also support the importance of substrate and propagation environment for root development. Abshahi et al. (2022) reported that rooting of *Juniperus sabina* was affected by substrate, season and IBA treatment, with the highest rooting percentage obtained in perlite–cocopeat (1:1). Güney et al. (2021) also found that rooting percentage, root length and number of roots varied among juniper cultivars under different greenhouse temperatures and auxin treatments. These studies indicate that root number in junipers is influenced by plant material, rooting medium and propagation environment, rather than by rooting medium alone.

Overall, the root number indicated that substrate effects depended on plant gender in two-year-old cuttings. This suggests that the biological characteristics of the cutting material and the physical characteristics of the rooting medium influenced the rooting ability of *J. communis.*

The biological condition of the cutting material may interact with the physical properties of the rooting medium because cuttings with greater capacity for adventitious root initiation may benefit more from substrates that provide adequate aeration, drainage and moisture availability, whereas cuttings with lower rooting capacity may be more sensitive to unfavourable air–water conditions in the rooting medium.

5.4 Root Length Responses

There were fewer statistically significant treatment effects for root length than for root number. This suggests that once roots were initiated, root elongation was less consistently affected by cutting age, plant gender, and substrate than root initiation was. Therefore, root number appeared to be a more responsive indicator of treatment effects than root length under the conditions of this study, although root length provided additional information on root elongation among surviving rooted cuttings.

At three, five and nine months, the root length of two-year-old cuttings was not significantly affected by plant gender or propagation substrate. Root length differences were noted but not significant. This indicates that the root elongation of the two-year-old cuttings that survived was inconsistent and did not differ distinctly between treatments under the conditions of this experiment.

The root-length data showed considerable variability among surviving cuttings, as reflected by the variation in root lengths within treatment combinations. This variability may have reduced the ability to detect treatment effects, particularly because root length was measured only on surviving rooted cuttings and the number of observations varied among treatments. Therefore, the absence of statistically significant differences in root length should be interpreted with caution, as relatively high variability among individual cuttings may have masked smaller treatment effects.

Substrate significantly affected root length at five months for one-year-old cuttings, with pumice having a longer mean root length than vermiculite: perlite (1:1, v/v). This result indicates that pumice provided more favourable conditions for root elongation at the time of this assessment. Pumice is well-drained and well-aerated, which can increase oxygen availability around growing roots. The water-holding capacity and the air-filled porosity of the rooting medium are important factors, since excessive water retention can limit oxygen diffusion and root growth (Soffer & Burger, 1988; Reinl et al., 1991; Raviv et al., 2019). At three and nine months, plant gender and substrate had no significant effect on root length of one-year-old cuttings. This means the substrate effect observed at five months was not sustained throughout the assessment period. This could be due to differences in the timing of root initiation. Cuttings that rooted earlier had longer roots, and later-rooting cuttings may have had shorter roots at the same time of evaluation. Only rooted cuttings that survived were measured for root length. Therefore, sample sizes varied among treatment combinations because dead and unrooted cuttings were not included in the analysis. This may have contributed to the variability in the root length measurements and reduced the ability to detect treatment effects.

Root length was a useful secondary indicator of root development, but was less responsive than root number. *For J. communis,* root number and survival seemed to be more reliable early measures of propagation success than root length alone. Similar variation in rooting responses has been reported in juniper propagation studies, where rooting responses were influenced by propagation substrate and other propagation conditions (Sanz Gallego et al., 2025).

This is broadly consistent with previous propagation research in Juniperus, although species-specific responses should be considered when making comparisons. Abshahi et al. (2022) studied Juniperus sabina, a species distinct from J. communis, and reported that rooting responses depended on the substrate, season, and IBA treatment. Therefore, differences in species biology and physiological characteristics may contribute to differences in rooting responses between J. sabina and J. communis. Güney et al. (2021) also found that root length and root number differed among juniper cultivars under varying greenhouse temperatures and auxin treatments. These studies provide evidence of the influence of propagation conditions, but rooting responses may differ among Juniperus species due to species-specific biological and physiological characteristics. Therefore, the findings of the present study should be interpreted specifically in relation to J. communis and should not be generalised to Juniperus species as a whole.

5.5 Shoot Growth Responses

Cuttings were taken in winter in New Zealand. Rooting ability may be affected by seasonal timing due to changes in carbohydrate reserves, hormone levels and tissue condition (McCrary, 1980; Denaxa et al., 2021; Khoshhal Sarmast et al., 2019). This study was conducted at a single collection time, and the results are valid only for the seasonal conditions under which the cuttings were collected and should not be generalised across seasons.

There were fewer treatment effects on shoot growth (shoot length) than on root number. During the whole study period, shoot length did not differ across several comparisons and was similar to the initial length. This suggests that shoot elongation did not advance during the early rooting phase. This is consistent with stem-cutting propagation, as cuttings need to survive, respond to wounding, and form adventitious roots before significant above-ground growth can be expected (Ragonezi et al., 2010; Druege et al., 2019).

In two-year-old cuttings, shoot length was not significantly affected by plant gender or substrate at five and nine months. In one-year-old cuttings, plant gender and substrate had no significant effect on shoot length at five and nine months. Results indicate that the root number was more responsive to the factors tested than the shoot length. Slow-growing conifers may show limited shoot elongation until adventitious roots are sufficiently developed to support new above-ground growth.

This interpretation is supported by the literature reviewed in Chapter 2, which indicates that adventitious rooting in conifers is a slow, multi-phase process that includes induction, initiation and expression before roots become functional (Ragonezi et al., 2010; Druege et al., 2016). Delayed and variable rooting in *J. communis* may result in early shoot elongation occurring only after cuttings have passed the root initiation and development stages. Thus, the limited shoot length response observed in the present study indicates that shoot growth was a later indicator of cutting performance than survival or root number.

This is important because rooting success does not necessarily indicate shoot growth. Rooting was more uniform in one-year-old cuttings, while two-year-old cuttings exhibited greater treatment effects for root number. Shoot length was not significantly affected by plant gender or propagation substrate, although some differences were observed between treatments. This means several parameters, including survival, root number, root length and shoot length, should be used to evaluate the success of propagation in the early stages. In *Juniperus* and other slow-rooting conifers, rooting and growth responses can take a long time, which is why multiparameter assessment is especially important (McCrary, 1980; Hazubska-Przybył, 2019).

5.6 Interaction between Plant Gender and Propagation Substrate

One of the most important findings of this study was that plant gender and propagation substrate did not act independently to affect two-year-old *Juniperus communis* cuttings. There were significant plant gender × propagation substrate interactions for both survival and root number, indicating that treatment responses depended on the interaction between plant gender and rooting substrate.

The different substrate responses of male and female cuttings showed this interaction. At some assessment times, male cuttings performed better in rockwool and peat: perlite (1:1, v/v), whereas female cuttings performed better in pumice. Therefore, the same substrate did not produce the same response in male and female cuttings. Similarly, the survival data showed that the effects of the treatments were more pronounced in two-year-old cuttings than in one-year-old cuttings, further highlighting the importance of accounting for both plant gender and substrate when evaluating propagation responses.

The plant gender × propagation substrate interaction suggests that differences in the physiological status of the cutting material, including tissue maturity, carbohydrate availability and cellular capacity for adventitious root initiation, may influence how cuttings respond to different propagation substrates (Kumar et al., 2022; Pincelli-Souza et al., 2024). These factors were not directly measured in the present study and therefore are considered possible explanations rather than demonstrated mechanisms. Rooting responses in conifers are also affected by the physical properties of the rooting medium, such as aeration, drainage and moisture retention.

The results indicate that propagation methods for *J. communis* should consider both plant gender and the propagation substrate to optimise survival and rooting performance.

5.7 Limitations of the Study

The interpretation of the results of this study should be viewed in light of the following limitations. First, the experiment was not replicated across multiple experimental runs; rather, it was conducted in a single greenhouse propagation environment. The mist bench conditions were similar, and all cuttings were maintained on the same mist bench. However, there may have been some differences in light, mist distribution, humidity and root-zone temperature within the propagation area. This environmental variation could have influenced cutting survival, adventitious root initiation and subsequent root and shoot growth.

2. There was a lack of independent experimental replication. Twenty-five cuttings were taken per treatment combination, and individual cuttings were the units of observation. However, treatments were not independently replicated on more than one greenhouse bench/tray or across repeated experimental runs. Conclusions regarding treatment responses should thus be viewed as responses to the greenhouse environment of this study and not as responses that can be easily extrapolated to other propagation environments.

3. Binary survival data were used to analyse survival, and percentages of survival/mortality were used to describe treatment responses. However, the same constraints of the experimental design, such as the single experimental run and limited independent replication, still affected this analysis. Thus, significant survival responses should be viewed in the context of the greenhouse environment and treatment design of this experiment.

4. Differences in survival affected the analyses of root length and shoot length. Root number was analysed for all cuttings, including unrooted and dead cuttings (root number = 0). Root length and shoot length, on the other hand, were measured only from the rooted cuttings that survived. This resulted in fewer observations for some treatment combinations and may have led to greater variation in the root-length and shoot-length responses. This greater variation may have reduced the ability to detect smaller treatment effects, particularly where the number of surviving rooted cuttings was low.

5. Two-way ANOVA without replication was used for some root-length and shoot-length comparisons, as the data were summarised at the treatment-mean level. These analyses were used to describe treatment patterns but were less robust than fully replicated analyses. Therefore, caution is warranted when interpreting both significant and non-significant results of these comparisons.

6. The assumptions of normality and homogeneity of variance were not fully met for the count data used in the ANOVA. This limitation should be considered when interpreting the statistical results, particularly because root number was recorded as count data and included zero values for unrooted or dead cuttings.

Finally, this study did not involve direct measurements of plant physiology or biochemistry, including carbohydrate and endogenous hormone levels, water status, or the degree of lignification. These measurements would have been useful for understanding the mechanisms underlying variation in survival, root number, root length, and shoot growth. Therefore, physiological explanations are limited to observed responses and published literature, rather than direct physiological measurements.

5.8 Future Directions

The experiment should be repeated in other seasons and greenhouse runs to determine whether the treatment responses observed in this experiment are repeatable. Comparisons between seasons would also be useful, as the timing of collection could affect tissue maturity and the rooting ability of woody cuttings (McCrary, 1980; Denaxa et al., 2021).

Future research should also be conducted with a fully balanced factorial design to test one-year-old and two-year-old cuttings on the same propagation substrates. This would allow a more precise evaluation of cutting age, plant gender, substrate and their interaction effects. Independent replication across trays, benches or greenhouse compartments would enhance statistical inference and minimise concerns about non-independence, particularly for survival and rooting responses.

In future studies, other physiological parameters, including endogenous hormone levels, water status, tissue lignification and carbohydrate concentration, should be measured directly. These measurements would help explain differences in rooting and survival success among cutting categories, and differences in substrate responses between cutting-age groups and plant genders.

Root quality, root dry weight and post-transplant performance should also be evaluated in future research. The establishment and survival of rooted cuttings after transplanting are important for commercial and conservation propagation, and root number, root length and survival are useful early indicators.

Future studies should also evaluate a wider range of *Juniperus communis* varieties, cultivars or genetic sources to determine whether the rooting responses observed in the present study are consistent across different plant materials. Variation among genotypes may contribute to differences in rooting ability and responses to propagation substrates and cutting age.

Future studies should also include cuttings from different Juniperus species to determine whether rooting responses vary among species. Different Juniperus species may respond differently to propagation conditions; therefore, the findings of the present study should not be assumed to apply broadly across the genus.

In general, the results of this study indicate that plant gender and propagation substrate may affect the survival and rooting ability of different cutting-age groups of *J. communis*. The results offer valuable data for the development of vegetative propagation techniques and indicate the need for additional replicated studies to maximise propagation success in this species.

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Appendix A: Additional Results Figures

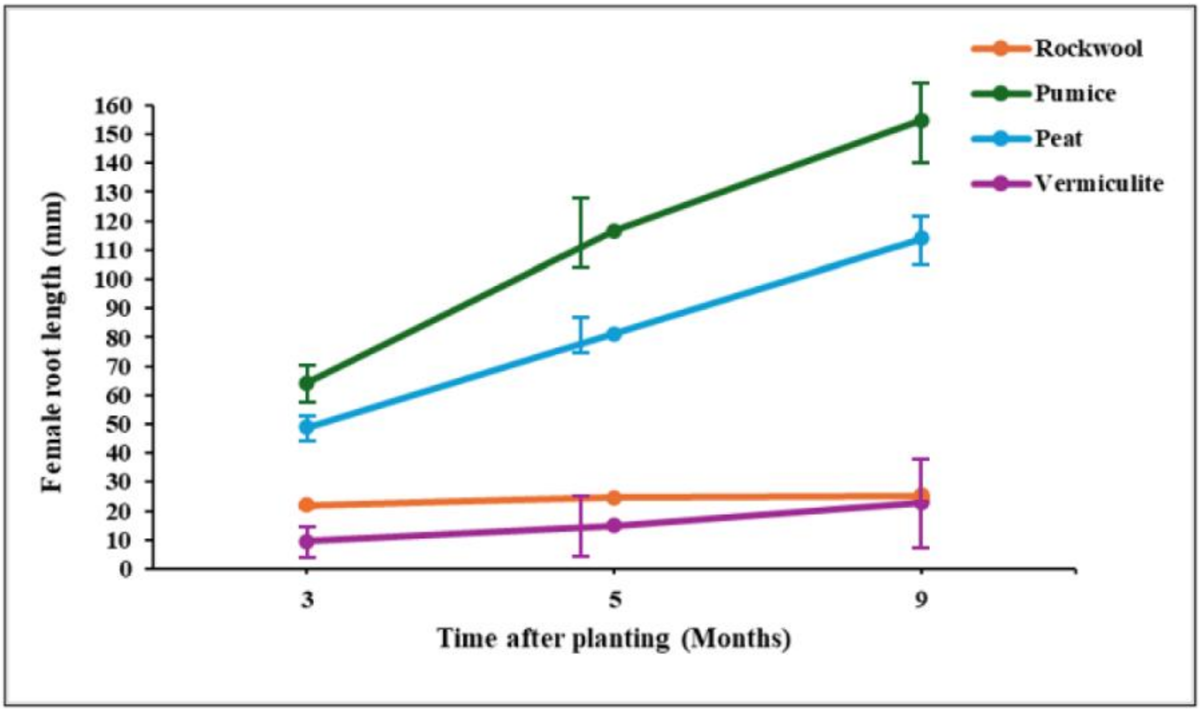

Figure A1: Mean root length (mm) of two-year-old female stem cuttings of *Juniperus communis* L. measured at three, five, and nine months after planting across different propagation substrates. Propagation substrates included rockwool cubes, pumice (2–4 mm particle size), peat: perlite (1:1, v/v), and vermiculite: perlite (1:1, v/v). Values represent mean root lengths of cuttings with recorded root-length measurements, including cuttings with zero root length. Sample size (n) was 25, 25, 13 and 9 at three months; 14, 19, 12 and 9 at five months; and 11, 17, 10 and 9 at nine months for rockwool, pumice, peat: perlite and vermiculite: perlite, respectively. There were no significant differences between treatments at $P < 0.05$ according to ANOVA tests. Error bars indicate 1 S.E.

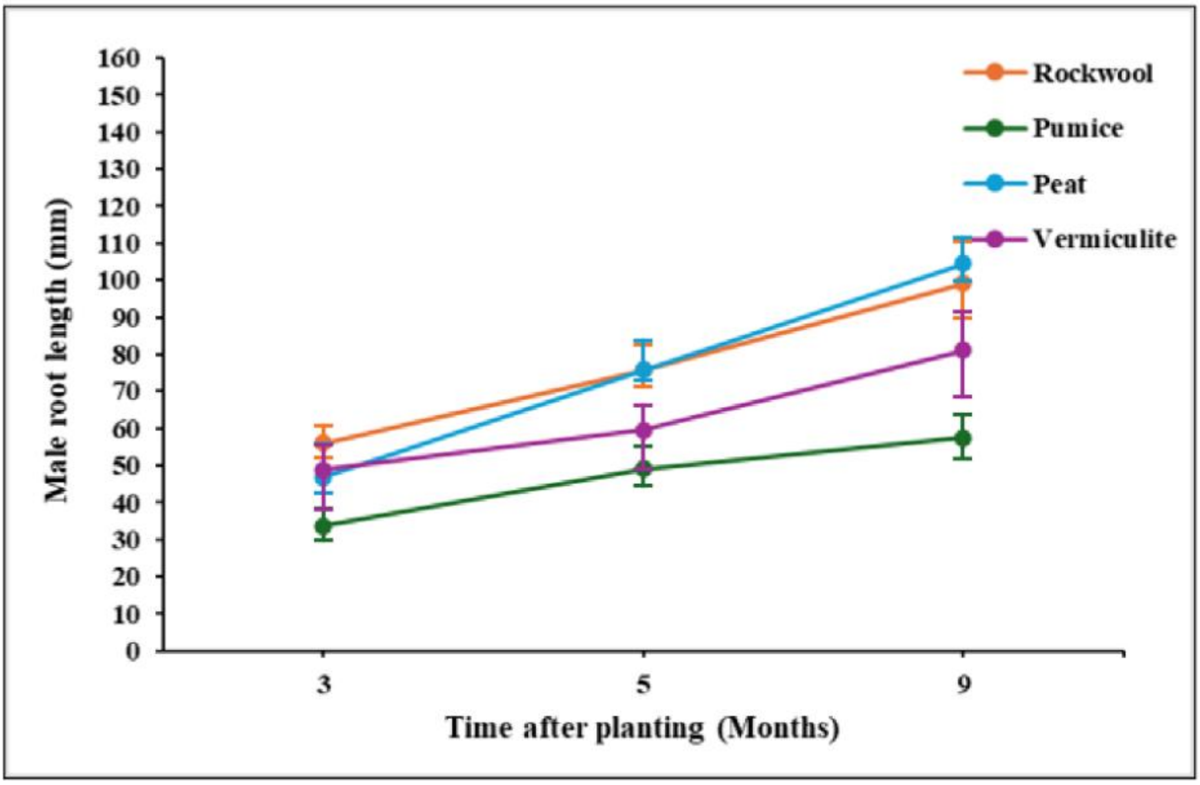

Figure A2: Mean root length (mm) of two-year-old male stem cuttings of *Juniperus communis* L. measured at three, five, and nine months after planting across different propagation substrates. Propagation substrates included rockwool cubes, pumice (2–4 mm particle size), peat: perlite (1:1, v/v), and vermiculite: perlite (1:1, v/v). Values represent means of surviving cuttings with recorded root-length measurements, including cuttings with zero root length. Sample size (n) was 25, 25, 14 and 18 at three months; 23, 20, 12 and 18 at five months; and 21, 20, 12 and 18 at nine months for rockwool, pumice, peat: perlite and vermiculite: perlite, respectively. There were no significant differences between treatments ($P > 0.05$) according to ANOVA tests. Error bars indicate 1 S.E.

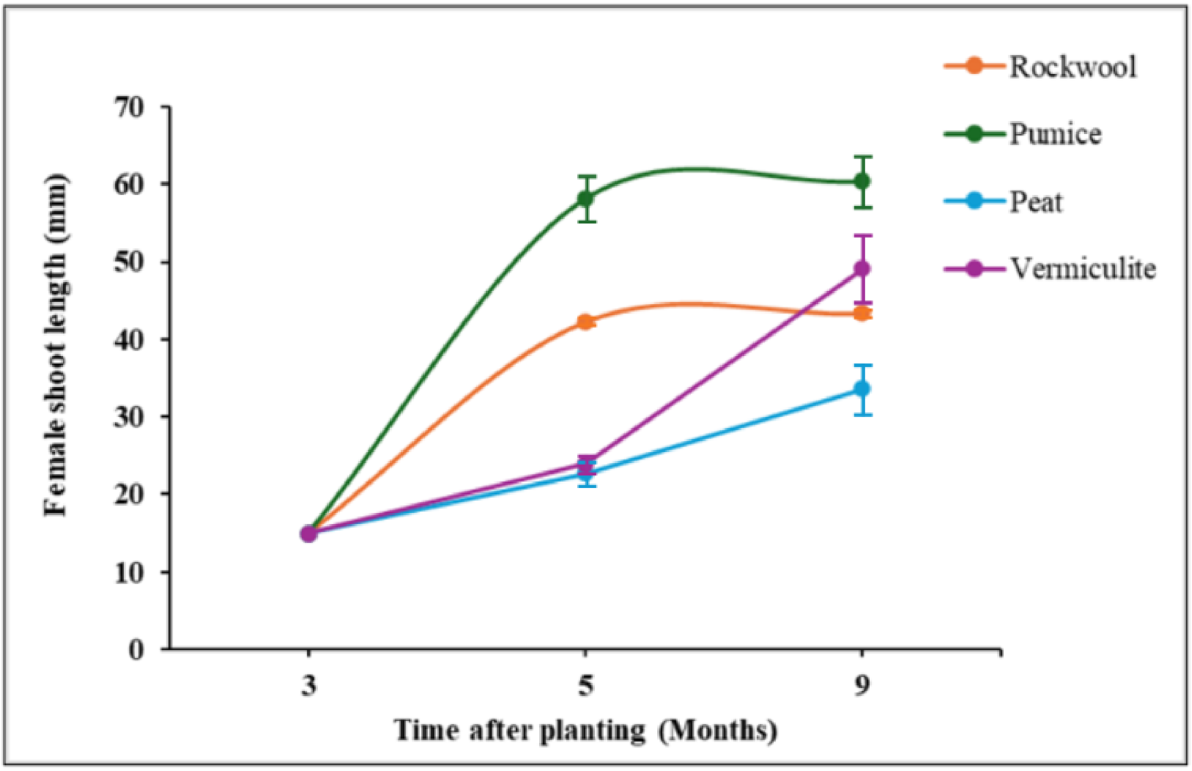

Figure A3: Mean shoot length (mm) of two-year-old female stem cuttings of *Juniperus communis* L. measured at three, five, and nine months after planting across different propagation substrates. Propagation substrates included rockwool cubes, pumice (2–4 mm particle size), peat: perlite (1:1, v/v), and vermiculite: perlite (1:1, v/v). Values represent means of surviving cuttings with recorded shoot-length measurements. Sample size (n) was 25, 25, 13 and 9 at three months; 14, 19, 12 and 9 at five months; and 11, 17, 10 and 9 at nine months for rockwool, pumice, peat: perlite and vermiculite: perlite, respectively. There were no significant differences between treatments ($P > 0.05$) according to ANOVA tests. Error bars indicate 1 S.E.

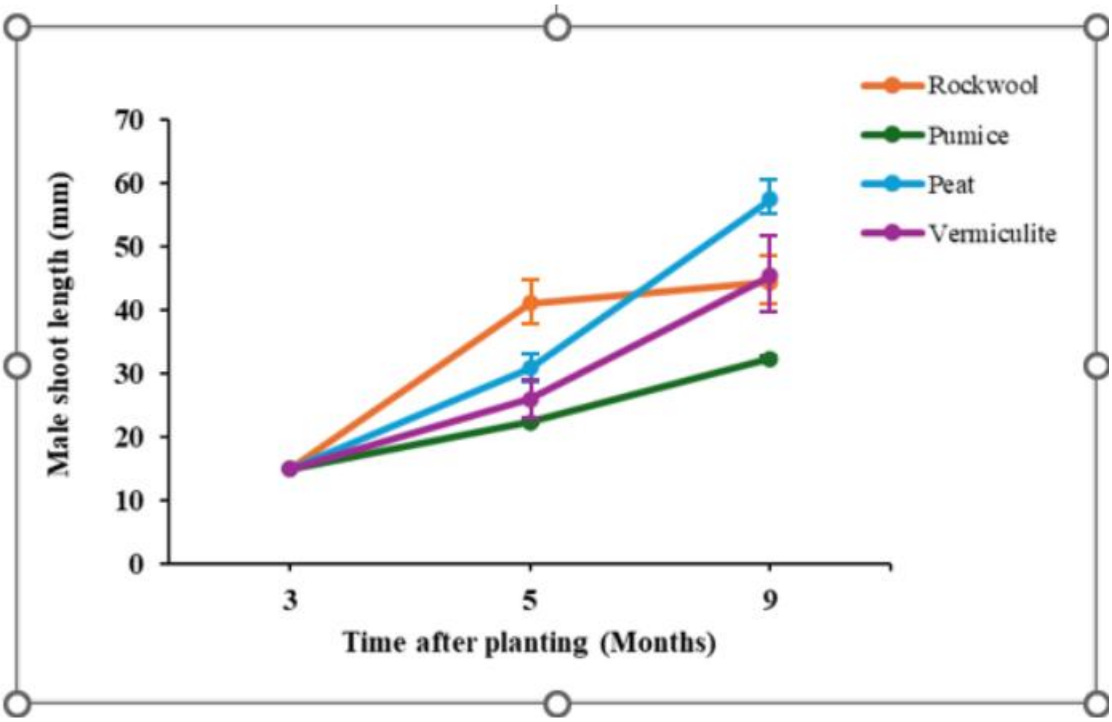

Figure A4: Mean shoot length (mm) of two-year-old male stem cuttings of *Juniperus communis* L. measured at three, five, and nine months after planting across different propagation substrates. Propagation substrates included rockwool cubes, pumice (2–4 mm particle size), peat: perlite (1:1, v/v), and vermiculite: perlite (1:1, v/v). Values represent means of surviving cuttings with recorded shoot-length measurements. Sample size (n) was 25, 25, 14 and 16 at three months; 23, 20, 12 and 16 at five months; and 21, 20, 12 and 16 at nine months for rockwool, pumice, peat: perlite and vermiculite: perlite, respectively. There were no significant differences between treatments ($P > 0.05$) according to ANOVA tests. Error bars indicate 1 S.E.

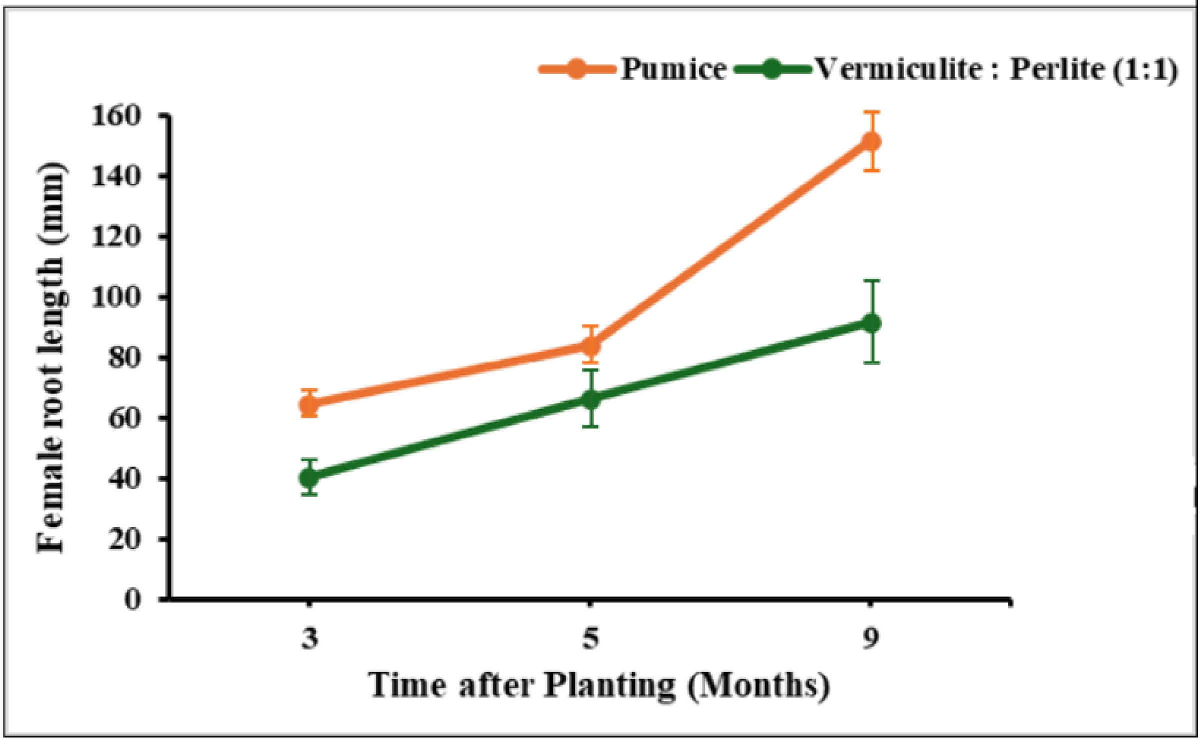

Figure A5: Mean root length (mm) of one-year-old female stem cuttings of *Juniperus communis* L. measured at three, five, and nine months after planting across different propagation substrates. Propagation substrates included pumice (2–4 mm particle size) and vermiculite: perlite (1:1, v/v). Values represent means of surviving rooted cuttings with recorded root-length measurements, including cuttings with zero root length. Sample size (n) was 25 and 20 at three months; 19 and 20 at five months; and 18 and 20 at nine months for pumice and vermiculite: perlite, respectively. There were no significant differences between treatments ($P > 0.05$) according to ANOVA tests. Error bars indicate 1 S.E.

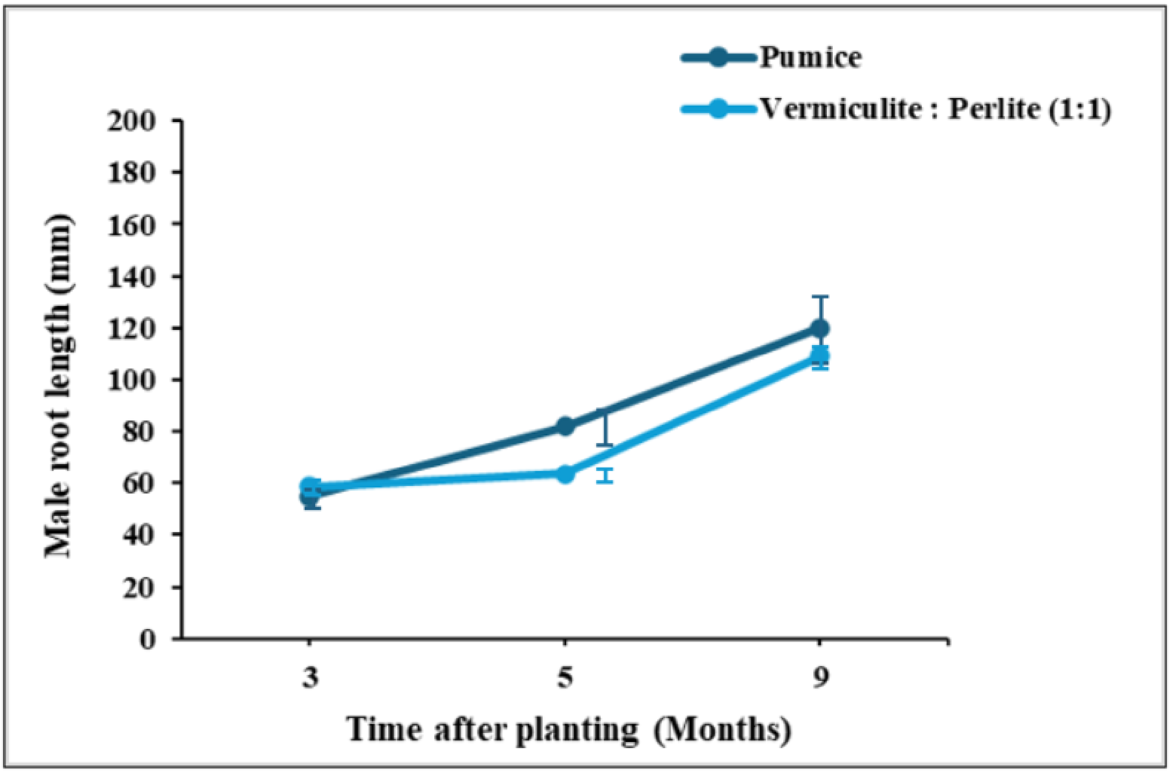

Figure A6: Mean root length (mm) of one-year-old male stem cuttings of *Juniperus communis* L. measured at three, five, and nine months after planting across two propagation substrates. Propagation substrates included pumice (2–4 mm particle size) and vermiculite: perlite (1:1, v/v). Values represent means of surviving rooted cuttings with recorded root-length measurements. Sample size (n) was 25 and 18 at three months; 20 and 18 at five months; and 15 and 18 at nine months for pumice and vermiculite: perlite, respectively. There were no significant differences between treatments ($P > 0.05$) according to ANOVA tests. Error bars indicate 1 S.E.

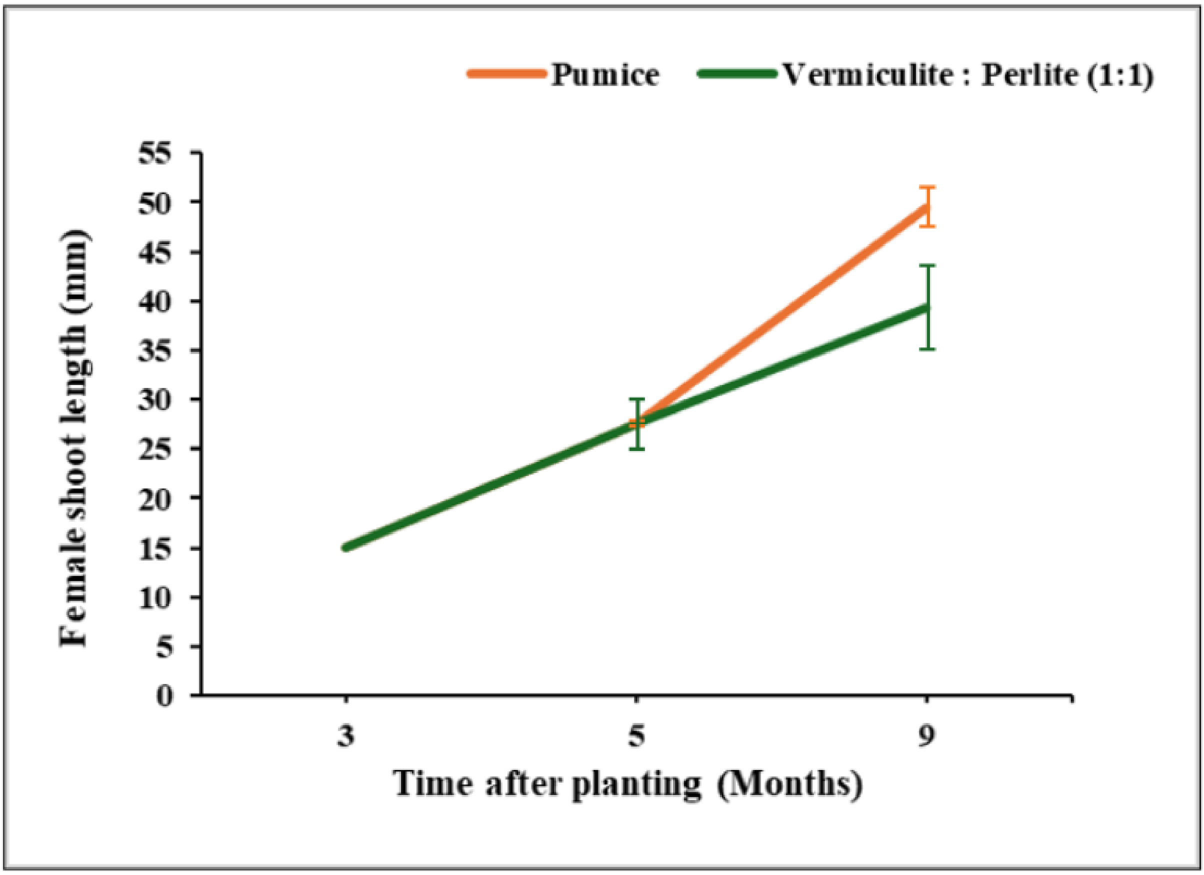

Figure A7: Mean shoot length (mm) of one-year-old female stem cuttings of *Juniperus communis* L. measured at three, five, and nine months after planting across two propagation substrates. Propagation substrates included pumice (2–4 mm particle size) and vermiculite: perlite (1:1, v/v). Values represent mean shoot lengths of cuttings with recorded shoot-length measurements. Sample size (n) was 25 and 18 at three months; 19 and 18 at five months; and 18 and 18 at nine months for pumice and vermiculite: perlite, respectively. No significant differences between propagation substrates were detected at $P < 0.05$ according to ANOVA tests. Error bars indicate 1 S.E.

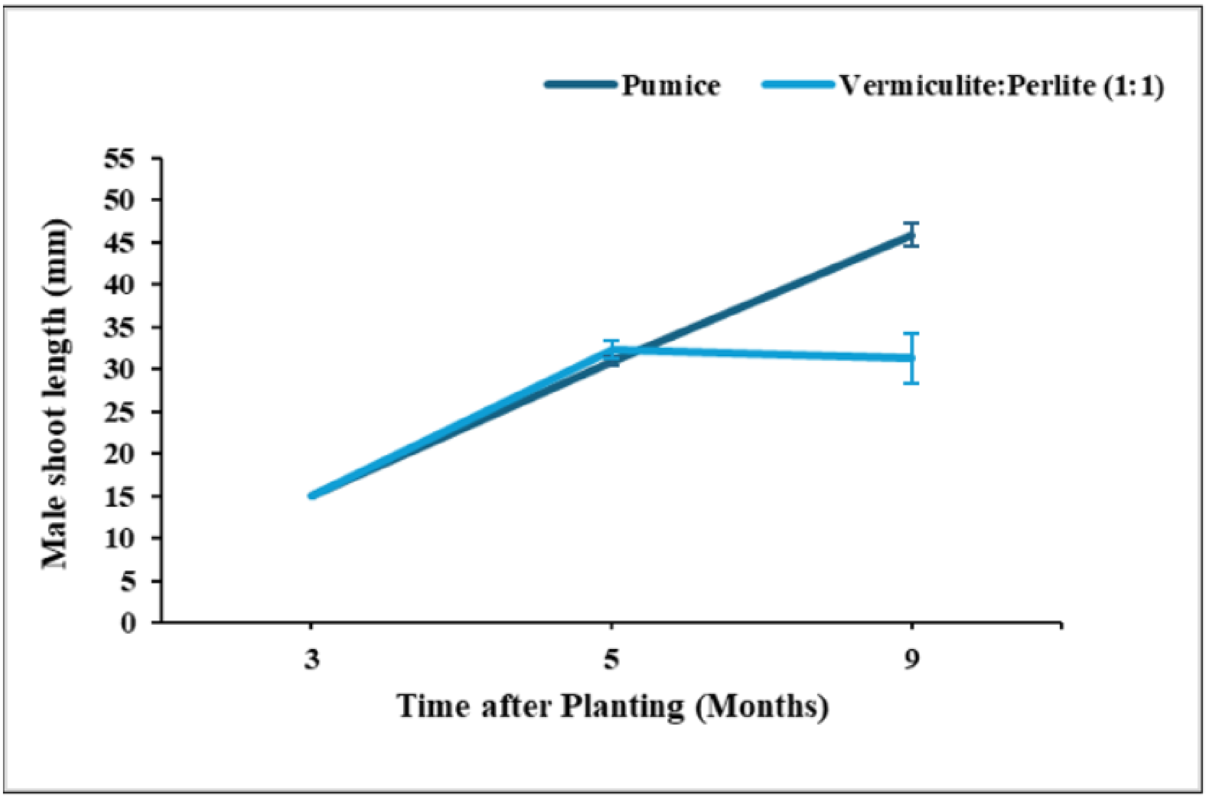

Figure A8: Mean shoot length (mm) of one-year-old male stem cuttings of *Juniperus communis* L. measured at three, five, and nine months after planting across two propagation substrates. Propagation substrates included pumice (2–4 mm particle size) and vermiculite: perlite (1:1, v/v). Values represent means of surviving rooted cuttings with recorded shoot-length measurements. Sample size (n) was 25 and 18 at three months; 19 and 16 at five months; and 15 and 18 at nine months for pumice and vermiculite: perlite, respectively. At three months, all treatment means were 15 mm, with no variation among observations; therefore, ANOVA was not performed. At five and nine months, there were no significant differences between substrates ($P > 0.05$) according to ANOVA tests. Error bars indicate 1 S.E.

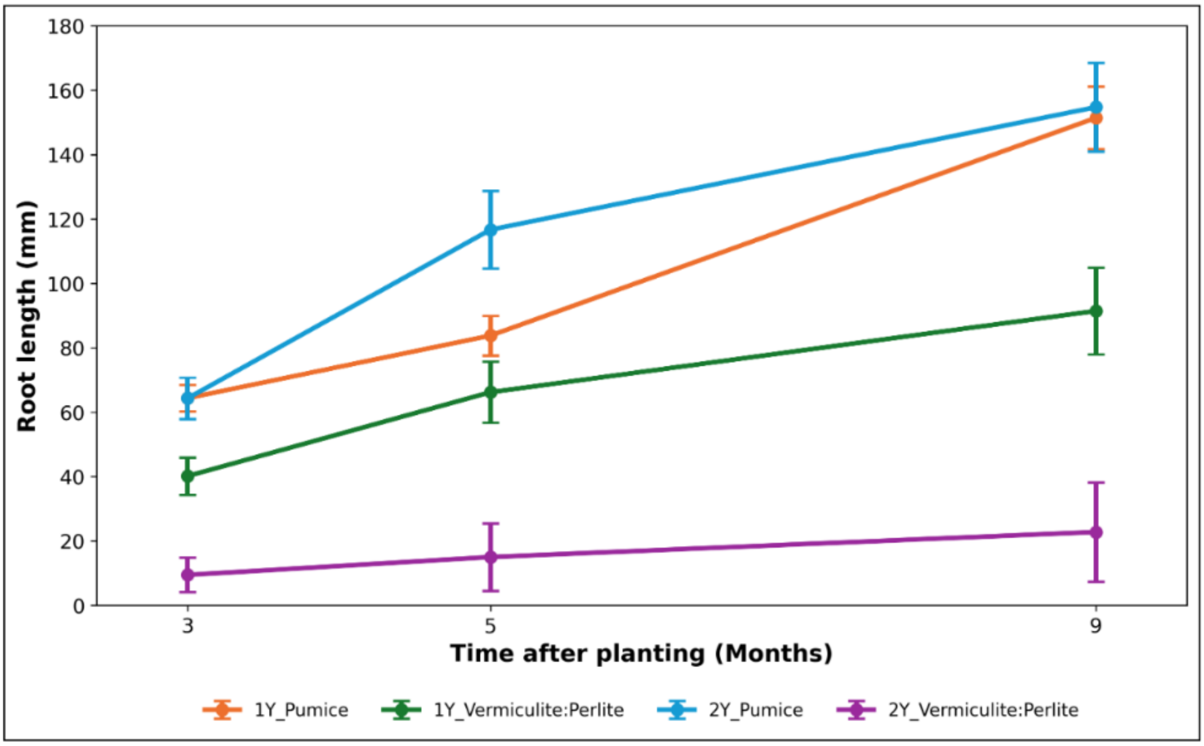

Figure A9: Mean root length (mm) of female stem cuttings of *Juniperus communis* L. derived from one-year-old and two-year-old stock plants, measured at three, five, and nine months after planting in pumice and vermiculite: perlite (1:1, v/v). Propagation substrates included pumice (2–4 mm particle size) and vermiculite: perlite (1:1, v/v). Values represent means of surviving rooted cuttings only. Sample size (n) was 25, 18, 25 and 3 at three months; 19, 18, 19 and 2 at five months; and 18, 18, 17 and 3 at nine months for one-year-old pumice, one-year-old vermiculite: perlite, two-year-old pumice, and two-year-old vermiculite: perlite, respectively. There were no significant differences between treatments at $P > 0.05$ according to ANOVA tests. Error bars indicate 1 S.E.

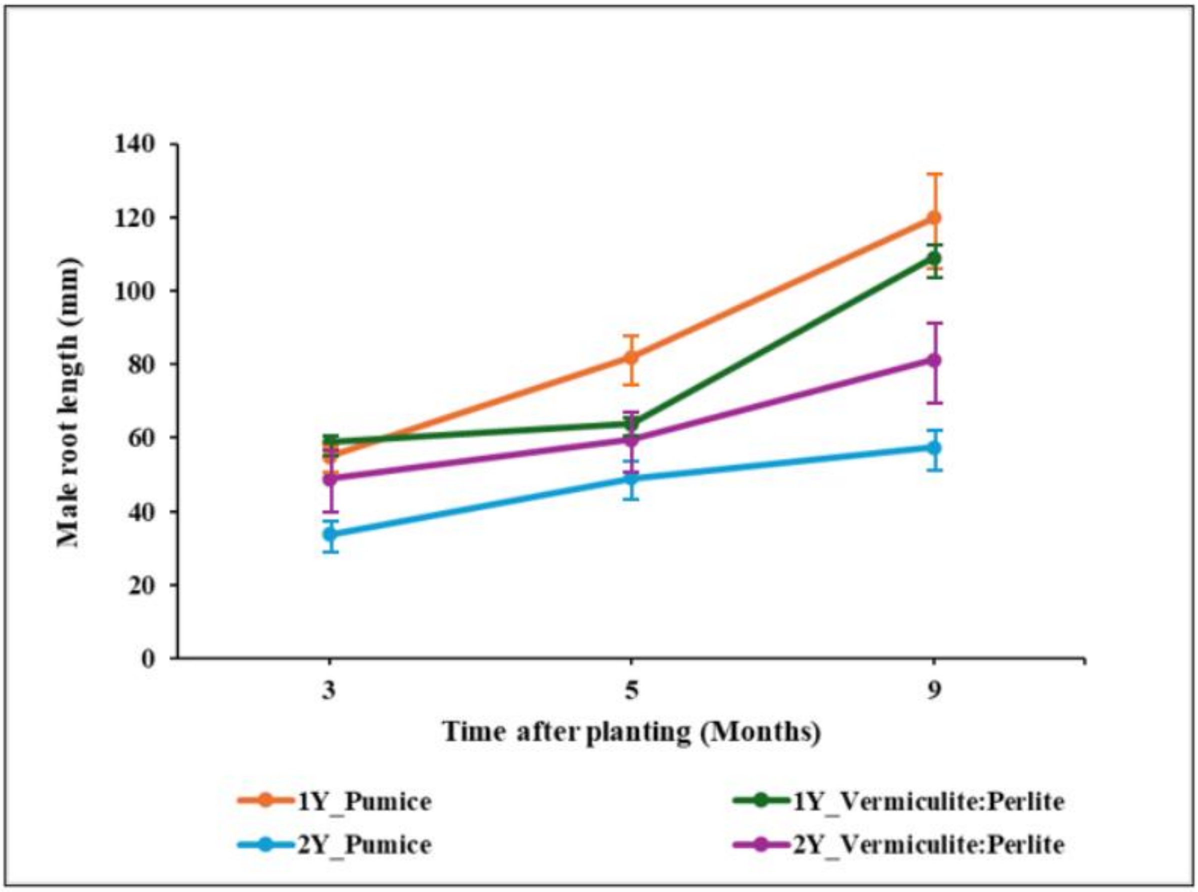

Figure A10: Mean root length (mm) of male stem cuttings of *Juniperus communis* L. measured at three, five, and nine months after planting and propagated in pumice and vermiculite: perlite (1:1, v/v) substrates. Cuttings were derived from one-year-old and two-year-old stock plants. Values represent means of surviving rooted cuttings only. Sample size (n) was 25, 18, 25 and 14 at three months; 20, 18, 23 and 12 at five months; and 15, 18, 21 and 12 at nine months for one-year-old pumice, one-year-old vermiculite: perlite, two-year-old pumice, and two-year-old vermiculite: perlite, respectively. There were no significant differences between treatments at P > 0.05 according to ANOVA tests. Error bars indicate 1 S.E.

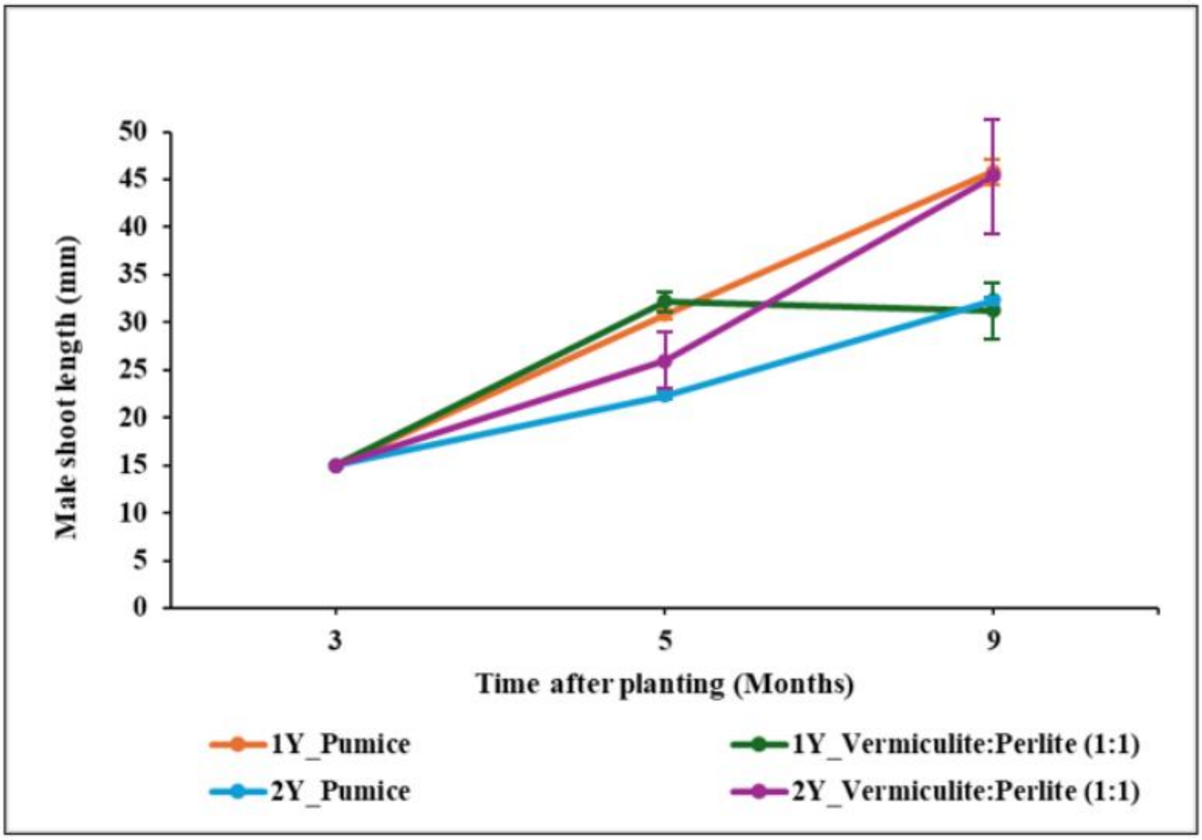

Figure A11: Mean shoot length (mm) of male stem cuttings of *Juniperus communis* L. derived from one-year-old and two-year-old stock plants, measured at three, five, and nine months after planting and propagated in pumice and vermiculite: perlite (1:1, v/v) substrates. Values represent means of surviving rooted cuttings only. Sample size (n) was 25, 18, 25 and 14 at three months; 20, 18, 23 and 12 at five months; and 15, 18, 21 and 12 at nine months for one-year-old pumice, one-year-old vermiculite: perlite, two-year-old pumice, and two-year-old vermiculite: perlite, respectively. No significant differences were detected between treatments ($P \geq 0.05$) according to ANOVA tests. Error bars indicate 1 S.E.

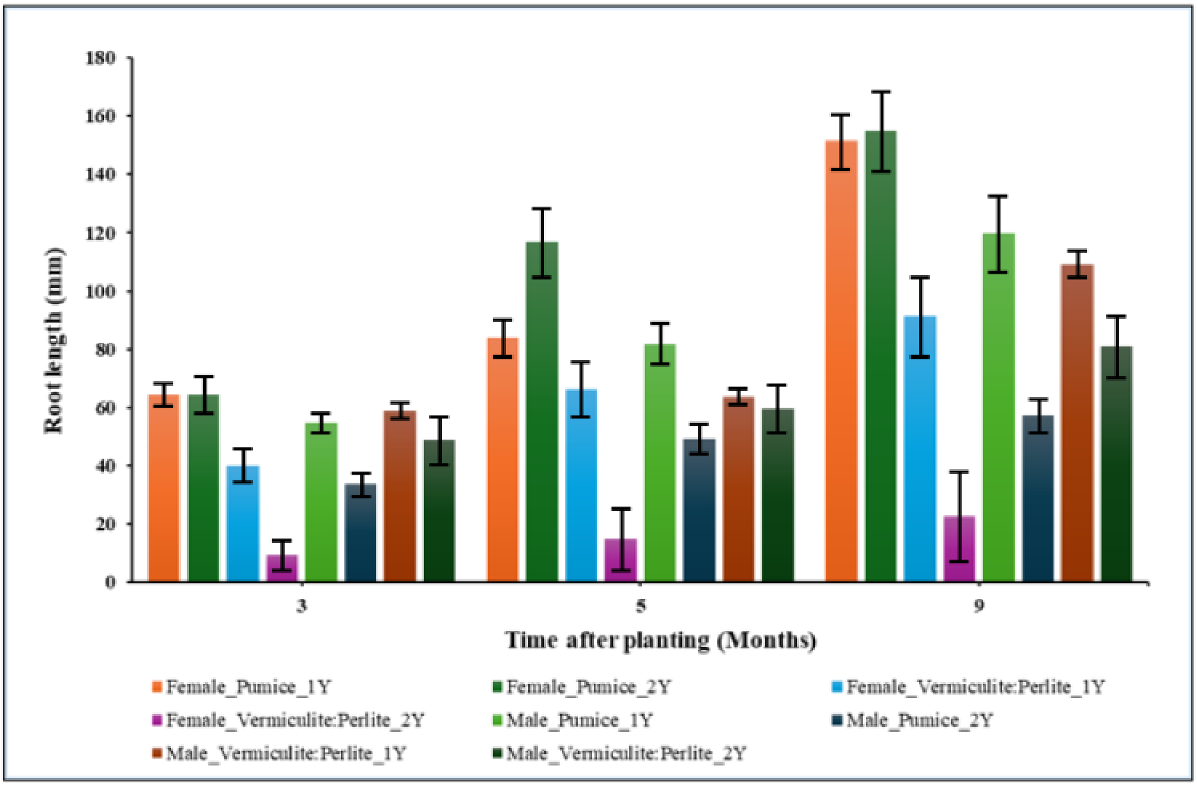

Figure A12: Mean root length (mm) of female and male stem cuttings of *Juniperus communis* L. derived from one-year-old and two-year-old stock plants and measured at three, five, and nine months after planting in pumice (2–4 mm particle size) and vermiculite: perlite (1:1, v/v) substrates. Values represent mean root lengths of surviving rooted cuttings for each treatment combination. Cuttings were maintained under controlled greenhouse conditions with bottom heat (18–25 °C) and intermittent mist irrigation. Data were analysed using two-way analysis of variance (ANOVA) separately for each assessment time point. No significant differences were detected between plant genders or among the age × substrate combinations at three, five, and nine months after planting ($P > 0.05$). Mean separation using Tukey's Honestly Significant Difference (HSD) test was not performed because the ANOVA was conducted without replication. Error bars indicate 1 S.E.

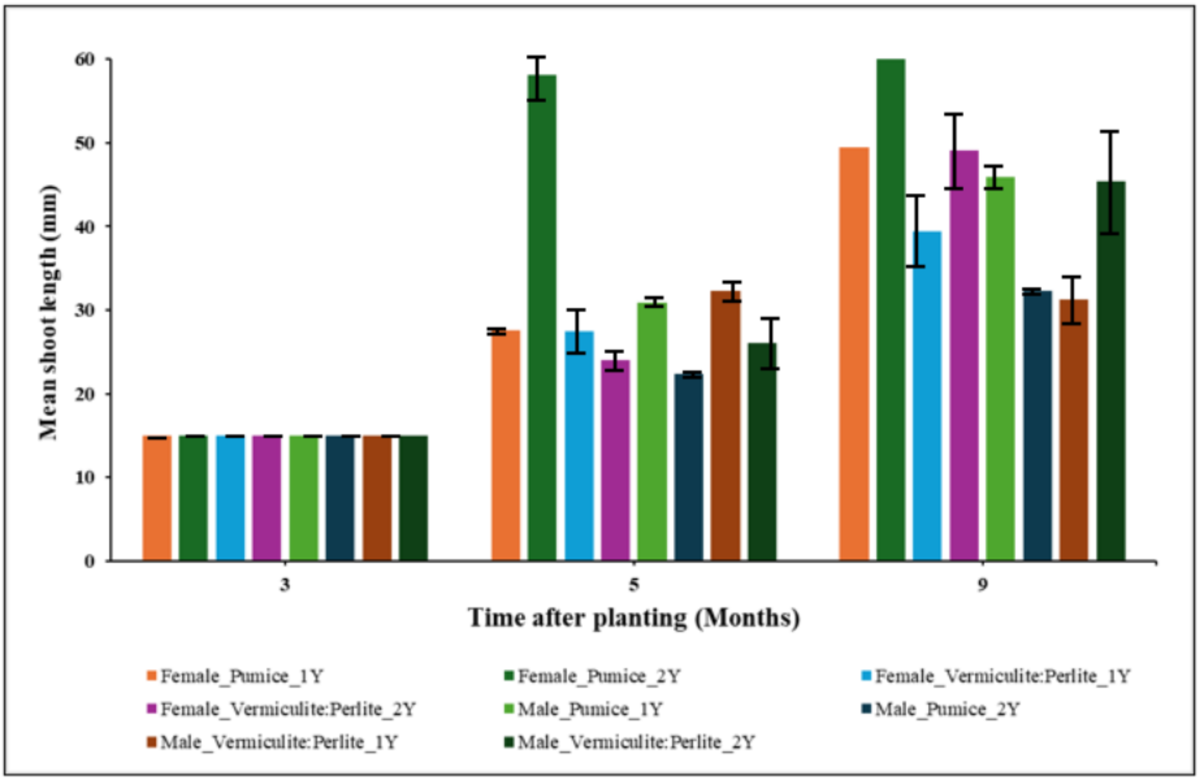

Figure A13: Mean shoot length (mm) at three, five, and nine months after planting in female and male stem cuttings of *Juniperus communis* L. derived from one-year-old and two-year-old stock plants. Propagation substrates included pumice (2–4 mm particle size) and vermiculite: perlite (1:1, v/v). Values represent the mean shoot length of surviving rooted cuttings. Cuttings were maintained under controlled greenhouse conditions with bottom heat (18–25 °C) and intermittent mist irrigation. At three months, all treatment combinations had a mean shoot length of 15 mm with no variation; therefore, ANOVA was not performed at this time point. At five months, no significant differences were detected between plant genders ($P = 0.558$) or among the stock plant age × propagation substrate combinations ($P = 0.743$). At nine months, no significant differences were detected between plant genders ($P = 0.158$) or among the stock plant age × propagation substrate combinations ($P = 0.488$). Mean separation using Tukey's Honestly Significant Difference (HSD) test was not performed because the ANOVA was conducted without replication. Error bars indicate 1 S.E.