ROOTS DYNAMIC IN URBAN WOODY LANDSCAPE SHRUBS AND THEIR INFLUENCE ON SHOOT MORPHOLOGY

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ABSTRACT

Roots are very much the 'hidden half' of any plant, and no more so that when grown as landscape plants in the urban environment. Root systems are expected to 'perform' in a variety of difficult and stressful environments, whether they are constrained in containers of limited volume, planted into shallow soils with restricted water / nutrient availability (e.g. green roofs) or even expected to grow in the absence of soil (e.g. within hydroponic systems commonly used in green facades). Lack of appreciation regarding root systems in general is coupled with an incomplete understanding of how root development might influence shoot growth and morphology in urban landscape plants. A limited range of model ornamental species (*Philadelphus* cv. Aureus, *Philadelphus* cv. Belle Etoile, *Euonymus* cv. Silver Queen and *Punica granatum*) were used, where plant root systems were exposed to a series of typical artificial urban environments such as limited area for growth, compacted soil, waterlogging and physical damage to the root systems in an attempt to identify modifications to root behaviour and the effects on shoot development. Among all the stress factors being studied, flooding was recorded to have the most detrimental effect on urban vegetation where there were a number of plant deaths recorded in *Philadelphus* cv. Aureus; whereas *Euonymus* cv. Silver Queen was observed to be more resistant toward flooding effect. Reduction of root and shoot biomass was an almost universal response when plants were exposed to stress, and this might be due to limited nutrient and water availability especially in compacted soil and smaller rootball geometry. Other commonly observed traits were reductions in height, reductions in leaf marginal area and in some circumstances changes in branching pattern. These physiological adaptations in plants changed the aesthetic character by producing more compact and smaller plants although this was not always significant for all species and environment stresses. In terms of direct root damage, data from one experiment using split-pots systems suggested a certain threshold of root damage needed to be crossed before significant levels of re-growth were activated. Careful species selection is required to ensure urban vegetation is resilient to the stresses commonly encountered, a factor that will be increasingly important with climate change and greater density of urban built infrastructure in future.

DECLARATION

No portion in this work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or institute of learning.

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CHAPTER 1

INTRODUCTION & REVIEW OF LITERATURE

1.1 Urban Green Infrastructure

With increasing awareness of the importance of quality green space within urban areas, greater attention is now being placed on the provision of effective green space within the urban matrix. Green space can provide a range of ecosystem services including urban cooling (Tyrväinen et al., 2005a), thermal insulation (Dwyer et al., 1992) storm water mitigation (Day and Dickinson, 2008) habitat for biodiversity (Jensen et al., 2005) and enhanced human health and well-being (Chiesura, 2004). Table 1.1 demonstrates the benefit of urban trees and forest based on 5 categories.

Table 1.1: Benefits and uses of urban forest and trees (Tyrväinen et al., 2005b)

Social benefits	Recreational opportunities, improvement of home and work environments on physical and mental health. Cultural and historical values of green areas
Aesthetic and architectural benefits	Landscape variation through different colours, textures, forms and densities of plants. Growth of trees, seasonal dynamics and experiencing nature. Defining open space, framing and screening views, landscaping buildings
Climatic and physical benefits	Cooling, wind control, impacts on urban climate through temperature and humidity control. Air pollution reduction, noise control, reduction of light glare and reflection, flood prevention and erosion control
Ecological benefits	Biotopes for flora and fauna in urban environment
Economic benefits	Products and markets (timber, berries, mushrooms etc.), increase property values, faster property re-sale retail turnover, tourism and employee productivity.

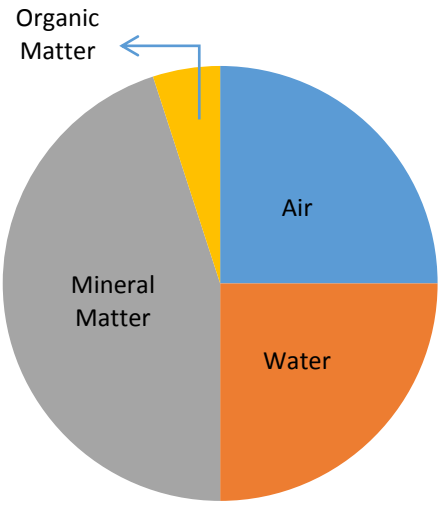
Urban trees and other woody plants are key components of this green space provision, but ironically many are considered to be in a poor state of health themselves, due to the difficult growing conditions encountered in the urban environment (Jim, 1998). Partially,

this may due to infrastructure changes over the last 30 years, including the development of new technologies that have required much alteration and construction within the urban matrix (Jim, 2003). These include the laying down of fibre optic cables for telecommunication purposes, the upgrading of water, sewage and power services, increased urbanisation through greater housing / building density including the construction of skyscrapers, and also the development for transportation services. Major changes in soil structure resulted in poor plant performance caused by poor root development. This may be due to the limited area that roots can spread into, poor aeration within the soil due to compaction, or unsuitable or poorly structured soil types that are not conducive to root development (e.g. stony, drought-prone soils) (Jim, 1998). Apart from changes in the characteristics of urban soils, pollutants within the soil contribute to poor plant performance. Evaluations of heavy metal contamination in an urban park in Guangzhou, China demonstrated various metals were concentrated in both soil and plants; with the highest concentrations within plants being found in the leaves of trees whilst overall highest levels were correlated with soils in the immediate vicinity of roads (Guan and Peart, 2006). Heavy metals impact on human health by the elements being leached into groundwater and / or absorbed by plant roots, concentrated in the tissues and eventually consumed by humankind (Ajmone-Marsan and Biasioli, 2010). For example in the UK, there have been incidences of vegetables such as radish, lettuce, spinach and cabbage having high concentrations of lead (Thornton, 1991).

1.2 Urban soil properties

In contrast to many urban soils, natural (brown) soils frequently have a balanced amount of water, air and the addition of mineral particles in various shapes, sizes and compositions; factors that are conducive to plant growth (Craul, 1992). Indeed the advantageous nature of natural soils is often a result of plant, invertebrate and microbial activity, in improving soil structure and recycling nutrients. Plants are tolerant of a wide range of soil conditions, but the optimum balance of the various elements is depicted in Figure 1.1.

Figure 1.1: The four major components of an ideal soil (Craul, 1992)



Pavao-Zuckerman (2008)mentions that urban soils are in a different taxonomic class due to their altered structure and functional capabilities compared to agricultural soils. In addition, intervention of human activities such as trampling, mixing of soil strata, and transporting alien material to, and then embedding with urban soils, adds to the complexities (De Kimpe and Morel, 2000, Rossiter, 2007). Similarly, (Bullock and Gregory, 1991) explain that disturbance and movement of urban soils in the past has resulted in the loss of soil structure, promoting the formation of compacted, soils and that this has become a major concern in maintaining fully biologically-active 'healthy' soils.

Changes in soil structure and the introduction of alien materials (concrete and brick rubble, plastics, metal or chemical contaminants etc.) to urban soil influences plant performance and development. Urban soils suffer physical, chemical and biological alterations resulting in general, in increased bulk densities, and reductions in soil organic matter, microbial activity and macro-biology biomass (Scharenbroch et al., 2005). These factors inter-relate. Increments in bulk density correspond to reductions in pore size and volumes, soil aeration, availability and root access to nutrients as well as altering soil chemistry.

Urban situations therefore, are considered to be challenging environments for effective root development, but the precise responses of root systems to the difficulties encountered is still not fully understood (Perry, 1982). Under ideal conditions it is thought roots will ramify to four to seven times the area of that of their normal crown area (Bassuk and Hawver, 2007). Lack of appreciation regarding root systems in general is coupled with an incomplete understanding of how root development might influence shoot growth and morphology in urban landscape plants.

1.3 Importance of urban landscape plants

Urban landscape plants provide a number of important benefits (eco-system services) within the urban matrix.

1.3.1 Microclimate modification

Urban areas have been reported to have higher temperatures than nearby rural areas (Streutker, 2002) due to the existence of buildings and other forms of hard surfaced infrastructure. The enlargement of urban centres, and the increased densification (more buildings for a given area) of towns and cities, combined with a warming climate will contribute to this Urban Heat Island (UHI) effect in future. Urban heat island events are dangerous as they have implications for human thermal comfort, with certain sections of society (the elderly, babies and those with respiratory or heat related medical conditions) suffering increased risk of heat stroke and similar physiological problems (Yu and Hien, 2006). UHI phenomena partially exist due to a lack of natural vegetation types and water bodies within many urban areas. These provide a cooling influence and offset the heat built up from buildings, roads, cars, and industrial machinery (Dixon and Mote, 2003). Dense, built up areas cause building structures, concrete and asphalt to absorb heat during the day and release it back to the atmosphere during the night. However, increasing the proportion of urban areas that have trees and other forms of vegetation can help mitigate the urban heat island effect.

Higher densities of plants help to create a better microclimate by reducing the air temperature in summer (Dimoudi and Nikolopoulou, 2003). Systematic planting design for urban environment especially below the canopy area help create a comfortable ambient pedestrian zone (Ng et al., 2012). In addition, plants function by providing direct shading thus reducing ground surface temperatures (Dimoudi and Nikolopoulou, 2003). In addition plants evapotranspire, thus reducing temperature increments as energy is preferentially consumed in latent heat (converting liquid water to vapour). Research from Hillsboro, Portland indicates that evaporation of water from irrigated vegetation helps to improve hidden heat exchange resulting in decreasing levels of surrounded air temperatures (House-Peters and Chang, 2011).

In contrast to this ability to cool urban areas in summer, the microclimatic effects of plants can also help provide insulation, thus providing a localised warming effect in winter and reducing energy losses through e.g. wind breaks (Bolund and Hunhammar, 1999).

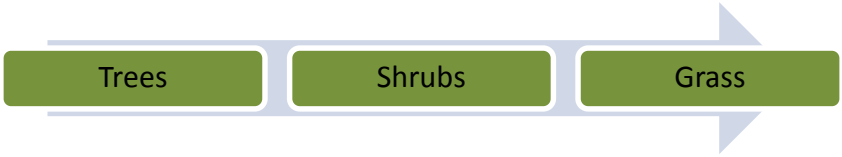
1.3.2 Improve air quality

Air pollution is among the major environmental issues and is a contributor to health problems in urban areas due to factors including transportation and heating of buildings (Bolund and Hunhammar, 1999). Many pieces of research have been done on proving how the existence of vegetation will help to reduce pollution. However, there are contradictory results in studies measuring nitrogen dioxide (NO_2) and volatile organic compound (VOCs) in Helsinki where vegetation was found to have little influence on gaseous removal from the environment (Setälä et al., 2013).

Among the studies that support the role of vegetation in improving air quality, Nowak et al., (2006) stated that environmental air quality and human health can be improved by having trees in the cities. Leaf stomata absorb pollutant gases which diffuse into intercellular spaces and are absorbed or deactivated by the plants. In addition leaves can attract larger particles of dust and smuts (particulate pollution) and remove these from the atmosphere. Such particles are thought to contribute to asthma and bronchitis as well as other respiratory and cardio-vascular health problems. Trees with denser

leaves are thought to be able to increase infiltration rates of pollutants and surface texture can play a role in attracting the particulate matter (Bolund and Hunhammar, 1999). In addition, infiltration capacity increases with the increasing surface size of the leaf, which can be summarized in diagram below (Givoni, 1991);

Diagram 1.1: Order of efficiency for pollutant infiltration.



On the other hand, bigger particles will be retained on the leaf surfaces rather than the atmosphere and will be washed off by the rain or dropped off to the soil when the leaves fall (Nowak, 2004).

1.3.3 Noise reduction

Noise pollution in urban environment can be reduced by vegetation. By acting as a barrier to the audio source of the noise such as traffic / major roadways etc, vegetation can deflect, absorb and break up sound waves. Noise is also perceived to be less in more green environments, suggesting a psychological benefit as well as physical benefit from the vegetation, and can aid in promoting the ambience of urban places (Bolund and Hunhammar, 1999). Fang and Ling (2003) suggested that noise reduction is greatest when high density shrub planting is employed.

1.3.4 Hydrology effects

Water flows in urban areas are disturbed by increasing built infrastructure and the loss of soil permeability (soil sealing) through extensive use of tarmac roads, concrete pavements etc. (Bolund and Hunhammar, 1999). Infrastructure development results in loss of vegetation, soil removal and natural ground cover being replaced by impermeable surfaces such as parking lots, roads and pathways. This results in poor infiltration of water into the soil, smaller water basins and increased surface runoff

(Konrad, 2003). In contrast, higher densities of vegetation in urban areas reduce surface run off, lower the storm water management costs, as well as improve the quality of water (Dwyer et al., 1992). In agreement, Nowak and Dwyer (2007) indicate that rain precipitation can be slowed down through interception and retention by tree leaves and reducing the energy within individual rain drops (e.g. reducing soil erosion).

1.3.5 Habitat for biodiversity

Greater urbanization causes habitat and biodiversity loss and species extinction in urban ecosystems together with urban animal and plant composition being strongly influenced by human activities (Kowarik, 2011, Sukopp, 2004). The awareness of protecting the remaining natural habitat increasing as well as the importance of conserving the biodiversity. Urban vegetation can contain relatively high level of biodiversity, and this can be achieved by the increased number and variety of trees and other plant types found in urban areas (Alvey, 2006). Increasing vegetation creates habitat and attracts wildlife into urban areas, and enables them to complete their life cycle within the boundaries of towns and cities.

1.4 Urban environment condition affected root and shoot system

Plants are immobile compared to most animals, and this makes them vulnerable to urban development, as since they cannot defend themselves from the associated environmental threats. Plants can only adapt to a degree of environmental stress and eventually will die if unfavourable conditions are prolonged, or thresholds exceeded. Limited area for root growth, compacted soil, poor nutrient availability and poor drainage are among the threats encountered by plants in urban conditions. Clark and Kjelgren (1990) described plant water stress and insufficient soil water content as key restriction factors in the growth of trees in urban areas. However, studies on the effect of urban soil condition (i.e. limited area for growth, compacted urban soil, root injury, water logging and water deficit) have been mostly discussed in the context of trees and further work is warranted on the impacts of such factors on shrub species.

1.4.1 Plant response to limited planting space

Plant roots are used to absorb nutrients, water and even some phytohormones from the soil as well as an anchor the plant for stability. Rhizosphere is the term used for describing the root and soil environment together (Gregory, 2006). Roots generally require adequate amounts of space in the soil for them to grow healthily. According toJim (2003), trees need large diameter volumes to grow lateral roots effectively; this is more important than greater depth as lateral roots can extend out to three times the distance of the tree crown area in normal conditions (without restrictions to root spread). However, the characteristics of tree root growth depend on the species in question. Studies on *Ficus retusa* 'Nitida' (Ficus) and *Schinus terebinthifolius* Raddi. (Brazillian pepper) demonstrated that greater rooting depth with a small diameter surface area enhanced root biomass in *Ficus* while the use of shallow and wide containers was more favourable for pepper root growth (Schuch et al., 2000).

In urban areas, street trees are affected by almost continuous engineering work that is required for the insertion and maintenance of infrastructure, such as pipes and telecommunication cables. As well as the disturbance these often result in limited space available below ground for tree root development, or the restricted space may encourage the roots to interfere with the service infrastructures. Jim (2003) claims that roadside trees are suffering due to their requirement to be grown in a minimum soil depth and being bounded by utilities' cables that are laid near the sidewalk surface. In agreement, Lindsey and Bassuk (1992), mention that urban trees have to compromise their root growth, by being constrained through both shallow depth of rhizosphere as well as the restricted width of available soil, which reduces lateral root extension, essentially contradicting the trees natural requirements. Grabosky and Bassuk (1995) state that plants require greater volumes of soil to reach their optimum size and function and provide the environmental benefits that they are planted for, yet this factor is not being adequately addressed in urban design. Even at a smaller scale, research findings support this notion; a previous study on the ornamental bedding plant, *Petunia x hybrid*a 'Orchid' showed that root mass was reduced when plants were grown in smaller containers, compared to that of plants in larger volume containers. The reduced root mass was correlated with shorter plant height (Haver and Schuch, 2001).

Apart from roots, restricted area / geometry also influenced above ground growth. Yong et al., (2010) showed that nutrient and water uptake under root restriction condition were limited and caused modification in shoot growth (leaf area, stem size and shoot biomass) and photosynthesis resulting in dwarf plants. Yong et al. (2010), point out that shoot reduction might also be affected by less nutrient availability. Dwarf plants have small leaves and short internodes and this occurs in plants grown in small pots as compared to larger pots (Carmi and Heuer, 1981). Smaller leaves associated with the more dwarf plants were linked to alterations in plant anatomy (Passioura, 2002) essentially there being fewer numbers of cells present, but not necessarily smaller individual cells (Korner et al., 1989).

Plants grown in limited soil volumes impact on biomass and yield (in crops). Studies conducted in agricultural crops and field vegetables indicate that limited land or area for growth results in yield penalties, including smaller and fewer items of production. Gross (1991) mentions that root growth can be harmful in poor soil and in some conditions can decrease crop production. Work with *Abutilon theoprasti* also points out that greater numbers of seed were produced as pot size / substrate volume increased (McConnaughay and Bazzaz, 1991)

NeSmith and Duval (1998), explain that photosynthetic rate is lower for plants grown in smaller containers due to reduction of plant biomass. Apart from that, Carmi and Heuer (1981) state that restricted space for root growth will reduce the amount of cytokinin exported from roots and this leads to poor fruit development. Cytokinin being transported by xylem vessels to shoots for development and reduction in biomass leading to smaller amounts of cytokinin synthesised.

Moreover, studies conducted on *Tagetes erects* L. 'Janie' seeds conclude that plants grown in smaller containers have smaller flower cover, decreased leaf growth and reduced shoot and root dry weight (Latimer, 1991). Research done on *Petunia* X *hybrida* Hort. Vilm,-Andr demonstrates that there are changes in morphology of those petunia grown in restricted volumes of media, with responses including reduced number of roots and shoot biomass, lateral branching as well as reduced leaf surface area (Haver

and Schuch, 2001). These morphological changes also appear to be the same in *Hydrangea* where plants grown in small pots showed reduced numbers of total nodes, leaf area, leaf number and shoot and root biomass when plants were controlled for fertilizer and water availability (Yeh and Chiang, 2001).

1.4.2 Plant response to compacted soil

Compacted soil is a common feature of urban environments. This is due to heavy vehicle and pedestrian traffic, but also because soils can be intentionally compacted during the process of laying the foundation to buildings, roadways and pathways (Day et *al*., 1995; Rhoades and Stipes, 1999; (Kirby and Bengough, 2002); Quigley, 2004). Root growth and spread is restricted in compacted soils with negative effects on plant stability and ability to access water and nutrients (Jim, 1998). Soil compaction happens both through natural processes and by human activities (Kozlowski, 1999). Tillage, growth of plants and forest fire can cause soil compaction as can the use of heavy machinery, traffic by pedestrians and animals. According to Kozlowski (1999), soil compaction has become an economical as well as ecological issue all over the world and Patterson (1977), Yingling et al. (1979) and Jim (1993) highlight the impacts within urban locations. Trees planted on construction sites commonly don't survive growing in compacted soil (Watson and Kelsey, 2006) and the process of planting mature specimen trees can even make the soil conditions worse as this itself involves heavy machinery and tramping of the soil. Irrespective of the direct impacts of compacted soil on root penetration of the substrate, it also can restrict plant growth by limiting the amount of water available (loss of pore space that holds water) or by puddling of water and making the soil anaerobic through waterlogging (Lindsey and Bassuk, 1992).

Soil compaction affects porosity and physical properties, e.g. reduction in volume of pores and water retention (Dexter, 2004).Richard et al. (2001), explains that compaction can effect hydraulic conductivity and water retention properties as well as reduces the volume of large pores; while changes in particle size distribution, organic carbon content and clay mineralogy affect the smaller pores. In addition, root penetration is harder in compacted soil due to the increasing mechanical resistance caused by high bulk densities

and the loss of pore spaces, along which roots may normally travel (Watson and Kelsey, 2006). Tracey et al. (2011), stated that root development in compacted soil was constrained by the increasing bulk density, and this limits the ability of roots to spread out.

In conjunction with high physical resistance to penetrate the soil, plants produce shorter root lengths compared to development under normal, non-compressed conditions. Previous studies using 7 –day old cereals, report that there was a reduction of 50% in root length for *Hordeum vulgare* (barley) and up to 79% in *Triticosecale wittmack* (triticale) (Lipiec et al., 2012). Also, plants grown in compacted soil tended to produce thicker roots with more root hairs present. Root diameter will increase in parallel to the penetration resistance. Kirby and Bengough (2002) explain that in the higher soil strength roots will expand cylindrically rather than longitudinally resulting in enhanced root thickness.

In soils with extreme compaction, decreases in stomatal conductance and slower shoot growth are not instantly noticeable, however, root growth is quickly affected with corresponding effects over the long term on crop yield (Roberts et al., 2002). In *Triticum aestivum* (wheat), soil compaction caused slower cell expansion rate and produced smaller mature leaves which then resulted in reduced plant growth (Andrade et al., 1993). Figure 1.2 shows leaf area as affected by soil bulk density.

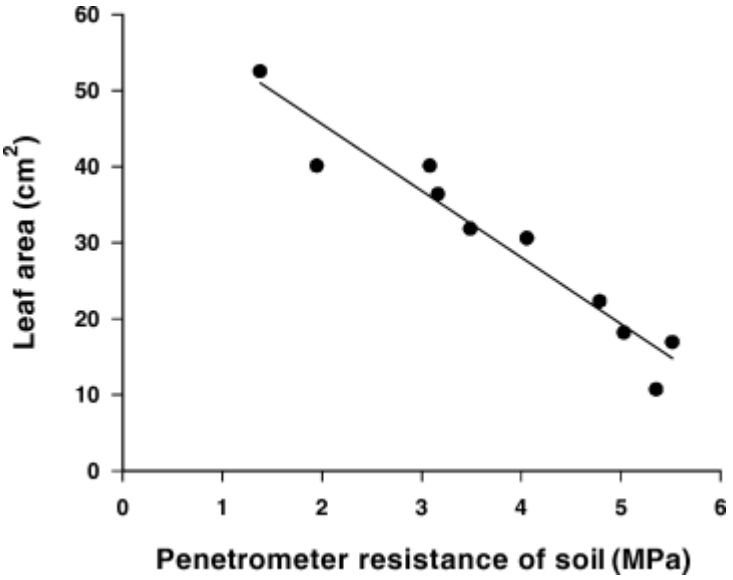


Figure 1.2 Effect of penetrometer resistance of soil on growth of young wheat plants (Tracy et al., 2011a).

In contrast, work on cassava (Maduakor, 1993) suggested leaf surface areas increase with increasing bulk density but optimum growth rates were not achieved across the range of densities investigated.

1.4.3 Plants response to partial and complete submergence

Urban soils are very sensitive to environmental changes and in the event of longer period of heavy rain, flooding conditions may occur. As discussed in plant response to soil compaction, changes in soil characteristic reduce infiltration of water, and can cause it stand at the surface and lead to waterlogging conditions. Smith et al., (2001) stated that poor subsoil and surface drainage as well as modifications in infiltration cause waterlogging conditions in urban areas. In addition, Craul (1992) explains that the hydrophobic character of the soil surface and compacted soil are the factors that cause reductions in infiltration rates.

Striker (2012) explains that flooding term used depending on the amount of excessive water ranging from water saturated soil (waterlogging) to complete submergence of plants.

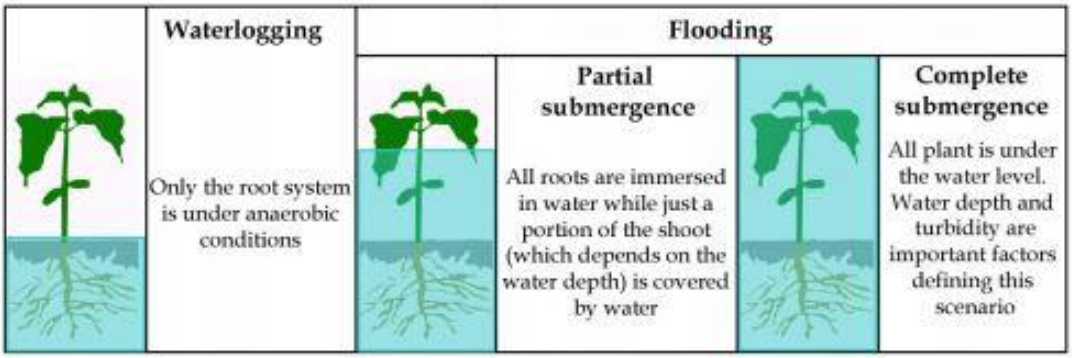


Figure 1.3 Scheme of the different scenarios encountered by plants to increasing levels of water excess, ranging from waterlogging to complete submergence (Striker, 2012).

The severity of effects on plant growth due to waterlogging depend on the time of occurrence (seasonal effects), the duration of flooding, the depth of waterlogging as well as the condition of the flood water (Kozlowski, 1984). Studies in *Triticum* indicate that short term waterlogging affects growth over the longer time, i.e. there can be delayed residual effects (Malik et al., 2002). Under partial submergence (waterlogging condition), leaves will continue with carbon fixation via photosynthesis but also lead to aerenchyma formation for gas diffusion to roots for elongation (Striker, 2012). In addition, the formation of adventitious roots near the aerated soil surface were stimulated by ethylene production when the roots / stems were submerged (Striker, 2012) and are a modification to allow for the continuity in water and nutrient uptake, and hence plant survival (Yamamoto et al., 1995).

Nevertheless, Striker (2012) also explains that when plants are completely submerged, they can either adapt to the situation by being in Low Oxygen Escape Syndrome (LOES) or Low Oxygen Quiescence Syndrome (LOQS). LOES is normally favourable in plants exposed to longer periods of flooding where there is a shallow water depth; this response results in upward shoot elongation. LOQS, however, is favourable in short duration deep floods where plants become inactive and recommence their growth when the flooding lessens.

Flooding affects the above ground tissues causing leaves to become yellow and then senesce (Ezin et al., 2010) due to reducing the total chlorophyll content of the leaf

(Kumar et al., 2013). According to (Kozlowski, 1984) most studies report that flooding injury is triggered by oxygen deficiency. Competition for oxygen between plant roots and micro-organisms; and slow diffusion of oxygen into the water body or submerged soil contribute to the development of anaerobic conditions (Visser et al., 2003, Jackson and Colmer, 2005). One characteristic of waterlogged plants is that they rarely achieve optimum growth, and for those plants that survive, growth parameters are curtailed. In *Eucalyptus camaldulensis* and *Eucalyptus globulus* waterlogged seedlings resulted in reduced numbers and size of leaves, and plants had shorter internode sections compared to controls (Sena Gomes and Kozlowski, 1980). Similar patterns were observed in *Triticum* where the final leaf length and leaf area of plants in waterlogged soil were less than those in normal soil conditions (Malik et al., 2002).

The impact of flooding on productivity can be striking. Due to the root and shoot injury, as well as direct effects (e.g. from ethylene) on flowering organs can result in reductions in total yield and delay crop maturity. Flowers per panicle and flower dry mass in *Triticum* were decreased by waterlogging at early flower development stages as well as prolonging the time to flower opening (Shao et al., 2013). The same story also has been reported by Kumar et al. (2013) in *Vigna radiata* (L.) Wilczek (mung bean) where waterlogging conditions resulted in decreasing number of pods per plants and pod setting contributing to lower yield; while studies on *Brassica oleracea* (broccoli) demonstrate a reduction in biomass production.

1.4.4 Plant response to physical root injury

Plant establishment after transplanting is a major consideration in landscaping due economics, logistics and customer / public perceptions of the landscaped site. Various reasons are reported as to what causes plant failure, for example the use of poor quality plant material, inappropriate planting or limited aftercare. Trees in urban areas are normally associated with poor growth and lower survival rates due to the roots being damaged. Ghani et al., (2009) state that most tree roots are found near the soil surface and root damage caused by trenching, excessive cultivation, or even increasing the depth of soil via re-alignment of the soil surface can lead to tree failure and death.

Trenching causes root damage during the fitting of utility infrastructure along roadways and pavements. Jim (2003) explains that plants take several years to show the effect of any root damage which by that time, the reasons behind the plant failure are already forgotten. Effects of trenching on plant growth varies depending on the season, distance from the trunk and how severe the damage is. Previous research on *Aesculus hippocastanum* (horse chestnut) and *Tilia × europea* (European limes) indicated that root trenching during the rainy season did not result in crown dieback and tree death, whereas activities during dry or drought periods were more likely to lead to tree death (Fini et al., 2013). A tree is unlikely to achieve optimum growth when injury due to the trenching is severe. Severe root damage will reduce tree growth over the longer term, with reduced trunk diameter (DBH) and shoot extension (Watson, 1998).

The type of root damage (or pruning) that occurs may influence the response. Carefully controlled root pruning in other areas of horticulture and agriculture can be used for positive effects such as controlling excessive shoot vigour and encourage a more branched fibrous root system. The ancient Japanese used root pruning in their art of Bonzai as a dwarfing practice (Schupp and Ferree, 1990) indeed primary branches and length in *Ziziphus jujube* Mill. trees were decreased by root pruning (Yang et al., 2010). In tree-fruit production root pruning is performed to enhanced yield. Studies done on *Malus domestica* 'Jonathan' (apple) found that fruit weight, colour and firmness were improved after root pruning but the total cumulative of yield depended on time of pruning (Ferree, 1992). Meanwhile, root pruning of two ornamental shrubs *Buddleja davidii* 'Summer Beauty' and *Cistus* 'Snow Fire' at time of planting into the ground from pots, indicated better establishment through the promotion of new roots, and enhanced root development compared to other manipulation techniques such as teasing out roots or leaving the roots in their original rootball (Blanusa et al., 2007).

1.5 Root to shoot communication in response to environmental stress

Roots have the ability to sense environmental changes in plants especially in the event of drying soil or nutrient shortage. The effect of environmental stress (drought, flooding,

mineral nutrient shortage, salinity or compaction) on plants, comprises responses such as stomatal closure, leaf epinasty, slower leaf elongation and leaf senescence (Jackson, 1997). Dodd (2005) states that shoot growth reduction in plants is generally associated with failure in providing enough water or nutrients from the roots. Roots detect changes in soil condition and send signals through hydraulic and chemical means to the shoots. These chemical signals involve hormonal action (Colebrook et al., 2014), where plants responsed to environmental modifications, growth and expansion by altering the hormonal signalling between different organs to help regulate growth in response to the stimuli.

1.5.1 Root to shoot signalling in compacted soil and water restriction

To grow optimally, plants need a root zone that holds moisture, air and nutrients in a balanced manner. However, urban soils are known for their poor soil characteristics in terms of aeration, poor drainage, high bulk density and lack of nutrients. Mullins (1991) states that optimum water uptake is essential for plant growth; and roots need to penetrate into the subsoil layer since water resources in topsoil are not adequate to support plants during dry periods, especially in lowland parts of UK.

To ensure plant survival, plant roots will response to any changes detected in soil. Tracy et al. (2011), mentions that plant roots will develop more root hairs to maximise water uptake in compacted soil. In addition, roots systems will develop more vigorous adventitious roots with longer, straighter and larger diameter in compacted soil, in contrary to normal soil where a tap root was found to penetrate deeper into the soil (Gilman et al., 1987). Many researchers reported that root diameter tends to increase in increasing mechanical impedance. Studies done in *Helianthus annuus* L. ('Vincent') (sumflower) and *Zea mays* L. (maize) proves that more roots were found near the surface area in higher bulk density soil with thicker roots diameter (Goodman and Ennos, 1999).

Plants roots sense and communicate any environmental changes to the shoots via a chemical signalling mechanism (Davies et al., 1993). This root to shoot signalling is important in understanding how plants adapt to environmental stresses and can

manipulate situations in an attempt to optimise growth and carbon gain. Schachtman and Goodger (2008), point out that in agriculture, root to shoot communication is vital especially as plants need to respond to mild and severe drought conditions and maximise their water use efficiency. Chaves et al. (2003), defined the range of plant responses when placed under water deficits (Figure 1.4).

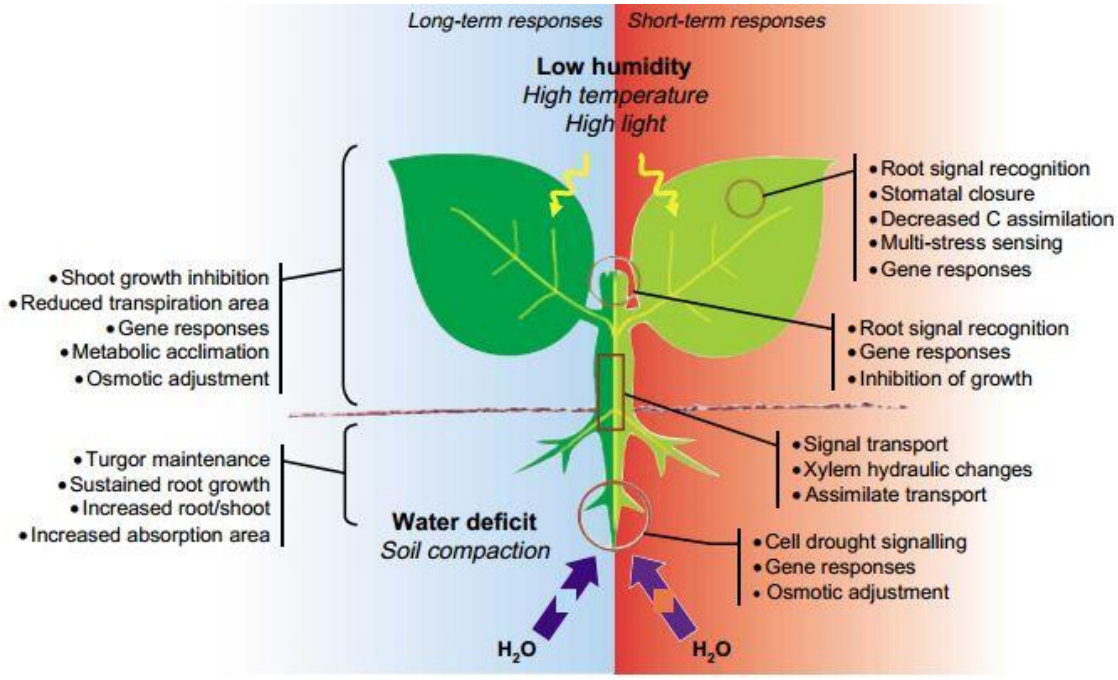


Figure 1.4 Whole-plant responses to drought stress. Left, long-term or acclimation responses; right, short-term responses (Chaves et al., 2003).

Chaves et al. (2003), described plant responses to short term and long term water stress and indicated there were slightly different strategies employed (Figure 1.4). Rapid water deficit will cause roots to send signals to the shoots that in turn minimize water loss by stomatal closure and inhibit further shoot extension and leaf expansion. On the other hand, when plants sense a water deficit due to soil compaction, plants tend to adapt to the situation by reducing the transpiration area, inhibit shoot growth and at the same time maximise water uptake. When roots sense drying soil, signals were sent to the shoots to reduce water loss via leaf transpiration and minimise water transportation between xylem and the growing soil to avoid plant growth (Davies and Zhang, 1991).

(Jackson, 1993) defines the four types of signal sent from root to shoot; positive message, negative message, accumulative message and debit message. Positive message will trigger additional hormonal production in shoots while negative message will instruct less production of a hormone. With an accumulation message, roots will become less active sinks resulting in hormone build-up in source tissues and lastly for debit message, root will become more active sinks for hormones.

1.5.2 Root to shoot signalling in flooding

Plants must act on stress situations as whole organisms, especially under low oxygen conditions where parts of the plant (i.e. stems and leaves) are not exposed to the flooding *per se* (Arru et al., 2013). This response is vital for plant survival and for adaptation as soil flooding could be harmful to plants. Many physiological dysfunctions affect plant growth in flooded soil including early leaf senescence, hormonal imbalances, low nutrient uptake, inconsistent distribution of carbohydrate, organ injuries and of course under prolonged or severe flooding ultimately plant death (Rodríguez-Gamir et al., 2011). The effects of flooding on plants, however, do depend on the severity of the flooding and the time/duration flooding occurs.

Oxygen (O_2) levels in flooded soil are generally lower than normal aerated soil due to the difficulty of gas diffusing through water. According to Visser and Voesenek (2005), depletion on oxygen in flooding soil is caused by slower oxygen transport from roots and loss of oxygen through transpiration process. Limited O_2 in flooded soil also results in accumulation of CO_2 which caused stomatal closure. Another responses to low O_2 level in flooding soil is leaf epinasty; where roots stimulates cell expansion on the adaxial surface of petioles after detecting lower oxygen level for more than 4 – 6 hours (Jackson, 2002).

Plants response to waterlogging regulated by phytohormones will be discussed in the next section.

1.5.3 The role of hormones in root to shoot signalling

Plant response to environmental stress is an evidence of communication within the plant. This communication known as plant signalling, specifically root to shoot signalling is utilized to counter the changes in soil status. Root to shoot signalling in plants is triggered by phytohormones including absicisic acid (ABA), ethylene (C_2H_4), gibberellin (GA) and cytokinins (CKs). Arru et al., (2013) state that generating signal transduction and stimulating plant physiological responses to biotic and abiotic stress is a main function of hormones. Apart from its function in stimulating signalling during stress, these phytohormones are also important in plant growth under normal conditions. For example, increasing ABA concentration in xylem sap is required to maintain shoot growth in barley (Mulholland et al., 1996) while ethylene promotes fruit ripening, flower opening and leaf abscission. In addition, gibberellin is important for seed germination and CKs were known for their function in cell division and shoot formation.

Plant response to soil water status is triggered by production of ABA in roots and transported to leaves as a chemical signal for stomatal closure (Schachtman and Goodger, 2008, Rodríguez-Gamir et al., 2011, Christmann et al., 2007). Plants will respond to water stress by closing the stomata to avoid the loss of excess water vapour. ABA is thought to be synthesized by the roots; and transported to the shoot via the phloem, although it is also feasible that ABA may occur in the rhizosphere and be absorbed from the surrounding soil water by roots (Wilkinson and Davies, 2002). However, according to Schachtman and Goodger (2008) ABA is not working alone as a signal for stomatal closure since ABA has been found in the conjugated form in xylem sap. The precise origin of ABA is also queried since there are studies that indicate that it can be synthesised in the roots, but also that it is produced in leaves and transported to the roots in a conjugated form, before being re-distributed elsewhere as ABA (Rodríguez-Gamir et al., 2011, Davies et al., 2005).

After ABA is produced, it can be transported from cell to cell through xylem vessels or transmitted to xylem apoplastically via the transpiration stream, and also can be stored or degraded (Wilkinson and Davies, 2002). Jian and Hartung (2008) explains that ABA formation in roots was higher when roots sensed lower levels of ammonium, phosphate,

or potassium but most of the ABA was released quickly into the xylem rather than in roots when the plants were under phosphorus and potassium shortage.

Apart from ABA, ethylene is also involved in plant signalling and under flooding situation there is build-up of ethylene in plant tissues (Reid and Bradford, 1984). Unlike ABA, ethylene is a gaseous hormone and can only move through diffusion which leads to the increasing level of ethylene in submerged plants. Plant adaptation induced by ethylene during flooding includes the inhibition or exhibition of roots, production of aerenchyma and the formation of adventitious roots (Steffens and Sauter, 2014). Increasing concentrations of ethylene results in leaf epinasty in flooded plants. Ethylene production in roots is triggered by the ethylene precursor 1 -Amynoacyl cyclopropane-1 –carbocylic acid (ACC) which is produced under anaerobic and/or anoxic conditions and later transported to aerial parts of shoot (Irfan et al., 2010).

Studies in tomato indicate that ethylene is also involved in plant signalling under compacted soil. Enhanced conversion of ACC in the leaf tissue results in increasing ethylene production which is influenced by ABA while it is being transported through the transpiration stream to shoots (Roberts et al., 2002). In addition, the presence of ethylene in compacted soil is thought to reduce shoot growth, whilst enhanced radial expansion in roots; resulting in thicker and shorter roots (Kays et al., 1974). Thicker root diameter helps roots penetrate the higher resistance soil structures. Coder (2007) explains that thicker roots can exert increased extension force per unit area in compacted soil which allow roots to penetrate farther as well as to reduce root bending.

GA and CKs role in plant signalling is still unclear. It is known that CKs are mainly produced in roots which could relate to plant response to drought stress (Anjum et al., 2011). Soil compaction alters soil structure which leads to slow water absorption; resulting in nutrient deficiency. CKs are known to control universal and local plant responses to phosphate deficiency. Studies in *Betula* (birch) seedlings indicate that phosphorus deficiency leads to reduced levels of CKs, which then restrict growth (Horgan and Wareing, 1980). Schachtman and Shin (2007) also explain that the

distribution of CKs under nutrient deficiency tends to be greater in the roots to promote further growth, with less emphasis on the leaves.

Dodd (2005) indicates that GA derived from root systems is able to support stem growth sufficiently but later can be synthesized in shoots. More research is required on how GA is involved in plant signalling, especially under stress scenarios.

1.6 Species selection

Plant responses towards urban soil condition were studied by conducting a series of experiments covering some of the key limiting factors associated with urban soil and using a limited number of model shrubs genotypes, namely *Philadelphus coronarius* 'Aureus', *Philadelphus* 'Belle Etoile' and *Euonymus* 'Silver Queen'. These shrubs were chosen due to their fast or intermediate growing characteristics and thus to accommodate the short term experimental durations of between 3 to 4 months. These shrubs represent examples of commonly used landscape plants (including deciduous i.e. *Philadelphus* cultivars and evergreen i.e. Euonymus) types.

Philadelphus coronarius is a plant native to Southern Europe and a popular ornamental plant for garden in temperate regions due to its profuse sweetly scented white blossom in early summer. This species can withstand very cold winter condition possibly down to -20 °C in all UK and Northern Europe. The golden foliage cultivar *Philadelphus coronarius* 'Aureus' is somewhat less vigorous, and in common with other yellow leaved foliage plants the leaves can scorch under high light condition. *Philadelphus* 'Belle Etoile' is a hybrid species from France developed from Chinese species by Victor Lemoine in the late 1800s and early 1900s. This hybrid known for a better fragrance with longer flowering period and more compact than other *Philadelphus*.

Euonymus fortunei was introduced into UK in 1860 by Scottish plant hunter, Robert Fortune. Its natural habitat is the forest area or natural area in mixed deciduous forest and low woodlands in China and other parts of temperate Asia. This species could withstand severe winter in most parts in UK (approx. up to -15 °C, however may not

withstand open/exposed site in central/northern locations. This derived cultivar Euonymus fortune 'Silver Queen' is a silver and green 'evergreen' genotype which is typically low growing and spreads quickly to inhibit weed growth on areas of bare soil.

1.6 Overall aim of the research

The overall aim of this research is to investigate the relationships between roots and shoot development within shrubs commonly used in urban settings.

1.7 Research question

a) How shrubs perform when exposed to typical urban environment soil conditions?
b) How does root growth in suboptimal conditions affect shoot development?

1.8 Research objectives

The objectives of the study are:

a) To study the root to shoot developmental relationship in young woody landscape plants particularly in response to environmental stresses common in the urban environment.
b) To study how roots respond to various conditions and to investigate is there any commonality in root response across different environment stress factors.
c) To study how root performance / response to environmental stress influences the subsequent shoot morphology (implications for plant development in ornamental plantings).
d) To evaluate a limited range of woody ornamental plants, in an attempt to develop 'principles' of how roots may perform under a range of environmental stresses. e.g. do species with contrasting shoot growth perform in a similar or dissimilar manner in term of root response to stress?

1.9 Research activities

Specimens of young shrubs were used within model systems that mimic the difficult and stressful environments encountered within the urban matrix. These young woody plants were exposed to artificial urban conditions such as limited space and volume, compacted soil, restricted water and nutrient availability and the impact on their development tested.

1.10 Thesis structure

Overall thesis contains seven chapters, details of each chapter as follow:-

a) **Chapter One : Background study and Review of Literature**
b) **Chapter Two: Materials and Methods (General)**
c) **Chapter Three : Rootball Geometry and effect on shoot development / morphology**

   Experiment 3a: Influence of contrasting rootball geometry on root and shoot development, when irrigation optimum

   Experiment 3b: Influence of contrasting rootball geometry on root and shoot development, when irrigation controlled

d) **Chapter Four**: **Compacted soils / media and influence on root and shoot development**

   Experiment 4a: The influence of different bulk density in an organic growing medium on root dynamics and shoot development

   Experiment 4b: The influence of different bulk density in three contrasting soil types (sand v clay v organic) on root dynamics and shoot development.

e) **Chapter Five: Root injury and effects on compensatory root growth and subsequent shoot development**

   Experiment 5a: Investigate the effect of root injury using a 'split-pot system' to determine root responses during the dormant phase in woody plants

   Experiment 5b: Investigate the effect of root injury using a 'split-pot system' to determine root responses during the active growth phase in woody plants

f) Chapter 6: The influence of depth of waterlogging on root behaviour and shoot development

Experiment 6a: Differential flooding (depth) in summer on root damage and subsequent development after draining

Experiment 6b: Differential flooding (depth) in winter on root damage and subsequent development after draining

g) Chapter 7: Conclusion

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 Chapter Overview

This chapter contains information on plant species and soils/media used, describing general methodology for experimental design, data collection methods and statistical analysis

2.2 Plant species

2.2.1. Philadelphus coronarius cv. Aureus

Philadelphus coronarius 'Aureus' (*Philadelphus* cv. Aureus) (Figure 2.1) is among the shrubs used in UK landscape. This species fall under Saxifragaceae family with green and yellow leaves in autumn and winter while yellow in summer. It can be grown in any reasonably fertile and well-drained soil, regardless in acid, alkaline or neutral soil condition. *Philadelphus* cv. Aureus is an upright and deciduous shrub which can survive under full sun or partial shade.

Philadelphus cv. Aureus can achieve 2.5 metre with 1.5 metres width with ovate leaves and creamy-white bowl-shaped fragrant flowers. This species can be propagated using both softwood and hardwood cuttings where softwood cuttings can be taken during summer and hardwood cuttings during autumn and winter.

2.2.2. Philadelphus cv. Belle Etoile

Philadelphus cv. Belle Etoile (Figure 2.2) is a deciduous shrub which can achieve up to 1.5 metres in height and spread up to 2.5 metres. It has green leaves colour with ovate shape and produces fragrant white flowers with tinged purple in the middle in spring

and summer. This species is under the same family with *Philadelphus cv. Aureus* and requires full sun or partial shade to grow.

Philadelphus cv. Belle Etoile can survive in various types of soil including chalk, clay sand or loam but most importantly is well-drained soil. *Philadelphus* cv. Belle Etoile can be propagate using both softwood and hardwood cuttings and suitable to be plants in coastal area and flower borders and bedding plants.

2.2.3. Euonymus fortunei cv. Silver Queen

Euonymus cv. Silver Queen (Figure 2.3) is a bushy evergreen shrub under *Celastaceae* family and can reach up to 2.5 metres height and 1.5 metres spread. It is, however, slower growing than the *Philadelphus* cultivars and has ovate shaped leaves with dark green colours margined with white and often tinged pink and produces white and pale green flowers in summer.

Euonymus cv. Silver Queen require well-drained soil or moist but well-drained soil condition to survive. It can be planted in chalk, clay, sand or loam soil types regardless it's acid, alkaline or neutral condition. This species can be propagated using semi-hardwood cuttings and suitable to be planted at banks and slopes, ground cover, hedging, flower border and etc. Full sun or partial shade is required for the species to grow.

2.2.4. Punica granatum

Punica granatum (Figure 2.5) is a bushy shrub with narrow oblong leaves and funnel-shaped flowers. The leaf colour is bronze in spring and green in summer and autumn while the colour of the flowers is red in summer. This species produce brown and red fruits in spherical shape in autumn. It requires full sun to grow and will only survive in well-drained soil condition.

Punica granatum can be propagated by seed and semi-hardwood cuttings. The seed need to be sown during spring at 13 to 18^{o}C and the cuttings need to be taken during

summer. This species can grow up to 2.5 metres in height and 2.5 metres spread and taken about 10 to 20 years to reach the ultimate height. This species has it origin in the region from the Mediterranean to central Asia.

Figure 2.1 *Philadelphus* cv. Aureus

Figure 2.2 *Philadelphus* cv. Belle Etoile

Figure 2.3 *Euonymus* cv. Silver Queen

Figure 2.4 *Punica granatum*

2.3 Stock Plants

2.3.1 Philadelphus cv. Aureus

Philadelphus cv. Aureus softwood cuttings were collected from the Harris Garden, University of Reading and propagated under mist within glasshouse at University of Reading at temperature 15 to 25^{o}C in April 2011. 200 cuttings were potted in plug trays contain a mixing of potting growing medium and Perlite (both William Sinclair Horticultural Ltd, Lincoln, UK). All of the cuttings were placed on a propagation bench under mist sprinkle and covered with plastic. After 6 weeks, 45 rooted cuttings with same size were selected to be potted in experimental pot (Exp. No. 1).

For the remaining experiment, 9 cm *Philadelphus cv. Aureus* liners were obtained from Northern Liners Company, Preston, Lancashire, UK.

2.3.2 Philadelphus cv. Belle Etoile

Philadelphus cv. Belle Etoile 9 cm liners were obtained from nursery supplier, The Northern Liner Company, Preston, Lancashire, UK. The plants were propagated under unheated glass house with overhead irrigation as the irrigation regime. Peat based compos with some dolomitic limestone for pH balance (around 6.5), a control release fertilizer Osmocote Pro 8-9 month and 12-14 month blended with 1:1 ratio and Met 52 for vine weevil control were used as a substrate for potting medium.

2.3.3 Euonymus cv. Silver Queen

Cuttings of *Euonymus* cv. Silver Queen were obtained from nursery supplier (The Northern Liner Company, Preston, Lancashire, UK) at the height of 9cm. The plants were propagated under unheated glass house in a pot with peat based composed with some dolomitic limestone for pH balance (around 6.5), a control release fertilizer Osmocote Pro 8-9 month and 12-14 month blended with 1:1 ratio and Met 52 for vine weevil control. Overhead irrigation was used as an irrigation scheme.

2.3.4 Punica granatum

Punica granatum cutting was collected from mother plants in Reading University glasshouse. 200 cuttings were propagated under mist within glasshouse at University of Reading in at temperature 15 to 25^{o}C in April 2011. 200 cuttings were potted in plug trays contain a mixing of potting growing medium and Perlite 50:50 (both William Sinclair Horticultural Ltd, Lincoln, UK). All of the cuttings were placed on a propagation bench under mist sprinkle and covered with plastic. Cuttings were left on the propagation bench until rooting and were used in experiment 1.

2.4 Growing medium

2.4.1 Sinclair Potting Medium

Potting medium from William Sinclair Holding PLC, Lincoln, UK was the most used medium among all the experiments. It is a 100% peat based medium (0-5mm 15% and 0-10mm 85%) with 6.0 pH. This peat also contains nitrogen (N_20_4), Phosphate (P_20_5) and Potassium (K_2O).

2.4.2 John Innes No. 1

John Innes No. 1 (Verve Brand) comes in a pack of 20kg from B&Q contains a mixture of loam, peat, sand and fertilisers (7:3:1). The loam base of the John Innes No. 1 contains clay, humus and traces elements which act as a natural reserve of plant foods.

2.4.3 Clay

Riverine clay was obtained from Goole, East Yorkshire (Grid Ref. SE771 243). A clay soil's fine texture is due the small size of particles (< 0.002 mm). This results in a high water and nutrient holding capacity but soil are usually poorly drained. These small particles result in clay having more than 1000 times more external surface area than coarse sand. The negative ion charges on clay attract and hold positive charged ions (including plant

nutrient such as magnesium, calcium and potassium) and allow clay soils to retain the highest nutrients of the main soil types.

2.4.4 Horticultural Sand and Grit

Horticultural sand and grit was obtained from B&Q store from Verve brand comes in 25 kg per pack.A mixture of horticulture sand and grit with 1:1 ratio were used in experiment 4b. Sand characteristic of free drainage is due to its bigger particle sizes (ranging from 0.0625 mm to 2mm for sand and 2mm to up to 62 mm for grit). Larger particle sizes in sand help plant roots penetrate the substrates easily, but are relatively ineffective at holding nutrients for plants growth.

2.4.5 Fertiliser

'Miracle Gro All Purpose Continuous Release Plant Food' (purchased from DIY chain B&Q) with N:P:K status of 17:9:10 were used in this research to provide a continuous supply of nutrients for plant growth. Ten granules of this slow release fertilizer were added to each pot to augment any deficiency of nutrient in the parent soil.

2.5 Experimental Design

2.5.1 Irrigation regime

All of the plants were irrigated manually using hand watering at least once a week but frequent in warmer weather. Frequent irrigation was done based on the soil and plants condition where irrigations were performed when the surfaces of the soil dry and when the plant's leaves wilted. Details on irrigation were explains in each of the experimental chapter under materials and methods section.

2.5.2 Plants arrangement

All of the experiments were arranged using Randomized Complete Block Design (RCBD).

2.5.3 Glasshouse temperature and relative humidity

All of the experiments were carried out in glasshouse at Reading University glasshouse and Sheffield City Council Nursery at Norton Lane, Sheffield, South Yorkshire. Glasshouse temperature and relative humidity for each experiment were recoded using Tiny Tag Data Logger (Gemini data Loggers Ltd, Chichester, UK) which continually logged from the start of the experiment. All of the logged data were downloaded at the end of the experiment. Daily minimum and maximum temperature data recorded by staff and the weather station was used in any case of logger failure.

2.6 Plant Data Collection

2.6.1 Plant Height

Plant height was measured from the base of the main stem (at the surface of growing medium) up to the base of terminal bud (not including the apical meristem) of the tallest branch or stem. The first heights were measured right after the experiment being set up and before destructive harvest took place.

2.6.2 Number of Leaves

Numbers of leaves were recorded by counting any visible leaf on the plants including the new emergence tips of leaf.

2.6.3 Number of Branches

Numbers of branches were recorded by counting any visible branch on the plants including the emergence of new bud.

2.6.4 Leaf Area

Leaf area was measured only once before the destructive harvest took place. Only one mature leaf from each branch was selected to be measured for each plant. Leaf area was measured using a formula as below,

$$Leaf\ surface\ area\ (cm^2) = Leaf\ length\ (cm) \times width\ (cm)$$

2.6.5 Whole Plant Leaf Area (L)

Whole Plant Leaf area was measured as estimation of total leaf area of the plant. L was calculated using the formula below:-

$$Whole\ Plant\ Leaf\ Area\ (L) = Individual\ Leaf\ Area\ x\ Number\ of\ Leaves$$

2.6.6 Internode Length

Internode length was measured by measuring the length of each branch individually and then divides by number of nodes in each branch.

$$Internode\ length\ (cm) = \frac{Length\ of\ branch\ (cm)}{No.of\ Nodes}$$

2.6.7 Shoot Fresh Weight

Shoot fresh weight of each sample were taken at the end of the experiment where all of the plants were harvested destructively. Each of the plants was cut into 2 parts where the shoot part is from the lowest trunk close to soil surface area up to the apical meristem. Each of the shoot sample were weighed and recorded as the fresh weight of the shoot.

2.6.8 Shoot Dry Weight

Once all of the fresh weights of shoots were recorded, all of them were place in the oven for 48 hours at 80^{o}C to let them dry. After the drying process ended, each of the samples was weighed once again to obtain their dry weight.

2.6.9 Root Fresh Weight

Root sample for each plants were washed carefully using bucket of water and running water to remove any soils. Clean root then were tap with tissue paper to get rid any excess of water before weighted and put into brown envelop to be dried.

2.6.10 Root Dry Weight

After all of the fresh weights were recorded, samples were placed in the oven for 48 hours at 80^{o}C (Gregory, 2006) to let them dry. After the drying process ended, each of the samples was weighted once again to record the dry weight

2.6.11 Root to Shoot Ratio (R:S)

Root to Shoot Ratio is a calculation to determine the relative source sink relationships between roots and shoots. When value of RS in plant is low, it often shows that plant used stored carbohydrates for their vegetative shoot growth. The ratio is calculated by the formula:

$$Root\ to\ Shoot\ Ratio\ (RS) = \frac{Root\ Dry\ Weight}{Stem\ and\ Leaf\ Dry\ Weight}$$

2.6.12 Root Score

Root score were given to record the root development and distribution in 2 experiments before destructively harvesting took place. Root scores were given based on the visibility of new roots at each of the lemonade bottle sides (4 sides per bottle).

2.6.13 Stomatal Conductance

Physiological changes in plants transpiration process were recorded via stomatal conductance using a Leaf Porometer AP-1 (Delta T-Devices Ltd., Cambridge, UK). The sensor head of the device was calibrated each time before the measurement took place for accurate reading and mmol /m^{2}s (milimoles per metre square per second).

A Leaf Porometer was used to detect water stress in plants by measuring the degree of stomatal opening (Shimshi, 1977). This Leaf Porometer AP-1 measured the stomatal

conductance by measuring the actual vapour flux from the leaf through the stomates and out to the environment. In essence dry air is fed over the leaf and the increases in humidity (moisture content) that ensue are due to water vapour being release from the stomatal pores. The stomatal conductance rate is then calculated by the porometer head.

2.6.14 Chlorophyll Fluorescence

Chlorophyll fluorescence was recorded using a Handy PEA Chlorophyll Fluorimeter (Hansatech Instrument Ltd – Norfolk, UK) to measure the maximum quantum efficiency of photosystem II (Fv/Fm) and internal forces of the plants to resist constrain (P.Index) to indicate plant health (Hansatech Instrument Ltd, 2014). Healthy plants normally will achieve values of 0.85 or more for Fv/Fm and lower values show that plants experienced biotic or abiotic stress factors.

Measuring the yield of Chlorophyll fluorescence provides information about changes in the efficiency of photochemistry energy and energy dissipation. Chlorophyll molecules in leaf absorb light and undergo one of the three process; i) used to drive photosynthesis; ii) dissipate the excess energy as heat or iii) re-emitted as light chlorophyll fluorescence (Maxwell & Johnson, 2000). In this experiment, Chlorophyll fluorescence was used to measure the efficiency of Photosystem II (PS II) which correlates to general photosynthetic performance. Reduction in Fv/Fm values and allied parameters are associated with loss of photosynthetic integrity and stress injury plant leaves (Krause & Weis, 1991).

Leaf clips were attached on the leaf sample for dark adaptation with the shutter plate closed for 20 -30 minutes. The head sensor was attached after 30 minutes to get the reading of Fo, Fm, Fv/Fm and P. Index. However, only Fv/Fm and P. Index values were used in the result section.

2.7 Statistical Analysis

Normality and homogeneity of error variance (using plots of residual values) of data was undertaken before being analysed using analysis of variance (ANOVA) with Genstat® software for Windows® (16th Edition).

CHAPTER 3

ROOTBALL GEOMETRY AND EFFECT ON SHOOT DEVELOPMENT / MORPHOLOGY

3.0 Introduction

Many urban plants are planted in containers, design planters or in restricted volumes of parent soil, such as tree planting pits or as part of a central barrier feature along roadways. The volume of soil / media available to the plant, and the particular dimensions however, are likely to influence both root and shoot development. Through studies on nursery-grown plants it is widely recognized (Warren and Blazich, 1991) that aspects such as root circling and kinked root shape associated with pot culture affects subsequent growth and development, including survival after transplanting. In addition, the initial form (shape) of the plant plays an important role in achieving the aesthetics desired in landscape design. Only limited research has been implemented on how root development influences the shape and vigor of shoot development in landscape plants *in situ* (largely due to the difficulty of accessing and viewing roots), although relevant information is available on root architecture in pot grown specimens and how this affects plant form and subsequent performance after planting in the soil.

Root morphology in container grown plants is different to that of field grown crops; and because of growth restriction roots compete between each other for resources which also affected shoot growth (NeSmith and Duval, 1998). Plants under root restriction will adapt to their condition (decreasing leaf size and photosynthesis) by controlling their nutrient and water uptake (Yong et al., 2010). Previous research demonstrates that container shape, dimension and volume of container grown trees influence factors such as plant longevity (Al-Zalzaleh, 2009).

It is recognized that the volume of media available to the root system can affect shoot growth performance due to limited nutrient and water availability (Poorter et al., 2012); what is less clear is how root development alters when the volume of accessible remains

the same but the dimensions of the rhizosphere (rootball) depth and width change. That is the objective of these experiments described here. Three different container geometries are exploited (providing contrasts in breadth, depth and surface area exposed to the atmosphere) to determine how these influence root and shoot behavior, whilst ensuring the same volume of media is used in each.

3.1 Experiment 3a: Influence of contrasting rootball geometry on root and shoot development, when 'optimum' irrigation is applied to each system

3.1.1 Hypotheses:

1. Shoot development (number and growth) of plants grown in equivalent volumes of media will be similar, irrespective of geometry of the rhizosphere (container shape).
2. Root development, however, will be affected by the dimension of the rhizosphere (size, shape and surface area of containers).

3.1.2 Objective:

- To study the effect of geometry on root and shoot growth

3.1.3 Materials and Methods:

Cuttings of two shrub species; *Philadelphus* cv. Aureus and *Punica granatum* were propagated under mist within a glasshouse at the University of Reading at temperature 15-25°C for rooting (Figure 3.1, 3.2 and 3.3). Rooted cuttings were then transferred into 3 different container types (treatment); conventional round 'Pots' (1 litre, 13 X 10 X 10.8 cm), 'Trays' (15 X 22 X 3 cm lbh) and 'Columns' (4.1 diameter X 80 cm height) (Figure 3.4, 3.5, and 3.6). There were three positional blocks within the glasshouse. In each block, each species was represented by 3 different container designs with 5 replicate plants for each design. Each of the containers was filled with equal volume of 1 liter of media (Sinclair potting media) and then were arranged in randomized complete block design on the glasshouse bench with temperature between 15 to 25^{o}C and full sunlight. Plants were hand watered at least once a week, but more frequently (e.g. 2-3 times) during warmer conditions. Watering was implemented on the basis 'of need' for the plants in the different treatments. For example, it was anticipated that plants grown in trays may need to be watered more frequently due to their greater surface area exposed to the atmosphere, drying out more quickly. Within a treatment, however, water was applied uniformly; all plants receiving the same duration of hand watering with hose and lance.

Plants were arranged using Randomize Complete Block Design for 3 blocks with five replicate plants per treatment. The initial data for plant height, branch and leaf number were recorded on 14th July 2011. Measurements for plant height, branch number and location, and leaf number were recorded weekly for 7 weeks. At week 8, all plants were harvested and samples divided into shoot and root sections. To determine root fresh weight, root samples were washed carefully through rinsing in both standing and running water to remove excess media; before drying the surface of roots by laying them within sections of paper towel. After 5 min root samples were placed on a balance to determine fresh weight.

After the fresh weight of roots and shoots were obtained, samples were put in a brown paper bag and labeled accordingly. Bags were then placed in an oven at 80^{o}C for 48 hours before re-weighted for dry weight. Analysis of Variance was used to determine any significant effects due to treatments on all the parameters recorded. .

Figure 3.1: *Philadelphus* cv. Aureus

Figure 3.2: *Punica granatum*

Figure 3.3 : Conventional Pot

Figure 3.4: Tray

Figure 3.5: Column

3.1.4 Results:

***Philadelphus* cv. Aureus**

Shoot and dry root weight associated with the pot grown plants tended to be twice that of either of the tray or column treatments (treatment effect for both shoots and roots highly significant, P = 0.005) (Figures 3.6 and 3.7). Root to Shoot ratios for this cultivar indicate that column grown plants were recorded to have the highest RS ratio.. Plant height for the *Philadelphus* cv. Aureus was greatest in the pot treatment, significantly taller than plants grown in columns. There were no significant differences in height between column grown and tray grown plants (Figure 3.9). Leaf number showed a similar pattern to height, with significantly more leaves in the pot treatment, and again, no significant different between tray and column (Figure 3.10).

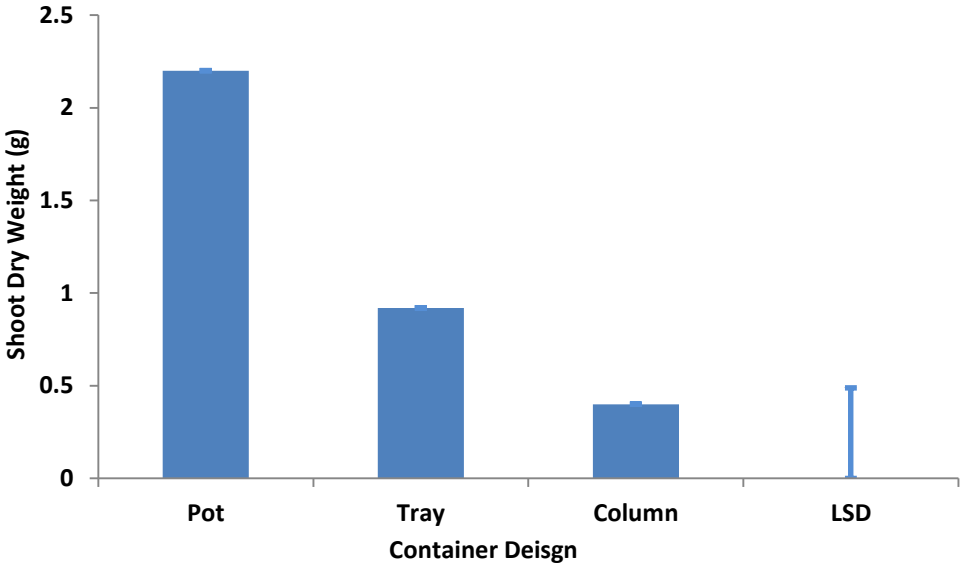


Figure 3.6 Shoot dry weight (g) of *Philadelphus* cv. Aureus in 3 different container designs. P ≤ 0.005, LSD: 0.489, d.f. = 42

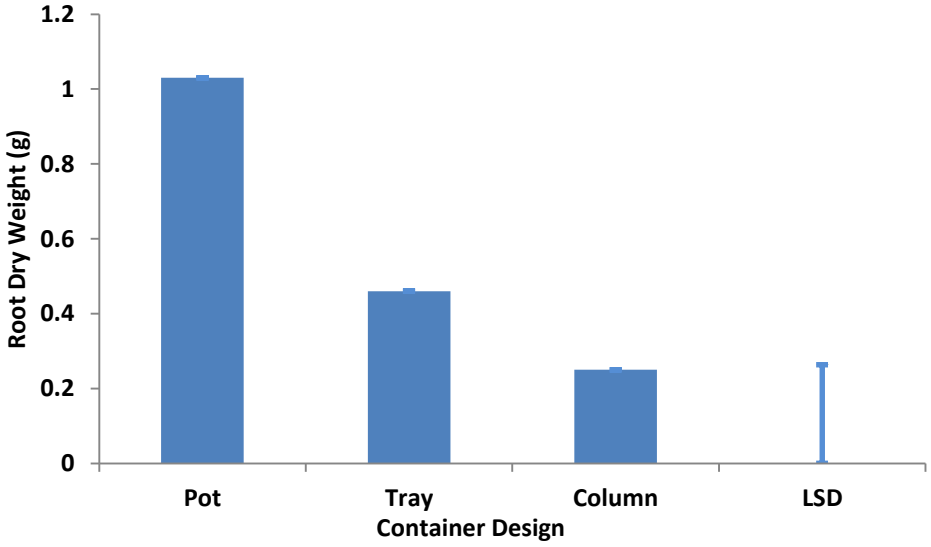


Figure 3.7 Root dry weight (g) of *Philadelphus* cv. Aureus in 3 different containers designs. P ≤ 0.005, LSD: 0.264, d.f. = 42

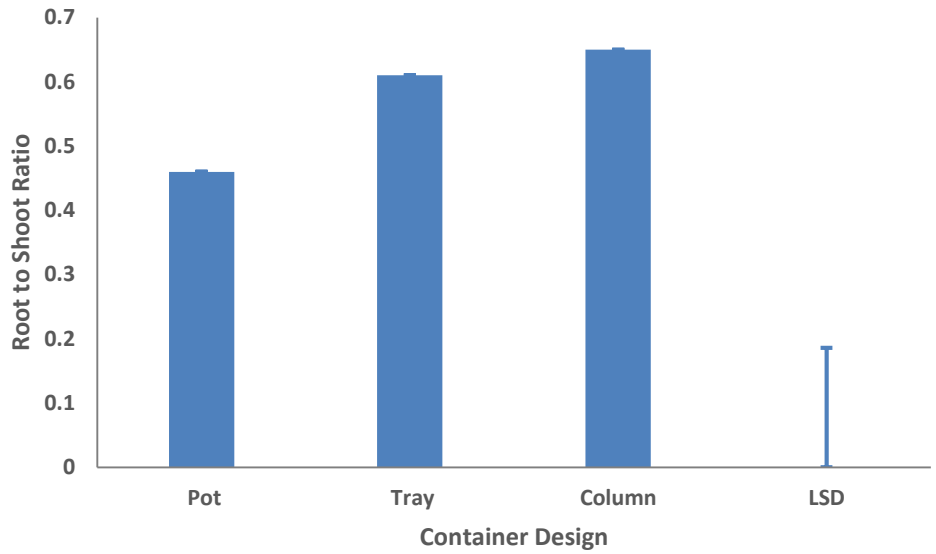


Figure 3.8 Root to Shoot ratios for *Philadelphus* cv. Aureus in 3 different container designs. P=0.005, LSD: 0.186, d.f. 42

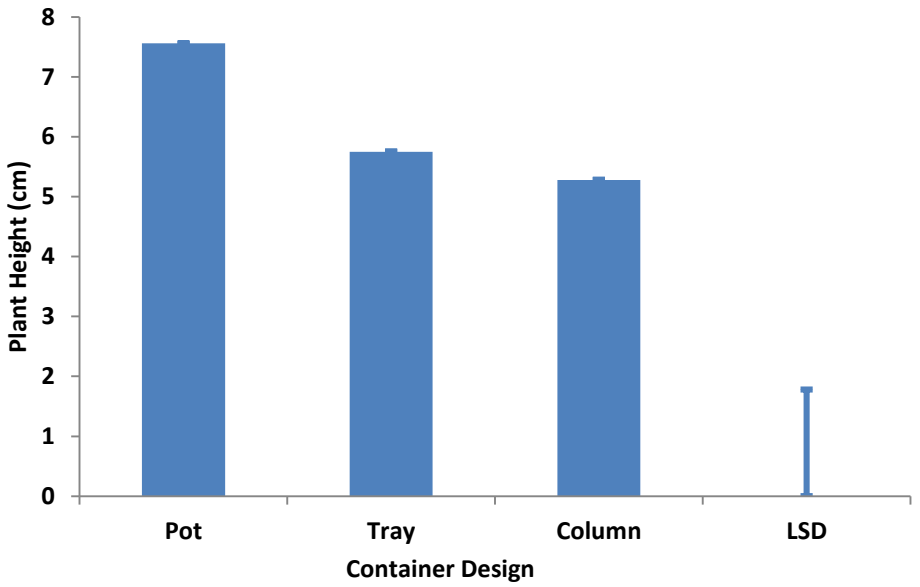


Figure 3.9 Plant height (cm) of *Philadelphus* cv. Aureus in 3 different containers designs. P ≤0.005, LSD: 1.781, d.f. 42

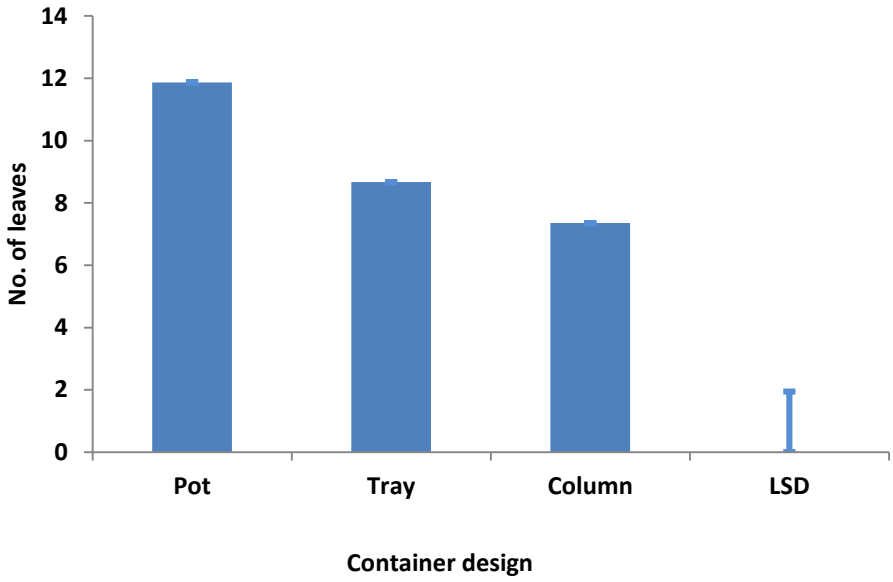


Figure 3.10 Number of leaves of *Philadelphus* cv. Aureus in 3 different container designs. P ≤0.005, LSD: 1.947, d.f.= 42

Punica granatum

Shoot, but not root dry weight was significantly greater with pot grown compared to tray plants (Figure 3.11 and 3.12). Column grown plants had least shoot dry weight (but not significantly less than Trays), although root dry matter was marginally significantly less (Fig. 3.12). Root to Shoot ratios for *Punica granatum* shows the same pattern as *Philadelphus* cv. Aureus with plants grown in a column recorded to have the highest RS ratio (3.13). In contrast to the *Philadelphus*, there were no significant differences in plant height for *Punica granatum* grown in pots compared to trays, but both significantly increased growth to those plants maintaining in the columns (Figure 3.14). There were, however, greater numbers of leaves in *Punica granatum* associated with the pot treatment compared to either of the treatments (Figure 3.15).

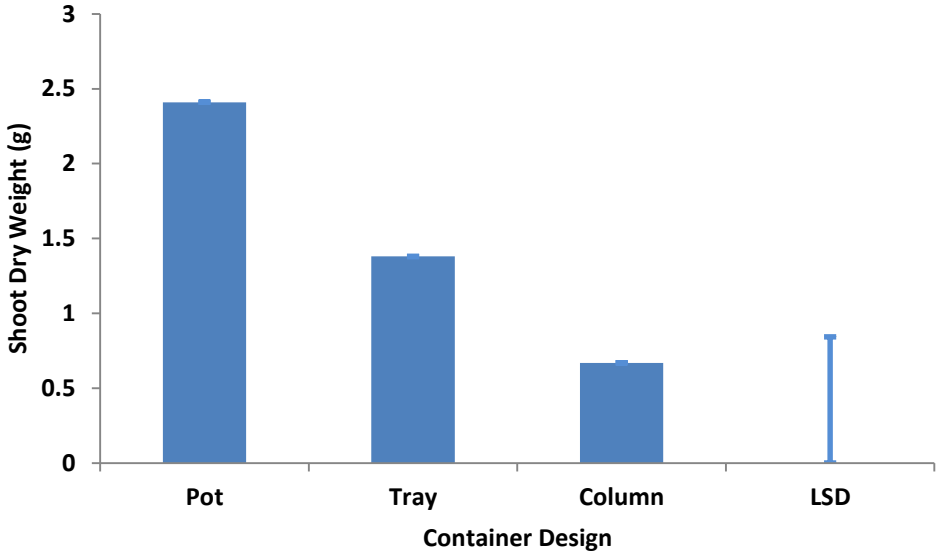


Figure 3.11 Shoot dry weight (g) of *Punica granatum* in 3 different container designs. P ≤ 0.005, LSD: 0.843, d.f. = 42

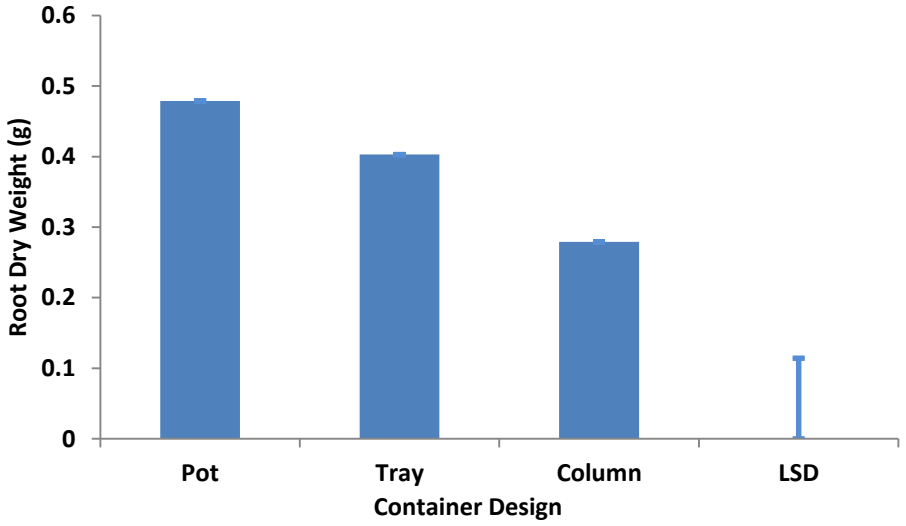


Figure 3.12 Root dry weight (g) of *Punica granatum* in 3 different container designs. P ≤ 0.005 LSD: 0.114, d.f. = 42

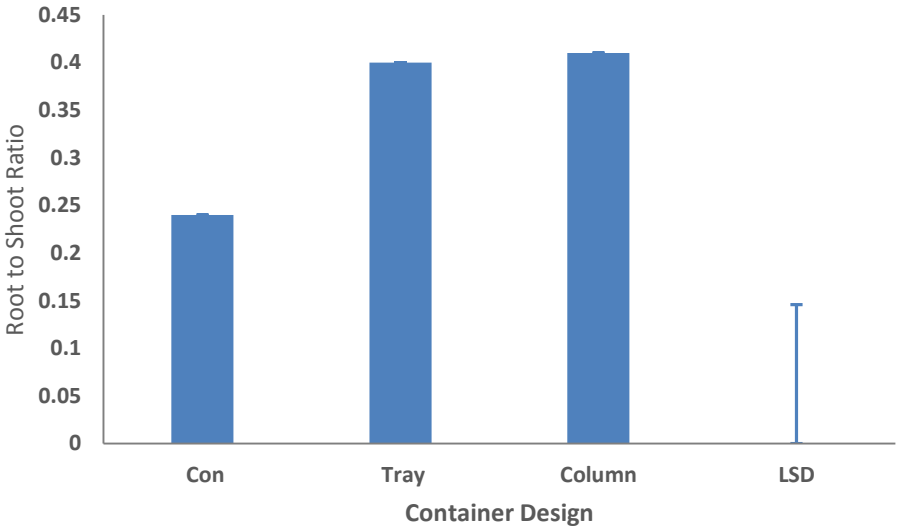


Figure 3.13 Root to Shoot ratios for *Punica granatum* in 3 different container designs. P ≤ 0.005, LSD: 0.146, d.f. 42

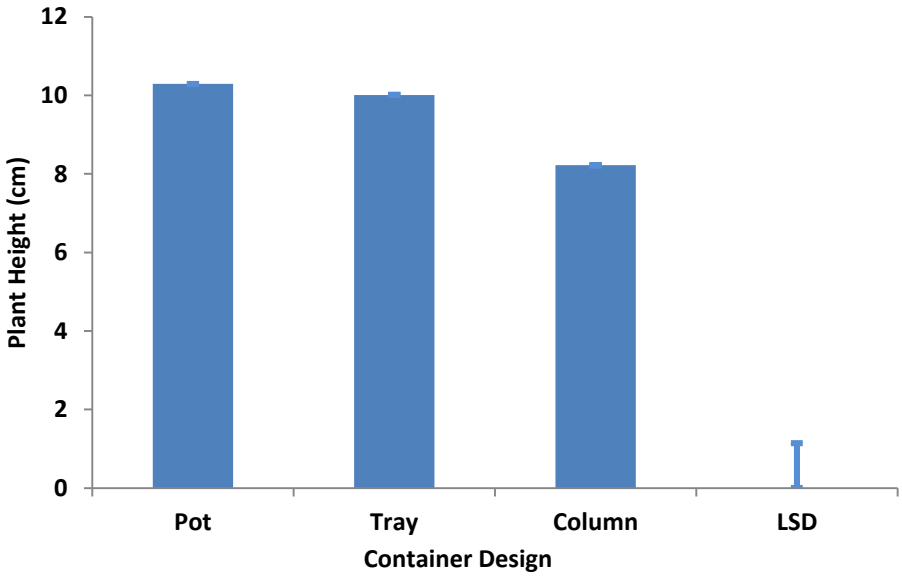


Figure 3.14 Plant height of *Punica granatum* in 3 different container designs. P ≤ 0.005, LSD: 1.139, d.f. 42

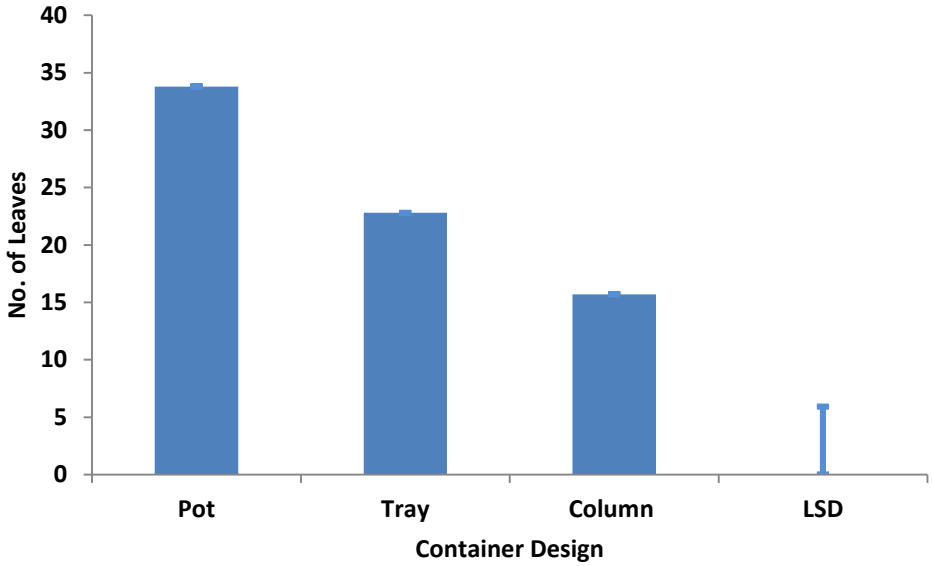


Figure 3.15 Number of leaves of *Punica granatum* in 3 different container designs. P ≤ 0.005, LSD: 5.93, d.f. 42

3.2 Experiment 3b: To study effect of rhizosphere volume / geometry on shoot growth and root development in three plant species, when irrigation application was 'equivalent' between pot designs.

This experiment will explore young shrubs growth and performance in three contrasting container with same irrigation regime for every container design.

3.2.1 Hypotheses:

1. Plants development (number and growth of roots and shoots) of plants grown in equivalent volumes of medium and supplied with the same volume of irrigation will be similar, irrespective of geometry of the rhizosphere (container shape).

3.2.2 Objective:

2. To study the relationship between container geometry on root and shoot growth, when equivalent volumes of irrigation water are applied.

3.2.3 Material and Methods:

This research was conducted in a glasshouse at Norton Nursery, Sheffield, South Yorkshire during spring 2013. Liners of *Philadelphus* cv. Aureus, *Philadelphus* cv. Belle Etoile and *Euonymus* cv. Silver Queen were potted on into 3 different geometrically designed containers; 1 liter conventional pot (15 X 22 X 3 cm), small size tray (22.5 x 16.5 x 5.5cm lbh) and column (4.1 d. x 80 cm h.). All of the containers were filled with 1 liter of Sinclair Potting Growing medium. There were three positional blocks within the glasshouse. In each block, each species was represented by 3 different container designs with 5 replicate plants for each design. Plants were arranged in randomized complete block design on the glasshouse bench. After potting up, plants were left to establish for two weeks before controlled irrigation was implemented. Once established, each plant was watered manually with 1 liter of water on each occasion. This was applied with a measuring jug, and water was applied slowly to ensure each container design could accommodate the volume with excess run-through, or spilling over the lip of the container. In essence more uniform application of irrigation was implemented than had been the case in Exp 3a.

Data collection

The initial data of plant height and branch number were recorded on 3rd June 2013. All of the plants then were left for 2 weeks to establish before the next data were recorded and then weekly recordings were conducted from that time on. Data were collected for 9 weeks before harvested by carefully taking it out from planting container and divided into 2 parts, root and shoot. Dry weight determinations followed the protocols in section 3.1.3. Plants were also assessed for height, total branch number, number of branches ≥ 10 cm; number between 5 and 10 cm and number ≤ 5 cm.

3.2.4 Results

***Philadelphus* cv. Aureus**

Shoot and root biomass was greatest in pot grown plants followed by those in trays with significantly lower values associated with column grown plants (Figure 3.16 and 3.17). Plant height, total number of branches, total branch length and number of branches ≥ 10 cm indicated the same treatment pattern as root and shoot biomass, however, values were not always significantly different (Figure 3.19 to 3.22). Although pot grown plants tended to have the greatest growth, there were no significant differences observed between pot and tray treatments, for any of the parameters recorded (in contrast to Exp. 1a. for this genotype). However, R:S ratios shows opposite pattern where column grown plant have the highest R:S ratios, significantly more than other two treatments (Figure 3.18).

With the number of branches ≤ 5 cm and between 5 cm to 10 cm, the trend in treatments changed, with lowest values being associated with those plants grown in trays and highest in pots, but there were no significant differences between treatments (Figure 3.23 and 3.24) The relatively large LSD bars for these parameters indicated a high degree of variability within any one treatment.

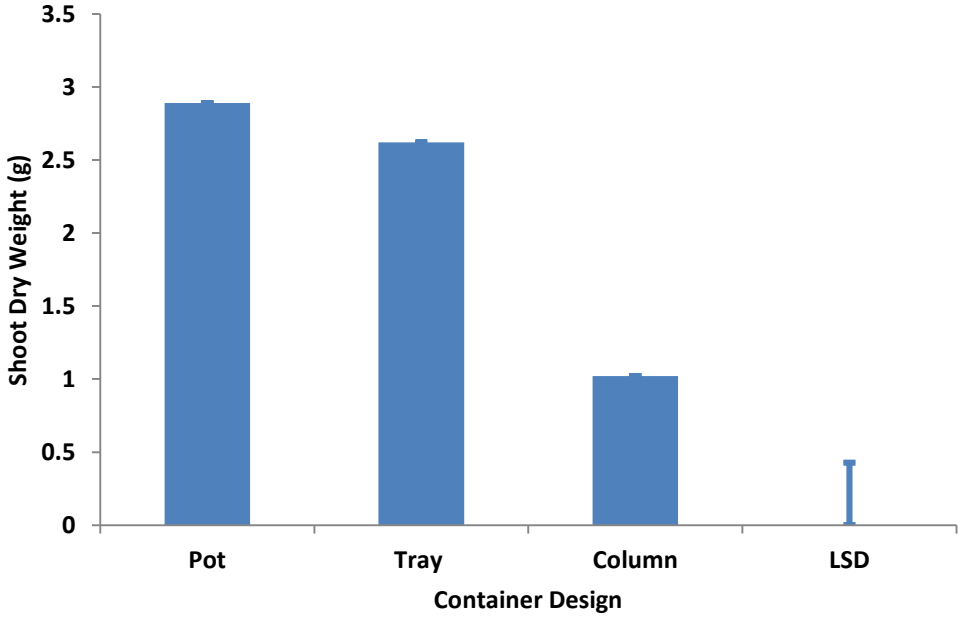


Figure 3.16 Shoot dry weight for *Philadelphus* cv. Aureus in three different container designs P ≤ 0.005, LSD: 0.427, d.f. = 42

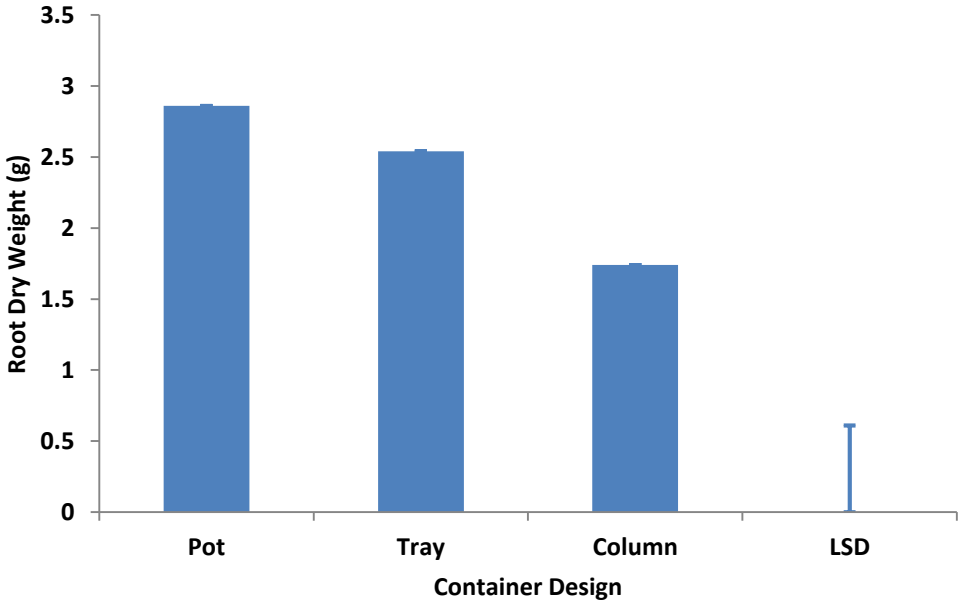


Figure 3.17 Root dry weight for *Philadelphus* cv. Aureus in three different container designs P ≤ 0.005, P: 0.61, d.f. = 42

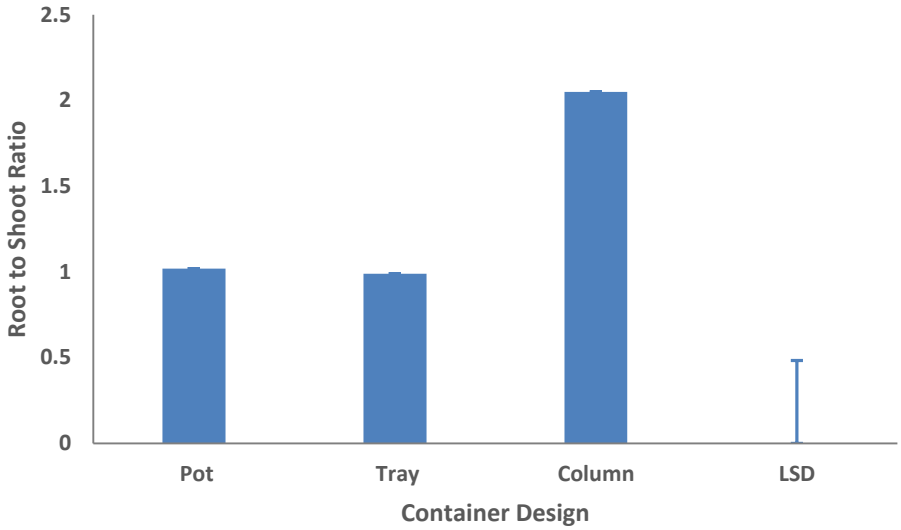


Figure 3.18 Root to Shoot ratios for *Philadelphus cv.* Aureus in 3 different container designs. P ≤ 0.005, LSD: 0.484, d.f:42.

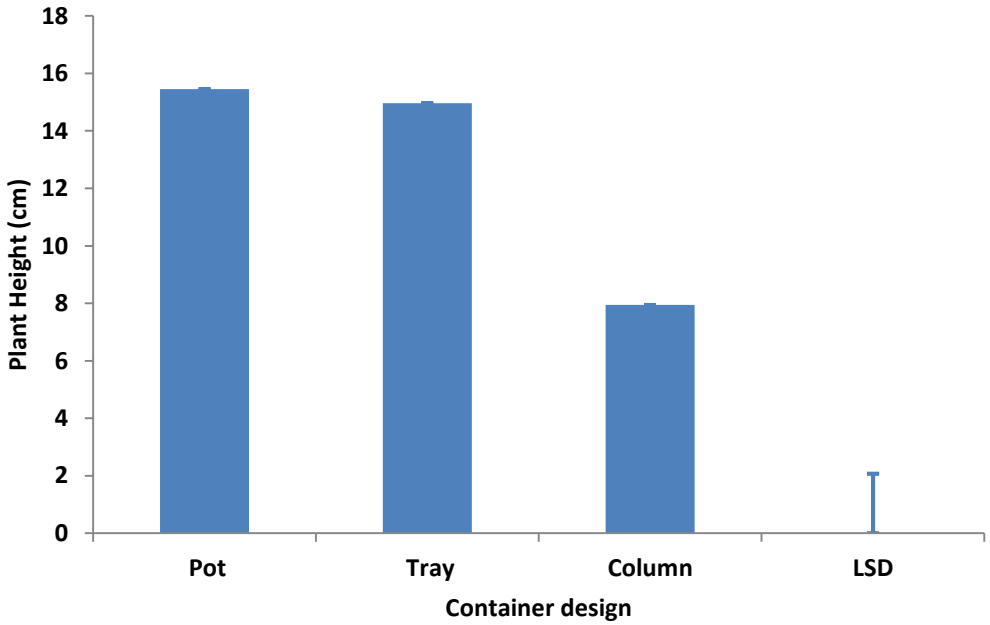


Figure 3.19 Plant height for *Philadelphus* cv. Aureus in three different container designs P ≤ 0.005, LSD: 2.072, d.f. = 42

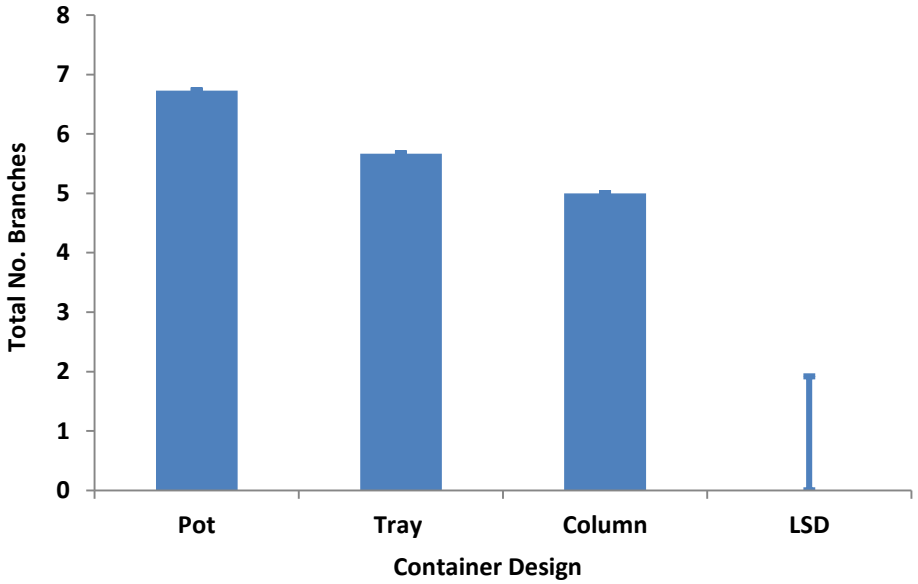


Figure 3.20 Total number of branches for *Philadelphus* cv. Aureus in three different container design P ≤ 0.005, LSD: 1.917, d.f. = 42

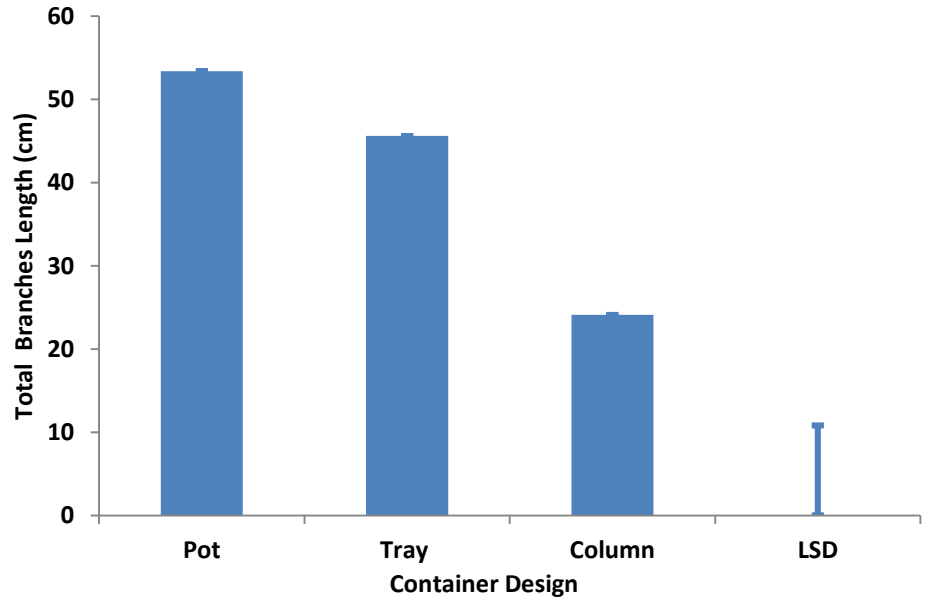


Figure 3.21 Total branches length for *Philadelphus* cv. Aureus in three different container design P ≤ 0.005, LSD: 10.81, d.f. = 42

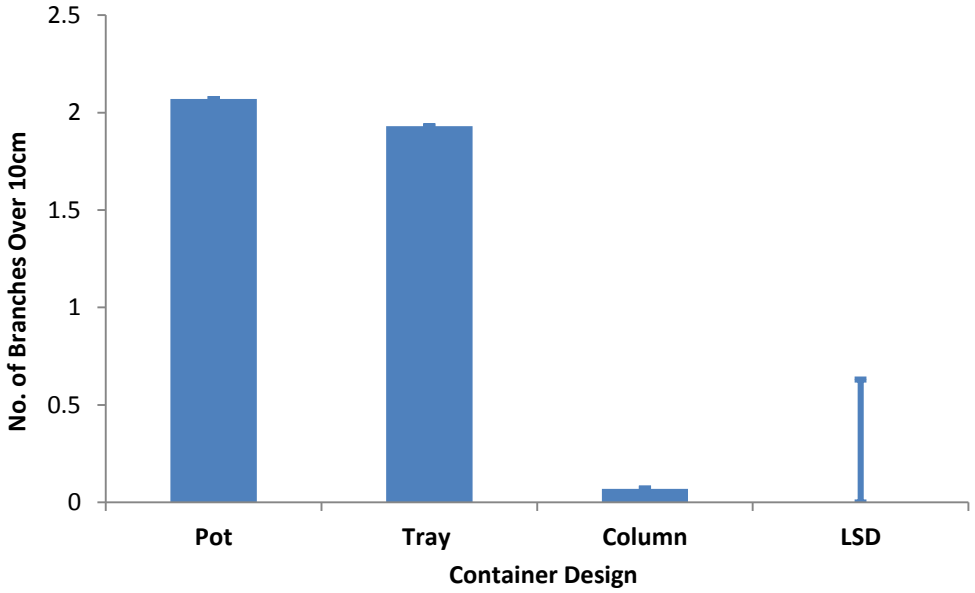


Figure 3.22 Total no of branches over 10cm for *Philadelphus* cv. Aureus in three different container designs P ≤ 0.005, LSD: 0.631, d.f. = 42

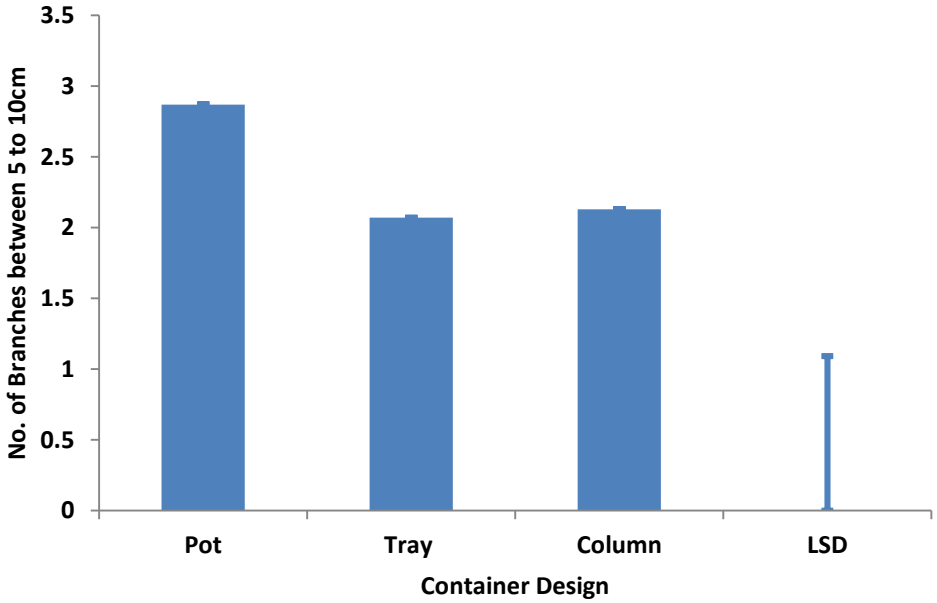


Figure 3.23 Total no of branches between 5cm to 10cm for *Philadelphus* cv. Aureus in three different container designs P ≤ 0.005, LSD: 1.093, d.f. = 42

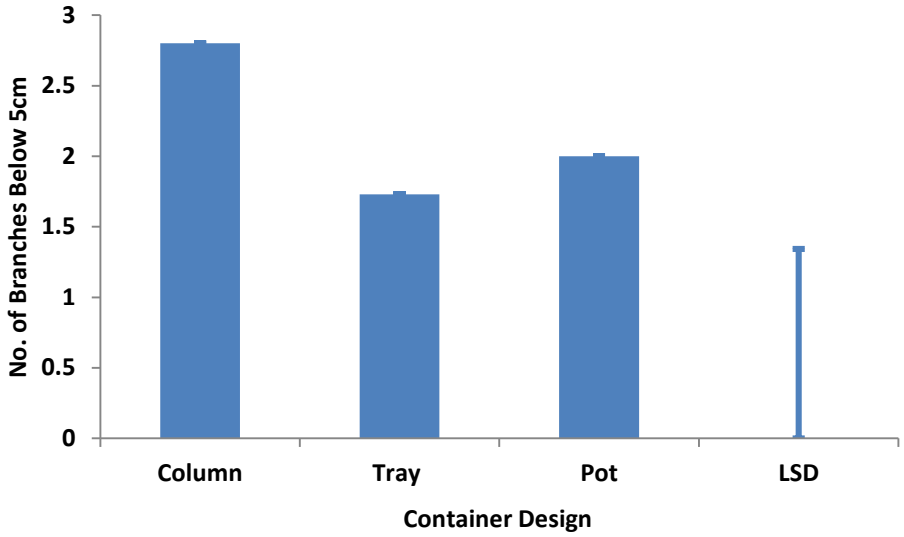


Figure 3.24 Total no of branches below 5cm for *Philadelphus* cv. Aureus in three different container designs P ≤ 0.005, LSD: 1.342, d.f. = 42

***Philadelphus* cv. Belle Etoile**

Shoot biomass for *Philadelphus* cv. Belle Etoile was greatest in pot grown plants significantly more biomass than either trays or column grown plants (Figure 3.25). For root biomass, however, greatest values were associated with pots, significantly greater than tray grown plants, but not those grown in the columns (Figure 3.26). Although R:S ratios for this cultivar shows the same pattern as *Philadelphus* cv. Aureus, increasing R:S ratios is not significantly different (Figure 3.27).

Plant height in this *Philadelphus* cultivar showed the same growth pattern as *Philadelphus* cv. Aureus with tallest plants in the pot treatment and significant reductions in height in both tray and column grown plants (Figure 3.28). Overall, there was no significant effect of treatment on total branch numbers (Figure 3.29), but total branch length shows that plant grown in pots had greatest shoot growth (matching data for shoot biomass and height) (Figure 3.30). Plants grown in the columns had fewer long shoots (≥ 10 cm), but more intermediate-sized shoots (5-10 cm) than other treatments (not significant) (Figures 3.31 and 3.32 respectively). Numbers of shoots ≤ 5cm was low across all treatments (Figure 3.33).

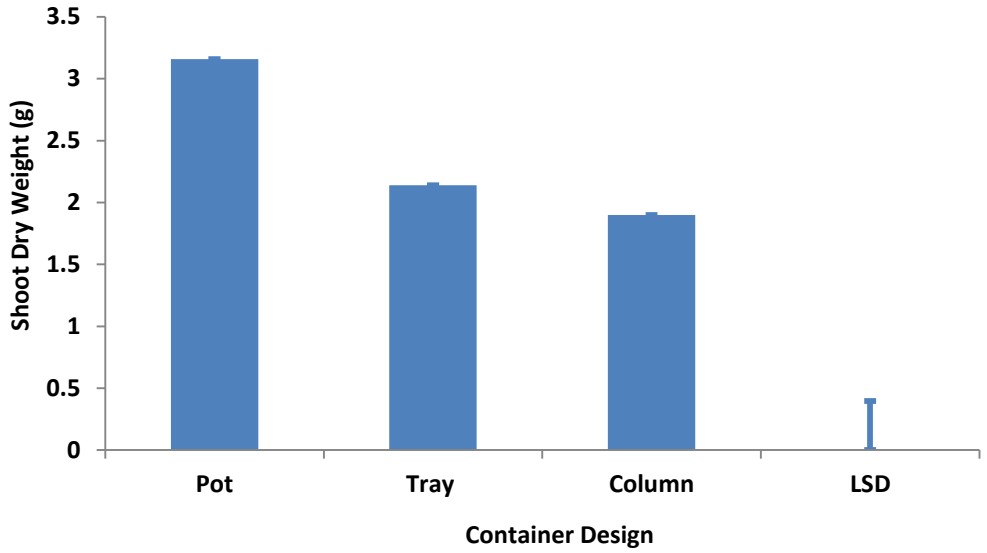


Figure 3.25 Shoot dry weight for *Philadelphus* cv. Belle Etoile in three different container designs P ≤ 0.005, LSD: 0.396, d.f. = 42

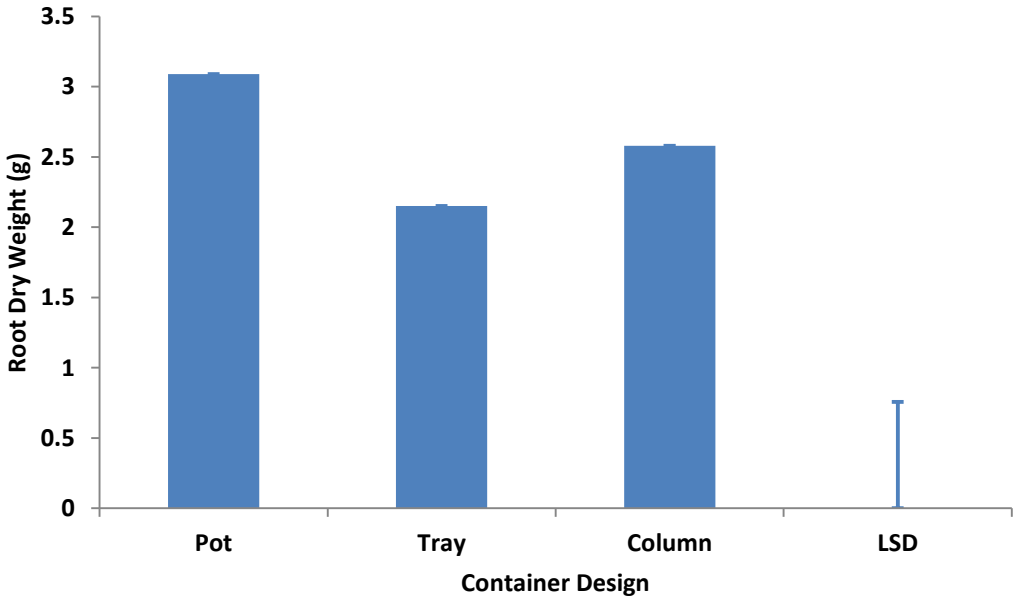


Figure 3.26 Root dry weight for *Philadelphus* cv. Belle Etoile in three different container designs P ≤ 0.005, LSD: 0.758, d.f. = 42

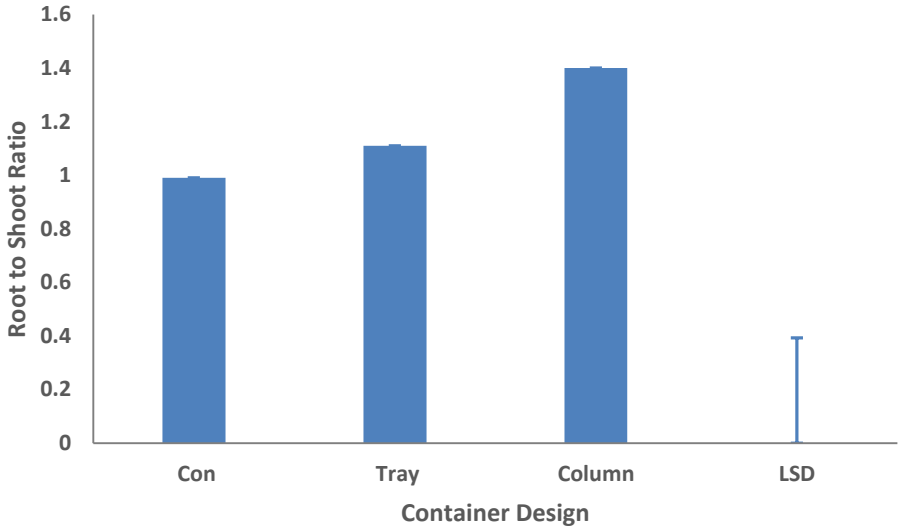


Figure 3.27 Root to Shoot ratios for *Philadelphus* cv. Belle Etoile in 3 different container designs. P ≤ 0.005, LSD: 0.393, d.f:42

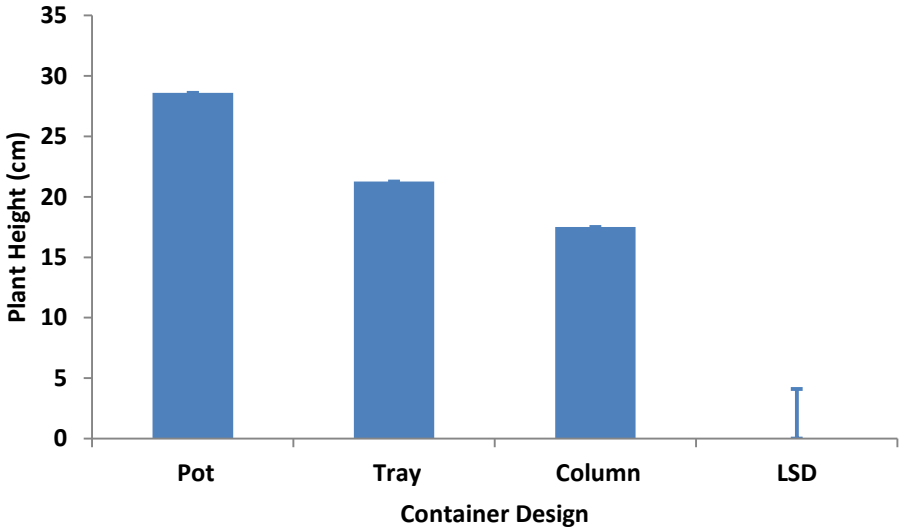


Figure 3.28 Plant height for *Philadelphus* cv. Belle Etoile in three different container designs P ≤ 0.005 LSD: 0.113, d.f. = 42

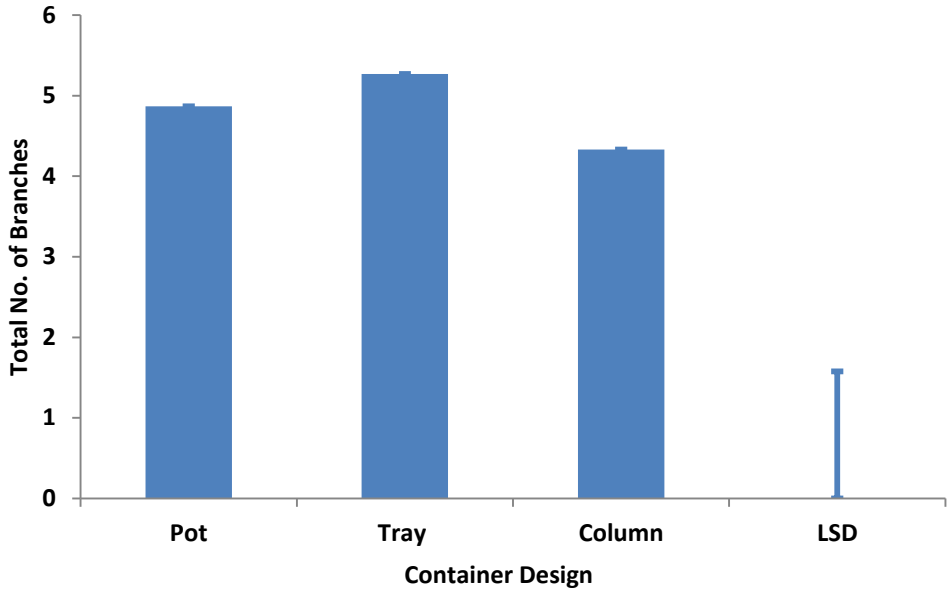


Figure 3.29 Total number of branches for *Philadelphus* cv. Belle Etoile in three different container designs P ≤ 0.005, LSD: 1.576, d.f. = 42

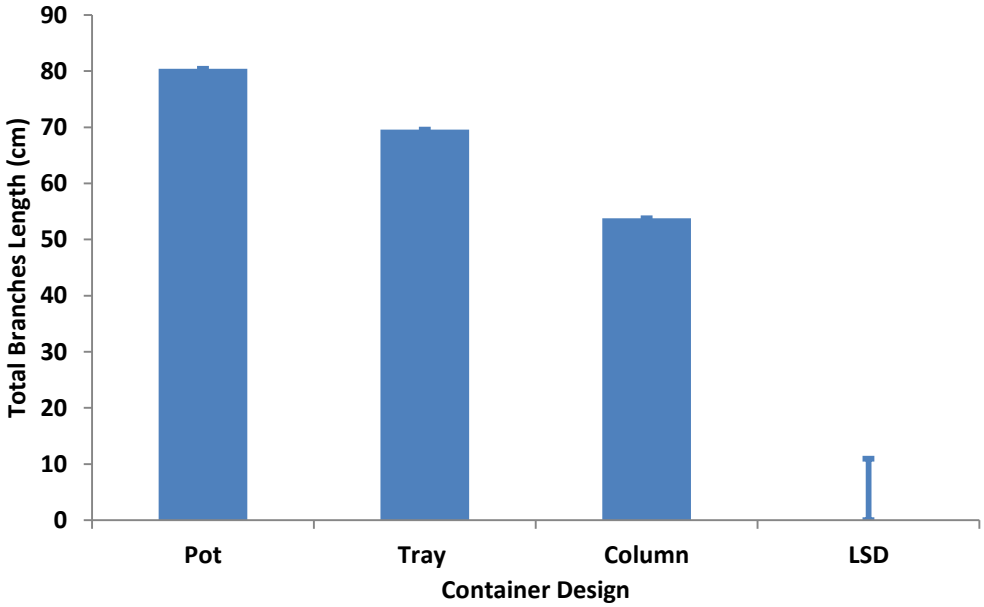


Figure 3.30 Total branches length for *Philadelphus* cv. Belle Etoile in three different container designs P ≤ 0.005, P: 10.94, d.f. = 42

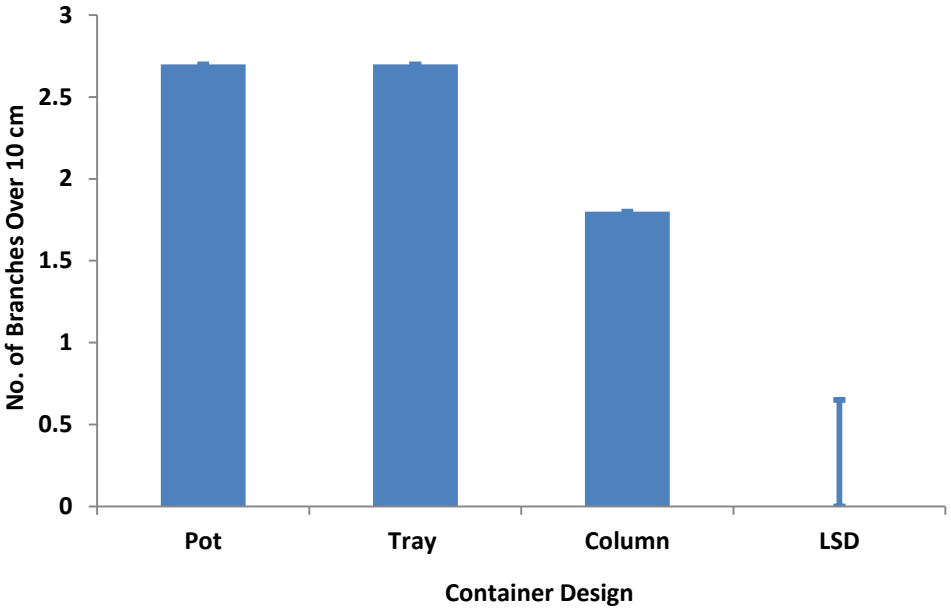


Figure 3.31 Total number of branches over 10cm for *Philadelphus* cv. Belle Etoile in three different container designs P ≤ 0.005, LSD: 0.65, d.f. = 42

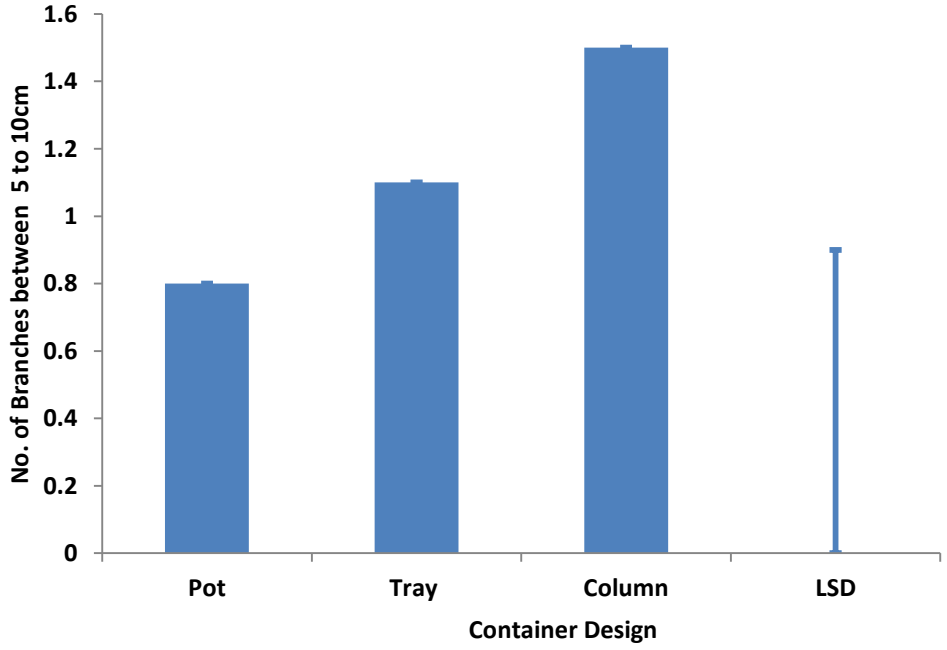


Figure 3.32 Total number of branches between 5 to 10cm for *Philadelphus* cv. Belle Etoile in three container designs P ≤ 0.005, LSD: 0.9, d.f. = 42

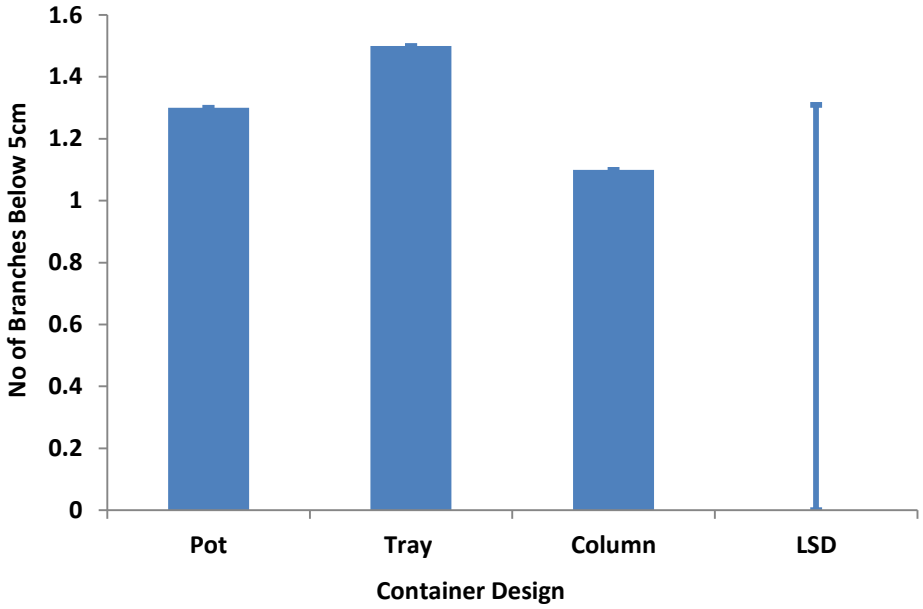


Figure 3.33 Total number of branches below 5cm for *Philadelphus* cv. Belle Etoile in three different container designs LSD P ≤ 0.005, LSD: 1.31, d.f. = 42

***Euonymus* cv. Silver Queen**

Shoot and root biomass associated with the pot grown plants tended to be significantly greater than tray plants and twice that of the column treatments (Figure 3.34 and 3.35). R:S ratios for *Euonymus* cv. Silver Queen shows a contrast pattern to both *Philadelphus* cultivars where pot grown plant have the highest R:S ratios (3.36). Plant height was less marked with pot grown specimens still largest, but not always significantly so (Figure 3.37).Pot grown plants, on the other hand, significantly increased the total number of branches (Figure 3.38) and their total length (Figure 3.39), the number of long branches present (Figure 3.40), but even the number of intermediate (Figure 3.41) and small (Figure 3.42) branches that occurred. In terms of branch size categories, there were no significant differences between tray and column grown plants.

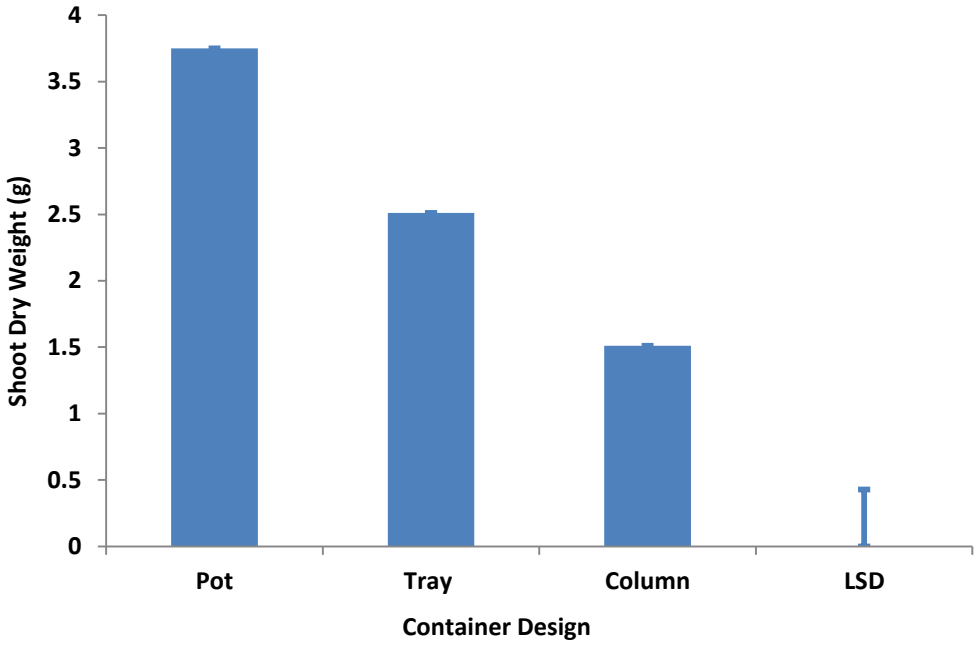


Figure 3.34 Shoot dry weight for *Euonymus* cv. Silver Queen in three different container design LSD P ≤ 0.005, LSD: 0.428, d.f. = 42

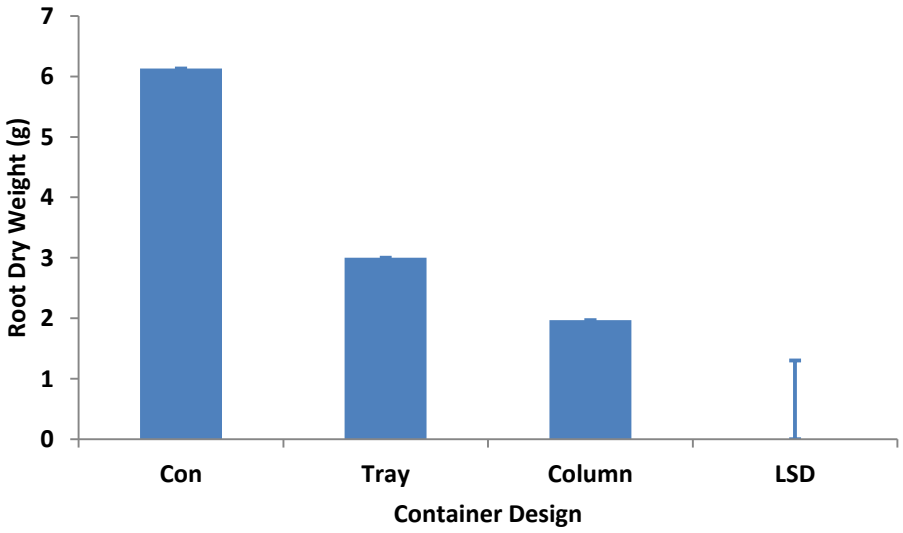


Figure 3.35 Root dry weight for *Euonymus* cv. Silver Queen in three different container designs P ≤ 0.005, LSD: 1.303, d.f. = 42

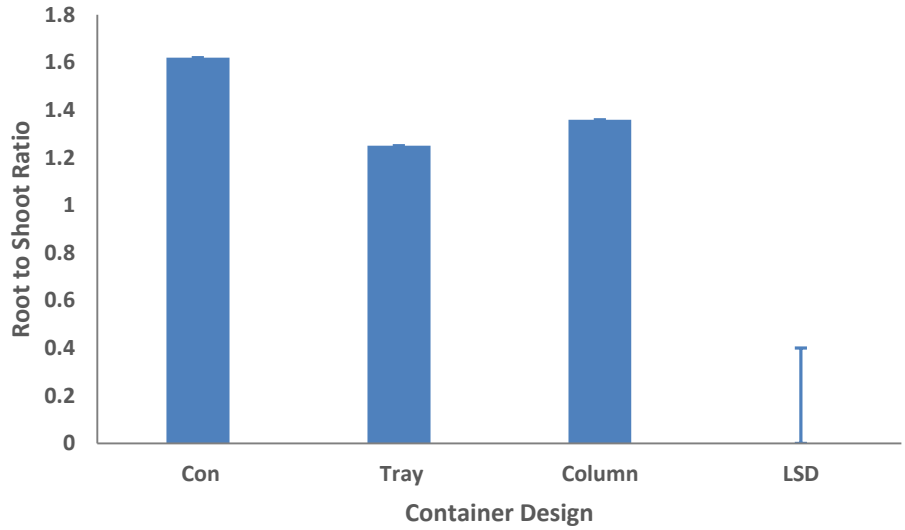


Figure 3.36 Root to Shoot ratios for *Euonymus* cv. Silver Queen in 3 different container designs. P ≤ 0.005, LSD: 0.4, d.f:42

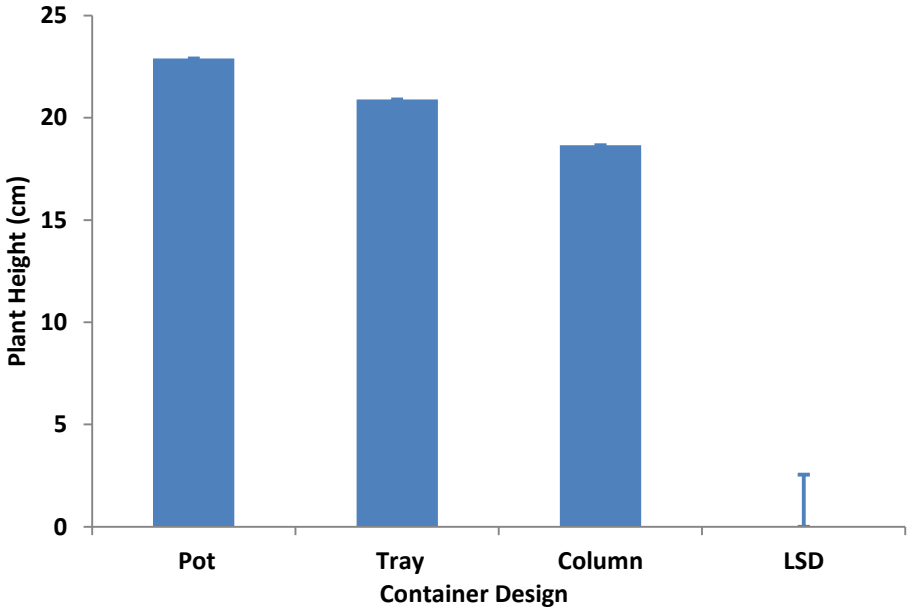


Figure 3.37 Plant height for *Euonymus* cv. Silver Queen in three different container design P ≤ 0.005, LSD: 2.554, d.f. = 42

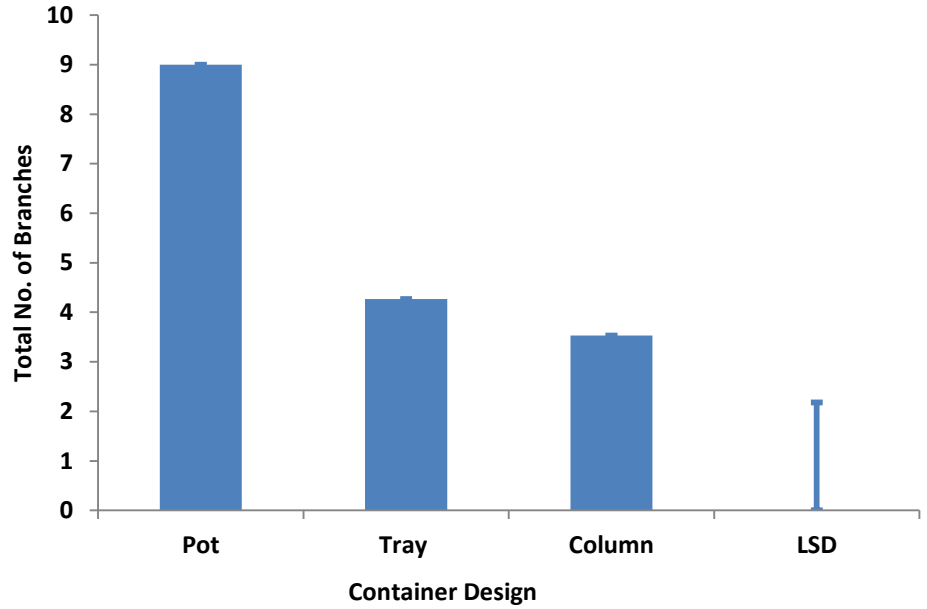


Figure 3.38 Total number of branches for *Euonymus* cv. Silver Queen in three different container designs P ≤ 0.005, LSD: 2.183, d.f. = 42

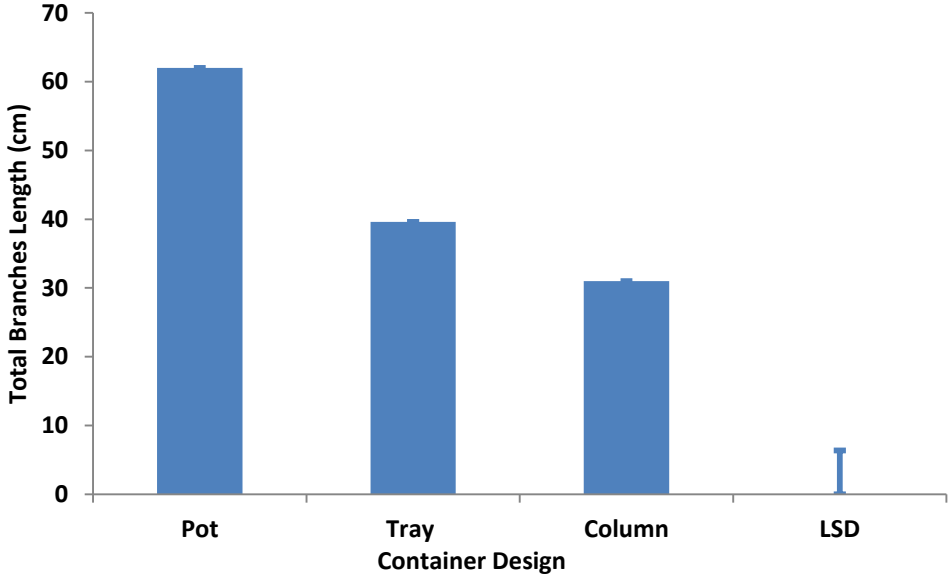


Figure 3.39 Total branch length for *Euonymus* cv. Silver Queen in three different container designs P ≤ 0.005, LSD: 6.38, d.f. = 42

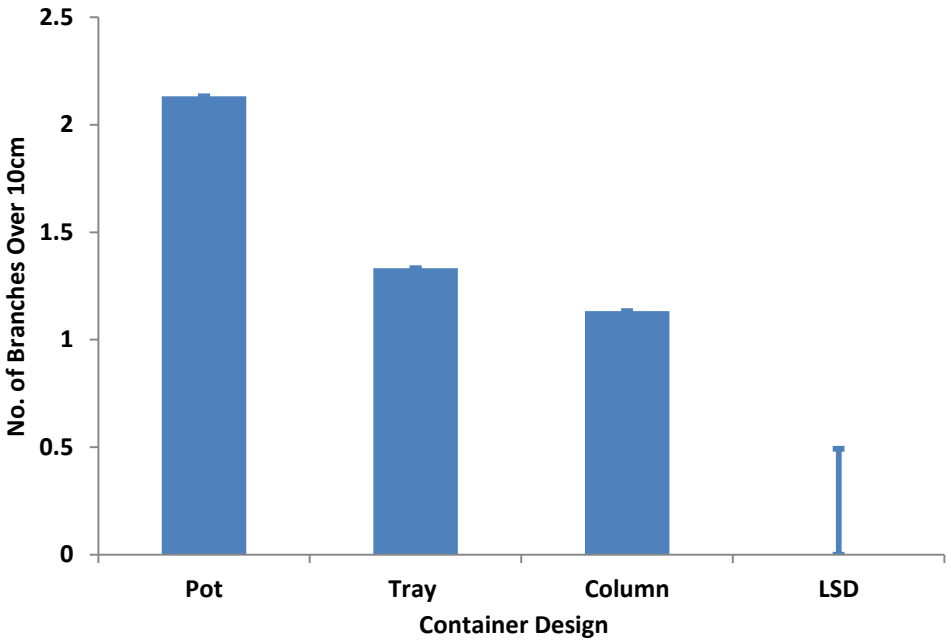


Figure 3.40 Number of branches over 10cm for *Euonymus cv. Silver Queen* in three container designs P ≤ 0.005, LSD: 0.493, d.f. = 42

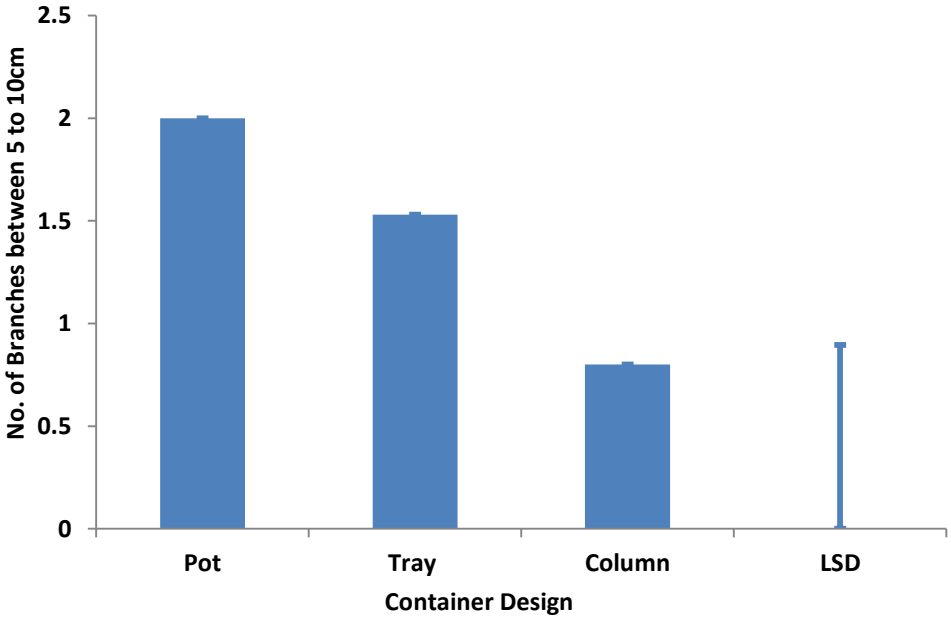


Figure 3.41 Number of branches between 5 to 10cm for *Euonymus* cv. Silver Queen in three different container designs P ≤ 0.005, LSD: 0.896, d.f. = 42.

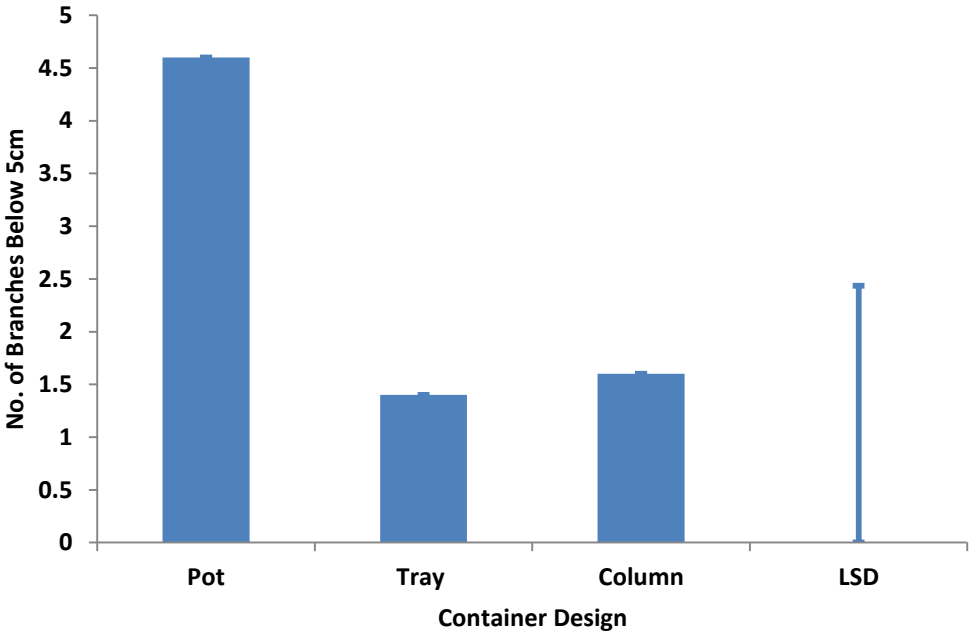


Figure 3.42 Number of branches below 5cm for *Euonymus* cv. Silver Queen in three different container designs LSD P ≤ 0.005, LSD: 2.436, d.f. = 42

3.3 Discussion

3.3.1 Plant growth

This chapter studied the effect of geometry / container design on plant growth in young shrubs. Root and shoot biomass for *Philadelphus* cv. *Aureus*, *Philadelphus* cv. Belle Etoile, *Punica granatum* and *Euonymus* cv. Silver Queen was greatest in conventional pot grown plants regardless if irrigation was optimal for each container type or controlled to provide equivalent volumes. Of the two non-conventional container shapes, growth parameters were frequently better in tray systems than columns, although differences were not necessarily significant. It is clearly evident from these results that container design / geometry is having a marked effect on plant morphology (irrespective of the volume of media available to exploit), with growth being optimised with a conventional pot design. The reasons, behind this, however, are less clear.

There would, however, appear to be two main criteria that are candidates for explaining the growth performances within the different treatments: 1. The geometry of the containers is influencing where roots can ramify and is affecting access to water, oxygen, nutrients and potentially the ability of the roots themselves to generate endogenous growth regulating phyto-hormones, and 2: the geometry of the different containers is influencing access to water by altering drainage and evaporation characteristics. This is despite water being applied based on perceived needs (Exp 1) and regulated to provide equivalent volumes (Exp 2).

Comparisons between the data for *Philadelphus cv. Aureus* in Exp 1 and Exp 2, particularly the relative differences in biomass and height between pot and tray treatments in the two experiments is notable. When the differences in water application are reduced (Exp 2) there is a narrowing in the differences within the growth parameters (compare figure 3.8 and 3.16). This might indicate that water availability, particularly more rapid moisture loss from the trays (large surface area, with little depth of substrate) was influencing results in Exp 1. Despite trays in Exp 1., being watered ‘on demand’, even temporary periods of reduced water availability may have affected

growth potential, for example through root chemical signalling (Peleg and Blumwald, 2011); with even just a few roots at the edge or surface of the media, experiencing a drying signal, and resulting in stomatal closure. Similarly, it is feasible that curtailed growth in the tall columns is due to excessive drainage and reduced ability to pull moisture up the media through capillary rise, due to the high tensions involved in the water column. As such, once moisture was exhausted in the upper regions of the column it may have been difficult for plants to access more.

There was no strong evidence, however, that moisture was limited in the columns, and reduced growth in these treatments may relate more to a physical constraint on the roots. Roots growth in limited volumes of soil will encourage competition for important resources (NeSmith and Duval, 1998) with overall effects on crop yield and root growth being compromised in such conditions (Rengasamy et al., 2003). R:S ratios for both of *Philadelphus* cultivars in Exp. 3a and 3b shows the same pattern where column grown plants possess the highest R:S ratios as compare to pot and tray but are not always significant. This relatively high root to shoot ratio probably reflects the difficulties these plants are having accessing water due to the large gravitational pull associated with the column. In effect they are having to invest in root development to reach down the column profile. This was less apparent however, for *Euonymus* cv. Silver Queen where R:S rattios were highest in pot grown plants, and where perhaps less constraint on roots in general were improving relative root development (roots in pots having less physical constraint compared to narrow columns or shallow trays).

The column system may also alter gas exchange, as there was only a very small surface area of media in direct contact with the atmosphere at the top of the column. Hence, it is theoretically possible that oxygen diffusion into the media and carbon dioxide release was compromised by this narrow aperture. Research on *Ficus benjamina* indicated that aeration was a limiting factor to root growth in narrow, deep containers due to its small surface area for oxygen absorption (Biran and Eliassaf, 1980) and root respiration and growth can be affected by the ventilation dynamics to the rhizosphere, e.g. in *Lycopersicon* (Niu et al., 2012).

However, in contrast studies done on *Tagetes* (Marigold) seedlings reports that plant height and width were not affected in smaller container but shoot dry weight was (Latimer, 1991). Total number of branches produced in conventional pot grown plants recorded to be greatest for *Philadelphus cv. Aureus* cultivar and *Euonymus* cv. Silver Queen. Somewhat in contrast, there appeared to be no proportional reduction in branch number in those plants of *Philadelphus* cv. Belle Etoile that were grown in the trays, despite overall penalties on growth in general. Perhaps having a lateral extended root system was promoting the generation of new shoots, rather than extension of existing shoots. Research in *Malus* cv. Queen Cox (apple) trees reported that shoot numbers and mean length of the shoots were reduced significantly in restricted root condition (Webster et al., 2000).

In summary, plant growth in different size of container / geometry implies that plants growth i.e. shoot and root dry weight, plant height and number of branches were reduced in smaller diameter container although it has the greatest depth to allow deeper root penetration. This would appear to be due to a lack of room for roots to ramify and low capillary rate for nutrient and water uptake resulting in less plant growth and smaller final specimens.

3.3.2 Future work

Soil aeration is important in root growth and future study on how soil aeration help in improving soil condition in restricted root zone will help to improve plant growth and performance in landscape especially urban area. The data presented here recorded root mass, but more refined experiments are required to understand how rhizosphere geometry affects root diameters and branching patterns. The numbers of roots as well as their relative location with respect to water and nutrients may influence both shoot extension and new shoot formation.

Calculation on minimum planting area for optimum shrubs performance is also crucial for landscape practise as well the suitable species which higher resistance to urban soil condition. This calculation can be done to make sure plants can obtain maximum

nutrients and water from the soil and also enough for rooting spaces for establishment and stability.

Key Points:-

1. **Alterations in root zone geometry, but not volumes influences the morphologically of the plant (i.e. root and shoot biomass, plant height and branching).**
2. **Plant under altered root zones tends to show similar symptoms to water deficit even though optimum irrigation was applied (particularly so in the narrow columns).**

Figure 3.38: Roots growth in conventional pot

Figure 3.39: Roots growth in tray

Figure 3.42: Roots growth in column

CHAPTER 4

COMPACTED SOILS / MEDIA AND INFLUENCE ON ROOT AND SHOOT DEVELOPMENT

4.0 Introduction

Compacted soil is a major problem for plant growth in urban areas. This is due to construction work during the development of urban areas, particularly through the use of heavy machinery, which compresses and compacts the soil. Compaction of soil interferes with number of plant developmental processes. Root growth is restricted due to high soil strength condition (high bulk density) and small pore size (Alberty et al., 1984). Compacted soil decreases the length of the roots, and frequently more roots are found in the surface layers of soil rather than in deeper zones, due to aeration and soil density problems (Lipiec et al., 2003). Changes in pore size cause more mechanical resistance or impedance to root extension as well as change the moisture characteristic of soil (Russell, 1977). Indeed, according to Soane and Ouwerkerk (1994), the major problem caused by soil compaction is the loss of soil fertility and difficulties in accessing moisture. This is due to a number of reasons, from the obvious for example, loss of nutrient-rich topsoil, mixture of other impurities, interruption of soil physical properties, through to indirect effects such as the volatilisation of nitrates as nitrogen gas under anaerobic conditions. Poor root development not only impairs nutrient / water uptake, but can interfere with hormone synthesis, and plant stability / anchorage (Bassuk and Hawver, 2007).

Plants will react through physiological and morphological modifications when in compacted soil, resulting in reduced growth and biomass production (Sadras et al., 2005). These alterations are due to the enhanced bulk density and smaller pore size in the soil which increases mechanical impedance to the roots, as well as reducing water availability (Bingham, 2001; Passioura, 2002). Plants grown in compacted soil will have lower stomatal conductance, reduced rates of cell division and expansion, which in turn results in slower root growth and less shoot extension (Sadras et al., 2005). Lack of cell

activity too, may impact on hormone synthesis, and this too may contribute to a 'vicious circle' with hormones such as abscisic acid (ABA) being activated and reducing growth further (Martin-Vertedor and Dodd, 2011).

This research investigates how shrubs respond to these factors (in contrast to trees) and indeed, whether there might be any advantages conferred in terms of shoot branching and more compact habit? Previous work has shown that compacted soils inhibit root development, and this has a secondary negative effect on shoot growth (caused by reduced nutrient and water uptake, but potentially also altered hormonal signal from the root (Sadras et al., 2005)). Most compaction has been recorded in natural mineral soils (heavy clay soils being particularly prone), but whether the same criteria applies to 'more' open organic soils and media requires verification.

4.1 Experiment 4a: The influence of different bulk density in an organic growing medium on root dynamics and shoot development

This experiment was done investigated the effect of different bulk density in the same organic media on plant growth across two different species.

4.1.1 Hypothesis:

1. Even in more 'open' organic growing media, greater compaction of the medium will inhibit root penetration and growth, thereby reducing shoot development and growth rate.
2. Greater compaction of organic growing media will result in smaller and more compact plants.

4.1.2 Objectives

1. To study the effect of severity on compaction in root growth
2. To study the effect of compaction on plant quality.

4.1.3 Materials and methods:

Seventy-two liners (small plants) of *Philadelphus* cv. Aureus and *Euonymus* cv. Silver Queen each were used as model plants in this experiment. All liners were potted into conventional round pots (13cm X 11.4cm) with 3 different volumes of medium acting as treatments. These three volume of media (1.4, 1.7 and 2.0 litres), as determined by volume on removal from the compost bag, were used to vary the degree of compression of the media. The 1.4 litres of media was loose filled into the pots, whereas the 1.7 litres and 2.0 litres volumes were compacted down by different levels of force to fill the same pot dimension. John Innes no.1 growing medium (Seedling and Young Plants) from B&Q (Bord na Mona Horticulture Limited, Ireland) was used as a substrate for this experiment. The 1.4 litres treatment was designated 'Control', whereas 1.7 and 2.0 litres were designated 'Medium' and 'High' bulk density treatment respectively. All of the plants were arranged using a Randomized Complete Block Design, with four replicate plants of each species placed within one of three positional blocks. Plant height and

branch number were recorded after potting and placement in the positional blocks, and then recorded at 2 weekly intervals. After 10 weeks of growth, plants were finally recorded with additional data on length of individual branches and number of nodes for each branch, mean internode, leaf number and mean leaf area being assessed prior to destructive harvesting for shoot and root dry weight.

To determine dry weight, all of the samples were placed in the oven at 80^{o}C for 48 hours before weighted. All of the data collected were analyzed using Analysis of Variance (ANOVA).

4.1.4 Results:

***Philadelphus* cv. Aureus**

Shoot fresh and dry weight of *Philadelphus cv*. Aureus was greatest in the High bulk density treatment (2.0 L), significantly more biomass than plants grown in the Control (1.4 L) treatment (Figures 4.1 and 4.2). There was also significantly enhanced root mass with the High bulk density treatment compared to the Control (Figure 4.3). There were no significant differences in shoot and root mass between plants grown in Medium bulk density (1.7 L) and High density (2.0 L) (Figures 4.1, 4.2 and 4.3). Despite alterations in biomass, there were no significant differences in plant height between treatments (Figure 4.4) although there was suppression in branch number with the High bulk density treatment (Figure 4.5). Plants grown in High bulk density (2.0 L) produced largest individual leaf sizes, marginally larger than that of the Medium density (1.7 L) treatments (Figure 4.6). There were no significant differences in mean internode length between treatments, although interestingly the trend changed, with longer internodes recorded in plants within the Control (1.4 L) compared to other two treatments (Figure 4.7). Media volumes for each treatments also being recovered to understand if there are any differences between treatments. It was observed that media volume was highest in the High bulk density treatment but lowest in Control treatment (Figure 4.8). This is likely to relate to the original volumes of media compressed into the pots, but also influenced by loss of media when irrigated and through natural oxidation of the organic components.

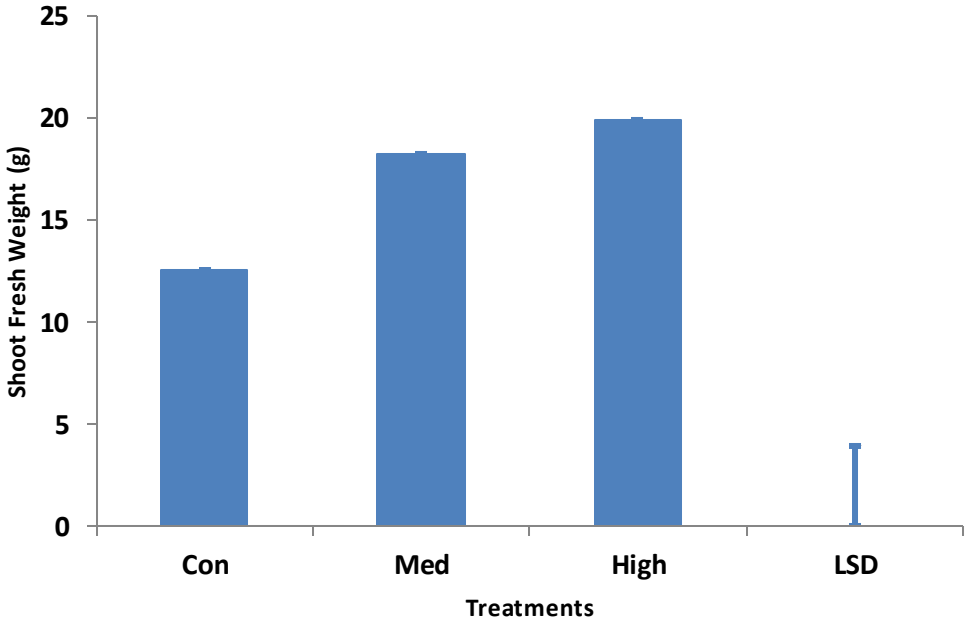


Figure 4.1 Shoot fresh weight (g) of *Philadelphus* cv. Aureus in 3 different bulk densities. P ≤ 0.005, LSD: 3.967, d.f. = 31

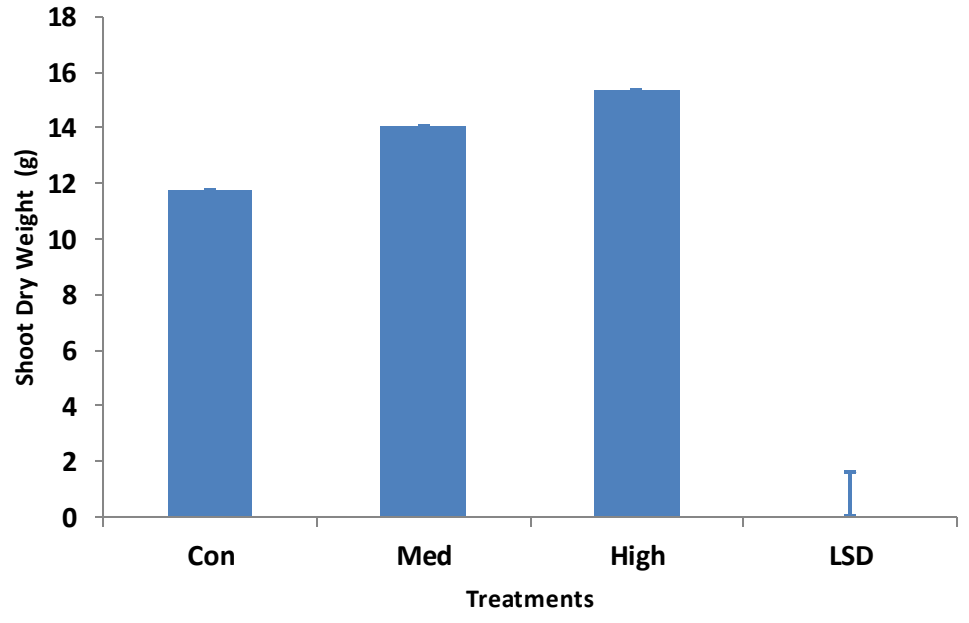


Figure 4.2 Shoot dry weight (g) of *Philadelphus* cv. Aureus in 3 different bulk densities. P ≤ 0.005, LSD: 1.597, d.f. = 31

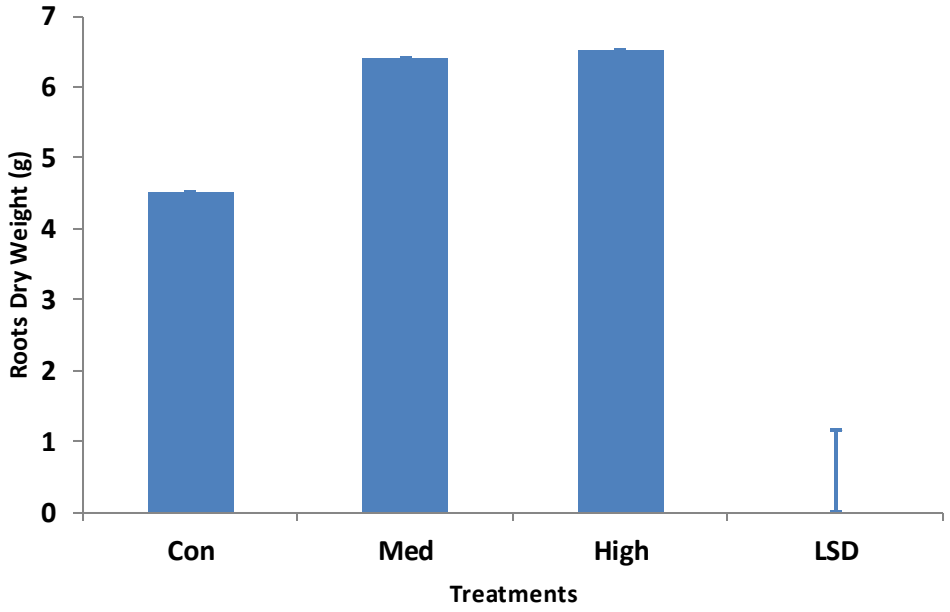


Figure 4.3 Root dry weight (g) of *Philadelphus* cv. Aureus in 3 different bulk densities. P ≤ 0.005, LSD: 1.146, d.f. = 31

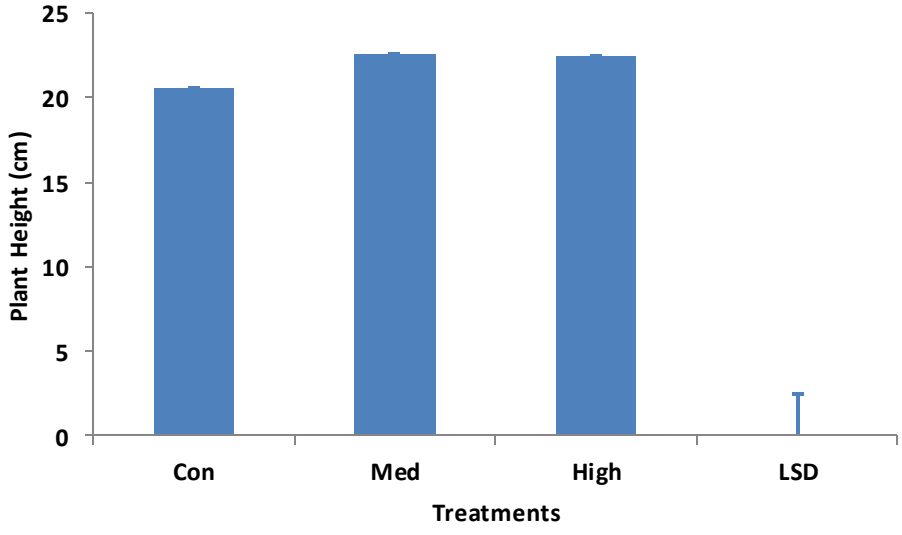


Figure 4.4 Plant height for *Philadelphus* cv. Aureus in 3 different bulk densities. P ≤ 0.005, LSD: 2.468, d.f. = 31

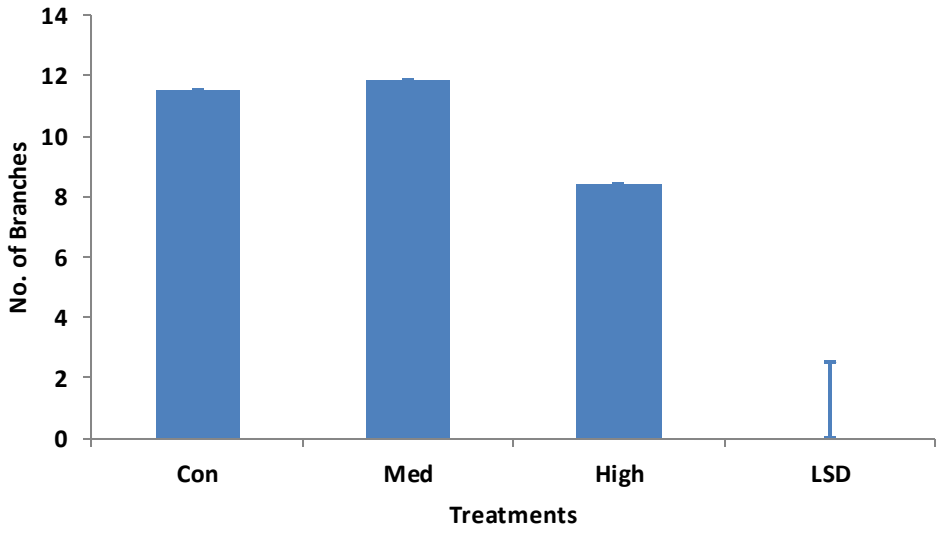


Figure 4.5 No. of branches for *Philadelphus* cv. Aureus in 3 different bulk densities. P ≤ 0.005, LSD: 2.552, d.f. = 31

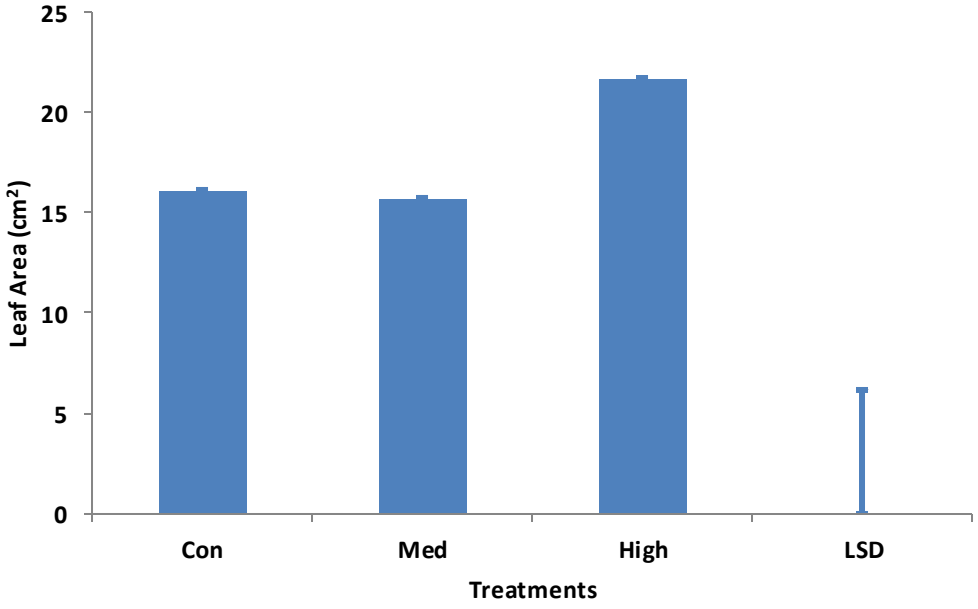


Figure 4.6 Leaf area (cm²) for *Philadelphus* cv. Aureus in 3 different bulk densities. P ≤ 0.005, LSD: 6.11, d.f. = 31

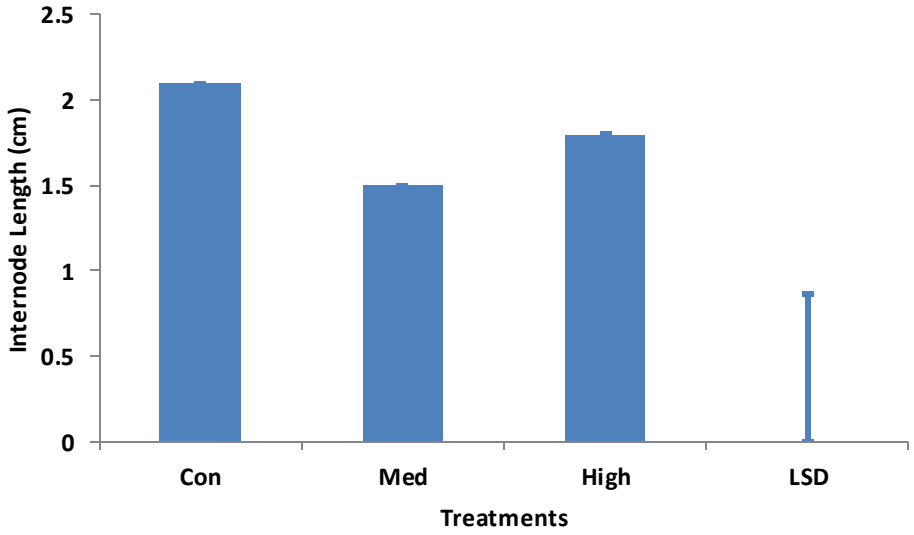


Figure 4.7 Internode length (cm) for *Philadelphus* cv. Aureus in 3 different bulk densities. P ≤ 0.005, LSD: 0.86, d.f. = 31

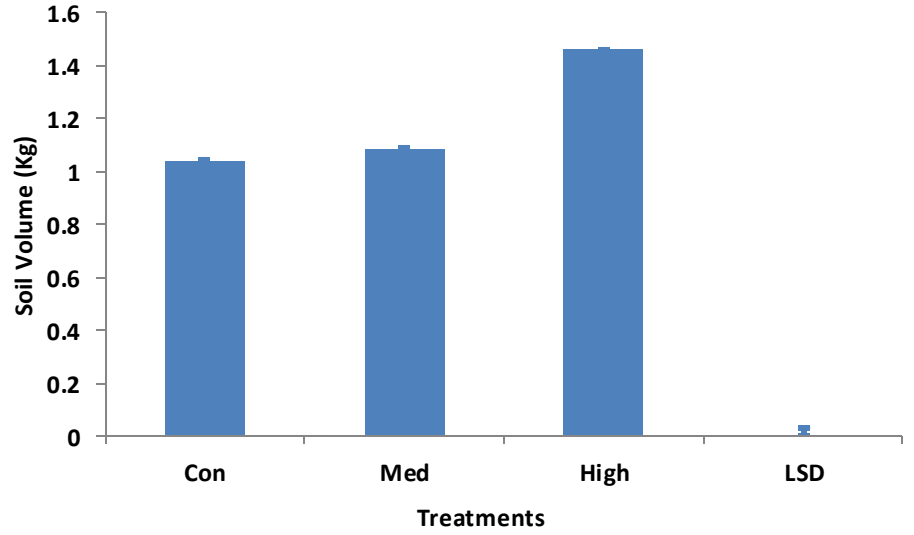


Figure 4.8 Media volumes in each treatment for *Philadelphus* cv. Aureus. P ≤ 0.005, LSD : 0.028, d.f. = 31

***Euonymus* cv. Silver Queen**

Euonymus cv. Silver Queen showed a contrasting pattern to that observed in *Philadelphus* cv. Aureus. In the *Euonymus*, shoot fresh and dry weight and root dry weight were significantly greater in the control (1.4 L) than the High density (2.0 L) treatment (Figures 4.9, 4.10 and 4.11). Controls values were also significantly greater than the Medium density (1.7 L) treatment for shoot fresh and dry weight, but not root dry weight, despite the volume of media subsequently being recovered from the pots being comparable (Figure 4.16). There were significant differences in plant height between treatments. Despite being lowest biomass, the High bulk density medium produced the tallest plants. However, there were no significant differences in total number of branches between treatments all treatments producing over 30 per plant (Figure 4.13). Leaf area and internode length showed no significant difference in each of the treatment but plants grown in the Medium bulk density treatment recorded the largest leaves and longest internode lengths (Figures 4.14 and 4.15).

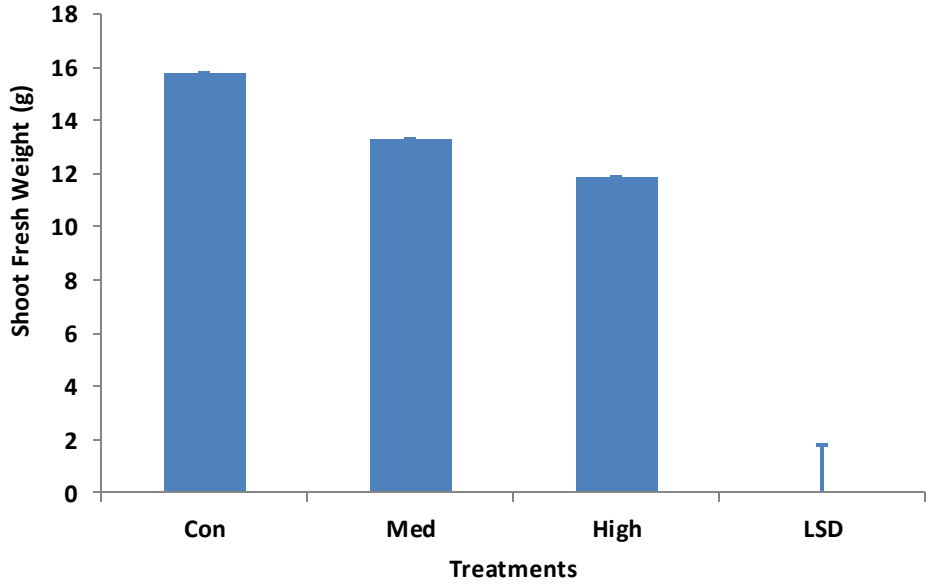


Figure 4.9 Shoot fresh weight (g) for *Euonymus* cv. Silver Queen in 3 different bulk densities. P ≤ 0.005, LSD: 1.77, d.f. = 31

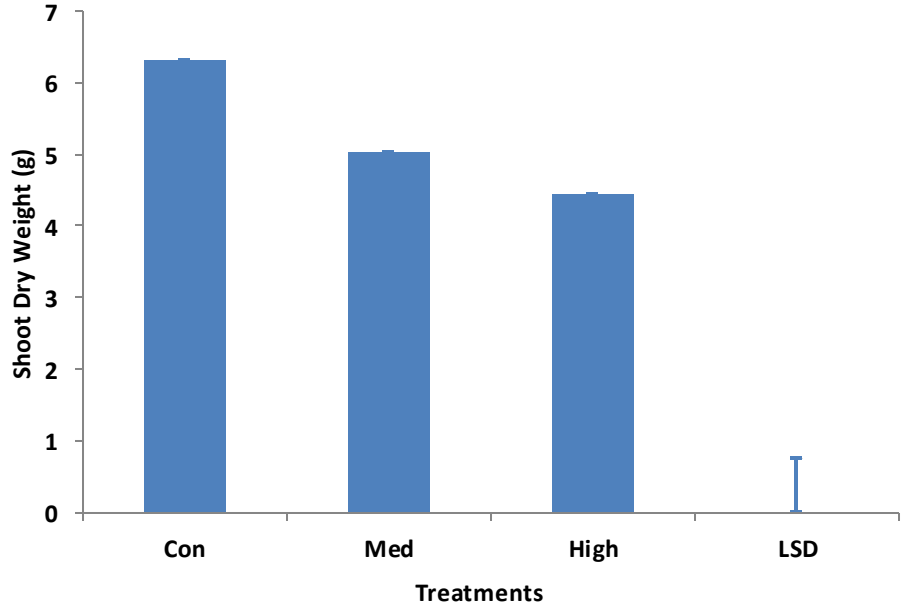


Figure 4.10 Shoot dry weight (g) *Euonymus* cv. Silver Queen in 3 different bulk densities. P ≤ 0.005, LSD: 0.75, d.f. = 31

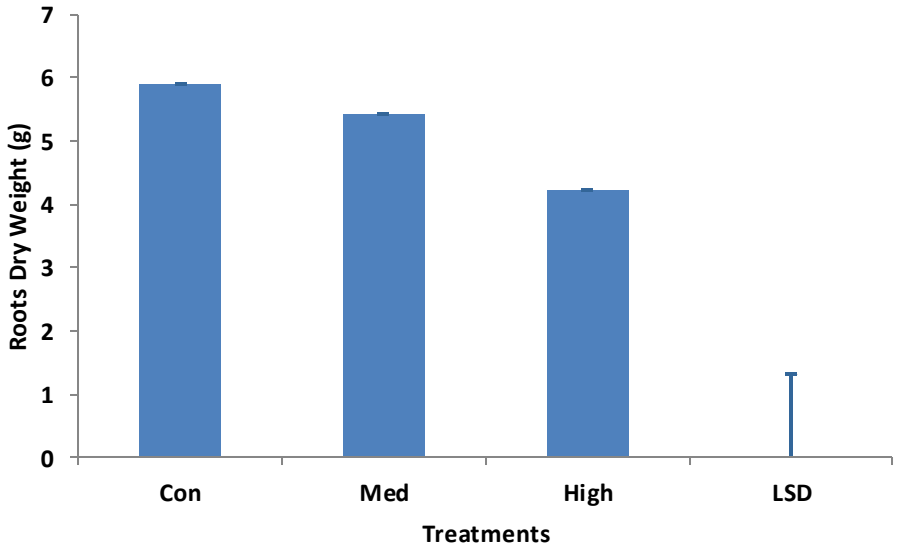


Figure 4.11 Roots dry weight (g) for *Euonymus* cv. Silver Queen in 3 different bulk densities. P ≤ 0.005, LSD: 1.32, d.f. = 31

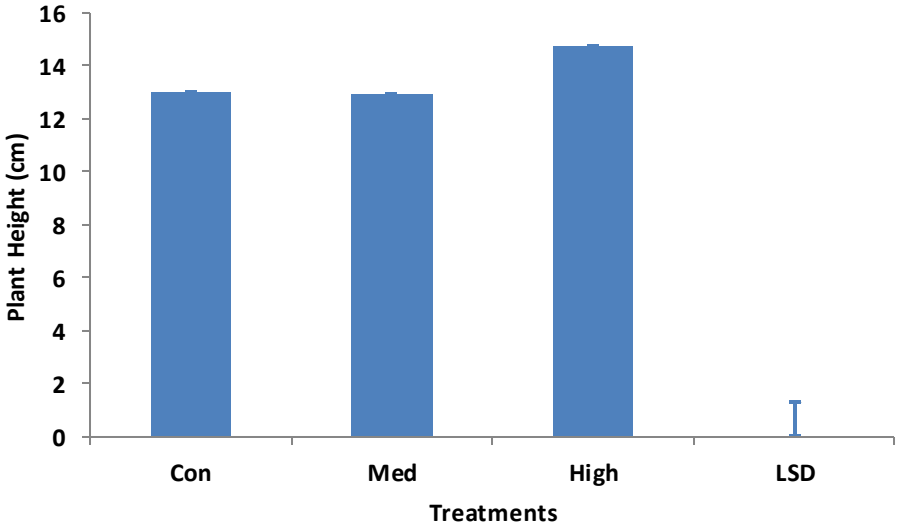


Figure 4.12 Plant height for *Euonymus* cv. Silver Queen in 3 different bulk densities. P ≤ 0.005, LSD: 1.285, d.f. = 31

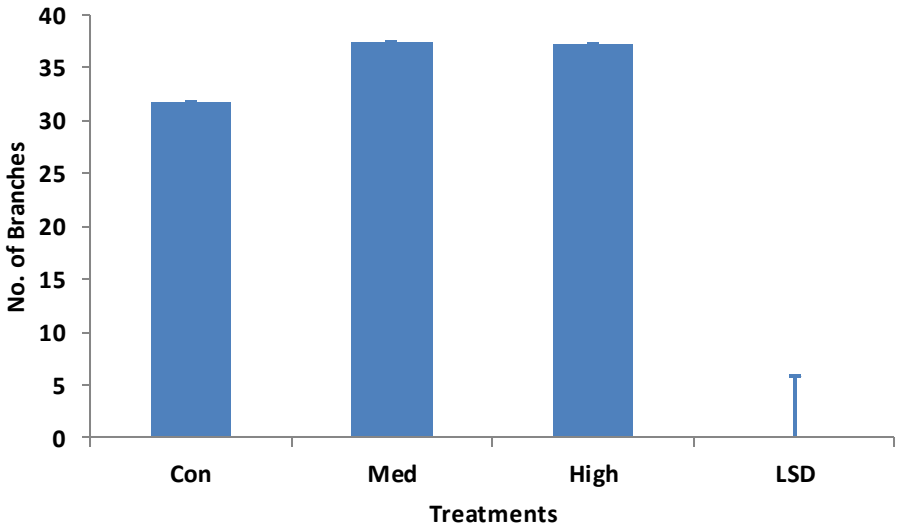


Figure 4.13 No. of branches for *Euonymus* cv. Silver Queen in 3 different bulk densities. P ≤ 0.005, LSD: 5.86, d.f. = 31

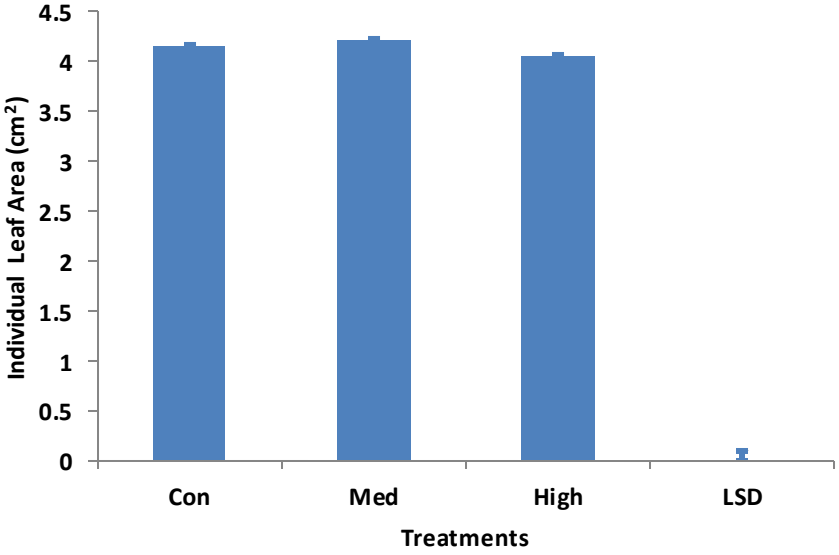


Figure 4.14 Leaf area (cm²) for *Euonymus* cv. Silver Queen in 3 different bulk densities. P ≤ 0.005, LSD: 0.839, d.f. = 31

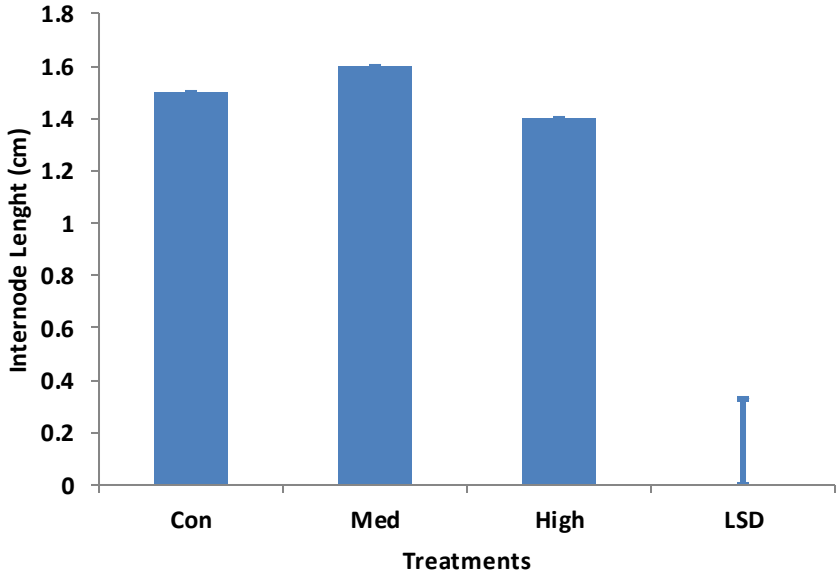


Figure 4.15 Internode length (cm) for *Euonymus* cv. Silver Queen in 3 different bulk densities. P ≤ 0.005, LSD: 0.33, d.f. = 31

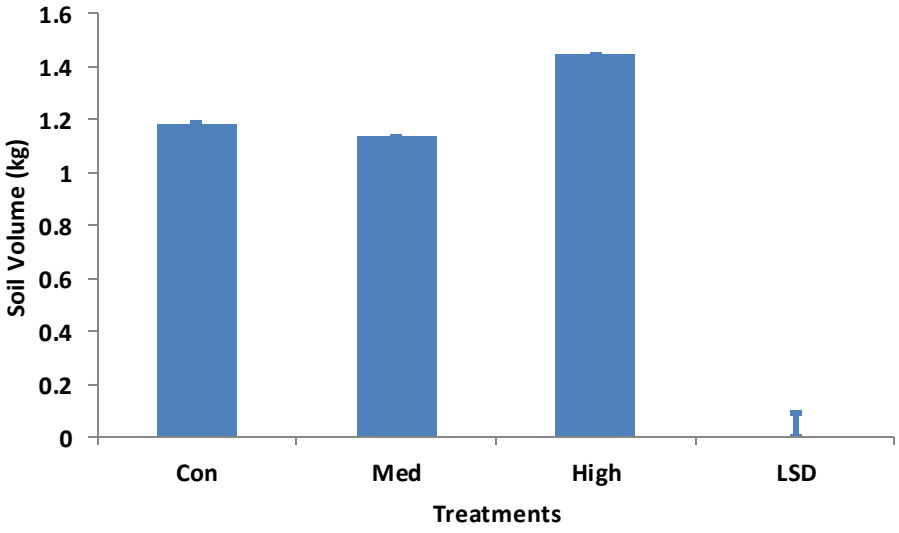


Figure 4.16 Media volumes in each treatment for *Euonymus* cv. Silver Queen in 3 different bulk densities. P ≤ 0.005, LSD: 0.0882, d.f. = 31

4.2 Experiment 4b: The influence of different bulk density in three contrasting soil types (sand v clay v organic) on root dynamics and shoot development

This experiment was conducted to investigate the effect of different level of compaction in three different media (organic, sand and clay) on plant growth across three different species.

4.2.1 Hypothesis:

The influence of substrate bulk density on plant development will vary with the nature of the substrate, i.e. type of soil.

4.2.2 Objectives

To determine how varying the level of compaction in three contrasting media (soil) types affect plant development, and whether the physiological effects caused by compaction are universal across the media types.

4.2.3 Materials and Methods:

This experiment evaluated the effect of increased compaction (bulk density) on plant shoot and root development, across 3 contrasting soil types; namely an organic loam as used in the previous experiment (John Innes No. 1 – 'Seedlings and Young Plants), clay soil (Riverine clay from Goole, East Yorkshire, Grid Ref. SE771 243)) and horticulture grit and sand Builder's Grade (B&Q, Sheffield). Taller pots (3 Litre, 15.9cm diameter X 20.5cm height) were used compared to experiment 4a to enable the media to be more effectively compressed (allowing for hammer blows). Saucers were placed at the base of each container to help ensure irrigation water was absorbed back into the media, especially for horticultural grit and sand which has free drainage compared to the organic medium and clay. For each growing media, different volumes of media were placed and, where necessary compacted into the pot.

Pots of the control treatment were filled with 3 Litre volume of one of the following media: - organic medium, sand or clay media without any compaction 12 representative pots per soil type. For the second treatment (Medium bulk density) pots were filled with 4.5 litre organic soil, 3.5 litre sand and 4.0 litre clay volume and compressed using 2.5kg Proctor hammer to ensure all of the medium will fit into the pot. Finally for the third treatment (High bulk density) pots were filled with 5.0 litre organic, 4.5 litre sand and 4.5 litre clay and compressed using 4.5kg Proctor hammer. The amounts of soil volumes were different in each treatment as each soil inherently starts with a different bulk density, and the volume / force of compression was used to increase the density relative to the control for each media type. By definition of course, altering the volume used may impact on water availability and the amount of nutrients stored in each pot.

The experiment evaluated three different genotypes. *Philadelphus* cv. Aureus and *Euonymus* cv. Silver Queen as before, but in addition an non-golden form of *Philadelphus*, *Philadelphus* cv. Belle Etoile to determine if the normal green form was more tolerant of the associated stresses associated with compaction. After the soils were compacted, a rooted cutting was placed in each pot, carefully creating a hole for the rootball without causing undue disturbance (i.e. radically altering the bulk density) of the media below / around it. Plants were arranged in a Randomize Complete Block Design on the glasshouse bench with 3 treatments, 3 types of soils and 4 replications for each species. Temperature of the glasshouse were recorded every 3 minutes using Tiny tag Data Logger. All of the plants were irrigated of using hand watering once a week or more depending on the environmental condition at the time. Plant height was recorded weekly.

Plants were left an arbitrary 3 weeks 'to establish' after planting in the pots and watering was monitored to avoid excessive or inadequate amounts being applied (the clay stayed wetter for longer than the sand and organic media). 10 granules of slow release fertilizer was used to top-dress the pots, as the sand and clay soils, particularly may have been deficient in the major nutrients. Any plants that failed to establish were replaced by fresh material during the establishment period. Plants were grown from 9th May 2013 until 11th July 2013.

Plant heights, number of leaves, number of branches, number of nodes, internode length were recorded every week. At the end of the experiment, leaf area of selected leaf (third leaf from shoot tip) from each plant was measured to see any differences of leaf expansion between treatments. Data of Chlorophyll Fluorescent and Stomatal Conductance were recorded once before the destructive harvesting took place, to measure the plants response to the environmental stresses. All of the plants were taken out from the pot and destructively harvested; dividing the tissues into shoots and roots. Both fresh and dry weights of shoots were recorded, as well as dry weight of roots. Two way analysis of variance (ANOVA) was conducted to investigate the interaction between the severity of compaction and types of media on plant growth.

a **b**

Figure 4.17: The process of compacting the media using Proctor Hammer 2.5kg and 4.5kg

4.2.4 Result

***Philadelphus* cv. Aureus**

Significant statistical interactions were observed between media type and bulk density for the following parameters:- plant height, shoot dry weight, root dry weight, total number of leaves, leaf area, number of nodes, internode length, branches number as well as, stomatal conductance and chlorophyll fluorescence. Mean data are depicted in Figures 4.18 to 4.29.

Trends between media types and compaction were not always consistent. Increasing the level of compaction in the organic and the clay media tended to reduce growth factors e.g. with significant response for at least one or other level of compaction against the control for plant height (organic and clay – Figure 4.18), shoot and root dry weight (organic – Figure 4.19 and 4.20), number of leaves (organic – 4.21) and total leaf area (clay – Figure 4.22). Whole plant leaf area shows the highest value in control plants for organic and clay media, but in medium in sand media (Figure 5.23).

One or other level of compaction also reduced the number of nodes (organic and clay – Figure 4.24), internode length (organic and clay - Figure 4.25), and number of branches (organic – Figure 4.26) present on the plants. There was a strong effect of media type on stomatal conductance, when this was measured at the end of the experiment. In this paradoxically, the lowest recorded values were associated with the organic media (Figure 4.27), despite plants in this media especially controls showing greatest growth (Figure 4.18). The sand based media corresponded to the highest stomatal conductance, with relatively high values still being noted in the most compacted treatment.

Despite the large growth differences observed, all treatments demonstrated mean chlorophyll fluorescent Fv/Fm values in excess of 0.7 (Figure 4.28) with the medium density organic media giving significantly higher P. Index values than all other treatments, bar the sand (Figure 4.29).

Taking the organic media in isolation, increasing the bulk density tended to reduce growth (as depicted by height, number of leaves, leaf area, shoot and root dry weight). Although the plants grown in clay, had less biomass compared to their counter parts in the organic medium, the trends were broadly similar, with increased compaction inhibiting growth. In the sand, however these trends were not repeated and often more favourable compaction level was the medium (and even sometimes in the highly compacted medium). Such differences, however, were not always significant.

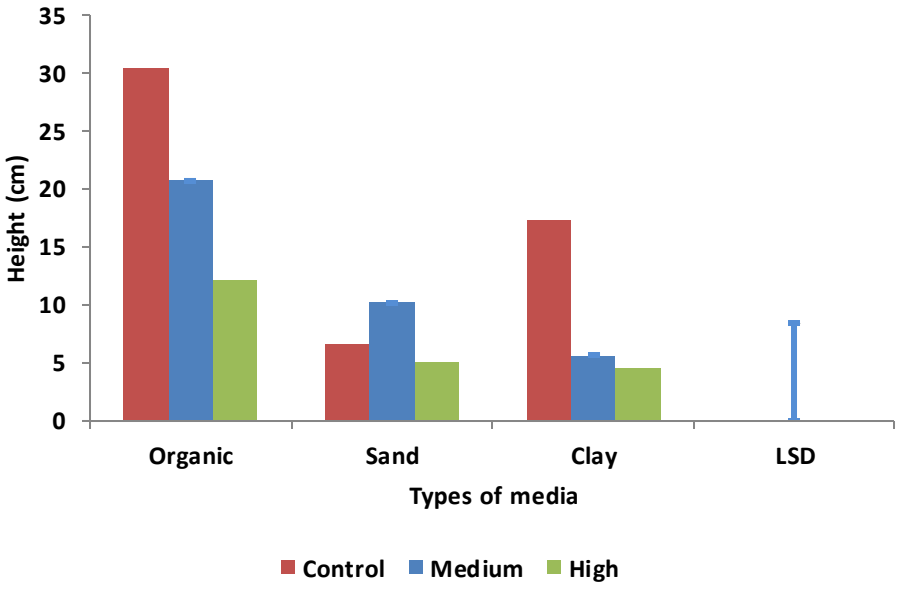


Figure 4.18 Plant height (cm) for *Philadelphus* cv. Aureus potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 8.362, d.f. = 27

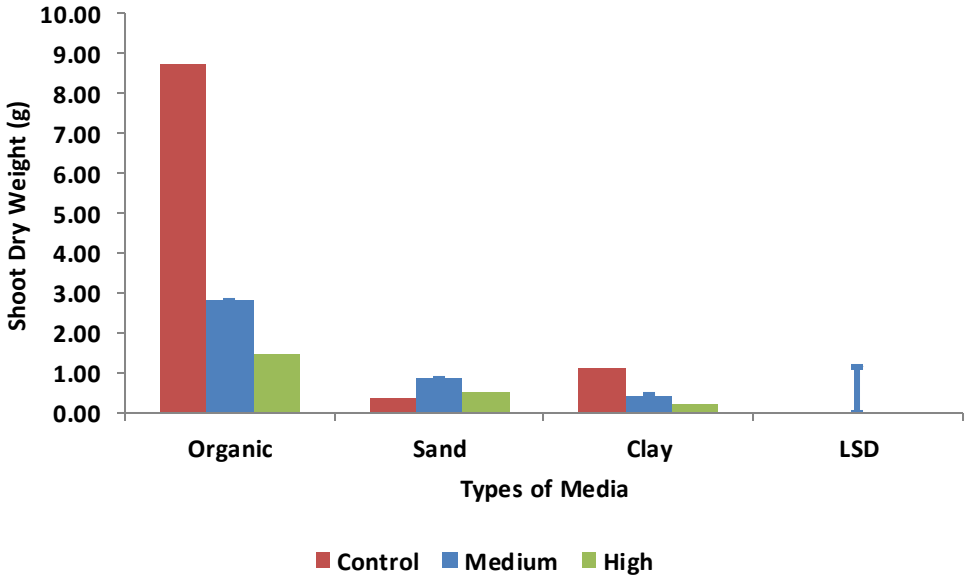


Figure 4.19 Shoot dry weight for *Philadelphus* cv. Aureus potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 1.12, d.f. = 27

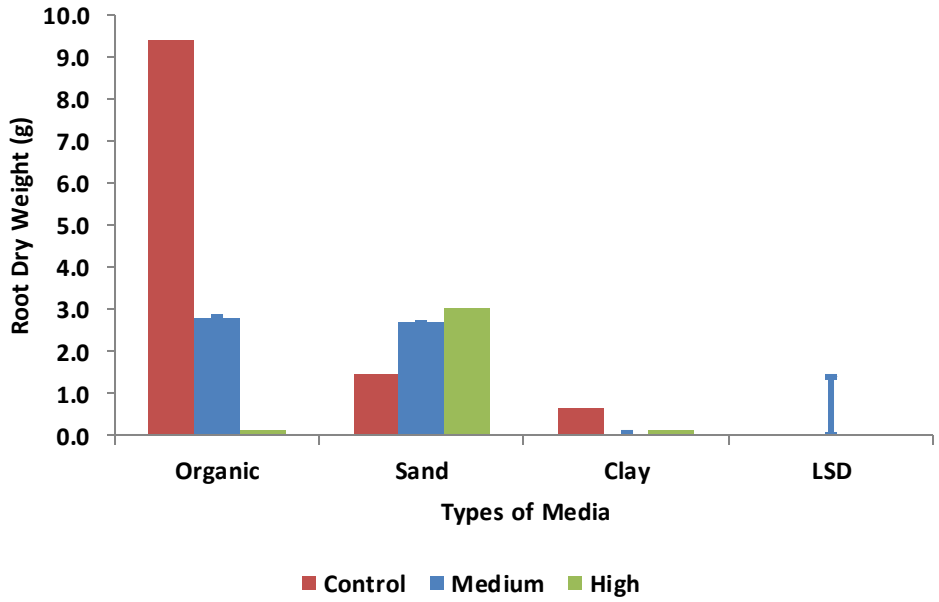


Figure 4.20 Root dry weight for *Philadelphus* cv. Aureus potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 1.379, d.f. = 27

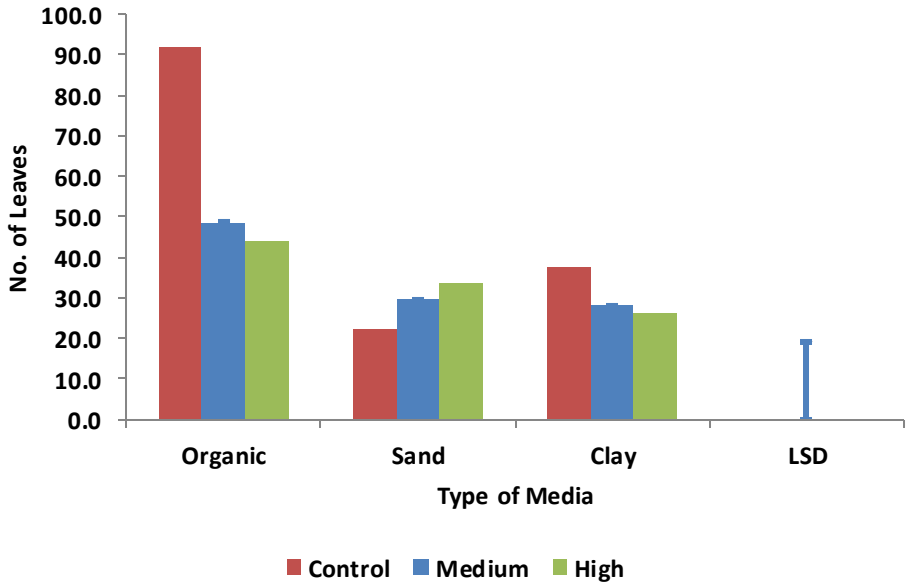


Figure 4.21 Number of leaves for *Philadelphus* cv. Aureus potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 18.89, d.f. = 27

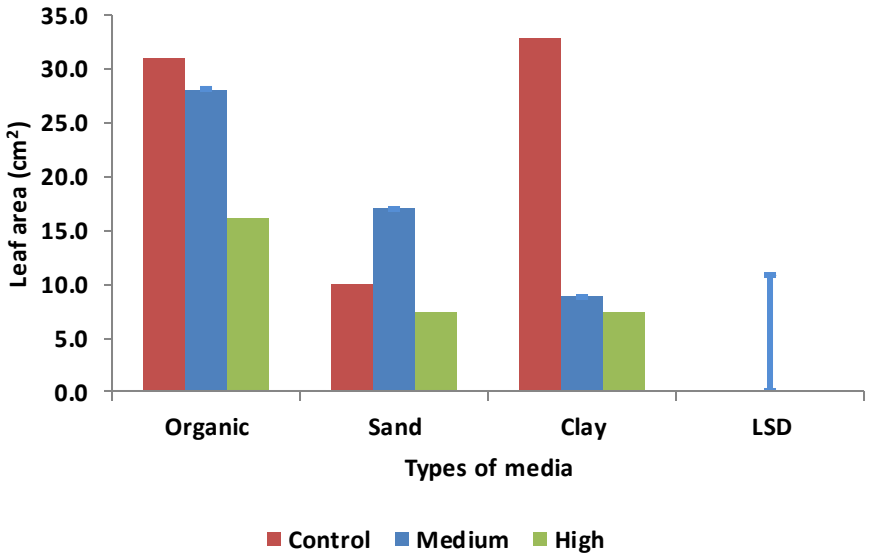


Figure 4.22 Leaf area (cm²) for *Philadelphus* cv. Aureus potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 10.91, d.f. = 27

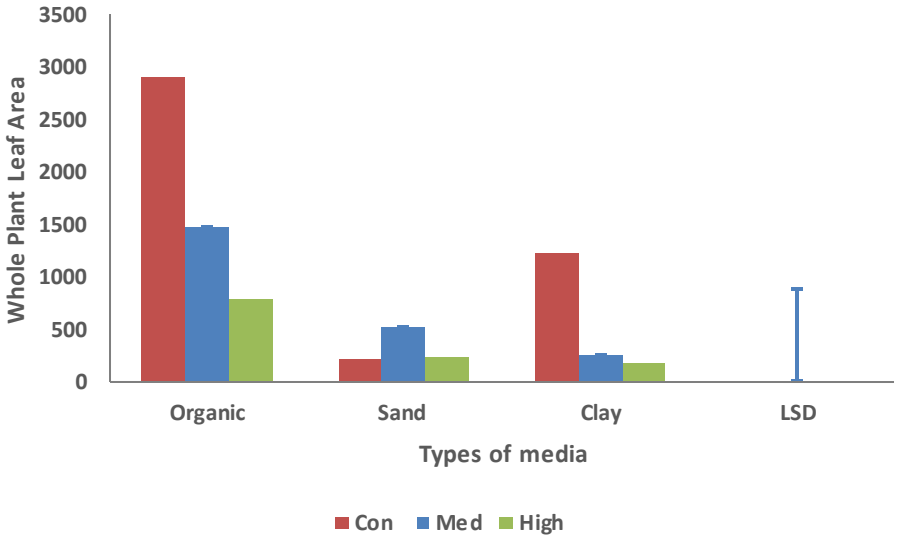


Figure 4.23 Whole plant leaf area for *Philadelphus* cv. Aureus in 3 different media with 3 different bulk desities. P ≤ 0,005, LSD: 880.1, d.f. = 27

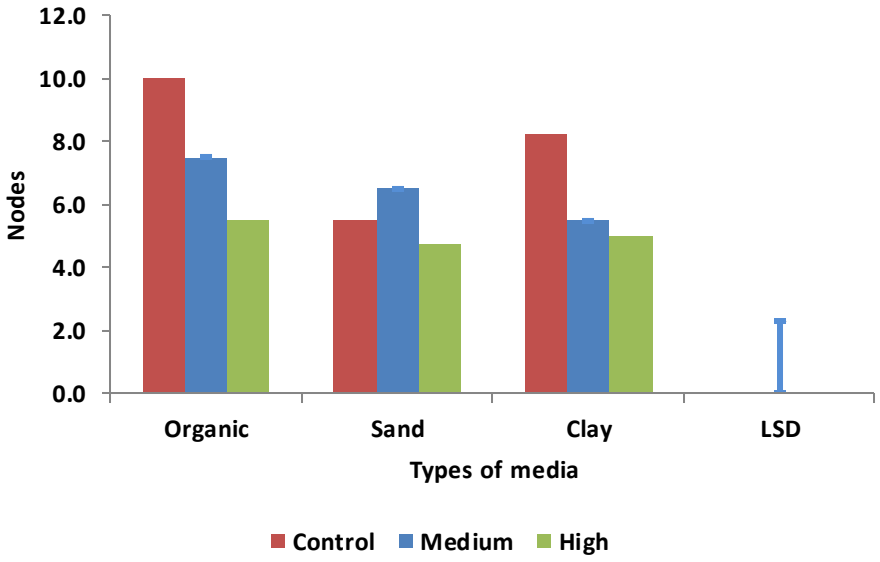


Figure 4.24 Number of nodes for *Philadelphus* cv. Aureus potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 2.227, d.f. = 27

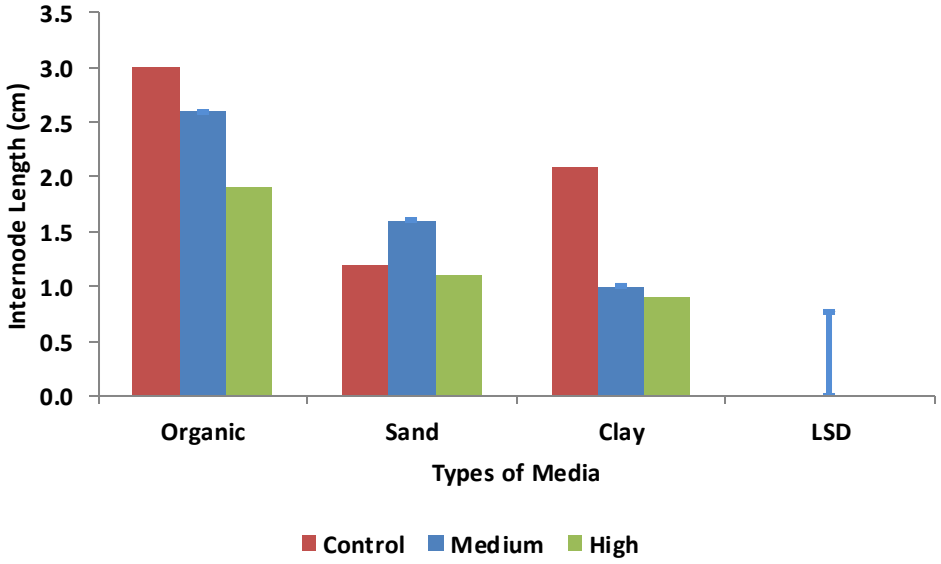


Figure 4.25 Internode length for *Philadelphus* cv. Aureus potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 0.78, d.f. = 27

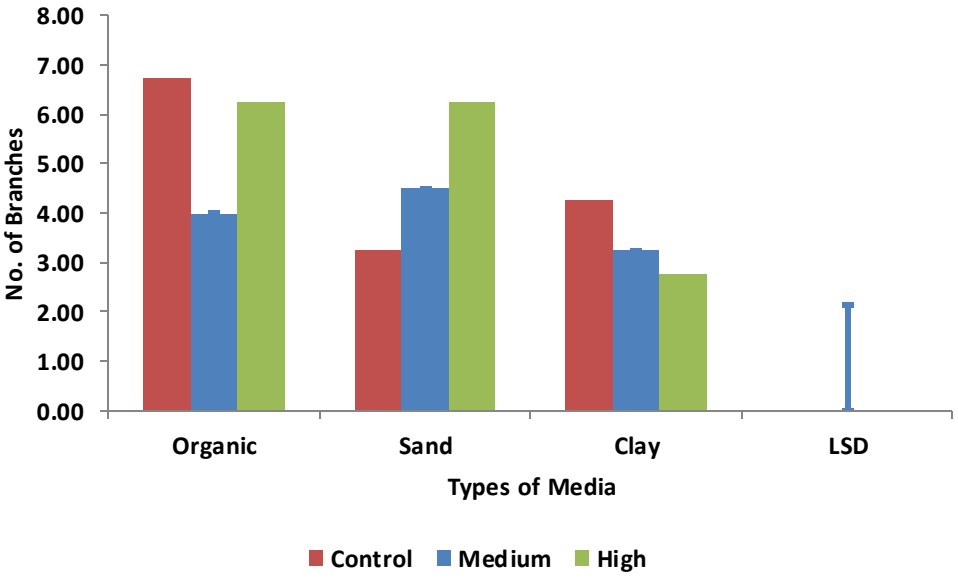


Figure 4.26 Number of branches for *Philadelphus* cv. Aureus potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 2.131, d.f. = 27

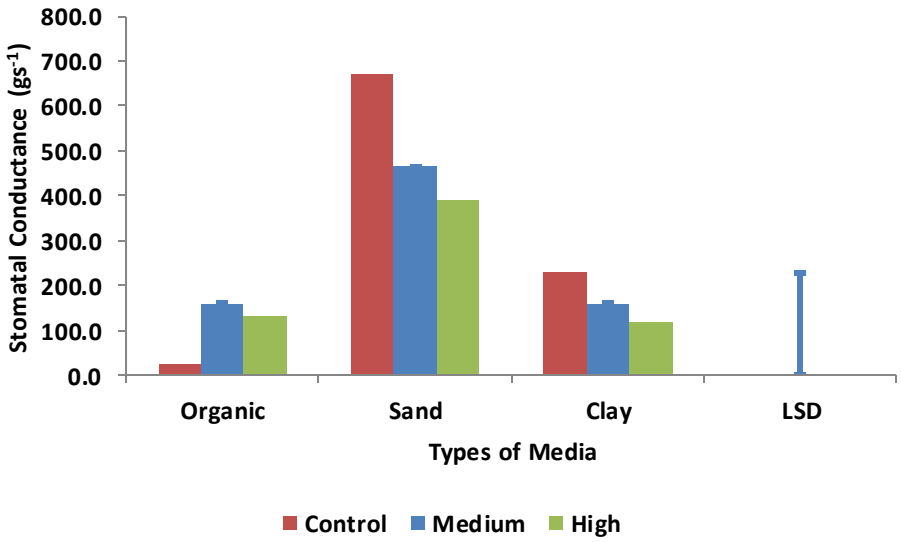


Figure 4.27 Stomatal Conductance (mmol m^{-2} s^{-1}) for *Philadelphus* cv. Aureus potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 225.2, d.f. = 27

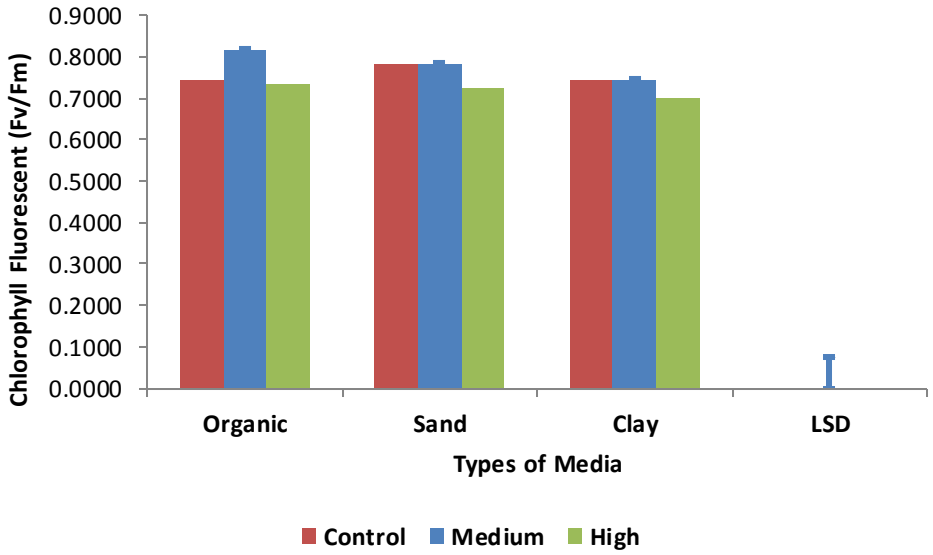


Figure 4.28 Chlorophyll Fluorescent (Fv/Fm) for *Philadelphus* cv. Aureus potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 0.0748, d.f. = 27

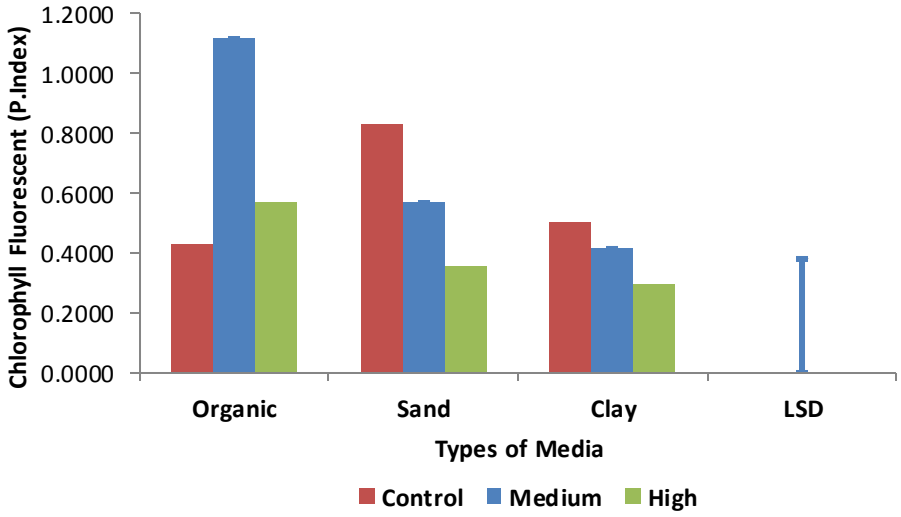


Figure 4.29 Chlorophyll Fluorescent (P. Index) for *Philadelphus* cv. Aureus potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 0.3769, d.f. = 27

***Philadelphus* cv. Belle Etoile**

Growth in this cultivar was strongly favoured by the non-compacted organic media, with significantly greater values for plant height (Figure 4.30) and shoot and root dry weight (Figure 4.31 and 4.32). Number of leaves per plant were reduced as compaction levels increased in the organic and clay media e.g. with a significant response (Figure 4.33) while leaf surface area were reduced with no significant response (Figure 4.34) towards at least one or other level of compaction against the control. Whole plant leaf area for this cultivar shows the same pattern with *Philadelphus* cv. Aureus with highest L recorded in control plant for organic and clay media; however in sand media, medium compacted sand show the highest L value (Figure 4.35 One or other level of compaction also reduced the number of nodes (organic and clay – Figure 4.36), internode length (Figure 4.37) and number of branches (Figure 4.38) present on the plants.

There was a strong effect of media type on stomatal conductance as measured in the final phase of the experiment, with organic and clay media generally being suppressed compared to the sand (Figure 4.97). The sand based media corresponded to greatest level stomatal conductance in control and medium compacted treatment (Figure 4.40), with both the sand and clay, but not the organic, showing a trend for lower values as compression increased. Mean for chlorophyll fluorescent Fv/Fm values demonstrate inconsistence pattern with values lower than 0.7 recorded in the organic control, clay medium and clay high bulk density treatments (Figure 4.41).

Increasing bulk density in organic and clay media tended to reduce growth (as demonstrate by height, shoot and root dry weight, number of leaves and leaf surface area). However, in sand media, these trends were not similar and often the more favourable compaction level was high (and sometimes the medium and control compacted media). Such differences, however, were not significant.

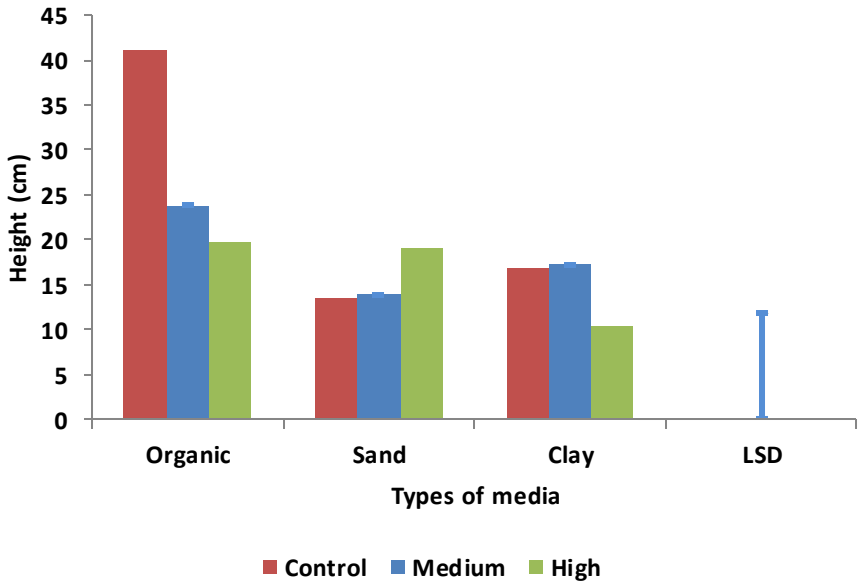


Figure 4.30 Height for *Philadelphus* cv. Belle Etoile potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 11.78, d.f. = 27

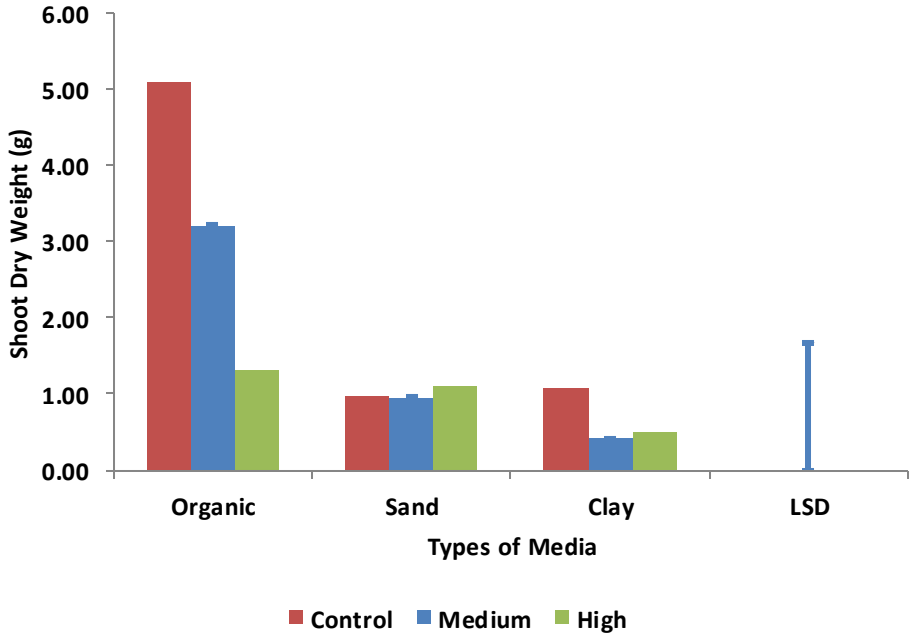


Figure 4.31 Shoot Dry Weight (g) *for Philadelphus* cv. Belle Etoile potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 1.682, d.f. = 27

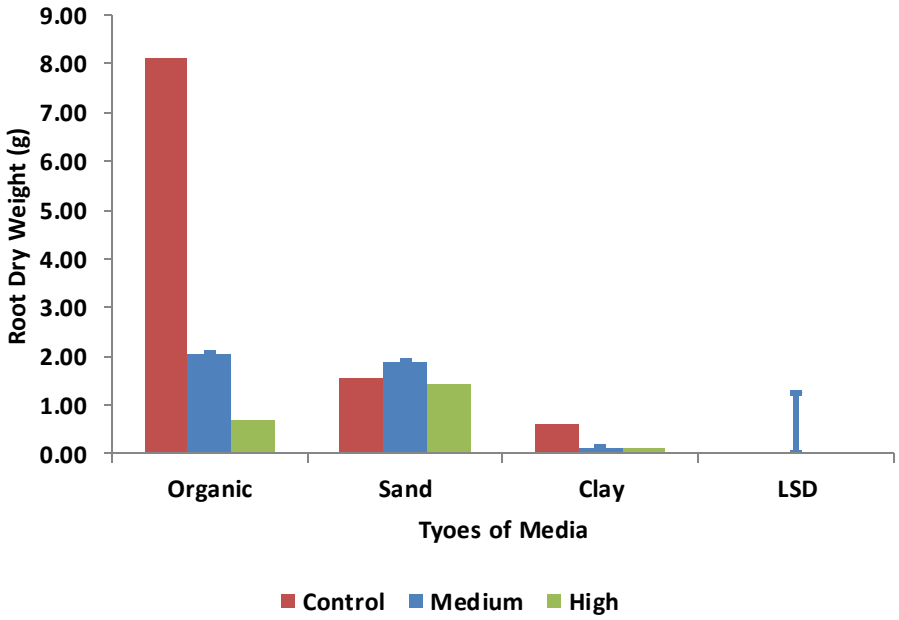


Figure 4.32 Root dry weight (g) for *Philadelphus* cv. Belle Etoile potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 1.248, d.f. = 27

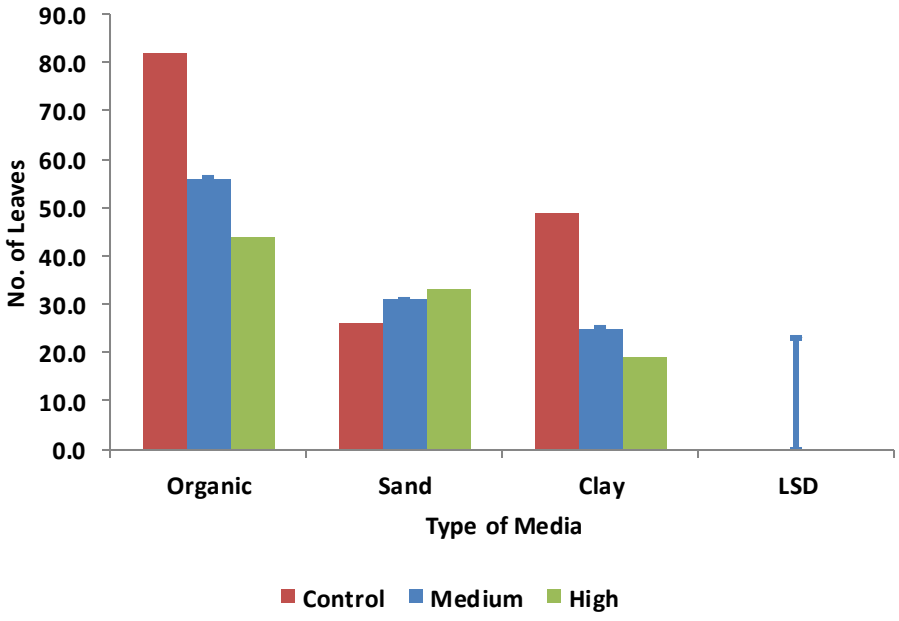


Figure 4.33 Number of leaves for *Philadelphus* cv. Belle Etoile potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 22.8, d.f. = 27

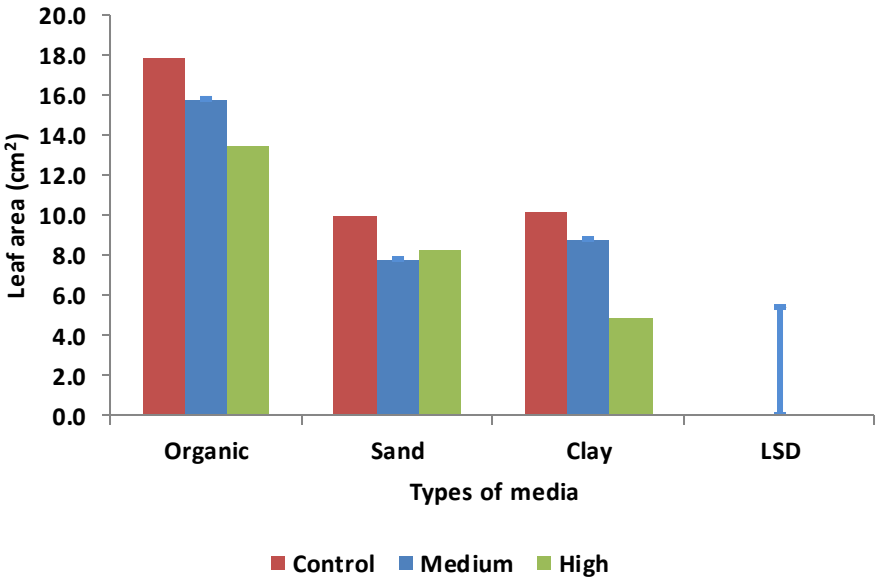


Figure 4.34 Leaf area (cm²) for *Philadelphus* cv. Belle Etoile potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 5.47, d.f. = 27

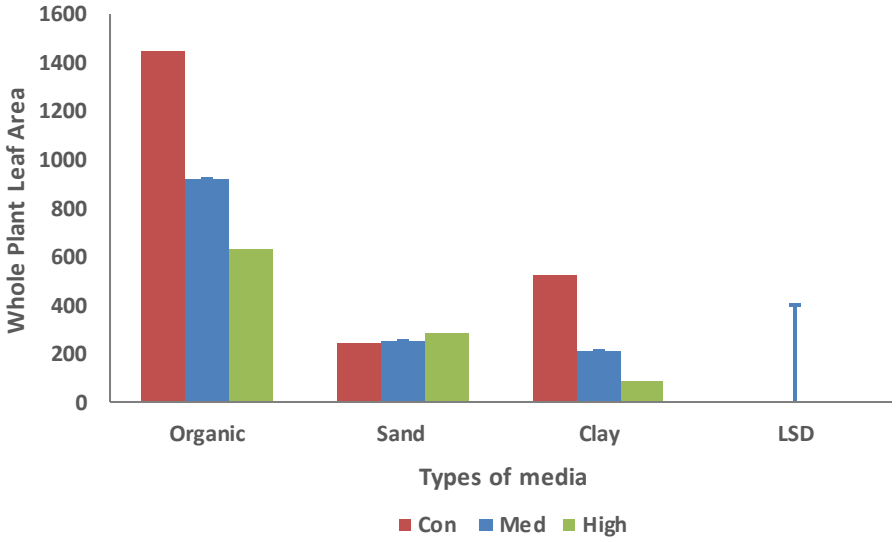


Figure 4.35 Whole plant leaf area for *Philadelphus* cv. Belle Etoile in 3 different media with 3 different bulk densities. P ≤ 0,005, LSD: 402.1, d.f. = 27

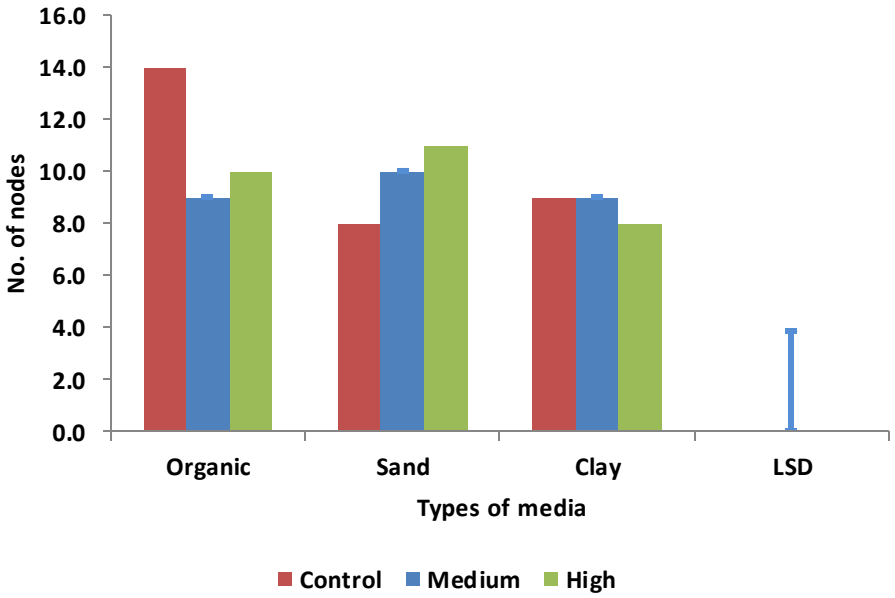


Figure 4.36 Number of nodes for *Philadelphus* cv. Belle Etoile potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 3.9, d.f. = 27

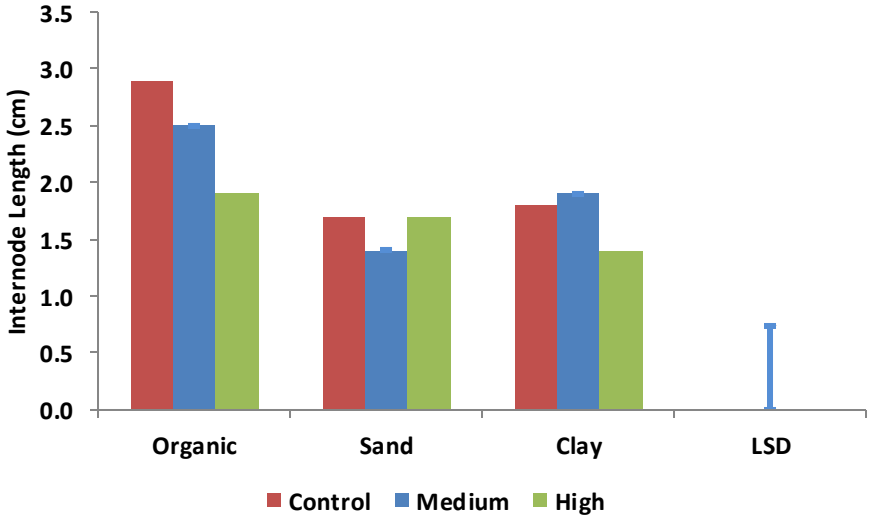


Figure 4.37 Internode Length (cm) for *Philadelphus* cv. Belle Etoile potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 0.74, d.f. = 27

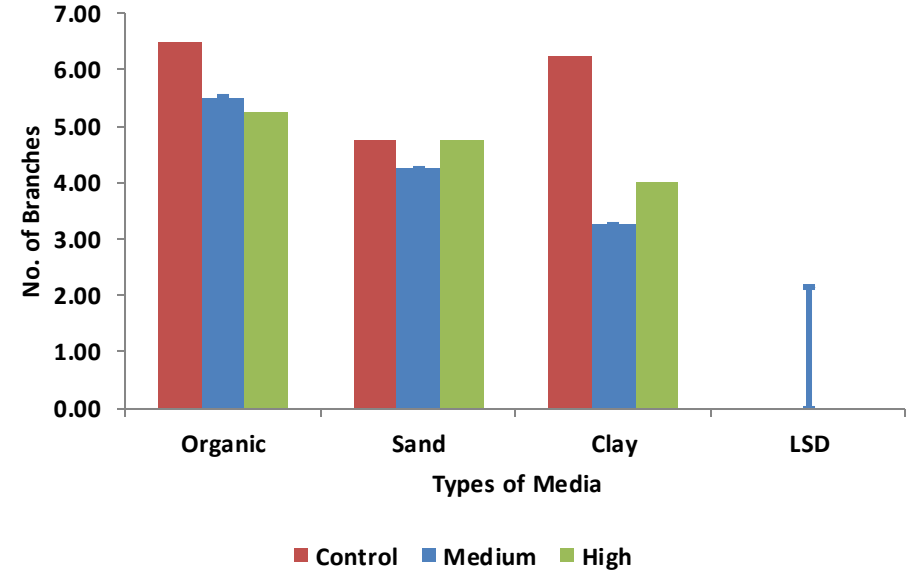


Figure 4.38Number of branches for *Philadelphus* cv. Belle Etoile potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 2.136, d.f. = 27

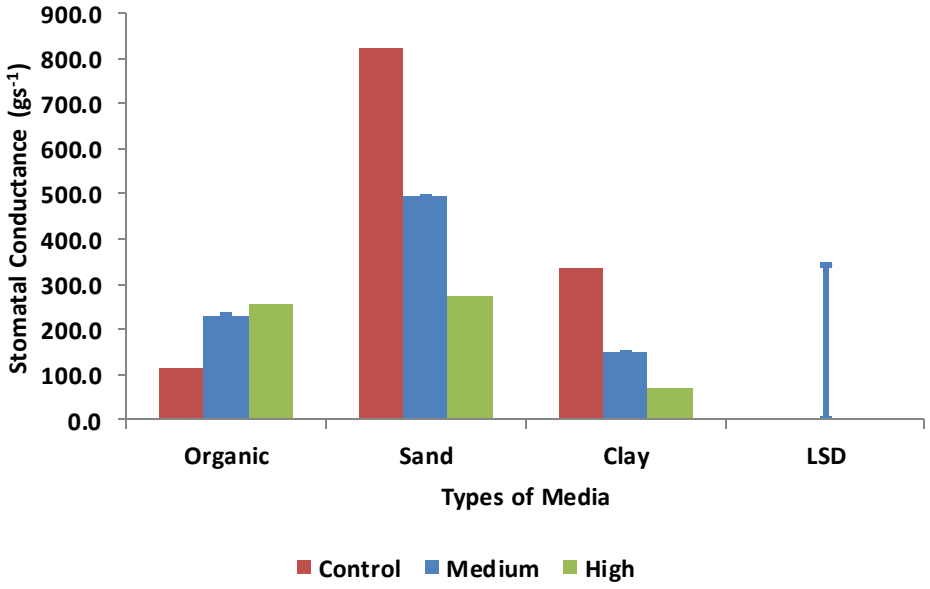


Figure 4.39 Stomatal Conductance (mmol m^{-2} s^{-1}) for *Philadelphus* cv. Belle Etoile potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 341.3, d.f. = 27

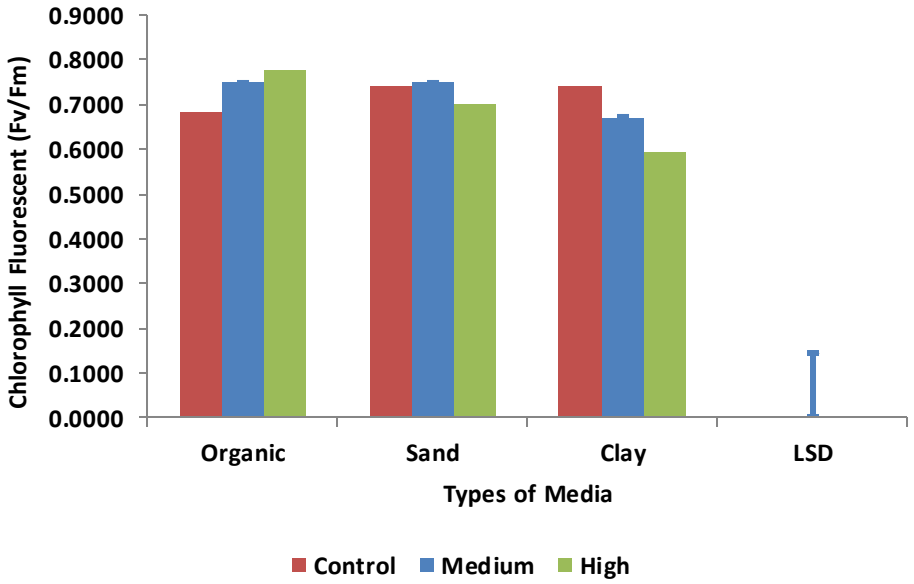


Figure 4.40 Chlorophyll Fluorescent (Fv/Fm) for *Philadelphus* cv. Belle Etoile potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 0.1443, d.f. = 27

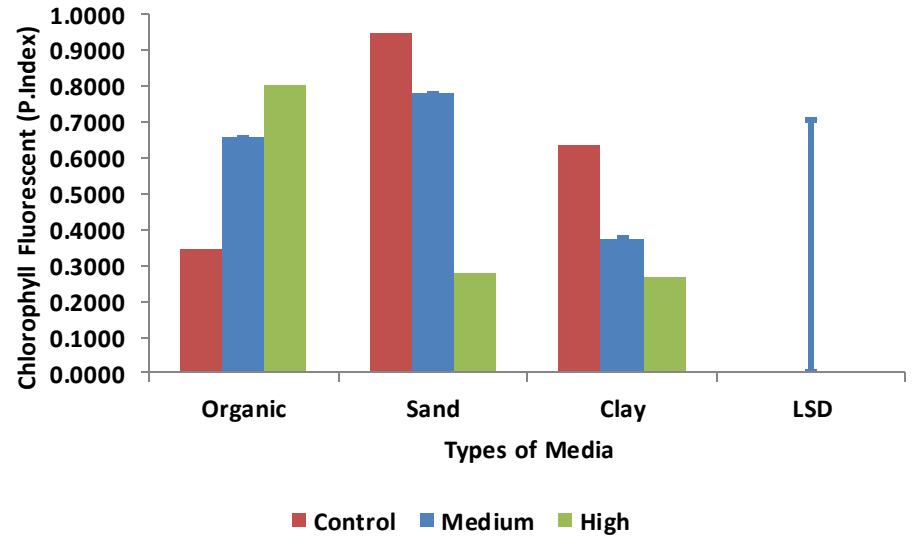


Figure 4.41 Chlorophyll Fluorescent (P. Index) for *Philadelphus* cv. Belle Etoile potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 0.7050, d.f. = 27

***Euonymus* cv. Silver Queen**

Significant interactions were observed between media types and bulk density for the parameters of plant height, shoot and root dry weight, total number of leaves, leaf area, number of nodes, internode length, and number of branches, stomatal conductance and chlorophyll fluorescent. Overall, there were inconsistence trends in plant growth between the different media. Clay tended to reduce shoot biomass and plant height compared to other substrates, with either level of compaction exacerbating the growth reductions (Figure 4.42 and 4.43). In sand, however, there was a positive growth response with increasing compaction (Figure 4.43), and root biomass was enhanced in sand in general (Figure 4.44). Highly compacted clay reduced leaf numbers (Figure 4.45) and the numbers of nodes laid done in a shoot (Figure 4.48). Leaf sizes tended to be greatest in plants grown in the organic medium (Control and Medium density treatments; Figure 4.46), and compaction of the organic medium increased number of nodes present (Figure 4.48), but reduced internode length (Figure 4.49). Whole plant leaf area for this cultivar was recorded to be highest in the medium treatment of organic media but lowest in the medium compacted sand media (Figure 4.47). The numbers of new branches varied between treatments but were rarely significantly different (Figure 4.47).

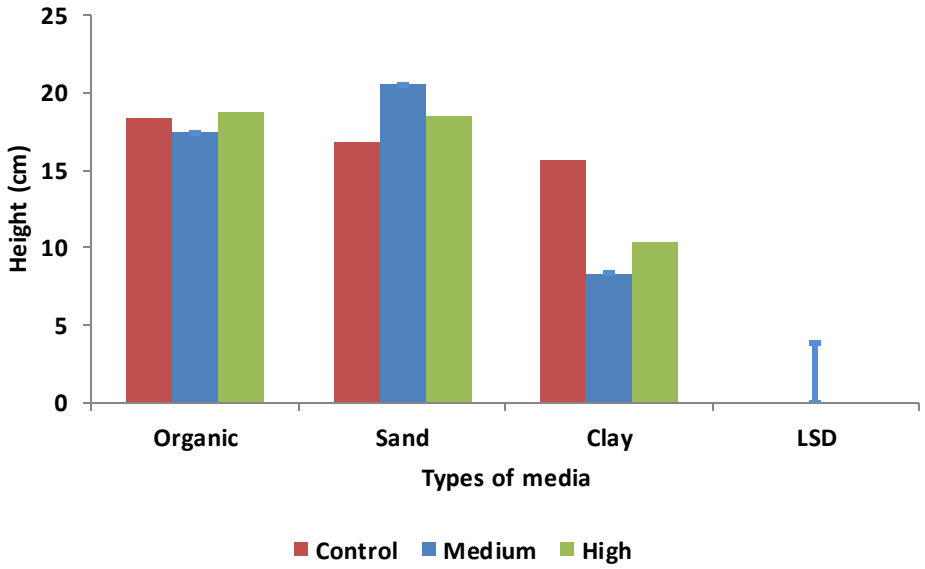


Figure 4.42 Plant height (cm) for *Euonymus* cv. Silver Queen potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 3.85, d.f. = 27

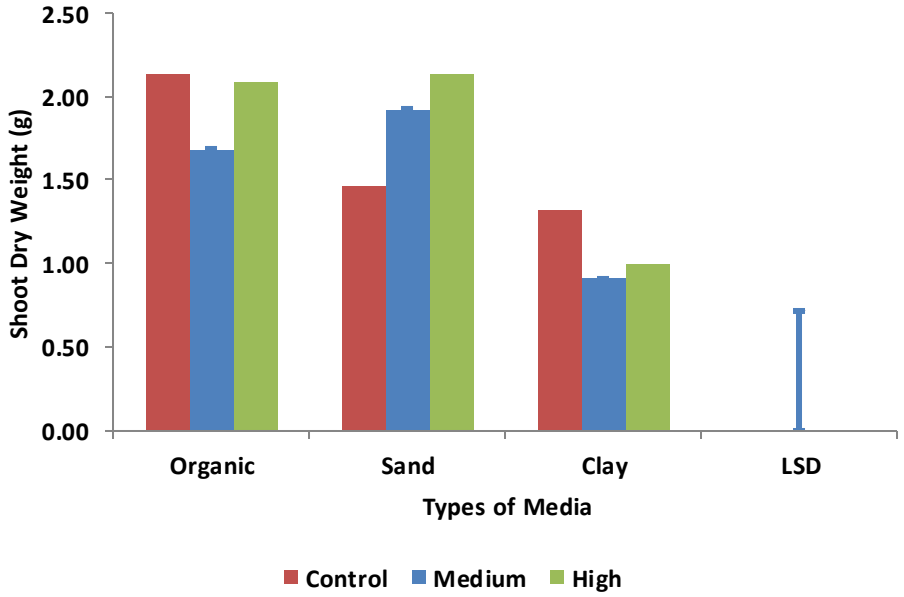


Figure 4.43 Shoot dry weight (g) for *Euonymus* cv. Silver Queen potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 0.701, d.f. = 27

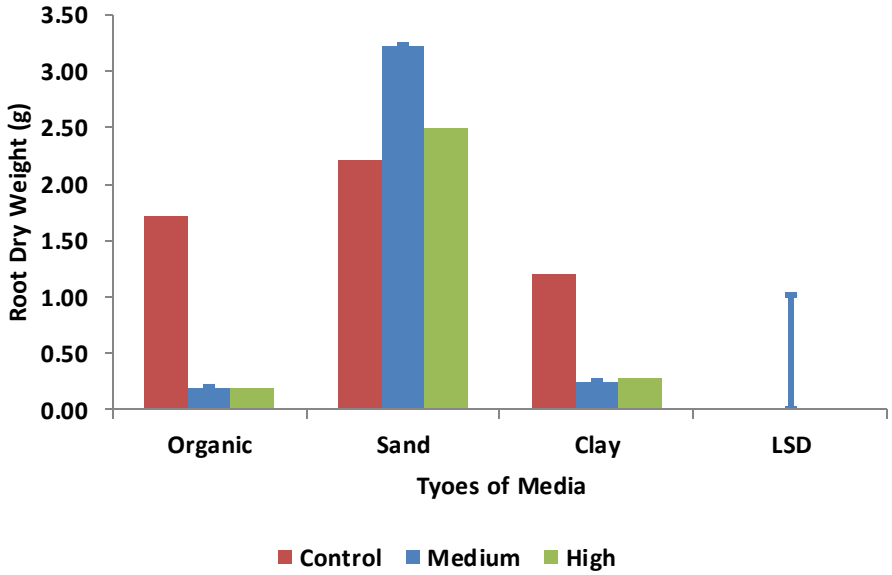


Figure 4.44 Root dry weight (g) for *Euonymus* cv. Silver Queen potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 1.012, d.f. = 27

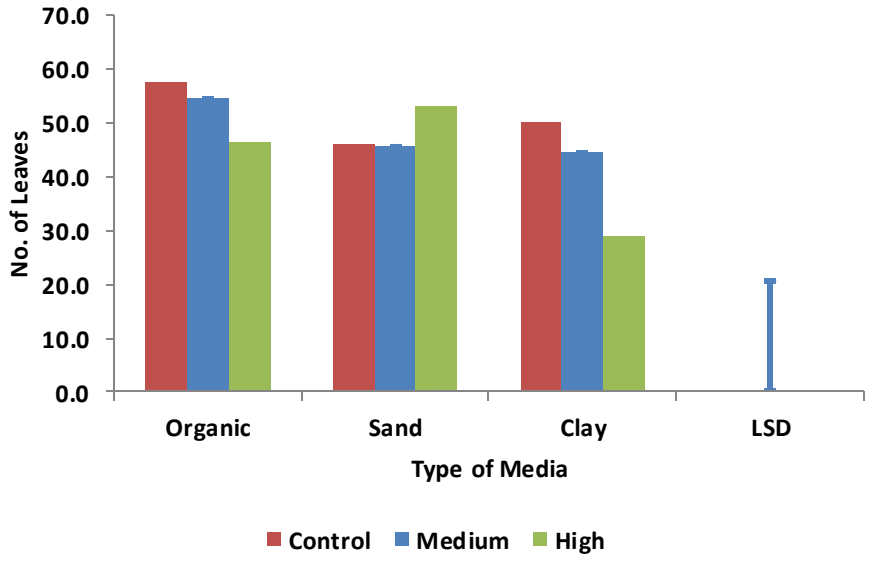


Figure 4.45 Number of leaves for *Euonymus* cv. Silver Queen potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 20.5, d.f. = 27

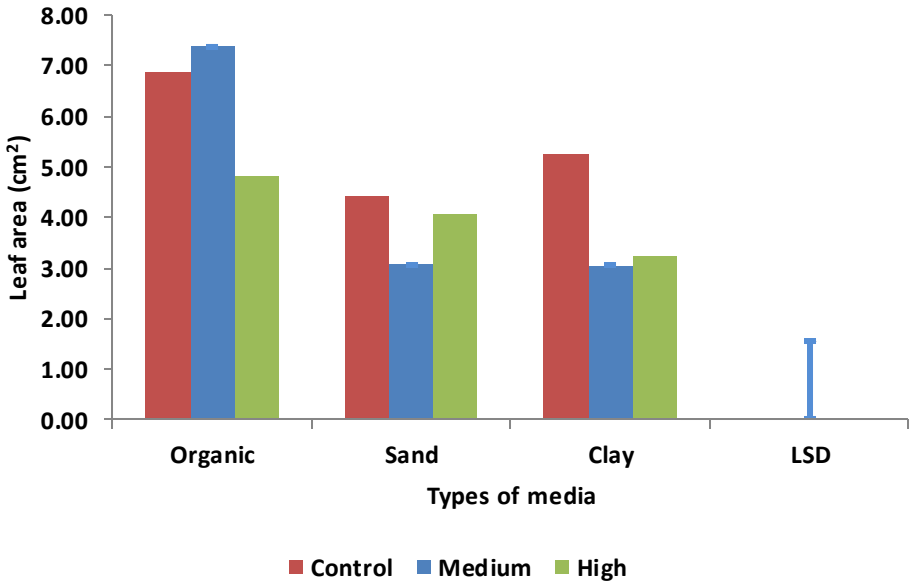


Figure 4.46 Leaf area (cm²) for *Euonymus* cv. Silver *Queen* potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 1.539, d.f. = 27

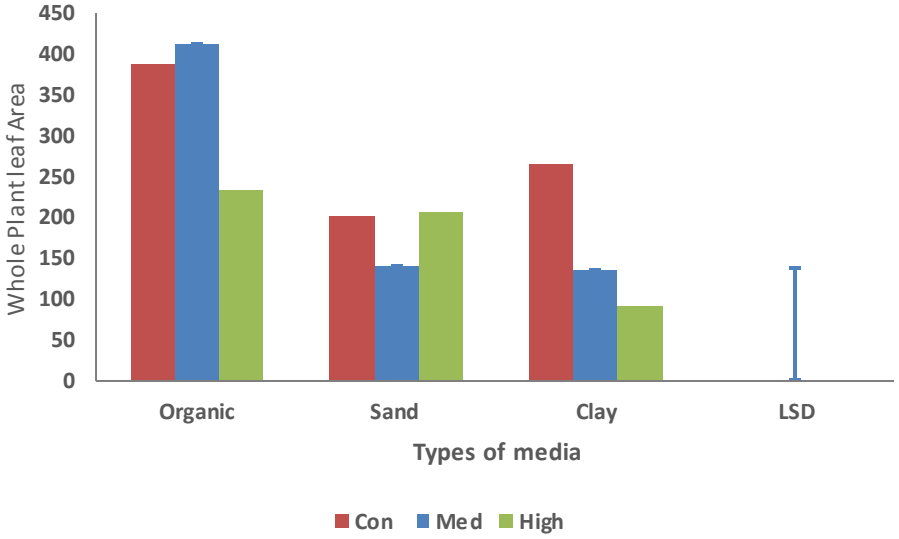


Figure 4.47 Whole Plant Leaf area for *Euonymus* cv. Silver Queen potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 137.1 d.f. = 27

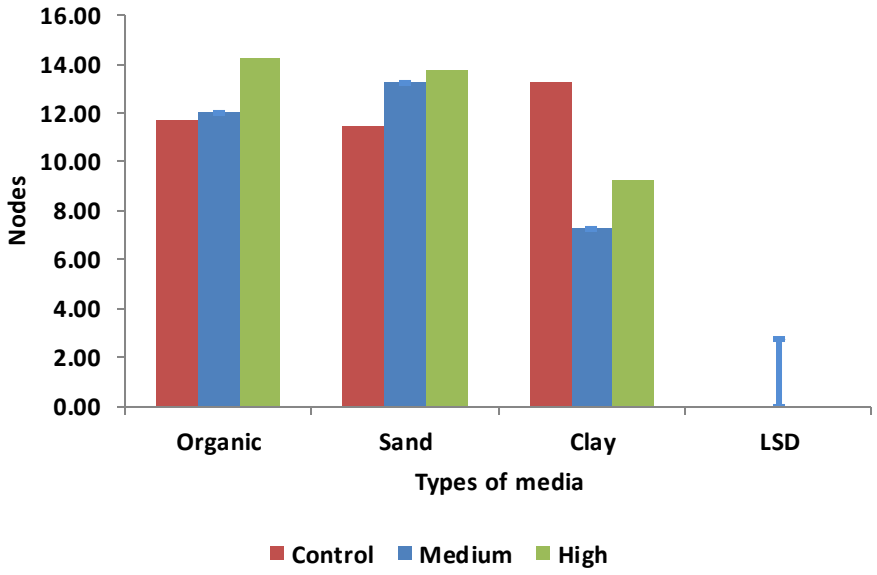


Figure 4.48 Number of nodes for *Euonymus* cv. Silver Queen potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 2.711, d.f. = 27

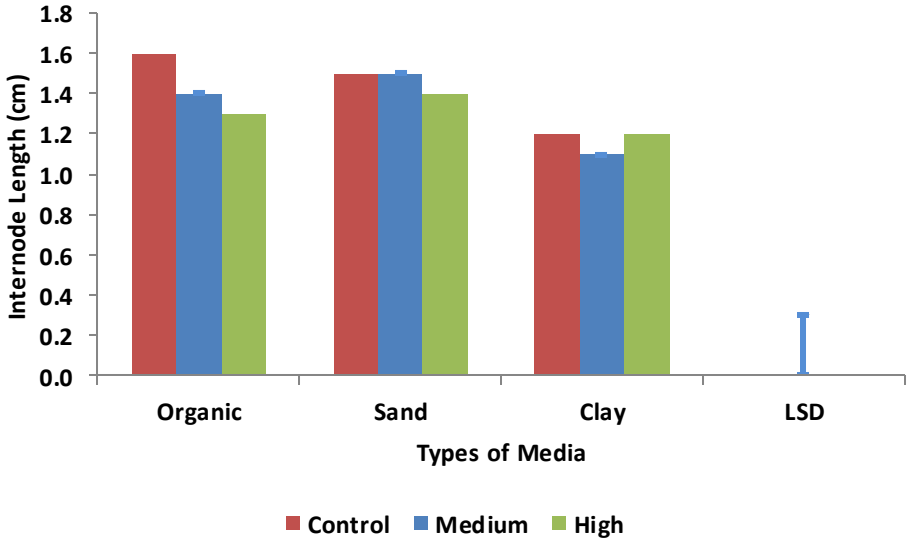


Figure 4.49 Internode length (cm) for *Euonymus cv. Silver Queen* potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 0.3, d.f. = 27

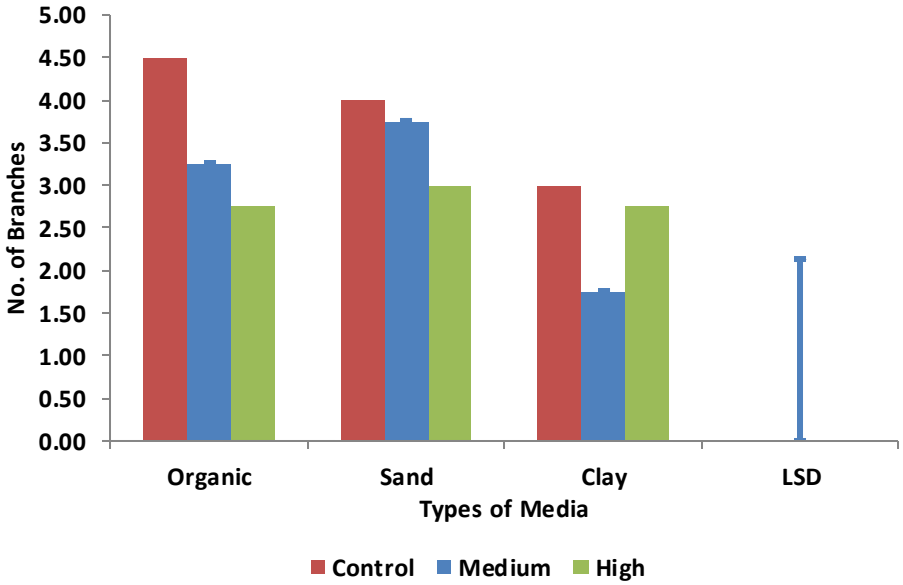


Figure 4.50 Number of branches for *Euonymus cv. Silver Queen* potted in 3 different media with 3 different bulk densities. P ≤ 0.005, LSD: 2.136, d.f. = 27

4.3 Discussion

This chapter studied the performance of young shrubs when grown in compacted soils of various media types. There were some contrasting results between the first (4.1) and the second (4.2) experiments for *Philadelphus* cv. Aureus and *Euonymus* cv. Silver Queen; even when comparisons are restricted to the organic medium alone within the second experiment. For *Philadelphus* cv. Aureus for example, treatments that aimed to increase the bulk density in Exp. 4.1 tended to enhance plant growth, whereas the opposite was true for similar treatments in Exp 4.2. In *Euonymus* cv. Silver Queen increasing compaction in Exp 4.1 reduced shoot and root biomass, but increased height by 20-30 mm per plant. The high compaction treatment in Exp 4.2 also marginally increased height in *Euonymus* cv. Silver Queen but in addition had a positive effect on shoot weight, although root biomass, as before was radically reduced. Such inconsistencies in response may have been due to other factors influenced by the compaction treatments (water and nutrient availability), or even differences in the setting up of the treatments between the two experiments. In experiment 4.1 compression was implemented by pressing the organic media into pots by hand, but the soft 'spongy' nature of the media may have resulted in inconsistent layers of compression; for example perhaps only the surface profile was compacted. The more thorough approach in Exp. 4.2 using the Proctor hammers, in contrast, seems more likely to have compressed the substrate throughout the entire profile of the media. Certainly, in Exp 4.1 the high compression treatment seems to provide *Philadelphus* cv. Aureus with some growth advantages, but this may relate to the fact that a larger volume of media was used in this treatment (as determined at the destructive harvest) and this may have supplied a greater amount of nutrients over the course of the experiment. Media volumes for both species was recovered and compared to the initial volumes placed in pots; although there was some loss of media during the experiment highest recovered amounts were still associated with the high bulk density treatment (Figures 4.8 and 4.16).

Some of variations in response to treatment between *Philadelphus* cv. Aureus and *Euonymus* cv. Silver Queen can possibly be attributed to differences in inherent growth

vigour. Indeed in Exp 4.2 both *Philadelphus* cultivars tended to showed proportionally greater reductions in height and shoot biomass with increases in bulk density compared to the less vigorous *Eunoymus*. A point re-enforced when placed in the less conducive growing media of the clay too.

Exp. 4.2 explored the effect of compaction in three different media; organic, clay and sand. Plants grown in organic and clay media showed consistent results in reduction of root and shoot biomass for *Philadelphus* cv. Aureus, *Philadelphus* cv. Belle Etoile and *Euonymus* cv. Silver Queen (marginally for shoot biomass in this latter species) as the bulk density increased. Such trends were not consistently apparent in the sand medium. Compression of the organic media resulted in reduced plant heights in *Philadelphus* cv. Aureus and *Philadelphus* cv. Belle Etoile, but not *Euonymus* cv. Silver Queen and compression of the clay media reduced height in *Philadelphus* cv. Aureus (See Figure 4.48) and *Euonymus* cv. Silver Queen but not *Philadelphus* cv. Belle Etoile. Again this implies some specific interactions between genotype and the environmental factors surrounding the different media. Overall growth and final plant height tended to be favoured in the organic medium compared to the clay.

Growing plants in sand reduced shoot growth in *Philadelphus* cv. Aureus and *Philadelphus* cv. Belle Etoile, but not the slower growing *Euonymus* cv. Silver Queen where plant heights were comparable for the organic medium (Figure 4.40). Growing plants in sand often had a relatively positive response in terms of root biomass, however, indicating that even after compression of the sand, roots could proliferate within this medium (Figure 4.50 and 4.51). This was not always mirrored by shoot extension, suggesting that plants in the sand were altering their root to shoot ratios; perhaps an indication of reduced water or nutrient availability.

Increase in bulk density is aligned with enhanced soil strength i.e. roots have to exert a greater force to pass through the soil particles, which results in turn, with greater resistance to root elongation (Clarke et al., 2003). Alterations in soil type that increase root impedance have been linked to suppressing shoot growth too, resulting in compressed growth habits (Merotto and Mundstock, 1999). Data here broadly confirms

that for the *Philadelphus* cultivars, where the use of sand and clay media reduced the mean internode length. This was also observed for these two cultivars as the bulk density increased within a given medium. In contrast, the less vigorous *Euonymus* did not readily display this response. This may indicate that soil factors that restrict root extension have a much more pronounced effect on shoot growth / habit in genotypes that tend to be naturally fast growing or more vigorous.

Increasing the level of compaction in the media, however did tend to reduce leaf size across all three genotypes, as well as reduce the number of leaves produced in many cases. Similar results have been observed in *Helianthus annuus,* L. where exposure to high bulk density in soil resulted in fewer and smaller leaves, and reduced rates of leaf expansion (Andrade et al., 1993). Other research in *Tricitum aestivum* L. suggests that growing in compacted soil leads to smaller mature cells in the leaf which subsequently affects final leaf length and width, but that leaf position on the stem can influence the degree to which these cells are reduced in size (Beemster and Masle, 1996).

Overall growth was poorest in the medium and highly compacted clay. This agrees with observations in field soils where clay is associated with a high bulk density and reduced root elongation (Chen et al., 2005). Although clay soils are known to be rich in plant nutrients, the small particle sizes and changes in their relationship to one another when compacted alters the water holding capacity and drainage characteristic of the soil. Håkansson and Lipiec (2000) points out that soil with heavy clay as a constituent have higher water contents and when compacted will resulted in higher bulk density. This induces greater resistance for plant roots to penetrate deep into the soil and thicker roots form due to more radial expansion in roots cortical cells (Clark et al., 2003). Future research with the cultivars used here warrants more detailed study on root size and length to give a clearer image on roots development in compacted soil and investigate cultivars differences in response.

Interestingly, the overall trend for treatments that optimised growth in Exp. 4.2, were not matched by stomatal conductance data in *Philadelphus* attained shortly before harvest. Indeed, control plants in the organic medium, which had shown greatest shoot

development, frequently showed poor stomatal response. The reasons for this are unclear, but may relate to water status near the termination of the experiment, as these large leafy specimens would be more prone to temporary water stress due to their larger canopy size than plants in other treatments. In contrast, plants in the sand treatments generally showed good stomatal response, possibly as a consequence of their more prolific and deeper root systems; hence a better ability to withstand any localised drying, or non-uniform distribution of irrigation water. Chlorophyll fluorescence rations (Fv/Fm) indicated little sign of stress effects in *Philadelphus* cv. Aureus, but the fact that some values dropped below 0.7 for *Philadelphus* cv. Belle Etoile in treatments such as control organic, and medium and high bulk density in clay, may indicate issues with water availability, or indeed nutrient deficiency becoming apparent at this stage of the experiment. The fact that neither stomatal conductance or chlorophyll fluorescence data was recorded consistently through the entire experiment indicates that relatively little importance should be attached to data that perhaps only represented the last day or so of the experiment (i.e. height biomass data more representative of the longer term trends of treatment effects).

Figure 4.51 *Philadelphus cv. Aureus* potted in clay media with 3 different treatments - Control (CC), Medium (CM) and High (CH) compaction

Figure 4.52 *Philadelphus* cv. Belle Etoile potted in clay media with 3 different treatments - Control (CC), Medium (CM) and High (CH) compaction

Figure 4.53 *Philadelphus* cv. Aureus potted in sand media with 3 different treatments - Control (CC), Medium (CM) and High (CH) compaction

Figure 4.54 *Philadelphus* cv. Belle etoile potted in sand media with 3 different treatments - Control (CC), Medium (CM) and High (CH) compaction

Key Points:

1. **High bulk density reduced root and shoot biomass and height.**
2. **However, different genotypes response differently to increasing bulk density.**
3. **Increased bulk density in different media have different effects on plants, where higher bulk density in sand have positive impact on root biomass in Euonymus but not both *Philadelpus* cultivars.**
4. **Compacted clay have the poorest growing rate for all cultivars as compared to other media.**

CHAPTER 5

THE INFLUENCE OF DEPTH OF WATERLOGGING ON ROOT BEHAVIOUR AND SHOOT DEVELOPMENT

5.0 Introduction

Urban soil characteristics such as poor physical structure, compaction, impaired subsoil drainage as well as alteration to natural infiltration patterns e.g. via soil sealing can account for waterlogging to occur in urban areas (Smith et al., 2001). Soils become waterlogged after heavy or prolonged rainfall events, and where the soil drainage capacity is exceeded by the volume of water inundating any given area. Low lying areas are particularly prone as surface run-off also accumulates in these depressions. Malik et al., (2001) indicate that the duration of waterlogging varies and is influenced by the amount of rain water, soil structure and evatranspiration. A waterlogged soil condition will give a negative impact on plant growth and development due to influences on the physical, chemical and biological properties of the soil (Armstrong, 1975; Ponnamperuma, 1984 as cited in (Voesenek et al., 1989).

When there are changes in soil water conditions, root growth and distribution are affected directly (McMicheal and Quisenberry, 1993). Oxygen diffusion into the soil from the atmosphere is crucial for plant growth and any changes detected by roots due to low oxygen supply affects the whole rhizosphere, with knock-on effects to the foliar parts of plants. Disturbance to gas flow into soil due to slower diffusion rates as occurs during waterlogging is deleterious to plants and extent of injury is strongly determined by time (season and growth phase), duration and other environmental factors including temperature, soil chemistry and biological activity and whether there is movement of the soil water with the capacity for oxygen rich water to flow towards plant roots (Gregory, 2006). Slower gas diffusion rate in soil will cause oxygen starvation leading to anaerobic effects on plant roots. Flooding in known to affect transpiration, water absorption, root hydraulic conductance and stomatal opening. In the event of flooding, one or two days of flooding will result in stomata closure and will continue to close for

a long time in flood intolerant species (Kozlowski, 1984). The precise mechanisms as to why flooding impairs plant water uptake is still open to debate a point made more complex in that the symptoms of water deficit are not always or consistently apparent. This may be due to subtle effects relating soil O_2 and CO_2 partial pressures as well as plant species (Blanke and Cooke, 2004, Araki, 2006). High CO_2 levels accumulated during flooding conditions by soil root respiration may be transformed to carbonic acid (H_2CO_3) which is transported to root cells and acidifies the cytoplasm. This is thought to inhibit aquaporin activity i.e. the pores by which water is moves between one cell and the next. (Tournaire-Roux et al., 2003) claim it is this transformation of CO_2 to H_2CO_3 and the resultant cytosolic acidification that inhibits root hydraulic conductivity under anaerobic conditions. Unfortunately, many studies on waterlogging document O_2 depletion, but do not monitor for CO_2 accumulation and activity.

Others argue that stomatal closure (and hence transpiration) is mediated by root derived chemical signals (Jackson et al., 2003, Araki, 2006, Else et al., 2001). However, the exact nature of the possible chemical signals is still unknown. Other studies argue that the toxic compounds regenerated in the roots during anaerobic conditions are responsible for interfering with water absorption and movement within the roots, for example ethanol, acetaldehyde (ACC), or lactic acid (Kamaluddin and Zwiazek, 2001, Tournaire-Roux et al., 2003). Irrespective of the mechanisms involved there are significant practical consequences to plant availability and productivity. For example, research done on *Triticum* indicates that there is a reduction of final grain yield due to winter flooding and the severity of the reduction depends on the time of the wheat development stages as well as the severity of the waterlogging itself (Shao et al., 2013).

Plant adaptation will begin when the desired environmental condition for growth changes for plants survival. Many studies conducted agree that plants will undergo morphological, physiological and anatomical changes to adapt with the waterlogging condition (Striker, 2012). When flooding happens, gas in soil is replaced by water which leads to oxygen deficiency (or CO_2 increase). (Parent et al., 2008) mentions that when plants encounter oxygen deficiency (hypoxia), stomatal conductance was reduced and water uptake is limited, resulting in internal water deficit and reduction of

photosynthesis rate. Another common adaptation of plants in longer flooding duration is the formation of aerenchyma roots (Takahashi et al., 2014). Aerenchyma roots are normally found at the stems located near the surface area to enable oxygen to diffuse through the internal tissues to the roots. (Perata et al., 2011) explain that aerenchyma acts as a medium to transport unsafe end products produced by roots as well as help CO_2 and ethylene emissions.

Many studies have investigated the effects of flooding on tree species, especially forestry and fruit tree crops. This research, however, investigates the effects of waterlogging in young specimens of ornamental shrubs and attempts to determine responses to flooding during different seasons (summer and winter); to help illustrate how timing and depth of flooding influences survival and adaptation.

5.1 Experiment 5a: The effect of differential flooding (depth) and duration of flooding in summer on root damage and subsequent development after draining

The first experiment was conducted during summer, i.e. plants were currently in active growth. Plant responses to soil waterlogging were investigate by exposing specimens to different depths of water over two different durations, and monitoring their ability to recover from these stress episodes.

5.1.1 Hypothesis:

Plant viability will be reduced by longer flooding durations, and by flooding to a greater depth.

5.1.2 Objective:

1. To study the effects of different flooding depth and duration on root and shoot growth.
2. To observe the recovery of plants after a series of waterlogging treatments, based on duration of flooding and depth of flooding.

5.1.3 Materials and methods:

Two common landscape shrubs species – *Philadelphus* cv. Aureus and *Euonymus* cv. *'*Silver Queen' were selected as model plants to study the effects of water logging on the root system and shoot growth. In total, 128 liners for each species were potted on into clear polypropylene bottles (5cm X 5cm X 20.5cm) filled with John Innes No. 1 growing medium. All the bottles were then covered with black polythene sheets (to exclude light from the roots) and then placed on a glasshouse bench for 8 weeks to establish. Plants were considered ready for flooding treatment, once it was evident that some roots had grown to the base of the bottle. This study took place from 18 July to 29 August 2013 with minimum glasshouse temperature of 10.6 ^{0}C and maximum temperature of 53.5 ^{0}C.

After the plants reached the desired stage, they were placed in 24 litre containers ('waterbaths') for the waterlogging treatments to take place; control plants being placed in similar containers but with drainage holes to allow free drainage to take place. Treated plants were left immersed in water within containers for either 14 days (short, S) or 28 days (long, L) where the water was monitored to maintain different level of immersion depth.

There were eight treatments:-

1. Treatment 1 – Controlled, freely drained 14 days **(ConS)**
2. Treatment 2 – Controlled, freely drained for 28 days **(ConL)**
3. Treatment 3 – Low (⅓ of container was filled with water), waterlogged for 14 days **(LowS)**
4. Treatment 4 – Low (⅓ of container was filled with water), waterlogged for 28 days **(LowL)**
5. Treatment 5 – Med, (⅔ of container was filled with water), waterlogged for 14 days **(MedS)**
6. Treatment 6 – Med, (⅔ of container was filled with water), waterlogged for 28 days **(MedL)**
7. Treatment 7 – Full (water was filled up to the top of the container) **(HighS)**
8. Treatment 8 – Full (water was filled up to the top of the container) **(HighL)**

Plants were arranged in Randomized Complete Block Design with 4 blocks and 16 replicates for each species. There were 8 containers per block which each of the water baths contained 4 plants with 2 plants per species in the same height and sizes. After 14 days, 8 replicates of each treatment and species were randomly chosen, removed from the water baths and placed on a glasshouse bench to drain, i.e. a 2 week 'recovery phase'. The remaining plants were exposed to their treatments for a further 14 days (i.e. 28 days in total) before also being removed to the bench and allowed to drain and recover; while the control plants were continued to be hand watered during the recovery period to avoid from drying.

5.1.4 Measurement

Measurements of Stomatal conductance (g_s) and Chlorophyll fluorescence were recorded on days 1, 4, 8, 11, 14, 18, 20, 25 and 28 of the experiment, with those on day 14 and day 28 corresponding to times just before the respective sub-samples of plants were removed from their waterlogging treatments. Stomatal conductance was used in this experiment as a tool to measure plant water stress while chlorophyll fluorescence was used to measure injury to the plants photosynthetic capacity.

Observations on root growth, leaf fall and senescence were recorded. After the recovery period, root systems were scored based on the degree of darkening (necrosis) of roots, with:-

1 = Dead, necrotic roots only

2 = Dark roots visible (dark 'water-soaked' appearance)

3 = Brown roots colour

4 = Yellowing roots colour

5 = Light roots colour

The number of new buds were also counted and recorded at the end of the recovery period as an indicator of plant viability after the waterlogging.

Figure 5.1: Control treatment **(Con)**

Figure 5.2: ⅓ waterlogged treatment **(Low)**

Figure 5.3: ⅔ waterlogged treatment **(Med)**

Figure 5.4: Full waterlogged treatment **(High)**

Note:

- **White line to denote water depth**

5.1.5 Results

Philadelphus cv. Aureus

Chlorophyll fluorescence (Fv/Fm)

Short Flooding

Values for chlorophyll fluorescence (Fv/Fm) were relatively uniform across all treatments on day 1 (Figure 5.5), but then decreased in **HighS** by day 4 and day 8 (not significant). By day 11 **HighS** values were significantly lower than **ConS** and **LowS**, with **MedS** being intermediate. Values continued to decrease for **HighS** and **MedS** from day 14, until the point at which the PEA chlorophyll fluorimeter could no longer record Fv/Fm value for **HighS** as leaves become increasingly necrotic until the end of experiment due to the dried and wilted leaf conditions. Although recordings for **ConS** and **LowS** remained significantly higher during the recovery phase (day 14 onwards) than the two more severely waterlogged treatments, there was a slight decline in values during day 25 and day 28.

Long Flooding

There was downward trend in Fv/Fm values over time, including with the **ConL** treatment, although rate and severity of reductions were greatest with the increasing severity of waterlogging (Figure 5.6). By day 11 values for **HighL** were significantly less than **ConL**, with reading being unattainable by day 18 due to severity of leave damage. **HighL** treatment values did not recover on draining following similar patterns to **HighS** in short term waterlogging due to the prevalence of wilted and dried leaf condition. Fv/Fm values **MedL** were significantly lower than **ConL** by day 18, and retained the mean values between 0.2 and 0.4 for the duration of the experiment and during the recovery period, increased at day 28 but continued to show decreasing pattern during the recovery period for **MedL** and fluctuate for **ConL** and **LowL** treatment.

Chlorophyll fluorescence (P. Index)

Short Flooding

P. Index readings after 24 hours of waterlogging varied between 0.3 to 0.8; such variability continued, reflecting relatively large LSD bars throughout for this parameter (Figure 5.7). As before, there was a general decline in mean values over time in all treatments, but a much more rapid decrease in those plants where much of the root mass was below the water level, i.e. **HighL** and **MedL.** .

Long Flooding

P. Index readings after 24 hours of waterlogging varied between 0.3 to 0.7 and continue to stayed at the same level in a fluctuate pattern for both **ConL** and **LowL** until Day 20. After Day 4, **MedL** and **HighL** showing a declined pattern in means values until Day 18 for both treatments, however means values for **MedL** did increased at the end of waterlogging. Recovery rate for all treatments were at poor stage where the mean values only ranging between 0.0 to 0.2.

Stomatal conductance (mmol m^{-2} s^{-1})

Short Flooding

Stomatal conductance values showed some degree of variability over the timecourse of the experiment, but overall tended to be higher with the **ConS**, intermediate with **Low S** and decrease and stay low in the **MedS** and **HighS** treatments from day 4 (Figure 5.9). Despite draining plants from day 14, there was no evidence of a return to normal stomatal behaviour in the **MedS** and **HighS** treatments.

Long Flooding

Stomatal conductance values showed the same pattern as short flooding with some degree of variability over the timecourse of the experiment, but overall tended to be higher with the **ConS**, intermediate with **LowS** and decrease and stay low in the **MedS** and **HighS** treatments from day 8 (Figure 5.10). There were not much changes of g_s over the recovery period for **ConL** and **LowL** and no evidence of a return to normal stomatal behaviour in the **MedS** and **HighS** treatments.

Root score, New Bud Growth and Leaf Fall

There were no new bud formations after the recovery period for **ConS** and **HighS**. **MedS** showed the highest number of new bud formations after the recovery period. Leaves of **HighS** plants dried out and the plants did not recover from the waterlogging treatment with 0% of survival rate while others treatments were still alive and survive the waterlogging events (Table 5.2).

Month	Glasshouse Temperature (°C)		Humidity (RH)	
	Highest	*Lowest*		
July	43.6	11.2	20.00%	100%
August	53.5	10.6	100%	15.40%

Table 5.1: Maximum and Minimum Temperature and Relative Humidity in Norton Nursery Glass House for July and August 2013 recorded by Tiny Tag Data Logger (Gemini data Loggers Ltd, Chichester, UK).

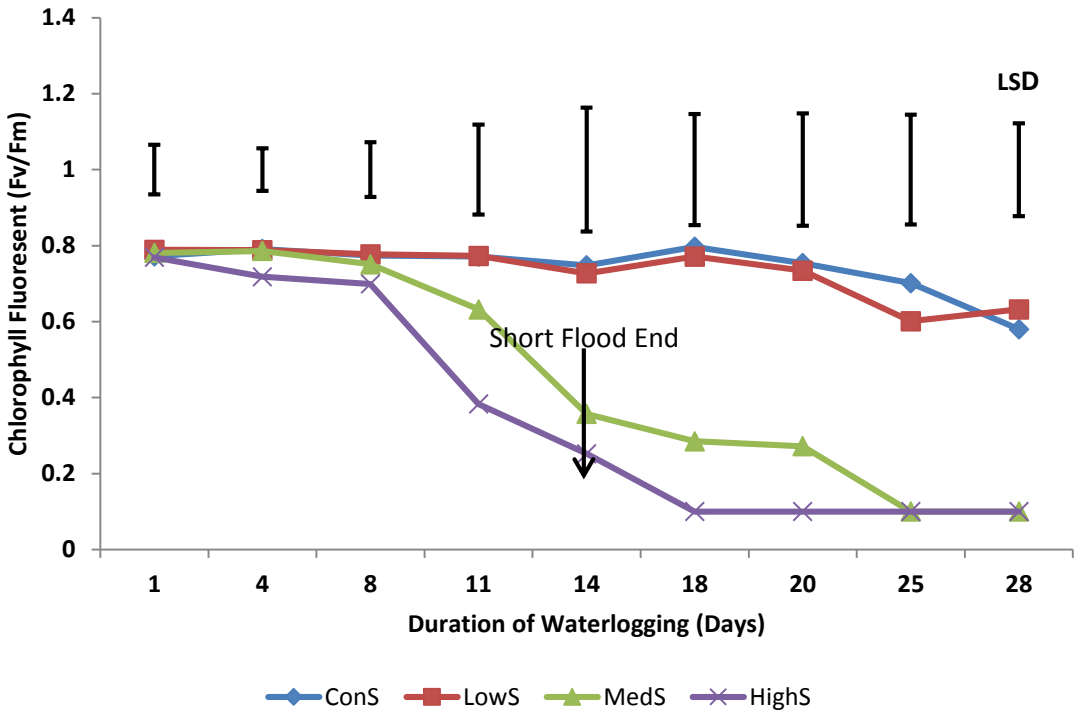


Figure 5.5: Chlorophyll Fluorescence (Fv/Fm) of *Philadelphus* cv. Aureus for 14 days of waterlogging. P ≤ 0.005, d.f.= 53

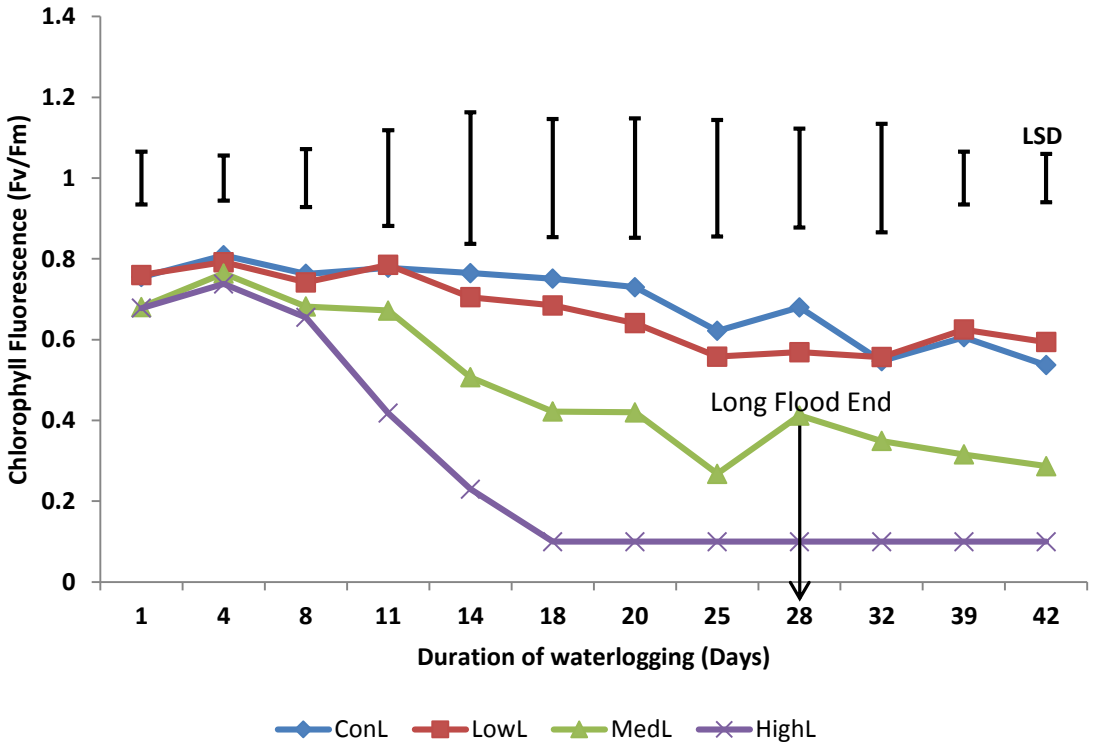


Figure 5.6: Chlorophyll Fluorescence (Fv/Fm) of *Philadelphus* cv. Aureus for 28 days of waterlogging. P ≤ 0.005, d.f.= 53.

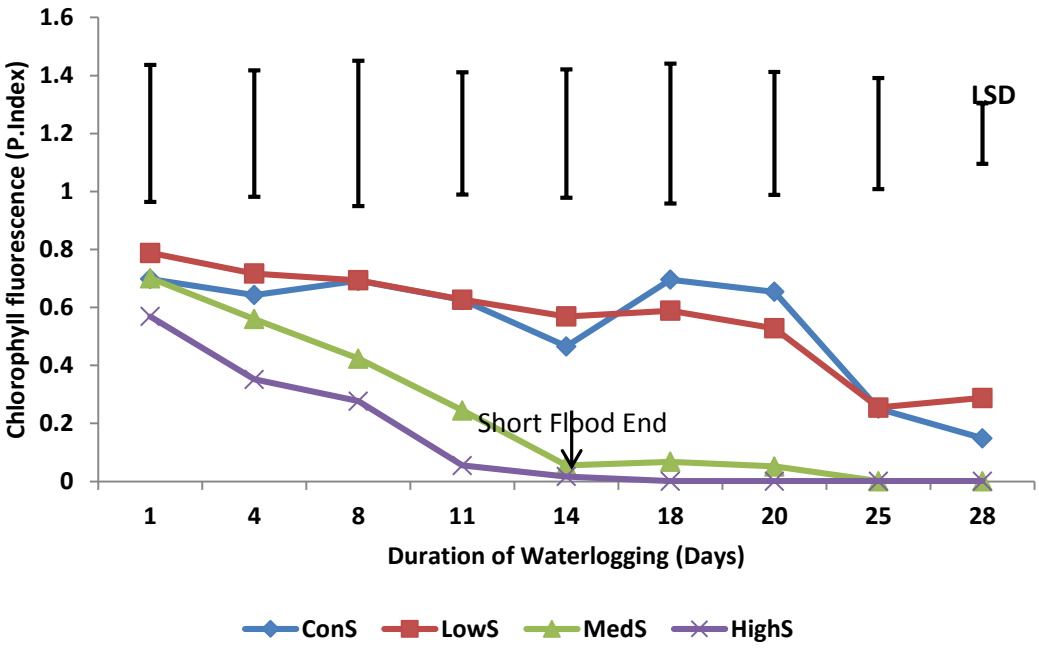


Figure 5.7: Chlorophyll Fluorescence (P.Index) of *Philadelphus* cv. Aureus for 14 days of waterlogging. P ≤ 0.005, d.f.= 53

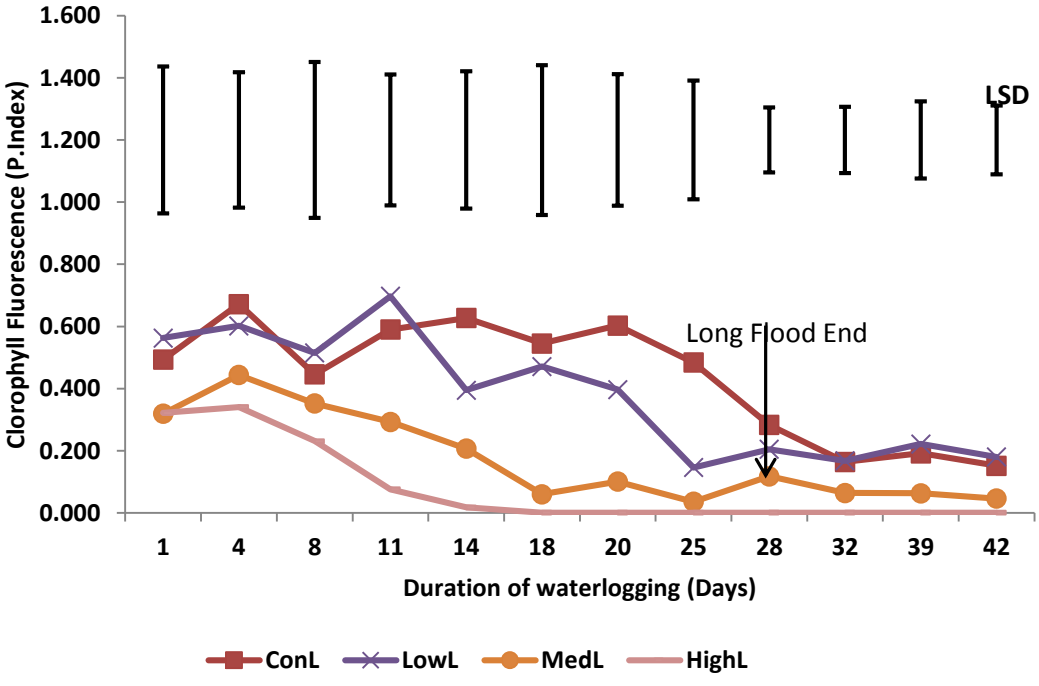


Figure 5.8: Chlorophyll Fluorescence (P.Index) of *Philadelphus* cv. Aureus for 28 days of waterlogging. P ≤ 0.005, d.f.= 53

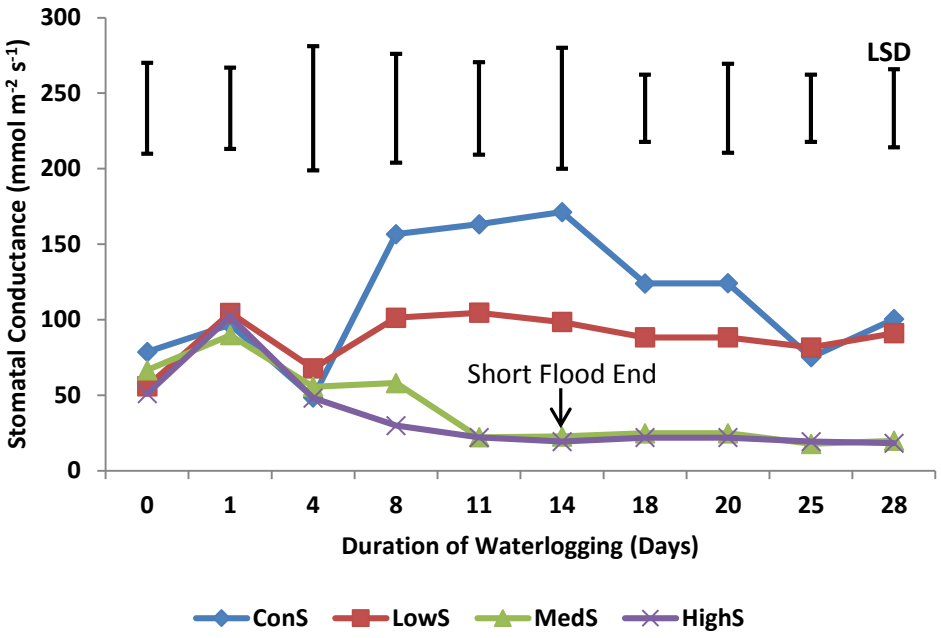


Figure 5.9: Stomatal Conductance (mmol m^{-2} s^{-1}) of *Philadelphus* cv. Aureus for 14 days of waterlogging. P ≤ 0.005, d.f.= 53

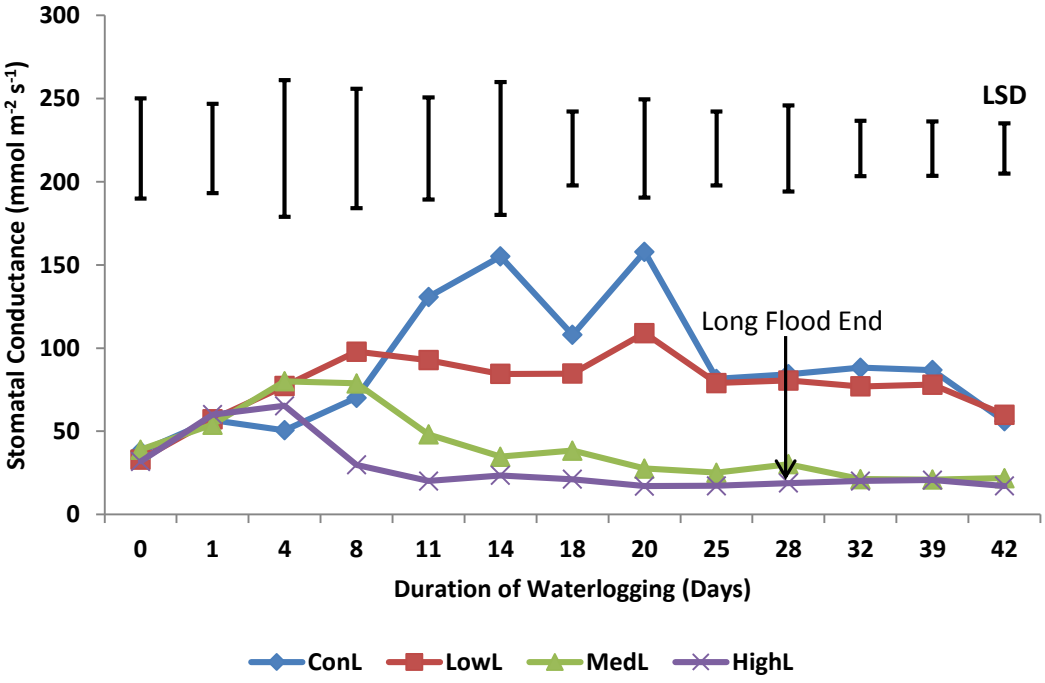


Figure 5.10: Stomatal Conductance (mmol m^{-2} s^{-1}) of *Philadelphus* cv. Aureus for 28 days of waterlogging. P ≤ 0.005, d.f.= 53

Treatment	% of survival	No. of New Bud	Root Score
ConS	100	0	8.75
ConL	100	0	8.75
LowS	100	2.2	7.50
LowL	100	4.9	7.50
MedS	100	22.5	6.38
MedL	100	21	6.50
HighS	0	0	5.50
HighL	0	0.1	5.63
LSD	*	11.6	0.53

**All 8 replicates for ConS, ConL, LowS, LowL, MedS and MedL treatment survive and still alive after the recovery. Number of new bud were counted after the recovery period ended for all treatments.*

Table 5.2: Percentage of plants survival and number of new buds for all treatments for *Philadelphus* cv. Aureus. P ≤ 0.005, d.f. = 53.

Euonymus cv. Silver Queen

The chlorophyll fluorescence and stomatal conductance (g_s) were not measured for this species because of its variegated leaves. The leaves for *E*. cv. 'Silver Queen' were yellowing before they dropped. Through observations, **HighL** dropped approximately 30% of the leaves and most of the leaves that dropped came from the mature leaves near the basal stem. The plants under **ConL** and **LowL** treatments remained the same whilst the **MedL** plants typically 10 – 15 leaves abscised per plant.

All of the plants survived waterlogging for both durations without any death recorded. At the end of the recovery period, the numbers of new buds formed were counted and it was evident that no new bud development was induced in **ConS**, **ConL** or **HighL**. The **MedL** plants produced more new buds during the recovery period ranging from 2 to 7 buds for each plant. However, for **LowL** only 2 to 3 new buds were observed but not always in every plant (Table 5.3).

Roots scores indicated greatest numbers of visible roots associated with Controls, with significant reductions in waterlogged plants, especially with the Med and High waterlogging treatments (Table 5.3).

Treatment	% of survival	No. of New Bud	Root Score
ConS	100	0	4
ConL	100	0	4.13
LowS	100	0.62	3.13
LowL	100	0.5	3.13
MedS	100	4.12	2
MedL	100	4.38	2
HighS	100	0	2
HighL	100	0	2
LSD		0.95	0.35

**All 8 replicates for all treatments survive and still alive after the recovery. Number of new bud was counted after the recovery period ended for all treatments.*

Table 5.3: Percentage of plants survival and number of new buds for all treatments for *Euonumys* cv. Silver Queen. P ≤ 0.005, d.f. = 53.

5.2 Experiment 5b: The Effects of prolonged flooding and differential of flooding for Philadelphus cv. Aureus *in winter and the development after draining during spring*

The second experiment was conducted during winter, i.e. plants were currently in passive growth. Plant responses to soil waterlogging were investigate by exposing specimens to different depths of water over four different durations, and monitoring their ability to recover from these stress episodes.

5.2.1 Hypothesis:

1. Plants will experience severe damage in winter
2. More new roots will develop during the recovery period in spring in the plants under low and medium treatments.

5.2.2 Objective

1. To investigate the effects of waterlogging on young shrubs in a dormant stage
2. To investigate young shrubs' recovery from winter waterlogging during an active period

5.2.3 Materials and Methods

Young plants of *Philadephus* cv. Aureus (36 in total) were obtained from a supplier in small pots (9cm diameter X 8.7 depth) were used as model plants to investigate the effects of winter waterlogging on young shrubs. Plants were divided equally into 4 groups of approximately same sizes and heights before the experiment was conducted. Plants were located in three positional blocks within a glasshouse, and divided into three treatments with 12 plants per treatment based on degree of waterlogging (Control = no waterlogging), (Medium = waterlogged until half of the pot) and (High = full waterlogged).

Waterlogged conditions were obtained by placing the plants in 24litre container with each container consist of 3 plants. For both Medium and High waterlogging condition,

each of the containers were filled with water according to the desired treatment. On the other hand, Control plants were placed in the same container with drainage holes at the bottom to allow water to drain out since plants were hand watered manually to avoid from dried out.

The remaining 24 plants were waterlogged for four durations which are 7, 14, 21 and 28 days and drained for two weeks after being waterlogged (based on the depths provided) on the glasshouse bench for recovery period. At the end of the experiment, plants were divided into separate root and shoot sections, before being dried and weighed.

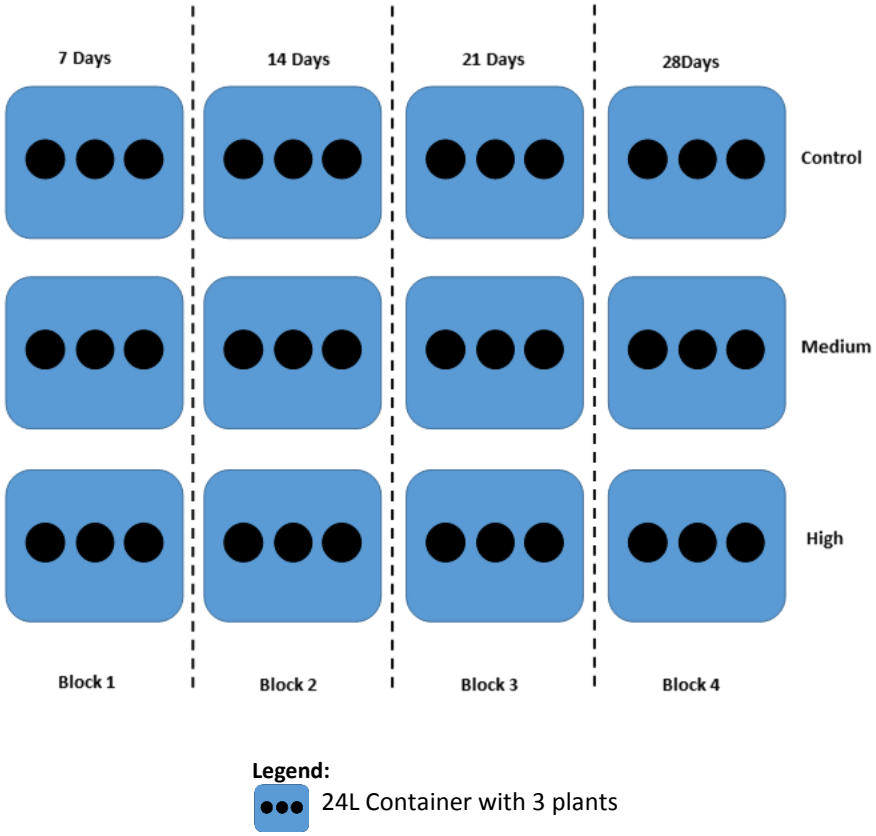


Figure 5.11 Layout of experiment 5b.

5.2.4 Measurements

Initial plant height was measured before treatment, at the end of waterlogging and after 14 days of recovery. There were few leaves (typically 5-10 leaves) present on plants at the onset of the experiments. The effect of waterlogging, however, on spring growth was recorded by counting the number of new emergent leaves present at the end of the recovery period as well as dry weight of new developing shoots without the original woody stem. Roots were carefully harvested at the end of the experiment and assessed for dry weight.

5.2.5 Results

Due to the existing lignified wood stem of all plants and circling roots, shoot and root biomass data was not reliable enough to show the differences between treatments based on the dry weight data (Figure 5.12 and 5.13). However, the treatments effects were notable in number of new shoots and bud break; and plant height recorded in the spring following treatments. Control treatment illustrates the increasing no. of new shoots and buds throughout the experiment. Assessment of new bud and shoot numbers showed a minimal reduction for medium and high treatment compared to controls after 7 days of waterlogging (Figure 5.14). Result indicates that there was a declining no. of new shoots and buds during the experiment but manage to recover back after two weeks of drain although not always exceeded the initial record.

High waterlogging depth indicates reduced number of new shoots growing during the waterlogging for 14, 21 and 28 days. By the end of recovery period, however, i.e. more shoots were produced than had been originally recorded initial data before being waterlogged for 21 and 28 days of waterlogging (Figure 5.15, 5.16 and 5.17).

All of the plants recorded to have increasing in height for all treatments after the recovery ended. This may be due to the new shoot growth at the top part of the stem resulting the increment in height (Table 5.4).

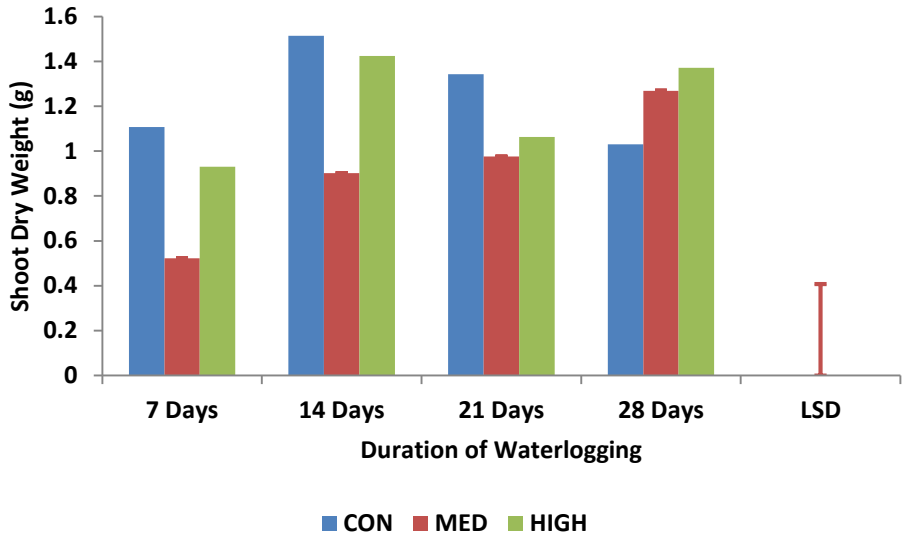


Figure 5.12 Shoot dry weight for *Philadelphus* cv. Aureus in three different depths and four duration of waterlogging. P ≤ 0.005, LSD: 0.4073, d.f: 22

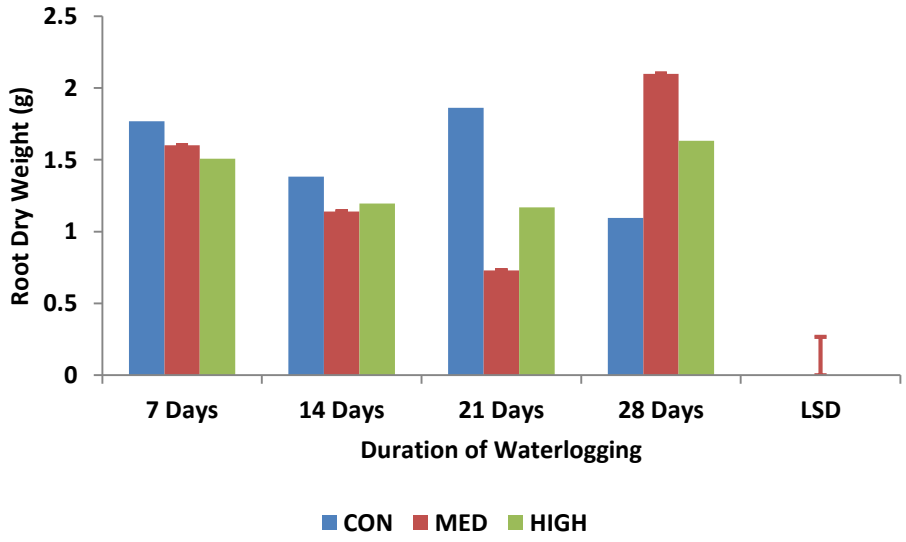


Figure 5.13: Roots dry weight for *Philadelphus* cv. Aureus in three different depths and four duration of waterlogging. P ≤ 0.005, LSD: 0.2665, d.f: 22

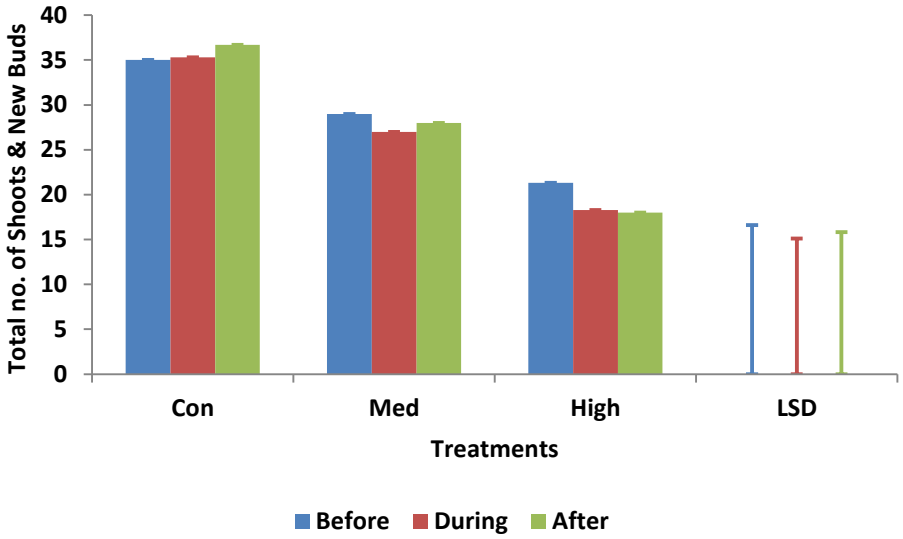


Figure 5.14 Total numbers of shoot and new buds for *Philadelphus* cv. Aureus in 7 days of waterlogging duration. P ≤ 0.005, LSD: (Con: 16.6, Med 15.11, High: 15.81), d.f: 22

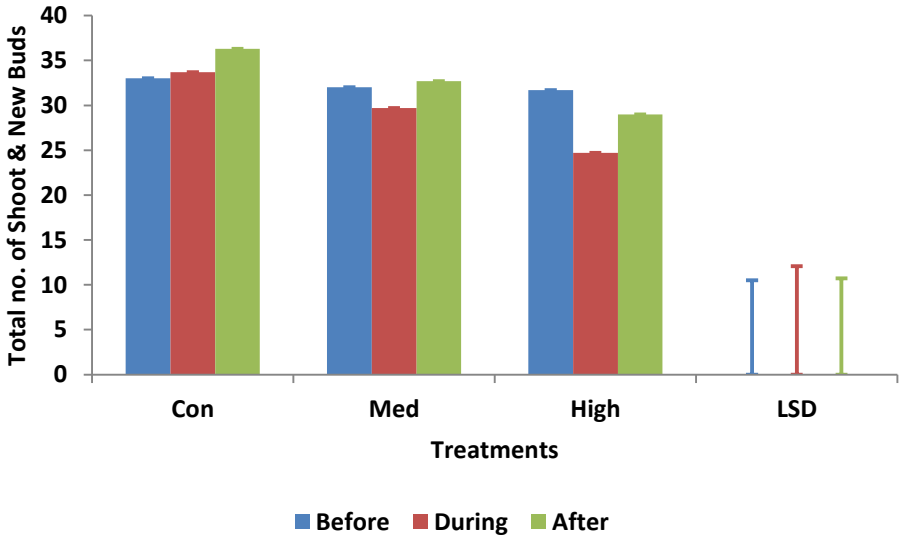


Figure 5.15 Total numbers of shoot and new buds for *Philadelphus* cv. Aureus in 14 days of waterlogging duration. P ≤ 0.005, LSD: (Con: 10.5, Med 12.07, High: 10.71), d.f: 22

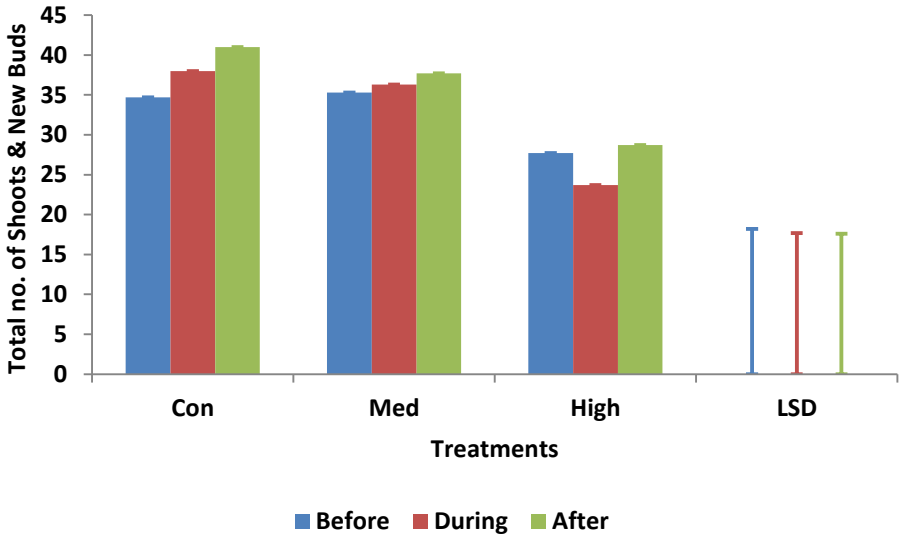


Figure 5.16 Total numbers of new buds for *Philadelphus* cv. Aureus in 21 days of waterlogging duration. P ≤ 0.005, LSD: (Con: 18.2, Med 17.66, High: 17.6), d.f: 22

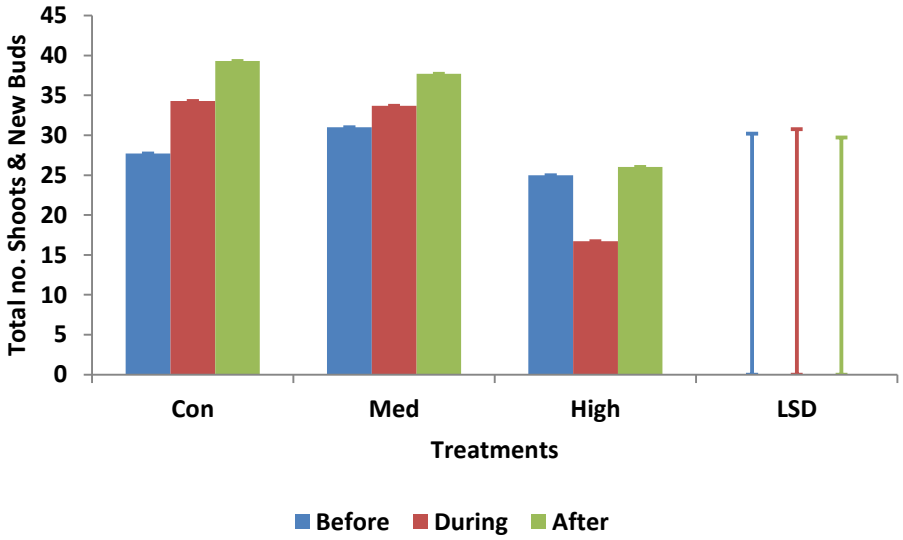


Figure 5.17 Total numbers of shoot and new buds for *Philadelphus* cv. Aureus in 28 days of waterlogging duration. P ≤ 0.005, LSD: (Con: 30.18, Med 30.76, High: 29.7), d.f: 22

Figure 5.18: *Philadelphus* cv. Aureus after 7 days of waterlogging in three different treatments (From left to right: Control, Medium and High).

	Control		Medium		High	
Duration	***Before***	***After***	***Before***	***After***	***Before***	***After***
7 Days	*12.77*	*12.83*	*11.93*	*11.97*	*11.7*	*11.8*
14 Days	*13.57*	*13.6*	*11.13*	*11.23*	*11.77*	*11.87*
21 Days	*14.5*	*15.07*	*12.17*	*12.5*	*9.97*	*10.07*
28 Days	*8.4*	*9.13*	*10.3*	*10.7*	*11.07*	*11.33*

Table 5.4: Plant height for *Philadelphus* cv. Aureus before and after waterlogging for 7, 14, 21 and 28 Days. P≤0.005, d.f: 22, LSD: 2.3

5.3 Experiment 5c: The effect of differential flooding (depth) and flooding duration on roots in winter and subsequent development after draining in spring for Euonymus cv. Silver Queen

Similar approaches were adopted to that Experiment 5a, with the exception the aim was to investigate the impact of waterlogging during winter months, when plant were not in active growth, and to determine how these treatments affected development in the following spring.

5.3.1 Hypothesis:

1. Plants will experience severe damage in winter
2. More new roots will develop during the recovery period in spring in the plants under low and medium treatments.

5.3.2 Objectives:

1. To investigate the effects of waterlogging on young shrubs in a dormant stage
2. To investigate young shrubs' recovery from winter waterlogging during an active period

5.3.3 Materials and methods:

This experiment was conducted under late winter (February to March 2014) to evaluate flooding responses before bud burst in this evergreen species. Thirty-six rooted cuttings of *Euonymus* 'Silver Queen' were used and placed in polypropylene bottles, with black polythene sleeves as before. Three treatments were imposed on the plants with 4 replicates on each treatment due to the limited numbers of experimental plants.

After plants were established, they were placed in 24 litre containers (water baths) as before for the treatment to take place. Plants being exposed to 14 days (short, **S**) and 28 days (long, **L**) waterlogging treatments. The water levels were monitored to ensure that the level was at the correct depth. With non-waterlogged control plants, holes were made at the bottom of the container for drainage and, plants were watered accordingly to avoid them from drying.

There are six treatments in this experiment:

Short term waterlogging (14 days)

1. Control Short: Freely drained (**CS**)
2. Low Short: ⅓ of water was filled in the container (**LS**)
3. High Short: Full waterlogged (**HS**)

Long term water logging (28 days)

4. Control Long: Freely drained (**CL**)
5. Low Long: ⅓ of water was filled in the container (**LL**)
6. High Long; Full waterlogged (**HL**)

After 14 days, 18 plants (2 plants X 3 treatment X 3 blocks) were randomly chosen to be placed in the glasshouse bench for drainage and undergo a recovery phase for 2 weeks. The remaining plants continued to be waterlogged for another 14 days. They were drained after this period ended and then went through another 2 weeks of recovery stage.

5.3.4 Measurements

Since *Euonymus* cv. 'Silver Queen' had variegated leaves, g_s and chlorophyll fluorescence were not measured in this experiment. Throughout the experiment, plants were assessed for root growth (number of visible roots on bottle surface) leaf abscission and necrosis, the number of shoot and buds; and plant height. Initial data of no. of shoots and roots were taken to determine any changes in new shoot and root growth, after both on termination of waterlogging and after the recovery period. Plants were finally assessed for dry biomass.

5.3.5 Results

Short term waterlogging (14 Days)

Plant development and response to short term waterlogging in winter for *Euonymus* cv. Silver Queen are depicted in figure 5.19 to 5.23. Shoot and root biomass shows the same pattern of reduction in biomass towards higher level of water logging (Figure 5.19 and 5.20). Number of shoots in **CS** and **MS** treatments were increased throughout the experiment but declined in **HS** treatment at the end of the experiment but bounced back during the recovery period (Figure 5.21). Number of visible roots on the bottle surface in **MS** and **HS** were the same before and during the waterlogging but increased after the recovery period ended (Figure 5.22). Interestingly, heights data showed increments in size with time in all treatments, with greatest increases associated with the **HS** (Figure 5.23). Comparison data between shoot and root number describes that waterlogging can cause shoot death and root development was slower in winter season. No necrosis and leaf fall were observed during the experiment.

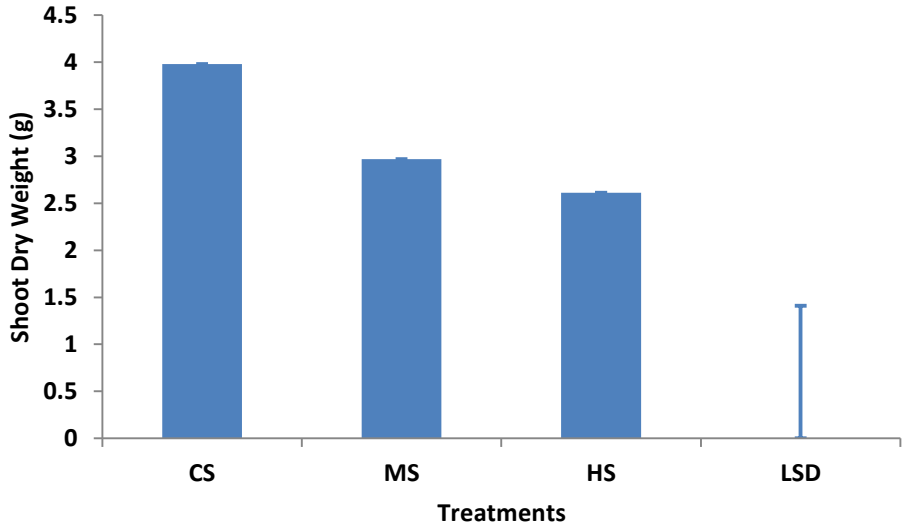


Figure 5.19: Shoot Dry Weight for *Euonymus* cv. Silver Queen after 3 weeks recovery from short waterlogging in 3 different depths. P ≤ 0.005, d.f: 22. LSD: 1.411

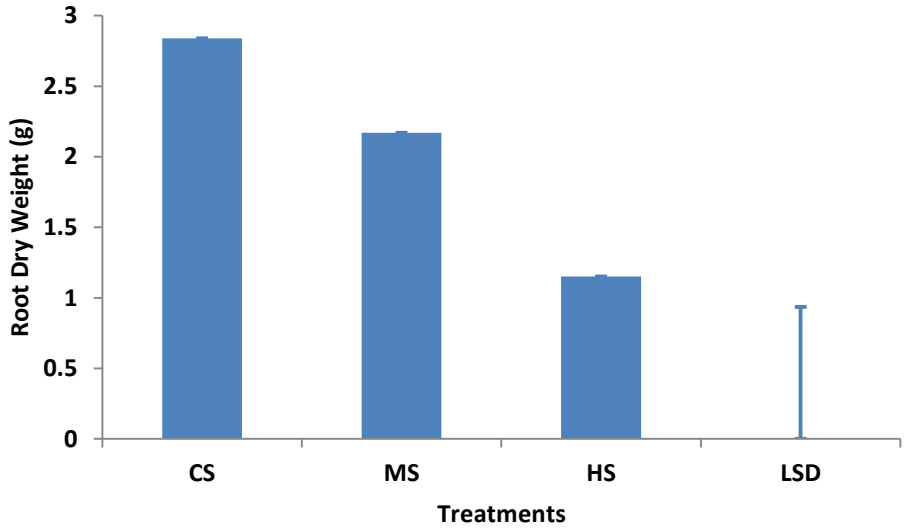


Figure 5.20 Root Dry Weight for *Euonymus* cv. Silver Queen after 3 weeks recovery from short waterlogging in 3 different depths. P ≤ 0.005, d.f: 22. LSD: 0.935.

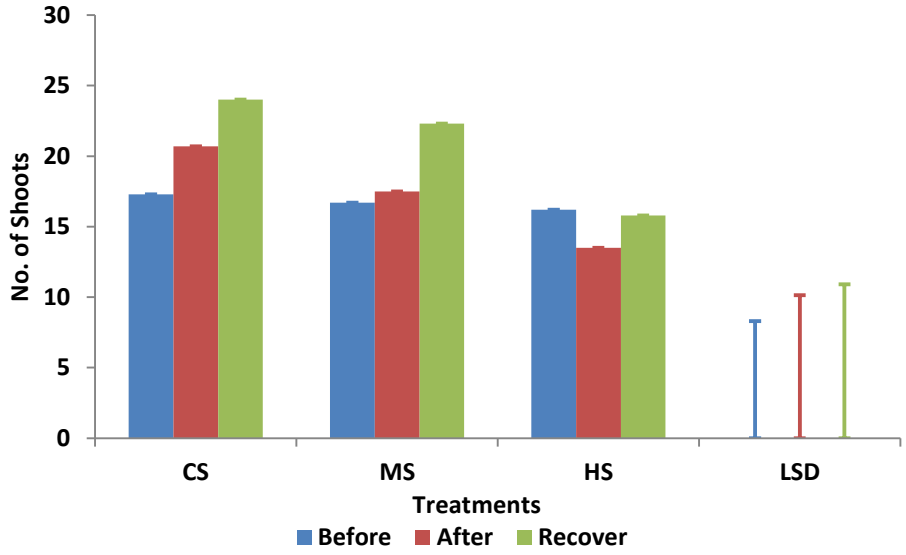


Figure 5.21 Number of shoots for *Euonymus* cv. Silver Queen before, after and after recovery during 14 days of waterlogging in 3 different depths. P≤0.005, d.f: 15. LSD: 8.31 (before), 10.14 (after) and 10.92 (recovery).

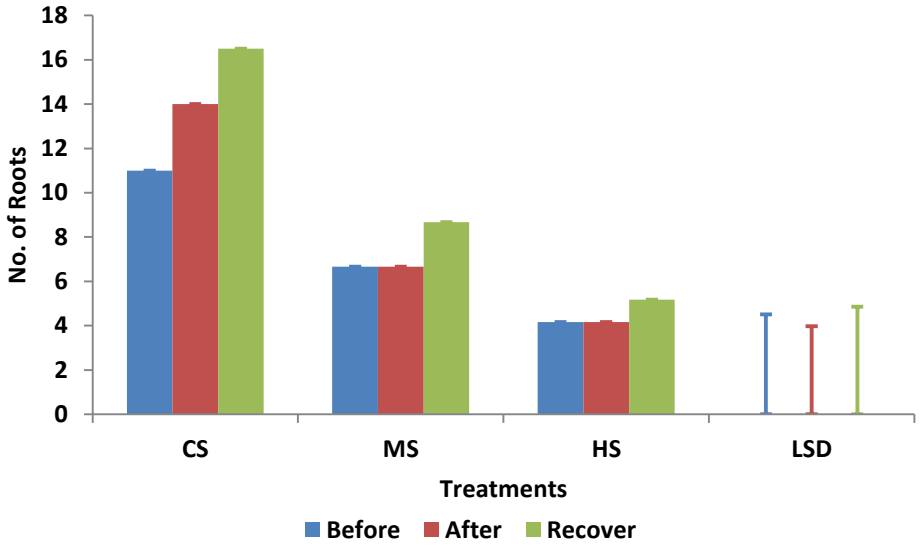


Figure 5.22 Number of visible roots for *Euonymus* cv. Silver Queen before, after and after recovery during 14 days of waterlogging in 3 different depths. P ≤ 0.005, d.f: 15. LSD: 4.518 (before), 3.971 (after) and 4.857 (recovery).

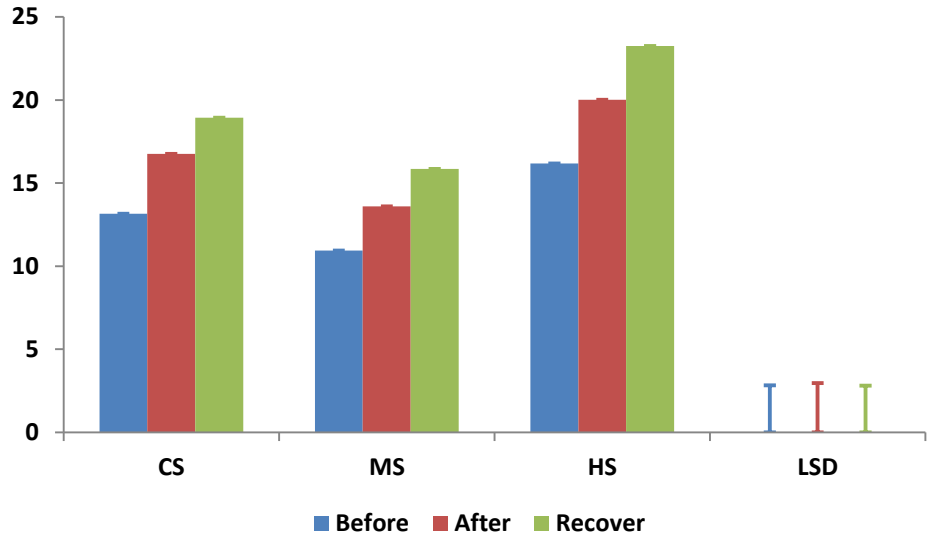


Figure 5.23 Plant heights for *Euonymus* cv. Silver Queen before, after and after recovery during 14 days of waterlogging in 3 different depths. P≤0.005, d.f: 15. LSD: 2.837 (before), 2.968 (after) and 2.811 (recovery).

Long term waterlogging (28 days)

Shoot and root biomass for *Euonymus* cv. Silver Queen in long term waterlogging illustrates the same pattern as short term waterlogging (Figure 5.24 and 5.25). However, the long term waterlogging effect on shoots were severe in **ML** and **HL** where shoot number decreased (due to die-back and necrosis) after the waterlogging ended but **ML** manage to produce more new shoot during the recovery period than **HL** (Figure 5.26). Number of roots also shows the same trends as number of shoots where there was no effects on control plants, but a slight reduction of roots count in **ML** (Figure 5.27). Plant height shows no effects on waterlogging (Figure 5.28) and no leaf fall or yellowing were observed in all treatments.

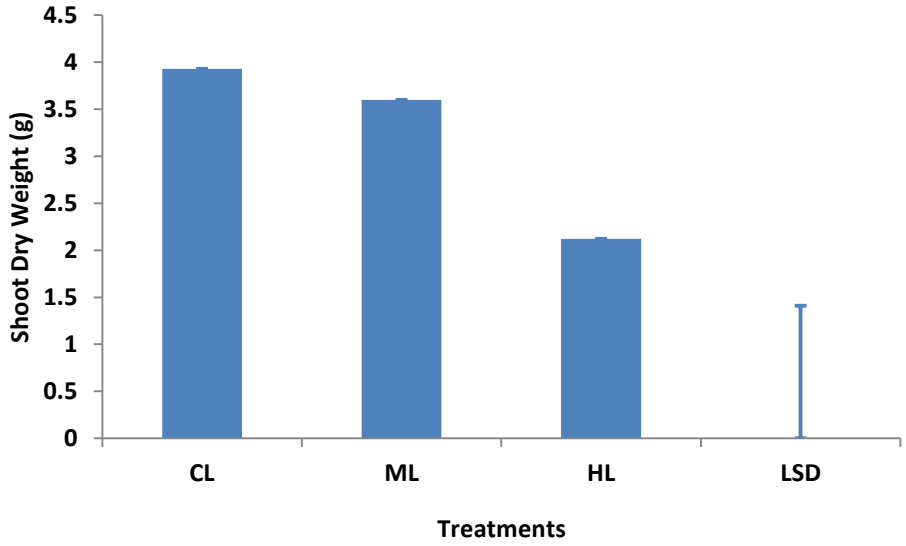


Figure 5.24 Shoot Dry Weight for *Euonymus* cv. Silver Queen after 3 weeks recovery from long waterlogging in 3 different depths. P ≤ 0.005, d.f: 22. LSD: 1.411

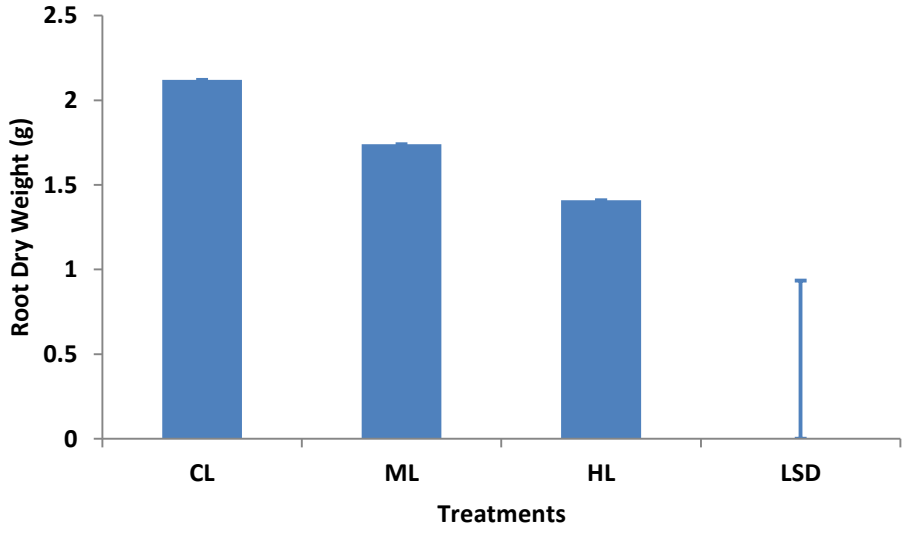


Figure 5.25 Root dry weight for *Euonumys cv. Silver Queen* after 3 weeks recovery from long waterlogging in 3 different depths. P ≤ 0.005, d.f: 22. LSD: 0.935

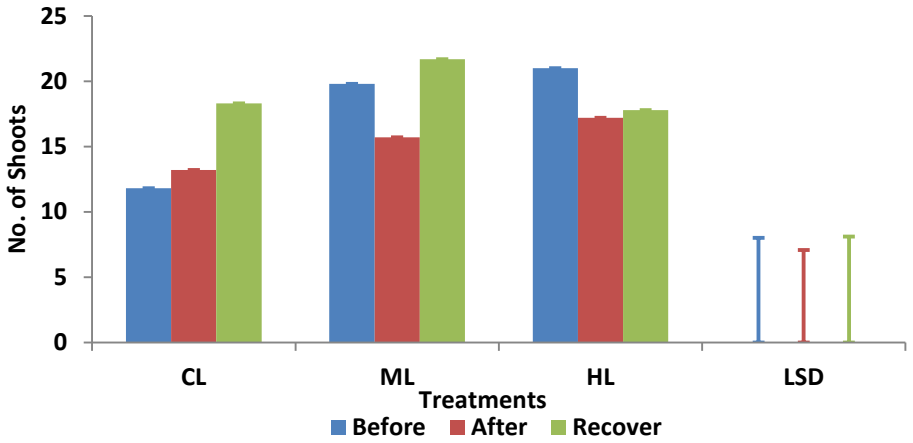


Figure 5.26 Number of shoots for *Euonumys* cv. Silver Queen before, after and after recovery during 28 days of waterlogging in 3 different depths. P ≤ 0.005, d.f: 15. LSD: 8 (before), 7.08 (after) and 8.11 (recovery)

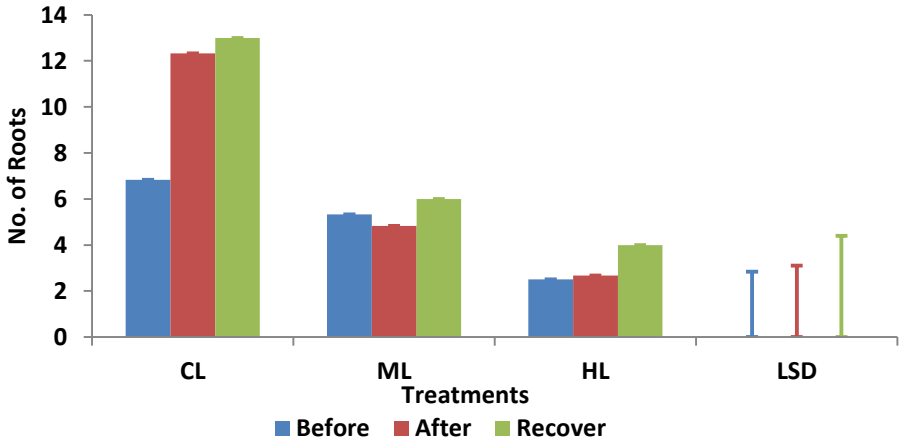


Figure 5.27 Number of roots for *Euonymus* cv. Silver Queen before, after and after recovery during 28 days of waterlogging in 3 different depths. P≤0.005, d.f:15. LSD: 2.836 (before), 3.105 (after), 4.403 (recovery)

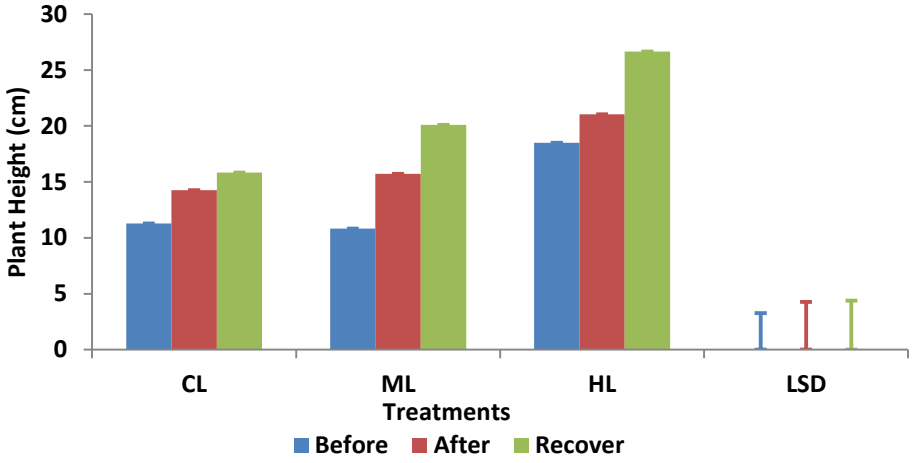


Figure 5.28 Plant height for *Euonymus cv.* Silver Queen before, after and after recovery during 28 days of waterlogging in 3 different depths. P≤0.005, d.f: 15. LSD: 3.286 (before), 4.264 (after) and 4.385 (recovery).

5.4 Discussion

This chapter examined the effect of different depths and durations of waterlogging in *Philadelphus* cv. Aureus and *Euonymus* cv. Silver Queen with separate experiments carried out in two different seasons, winter and summer. The present study clearly demonstrated that the longer duration and full waterlogging level in *Philadelphus* severely harmed the plants for both seasons but varied in recovering rate for post waterlogging. *Philadelphus* did not recover from full waterlogging condition during summer after exposure to either the short (14 days) or long (28 days) durations with clear evidence of significant leaf damage (wilted and desiccated leaves, Figure 5.29) and no new bud formation after 2 weeks recovery period. However, in winter waterlogging, both *Philadelphus* and *Euonymus* survived the waterlogging event with quite numbers of new bud emergences (See figures 5.14, 5.15, 5.16, 5.21 and 5.26).

Figure 5.29 Dry and wilted leaf condition in *Philadelphus* cv. Aureus at the end of waterlogging.

The desiccated and wilting leaf effect associated with waterlogging has been observed in cotton plant where this condition also known as sudden wilt, new wilt and parawilt, and according to (Hebbar and D Mayee, 2011) soil waterlogging / soil saturation, rapid growth rate during an active development phase and; high temperature and full exposure to sunlight were the three factors that has been suggested as causing the leaf wilting condition in cotton. This happened due to high water loss during transpiration under full sun and high temperature; and limited water uptake and transport from roots. In agreement, rapid wilting of upper leaves reported to cause sudden death in Japanese Soybean (*Glycine max* Merr.), and this condition been linked to the increasing temperature during waterlogging which resultant to induce higher transpiration in leaf (Jitsuyama, 2013).

Similar responses have been reported in *Citrus reshni* Hort. Ex., Tan (Cleopatra mandarin), *Poncirus trifoliata* L. Raf. *X Citrus paradisi* L. Macf. (Citrumelo CPB 4475) and *Poncirus trifoliata* L. Raf. *X Citrus sinensis* L. Osb. (Carizzo citragne) where midrib vein yellowing, curling and wilting were visible after 20 days of flooding (Arbona and Gómez-Cadenas, 2008).

Changes in stomatal conductance, g_s and chlorophyll fluorescence, Fv/Fm were evident in *Philadelphus* under waterlogged conditions. Fv/Fm and g_s values progressively decreased as waterlogging was prolonged during both the short term and long term waterlogging durations. Reduction in g_s values in waterlogging is generally associated with the depletion of oxygen in soil (or increases in CO_2) and is an attempt to avoid excessive water loss through transpiration. High temperature during summer induce higher photosynthesis rate and as stated by (Aroca et al., 2011, Aroca et al., 2012) imbalance between root water uptake and leaf transpiration results in tissue dehydration which in turn induces stomatal closure. Reduction in g_s has also been linked to hormonal root signalling in plants as a communication tools in response to environmental changes. Studies in Citrus seedling, *Carizzo citrange,* however, concludes that ABA accumulation in leaf transported from roots was not the main reason of stomatal closure in flooded plants since stomatal closure was detected earlier (Day 7) than increasing ABA in leaves (Day 21) (Rodríguez-Gamir et al., 2011).

In contrast, stomatal closure has been associated with decreased root hydraulic conductivity directly, and rapid reductions in root hydraulic conductance during flooding are thought to affect leaf water potential and xylem hydrostatic potential, with a resultant closure of stomata (Else et al., 2001). (Comstock, 2002) explains that soil flooding and drying that require stomatal closure will cause reduction in hydraulic conductance to maintain the stability of leaf water potential. Research on castor oil plants (*Ricinus communis*) explains that 2-6 hours of flooding caused stomatal closure and reduced leaf expansion triggered by increase of CO_2 and /or O_2 depletion. This responses is in regard to the reduction in hydraulic conductance that limits water uptake, and restricted water loss via transpiration (Else et al., 2001).

Chlorophyll fluorescence values, Fv/Fm also were measured to detect the changes of photosynthetic efficiency in plants. Fv/Fv decreased in the longer duration waterlogging event in this study. Research on *Jatropha curcas* L. also demonstrated a decreasing pattern of Fv/Fm during waterlogging which suggests some loss of photosynthetic efficiency of PS II due to the stress factors imposed (Verma et al., 2014). Reduction in net photosynthesis is caused by a decrease in CO_2 fixation which is very much related to reductions in stomatal aperture (Pociecha et al., 2008); as well as changes in photo inhibition (Ahmed et al., 2002), and this eventually has a negative effect on PS II.

Plant can recover from waterlogging depending on time and age of plants when the flooding events happen. At early developmental stages, the event of flooding will affect growth and yield more severely than at later development stages according to (Watson et al., 1976, Kozlowski, 1984).Waterlogging during summer is more detrimental to plants due to losses in leaf hydration caused by high temperature and exposure to sunlight (Jitsuyama, 2013). This is exacerbated by the loss of oxygen / increase in carbon dioxide in the rhizosphere being accelerated at higher temperatures. Differences in new bud and new root development between two seasons were observed, with new shoot and root development being more rapid after recover from the winter waterlogging. This might be due to the changing in season from winter to spring which marks the starting point of natural active growth period. When plants experienced waterlogging during

summer, the damage was severe and plants took a longer time to recover from the non-lethal waterlogging events. (Kozlowski, 1984) stated that waterlogging during active periods of plant growth is much more detrimental compared to waterlogging during passive or quiescent phases.

Among the two species tested, *Philadelphus* and *Euonymus* it is obvious that *Euonymus* is more resistant to waterlogging in both seasons. This may be due to the this genotype possessing a slower growing characteristics, perhaps with less demand for oxygen due to lower respiration rates, or slower rates of cell division. It may also have physiological adaptations not detected in this study – e.g. aerenchyma formation. Also the thicker evergreen leaves may provide some degree of resilience to the stresses imposed. (Chapin, 1980), concluded that evergreen leaves have prolonged leaf longevity and low relative growth which can help these species survive poor nutrient sites and other environmental stresses (Mooney and Rundel, 1979).

Key Points:-

1. **Greater depth and longer duration of waterlogging is more harmful to plants**
2. **Summer waterlogging is more detrimental to *Philadelphus* due to wilted and dry leaves condition but no significant effect on *Euonymus*.**
3. **Both species survive winter waterlogging due and have higher survival rate than summer waterlogging.**

CHAPTER 6

ROOT INJURY AND EFFECTS ON COMPENSATORY ROOT GROWTH AND SUBSEQUENT SHOOT DEVELOPMENT

6.0 Introduction

Plants in urban areas are exposed to a range of environmental threats such as anaerobic soils, compacted soils with high bulk density, poor soil structure and nutrient availability and severance of roots through cabling and trenching activities. Many of these key stresses impact on roots directly.

Root damage by trenching is nearly always associated with a reduction in tree stability, lower resistance to uprooting (i.e. during strong wind), increased tree mortality and reduction in plant growth. A previous inventory conducted on street trees in Milwaukee, Wisconsin, USA suggested that tree damage caused by the construction industry exacerbated decline for example, construction activities accounted for 22.7 percent of tree deaths between five to eight years after an incident, compared to 18.6 percent decline in the control group (Hauer et al., 1994). In addition, root damage also inhibited subsequent root growth. This may have been due to the damaged roots having an adverse effect on water and nutrient uptake (and perhaps root-derived phytohormones?), which had a consequential adverse effect on the trees photosynthetic capacity (Hamilton, 1988). It is also feasible, however, that large old main lateral roots, once severed, have only limited potential to generate new primary roots.

The severity of the root damage is dependent on the distance of trenching from the tree trunk; with severity of damage tending to increase as the trench location becomes closer to the tree trunk (Miller and Neely, 1993). Large amount of root loss will have a more adverse effect on survival and subsequent plant re-growth compared to minimum amounts of root loss. Watson (1998) reported that greater extents of root injury resulted in longer recovery periods.

In the field of top-fruit and other orchard based crops, tree management tends to be more favourable as growers can choose the species be cultivated and have greater opportunities to modify/manage the soil Indeed, special rootstocks have been cultivated to help plants adapt to soil conditions and to regulate growth and cropping (Wajja-Musukwe et al., 2008). Root damage has been purposely done by pruning the tree roots to optimise yield and control excessively vigorous shoot growth. Research on *Malus domestics* Borkh. indicated that root pruning reduced the preharvest fruits from abscising early; and produced firmer fruits with an increase in soluble solids concentration (Ferree, 1992); while root pruning in *Pyrus* (pear) proves to be effective in controlling shoot growth and improving fruit yield and quality when combined with appropriate irrigation (Wang et al., 2014).

In horticulture, root pruning is a cultural practise used in nursery to produce compact trees and increase plant survival and establishment. Research done in Douglas-fir seedlings indicates that root regeneration is different depending on the severity of the pruning, rooting condition and area of the pruning (Eis, 1968). Greater re-generation of new roots was found in seedlings which initially had poor root systems but not in those seedlings which already possessed a good root system. In addition, severe pruning in Douglas-fir (pruning of both sides of the root) generated better root systems rather than just light pruning on one side only. Research in southern *Magnolia grandiflora* L. (Magnolia) points out that there were reductions in leaf number, tree height and trunk calliper in root pruned plant, irrespective of the seasons that root pruning took place as compared to controls (Gilman and Kane, 1990).

In contrast to tree work, relatively little attention has been paid to root pruning in shrubs, at least in a landscape context. [There has been some research and anecdotal observations on how root damage e.g. during potting-on may affect growth of container grown shrubs during commercial production]. This chapter, however, investigates the effect of root damage in young shrubs where root pruning treatments were imposed to imitate the root damage in urban plants artificially. Two experiments were conducted covering two different growth phases, i.e. during dormancy (passive) and during late season shoot development (active) phase. Both experiments were designed to study the

effect of root pruning on the top growth and also the root system of the root pruned plants. It is interesting to evaluate new root development after pruning, to determine whether preference is given with respect to the location of root damage and extent of injury (severe v light pruning). In parallel the experiments wishes to assess whether there was a locational effect on new shoot development after root pruning.

6.1 Experiment 6a: Investigate the effect of root injury using a 'split-pot system' to determine root responses during the passive (dormant) phase in woody plants

This experiment studied the effect of root damage / injury on shoot growth and root growth after pruning when injury was induced during the passive / dormant stage of plant development. Observations were based on two parameters:- 1) the extent of new shoot and root growth after root pruning and 2) was there any influence of location and severity of pruning. Does more severe pruning stimulate greater amounts of root re-growth, or is there a relationship with which buds / shoots are activated to grow, based on response to root pruning.

6.1.1 Hypothesis:

1. Severe root pruning will decrease shoot growth in young shrubs to a greater extent than light root pruning.
2. Root regeneration is higher in severely injured parts of the root system compared to those more lightly damaged.
3. Fertilizer addition will stimulate more root development in the zone that is damaged, irrespective of where it is applied

6.1.2 Objectives:

1. To study the effect of root pruning on shoot growth.
2. To determine whether fertilizer will help encourage root growth in the damaged part of the root system.
3. To determine the effect of injury induced to selective parts of the root system on top growth and root system growth.

6.1.3 Material and Methods:

This experiment was conducted in a glasshouse at Norton Nursery during from 28th May 2012 until 18 March 2013. The initial experiment aimed to observe the effect of root damage done in Autumn and the recovery during Winter, however due to no different of growth observed during the winter; this experiment was prolonged to Spring 2014 to study the plant recovery (root and shoot) after being root pruned. A total of 56 liners of *Philadelphus coronarius* 'Aureus' and 56 liners of *Euonymus* 'Silver Queen' were used in this study where each of liner plant was grown in a split pot (two cut down clear polypropylene [lemonade] bottles stapled together) **(Figure 6.1)**. Sinclair potting growing medium was used as a growing medium. Each plant's root ball was divided into two equal sections with each side being re-potted into their individual containers; left and right sides were labelled and linked to treatments to ensure subsequent recording corresponded with the appropriate sub-treatments **(Figure 6.2)**.

Since all plants were potted in clear split pot, black polythene sheet were used to cover the pots to avoid phototropism in roots growth **(Figure 6.4)**; and then were placed on the glass house bench. All of the plants were left to establish for 4 months until the roots come to the base of the containers **(Figure 6.5)**.

Plants of each genotype were graded and divided into seven groups to provide comparable populations in each treatment. On 27th September 2012, seven treatments were imposed to each plant as below:-

1: Light pruning both sides **(Light & Light)**	: ⅓ of the roots were pruned - both sides;
2: Severe pruning **(Sev & Sev)**	: ⅔ of the roots were pruned - both sides;
3: Light and severe pruning **(Light & Sev)**	: ⅓ and ⅔ of roots were pruned each side, respectively;
4: Light pruning with fertilizer **(Light & Light Fert)**	: ⅓ of the roots were pruned both sides with slow release fertilizer applied to one side only;
5: Severe pruning with fertilizer **(Sev & Sev Fert)**	: ⅔ of the roots were pruned both sides with slow release fertilizer in one side only;

6: Light and Severe pruning with fertilizer **Light Fert & Sev)** : ⅓ and ⅔ of the roots were pruned with fertilizer in the ⅓ pruned side

7: Light and Severe pruning with fertilizer **(Light & Sev Fert)** : ⅓ and ⅔ of the roots were pruned with fertilizer in the ⅔ pruned side.

To maintain the moisture condition of the soil, all of the pots were hand watered once a week or more frequently in warmer weather. Care was taken to provide both sides of the pot with the same volume of water to avoid any roots on either side become excessively dry.

Data collection

Plant growth and development after root loss in *Philadelphus* cv. Aureus were observed by the following parameters; total shoot and root biomass, shoot and root biomass by treatment side, total new shoot, new shoot by treatment side, total new root, new roots for each side and root score in each side of the treatment. At the end of the experiment, new shoot and root numbers of each side were counted and scores were given based on the root distribution. Finally, to obtain the dry weight, plants were harvested and divided into root and shoot sections and dried in the oven.

Figure 6.1: Lemonade bottles stapled together as container

Figure 6.2: Roots were divided equally both sides

Figure 6.3: Plants sample

Figure 6.4: Bottles were covered with black polythene sheet

Types of treatments

Figure 6.5: Established plant before pruning

Figure 6.6: Light pruning both sides

Figure 6.7: Severe pruning both sides

Figure 6.8: Light and severe pruning in one s

6.1.4 Result

***Philadelphus* cv. Aureus**

Without fertilizer effect

There were no significant difference between total shoot biomass for **LL**, **LS** and **S S** (Figure 6.9). Shoot dry weight for each treatment side shows no significant difference although there were slightly different data between left and right in the same treatment, **LL** and **S S** (Figure 6.10). These differences being largely due to the difficulty of dividing an entire shoot system is a symmetrical manner, i.e. there was no obvious bias associated with shoot development based on the location of root pruning. In contrast, root biomass in **LS** treatment were significantly reduced with greater reduction of root dry weight in severe damage part (Figure 6.11and 6.12). Number of new shoot also show the same pattern like root biomass with greater reduction observed in severe damage part for **L S** treatment (Figure 6.13 and 6.14). For number of new roots data and root score, there were no significant differences demonstrates for both total new roots and number of new roots by treatment side (Figure 6.15, 6.16, 6.17 and 6.18).

With fertilizer effect

Overall adding fertilizer seemed to have a positive growth effect to plants that had one side of their root system lightly pruned and the other severely pruned (I.e. LSF and LFS) compared to equivalent treatments without the fertilizer. In contrast, adding fertilizer to plants where both sides were severely pruned does not seem to have encouraged new root growth (Fig. 6.15), although shoot mass is equivalent to non- fertilized plants treated in this way (Fig. 6.9).

As indicated above adding fertilizer did not radically alter root dry weight or number, except to the plants where there was a differential pruning level, i.e. there seemed to be some compensation for the loss of roots here, although differences not always significantly different (compare LS to LSF and LFS; Fig 6.12 and Fig. 6.16). It also seemed to support new shoot development in this particular treatment (Fig. 6.14).

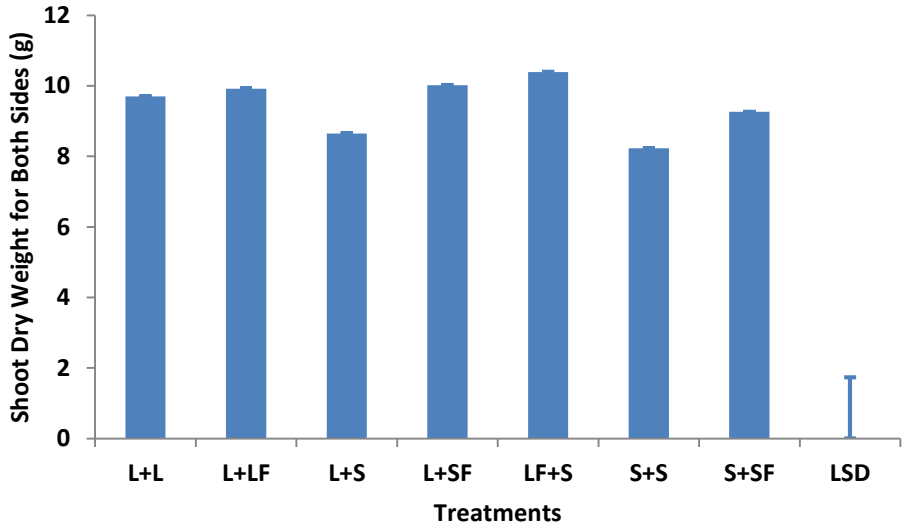


Figure 6.9 Total shoot dry weight for *Philadelphus* cv. Aureus for all treatments. P ≤ 0.005, d.f: 49. LSD: 1.736. L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

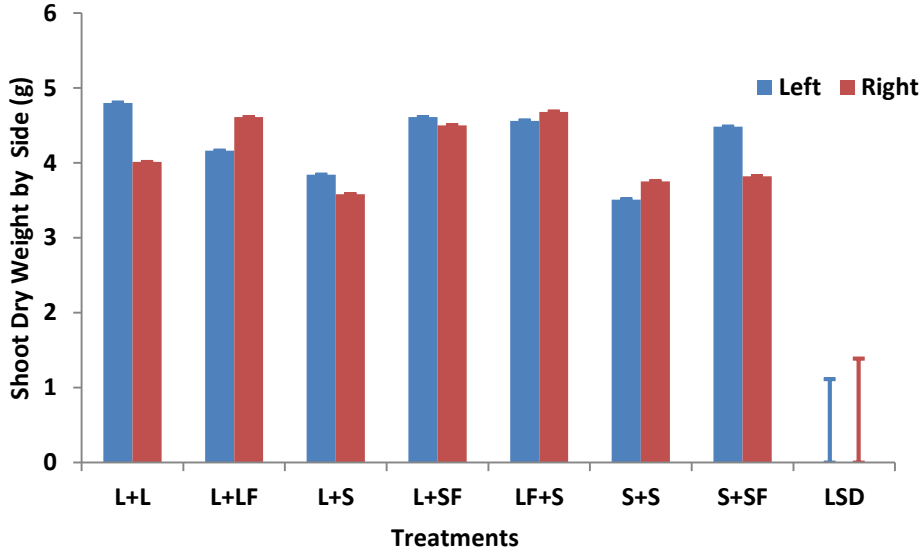


Figure 6.10 Shoot dry weight by treatment side for *Philadelphus* cv. Aureus for all treatments. P ≤ 0.005, d.f: 49. LSD: 1.114 (Left), 1.387 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

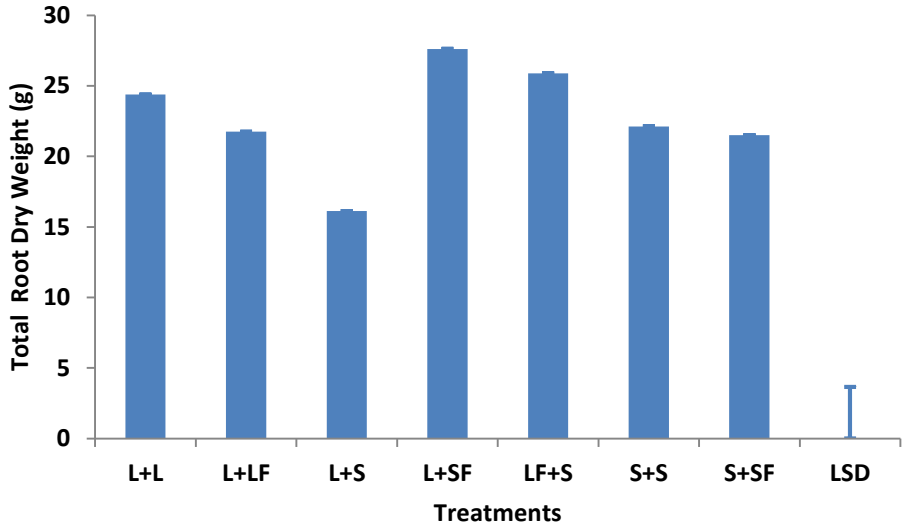


Figure 6.11 Total root dry weight for *Philadelphus* cv. Aureus for all treatments. P ≤ 0.005, d.f: 49. LSD: 3.648. L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

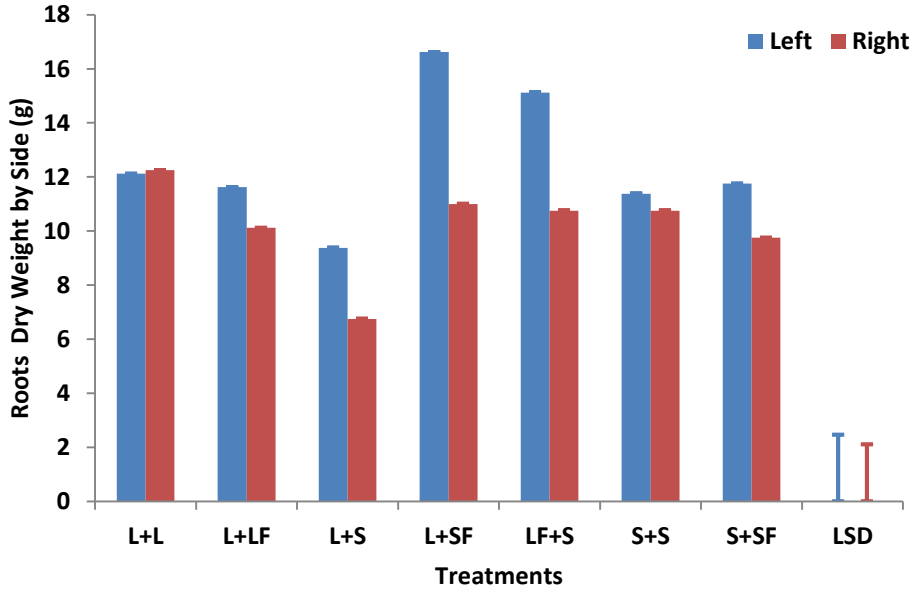


Figure 6.12 Root dry weight by treatment side for *Philadelphus* cv. Aureus for all treatments. P ≤ 0.005, d.f: 49. LSD: 2.464 (Left), 2.111 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

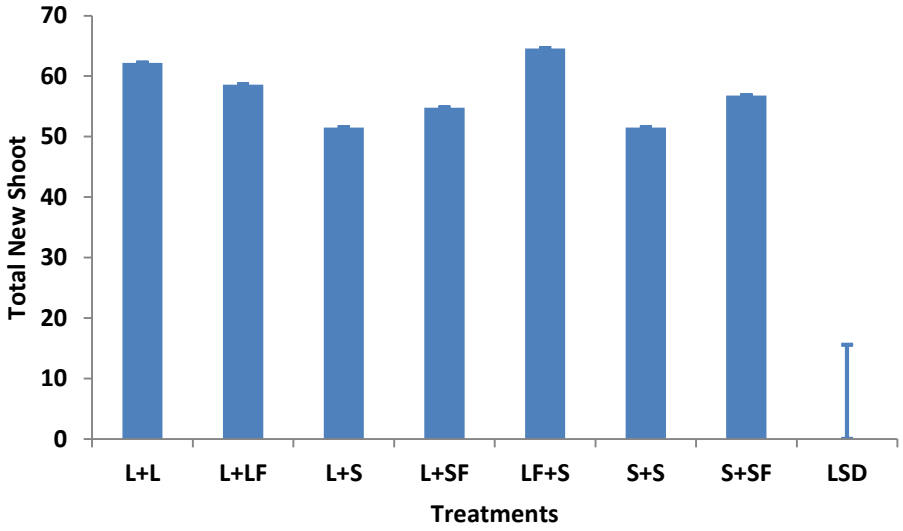


Figure 6.13 Total numbers of new shoots for *Philadelphus* cv. Aureus for all treatments. P ≤ 0.005, d.f: 54. LSD: 15.56. L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

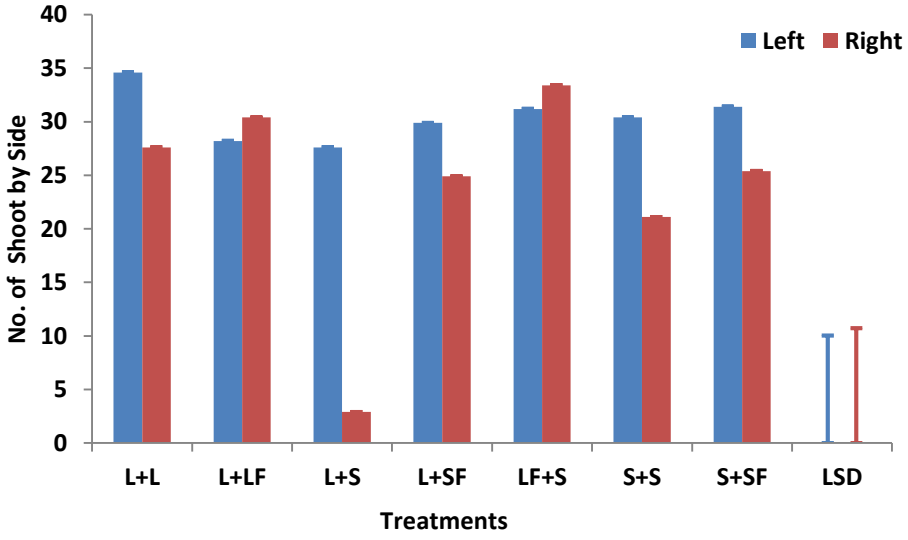


Figure 6.14 Number of new shoots for *Philadelphus* cv. Aureus each side for every treatments. P ≤ 0.005, d.f:54. LSD: 10.04 (Left), 10.71 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

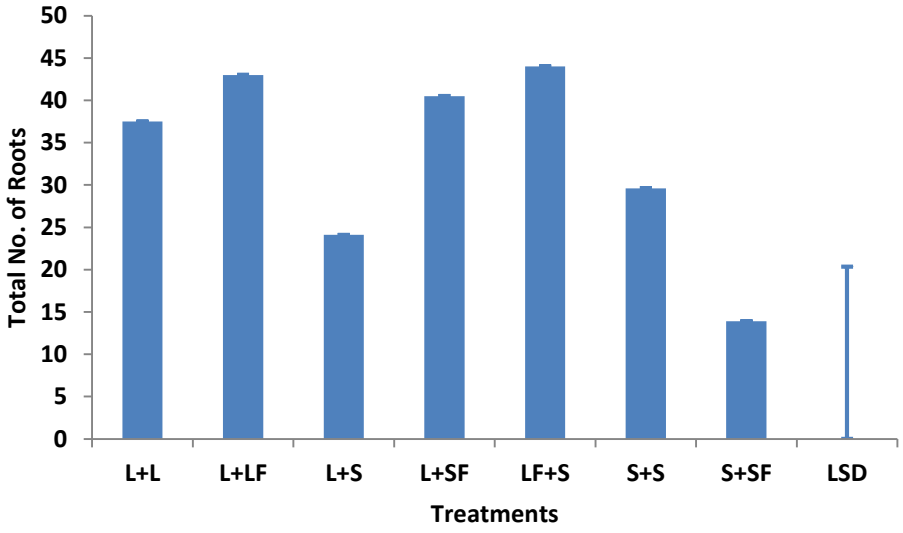


Figure 6.15 Total of new roots visible on bottle surface for *Philadelphus* cv. Aureus in 7 different treatments. P ≤ 0.005, d.f: 49. LSD: 20.43. L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

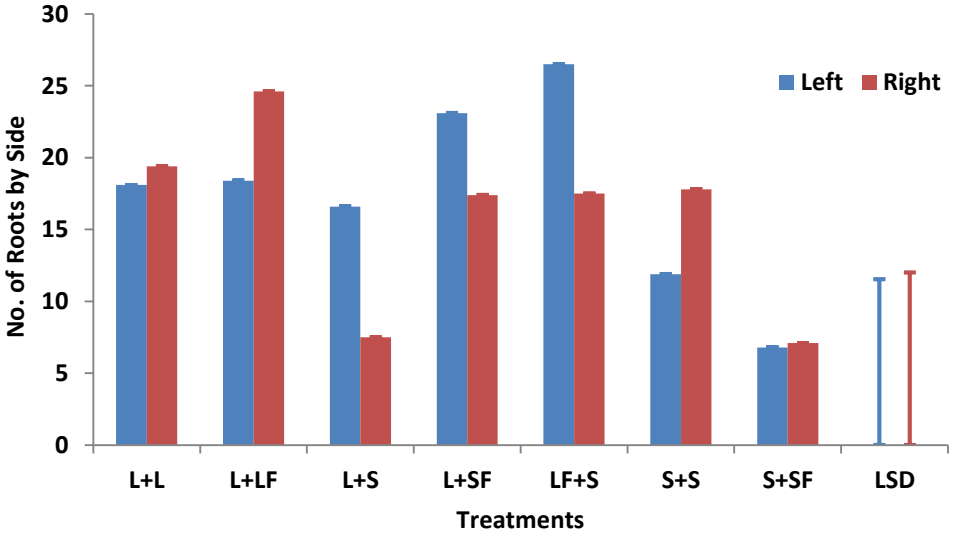


Figure 6.16 Number of new roots on each side of the treatment for *Philadelphus* cv. Aureus in 7 different treatments. P ≤ 0.005, d.f: 49. LSD: 11.55 (Left), 12.01 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

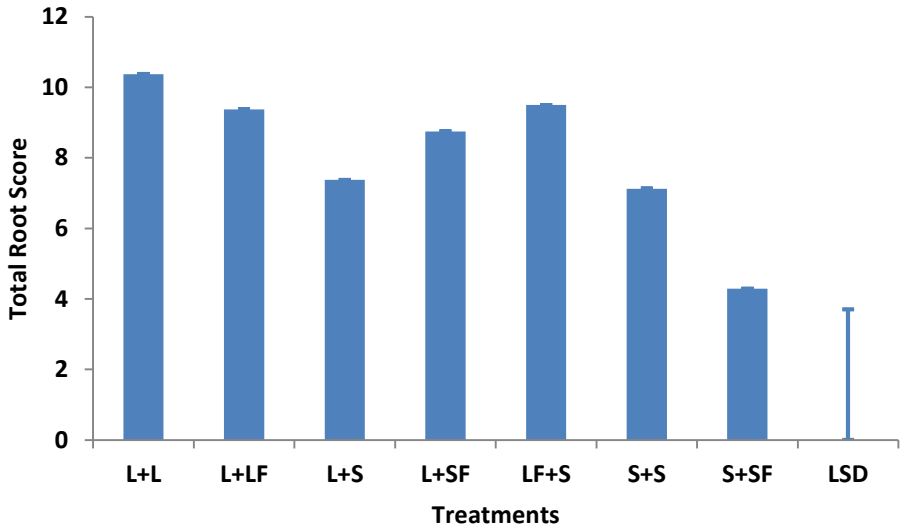


Figure 6.17 Total root score for *Philadelphus* cv. Aureus for all treatments. P ≤ 0.005, d.f: 49. LSD: 3.704 (Left), 2.285 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

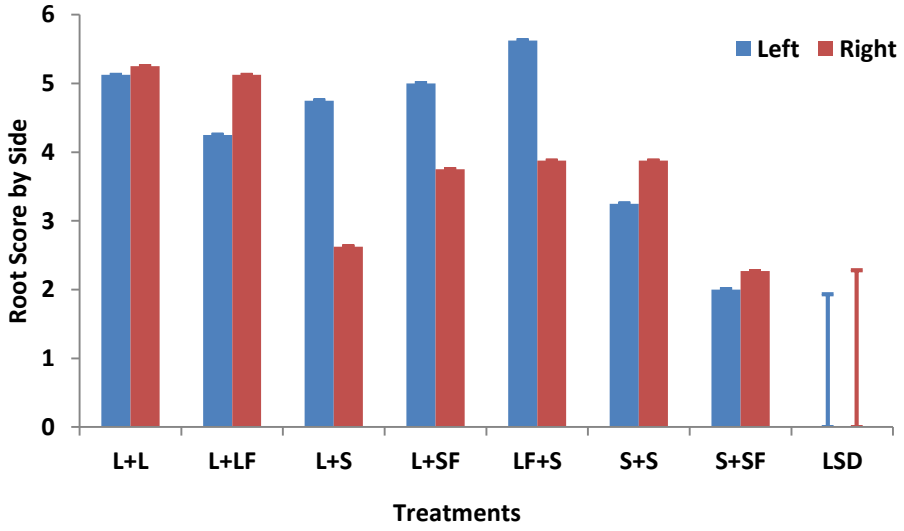


Figure 6.18 Root score on each side for *Philadelphus* cv. Aureus for all treatments. P ≤ 0.005, d.f: 49. LSD: 1.932 (Left), 2.28 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

***Euonymus* cv. Silver Queen**

Without fertilizer effect

Pruning treatments had no significant effect on *total* shoot dry weight (Fig. 6.19), new shoots (Fig. 6.23) new roots (Fig. 6.25) or root score (Fig, 6.27); although total root dry weight in L+L was marginally significantly less than either L+S or S+S (Fig. 6.21). To some extent this disguises however, the responses on the individual sides of the plants in terms of number and score of roots, with the severely pruned side of the L+S treatment, not recovering well (Figs. 6.26 and 6.28) This was not the case in the S+S treated plants.

With fertilizer effect

The addition of fertilizer did not provide a significant benefit to any of the pruning treatments for parameters relating to the total plant in *Euonymus*. When data is broken down by side of plant, however more subtle trends become apparent. Fertilizer seemed to boost the number of roots recorded (not significant) (Fig 6.26) and root scores (Fig. 6.28) in the L+S treated plants, especially on the side it was placed. This did not enhance, however, shoot biomass or number of new shoots recorded (Figs. 6.20 and 6.24).

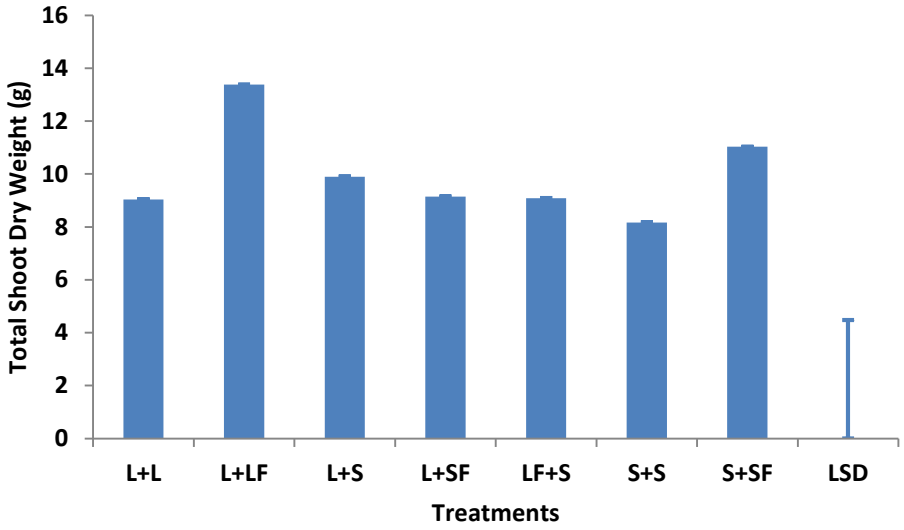


Figure 6.19 Total shoot dry weight for *Euonymus* cv. Silver Queen for all treatments. P≤ 0.005, d.f: 49. LSD: 4.484. L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

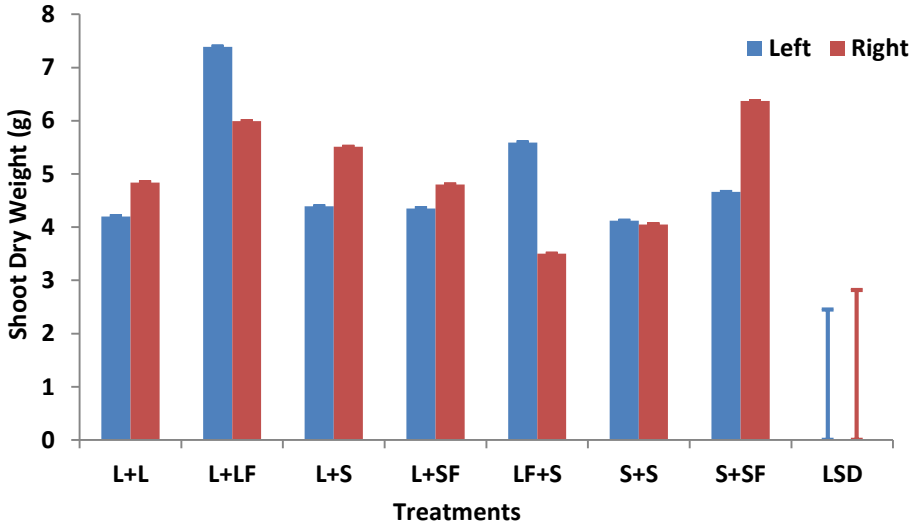


Figure 6.20 Shoot dry weight by treatment side for *Euonymus* cv. Silver Queen for all treatments. P ≤ 0.005, d.f: 49. LSD: 2.45 (Left), 2.819 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

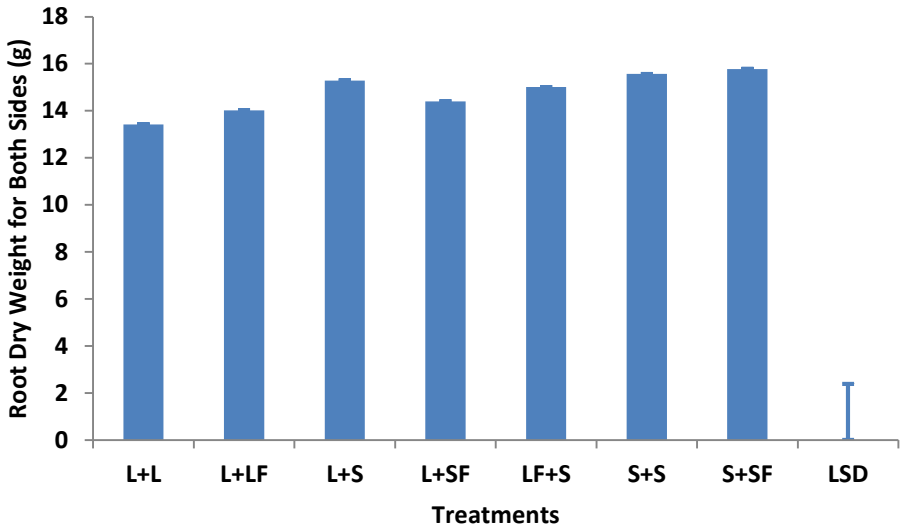


Figure 6.21 Total root dry weights for *Euonymus* cv. Silver Queen for all treatments. P ≤ 0.005, d.f: 49. LSD: 2.739. L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

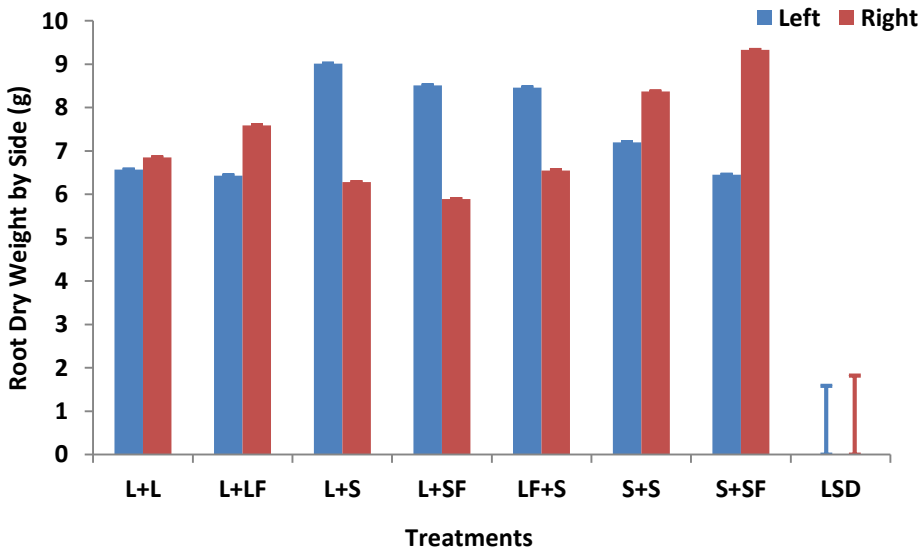


Figure 6.22 Root dry weights by treatment side for *Euonymus* cv. Silver Queen for all treatments. P ≤ 0.005, d.f: 49. LSD: 1.584 (Left), 1.821 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

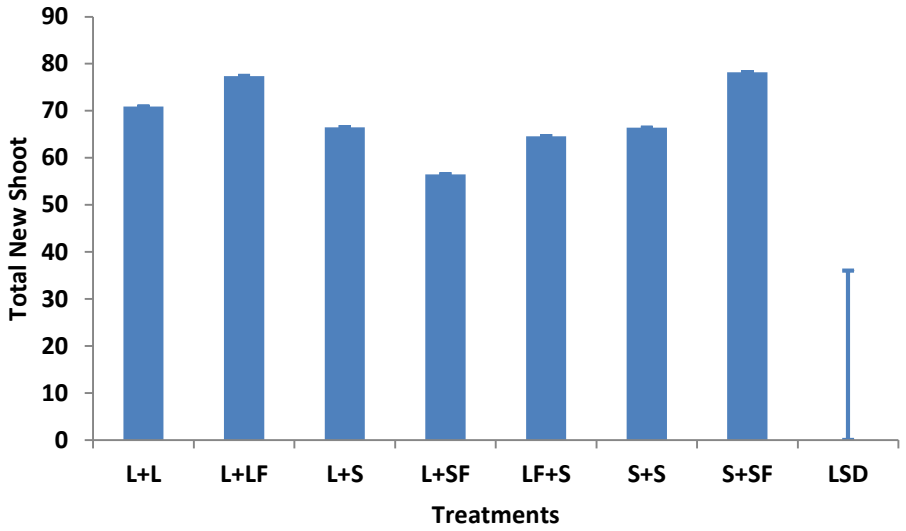


Figure 6.23 Total number of new shoot for *Euonymus* cv. Silver Queen for all treatments. P≤ 0.005, d.f: 49. LSD: 36.03. L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

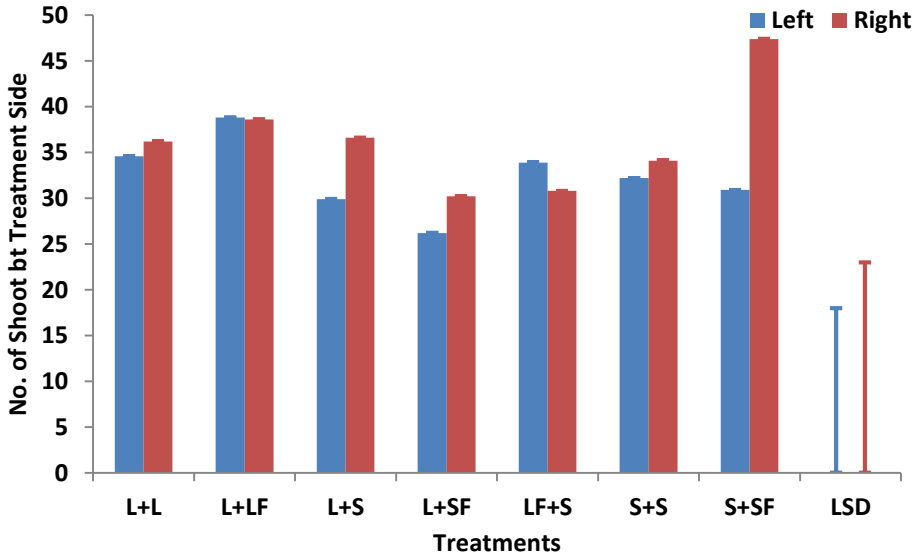


Figure 6.24 Number of new shoot by treatment side for *Euonymus* cv. Silver Queen for all treatments. P≤ 0.005, d.f: 49. LSD: 18.00 (Left), 23.00 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

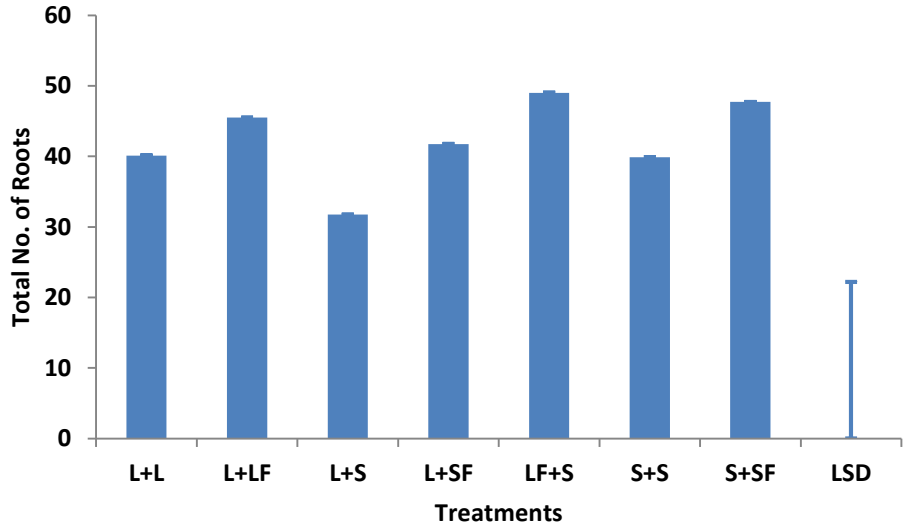


Figure 6.25 Total of new roots visible on bottle surface for *Euonymus* cv. Silver Queen in 7 different treatments. P ≤ 0.005, d.f: 49. LSD: 21.83. L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

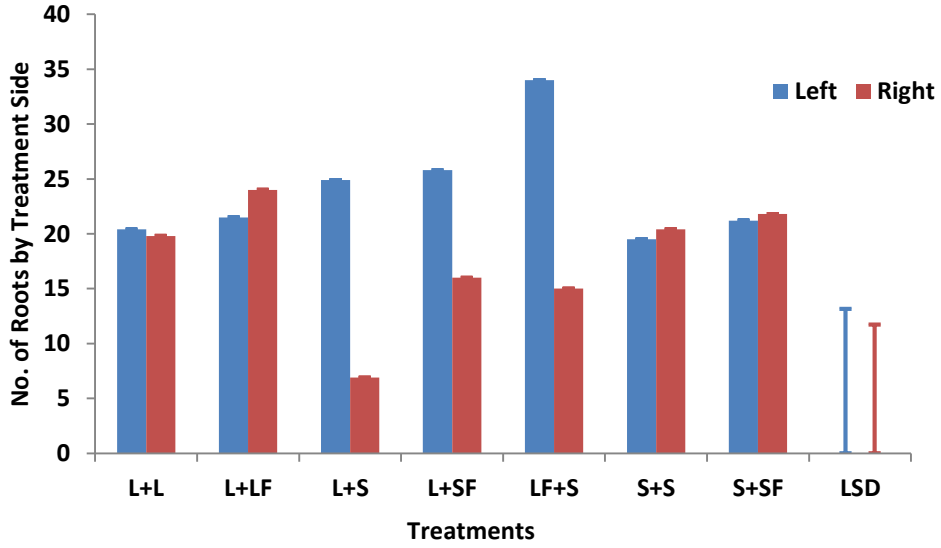


Figure 6.26 Number of new root on each side of the treatment for *Euonymus* cv. Silver Queen for all treatments. P ≤ 0.005, d.f: 49. LSD: 13.16(Left), 11.73 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

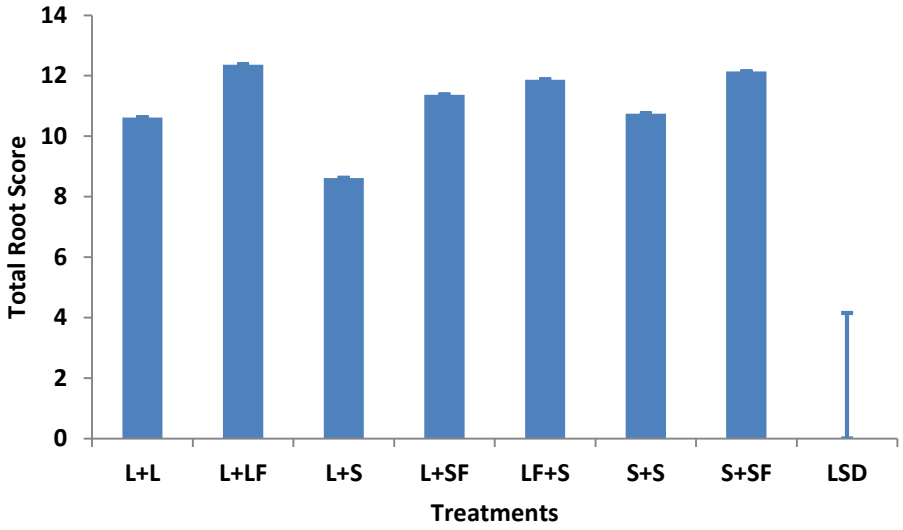


Figure 6.27 Total root score for *Euonymus* cv. Silver Queen in 7 different treatments. P ≤ 0.005, d.f: 49. LSD: 4.157. L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

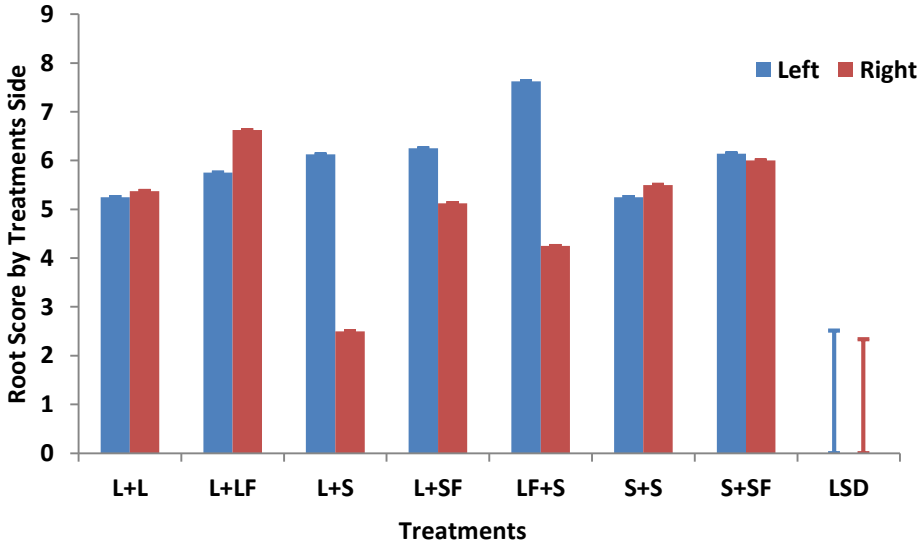


Figure 6.28 Root score on each side for *Euonymus* cv. Silver Queen in 7 different treatments. P ≤ 0.005, d.f: 49. LSD: 2.512 (Left), 2.34 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

6.2 Experiment 6b: Investigate the effect of root injury using a 'split-pot system' to determine root responses during the active (summer) growth phase in woody plants

This experiment was conducted to study the effect of root injury / damage on young shrubs and its compensatory growth when injury was applied during the active growth phase, i.e. late summer. The same parameters were measured as in the previous experiment to compare the commonality or differences in plant growth and response to root damage / loss between the two seasons.

6.2.1 Hypothesis:

1. Severe root pruning will decrease the growth of shoots in young shrubs as compared to light root pruning.
2. Root regeneration is higher in severely injured part rather than lighter part and fertilizer will help to induce more root growth in the part with greater injury.

6.2.2 Objectives:

1. To study the effect of root pruning on shoot growth.
2. To determine whether fertilizer will help encourage root growth after injury.
3. To determine the effect on top growth and root regeneration by damaging selective parts of the roots.

6.2.3 Material and Method

This study was conducted in a glasshouse at Norton Nursery during from 15th May 2013 until 18 March 2014. A total of 56 liners of *Philadelphus* cv. Aureus and 56 liners of *Euonymus* cv. Silver Queen were used in this study where each liner plant was grown in a split pot (two cut down clear polypropylene bottles stapled together). Plants were grown in a peat based medium (Sinclair potting). Each of the plant's roots were divided into two sides equally before potted into the split pot for each side and labelled with left and right.

Since all plants were potted in clear split pot, black polythene sheet were used to cover the pot to avoid phototropism affecting root behaviour. All of the plants were left to establish for 4 months until it was evident roots had reached the base of the containers. Plant were grouped into seven groups with seven replicate plants per treatment. Same treatments as Exp. 6a were imposed on all plants on 5th August 2013.

1: Light pruning both sides **(L+L)** : ⅓ of the roots were pruned both side;
2: Severe pruning **(S+S)** : ⅔ of the roots were pruned both side;
3: Light and severe pruning **(L+S)** : ⅓ and ⅔ of roots were pruned each side;
4: Light pruning with fertilizer **(L+LF)** : ⅓ of the roots were pruned both sides with slow release fertilizer in one side;
5: Severe pruning with fertilizer **(S+SF)** : ⅔ of the roots were pruned both sides with slow release fertilizer in one side;
6: Light and Severe pruning with fertilizer **(LF+S)** : ⅓ and ⅔ of the roots were pruned with fertilizer in ⅓ sides
7: Light and Severe pruning with fertilizer **(L+SF)** : ⅓ and ⅔ of the roots were pruned with fertilizer in ⅔ sides.

Similar irrigation regime and data measurement in Exp. 6a were applied in this experiment.

6.2.4 Result

***Philadelphus* cv. Aureus**

Without fertilizer effect

Greater reduction in total shoot and root biomass were observed in **L+S** treatments with less shoot and root dry weight in severe part of **L+S** treatment (Figure 6.29, 6.30, 6.31 and 6.32). The **L+S** treatment significantly under-performs compared to either the **L+L** (less overall damage) and the **S+S** (more overall damage). Similar patterns are observed in the total number of active new shoots, total new roots and root score; less visible new root were notable in **L+S** treatment significantly in severe pruned side (Figure 6.33, 6.34, 6.35, 6.36, 6.37 and 6.38). From this result we can assume that severe root loss inhibit root and shoot growth when the root damage happened during the active growing phase.

With fertilizer effect

There was an intriguing response to fertilizer addition in this *Philadelphus* cultivar, with interactions being observed between the pruning treatments and the addition of fertilizer. In general the addition of fertilizer significantly enhanced the growth of the plants in the differential severity of root-pruning treatment i.e. **LF+S** and **L+SF** had much greater growth responses than **L+S** alone (Figs. 6.29 to 6.32 and Figs. 6.35 to 6.38). In contrast, there was no significant advantage to adding fertilizer to the severe-severe treatment (i.e. **S+SF** not significantly better than **S+S**), and if anything, there was a slight negative (differences not always significant) response to adding fertilizer to those plants only light root-pruned (i.e. **L+LF** less growth than **L+L**, e.g. Fig 6.29). In essence the addition of fertilizer appeared to be inducing different responses, depending on how badly damaged the root systems were.

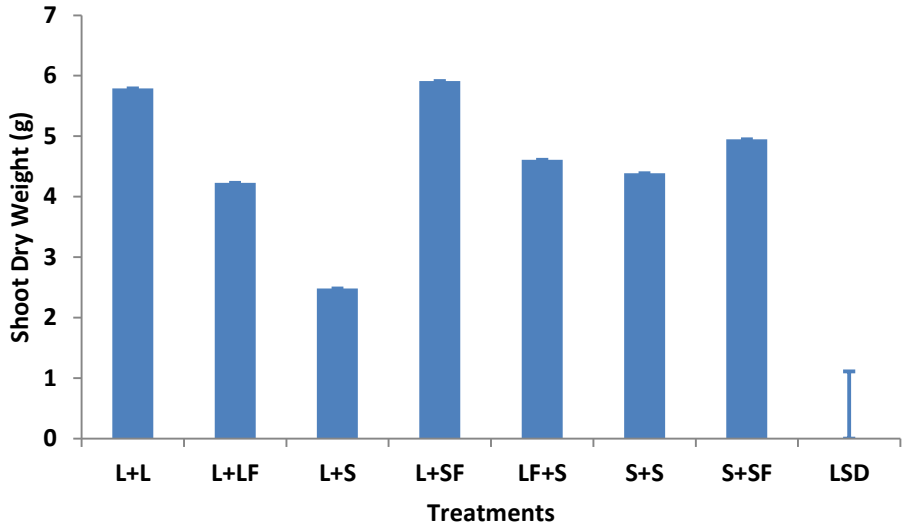


Figure 6.29 Total shoot dry weights for *Philadelphus* cv. Aureus for all treatments. P ≤ 0.005, d.f: 49. LSD: 1.11. L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

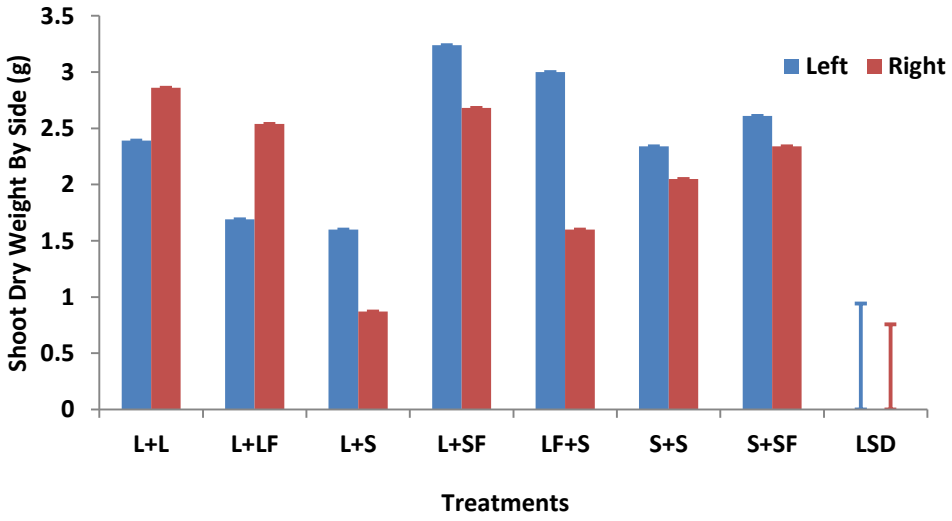


Figure 6.30 Shoot dry weights by treatment side for *Philadelphus* cv. Aureus for all treatments. P ≤ 0.005, d.f: 49. LSD: 0.941 (Left), 0.756 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

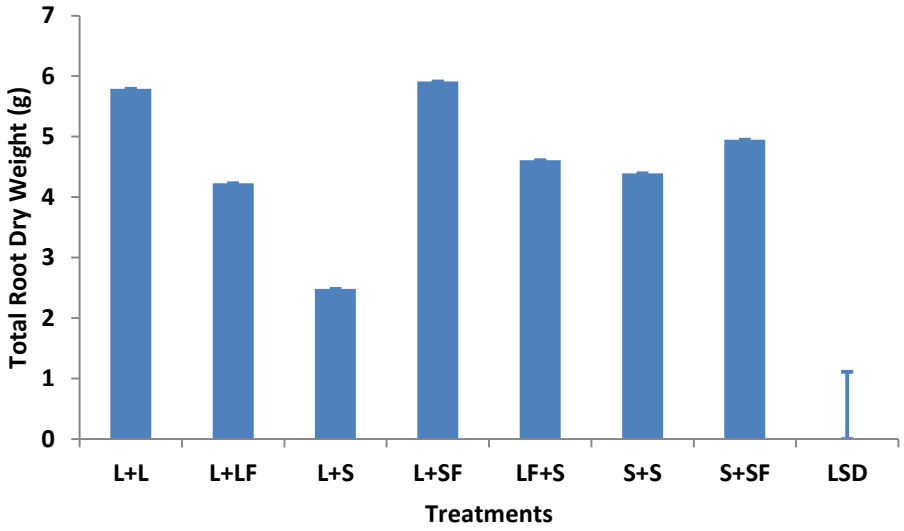


Figure 6.31 Total root dry weight for *Philadelphus* cv. Aureus for all treatments. P≤0.005, d.f: 49. LD: 1.11. L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

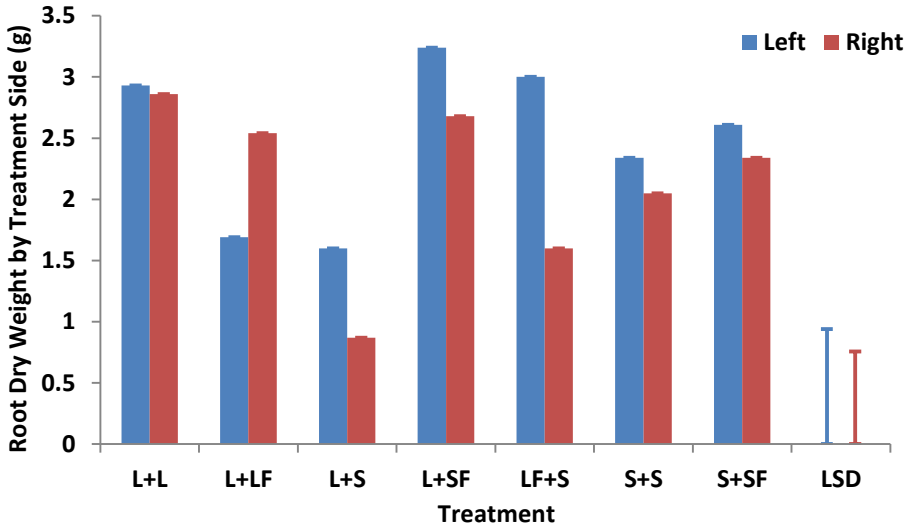


Figure 6.32 Root dry weights by treatment side for *Philadelphus* cv. Aureus for all treatments. P ≤ 0.005, d.f: 49. LSD: 0.941 (Left), 0.756 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

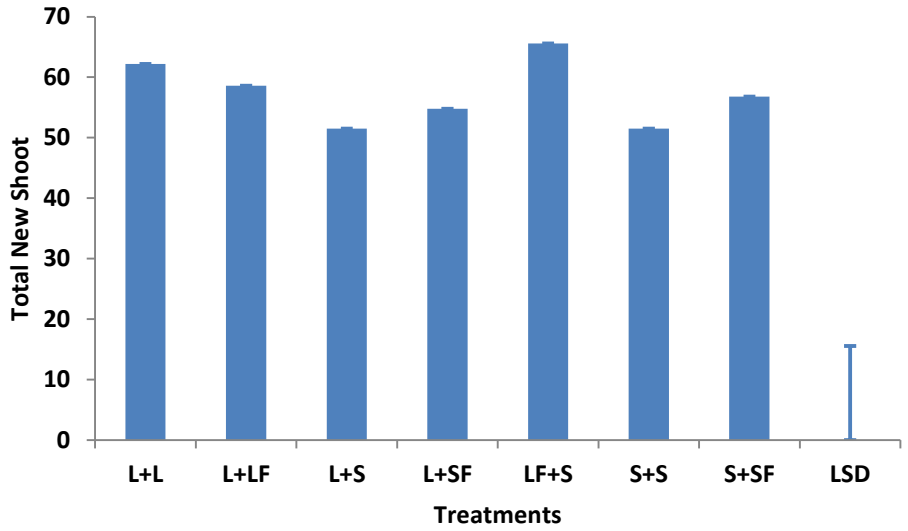


Figure 6.33 Total numbers of new shoot for *Philadelphus* cv. Aureus for all treatments. P ≤ 0.005, d.f: 49. LSD: 15.56. L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

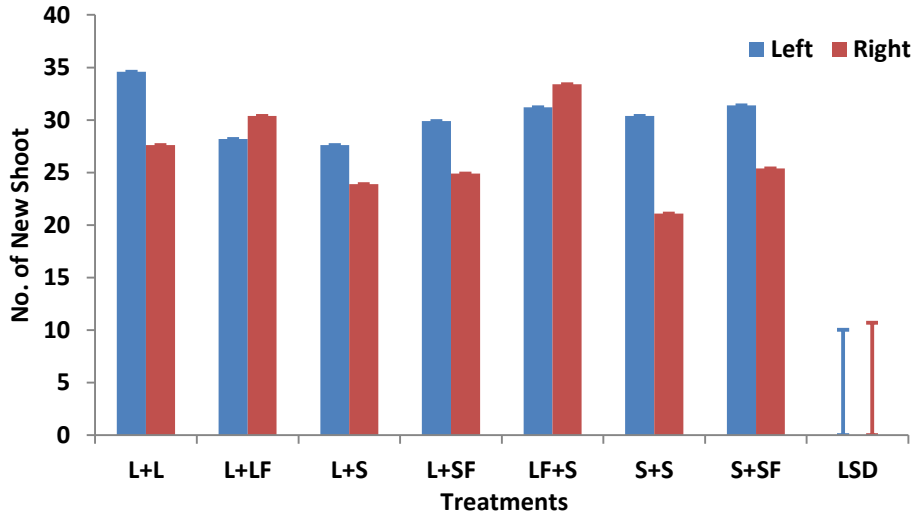


Figure 6.34 Number of new shoot for *Philadelphus* cv. Aureus each side for every treatments. P ≤ 0.005, d.f: 49. LSD: 10.04 (Left), 10.71 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

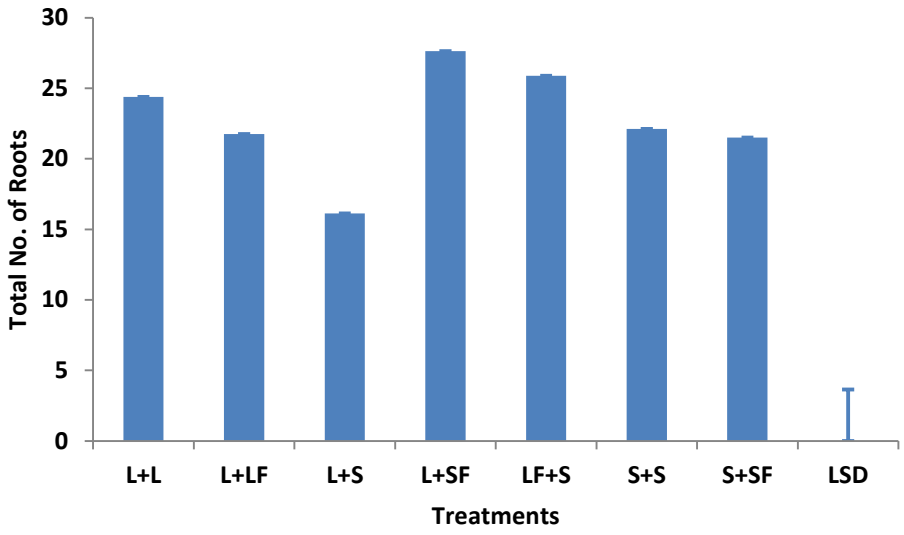


Figure 6.35 Total of new roots visible on bottle surface for *Philadelphus* cv. Aureus in all treatments. P ≤ 0.005, d.f: 49. LSD: 3.648. L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

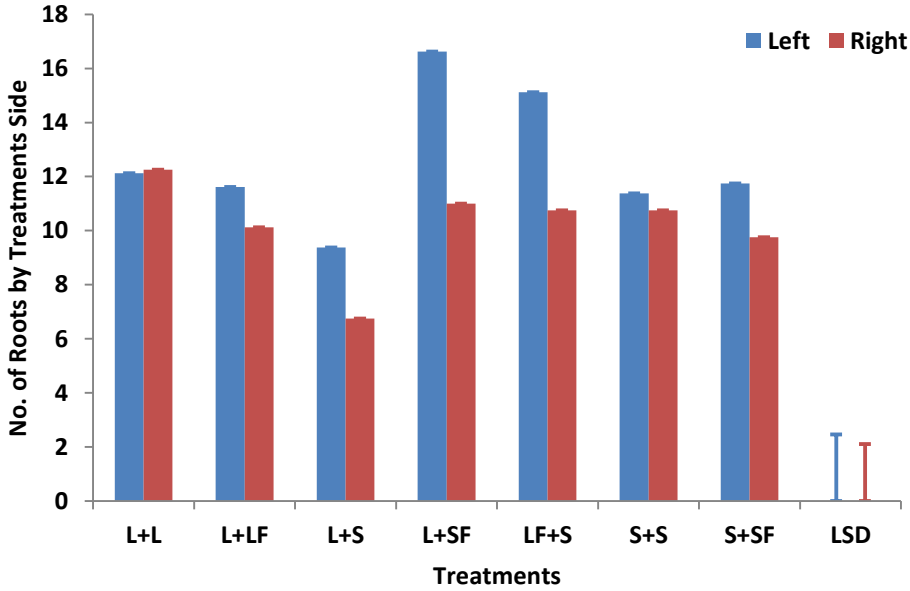


Figure 6.36 Number of new roots on each side of the treatment for *Philadelphus* cv. Aureus in 7 different treatments. P ≤ 0.005, d.f: 49. LSD: 2.464 (Left), 2.111 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

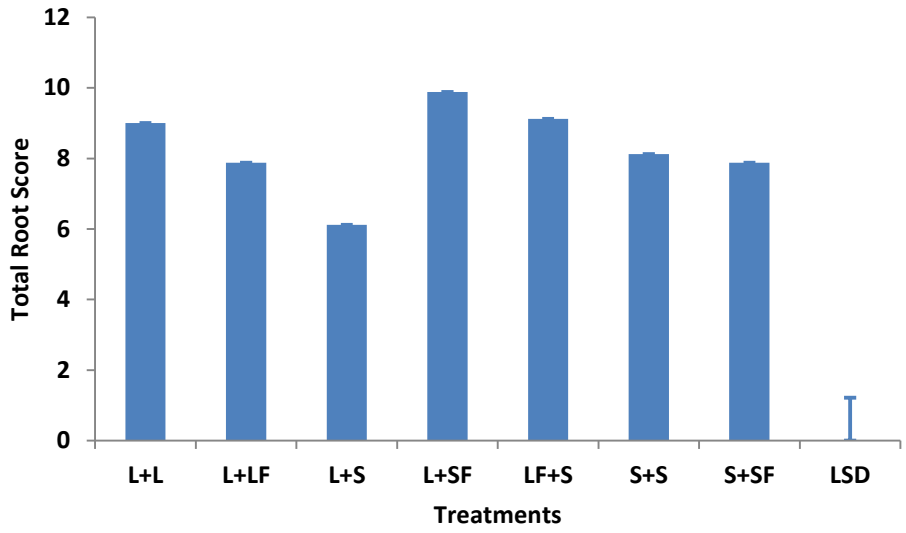


Figure 6.37 Total root score for *Philadelphus* cv. Aureus in 7 different treatments. P ≤ 0.005, d.f: 49. LSD: 1.212. L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

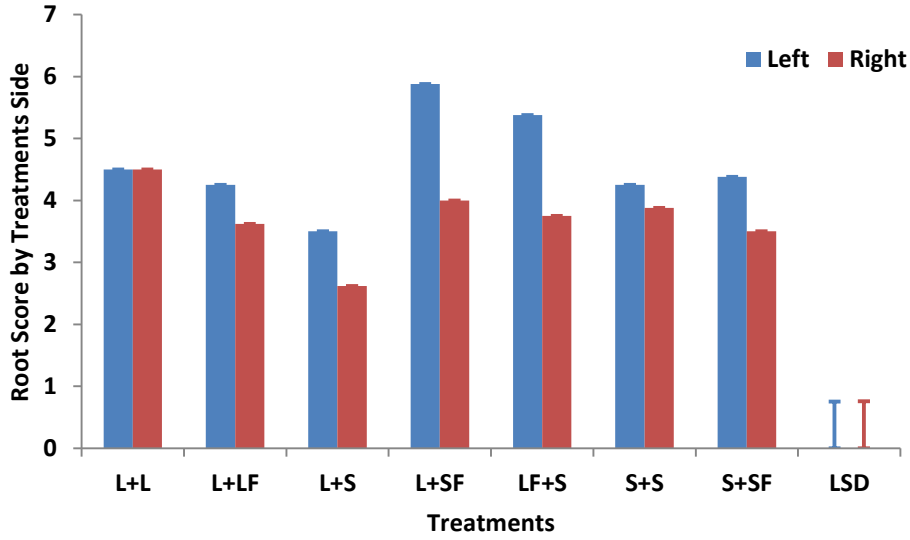


Figure 6.38 Root score on each side for *Philadelphus* cv. Aureus in 7 different treatments. P ≤ 0.005, d.f: 49. LSD: 0.754 (Left), 0.761 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

***Euonymus* cv. Silver Queen**

Without fertilizer effect

There were no significant differences in *total* values for shoot growth (Figure 6.39) root weight (Figure 6.41), new shoots (Figure 6.43), new roots (Figure 6.45) or root score (Figure 6.47), due to the different root pruning treatments alone. Again differences were not usually significant, when the parameters were assessed due to plant symmetry (i.e. right v left side) (Figures 6.40, 6.42, 6.46 and 6.48), although there was a reduction (marginally non-significant) on the number of new shoots generated in plants where the roots had been differentially pruned (**L+S**) compared to the **L+L** (on the light pruned side only Figure 6.44).

With fertilizer effect

Plants that were lightly root-pruned on both sides, generally responded well to fertilizer addition (significantly greater shoot weight Figure 6.39, especially on the fertilized side Figure 6.40) and enhanced root weight (Figures 6.41 and 6.42). Similarly, there was some positive response to fertilizer when added to the Light-Severe pruning regime, with most advantage noted when the fertilizer was added to the side with the severely pruned roots (e.g. Figure 6.44). Somewhat in contrast, for those plants that received severe root pruning on both sides of the rootball, supplementary additions of fertilizer had little overall effect on growth responses, or had a slight negative effect (e.g. on root dry weight; Figure 6.42).

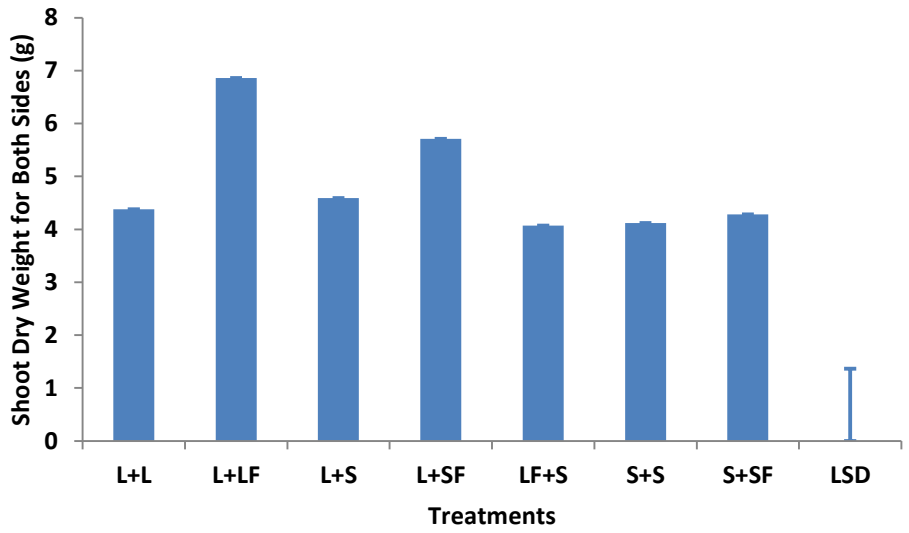


Figure 6.39 Total shoot dry weights for *Euonymus* cv. Silver Queen for all treatments. P ≤ 0.005, d.f: 49. LSD: 1.367. L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

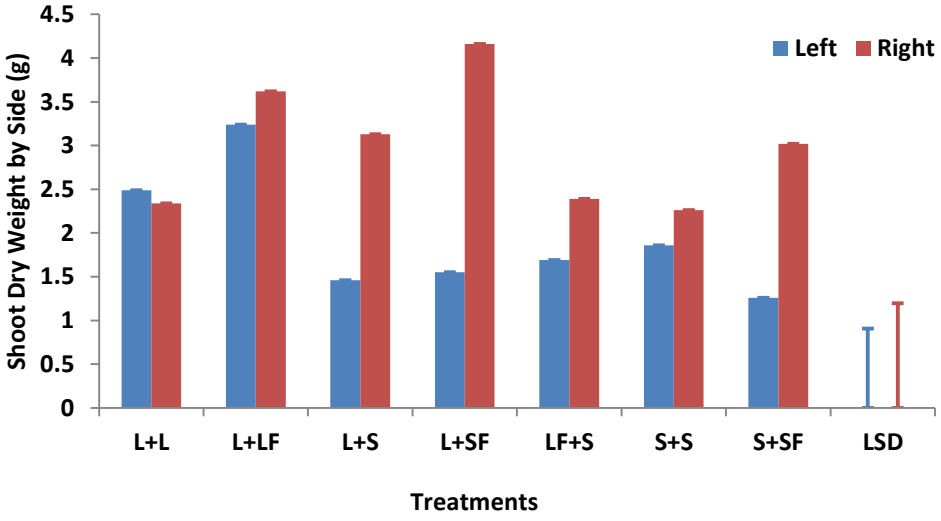


Figure 6.40 Shoot dry weights by treatment side for *Euonymus* cv. Silver Queen for all treatments. P≤0.005, d.f: 49. LSD: 0.908 (Left), 1.197 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

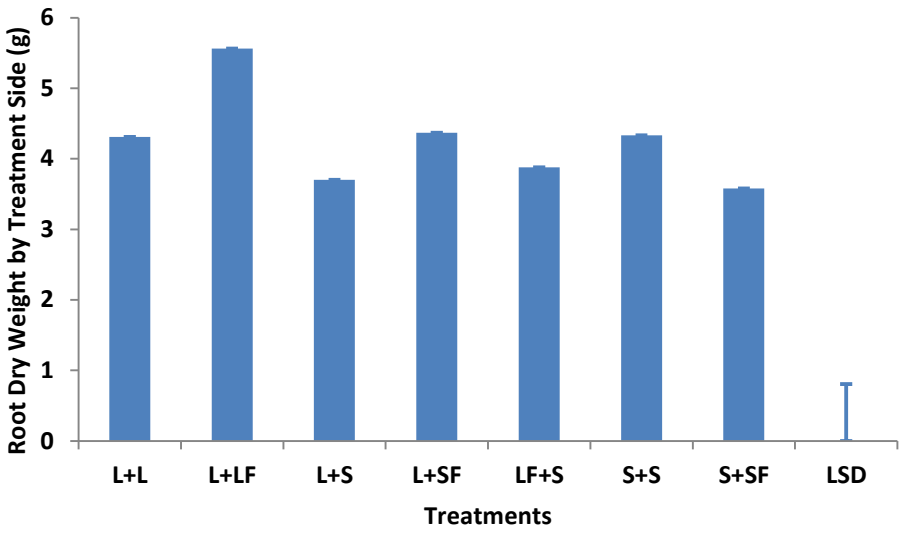


Figure 6.41 Total root dry weights for *Euonymus* cv. Silver Queen for all treatments. P ≤ 0.005, d.f: 49. LSD: 0.807. L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

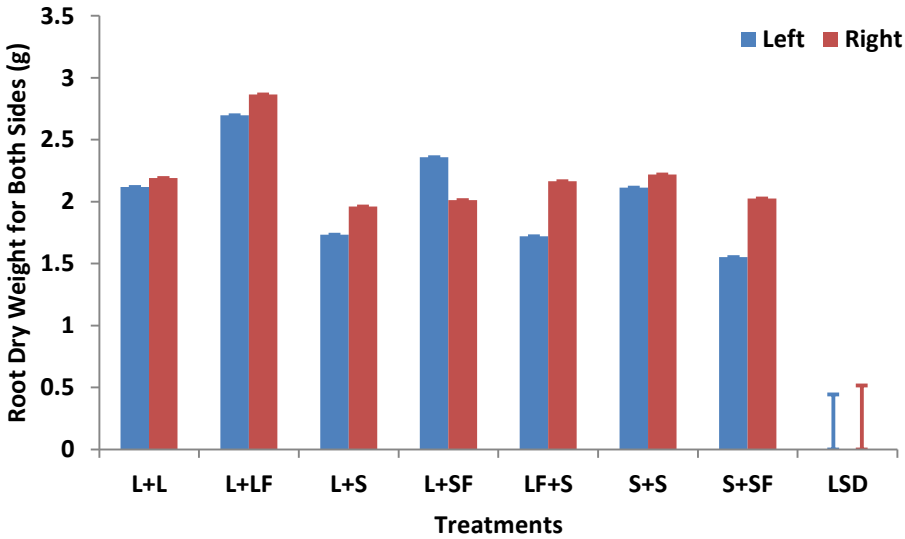


Figure 6.42 Root dry weights by treatment side for *Euonymus* cv. Silver Queen for all treatments. P ≤ 0.005, d.f: 49. LSD: 0.4456 (Left), 0.5175 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

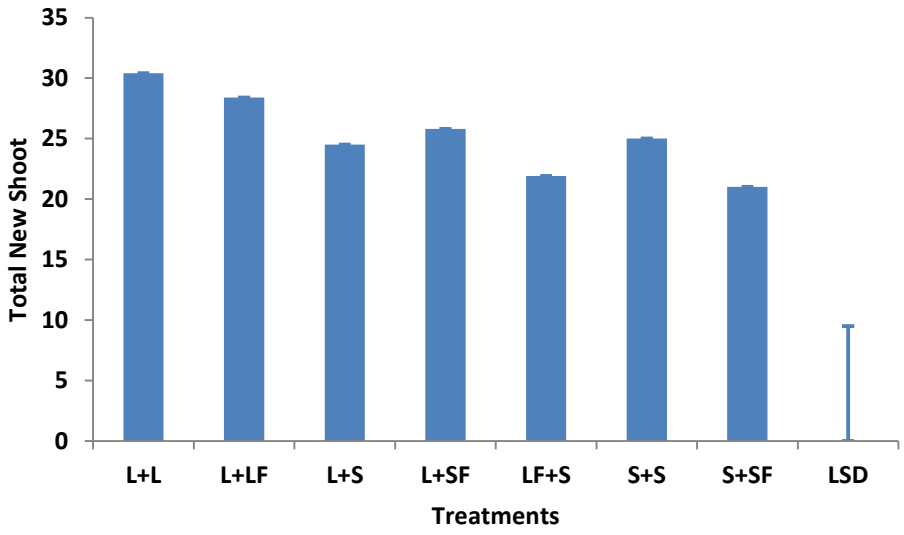


Figure 6.43 Total number of new shoot for *Euonymus* cv. Silver Queen for all treatments. P ≤ 0.005, d.f: 49. LSD: 9.5. L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

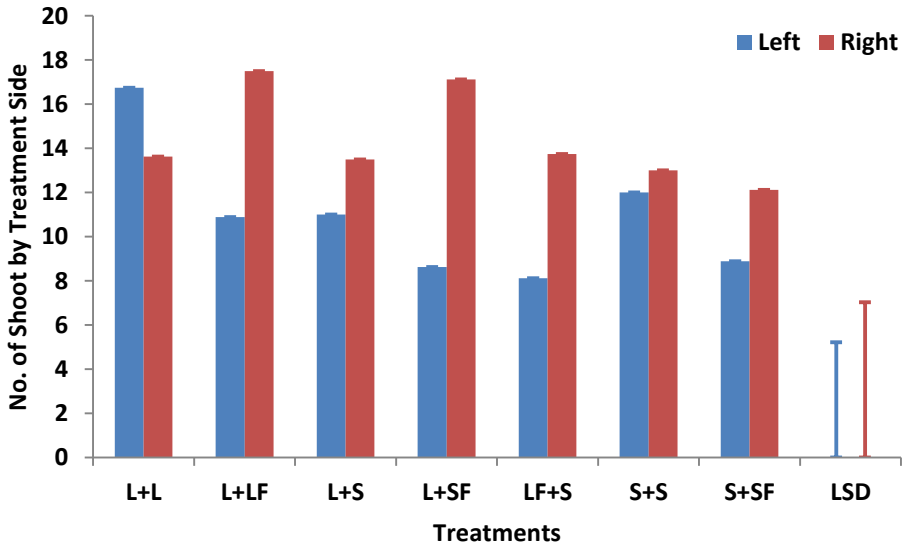


Figure 6.44 Number of new shoot by treatment side for *Euonymus* cv. Silver Queen for all treatments. P ≤ 0.005, d.f: 49. LSD: 5.215 (Left), 7.031 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

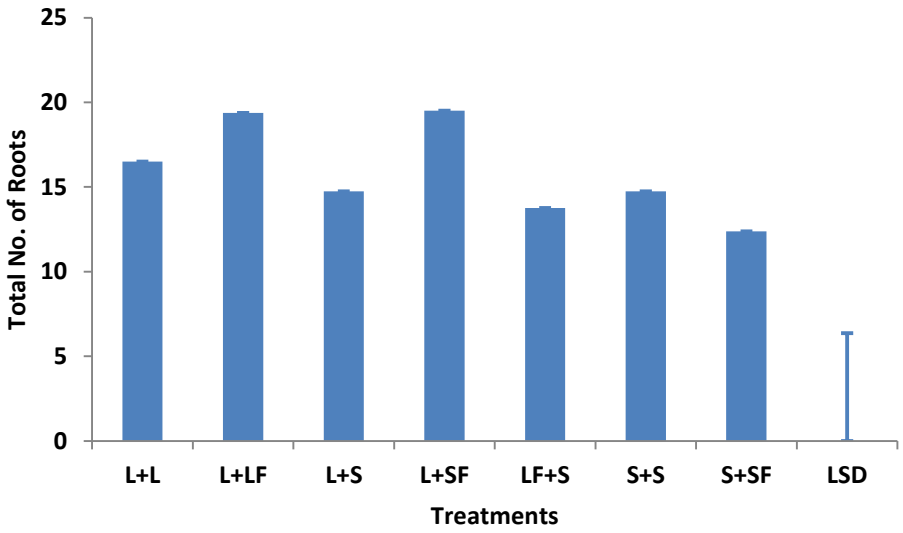


Figure 6.45 Total numbers of new roots visible on bottle surface for *Euonymus* cv. Queen for all treatments. P ≤ 0.005, d.f: 49. LSD: 6.355. L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

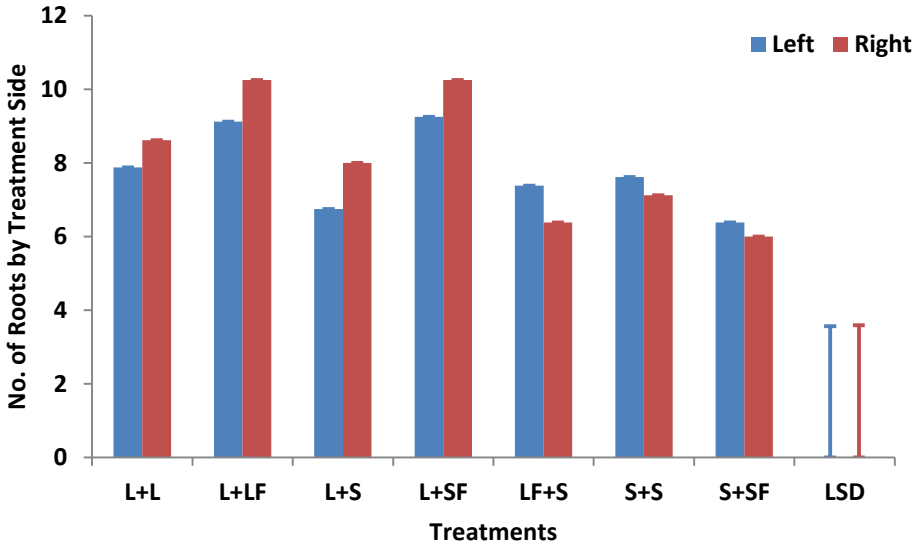


Figure 6.46 Number of new root on each side of the treatment for *Euonymus* cv. Silver Queen for all treatments. P≤0.005, d.f: 49. LSD: 3.564 (Left), 3.59 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

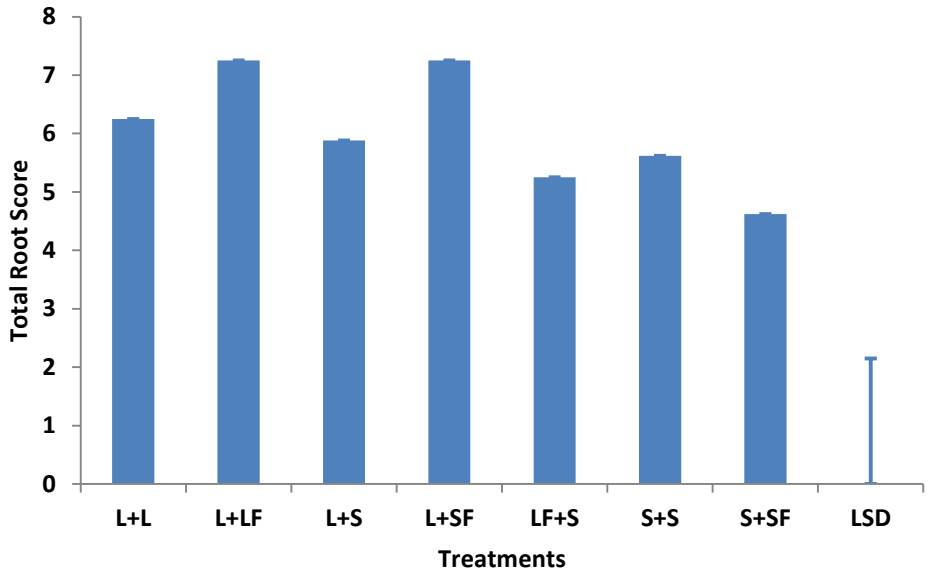


Figure 6.47 Total root score for *Euonymus* cv. Silver Queen for all treatments. P ≤ 0.005, d.f: 49. LSD: 2.151. L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

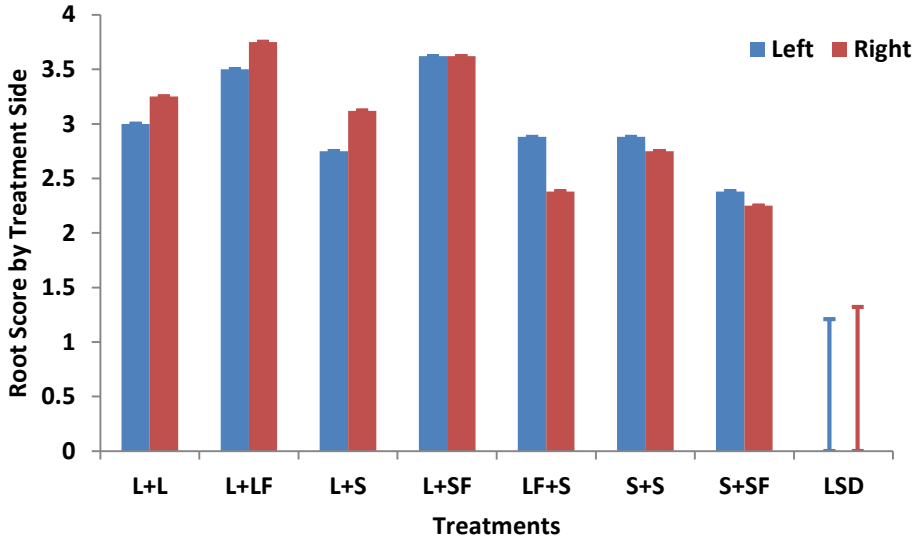


Figure 6.48 Root score for each side of the treatment for *Euonymus* cv. Silver Queen for all treatments. P ≤ 0.005, d.f: 49. LSD: 1.209 (Left), 1.32 (Right). L+L=Light & Light, L+LF= Light & Light with Fertilizer, L+S= Light & Severe, L+SF= Light & Severe with Fertilizer, LF+S= Light with Fertilizer & Severe, S+S= Severe & Severe and S+SF= Severe & Severe with Fertilizer.

6.3 Discussion

This chapter studied the effect of root damage on young specimens of - *Philadelphus* cv. Aureus and *Euonymus* cv. Silver Queen across two different seasons (autumn - passive growing phase and summer – active growing phase); exploring the impact of different severities of damage and subsequent root regeneration and shoot development . For clarity the results are discussed in two sections – the first dealing with impacts without fertilizer addition, and the second determining how the addition of nutrients interacted with the pruning severity / location responses. Slow release fertilizer (Miracle Gro All Purpose Continuous Release Plant Food) with N:P:K status of 17:9:10 were used to provide additional nutrients to root pruned parts. Fertilizer addition to root pruned part was undertaken to understand more on root compensatory growth, which may be aided by adding fertilizer in some cases.

Philadelphus

It was noted that in *Philadelphus* without fertilizer, greatest growth penalties were observed in the **L+S** treatment; total root and shoot biomass being reduced in both summer and autumn experiments, compared to the **L+L** and **S+S** treatments. This seems illogical, as the **L+S** treatment would have been intermediate in terms of overall root damage incurred. However, it may be that the plants in this treatment did not lose enough root biomass to stimulate a strong 'wound' response and divert resources from elsewhere to help regenerate roots on the damaged side. Conversely, the fact that one half of the root system was relatively speaking, considerably less damaged, may also suggest that some sort of signalling mechanism was indicating the root provision was adequate and re-allocation of reserves away from shoot growth was not required. Certainly, the root volume on the L side may be considered sufficiently great to ensure plants remain viable, if perhaps not with the optimum complement of roots. This would not appear to be the case with plants exposed to the S+S treatment, where the significant loss of root on both sides, may have encouraged significant re-growth to the root system. Potentially this high level of root regeneration and growth activity, may

also have encouraged shoot growth in due time (e.g. via phytohormone activity) to provide photosynthates that would help support the new root biomass.

Any detrimental growth response associated with the **L+S** treatment in *Philadelphus*, could to some extent be compensated for by the addition of fertilizer. In both seasons the addition of extra nutrition to encourage more positive growth responses compared to the **L+S** treatment in the absence of fertilizer. During the autumn dormant phase (Exp. 6a) the location of the added fertilizer had little impact on growth; whereas there was a suggestion that during active growth in summer, placing the fertilizer on the side of the severely damaged roots had some benefit for regenerating roots, if perhaps not have a similar positive effect on shoots.

There did not appear to be a seasonal difference in *Philadelphus*, with respect to how plants responded to uniform light pruning **L+L** and severe root pruning **S+S**, with on average the **L+L** treatment demonstrating stronger growth than its more severely injured counterpart. However, this result was somewhat in contrast with studies on the *Buddleja davidii* 'Summer Beauty' (butterfly bush) and *Cistus* 'Snow Fire' (rock rose), where light pruning during active phase for both species was also favourable in encouraging root growth but conversely decreased shoot dry weight over the time (Blanusa et al., 2007).

Response to fertilizer, however, demonstrated some more subtle responses, based on both pruning and season. Applying fertilizer to severe-root-pruned plants in the non-active autumn period, subsequently aided shoot development, but not necessarily root development, i.e. new root growth did not appear to be particularly activated by higher nutrient status levels. When applied in the summer period, additional fertilizer had only a marginal positive, or no overall effect on the **S+S** treatment.

In the plants only lightly pruned (**L+L**), additional nutrition made little difference to growth responses during autumn, and if any trend was apparent in summer, it was to actually reduce growth in roots and shoots compared to the non-fertilized plants. However, this result is contrary to studies in *Ilex cornuta* 'Burfordii Nana (Burford Holly)

where there is no significant growth in new shoot and new root growth by adding fertilizer in pruned roots (Gilman et al., 1996).

Euonymus

In contrast to *Philadelphus*, the *Euonymus* was overall rather unresponsive to the root pruning treatment (in either season). Perhaps this reflects the slower growing nature of this genotype; or its greater capacity to accommodate a significant stress. Certainly the significant reduction in root biomass induced by **S+S** did not impair subsequent development and plants seemed to respond fairly well to even this level of stress. If anything, the differential pruning in summer (**L+S**) was somewhat more detrimental than **S+S** (in line, but not to the same noticeable degree as the *Philadelphus*). In line with this light pruning alone did not induce any strong activation of new root development *per se*.

The impact of fertilizer additions was relatively low in terms of overall plant development (e.g. total values), but could influence responses at the more local level within individual containers. Overall, fertilizer additions to *Euonymus* tended to enhance growth (albeit marginally) to those plants that had the least traumatic root pruning, with some localized benefits for plants exposed to (**L+S**) in the dormant autumn or active summer phases and to plants lightly pruned in summer (**L+L**). There was some data to suggest that adding nutrition to the damaged side of the root system in the differential pruning treatment, i.e. **L+SF** was particularly beneficial. It did not always compensate for the loss of root encountered in the **S+S** treatments, however.

Key Points:-

1. **Light pruning encourage more root growth than severe pruning**
2. **Severe pruning in both sides have more root biomass than mix pruning (L+S)**
3. **Addition of fertilizer at severe root pruned parts of *Philadelphus* aided shoot growth in autumn but no overall effect in summer.**
4. **Addition of fertilizer at lighter root pruned parts of *Philadelphus* have little different growth in autumn but reduced root and shoot growth in summer.**

5. **Addition of fertilizer at lighter root pruned parts of *Euonymus* tended to enhance growth in dormant or active but only to lightly pruned plants in summer.**

CHAPTER 7

OVERALL DISCUSSION AND CONCLUSION

This research investigated the performance of young shrubs when exposed to conditions that mimicked urban soil conditions. With the exception of *Punica granatum* (a Mediterranean species) the genotypes used in this research are commonly used in the UK landscape. *Philadelphus* was chosen to represent a fast growing vigorous landscape specimen (potentially growing up to 1-1.5m per year once established), but with two contrasting foliage colours to determine if this affected resilience to soil-based stresses. However, both cultivars have similar response overall in soil compaction. In contrast, a slower growing, small evergreen shrub with variegated leaves *Euonymus* cv. Silver Queen was used to represent the lower growing shrub groups often used as ground cover or where space is restricted in the urban landscape (containers, planters etc.) Studies encompassed both periods in which plants were in active growth (spring/summer seasons) and passive dormant phases (autumn/winter seasons) to determine how seasonal affects influences root injury and regeneration capacity. This final chapter provides an overview of the main findings within the research and attempts to identify the extent to which root and shoot systems have common responses and adaptations to a wide range of soil based stress factors.

7.1 How plant response to urban environment stress?

Findings from this research clearly demonstrate that environmental stress at the rootzone alters the physiology of plants and impacts on biomass accumulation. Despite the research relying on semi-controlled conditions rather than field evaluations *per se* there are strong implications for future urban landscape design, planting and management. Information reviewed in Chapter One demonstrated that the poor state of health often found with urban vegetation is largely due to inadequate soil condition and that some degree of soil modification is desirable (Jim, 1998). This would, ideally, include factors such as improving the volume of soil accessible to roots, improving the soil structure and aiding soil crumb formation, enhancing organic matter content and

reducing the likelihood of soil compaction damaging the pore structure and altering aeration and drainage parameters. In addition, attention should be paid in the future to the shape of the rhizosphere. Some of the results from this research can be used to challenge established principles and provide opportunities to better accommodate plants needs and requirement for optimum growth and performance. Data from Chapter 3 for example suggests that the orientation of the potential rootzone may influence root dynamics. Root development was greater when roots were encourage to ramify through a shallow horizontal substrate volume (tray system), compared to a narrow vertical 'column' of substrate, despite the volume of substrate available being the same. The precise reasons for this are not clear, with potential factors being differences in water drainage / holding parameters, aeration and physical restrictions on roots and limited ability to form secondary / tertiary root branches in the column system. Nevertheless, this demonstrates that the geometry of the planting pit / rootzone can influence the volume of roots generated and their direction of growth. Currently some tree pit systems in practice are encouraging root growth vertically down the soil profile and away from urban infrastructure such as flag stones, paving etc. The data from this research, however, would indicate that this should not be promoted indefinitely as root development may be stronger when encouraged to grow laterally along a horizontal direction. This has implications for practice, in that early root development may need to be directed in a vertical direction, but then planting systems may need to accommodate the potential for roots to explore horizontally, but perhaps at a greater depth than would have occurred in the past (i.e. horizontal development, but not necessarily immediately below the soil surface.

The results with the column system are analogous to previous work where the soil volume has actually been decreased. Research in *Zea mays* (maize) demonstrated plants grown in smaller pots (2.4 litre) only produced 44% of the plant biomass compared to those in larger volume pots (16.2 litre pot) under optimum irrigation (Ray and Sinclair, 1998). Smaller root volumes have been linked to lower nutrient content and supply to shoots (Yong et al., 2010), direct reductions in photosynthesis rates (Poorter et al., 2012) and lower transfer rate of cytokinin from root to shoot (Yeh and Chiang, 2001).

Although a shallow, narrow rootzone was superior to a tall column, neither compared favourably to the conventional pots shape (Table 7.1). This was despite trying to manage the water availability through two different methods, apply what plants appeared to require (Exp 3a) and ensuring the same volumes of water was delivered irrespective of apparent need (Exp 3b). Both irrigation strategies resulted in C reductions in shoot and root biomass for all species in column and tray treatments compared to the pot, despite volume of media available being uniform. As discussed above, some of the growth reductions in the column, may relate to the physical restraint on roots or competition to fill the limited volume of media available at the top of the column. In contrast, reductions in growth in the tray system (compared to controls) may relate more to water availability and distribution. In the genotype that was in both Exp 3a and 3b – *Philadelphus* cv. Aureus almost double the biomass was generated when water was applied in a controlled manner with a uniform volume (Exp 3b) to each container type. Any reductions in growth compared to the conventional pot shape, may simply relate to the greater surface area of the media exposed to the atmosphere in the tray system (i.e. a greater proportion of the water may be evaporated through the media surface than would be the case in the pot). Further research may requires some form of soil / media 'sealing' to minimise the influences of this, and help determine how geometry of the rootzone alone interacts with root behaviour.

	Optimum irrigation (Exp. 3a)			Similar irrigation (Exp. 3b)	
	Column	Tray		Column	Tray
Species			Species		
	Shoot			Shoot	
***Philadelphus* cv. Aureus**	17.70%	40.71%	***Philadelphus* cv. Aureus**	35.29%	90.66%
Punica granatum	40.25%	57.26%	***Philadelphus* cv. Belle Etoile**	60.13%	67.72%
			***Euonymus* cv. Silver Queen**	40.27%	66.93%
	Root			Root	
***Philadelphus* cv. Aureus**	24.27%	44.66%	***Philadelphus* cv. Aureus**	59.79%	88.81%
Punica granatum	58.25%	84.13%	***Philadelphus* cv. Belle Etoile**	83.50%	69.58%
			***Euonymus* cv. Silver Queen**	32.18%	48.94%

**Plants grown in conventional pot produced the maximum shoot dry weight in all species.*

Table 7.1 Shoot and root biomass percentage for both experiments in all species as compared to conventional pot grown plants

7.2 Plant response to abiotic stress

One objective of this research was to determine how plants responded to the wide variety of stress factors that can occur in urban soils. Most stress factors at least at the more extreme end of their spectra, had a negative effect on overall biomass (Figure 7.2), with those plants experiencing stress generally being smaller than their non-stressed counterparts. Stress tended to make plants more compact with shorter branch lengths and internode lengths. Stress factors for the most part did not appear to increase the numbers of branches produced on plants, contrary to some anecdotal reports of slower shoot extension corresponding with a reduction in the apical dominance of the plant and encouragement of lateral branching (Foo et al., 2001). A couple of possible exception to this, however, was in the compression of the organic based media (Exp. 4b) where high compression encouraged shoot branching in *Philadelphus* cv. Aureus (Figure. 4.25), and where reduction in branching habit was not proportional to reductions in shoot biomass in *Philadelphus* cv. Belle Etoile (compare organic data in Figures 4.30 and 4.36). Similarly, there was some suggestion that a moderate level of water logging promoted new bud activation and shoot development in *Philadelphus* cv. Aureus and *Euonymus* cv. Silver Queen, whereas no stress or full stress (full waterlogging) did not achieve the same results. Perhaps, one reason for the lack of a 'generic' promotion of side branches in all experiments, may be due to the fact that the some stress factors directly affected photosynthetic capacity (and hence reduced energy sources to support lateral bud development) whereas others just disrupted an apical auxin signal.

In a number of incidences, plants appeared highly resilient to the stresses imposed on them. Compressing a sand based media appeared to encourage root growth in *Philadelphus* cv. Aureus (Figure 4.20) and shoot and root development in *Euonymus* (Figures 4.40, 4.41 4.42); perhaps by improving water relations within the substrate. *Euonymus* cv. Silver Queen also did not suffer severe penalties in terms of overall shoot biomass despite having either one side of its root system lightly pruned and the other severely pruned, or indeed both sides severely pruned (Figure 6.39).

Figure 7.2 below summarise the effect of environment stresses on plant growth in four stresses as imposed in this research. Environmental stresses demonstrates to reduce plant root and shoot biomass, plant height, branches, number of leaves, induced stomatal closure, affect leaf expansion and in extreme condition could lead to plant death.

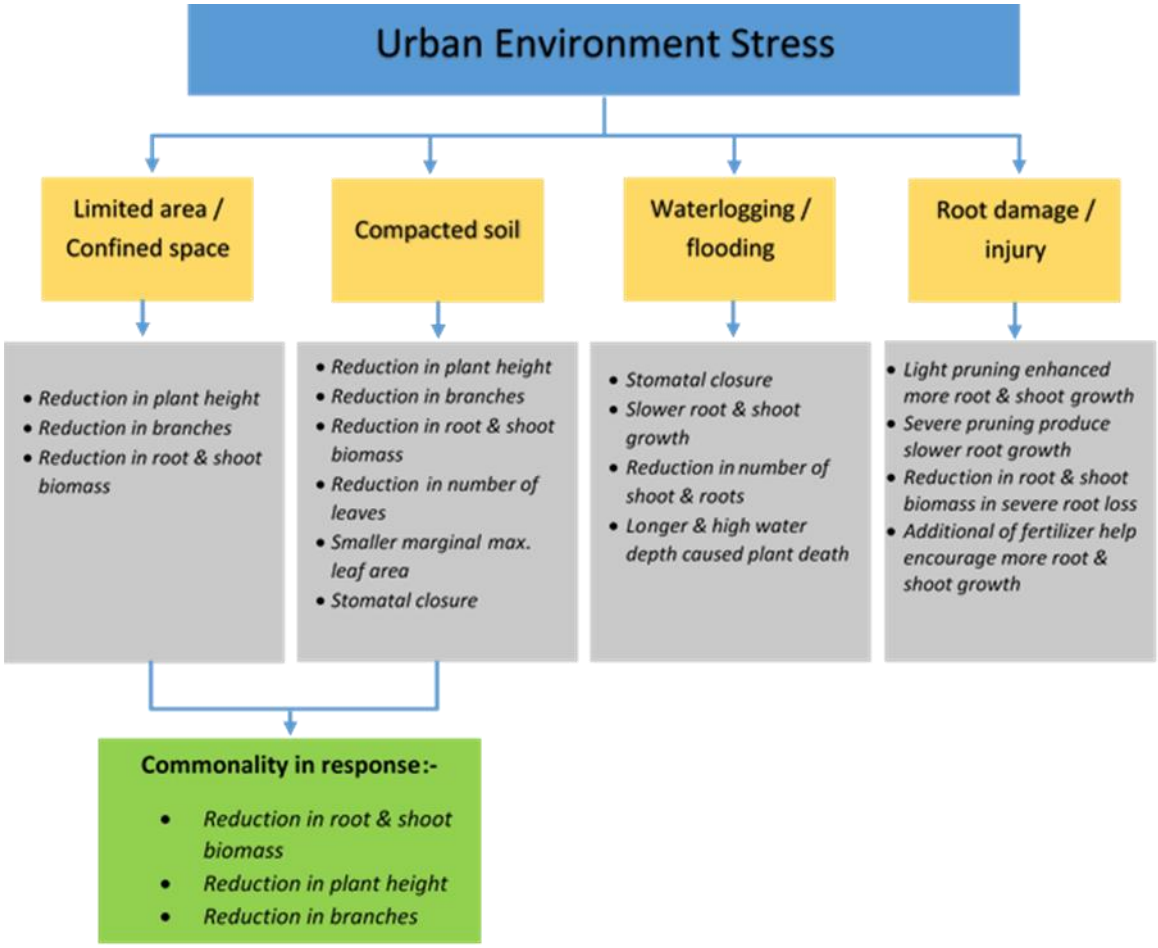


Figure 7.1 Summary of plant response to urban environment stresses studied in this research

Resilience was often genotype dependant. The research highlighted waterlogging conditions during summer affected young *Philadelphus* cv. Aureus severely due to the lower percentage of plant's survival in post flooding (High treatment, Table 5.2) as compared to *Euonymus* cv. Silver Queen with 100% survival rate (Table 5.3). The first response observed in *Philadelphus* under medium and high flooding condition is the reduction in g_s on day 4 of waterlogging. However, flooded tomato plant (*Lycopersicon esculentum* Mill, cv. Ailsa Craig) experienced decreasing g_s as early as 4 hours after

flooding during photoperiod time (Else et al., 1996). This may reflect the sensitivity of different species to the waterlogging event, nature of the chemical / biochemical alterations taking place and differences in physiological responses to these. The g_s data in this experiment was quite variable, however, with control values oscillating considerably between days. Normally, it might be expected that values would increase as temperatures rise e.g. data recorded show the maximum temperature on day 4 is 19.8°C which was an overcast day; compared to day 1 which is 35.6°C and predominately sunny day (See Figures 5.9 and 5.10 in chapter 5). This could be an example, however of where temperatures and irradiance were supra-optimal, with consequential reductions in g_s. Overall, however, g_s values decreased with time in the waterlogged treatments, and be in line with the severity of stress imposed (depth of water). This result supports previous findings on waterlogging causing reductions in stomatal conductance (Kozlowski, 1984, Jackson and Hall, 1987, Bradford and Hsiao, 1982).

Responses to waterlogging

Summer flooding appeared to be more severe and induce greater injuries (in *Philadelphus* at least) than winter flooding events. This corresponds to previous research in shrubs (King et al., 2012) which showed injury levels increasing progressively as flood events progressed from winter through spring to summer. High temperatures in summer being considered detrimental by reducing oxygen availability more rapidly; less oxygen held within water, but also more microbial competition for O_2. Flooding response was typified in *Philadelphus* cv. Aureus during summer flooding by wilting and subsequent desiccation of leaves on plants within the high waterlogging treatment; symptoms becoming apparent from day 11. This condition continued through the post flooding 'recovery' period, during which there was no evidence of new shoot development. Such leaf symptoms have been recorded in other waterlogging studies e.g. in *Gossypium* (cotton plants) (Hebbar and D Mayee, 2011), *Glycine max* Merr. Japanese Soybean (Jitsuyama, 2013) and *Citrus rehsni* Hort. Ex Tan., CM (Cleopatra mandarin) (Arbona and Gómez-Cadenas, 2008). In contrast to the data on *Philadelphus*

here, however, in cotton there was a recovery from the wilted and desiccated leaf conditions as plants recovered once the flooding ended. Results from *Philadelphus* support the hypothesis set out for Exp 5a. in that within summer flooding, prolonged and deeper flooding induced greater injury that shorter periods, or in situations where some of the roots remained above the 'water table'.

One interesting response in *Philadelphus* was that in the non-fatal water logging treatments of Low and Medium water levels, new shoot growth was evident after the flooding events, in contrast to non-flooded control plants. This may be a response to a non-fatal stress inducing 'out of season' re-growth. Whether this is due to the stress deactivating some of the natural quiescence / dormancy induction signals that these woody plants may receive late in the summer, or again, whether this has been induced by some deficit in carbohydrates and other resources is not clear. Understanding of root derived signals within woody plants, where plants are inured but not killed, requires further investigations.

In contrast to *Philadelphus*, *Euonymus* was more resistant to flooding condition and there was a 100% survival rate. Responses within *Euonymus* however, were associated with approximately 5-15% of its leaves becoming chlorotic (in **MedL** and **HighL** treatments) especially near the basal part of the stem, with those in the full depth of waterlogging treatment having approx. 30% of their leaves abscise eventually. It was evident that the slower growing evergreen *Euonymus* was deemed more resilient that the faster growing *Philadelphus* in tolerating water logging during the summer months. Whether this was due to the oxygen demand being greater in the *Philadelphus* roots (e.g. higher respiration rates due to more active cell division and thereby depleted available oxygen more quickly) or some other factor e.g. tolerance to the phytotoxic by-products of anaerobic respiration (ethylene, ACC etc.) is not clear, but warrants further research.

Interestingly, exposure to waterlogging condition in winter for *Philadelphus* and *Euonymus* did not affect both species markedly, with 100% survival rate and greater post flooding recovery in terms of new shoot development. This result therefore rejects the

original hypothesis that winter flooding will cause severe damage to plants. Even prolonging the duration of the waterlogging had limited negative effects on Medium and High level water treatments, as a greater proportion of those buds present developed as durations increased (note the trends between blue, red and green columns within a treatment as durations [but also as progress of the growing season] increases, Figures 5.14 to 5.17). This data is confounded by warmer conditions as the season progressed and the timing when plants were measured for re-growth. Nevertheless, even plants exposed to 28 days of full waterlogging showed a net increase in active buds and developing shoots compared to what had been measured as viable buds at the start of treatment (Figure 5.17). This would suggest plants were relatively unaffected by the waterlogging treatments in winter and early spring.

Soil type and compaction

The research illustrated that specific criteria of urban soil factors could alter responses. Both *Philadelphus* cultivars had poor growth rates when grown in sand or clay, whereas *Euonymus* showed a marginal positive response to the sand based media, under increasing levels of compaction (Chapter 4). It is feasible that the more compacted sand treatment had better water retention/availability than control values for this medium, and that the *Euonymus* was better placed to exploit this, or at least was less negatively affected by some other artefact of the sand e.g. localised nutrient deficiencies, which may have impacted on the more vigorous *Philadelphus*. Possible reasons for the relatively positive response of Euonymus in sand may relate to the fact that the physical nature of the sand means there is still a viable pore structure present even after compaction (Sands et al., 1979), and somewhat counter-intuitive to this, the compression of the sand slows the rate of irrigation water through the substrate (Laboski et al., 1998), allowing better diffusion of moisture into the micro-pores and adhering to individual sand particles.

In addition to altering overall growth potential, substrate type also has some influence over form. Plants grown in sand and clay, irrespective of the level of compaction often

had shorter internodes and smaller individual leaf sizes (more compact habit) than counterparts in the non-compacted organic based medium. In addition, higher levels of compaction also suppress plant height, reduced amount of leaves and produced smaller leaf areas. These results reflect other studies on the effect of soil compaction to plant growth (Sadras et al., 2005, Andrade et al., 1993) where increasing bulk density, increases resistance to root elongation and affects cell division and expansion.

Growth differences between species for example, was evident when the type of substrate they were grown in altered (Chapter 4). *Euonymus* was unaffected by growing in either organic media or sand (at any level of compaction), but was noted to have a reduction in growth and height when grown in clay, for example, with increasing compaction in clay.

Physical Root Injury

Root damage / injury cause by utilities installation was a major concern in the 1980's and 1990's when television cabling companies dug-up roadways and pavements to place cables along streetscapes. Although the extent of damage is not so great today, the placing of cables, pipes and other infrastructure below street level is still a significant threat to trees. Root loss and damage always is associated with poor plant stability particularly in urban trees. This research examined the effect of different severity of root loss in young shrubs on root and shoot development. In contrast to the scenarios around trees where severe root loss can cause plant death within 5 to 8 years (Hauer et al., 1994)., no plant failure due to root loss was recorded in the shrubs specie employed here. From this it may be postulated that older, larger plants such as trees cannot withstand severe root loss in the same manner (proportion) as smaller, younger plants represented by the shrubs here. To some extent, age and the retention of some 'juvenility factor' may help less mature specimens respond to severe damage in a more constructive way. Certainly horizontal root trimming (undercutting) in the field does little damage to young nursery trees, other than provide a temporary check to growth. No equivalent treatment has been implemented on mature trees as far as can be

ascertained, but lateral damage of an equivalent extent in a mature specimen is likely to have a highly detrimental factor. So, age may well be an important factor. There may, however, also be elements fundamental to shrub growth that make them more resilient than trees. By definition they tend to have a greater shoot branching habit than trees (i.e. multi—stemmed from / near the base). Similarly, they may have a greater propensity to develop lateral roots and promote a more branched root system. This may aid recovery once a traumatic injury to the root system has been inflicted. Certainly, even in young trees, once a main primary root is cut the development of side lateral roots from the wound side is not always forthcoming, yet this does to seem to be true of shrubs (Blanusa et al., 2007). Another factor that may put street trees under greater risk from root injury is that they often have huge leaf canopies, so any rapid and significant loss of their root system in spring / summer (If deciduous) will place immediate pressure on water availability to their extensive area of foliage. Failure to supply these large canopies with water (e.g., even a small tree species such as *Betula* may require 300 l water per day) will result in leaf abscission and branch die-back.

In the shrub species studied here, it was observed there was a differential response based on the degree of injury inflicted on the root system. Greatest reductions in shoot and root biomass in *Philadelphus* cv. Aureus was linked with intermediate levels of damage Light+Severe, growth being penalised more than even a Severe+Severe root pruning treatment. The fact that almost half the root system was left intact in the L+S suggests there was not enough of a wound signal to stimulate a radical re-allocation of resources and promote new root and shoot development. Blanusa et al., (2007) found something similar in *Buddleia* and *Cistus*, in that root pruning (severe injury) encouraged more root growth than root teasing (light injury) when plants were being planted out into the landscape. The mechanisms behind this are not well understood. Is there a hormonal signal activating the response to wounding, and does the wounding need to be about a certain threshold before this signal is induced or become effective? Alternatively are the promoting factors for new root / shoot generation promoted simply buy a lack of nutrients / water brought on by the much depleted root system? In the case of the L+S treatment, perhaps the lightly pruned side is sufficiently functional to meet the plants entire requirements for water and nutrients, and no regeneration of

roots on the severe side is required. In contrast, in the context of street trees, root loss in one side is enough to cause damage and urban environmental stresses (small volumes of soil and often poor quality of soil) would make this damage worse and limit potential to rejuvenate.

Reduction of shoot and root growth in L+S treatment also may be related to more efficient water use in plants which might be caused by limited water uptake to shoots. This response is similar to partial root drying (PRD) where half of the root zone were irrigated while the other half were left to dry out (Cameron et al. 2006; 2008). In grapevines this PRD technique could reduce the plant vigour but increase the quality and yield of fruits as well as research done in tomato plants (*Lycopersicon esculentum* Mill.) where there were reductions in fruit number but not fruit biomass and diameter (Stikic, et al., 2003).

Data from *Philadelphus* where fertilizer was added to the severely damaged side of the root systems in summer (Figures 6.29-6.32), supports the notion that root regeneration is stimulated by negative feedback mechanism (not enough nutrients / water to support shoot growth). This resulted in new shoot growth (required for photosynthetic energy capture) but not much new root growth (nutrients freely available, so no stimulus for root extension and division). This has implications for remedial treatments for plants in the landscape – adding fertiliser to damaged root systems may help support new shoot development and help ensure the plant remains competitive in terms of carbon capture, however a lack of new root growth deep into or across the soil profile may undermine the ability to extract water when it is required. Eventually, plants might be over-produce shoot biomass but with a resultant failure of roots to accommodate demands from the shoots in future. (Gilman et al., (1996) suggested that applying generous amounts of irrigation after root pruning will help plants produce both more root and shoot growth rather than just applying fertilizer alone or indeed applying fertilizer with irrigation.

The differences in response across the genotypes was again evident in the root pruning experiments. Being a slower growing, evergreen species seemed to provide *Euonymus* with some advantages when it encountered root loss. No significant response were

observed in root pruned *Euonymus* for both seasons with overall, plants somewhat coping with the stresses imposed. This perhaps can be explained by a better ability to survive low nutrient condition, as well as a slower metabolism (slower growth) that allows it to cope with abiotic stress (Mooney and Rundel, 1979, Grime, 1977, Chapin, 1980).

7.3 Application in landscape design

It is anticipated that results from this research will be useful to landscape designers as they attempt to match appropriate species choice to urban localities, or to modify the conditions plants are placed under. As most existing urban soil conditions reduce plant growth, developing protocols that optimise soil condition have to be taken into consideration e.g. minimal planting space for optimum root growth, soil moisture provision and proper drainage for maximum water and nutrient uptake as well as alteration of topsoil physical properties. According to results obtained from this research, larger and deeper planting areas are best for rooting in shrubs. For example, 1m X 1m X 1m planting space is ideal for planting shrubs in rather than 0.5m X 0.5m X 1.0 m area.

Furthermore, improvement of soil aeration in compacted soil will do much to aid drainage and promote root penetration for urban soils, allowing for effective plant development. This could also reduce the risk of waterlogging which was shown to be so harmful to plants (*Philadelphus*) during the warmer summer months. Scheduled maintenance and installation of new infrastructures also could help to minimize root damage impacts as root damage in autumn / winter proves to have the lower impact on plants. This is due to the dormant phase where roots are under less pressure to supply the developing shoots with resources, and so can preferentially supply resources towards new root regeneration (this assumes that reserve carbohydrates are not in short supply).

Appropriate species selection for urban landscape also plays an important role for plant establishment. Species with lower resistant towards environmental stresses could survive longer and in this research, evergreen species like *Euonymus* appear a better choice than *Philadelphus* due to their slow growth and ability to survive low nutrient soil condition. However, different cultivar may have different adaptation and resistance towards these stresses and future research in species with different cultivar could help in better species selection.

7.4 Recommendation for future research

This research was conducted to better understand the responses and inter-relationships between shoots and roots in young shrubs species when exposed to suboptimal conditions in urban setting. Evidence provided here is based on mimicking artificial urban environmental conditions using glasshouses which may of course, provide different results to full scale experiments genuine urban settings. Nevertheless this work had the advantage of 'teasing out' individual stress factors and studying these in isolation, which is not always feasibly in the field. Eight experiments conducted to cover four different stress factors, but these were conducted over relatively short periods of time, and further experiments are required to more closely match conditions *in situ* and which are maintained over more realistic durations. This will make the findings in this thesis more robust and applicable to practical applications.

Nevertheless, the results provided here begin to highlight the complexities of fitting species to urban conditions. Factors such as genotype, soil type / condition, degree of stress imposed all have large and significant impacts on how plants respond. Therefore this data helps to set the scene for further work, and be used to investigate further work in a real urban settings with a better comprehension of the main factors influencing responses, and some understanding of the variations in genotypic response /adaptation to urban environmental stress. Indeed, future work will need to evaluate further species / genotypes traits to help identify truly resilient species (able to cope with existing environmental conditions, but also those in future brought on by climate change). Trials

involved a larger selection of species and varieties with in situ plots are warranted. This will help practical guidelines for practitioners to use in consideration of species selection and planting requirements. In addition, the differences between growth rates (slow growing, moderate growing and fast growing), final plant product and survival rates in different species will help to recognise the advantages or disadvantages of their adaptation in landscape establishment and aesthetic values.

In addition, research done in understanding the effect of soil compaction to plants in Chapter 4 with different result between Exp. 4a and 4b explained that there might be differences in the soil bulk density. Measuring the bulk density using penetrometer in future research will help to understand more about how compacted soil affect plant growth as well as will guide researcher in setting up the experiment especially in differentiating the treatments.

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