WATER USE AND PRODUCTION POTENTIAL OF KAROO SHRUBS

by

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DECLARATION

I declare that the thesis hereby submitted by Paul Johannes Malan for the degree Philosophiae Doctor at the University of the Free State, is my own independent work and has not previously been submitted by me at another University/Faculty. I further cede copyright of the thesis in favor of the University of the Free State.

Signed: ……………………………..
Paul Johannes Malan

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Proverbs 3: 5 & 6: Trust in the Lord with all your heart, and lean not on your own understanding; in all your ways acknowledge Him, and He will make your paths straight.

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ABSTRACT

Water use and production potential of Karoo shrubs

Variation in, and changing of climatological patterns, especially rainfall, as well as grazing intensity and frequency has the biggest influence on rangeland productivity and sustainable animal production in the Nama-karoo Biome. This study was conducted to quantify whole-plant productivity, nutritive value and morphological adaptations of Karoo shrubs to defoliation along a soil-water deficit gradient. Two Karoo shrubs, *Nenax microphylla* and *Pentzia incana* were investigated. The watering treatments included the following: 0 - 25% depletion (non-stressed), 25 – 50% depletion (mildly stressed), 50 – 75% depletion (moderately stressed) and 75 – 100% depletion (severely stressed) of field capacity. The defoliation treatments were defoliation intensity to a height of 50 mm, 125 mm and 200 mm; and defoliation frequencies of three-monthly, six-monthly and twelve-monthly which was also used as the control. Water availability proved to be the single most important factor influencing both above- and belowground rangeland productivity. Defoliation intensity had the lowest impact on productivity, while the impact of defoliation frequency was markedly higher on both above- and belowground phytomass production. The root:shoot ratio increased with increased water deficit as a means to improve the water absorption of the shrubs. Determination of water-use efficiency (WUE) included both above- and belowground phytomass, while it excluded evaporation which gave a more accurate estimation of WUE. This is of the few studies where root growth is also included in calculating WUE. The expression of WUE in terms of transpiration as was done in this study, is more sensitive for describing ecosystem functioning than evapotranspiration, as was done in most studies in the past. In general, the WUE of the shrubs increased when exposed to water stress and higher grazing pressure. The more frequent and intensely the plants were defoliated, the higher the nutritive value of the produced edible phytomass. The quantity of this produced phytomass was, however, very low. The increased CP (N-content) of the water stressed plants could have contributed to the increased WUE. Strong evidence of compensatory growth and WUE were recorded for both species. This compensatory ability especially enabled the shrubs to display increased recovery after defoliation when water is not limited. Water stress had no marked influence on the reproductive ability of the investigated shrubs. It was, however, proved that both defoliation frequency and intensity had a bigger influence on seed production and germination percentage, than water stress. *Pentzia incana* has a high density of reflective trichomes that provides protection against heat and solar radiation. It also has a high stomatal density which allows increased photosynthetic rates when growth conditions are favourable. The stomata of *N. microphylla* occur only on the abaxial (lower) side of the leaf which protects it from direct sunlight and heat. It also has a very high stomatal density which could contribute to its ability of compensatory growth when adequate water is available. Furthermore, the leaves have a shiny appearance which enables them to reflect solar radiation to reduce leaf temperatures. This study highlighted the complexity of the effect of external influences, like rainfall and grazing (defoliation) on the functioning of the rangeland ecosystem in the arid and semi-arid Nama-karoo Biome. Although the land user does not have control over plant water availability, control over defoliation is possible. Defoliation therefore should be the most important key aspect in sustainable ecosystem utilization.

Keywords: Above- and belowground phytomass, compensatory growth, defoliation intensity and frequency, leaf morphology, nutritive value, water-use efficiency

UITTREKSEL

Waterverbruik en produksiepotensiaal van Karoo bossies

Variasie, en verandering in klimaatspatrone, veral reënval, sowel as intensiteit en frekwensie van beweiding het die grootste invloed op weiveldproduktiwiteit en volhoubare diereproduksie in die Nama-karoo Bioom. Hierdie studie is uitgevoer om die geheel plant se produktiwiteit, voedingswaarde en morfologiese aanpassings van Karoo bossies tydens ontblaring en blootstelling aan ‘n grondwaterstremming gradiënt te kwantifiseer. Twee Karoo bossies, naamlik *Nenax microphylla* en *Pentzia incana* is ondersoek. Die waterbehandelings het die volgende ingesluit: 0 - 25% versadiging (nie gestrem), 25 - 50% versadiging (effens gestrem), 50 - 75% versadiging (redelik gestrem) en 75 - 100% versadiging (volkome gestrem) van veldkapasiteit. Die ontblaringsbehandelings het ingesluit, intensiteite van 50 mm, 125 mm en 200 mm hoogtes; en ontblaring frekwensies van drie-maandeliks, ses-maandeliks en twaalf-maandeliks wat ook as die kontrole beskou is. Beskikbaarheid van water is die enkele faktor wat die grootste impak op beide bo- en ondergrondse weiveldproduktiwiteit gehad het. Ontblaring intensiteit het produktiwiteit die minste beïnvloed, terwyl die impak van ontblaring frekwensie merkbaar hoër is op beide bo- en ondergrondse fitomassaproduksie. Die wortel:stingel verhouding het toegeneem met toename in waterstremming om sodoende die water opname van die bossies te verbeter. Vir die berekening van waterverbruiksdoeltreffendheid (WVD) is beide die bo- en ondergrondse fitomassa gebruik, terwyl evaporasie ook uitgeskakel is, wat sodoende ‘n meer akkurate WVD bepaling tot gevolg het. Hierdie is een van die min studies waar wortelproduksie tydens die bepaling van WVD in berekening gebring is. Die uitdrukking van WVD in terme van transpirasie, in plaas van evapotranspirasie soos in meeste studies in die verlede uitgevoer, lewer ‘n akkurater bydrae tot ekosisteem funksionering. Oor die algemeen is daar ‘n toename in WVD indien plante aan waterstremming en beweidingsdruk blootgestel is. Die vreetbare plantmateriaal se voedingswaarde het toegeneem met toename in intensiteit en frekwensie van ontblaring. Die hoeveelheid geproduseerde vreetbare materiaal is egter baie laag. Die toename in ru-proteïn (N-inhoud) van die watergestremde plante het moontlik bygedra tot verbeterde WVD. Sterk tekens van kompensatoriese groei en WVD is waargeneem vir beide plant spesies. Hierdie kompensatoriese vermoë stel die plante in staat tot versnelde hergroei na ontblaring, mits voldoende water beskikbaar is. Waterstremming toon geen merkbare invloed op die reproduksievermoë van die plante nie. Beide frekwensie en intensiteit van ontblaring toon ‘n groter invloed op saadproduksie en persentasie ontkieming as die geval met waterstremming. *Pentzia incana* beskik oor ‘n hoë digtheid weerkaatsende trigome wat die plant teen hitte en bestraling beskerm. Die plant het ook ‘n hoë huidmondjie digtheid wat versnelde fotosintese onder gunstige klimaatstoestande toelaat. By *Nenax microphylla* kom huidmondjie slegs op die abaksiale (onder) kant van die blare voor, wat dit teen hitte en direkte sonlig beskerm. Hierdie plant het ook ‘n baie hoë huidmondjie digtheid wat waarskynlik bydrae tot die kompensatoriese groeivermoë wanneer voldoende water beskikbaar is. Die blare van *N. microphylla* het ‘n blink voorkoms wat dit in staat stel om sonstrale te weerkaats en sodoende blaartemperatuur te verlaag. Hierdie studie het die kompleksiteit van die effek van eksterne faktore, soos reënval en beweiding op die funksionering van die weidingekosisteem in die ariede en semi-ariede Nama-karoo Bioom, beklemtoon. Alhoewel die boer nie beheer oor water beskikbaarheid vir plante het nie, kan ontblaring (beweiding) wel beheer word. Ontblaring is dus die mees belangrike sleutel aspek in die volhoubare benutting van die weidingekosisteem.

CHAPTER 1

INTRODUCTION

1.1 General

The mechanisms behind, and the quantification of ecosystem responses to global environmental change, is a central theme in today's ecological research (Reed et al. 2012; Ruppert and Linstadter 2014). Human actions, causing ecosystem and biodiversity declines, created an increasing worldwide need to assess environmental changes (Yao and Xinzhi 2014). Our understanding of how the dynamics and structure of the world's dryland ecosystems (roughly 40% of Earth's terrestrial landmass) will respond to changing climate and land use is still surprisingly poor (Thornton et al. 2009; Maestre et al. 2012). Drylands comprise arid, semi-arid and dry-subhumid ecosystems, which are characterized by water-deficiency during prolonged dry periods (Asner and Heidebrecht 2005). Low and highly variable precipitation mainly directs plant growth (Ruppert et al. 2012), which leaves no other land use option other than extensive livestock production. The larger portion of these drylands is used as rain-fed rangelands (MEA 2005), where livelihood security mainly relies on livestock production off the forage resources from natural vegetation.

With the relatively new debate on the potential effects of global warming (Ash et al. 2012; Dai 2013), the need for water utilization studies in arid and semi-arid areas becomes strategically important. DeMalach et al. (2014) noted an increasing frequency in dry seasons in the Negev Desert in Israel, while Dai (2011) predicts that precipitation may become more intense but less frequent under greenhouse gas induced global warming. This is predicted for most of Africa, most of the Americas, Australia, Southeast Asia, southern Europe and the Middle East. Such adverse conditions might increase and surpass current coping mechanisms, forcing farmers to implement more innovative measures to counter heightened risks (Muller and Shackleton 2014). A better understanding and unlocking of current responses of productivity to climate variability in arid lands, might be useful to predict future responses of these systems to environmental change (Xia et al. 2010).

Roughly 70% of agricultural land in South Africa can only be utilized by game and livestock (Meissner et al. 2013). The Karoo ecosystem accounts for 31% of the total surface area of South Africa (Rutherford and Westfall 1986), which is described as arid to semi-arid. In these ecological sensitive areas, the main farming activity is extensive livestock production. The unpredictability of the weather, together with the unique vegetation of the region, makes livestock farming more challenging in the Karoo than in other parts of South Africa (Esler et al. 2006). Meissner et al. (2013) express their concern about the losses of natural systems and biodiversity of most rangeland areas of South Africa due to rangeland miss management.

More or less 65% of South African rangelands receive less than 500 mm rainfall per year and drought is more the rule than the exception (Snyman 1998). In these areas where rainfall is one of the limiting factors influencing plant production, understanding the water use of native plants is critically important. According to Blignaut et al. (2009), the average rainfall of South Africa during the 2000$^{'s}$ was 6% lower than in the 1970$^{'s}$, while Meissner et al. (2013) also describes South Africa as a water scarce country. Muller and Shackelton (2014) emphasize that other factors like an increase in the frequency of droughts and more prolonged dry periods, will have major implications for future farming activity in South Africa. With the above as background, there is a serious need for intensive water balance studies to ensure sustainable animal production for specifically the drier rangeland areas. The more one knows about how this extraordinary ecosystem works, the better one can adapt the management to avoid drought disasters and veld degradation and maintain animal productivity without loss of species diversity and natural resources (Esler et al. 2006).

1.2 Water balance studies

In South Africa, intensive water balance studies were conducted over the past three decades (Opperman 1970 to 1983, and more recently by Oosthuizen 2003, Venter 2003, Snyman 2000 to 2009 and Marais 2005), on different grass species and over different veld condition stages for arid and semi-arid climates (Opperman 1975; Oosthuizen and Snyman 2003a, b; Venter 2003; Marais 2005; Snyman 2000, 2009a, b). Water balance studies on *Opuntia* species, a South American succulent sometimes planted for fruit production or as a green forage bank, were also conducted by Ramakatane (2003) and Snyman (2006, 2013, 2014a). By contrast, to date very little research has been done on specific water use-efficiency (WUE) of Karoo shrubs of the Nama-karoo. Water use-efficiency entails the amount of water used to produce plant phytomass. Although some water stress studies were done on different Karoo shrub species (Gerber 1993; Midgeley and Moll 1993), to date their WUE has not yet been quantified. On a landscape scale, Palmer and Yunusa (2011) quantified the WUE of the vegetation of the Northern Cape Province, which included parts of both the Nama-karoo and Savanna Biomes. They used remote sensing techniques to predict standing green biomass and to estimate actual evapotranspiration, which were used to calculate annual WUE for the whole vegetation component.

A thorough understanding of the dynamics of these dry Nama-karoo ecosystems and more specifically water utilization and adaptability of shrubs is lacking. If the water utilization of some key Karoo shrubs can be quantified, it can contribute to the conservation and sound management of these arid and semi-arid ecosystems for future sustainable animal production (Esler et al. 2006). Water utilization quantification for different Karoo shrubs might serve as an important indicator for quantifying WUE of these plants. Such data can be successfully used to compare the water use of different Karoo shrubs, as well as that of other fodder crops, for implementation in veld condition assessment studies (Venter 2003). This information can also be incorporated into grazing management strategies and drought assessment models for the arid and semi-arid grassland and Karoo shrubland areas, that can be applied at farm level (Lane and Stone 1983).

The National Livestock Strategy highlighted the future importance of the livestock sector, focusing on its contribution to the national economy, food security and rural development (Meissner 2006; Meissner et al. 2013). One of the important aims of this strategy, which is also applicable to Karoo vegetation and was also highlighted by Esler et al. (2006), was to ensure that land use practices do not result in over utilization of the natural resource, but follow sound management and sustainability directives. The Nama-karoo, as the second largest biome in South Africa (Low and Rebelo 1996), represents a large portion of the 80% of agricultural land in South Africa, where stock farming is the main land use enterprise. Given the fact that there is a constant increase in the demand for animal products in southern Africa (Fynn 2012; Meissner et al. 2013; Snyman 2014b), the dry Nama-karoo has an important role to play in this regard. As rainfall is the single most limiting factor for vegetation production in these arid and semi-arid areas, higher animal production can only be realized through better genetic (animal) material, combined with an increase in fodder production through more efficient water utilization by the correct fodder plant. The complexity of the interaction between grazing and rainfall variability (annual and seasonal) is also an important factor to bear in mind for sustainable utilization of the ecosystem (O'Connor and Roux 1995). Due to constraints and limitations of the natural agricultural resources (Kraaij and Milton 2006), and an increase in environmental degradation, especially a decrease in the density of palatable plants (Milton and Hoffman 1994), an unassisted increase in fodder production is highly unlikely. However, Wilcock et al. (2004), van den Berg and Kellner (2005), as well as Visser et al. (2007) demonstrated that restoration of degraded rangelands in the Nama-karoo is attainable through revegetation, over sowing, ripping and brush packing.

Although du Toit (1986) studied water infiltration and soil compaction of the Eastern Mixed Karoo under different grazing pressures, he did not evaluate its direct effect on the vegetation itself. In a Succulent Karoo study (Rundel et al. 1999), some aspects concerning the WUE of some vascular plant species were quantified. The finding was that WUE not only varied between and within species, but also between seedlings and mature shrubs as well as between sites along an aridity gradient. It can be postulated that a combination of opportunistic water-use patterns, with water-storage capacity (in succulents) or drought tolerance (in non-succulents) may have a powerful effect on Karoo plant community structure and composition (Dean and Milton 1999).

1.3 Defoliation of Karoo shrubs

Management of the Karoo ecosystem for sustainable animal and plant production requires the application of the correct frequency and intensity of defoliation. Unfortunately scientific studies on this aspect are limited. The few defoliation studies on Karoo vegetation were discussed in detail by Bosch (1987), while methods for the scientific measurement of shrub defoliation were tested by Hobson (1988). Hobson (1989) demonstrated from his study of different intensities and frequencies of defoliation of Karoo shrubs, that the more severely plants are defoliated, the less frequently they can tolerate such heavy defoliation. It was also argued that seedlings up to a certain age are negatively influenced by defoliation. This emphasizes the importance of taking plant age, intensity and frequency of grazing in consideration before conducting defoliation studies. Transpiration after defoliation at different soil-water levels of Karoo plants was investigated by du Preez (1964) and also highlighted the importance of sound scientific rangeland management.

Hobson (1985) and Gerber (1993) both used the Karoo shrub, *Pentzia incana*, in their defoliation studies. It was shown that the more severely *P. incana* is defoliated; the longer the recovery period required before a subsequent defoliation. Van der Heyden and Stock (1996) found that the Karoo shrub, *Osteospermum sinuatum*, depends on growth reserves for the first two weeks after defoliation, after which leaf growth relied on photosynthesis by new and remaining leaves. On the other hand, water stressed plants would not regrow easily as photosynthesis cannot take place due to the closed stomata of wilted leaves (Letts et al. 2010). Such stressed plants will therefore have to rely much more on stored growth reserves. However, Gerber (1993) indicated that water stressed *Pentzia incana* plants could utilize all excess non-structural carbohydrates reserves from above- and belowground sources, during regrowth. There was, however, a lack of extension growth due to insufficient turgor pressure.

Gerber (1993) argued that the complexity of this kind of defoliation studies on Karoo shrub species is confounded by too many variables. This might explain why very little research has been done on individual species in recent time. Van der Westhuyzen and Joubert (1983) found that defoliation of *O. sinuatum* during anthesis led to a reduction of carbohydrate content of the remaining flowering organs, with an increase in the remaining foliage for the production of new photosynthetic material. This phenomenon was more pronounced under deteriorating climatic conditions. Stock et al. (1993) explained the responses of Karoo shrubs to simulated browsing to be the result of passive alterations in plant chemistry rather than as active defense responses to herbivores as found in some woody species. According to van der Heyden and Stock (1995), it appears that regrowth of semi-arid shrubs in response to browsing, is not limited by growth reserves, but by availability of resources like water and nutrients.

Van der Heyden et al. (1999) found that certain ecophysiological browsing responses provide evidence that some unpalatable Karoo shrubs, like *Pteronia tricephala*, respond mechanistically to heavy browsing in such a way that their survival is guaranteed. Other Karoo shrubs, like *Eriocephalus ericoides* on the contrary, have no such compensatory mechanisms and it appears that heavy browsing might weaken their survival ability. It was found that the Karoo shrub *O. sinuatum* responds to heavy browsing by rapid regrowth which is a sign of compensatory growth, only when enough water is available (van der Heyden and Stock 1995). In terms of animal grazing, du Toit (1996) highlighted the importance of browsable shoot diameter that should be taken into account during defoliation studies where edible dry matter is to be measured.

More recent literature, like that of Beukes et al. (2002) and Kraaij and Milton (2006) focused more on the influence of defoliation on all species present in the Karoo plant community as a whole. Changes over time in plant composition and cover of shrub vegetation, might be explained by annual and short term rainfall, rather than by grazing impacts (Beukes and Cowling 2000). This indicates the importance of understanding water use of individual Karoo bush species. On the other hand, *Pentzia incana*, one of the species included in this study, showed resilience to high grazing intensities together with signs of compensatory growth (Gerber 1993). By contrast, some grass species showed a decline in photosynthetic growth and WUE at an increased level of grazing (Peng et al. 2007). Different *Indigofera* species can react differently to water stress conditions, which also include the root mass fractions (Hassen et al. 2007). Investigations on root development of fodder plants are becoming increasingly important as was discussed by Smit (2005). For example, Hobson and Sykes (1980) reported on the root development of three Karoo shrub species (*Eriocephalus ericoides*, *Felicia muricata* and *Pentzia incana*) under different defoliation treatments. They indicated that no significant difference in root mass and volume was found between a 14 day and a 60 day defoliation treatment, while both were significantly less than that of no defoliation. The above- and belowground dry matter production of *Opuntia* spp. (Snyman 2006, 2013, 2014a) and rangeland in different condition classes (Snyman 2005; Oosthuizen et al. 2006) were studied in a semi-arid climate of South Africa, where the importance of including belowground dry matter production in WUE studies were highlighted. Palacio and Montserrat-Martí (2007) also highlighted the importance of water availability on the dynamics of Mediterranean shrub root growth, as well as the relation between shoot and root growth processes.

1.4 Aim and justification

Through investigating the WUE and reaction of indigenous plant species of the Nama-karoo Biome to defoliation, decision making regarding sustainable management of this ecosystem might be improved. Therefore, in this study the influence of defoliation was combined with different water regimes. This might relate the influence of water stress to the above- and belowground plant production of Karoo plant species under different defoliation treatments. According to reviewed literature, to date this aspect of Karoo plant dynamics has not been fully studied. The outcome of this study may give direction to future investigations where different Karoo shrub species could be incorporated into water balance studies to quantify the dynamics concerning WUE of Karoo vegetation. The results may also contribute to a more scientific rangeland management approach under different climatic conditions for Nama-karoo ecosystems. By managing rangeland to conserve certain plant species, the species richness of the Nama-karoo can be protected, an improvement of rangeland condition can take place and at the end ensure sustainable plant and animal production.

In response to the lack of scientific information the following research questions were set to investigate the dynamics of two keystone Nama-karoo shrubs, namely: *Nenax microphylla* (Sond.) Salter and *Pentzia incana* (Thunb.) Kuntze, following different water and defoliation treatments:

How efficiently can Karoo shrubs utilize water following a soil-water gradient in terms of productivity (above- and belowground phytomass), WUE and nutritive value of Karoo shrubs?

Does intensity and frequency of defoliation influence the productivity (above- and belowground phytomass), WUE and nutritive value of Karoo shrubs?

How do Karoo shrubs adapt to the interaction between different combinations of defoliation and water availability?

Can water stress and defoliation influence the reproductive potential of Karoo shrubs?

How does leaf morphology of Karoo shrubs influence the adaptability of these plants to water stress?

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CHAPTER 2

LITERATURE STUDY

2.1 Introduction

The Nama-karoo Biome with its diverse ecosystems and complex vegetation structure has been under investigation for many decades (Henrici 1931, 1948, 1951, Scott and van Breda 1937a, Roux 1966, 1976, 1981, Acocks 1976, Hobson 1983, Midgley and Bosenberg 1990, Milton 1990, Milton and Dean 1995, O'Connor and Roux 1995, Dean and Milton 1999, Beukes and Cowling 2000, Esler et al. 2006, Kraaij and Milton 2006, Todd 2006, Seymour et al. 2010, Rutherford et al. 2012, Masubulele et al. 2013, 2014). The two most important factors that play a role in the sustainability of these ecosystems are rangeland utilization (defoliation by livestock and game) and influence of climate (mainly rainfall related), (Figure 2.1). The combination of these two factors could be an advantage for better functioning of the ecosystem, but unfortunately is mostly detrimental to both the sustainable vegetation production (Kraaij and Milton 2006), and to the resilience of the vegetation (Seymour et al. 2010). Currently there is an increasing demand for red meat production due to the growing world population (Meissner 2006, Meissner et al. 2013), while the impending threat of climate change, desertification and global warming put serious pressure on our natural resources (Dai 2013, DeMalach et al. 2014). A better understanding of the water utilization, adaptability and productivity of Karoo vegetation under variable water availabilities and defoliations, might contribute to better future management of the Nama-karoo biome.

In arid and semi-arid rangelands, rainfall is one of the most limiting environmental factors, where a drought is more the rule than the exception (Snyman 1998). Synergistic interactions between drought and grazing could therefore contribute to further rangeland degradation (Milton and Hoffman 1994). Investigations to better understanding of the dynamics of the Nama-karoo vegetation, especially the shrub component, are essential for sustainable utilization of these drier ecosystems.

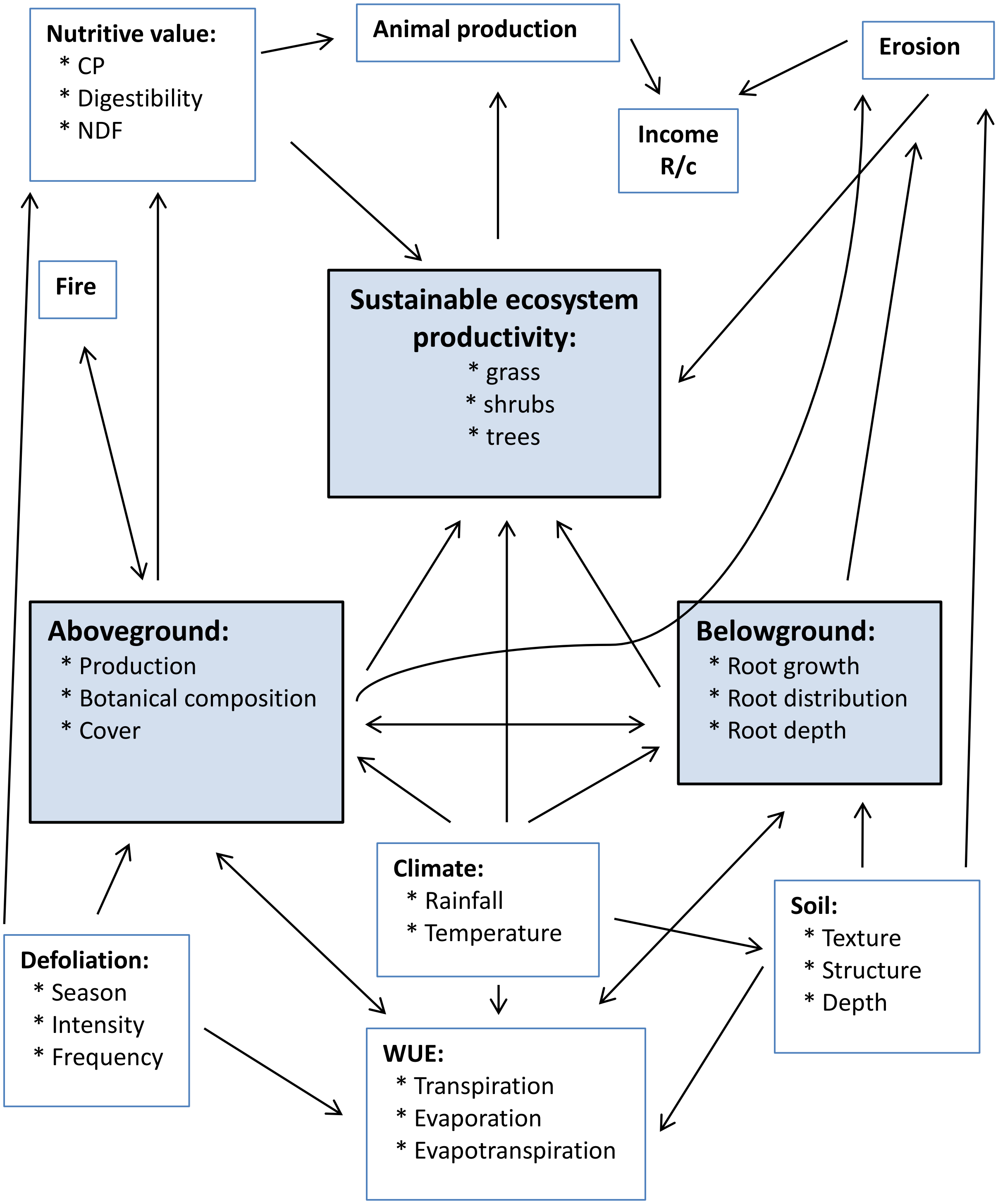
Nutritive value:
* CP
* Digestibility
* NDF
Animal production
Erosion
Income
R/c
Fire
Sustainable ecosystem productivity:
* grass
* shrubs
* trees
Aboveground:
* Production
* Botanical composition
* Cover
Belowground:
* Root growth
* Root distribution
* Root depth
Climate:
* Rainfall
* Temperature
Soil:
* Texture
* Structure
* Depth
Defoliation:
* Season
* Intensity
* Frequency
WUE:
* Transpiration
* Evaporation
* Evapotranspiration

Figure 2.1: Interaction of all plant, soil and climate parameters with the grazing ecosystem, to ensure sustainability.

The productivity of arid-land plants might increase with rising concentrations of atmospheric carbon dioxide (CO_2) as a result of enhancement in plant water-use efficiency (WUE) (Housman et al. 2006). Quantification of water utilization of different Karoo shrubs will give an indication of the WUE of these plants, as well as of the whole ecosystem. Such information can be used to compare the water use of different Karoo shrubs with each other and also with that of other fodder crops. This information can also be incorporated into grazing management systems of the arid and semi-arid areas of South Africa, to ensure sustainable animal production.

2.2 Nama-karoo Biome

The well-known ecologist John Acocks subdivided the Karoo into 46 veld types (Acocks 1988). Parts of what Acocks described as the Karoo were later subdivided by Low and Rebelo (1996) into the Thicket Biome, Succulent Karoo Biome, Fynbos Biome and Nama-karoo Biome. They further subdivided the Nama-karoo into 10 sub-regions. Low and Rebelo (1996) described the vegetation of South Africa within different Biomes in detail. The latest subdivision by Mucina and Rutherford (2006) shows 16 different types for the Nama-karoo Biome. The map in Figure 2.2 gives an indication of the specific boundaries of the Nama-karoo Biome.

The Nama-karoo Biome is the second largest Biome in South Africa (248 284 km^2) and altitudes range from 500 to 2000 m. Most of the rain falls in the summer, especially late summer and varies between 100 and 520 mm per annum. The vegetation is diverse and dominated by dwarf shrubs and grasses, while succulents, geophytes and annual forbs also occur (Low and Rebelo 1996, Mucina and Rutherford 2006).

Vegetation change over time is a continuous topic of discussion (Roux and Vorster 1983, Cowling and Roux 1987, O'Connor and Roux 1995, Kraaij and Milton 2006, Masubelele et al. 2013, 2014). This change mostly entails a shift in dominance between grass and karoo shrubs, of which the percentage species composition is driven by a combination of rainfall and herbivory by both livestock and game. Rainfall arguably has the greatest impact on vegetation change, but its impact is further influenced by grazing treatments, especially over long time periods (Roux 1966, O'Connor and Roux 1995). Above normal summer rainfall favours grass growth and establishment, while higher autumn rainfall favours karoo shrub growth. With global warming a reality, there is the perception amongst farmers and scientists that the normal rainfall distribution might have changed over years to be more favourable to grasses than shrubs in the Nama-karoo Biome – a reason for concern. Summer overgrazing of the grass component causes deterioration of the grass stand with a shift to shrub dominance of the vegetation (Milton and Dean 1996).

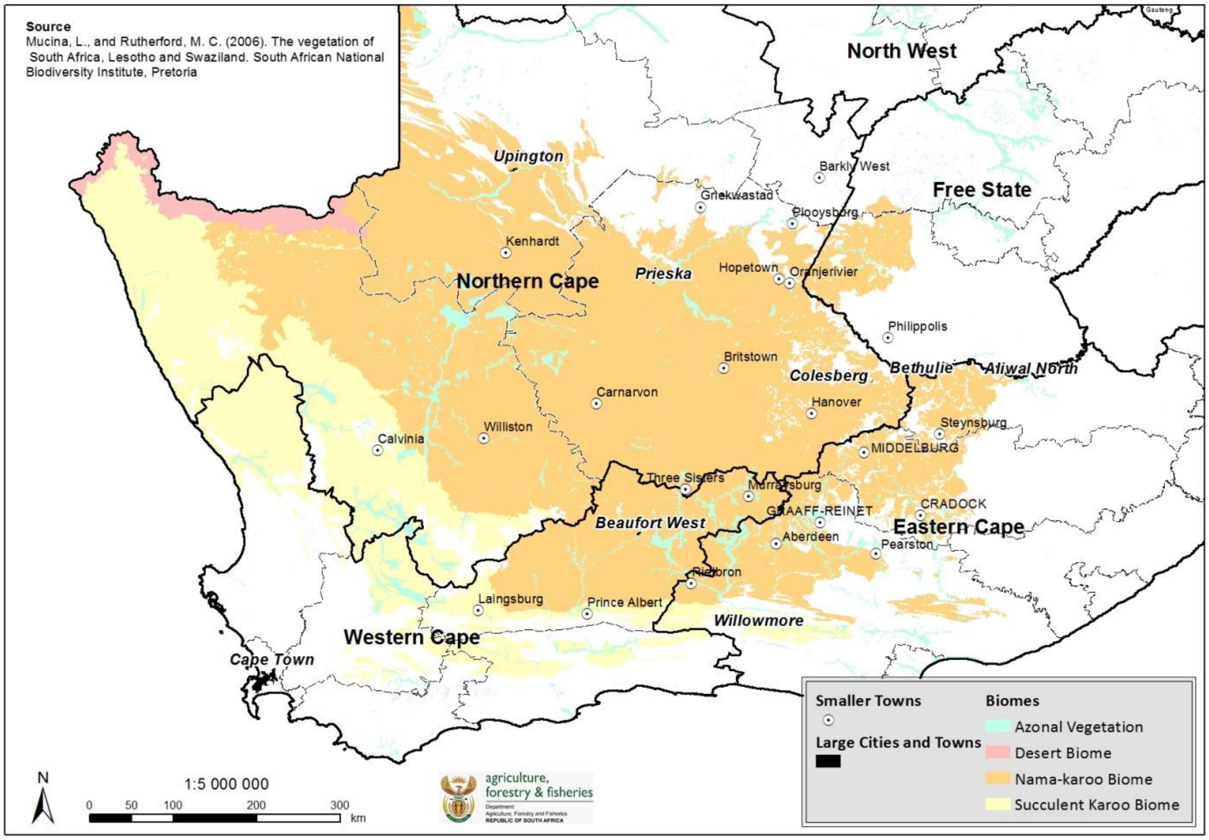
Source
Mucina, L., and Rutherford, M. C. (2006). The vegetation of South Africa, Lesotho and Swaziland. South African National Biodiversity Institute, Pretoria

Figure 2.2: Larger Karoo area of South Africa.

Climatic influence, like rainfall and fire (lightning), might have a greater effect on species composition and their productivity than management aspects (Hart and Norton 1988). This might, however, differ between higher and lower rainfall areas. By contrast, Koerner and

Collins (2014) argued that grazing, fire and climate (rainfall) are of equal importance in controlling mesic grassland ecosystems. On the contrary, Rutherford et al. (2012) highlight that over the past decades it was proved that rainfall had a greater effect in controlling plant growth and species composition in the Nama-karoo, than grazing. Du Toit et al. (2015) discussed the enormous negative impact of unplanned fire on the shrub component of the Nama-karoo in favour of the grass component (Figure 2.1). Hanke et al. (2014) also explains that inter-annual variability in species richness in arid rangelands of Southern Africa is mainly driven by precipitation variability. The projected future higher rainfall variability (Dai 2011) could further increase these discussed fluctuations, which can be exacerbated by improper grazing management.

As discussed above, in both conservation and agricultural communities, the environment is seldom optimal for plant growth, with a clear interaction between the different components of the ecosystem (Figure 2.1). Environmental stress limits the overall productivity of rangelands to its full potential. The Nama-karoo Biome especially, experiences large seasonal fluctuations in soil-water and nutrients, often to levels sub-optimal for plant growth (Kraaij and Milton 2006). Grazing adds another component where the plant is continuously encountering new combinations of environmental stresses (Dean and Milton 1999). The nature of control over plant growth is of particular interest, because these are the only habitats into which agriculture can expand in most developing countries and impending global climate change will alter the suitability of most terrestrial habitats for plant growth (Chapin 1991). Consequently, we need to understand the ecological and physiological mechanisms that enable plants to survive and reproduce under suboptimal conditions (Esler et al. 2006). It is therefore important to quantify the adaptability of different karoo shrubs to these changeable environmental factors, as well as the impact of utilization on their productivity. A better understanding of the ecosystem functioning in specifically these drier areas is essential to ensure future sustainable animal production (Figure 2.1).

2.3 Water utilization and water-use efficiency of arid land vegetation

Investigations on rainfall to productivity relationships began as early as the 1930's in the USA and have become increasingly popular since the 1960's (Le Houérou 1984). Le Houérou (1984) defines WUE as how efficiently the individual plant or landscape uses precipitation to produce a certain amount of biomass, while Golluscio and Oesterheld (2007) defines it as the amount of C fixed per unit of transpired water. Over the years WUE for fodder crops was expressed in different ways, which included mainly: production (above- and belowground) ha^{-1} or $plant^{-1}$ for the amount of water (mm or g) used (evaporated, transpired or evapotranspired) (Table 2.1).

Table 2.1: Expression of WUE in different ways, as well as the scale of investigation.

Author	Expression
Landscape scale	
Le Houérou (1984)	g kg^{-1} DM (rain-use efficiency)
Snyman (1989, 1994, 1999, 2000, 2005, 2009a)	kg DM ha^{-1} mm^{-1} yr^{-1} (evapotranspiration)
Snyman (1994, 1999)	kg CP mm^{-1} ha^{-1} yr^{-1} (evapotranspiration)
Marais et al. (2006)	kg DM ha^{-1} mm^{-1} (transpiration)
Palmer and Yunusa (2011)	kg DM mm^{-1} ha^{-1} yr^{-1} (evapotranspiration)
Individual species	
Le Houérou (1996)	Units of DM per 1000 units of water: mg g^{-1}, g kg^{-1}, kg t^{-1}
Xu and Li (2006)	mmol CO_2 $mmol^{-1}$ H_2O (transpiration)
Emmerich (2007)	g CO_2 mm^{-1} (evapotranspiration)
Golluscio and Oesterheld (2007)	C per water transpired
Hassen et al. (2007)	g DM kg^{-1} water used (transpiration)
Xu et al. (2011)	g DM kg^{-1} water used (transpiration)

In a relatively limited number of cases the correlation between annual productivity and annual rainfall is poor; these exceptions are usually depressions benefitting from runoff, or soil having a water table within reach of the roots (Figure 2.1). It usually depends on the type of vegetation: some shrubs and trees being able to reach levels of soil water 50 – 100 m below soil surface and quite commonly 10 to 20 m, while herbaceous vegetation rarely reaches 2 m (Le Houérou 1984). Hassen et al. (2007) and Snyman (2009a) were of the few that included both above- and belowground phytomass production in determining WUE gives more accurate results. Xu et al. (2011) used the term transpiration water-use efficiency (TWUE), where evaporation was subtracted from evapotranspiration (ET) to quantify transpiration. Ecosystem water-use efficiency (EWUE), which can be compared to WUE at landscape level, is expressed by Emmerich (2007) as g CO_2 mm^{-1} ET. For the purpose of this study, the term WUE will be defined as g DM mm^{-1} transpiration, where transpiration = (evapotranspiration – evaporation).

Over the past four decades various water balance studies on grass species from the semi-arid areas of South Africa were conducted, firstly by Opperman (1975, 1977a, 1977b), and later by Oosthuizen and Snyman (2003a, 2003b), Venter (2003), Snyman (2000 – 2006) and Marais (2005). Ramakatane (2003) and Snyman (2000-2006, 2013, 2014) conducted water balance studies on *Opuntia* species, which is also an adapted aridland crop. On the contrary, very little research has been done on water use of Nama-karoo shrubs. Du Preez (1964) was the first to investigate the reaction of Karoo shrubs to different soil-water levels. More recent water stress studies that were conducted on Karoo shrub species (Gerber 1993, Midgeley and Moll 1993), did unfortunately not quantify the WUE of the studied plants. Many efforts have been devoted to the development of Karoo rangeland drought models, while there is still a need for a drought assessment method that can be applied at farm level (Venter 2003). In an attempt to establish an objective basis to pricing for ecosystem services, an approach that combines the potential evapotranspiration (ET_0) and the MODIS f_{PAR} product to predict WUE for the arid rangelands at landscape level of the Northern Cape, which largely includes the Nama-karoo, was implemented by Palmer and Yunusa (2011). In these areas water is the most limiting environmental factor influencing plant production and should therefore be utilized wisely by the vegetation. Water-use efficiency data for individual Karoo shrubs are at this stage lacking.

Water infiltration and soil compaction of the Eastern Mixed Karoo was studied under different grazing intensities, but did not evaluate its direct effect on the vegetation (du Toit 1986).

Rundel et al. (1999), mentioned some relations concerning the WUE of some vascular plant species of the Succulent Karoo. The indication was that WUE not only varied between species and within species, but also between seedlings and mature shrubs and between sites along an aridity gradient. The WUE was, however, not quantified for these plants. It is suspected that a combination of opportunistic water-use patterns with water-storage capacity (in succulents) or drought tolerance (in non-succulents) may have a powerful effect on Karoo plant community structure and composition (Dean and Milton 1999).

Water-use efficiency of three desert shrubs (Gurbantonggut Desert – Central Asia) was compared under drought conditions with that of WUE after rain (Xu and Li 2006). Two of the species showed no difference while for the third one, WUE was significantly higher during a dry period. Lane and Stone (1983) used a water balance equation, together with WUE factors and soil data, to estimate annual aboveground net primary production of perennial shrubland in the United States. Research by Marais et al. (2006) also indicated that five native South African grasses tended to use water more efficiently under moderate to severe water limiting conditions, but with differences between the species. It was, however, argued that although these treatments had better WUE rates, significantly lower DM yields were produced. It can be argued whether there are any advantages of producing less dry matter, while using water more efficiently. If irrigation is considered, it might be useful to establish irrigation levels for specific plants with the potential to produce optimum yields. The WUE factor is currently widely used among crop physiologists, production ecologists and agronomists.

Smaller juvenile shrubs showed lower WUE than larger adult shrubs of the same species at the Tintic Range Experimental station in Utah (Donovan and Ehleringer 1992, 1994). It can therefore be concluded that strong evidence indicates differences of WUE between different plant species and their growth stages, as well as between different levels of water stress. All these variations in more resent WUE'^{s} for different veld types, species and in different drier areas are shown in Table 2.2. The WUE factor is usually of the order of magnitude of 1.0 – 8.0 kg DM ha^{-1} mm^{-1} yr^{-1} in arid and semi-arid natural rangelands. These figures correspond to aboveground primary productions of 100 – 2500 kg ha^{-1} yr^{-1}, as reported by several authors. Water-use efficiency of maize and sugar cane can be as high as 75 and 63 kg DM ha^{-1} mm^{-1}, respectively (Finca et al. 2015). According to Le Houérou (1984) and Snyman (1998, 1999, 2000) WUE may be substantially lower in degraded ecosystems or considerably higher in pristine conditions, or under good management. A very detailed summary of WUE'^{s} for worldwide fodder species in drier areas before 1984 was done by Le Houérou (1984). In this summary he identified the different ways in which WUE was expressed worldwide over years, as well as the practical value for quantifying it.

Plants in a monoculture or grown individually might have different WUE values than plants which are in competition with other species or individuals of the same species (Xu et al. 2010). Field et al. (1983), however, argued that sometimes potential resource-use efficiency needs to be measured under controlled environments, and are not expected to explain resource-use efficiency under varying and complex natural conditions. Emmerich (2007) found that C_4-grass-dominated ecosystems had higher WUE than C_3-shrub-dominated ecosystems under the same environmental conditions. The same conclusion was made by February et al. (2011) for single species in an ecotone between the Nama and Succulant Karoo of South Africa.

In conclusion, the WUE factor seems to be a useful tool for assessing the health and productivity of arid zone ecosystems (Figure 2.1), particularly when actual evapotranspiration data are missing, as often happens. The concept also makes it possible to compare the productivity of different types of vegetation, or environments in a given geographical area or of similar types in different areas in a valid way. It further tends to underline the extreme importance of ecosystem management on overall productivity, since ground cover and aboveground phytomass seems often to override the effects of soil and climate in the determination of productivity per unit of water available (Le Houérou 1984). The WUE concept may furthermore be useful in attempting to predict long term productivity of vegetation, knowing the condition and trend.

Table 2.2: Example of WUE values for different vegetation types, species and areas.

Vegetation species / area	**WUE**	**Author**
South Africa		
Grasses, shrubs and *Opuntia* species	kg DM ha^{-1} mm^{-1} yr^{-1}	Snyman (1989)
Panicum stapfianum	4.7	
Cymbopogon pospischilii	7.8	
Chloris gayana	7.2	Snyman (1994)
Anthepora pubescens	4.0	
Panicum maximum	4.2	
Semi-arid grassland		Snyman (2000)
Poor condition	1.4	
Good condition	3.5	
Pennisetum clandestinum	7.2	Marais et al. (2006)
Cenchrus cilliaris	22.5	
Arid rangeland (mixture grass, shrubs, trees)	1.6	Palmer and Yunusa (2011)
Opuntia species (four years old)	7.7	Snyman (2013)
Karoo shrubs	mmol CO_2 mol^{-1} H_2O	Midgley and Moll (1993)
Eriocephalus ericoides		
Non-stressed	4.0	
Water-stressed	0.05	
Pentzia incana		
Non-stressed	3.0	
Water-stressed	0.1	
Other parts of the world	kg DM mm^{-1} ha^{-1} yr^{-1}	
Arid rangelands	0.5 to 10.0	Le Houérou (1984)
Haloxylon ammodendron	mmol CO_2 mmol^{-1} H_2O	Xu and Li (2006)
Non-stressed	0.026	
Water-stressed	0.039	
Tamarix ramosissima		
Non-stressed	0.0033	
Water-stressed	0.0031	
Indigofera species (shrubs)	g DM kg^{-1} water	Hassen et al. (2007)
Non-stressed	1.4	
Water-stressed	1.8	

2.4 Impact of defoliation on arid land vegetation

Management of the Karoo ecosystems for sustainable animal and plant production requires the application of the correct frequency, intensity and season of defoliation (Figure 2.1). Scientific studies on the defoliation of individual Karoo shrubs for sustainable production are limited. Defoliation studies on Karoo vegetation were discussed by Bosch (1987) in general, while methods for the scientific measurement of defoliation were tested in detail by Hobson (1988). Although Karoo vegetation is relatively resilient to livestock grazing, heavy grazing of highly palatable shrubs may cause their numbers to decline, due to death or repeated reproductive failure (Todd 2006). Hobson (1989) discussed the intensity and frequency of defoliation of Karoo shrubs, demonstrating that the more severely plants are defoliated, the less frequently they can tolerate such heavy defoliation. It was also mentioned that seedlings up to a certain age are negatively influenced by defoliation. This emphasizes the importance of taking plant age, intensity and frequency of grazing into consideration before conducting defoliation studies or applied rangeland management strategies. Season of defoliation of Karoo shrubs is also important. Hobson (1985) distinguishes between physiological, vegetative and reproductive damage to Karoo shrubs in different seasons. Summer and winter grazing have the lowest impact, while autumn has the largest impact on production, especially vegetative and reproductive damage.

Grasses and shrubs (Karoo shrubs included) react differently to defoliation. The Karoo shrub *Osteospermum sinuatum* used accumulated carbohydrates from the leaves and twigs prior to that stored in the stems and belowground, for regrowth after defoliation (van der Heyden and Stock 1996). For *Themeda triandra* on the contrary, the growth reserves retrieved for regrowth after defoliation, were greater from the roots than the stems (Danckwerts and Gordon 1990).

Transpiration after defoliation at different soil-water levels of karoo plants was investigated by du Preez (1964), who concluded that transpiration rate declined with lower soil water levels. Hobson (1985) and Gerber (1993) both used *Pentzia incana* in their defoliation studies. It was shown that the more severely *P. incana* was defoliated, the longer recovery period is required before a subsequent defoliation. Van der Heyden and Stock (1996) found that *Osteospermum sinuatum* depended initially on carbon reserves (first two weeks) after clipping, after which leaf growth relied on photosynthates produced by new and remaining leaves. Water stressed plants will not regrow easily as photosynthesis cannot take place due to the closed stomata of wilted leaves. Such stressed plants will therefore have to rely much more on stored growth reserves. However, Gerber (1993) indicated that water stressed *P. incana* plants could utilize all excess non-structural carbohydrates reserves from above- and belowground sources, during regrowth. There was, however, a lack of extension growth due to insufficient turgor pressure. Gerber (1993) argued that the influence of too many variables increases the complexity of these kind of studies on Karoo plant species. This might explain why very little research has been done on individual species in recent time.

Defoliation of the Karoo shrub *Osteospermum sinuatum* during anthesis, led to a reduction of carbohydrate content of the remaining flowering organs with an increase in the remaining foliage for the production of new photosynthetic material (van der Westhuyzen and Joubert 1983). This phenomenon was more pronounced under deteriorating climatic conditions. Stock et al. (1993) explained the responses of karoo shrubs to simulated browsing to be the result of passive alterations in plant chemistry rather than an active defence response to herbivores as found in some woody species. According to van der Heyden and Stock (1993) it appears that regrowth of semi-arid shrubs in response to browsing is not limited by carbon reserves. Richards (1993) argued that the success of rapid refoliation lies in the presence of active shoot meristematic regions that should remain on a plant after defoliation. Furthermore the size of the photosynthetic canopy that remains on the plant after defoliation will also contribute to the rate of refoliation.

It was discovered that certain ecophysiological browsing responses provide evidence that some unpalatable shrubs, like *Pteronia tricephala*, respond mechanistically to heavy browsing in such a way that their survival is guaranteed (van der Heyden et al. 1999). Palatable Karoo shrubs, like *Eriocephalus ericoides*, on the other hand, have no such compensatory mechanisms and it appears that heavy browsing impairs their survival ability. These shrubs may respond to heavy browsing by growing rapidly, as was shown for *Osteospermum sinuatum* (van der Heyden and Stock 1995, van Heerden et al. 1996). du Toit (1996) highlighted the importance of browsable shoot diameter that should be taken into account during defoliation studies where edible dry matter is to be measured.

Literature from the past decade, like that of Beukes et al. (2002) and Kraaij and Milton (2006) focused more on the influence of defoliation on all species present in the plant community (landscape scale). Changes over time in plant composition and cover are explained by annual and short term rainfall rather than by grazing impacts (Beukes and Cowling 2000). This indicates the importance of the understanding of water-use efficiency of individual Karoo bush species. On the other hand, *P. incana*, one of the species to be included in this study, showed resilience to high grazing intensities together with signs of compensatory growth. Peng et al. (2007) studied the water-use efficiency of some grass species under different grazing intensities. The results clearly indicated that, while there was a sharp decrease in leaf area and aboveground biomass, photosynthetic growth and water-use efficiency were enhanced under a moderate level of grazing, while these plant functions declined with an increased level of grazing. Different *Indigofera* species, as evaluated by Hassen et al. (2007), showed different responses to water stress conditions, indicating the variation between different species of the same genus. Root mass fraction in relation to water stress also formed part of this study.

The importance of root development investigations is becoming increasingly important for understanding the whole picture of ecosystem functioning (Smit 2005). Hobson and Sykes (1980) reported on the root development of three Karoo shrub species under different defoliation treatments, indicating that no statistical difference in root mass and volume was found between a 14 day and a 60 day defoliation interval treatment, while both were significantly less than that of the control treatment.

2.5 Root studies

The interaction between above- and belowground plant production must always be considered in applying rangeland management systems (Snyman 2009a, Figure 2.1). Most studies on vegetation dynamics focused mainly on DM yield of the aboveground plant fractions (Milton 1994, Beukes and Cowling 2000, du Toit 2001, Hoffman 2003) with the belowground biomass yield often neglected (Snyman 2009a). Oba et al. (2006) also emphasise the lack of investigations into belowground biomass partitioning for shrubs, like *Indigofera spinosa* and *I. cliffordiana*. When expressing WUE as DM yield per unit water transpired, it would be more accurate to also include belowground biomass into total biomass yield, as was done by Hassen et al. (2007). Snyman (2009a) highlights the need to also include root production to determine water-use efficiency and therefore determine the whole picture of ecosystem functioning on rangelands. To improve predictions of plants response to grazing, root studies, which have been largely ignored in the past, should definitely be included in empirical trait surveys (May et al. 2009).

The importance of including belowground phytomass production in water utilization studies was further highlighted by Snyman (2005, 2006, 2007, 2014) and Oosthuizen et al. (2006), whom studied belowground phytomass production of *Opuntia spp*. and semi-arid grasses, respectively. An underestimation of the productivity of an ecosystem is the reality when excluding the root component. This is supported by Palacio and Montserrat-Martí (2007) who highlighted the importance of water availability on the dynamics of root growth, as well as the ratio between shoot and root growth processes. Xu and Li (2006) also emphasize the neglect of root studies when plant growth is studied in relation to water availability. The differences in soil-water use by different plant species might be influenced by their different root distribution within the soil, especially where the soil water content also differs, for example at different soil depths (Carrick 2003, February et al. 2011, Zhu et al. 2011; Figure 2.1). Xu and Li (2006) mentioned the importance of the description of root architecture in relation to water availability in their natural habitats.

Root:shoot ratio is often used to express the impact of defoliation and/or water stress on plant biomass portioning (Chaves et al. 2002, Snyman 2009a, Xu et al. 2011, Evans et al. 2013). Plants with a higher ability to withstand water stress usually exhibit a high root to shoot ratio (Dube 1999). Unfortunately there is an uncertainty on the correlation between root:shoot ratios and drought resistance (Hoffman and Cowling 1987). Some evidence exists that a high root:shoot ratio is an adaptive mechanism for drought resistance, while others evidence indicate the opposite. Evans et al. (2013) explains that root:shoot ratio generally tends to increase as water becomes limited, but also mentions that this is not the case for all plant species. Snyman (2009a) states that rangelands in moderate to poor condition had slightly higher root:shoot ratios than that in a good condition. By contrast, other results indicated that water stress did not cause big differences in root:shoot ratios, due to above- and belowground growth that were adjusted in the same way (Snyman 2009b). For the encroacher shrub, *Seriphium plumosum*, the root:shoot ratio increased significantly as the plant reached maturity (Snyman 2012), which could also be the case for most other shrub species.

Information on root architecture of Nama-karoo vegetation is limited. Preliminary studies by Scott and van Breda (1937a, 1937b, 1938, 1939) was pioneer work that was unfortunately not elaborated on in recent years. Carrick (2003) described the root architecture of two leaf-succulent species (*Leipoldtia schultzei* and *Ruschia robusta*) and a non-succulent (*Hirpicium alienatum*). His findings indicated that most of the roots of the leaf-succulents occurred in the top 50 mm of the soil layer, while that of the non-succulent were found at greater depths. This indicates how plants with differentiation in rooting depths are structured together without being in competition with one another. February et al. (2011) revealed that for *Stipagrostas brevifolia* (grass) and *Ruchia robusta* (shrub) (on the ecotone between the Nama- and Succulent Karoo, South Africa) the highest root biomass is found where soil nitrogen is the highest and therefore conclude that root distribution in the soil layer may be primarily responding to nitrogen availability rather than water availability.

In general, there is a decrease in root proportions with increasing depth for most rangelands (Shackleton et al. 1988, Snyman 2009a). Most researchers found more than 85% of roots in the top 300 mm of soil (Tainton 1981, Dormaar et al. 1984, Moore 1989, Snyman 1998, 2005). Distel and Fernández (1986) reported a 67% root mass in the first 200 mm soil layer of a semi-arid grassland in Argentina. A drastic decrease in root distribution with depth occurred from 150 mm downward in a poor condition rangeland (Snyman 2009a), while a more even distribution was found in rangeland in a good condition. The high concentrations of roots in the top soil layers could be due to increased nutrient availablity in these soil layers (Montani et al. 1996, Ingram 2002). Restraining of growth due to water limitations not only depends on climatic conditions and soil properties, but also on species difference, e.g. rooting depth. Franco et al. (2006) also gave a brief review on the adaptabilities of plants to semi-arid environmental conditions, such as a reduction in the root:shoot ratio under water stress conditions.

Results on perennial grasses indicated that water stress had a greater impact on root growth than defoliation (Flemmer et al. 2002). Oosthuizen and Snyman (2009) also found that water stress had a greater effect on root growth of *Themeda triandra* than defoliation. Abundant soil water could buffer the negative effects of defoliation Volesky et al. (2011).

2.6 Nutritive value

Nutritive value of fodder plants has an enormous impact on animal production and therefore influences the sustainability of the ecosystem (Figure 2.1). Although the nutritive value of Nama-karoo vegetation was studied and described by Henrici (1945), Louw et al. (1968a, 1968b, 1968c), Louw (1969), Botha et al. (1990a, 1990b, 1990c), Botha and Nash (1990), and du Toit (1998) in the earlier years, it was not reported on frequently over the past few decades. Cultivated pastures are better known for investigations on the quality of the pasture mixtures (Meissner et al. 1989) or individual species (Marais et al. 2002, Theron et al. 2002, Theron and Snyman 2004,), often where diet selection of animals are incorporated (Meissner et al. 1989).

Initial work on Nama-karoo vegetation by Henrici (1945) focused on digestibility of individual shrubs, while Louw et al. (1968a, 1968b, 1968c) grouped species together by determining crude-protein (CP)-, fibre and mineral contents. Botha et al. (1990a, 1990b, 1990c, 1990d) did similar work, but focused on a larger number of individual species. They found difference between different plant species, as well as differences within the same species between different seasons. The quality of diet selection by grazing animals was later researched (Zeeman et al. 1983, Zeeman and Fourie 1984).

The influence of defoliation on nutritive value of shrub species in Morocco was reported by Alami et al. (1997) and shrub species from Spain by Mancilla- Leytón et al. (2014). Defoliation stimulates new growth, which usually has a higher nutritive value than mature growth (Alami et al. 1997). Although browsing of shrubs leads to increased CP content of the new growth, there could also be an increase in tannins, which impacts negatively on the utilization of some shrubs (Mancilla- Leytón et al. 2014). Stock et al. (1993) also observed slight increases of tannins in *Ruschia spinosa*. Variations in CP content between seasons were also reported (Stock et al. 1993). Seasonal nutrient fluctuations were also observed by Stapelberg et al. (2008) for various plant species in the Kalahari, South Africa. Chapin and Slack (1979) found that severely defoliated grasses had higher phosphate absorption rates than less defoliated plants.

Different irrigation levels can also influence the nutritive value of annual subtropical fodder crops (Marais et al. 2002). Lower irrigation levels can create lower in vitro digestibility, but slightly higher CP contents. Oosthuizen et al. (2006) investigated CP content in response to water stress in *Themeda triandra* where the CP content showed a clear increase under water stress conditions.

Some researchers showed interest in using the nitrogen content of leaves (Donovan and Ehleringer 1992) and in some cases roots (Rodríguez et al. 2007) of plants to explain nitrogen-use efficiency, which is related to photosenthetic activity and thus resource-use efficiency. Younger plants tend to have a higher nitrogen-use efficiency for increased growth, while the root system is shallow and less expanded (Donovan and Ehleringer 1992), while leaf succulents from the Succulent Karoo of South Africa have higher root concentrations in the upper 50 mm soil layer, where more nitrogen is available and which leads to better nitrogen-use efficiency (February et al. 2011)

Information on the influence of water stress and/or defoliation on nutritive value of Nama-karoo vegetation is currently lacking. Such data could contribute to a better understanding of sustainable utilization of this complex and diverse ecosystem.

2.7 Compensatory growth

Compensatory growth was at first described as a phenomenon found in livestock (Bohman 1955, Bohman and Torell 1956). Later it was also found in plants, but was not as well researched and documented as is the case with livestock (Crossett et al. 1975). There is, however, an increasing interest in compensatory growth in plants over the past two decades (Oesterheld and McNaughton 1991, Richards, 1993, Stock et al. 1993, Hamilton et al. 1998, van Staalduinen and Anten 2005, Oba et al. 2006, Zhao et al. 2008, Rea and Massicotte 2010, Chauhan et al. 2011, Gruntman and Novoplansky 2011, Zheng et al. 2012).

Plant compensatory growth can be described as exaggerated vegetative growth in reaction to mechanical damage to plants, for example cutting and browsing by animals (Rea and Massicotte 2010). When plants are defoliated they suffer a loss of photosynthetic tissue (Zhao et al. 2008). Such plants have the ability to compensate for that loss through a number of mechanisms, of which one is the ability to produce more biomass than non-defoliated plants. This can be described as compensatory growth. Richards (1993) found that compensatory carbon and nitrogen allocation after defoliation showed preferential allocation to actively growing meristematic regions in plant shoots. These meristematic tissues act as strong enough sinks to deprive roots and growing stems (other sink regions) of photosynthate until the source tissue is of such an amount that it exceeds the demands of other actively growing sinks.

Compensatory photosynthesis also develops in the leaves of defoliated plants, which is an increased photosynthetic rate compared to that of undefoliated plants.

McNaughton (1983) distinguishes between under-compensation, equal-compensation and over-compensation. Only over-compensation will be discussed, but under the commonly used term: compensatory growth. Chauhan et al. (2011) described the ability of certain weeds to out compete crops also as compensatory growth. Compensatory growth at seedling level was discussed by Zheng et al. (2012) for dicotyledonous species, which developed a compensatory growth strategy to resist leaf loss. Xu et al. (2010) mentioned the compensatory ability, which includes better biomass production and water-use efficiency, of annual crops like: *Triticum aestivum* (wheat) and *Setaria italica* (foxtail millet) as well as in perennial crops like: *Lolium perenne* (ryegrass), *Festuca arundinacea* (tall fescue) and *Trifolium repens* (white clover). The compensatory ability of some *Indigofera* species was described by Oba et al. (2006) as resistance against grazing pressure. This indicates that some vegetation types implement compensatory growth to ensure its survival against external impacts.

Usually physical damage, like defoliation, causes an increase in the root:shoot ratio due to the loss of aboveground biomass. Root reserves are therefore allocated to fewer buds, resulting in exaggerated vegetative growth (Rea and Massicotte 2010). Archer and Detling (1986) argued that clipping removes transpiring leaf area which improves the plant-water potential due to conservation of soil water (less transpiration).

Compensatory growth is largely influenced by grazing intensity, soil water and nutrient availability. Plants growing at low resource availability, like water stress, are more likely to increase production following defoliation, than plants growing under optimal conditions (van Staalduinen and Anten 2005, Xu et al. 2010). According to van Staalduinen and Anten (2005) some mechanisms to compensate for loss in leaf area includes: increased biomass allocation to production of new leaves, reallocation of stored carbohydrates or the activation of dormant buds.

It was found that grasses growing at low water and nutrient availability exhibited stronger compensatory growth than those growing under favourable conditions (Oesterheld and McNaughton 1991). Hamilton et al. (1998), however, discovered that nitrogen has to be above a critical level for certain grass species to compensate for leaf removal. Although nitrogen only slightly improved biomass yield of a grass species, different phosphorus and water levels did not influence defoliated plants (Zhao et al. 2008). Van Staalduinen and Anten (2005) compared two grass species, exposed to clipping under water stress conditions. Although there was a clear difference in the compensatory ability between the two species, both showed higher biomass productivity and leaf productivity than under non water stressed treatments.

Compensatory growth can be measured (detected) by comparing final biomass production of, for example, clipped (defoliated) plants with non-compensating (unclipped) plants (van Staalduinen and Anten 2005). Xu et al. 2010 also stated compensatory growth as greater biomass production and water-use efficiency. Van Staalduinen and Anten (2005) stressed the importance of investigating the effect of drought (water stress) on the amount of compensatory growth in semi-arid rangelands that are exposed to grazing by livestock.

Although most research on compensatory ability of plants focused on grasses (Lacery and van Poollean 1981, Oesterheld and McNaughton 1991, Hamilton et al. 1998, van Staalduinen and Anten 2005, Zhao et al. 2008), some work on dicotyledonous plants was also documented (Gruntman and Novoplansky 2011, Zheng et al. 2012). Oba et al. (2006) discovered that *Indigofera cliffordiana* (a dwarf shrub in arid zone habitats in central Africa) compensate for heavy defoliation by increasing its belowground biomass fraction in relation to its combined biomass. The Karoo shrub, *Tripteris sinuatum* (previously named *Osteospermum sinuatum*), used compensatory growth as method of rapid recovery after defoliation (Stock et al. 1993).

2.8 Nama-karoo shrubs used in study

2.8.1 *Pentzia incana* (Thunb.) Kuntze (Anchor karoo or ankerkaroo) Asteraceae

Pentzia incana is a dwarf shrub with split axes that can grow to 500 mm but are generally 200 to 250 mm tall due to being well grazed (Hoffman and Cowling 1987, Le Roux et al. 1994, Shearing and van Heerden 2008). It is recognized by curved branches that anchor in the soil (Figure 2.2). Leaves are small (2-3 x 0.5-1 mm), vary from green to grey (during dry periods), alternate, fleshy with short finger-like lobes with smooth margins (du Toit 2003). Flowers are carried at branch tips in the form of daisy heads (10-15 mm in diameter). The seeds are cigar–shaped, 1.5-2 mm long with a yellow to buff colour. Scott and van Breda (1938) measured *P. incana* roots to a depth of 3 m in a deep soil in the Worcester-Robertson Karoo.

Figure 2.2: *Pentzia incana* is well-known for curved branches that are anchored to the soil by roots.

It is the main host of the Karoo caterpillar (*Loxostege frustalis*) and is often attacked by it. *Pentzia incana* forms an important part of small stock’s diet where abundant. Its quality is not very high, but acceptable with varied palatability depending on the region. Du Toit (2003) compiled a list of different index values, (ecological and grazing), for Karoo shrubs, which ranged from 0 - 10. For *Pentzia incana* these values are: ecological index value (EIV) 4, subjective grazing index value (SGIV) 5.7 and linear regression grazing index value (LNREGGIV) 2.88.

Figure 2.3 *Pentzia incana* in flower – in this potting trial in glasshouse.

2.8.2 *Nenax microphylla* (Sond.) Salter (daggapit) Rubiaceae

This species is a dwarf, compact, woody, spreading shrub 200-300 mm tall with a diameter of up to 300-500 mm. Some stems might curve over to anchor in the soil. Leaves are small (2.5-4 mm x 0.8-1.2 mm), pinnate, shiny and curved backward on the stems. They are dark green above and pale green underneath and carried in whirls around the stems. Flowers are solitary in leaf axils, while the fruits are a red to brown, rounded oblong capsule (2 x 1 mm) and very noticeable during seasons with high rainfall (Le Roux et al. 1994, Shearing and van Heerden 2008).

Figure 2.4 *Nenax microphylla* in potting trial in glasshouse.

Nenax microphylla is one of the most valuable and palatable Karoo shrubs with a high production and is well utilized by most small stock. Its index values are: ecological index value (EIV) 7, subjective grazing index value (SGIV) 7 and linear regression grazing index value (LNREGGIV) 3.52 (du Toit 2003).

2.9 Conclusion

Sustainable rangeland productivity depends on various factors such as climate, nature of soil, botanical composition, vegetation structure and plant management (grazing patterns and stocking rate). Since all these factors or group of factors can limit yield, one should be able to integrate them all for sustainable animal production. From this literature review it is clear that all plants respond to environmental stress at basically the same way: through decline in growth rate and in the rate of acquisition of all resources. Further studies of these stress responses should consider the integrated nature of these different systems rather than focusing on a single environmental resource. This integration will require a broad interdisciplinary approach that draws on the skills of different physiologists, soil scientists and ecologists.

As mentioned before, drought has always been a normal recurrent event in arid and semi-arid rangelands. Additionally, the possible effect of climate change can have a meaningful consequence on arid environments, as compared with the past and present impact of humans and their livestock. Strategies and tactics to mitigate its consequences via improved land use and management practices are therefore essential to ensure future sustainable animal production.

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CHAPTER 3

PROPAGATION OF KAROO SHRUBS – PILOT TRIALS

In preparation for the main greenhouse trial to determine the water use and productivity of Karoo shrubs, two pilot trials were firstly conducted. Due to the scope of the bigger greenhouse trial, a few valuable key Karoo shrubs were tested for their ability to be propagated and multiplied vegetatively, as well as their ability to grow in a controlled environment. After satisfactory results were obtained, two soil types were also tested as growth medium. The one was a typical Karoo soil, while the other was a soil found closer to the University of the Free State campus where the trial was conducted. These soils were compared purely for logistical purposes as almost 15 t was needed for the study. If the soil had to be transported from the Karoo region it would have been impractical and expensive, therefore the use of a closer soil source was investigated.

3.1 Rooting of Karoo shrub cuttings

3.1.1 Introduction

The Nama-karoo Biome of South Africa is dominated by Karoo shrubs (bushes), which are mainly utilized as extensive sheep farming enterprises (Kraaij and Milton 2006). Unfortunately, seed of these Karoo shrubs is not commercially readily available (Esler et al. 2006), and the germination dynamics of the seeds could also be very complex (Hoffman and Cowling 1987; Esler 1999). According to Everett et al. (1978), rooting of plant material is generally used for mass production of vegetative material of plant species that usually have poor seed germination or for the production of clones of selected individual plants of the same species. In this study a large number of plants were needed for the greenhouse trial for quantifying the water use of Karoo shrubs, exposed to different water and defoliation treatments. Another reason for deciding on clones of one mother plant, was to eliminate any genetic differences between individual plants of the same species.

A pilot trial was therefore conducted for testing the viability of using stem cuttings of different Karoo shrub species for vegetative propagation. It was decided that if enough clones could be propagated from two of the selected species, those species could be used in the main trial. *Pentzia incana*, with its creeping growth form and acceptable grazing index value (du Toit 2003) was a first and definite choice, included as highly likely to be used in the main trial. It is known for its rooting ability where curved branches anchors to the soil (le Roux et al. 1994). Cuttings of this species are also commercially available at Renu Karoo (Milton-Dean and Dean, 2014), a company producing and distributing seed and plant material for, amongst others, rehabilitation purposes in the Nama-karoo. It was also postulated that successful rooting of cuttings from some Karoo shrubs might be a handy tool for production of plants that might be useful for reclamation purposes. The choice of a second shrub would depend on its rooting potential, despite its grazing index value.

Knowledge on vegetative propagation of Karoo shrubs by stem cuttings is limited. According to Codd and March (1983) the propagation of *Aptosimum procumbens* plants for example, is easier with cuttings than establishing it by seed. This is supported by Vorster and Kimpton (1986) who found the same for *Pelargonium quercifolium*. The above mentioned examples are unfortunately not supported by clear scientific research results.

On the other hand, numerous studies were done on rooting of trees (forestry) (Chen et al. 2012), fruit trees (Kareem et al. 2013), horticultural shrubs (Haile et al. 2011), ornamental plants (Misra et al. 2012) and also desert shrubs (Dessena and Mulas 2012). The results achieved in many of these mentioned studies highlight the possibility that many Karoo shrubs might also possess the ability to be vegetatively propagated as need arises in future.

Karoo shrubs are generally low producing species (DM yield per plant), which makes them less favourable as cultivatable fodder crops. The establishment of rooted cuttings in the veld will be a challenge, as soil water is usually limited in the Karoo areas. The young plants should also be excluded from grazing and defoliation by animals to allow successful establishment.

3.1.2 Materials and Methods

Plant material was collected during March 2006 (autumn) at Grootfontein Agricultural Development Institute situated in the False Upper Karoo (South Africa), also described as the Eastern Mixed Nama Karoo (Nama-karoo Biome) (Hoffman 1996). Grootfontein is situated adjacent to the town of Middelburg in the Eastern Cape Province (25° 06′ E; 31° 40′ S, at an altitude of 1400 m). Rainfall is almost exclusively during spring (September-November) and autumn (March-April), with a mean annual rainfall of 366 mm. Mean monthly minimum and maximum temperatures range from -12 °C in June to 38°C in January, with a mean of 150 frost free days per annum (Schulze 1979).

The Eastern Mixed Nama-karoo is a complex mix of grass- and subshrub-dominated vegetation types, where seasonal rainfall events cause dynamic changes in species composition (Hoffman 1996). Grasses like *Aristida* spp., *Eragrosis* spp. and *Themeda triandra* may dominate during above average summer rains, while common shrubs or Karoo bushes include *Pentzia incana*, *Ericephalus ericoides* and *Hermannia* species. Of all the Nama-karoo veld types, this vegetation type has the highest cover of herbs and geophytes. Soils in this area are mostly of the Valsrivier Form (mostly clay loam soils) (Soil classification Working Group 1991).

Over the last three decades index values, scores and ratings were allocated to Karoo shrubs by various researchers (le Roux et al. 1994, Shearing and van Heerden 1994, Botha and du Toit 2001, du Toit 2003). These ratings mostly included the grazing value (palatability, nutritive value, tolerance to drought and defoliation, preference/selection by herbivores) and ecological value (ability to protect the soil against erosion) of the different Karoo shrubs. For this trial eight shrub species with above average and two with below average grazing values were selected (Table 3.1).

Plant material was cut in the veld and placed in plastic containers, filled to a depth of 20 mm with water. The water was added to keep the plant material moist and prevent excessive plant water loss during transportation to Bloemfontein, where the trial was conducted. More than one plant of a single species was sampled. Only plants of the same species within a 2 m radius were chosen. This was done to ensure that all plant material of the same species was genetically as closely related as possible. Cuttings were prepared and placed in the growth media within 36 hours after collection.

Table 3.1: List of Karoo shrub species chosen for stem cutting trial, as well as their relative grazing index values (le Roux et al. 1994)

Karoo shrub species	Relative grazing index value
Eriocephalus ericoides (L.f.) Druce	4
Hermannia cuneifolia Jacq. *Var. glabrescens* (Harv.) I. Verd.	7
Limeum aethiopicum Burm.	10
Nenax microphylla (Sond.) Salter	10
Pentzia incana (Thunb.) Kuntze	6
Phymaspermum parvifolium (DC.) Benth. & Hook. Ex Jackson	6
Plinthus karooicus I. Verd.	8
Salsola calluna Fenzl. Ex C.H. Wright	8
Tripteris sinuata DC.	10
Walafrida saxatilis (E. Mey.) Rolfe	1

Stem cuttings with a length of 100 mm were made of both terminal and sub-terminal portions of shoots. The bottom end (20 mm of each cutting) was stripped from all leaves and the bark partially removed with a knife to expose the plant tissue to the growth hormone. The auxin, Butyric acid [IBA - [4-(indol-3-yl)- butyric acid]], was used as growth hormone (Everett et al. 1978). Butyric acid (IBA) is a plant hormone (auxin) which is known to induce root development. Three hormonal applications were used, namely: control at 0 g kg^{-1} (T1), 1 g kg^{-1} (T2) and 3 g kg^{-1} (T3) IBA [4-(indol-3-yl)- butyric acid]. Cuttings were first dipped in water, tapped on the side of the container to remove excess water and then dipped in the commercial hormone powder (Seradix B No. 1 and No. 2 - Bayer), containing IBA, at the mentioned concentrations (Richardson et al. 1979). Treated cuttings were placed (20 mm deep) in two growth media (filter sand and hygro seedling mix) in a mistbed for one month. A completely random design was laid out in the mist bed, with 10 cuttings per treatment and three replications. Water was applied as a mist spray every three minutes for 15 seconds. This ensured high humidity and kept the cuttings moist to prevent water loss and drying out of plant material (Malan and Rethman 2001).

3.1.3 Statistical Analysis

The experimental layout consisted of a randomized block design (growth media) with three hormone treatments (control, 1 g kg^{-1} and 3 g kg^{-1} IBA) and three replicates (Menderhall and Sincich 1996). The rooting percentage data were analysed by analysis of variance (ANOVA) for a completely random design using the statistical program GenStat® (Payne et al. 2011). The ANOVA tested for differences between the main effects of two growth media and three hormone treatments, as well as the medium by treatment interaction. Means were separated by Fisher's protected least significant difference (LSD) t-test at the 5% level.

3.1.4 Results and discussion

Limeum aethiopicum and *Salsola calluna* cuttings did not root in any of the treatments (Table 3.2). The other species showed varied rooting percentages which ranged from 7.38% for untreated *Eriocephalus ericoides* to 64.92% for IBA treated *Nenax microphylla*. Regardless of the IBA treatment, *N. microphylla* and *Pentzia incana* showed the highest ($P < 0.05$) rooting percentages.

On average T3 showed the highest rooting at 28.38% and was therefore more effective, with T1 the lowest average at 23.65% (Table 3.2). The rooting of T2 was intermediate with an average of 25.69%. Amri et al. (2010) also noted a strong IBA influence (at 3 g kg^{-1}) on rooting of cuttings in comparison with untreated (control) cuttings for the woody species *Dalbergia melanoxylon*.

Table 3.2: Mean rooting (% ± SE) of each Karoo shrub species for the different IBA [4-(indol-3-yl)-butyric acid] treatments. Data are means and standard deviations. Least significant differences (LSD) were calculated at the 5% level. Means (n = 10) in rows with different superscript letters indicate significant ($p < 0.05$) differences among treatments, based on Tukey's test.

Species	Rooting (%) IBA [4-(indol-3-yl)- butyric acid] T1 (0 g kg^{-1})	T2 (1 g kg^{-1})	T3 (3 g kg^{-1})	**Mean rooting per species (%)**	LSD 0.05
Limeum aethiopicum	0.00	0.00	0.00	**0.00**	
	± 0.00	± 0.00	± 0.00		
Salsola calluna	0.00	0.00	0.00	**0.00**	
	± 0.00	± 0.00	± 0.00		
Phymaspermum parvifolium	16.72	11.93	23.89	**17.50**	13.69
	± 8.81	± 13.40	± 8.08	± 10.97	
Eriocephalus ericoides	7.38^{a}	18.99^{b}	30.12^{b}	**18.80**	11.59
	± 8.33	± 11.59	± 7.53	± 12.96	
Walafrida saxatilis	14.37	15.73	26.55	**18.90**	15.01
	± 11.54	± 12.54	± 7.47	± 11.55	
Hermannia cuneifolia var glabrescens	9.02^{a}	25.60^{b}	25.90^{b}	**20.18**	4.58
	± 10.34	± 9.07	± 28.52	± 19.00	
Tripteris sinuata	23.32^{b}	30.69^{c}	11.69^{a}	**21.90**	6.85
	± 3.03	± 7.85	± 6.14	± 9.83	
Plinthus karooicus	17.76	25.52	25.80	**23.00**	6.51
	± 5.89	± 9.64	± 8.31	± 8.52	
Pentzia incana	58.11	61.73	58.59	**59.50**	9.13
	± 6.30	± 10.95	± 5.82	± 7.72	
Nenax microphylla	58.56	64.92	63.80	**62.40**	8.65
	± 5.21	± 14.32	± 5.69	± 9.27	
Mean rooting per treatment (%)	23.65	25.69	28.38	24.22	

The different species, however, reacted differently to the different IBA treatments. Other than most of the species, *Tripterus sinuata* showed higher rooting percentages, namely 23.32 and 30.69 for T1 and T2, respectively, than for T3 (11.69). *Eriocephalus ericoides* showed a strong increase in rooting percentage with an increase in IBA concentration. It might therefore be that a higher IBA concentration can increase rooting percentage of these shrubs even further.

Table 3.3: Mean rooting (% ± SE) of each Karoo shrub species for the different growth media. Data are means (n = 10) and standard deviations. Least significant differences (LSD) are calculated at the 5% level. Means (in rows and in mean rooting colomn) with different superscript letters indicate significant ($p < 0.05$) differences among treatments, based on Tukey's test.

Species	Rooting %		Mean rooting per species (%)	LSD 0.05
	Filter sand	Hygro mix		
Limeum aethiopicum	0.00	0.00	0.00	
	± 0.00	± 0.00	± 0.00	
Salsola calluna	0.00	0.00	0.00	
	± 0.00	± 0.00	± 0.00	
Phymaspermum parvifolium	16.50	18.53	17.51^{a}	11.18
	± 10.29	± 12.16	± 10.97	
Eriocephalus ericoides	18.14	19.51	18.83^{a}	9.47
	± 11.71	± 14.78	± 12.96	
Walafrida saxatilis	18.33	19.43	18.88^{a}	12.25
	± 18.33	± 12.45	± 11.55	
Hermannia cuneifolia var glabrescens	6.02^{a}	34.34^{b}	20.18^{a}	3.74
	± 9.36	± 15.11	± 19.00	
Tripteris sinuata	19.93	23.86	21.90^{a}	5.59
	± 8.70	± 11.01	± 9.83	
Plinthus karooicus	28.07^{a}	17.98^{b}	23.03^{a}	5.32
	± 8.67	± 4.67	± 8.52	
Pentzia incana	60.76	58.19	59.48^{b}	7.45
	± 8.65	± 6.95	± 7.72	
Nenax microphylla	63.95	60.90	62.42^{b}	7.06
	± 10.91	± 7.62	± 9.27	
Mean rooting per growth medium (%)	24.70	25.05	24.22	

Pentzia incana and *Nenax microphylla* showed spontaneous rooting of cuttings and responded only slightly to the different IBA concentrations. Cuttings of these species could therefore even be rooted without any IBA treatment. In comparing the mean rooting percentage of the two growth media (Table 3.3) no significant (P > 0.05) difference was found (Table 3.2). There were, however, differences within individual species, as illustrated in Table 3.3. *Plinthus karooicus* showed a higher rooting percentage (28.07%) in the filter sand than in the hygro seedling mix (17.98%), while *Hermannia cuneifolia* var. *glabrescens* rooted better in the hygro seedling mix (34.34%) (Figure 3.1 A – left) than in the filter sand (6.02%) (Figure 3.1 A – right).

Figure 3.1: Pictures indicating various cuttings in the mistbed (A), as well as a rooted *N. microphylla* (B) and *P. incana* (C) cutting.

The rooting percentage of *N. microphylla* (62.40%) (Figure 3.1 – B) and *P. incana* (59.50%) (Figure 3.1 – C), was significantly (P<0.05) higher than that of the other species, while *L. aethiopicum* and *S. calluna* showed no rooting at all. The rooting of the other six species was much lower and varied from 17.51% for *P. parvifolium* to 23.03% for *P. karooicus*. These results revealed that other ways to instigate rooting of *L. aethiopicum* and *S. calluna* should be considered for future studies. For example to try different seasons (Haile et al. 2011) or even different months and also to try different lengths of shoots or different plant parts (Palanisamy and Kumar 1997, Morini et al. 2003) for cuttings. For most species IBA treatment had a more positive influence on rooting than the impact of growth medium.

3.1.5 Conclusions and recommendations

Although this trial was conducted during the autumn, its repetition during the other three seasons might show different results. It is therefore recommended to repeat this trial in future also during the other seasons, while the Karoo shrubs are in different phenological and physiological stages. Other concentrations of IBA might also be investigated, as well as different portions of stems used for cuttings and age of stems (Malan and Rethman 2001). From this study it was clear that different cutting lengths, various placement depths in growth media, different seasons, could have a definite influence on the success of future propagation of Karoo shrubs. Although all abovementioned additional aspects of shrub propagation can be applied or tested in future, sufficient information was obtained for the bigger planned study.

Rooting of *N. microphylla* and *P. incana* stem cuttings was satisfactory and might even be increased with further refinement of the rooting procedure. These two species were identified as the most suitable, among the tested ones, for incorporation into the bigger greenhouse trial to follow. For the purpose of this pilot trial, these two Karoo shrub species were identified and successfully multiplied for further use.

Although it was not the main aim of this study, future trials to explore the possibilities of Karoo bush species for vegetative propagation should be much more expansive and intensive. It should include different collection dates (for example monthly), different cutting times per day (morning versus afternoon), different plant parts, and different cutting sizes. Furthermore, the use of different humidity's in the mistbed can be tested; different growth media and different temperatures of the growth media are also aspects for future research. Other variables to consider are soil depth and soil texture as these influence oxygen availability to the developing roots. It might be that Karoo shrubs will root better at lower humidity due to their origin from desert-like climates. Much higher rooting percentages could therefore be achieved in future studies if the identified short-comings of this study are addressed.

3.2 Influence of soil type on *Pentzia incana* and *Nenax microphylla* adaptability.

3.2.1 Introduction

It is taken for granted that Karoo shrubs are well adapted to only the soils and climate of the Nama-karoo Biome in South Africa (Mills et al. 2009). Although easterly movement of Karoo vegetation into the Grassland biome (dominated by grasses) is well documented (Acocks 1988), ecosystems response to global climate change is a new debate. Therefore there is an arising need for studies on the adaptability of Karoo shrubs to different soil types and climatic conditions. Such information should be of value for future veld management planning.

Soil was needed for the main glasshouse pot trial in Bloemfontein in the central Free State of South Africa. The plants to be grown were two Karoo shrub species, namely *Nenax microphylla* and *Pentzia incana*, as identified previously. Both these two species are fairly widespread throughout the Nama-karoo Biome. Both species are however also found in specific grass dominated areas in the Free State province, as far as Bultfontein in the North West Free State. *Pentzia incana* is usually found in soils with a higher clay content, but also grows well in more sandy soil types. While *N. microphylla* usually prefers well drained, more sandy soils.

Due to the scope of the main trial, as much as 15 t of soil was needed to fill the pots for the glasshouse trial. It was logistically difficult to gather and transport such an amount of soil from the Karoo region to Bloemfontein. It was therefore decided to compare the growth and survival of clones (discussed previously) of the two Karoo shrub species in two different soil types. The one soil from Bloemfontein (Free State province) in the Grassland Biome, and the other one from Middelburg (Eastern Cape Province) in the Nama-karoo Biome. This was done in a smaller pilot trial. If the shrubs grew "equally well" in both soil types, the nearer soil from Bloemfontein could therefore be used for the main Bloemfontein greenhouse trial. Parameters to compare shrub growth were aboveground phytomass production, which includes edible and inedible fractions as well as belowground phytomass production (root growth).

3.2.2 Materials and Methods

Established plants of *N. microphylla* and *P. incana*, as described previously, were evaluated using two soil types. The first soil type was collected from Grootfontein Agricultural Development Institute at Middelburg Eastern Cape in the Nama-karoo Biome (Valsrivier form - 40 % clay content, annual rainfall 366 mm), while the other soil type was from Bloemfontein in the Grassland Biome (Bainsvlei form - 14 % clay content, annual rainfall 530 mm) (Soil classification Working Group 1991). One mature plant of each species was vegetatively multiplied by means of stem cuttings as described previously. This means that six clones of each species were used and therefore the different plants from a species were genetically identical. Three plants per species were therefore evaluated in each soil type. The plants were planted in cylindrical pots with a depth of 540 mm and a radius of 105 mm (volume of 0.019 m^3). Six pots were filled with each of the two soil types and kept in a greenhouse at a day/night temperature of 25°C and 10°C, respectively. Pots were watered once a week, with the same volume of water, for eight weeks during the spring. Pots were rotated twice per week to account for variation in growing condition within the greenhouse.

Phytomass production and structure (roots, inedible stems, edible stems and leaves) were determined by harvesting all plants destructively after six months (Malan 2000). Edible stems were those with a diameter of less than 2 mm (du Toit 1996), and the rest taken was inedible. The roots of all plants were washed from the soil through a 1 mm mesh (Oosthuizen and Snyman 2003).

3.2.3 Statistical analyses

Data were analysed by analysis of variance (ANOVA) for a completely random design using the statistical program GenStat® (Payne et al. 2011). The ANOVA tested for differences between the two soil types. The t-test for two independent samples, testing differences between two soil type effects at the 5% significance level, were used.

3.2.4 Results and discussion

No significant ($P > 0.05$) differences were found for phytomass production of any of the plant components. Root development and production of both species were slightly higher ($P > 0.05$) in the Grassland soil (Bainsvlei form) with the lower clay content (Tables 3.4 and 3.5). Plants in the Grassland soil wilted earlier than those in the Karoo soil, which might be due to the higher clay content (40%) of the Karoo soil (Valsrivier soil). Even though the mineral content of the grassland soil was slightly lower, it still showed the highest production (roots and aboveground). The higher soil pH level of the clay soil might inhibit the availability of the soil nutrients. Van Heerden et al. (1996) found similar results for *Osteospermum sinuatum* which showed higher yields in a sandy than in a soil with a high clay content.

Table 3.4: Soil analysis results for the two soil types

Soil type	Ca	K	Mg	Na	P	Clay	pH (water)
			ppm			(%)	
Grassland soil (Bainsvlei form)	486	200	154	254	22	14	6.1
Karoo soil (Valsrivier form)	1908	320	900	320	11	40	7.8

The total phytomass production (aboveground plus belowground phytomass) of *P. incana* was on average 58.97 g plant^{-1} on the Grassland soil and only 44.74 g plant^{-1} on the Karoo soil, while that of *N. microphylla* was also higher in the Grassland soil at 63.93 g plant^{-1} compared to 51.53 g plant^{-1} on the Karoo soil (Table 3.5). The percentage contribution of different plant components were virtually the same for each species in both soil types, while *N. microphylla* produced a higher percentage of edible material than *P. incana* (Table 3.6). The inedible component of *P. incana* was, regardless of the soil type, twice that of *N. microphylla*.

No significant (P > 0.05) differences were found for root:shoot ratio. It was however lower in the Karoo soil for both species. *Pentzia incana* showed a slightly higher root:shoot ratio than *N. microphylla* in both soil types. As the plants were relatively young during this trial, it might be the result of the unusually low root:shoot ratios and perhaps even unrealistic dry matter fractions (edible and inedible stems).

Table 3.5: Mean (n = 3) phytomass production (g plant^{-1} ± SEM) of the different plant components.

Species	Roots	Leaves and edible twigs	Inedible twigs	Total
Grassland soil				
N. microphylla	9.78 ± 0.59	51.51 ± 5.14	2.64 ± 0.64	63.93 ± 6.25
P. incana	10.07 ± 2.53	42.96 ± 4.54	5.94 ± 0.93	58.97 ± 5.53
Karoo soil				
N. microphylla	6.45 ± 1.09	42.10 ± 3.28	2.98 ± 0.36	51.53 ± 1.94
P. incana	5.48 ± 0.56	34.09 ± 12.04	5.17 ± 0.69	44.74 ± 11.73

According to Hoffman and Cowling (1987) high root:shoot ratios are expected from Karoo shrubs. As the plants mature, in its natural environment, it would be expected that the root percentage might increase, especially for survival during water scarce periods and with potentially unrestricted soil depths.

Table 3.6: Percentage contribution (%) of different plant components to the average total phytomass production per species. The root:shoot ratio is means (n = 3) ± SEM.

Species	Roots	Leaves and edible stems (%)	Inedible stems	Root:shoot ratio
		Grassland soil		
N. microphylla	15	81	4	0.18 ± 0.01
P. incana	17	73	10	0.20 ± 0.04
		Karoo soil		
N. microphylla	13	82	6	0.15 ± 0.03
P. incana	12	76	12	0.17 ± 0.05

3.2.5 Conclusions

Surprisingly, plants generally grew better on the Grassland soil than on the Karoo soil, which might be ascribed to the better root development in the Grassland soil. The plants might persist longer in the Karoo soil with its higher clay content, which will enhance water retention, and higher nutritional status. Although a lower phytomass production in the Karoo soil over the short term seems evident, the tested plant species might survive better in the Karoo soil over a longer period. This study clearly indicated the adaptability of Karoo shrubs to different soil types, which might be important for vegetation changes and movements due to global warming.

The practical and economical aspects regarding soil collection for the main (bigger) greenhouse trial was clearly solved from these results. It was concluded that the Grassland soil could be used, as no important differences in plant growth were registered between the two soil types. The other positive outcome from this trial was that the out process of washing the shrubs' roots from the pots, is much easier and more accurate from the Grassland soil with the lower clay content.

3.3 References

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CHAPTER 4

MATERIALS AND METHODS – GREENHOUSE TRIAL
(above- and below ground phytomass production)

4.1 Introduction

As explained in Chapter 3, plant material was collected from Middelburg in the Eastern Cape and multiplied using stem cuttings. The two species that rooted best were *Nenax microphylla* and *Pentzia incana*. There were sufficient numbers of rooted cuttings of these two species to be used for the follow up trial in the greenhouse. A comprehensive greenhouse trial was conducted to investigate the water use and production potential of these two Karoo shrubs. In this chapter all procedures followed for the greenhouse trial will be discussed.

4.2 Propagation of plant material

The rooted material was transplanted into 1L plastic bags, filled with a mixture of compost, coarse sand and Hygrotech (Canadian peat, vermiculite and mono-ammonium phosphate) growth medium. These plants were kept in a plastic tunnel and irrigated every four minutes to be hardened off. Irrigation intervals were decreased gradually until the plants were irrigated only once per day. Plants were allowed to grow out for eight months before being transplanted into the trial pots.

4.3 Soil collection

After deciding on the soil type, according to results from the Pilot trial as discussed in Chapter 3, soil (a sandy-loam soil, Hutton form; Soil Classification Working Group 1991) was collected from the Sydenham Experimental farm of the University of the Free State, 20 km south of the city of Bloemfontein. The clay content of the soil was only 14% which facilitates the process of washing out the roots from the soil. Only the top 200 to 300 mm soil layer was removed and approximately 20 t transported to Bloemfontein. The soil was spread out on concrete floor, left in the sun and turned over daily to dry out (Snyman 2004). The soil was sealed off from rain by covering it from time to time with a plastic sheet as needed. After two weeks of drying, the soil was sieved through a 2 mm sieve to get rid of any stones, seed, and plant material. The sieved soil was therefore more or less uniform in terms of particle size. This was necessary to evenly pack/fill the pots with soil of uniform bulk density.

4.4 Pot selection

One of the main aspects under investigation was the dynamics of belowground phytomass production, or root production. In order to allow enough space (volume) for root growth, it was decided to use cylindrical pots of a 540 mm depth and radius of 105 mm (volume of 0.019 m^3). The unpainted pots were made from nutec fiber cement, with three holes (15 mm) at the bottom of each pot. As the pots were not sealed water tight, they were lined with 0.85 micron clear plastic bags. Holes were melted into the plastic bags at exactly the same place as the holes in the pots. The plastic bags made the washing out of the roots from the soil much easier, which will be explained in more detail later. The holes were made to prevent water logging at the bottom of the pots.

4.5 Filling of pots with soil

As the empty pots all differed slightly in mass, they were numbered and weighed to register the weight of the empty pots, as well as total weight of each filled pot. After lining out the pots with plastic bags, each were firstly filled with 1 kg gravel with a size of approximately 10 x 10 mm. Soil was packed in portions of 2.5 kg pot^{-1} (to remove as much air as possible) on top of the gravel, to a total of 27.5 kg pot^{-1}. After adding each soil portion, the soil was slightly tamped with a small wooden pole to ensure a firm bulk density.

4.6 Determining soil-water content

To determine field water capacity, five pots were filled with the same mass of dry soil and weighed. As the soil was still completely dry, these values were taken as the permanent wilting point (PWP) of the soil (Snyman 2007). To determine field water capacity (FWC) the pots were saturated with water and left for 48 hours before being weighed again. Plant available water (PAW) was determined as the difference between the dry weight and the water saturated weight at FWC. The average dry weight of 29.25 kg, and the average saturated weight of 35.21 kg, resulting in the PAW of 5.96 l pot^{-1}, is shown in Table 4.1.

Table 4.1: Determination of field water capacity (FWC) and plant available water (PAW), as well as volumetric soil water (VSW).

Pot nr.	Dry weight (kg)	FWC (kg)	PAW (l)	VSW (%)
273	29.58	35.62	6.04	17
274	28.78	34.74	5.96	17
322	28.78	34.52	5.74	17
409	29.04	35.06	6.02	17
462	30.08	36.10	6.02	17
Average	**29.25**	**35.21**	**5.96**	**17**

4.7 Preparation and planting of plants

As mentioned, plants of the selected two species were gradually hardened off in the plastic tunnel until they were watered only once per day. During late November 2006, eight month old plants were removed from the plastic bags, excess growth medium was carefully removed from the roots and the plants transplanted into the pots. These plants were allowed for two months to establish well before commencement of the water gradient trial in February 2007. Care was taken that the planted plants for each species were more or less the same size.

4.8 Managing the greenhouse

The day and night temperatures in the greenhouse were changed monthly according to the long-term averages for Middelburg (Eastern Cape) from where the plant material was collected (Table 4.2). Both the temperature settings and the filtering of the water were to duplicate growing conditions as close as possible to the natural conditions of the two species.

Table 4.2: Long-term average daily minimum (night) and maximum (day) temperatures for Grootfontein, Middelburg Eastern Cape (South African Weather Services 2002).

	Month											
	J	F	M	A	M	J	J	A	S	O	N	D
	Temperature (°C)											
Night	13	13	11	7	3	1	0	2	4	7	9	12
Day	30	29	26	22	19	16	16	19	22	24	27	29

During greenhouse trials pests like aphids and bryobia mites can be a problem. For this reason plants were sprayed with Metasystox that was alternated with Chlorpirifos, whenever some of these pests were noticed. Fortunately not many such problems occurred over the study period.

4.9 Different treatments

4.9.1 Water treatments

Four water stress treatments, namely W1 = 0-25% depletion (non-stressed) of plant available water (PAW), W2 = 25-50% depletion (mildly stressed) of PAW, W3 = 50-75% depletion (moderately stressed) of PAW and W4 = 75-100% depletion (severely stressed) of PAW were applied (Snyman 2007). As pots weighed up to 37 kg they were weighed with a Nagata HTR industrial hanging balance (Figure 4.1). Four weight ranges were determined to serve as weight increments, giving the maximum allowable water loss for each watering treatment. The water loss was therefore used to determine the depletion of PAW within a specific water stress treatment. The volume of water to be added to the pots of each of the different water treatments, to restore it to its appropriate level of PAW, were periodically determined and added throughout the trial. It was accepted that 1 L of water weighs 1 kg. Pots were initially weighed daily for the first two weeks, after which it was decided, that weighing of pots twice per week would be sufficient to determine water loss. The pots were therefore weighed twice per week during summer and once per week during winter to determine the soil-water content, and therefore, when and how much water should be applied for each treatment.

Figure 4.1: Weighing of pots in greenhouse

For T1 the pots were watered as soon as 1.5 kg reduction in weight was registered. For T2 to T4 the pots were first depleted to their correct levels (as % of PAW), after which watering also took place as soon as a 1.5 kg reduction in weight was registered. This method is the same as described by Oosthuizen and Snyman (2003) and Snyman (2004, 2007, 2014). These watering treatments were applied over a period of 12 months. Municipal tap water, which was filtered through a carbon filter and a dechlorinator to remove the added chemicals for purification, was used. This was done to prevent the buildup of unwanted chemicals in the soil.

4.9.2 Defoliation treatments

Plants were defoliated by cutting with secateurs. The trial started on 2 February 2006 on which date all plants were defoliated to their different defoliation intensities. The defoliation intensities were to heights of 50 (I), 125 (II) and 200 mm (III) from soil level. Three dowel sticks of the appropriate heights were used to cut the plants according to the treatment heights. Branches were bunched together and cut to the specified heights, which more or less simulated browsing by animals (Figures 4.2 and 4.3). Thereafter, plants were defoliated at three different frequencies, namely: intervals of three (A), six (B) and 12 months (C) (Table 4.3). The defoliation at 12 months (C) was at the end of the trial and can be seen as the control.

Table 4.3: Three different defoliation frequencies

	Date				
Frequency intervals	2 February 2007	4 May 2007	3 August 2007	5 November 2007	11 February 2008
A (3 monthly)	A	A1	A2	A3	A4
B (6 monthly)	B		B1		B2
C (after 12 months)	C				C

Figure 4.2: Defoliation of *Pentzia incana*.

Figure 4.3: Defoliation of *Nenax microphylla*.

4.9.3 Experimental layout

The two species were planted in adjacent greenhouses, separated by a glass panel. The greenhouse environment was thus not exactly the same for both species. The species were therefore not statistically compared although they received the same treatments. The experimental layout was a randomized block design. The four watering treatments, together with the three defoliation intensities and three defoliation frequencies gave a total of 36 treatment combinations. Each treatment combination was replicated six times. This resulted in 216 pots per species. An additional 12 and 6 pots per species were included to measure soil evaporation and root development respectively. Pots were numbered and different replications of different treatment combinations were allocated to the 216 pots. The pots were positioned randomly and placed in double rows on five rows of steel tables in the greenhouse. Pots were rotated twice per week for the duration of the trial to account for variation in growing condition within the greenhouse (Hassen et al. 2007).

4.10 Data collection

4.10.1 Aboveground phytomass

Defoliated plant material was oven dried at 75 °C to constant weight, after which it was separated into edible (leaves and twigs with diameter smaller than 2.5 mm) and inedible portions (thicker than 2.5 mm) (du Toit 1996). The different portions were weighed to obtain aboveground phytomass production, which was expressed as g plant^{-1}. The problem in determining aboveground phytomass of shrubs at specific heights is that only the outer proportion of growth is cut and measured. The branches and side-branches developing and growing to the sides and to the inside, below the cutting height, are due to practical reasons, not measured. Therefore there might be a slight under estimation of the edible aboveground phytomass.

4.10.2 Water-use efficiency

Transpiration (T) was determined by the soil-water balance equation (Hillel 1971). Irrigation (I) was measured by the adding of water to the pots. The change in soil water (ΔW) was calculated according to the weighing as discussed previously (4.9.1), where (+) indicated an increase and (-) a decrease in the quantity of water within the root zone. Percolation is usually measured from water drained from the pot at the bottom. Percolation can be, in this case, discarded in terms of calculating the water balance since no water drained from the pots. Runoff was negligible for the water balance equation because water was added to the pots and no runoff took place. Evaporation (E) accounts for the water that evaporates through the soil surface. In the 12 pots (four per water treatment) used to measure evaporation (Hassen et al. 2007, Xu et al. 2011), wire domes, covered with different layers of shade cloth, to imitate the shade of shrub canopies were used. These pots were then also weighed and watered under the different watering treatments. As the plants grew bigger and denser, the shade cloth layers were increased. By weighing these pots, evaporation was estimated and subtracted from irrigation in the water balance equation.

Transpiration was therefore calculated using the following equation:

$T = I \pm \Delta W - E$

One of the aims of the study was to determine water-use efficiency (WUE). The WUE was defined as the amount of aboveground phytomass produced per unit of water transpired (WUEa), as well as expressed in terms of above- and belowground phytomass (WUEab) per unit of water transpired.

The roots in the six pots allocated for root determination, before the different treatments were applied, were washed out from the soil without separating it in different root fractions (washing out procedure described in detail later). This was done to be subtracted from the total root mass at the end of the trial to get an accurate measurement of only the root mass produced during the trial period. This adjusted root mass was used for calculating WUEab.

4.10.3 Root sampling

The roots of all plants were washed from the soil through a 1 mm mesh, and separated into coarse (≥ 3 mm), medium (1 – 3 mm) and fine (≤ 1 mm) fractions. Photographs of the washing out of the roots are presented in Figure 4.5. Belowground phytomass production was also expressed as g plant^{-1}. Root length, for each fraction, was measured by using a modified infrared root length counter (Rowse and Pillips, 1974; Snyman 2004). The root counter was calibrated using five different known lengths of string, ranging from 1 to 5 m. The diameter of the string was more or less the same as that of the average root thickness. The regression that was used to calculate root length is shown in Figure 4.4.

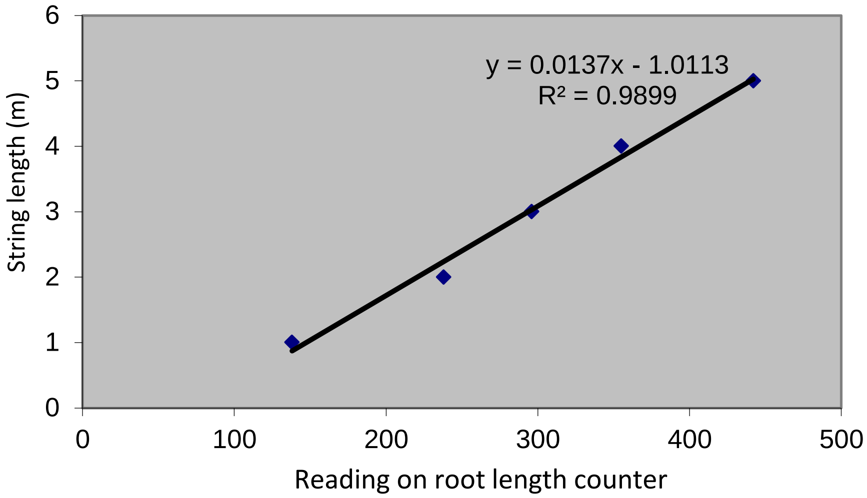

Figure 4.4: Regression of the different string lengths that were used to calibrate the modified infrared root counter.

From the regression equation y = root length, while x = root length counter reading. For each of the root fractions, a subsample (more or less 10%) was taken and kept fresh in a cooler. The rest of the sample was oven dried at 75 °C to constant weight and weighed. Lengths of the fresh sub-samples were measured, after which they were also oven dried at 75 °C to constant weight and weighed. The percentage of the sub-sample in relation to the whole sample was determined and used to calculate the total root length.

Figure 4.5: Washing out of roots.

4.10.4 Measurement of shoot length and leaf dimensions

For the measurement of shoot length two stems per plant (pot) were marked by attaching a very small, light, coloured, plastic marker to it. Measurements were done with a ruler a day before each defoliation date. For the three- and six-monthly defoliation frequencies new shoots were marked two weeks after defoliation took place, to start measuring a new shoot in the place of the one that was harvested.

Leaf dimensions were measured as a once off measurement during November. Three mature leaves (at least 80 mm from growth point of shoot) were measured per plant (pot), using a calliper. Leaf width was measured at the point where the leaf was at its widest, and leaf length was measured from the base to the tip of the leaf.

4.10.5 Determination of nutritive values

The determination of nutritive values was done only on the edible plant fraction, as defoliated at each defoliation date. The edible plant samples were milled through a 1 mm sieve after which the chemical analyses were done on these milled samples. The following chemical analyses were conducted to determine nutritive values of the edible plant material, harvested at each defoliation date. Determination of *in vitro* DM digestibility (IVDMD) was done using the Tilley and Terrey technique as adapted by van der Merwe (van der Merwe and Smith, 1991). Neutral detergent fiber (NDF) was determined using the Dosi Fibre system with the Robbertson and van Soest method (van Soest et al. 1991). For calculation of crude protein (CP), Nitrogen (N) was determined in a Leco Nitrogen analyzer (Leco, 2001) and crude protein (CP) calculated by multiplying the N content by a factor of 6.25.

4.11 Statistical analyses

The layout of the trial was a complete randomized block design. All data were analysed using the statistical program GenStat® (Payne 2012). Data such as phytomass production, root length and WUE were analysed with factorial ANOVA (unblocked) testing for differences between all main effects (Water, defoliation intensity, defoliation frequency) and all their interactions (Snedecor and Cochran 1980). The residuals were not skew distributed, but treatment variances were heterogeneous, thus Fisher's protected LSD test was used to compare means at the 1% level (Glass et al. 1972). *Pentzia incana* analyses were based on the 6 pots, but *N. microphylla* analyses were based on 4 pots only, because of too many missing values.

For measurements over time (defoliation frequencies), repeated measurements linear mixed model analysis, or REML, was used to test for differences between different factors (Payne et al. 2012). The fixed effects were specified as the factors and all their interactions, while the random effect was specified as the pot by time interaction, allowing for time variances to change.

Shoot length differences were analysed with linear mixed model, or REML, analysis as the data was very unbalanced (Payne 2012). The fixed effects were specified as the main effects and the interactions. Fisher's protected LSD test was used to compare means at the 1% level as the treatment variances were heterogeneous.

4.12 References

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CHAPTER 5

ABOVE- AND BELOWGROUND PHYTOMASS PRODUCTION OF *Nenax microphylla* UNDER DIFFERENT DEFOLIATION FREQUENCIES AND INTENSITIES ALONG A WATER DEFICIT GRADIENT

5.1 Introduction

This chapter focusses on the above- and belowground phytomass production of *Nenax microphylla*, as influenced by the different defoliation and water treatments and their interactions. The importance of this part of the study was explained and discussed in detail in previous chapters (Chapters 1, 3 and 4). The general discussion of Chapter 5 (*N. microphylla*) and Chapter 6 (*P. incana*) will follow in Chapter 7 to avoid duplication, as the two plants reacted similarly to some of the treatments.

Three main effects form the core of the results to be discussed, namely: water (W), defoliation intensity (Di) and defoliation frequency (Df). The water treatments were as follows: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Three defoliation intensity treatments included defoliation to heights of: I = 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency treatments were: A = every three months (May, August, November and February), B = every six months (August and February) and C = every 12 months (February). On the date the trial started, all plants were defoliated to their specific cutting heights, within the different defoliation intensity treatments. As the trial ran over only 12 months, the C defoliation frequency treatment was applied on the day the trial started and again, 12 months later at the end of the trial. It can therefore also be regarded as the control. Four interactions occurred as result of the treatment combinations. These interactions included: W x Di, W x Df, Di x Df and W x Di x Df. For the presentation and discussion of results emphasis was placed only on those interactions which tested highly significant ($P < 0.001$) and explains the highest amount of variation in the data. To better explain the results, ANOVA summaries are also presented.

The discussion will be structured according to the main effect, defoliation frequency, under headings Frequency A to Frequency C. Within each frequency, the other two main effects, water and defoliation intensity, plus their interactions (where appropriate) will be presented and discussed. All harvested plant material was separated into the following fractions: edible phytomass (EP), inedible phytomass (IEP), total aboveground phytomass (TAP), belowground phytomass (BP) and total above- and belowground phytomass (TA&BP). Edible phytomass (EP) comprised of leaves and edible twigs (twigs less than 2 mm in diameter, du Toit 1996). For Frequencies A and B, the results and discussions comparing treatments and defoliation dates, includes only edible phytomass.

5.2 Results and discussion

Water accounted, by far, for the highest variation in most data sets, regardless of the defoliation treatments and therefore had the greatest effect on aboveground phytomass production (Tables 5.1 and 5.2).

5.2.1 Defoliation frequency A (three-monthly interval)

The defoliation frequency data for the three-monthly intervals (May = A1, August = A2, November = A3 and February = A4) are illustrated as means for the main effects, namely water, defoliation intensity and time (Figure 5.1). Harvested plant material comprised of leaves and edible twigs (twigs less than 2 mm in diameter, du Toit 1996) and are indicated and discussed as edible phytomass (EP). Other than being a three-monthly defoliation treatment, season (time/date) might also have had a notable effect on the results, as the defoliation dates followed more or less the four seasons. To compare the four defoliation dates a linear mixed model repeated measurements analysis (REML) was done (Table 5.1).

Table 5.1 REML variance components analysis for frequency A (A1, A2, A3, A4 compared).

A1, A2, A3, A4 compared		T	W	I	T x W	T x I	W x I	T x W x I
Variable	Treatment	F statistic and significance						
EP	A1vs A2 vs A3 vs A4	211 ***	175 ***	9 ***	26 ***	5 ***	12 ***	3 ***

REML = Linear mixed model repeated measurement analysis, T = time (month), W = water, I = defoliation intensity, NS = non-significant, EP = edible phytomass, Frequency A: A1 = May, A2 = August, A3 = November, A4 = February, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

Time and water accounts by far for most of the variance in the data (Table 5.1) and both tested to having a highly significant ($P < 0.001$) influence on edible phytomass production. For both time and water, phytomass production for all treatments differed significantly ($P < 0.001$). The difference in phytomass production for defoliation intensity was only significant for treatments I and II which differed significantly ($P < 0.001$) from each other.

The four defoliation dates roughly followed the seasons: May = end of autumn, August = end of winter, November end of spring and February end of summer. Phytomass production at all four these defoliation dates differed significantly ($P < 0.001$), with phytomass production very low at the end of winter (A2) and very high at the end of summer (A4) (Figure 5.1).

The edible phytomass production for February (A4) was significantly higher ($P < 0.001$) than that of any of the other defoliation months, mostly due to the three months of optimal conditions for plant growth from November to February (Figure 5.1). By contrast, the August harvested (A2) phytomass was significantly ($P < 0.001$) lower than any of the other defoliation month, as virtually no growth took place during the winter months (June to August). The difference in edible phytomass production between A2 and A4 was 715%, which is a much bigger difference than between the extreme water or defoliation treatments. The non-stressed (W1) water treatment gave a 556% higher phytomass production than the severely stressed (W4) treatment.

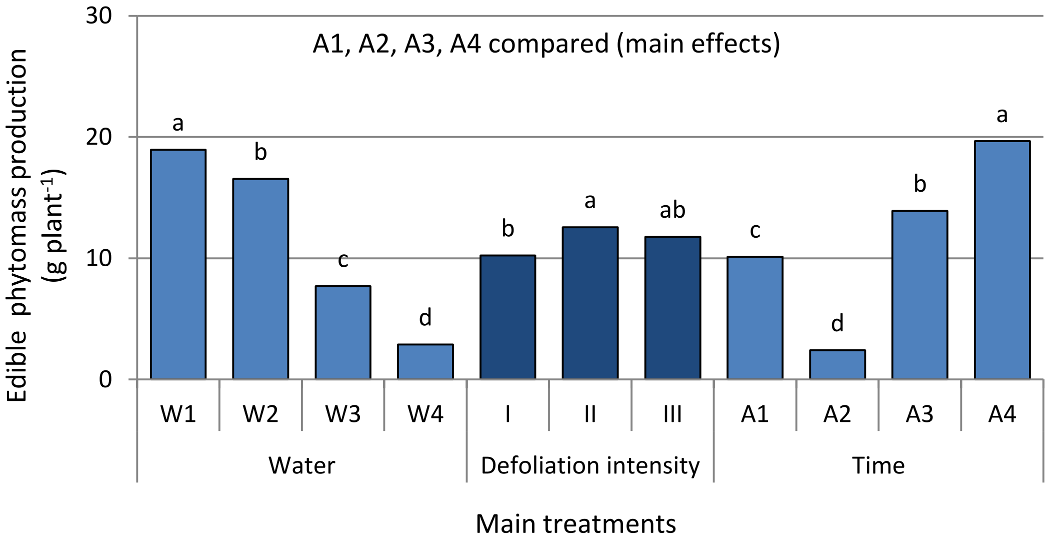

Figure 5.1: Mean (n = 4) cumulative edible aboveground phytomass production (g plant^{-1}) for *Nenax microphylla* for the three-monthly defoliation frequency, by comparing the four defoliation dates. The three main effects (water, defoliation intensity and time) are indicated. The defoliation dates are: A1 = May, A2 = August, A3 = October and A4 = February. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences ($P < 0.001$) within treatments.

Water clearly accounted for most of the variation ($P < 0.001$) in edible phytomass production for A1, A3 and A4 (Table 5.2). With virtually no growth during the winter months (A2), water and defoliation treatments both accounted for very little ($P < 0.01$) variation in the data.

Table 5.2: ANOVA summary for edible phytomass production of *Nenax microphylla* for the three-monthly frequency (Frequency A).

Frequency A		W		I		W x I	
Variable	Defoliation date	SS% and significance					
EP	A1 (May)	71	***	1	NS	9	**
EP	A2 (August)	19	**	17	**	16	*
EP	A3 (November)	70	***	6	***	10	***
EP	A4 (February)	75	***	1	NS	11	***

EP = edible phytomass, W = water, I = defoliation intensity, NS = non-significant, SS% = sum of squares percentage, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

It can be concluded that for the three-monthly defoliation frequency, defoliation date had a greater effect on edible phytomass production than the defoliation intensity.

5.2.1.1 Main effect: water

In August, at the end of winter, there was not much ($P > 0.05$) variation in aboveground phytomass production between the watering treatments, while in spring (November) all four water treatments had a significant ($P < 0.001$) influence on edible phytomass production. With the exception of August, water treatments W3 and W4 had lower ($P < 0.001$) edible phytomass productions than W1 and W2 (Figure 5.2). The plants reacted the best ($P < 0.001$) in terms of production for all water levels at the February defoliation. The lowest water treatment (W4) constantly gave very low phytomass productions, with no difference ($P > 0.05$) between defoliation date treatments. The August defoliation showed by far ($P < 0.001$) the lowest phytomass production, regardless the water treatment. The difference in edible phytomass between W1 and W4 was the largest at the February defoliation. This indicates both the possible high edible phytomass production during high summer rainfall in contrast to the very low expected edible phytomass production under water deficit conditions in the same season.

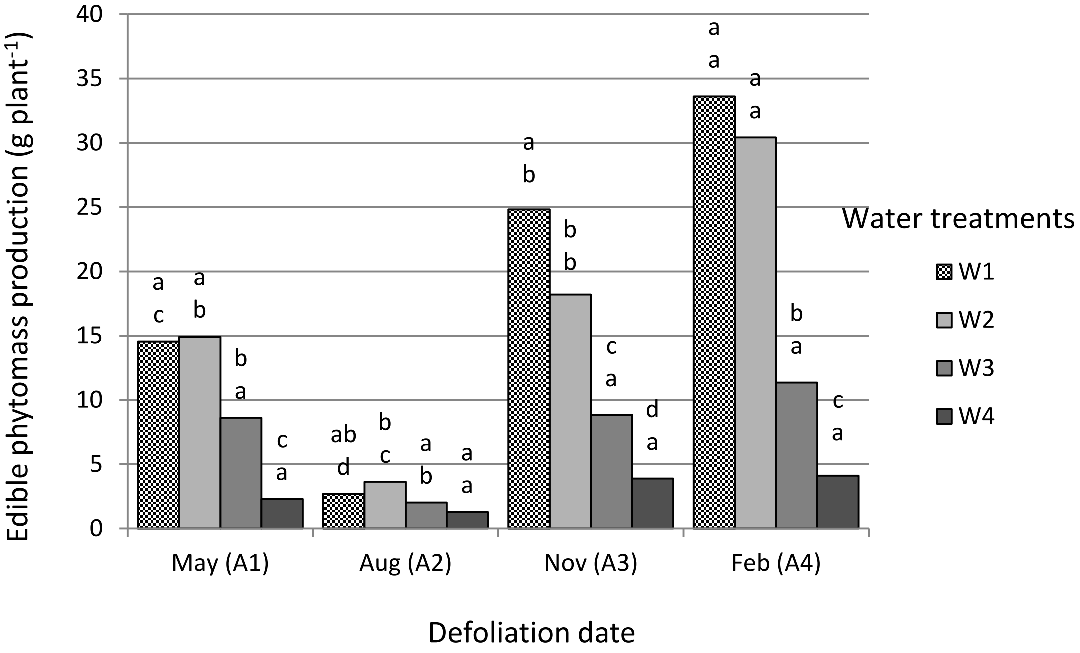

Figure 5.2: Mean (n = 4) edible phytomass production (g plant^{-1}) of *Nenax microphylla* at a three-monthly defoliation frequency with four different water levels. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Different top letters at each month (defoliation treatment) show significant differences ($P < 0.001$), between water treatments. Different bottom letters show significant differences ($P < 0.001$) between months within a water treatment.

5.2.1.2 Cumulative productions at different time intervals

The cumulative aboveground phytomass production for the three-monthly defoliation frequency was determined by adding the phytomass productions of May (A1) and August (A2), as well as A1 plus A2 plus November (A3) and lastly by adding A1 plus A2 plus A3 plus February (A4). This was done to quantify the cumulative phytomass productions over time (Figure 5.3). The defoliation intensities showed no significant ($P > 0.05$) differences in edible phytomass production, while water treatments significantly ($P < 0.001$) influenced production (Figure 5.3, Table 5.3).

Table 5.3: ANOVA summary for phytomass production of *Nenax microphylla* for the cumulative three-monthly frequency (Frequency A).

Frequency A		W		I		W x I	
Variable	Treatment	SS% and significance					
EP	A1	71	***	1	NS	9	**
EP	A1+A2	69	***	2	NS	11	***
EP	A1+A2+A3	73	***	3	**	10	***
EP	A1+A2+A3+A4	78	***	2	*	10	***

W = water, I = defoliation intensity, NS = non-significant,
SS% = sum of squares percentage, EP = edible phytomass, Frequency A: A1 = May,
A2 = August, A3 = November, A4 = February, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

There were no significant ($P > 0.05$) differences in production between W1 and W2, while they both differed significantly ($P < 0.001$) in terms of production from W3 and W4, which also differed significantly from each other. The lowest water level (W4) resulted in a cumulative total phytomass production that was 173% lower than that of W3 and 504% lower than W1 and W2. Phytomass production of W3 was 130% lower than that of W1 and W2.

These results clearly indicate that defoliation intensity had a small ($P > 0.05$) influence on phytomass production for the three-monthly defoliation intensity treatment. Availability of water had a highly significant ($P < 0.001$) influence on production, especially for W4, where very little cumulative production took place over time in comparison with all three other watering treatments.

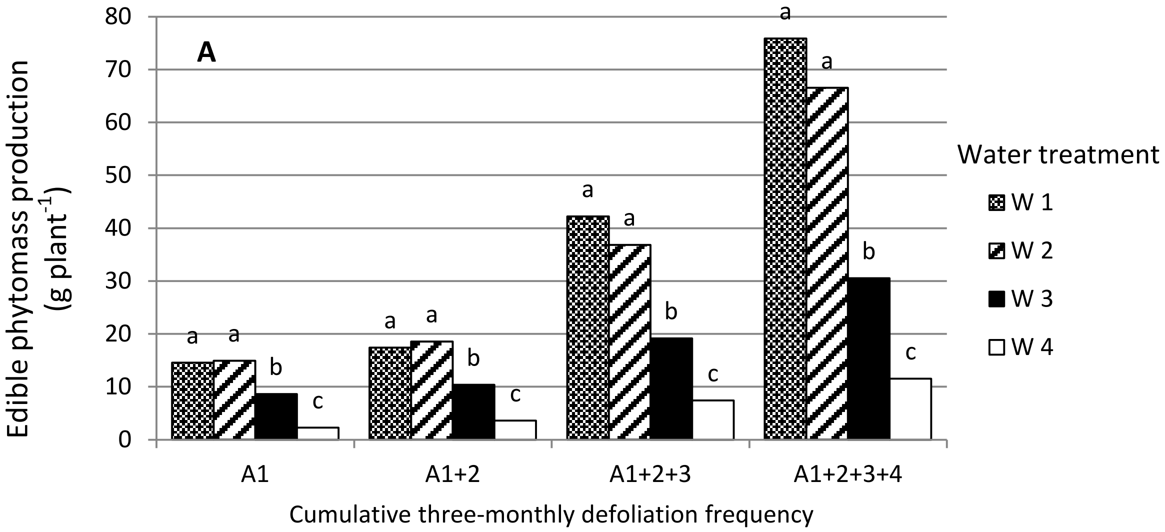

Figure 5.3: Cumulative mean (n = 4) edible phytomass production (g plant^{-1}) for *Nenax microphylla* at a three-monthly defoliation frequency with four different water treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation dates are: A1 = May, A2 = August, A3 = November and A4 = February. Different letters within each cumulative time interval show significant differences ($P < 0.001$) between water treatments and defoliation intensities.

5.2.1.3 First six months (A1 + A2) (autumn, winter) compared with second six months (A3 + A4) (spring, summer)

In comparing the phytomass production for the first six months (A1 + A2) with that of the second six months (A3 + A4), the same tendency was found, with defoliation intensity having no significant ($P > 0.05$) influence on phytomass production (Figure 5.4). By contrast, water stress decreased phytomass production ($P < 0.001$). Phytomass production of W4 was 85% lower than that of W1 and W2 and 66% lower than that of W3, indicating the huge detrimental influence of severe water stress on phytomass production (Figure 5.4). Time (season) had an even bigger effect on phytomass production than water (Table 5.4), by accounting for much more variation in the data than water.

Table 5.4 REML variance components analysis (A1 + A2 compared to A3 + A4).

A1+A2 and A3+A4 compared		T		W		I		T x W		T x I		W x I		T x W x I	
Variable	Treatment	F statistic and significance													
EP	A1+A2 vs A3+A4	516	***	95	***	5	*	67	***	3	NS	7	***	4	**

REML = Linear mixed model repeated measurement analysis, T = time (month), W = water, I = defoliation intensity, NS = non-significant, SS% = sum of squares percentage, EP = edible phytomass, Frequency A: A1 = May, A2 = August, A3 = November, A4 = February *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

Phytomass production of the second six months was more than 50% higher than that of the first six months, mainly due to the spring and summer growing season's impacts on production (Figure 5.4).

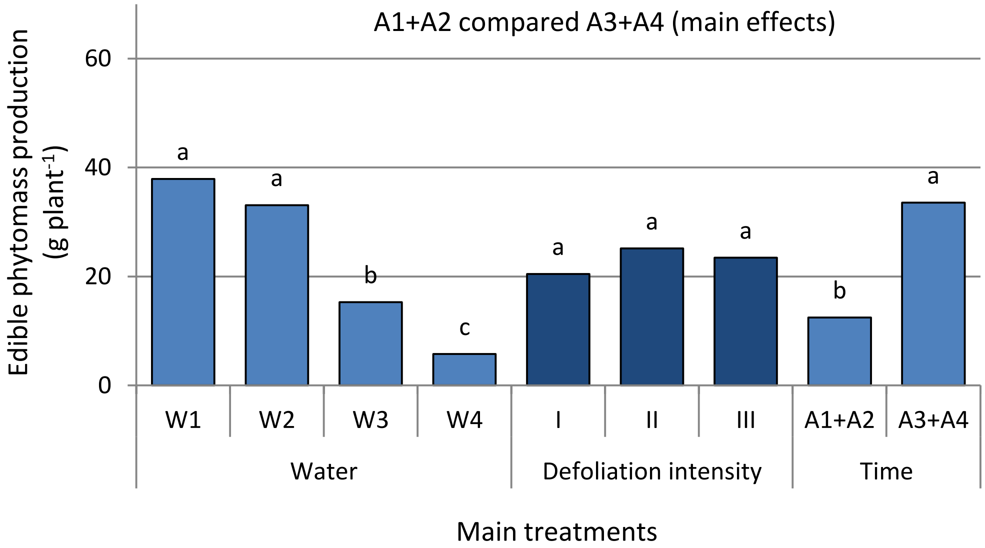

Figure 5.4: Mean (n = 4) cumulative edible phytomass production (g plant^{-1}) for *Nenax microphylla* for the three-monthly defoliation frequency, by comparing the first six months (A1 + A2) with that of the second six months (A3 + A4). The three main effects (water, defoliation intensity and time (season)) are indicated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences ($P < 0.001$) within treatments.

5.2.1.4 Total cumulative above- and belowground phytomass production of defoliation frequency A (after 12 months)

The total cumulative above- and belowground phytomass production, after 12 months for all treatments that was defoliated every three months (the three-monthly defoliation frequency) is presented in Figure 5.5. At the end of the trial, when plants were harvested destructively, plant material was separated into different fractions, namely: aboveground (edible- and inedible phytomass) and belowground (roots), as explained earlier. Total cumulative phytomass production (A1 + A2 + A3 + A4) was used for Figure 5.5.

Water availability accounted for the majority of variation in data for EP, TAP and TA&BP (Table 5.5). By contrast, defoliation intensity accounted for most variation in data for IEP and BP and thus had a bigger influence than water.

Table 5.5: ANOVA summary for the cumulative above- and belowground phytomass production of *Nenax microphylla* for the three-monthly frequency (Frequency A).

Frequency A		W		I		W x I	
Variable	Treatment	SS% and significance					
EP	A cum at end	**78**	***	2	*	10	***
IEP	A cum at end	13	***	**77**	***	6	***
TAP	A cum at end	**64**	***	19	***	10	***
BP	A cum at end	11	**	**53**	***	8	NS
TA&BP	A cum at end	**57**	***	25	***	10	***

W = water, I = defoliation intensity, EP = edible phytomass, IEP inedible phytomass, TAP = total aboveground phytomass, BP = belowground phytomass, TA&BP = total above- and belowground phytomass, A cum at end = cumulative phytomass production of frequency A after 12 months, NS = non-significant, SS% = sum of squares percentage, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

For all plant fractions, water stress showed a decrease in aboveground phytomass production (Figure 5.5). Production for edible phytomass (EP), inedible phytomass (IEP) and total aboveground phytomass (TAP) did not differ significantly ($P > 0.05$) between W1 and W2, but showed significant ($P < 0.001$) differences between the other water treatments (Figure 5.5). Belowground phytomass (BP) showed very few and rather small differences ($P > 0.05$) between water treatments, while differences attributed to defoliation intensities were more significant

(P < 0.001). Looking at total above- and belowground (TA&BP) phytomass production, significant (P < 0.001) differences in production were measured between all water treatments. Water availability accounted for a 221% increase in phytomass production, while defoliation intensity differed 96% between extremes.

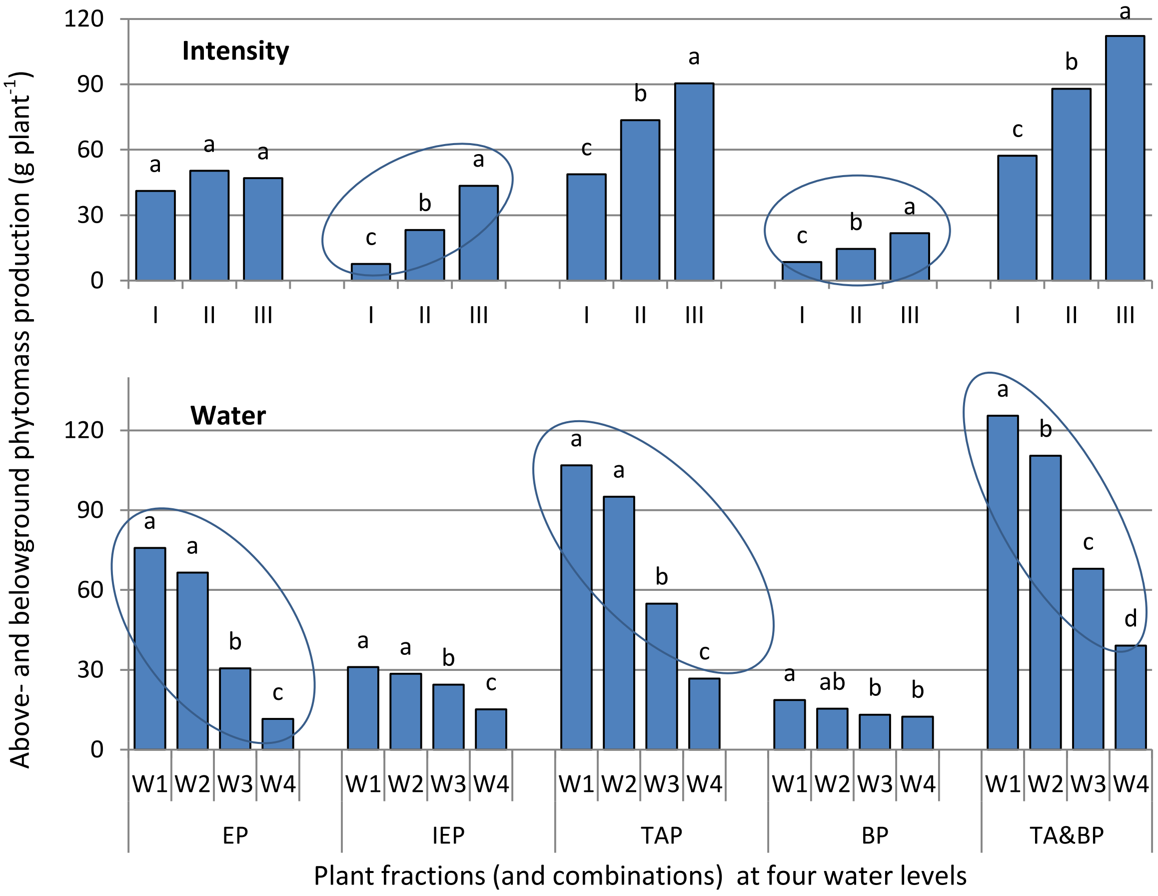

Figure 5.5: Mean (n = 4) cumulative above- and belowground phytomass production (g plant^{-1}) for *Nenax microphylla* (three-monthly defoliation frequency) for different plant fractions (and combinations) at different defoliation intensities and water levels after 12 months, when all plants were destructively defoliated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. EP (edible phytomass), IEP (inedible phytomass), TAP (total aboveground phytomass), BP (belowground phytomass), TA&BP (total above- and belowground phytomass). Different letters show significant differences (P < 0.001), between water treatments and between defoliation intensities, within each plant fraction.

It was clear that increased defoliation intensity showed a decrease in above- and belowground phytomass production (Figure 5.5). For TA&BP defoliation intensity played an important role in accounting for variation in data (Table 5.5), which is highlighted by intensity III having a 96% higher phytomass production than intensity I. Significant differences ($P < 0.001$) were found for all plant fractions, in terms of production for different defoliation intensities, with exception of EP (Figure 5.5). As the plants were defoliated every three months the accumulated regrowth (EP) did not differ much ($p > 0.05$).

5.2.1.5 Summary and discussion of defoliation frequency A

Defoliation date (time) and water had a highly significant ($P < 0.001$) influence on edible phytomass production, as well as on total above- and belowground phytomass production, while defoliation intensity had no significant ($P > 0.05$) influence. Defoliation intensity, however, had the highest ($P < 0.001$) influence on inedible and belowground phytomass production. For almost all the data, W1 and W2 did not differ significantly ($P > 0.05$). In comparing the four defoliation dates (seasons), the edible phytomass production for W3 and W4 did not differ significantly ($P > 0.05$) from each other.

In general, season of defoliation for the three-monthly defoliation frequency had a major impact on edible phytomass production. Edible phytomass production (growth) during the winter months was extremely low (below 5 g plant^{-1}) for all watering treatments, with autumn second lowest, spring higher than autumn and summer the highest. Edible phytomass production for the most severely water stressed treatment (W4) was equally low (< 5 g plant^{-1}) over all four seasons.

5.2.2 Defoliation frequency B (six-monthly interval)

5.2.2.1 Main effects: water and defoliation intensity

For B1 and B2, as well as B1+B2, water accounted for most of the variation (> 65%) in the data, while defoliation intensity, although tested significantly ($P < 0.001$), accounted for only around 7% of the variation in data (Table 5.6).

Table 5.6: ANOVA summary for the aboveground phytomass production of *Nenax microphylla* for the six-monthly frequency (Frequency B).

Frequency B		W		I		W x I	
Variable	Treatment	SS% and significance					
EP	B1	66	***	5	*	2	NS
EP	B2	82	***	7	***	5	***
EP	B1+B2	81	***	8	***	2	NS

W = water, I = defoliation intensity, NS = non-significant,
SS% = sum of squares percentage, EP = edible phytomass, B1 = first six months,
B2 = second six months, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

Phytomass production for frequency B only showed significant differences ($P < 0.001$) between the first six months and the second six months for W1 and W2, with phytomass production of W1 of the second six months almost double the phytomass of that of the first six months (Figure 5.6). During the first six months, phytomass production at W1 and W2 were almost similar ($P > 0.05$), but both higher ($P < 0.001$) than W3 and W4 for which phytomass production also differed significantly ($P < 0.001$) from each other. In the second 6 months the phytomass production differed significantly ($P < 0.001$) between all four water treatments with the edible phytomass production of W1 552% higher than that of W4. Defoliation intensity during the first six months did not show any significant ($P > 0.05$) differences in phytomass production, while it showed significant differences ($P < 0.001$) between all three treatments at the second six months (Table 5.6 and Figure 5.6).

The first six months were from February until the beginning of August, with relatively slow growth, especially during the cooler winter months. Growth during the second six months (September to the beginning of February) was much better during the warmer, mainly summer, months. As was the case with frequency A, the differences within treatments were bigger during the period of good growth, compared to the period of slower growth.

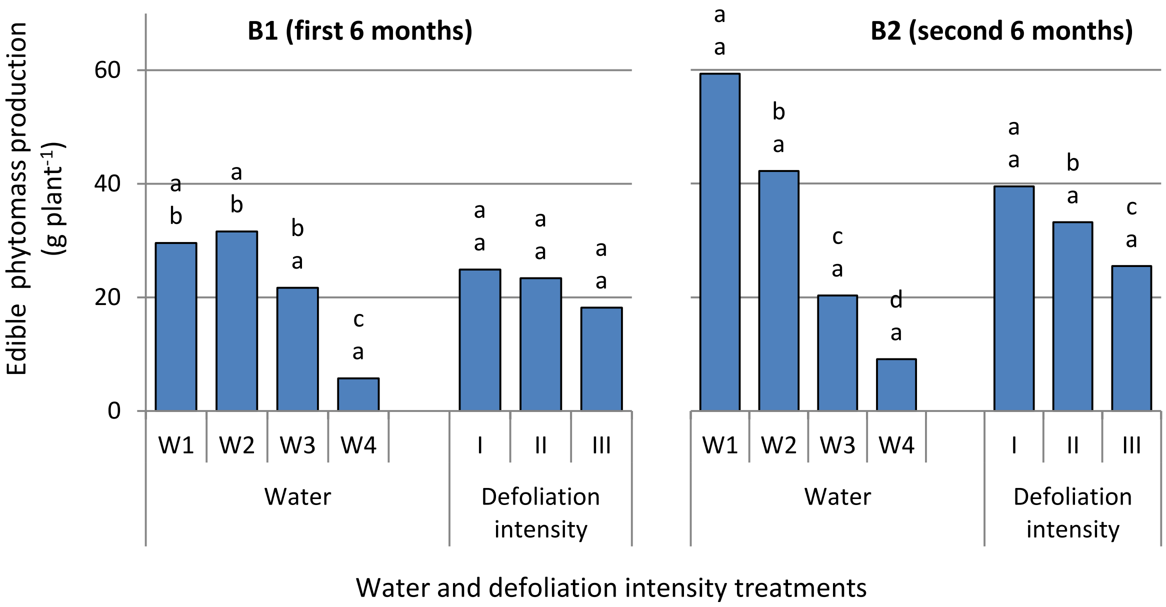

Figure 5.6 Mean (n = 4) edible phytomass production (g plant^{-1}) for *Nenax microphylla* (six-monthly defoliation frequency) at different water treatments and defoliation intensities. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different top letters, at each date, show significant differences ($P < 0.001$), between water- and between defoliation treatments at each date. Different bottom letters show significant differences ($P < 0.001$) between dates for similar water- and defoliation treatments.

Cumulative edible phytomass production (B1 + B2) showed significant ($P < 0.001$) differences with increased water stress, as well as for increased defoliation intensity (Figure 5.7). The edible phytomass production of W1 is more than five times the phytomass production of W4. Water by far accounted for most of the variation in data (81%), compared to the only 8% of defoliation intensity (Table 5.6).

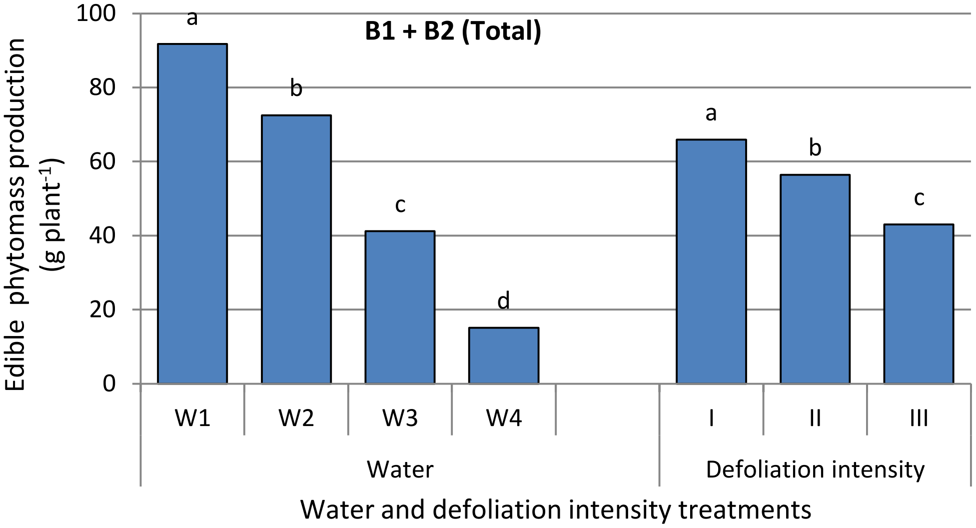

Figure 5.7: Mean (n = 4) cumulative edible phytomass production (g plant^{-1}) for *Nenax microphylla* (six-monthly defoliation frequency) at different water treatments and defoliation intensities. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences ($P < 0.001$) within treatments.

5.2.2.2 B1 compared to B2 at two different seasons (as two different time intervals)

In comparing B1 and B2 as two different time intervals (REML analysis), time (defoliation date/season) accounted for more variation in data than water, with intensity very low (Table 5.7). The time x water interaction accounted for more variation than defoliation intensity and is therefore presented in Figure 5.9.

Table 5.7: REML variance components analysis (B1 compared to B2)

B1 and B2 compared		T		W		I		T x W		T x I		W x I		T x W x I	
Variable	Treatment	F statistic and significance													
EP	B1 vs B2	128	***	92	***	11	***	55	***	4	*	1	NS	5	***

REML = Linear mixed model repeated measurement analysis, T = time (month), W = water, I = defoliation intensity, NS = non-significant, SS% = sum of squares percentage, EP = edible phytomass, B1 = first six months, B2 = second six months, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

The edible phytomass production of B2 was higher ($P < 0.001$) than that of B1 (Figure 5.8). This can also be attributed to B1 (first six months) being the autumn and winter months' growth, which was proved at frequency A as the seasons with the lowest edible phytomass production. The edible phytomass production of defoliation intensities I and II did not differ significantly ($P > 0.05$) from each other, but was higher ($P < 0.001$) than that of intensity III. Water stress had a negative ($P < 0.001$) influence on edible phytomass production, with the edible phytomass of W1 485% higher than that of W4.

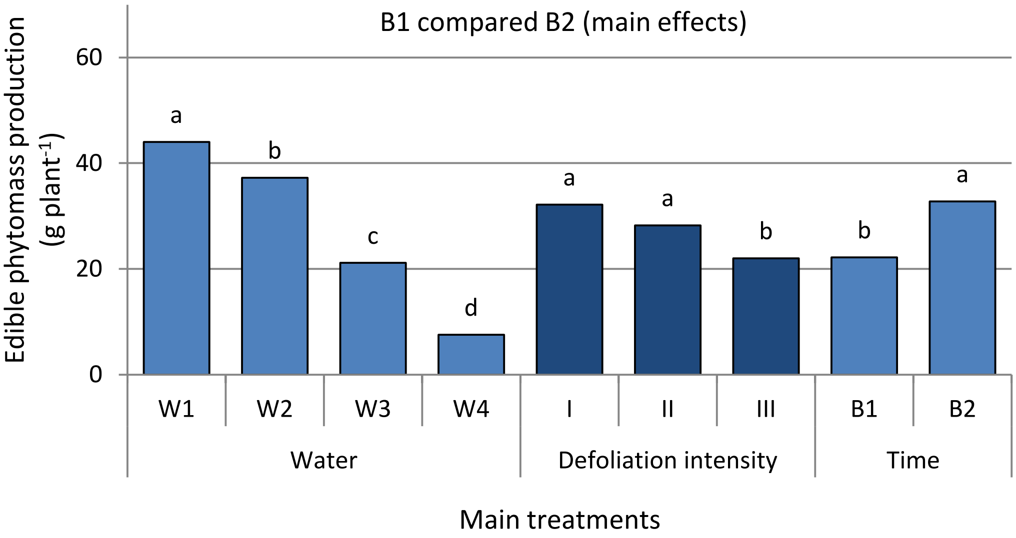

Figure 5.8: Mean (n = 4) edible phytomass production (g plant^{-1}) for *Nenax microphylla.* Comparison between phytomass production of B1 (first six months) and B2 (second six months). The three main effects (water, defoliation intensity and time) are indicated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences ($P < 0.001$) within treatments.

Water x time (season) interaction tested significantly ($P < 0.001$) as well as accounted for a high amount of the variation in data (Figure 5.9). Phytomass production differed significantly ($P < 0.001$) between defoliation dates for W1 and W2 (unstressed conditions), while it did not differ significantly ($P > 0.05$) for W3 and W4 (water stress conditions). Phytomass production for B2 (spring and summer seasons) showed a more rapid reduction with increasing water stress, while the decline in phytomass production for B1 (autumn and winter seasons) was less rapid.

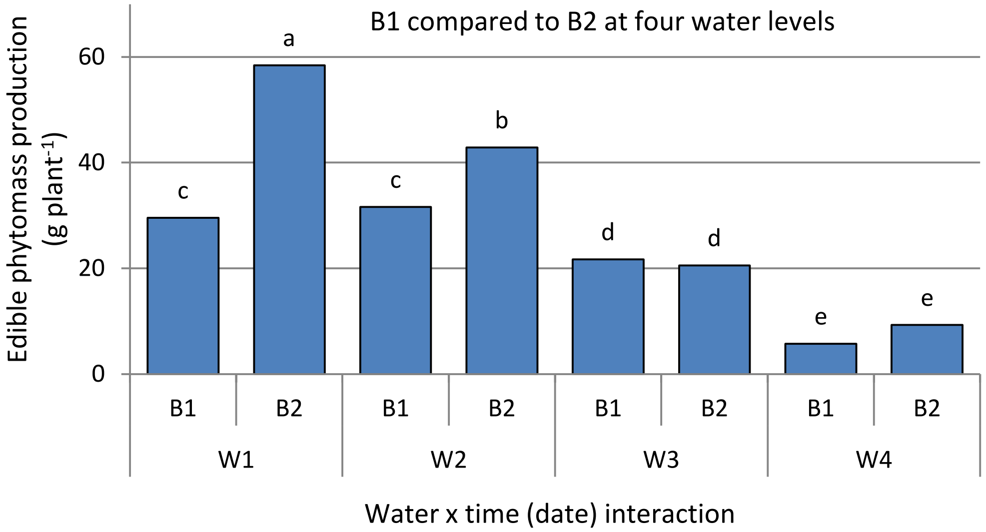

Figure 5.9: Mean (n = 4) aboveground edible phytomass production (g plant^{-1}) for *Nenax microphylla* at the water x time interaction. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). B1 = first six months and B2 = second six months. Different letters show significant differences ($P < 0.001$) for all treatment combinations.

5.2.2.3 Total cumulative above- and belowground phytomass production for frequency B. (after 12 months)

For the cumulative phytomass production of the different plant fractions and their combinations at Frequency B, water accounted by far for most of the variation (80%+) in data for EP, TAP and TA&BP (Table 5.8). For BP water accounted for more variation than defoliation intensity, but defoliation intensity did play a significant ($P < 0.001$) role in variation of BP. Defoliation intensity accounted for most of the variation (69%) in data for IEP (Table 5.8). Although the water x intensity interaction tested highly significant ($P < 0.001$) for IEP and TA&BP, the SS% was so low ($P > 0.05$) that it will not be discussed further.

Table 5.8: ANOVA summary for the cumulative above- and belowground phytomass production of *Nenax microphylla* for the six-monthly frequency (Frequency B).

Frequency B		W		I		W x I	
Variable	Treatment	SS% and significance					
EP	B cum at end	**81**	***	8	***	2	NS
IEP	B cum at end	16	***	**69**	***	11	***
TAP	B cum at end	**89**	***	3	***	2	NS
BP	B cum at end	**39**	***	**27**	***	8	NS
TA&BP	B cum at end	**83**	***	6	***	4	***

W = water, I = defoliation intensity, EP = edible phytomass,
IEP inedible phytomass, TAP = total aboveground phytomass, BP = belowground phytomass, TA&BP = total above- and belowground phytomass, B cum at end = cumulative phytomass production of frequency B after 12 months, NS = non-significant,
SS% = sum of squares percentage, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

Phytomass production for all plant fractions showed little variation between treatments for defoliation intensity, but greater and more significant ($P < 0.001$) variation in phytomass production between all water treatments (Figure 5.10). The only exception where defoliation intensity had a bigger influence on phytomass production was for IEP, where phytomass production of intensity III was 422% higher than that of intensity I.

When looking at TA&BP, defoliation intensity III had only 27% higher phytomass production than intensity I, while W1 had a 261% higher phytomass production than W4. This indicates that water stress had a bigger influence on phytomass production than defoliation intensity.

Belowground phytomass production for defoliation intensity III was 38% higher than for intensity I, while BP for W1 was 51% higher than W4 (Figure 5.10). Thus, both water deficit and extreme defoliation intensity lowered ($P < 0.001$) belowground phytomass (Table 5.8 and Figure 5.10).

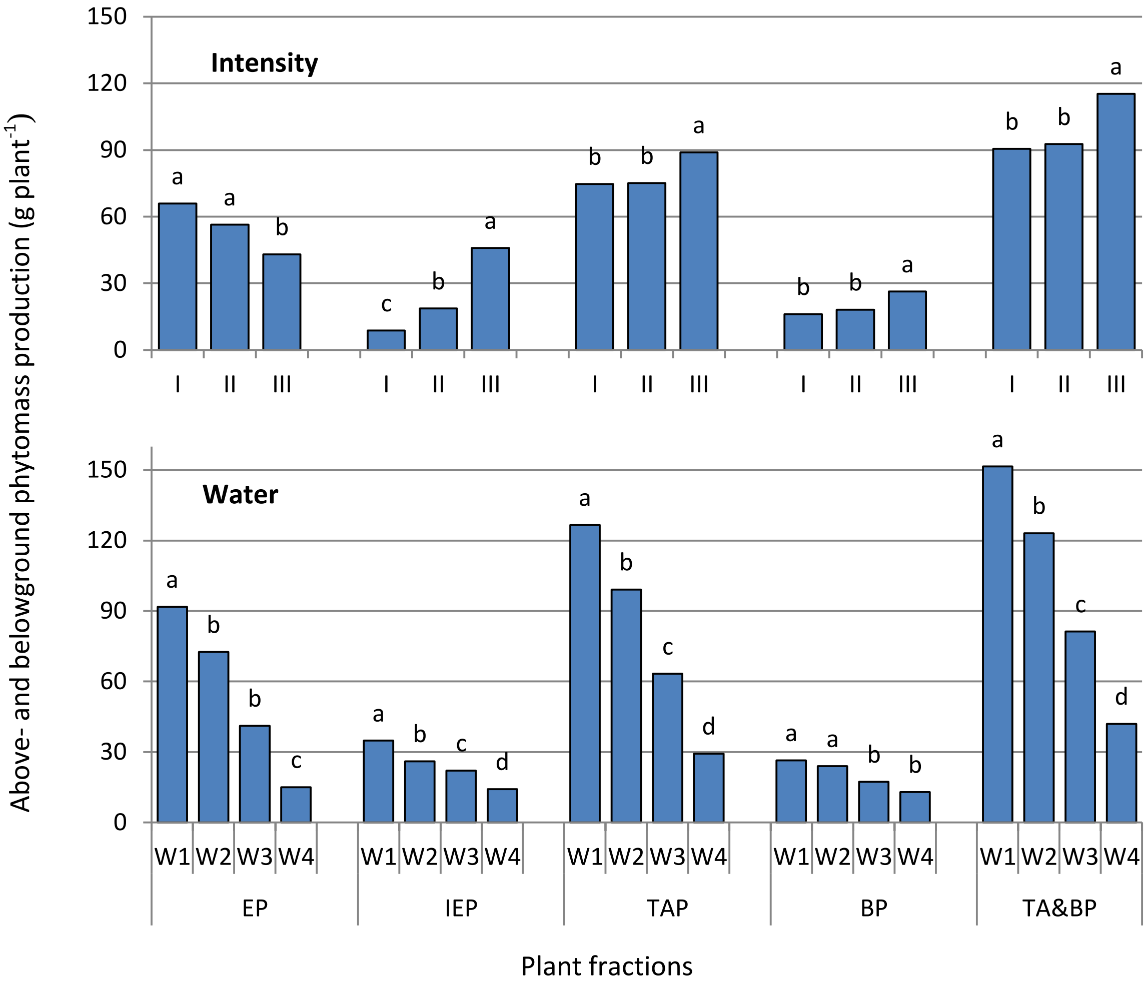

Figure 5.10: Mean (n = 4) cumulative above- and belowground phytomass production (g plant^{-1}) for *Nenax microphylla* (six-monthly defoliation frequency) for different plant fractions (and combinations) at different defoliation intensities and water levels after 12 months, when all plants were destructively defoliated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. EP (edible phytomass), IEP (inedible phytomass), TAP (total aboveground phytomass), BP (belowground phytomass), TA&BP (total above and belowground phytomass). Different letters show significant differences ($P < 0.001$), between water treatments and between defoliation intensities, within each plant fraction.

5.2.2.4 Summary and discussion of defoliation frequency B (six-monthly defoliation)

As was the case with the three-monthly defoliation frequency, defoliation time (season) and water influenced edible phytomass production the most ($P < 0.001$), and also all other plant fractions and combinations. Defoliation intensity had the greatest ($P < 0.001$) influence on both inedible and belowground phytomass production and also, but to a lesser extent than water, on the other plant fractions and combinations. In comparing the two defoliation dates (B1 and B2), the edible phytomass production for the most severe water stress treatments did not differ much ($P > 0.05$), while it did occur between the less stressed plants, which both had significantly ($P < 0.001$) higher phytomass productions than the stressed plants.

The edible phytomass production of the second six months, spring plus summer (B2) was significantly ($P < 0.001$) higher (48%) than that of the first six months, autumn plus winter (B1). There was a much more rapid decline in edible phytomass production under water deficit at B2 (552%) than B1 (396%), indicating bigger differences between water treatments under more favourable growing conditions (spring plus summer).

Looking at total above- and belowground phytomass production, the difference between the two extreme water treatments (W1 and W4) was 261%, while only 27% between the two most extreme defoliation intensity treatments. It follows the same pattern as at the three-monthly defoliation frequency where water treatment differed by 221% and defoliation intensities by 96%.

5.2.3 Defoliation frequency C (after 12 months)

For defoliation frequency C, water accounted for the majority of variation in the data for all plant part fractions and combinations, with the exception of IEP where intensity accounted for the most variation in data (Table 5.9). Although the water x intensity interaction tested highly significant, it will not be discussed as it accounts for only 4% of the variation in the data.

Table 5.9: ANOVA summary for the cumulative above- and belowground phytomass production of *Nenax microphylla* after 12 months (Frequency C).

Frequency C		W		I		W x I	
Variable	Defoliation frequency	SS% and significance					
EP	C	**<u>81</u>**	***	8	***	4	***
IEP	C	**24**	***	**<u>59</u>**	***	4	*
TAP	C	**<u>93</u>**	***	1	*	1	*
BP	C	**<u>78</u>**	***	1	NS	5	*
TA&BP	C	**<u>92</u>**	***	1	NS	2	*

W = water, I = defoliation intensity, EP = edible phytomass, IEP inedible phytomass, TAP = total aboveground phytomass, BP = belowground phytomass, TA&BP = total above- and belowground phytomass, NS = non-significant, SS% = sum of squares percentage, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

With the exception of IEP, where the phytomass production of all three defoliation intensity treatments differed significantly ($P < 0.001$), phytomass production of defoliation intensity did not differ much ($P > 0.05$) for the other plant part fractions. Phytomass production for the different water treatments, however, differed significantly ($P < 0.001$) between all treatments for all plant part fractions and combinations, with only W1 and W2 for IEP, which did not differ significantly ($P > 0.05$) (Figure 5.11). For TA&BP, W1 had a 377% higher phytomass production than W4. Water had a great influence also on BP, with W1 having a 267% higher production than W4.

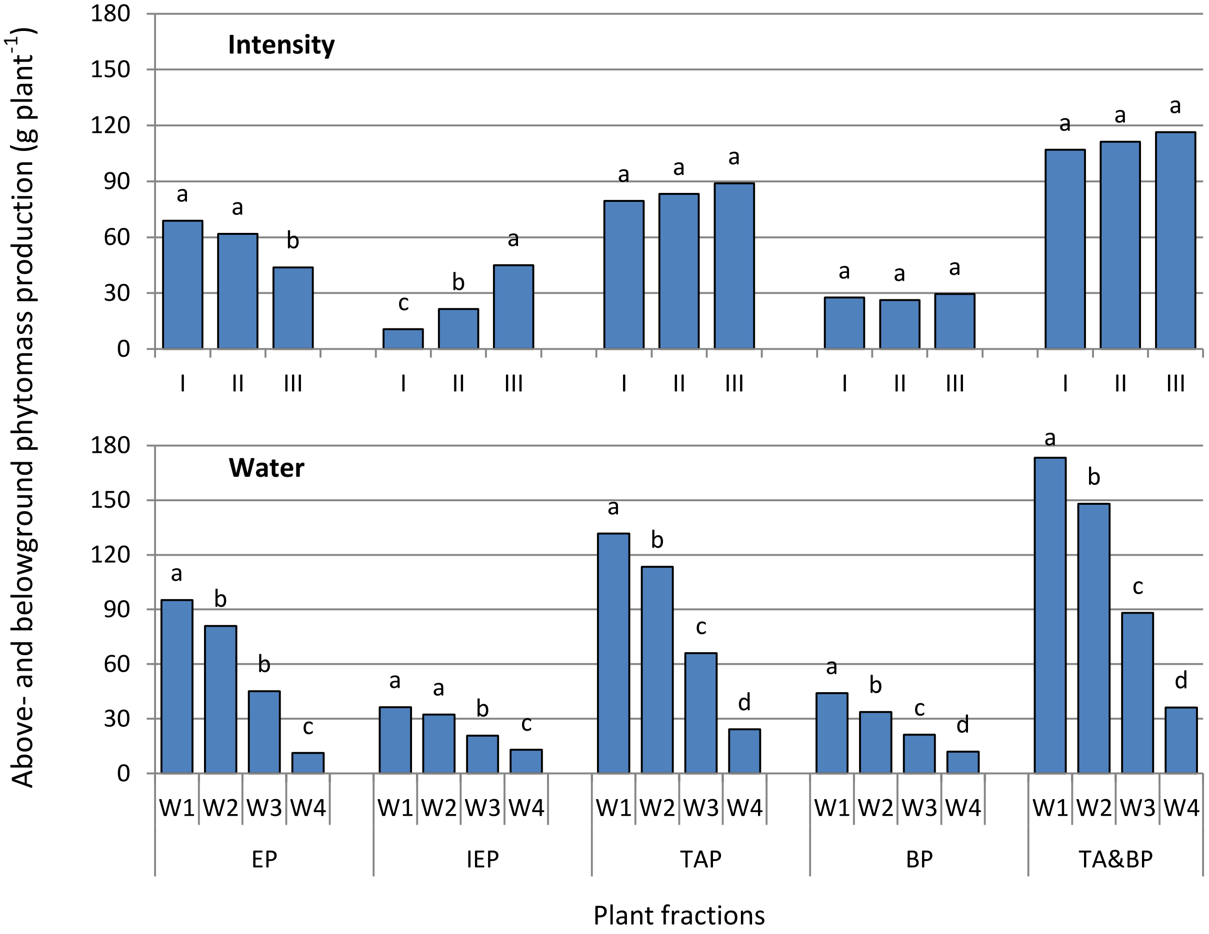

Figure 5.11: Mean (n = 4) cumulative above- and belowground phytomass production (g plant^{-1}) for *Nenax microphylla* (twelve monthly defoliation frequency) for different plant fractions (and combinations) at different defoliation intensities and water levels after 12 months, when all plants were destructively harvested. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. EP (edible phytomass), IEP (inedible phytomass), TAP (total aboveground phytomass), BP (belowground phytomass), TA&BP (total above and belowground phytomass). Different letters show significant differences (P < 0.001), between water treatments and between defoliation intensities, within each plant fraction.

5.2.3.1 Summary and discussion of defoliation frequency C (control)

The plants from defoliation frequency C were defoliated once on the date the trial started and a second time exactly 12 months later when the trial was terminated. It seems as if the first defoliation might have had small influences on phytomass production. Frequency C can also be regarded as a control treatment, regarding defoliation frequency.

Water had by far the most significant influence on all plant part fractions and combinations, with the exception of defoliation intensity, which had a bigger influence on inedible phytomass production than water. The differences in total above- and belowground phytomass production between extremes for water and defoliation intensities were 377% and 7%, respectively. The comparison between the three frequencies are illustrated in the table below, showing that the least frequent the plants was defoliated, the higher the water influence and lower the influence of intensity (Table 5.10).

Table 5.10: Percentage (%) differences between water and defoliation intensity treatment extremes, within each of the three defoliation frequencies.

Defoliation frequency	Water	Intensity
	% differences between treatment extremes	
A	221	96
B	261	27
C	377	7

5.2.4 Comparison of Frequency A, B and C

5.2.4.1 Frequency A compared to frequency B

First six months (A1 + A2 compared to B1)

Water (51%) and frequency (18%) accounted for most of the variation in the data (Table 5.10). Intensity and most interactions tested as non-significant (P > 0.05).

Table 5.11: ANOVA summary for the cumulative aboveground phytomass production of *Nenax microphylla* after the first and second six months for frequency A compared to that of frequency B for the same time intervals.

A and B compared		W		I		F		W x I		W x F		I x F		W x I x F	
Variable	Treatment	SS% and significance													
EP	A1+A2 compared B1	**51**	***	1	NS	**18**	***	2	NS	4	***	2	**	1	NS
EP	A3+A4 compared B2	**80**	***	1	**	0	NS	2	**	0	NS	3	***	5	***

W = water, I = defoliation intensity, F = defoliation frequency, EP = edible phytomass, IEP inedible phytomass, TAP = total aboveground phytomass, BP = belowground phytomass, TA&BP = total above- and belowground phytomass, A = Frequency A, B = frequency B, A1 = May, A2 = August, A3 = November, A4 = February, B1 = first six months, B2 = second six months, NS = non-significant, SS% = sum of squares percentage, *** = P < 0.001, ** = P < 0.01, * = P < 0.05

After the first six months the edible phytomass production of frequency B was higher (P < 0.001) than that of frequency A (Figure 5.12). The one extra defoliation (A1) of frequency A caused a reduction (P < 0.001) in phytomass production, compared to frequency B which was defoliated for the first time at that stage. Defoliation intensity showed no significant differences (P > 0.05) regarding phytomass production. Phytomass production for the severely stressed water treatment (W4) was lower (P < 0.001) than that of any other water treatment, with phytomass production of W1 and W2 virtually (P > 0.05) the same.

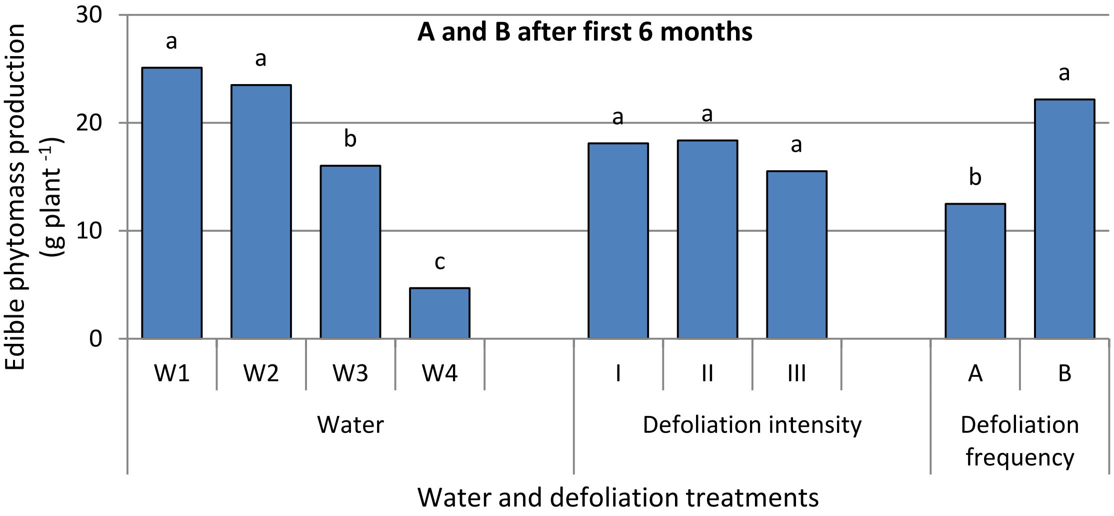

Figure 5.12: Mean (n = 4) edible phytomass production (g plant^{-1}) for *Nenax microphylla.* Comparison between the phytomass production of frequency A and B after the first six months. The three main effects (water, defoliation intensity and defoliation frequency) are indicated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. A = cumulative phytomass production for the first six months of the three-monthly defoliation frequency, B = First six months of the six-monthly defoliation frequency. Different letters show significant differences (P < 0.001) within treatments.

Second six months (A3 + A4 compared to B2)

After the second six months (after the spring and summer growing seasons), water accounted for most of the variation in data, with defoliation intensity and frequency not testing significantly (P > 0.05) (Table 5.10 and Figure 5.13). Frequencies A and B which shows similar phytomass productions after the six months (P > 0.05), indicates that Frequency A caught up with frequency B, as far as phytomass production is concerned, even though it had an extra defoliation (A3) compared to frequency B. This might be attributed to compensatory growth, with the plants of frequency A giving comparable phytomass production to frequency B during the favourable growth season, even though they were exposed to more stress. Phytomass production was significantly ($P < 0.001$) decreased by water stress, with W1 showing an 590% higher production than W4.

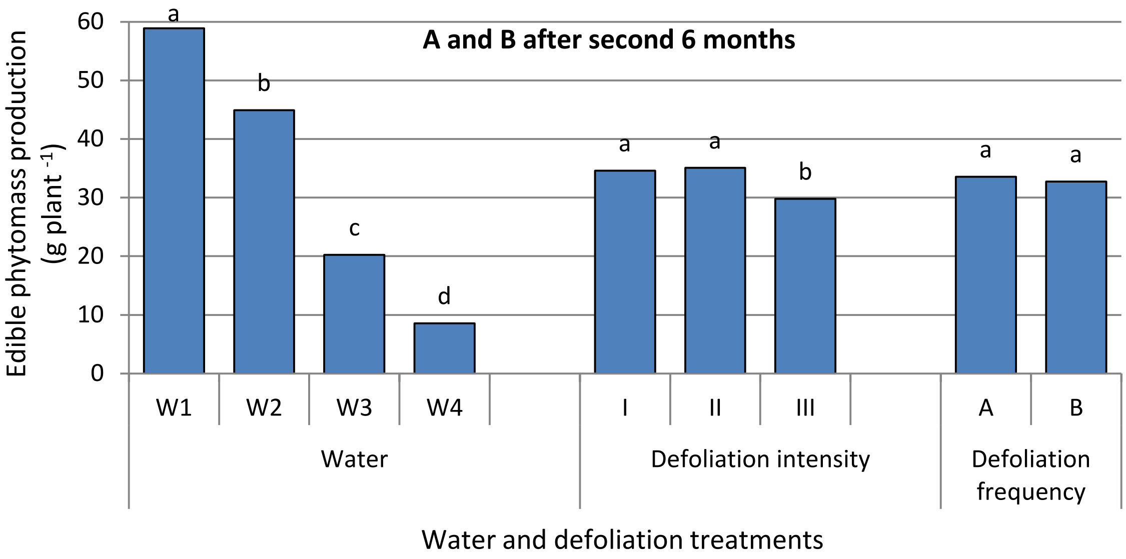

Figure 5.13: Mean (n = 4) edible phytomass production (g plant^{-1}) for *Nenax microphylla.* Comparison between the phytomass production of frequency A and B after the second six months. The three main effects (water, defoliation intensity and defoliation frequency) are indicated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. A = cumulative phytomass production for the second six months of the three-monthly defoliation frequency, B = Second six months of the six-monthly defoliation frequency. Different letters show significant differences ($P < 0.001$) within treatments.

The water x intensity x frequency interaction tested significantly ($P < 0.001$), but accounted for only 5% of the variation in the data (Table 5.10). Figure 5.14 is however presented to indicate that for W1 and W2 (intensities II and III), frequency A had a higher ($P < 0.001$) edible phytomass production than frequency B. Frequency A, out producing frequency B, might be due to compensatory growth. At the lower water levels, water is just too limited for the plants to respond to the negative impact of defoliation. Defoliation intensity I (most severely defoliated) had a lower ($P < 0.001$) edible phytomass production at W1 for frequency A than defoliation frequency B. Contrary, at W3 and W4, differences in edible phytomass between defoliation intensities were very small ($P > 0.05$) or did not occur.

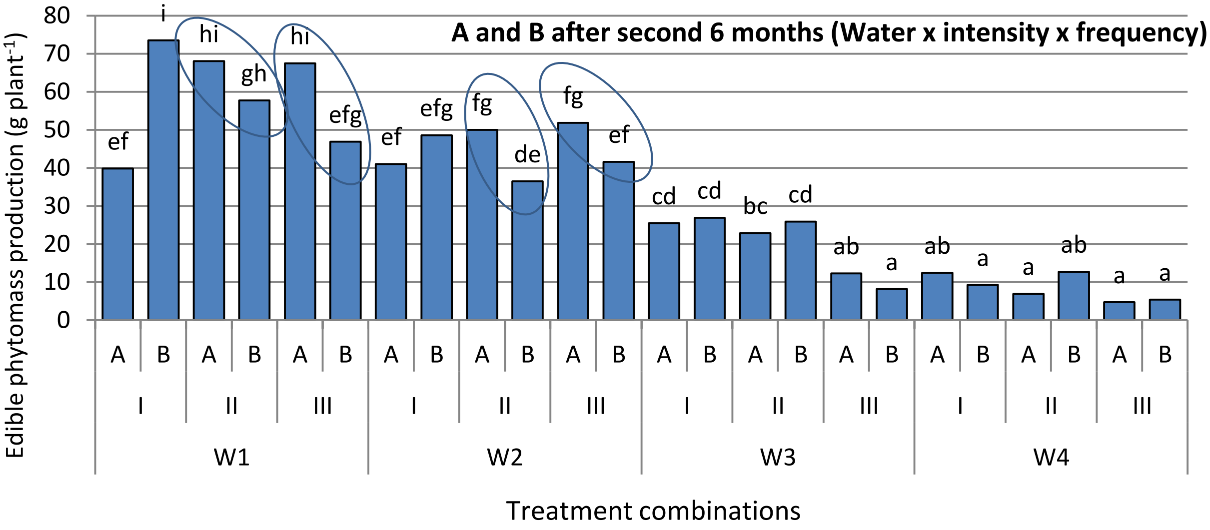

Figure 5.14: Mean (n = 4) aboveground phytomass production (g plant^{-1}) for *Nenax microphylla* for the water x intensity x frequency interaction after the first six months. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. A = cumulative phytomass production for the first six months of the three-monthly defoliation frequency, B = First six months of the six-monthly defoliation frequency. Different letters show significant differences ($P < 0.001$) between all treatments.

5.2.4.1.1 Summary and discussion of frequency A and B compared

In comparing the first six months, the production of the first three months (A1 – May) added to that of the second three months (A2 – August), therefore A1+A2, compared to that of only B1 (the first six months), both water and frequency influenced edible phytomass significantly ($P < 0.001$). At that stage, the edible phytomass of B1 was 78% higher than that of A1+A2. This indicates that the one extra defoliation of frequency A (autumn) almost halved the production potential of *N. microphylla* compared to B1.

The results from the second six months were even more interesting. At the end of the second six months, B2 was compared to the third three months (A3 – November) added to the fourth three months (A4 – February), therefore A3+A4. This time there was no significant ($P > 0.05$) difference between frequencies, but water had still a high ($P < 0.001$) influence on edible phytomass production. At the most severe defoliation intensity, the cumulative edible phytomass production of defoliation frequency A (A3+A4) was lower than that of frequency B (at B2), while the opposite happened at the less severe defoliation intensities (intensities II and III), where edible phytomass production was higher ($P < 0.001$) at A3+A4 than at B2.

At water treatment one in combination with defoliation intensity III, B1 had a 40% higher edible phytomass production than A1+A2, while almost the opposite was true for A3+A4 which was 44% higher than B2. This resulted in an 84% switch in edible phytomass production between the first and second six-month defoliation for frequencies A and B. From these results it can be argued that a plant that receives two moderate defoliations during spring and summer can give significantly higher edible phytomass than a plant that receives one moderate defoliation. This might, however, only be the case when sufficient water is available. It can also be reasoned that if a more frequently defoliated plant came through the winter with a, for example, 78% deficit in edible production to a less defoliated plant, it can totally outperform the less defoliated plant during the spring and summer growth period. This is strong evidence of compensatory ability and might also be a sign of resilience for which Nama-karoo vegetation is known (Todd 2006, Vetter 2009). The plant makes up for lost growth by showing extremely rapid growth, even outperforming the plants that are less frequently defoliated. Compensatory growth is only possible when for example water is not limited – as during periods of high rainfall. The lower water treatments (W3 and W4) did not show this ability, with edible phytomass productions remaining low, even during the spring and summer which proved to be the seasons more favourable for phytomass production of *N. microphylla*.

To conclude, autumn grazing, just before winter normally causes a major setback in individual plant performance during the winter months, but *N. microphylla* has the ability to recover and even significantly outperform, in terms of edible phytomass, the plants that were defoliated less frequently, under favourable soil moisture conditions.

5.2.4.2 Frequency A, B, C compared

In comparing frequencies A, B and C, water accounted for most variation in the data for EP (77%), TAP (80%) and TA&BP (73%) (Table 5.12). Intensity accounted for most variation in the data for IEP (67%), while frequency (21%) and water (33%) accounted for most variation in data for BP. Although most of the interaction tested highly significant ($P < 0.001$), the SS% was too low to be discussed. The interaction of I x F and W x F are however presented to explain some notable findings.

Table 5.12: ANOVA summary for the cumulative above- and belowground phytomass production of *Nenax microphylla* for all frequencies at the end of the trial.

A,B,C compared		W		I		F		W x I		W x F		I x F		W x I x F	
Variable	Treatment	SS% and significance													
EP	A, B, C compared	**77**	***	3	***	2	***	1	NS	1	*	3	***	4	***
IEP	A, B, C compared	17	***	**67**	***	0	NS	6	***	1	*	1	NS	1	NS
TAP	A, B, C compared	**80**	***	5	***	2	***	1	***	1	***	2	***	3	***
BP	A, B, C compared	**33**	***	9	***	**21**	***	1	NS	**13**	***	3	***	4	**
TA&BP	A, B, C compared	**73**	***	6	***	4	***	1	*	3	***	3	***	4	***

W = water, I = defoliation intensity, F = defoliation frequency, EP = edible phytomass, IEP inedible phytomass, TAP = total aboveground phytomass, BP = belowground phytomass, TA&BP = total above- and belowground phytomass, A = Frequency A, B = Frequency B, C = Frequency C, NS = non-significant, SS% = sum of squares percentage, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

Phytomass production was decreased ($P < 0.001$) by water stress for all plant part fractions (Figure 5.15). For the water treatments, the biggest difference in phytomass production was found with edible phytomass, where W1 had a 594% higher edible phytomass production than W4.

All three defoliation intensity treatments differed significantly ($P < 0.001$), regarding phytomass production, for IEP and TA&BP. The biggest variation in phytomass production was recorded for IEP, where intensity III had a phytomass production of 395% higher than intensity I.

All three defoliation frequencies differed significantly ($P < 0.001$) for BP and TA&BP with regard to phytomass production. Belowground phytomass production showed the biggest variation of all plant part fractions between defoliation frequencies, with phytomass production of frequency C being 86% higher than that of frequency A.

These results indicate that in general, rainfall (water availability) has a greater influence on the phytomass production of *N. microphylla* than grazing (defoliation). Furthermore, root production (belowground phytomass) is highly influenced by grazing frequency, but not as much as by water.

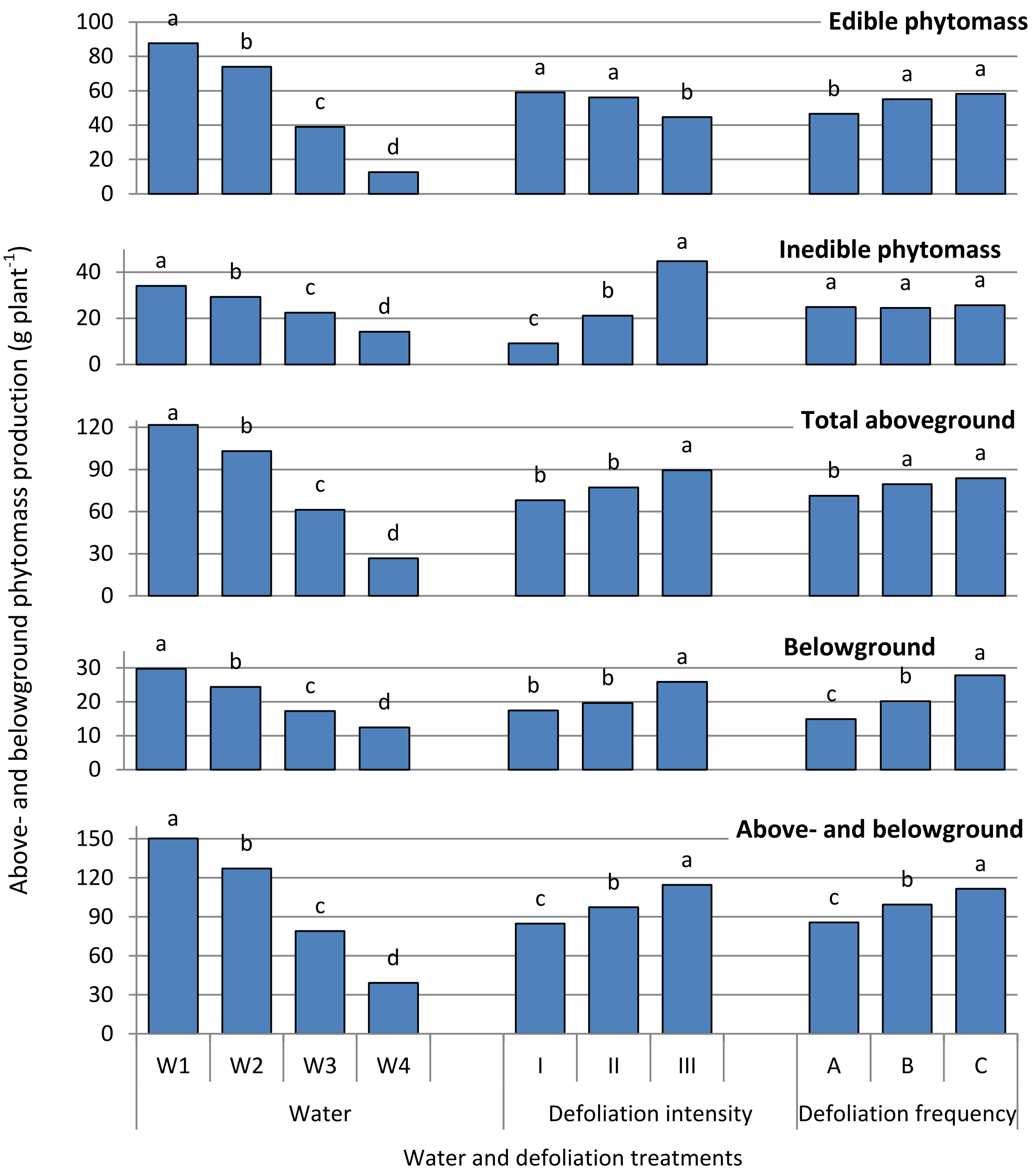

Figure 5.15: Mean (n = 4) cumulative above- and belowground phytomass production (g plant^{-1}) for *Nenax microphylla* for different plant fractions (and combinations) after 12 months, when all plants were destructively harvested. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences ($P < 0.001$), within treatments at each fraction. Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve monthly or control.

The highest variation in phytomass production for defoliation intensities was found for frequency A, where all plant part fractions differed significantly ($P < 0.001$), with the exception of EP (Figure 5.16). On the contrary, phytomass production at frequency C showed the least variation between defoliation intensity treatments. Inedible phytomass production differed significantly ($P < 0.001$) at all three frequencies with regards to defoliation intensity treatments, which can be attributed to the three different cutting heights for the three different defoliation intensity treatments.

With the exception of edible phytomass, the significant differences between defoliation intensities at frequency A indicates significant influences on phytomass production for repeated (three-monthly) severe defoliations. The fact that edible phytomass did not differ significantly might be ascribed to the compensatory ability of *N. microphylla*. The compensatory growth of the more severely defoliated plants was so high that it equaled the phytomass production of the less severe defoliation treatments. The regrowth was also the edible phytomass which was repeatedly defoliated at every defoliation date, with virtually no growth (expansion) of inedible phytomass, therefore the constant significant ($P < 0.001$) difference in inedible phytomass production (Figure 5.16). It could even be postulated that the rapid (compensatory) regrowth was at the cost of inedible (thicker twigs) and root growth, with the plant trying to rapidly increase its photosynthetic surface. Unfortunately, before an adequate photosynthetic surface is reached and maintained to redirect growth to stems (branches) and roots, the plant gets defoliated again, resulting in the negative growth cycle starting again. Defoliation at repeated short intervals, might therefore mask the possible positive effect of compensatory growth in the process of recovery after grazing.

At frequency B the phytomass production of defoliation intensity III was significantly ($P < 0.001$) higher than that of treatments I and II for TAP, BP and TA&BP. This indicates the detrimental influence of a more severe defoliation intensity, repeated at six-monthly intervals. For edible phytomass, however, the two more severely defoliated treatments (intensity I and II) gave higher (P < 0.001) phytomass productions than the most lenient defoliation (intensity III). This strengthens the argument of the compensatory ability of *N. microphylla* as discussed above.

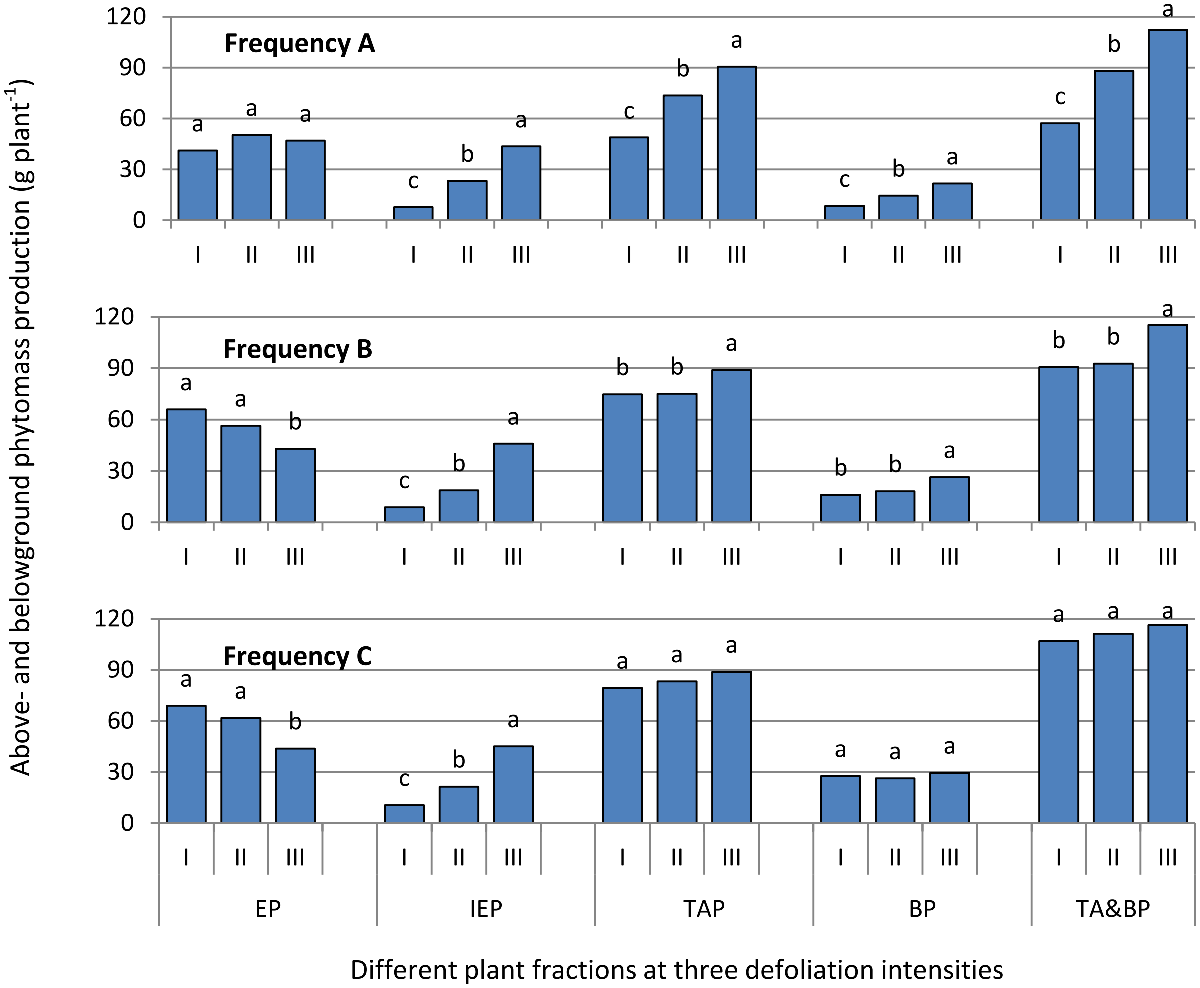

Figure 5.16: Mean (n = 4) cumulative above- and belowground phytomass production (g plant^{-1}) for *Nenax microphylla* (frequency A, B and C) for different plant fractions (and combinations) at different defoliation intensities after 12 months, when all plants were destructively defoliated. Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. EP (edible phytomass), IEP (inedible phytomass), TAP (total aboveground phytomass), BP (belowground phytomass), TA&BP (total above and belowground phytomass). Different letters show significant differences (P < 0.001), between water treatments within each fraction for each frequency.

A notable finding was the slight differences between the defoliation intensities for both total aboveground and total above- plus belowground phytomass production, at frequency C, showing a decrease in phytomass production with increasing defoliation intensity. Although these slight differences in phytomass production are not significant, they can be attributed to the first defoliation, 12 months prior to the current defoliation results under discussion. It can therefore be argued that even after a 12 month rest period, the effect of the previous defoliation treatment was still evident.

The water x frequency interaction showed significant ($P < 0.001$) differences in phytomass production for all four watering treatments at all three defoliation frequencies for TA&BP (Figure 5.17). The production differences for frequency A were smaller for all plant parts than at frequencies B and C. This might be an indication that the severe (three-monthly) defoliation frequency, impacted so much on phytomass production that the effect of water was diluted to some extent, compared to the bigger influence of water at the other defoliation frequencies.

Belowground phytomass production accounted for 13% variation in the water x frequency interaction data, more than any other of these interactions. For W4 no significant ($P > 0.05$) differences were evident in TAP, BP and TA&BP between defoliation frequencies (Figure 5.17). This indicates that when plants are severely water stressed, the water stress has a bigger impact on phytomass production than defoliation frequency itself.

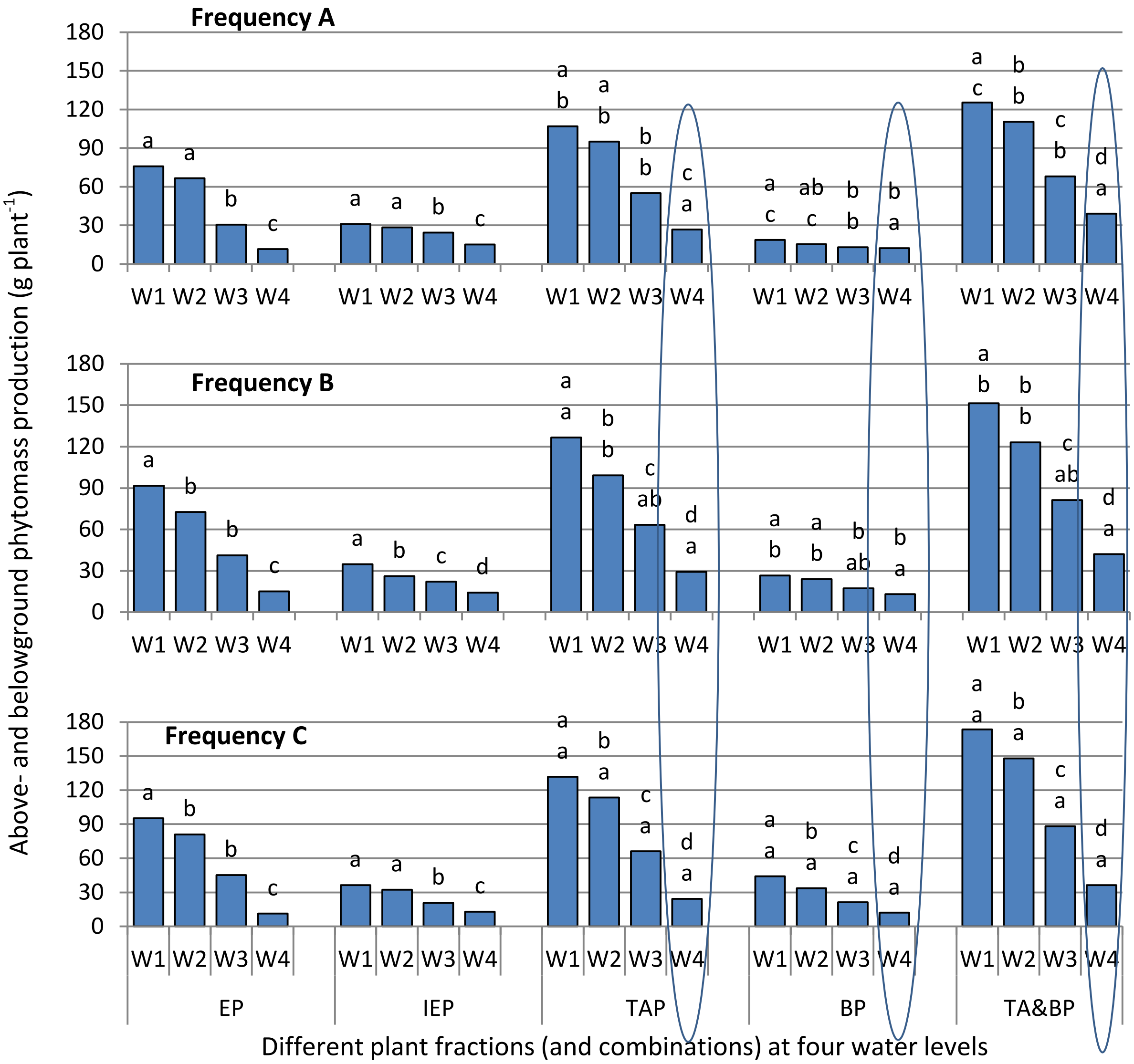

Figure 5.17: Mean (n = 4) cumulative above- and belowground phytomass production (g plant^{-1}) for *Nenax microphylla* (frequency A, B and C) for different plant fractions (and combinations) at different water levels after 12 months, when all plants were destructively defoliated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). EP (edible phytomass), IEP (inedible phytomass), TAP (total aboveground phytomass), BP (belowground phytomass), TA&BP (total above- and belowground phytomass). Different top letters show significant differences (P < 0.001), between water treatments within each fraction for each frequency. Different bottom letters shows significant differences (P < 0.001) between frequencies for similar water treatments.

Figure 5.18 was included to, amongst others, better explain the effect of water stress on root growth. This figure indicates the percentage difference in phytomass production between defoliation intensity treatment extremes (intensities I and III) at the three defoliation frequencies for all plant part fractions. It also indicates the percentage difference in phytomass production between the water treatment extremes (W1 and W4) at the three defoliation frequencies for all plant part fractions.

Defoliation intensity showed the lowest percentage differences between treatment I and III at defoliation frequency C (least frequent defoliation). At frequency A (most frequent defoliation) the percentage difference between intensity I and III is much greater, indicating the large influence of defoliation intensity in combination with the most frequent defoliation treatment, namely frequency A on phytomass production. With the exception of IEP, water caused far greater differences between treatment extremes than intensity (Figure 5.18).

Defoliation frequency C showed the greatest percentage difference between water treatment W1 and W4. This is an indication that the more frequently (frequencies A and B) the plants were defoliated, the smaller the effect of water on phytomass production. Thus, although the greater majority of data indicated water as the major determinator of phytomass production, defoliation frequency also has a definite effect on phytomass production. Edible phytomass had the largest percentage differences between watering treatments W1 and W4 for all three frequencies, while belowground phytomass production showed the biggest variation between defoliation frequencies.

The most notable phenomenon was that BP showed clear opposite reactions between defoliation intensity and water treatments. When a plant is defoliated frequently (frequency A), the difference in BP was 156% between treatment I and III. The opposite was true for water, where the difference between W1 and W4 was low (51%) at frequency A. The contrary was true for frequency C where the plants were defoliated at 12 months. The difference in BP between intensity I and III were only 7%, while the difference between W1 and W4 was 267%. This is another illustration of water having a much greater effect on phytomass production than for instance defoliation intensity.

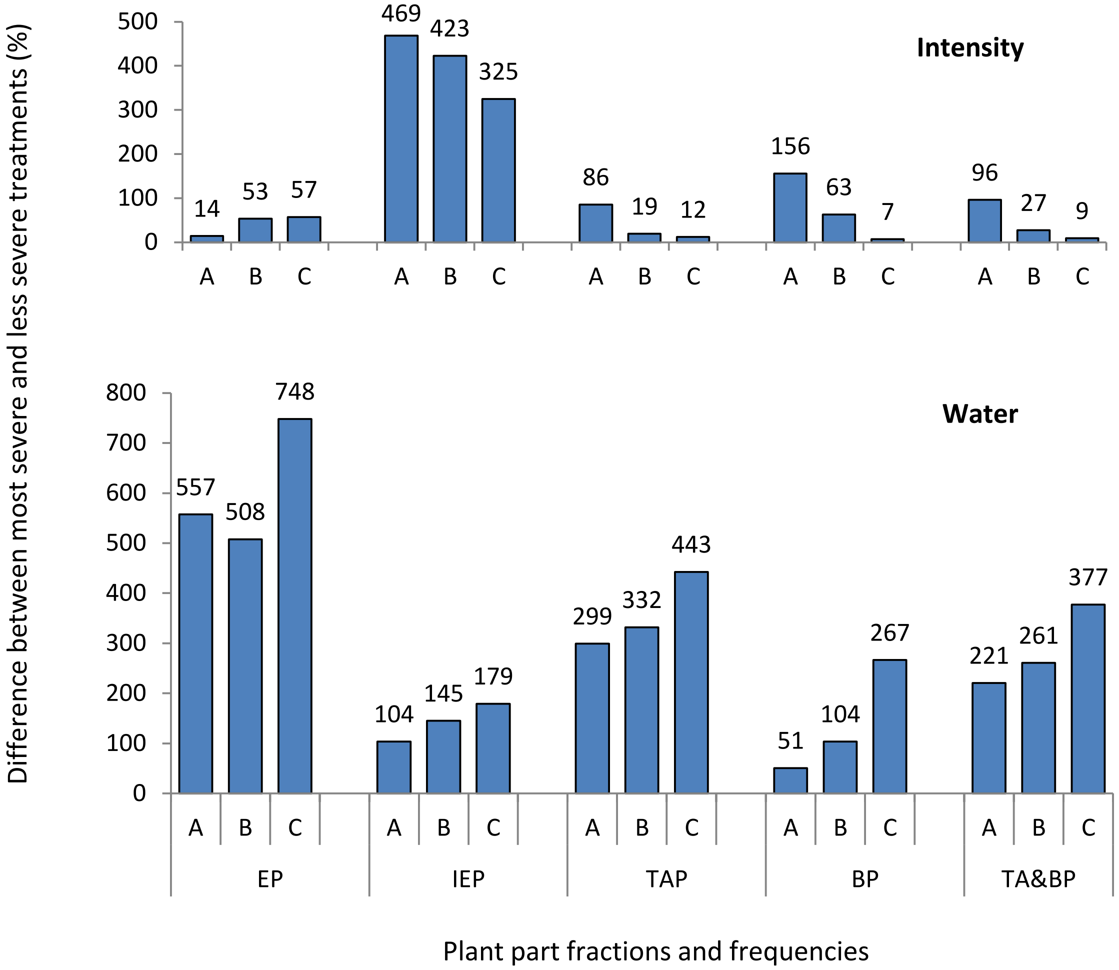

Figure 5.18: Summary of the percentage difference between the most severe and least severe defoliation and water treatments for the different plant part fractions and combinations of *Nenax microphylla* at the three defoliation frequencies. EP (edible phytomass), IEP (inedible phytomass), TAP (total aboveground phytomass), BP (belowground phytomass), TA&BP (total above- and belowground phytomass). Defoliation frequency: A = (three-monthly, B = six-monthly and C = 12 monthly or control.

5.2.4.3 **Summary** and **discussion of comparison between defoliation frequencies A, B and C**

Overall, water had the most significant (P < 0.001) influence on phytomass production, greater than that of defoliation intensity and frequency, which also had significant influences on phytomass production, but to a lesser extent than that of water.

Table 5.13 gives a summary of the SS% for the different plant part fractions and combinations, to indicate which main effect accounted for the most variation in data, thus having the biggest influence on phytomass production, at the three defoliation frequencies. At frequency A, water had the biggest effect on edible phytomass, total aboveground phytomass and total above- plus belowground phytomass, while intensity had the biggest influence on inedible phytomass production and belowground phytomass production. For phytomass production of all plant fractions and plant fraction combinations, there is an increase in SS% for water and a decrease in the SS% for intensity, with decreased defoliation frequency. At frequency C, with the exception of inedible phytomass, water by far had the biggest influence on the phytomass production of all plant part fractions.

Table 5.13: ANOVA summary of sum of square percentages (%) of the main effects, water and defoliation intensity at the three defoliation frequencies.

Variable	A		B		C	
	W	I	W	I	W	I
EP	**78**	2	**81**	8	**81**	8
IEP	13	**77**	16	**69**	24	**59**
TAP	**64**	19	**89**	3	**93**	1
BP	11	**53**	**39**	**27**	**78**	1
TA&BP	**54**	25	**83**	6	**92**	1

EP = edible phytomass, IEP inedible phytomass, TAP = total aboveground phytomass,
BP = belowground phytomass, TA&BP = total above- and belowground phytomass,
W = water, I = defoliation intensity, A = Frequency A, B = Frequency B, C = Frequency C

At the end of the study, in comparing all three frequencies, frequency had a bigger effect on BP than intensity. Water had a slightly bigger effect on BP than frequency. While water is not limited (W1 and W2), differences between intensities and frequencies (and time/date) do occur, but when water is limited (W3 and W4) all treatments gave equally low phytomass productions.

Table 5.14 summarizes the percentage difference between treatment extremes in interaction with each of the main effects. Firstly, considering defoliation intensities and frequencies at increased water deficit, it is clear that as the water deficit increased, there was a decrease in differences between frequencies A and C. For defoliation intensity, the difference between intensity extremes stayed more or less the same for W1, W2 and W3, while a large difference (70%) was recorded at W4.

Secondly, considering the defoliation frequency and water at increased defoliation intensities, there was a definite increase in percentage difference between extremes for both defoliation frequency and water, with an increase in defoliation intensity. The percentage differences between water extremes were much higher than that of defoliation frequency at all defoliation intensities.

Table 5.14: Percentage difference between treatment extremes in interaction with each of the main effects for total above- plus belowground phytomass production.

Water	A - C	I - III	**Intensity**	A - C	W1 - W4	**Frequency**	I - III	W1 - W4
W1	**38**	33	I	**87**	**344**	A	**96**	221
W2	34	33	II	26	285	B	27	261
W3	29	27	III	5	248	C	9	**377**
W4	8	**70**						

Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters shows significant differences ($P < 0.001$), within treatments at each fraction. Defoliation frequency: A = (three-monthly, B = six-monthly and C = twelve monthly or control.

Thirdly, looking at what happened at the defoliation intensities and water at increased defoliation frequencies, the percentage difference between treatment extremes for defoliation intensity increased with increased defoliation frequency, while that of water decreased with increased defoliation frequency. Again the percentage differences between water extremes were much higher than that for defoliation intensity at all defoliation frequencies.

5.2.5 Practical implication of phytomass findings

All phytomass findings are summarized in Figure 5.19 to highlight the three way interaction (all 36 treatment combinations) for the aboveground, belowground and total above- plus belowground phytomass production. This figure summarizes the influence of water and defoliation interactions on phytomass production. The aim is to derive the possible influences of grazing and rainfall interactions on phytomass production of *N. microphylla*. Although the trial was carried out *in sito*, it might explain how rainfall and grazing interaction could impact on the productivity of *N. microphylla in vivo*.

The vertical lines to the right hand side of the graph (Figure 5.19) indicate how water treatment dominated the distribution of the data. The data were ranked from the lowest to the highest phytomass production and roughly followed the water treatments, from the most severely stressed water treatment (W4) tot the unstressed plants (W1).

The darker black horizontal lines in Figure 5.19 were inserted to indicate the best and worse set of treatments, selected according to the multiple t-distribution test procedure of Gupta and Panchapakesan (1979). The treatment combinations below the last horizontal lines at the bottom of the figure indicate the best group regarding phytomass production. This is the group of treatment combinations that gave the highest phytomass productions without differing significantly from each other ($P < 0.001$). Watering treatment W1 (least water stressed) in combination with defoliation intensity III (less severe at 200 mm height), for all three defoliation frequencies, are present in this group for aboveground, belowground and total above- plus belowground phytomass production. This illustrates that above normal rainfall with a lenient defoliation might give the highest phytomass production, regardless of the defoliation frequency. Thus, in high rainfall years, *N. microphylla* might be defoliated more frequently, but at a moderate intensity.

The treatment combinations above the top horizontal lines in Figure 5.19 indicate the weakest group (Gupta and Panchapakesan 1979) regarding phytomass production (ignore alphabet letters). This is the group of treatment combinations that gave the lowest phytomass production without differing significantly ($P < 0.001$) from each other. Water treatment W4 (severely stressed) totally dominates this group for aboveground, belowground and total above- plus belowground phytomass production. Defoliation intensity I (most severe defoliation – 50 mm height) in combination with the most stressed plants gave the lowest phytomass production, although it did not differ significantly ($P > 0.05$), regarding phytomass production, from the other treatment combinations in the same group. This explains that during droughts the impact of water stress is so high on phytomass production that none of the defoliation treatments resulted in any significantly higher phytomass production. Although not significantly higher, a light defoliation intensity (defoliation intensity III – 200 mm height), might give higher phytomass productions, regardless of the defoliation frequency.

Looking at the influence of defoliation treatments on phytomass production, defoliation intensity I (most severe – 50 mm height) in combination with defoliation frequency A (three-monthly defoliation) proved to be the most detrimental, regardless of water treatment (encircled in red in Figure 5.19). This indicates that frequent severe grazing should always be avoided, irrespective of the amount of rainfall. On the contrary, a lighter (defoliation intensity III – 200 mm height), less frequent (defoliation frequency C – once per year) gave the highest phytomass production (encircled in green in Figure 5.19). This indicates that a light grazing, once per year, even at W3 (the second most severely stressed water treatment) is most beneficial for phytomass production of *N. microphylla*.

It should, however, be taken into account that for survival and sustainability farmers need to stock their farms and utilize the vegetation according to the long term grazing capacity of the rangeland, not only to the benefit of the vegetation but also for their own economic benefit. This might cause them to sometimes apply a more severe defoliation, which can be lowered by applying longer resting periods through appropriate grazing management systems. The Nama-karoo Biome is however known for its diverse botanical composition, mostly consisting of various preferred shrub and grass species with variable palatability. This complex and delicate interaction between animal and plant is extremely difficult to manage for sustainable animal production. Awareness of the reaction of single shrub species to different defoliation intensities and frequencies under variable rainfall occurrence might shed a little bit of light on which defoliation interactions to avoid and still ensure higher and sustainable phytomass production.

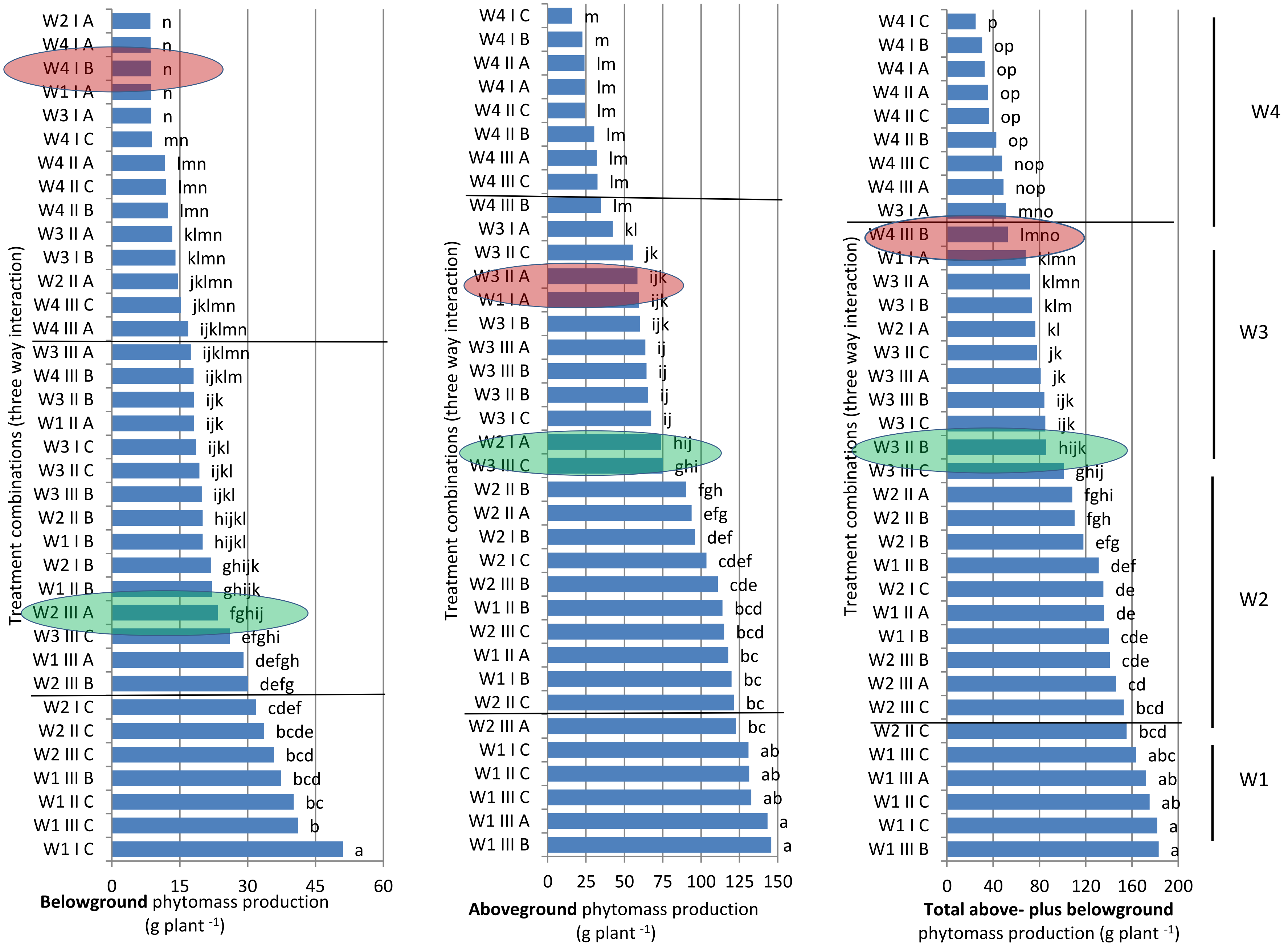

Figure 5.19: Phytomass production (g plant^{-1}) for the three way interaction (water x intensity x frequency) of *Nenax microphylla*. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = (three-monthly, B = six-monthly and C = twelve monthly or control. Different letters shows significant differences (P < 0.001), within treatments at each fraction.

5.2.6 Plant part fractions ratio (including root:shoot ratio)

The plant fractions ratio gives an indication of how the plant allocates phytomass to the different plant parts (fractions), namely: edible phytomass (EP), inedible phytomass (IEP) and belowground phytomass (BP). The more commonly used term, root:shoot ratio will also be discussed under this heading.

Variation in edible phytomass were mostly accounted for by defoliation intensity (54%) and secondly by water availability (32%) (Table 5.15). Most (74%) of the variation in inedible phytomass was accounted for by defoliation intensity, with water explaining 11% of the variation in data. Water accounted for most (52%) of the variation in data for belowground phytomass production, with frequency the second highest at 15%. Although most of the interaction tested to have a significant (P < 0.001) influence on phytomass production, it will not be discussed, due to very low sum of square percentages.

Table 5.15: ANOVA summary for the different plant fraction ratios for the phytomass production of *Nenax microphylla* for all frequencies at the end of the trial.

Plant part fractions ratio		W		I		F		W x I		W x F		I x F		W x I x F	
Variable	Treatment	SS% and significance													
EP	A, B, C compared	**32**	***	**54**	***	1	***	2	***	2	***	1	***	1	NS
IEP	A, B, C compared	11	***	**74**	***	2	***	3	***	1	**	1	*	1	NS
BP	A, B, C compared	**52**	***	2	**	**15**	***	1	NS	3	*	3	**	2	NS

W = water, I = defoliation intensity, F = defoliation frequency, EP = edible phytomass, IEP inedible phytomass, BP = belowground phytomass, A = Frequency A, B = Frequency B, C = Frequency C, NS = non-significant, SS% = sum of squares percentage, *** = P < 0.001, ** = P < 0.01, * = P < 0.05

Defoliation frequency had the lowest impact on plant fraction ratio, with only belowground phytomass differing significantly (P < 0.001) between all three frequencies, from 19% for frequency A to 26% for frequency C (Figure 5.20). Defoliation intensity caused a significant (P < 0.001) variation in the percentage edible and inedible phytomass allocation between all three intensities, with little variation in belowground phytomass allocation. Phytomass allocation was the same for the least stressed water treatments (W1 and W2) with the edible fraction at 60% and the belowground (root) fraction 18%. As water stress increased, a more even distribution of phytomass allocation occurred between edible, inedible and belowground phytomass, with an almost equal distribution for the most severe water stressed treatment (W4) (Figure 5.20). Belowground phytomass allocation of this severest water stress treatment was significantly (P < 0.001) higher than any of the other water treatments.

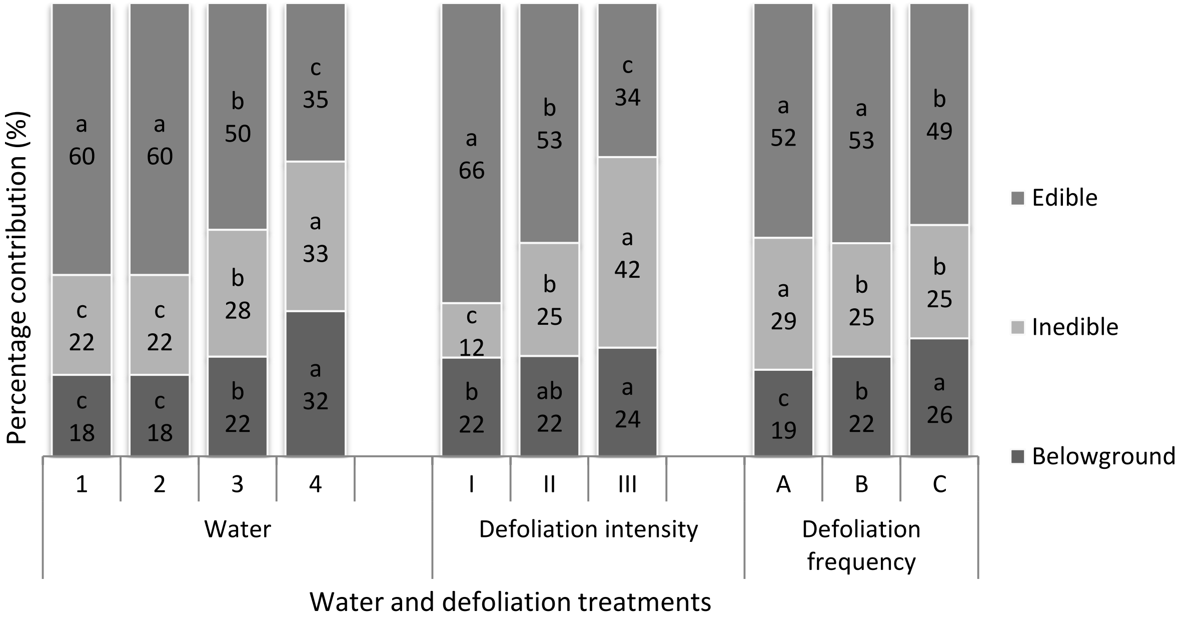

Figure 5.20: Percentage contribution of different plant fractions to total phytomass production of *Nenax microphylla* at different water and defoliation treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequencies are: A = three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences (P < 0.001), between the same plant part fraction within treatments.

Root:shoot ratio is widely used to indicate biomass partitioning of plants by expressing the ratio of below- to aboveground phytomass allocation. Water had the largest influence on root:shoot ratio by accounting for 54% in the variation in data, with frequency second highest, but rather low, at 12% (Table 5.16). Intensity as well as its interaction with the other treatments had very low sum of square percentages and will therefore not be discussed.

Table 5.16: ANOVA summary for the root:shoot ratio for the phytomass production of *Nenax microphylla* for all frequencies compared at the end of the trial.

Root:shoot ratio		W		I		F		W x I		W x F		I x F		W x I x F	
Variable	Treatment	SS% and significance													
Ratio	A,B, C compared	**54**	***	1	*	**12**	***	1	NS	1	NS	3	**	2	NS

W = water, I = defoliation intensity, F = defoliation frequency, A = Frequency A, B = Frequency B, C = Frequency C, NS = non-significant, SS% = sum of squares percentage, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

No significant ($P > 0.05$) differences in root:shoot ratio were recorded for the different defoliation intensities (Figure 5.21). Root:shoot ratio differed significantly ($P < 0.001$) between all three defoliation frequencies, with frequency C the highest (0.38) and frequency A the lowest (0.26). The highest root:shoot ratio was registered at water treatment W4, which differed ($P < 0.001$) from the other water treatments and was at 0.48 almost double that of water treatment W1 and W2 at 0.24 (Figure 5.21). It is interesting to note the difference between water and frequency treatments, where increased water stress caused increased root:shoot ratios, while the opposite happened at defoliation frequency, where an increase in frequency (more frequent defoliation) caused a decrease in root:shoot ratios.

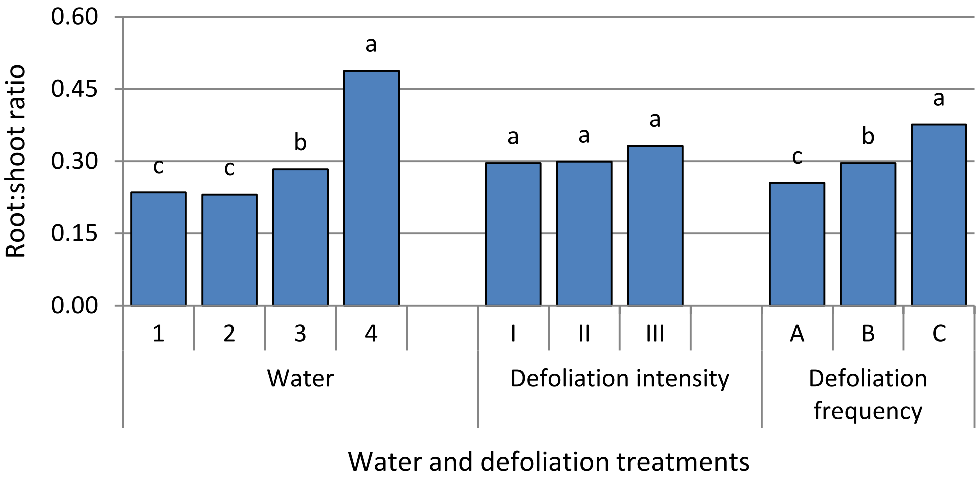

Figure 5.21: Root:shoot ratio of *Nenax microphylla* at different water and defoliation treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequencies are: A = three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences ($P < 0.001$), between treatment levels within each treatment.

5.2.7 Root length

Root length is not as frequently used to describe root growth as root mass, although it might be a more useful measurement, indicating the total root abundance (Volesky 2011). Measuring root length is more difficult than determining root mass, this possibly explains the lack of such information for fodder plants (Oosthuizen and Snyman 2009). Unfortunately the variation in root length data was slightly higher than expected, but never the less yielded data that is still acceptable enough to be used. Roots were separated into three fraction classes based on root diameter, where: R1 = roots with diameter ≥ 3 mm, R2 = roots with diameter 1 to 3 mm and R3 = roots with diameter < 1 mm.

5.2.7.1 Root length over all treatments

Water accounted for most of the variation in total root length data (25%), with defoliation frequency at 16% (Table 5.17). The water x frequency interaction accounted for more variation in total root length data than any of the other interactions.

Table 5.17: ANOVA summary for the root length of *Nenax microphylla* for all frequencies compared at the end of the trial.

Root length		W		I		F		W x I		W x F		I x F		W x I x F	
Variable	Treatment	SS% and significance													
R1 (≥ 3 mm)	A, B, C compared	**25**	***	2	NS	5	**	1	NS	3	NS	3	NS	6	NS
R2 (1 - 3 mm)	A, B, C compared	**19**	***	2	NS	**13**	***	1	NS	7	**	2	NS	3	NS
R3 (< 1 mm)	A, B, C compared	**15**	***	5	***	**15**	***	1	NS	**19**	***	4	*	1	NS
Total length	A, B, C compared	**25**	***	7	***	**16**	***	1	NS	**14**	***	4	**	2	NS

W = water, I = defoliation intensity, F = defoliation frequency, A = Frequency A, B = Frequency B, C = Frequency C, NS = non-significant, SS% = sum of squares percentage, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, R1 = coarse roots (≥ 3 mm), R2 = medium roots (1 – 3 mm), R3 = fine roots (< 1 mm).

Defoliation intensity had the lowest variation in root length between treatments, with intensity I and II both lower ($P < 0.001$) than intensity III, but did not differ ($P > 0.05$) from each other (Figure 5.22). Root length of defoliation frequency C was higher ($P < 0.001$) than that of frequencies A and B by around 70%, while frequencies A and B also differed significantly, but only by 18% (Figure 5.22). Water treatments W1 and W2, (least water stressed treatments) gave the highest total root length of around 250 m plant^{-1}, while water treatment W4, (the most stressed water treatment) was significantly lower at 125 m plant^{-1}. Root length of the different fraction proportionally followed the same pattern as total root length, while R 3 (< 1 mm) made up around 80% of total root length for all treatments. Root fraction R2 (1 – 3 mm) were second highest (13%) with R1 (≥ 3mm) the lowest (7%). Fraction lengths of R1, as well as R2 did not differ ($P > 0.05$) within fraction type between the three defoliation intensities.

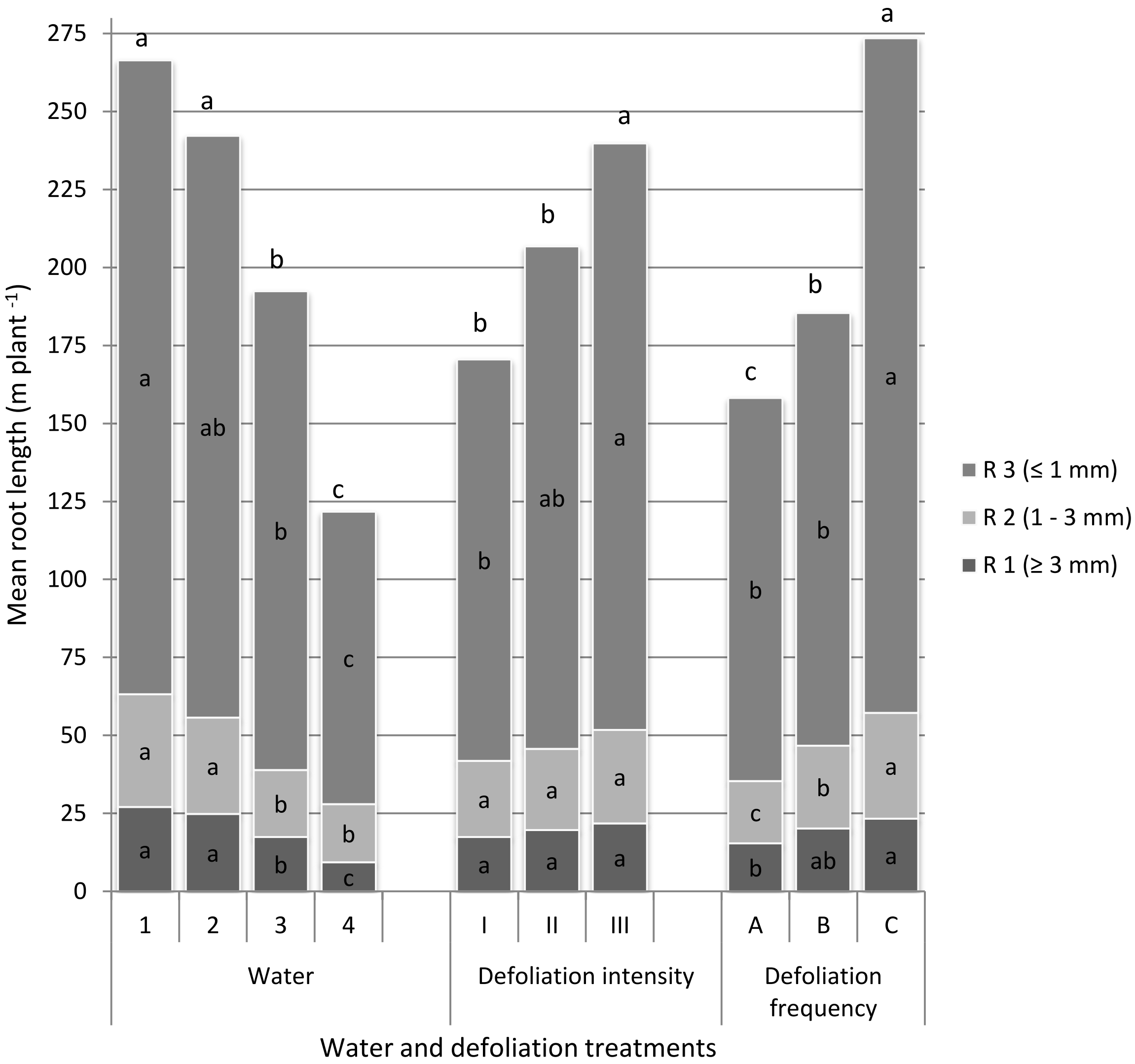

Figure 5.22: Root length of different root fractions and total root length for *Nenax microphylla* at different water and defoliation treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequencies are: A = three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences ($P < 0.001$), within treatments at each fraction for total root length.

5.2.7.2 Root length (defoliation frequencies separately illustrated)

As defoliation frequency influenced (P < 0.001) root length, it was decided to illustrate and discuss root length for each of the three defoliation frequencies. For frequency A, intensity accounted by far for most of the variation in root length data (Table 5.18). For frequency B water accounted for most variation in root length data, while intensity also contributed significantly (P < 0.01) but not as much as water (Table 5.18). Water again (P < 0.001) accounted for most variation in root length at frequency C.

Table 5.18: ANOVA summary for the root length of *Nenax microphylla* for each frequency.

Root length		W		I		W x I	
Variable	**Frequency**	SS% and significance					
R1 (≥ 3 mm)	**A**	19	**	15	**	14	NS
R2 (1 - 3 mm)	**A**	6	NS	28	***	9	NS
R3 (< 1 mm)	**A**	5	NS	47	***	2	NS
Total length	**A**	7	NS	**50**	***	4	NS
R1 (≥ 3 mm)	**B**	32	***	1	NS	4	NS
R2 (1 - 3 mm)	**B**	14	NS	1	NS	2	NS
R3 (< 1 mm)	**B**	26	***	3	NS	4	NS
Total length	**B**	**38**	***	**11**	**	8	NS
R1 (≥ 3 mm)	**C**	34	***	2	NS	6	NS
R2 (1 - 3 mm)	**C**	57	***	0	NS	4	NS
R3 (< 1 mm)	**C**	54	***	1	NS	1	NS
Total length	**C**	**65**	***	0	NS	1	NS

W = water, I = defoliation intensity, A = Frequency A, B = Frequency B, C = Frequency C, NS = non-significant, SS% = sum of squares percentage, *** = P < 0.001, ** = P < 0.01, * = P < 0.05, R1 = coarse roots (≥ 3 mm), R2 = medium roots (1 – 3 mm), R3 = fine roots (< 1 mm).

At frequency A no significant (P > 0.05) differences in root length occurred between water treatments, while the root length of all three defoliation intensities differed significantly (P < 0.001) (Figure 5.23). For frequency C the opposite was true, with the root length of all four water treatments differing significantly, while defoliation intensity showed no significant (P > 0.05) differences. Influence of water and defoliation intensities at frequency B was intermediate, compared to the other two frequencies, with both water and intensity showing some significant (P < 0.001) differences in root length (Figure 5.23).

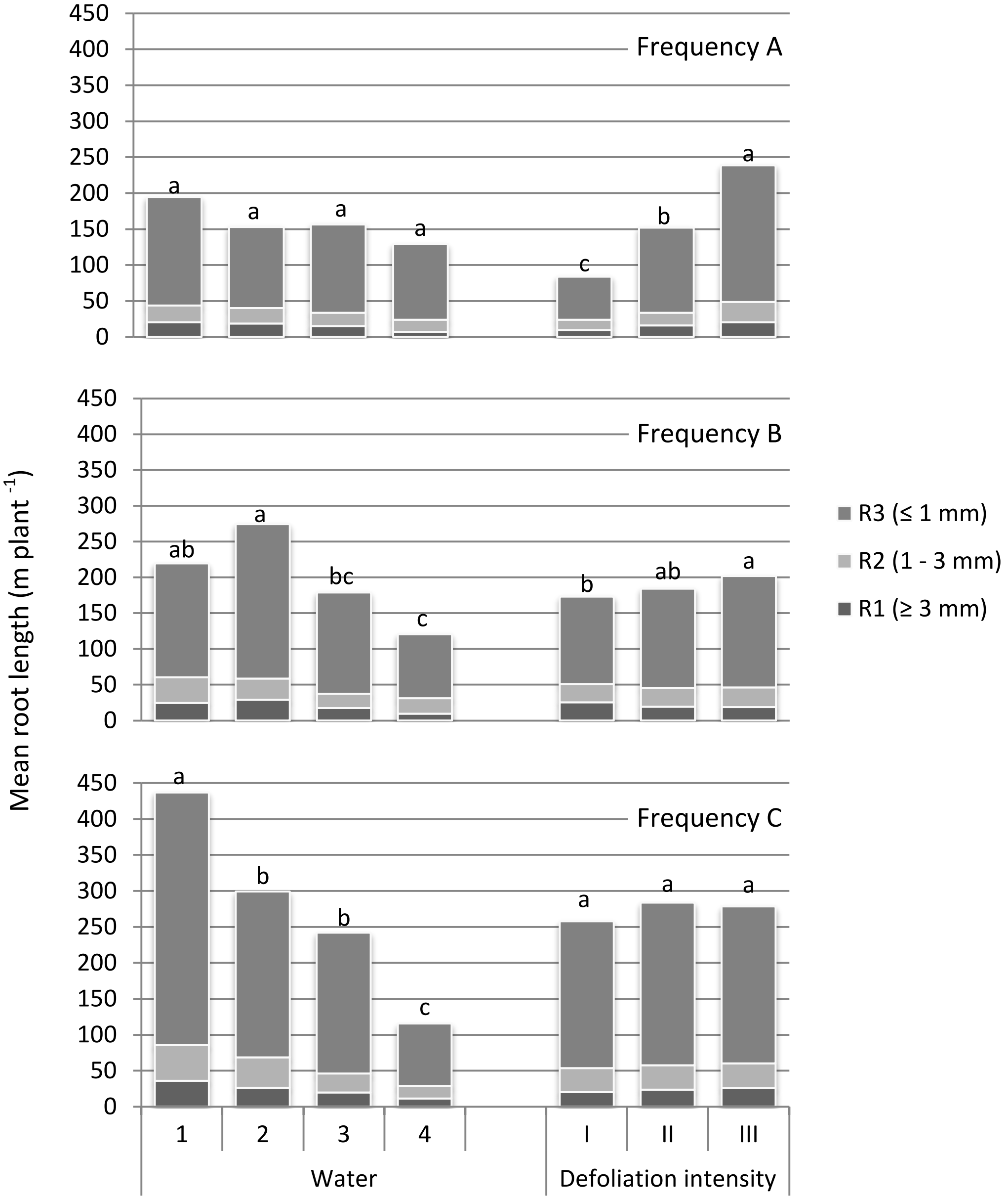

Figure 5.23: Root length of different root fractions and total root length for *Nenax microphylla* at different water and defoliation intensities in interaction with three different frequencies. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequencies are: A = three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences ($P < 0.001$), within treatments at each frequency for total root length.

5.2.8 Shoot length

Shoot length was only measured and presented for defoliation frequencies A and B. With the shoots forming more and more side branches as they grow longer and older over time, this measurement became more complicated and could be inaccurate. If this was true, the same inaccuracy would be repeated over all measured treatments, validating the usefulness of this data. Shoot length for frequency C was also measured, but after seven to eight months no increase in shoot length occurred as many side branches were formed. Shoot mass might have been a better option for measurement of shoot growth. For defoliation frequencies A and B, after each defoliation treatment, new shoots were marked of which the length was measured monthly.

5.2.8.1 Shoot length of defoliation frequency A

Defoliation intensity had a significant ($P < 0.001$) influence on shoot length and accounted for most of the variation in shoot length data at frequency A (Table 5.19). Water also had a significant ($P < 0.001$) influence on shoot length, but accounted for less variation in data than defoliation intensity.

Table 5.19: REML variance components analysis for shoot length of *Nenax microphylla* at defoliation frequency A.

Shoot length		W		I		W x I	
Date	Frequency	F statistic and significance					
May (Autumn) (A1)	A	10	***	**17**	***	1	NS
August (Winter) (A2)	A	4	*	**19**	***	6	***
November (Spring) (A3)	A	21	***	**54**	***	4	**
February (Summer) (A4)	A	13	***	**18**	***	1	NS

REML = Linear mixed model repeated measurement analysis, W = water, I = defoliation intensity, NS = non-significant, SS% = sum of squares percentage, EP = edible phytomass, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

For all four defoliation dates, shoot length between water treatment W1 and W2 did not differ much ($P > 0.05$) (Figure 5.24). For the August defoliation, after the winter growth period, water had no significant ($P > 0.05$) influence on shoot length. For the May defoliation shoot length at water treatment W4 differed significantly ($P < 0.001$) from treatments W1 and W2, but not from treatment W3, while shoot length at water treatments W3 and W4 differed significantly from treatments W1 and W2 at both the November and February defoliation dates (Figure 5.24). To summarize, with the exception of the August defoliation date, water stress caused a significant ($P < 0.001$) decline in shoot length.

With the exception of the February defoliation date, where shoot length of defoliation intensity I and II did not differ significantly ($P > 0.05$), shoot length at all three defoliation intensities differed significantly ($P < 0.001$) at the May, August and November defoliation dates. Intensity I, the most severe defoliation, gave the highest shoot lengths. This might be a way of the plants to compensate for the severe defoliation treatment, by showing rapid regrowth.

The longest shoot lengths for both water and intensity treatments were recorded at the May (autumn) and November (spring) defoliations. The biggest differences between extremes of water and defoliation treatments were, however, registered at the February defoliation. This might be an indication that the plant grows more rapidly during spring and autumn, but during the warmer summer months, while growth is a bit slower, water stress and defoliation severity have a bigger impact on shoot growth than during the cooler spring and autumn growth periods. This relates well to the general tendency of shrubs form the Nama-karoo to grow more actively during the cooler (and wetter) spring and autumn months (Esler et al. 2006).

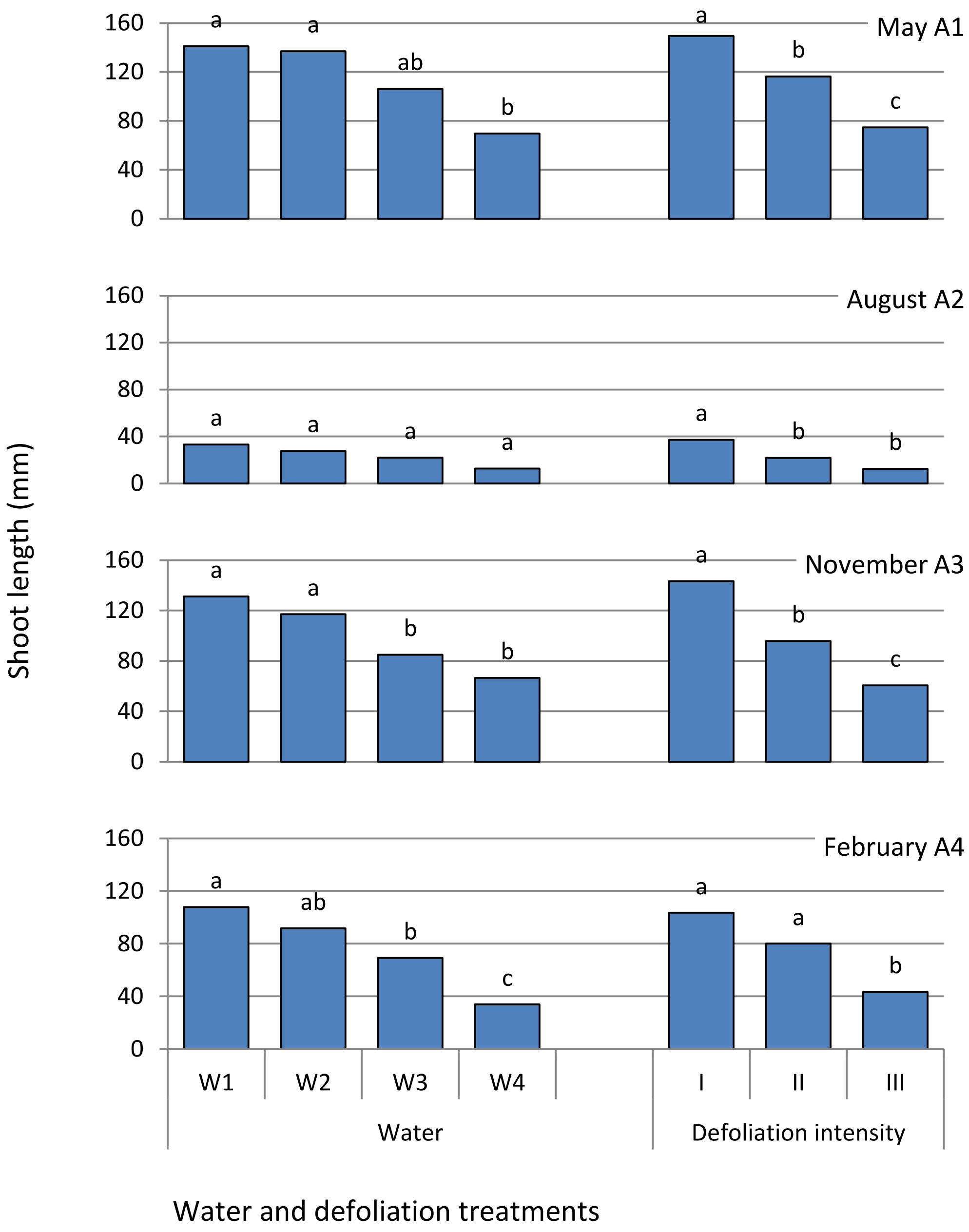

Figure 5.24: Shoot length (mm) for *Nenax microphylla* at defoliation frequency A at different water and defoliation intensity treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences ($P < 0.001$), within treatments.

5.2.8.2 Shoot length of defoliation frequency B

For the six-monthly defoliation frequency, both water and defoliation intensity had significant (P < 0.001) influences on shoot length for B1 and B2. The influence of the water treatment on shoot length was, however, much lower at B1 than at B2 (Table 5.20).

Table 5.20: REML variance components analysis for shoot length of *Nenax microphylla* at defoliation frequency B.

Shoot length		W		I		W x I	
Date	Frequency	F statistic and significance					
August (B1)	B	8	***	**35**	***	1	NS
February (B2)	B	**34**	***	**35**	***	3	*

REML = Linear mixed model repeated measurement analysis, W = water, I = defoliation intensity, NS = non-significant, SS% = sum of squares percentage, EP = edible phytomass, B1 = first six months, B2 = second six months, *** = P < 0.001, ** = P < 0.01, * = P < 0.05

Water treatment showed little variation in shoot length between treatments W1, W2 and W3 (P > 0.05), with treatment W4 significantly (P < 0.001) lowered at B1, which might be a result of it being the autumn and winter growing seasons (Figure 5.25). At B2 water stress had a greater impact (P < 0.001) on shoot length. Defoliation intensity, however, caused significant (P < 0.001) differences in shoot length between all three defoliation treatments at both B1 and B2 (Figure 5.25).

Defoliation intensity I gave longer (P < 0.001) shoot lengths than the other defoliation treatments. It is similar to the results from frequency A, indicating signs of compensatory growth as a means to compensate for loss on vegetative material due to severe defoliation.

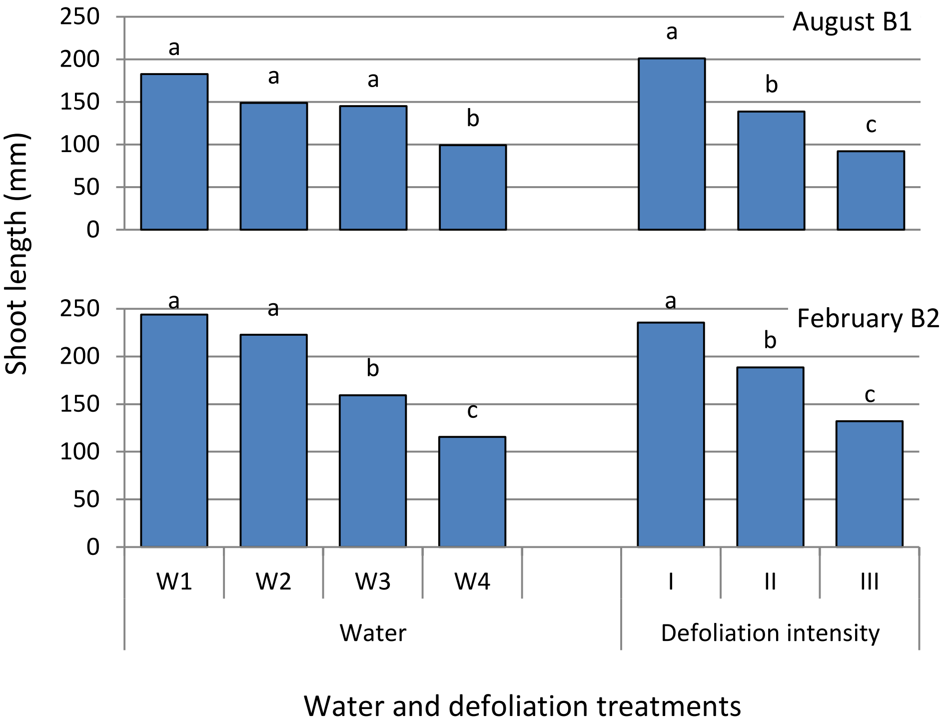

Figure 5.25: Shoot length (mm) for *Nenax microphylla* at defoliation frequency B at different water and defoliation intensity treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences (P < 0.001), within treatments.

5.2.9 Leaf dimensions (length, width and surface)

Leaf dimensions (length and width) were measured to quantify the possible effect the different treatments might have on the growth dynamics of the shrub. Leaf surface was measured by simple multiplying leaf length with leaf width.

Both leaf length and surface were significantly influenced by water treatments as well as by both defoliation intensity and frequency, with water having the lesser influence (Table 5.21).

Leaf width was also influenced (P < 0.001) by all three main treatments, but to almost equal amounts.

Table 5.21: ANOVA summary for the leaf dimensions of *Nenax microphylla*.

Leaf measurements	W		I		F		W x I		W x F		I x F		W x I x F	
Variable	SS% and significance													
Leaf length	**8**	***	**20**	***	**21**	***	5	**	7	***	8	***	3	NS
Leaf width	**11**	***	**9**	***	**8**	***	2	NS	2	NS	6	*	4	NS
Leaf surface (length x width)	**9**	***	**17**	***	**20**	***	4	*	5	**	8	***	3	NS

W = water, I = defoliation intensity, F = defoliation frequency, NS = non-significant, SS% = sum of squares percentage, *** = P < 0.001, ** = P < 0.01, * = P < 0.05

Leaf length, -width and –surface were lower (P < 0.001) at water treatment W4 than at the other three water treatments (Figure 5.26). This could possibly be due to the leaves being wilted rather than water having an influence on leaf growth. On the other hand, for the non-stressed (W1) plants leaf sizes were the highest, compared to watering treatments W2 and W3, which might be due to higher turgor pressure at high water levels.

With the exception of leaf width, the other leaf dimensions showed significant (P < 0.001) differences between all three defoliation intensity treatments, with defoliation intensity III having smaller (P < 0.001) leaves (Figure 5.26) than the other two intensities. Defoliation intensity I, the most severe defoliation intensity treatment, had a larger (P < 0.001) leaf surface, almost twice the size than that of intensity III. This might be ascribed to compensatory growth in the sense that the severely defoliated plant increases its leaf surface to instigate rapid growth, since the source of growth reserves (majority of the stems) was removed during the defoliation process.

With the exception of leaf width, defoliation frequency B had greater (P < 0.001) leaf dimensions than frequency A and C which also differed significantly from each other (Figure 5.26). The same can be argued as for intensity, where frequency C (control / defoliated every 12 months), had the smallest leaves, while frequencies A and B had bigger leaves as a compensatory mechanism to increase photosynthetic ability.

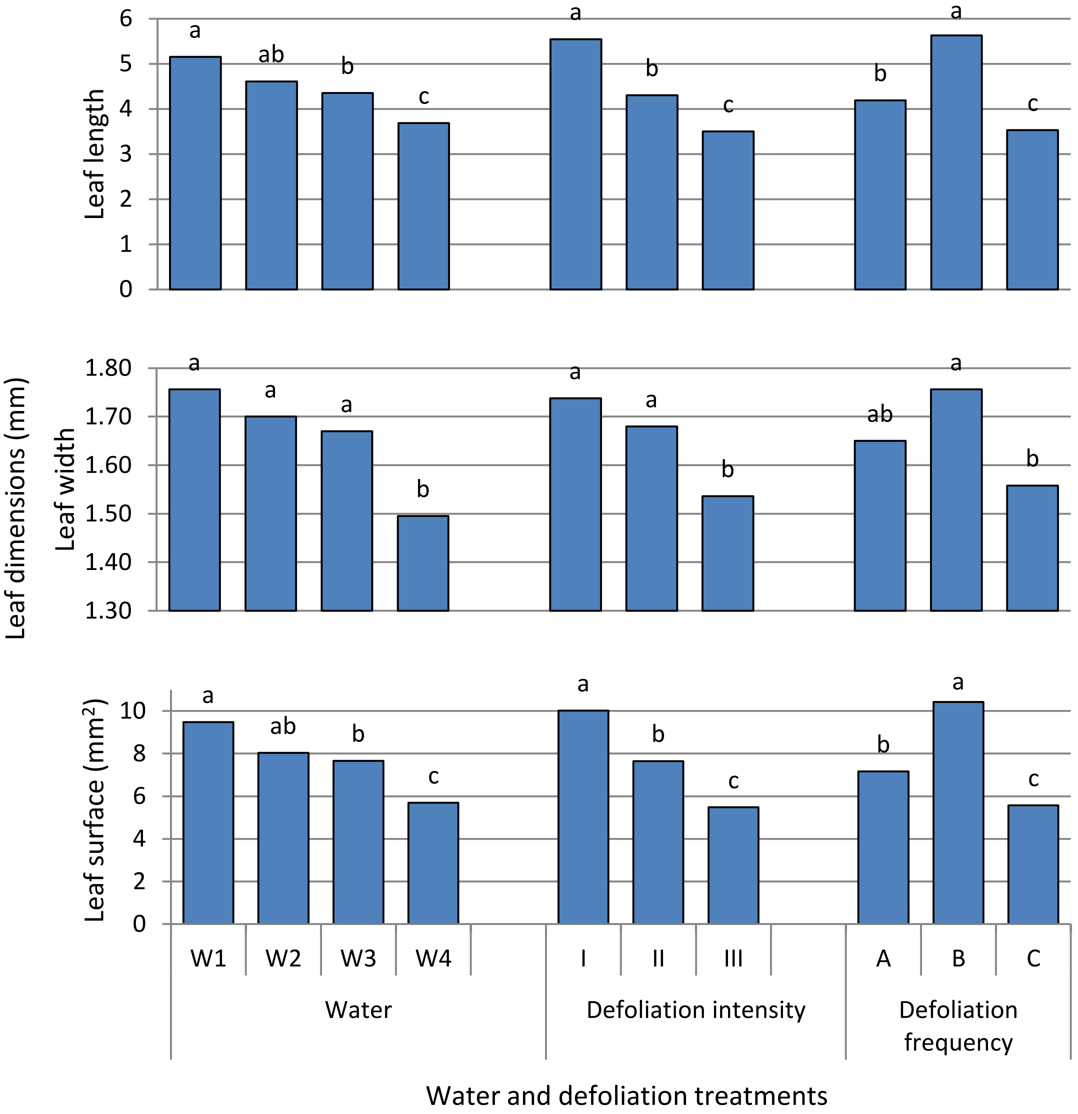

Figure 5.26: Leaf dimension measurements (mm) for *Nenax microphylla* at different water and defoliation intensities and –frequencies treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequencies are: A = three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences (P < 0.001), within treatments.

5.2.10 References

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CHAPTER 6

ABOVE- AND BELOWGROUND PHYTOMASS PRODUCTION OF *Pentzia incana* UNDER DIFFERENT DEFOLIATION FREQUENCIES AND INTENSITIES ALONG A WATER DEFICIT GRADIENT

6.1 Introduction

This chapter focusses on the above- and belowground phytomass production of *Pentzia incana*, as influenced by the different defoliation and water treatments and their interactions. The importance of this part of the study was explained and discussed in detail in previous chapters (Chapters 1, 3 and 4). The general discussion of Chapter 5 (*N. microphylla*) and Chapter 6 (*P. incana*) will follow in Chapter 7 to avoid duplication, as the two plants reacted similarly to some of the treatments.

Three main effects form the core of the results to be discussed, namely: water (W), defoliation intensity (Di) and defoliation frequency (Df). The water treatments were as follows: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Three defoliation intensity treatments included defoliation to heights of: I = 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency treatments were: A = every three months (May, August, November and February), B = every six months (August and February) and C = every 12 months (February). On the date the trial started, all plants were defoliated to their specific cutting heights, within the different defoliation intensity treatments. As the trial ran over only 12 months, the C defoliation frequency treatment was applied on the day the trial started and again, 12 months later at the end of the trial. It can therefore also be regarded as the control. Four interactions occurred as result of the treatment combinations. These interactions included: W x Di, W x Df, Di x Df and W x Di x Df. For the presentation and discussion of results emphasis was placed only on those interactions which tested highly significant ($P < 0.001$) and explains the highest amount of variation in the data. To better explain the results, ANOVA summaries are also presented.

The discussion will be structured according to the main effect, defoliation frequency, under headings Frequency A to Frequency C. Within each frequency, the other two main effects, water and defoliation intensity, plus their interactions (where appropriate) will be presented and discussed. All harvested plant material was separated into the following fractions: edible phytomass (EP), inedible phytomass (IEP), total aboveground phytomass (TAP), belowground phytomass (BP) and total above- and belowground phytomass (TA&BP). Edible phytomass (EP) comprised of leaves and edible twigs (twigs less than 2 mm in diameter, du Toit 1996). For Frequencies A and B, results and discussions, comparing treatments and defoliation dates, includes only edible phytomass.

6.2 Results and discussion

As was the case for *Nenax microphylla*, water accounted, by far, for the highest variation in most data sets, regardless of the defoliation treatments and therefore had the greatest effect on aboveground phytomass production (Tables 6.1 and 6.2).

6.2.1 Defoliation frequency A (three-monthly interval)

The defoliation frequency data for the three-monthly intervals (May = A1, August = A2, November = A3 and February = A4) are illustrated as means for the main effects, namely water, defoliation intensity and time (Figure 6.1). All harvested plant material comprised of leaves and edible twigs and are indicated and discussed as edible phytomass (EP). Other than being a three-monthly defoliation treatment, season (time/date) might also have had a notable effect on the results, as the defoliation dates followed more or less the four seasons. A linear mixed model repeated measurements analysis (REML) was used to compare the four defoliation dates (Table 6.1).

Time accounted for most of the variation in data and together with water accounts by far for most of the variance in the data (Table 6.1) and both tested to having a highly significant (P < 0.001) influence on edible phytomass production.

Table 6.1 REML variance components analysis for frequency A (A1, A2, A3, A4 compared)

A1, A2, A3, A4 compared		T	W	I	T x W	T x I	W x I	T x W x I
Variable	Treatment	F statistic and significance						
EP	A1vs A2 vs A3 vs A4	179 ***	47 ***	1 NS	16 ***	7 ***	1 NS	2 NS

REML = Linear mixed model repeated measurement analysis, T = time (month), W = water, I = defoliation intensity, NS = non-significant, EP + edible phytomass, A1 = May, A2 = August, A3 = November A4 = February, *** = P < 0.001, ** = P < 0.01, * = P < 0.05

For time, edible phytomass production for all treatments differed significantly (P < 0.001), while it did not differ significantly (P > 0.05) between W1 and W2 which were both higher (P < 0.001) than W3 and W4, which also differed significantly from each other (Figure 6.1). Defoliation intensity had no influence (P > 0.05) on edible phytomass production. Edible phytomass production differed significantly (P < 0.001) between all four defoliation dates, with the lowest production at A2 (August – winter growth period) and the highest production at A4 (February – summer growth period), with that of A4, 193% higher than A2. Although time (defoliation date) accounted for most of the variation in edible phytomass data (Table 6.1), the difference between treatment extremes were the biggest at the water deficit treatments, with W1 being 300% higher than W4 (Figure 6.1).

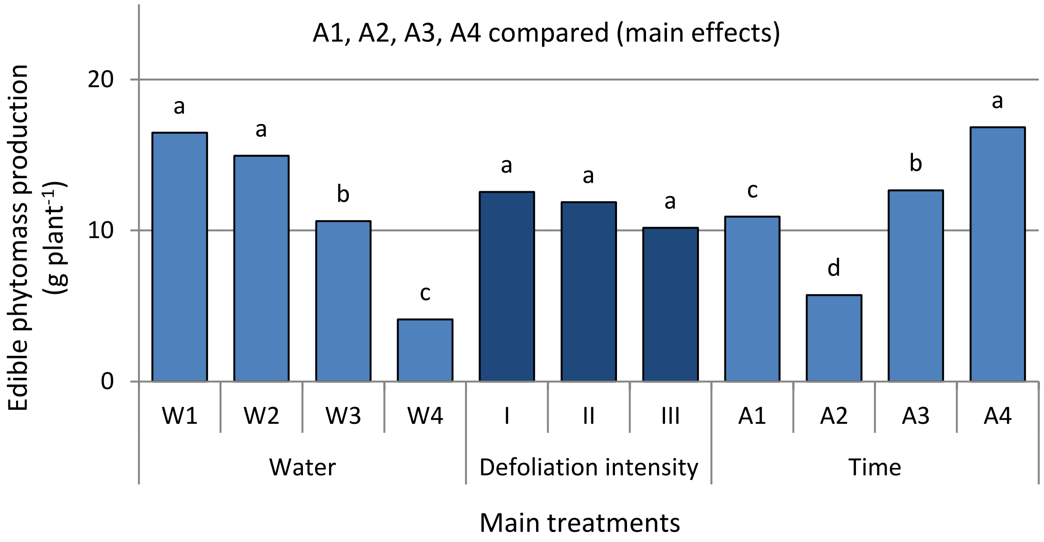

Figure 6.1: Mean (n = 6) cumulative aboveground phytomass production (g plant^{-1}) for *Pentzia incana* for the three-monthly defoliation frequency, by comparing the four defoliation dates. The three main effects (water, defoliation intensity and time) are indicated. The defoliation dates are: A1 = May, A2 = August, A3 = October and A4 = February. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences (P < 0.001) within treatments.

Although defoliation intensity also tested highly significant (P < 0.001) at A1 and A3, water by far accounted for most of the variation at all four defoliation dates (Table 6.2)

Table 6.2: ANOVA summary of the edible phytomass production of *Pentzia incana* for the three-monthly frequency (Frequency A).

Frequency A		W		I		W x I	
Variable	Treatment	SS% and significance					
EP	A1 (May)	75	***	6	***	4	*
EP	A2 (August)	36	***	8	*	5	NS
EP	A3 (November)	56	***	9	***	2	NS
EP	A4 (February)	71	***	3	*	5	*

W = water, I = defoliation intensity, F = defoliation frequency, NS = non-significant, SS% = sum of squares percentage, *** = P < 0.001, ** = P < 0.01, * = P < 0.05

It can be concluded that at the three-monthly defoliation frequency, water and defoliation frequency had the most significant influence on edible phytomass production, with defoliation intensity not having a mentionable impact.

6.2.1.1 Main effect: water

For all four defoliation dates, edible phytomass at W1 and W2 did not differ significantly ($P < 0.001$), while edible phytomass at both watering treatments differed significantly ($P < 0.001$) from W3 and W4 which also differed significantly ($P < 0.001$) from each other (Figure 6.2).

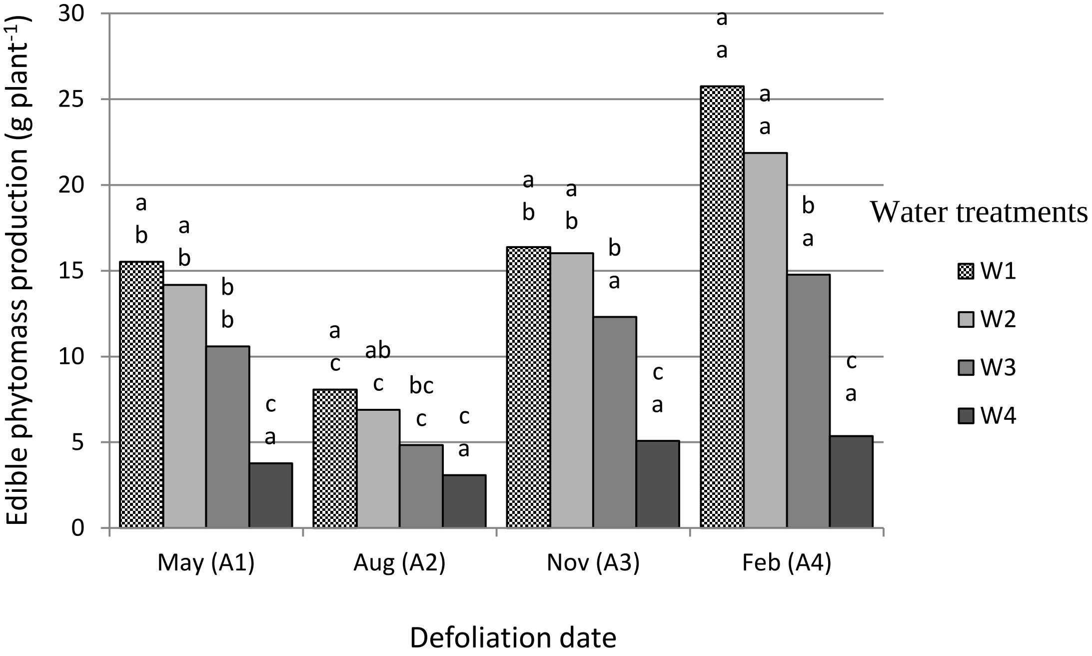

Figure 6.2: Mean (n = 6) edible phytomass production (g $plant^{-1}$) of *Pentzia incana* at a three-monthly defoliation frequency with four different water levels. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Different top letters at each month (defoliation treatment) show significant differences ($P < 0.001$), between water treatments. Different bottom letters show significant differences ($P < 0.001$) between months within a water treatment.

With the exception of water treatment four (W4), A4 (February) had a higher edible phytomass production than the other defoliation dates, with the August (A2) defoliation showing the lowest (P < 0.001) edible phytomass production, regardless the water treatment. The lowest water treatment (W4) constantly gave very low phytomass productions, with no difference (P > 0.05) between defoliation date treatments. The difference in edible phytomass between W1 and W4, of 380%, was the largest at the February defoliation. This indicates both the possible high edible phytomass production during high summer rainfall in contrast to the very low expected edible phytomass production under water deficit conditions in the same season.

6.2.1.2 Cumulative productions at different time intervals

As was the case with *Nenax microphylla*, the cumulative aboveground phytomass production for the three-monthly defoliation frequency was determined by adding the phytomass productions of May (A1) and August (A2), as well as A1 plus A2 plus November (A3) and lastly by adding A1 plus A2 plus A3 plus February (A4). This was done to quantify the cumulative phytomass productions over time (Figure 6.3). The defoliation intensities showed no significant (P > 0.05) differences in edible phytomass production, while water treatments significantly (P < 0.001) influenced edible phytomass production (Figure 6.3, Table 6.3).

Table 6.3: ANOVA summary for phytomass production of *Pentzia incana* for the cumulative three-monthly frequency (Frequency A).

Frequency A		W		I		W x I	
Variable	Treatment	SS% and significance					
EP	A1	75	***	6	***	4	*
EP	A1+A2	77	***	1	NS	2	NS
EP	A1+A2+A3	77	***	3	*	2	NS
EP	A1+A2+A3+A4	79	***	3	**	3	NS

W = water, I = defoliation intensity, F = defoliation frequency, NS = non-significant, SS% = sum of squares percentage, EP = edible phytomass, A1 = May, A2 = August, A3 = October, A4 = February, *** = P < 0.001, ** = P < 0.01, * = P < 0.05

No significant ($P > 0.05$) differences in edible phytomass production were registered between W1 and W2, while both differed significantly ($P < 0.001$) in terms of production from W3 and W4, which also differed significantly ($P < 0.001$) from each other. Water level three had a cumulative total edible phytomass production of 143% higher than that of W4 and that of W1 and W2 was 270% higher of W4. Edible phytomass production of W3 was 50% lower than that of W1 and W2. Water availability had a highly significant ($P < 0.001$) influence on edible phytomass production, especially for W4, where very little cumulative production took place over time in comparison with all three other watering treatments.

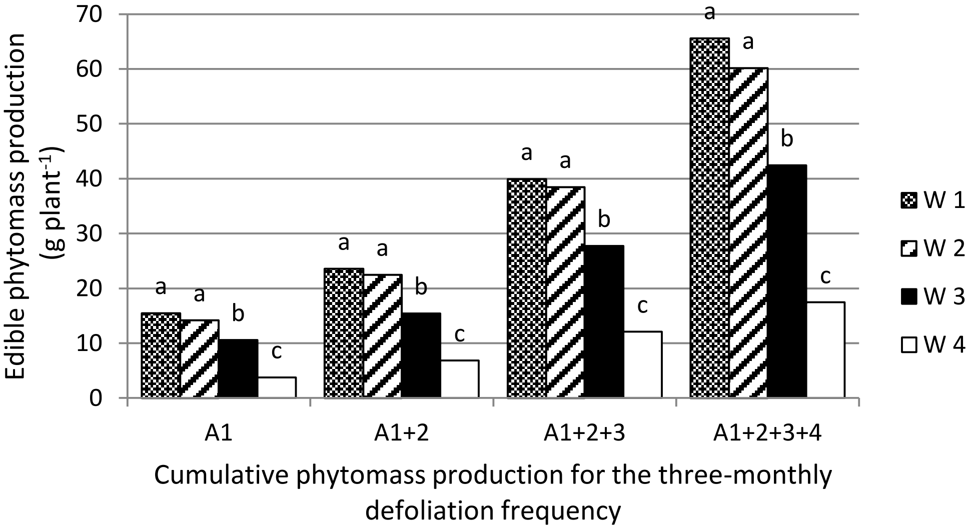

Figure 6.3: Cumulative mean (n = 6) edible phytomass production (g plant^{-1}) for *Pentzia incana* at a three-monthly defoliation frequency with four different water treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation dates are: A1 = May, A2 = August, A3 = November and A4 = February. Different letters within each cumulative time interval show significant differences ($P < 0.001$) between water treatments.

6.2.1.3 First six months (A1 + A2) (autumn, winter) compared with second six months (A3 + A4) (spring, summer)

In comparing the cumulative edible phytomass production for the first six months (A1 + A2) with that of the second six months (A3 + A4), the same tendency was found, with defoliation intensity having no significant ($P > 0.05$) influence on phytomass production (Figure 6.4). By contrast, water stress decreased edible phytomass production significantly ($P < 0.001$), with that of W1 and W2 being 260% higher than that of W4 and that of W3 147% higher than that of W4, indicating the huge detrimental influence of severe water stress on edible phytomass production (Figure 6.4). Time (season) had an even bigger effect on phytomass production than water (Table 6.4), by accounting for much more variation in the data than water.

Table 6.4 REML variance components analysis (A1 + A2 compared to A3 + A4)

A1+A2 and A3+A4 compared		T		W		I		T x W		T x I		W x I		T x W x I	
Variable	Treatment	F statistic and significance													
EP	A1+A2 vs A3+A4	415	***	101	***	6	**	28	***	12	***	2	NS	3	*

REML = Linear mixed model repeated measurement analysis, T = time (month), W = water, I = defoliation intensity, NS = non-significant, SS% = sum of squares percentage, EP = edible phytomass, A1 = May, A2 = August, A3 = October, A4 = February *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

Edible phytomass production of the second six months (A3 + A4) was higher ($P < 0.001$) than that of the first six months (A1 + A2) (Figure 6.4). It could therefore be assumed that the cumulative spring and summer production of *P. incana* is higher ($P < 0.001$) than that of the autumn and winter production period.

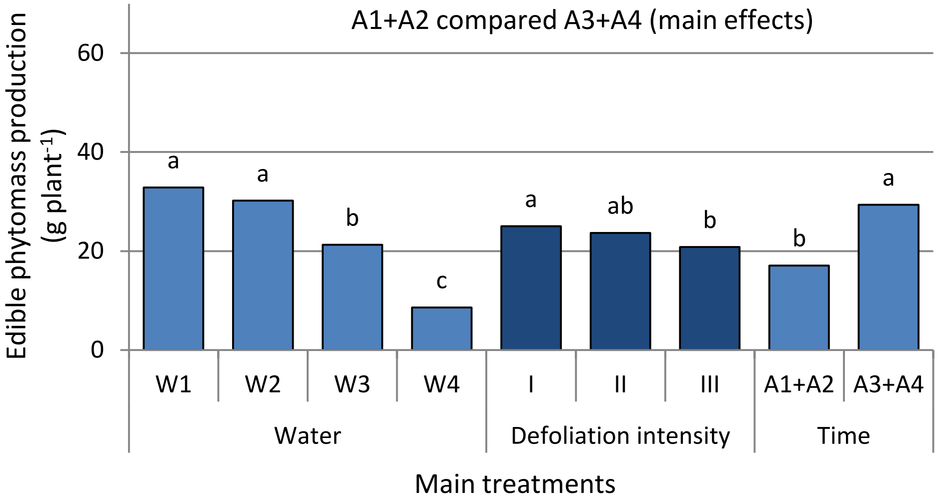

Figure 6.4: Mean (n = 6) cumulative edible phytomass production (g plant^{-1}) for *Pentzia incana* for the three-monthly defoliation frequency, by comparing the first six months (A1 + A2) with that of the second six months (A3 + A4). The three main effects (water, defoliation intensity and time (season)) are indicated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences (P < 0.001) within treatments.

6.2.1.4 Total cumulative above- and belowground phytomass production of defoliation frequency A (after 12 months)

At the end of the trial, when plants were harvested destructively, plant material was separated into different plant fractions, namely: aboveground (edible- and inedible phytomass) and belowground (roots), as explained earlier. Total cumulative edible phytomass production (A1 + A2 + A3 + A4) was used in Figure 6.5.

With the exception of IEP, water accounted for the majority of variation in data for all plant fractions (Table 6.5). Defoliation intensity accounted for most variation in data for IEP and for a large amount of variation in BP data. The water x intensity interactions had no significant (P > 0.05) influence on the variation data for all plant fractions.

Table 6.5: ANOVA summary for the cumulative above- and belowground phytomass production of *Pentzia incana* for the three-monthly frequency (Frequency A).

Frequency A		W		I		W x I	
Variable	Treatment	SS% and significance					
EP	A cum at end	**79**	***	3	**	3	NS
IEP	A cum at end	27	***	**56**	***	8	NS
TAP	A cum at end	**84**	***	3	***	1	NS
BP	A cum at end	**34**	***	**27**	***	1	NS
TA&BP	A cum at end	**78**	***	8	***	2	NS

W = water, I = defoliation intensity, F = defoliation frequency, EP = edible phytomass, IEP inedible phytomass, TAP = total aboveground phytomass, BP = belowground phytomass, TA&BP = total above- and belowground phytomass, A cum at end = cumulative phytomass production of frequency A after 12 months, NS = non-significant, SS% = sum of squares percentage, *** = P < 0.001, ** = P < 0.01, * = P < 0.05

With the exception of EP, increased defoliation intensity showed a decrease (P < 0.001) in all other plant fractions (Figure 6.5). The greatest decrease in phytomass was for IEP where intensity III was 247% higher than that of intensity I. By contrast, water deficit caused a much bigger decrease in above- and belowground phytomass production. There was a decline (P < 0.001) in phytomass production, with increased water stress for all plant part fractions. For EP, BP and TA&BP the difference in phytomass production between W1 and W2 was low (P > 0.05) while all other water treatments differed significantly (P < 0.001) within plant part fraction, with regard to phytomass production. To summarize, both increased defoliation intensities and increased water stress caused noteworthy decreases in phytomass production. Water, however, caused bigger differences in phytomass production between treatment extremes, than was the case at defoliation intensity.

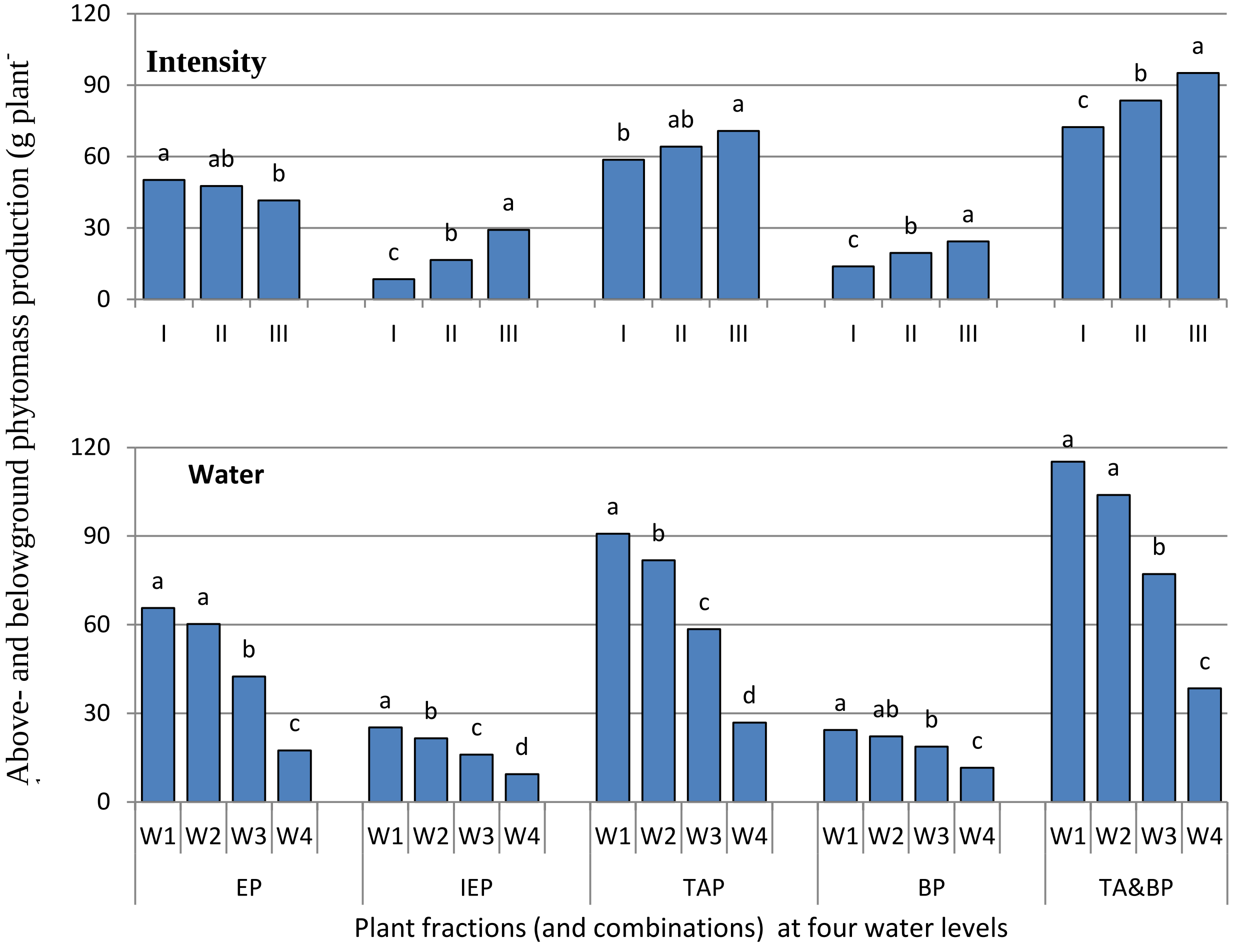

Figure 6.5: Mean (n = 6) cumulative above- and belowground phytomass production (g plant^{-1}) for *Pentzia incana* (three-monthly defoliation frequency) for different plant fractions (and combinations) at different defoliation intensities and water levels after 12 months, when all plants were destructively defoliated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. EP (edible phytomass), IEP (inedible phytomass), TAP (total aboveground phytomass), BP (below ground phytomass), TA&BP (total above and below ground phytomass). Different letters show significant differences ($P < 0.001$), between water treatments and between defoliation intensities, within each plant fraction.

6.2.1.5 Summary and discussion of defoliation frequency A

At all four defoliation dates (times) as well as for the cumulative data, water had the biggest ($P < 0.001$) influence on edible phytomass production, while defoliation intensity did not cause any significant ($P > 0.05$) differences. For most of the results, edible phytomass at W1 and W2 did not differ much ($P > 0.05$), while both differed ($P < 0.001$) form W3 and W4 which also differed significantly ($P < 0.001$) from each other.

At the destructive harvest at the end of the trial, water deficit caused a significant ($P < 0.001$) decrease in phytomass production at all plant fractions, while increased defoliation intensity caused a highly significant ($P < 0.001$) decrease in phytomass production at both inedible and belowground phytomass production. The water x intensity interaction did not test significantly ($P > 0.05$).

6.2.2 Defoliation frequency B (six-monthly interval)

6.2.2.1 Main effects: water and defoliation intensity

Water by far accounted for most of the variation (around 80%) in edible phytomass data for B1, B2 and the cumulative data of B1 + B2 (Table 6.6). Intensity also tested to have a significant influence on edible phytomass, but with much less influence than that of water treatments.

Table 6.6: ANOVA summary for the aboveground phytomass production of *Pentzia incana* for the six-monthly frequency (Frequency B).

Frequency B		W		I		W x I	
Variable	Treatment	SS% and significance					
EP	B1	78	***	11	***	4	***
EP	B2	82	***	5	***	2	NS
EP	B1+B2	84	***	8	***	2	**

W = water, I = defoliation intensity, F = defoliation frequency, NS = non-significant, SS% = sum of squares percentage, EP = edible phytomass, B1 = first six months, B2 = second six months, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

Edible phytomass production of B2 was higher (P < 0.001) than that of B1 at W1 and W2, while that of W3 and W4 did not differ (P > 0.05) (Figure 6.6). At both dates (B1 and B2), water deficit caused a significant (P < 0.001) decrease in edible phytomass production, with that of W4 302% lower than W1 at B2. For both B1 and B2 an increase in edible phytomass resulted from increased defoliation intensity, with both intensity I and II, higher (P < 0.001) than intensity III.

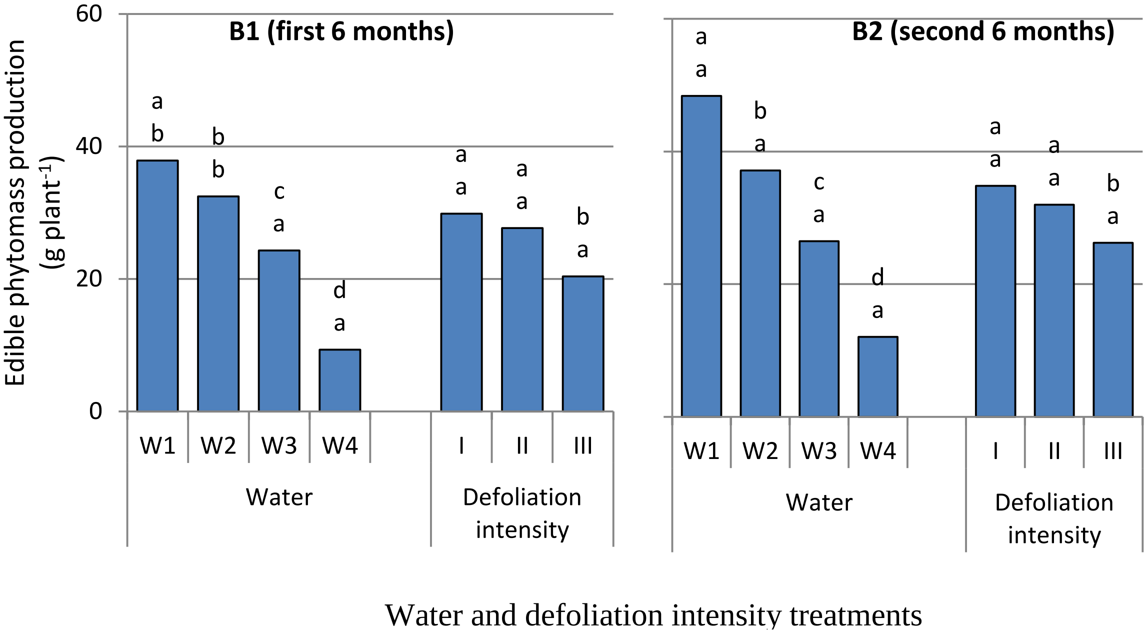

Figure 6.6 Mean (n = 6) edible phytomass production (g plant^{-1}) for *Pentzia incana* (six-monthly defoliation frequency) at different water treatments and defoliation intensities. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different top letters, at each date, show significant differences (P < 0.001), between water- and between defoliation treatments at each date. Different bottom letters show significant differences (P < 0.001) between dates for similar water- and defoliation treatments.

For the cumulative edible phytomass production (B1 + B2), water by far accounted for most of the variation in data (84%), compared to the only 8% of defoliation intensity (Table 6.6). There was a decline ($P < 0.001$) in edible phytomass production, with increased water stress, with W1 having a 301% higher production than W4 Figure 6.7). Edible phytomass production did not differ ($P > 0.05$) between defoliation intensity I and II, but both differed significantly ($P < 0.001$) form intensity III. Differences in edible phytomass between defoliation intensities were, however, much smaller than between water treatments.

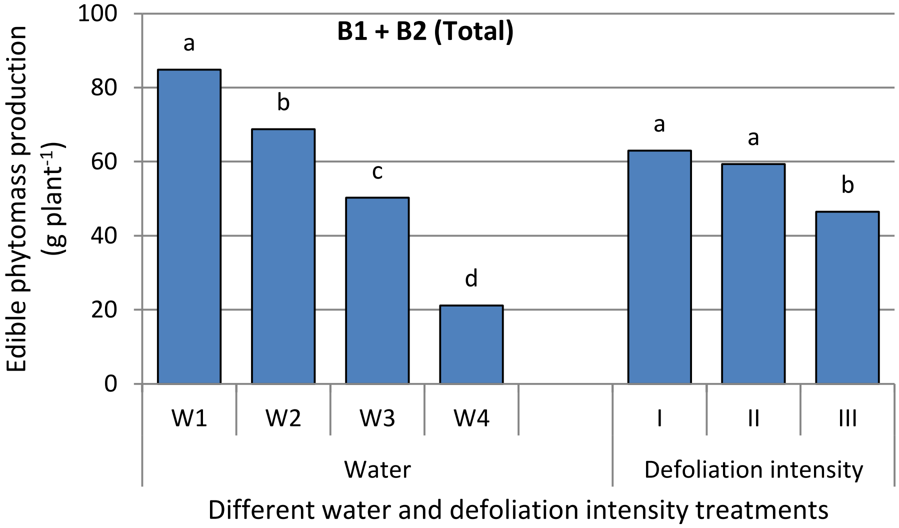

Figure 6.7: Mean (n = 6) cumulative edible phytomass production (g plant^{-1}) for *Pentzia incana* (six-monthly defoliation frequency) at different water treatments and defoliation intensities. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences ($P < 0.001$) within treatments.

6.2.2.2 B1 compared to B2 at two different seasons

In comparing B1 and B2 as two different time intervals (REML analysis), water by far accounted for more variation in the data, than time (defoliation date/season) and defoliation intensity (Table 6.7).

Table 6.7: REML variance components analysis (B1 compared to B2)

B1 and B2 compared		T	W	I	T x W	T x I	W x I	T x W x I
Variable	Treatment	F statistic and significance						
EP	B1 vs B2	60 ***	254 ***	38 ***	9 ***	1 NS	3 **	1 NS

REML = Linear mixed model repeated measurement analysis, T = time (month), W = water, I = defoliation intensity, NS = non-significant, SS% = sum of squares percentage, EP = edible phytomass, B1 = first six months, B2 = second six months, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

Water stress had a negative ($P < 0.001$) influence on edible phytomass production, with the edible phytomass of W1 being 304% higher than that of W4 (Figure 6.8). The edible phytomass production of defoliation intensities I and II did not differ significantly ($P > 0.05$) from each other, but was higher ($P < 0.001$) than that of intensity III. The edible phytomass production of B2 was higher ($P < 0.001$) than that of B1 (Figure 6.8). This can also be attributed to B1 (first six months) being the autumn and winter months' growth, which was proved at frequency A as the seasons with the lowest edible phytomass production.

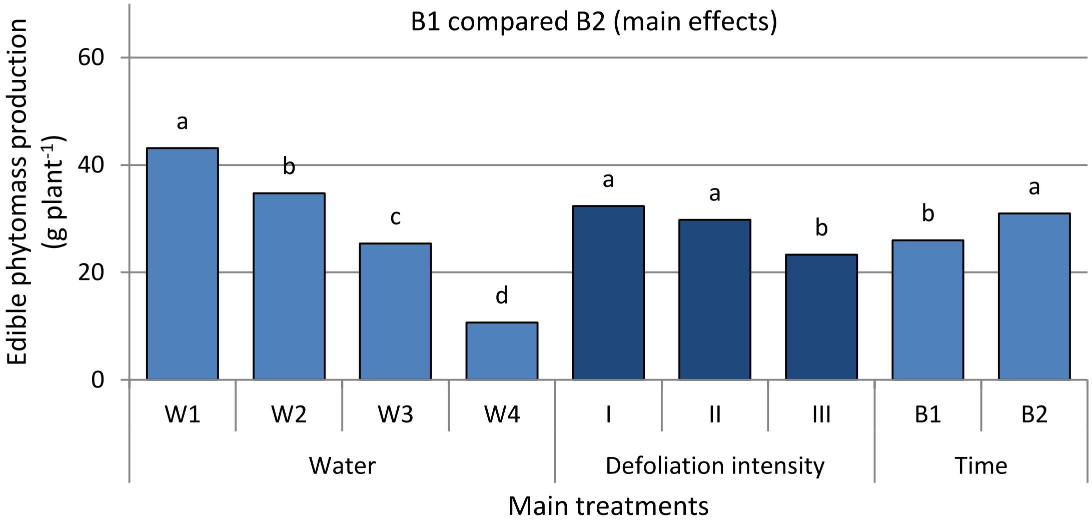

Figure 6.8: Mean (n = 6) edible phytomass production (g plant^{-1}) for *Pentzia incana.* Comparison between phytomass productions of B1 (first six months) and B2 (second six months). The three main effects (water, defoliation intensity and time) are indicated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences (P < 0.001) within treatments.

6.2.2.3 Total cumulative above- and belowground phytomass production for Frequency B. (after 12 months)

With the exception of IEP, water accounted by far for most of the variation in data for all plant fractions (Table 6.8). Defoliation intensity accounted for most variation in phytomass data only at IEP.

Table 6.8: ANOVA summary for the cumulative above- and belowground phytomass production of *Pentzia incana* for the six-monthly frequency (Frequency B).

Frequency B		W		I		W x I	
Variable	Treatment	SS% and significance					
EP	B cum at end	**<u>84</u>**	***	8	***	2	**
IEP	B cum at end	**21**	***	**<u>60</u>**	***	4	*
TAP	B cum at end	**<u>92</u>**	***	1	NS	2	*
BP	B cum at end	**<u>54</u>**	***	3	NS	4	NS
TA&BP	B cum at end	**<u>88</u>**	***	1	NS	2	NS

W = water, I = defoliation intensity, F = defoliation frequency, EP = edible phytomass, IEP inedible phytomass, TAP = total aboveground phytomass, BP = belowground phytomass, TA&BP = total above- and belowground phytomass, NS = non-significant, SS% = sum of squares percentage, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

Phytomass production for all plant fractions showed little to no variation between defoliation intensity treatments, but greater and more significant ($P < 0.001$) variation in phytomass production between all water treatments (Figure 6.9). The only exception was for IEP where defoliation intensity caused more variation ($P < 0.001$) in phytomass production, than water treatments, with phytomass production of intensity III 232% higher than that of intensity I.

For the total aboveground plus belowground phytomass production water availability caused a 216% increase in production, while increased defoliation intensity caused only a 5% decline in production. This indicates that water stress had a much greater influence on phytomass production than defoliation intensity (Figure 6.9).

Belowground phytomass production increased by 146% with reducing water deficit, while increased defoliation intensity lowered belowground phytomass production by only 17%. This also highlights to greater influence of water stress than defoliation intensity on phytomass production at the six-monthly defoliation frequency.

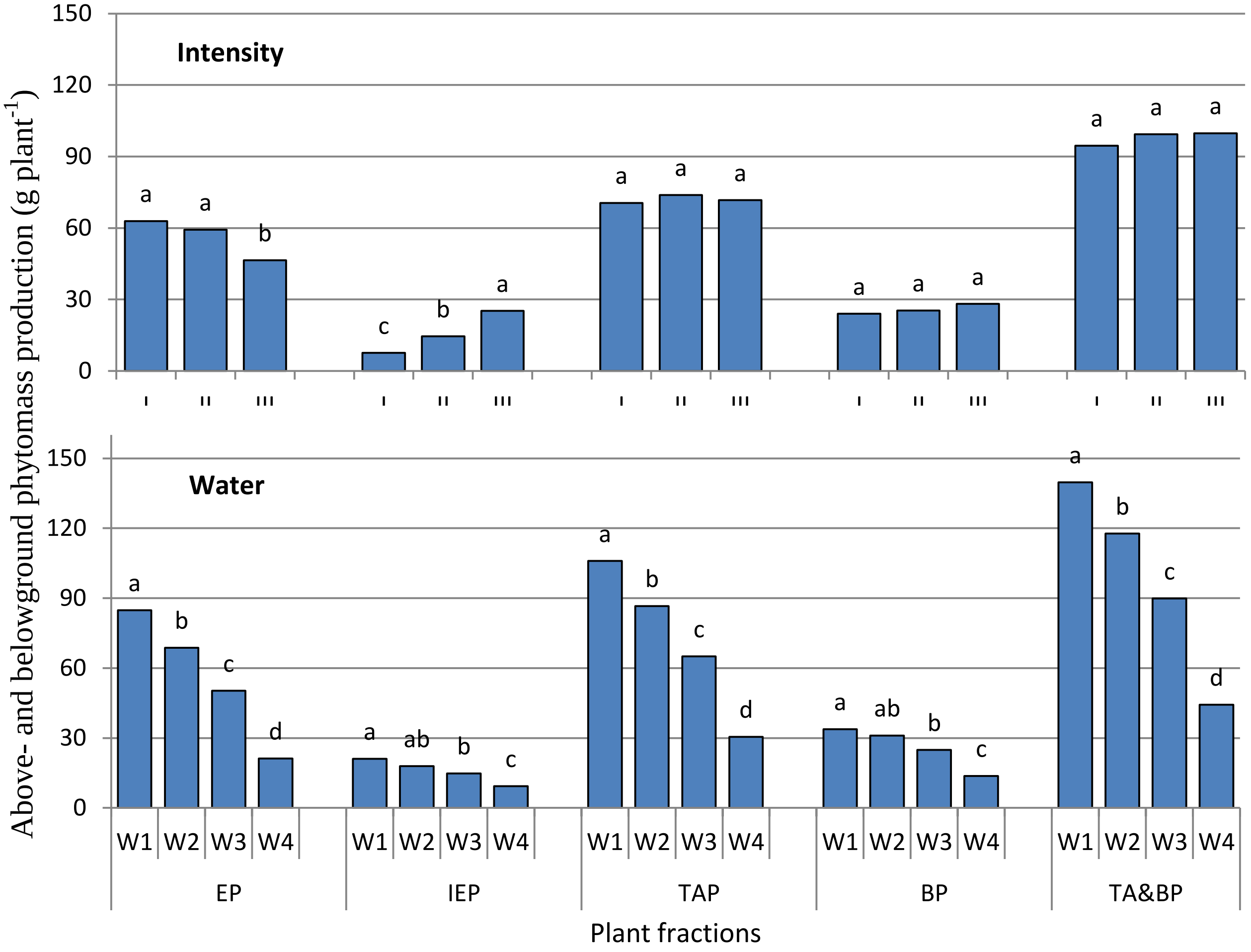

Figure 6.9: Mean (n = 6) cumulative above- and belowground phytomass production (g plant^{-1}) for *Pentzia incana* (six-monthly defoliation frequency) for different plant fractions (and combinations) at different defoliation intensities and water levels after 12 months, when all plants were destructively defoliated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. EP (edible phytomass), IEP (inedible phytomass), TAP (total aboveground phytomass), BP (below ground phytomass), TA&BP (total above and below ground phytomass). Different letters show significant differences ($P < 0.001$), between water treatments and between defoliation intensities, within each plant fraction.

6.2.2.4 Summary and discussion of defoliation frequency B (six-monthly defoliation)

Water had the greatest influence on edible phytomass of *P. incana*, with time (season) having the second highest influence. With the exception of inedible phytomass production, increased defoliation intensity had no influence ($P > 0.05$) on phytomass production.

Edible phytomass production was 19% higher ($P < 0.001$) after the second six months, spring plus summer (B2), than that of the first six months, autumn plus winter (B1). In comparing the two defoliation dates (B1 and B2), the edible phytomass production for the most severe water stressed treatments (W3 and W4) did not differ much ($P > 0.05$), while it did differ between the unstressed plants (W1 and W2), which both had significantly ($P < 0.001$) higher edible phytomass productions after the second six months (B2) than after the first six months (B1). The decline in edible phytomass under water deficit was almost similar, being 307% at B1 and 302% at B2, indicating equal response of *P. incana* to water stress over both time periods (autumn plus winter and spring plus summer).

Water sufficiency resulted in a 216% increase in total above- plus belowground phytomass production, while increased defoliation intensity lowered phytomass production by only 5%. For this six-monthly defoliation frequency water deficit played a slightly more important role than at the three-monthly frequency, while the influence of defoliation intensity is insignificant, compared to the three-monthly frequency where it had a much bigger influence on phytomass production.

6.2.3 Defoliation frequency C (after 12 months)

With the exception of IEP, where intensity accounted for the most variation in data, water accounted for the majority of variation in the data for all plant fractions and combinations (Table 6.9). The water x intensity interaction tested non-significant (P > 0.05) for most plant fractions and will therefore not be discussed.

Table 6.9: ANOVA summary for the cumulative above- and belowground phytomass production of *Pentzia incana* after 12 months (Frequency C).

Frequency C		W		I		W x I	
Variable	Treatment	SS% and significance					
EP	C	**<u>78</u>**	***	9	***	2	*
IEP	C	**16**	***	**<u>60</u>**	***	5	*
TAP	C	**<u>88</u>**	***	1	NS	2	NS
BP	C	**<u>58</u>**	***	2	NS	7	NS
TA&BP	C	**<u>83</u>**	***	1	NS	2	NS

W = water, I = defoliation intensity, F = defoliation frequency, EP = edible phytomass, IEP inedible phytomass, TAP = total aboveground phytomass, BP = belowground phytomass, TA&BP = total above- and belowground phytomass, NS = non-significant, SS% = sum of squares percentage, *** = P < 0.001, ** = P < 0.01, * = P < 0.05

Phytomass production of defoliation intensity treatments did not differ much (P > 0.05) for most of the plant fractions. It was only for inedible phytomass production where increased defoliation intensity caused a significant (P < 0.001) decline in production. Phytomass production for the different water treatments, however, decreased significantly (P < 0.001) with increased water stress for all plant fractions and combinations, with only W1 and W2 for belowground phytomass production (BP), which did not differ significantly (P > 0.05) (Figure 6.10). For TA&BP, W1 had a 228% higher phytomass production than W4. Water also had a greater influence on BP, with W1 having a 202% higher production than W4.

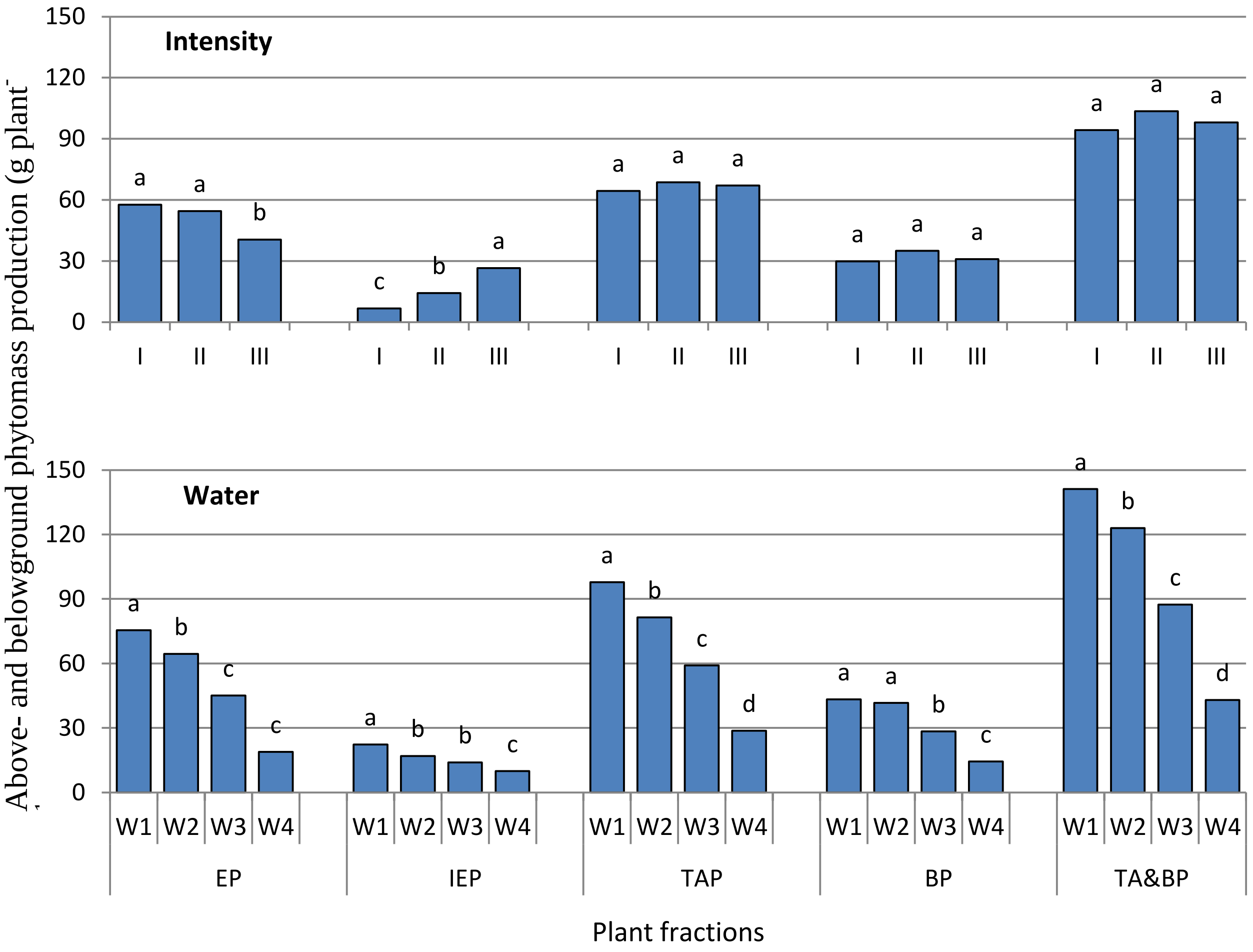

Figure 6.10: Mean (n = 6) cumulative above- and belowground phytomass production (g plant^{-1}) for *Pentzia incana* (twelve-monthly defoliation frequency) for different plant fractions (and combinations) at different defoliation intensities and water levels after 12 months, when all plants were destructively harvested. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. EP (edible phytomass), IEP (inedible phytomass), TAP (total aboveground phytomass), BP (below ground phytomass), TA&BP (total above and below ground phytomass). Different letters show significant differences (P < 0.001), between water treatments and between defoliation intensities, within each plant fraction.

6.2.3.1 Summary and discussion of defoliation frequency C (control)

The plants from defoliation frequency C were defoliated once on the specific date when the trial started and a second time exactly 12 months later when the trial was terminated. It seems as if the first defoliation might have had small influences on phytomass production. Frequency C can also be regarded as a control treatment, in terms of defoliation frequency.

With the exception of defoliation intensity, which had the greatest influence on inedible phytomass production, water had by far the most significant influence on all plant fractions and combinations. The differences in total above- and belowground phytomass production between extremes for water and defoliation intensities were 228% and 4%, respectively. The comparison between the three frequencies are illustrated in Table 6.10, showing that the least frequent *P. incana* was defoliated, the higher the water influence and lower the influence of intensity. This phenomenon was however much more prominent for *N. microphylla*.

Table 6.10: Percentage (%) differences between water and defoliation intensity treatment extremes, within each of the three defoliation frequencies.

Defoliation frequency	Water	Intensity
	% differences between treatment extremes	
A	200	31
B	216	5
C	228	4

6.2.4 Comparison of Frequency A, B and C

6.2.4.1 Frequency A compared to frequency B

First six months (A1 + A2 compared to B1)

For the first six months (A1 + A2 compared to B1), water and frequency accounted for most of the variation in edible phytomass data (Table 6.11). Although defoliation intensity and some of the interactions tested to have a significant ($P < 0.001$) influence on edible phytomass, the SS% of these is too low to be discussed further.

Table 6.11: ANOVA summary for the cumulative aboveground phytomass production of *Pentzia incana* after the first and second six months for frequency A compared to that of frequency B for the same time intervals.

A and B compared		W		I		F		W x I		W x F		I x F		W x I x F	
Variable	Treatment	SS% and significance													
EP	A1+A2 compared B1	**<u>61</u>**	***	4	***	**<u>16</u>**	***	1	NS	4	***	3	**	2	***
EP	A3+A4 compared B2	**<u>77</u>**	***	6	***	0	NS	1	NS	1	*	0	NS	1	NS

W = water, I = defoliation intensity, F = defoliation frequency, EP = edible phytomass, IEP inedible phytomass, TAP = total aboveground phytomass, BP = belowground phytomass, TA&BP = total above- and belowground phytomass, A = Frequency A, B = frequency B, A1 = May, A2 = August, A3 = November, A4 = February, B1 = first six months, B2 = second six months, NS = non-significant, SS% = sum of squares percentage, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

After the first six months the edible phytomass production of frequency B was higher ($P < 0.001$) than the cumulative edible phytomass of frequency A (A1 + A2) (Figure 6.11). The one extra defoliation (A1) of frequency A caused a 52% reduction ($P < 0.001$) in phytomass production, compared to frequency B which was defoliated for the first time at that stage. The edible phytomass production of the most lenient defoliation intensity (III) was lower ($P < 0.001$) than that of the more severe intensity treatments (I and II). The edible phytomass production for the most severely stressed water treatment (W4) was much lower ($P < 0.001$) than that of any other water treatment, which all also differed ($P < 0.001$) from each other.

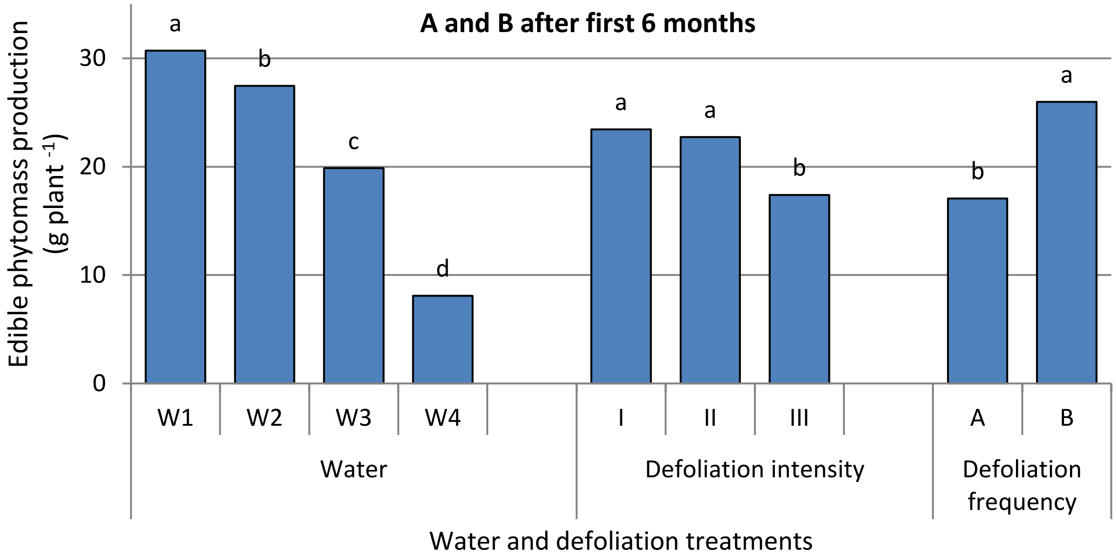

Figure 6.11: Mean (n = 6) edible phytomass production (g plant^{-1}) for *Pentzia incana.* Comparison between the phytomass production of frequency A and B after the first six months. The three main effects (water, defoliation intensity and defoliation frequency) are indicated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. A = cumulative phytomass production for the first six months of the three-monthly defoliation frequency, B = First six months of the six-monthly defoliation frequency. Different letters show significant differences (P < 0.001) within treatments.

Second six months (A3 + A4 compared to B2)

After the second six months (A3 + A4 compared to B2) (spring and summer growing seasons), defoliation intensity and frequency did not test significantly (P > 0.05), while water accounted for most of the variation in data (Table 6.11 and Figure 6.12). Edible phytomass production at frequencies A and B were similar (P > 0.05), indicating that phytomass production of frequency A caught up with that of frequency B, even though it had an extra defoliation (A3) compared to frequency B. This might be attributed to compensatory growth, with the plants of frequency A giving comparable phytomass production to frequency B during the more favourable growth season, even though some plants were exposed to water stress. Water stress significantly ($P < 0.001$) decreased edible phytomass production, with W1 showing 303% higher edible phytomass production than W4.

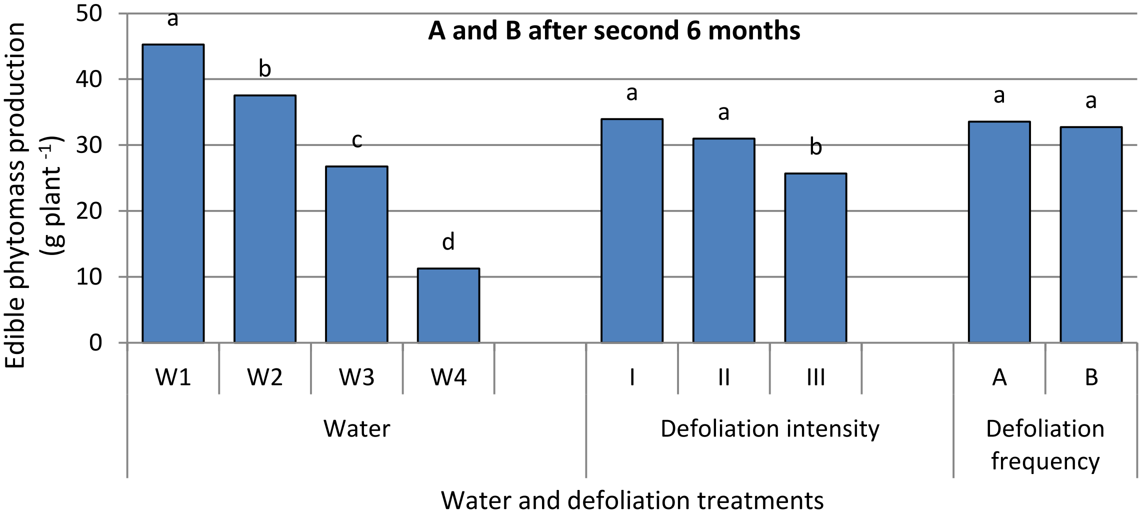

Figure 6.12: Mean (n = 6) edible phytomass production (g plant^{-1}) for *Pentzia incana.* Comparison between the phytomass production of frequency A and B after the second six months. The three main effects (water, defoliation intensity and defoliation frequency) are indicated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. A = cumulative phytomass production for the second six months of the three-monthly defoliation frequency, B = Second six months of the six-monthly defoliation frequency. Different letters show significant differences ($P < 0.001$) within treatments.

6.2.4.1.1 Summary and discussion of frequency A and B compared

At the end of the first six months, in comparing the cumulative edible phytomass of frequency A (A1 + A2) with that of B1, both water and frequency influenced edible phytomass significantly ($P < 0.001$). The edible phytomass of B1 was 52% higher than that of A1+A2. This indicates that the one extra defoliation of frequency A (autumn) caused a 52% decrease in the production potential of *P. incana* compared to B1 which were defoliated only once, at the end of the first 6 months.

At the end of the second six months, edible phytomass of B2 was compared to the cumulative edible phytomass of frequency A (A3 + A4). There was no difference ($P > 0.05$) in edible phytomass between frequencies, while water still had a high ($P < 0.001$) influence on edible phytomass production.

The cumulative edible phytomass production of frequency A was 97% higher after the second six months than from what it was after the first six months, compared to that of B2 which was only 27% higher than B1. This increased growth after defoliation stress could be regarded as compensatory growth, where the more frequently defoliated plants (A) showed increased productivity compared to the less frequently defoliated plants (B). This might, however, only be the case when sufficient water is available. Another explanation can be that if a more frequently defoliated plant came through the winter with, for example a deficit in edible production to a less defoliated plant, it can catch up with and equal the production of the less defoliated plant during the spring and summer growth period. Compensatory growth is only possible under favourable growth condition, for example when sufficient rainfall occurs.

It can therefore be concluded that autumn grazing, just before winter could cause a major setback in individual plant performance of *P. incana* during the winter months. By contrast, *P. incana* has the ability to show rapid regrowth, in terms of edible phytomass, equalling the edible phytomass production of less frequently defoliated plants.

6.2.4.2 Frequency A, B, C compared

Water accounted for most variation in phytomass data for EP (78%), TAP (87%), BP (40%) and TA&BP (80%), while intensity accounted for most variation in the data for IEP (58%) (Table 6.12). Frequency also accounted for some (16%) of the variation in data for BP. Although some of the interaction tested highly significant ($P < 0.001$), the SS% was too low to be discussed. The I x F and W x F interactions are, however, presented to explain some notable findings.

Table 6.12: ANOVA summary for the cumulative above- and belowground phytomass production of *Pentzia incana* for all frequencies at the end of the trial.

A,B,C compared		W		I		F		W x I		W x F		I x F		W x I x F	
Variable	Treatment	SS% and significance													
EP	A, B, C compared	**78**	***	6	***	3	***	1	*	1	**	1	NS	1	*
IEP	A, B, C compared	22	***	**58**	***	1	**	5	***	1	NS	1	NS	1	NS
TAP	A, B, C compared	**87**	***	1	**	1	***	1	NS	1	NS	1	*	1	NS
BP	A, B, C compared	**40**	***	3	***	**16**	***	1	NS	5	***	2	*	4	*
TA&BP	A, B, C compared	**80**	***	1	***	3	***	1	NS	1	*	1	**	2	*

W = water, I = defoliation intensity, F = defoliation frequency, EP = edible phytomass, IEP inedible phytomass, TAP = total aboveground phytomass, BP = belowground phytomass, TA&BP = total above- and belowground phytomass, A = Frequency A, B = Frequency B, C = Frequency C, NS = non-significant, SS% = sum of squares percentage, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

Water stress decreased ($P < 0.001$) phytomass production of all plant fractions (Figure 6.13). The difference between the edible phytomass production of the unstressed and severely stressed water treatments was 292%. Increased defoliation intensity caused a decrease ($P < 0.001$) in phytomass production only at the inedible plant fraction, with most lenient defoliation (intensity III) being 147% higher than the most severe defoliation intensity (intensity I). With the exception of inedible phytomass, defoliation frequency A showed the lowest ($P < 0.001$) phytomass production of the three frequencies at all other plant part fractions. Interestingly frequency B had significantly ($P < 0.001$) higher phytomass productions for edible phytomass production and total aboveground phytomass production than the other frequencies and was equal to frequency C at total above- plus belowground phytomass production. These results indicate that in general, rainfall (water availability) has a greater influence on the phytomass production of *P. incana* than grazing (defoliation). Furthermore, root production (belowground phytomass) is highly influenced by grazing frequency, but not as much as by water.

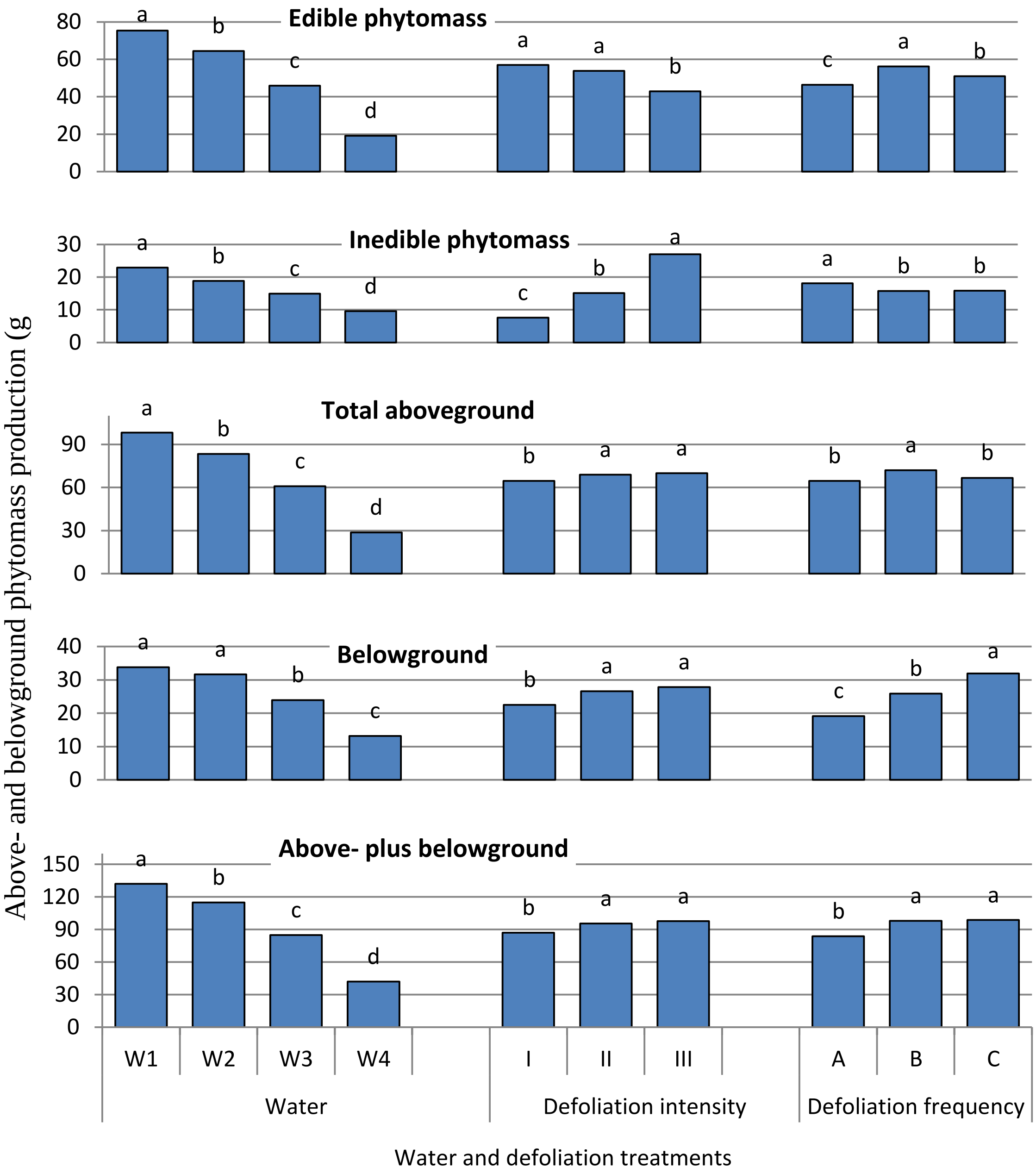

Figure 6.13: Mean (n = 6) cumulative above- and belowground phytomass production (g plant^{-1}) for *Nenax microphylla* for different plant fractions (and combinations) after 12 months, when all plants were destructively harvested. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = (three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences (P < 0.001), within treatments at each fraction.

Defoliation frequency A showed higher variation in phytomass production for defoliation intensities over all plant fractions, than the other defoliation frequencies (Figure 6.14). With the exception of edible phytomass production which increased with increased defoliation intensity, all other plant fraction showed a decrease ($P < 0.001$) (Figure 6.14). On the contrary, phytomass production at frequencies B and C only decreased significantly ($P < 0.001$) for inedible phytomass, while both frequencies also showed an increase in edible phytomass production with increased defoliation intensity.

The fact that edible phytomass of both frequency A and B increased ($P < 0.001$) with increased defoliation intensity might be ascribed to the compensatory ability of *P. incana*. The compensatory growth of the more severely defoliated plants was so high that it outperformed the phytomass production of the less severe defoliation treatments. The regrowth was also the edible phytomass which was repeatedly defoliated at every defoliation date, with virtually no growth (expansion) of inedible phytomass, therefore the constant significant ($P < 0.001$) difference in inedible phytomass production (Figure 6.14). It could even be postulated that the rapid (compensatory) regrowth was at the cost of inedible (thicker twigs) and root growth, with the plant trying to rapidly increase its photosynthetic surface. Unfortunately, before an adequate photosynthetic surface is reached and maintained to redirect growth to stems (branches) and roots, the plant gets defoliated again, resulting in the negative growth cycle starting again. Defoliation at repeated short intervals, might therefore mask the possible positive effect of compensatory growth in the process of recovery after grazing.

With the exception of edible phytomass, the significant ($P < 0.001$) decrease in phytomass production with increased defoliation intensity at frequency A, indicates the detrimental influence of repeated (three-monthly) severe defoliations on phytomass production. The slight differences between the defoliation intensities for both total aboveground and total above plus belowground phytomass production, at frequency C, showing a slight decrease in phytomass production with increasing defoliation intensity, was a notable finding. Although these slight differences in phytomass production are not significant, they can be attributed to the first defoliation, 12 months prior to the current defoliation results under discussion. It can therefore be argued that even after a 12 month rest period, the effect of the previous defoliation treatment was still evident.

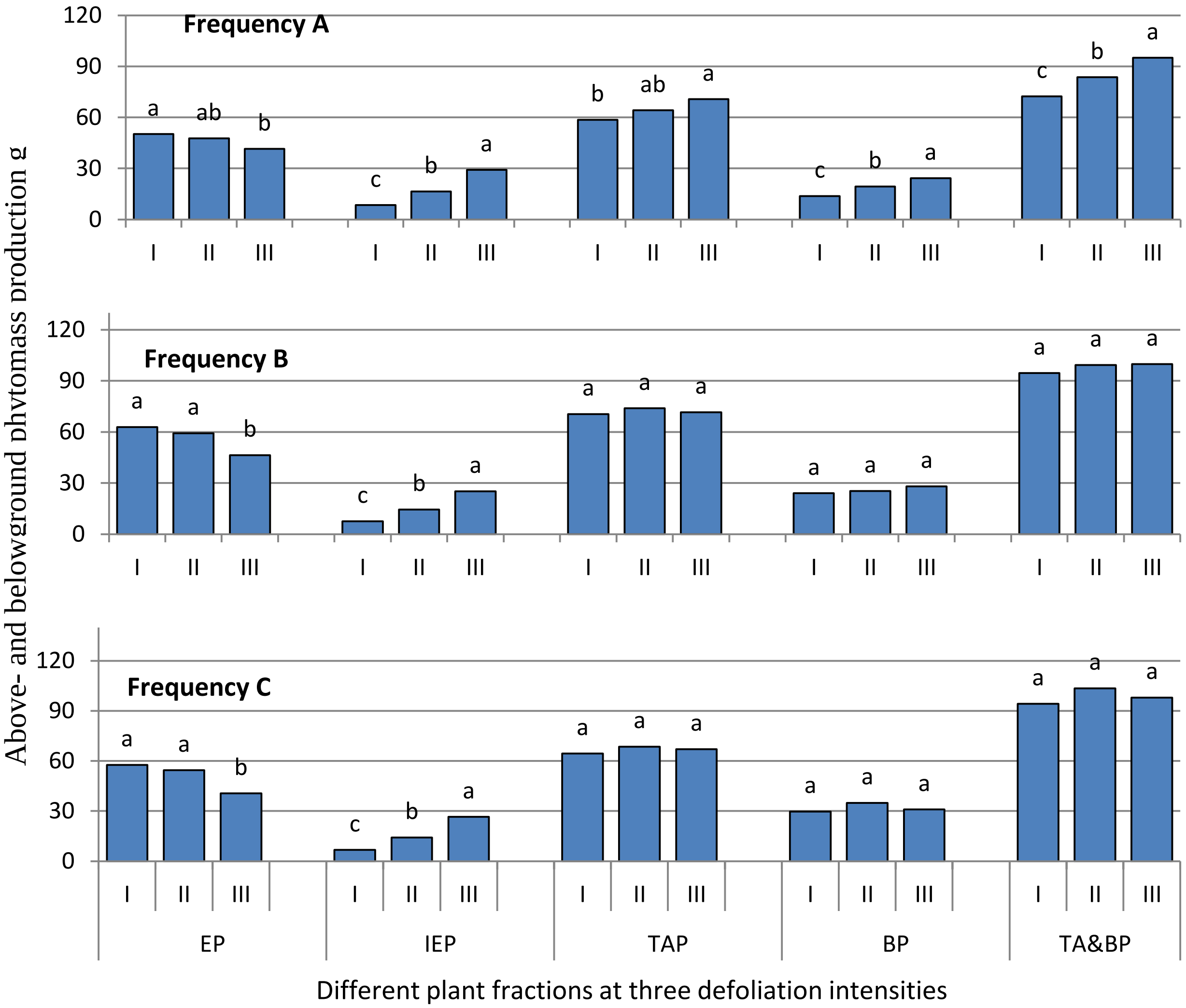

Figure 6.14: Mean (n = 6) cumulative above- and belowground phytomass production (g plant^{-1}) for *Pentzia incana* (frequency A, B and C) for different plant fractions (and combinations) at different defoliation intensities after 12 months, when all plants were destructively defoliated. Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. EP (edible phytomass), IEP (inedible phytomass), TAP (total aboveground phytomass), BP (belowground phytomass), TA&BP (total above- and belowground phytomass). Different letters show significant differences (P < 0.001), between water treatments within each fraction for each frequency.

For the water x frequency interaction, water deficit caused a decrease ($P < 0.001$) in phytomass production for all plant fractions, at all three defoliation frequencies (Figure 6.15).

Only edible ($P < 0.05$) and belowground ($P < 0.001$) plant fractions had a significant influence on phytomass production at the water x frequency interaction (Table 6.12). For both these plant fractions, as well as the other plant fractions, phytomass production at the most severely stressed water treatment (W4) was equally low at all three defoliation frequencies (Figure 6.15). This indicates that when plants are severely water stressed, the water stress has a bigger influence on phytomass production than defoliation frequency itself. By contrast, the phytomass production of the belowground fraction showed a decrease ($P < 0.001$) in with increased defoliation frequency for the less stressed water treatments. Interestingly, edible phytomass showed the highest ($P < 0.001$) production at frequency B for the unstressed water treatment.

This can also be attributed to compensatory growth. Plants have the best opportunity to show compensatory growth when ample resources are available (Stock et al. 1993). The plants at frequency B are less defoliated than the plants at frequency C, which might be the reason for better compensatory ability at the same, unstressed water treatments.

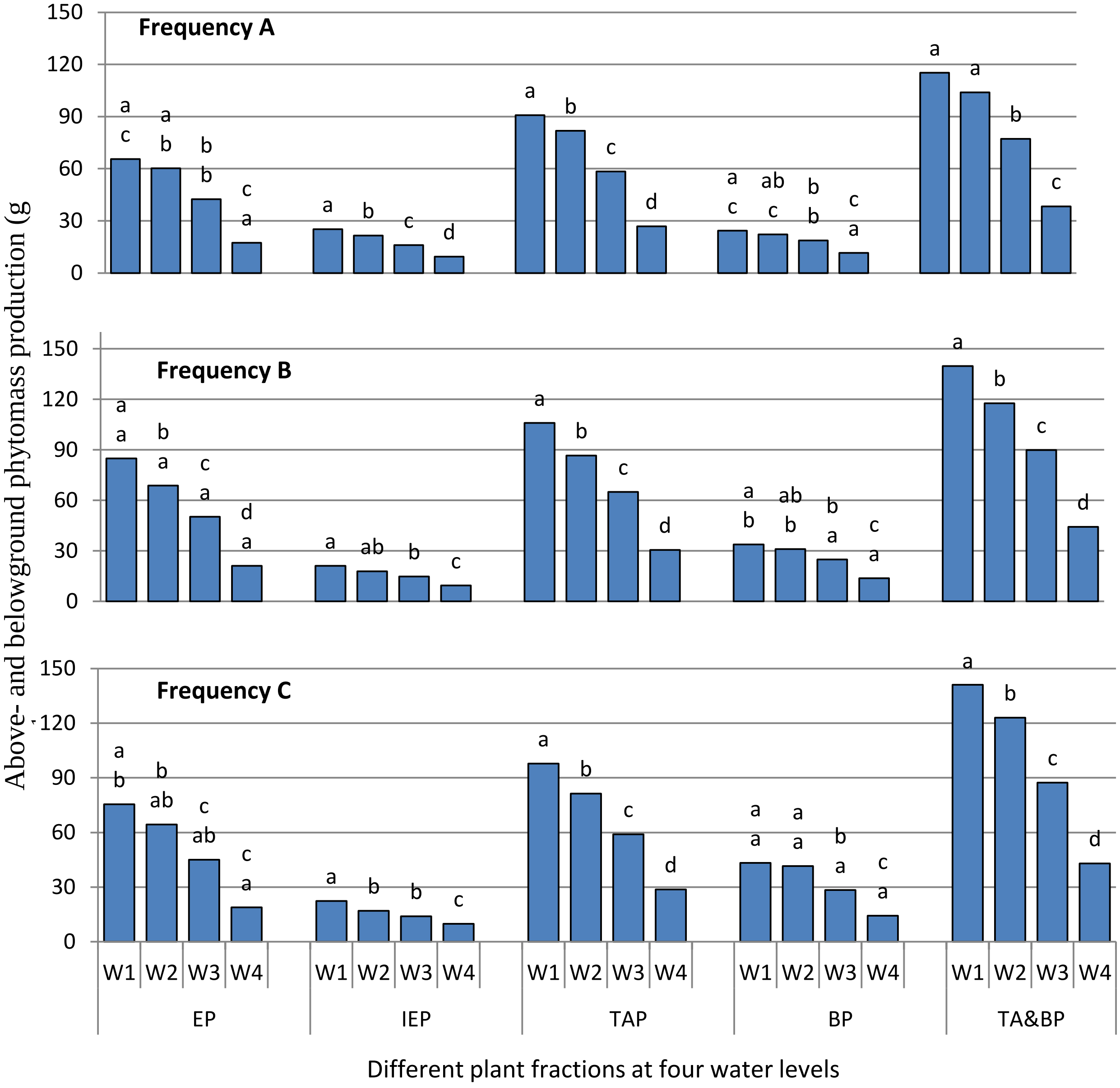

Figure 6.15: Mean (n = 6) cumulative above- and belowground phytomass production (g plant^{-1}) for *Pentzia incana* (frequency A, B and C) for different plant fractions (and combinations) at different water levels after 12 months, when all plants were destructively defoliated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). EP (edible phytomass), IEP (inedible phytomass), TAP (total aboveground phytomass), BP (belowground phytomass), TA&BP (total above- and belowground phytomass). Different top letters show significant differences ($P < 0.001$), between water treatments within each fraction for each frequency. Different bottom letters show significant differences ($P < 0.001$) between frequencies for similar water treatments.

Figure 6.16 indicates the percentage difference in phytomass production between defoliation intensity treatment extremes (intensities I and III) at the three defoliation frequencies for all plant fractions. It also indicates the percentage difference in phytomass production between the water treatment extremes (W1 and W4) at the three defoliation frequencies for all plant fractions. It was also included, amongst others, for better explaining the effect of water stress on root growth. With the exception of IEP, water caused far greater differences between treatment extremes than intensity (Figure 6.16). This is another illustration of water having a much greater effect on phytomass production than, for instance, defoliation intensity and defoliation frequency.

Defoliation intensity showed the lowest percentage differences between treatment I and III at defoliation frequency C (least frequent defoliation) and frequency B (six-monthly defoliation) for total aboveground phytomass, belowground phytomass and total above- plus belowground phytomass. At frequency A (most frequent defoliation) the percentage difference between intensity I and III is much greater, indicating the large influence of defoliation intensity in combination with the most frequent defoliation treatment, namely frequency A on phytomass production. By contrast, frequency A (three-monthly defoliation) caused less variation in both edible and inedible phytomass than frequency C (defoliated after 12 months). This is partly due to compensatory growth which resulted in abnormal vigorous regrowth of the most severely (intensity I) defoliated plants.

Water treatments caused great, but almost equal (amongst frequencies), differences for edible phytomass, total aboveground phytomass and total above- plus belowground phytomass. For inedible phytomass, the most severely defoliated plants (frequency A) showed a higher percentage difference between water extremes than the less frequently defoliated plants.

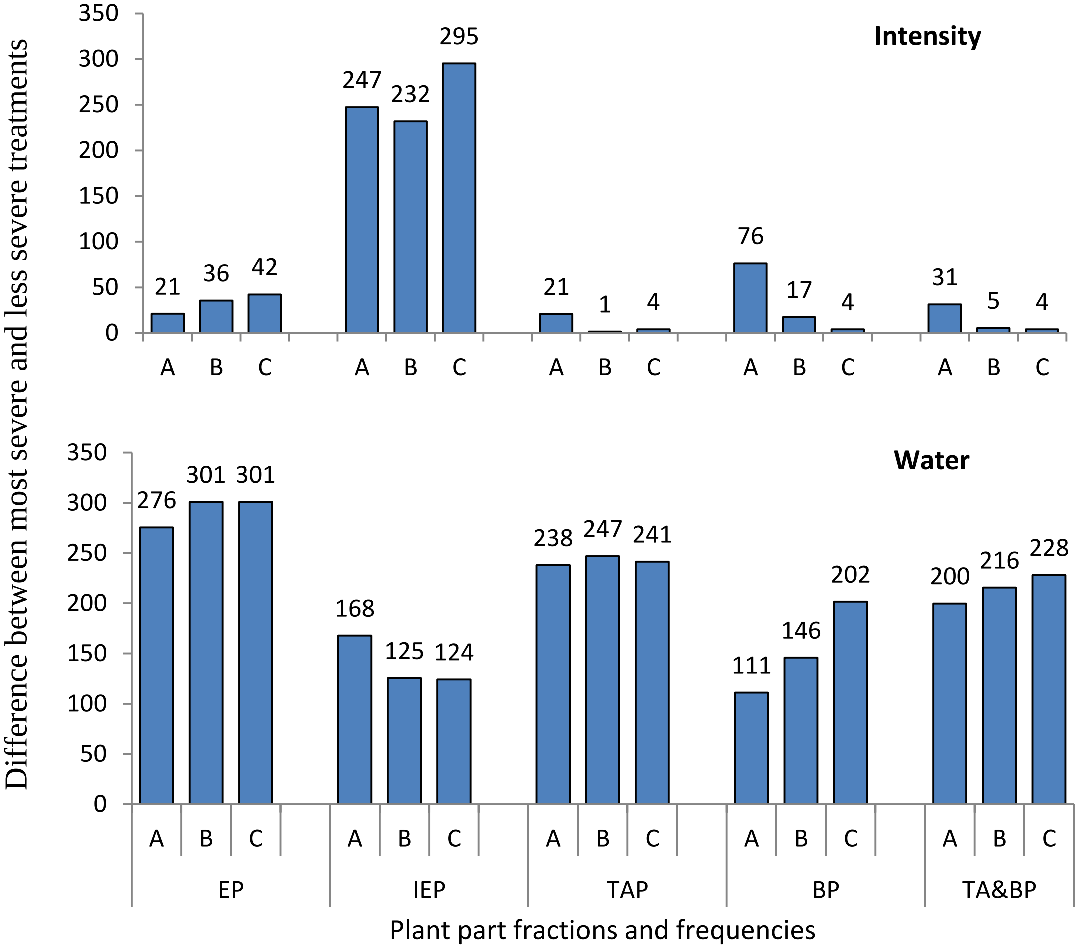

Figure 6.16: Summary of the percentage difference between the most severe and least severe defoliation and water treatments for the different plant part fractions and combinations of *Pentzia incana* at the three defoliation frequencies. EP (edible phytomass), IEP (inedible phytomass), TAP (total aboveground phytomass), BP (belowground phytomass), TA&BP (total above- and belowground phytomass). Defoliation frequency: A = (three-monthly, B = six-monthly and C = twelve monthly or control.

The most notable phenomenon was belowground phytomass (BP) which showed clear opposite reactions between defoliation intensity and water treatments. The more frequently defoliated plants (frequency A), showed the highest percentage difference between intensity extremes, but the lowest percentage difference between the water extremes. The opposite was true for frequency C where the plants were defoliated at 12 months. The greatest difference between intensity extremes at frequency A is an indication of the extremely detrimental effect of a combination of high frequency in combination with high intensity of grazing on plant root growth. For water treatments, where the difference between water treatments were the smallest at the highest defoliation frequency (frequency A), the plants are to some extent compensating for the water stress in combination with increased defoliation frequency by reduced termination of root growth. The plants can therefore better adapt their root survival under water stress conditions, than at under high intensity grazing situations, when frequently defoliated.

6.2.4.3 Summary and discussion of comparison between defoliation frequencies A, B and C

In general, water had the most significant ($P < 0.001$) influence on phytomass production, greater than that of defoliation intensity and frequency, which also had significant influences on phytomass production, but to a lesser extent than that of water.

A summary of the SS% for the different plant part fractions and combinations is presented in Table 6.13, to indicate which main effect accounted for the most variation in data, thus having the biggest influence on phytomass production, at the three defoliation frequencies.

Table 6.13: ANOVA summary of sum of square percentages (%) of the main effects, water and defoliation intensity at the three defoliation frequencies.

Variable	A		B		C	
	W	I	W	I	W	I
EP	**79**	3	**84**	8	**78**	9
IEP	27	**56**	21	**60**	16	**60**
TAP	**84**	3	**92**	1	**88**	1
BP	**34**	27	**54**	3	**58**	2
TA&BP	**78**	8	**88**	1	**83**	1

EP = edible phytomass, IEP inedible phytomass, TAP = total aboveground phytomass,
BP = belowground phytomass, TA&BP = total above- and belowground phytomass,
W = water, I = defoliation intensity, A = Frequency A, B = Frequency B, C = Frequency C

At frequency A, water had the biggest effect on edible phytomass, total aboveground phytomass and total above- plus belowground phytomass, while intensity had the biggest influence on inedible phytomass production. Intensity also had a notable influence on belowground phytomass production, but to a lesser extent than water. For phytomass production of all plant fractions and plant fraction combinations, there was some variation in SS% for water and, with the exception of edible phytomass, a decrease in the SS% for intensity, with decreased defoliation frequency. At both frequency B and frequency C (the two less frequently defoliations), with the exception of inedible phytomass, water, by far, had the biggest influence on the phytomass production of all plant part fractions.

When the three main effects are compared, frequency had a bigger effect on BP than intensity, while water had a greater effect on BP than frequency. While water is not limited (W1 and W2) and under mildly stressed (W3) conditions, differences between intensities and frequencies (and time/date) do occur, but when water gets limited (W4) all treatments gave equally low phytomass productions.

The percentage difference between treatment extremes in interaction with each of the main effects is summarized in Table 6.14. Firstly, considering defoliation intensities and frequencies at increased water deficit, it is clear that as water deficit increased, there was a decrease in differences between frequencies A and C. At the defoliation intensity, the difference between intensity extremes stayed the same for W1, and W3, while a larger difference was recorded at W2 and W4.

Secondly, considering the defoliation frequency and water at increased defoliation intensities, there was a definite increase in percentage difference between extremes for defoliation frequency with an increase in defoliation intensity. Water caused the lowest (although much higher than at frequency) variation at the most severe intensity (intensity I) and the most variation at intensity II the intermediate defoliation intensity. The percentage differences between water extremes were much higher than that of defoliation frequency at all defoliation intensities.

Thirdly, looking at what happened at the defoliation intensities and water at increased defoliation frequencies, the percentage difference between treatment extremes for defoliation intensity increased with increased defoliation frequency, while that of water decreased with increased defoliation frequency. Again the percentage differences between water extremes were much higher than those for defoliation intensity at all defoliation frequencies.

Table 6.14: Percentage difference between treatment extremes in interaction with each of the main effects for total above- plus belowground phytomass production.

Water	A - C	I - III	**Intensity**	A - C	W1 - W4	**Frequency**	I - III	W1 - W4
W1	**23**	27	I	**30**	247	A	**31**	200
W2	18	39	II	24	**342**	B	5	216
W3	13	27	III	3	307	C	4	**228**
W4	12	**49**						

Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters shows significant differences (P < 0.001), within treatments at each fraction. Defoliation frequency: A = (three-monthly, B = six-monthly and C = twelve monthly or control.

6.2.5 Practical implication of phytomass findings

The three way interaction (all 36 treatment combinations) of all phytomass findings is presented in Figure 6.17 to summarize the aboveground, belowground and total above- plus belowground phytomass production. This figure summarizes the influence of water and defoliation interactions on phytomass production. The aim is to derive the possible influences of grazing and rainfall interactions on phytomass production of *P. incana*. Although the trial was carried out *in sito*, it might explain how rainfall and grazing interaction could impact on the productivity of *P. incana in vivo*.

All data were ranked from the lowest to the highest phytomass production. The ranked data roughly followed the water treatments, from the most severely stressed water treatment (W4) tot the unstressed plants (W1) (Figure 6.17). The vertical lines to the right hand side of the graph indicate how water treatment dominated the distribution of the data.

The darker black horizontal lines in Figure 6.17 were inserted to indicate the best and worse set of treatments (not differing significantly from each other – within the same least significant difference (LSD) range), selected according to the multiple t-distribution test procedure of Gupta and Panchapakesan (1979). The treatment combinations below the last horizontal lines at the bottom of the figure indicate the best group regarding phytomass production. This is the group of treatment combinations that gave the highest phytomass production without differing significantly from each other ($P < 0.001$). Watering treatment W1 (least water stressed) in combination with defoliation intensity II and III (II = intermediate height of 125mm and III = less severe at 200 mm height), for defoliation frequencies B and C (less frequently defoliated), are present in this group for aboveground and total above- plus belowground phytomass production. This illustrates that above normal rainfall with a lenient defoliation could give the highest phytomass production, at a moderate to intermediate defoliation frequency. Thus, under high rainfall years, *P. incana* might be defoliated a bit more frequently, but at a moderate intensity.

The weakest groups (Gupta and Panchapakesan 1997) of treatment combinations regarding phytomass production, are situated above the top horizontal lines in (Figure 6.17). This is the group of treatment combinations that gave the lowest phytomass productions without differing significantly ($P < 0.001$) from each other. Water treatment W4 (severely stressed) totally dominates this group for aboveground and total above- plus belowground phytomass production. Defoliation intensity I and II (most severe defoliation – 50 mm height and moderate defoliation 125 mm height) in combination with the most water stressed plants gave the lowest phytomass production, although it did not differ significantly ($P > 0.05$), regarding phytomass production, from the other treatment combinations in the same group. This explains that during droughts the impact of water stress is so high on phytomass production that none of the defoliation treatments resulted in any significantly higher phytomass production. Although not significantly higher, a light defoliation intensity (defoliation intensity III – 200 mm height), might give higher phytomass production, regardless of the defoliation frequency. For the belowground fraction this group was a bit larger and included the most frequent (frequency A) and/or most severely (intensity I) defoliated treatments of W1, W2 and W3 (bars marked in red in graph).

Looking at the influence of defoliation treatments on phytomass production, defoliation intensity I (most severe – 50 mm height) in combination with defoliation frequency A (three-monthly defoliation) proved to be the most detrimental, at each of the water treatments (one example encircled in red in Figure 6.17). This indicates that frequent severe grazing should always be avoided, irrespective of the amount of rainfall. On the contrary, a lighter (defoliation intensity III – 200 mm height), less frequent defoliation (defoliation frequency C – once per year) gave the highest phytomass production (one example encircled in green in Figure 6.17). This indicates that a light grazing, once per year, even at W3 (the second most severely stressed water treatment) is most beneficial for phytomass production of *P. incana*.

It should, however, be taken into account that for survival and sustainability farmers need to stock their farms and utilize the vegetation according to the long term grazing capacity of the rangeland, not only to the benefit of the vegetation but also for their own economic benefit. This might cause them to sometimes apply a more severe defoliation, of which the impact can be reduced by applying longer resting periods through appropriate grazing management systems. The Nama-karoo Biome is, however, known for its diverse botanical composition, mostly consisting of various preferred shrub and grass species with variable palatability. This complex and delicate interaction between animal and plant is extremely difficult to manage for sustainable animal production. Awareness of the reaction of single shrub species to different defoliation intensities and frequencies under variable rainfall occurrence might clarify which defoliation interactions to avoid and still ensure higher and sustainable phytomass production.

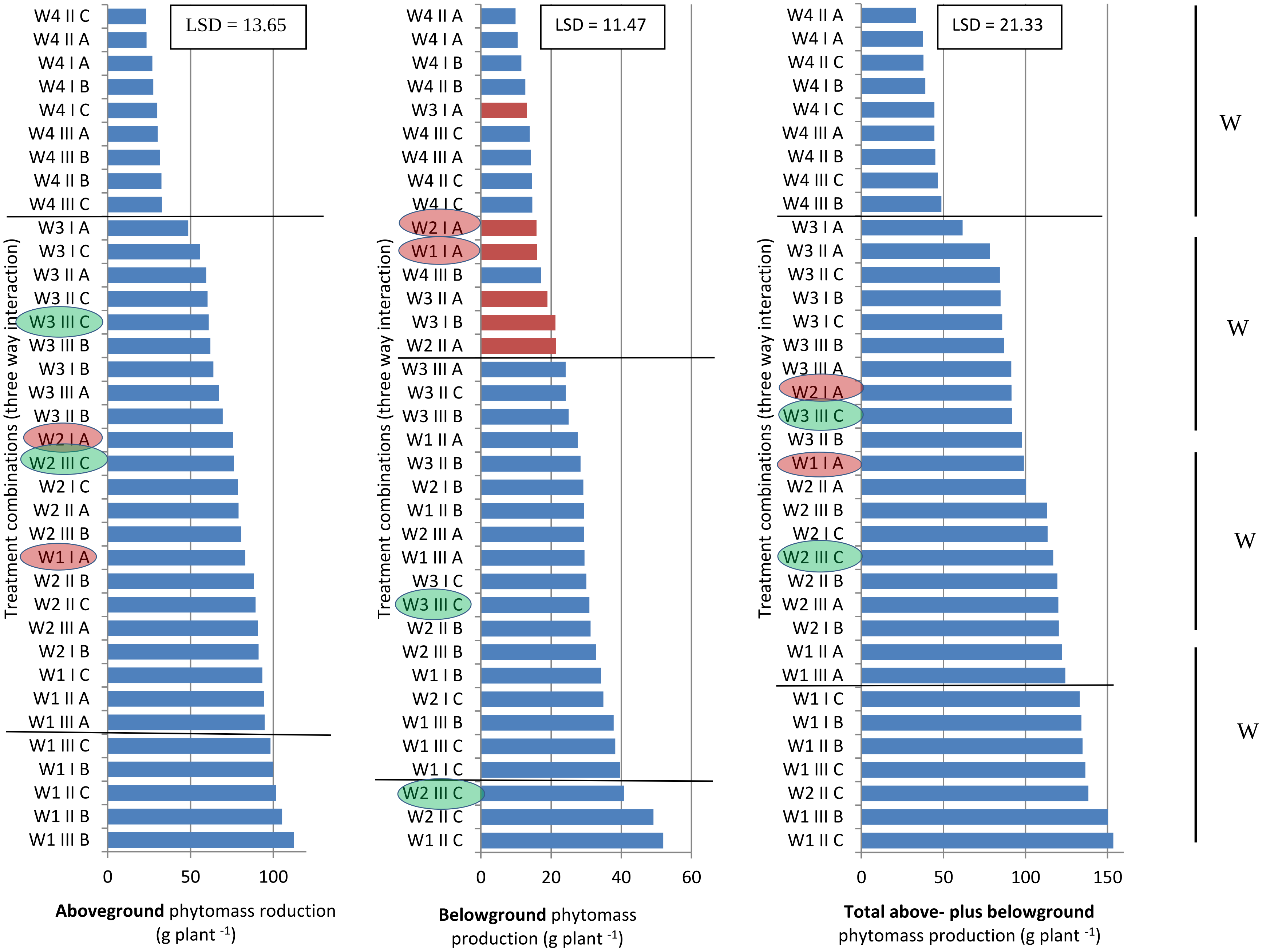

Figure 6.17: Phytomass production (g plant-1) for the three way interaction (water x intensity x frequency) of *Pentzia incana*. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = (three-monthly, B = six-monthly and C = twelve monthly or control.

6.2.6 Plant fraction ratio (including root:shoot ratio)

The plant fractions ratio gives an indication of how the plant allocates phytomass to the different plant parts (fractions), namely: edible phytomass (EP), inedible phytomass (IEP) and belowground phytomass (BP). The more commonly used term, root:shoot ratio will also be discussed under this heading.

Most (57%) of the variation in edible phytomass (EP) data was accounted for by defoliation intensity, with water availability explaining 15% of the variation in data (Table 6.15). Variation in inedible phytomass (IEP) was mostly accounted for by defoliation intensity (69%). Defoliation frequency accounted for most (29%) of the variation in data for belowground phytomass production, with water the second highest at 14%. Most of the interaction tested to have a non-significant (P > 0.05) influence on phytomass production.

Table 6.15: ANOVA summary for the different plant fraction ratios for the phytomass production of *Pentzia incana* for all frequencies at the end of the trial.

Plant part fraction ratio		W		I		F		W x I		W x F		I x F		W x I x F	
Variable	Treatment	SS% and significance													
EP	A, B, C compared	**15**	***	**57**	***	4	***	1	NS	1	NS	1	NS	2	NS
IEP	A, B, C compared	6	***	**69**	***	4	***	1	NS	1	NS	1	NS	1	NS
BP	A, B, C compared	**14**	***	3	**	**29**	***	1	NS	3	*	3	**	6	**

W = water, I = defoliation intensity, F = defoliation frequency, EP = edible phytomass, IEP inedible phytomass, BP = belowground phytomass, A = Frequency A, B = Frequency B, C = Frequency C, NS = non-significant, SS% = sum of squares percentage, *** = P < 0.001, ** = P < 0.01, * = P < 0.05

Water deficit caused a decrease (P < 0.001) in percentage edible phytomass allocation, with an increase (P < 0.001) in phytomass allocation to belowground phytomass (Figure 6.18). Increased defoliation intensity caused an increase (P < 0.001) in the percentage edible phytomass and a decrease (P < 0.001) in the percentage inedible phytomass. Increased defoliation frequency caused a significant decrease (P < 0.001) in the percentage belowground phytomass allocation (Figure 6.18).

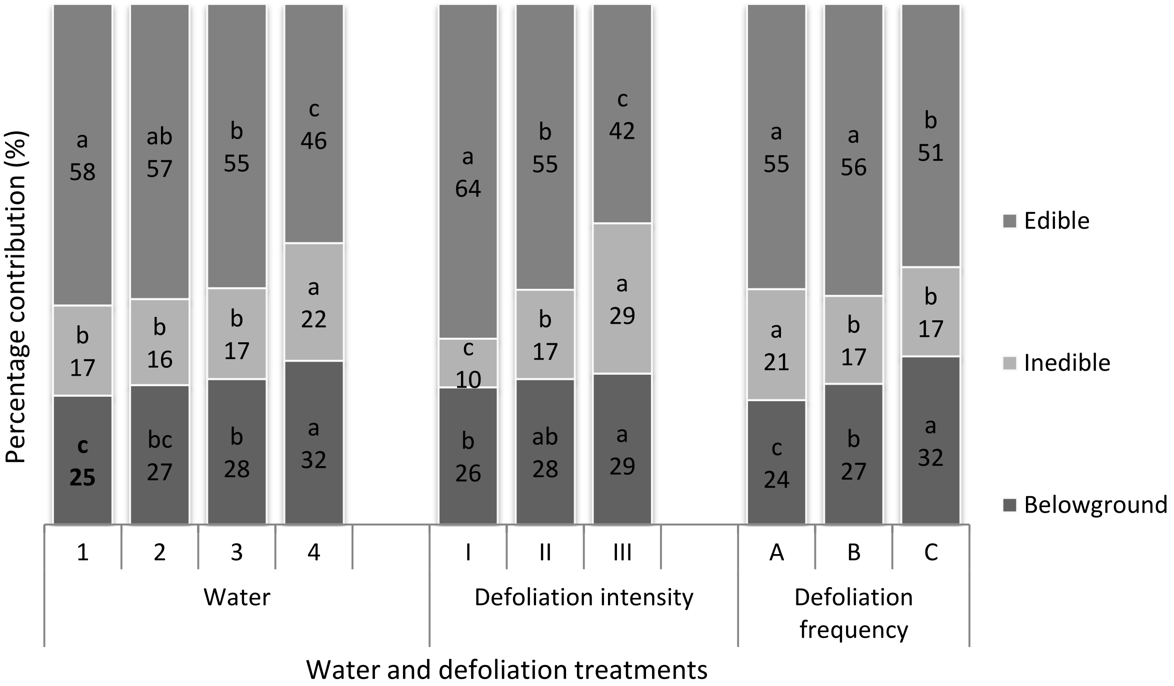

Figure 6.18: Percentage contribution of different plant fractions to total phytomass production of *Pentzia incana* at different water and defoliation treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequencies are: A = three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences ($P < 0.001$), between the same plant fraction within treatments.

Root:shoot ratio is widely used to indicate biomass partitioning of plants by expressing the ratio of below- to aboveground phytomass allocation. Defoliation frequency had the largest influence on root:shoot ratio by accounting for 28% of the variation in data, with water second highest, but rather low, at 13% (Table 6.16). Intensity as well as the treatment interactions had very low sum of square percentages and will therefore not be discussed.

Table 6.16: ANOVA summary for the root:shoot ratio for the phytomass production of *Pentzia incana* for all frequencies compared at the end of the trial.

Root:shoot ratio		W		I		F		W x I		W x F		I x F		W x I x F	
Variable	Treatment	SS% and significance													
Ratio	A,B, C compared	**13**	***	2	*	**28**	***	1	NS	3	NS	3	**	7	**

W = water, I = defoliation intensity, F = defoliation frequency, A = Frequency A, B = Frequency B, C = Frequency C, NS = non-significant, SS% = sum of squares percentage, *** = P < 0.001, ** = P < 0.01, * = P < 0.05

Increased defoliation intensity did not have an impact (P > 0.05) on the root:shoot ratio (Figure 6.19). Water deficit caused a significant (P < 0.001) increase in root:shoot ratio, with the ration at W4 being 39% higher than at W1. By contrast, increased defoliation frequency resulted in a decrease (P < 0.001) in the root:shoot ratio, with the root:shoot ratio at frequency A being 52% lower than that of frequency C. This indicates that defoliation frequency had a greater and opposite (more negative) impact on root:shoot ratio than water stress.

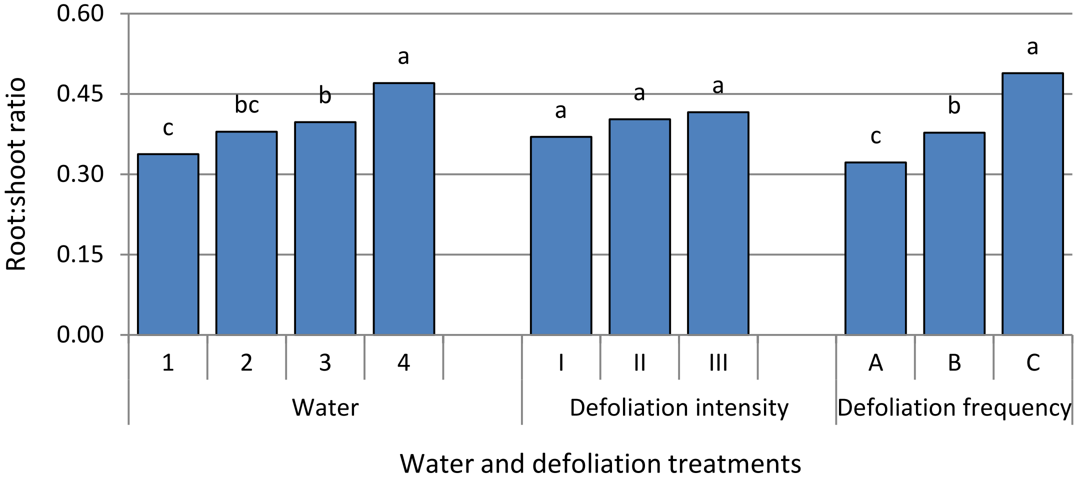

Figure 6.19: Root:shoot ratio of *Pentzia incana* at different water and defoliation treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequencies are: A = three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences (P < 0.001), between treatment levels within each treatment.

6.2.7 Root length

Although it might be a more useful measurement, root length is not as frequently used to describe root growth as root mass (Volesky 2011). Measuring root length is more difficult than determining root mass, this possibly explains the lack of such information for fodder plants (Oosthuizen and Snyman 2009). Unfortunately the variation in root length data was slightly higher than expected, but never the less yielded data that is still acceptable enough to be used. Roots were separated into three fraction classes based on root diameter, where: R1 = roots with diameter ≥ 3 mm, R2 = roots with diameter 1 to 3 mm and R3 = roots with diameter < 1 mm.

6.2.7.1 Root length over all treatments

Although the influence of defoliation intensity on total root length was significant (P < 0.001), it was very low. Water, on the contrary, accounted for most of the variation in total root length data (30%), with defoliation frequency at 13% (Table 6.17). The water x frequency interaction accounted for more variation in total root length data than any of the other interactions.

Table 6.17: ANOVA summary for the root length of *Pentzia incana* for all frequencies compared at the end of the trial.

Root length		W		I		F		W x I		W x F		I x F		W x I x F	
Variable	Treatment	SS% and significance													
R1 (≥ 3 mm)	A, B, C compared	**17**	***	1	NS	3	*	2	NS	2	NS	2	NS	4	NS
R2 (1 - 3 mm)	A, B, C compared	**33**	***	1	NS	**11**	***	3	NS	3	*	3	*	9	***
R3 (< 1 mm)	A, B, C compared	**21**	***	5	***	**10**	***	2	NS	**8**	***	2	NS	3	NS
Total length	A, B, C compared	**30**	***	4	***	**13**	***	1	NS	**7**	***	2	*	4	NS

W = water, I = defoliation intensity, F = defoliation frequency, A = Frequency A, B = Frequency B, C = Frequency C, NS = non-significant, SS% = sum of squares percentage, *** = P < 0.001, ** = P < 0.01, * = P < 0.05, R1 = coarse roots (≥ 3 mm), R2 = medium roots (1 – 3 mm), R3 = fine roots (< 1 mm).

Increased defoliation intensity caused a decrease in total root length, with the total root length of intensity I being significantly ($P < 0.001$) lower than that of intensity II and III which did not differ much ($P > 0.05$) (Figure 6.20). Root length decreased significantly ($P < 0.001$) with increased defoliation frequency. Water deficit caused the greatest decrease in root length, with W1 being 123% higher than W4. Treatments W1 and W2, (least water stressed treatments) gave the highest total root length of around 375 m plant^{-1}, while water treatment W4, (the most stressed water treatment) was lower ($P < 0.001$) at 175 m plant^{-1}.

Root length of the different fractions proportionally followed the same pattern as total root length, while R 3 (< 1 mm) made up around 68% of total root length for all treatments. Root fraction R2 (1 – 3 mm) was second highest (24%) with R1 (≥ 3mm) the lowest (8%). Fraction lengths of R1, as well as R3 did not differ ($P > 0.05$) within fraction type between the three defoliation intensities.

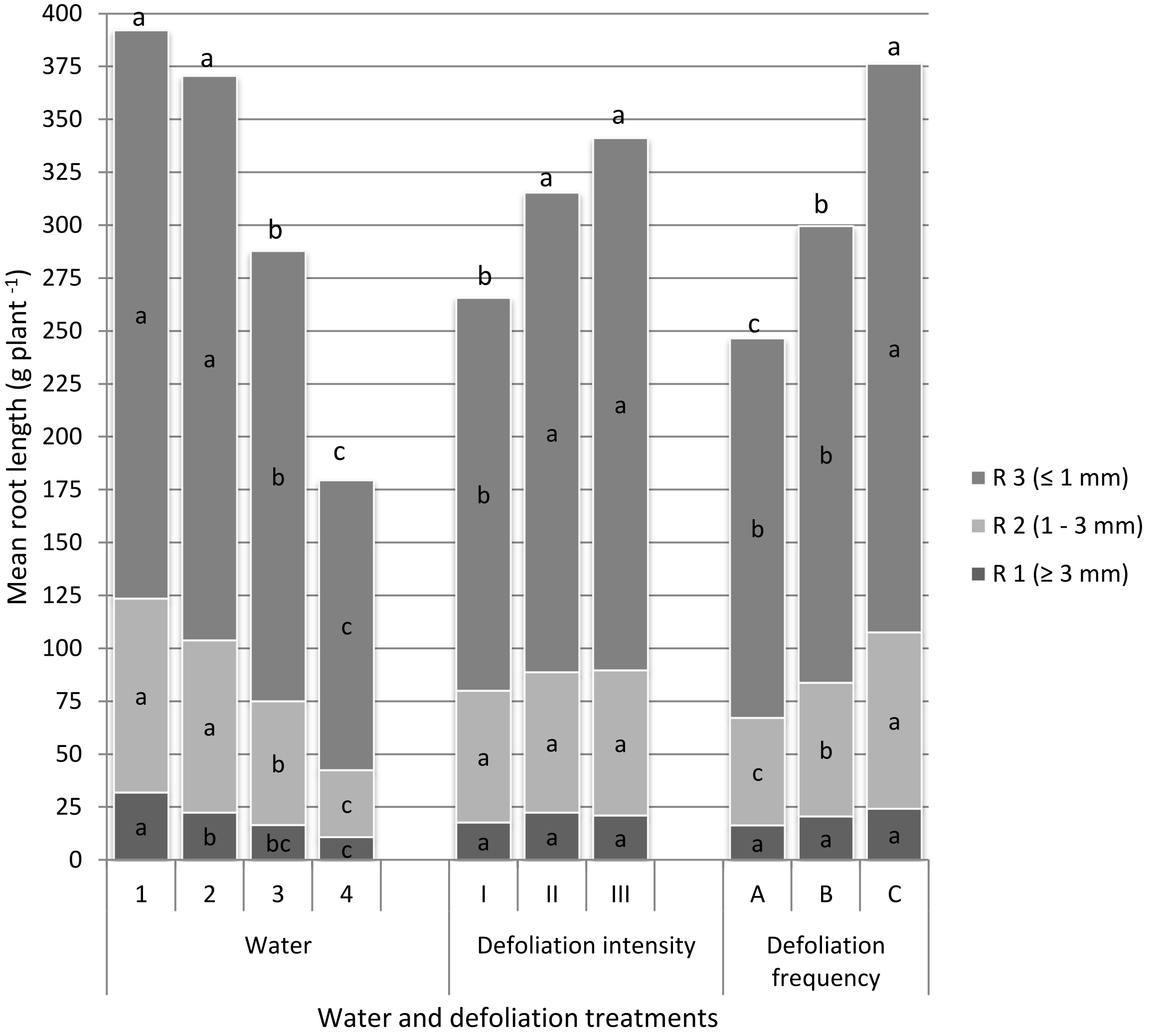

Figure 6.20: Root length of different root fraction and total root length (per plant) for *Pentzia incana* at different water and defoliation treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequencies are: A = three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences (P < 0.001), within treatments at each fraction and total root length.

6.2.7.2 Root length (defoliation frequencies separately illustrated)

Due to the significant (P < 0.001) influence of defoliation frequency on root length, it was decided to illustrate and discuss root length for each of the three defoliation frequencies. For frequency A, water accounted for most the variation in root length data (Table 6.18), with defoliation intensity also contributing significantly (P < 0.001). For frequency B water accounted for most variation in root length data, while intensity also contributed significantly (P < 0.05) but not as much as water (Table 6.18). Water again (P < 0.001) accounted for most variation in root length at frequency C.

Table 6.18: ANOVA summary for the root length of *Pentzia incana* for each frequency.

Root length		W		I		W x I	
Variable	**Frequency**	SS% and significance					
R1 (≥ 3 mm)	**A**	18	**	7	NS	9	NS
R2 (1 - 3 mm)	**A**	34	***	11	***	21	***
R3 (< 1 mm)	**A**	27	***	20	***	10	NS
Total length	**A**	**35**	***	**21**	***	10	**
R1 (≥ 3 mm)	**B**	23	**	1	NS	1	NS
R2 (1 - 3 mm)	**B**	39	***	2	NS	7	NS
R3 (< 1 mm)	**B**	24	***	13	**	6	NS
Total length	**B**	**37**	***	**10**	*	5	NS
R1 (≥ 3 mm)	**C**	19	**	3	NS	7	NS
R2 (1 - 3 mm)	**C**	45	***	1	NS	12	*
R3 (< 1 mm)	**C**	39	***	1	NS	4	NS
Total length	**C**	**48**	***	1	NS	4	NS

W = water, I = defoliation intensity, F = defoliation frequency, A = Frequency A, B = Frequency B, C = Frequency C, NS = non-significant, SS% = sum of squares percentage, *** = P < 0.001, ** = P < 0.01, * = P < 0.05, R1 = coarse roots (≥ 3 mm), R2 = medium roots (1 – 3 mm), R3 = fine roots (< 1 mm).

Most variation between treatments was recorded at frequency A (Figure 6.21). Root length decreased (P < 0.001) with water deficit, while W2 and W3 did not differ (P > 0.05). Increased intensity also resulted in decreased (P < 0.001) root length. At frequency B, the most stressed water treatment had a lower (P < 0.001) root length than the other three water treatments, while increased defoliation intensity gave a slight decrease in root length. Defoliation intensity had no influence ($P > 0.05$) on root length for frequency C, while root length at W1 and W2 was significantly ($P < 0.001$) higher than that of W3 and W4.

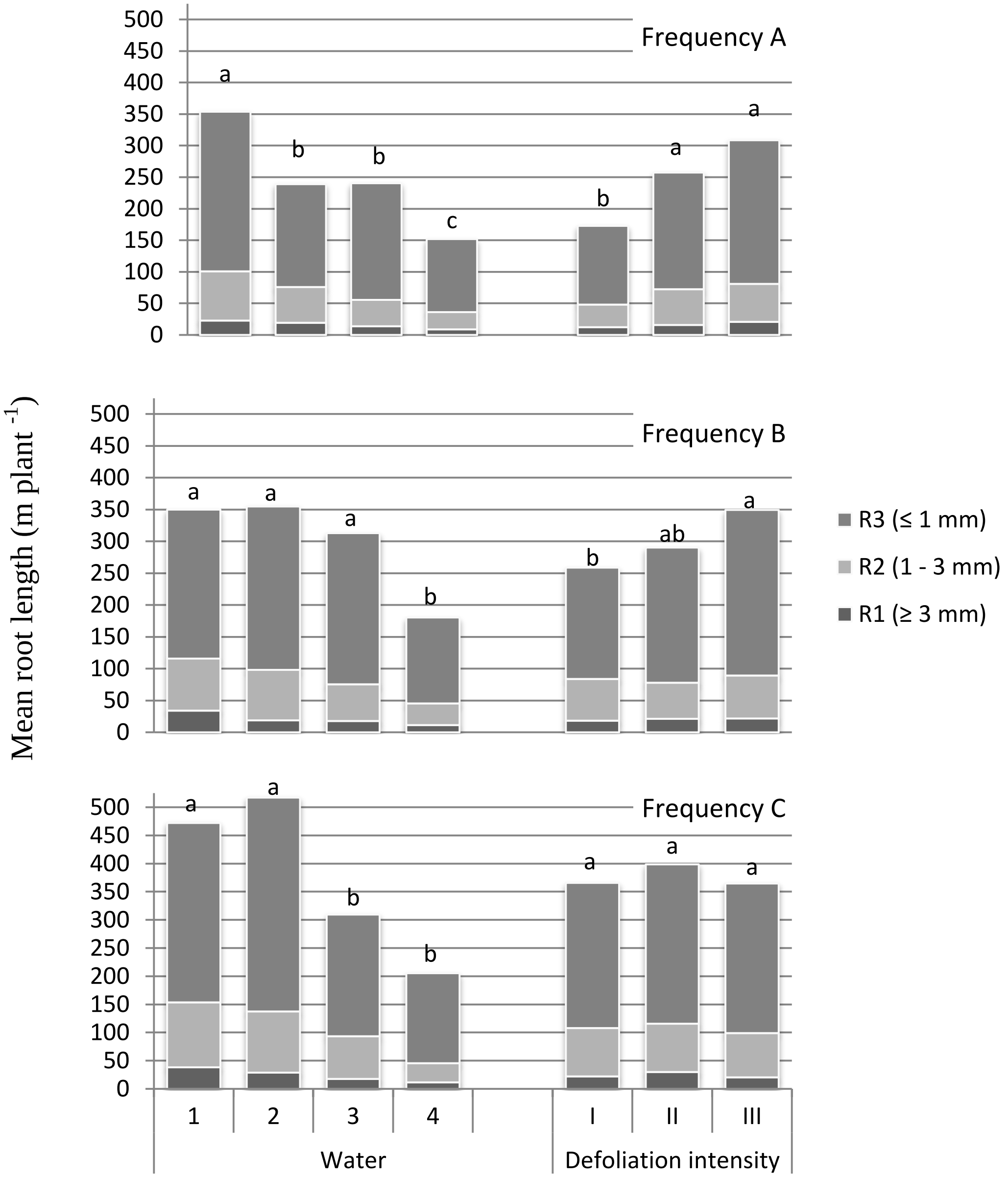

Figure 6.21: Root length of different root fractions and total root length for *Pentzia incana* at different water and defoliation intensities in interaction with three different frequencies. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequencies are: A = three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences ($P < 0.001$), within treatments at each frequency for total root length.

6.2.8 Shoot length

As was the case for *Nenax microphylla*, shoot length was only measured and presented for defoliation frequencies A and B. With the shoots increasingly forming side branches as they grow longer and older over time, this measurement became more complicated and could be inaccurate. If this was true, the same inaccuracy would be repeated over all measured treatments, validating the usefulness of this data. Shoot length for frequency C was also measured, but after seven to eight months no increase in shoot length occurred as many side branches were formed. Shoot mass might have been a better option for measurement of shoot growth. For defoliation frequencies A and B, after each defoliation treatment, new shoots were marked of which the length was measured monthly.

6.2.8.1 Shoot length of defoliation frequency A

For the May and November defoliations, water treatments and defoliation intensity accounted for equal amounts of variation in the shoot length data, while water accounted for most variation in August and defoliation intensity at the February defoliation (Table 6.19). The low sum of square percentages (SS% values) is an indication of high residual values due to a lot of unaccounted for variation in data.

Table 6.19: REML variance components analysis for shoot length of *Pentzia incana* at defoliation frequency A.

Shoot length		W		I		W x I	
Date	Frequency	F statistic and significance					
May (A1)	A	4	*	4	*	1	NS
August (A2)	A	**11**	***	4	*	2	NS
November (A3)	A	**14**	***	**14**	***	1	NS
February (A4)	A	11	***	**<u>41</u>**	***	0	NS

REML = Linear mixed model repeated measurement analysis, W = water, I = defoliation intensity, NS = non-significant, SS% = sum of squares percentage, EP = edible phytomass, *** = P < 0.001, ** = P < 0.01, * = P < 0.05

For all four defoliation dates, shoot length between water treatment W1 and W2 (for May, August and February – W3 also included) did not differ much ($P > 0.05$) (Figure 6.22). For all four defoliation dates the most stressed water treatment (W4) had significantly ($P < 0.001$) lower shoot lengths than the less stressed and unstressed water treatments. Although not always over all water treatments, in general water stress caused a significant ($P < 0.001$) decline in shoot length.

At the August defoliation (after the winter growth period), an increased defoliation intensity had no effect ($P > 0.05$) on shoot length. At the May, November and February defoliations, the most severe defoliation intensity (intensity I) gave significantly ($P < 0.001$) higher shoot lengths than the most lenient defoliation intensity (intensity III). This might be a way of the plants to compensate for the severe defoliation treatment, by showing rapid regrowth (compensatory growth).

In general, shoot lengths were highest at the May defoliation (autumn growth period) for all water and intensity treatments, with November (spring growth period) second highest. The biggest differences between extremes of water treatments were equal for November and February and for defoliation intensity treatments highest at the February defoliation. This might be an indication that the plant grows more rapidly during spring and autumn, but during the warmer summer months, while growth is a bit slower, water stress and defoliation severity have a bigger impact on shoot growth than during the cooler spring and autumn growth periods. This relates well to the general tendency of shrubs form the Nama-karoo to grow more actively during the cooler (and wetter) spring and autumn months (Esler et al. 2006).

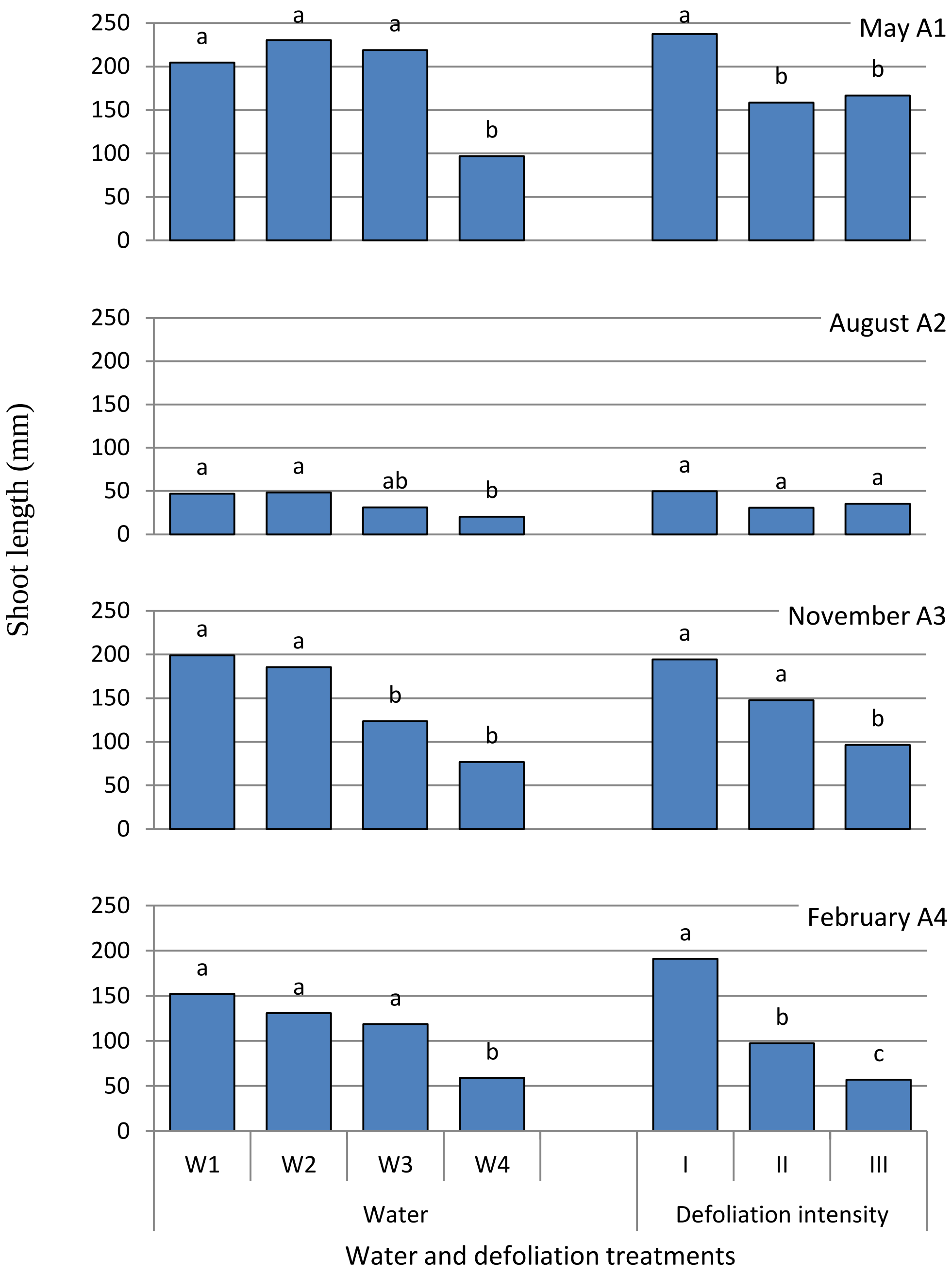

Figure 6.22: Mean (n = 6) shoot length (mm) for *Pentzia incana* at each of the four cutting times for frequency A. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences (P < 0.001), within treatments at each defoliation date.

6.2.8.2 Shoot length of defoliation frequency B

Both water and defoliation intensity had significant (P < 0.001) influences on shoot length. Defoliation intensity accounted for most of the variation on shoot length data for B1 and B2, with water accounting for the second most variation in data (Table 6.20).

Table 6.20: REML variance components analysis for shoot length of *Pentzia incana* at defoliation frequency B.

Shoot length		W		I		W x I	
Date	Frequency	F statistic and significance					
August (B1)	B	**10**	***	**16**	***	1	NS
February (B2)	B	**14**	***	**22**	***	1	NS

REML = Linear mixed model repeated measurement analysis, W = water, I = defoliation intensity, NS = non-significant, SS% = sum of squares percentage, EP = edible phytomass, B1 = first six months, B2 = second six months, *** = P < 0.001, ** = P < 0.01, * = P < 0.05

The most severe water stress treatment (W4) had lower (P < 0.001) shoot lengths than the other water treatments for both B1 and B2 (Figure 6.23). The shoot length of the most severe defoliated treatment (intensity I), was around 80% higher (P < 0.001) than that of the less severe intensities that had almost equal (P > 0.05) shoot lengths.

The longer (P < 0.001) shoot lengths of defoliation intensity I, compared to the less severe defoliation treatments, is similar to the results from frequency A, indicating signs of compensatory growth as a means to compensate for loss on vegetative material due to severe defoliation.

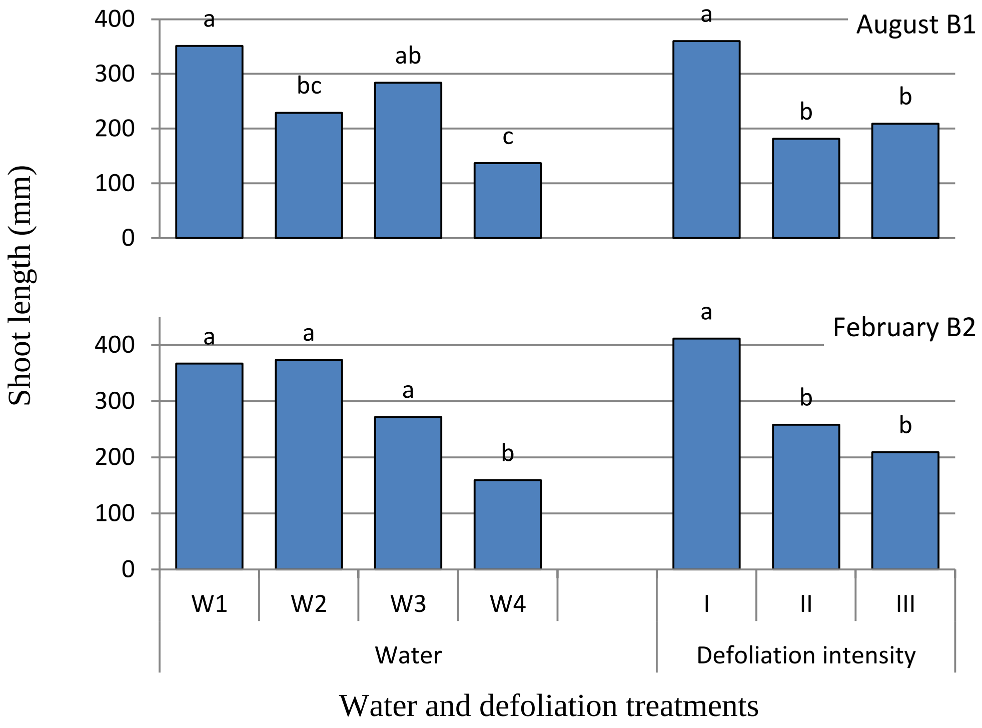

Figure 6.23: Mean (n = 6) shoot length (mm) for *Pentzia incana* at each of the two cutting times for frequency B. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences ($P < 0.001$), within treatments at each defoliation date.

6.2.9 Leaf dimensions (length, width and surface)

Leaf dimensions (length and width) were measured to quantify the possible effect the different treatments might have on the growth dynamics of the shrub. Leaf surface was measured by simply multiplying leaf length with leaf width.

Frequency accounted for most of the variation ($P < 0.001$) in leaf length, -width and –surface (Table 6.21). Defoliation intensity also tested significantly ($P < 0.001$) for leaf length and leaf surface, but accounted for less variation in data than frequency. Interestingly, water had no significant ($P > 0.05$) influences on any of the measured leaf dimensions.

Table 6.21: ANOVA summary for the different leaf measurements of *Pentzia incana*.

Leaf measurements	W		I		F		W x I		W x F		I x F		W x I x F	
Variable	SS% and significance													
Leaf length	1	NS	**18**	***	**36**	***	1	NS	1	NS	6	***	2	NS
Leaf width	1	NS	2	*	**7**	***	4	NS	2	NS	1	NS	8	NS
Leaf surface (length x width)	1	NS	**12**	***	**23**	***	3	NS	1	NS	4	*	6	NS

W = water, I = defoliation intensity, F = defoliation frequency, NS = non-significant, SS% = sum of squares percentage, *** = P < 0.001, ** = P < 0.01, * = P < 0.05

None of the measured leaf dimensions were affected ($P > 0.05$) by water deficit (Figure 6.24). With the exception of leaf width, the other leaf dimensions showed a significant ($P < 0.001$) decrease with decreased defoliation intensity treatments, with defoliation intensity III having smaller ($P < 0.001$) leaves (Figure 6.24) than the other two intensities. Defoliation intensity I, the most severe defoliation intensity treatment, had a 77% larger ($P < 0.001$) leaf surface, than that of intensity III. This might be ascribed to compensatory growth in the sense that the severely defoliated plant increases its leaf surface to instigate rapid growth, since the source of growth reserves (majority of the stems) was removed during the defoliation process.

Defoliation frequency B had bigger ($P < 0.001$) leaf dimensions than frequency A and C for all leaf dimensions. Defoliation frequency C resulted in smaller leaf dimensions than frequency A for leaf length and leaf surface (Figure 6.24). The same can be argued as for intensity, where frequency C (control / defoliated every 12 months), had the smallest leaves, while frequencies A and B had bigger leaves as a compensatory mechanism to increase photosynthesis. It might be that defoliation frequency A, the three-monthly intervals, had a more detrimental effect than the six-monthly defoliation interval, resulting in smaller leaves than the latter.

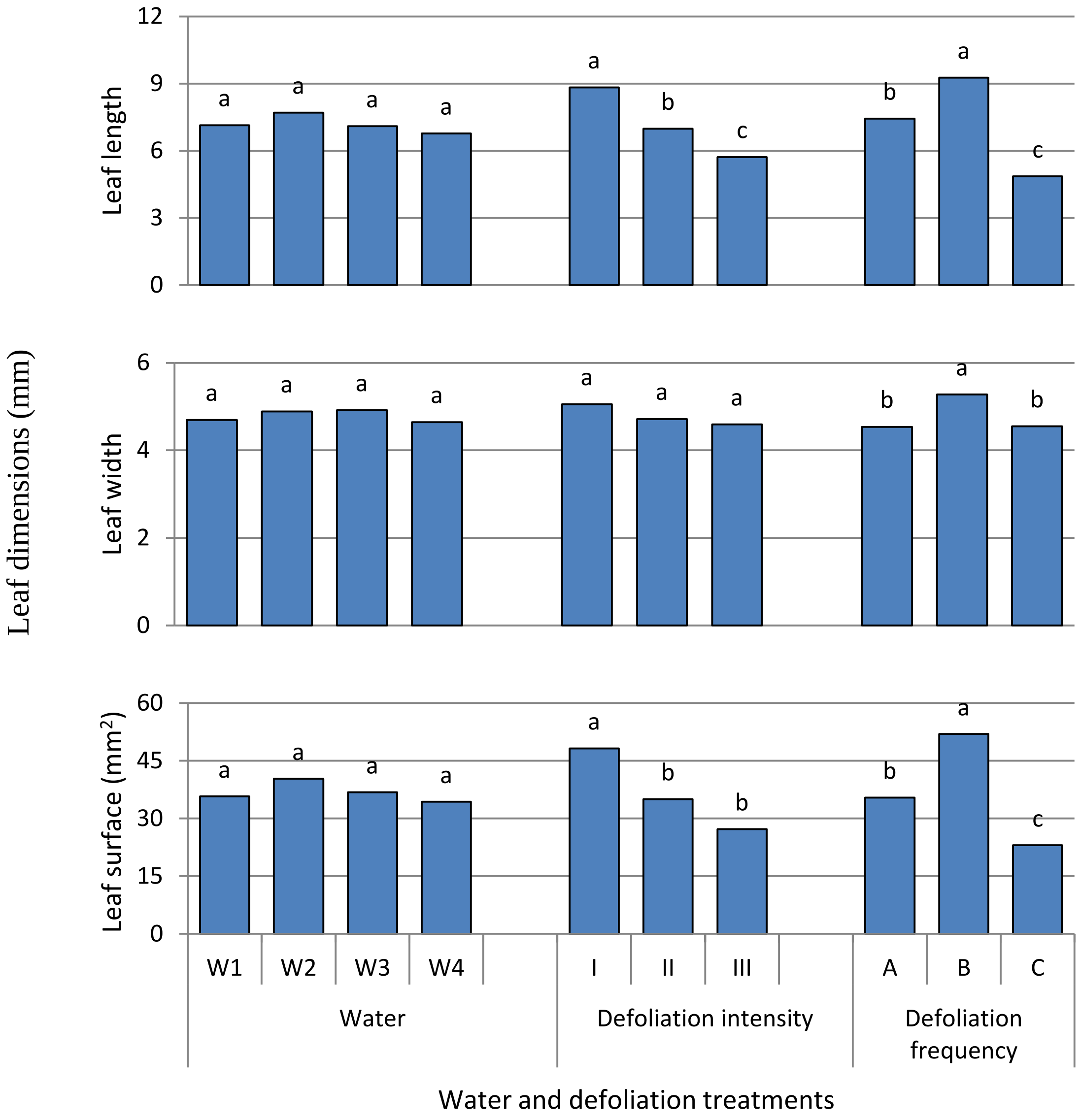

Figure 6.24: Mean (n = 6) leaf measurement (mm) for *Pentzia incana* at all the different treatments. W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequencies are: A = three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences (P < 0.001), within treatments at each leaf measurement.

6.2.10 References

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CHAPTER 7

GENERAL DISCUSSION AND COMPARISON: PHYTOMASS PRODUCTION RESULTS OF *N. microphylla* AND *P. incana*

7.1 Introduction

The general discussion of all presented phytomass data in Chapters 5 and 6 will be done separately in this chapter for more clarity. The discussion will start off with a short comparison between the two species, after which a general detailed discussion and concluding remarks regarding phytomass production will follow.

7.2 Comparison of main treatments between the two species

As the two plants are unrelated and belong to two different plant families, *P. incana* to Asteraceae and *N. microphylla* to Rubiaceae, different reactions to the applied water and defoliation treatments were inevitable. Surprisingly, these two Karoo shrubs reacted mostly similarly to the main treatments with a few minor differences which will be highlighted in this section.

Firstly, some differences in the edible phytomass (g plant^{-1}) is evident, where *P. incana* had slightly lower production than *N. microphylla* at higher water availability, while the opposite was true for the water treatments causing more stress (Figure 7.1). This is a subtle indication that *N. microphylla* can exploit higher soil-water condition better, while *P. incana* has a greater ability to produce phytomass when soil-water gets limited.

Both species showed a similar increase in edible phytomass with increased defoliation intensity (Figure 7.1). Edible phytomass production of *Pentzia incana* was the highest at the six-monthly defoliation frequency, while that of *N. microphylla* showed a steady increase from the three-monthly to the twelve-monthly defoliation frequency. It can therefore be argued that *P. incana* needs to be defoliated at least every six months for optimum edible phytomass production, while *N. microphylla* will thrive at a 12 monthly defoliation frequency.

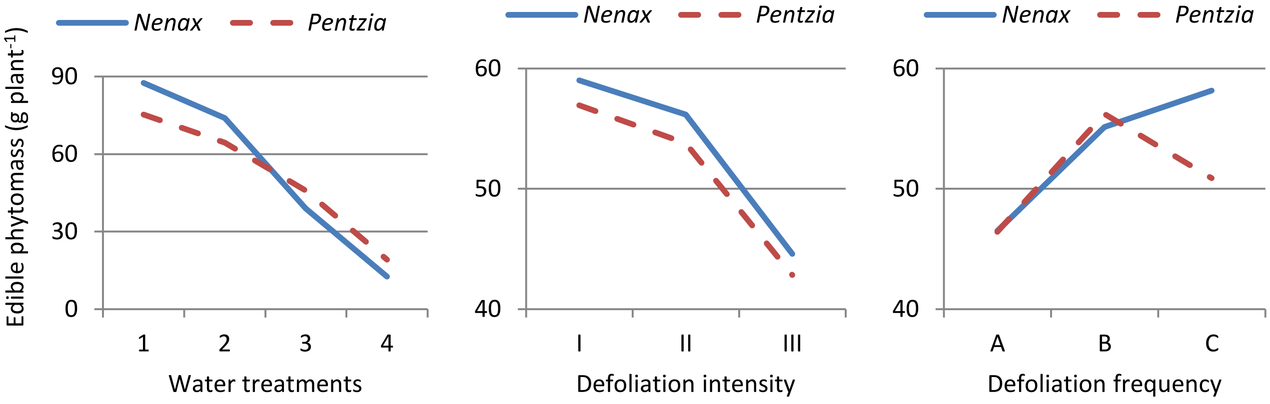

Figure 7.1: Comparison between the edible phytomass (g plant^{-1}) production of *Nenax microphylla* and *Pentzia incana* at the different water and defoliation treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve-monthly or control.

The edible phytomass production of the three-monthly and six-monthly defoliation treatments revealed some interesting differences between the two species (Figure 7.2). It seems that *N. microphylla* tends to produce slightly more edible phytomass during spring (November) and summer (February) than *P. incana*, while the latter produced more edible phytomass during autumn (May) and winter (August) than *N. microphylla*. This variation in seasonal phytomass production between Karoo shrub species is a common phenomenon in the Nama-karoo Biome (Roux 1966, Vorster et al. 1983).

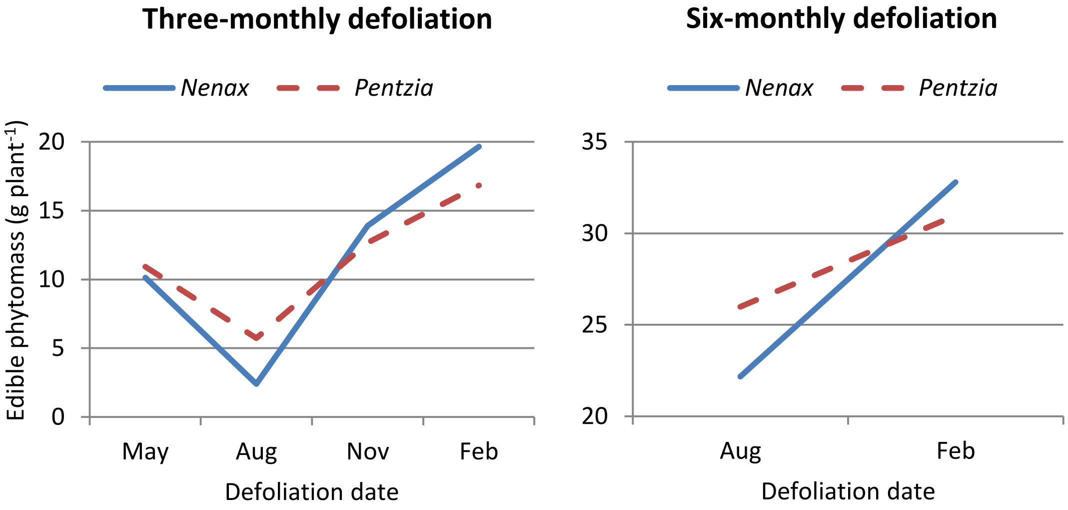

Figure 7.2: Comparison between the edible phytomass (g plant^{-1}) production of *Nenax microphylla* and *Pentzia incana* at the three-monthly and six-monthly defoliation frequencies.

The effect of the different water and defoliation treatments on total above- and belowground phytomass productions did not differ significantly between the two species (Figure 7.3). The only notable difference is that *N. microphylla* in general has a higher aboveground phytomass production than *P. incana*, while *P. incana* had a slightly higher belowground phytomass production than that of *N. microphylla*.

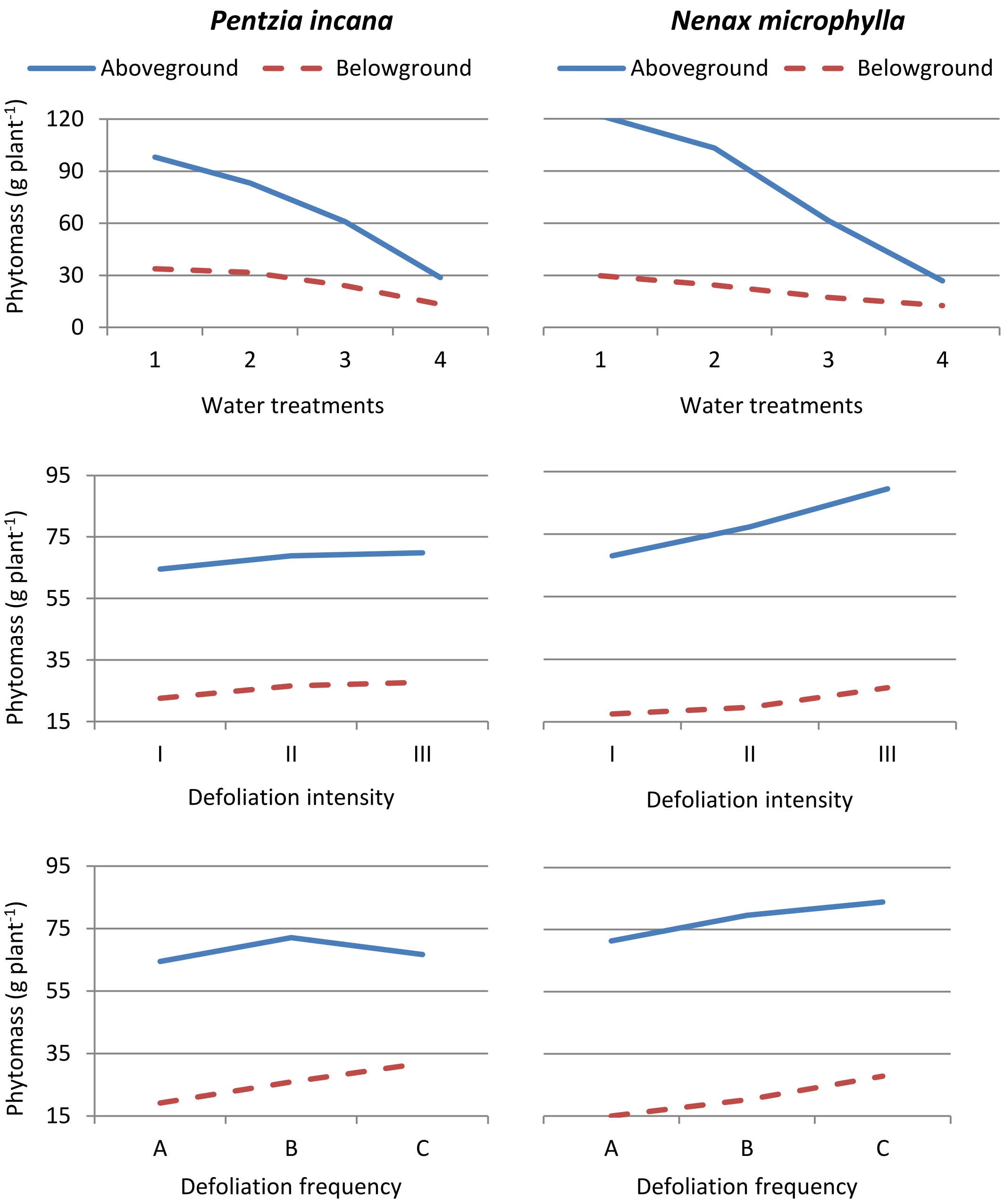

Figure 7.3: Comparison of the total above- and belowground phytomass (g plant^{-1}) production between *Nenax microphylla* and *Pentzia incana* at the different water and defoliation treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve-monthly or control.

Total root length of both species followed the same pattern along the different water and defoliation treatments (Figure 7.4). Total root length of *P. incana* was markedly higher than that of *N. microphylla*.

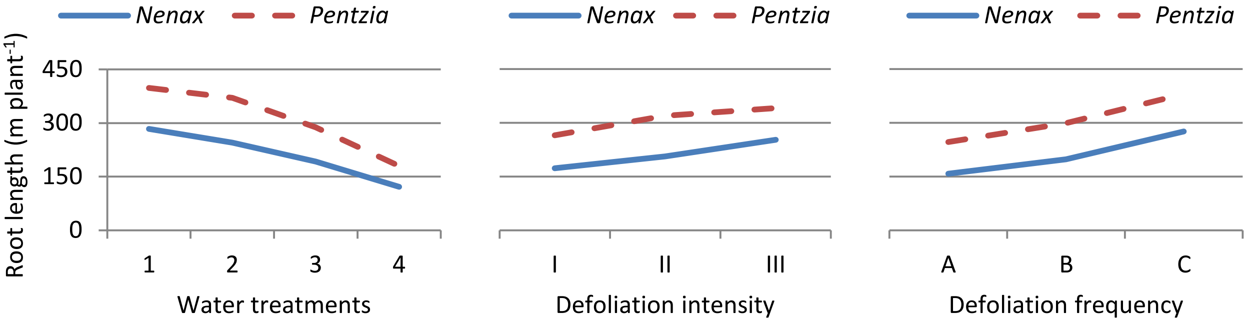

Figure 7.4: Comparison between the total root lengths (m plant^{-1}) of *Nenax microphylla* and *Pentzia incana* at the different water and defoliation treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve-monthly or control.

7.3 General discussion on phytomass results

7.3.1 Main effect: water

In arid and semi-arid climatical conditions water, temperature and nutrient stress may act in concert to constrain growth by inhibition of physiological and biochemical process. Of these, water availability exerts the greatest control over plant physiological processes in all arid and semi-arid environments (Ingram 2002). For sound rangeland management it is essential to develop a better understanding of patterns of plant growth, production and how they relate to the driving influences of water (Snyman 2009a). Therefore, one of the most important principles in sustainable utilization of the drier rangeland areas is effective soil-water management (Snyman 1998). These abovementioned statements are fully supported by findings of this study. Although all three main effects tested to have significant influences on phytomass production for both *N. microphylla* and *P. incana*, water by far accounted for most of the variation in data, by amongst others causing the greatest differences between treatment extremes. Both Cambell et al. (1997) and Snyman (1999) emphasized that water (annual precipitation) accounts for up to 90% variance in phytomass production of rangeland ecosystems (globally). Other researchers also speculated around this statement that variation in annual rainfall might have a greater effect on especially growth form composition, than grazing (defoliation) (Snyman et al. 1987, Snyman 1993, Oosthuizen and Snyman 2009, Rutherford et al. 2012). This is very much in line with the findings of this study, that the impact of water (rainfall) is greater than that of defoliation (grazing). It is also supported by studies done on *Lezpedeza davurica* (subshrub) and *Borthriochloa ischaemum* (grass), which also showed a decreased phytomass production over a soil water deficit (Xu et al. 2011). Although *P. incana* grew better during winter than *N. microphylla*, it was the only season during which water did not significantly influence phytomass production, which is in line with findings of Snyman (2009a) and Evans et al. (2013) stating that winter water availability is of lesser significance since growth is not really taking place.

For both *N. microphylla* and *P. incana*, phytomass production still followed the water deficit range, with the highest production at the least water stressed treatment, with field capacity of 80% and higher, which corresponds to results for *P. incana* by Gerber (1993). The initial theory was, however, that water treatment one (> 80% of field capacity), might give lower phytomass, as too wet soil conditions might be detrimental to phytomass production. By contrast, *Calotropis procera*, known as a drought resistant desert shrub, grown in a pot experiments under 30%, 50% and 80% field capacity, gave the highest biomass production at 50% of field water capacity (Boutraa 2010). It is an indication that individual plant species from arid regions might react differently to wetter soil-water conditions and therefore each species should be studied separately in future.

A more comprehensive discussion on root growth will follow later in this chapter. It can however be mentioned that water deficit caused a decrease in root production (mass) for both species. Similar findings were reported by Busso and Bolletta (2007) and Snyman (2009a), who also found increased root growth with increasing soil-water content. Although the root production was lower at lower water levels for *N. microphylla* and *P. incana*, it was higher in proportion to aboveground phytomass production than at the less water stressed treatments, which correlates with general explanations by Chaves et al. (2002) on how plants cope with water stress.

7.3.2 Main effect: defoliation frequency and intensity

Ecologically sensitive arid and semi-arid rangelands are increasingly subjected to severe grazing pressure, which can cause their rapid degradation (Snyman 2009a). Defoliation intensity, however, had the lowest impact on phytomass production on both species in this study, which was similar to findings of Volesky et al. (2011) done on *Elymus trachycalus* (grass) and *Lotus corniculatus* (shrub), where the impact of defoliation frequency was also higher. Although defoliation intensity accounted for the least variation in data, compared to frequency and water, it might however still impact on the physiological functioning of the plant. In general, Karoo shrubs use accumulated non-structural carbohydrates essentially for initiating regrowth after dormant periods, for example drought (Noy-Meir 1993, Stock et al. 1993). Regrowth after defoliation will therefore be fuelled from photosynthate of the residual leaves rather than from accumulated non-structural carbohydrates (Hobson and Sykes 1980). Harsher defoliation actions, produce plants with smaller canopies and therefore smaller photosynthetic factories. This stresses the value of a more lenient defoliation, as seen from this study that defoliation intensity III (the most lenient defoliation) showed the highest productivity.

Similar results to this study were found by Hobson and Sykes (1980) for three Karoo shrubs (*Eriocephalus ericoides*, *Felicia muricata* and *Pentzia incana*), which also showed increased production with decreased defoliation frequency. Van Heerden et al. (1996) also reported a declining yield with increased cutting frequency for the Karoo shrub *Osteospermum sinuatum*.

Many researchers suggested that any defoliation frequency more than twice per year is highly detrimental to phytomass production (Matches 1966, Opperman et al. 1970, Fulkerson et al. 1993, Snyman 1999, Gittins et al. 2010, Volesky et al. 2011, Snyman and Malan 2015), which correlates with the findings from this study, where a three-monthly (four times per year) defoliation frequency, were the most detrimental to phytomass production.

Corresponding results to this study were reported by van Heerden et al. (1996) on the shrub *Osteospermum sinuatum*, where a decreased defoliation frequency (six-monthly and annually) showed no differences in phytomass production between defoliation intensity treatments. Therefore, infrequent defoliation may result in shrubs being less sensitive to defoliation intensity, while a light defoliation may result in a lower sensitivity to frequent defoliation. The results from this study emphasised the extremely negative impact, in terms of phytomass production of Karoo shrubs, of a high frequency of defoliation in combination with high intensity of defoliation, which is in line with work done by Vorster et al. (1983).

7.3.3 Compensatory growth

To maximize growth in drier ecosystems, like the Nama-karoo Biome, where rainfall is often low and infrequent, shrubs have adapted to these conditions through the rapid initiation of photosynthesis and transpiration with the onset of rain (Henrici 1937 and 1951). The ability of rapid regrowth as described by Henrici (1951) for *P. incana* after grazing was at that stage not recognized as a compensatory ability, while the results of this trial confirms that it could easily have been attributed to the compensatory growth ability of *P. incana*. As well as being capable of rapid rates of photosynthesis and transpiration, arid and semi-arid shrubs can maximize growth periods by maintaining photosynthesis and transpiration as soil-water content declines (Ingram 2002). Transpiration in these species remains relatively unaffected by declining soil-water content until leaf water potential drops below a certain critical point (Ingram 2002).

Rapid recovery (regrowth) after defoliation was ascribed to compensatory growth, especially for the unstressed plants (W1 and W2) at the three-monthly defoliation frequency. It could therefore be regarded as a reaction to the defoliation treatment, rather than a reaction to water stress. Stock et al. (1993) stresses that compensatory growth, as enhanced growth in response to defoliation, is only possible where amongst others, water is abundantly available. Plants cannot show compensatory growth when elements, like water, which is necessary for replacement growth, are not available. This might be the reason for the unstressed plants showing this ability, and the water stressed plants not. This rapid regrowth can also be regarded as a defence mechanism of more palatable plants against grazing, while other less palatable plants might increase some chemical substances, like tannins, as a defence mechanism (Stock et al. 1993). The presence of tannins, phenolics (Dini and Owen-Smith 1995) or silica (McNaughton et al. 1985) strongly influences the extent to which plants are grazed.

Oba et al. (2006) highlighted the important role of remaining biomass after defoliation on the compensatory growth ability of plants. However, heavy grazing and the resultant large loss of leaf area can result in an overall decline in photosynthesis (Parsons et al. 1983). Leaf loss due to grazing may lead to a decline in whole plant transpiration, even if rates of leaf transpiration increase (Detling and Painter 1983, Wallace et al. 1985, Gold and Caldwell 1989). This can explain why the plants from the most intense defoliation (50 mm height) did not show compensatory growth in this study, while intensity heights of 125 and 200 mm did. The proportion of the biomass that remains after defoliation will not only influence the compensatory ability of shrubs, but also the compensatory rate (Richards 1993). The compensatory process contributes to a more rapid re-establishment of the photosynthetic canopy after defoliation (Noy-Meir 1993). The actively growing meristematic tissues, like shoot growth points, are stronger sink regions than for example roots, for compensatory growth (White 1973). Van der Heyden and Stock (1996) explained that *Osteospermum sinuatum* initially depends on carbon reserves for regrowth after which photsynthates from new and remaining leaves take over that function. They further argues that Karoo-shrubs have a more effective dependence on carbon reserves after defoliation than grasses, which could explain the persistence of shrubs in graminoid-shurb communities subjected to severe grazing pressures in the Nama-karoo.

7.3.4 General remarks

Grazing is a common feature of many arid and semi-arid ecosystems. However, the detrimental impacts associated with grazing may make it unsustainable both agronomically and ecologically. The detrimental impacts of grazing include soil compaction, plant trampling, or severe and/or frequent defoliation that reduces a plants ability to cope with other environmental stresses (Holechek et al. 1995).

It is clear that water has the greatest impact on phytomass production of both *N. microphylla* and *P. incana*. Unfortunately land users do not have control over the rainfall, and therefore the water available to Karoo shrubs, like *N. microphylla* and *P. incana*. On the contrary, two of the most manageable variables that influence plant response to grazing are frequency and intensity of grazing (Trlica and Rittenhouse 1993, Snyman and Malan 2015), which the land manager has control over. More emphasis should therefore be given to factors that one can control, such as defoliation, taking into account the fact that semi-arid to arid regions are prone to frequent dry spells, and should be manage therefore as if dry spells are always present (Esler et al. 2006, Kraaij and Milton 2006, Masubelel et al. 2014). Rainfall exacerbates the impact of defoliation frequency and intensity (van Heerden et al. 1996), and should therefore not be neglected. It is more detrimental to graze Karoo shrubs under unfavourable soil-water conditions (Vorster et al. 1983). This was also highlighted in this study where severely water stressed plants that were defoliated every third month and at a severe intensity (50 mm height) gave the lowest phytomass production, supporting results of Gerber (1993) for *P. incana*.

Unfortunately this study being a glasshouse trial which was terminated after one season might differ from results obtained in rangelands. Van Heerden and Stock (1996) suggests that the negative influence of a combination of both high frequency and intensity of defoliation seems to be cumulative over seasons, becoming more severe when repeated over a number of seasons. Vorster et al. (1983) states that over the long-term, it is more detrimental to graze plants during the season in which they are actively growing.

The possible compensatory ability could be exploited with strategic resting periods of certain areas directly after good rains, which is mostly during spring and autumn in the Nama-karoo Biome. Knowing that *N. microphylla* and *P. incana* do show compensatory growth after defoliation under favourable soil-water conditions can be used to increase recovery by strategically withdrawing camps from grazing directly after good rains during especially spring, summer and autumn months. Spring and autumn has proved to be the two seasons during which Karoo shrubs grow most actively (Vorster et al. 1983, Dean and Milton 1999). It is also important to place grazing effects within the context of the physiological status during the period when they are most likely to be grazed (Ingram 2002). Grazing of Karoo shrubs is generally most often in spring and autumn when they are actively growing due to the better rainfall events. Severe defoliation of Karoo shrubs during these two seasons can significantly reduce total aboveground phytomass production of the shrub component and over the long-term can change the botanical composition to more dominant grassy sward.

In a study of desert shrubs under variable rainfall it was found that in some instances, during water deficit, increased photosynthesis occurred without increased phytomass production, resulting in photosynthate assimilation still taking place (Houseman et al. 2006). Much more work can be done in future to determine whether the same is true for Karoo shrubs, and if so, where the assimilates are translocated to, or stored. Oba et al. (2006), Hassen et al. (2007) and Boutraa (2010) found that different species of the same genus of the shrubs from their studies, reacted differently to defoliation and water treatments. It might thus be dangerous to generalize by arguing that all Karoo shrubs will react similar to *N. microphylla* and *P. incana* to water deficit stress and defoliation treatments. A wider variety of Karoo shrubs should therefore be investigated in future similar studies.

Over the lifespan of an individual plant, grazing can be seen as a long-term disturbance, while drought events have a relatively shorter impact on the plant (DeMalach 2014). Separation of defoliation impact and water deficit trends might therefore give different results and should be investigated in separate studies in future. Normally, after droughts a great number of annuals (ephemerals) might outcompete some perennials, which might have a negative impact on the recovery potential of specific, many times overgrazed, Karoo shrubs (Todd 2006, Anderson and Hoffman 2007). Another interesting inclusion for future studies would be quantifying seasonal root growth, for example autumn versus spring growth (Palacio and Monserrat-Marti 2007), which are the normal rainy periods for the Nama-karoo Biome (Esler et al. 2006).

The Nama-karoo Biome remains a very complex ecosystem with a variety of plant species competing to co-exist under variable water availability and grazing impacts (Kraaij and Milton 2006). This indicates a much more complex environment than in the case of a pot experiment in a glasshouse. Other factors, like plant competition (Carrick 2003, May et al. 2009, Everson et al. 2009, Shiponeni et al. 2011, DeMalach et al. 2014) might therefore result in different findings, although the findings of this study related well to similar defoliation trials on Karoo shrubs.

7.4 Root:shoot ratio

In the past, plant ecological studies have largely concentrated on aboveground parts of the rangeland ecosystem (Snyman 1998), with less emphasis on root growth and development. However, belowground information is essential for predicting rangeland responses to seasonal rainfall patterns (Snyman 2009b).

The tendency of more phytomass allocation to belowground than aboveground plant parts, as experienced in this study under water deficit conditions, were found in several other studies (Oba et al. 2006, Morat 2008, Xu et al. 2011, Evans et al. 2013). By contrast, the opposite was reported by Snyman (2009b) in a semi-arid rangeland and on cactus pear (Snyman 2013). *Lespedeza davurica* (shrub) also increased root:shoot ratios as water deficit increased (Xu et al.

2011). Desert shrubs and cactus pear tend to have a low root:shoot ratio of less than one (Snyman 2013), compared to grass species that can have a root:shoot ration of three to six (Snyman 2009b). In this study the root:shoot ratio of *N. microphylla* ranged only between 0.24 and 0.49, while that of *P. incana* ranged from 0.32 to 0.49. This correlates well with values of 0.31 and 0.37 that were reported for two other Karoo shrubs from the Succulent Karoo, namely *Ruschia robusta* and *Leipoldtia schultzei*, respectively (Carrick 2003).

Root:shoot ratios of this study also relate very well to that of a study done by Boutraa (2010) on the desert shrub *Calotropis procera*. Although an increased soil-water content increased root mass, the most severely stressed plants gave a higher root:shoot ratio. Roots play a more important role in plant survival than the aboveground plant parts (Shackleton et al. 1988, Snyman 2009a, Snyman 2009b), therefore the shift in phytomass allocation to the roots under water stress conditions. Thus, extrapolating from aboveground observations to belowground functions can be misleading (Snyman 2009b). The Mediterranean subshrub, *Echinispartum horridum* had higher root growth in autumn, while shoot growth was higher in spring (Palacio and Monserrat-Marti 2007). This indicates that some shrubs tend to separate root and shoot growth processes throughout the year, something to be investigated in future on Karoo shrubs.

In most published work the tendency is shown that root:shoot ratio decrease with plant age over time (Snyman 2009b). The age of the shrubs in this study was approximately two years on the date of destructive harvesting where the roots were also washed out. Older plants might therefore show lower root:shoot ratios than what was measured in this study.

7.5 Root growth

Root growth was less studied in the past than aboveground growth of shrubs. The main reason for this is could be the difficulty of doing root studies compared to that of aboveground components (Snyman 2009b). Shrub roots tend to show a decrease in root growth under water deficit conditions (Palacio and Monserrat-Marti 2007) and defoliation (Oba et al. 2006, Snyman 2014, Snyman and Malan 2015). The same tendency was evident for *N. microphylla* and *P.*

incana in this study. In other water balance studies total root length was shown to be reduced under water stress compared with irrigated conditions in several perennial tussock grasses (Simoes and Baruch 1991, Busso and Bolletta 2007). The opposite was found by Oosthuizen and Snyman (2009) on the semi-arid climax grass, *Themeda triandra*. Roots can, however, react differently than aboveground plant parts under certain treatments, for example root production of desert shrubs might not show significant differences under elevated CO_2 concentrations, while the aboveground production can (Houseman et al. 2006). According to Oosthuizen and Snyman (2009) root length and mass can be higher during the reproductive growth stage for *Themeda triandra*, regardless the soil-water content.

Root growth was not markedly affected by defoliation intensity, while increased defoliation frequency caused a significant decrease in root growth, for both *N. microphylla* and *P. incana*. Similar results were reported by Volesky et al. (2011) and Snyman and Malan (2015) for fodder grasses.

Some fodder plants have an extended root system to reach deeper soil water from the soil profile, which is just as effective as a long rope in a deep well (Blum 2005). The rooting patterns of Karoo shrubs enable plants to access both surface and deeper sub-surface soil water. Roots near the soil surface produce fine rootlets that are maintained under moist soil conditions but die as the soil dries out (Henrici 1937). In this study the roots of a single *N. microphylla* and *P. incana* plant was excavated in the veld where these shrubs are normally found in the Nama-karoo Biome (not mentioned under material and methods because not part of initial study). As it was difficult to quantify the root architecture no quantitative data is presented. What can, however, be mentioned was that the majority of the roots were found in the upper 300 mm soil layer and that there was more than one thick tap root that extended into the bedrock that was reached at a depth of about 400 mm (Figure 7.1). Morat et al. (2008) explains that plants with prolific root systems enhance their ability to capture water, and this can be seen as a drought avoidance mechanism. In this study the total root length of *P. incana* of almost up to 400 m plant^{-1} is a good example.

Figure 7.1: Root system of *Nenax microphylla* as excavated in the veld. The circles indicate the main roots of two separate plants, penetrating through the bedrock to unknown depths.

Scott and van Breda (1938) also studied the root system of *P. incana* and described the roots up to a depth of 2.9 m. Shrubs like these normally use the roots in the upper soil layers to utilize soil water for rapid growth (production), while the few roots that penetrate the soil to unknown depths, mostly serve as means for plant survival during droughts. This is further explained by Carrick (2003) as a phenomenon found in non-succulent Karoo shrubs which do not have the ability to store water, to access larger pools of soil-stored water, compared to succulents which have the ability to store water.

It is a general fact that root proportions of grasses and shrubs decreases with increasing depth (Snyman 2009b). As with observations in the current study, other studies also found more than 85% of roots in the top 300 mm of soil layer (Snyman 1998, Baer et al. 2002, Snyman 2009b).

Such high root concentrations in the top soil layers could also be due to increased nutrient availability in these upper soil layers (Ingram 2002, Franco et al. 2006).

7.6 Shoot growth

Inhibition of shoot growth under water stress, as measured in this study, is a drought adaptive mechanism of plants to reduce water loss by transpiration (Xu et al. 2011). It was clear from desert shrubs, exposed to water stress, that they compensated by abscission of smaller stems (Houseman et al. 2006), which was not the case in this study. It was however noted during field visits that older *N. microphylla* plants have a large number of dead stems. These stems could have died during prolonged drought periods. Figure 7.2 indicates a few such dead stems on a plant that was photographed in the Nama-karoo.

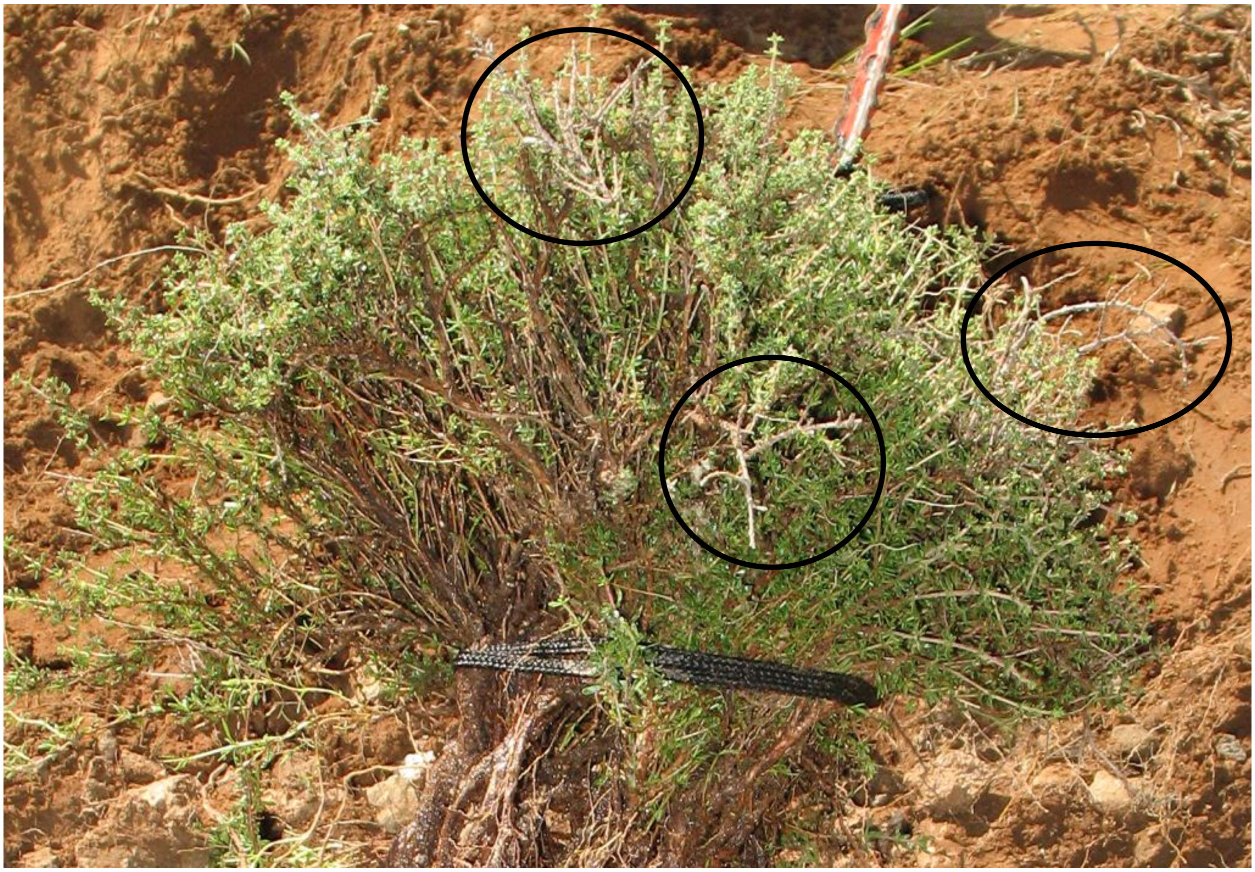

Figure 7.2: *Nenax microphylla* plant with dead stems, encircled in black.

Compensatory shoot growth, as recorded in this study, was also reported by Gadd et al. (2001) for *Acacia drepanolobium*.

In future other, perhaps more comprehensive methods should be used to measure shrub growth. A destructive method by measuring shoot mass could be considered (Xu et al. 2011), or a non-destructive method of measuring shoot diameter with addition to counting of side branches (Houseman et al. 2006).

7.8 Leaf dimensions

In this study water stress significantly reduced single leaf area of *N. microphylla*, while Hassen et al. (2007) reported a reduction in total leaf area with water stress for *Indigofera* species, and Morat et al. (2008) mentions similar findings for various pasture crops. A reduction in total leaf length during dry periods was recorded by Smith and Nobel (1977) for desert shrubs, as was the case for both *N. microphylla* and *P. incana* in this study. A positive plant carbon balance is required after defoliation to restore plant growth. One such process is re-establishment of the photosynthetic canopy by leaf and shoot growth (Richards 1993). For this study an increased leaf area was recorded with increase in defoliation intensity, which correlates with an increased leaf area after herbivory that was experienced for *Indigofera cliffordiana* (Oba et al. 2006). Similar results were found for *Buddleja davidii* (shrub) where defoliated plants had a leaf area of 52% greater than that of control plants, which were described as compensational leaf growth (Thomas et al. 2008). Compensatory leaf elongation after defoliation of *Kyllinga nervosa* (an African sedge) was reported by McNaughton et al. (1983). The increase in leaf growth of *N. microphylla*, with increased defoliation intensity forms part of the compensatory growth which was discussed previously. The increase in leaf size acts as a means of increasing the photosynthetic area to contribute to rapid regrowth after defoliation to restore the photosynthetic capacity of the plant as quickly as possible, while adequate water is available. Thomas et al. (2008) found that the compensation ability of frequently defoliated plants was reduced after two years due to a reduction in leaf size.

7.9 Summary of shoot length and leaf dimensions

Although the shoot length for the severely defoliation intensity (50 mm height) was significantly higher than the other intensities, the edible phytomass (and other plant part fractions) for the 50 mm height was lower than for the other defoliation intensities. Therefore although these plants try to compensate with larger shoot lengths and bigger leaf areas, the total plant size, compared to the other defoliation intensities is to low (to small) to be able to outperform the other treatments.

7.10 Conclusion

The two most important factors determining sustainable livestock production in the Nama-karoo is rainfall and grazing. Rainfall is arguably the more important of the two, with the biggest influence on rangeland productivity, but unfortunately the land user has no control over it. The land user, however, has a great amount of control over the frequency and intensity of the defoliation of the Karoo shrubs through grazing management systems and animal numbers. The critical objective of grazing management is to sustain long-term rangeland productivity. The more severely *N. microphylla* and *P. incana* gets defoliated, the less frequently it can tolerate such an action, while the less severely (more lenient) it gets defoliated, the more frequently it can tolerate such an action. Under extreme water deficit conditions, the abovementioned statement is however not true as regrowth is almost zero due to lack of soil water. Drought is a natural phenomenon in the Nama-karoo and therefore the correct management of these ecological sensitive plants is critical. Awareness of the codependent effect of water deficit and defoliation on whole shrub productivity should always drive management strategies.

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CHAPTER 8

WATER-USE EFFICIENCY OF *Nenax microphylla* AND *Pentzia incana* AT DIFFERENT DEFOLIATION TREATMENTS ALONG A WATER DEFICIT GRADIENT

8.1 Introduction

Water-use efficiency (WUE) determination and approach (procedure) for *Nenax microphylla* and *Pentzia incana* in the glasshouse are explained in detail in Chapter 4 under materials and methods. Water-use efficiency quantification for single plants was expressed as grams of phytomass produced per litre of water transpired per plant, stated as the mean value of the replications for each treatment. The units expressed in the data presentation and discussion will, however, only be grams of phytomass per litre of water (g l^{-1}). When WUE is expressed only for the aboveground phytomass production it is indicated as WUEa and when expressed for total above- plus belowground phytomass, it is indicated as WUEab. The same water and defoliation treatments as discussed in the previous chapters will be evaluated for phytomass production, but, only at the final destructive harvest, when the roots (belowground phytomass) were also harvested. The aboveground phytomass therefore includes both edible and inedible plant fractions. In other words, the whole plant was destructively harvested and not only the regrowth (edible fraction). The main reason for the above mentioned aspect, of including both fractions (not only edible) in calculating WUE, will be discussed in detail.

For both defoliation frequency A (three-monthly) and frequency B (six-monthly), the cumulative edible phytomass plus the inedible phytomass (total aboveground phytomass produced over the 12 month period) were used in determining WUEa and WUEab. Although the WUE on the different defoliation dates was, however, also calculated, it was only done for the edible (defoliated) plant fraction. The problem arises that the applied water up to that stage (date) was also used by the plant for growth of the inedible plant fraction. Over time side branches were formed and stems of the inedible fraction gained mass. The other problem was that for determination of the WUE for different defoliation intensities, edible phytomass was defoliated at three heights of: 50, 125 and 200 mm. The data showed that on each defoliation date, the phytomass from the 50 mm height was generally higher than that of the 200 mm height. This gave a skew picture of WUE because the total amount of water applied was used to determine WUE when a much smaller portion of the plant was defoliated. Due to the above mentioned problem, it was decided not to include the WUE of the edible plant fraction of the different defoliation dates for defoliation frequencies A and B. This data will not give a true reflection of WUE dynamics in practice, as plant growth is exponential it is not possible to measure only the amount of water that was used for regrowth.

8.2 Total amount of water applied to both *N. microphylla* and *P. incana*

Before starting with WUE results and discussion, the total volume of water applied over the 12 month trial period for the various treatments is shown and briefly discussed. This will give a clear indication of which treatment combination used the greatest and the least amount of water. Water application was done by keeping the soil-water contents of the different watering treatments within the pre-determined water ranges as explained in Chapter 4. The water treatments were as follows: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed).

As expected, water overwhelmingly accounted for most variation in the data for both species (Table 8.1). Defoliation intensity and frequency, as well as all the intensity x frequency interaction had a significant ($P < 0.001$) influence on the total amount of water applied for both species. The only exception was for the three way interaction (W x I x F) of *P. incana*, which influence was insignificant ($P > 0.05$). As expected water deficit showed a decline ($P < 0.001$) in total water applied for both species (Figure 8.1). Increased defoliation intensity, as well as increased defoliation frequency resulted in decreased water use for both species (Figure 8.1). On the one hand it could be that less water was used for more frequent and more severe defoliation, due to smaller photosynthetic (transpiration) surfaces, or secondly more effective water use as a compensatory mechanism.

Table 8.1: ANOVA summary of the total water applied (l plant^{-1}) for *Nenax microphylla* and *Pentzia incana* over the 12 month trial period.

Karoo shrub species	W		I		F		W x I		W x F		I x F		W x I x F	
	SS% and significance													
Nenax microphylla	77	***	7	***	7	***	1	***	3	***	2	***	2	***
Pentzia incana	82	***	3	***	6	***	1	***	2	***	1	***	0	NS

W = water, I = defoliation intensity, F = defoliation frequency, NS = non-significant, SS% = sum of squares percentage, *** = P < 0.001, ** = P < 0.01, * = P < 0.05

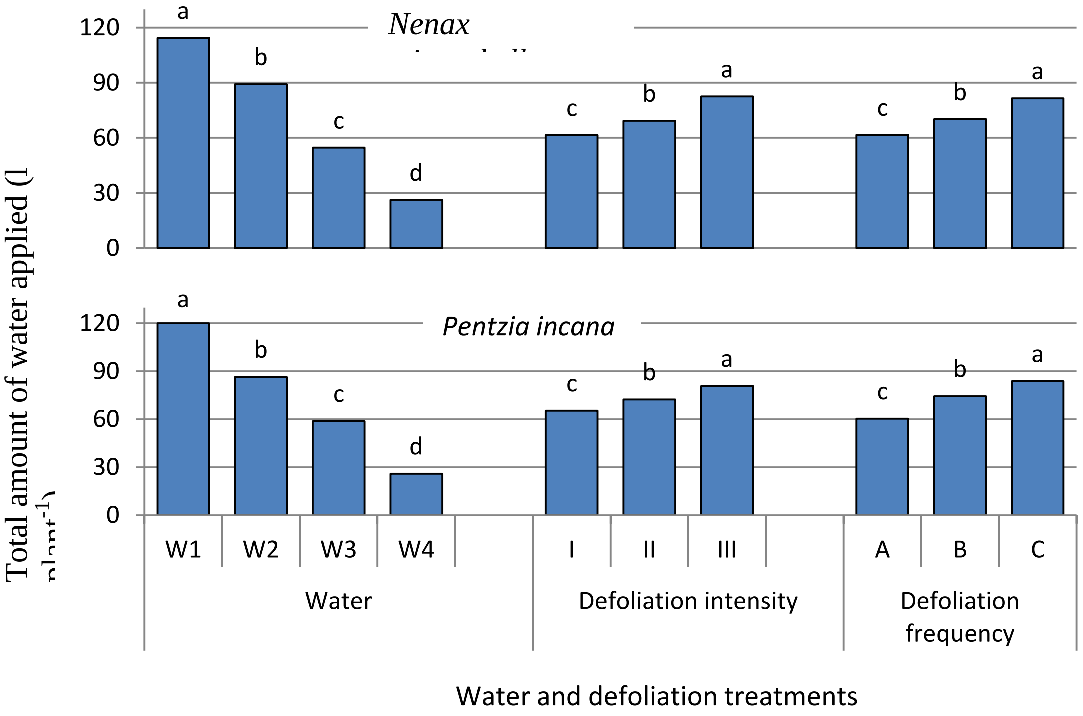


Figure 8.1: Mean total water applied (l plant^{-1}) for *Nenax microphylla* and *Pentzia incana* at the different water and defoliation treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences (P < 0.001) within treatments.

The darker black horizontal lines in Figure 8.2 were inserted to indicate the best and worst set of treatments, selected according to the multiple t-distribution test procedure of Gupta and Panchapakesan (1979). It represents all interactions within one LSD value increment. The treatment combinations below the last horizontal lines at the bottom of the figure indicate the best group regarding phytomass production. This is the group of treatment combinations that gave the highest WUEa and WUEab without differing significantly from each other ($P < 0.001$). These black horizontal lines, indicating LSD groupings will also be used for the rest of this chapter.

In general *P. incana* used more water than *N. microphylla*, with only a few exceptions. As expected, for both species the best group (group that used the most water) consist of unstressed water treatments (W1) mostly in combination with defoliation frequency C (control – defoliated at 12 months) and the most lenient defoliation intensity (intensity III). These were the plants with the largest canopy cover, giving the greatest photosynthetic (transpiration) surface.

The worst group (group that used the smallest amount of water) was dominated by the most severely stressed water treatment (W4) in combination with all defoliation treatments, for both species. This indicates that no defoliation treatment or defoliation treatment combinations had a significantly positive influence on the water deficit applied.

At water treatment W1, W2 and W3, defoliation treatments had a much bigger effect on water use than at W4 (most severely stressed treatment) (Figure 8.2). The W2 X I X A interaction (marked with green bars on graph) was for both species the least stress water treatment that used the smallest amount of water. This interaction will also prove to be giving the best WUE as explained later in this chapter. Of the more water stressed treatments, the W3 X III X C interaction used the most water for both species (encircled on graph), which was a similar amount of water as the W1 X I X A interaction.

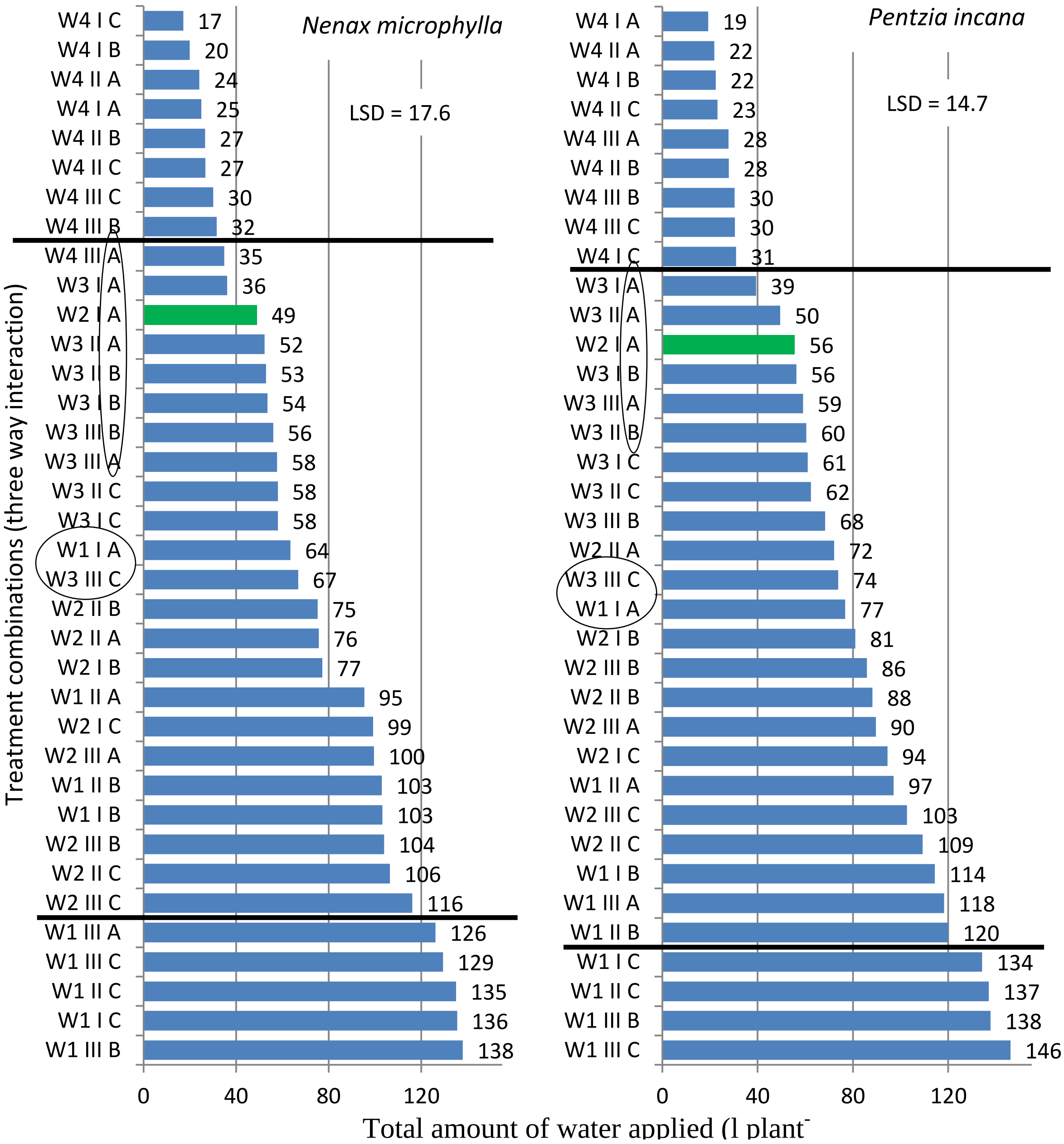


Figure 8.2: Mean total water applied (l plant^{-1}) for *Nenax microphylla* and *Pentzia incana* at the different water and defoliation treatment combinations. LSD = least significant difference. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve monthly or control.

8.3 Results and discussion of WUE

The WUE results of both species, *N. microphylla* and *P. incana*, will be presented and discussed in this chapter, but under separate headings.

8.3.1 *Nenax microphylla*

8.3.1.1 Water-use efficiency for the three-monthly defoliation frequency (Frequency A)

Water availability had a significant (P < 0.001) influence on WUE and accounted for most of the variation in the WUE data for both WUEa and WUEab (Table 8.2). Defoliation accounted for some variation in WUEa data (P < 0.05), but not as significant as water.

Table 8.2: ANOVA summary for the WUE of *Nenax microphylla* for the three-monthly defoliation frequency (frequency A).

Frequency A		W		I		W x I	
Variable	Treatment	SS% and significance					
WUEa	A cum at end	50	***	7	*	5	NS
WUEab	A cum at end	26	***	4	NS	9	NS

WUEa = water-use efficiency for total aboveground phytomass, WUEab = water-use efficiency for total above- plus belowground phytomass, A cum at end = cumulative aboveground phytomass at the end of the trial, W = water, I = defoliation intensity, F = defoliation frequency, NS = non-significant, SS% = sum of squares percentage, *** = P < 0.001, ** = P < 0.01, * = P < 0.05

Water deficit caused a decline (P < 0.001) in WUEa, with the exception of W2 (Figure 8.3). The reason for WUE of W2 being slightly higher than that of W1 might be an indication that shrubs from semi-arid areas, like *N. microphylla*, cannot exploit very high soil-water conditions (0 – 25 % depletion of soil capacity) to its full potential. The less water stressed treatments, namely W1 and W2, gave the highest WUEa, with that of the moderately stressed water treatment (W3) significantly (P < 0.001) lower and the severely stressed treatment (W4) lower (P < 0.001) than any of the other water treatments. By contrast, increased defoliation intensity caused an increase in WUEa, which was, however, not significantly (P > 0.05) different from each other. This increase in WUE under defoliation stress might be an expression of the compensatory growth ability of *N. microphylla* under defoliation stress.

When the belowground phytomass production was included in the calculation of WUE to quantify WUEab, water deficit caused a smaller decline in WUEab. Only WUEab of W2 was significantly ($P < 0.001$) higher than the two water stressed treatments (W3 and W4). Although WUEab still showed and increase with harsher defoliation intensity, the increase was not significant ($P > 0.05$).

The WUEab was in general higher than WUEa for water availability and defoliation intensity treatments. Higher root production at W4 increased the WUEab of W4, compared to that of WUEa, resulting in overall better water utilization of these water stressed plants.

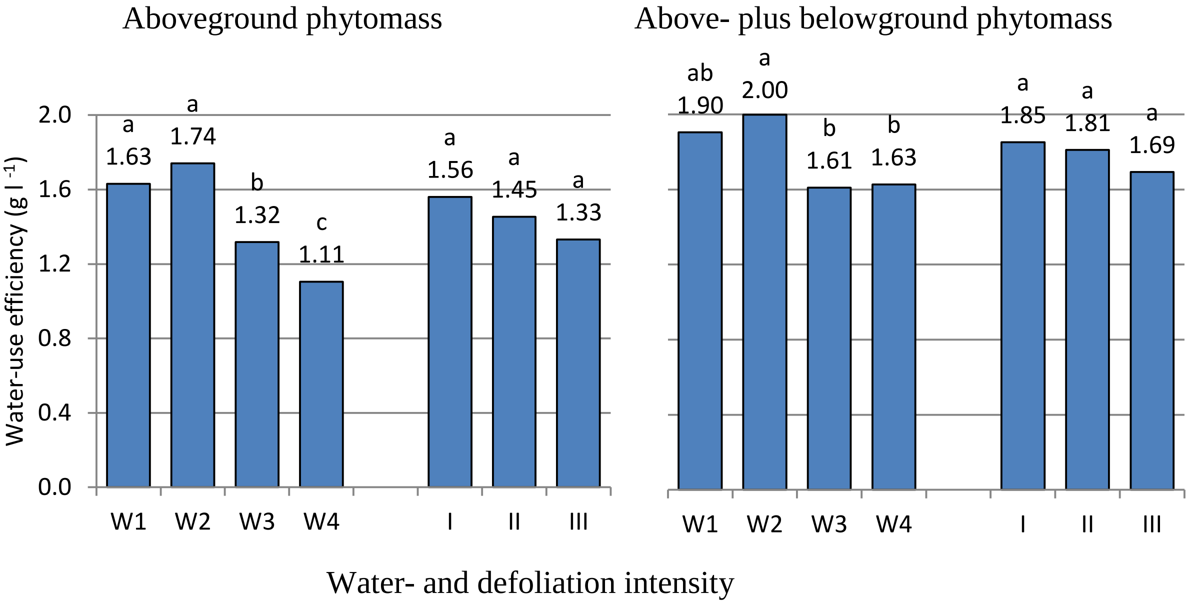


Figure 8.3: Mean (n = 4) water-use efficiency (g l^{-1}) for *Nenax microphylla* for the three-monthly defoliation frequency (frequency A). The two main effects (water and defoliation intensity) are indicated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences ($P < 0.001$) within treatments.

Although the water x intensity interaction did not influence WUE significantly ($P > 0.05$) (Table 8.2), it is still presented and discussed due to an almost 100% difference between the lowest and highest WUEa values (Figure 8.4). This discussion, of insignificant interactions, will be the case for the rest of this chapter, where appropriate. The darker black horizontal lines in Figure 8.4 indicate the best LSD groupings (t-distribution test, Gupta and Panchapakesan (1979)). The treatment combinations below the horizontal line represent the best group regarding WUE. This is the group of treatment combinations that gave the highest WUEa and WUEab without differing significantly from each other (P < 0.001).

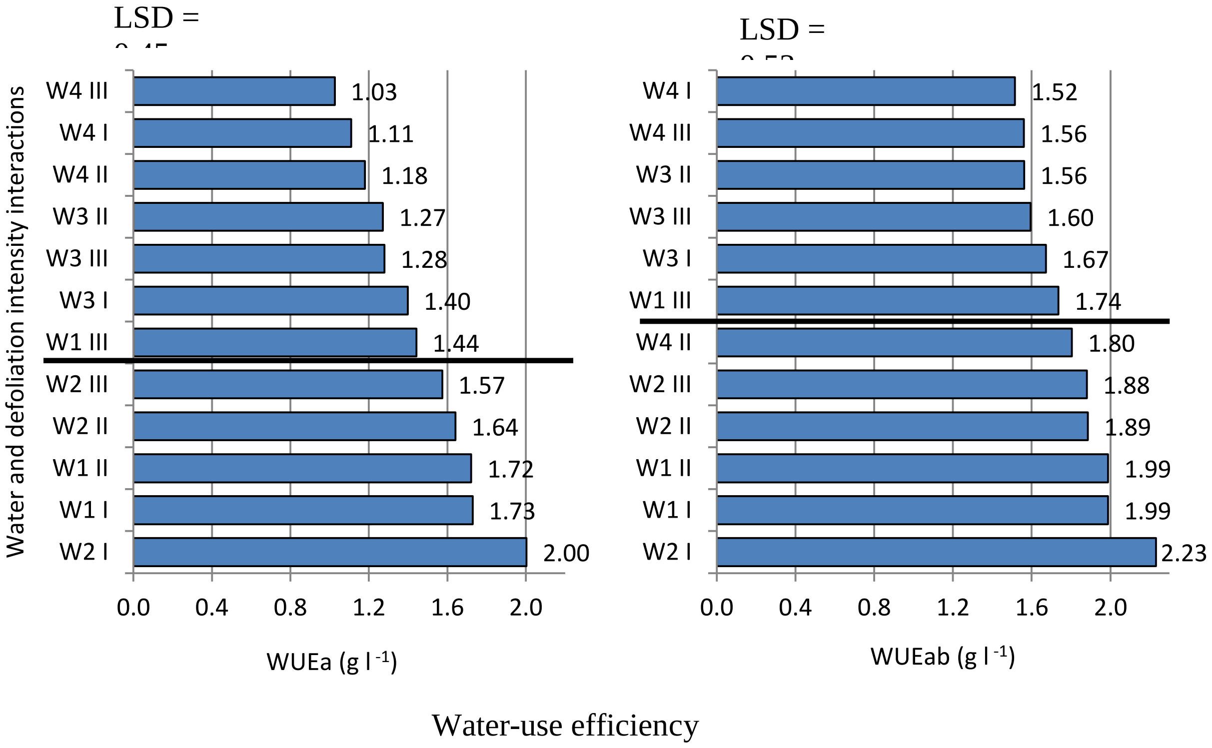


Figure 8.4: Mean (n = 4) water-use efficiency (WUE) (g l^{-1}) for *Nenax microphylla* for the three-monthly defoliation frequency (frequency A). The interactions between water and defoliation intensity treatments are indicated. LSD = least significant difference. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm.

Regardless of defoliation intensity, the more severely water stressed treatments (W3 and W4) dominates the weaker group. The best group, below the black horizontal line through the graph, is dominated by the unstressed water treatments (W1 and W2), in combination with a distinct decrease in defoliation intensity treatments. It is interesting to note that the second highest watering treatment (W2) in combination with defoliation intensity I (most severe defoliation intensity), although not significantly ($P > 0.05$) higher than interaction from the same group, gave the best WUEa and WUEab.

8.3.1.2 Water-use efficiency for the six-monthly defoliation frequency (Frequency B)

Water availability and defoliation intensity influenced WUE significantly ($P < 0.01$) and accounted for equal amounts of variation in the WUEa data (Table 8.3). For WUEab only defoliation intensity had a small, but significant ($P < 0.05$) influence on the data.

Table 8.3: ANOVA summary for the WUE of *Nenax microphylla* for the six-monthly defoliation frequency (frequency B).

Frequency B		W		I		W x I	
Variable	Treatment	SS% and significance					
WUEa	B cum at end	19	**	19	**	10	NS
WUEab	B cum at end	10	NS	11	*	12	NS

WUEa = water-use efficiency for total aboveground phytomass, WUEab = water-use efficiency for total above- plus belowground phytomass, B cum at end = cumulative aboveground phytomass at the end of the trial, W = water, I = defoliation intensity, F = defoliation frequency, NS = non-significant, SS% = sum of squares percentage, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

Water deficit had no distinct influence on WUEa (Figure 8.5). The most severely water stressed treatment (W4) was significantly ($P < 0.001$) lower than that of W2 and W3, but did not differ ($P > 0.05$) from W1 (unstressed water treatment). Increased defoliation intensity resulted in an increase in WUEa, with the WUEa of defoliation intensity III (most lenient intensity) being significantly ($P < 0.001$) lower than the more severely defoliated intensities I and II.

The small variation in data registered for WUEab between the different watering and defoliation intensities, showed no significant ($P > 0.05$) differences. With water stress increase the WUEab increased ($P > 0.05$), while it also increased ($P > 0.05$) with increased defoliation intensity. The slightly lower water-use efficiency of the unstressed water treatment (W1) for both WUEa and WUEab indicates that *N. microphylla* cannot make optimal use of the abundantly available water.

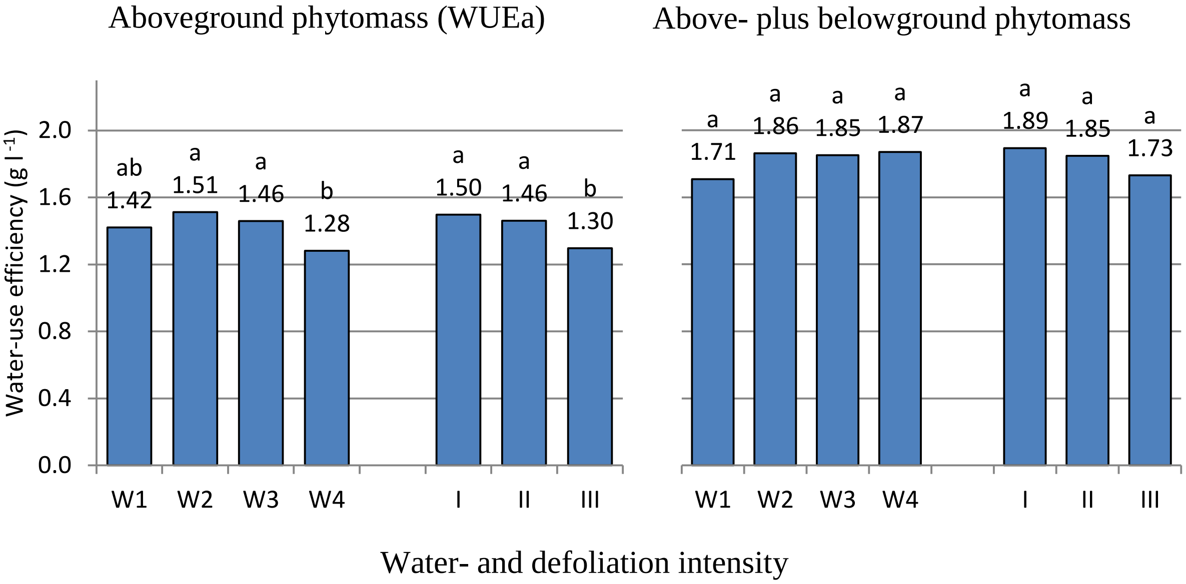


Figure 8.5: Mean (n = 4) water-use efficiency (WUE) (g l^{-1}) for *Nenax microphylla* for the six-monthly defoliation frequency (frequency B). The two main effects (water and defoliation intensity) are indicated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences ($P < 0.001$) within treatments.

Although the water x intensity interaction did not test significantly ($P > 0.05$), the data is presented in Figure 8.6 to show the change in ranked order of the interaction when the root production (belowground phytomass) is included in the calculation of water-use efficiency. As was the case at defoliation frequency A, the W2 X I interaction was again the interaction with the best WUEa and WUEab, although not by much. When belowground phytomass is added to determine WUEab, all the severely stressed water treatments (W4) shifted to the group with the better WUE, with only W1 X II and W2 X III outside that group. The black horizontal line only indicates the best group, which are all the treatment combination below the line. When the root fraction was added to the WUEab calculation, almost all the treatment combinations did not differ significantly ($P > 0.05$).

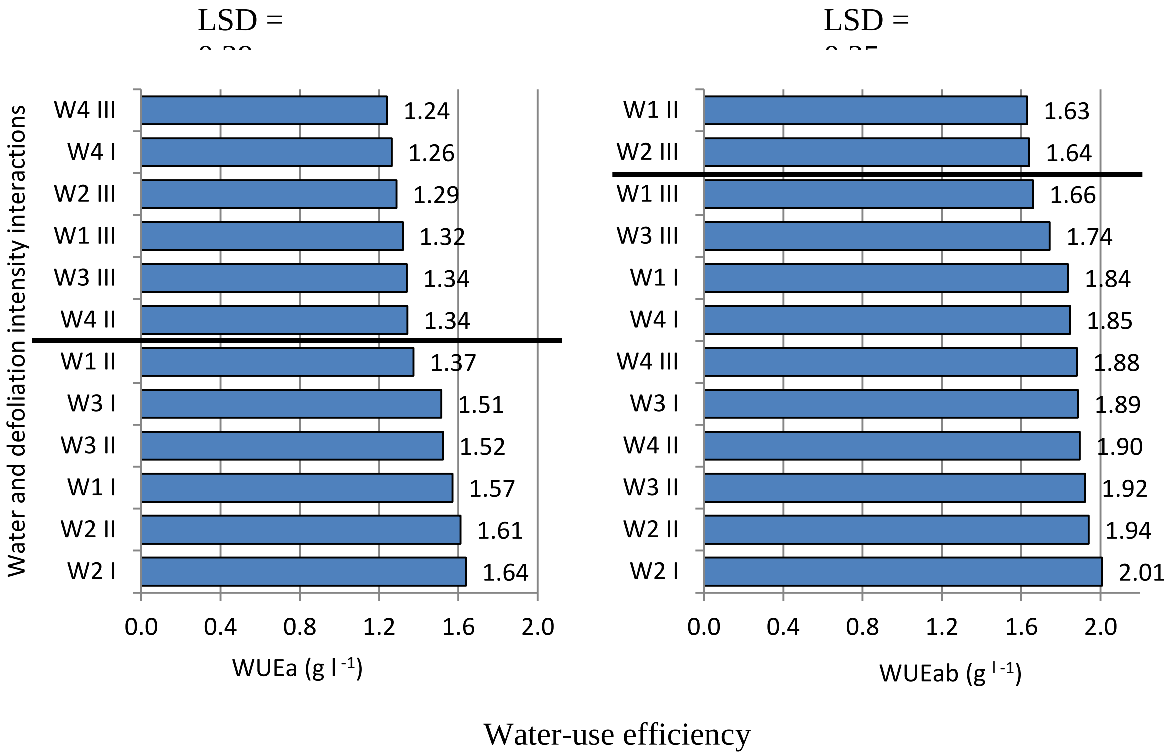


Figure 8.6: Mean (n = 4) water-use efficiency (WUE) (g l^{-1}) for *Nenax microphylla* for the six-monthly defoliation frequency (frequency B). The interactions between water and defoliation intensity treatments are indicated. LSD = least significant difference. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm.

8.3.1.3 Water-use efficiency for the twelve-monthly defoliation frequency (Frequency C)

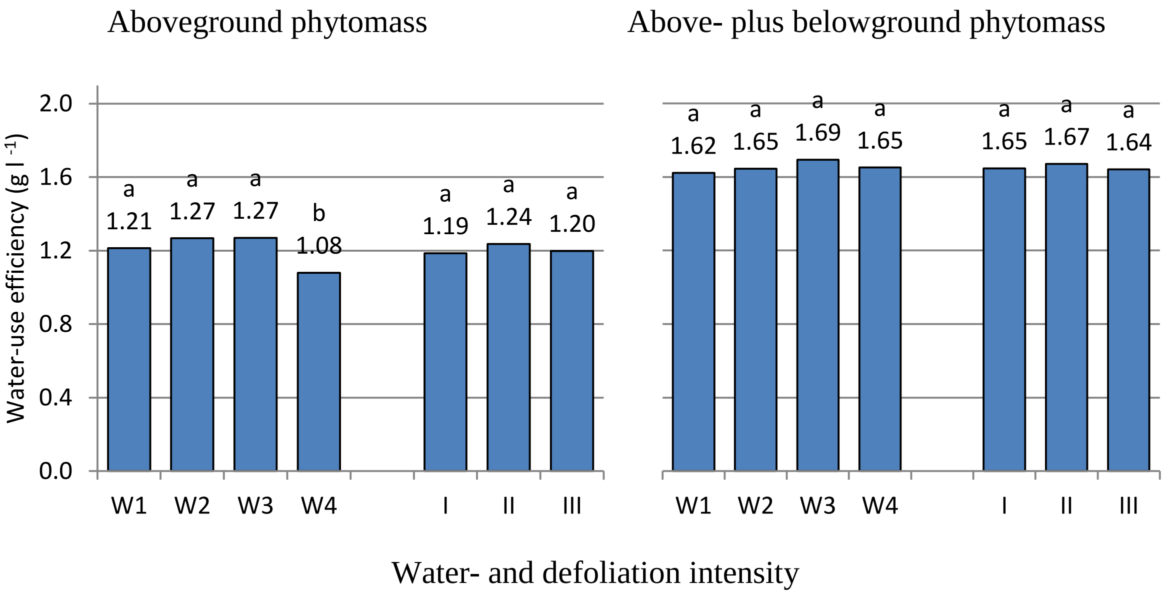
As explained before, the plants from this treatment were defoliated once at the beginning of the trial and once 12 months later, at the end of the trial, and can therefore also be seen as the control, regarding defoliation treatments. Water and the water x intensity interaction accounted for a significant ($P < 0.001$) amount of variation in the WUEa data, while none of the treatments tested to have a significant influence ($P > 0.05$) on the data at WUEab (Table 8.4).

Table 8.4: ANOVA summary for the WUE of *Nenax microphylla* for the twelve-monthly defoliation frequency (frequency C) or control.

Frequency C		W		I		F		W x I	
Variable	Treatment	SS% and significance							
WUEa	C	30	***	2	NS			26	***
WUEab	C	2	NS	1	NS			23	NS

WUEa = water-use efficiency for total aboveground phytomass, WUEab = water-use efficiency for total above- plus belowground phytomass, C = phytomass at the end of the trial after 12 months, W = water, I = defoliation intensity, F = defoliation frequency, NS = non-significant, SS% = sum of squares percentage,
*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

The only significant difference in WUE within water treatments was at WUEa, where the most severely stressed (W4) plant's WUEa was lower ($P < 0.001$) than that of any of the other water treatments (Figure 8.7). Water-use efficiency for the defoliation intensity treatments were all virtually equal ($P > 0.05$) for both aboveground and above- plus belowground phytomass production. The average WUE increased form 1.21 g l^{-1} for WUEa to 1.65 g l^{-1} for WUEab, a 36% increase. This is an indication of the importance of also including root phytomass in WUE calculations, giving a more reliable indication of what is happening in practice. Furthermore, the mostly similar WUE values of this treatment (frequency C – defoliated only once in 12 months), compared to the occurrence of some variation (mostly a decrease with water deficit) in frequencies A and B, indicates that WUE is more affected by water deficit when more frequently defoliated.



Aboveground phytomass

Above- plus belowground phytomass

Figure 8.7: Mean (n = 4) water-use efficiency (WUE) (g l^{-1}) for *Nenax microphylla* for the twelve-monthly defoliation frequency (frequency C). The two main effects (water and defoliation intensity) are indicated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences ($P < 0.001$) within treatments.

The different treatment combinations at the water x intensity interaction did not vary much regarding WUEab, but showed some more important evident differences at WUEa (Figure 8.8). Although no clear pattern could be identified, the treatment combinations below the horizontal lines represent the best group which did not differ ($P > 0.05$) from each other. When root phytomass is included in calculating WUEab, almost all treatment combination was in the same group.

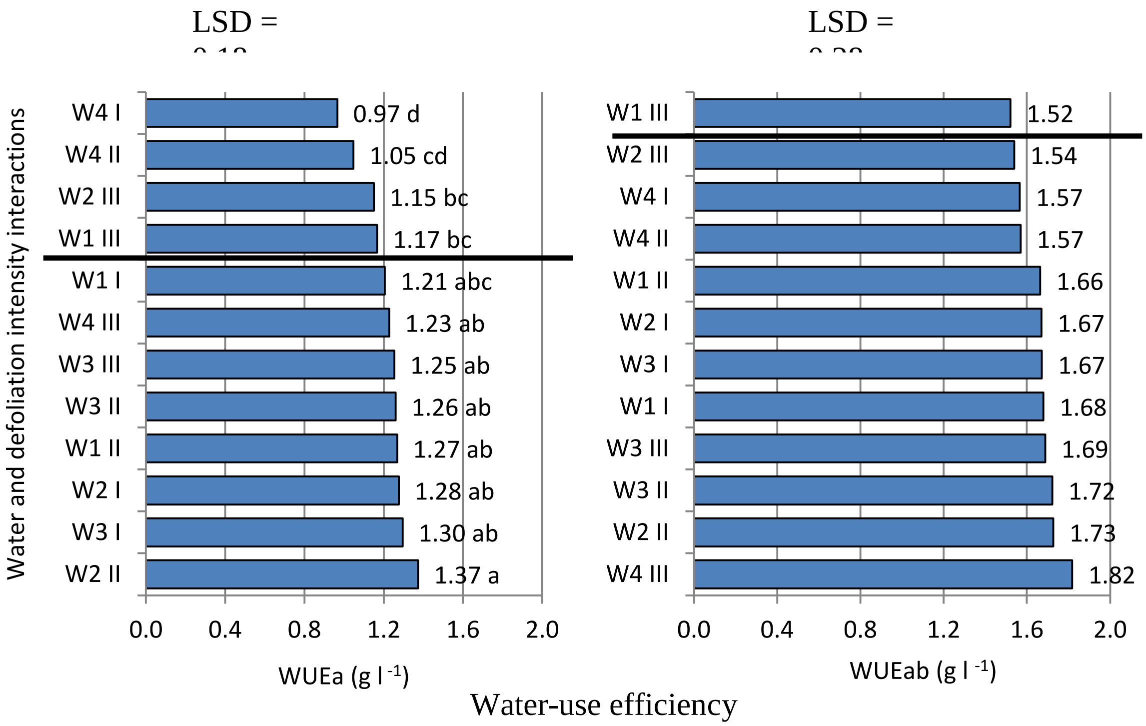


Figure 8.8: Mean (n = 4) water-use efficiency (WUE) (g l^{-1}) for *Nenax microphylla* for the 12 monthly defoliation frequency (frequency C). The interactions between water and defoliation intensity treatments are indicated. LSD = least significant difference. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences (P < 0.001) within treatments.

8.3.1.4 Comparison of WUE for the three defoliation frequencies

Water, defoliation frequency together with the water x frequency interaction accounted for most (P < 0.001) of the variation in WUEa data, while defoliation intensity also had a significant, but lower influence (Table 8.5). Defoliation frequency and water x frequency interaction accounted for most (P < 0.001) variation in WUEab data.

Table 8.5: ANOVA summary for the WUE of *Nenax microphylla* for a comparison between the three defoliation frequencies (A, B and C).

A,B,C compared		W		I		F		W x I		W x F		I x F		W x I x F	
Variable	Treatment	SS% and significance													
WUEa	A, B, C compared	**23**	***	5	***	**16**	***	4	*	**12**	***	3	*	3	NS
WUEab	A, B, C compared	4	*	3	*	**8**	***	6	NS	**13**	***	1	NS	5	NS

WUEa = water-use efficiency for total aboveground phytomass, WUEab = water-use efficiency for total above- plus belowground phytomass, A = Frequency A, B = Frequency B, C = Frequency C, W = water, I = defoliation intensity, F = defoliation frequency, NS = non-significant, SS% = sum of squares percentage, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

The WUE data in this comparison of defoliation frequencies A, B and C follows the same tendency as in the previous discussions of water and defoliation intensity, with WUEa showing more variation between treatments than WUEab where the root phytomass is included in the WUE calculation (Figure 8.9). Water-use efficiency of W2 was slightly higher ($P > 0.05$) than at W1 and significantly ($P < 0.001$) higher than W3 and W4, almost as if it is the optimum water treatment regarding WUEa. At WUEab, W2 was also only slightly higher than the other water treatments. A steady decline in WUEa ($P < 0.001$) and WUEab ($P > 0.05$) is visible with increased water stress. On the contrary, WUEa ($P < 0.001$) and WUEab ($P > 0.05$) showed a steady increase with increased defoliation intensity and increased defoliation frequency. Both the most lenient defoliation frequency and defoliation intensity had significantly ($P < 0.001$) but lower WUEa than the more severe treatments. For both WUEa and WUEab defoliation frequency C had lower ($P < 0.001$) WUEab than at frequency A and B (Figure 8.9).

It seems as if water availability impacted negatively on WUE, while increased defoliation had a positive influence on WUE. This might be an expression of the compensatory ability, as a protective mechanism, where the plant increases its WUE for improved regrowth after defoliation. Frequencies A and B that were defoliated more often than frequency C had higher WUEab values than frequency C. These values could even have been higher if the roots that died back (Xu and Li 2006; Snyman and Malan 2015) after each defoliation were to be included in the WUE calculations.

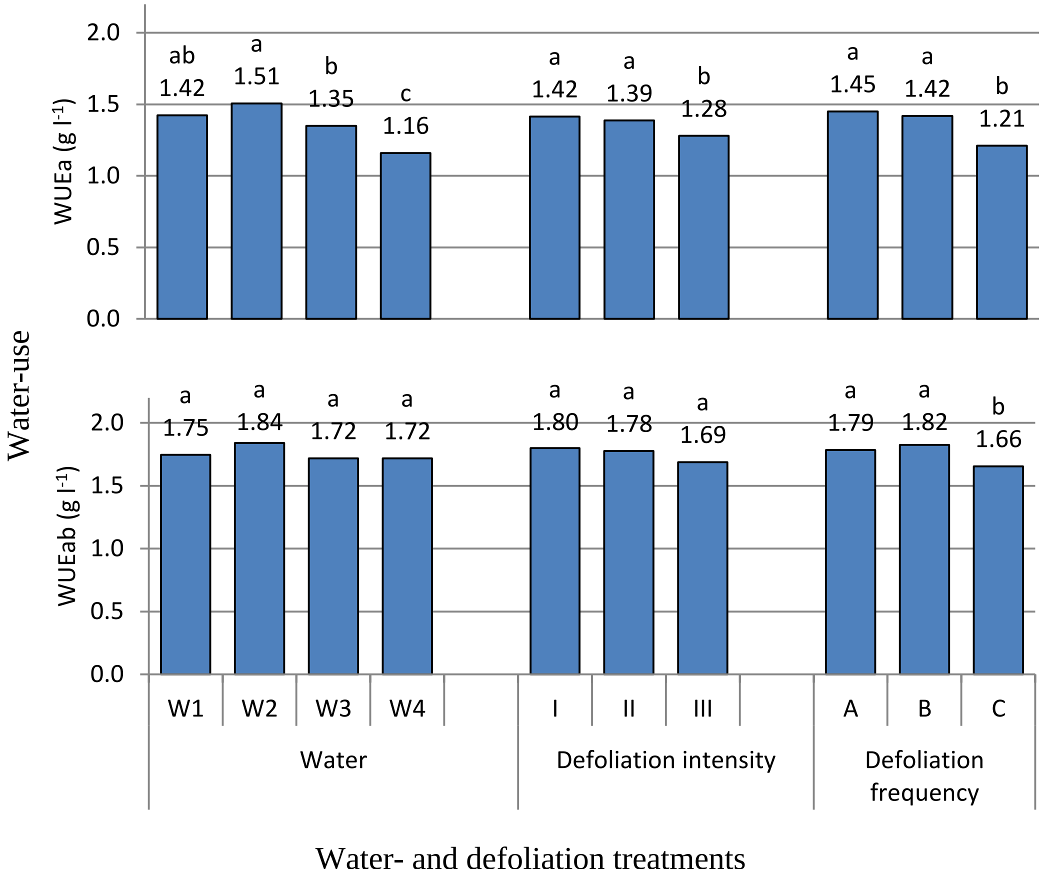


Figure 8.9: Mean (n = 4) water-use efficiency (WUE) (g l^{-1}) for *Nenax microphylla* in comparing the three defoliation frequencies. The interactions between water and defoliation intensity treatments are indicated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences ($P < 0.001$) within treatments.

The water x defoliation frequency interactions results for WUEa and WUEab strengthens the compensatory ability argument (Figure 8.10). The best group of W X F interactions, situated below the bottom horizontal lines, includes more severely defoliated plants in combination with mostly higher water availability. On the contrary the weaker group (lower WUE) includes most of the leniently defoliated treatment combinations.

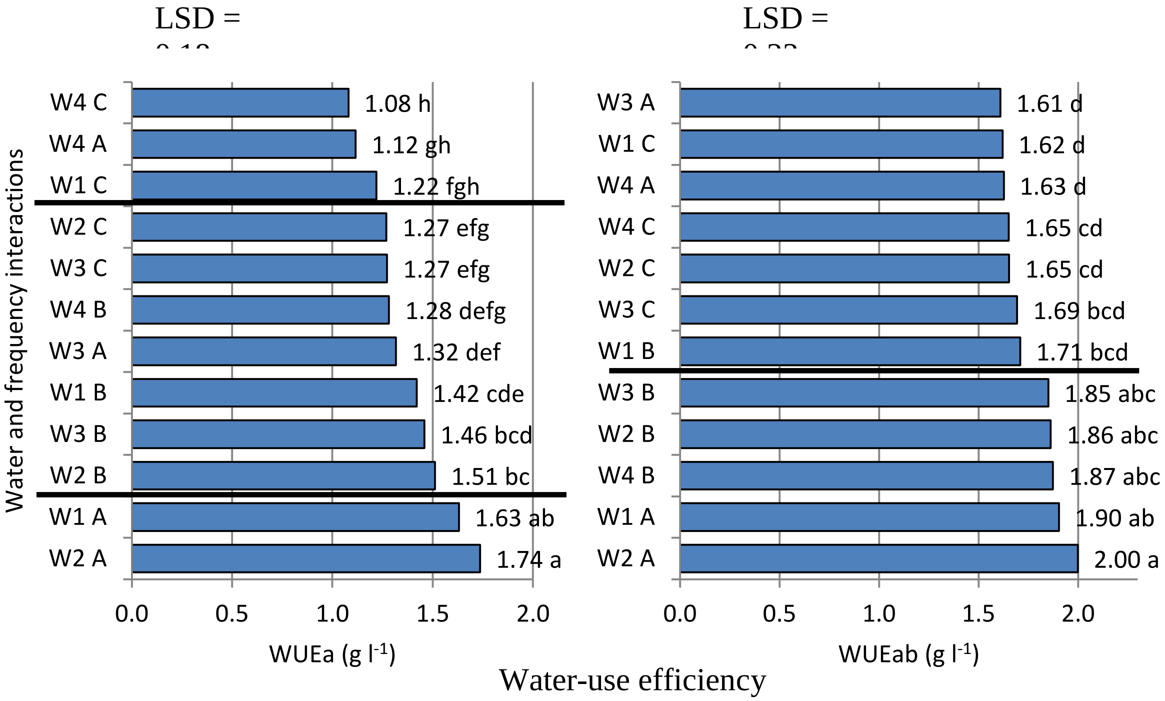


Figure 8.10: Mean (n = 4) water-use efficiency (WUE) (g l^{-1}) for *Nenax microphylla* for in comparing defoliation frequencies A, B and C. The interactions between water and defoliation frequency treatments are indicated. LSD = least significant difference. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences (P < 0.001) within treatments.

The results for WUEa and WUEab of all 36 water and defoliation treatments combinations are presented in Figure 8.11. Water-use efficiency was in general much higher at WUEab where the root phytomass was also included in the WUE calculation. This increase was 29% on average over all treatment combinations, which is reasonably high. If this is compared to the root fraction, it is interesting to note that on average the roots made up 23% of the total phytomass over all treatment combinations.

This increase was greater for the more water stressed treatments than the unstressed treatments. Examples are W4 I C (most severely water stressed, high intensity and low frequency defoliation) which increased form a WUEa of 0.97 g l^{-1} to WUEab of 1.57 g l^{-1} while the increase of W2 I A (sufficient water, most severely defoliated) was from 2.00 g l^{-1} at WUEa to only 2.23 g l^{-1} at WUEab. This can be ascribed to a higher root:shoot ratio for the water stressed plants as discussed in Chapter 5.

Defoliation frequency C (defoliated only every 12 months), was not present in the group of best (group below black horizontal line) WUE for both WUEa and WUEab (indicated with the green dotted line in Figure 8.11), although defoliation frequency C had the highest phytomass production (Chapter 5). The reason could be that these plants were exposed to less stress, which allowed it to use water less economically.

In the group of best WUEab, only two treatment combinations, consisting of defoliation intensity III (most lenient intensity), were present, the rest were all the more severely defoliated (intensities I and II) treatments. Another indication that the less the plants were stressed, the lower the WUE. Under these drier environmental conditions the Karoo shrubs are therefore more adapted to use water more efficiently during drought periods.

One interesting aspect at WUEab, was that the only severely water stressed treatment combinations that were present in the best group was W4 I B, W4 II B and W4 III B (most severely water stressed, at all three defoliation intensities, of the six-monthly defoliation frequency). This indicates that a six-monthly defoliation frequency, at any intensity resulted in a high WUEab, even under severely water stressed conditions. These same three treatment combination were however in the lowest group regarding phytomass production (Chapter 5). Another mentionable result is the W2 I A interaction (sufficient water, most severely defoliated) which had, although not significant, a noteworthy higher WUEa and WUEab than all of the other interactions. But it also did not show the same tendency regarding phytomass production (Chapter 5).

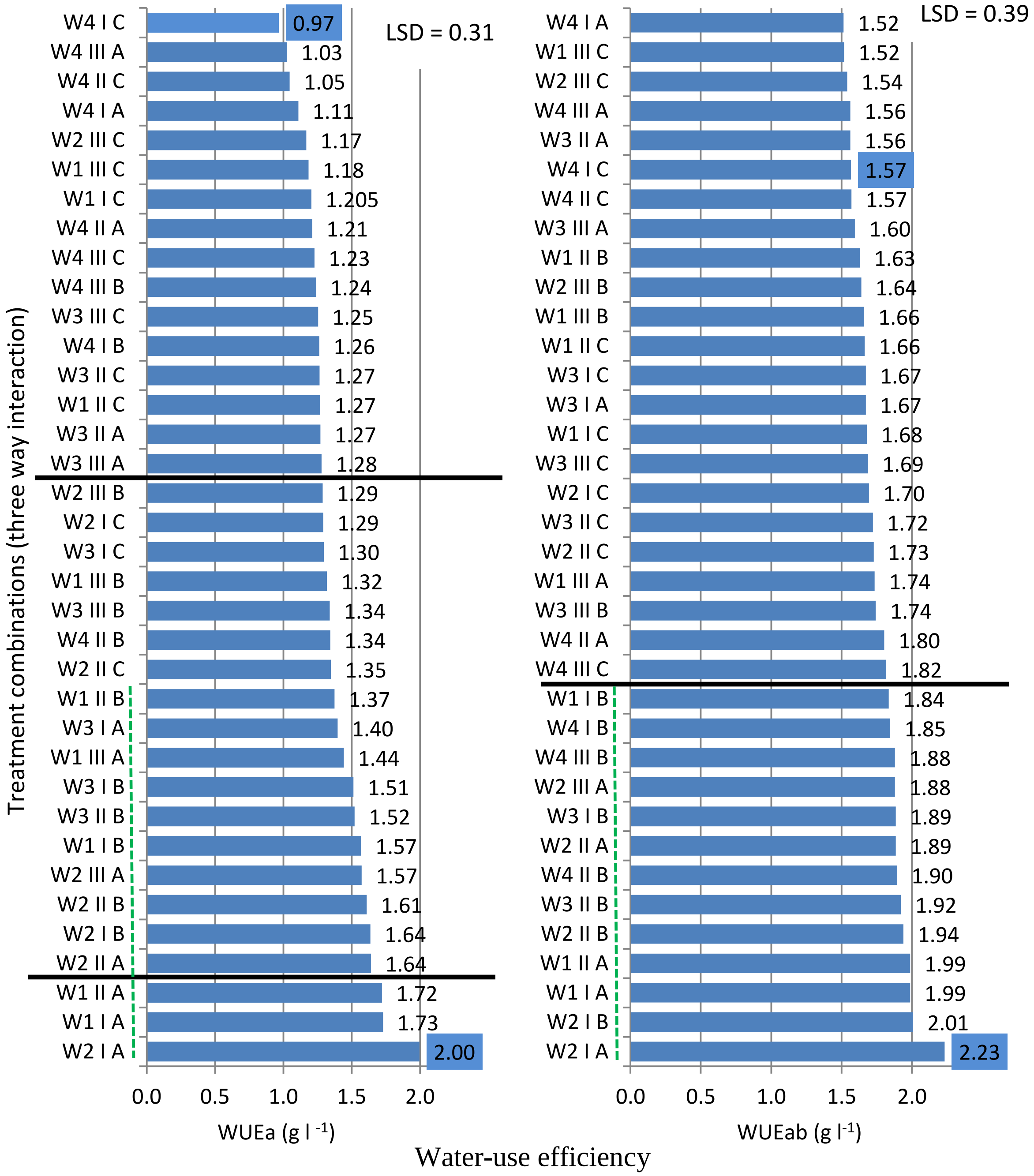


Figure 8.11: Mean (n = 4) WUE (g l^{-1}) for *Nenax microphylla* at the different water and defoliation treatment combinations. LSD = least significant difference. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve monthly or control.

8.3.2 *Pentzia incana*

8.3.2.1 Water-use efficiency for the three-monthly defoliation frequency (Frequency A)

Defoliation intensity accounted for most of the variation ($P < 0.001$) in WUEa and WUEab data (Table 8.6). Surprisingly, the influence of water on WUEa was insignificant ($P > 0.05$) and also very small ($P < 0.05$) on WUEab. The water x defoliation intensity interaction accounted for some of the variation ($P < 0.05$) in WUEa data, but not at WUEab ($P > 0.05$).

Table 8.6: ANOVA summary for the WUE of *Pentzia incana* for the three-monthly defoliation frequency (frequency A).

Frequency A		W		I		W x I	
Variable	Treatment	SS% and significance					
WUEa	A cum at end	5	NS	44	***	11	*
WUEab	A cum at end	11	*	26	***	9	NS

WUEa = water-use efficiency for total aboveground phytomass, WUEab = water-use efficiency for total above- plus belowground phytomass, A cum at end = cumulative aboveground phytomass at the end of the trial, W = water, I = defoliation intensity, F = defoliation frequency, NS = non-significant, SS% = sum of squares percentage, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

Although WUE at W2 was slightly higher than that of the other water treatments, water deficit had no significant ($P > 0.05$) influence on WUEa (Figure 8.12). Water deficit caused an increase in WUEab where the most severely water stressed (W4) plants had a significantly ($P < 0.001$) higher WUE than that of the unstressed (W1) plants. Water-use efficiency increased significantly ($P < 0.001$) with increased defoliation intensity for both WUEa and WUEab.

Water-use efficiency at WUEab, where the root fraction was included in WUE calculation, was higher than at WUEa. Inclusion of the root phytomass exponentially improved WUEab along the water deficit gradient. This could be related to higher root:shoot ratio's with increased water deficit, as reported in Chapter 6. Both the severest stressed water and defoliation intensity treatments lead to higher WUE, indicating the ability of *P. incana* to compensate for water stress conditions by improving its WUE.

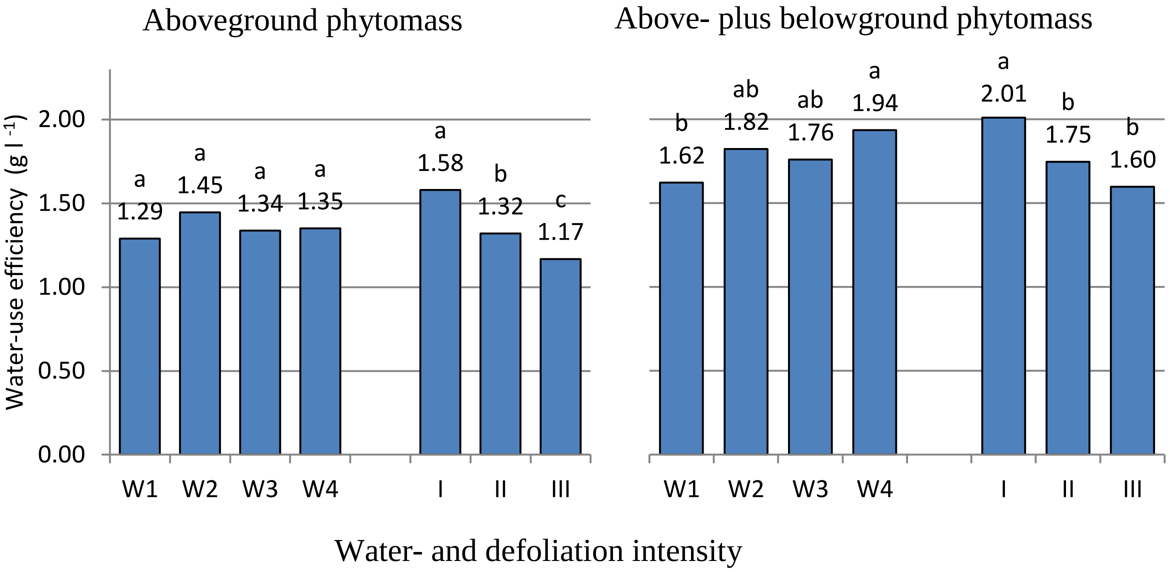


Figure 8.12: Mean (n = 6) water-use efficiency (g l^{-1}) for *Pentzia incana* for the three-monthly defoliation frequency (frequency A). The two main effects (water and defoliation intensity) are indicated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences (P < 0.001) within treatments.

The water x defoliation intensity interaction had a small (P > 0.05), but notable influence on WUEa (Figure 8.13). Although this interaction was non-significant (P > 0.05) for WUEab it is also presented (Figure 8.13). The darker black horizontal lines in Figure 8.13 indicate the best LSD groupings (t-distribution test, Gupta and Panchapakesan 1979). The treatment combinations below the horizontal line represent the best group regarding WUE. This is the group of treatment combinations that gave the highest WUEa and WUEab without differing significantly from each other (P < 0.001).

For WUEa, where water deficit had no influence on WUE, defoliation intensity clearly dominated the ranking of this data from the largest to the smallest (Figure 8.13). Defoliation intensity I (most severely defoliated) had the highest WUE and was present in the best WUE group in combination with the unstressed water treatments (W1 and W2), while the weakest group with the lowest WUE contained all the water treatments in combination with defoliation intensity III (the least severe intensity).

The best WUEab group (below the black line) included all the W4 (most severely water stressed) treatments and all the defoliation intensity I (most severe defoliation intensity) treatments. This phenomenon strengthens the perception that *P. incana* has the ability to improve its WUE under water and defoliation stress conditions.

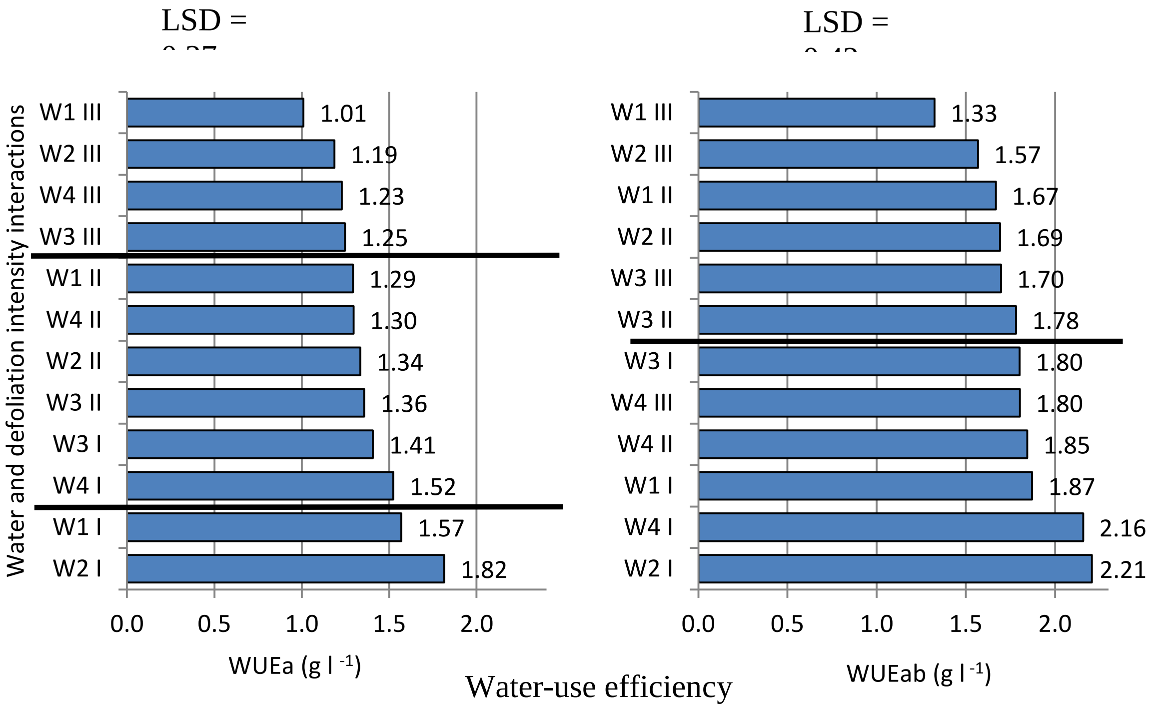


Figure 8.13: Mean (n = 4) water-use efficiency (WUE) (g l^{-1}) for *Pentzia incana* for the three-monthly defoliation frequency (frequency A). The interactions between water and defoliation intensity treatments are indicated. LSD = least significant difference. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm.

8.3.2.2 Water-use efficiency for the six-monthly defoliation frequency (Frequency B)

Water availability and defoliation intensity accounted for almost equal amounts of variation in the WUEa data (Table 8.7). For WUEab the impact of Intensity was much lower ($P < 0.05$), while water had the greatest ($P < 0.001$) influence by accounting for most of the variation in WUEab data. The influence of the water x intensity interaction was insignificant ($P > 0.05$).

Table 8.7: ANOVA summary for the WUE of *Pentzia incana* for the six-monthly defoliation frequency (frequency B).

Frequency B		W		I		W x I	
Variable	Treatment	SS% and significance					
WUEa	B cum at end	23	***	22	***	5	NS
WUEab	B cum at end	35	***	10	*	5	NS

WUEa = water-use efficiency for total aboveground phytomass, WUEab = water-use efficiency for total above- plus belowground phytomass, B cum at end = cumulative aboveground phytomass at the end of the trial, W = water, I = defoliation intensity, F = defoliation frequency, NS = non-significant, SS% = sum of squares percentage, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

Water-use efficiency shows a slight increase ($P < 0.001$) with decreased water availability, for both WUEa and WUEab (Figure 8.14). Increased defoliation intensity also resulted in increased ($P < 0.001$) WUEa and WUEab. The WUE of the unstressed water treatment was significantly lower ($P < 0.001$) than that of the most severely stressed water treatment, while the WUE of the highest defoliation intensity was significantly higher ($P < 0.001$) than that of the most lenient intensity.

There is a shift in impact of treatments on WUE in frequency B where the impact of water availability is getting more important than that of defoliation intensity, compared to frequency A, where the opposite happened. Plants defoliated four times at frequency A, caused higher defoliation stress than at frequency B where only two defoliations were applied. It seems that the plants reacted against the most detrimental treatment, by increasing WUE as a compensatory mechanism to improve survival ability.

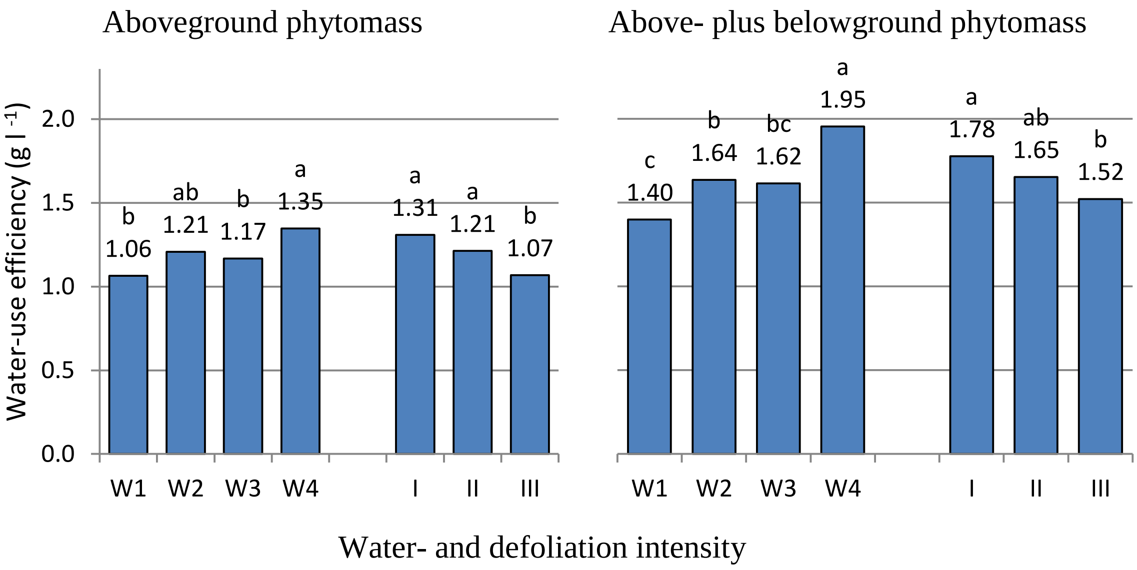


Figure 8.14: Mean (n = 6) water-use efficiency (WUE) (g l^{-1}) for *Pentzia incana* for the six-monthly defoliation frequency (frequency B). The two main effects (water and defoliation intensity) are indicated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences ($P < 0.001$) within treatments.

Water-use efficiency of the above- plus belowground phytomass was much higher ($P < 0.001$) than that of only the aboveground phytomass at the water x intensity interactions (Figure 8.15), emphasizing the importance of including root phytomass in WUE studies. The best group regarding WUEab (below black line) consisted of the most severe water stress (W4) treatments at all three defoliation intensities.

The very high WUEab at the W4 X I interaction, of 2.20 g l^{-1} is worth mentioning, as this is the combination of the two most harsh water and defoliation treatments. When the root phytomass was included in the calculation of WUE at this interaction, WUE increased from 1.54 g l^{-1} at WUEa to 2.20 g l^{-1} at WUEab. The plants exposed to this treatment combination, increased their root:shoot ratio (Chapter 6) as well as its WUE as a compensatory mechanism to counteract the applied stress from both water and defoliation treatments.

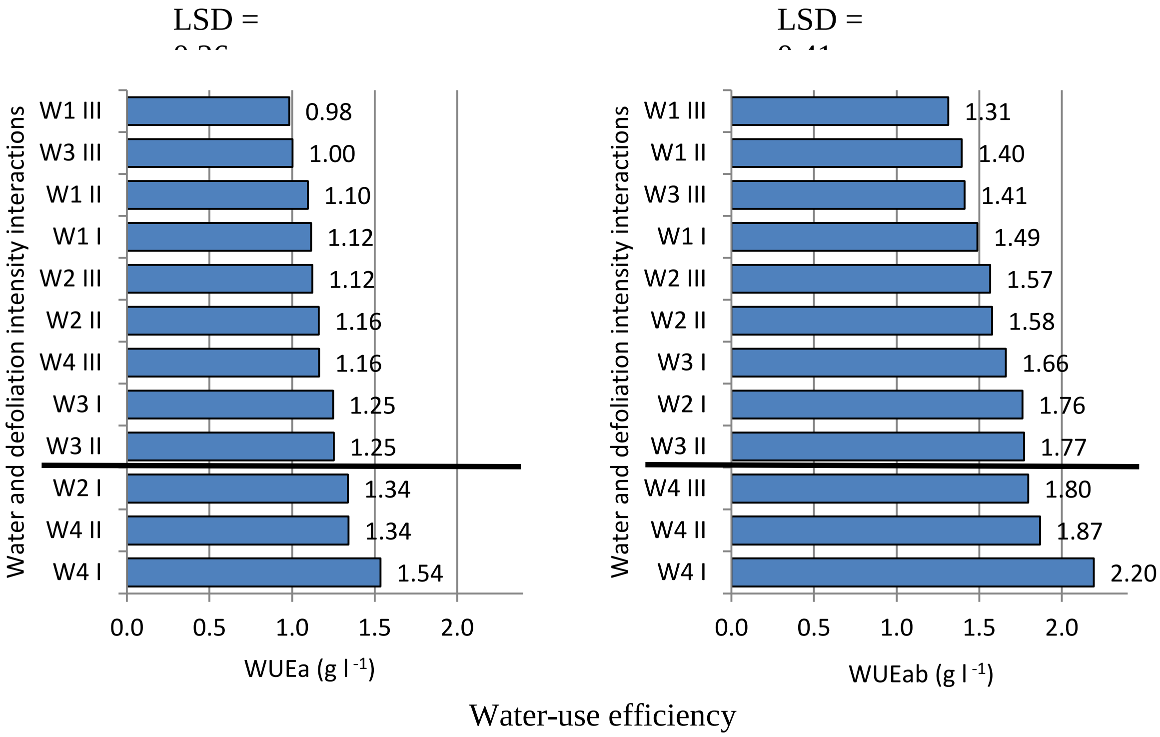


Figure 8.15: Mean (n = 6) water-use efficiency (WUE) (g l^{-1}) for *Pentzia incana* for the six-monthly defoliation frequency (frequency B). The interactions between water and defoliation intensity treatments are indicated. LSD = least significant difference. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm.

8.3.2.3 Water-use efficiency for the twelve-monthly defoliation frequency (Frequency C)

Water by far accounted for most of the variation in the WUE data for both WUEa and WUEab (Table 8.8). It is interesting to note that at frequency A, intensity accounted for most of the variation and at frequency B, both water and intensity played a role but with a subtle shift to water availability getting more important. By contrast, at frequency C water played the most significant role in WUE.

Table 8.8: ANOVA summary for the WUE of *Pentzia incana* for the twelve-monthly defoliation frequency (frequency C or control).

Frequency C		W		I		W x I	
Variable	Treatment	SS% and significance					
WUEa	C	49	***	4	NS	6	NS
WUEab	C	47	***	6	*	5	NS

WUEa = water-use efficiency for total aboveground phytomass, WUEab = water-use efficiency for total above- plus belowground phytomass, C = phytomass at the end of the trial after 12 months, W = water, I = defoliation intensity, F = defoliation frequency, NS = non-significant, SS% = sum of squares percentage,
*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

The plants from frequency C were defoliated once at the start of the trial and again 12 months later at the end of the trial. For that reason both WUEa and WUEab showed no significant ($P > 0.05$) differences between defoliation intensity treatments (Figure 8.16). It is however worth mentioning that WUEab was slightly lower at the most lenient defoliation intensity (III) than at the two harsher defoliation intensities, which might be a carry-over effect of the defoliation treatment of 12 months earlier. If this is true, it indicates that the plants compensated for the defoliation stress over the twelve month period, by increasing WUE.

Increased water deficit, however, caused increased ($P < 0.001$) WUEa and WUEab. For both WUEa and WUEab the most severely stressed water treatment (W4), had a significantly ($P < 0.001$) higher WUE than any of the other (less stressed) water treatments. With the root phytomass included in the calculation of WUE, it increased from 1.19 g l^{-1} at WUEa to 1.81 g l^{-1} at WUEab at W4 (the most severely stressed water treatment).

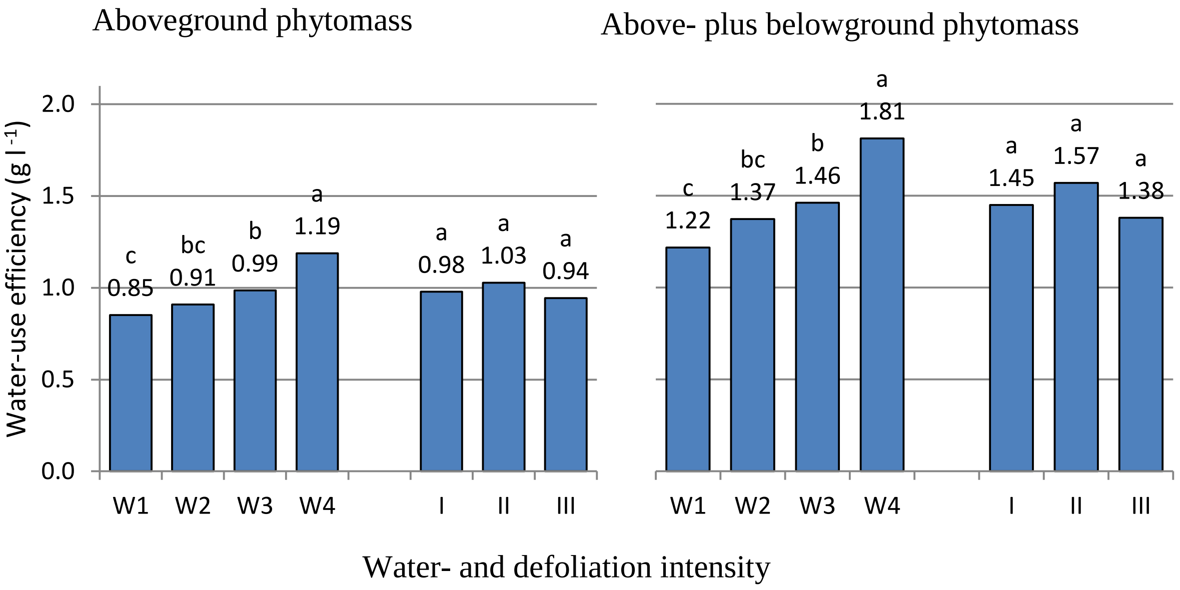


Figure 8.16: Mean (n = 6) water-use efficiency (WUE) (g l^{-1}) for *Pentzia incana* for the twelve-monthly defoliation frequency (frequency C). The two main effects (water and defoliation intensity) are indicated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences ($P < 0.001$) within treatments.

Water-use efficiency of the above- plus belowground phytomass was much higher than that of only the aboveground phytomass at the water x intensity interactions (Figure 8.17), highlighting the importance of including root phytomass in WUE studies. The best group, regarding WUEa and WUEab (below the black line), mainly consisted of the most severe water stress (W4) treatments in interaction with all three defoliation intensities. The unstressed water treatments (W1 and W2) in interaction with the most lenient defoliation intensity (intensity III) had the lowest values in the weaker WUE groups (above the black horizontal lines).

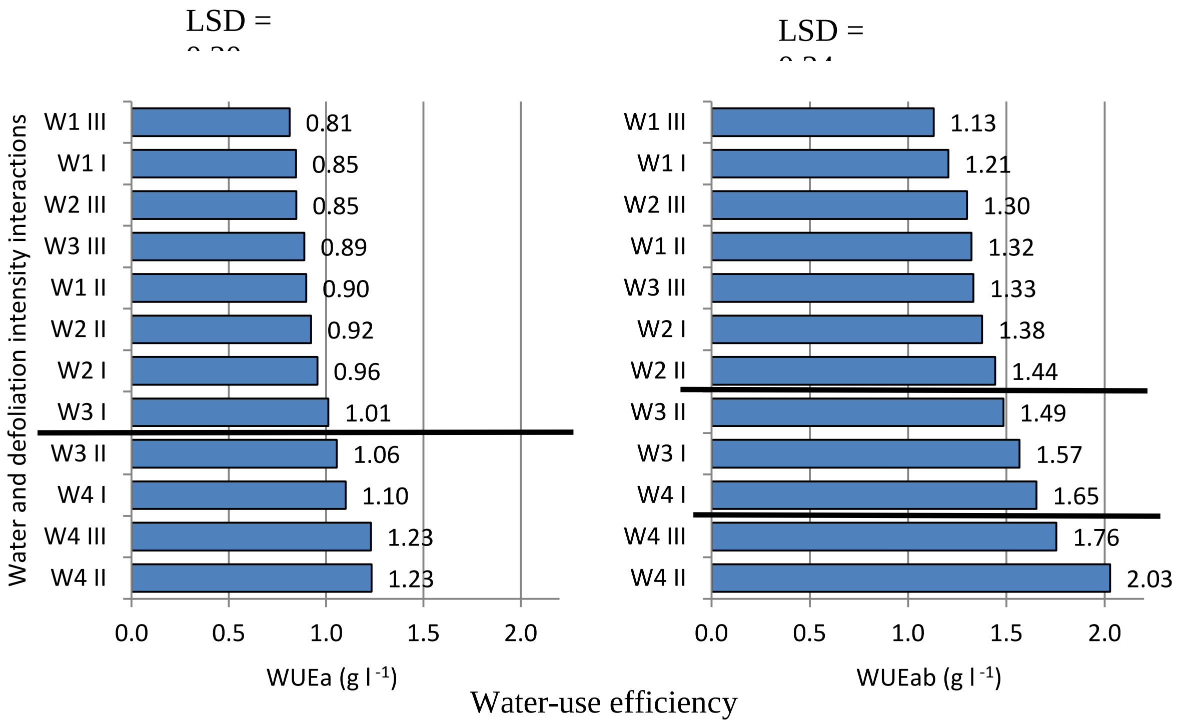


Figure 8.17: Mean (n = 6) water-use efficiency (WUE) (g l^{-1}) for *Pentzia incana* for the 12 monthly defoliation frequency (frequency C). The interactions between water and defoliation intensity treatments are indicated. LSD = least significant difference. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences (P < 0.001) within treatments.

8.3.2.4 Comparison of WUE for the three defoliation frequencies

Defoliation frequency accounted for most of the variation in WUEa data, while water availability and defoliation intensity also had a significant (P < 0.001) influence (Table 8.9). Water accounted for most of the variation in WUEab data, with defoliation frequency and intensity the second and third highest, respectively. For WUEa defoliation frequency had the highest influence on WUE, but at WUEab, when the root phytomass is included in WUE calculation, water availability became much more important.

Table 8.9: ANOVA summary for the WUE of *Pentzia incana* for a comparison between the three defoliation frequencies (A, B and C).

A,B,C compared		W		I		F		W x I		W x F		I x F		W x I x F	
Variable	Treatment	SS% and significance													
WUEa	A, B, C compared	9	***	**12**	***	**33**	***	2	NS	5	***	6	***	4	*
WUEab	A, B, C compared	**24**	***	8	***	**14**	***	1	NS	2	NS	4	*	4	NS

WUEa = water-use efficiency for total aboveground phytomass, WUEab = water-use efficiency for total above- plus belowground phytomass, A = Frequency A, B = Frequency B, C = Frequency C, W = water, I = defoliation intensity, F = defoliation frequency, NS = non-significant, SS% = sum of squares percentage, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

Water-use efficiency increased ($P < 0.001$) when water availability decreased for both WUEa and WUEab (Figure 8.18). The two intermediate water treatments (W2 and W3) did not differ ($P > 0.05$) regarding WUEa and WUEab, while all other water treatments differed significantly ($P < 0.001$). Increased defoliation intensity and defoliation frequency caused a significant ($P < 0.001$) increase in both WUEa and WUEab.

The general tendency in this comparison of the three defoliation frequencies was that WUE increased considerably with increased water stress and increased defoliation intensity and frequency (defoliation pressure). This indicates the ability of *P. incana* to use water more efficiently ($P < 0.001$) under unfavourable conditions regarding water availability and defoliation application.

When the root phytomass was included in WUE calculations, WUEab was much higher (better) than WUEa. The tendency of increased WUE efficiency under water deficit and grazing pressure remained the same. The WUEab of the most severely stressed water treatment (W4) was 35% higher than that of the unstressed treatment (W1), compared to the 22% difference at WUEa, indicating that the effect of water availability is more distinct when the root phytomass forms part of WUE calculations.

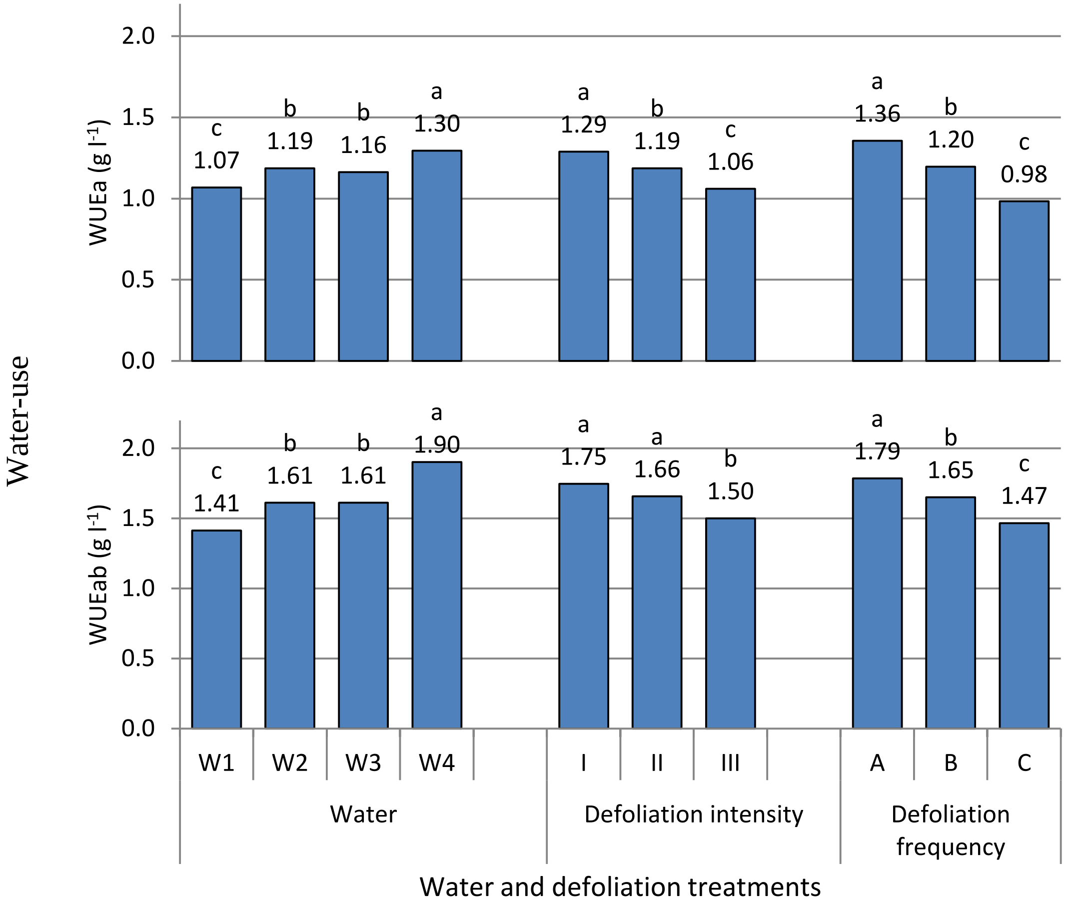


Figure 8.18: Mean (n = 6) water-use efficiency (WUE) (g l^{-1}) for *Pentzia incana* in comparing the three defoliation frequencies. The interactions between water and defoliation intensity treatments are indicated. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences (P < 0.001) within treatments.

The worst group (above the top black line) regarding WUEa includes almost all the defoliation frequency C (12-monthly) X water interactions, while the best group (below the bottom black line) includes almost all the defoliation frequency A (three-monthly) x water interactions (Figure

8.19). The same tendency occurred at WUEab, which has slightly more treatment combination in the best group, and which includes all the most severely water stressed (W4) combinations. The WUE of WUEab was once again far higher than that of WUEa.

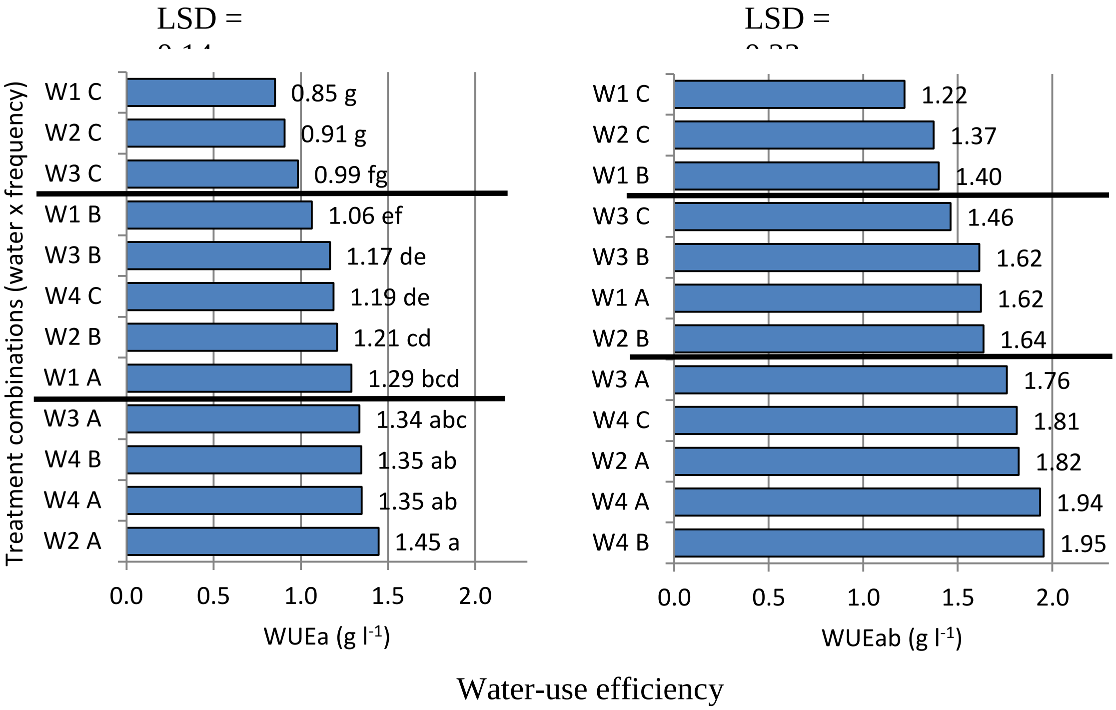


Figure 8.19: Mean (n = 6) water-use efficiency (WUE) (g l^{-1}) for *Pentzia incana* when comparing defoliation frequencies A, B and C. The interactions between water and defoliation frequency treatments are indicated. LSD = least significant difference. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences (P < 0.001) within treatments.

The results for WUEa and WUEab of all 36 water and defoliation treatments combinations are presented in Figure 8.20. Water-use efficiency was in general much higher at WUEab where the root phytomass was also included in the WUE calculation. This increase was roughly the same over all treatment combinations at an average of 32%, which is reasonably high. If this is compared to the root fraction (Chapter 6), it is interesting to note that on average the roots made up 28% of the total phytomass over all treatment combinations.

The W2 I A interaction (sufficient water, most severely defoliated) had a significantly ($P < 0.001$) higher WUEa than any other treatment combination, and although not significant, also the highest WUEab. This interaction did not show the same tendency regarding phytomass production (Chapter 6), where it had the lowest phytomass production of all the unstressed water treatments (W1 and W2).

No treatment combinations consisting of defoliation intensity III (most lenient intensity) were present in the group of best WUEab, while this group was also dominated by treatment combinations consisting of the most severely stressed water treatment (W4). This highlights the ability of *P. incana* to improve its WUE under water stress and grazing (defoliation) pressure.

With one exception, defoliation frequency C (defoliated only every 12 months), was not present in the group of best (group below black horizontal line) WUE for both WUEa and WUEab (indicated with the green dotted line in Figure 7.20), although defoliation frequency C had the highest phytomass production (Chapter 6). The reason could be that these plants were exposed to less stress, which allowed them to use water less economically.

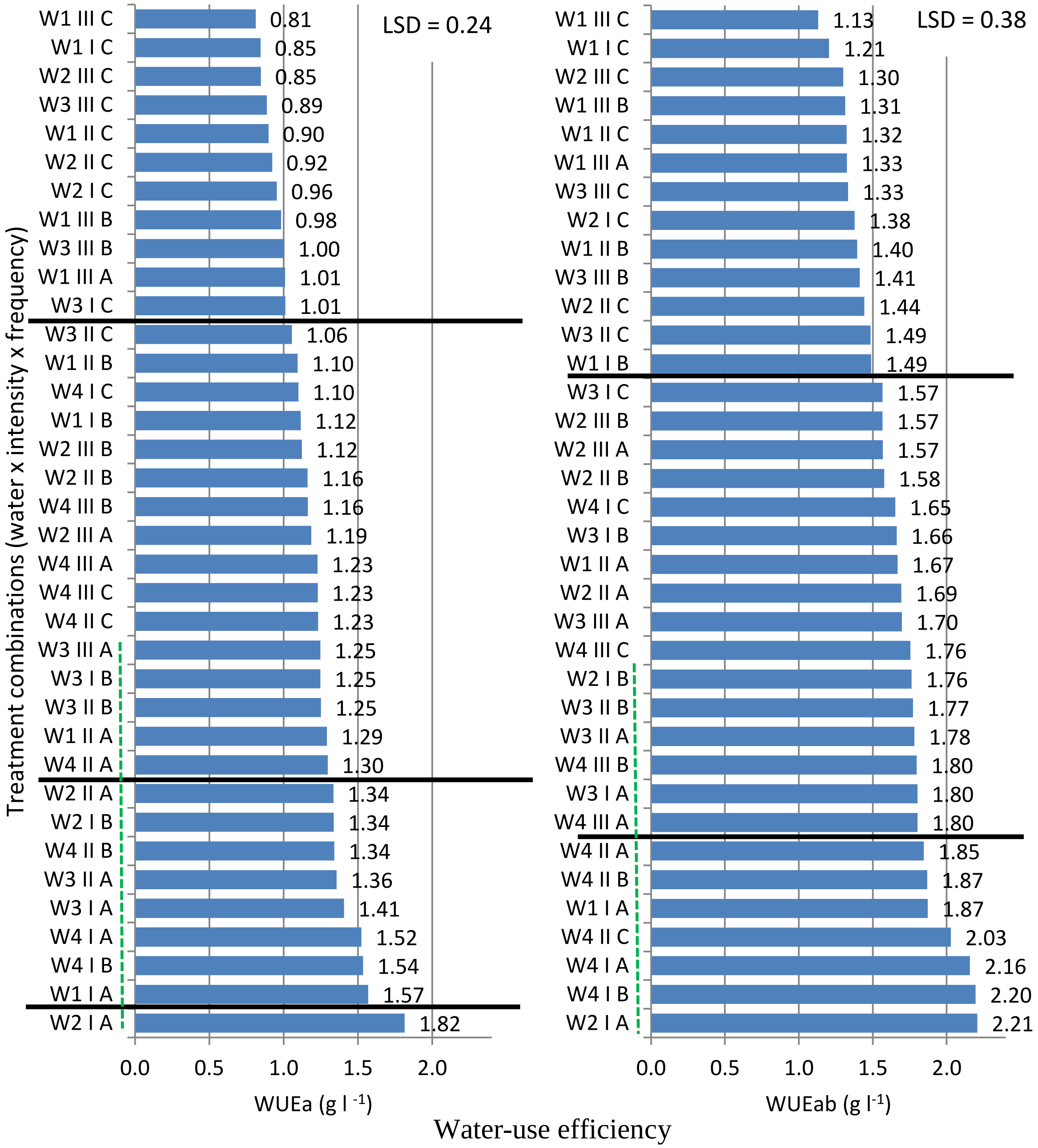


Figure 8.20: Mean (n = 4) WUE (g l^{-1}) for *Pentzia incana* at the different water and defoliation treatment combinations. LSD = least significant difference. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve monthly or control.

8.4 General discussion on the WUE for both *N. microphylla* and *P. incana*

In arid and semi-arid areas, where plants do not have any guaranteed water supply throughout the year, the utilization of water must be carefully planned (Snyman et al. 1997). For practical reasons, only evapotranspiration was measured in most rangeland areas, which makes the expression of WUE in terms of transpiration, as done in this study, one of the few available data sets for the drier areas.

In the past, researchers only included aboveground production of rangeland in estimating WUE and therefore this study is unique with regards to the root fraction also being included in WUE quantification. Water-use efficiency, for both shrub species, was in general much higher at WUEab where the root phytomass was also included in WUE calculation. This increase was roughly the same over all treatment combinations (water and defoliation) at an average of 32% for *P. incana* and 29% for *N. microphylla*, which is reasonably high. This indicates the importance of root studies to present the whole water use dynamics for clarifying the functioning of the rangeland ecosystem.

The grand WUE mean (mean over all treatments) per plant of *N. microphylla* was 1.76 g l^{-1}, which was slightly higher than the 1.63 g l^{-1} of *P. incana*. Unfortunately there is a lack of WUE data on Karoo shrubs to compare the two studied shrubs with. These values are however in line with findings of other researchers, for example: Hassen et al. (2007) measured WUEab of *Indigofera coerulea* at 1.18 g l^{-1} and that of *I. arrecta* at 1.67 g l^{-1}, while Stabler and Martin (2004) reported WUE values of 1.19 g l^{-1} for *Nerium oleander* and 0.52 g l^{-1} for *Leuclophyllum frutescens*.

The fact that WUE is expressed in so many different ways complicates/restricts the ability to properly relate the findings of this study with that of many other WUE studies over the world (Le Houérou 1984, Chapter 2 of this study). The different reactions of different plants, even within the same genus (Hassen et al. 2007), complicate the matter even further. Water-use efficiency values for grassland communities that were reported over the world ranged between

2.5 and 6.0 kg ha^{-1} mm^{-1} (Sala et al. 1988, Guevera et al. 2000, O'Connor et al. 2001, Holm et al. 2002, Ingram 2002, Snyman 2009). Palmar and Yunusa (2011) estimated WUE of South African arid rangelands (which largely include the Nama-karoo) at 1.6 kg ha^{-1} mm^{-1}, which is much lower than the abovementioned values for grasslands. Data from a very comprehensive review paper by Le Houérou (1984) also indicates a generally slightly higher rain-use efficiency (RUE) for grasslands than for arid rangelands.

From the literature there is however contradicting arguments in comparing WUE of grasses with that of shrubs, where Colluscio and Oesterheld (2007) argued that WUE of shrubs is generally better than that of grasses. By contrast, Emmerich (2007) reported that grass dominated ecosystems are more water use efficient than shrub dominated ecosystems. Other data indicated almost similar WUEab values for trees (*Acacia karroo*, *Colophospermum mopane*, *Combretum apiculatum*, *Dichrostachys cineria*) and grasses (Cymbopogon plurinoides, *Heteropogon contortus*, *Themeda triandra*) of around 4.5 g kg^{-1} (Illius et al. 2000). The large variation in WUE between individual grass species and between different rangeland condition classes could be a possible reason for these contradicting and confusing results. Snyman (2000) reported that semi-arid rangeland in good condition could have a WUE of more than two times higher than that of semi-arid rangeland in poor condition. Snyman (1994) reported that the WUE of the cultivated pasture species *Chloris gayana* can be as high as 7.2 kg DM ha^{-1} mm^{-1} yr^{-1}, compared to the 4.0 kg DM ha^{-1} mm^{-1} yr^{-1} of *Anthepora pubescens*, while Marais et al. (2006) measured the WUE of *Pennisetum clandestinum* at 7.2 kg DM ha^{-1} mm^{-1} yr^{-1}, compared to 22.5 kg DM ha^{-1} mm^{-1} yr^{-1} for *Cenchrus cilliaris*. These cultivated pasture species WUE values compared well with that of cactus pears of 7.87 kg DM ha^{-1} mm^{-1} yr^{-1} (Snyman 2013).

In further scrutinising the literature another possible and maybe a more relevant reason for the differences in WUE between grass and woody vegetation was found. Plants following the C_4 photosynthetic pathway usually have a higher WUE than the C_3 plants (Ripley et al. 2007, Hendrickson et al. 2013). Although some grass species make use of the C_3 photosynthetic pathway, most grass species are reckoned as C_4 plants. Most Nama-karoo shrubs are C_3 plants

(Midgley and van der Heyden 1999). Furthermore, CAM (crassulacean acid metabolism) plants possess the ability to use water even more efficiently than C_3 and C_4 plants (von Willert et al. 1985, Delatorre-Herrera et al. 2010), because they can shift between the different photosynthetic pathways. Although grouping plants as C_3 or C_4 plants might better explain WUE differences between plant life forms, differences might still occur, for example water (Ripley et al. 2007) and nitrogen (Felker et al. 1980, Golluscio and Oesterheld 2007) availability might further influence the plants metabolism. In comparing a C_3 and C_4 subspecies of *Alloteropsis semialata* (grass species from South Africa), Ripley et al. (2007) found that WUE was significantly higher in the C_4 than in the C_3 subspecies. This advantage of the C_4 subspecies was, however, diminished during severe drought conditions, due to greater metabolic limitation. This may partially explain the paradox of decreasing numbers of C_4 species during severe droughts, while the C_3 Karoo shrubs survive such conditions better.

In general, the two shrub species in this study also reacted differently to the three main effects, namely: water availability, defoliation intensity and defoliation frequency, regarding WUE. This discussion will therefore start with a short comparison between the WUE of the two species, regarding the three main effects.

Main effect: water deficit gradient

The WUE of the two species showed clear opposite trends along a water deficit gradient. Water-use efficiency of *N. microphylla* decreased ($P < 0.001$) with increased water stress, while that of *P. incana* increased ($P < 0.001$) with increased water stress.

The increased WUE of *P. incana* under water and defoliation stress, could be regarded as a compensatory mechanism to counteract the applied stress. Deng et al. (2006) also highlights that limited irrigation could induce compensatory WUE of some crops. The improved WUE in this study is rather to improve the survival ability than to impose higher phytomass production, as the phytomass production data does not show the same tendencies. Midgley and Mol (1993), however, reported an initial increase in water-use efficiency with increased water stress for *P. incana*, followed by a decrease in WUE the moment soil-water content drops below 5%. Their soil water ranged from 20% gravimetric soil-water content down to almost 0%. In this study, the lowest range (severely water stressed) was from 0 – 25 %, within which the WUE was always significantly higher than the less stressed water treatments (W1, W2, and W3). Midgley and Mol (1993) argues that the reduction in WUE at this extremely low soil-water content is a mechanism to defer lethal water stress.

Increased WUE under increased water stress is a common phenomenon in fodder plants, as reported by various researchers (Blum, 2005, Yin et al. 2005, Golluscio and Oesterheld 2007, Hassen et al 2007, Xu et al. 2011). Blum (2005), however, emphasizes the fact that higher WUE many times goes hand in hand with reduced yield potential. In this study it was also experienced for *P. incana* where the treatment combinations that had the highest WUE were also those with the lowest phytomass production.

Main effect: defoliation intensity and frequency

For *N. microphylla*, WUE was not that much affected by increased defoliation intensity, and tend to show a small but mostly non-significant ($P > 0.05$) increase in WUE with increased defoliation intensity. For *P. incana* there was a steep increase ($P < 0.001$) in WUE with increased defoliation intensity, especially at three- and six-monthly defoliations. At the twelve-monthly defoliation frequency the increased WUE with increased defoliation intensity was still significant ($P < 0.001$), but less prominent.

The WUE of *N. microphylla* was less affected by defoliation frequency, than that of *P. incana*. Water-use efficiency of *N. microphylla* increased slightly with increased defoliation frequency, with WUE at the most moderate defoliation frequency (every 12 months), being significantly lower than that of the two more frequently defoliated treatments (every three and six months). Water-use efficiency of *P. incana* increased significantly ($P < 0.001$), with increased defoliation frequency, over all three frequencies. Peng et al. (2007) explained that some plants have the ability to use water more economically, in an attempt to accommodate stress, when exposed to higher grazing intensities.

For *P. incana*, there was a distinct shift in WUE, with decreased defoliation frequency. At the three-monthly defoliation frequency (frequency A), defoliation intensity played a bigger role than water availability, while water availability had the biggest effect on WUE at frequency C (defoliated every 12 months). This indicates how the plant protects itself, by increased WUE, against the factor that is causing the most harm to its survival at a given time.

The different reactions, regarding WUE especially at increased water stress, of *N. microphylla* (WUE decreased) and *P. incana* (WUE increased) corresponds well with the fact that the WUE of different plant species are affected differently by varying watering and defoliation treatments (Snyman et al. 1997, Stabler and Martin 2004, Housman et al. 2006, Marais et al. 2006, Hassen et al. 2007, Xu et al. 2011, Evans et al. 2013). This might question expression of WUE at landscape level (Le Houérou 1984, Palmer and Yunusa 2011). In the Nama-karoo a single paddock may easily consist of more than 50 Karoo shrub species (Roux and Cowling 1987), with potentially varying water-use efficiencies, especially at varying water availability and defoliation pressure. With most of the plants having different WUE abilities and being present at different percentages (densities), it would be difficult to give an estimation of WUE at landscape level.

In this study both *N. microphylla* and *P. incana* showed increased WUE under increased defoliation stress, regardless of the watering treatment. The contrary was found by Stabler and Martin (2004) for two desert shrubs of which WUE was lowered by defoliation of well watered plants. Midgley and Mol (1993) also found decreased WUE for *P. incana* at higher water availability, which they postulate as a means in which the plant raises its competitive success. This rapid use of soil-water resources might divert the plants from its competing neighbours. Higher phytomass productions at increasing irrigation, many times go hand in hand with lower WUE for some cash crops (Deng et al. 2006).

With various factors influencing WUE, its measurements could be more refined in future studies, by comparing adult plants and seedlings (juveniles) (Donovan and Ehleringer 1992), monitoring different phenological stages of the plants (Snyman et al. 1997), including nitrogen availability and rooting depth (Golluscio and Oesterheld 2007), different times during the growing season (Zhu and Yang 2011) and the length at which plants were exposed to water stress (Ruppert et al. 2012).

The different efficiencies at which, for example *P. incana*, uses water is very interesting. It can arguably use a low WUE at high water availability to possibly outcompete neighbouring plants, through rapid growth with uneconomical water use. By contrast, it can increase its WUE when water gets limited, to increase its survival potential. This indicates how the plant can adapt through its WUE under varying water availability, to always ensure the optimal position for surviving any unfavourable climatic and defoliation conditions.

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CHAPTER 9

NUTRITIVE VALUES OF *Nenax microphylla* AND *Pentzia incana* AT DIFFERENT DEFOLIATION TREATMENTS ALONG A WATER DEFICIT GRADIENT

9.1 Introduction

Karoo vegetation is in general well known for its high nutritive value and its ability to produce marketable lambs straight from the veld. The benefit of a shrub/grass mixture vegetation is that highly nutritive forage is obtained from the shrub component in autumn and winter when the nutritive value of the grasses are very low. In the past nutritive values were measured for several Karoo shrub species, of which the data was used for development of grazing management systems (Vorster and Roux 1983). Most of these measurements were done as a once off study by just selecting and harvesting some plants randomly for laboratory analyses of certain nutritive qualities, while others repeated these measurements on plant material harvested over the four seasons. There is a lack of nutritive value information where different intensities and frequencies of defoliation are built into the management of Karoo shrub vegetation. In this study, analyses for certain nutritive qualities were done on edible plant material that was harvested at different defoliation intensities and defoliation frequency, along a water deficit gradient.

As in the case of most plant nutritive value studies, the measured nitrogen content is also expressed in this study as crude protein (CP) percentage. This was analysed for the edible phytomass (leaves and stems with a diameter of 2 mm and smaller (Botha and Nash 1990, du Toit 1996)). This percentage, together with the edible phytomass production itself was used to determine the CP production per plant (g CP plant^{-1}) for the edible fraction. Crude protein (%) was also determined for the fine roots (diameter ≤ 1 mm).

Neutral detergent fibre (NDF) and in vitro dry matter digestibility (IVDMD) were also measured as percentages. Unfortunately the nutritive value information cannot also be expressed in terms of nitrogen-use efficiency where for example CP production is expressed in terms of the amount of water used. The reason for this is that only the edible fraction of the shrubs was analysed for N and not the whole plant, as is usually done for nitrogen-use efficiency studies (Field et al. 1983).

The procedures that were followed for these analyses are described in detail in Chapter 3 (Material and Methods). Results are presented firstly for *N. microphylla* and then for *P. incana*, after which a general discussion will follow where both species will be included.

9.2 Results and discussion

Results are presented on the basis of defoliation frequency. Within each frequency the nutritive values will be presented for the different water treatments and for the different defoliation intensity treatments. Frequencies cannot be compared statistically, as analyses were done only on the harvested phytomass on each predetermined defoliation date. When the defoliation treatments were applied, the amount of edible phytomass was very low for the most severely water stressed treatment (W4) and also for the defoliation after the winter (A3), with little or no growth taking place during the winter. There was therefore only enough plant material to determine CP content, but not enough to determine NDF and IVDMD. The tables for data presentation of NDF and IVDMD will therefore not contain any data for treatments W4 and A2 (Tables 9.2 and 9.9).

9.2.1 *Nenax microphylla*

9.2.1.2 Nutritive values for the three-monthly defoliation frequency (Frequency A)

The mean CP content of the edible phytomass for the tree-monthly defoliation frequency was 13.28%. There was a significant ($P < 0.001$) increase in CP with increased water stress for the autumn (A1) and summer (A4) defoliations (Table 9.1). By contrast, CP decreased ($P < 0.001$) with increased water stress at the winter defoliation, while CP content was not affected ($P > 0.05$) by water availability at the spring defoliation. Despite the general tendency of increased CP content with increased water stress, the CP production (g CP plant^{-1}) decreased significantly with increased water stress (Table 9.1).

In general, CP content increased ($P < 0.001$) with increased defoliation intensity, while the CP production was not noticeably affected (Table 9.1). Crude protein content was the highest at autumn (A1) and winter (A2) ($P < 0.001$) defoliations and the lowest ($P < 0.001$) at the summer (A4) defoliation. The edible phytomass production (Chapter 6) was however the highest at A4, which is reflected in the CP production of 2.1 g plant^{-1} which is significantly ($P < 0.001$) higher than any of the other defoliation dates.

The CP content of the fine roots (diameter ≤ 1 mm) was on average 10.70% compared to 13.28% for the edible phytomass. Crude protein content of the roots at W1 was lower ($P < 0.001$) than that of the other water treatments, while defoliation intensity caused no marked differences ($P > 0.05$) in root CP (Table 9.1). The root CP content of defoliation intensity III was slightly ($P > 0.05$) lower than that of the other defoliation intensities.

Table 9.1: Crude protein (mean ± SD) values (% and g plant^{-1}) for *Nenax microphylla* along a water deficit gradient at three defoliation intensities for the three-monthly defoliation frequency.

Variable	Water treatments				Defoliation intensity			Defoliation date
	W1	W2	W3	W4	I	II	III	Time
A1								**A1**
CP (%)	11 c	11 c	14 b	20 a	15 a	14 ab	13 b	14 b
	(±2.52)	(±1.74)	(±1.36)	(±1.45)	(±2.90)	(±4.18)	(±3.46)	(±3.82)
CP (g)	1.5 ab	1.7 a	1.2 b	0.5 c	1.3 a	1.3 a	1.1 a	1.3 c
	(±0.48)	(±0.36)	(±0.32)	(±0.40)	(±0.53)	(±0.61)	(±0.59)	(±0.57)
A2								**A2**
CP (%)	17 a	16 a	13 b	14 b	18 a	14 b	13 b	15 a
	(±2.43)	(±2.78)	(±3.16)	(±1.71)	(±2.34)	(±2.23)	(±2.22)	(±2.88)
CP (g)	0.4 ab	0.5 a	0.2 b	0.2 b	0.3 a	0.4 a	0.4 a	0.4 d
	(±0.22)	(±0.36)	(±0.12)	(±0.08)	(±0.16)	(±0.24)	(±0.36)	(±0.27)
A3								**A3**
CP (%)	12 a	13 a	13 a	14 a	14 a	12 b	12 b	13 c
	(±1.76)	(±1.62)	(±1.31)	(±0.92)	(±1.28)	(±1.36)	(±1.36)	(±1.52)
CP (g)	2.8 a	2.3 a	1.1 b	0.5 b	1.7 ab	2.0 a	1.4 b	1.8 b
	(±0.77)	(±0.78)	(±0.67)	(±0.34)	(±0.95)	(±1.32)	(±1.01)	(±1.10)
A4								**A4**
CP (%)	10 b	10 b	12 a	13 a	12 a	11 a	11 a	11 d
	(±1.75)	(±1.30)	(±1.32)	(±1.03)	(±1.70)	(±1.75)	(±2.27)	(±1.94)
CP (g)	3.1 a	3.1 a	1.2 b	0.5 c	1.9 a	1.9 a	2.2 a	2.1 a
	(±0.79)	(±0.93)	(±0.60)	(±0.35)	(±0.96)	(±1.13)	(±1.77)	(±1.30)
CPr (%)	10 b	11 a	11 a	11 a	11 a	11 a	10 a	
	(±1.09)	(±0.88)	(±0.77)	(±0.54)	(±0.47)	(±0.89)	(±0.76)	

CP = crude protein, CPr = crude protein roots, A1 = May (autumn) defoliation, A2 = August (winter) defoliation, A3 = November (Spring) defoliation, A4 = February (summer) defoliation, W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed), I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences ($P < 0.001$) within a treatment

The mean NDF and IVDMD values for three-monthly frequency were 44% and 61%, respectively. In general both water deficit and defoliation intensity did not influence NDF and IVDMD significantly ($P > 0.05$) (Table 9.2).

Table 9.2: Neutral detergent fibre (mean ± SD) and *in vitro* dry matter digestibility (mean % ± SD) for *Nenax microphylla* along a water deficit gradient at three defoliation intensities for the three-monthly defoliation frequency.

Variable	Water treatments			Defoliation intensity			Defoliation date
	W1	W2	W3	I	II	III	Time
A1							
NDF (%)	43 a (±3.62)	40 a (±3.79)	39 a (±0.59)	40 a (±2.73)	41 a (±3.95)	42 a (±3.85)	41 b (±3.82)
IVDMD (%)	60 a (±3.31)	60 a (±2.88)	61 a (±1.58)	62 a (±2.05)	62 a (±2.85)	60 a (±2.48)	60 a (±2.15)
A3							
NDF (%)	46 a (±4.99)	45 a (±4.16)	41 a (±5.71)	46 a (±3.30)	45 a (±5.52)	40 a (±4.44)	44 b (±3.96)
IVDMD (%)	53 a (±3.82)	54 a (±4.57)	59 a (±1.99)	55 a (±4.15)	55 a (±5.25)	56 a (±4.20)	55 b (±5.00)
A4							
NDF (%)	53 a (±6.02)	50 a (±4.74)	42 b (±4.14)	51 a (±6.60)	49 ab (±5.34)	45 b (±6.96)	48 a (±5.14)
IVDMD (%)	49 b (±3.54)	52 b (±4.01)	59 a (±3.24)	53 a (±5.70)	53 a (±3.91)	54 a (±5.99)	53 b (±4.06)

NDF = neutral detergent fibre, IVDMD = *in vitro* dry matter digestibility, A1 = May (autumn) defoliation, A2 = August (winter) defoliation, A3 = November (Spring) defoliation, A4 = February (summer) defoliation, W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed), I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences (P < 0.001) within a treatment

For the summer defoliation (A4), the severely water stressed (W3) plants had a significantly lower (P<0.001) NDF and higher (P < 0.001) IVDMD than the other water treatments (Table 9.2). The less severe defoliation intensity (III) also had a lower (P < 0.001) NDF value at 45% than that of defoliation intensity I at 51%. Defoliation intensity I, however, could have contained a higher stem fraction and less leaves, as it was only defoliated at a height of 50 mm. The summer defoliation also resulted in significantly higher (P < 0.001) NDF and lower (P < 0.001) IVDMD than that of the autumn defoliation.

9.2.1.3 Nutritive values for the six-monthly defoliation frequency (Frequency B)

The mean CP content was slightly lower than that of the three-monthly defoliation frequency (A), at 10.36% (Table 9.3). There was a slight, but significant ($P < 0.001$), increase in CP content with increased water stress. By contrast, there was a highly significant ($P < 0.001$) decrease in CP production with increased water stress.

Table 9.3: Crude protein (mean ± SD) values (% and g plant^{-1}) for *Nenax microphylla* along a water deficit gradient at three defoliation intensities for the six-monthly defoliation frequency.

Variable	Water treatments				Defoliation intensity			Defoliation date
	W1	W2	W3	W4	I	II	III	Time
B1								
CP (%)	11 b	11 b	11 b	13 a	12 a	11 ab	10 b	11 a
	(±2.84)	(±1.04)	(±1.55)	(±0.64)	(±1.16)	(±1.67)	(±2.08)	(±1.87)
CP (g)	3.3 a	3.3 a	2.6 a	0.8 b	3.0 a	2.5 ab	2.0 b	2.5 a
	(±1.16)	(±0.95)	(±0.88)	(±0.54)	(±1.49)	(±1.26)	(±1.10)	(±1.10)
B2								
CP (%)	8 c	8 c	10 b	12 a	9 a	9 a	10 a	9 b
	(±0.87)	(±0.73)	(±1.55)	(±1.51)	(±1.55)	(±1.83)	(±2.40)	(±1.88)
CP (g)	4.5 a	3.4 b	1.9 c	1.0 d	3.2 a	2.8 b	2.0 c	2.7 a
	(±1.03)	(±0.72)	(±0.87)	(±0.44)	(±1.76)	(±1.22)	(±1.34)	(±1.16)
CPr (%)	10 b	11 c	12 a	12 a	11 a	11 a	10 a	
	(±1.17)	(±1.09)	(±1.18)	(±0.91)	(±1.24)	(±1.13)	(±1.21)	

CP = crude protein, CPr = crude protein roots, B1 = August defoliation (after the first six months), B2 = February defoliation (after second six months), W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed), I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences ($P < 0.001$) within a treatment

The summer defoliation (B2) had significantly ($P < 0.001$) lower CP (9%) than B1 (11%), but the CP production was virtually the same ($P > 0.05$) (Table 9.3). The CP content of the fine roots was not ($P > 0.05$) influenced by defoliation intensity, although that of intensity III was slightly lower than the more severe defoliation intensities I and II. Increased water stress resulted in a distinct increase ($P < 0.001$) in fine root CP. The mean CP content of the fine roots was 11.25%, which was slightly higher than the 10.36% of the edible phytomass.

The edible phytomass production of W4 at B1 was very small and only enough for CP content analyses, therefore there are no NDF and IVDMD values available (Table 9.4). Defoliation intensity had no significant ($P > 0.05$) influence on NDF and IVDMD. Neutral detergent fibre declined significantly ($P < 0.001$) with increased water stress at B2 (summer defoliation). The IVDMD of W4 (most severely water stressed) was the highest at 55%.

Table 9.4: Neutral detergent fibre (mean % ± SD) and *in vitro* dry matter digestibility (mean % ± SD) for *Nenax microphylla* along a water deficit gradient at three defoliation intensities for the six-monthly defoliation frequency.

Variable	Water treatments				Defoliation intensity			Defoliation date
	W1	W2	W3	W4	I	II	III	Time
B1								
NDF (%)	41 a (±5.30)	45 a (±4.77)	37 a (±2.74)		42 a (±5.34)	40 a (±6.26)	41 a (±4.56)	41 b (±4.86)
IVDMD (%)	57 a (±4.57)	55 a (±4.47)	60 a (±2.57)		58 a (±5.50)	58 a (±4.65)	57 a (±3.15)	57 a (±3.86)
B2								
NDF (%)	54 a (±4.78)	56 a (±5.80)	49 b (±4.22)	45 c (±1.63)	53 a (±5.66)	50 a (±5.01)	50 a (±7.03)	53 a (±5.55)
IVDMD (%)	45 b (±3.94)	46 b (±6.05)	48 b (±5.38)	55 a (±1.47)	47 a (±6.04)	50 a (±5.12)	49 a (±6.23)	46 b (±5.84)

NDF = neutral detergent fibre, IVDMD = *in vitro* dry matter digestibility, B1 = August defoliation (after the first six months), B2 = February defoliation (after second six months), W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed), I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences ($P < 0.001$) within a treatment

In comparing the two defoliation dates, the NDF content was higher ($P < 0.001$) at B2 than at B1, while IVDMD was lower ($P < 0.001$). In general, NDF was slightly higher ($P > 0.05$) and IVDMD slightly lower ($P > 0.05$) than at the three-monthly defoliation frequency. This is an indication of the older growth (six months compared to three months of growth) containing more fibre, due to a higher woody (stem) component in the edible phytomass production.

9.2.1.4 Nutritive values for the defoliation after 12 months (Frequency C)

The mean CP content for frequency C was 8.10% which was much lower than that of frequency A (13.28%) and frequency B (10.36%). There was a notable increase ($P < 0.001$) in CP content with increased water stress (Table 9.5). The CP content of W1 was very low at 6%, almost half that of W4 (11%). The higher edible phytomass production (Chapter 5) of W1 contained a higher stem component than W4, as it was observed (but not recorded) that the internode lengths of the unstressed water treatments was much longer than that of the water stressed plants. This could be one possible reason for the lower CP values of the faster growing plants. The CP production of W1 and W2 was, however, higher ($P < 0.001$) than that of W3 and W4, which had a higher ($P < 0.001$) CP content.

Table 9.5: Crude protein (mean ± SD) values (% and g plant^{-1}) for *Nenax microphylla* along a water deficit gradient at three defoliation intensities for the twelve-monthly defoliation frequency.

Variable	Water treatments				Defoliation intensity		
	W1	W2	W3	W4	I	II	III
CP (%)	6 c	7 c	8 b	11 a	8 b	8 b	9 a
	(±0.83)	(±0.66)	(±0.82)	(±1.42)	(±1.70)	(±2.06)	(±1.86)
CP (g)	6.0 a	5.7 a	3.8 b	1.1 c	4.7 a	4.5 a	3.3 b
	(±1.10)	(±1.16)	(±1.14)	(±0.47)	(±2.38)	(±2.18)	(±1.80)
CPr (%)	8 b	9 b	10 a	11 a	10 a	10 a	9 a
	(±0.75)	(±1.36)	(±0.94)	(±0.60)	(±1.47)	(±1.53)	(±1.49)

CP = crude protein, CPr = crude protein roots, W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed), I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences ($P < 0.001$) within a treatment

Crude protein content of the fine roots increased ($P < 0.001$) with increased water stress, but stayed the same ($P > 0.05$) for all defoliation intensities (Table 9.5). The mean CP content of the fine roots at 9.50% was slightly higher ($P > 0.05$) than that of the edible phytomass at 8.10%.

Defoliation intensity had no marked ($P > 0.05$) influence on NDF and IVDMD. Water deficit stress caused a decrease ($P < 0.001$) in NDF, accompanied by a slight increase in IVDMD (Table 9.6). In general NDF was higher and IVDM lower than at defoliation frequencies A and B. This indicates that the least frequently defoliated plants (every 12 months) produced edible phytomass with a reduced quality, compared to the more frequently defoliated plants. The same argument is also true for CP content.

Table 9.6: Neutral detergent fibre (mean % ± SD) and *in vitro* dry matter digestibility (mean % ± SD) for *Nenax microphylla* along a water deficit gradient at three defoliation intensities for the twelve-monthly defoliation frequency.

Variable	**Water treatments**				Defoliation intensity		
	W1	W2	W3	W4	I	II	III
NDF (%)	59 a (±4.08)	58 a (±7.01)	55 ab (±3.07)	51 c (±1.72)	57 a (±4.58)	56 a (±4.57)	53 a (±4.62)
IVDMD (%)	42 ab (±4.33)	40 b (±5.86)	45 ab (±3.86)	47 a (±2.47)	44 a (±4.86)	42 a (±4.58)	45 a (±4.55)

NDF = neutral detergent fibre, IVDMD = *in vitro* dry matter digestibility, W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed), I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences ($P < 0.001$) within a treatment

9.2.1.5 Comparison between the three defoliation frequencies

Data of the three defoliation frequencies are summarized in Table 9.7 as a descriptive summary to indicate the differences in nutritive values between the frequencies. In general, the edible phytomass of the more frequently defoliated plants (frequency A) had a higher total nutritive value than that of the least frequently (frequency C) defoliated plants. The CP content of the fine roots was slightly higher at the more frequently defoliated plants.

Table 9.7: Summary of the average nutritive values (% and g plant^{-1}) of *Nenax microphylla* at three defoliation frequencies

Variable	Defoliation frequency		
	A	B	C
CP (%)	13.3	10.4	8.1
CP (g)	7.8	7.6	6.0
CPr (%)	10.7	11.3	9.5
NDF (%)	44	46	56
IVDMD (%)	56	53	43

CP = crude protein, CPr = crude protein roots, NDF = neutral detergent fibre, IVDMD = *in vitro* dry matter digestibility, A = three-monthly, B = six-monthly, C = twelve-monthly

9.3.1 *Pentzia incana*

9.3.1.2 Nutritive values for the three-monthly defoliation frequency (Frequency A)

The mean CP content of the edible phytomass for the tree-monthly defoliation frequency was 14.85%. With the exception of the winter defoliation (A2), CP content increased significantly ($P < 0.001$) with increased water stress (Table 9.8). The increased CP content coincides with a general decrease in CP production, which was most noticeable ($P < 0.001$) at the summer defoliation (A4). Crude protein content at defoliation intensity I was higher ($P < 0.001$) than intensities II and III at all defoliation dates, but not for the autumn (A2) defoliation where it was the same ($P > 0.05$) for all three defoliation intensities. The CP production was also the highest ($P < 0.001$) at intensity I and decreased ($P < 0.001$) with decreased defoliation intensity (Table 9.8).

There was a significant ($P < 0.001$) increase in CP production from A1 (autumn defoliation) to A4 (summer defoliation), while the CP content varied between dates, but without a clear trend (Table 9.8). The increased CP production correlates well with the increased edible phytomass production at the summer (A4) defoliation (Chapter 6).

Table 9.8: Crude protein (mean ± SD) values (% and g plant^{-1}) for *Pentzia incana* along a water deficit gradient at three defoliation intensities for the three-monthly defoliation frequency.

Variable	Water treatments				Defoliation intensity			Defoliation date
	W1	W2	W3	W4	I	II	III	Time
A1								**A1**
CP (%)	9 c	9 c	12 b	17 a	12 a	12 a	12 a	12 d
	(±1.03)	(±0.78)	(±2.50)	(±1.93)	(±3.73)	(±3.62)	(±3.86)	(±3.69)
CP (g)	1.4 a	1.3 a	1.3 a	0.6 b	1.3 a	1.2 b	1.0 c	1.1 c
	(±0.35)	(±0.23)	(±0.27)	(±0.24)	(±0.42)	(±0.36)	(±0.39)	(±0.41)
A2								**A2**
CP (%)	20 a	20 a	20 a	20 a	22 a	20 b	17 c	20 a
	(±3.92)	(±3.08)	(±1.95)	(±2.61)	(±1.99)	(±2.47)	(±2.20)	(±2.96)
CP (g)	1.5 a	1.5 a	1.0 b	0.6 b	1.0 a	1.3 a	1.2 a	1.2 c
	(±0.49)	(±0.37)	(±0.36)	(±0.22)	(±0.5)	(±0.49)	(±0.58)	(±0.53)
A3								**A3**
CP (%)	13 c	14 bc	15 b	17 a	16 a	15 a	14 b	15 b
	(±1.55)	(±1.65)	(±1.07)	(±1.78)	(±1.90)	(±1.91)	(±2.13)	(±2.11)
CP (g)	2.2 a	2.2 a	1.8 a	0.9 b	2.2 a	1.8 b	1.4 c	1.8 b
	(±0.65)	(±0.71)	(±0.62)	(±0.37)	(±0.82)	(±0.76)	(±0.58)	(±0.80)
A4								**A4**
CP (%)	10 c	11 c	14 b	18 a	15 a	13 b	13 b	13 C
	(±2.18)	(±2.09)	(±1.92)	(±1.81)	(±3.08)	(±4.02)	(±3.66)	(±3.66)
CP (g)	2.5 a	2.3 ab	2.1 b	1.0 c	2.4 a	1.9 b	1.6 b	2.0 a
	(±0.83)	(±0.61)	(±0.63)	(±0.42)	(±0.99)	(±0.80)	(±0.61)	(±0.87)
CPr (%)	11 a	11 a	11 a	11 a	11 a	11 a	11 a	
	(±0.97)	(±1.18)	(±1.15)	(±1.10)	(±1.17)	(±0.76)	(±1.24)	

CP = crude protein, CPr = crude protein roots, A1 = May (autumn) defoliation, A2 = August (winter) defoliation, A3 = November (Spring) defoliation, A4 = February (summer) defoliation, W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed), I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences (P < 0.001) within a treatment

Crude protein content of the fine roots was the same at 11% for all water and defoliation treatments (Table 9.8). This was lower than the average CP content of 13.25% of the edible phytomass at the same defoliation date (A4).

In general, with one or two exceptions, both NDF and IVDMD were mostly not significantly (P > 0.05) influenced by the water and defoliation treatments (Table 9.9). The general trend was however a decreased NDF percentage with increased water deficit. The decreased NDF resulted in increased IVDMD.

Table 9.9: Neutral detergent fibre (mean % ± SD) and *in vitro* dry matter digestibility (mean % ± SD) for *Pentzia incana* along a water deficit gradient at three defoliation intensities for the three-monthly defoliation frequency.

Variable	Water treatments			Defoliation intensity			Defoliation date
	W1	W2	W3	I	II	III	Time
A1							
NDF (%)	46 a (±4.08)	45a (±5.11)	41 b (±3.06)	47 a (±5.20)	45 ab (±4.69)	41 b (±2.27)	44 a (±4.55)
IVDMD (%)	61 b (±3.86)	61 b (±5.21)	65 a (±2.39)	59 b (±4.65)	62 ab (±4.16)	65 a (±1.65)	62 b (±4.26)
A3							
NDF (%)	45 a (±3.77)	42 ab (±2.64)	40 b (±2.68)	44 a (±4.61)	42 a (±3.06)	42 a (±3.21)	42 a (±3.12)
IVDMD (%)	62 b (±3.44)	64 a (±1.43)	67 a (±1.68)	63 a (±3.46)	64 a (±3.00)	65 a (±3.03)	64 a (±2.86)
A4							
NDF (%)	46 a (±5.42)	49 a (±5.80)	43 a (±4.38)	50 a (±6.14)	44 ab (±3.58)	43 b (±4.34)	46 a (±5.14)
IVDMD (%)	57 a (±5.80)	56 a (±5.54)	61 a (±3.39)	55 a (±5.78)	58 a (±4.60)	61 a (±3.78)	58 c (±4.98)

NDF = neutral detergent fibre, IVDMD = *in vitro* dry matter digestibility, A1 = May (autumn) defoliation, A2 = August (winter) defoliation, A3 = November (Spring) defoliation, A4 = February (summer) defoliation, W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed), I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences (P < 0.001) within a treatment

For defoliation intensity, the general trend was increased NDF and decreased IVDMD with increased defoliation intensity (Table 9.9). The NDF values did not differ (P > 0.05) over seasons (defoliation dates), while the IVDMD was significantly (P < 0.001) lower for the summer (A4) defoliation.

9.3.1.3 Nutritive values for the six-monthly defoliation frequency (Frequency B)

The mean CP was slightly lower than that of the three-monthly (14.85%) defoliation frequency at 11.86%. There was a very distinct increase ($P < 0.001$) in CP with increased water stress, with CP at W4 almost double that of water treatment one (Table 9.10). The CP production was, however, more than two times less at W4 than at W1. The influence of defoliation intensity was mostly non-significant ($P > 0.05$), with CP production at defoliation intensity I being the highest 3.3 g plant^{-1}.

Table 9.10: Crude protein (mean ± SD) values (% and g plant^{-1}) for *Pentzia incana* along a water deficit gradient at three defoliation intensities for the six-monthly defoliation frequency.

Variable	Water treatments				Defoliation intensity			Defoliation date
	W1	W2	W3	W4	I	II	III	Time
B1								
CP (%)	10 d	11 c	13 b	17 a	12 b	12 b	14 a	13 a
	(±0.75)	(±1.56)	(±0.95)	(±2.59)	(±2.47)	(±2.64)	(±4.03)	(±3.15)
CP (g)	3.7 a	3.6 a	3.1 b	1.6 c	3.3 a	3.2 a	2.4 b	3.0 a
	(±0.49)	(±0.91)	(±0.76)	(±0.42)	(±1.11)	(±1.08)	(±0.82)	(±0.91)
B2								
CP (%)	8 c	9 c	12 b	16 a	11 a	11 a	11 a	11 b
	(±0.92)	(±2.27)	(±2.46)	(±1.71)	(±3.73)	(±3.99)	(±2.74)	(±3.77)
CP (g)	3.6 a	3.3 a	3.1 a	1.9 b	3.3 a	3.1 ab	2.5 b	3.0 a
	(±0.57)	(±0.62)	(±1.30)	(±0.54)	(±1.06)	(±1.15)	(±0.76)	(±1.02)
CPr (%)	10 a	10 a	11 a	11 a	11 a	11 a	10 a	
	(±0.88)	(±1.21)	(±0.96)	(±0.99)	(±1.03)	(±1.12)	(±1.25)	

CP = crude protein, CPr = crude protein roots, B1 = August defoliation (after the first six months), B2 = February defoliation (after second six months), W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed), I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences ($P < 0.001$) within a treatment

The winter defoliation (B1) had a higher ($P < 0.001$) CP content than that of the summer defoliation (B2), while the CP production was the same ($P > 0.05$) (Table 9.10). Crude protein content of the fine roots was not influenced by water availability or defoliation intensity (P > 0.05), but was at 10.50% slightly lower than that of the edible phytomass at 11.25%.

Neutral detergent fibre decreased (P < 0.001) with increasing water stress and with decreasing defoliation intensity (Table 9.11). By contrast, the IVDMD increased (P < 0.001) with water stress and decreased (P < 0.001) with increased defoliation intensity. Neutral detergent fibre was lower and IVDMD higher at B1 than at B2.

Table 9.11: Neutral detergent fibre (mean % ± SD) and *in vitro* dry matter digestibility (mean % ± SD) for *Pentzia incana* along a water deficit gradient at three defoliation intensities for the six-monthly defoliation frequency.

Variable	Water treatments				Defoliation intensity			Defoliation date
	W1	W2	W3	W4	I	II	III	Time
B1								
NDF (%)	48 a	48 a	47 a	40 b	50 a	46 ab	43 b	46 b
	(±2.42)	(±5.64)	(±4.74)	(±3.19)	(±4.91)	(±3.94)	(±4.17)	(±4.00)
IVDMD (%)	55 b	56 b	58 b	64 a	55 b	59 a	62 a	58 a
	(±3.07)	(±5.93)	(±4.56)	(±2.77)	(±5.26)	(±3.46)	(±3.90)	(±3.98)
B2								
NDF (%)	55 a	52 ab	48 bc	44 c	53 a	50 a	46 b	50 a
	(±3.54)	(±6.28)	(±3.77)	(±5.82)	(±4.88)	(±5.60)	(±6.93)	(±4.90)
IVDMD (%)	49 a	51 a	57 b	61 b	52 a	54 a	57 a	54 b
	(±3.35)	(±5.66)	(±3.41)	(±5.00)	(±5.45)	(±6.02)	(±7.05)	(±5.84)

NDF = neutral detergent fibre, IVDMD = *in vitro* dry matter digestibility, B1 = August defoliation (after the first six months), B2 = February defoliation (after second six months), W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed), I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences (P < 0.001) within a treatment

9.3.1.4 Nutritive values for the defoliation after 12 months (Frequency C)

The mean CP content of the edible phytomass was 9.47%, which is lower than that of defoliation frequency A (14.85%) and B (11.86%). This is probably due to the age of the fractions of material harvested. Crude protein increased significantly (P < 0.001) with increased water stress, while the CP production decreased (P < 0.001) with increased water stress (Table 9.12). Defoliation intensity had no influence (P > 0.05) on CP content, but CP production increased (P < 0.001) with increased defoliation intensity, mainly due to higher edible phytomass production (Chapter 6).

Crude protein of the fine roots stayed the same (P > 0.05) over all treatments, although it was slightly lower for the less severely water stressed and defoliation intensity treatments (Table 9.12). The average CP value of 10.5% for the fine roots is slightly higher than the average of 9.5% for the edible phytomass.

Table 9.12: Crude protein (mean ± SD) values (% and g plant^{-1}) for *Pentzia incana* along a water deficit gradient at three defoliation intensities for the twelve-monthly defoliation frequency.

Variable	Water treatments				Defoliation intensity		
	W1	W2	W3	W4	I	II	III
CP (%)	7 c (±0.61)	7 c (±0.90)	10 b (±1.68)	14 a (±1.46)	9 a (±3.15)	9 a (±3.63)	10 a (±2.96)
CP (g)	4.9 a (±0.59)	4.7 ab (±0.10)	4.3 b (±0.91)	2.6 c (±1.03)	4.8 a (±1.19)	4.2 b (±1.26)	3.4 c (±0.96)
CPr (%)	10 a (±0.69)	10 a (±1.11)	11 a (±1.21)	11 a (±0.79)	11 a (±1.10)	10 a (±1.05)	10 a (±1.15)

CP = crude protein, CPr = crude protein roots, W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed), I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences (P < 0.001) within a treatment

Increased water stress decreased (P < 0.001) NDF and increased (P < 0.001) IVDMD (Table 9.14). Neutral detergent fibre was increased (P < 0.001), while IVDMD (P < 0.001) was decreased by increased defoliation intensity.

Table 9.13: Neutral detergent fibre (mean % ± SD) and *in vitro* dry matter digestibility (mean % ± SD) for *Pentzia incana* along a water deficit gradient at three defoliation intensities for the twelve-monthly defoliation frequency.

Variable	Water treatments				Defoliation intensity		
	W1	W2	W3	W4	I	II	III
NDF (%)	57 a (±3.51)	57 a (±4.49)	53 b (±3.18)	47 c (±3.46)	56 a (±4.22)	54 ab (±6.42)	51 c (±4.86)
IVDMD (%)	46 c (±4.42)	44 c (±5.31)	50 b (±3.39)	58 a (±2.93)	47 b (±5.86)	48 b (±7.27)	53 a (±5.18)

NDF = neutral detergent fibre, IVDMD = *in vitro* dry matter digestibility, W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed), I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Different letters show significant differences (P < 0.001) within treatments

9.3.1.5 Comparison between the three defoliation frequencies

Data of the three defoliation frequencies are summarized in Table 9.14 as a descriptive summary to indicate the differences in nutritive values between the frequencies. In general, the edible phytomass of the more frequently defoliated plants (frequency A) had a better total nutritive value than that of the least frequently (frequency C) defoliated plants. The CP content of the fine roots was slightly higher at the more frequently defoliated plants.

Table 9.14: Summary of the average nutritive values (% and g plant^{-1}) of *Pentzia incana* at three defoliation frequencies.

Variable	Defoliation frequency		
	A	B	C
CP (%)	14.9	11.9	9.5
CP (g)	8.6	7.3	4.9
CPr (%)	11.0	10.5	10.5
NDF (%)	44	48	54
IVDMD (%)	61	56	49

CP = crude protein, CPr = crude protein roots, NDF = neutral detergent fibre, IVDMD = *in vitro* dry matter digestibility, A = three-monthly, B = six-monthly, C = twelve-monthly

9.4 General discussion

While working through the data of this chapter and reading through the literature it was realised that these types of nutrient analyses are usually done for different reasons. The reason it was done in this study was initially to quantify the impact of different watering and defoliation treatments on the nutritive value of Karoo shrubs, as fodder plants for animals. This information can be of enormous benefit to the farmer in utilizing the shrubs for sustainable animal production. From the previous phytomass production chapter's results, it was realised that especially the CP values (which were derived from measured N values) should also be evaluated from the viewpoint of the plant and not only from the animal's side. The uncertainty was if these Karoo shrubs could increase their CP contents when exposed to water and defoliation stress? It may be a morphological adaptation that slower growing plants have a higher leaf percentage, due to shorter internodes – causing higher CP contents. It could also be attributed to a deliberate increase in CP (nitrogen) to improve the plant's survival ability during stress conditions, to be able to apply compensatory growth the moment conditions for growth get more favourable again? It might even be a combination of the two theories. This discussion will firstly focus on the nutritive value as fodder shrubs for animals and will end off with some speculation around the benefits of increased CP (N) content to the shrub itself.

Nutritive value for *P. incana* was previously quantified by many researchers (Henrici 1945, Louw 1969, Botha et al 1990), but no information regarding nutritive values of *N. microphylla* could be found. It is important to note that the portion of a plant which is chosen and harvested for chemical analysis could have a major impact on the results (Louw 1969, de Waal et al. 1980, Bransby et al. 1981, Botha and Nash 1990). Some researchers for example harvested and analysed the whole plant, while others distinguished between edible and inedible portions of the plant as well as between leaves and twigs (Wilcock et al. 2004). To complicate the matter even further, some animals have the ability to select specifically for more nutritious plant parts and mostly select a diet with a higher nutritive value than that measured for harvested plant material (Zeeman et al. 1983, Botha et al. 1983, Zeeman and Fourie 1984, Stapelberg et al. 2008).

Bringing the nutritive value results in context with the phytomass production, can better explain the feed value of these shrubs in terms of animal production. Higher edible phytomass productions have lower CP percentages but higher CP production values (per plant or area) than those with the lower phytomass production but with higher CP percentages. By contrast the higher edible phytomass productions resulted in higher NDF values which caused lower INVDMD. Therefore, some compromise between CP production and digestibility is needed to obtain the optimum quality for sustainable animal production. This indicates that the most valuable defoliation would be an intermediate intensity and frequency which will benefit both the animal and concurrently also the plant.

Crude protein levels of *P. incana* ranging from 10 to 15% obtained in this study, compares well with levels of 13% measured by Louw (1969) and 12% by Zeeman and Coetsee (1981). Increase of CP content with increased defoliation frequency is similar to results from other researchers on various fodder plant species (Motazedian and Sharrow 1990, Illius et al. 1995, Theron et al. 2002). Botha et al. (1990) determined the chemical composition of a number of Karoo shrubs over different growing seasons. Their results indicated a decline in the CP content of *P. incana* during the summer, which coincides with the results of this study. Research by Stapelberg et al. (2008) showed a significant increase in CP content for shrubs and grasses from the Kalahari veld type in South Africa during droughts, which is similar to the findings for the two Karoo shrubs in this study. Marais et al. (2002) also noted a slight increase in CP content when some annual fodder crops were exposed to water stress. Snyman (2000, 2006) explained that over utilized rangeland generally had a higher CP content, but lower CP production, than that of rangeland in a good condition. These results corresponds with findings from this study that plants, exposed to defoliation and water stress also had higher CP contents, but with lower CP production than unstressed plants.

The higher NDF and lower IVDMD when the shrubs were water stressed in this study, could be a result of shorter internode lengths compared to the unstressed, faster growing plants.

Although the leaf:shoot ratio was not measured in this study, the observed increase of this ratio in the water stressed plants could have caused higher NDF and lower IVDMD. The higher NDF and lower IVDMD of the less frequently defoliated and therefore older harvested plant material could also be partially due to the slightly older stems which could have a higher fibre content. The increased digestibility that was recorded under water stress conditions in this study, is similar to findings of Zeeman et al. (1983) on Karoo shrubs in general. Furthermore lower digestibility during late summer compared to that of autumn and spring is in line with findings from other researchers (Alcaide et al. 1997). Decreased NDF and increased IVDMD with increased defoliation frequency were also reported for fodder crops by Theron and Snyman (2004). The increased IVDMD under water stress was also reported by Ngugi et al. (2004) for various aridland shrubs and grasses from eastern Africa. On the contrary, Marais et al. (2002) found that water availability had no significant influence on the digestibility of some annual fodder crops. Lower NDF and higher digestibility has a marked influence on the voluntary intake, therefore acceptability, of fodder plants to animals (Botha et al. 1983, Meissner et al. 1989, Theron and Snyman 2004, van Niekerk et al. 2009). On the contrary, lower digestibility can decrease the palatability of some plants (Quiroga et al. 2010).

The indication from this study is that the more frequent and intense the plants were defoliated, the more nutritious the produced edible phytomass. It must be borne in mind that the quantity of this produced phytomass is unfortunately low. The veld manager should therefore aim to compromise to a more lenient defoliation and frequency to get a higher phytomass production even though the nutritive value will be a bit lower. The nutritive values of both *P. incana* and *N. microphylla* met the standards for optimal animal production when leniently defoliated. The danger exists that these already overgrazed plants will be over utilized recurrently due to their high nutritive value and high palatability. Some fodder plants has the ability to increase certain chemicals (Stock et al. 1993), like tannins as a defence mechanism, while others, like *P. incana*, has other survival skills (deep root system, compensatory growth ability, high seed production) to survive harsh defoliations. To complicate matters further, the plants also have a higher nutritive value during dry spells, but at low phytomass productions. The Karoo farmer should rely on his good judgement to protect the natural resource and especially the vulnerable Karoo shrubs against over utilization.

Data from the literature on the CP or N content of plant roots is very limited. The CP content of the fine roots were differently influenced by the different water and defoliation treatments. For *P. incana* the fine roots CP was not affected, while the fine root CP of *N. microphylla* increased slightly when exposed to both water and defoliation stress. Oosthuizen et al. (2006) found similar results for *Themeda triandra* (grass), which also showed a slight increase in CP with increased water stress. Some shrubs allocates a higher N percentage to their roots than to aboveground parts during seedling establishment, to instigate faster root growth for quicker exploration of deeper soil layers for water and nutrients (Lloret et al. 1999). The slight increase of CP in the roots of plants under water stress in this study might therefore be to improve the growth and functioning of these roots to improve plant survival ability. Garcia-Moya and McKell (1970) as well as Yang et al. (2014) also reported very little variation in root N content within desert shrub species and some variation between species, while the N content of the roots was in general lower than that of the aboveground plant parts, which correlates well with findings from this study. Martínez et al. (2002) reported a strong correlation between root chemical composition and soil chemical composition, suggesting the role that habitat plays is usually underestimated and neglected.

From this study the question can be raised that if the increased CP content with increased water stress might contribute to the compensatory growth and increased WUE that were discovered in previous chapters? In the literature strong evidence was found that this could indeed be the case. Unfortunately nitrogen-use efficiency (CP production in relation to water used) was not stated in this study. This could perhaps have explained some of these uncertainties. Many researchers, however, reported that higher leaf N (or CP) content increased WUE for a variety of plant life forms (Felker et al. 1980, Field et al. 1983, Toft et al. 1989, Coughenour et al. 1990, Richards 1993, Chaves et al. 2002, Peng et al. 2007). Furthermore, Peng et al. (2007) suggests that increased N could allow compensatory photosynthetic growth. It therefore seems that the increased CP content of water and defoliation stressed plants could partially be seen as a compensatory ability to increase growth and WUE the moment more favourable growth conditions occurs again.

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CHAPTER 10

REPRODUCTIVE DYNAMICS OF *Nenax microphylla* AND *Pentzia incana* ALONG A WATER AND DEFOLIATION GRADIENT

10.1 Introduction

Although Karoo shrubs are adapted to the drier areas of the Nama-Karoo, there is limited information regarding the impact of climatical conditions on flowering, seed production and germination potential of these shrubs. In the past concerns were also expressed about seed set ability of Karoo shrubs following grazing (Dean et al. 1995). Quantitative evidence of the influence of water stress and defoliation on seed production and germination of specific key arid land shrubs, like *Pentzia incana*, is also lacking (Hoffman 1988, Milton 1992, Milton 1994, Todd 1999). Such information might, however, contribute towards a better understanding of the influence of rangeland management on future survival and management of these species.

With rangeland restoration, especially in the Nama-karoo, being a topic under investigation for many years (Dean et al. 1993, Anderson et al. 2004, van den Berg and Kellner 2005, Visser et al. 2007, Dreber et al. 2011), information on seed production ability of Karoo shrubs can be useful for future, more intensive rehabilitation efforts. This can be useful for the introduction of seed to areas where the soil seed bank could be lacking of adapted palatable species (Milton 1995, Jones and Esler 2004, Dreber and Esler 2011).

10.2 Material and methods

Nenax microphylla and *P. incana* plants as described and discussed in previous chapters were watered at different levels of field water capacity, namely: T1 = 0-25% depletion (non-stressed) of plant available water (PAW), T2 = 25-50% depletion (mildly stressed) of PAW, T3 = 50-75% depletion (moderately stressed) of PAW and T4 = 75-100% depletion (severely stressed) of PAW. Defoliation treatments were applied every three and six months, with a 12 month control, while defoliation intensities were to 50 mm, 125 mm and 200 mm. All treatments were replicated six times. All these treatments were described in detail in previous chapters.

Pentzia incana flowered for almost the whole trial period of 12 months with two peaks in autumn and spring. Flowers for *P. incana* were therefore counted at the end of autumn (beginning of June) and also during the spring (November). *Nenax microphylla* flowered only once, which was during spring (November) when all flowers on each plant were counted. Interestingly, *N. microphylla* produced only female flowers and no seed was therefore produced. This might be attributed to, without being aware, only female plants being cloned for the glasshouse trial, as explained in Chapter 3.

For *P. incana* the number of seed (fruit = achenees) per flower head (inflorescence = capitulum) was also counted for one flower head per plant (the common terms will be used instead of the scientific terms). This information together with the number of flower heads per plant was used to determine the number of seeds produced by each plant. Furthermore, 30 seeds of each plant were tested for germination potential. Germination was done in an incubator at 25°C for twenty days. Seeds were placed on germination paper in a petri dish and watered every second day with 2 ml distilled water. The germination trial was done with seed harvested after spring flowering (November 2007 flowering) and was repeated with the same seed source three years later in 2010. The germination percentages for the 2007 germination trial were used to determine the number of viable seeds (seeds with the ability to germinate) produced in the main treatments.

10.3 Statistical analysis

The layout of the trial was a complete randomized block design. All data were analysed using the statistical program GenStat® (Payne et al. 2012). Data were analysed with factorial ANOVA (unblocked), with the Logit transformation for percentages, testing for differences between all main effects (water, defoliation intensity, defoliation frequency) and all interactions thereof (Snedecor and Cochran 1980). Fisher's protected LSD test was used to compare means at the

1% level (Glass et al. 1972). The variation of the data was extremely high and almost no significant differences were found.

10.4 Results

10.4.1 Number of flowers per plant – *N. microphylla* and *P. incana*

10.4.1.1 *Nenax microphylla*

Nenax microphylla flowered only once over the 12 month trial period as previously mentioned. Unfortunately all flowers were female flowers (Figures 10.1 and 10.2). Looking at other plant material that was not included in the glasshouse study, it was discovered that *N. microphylla* plants can bear only female flowers, only male flowers and some plants can bear both male and female flowers.

Figure 10.1: Male and female flowers of *Nenax microphylla*.

In general the plants had high numbers of flowers, which ranged between 50 and 350 flowers per plant depending on the different treatments (Figures 10.2 and 10.3)

Figure 10.2: Flowering of a *Nenax microphylla* plant in the glasshouse.

The variation in flowering data between the treatments was very high, which made it difficult to identify clear trends. Due to the high variation in the data, it had to be transformed and the back transformed means are uses for the graphs. There were unfortunately no standard errors or deviations included to use for indication of variation in the graphs. Defoliation intensity showed a clear, but insignificant ($P > 0.05$), decreasing trend in number of flowers per plant with increased defoliation intensity. This is, however, mostly influenced by the plant size, where the more intensely defoliated plants are smaller and therefore produce fewer flowers than the less intensely defoliated treatment, namely intensity III. Water deficit had a very slight, but insignificant ($P > 0.05$) influence on flower numbers. Defoliation frequency C, which was defoliated only once in 12 months, and therefore also resulted in larger plants, had significantly ($P < 0.001$) more flowers per plants than the other defoliation frequencies.

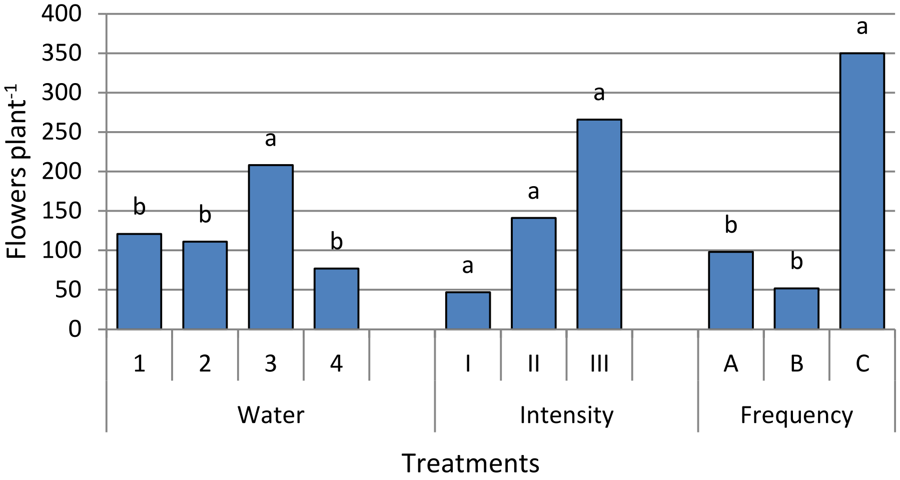

Figure 10.3: Number of flowers plant^{-1} for *Nenax microphylla* at the different water and defoliation treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences ($P < 0.001$) within treatments.

When the water by intensity and water by frequency interactions are investigated it becomes evident that water availability does not have as big an influence on number of flowers per plant as do defoliation frequency and defoliation intensity (Figure 10.4). The less frequently and less intensely defoliated plants had significantly ($P < 0.001$) higher flower numbers than the more frequently and intensely defoliated plants, regardless of the water treatments. This indicates that the physical damage, due to defoliation, has a greater impact on flower numbers than water availability.

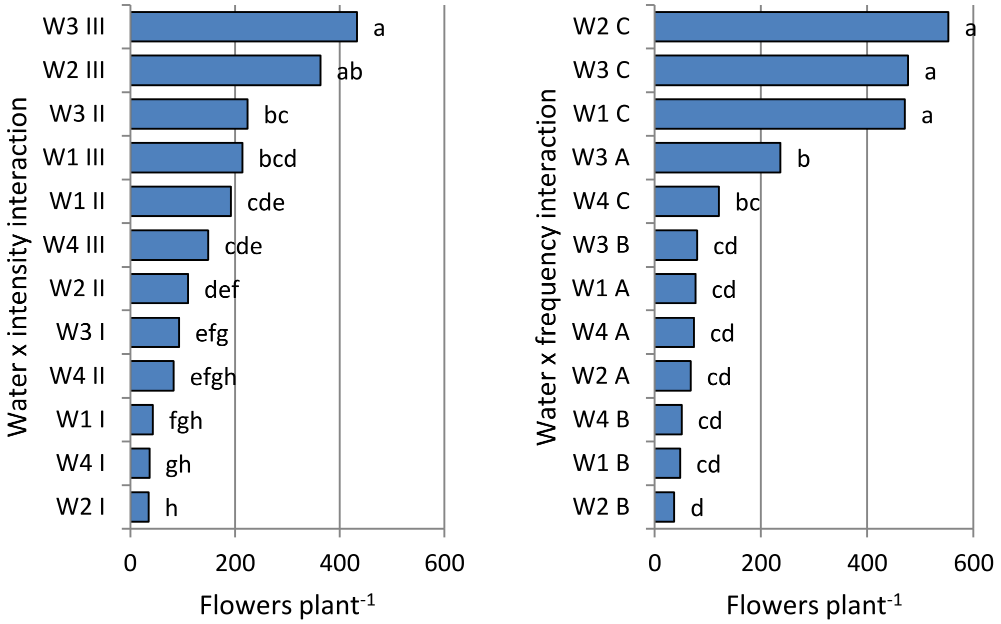

Figure 10.4: Number of flowers plant^{-1} for *Nenax microphylla* at the different water by defoliation intensity interaction as well as the water by defoliation frequency interactions. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences ($P < 0.001$) within treatments.

10.4.1.2 *Pentzia incana*

Pentzia incana flowered for most of the 12 month study period, as was also experienced by Gerber (1993) during a pot trial. The number of flowers per plant were fewer than that of *N. microphylla* and ranged from roughly 60 to 80 depending on the different treatments (Figures 10.5 and 10.6).

Figure 10.5: Flowering *Pentzia incana* plants in the glasshouse.

There was much variation in the flower head number data which hampered the statistical analyses with no definite trends being identified. Flower head numbers per plant decreased ($P > 0.05$) with increased water stress, while both defoliation frequency and intensity did also not result in reliable differences ($P > 0.05$) in flower numbers between treatments. The number of flowers per plant for the most intensely defoliated plants was, however, significantly lower ($P < 0.001$) than that of the other defoliation intensity treatments, most probably due to the plants being smaller.

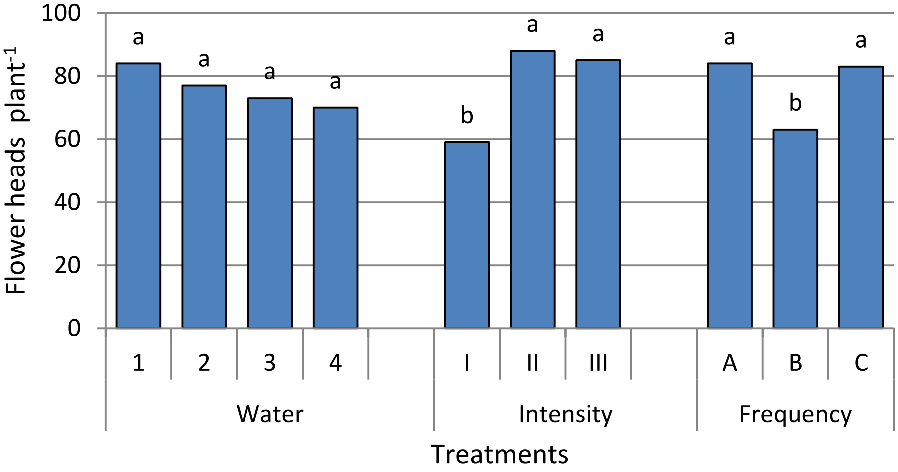

Figure 10.6: Number of flowers plant^{-1} for *Pentzia incana* at the different water and defoliation treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences ($P < 0.001$) within treatments.

The water by defoliation intensity and water by defoliation frequency interactions of flower numbers for *P. incana* showed similar results to that of *N. microphylla* thus indicating that water treatments had no clear effect on flower numbers. By contrast, the defoliation intensity and frequency had a very small ($P > 0.05$) impact on flower numbers which could probably be attributed to structural damage to the plant due to the defoliation treatments.

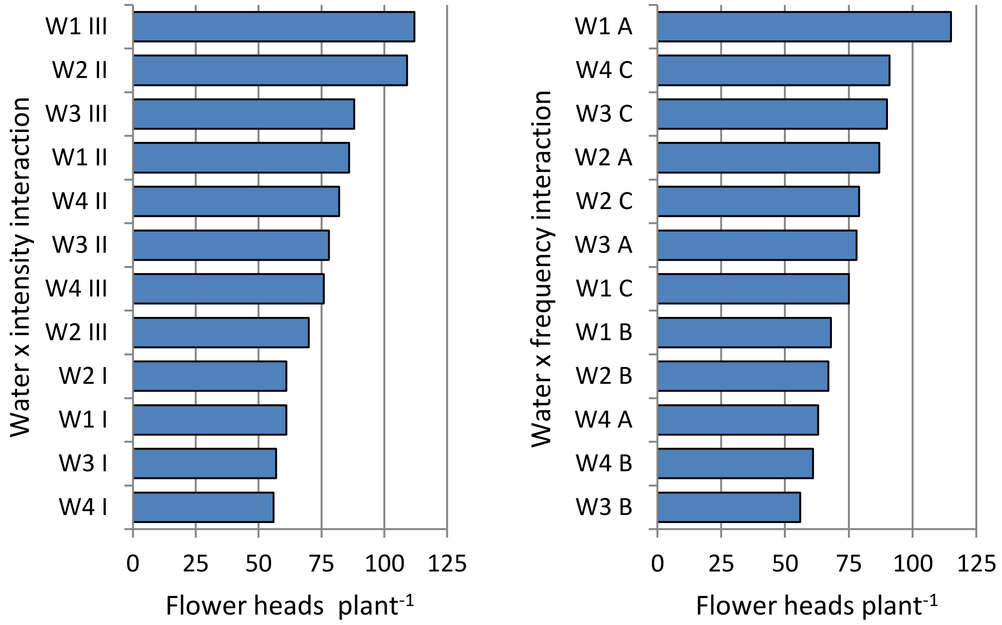

Figure 10.7: Number of flower heads plant^{-1} for *Pentzia incana* at the different water by defoliation intensity interaction as well as the water by defoliation frequency interactions. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve monthly or control.

10.4.2 Number of seeds per flower head – *P. incana*

The seed numbers per flower head for *P. incana* was determined after autumn (June) and by the end of spring (November). The June count was done to soon after the May defoliation, which resulted in no flowers being available for seed counting for defoliation frequency A. Seed numbers were slightly higher for the November count than for the June count (Figure 10.8). Seed number per flower varied on average from 80 to 90 seeds per flower head. As with the flower numbers, the seed numbers also did not result in statistically significant differences between treatments. What was, however, interesting is that the plants that were more harshly defoliated (both intensity and frequency wise) tended to produce more seed per flower than those of the more leniently defoliated plants. Although this is not significant ($P > 0.05$), it is worth noticing and could be seen as a means of the plant to compensate for the treatments that produced fewer flowers, by increasing the number of seed per flower.

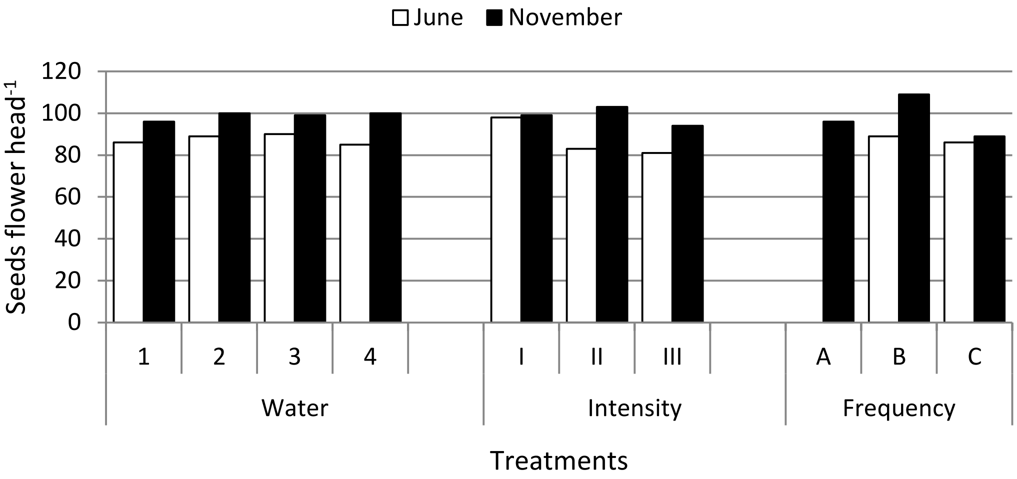

Figure 10.8: Number of seeds flower head^{-1} for *Pentzia incana* at the different water and defoliation treatments for June and November. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve-monthly or control.

The water by frequency interaction had a much bigger and clearer influence on seed production during November than during June (Figure 10.9). For June no clear trend or notable differences between treatments, regarding seeds per plant was evident. At the November count seed numbers for frequency A (three-monthly defoliated) and frequency B (six-monthly defoliated) were above 100 seed per flower, while those of the C (12-monthly defoliated) treatment was just above 80 seeds per flower.

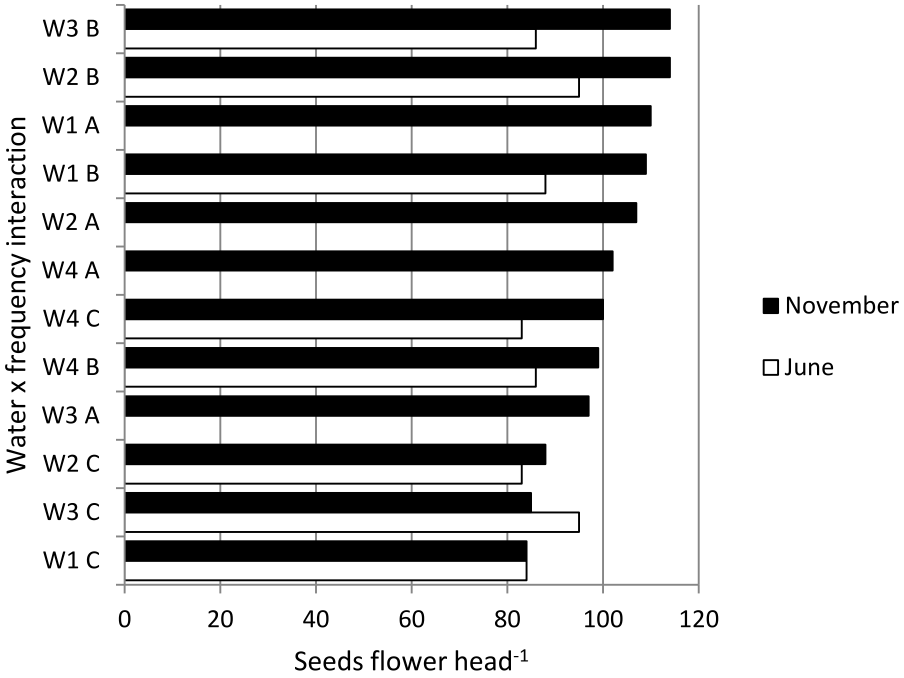

Figure 10.9: Number of seeds flower head^{-1} for *Pentzia incana* at the different water by defoliation frequency interactions. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences ($P < 0.001$) within treatments.

10.4.3 Number of seeds per plant – *P. incana*

The number of seed per flower head was multiplied by the number of flowers per plant to determine the number of seeds per plant. The number of seeds per plant was extremely high and ranged from roughly 6000 to 8500 between the different treatments (Figure 10.10). These results showed a slight, but insignificant ($P > 0.05$) increase in seeds per plant with increased water deficit. The most leniently defoliated intensity and frequency did not produce higher ($P > 0.05$) seed numbers than those of the more severely defoliated plants.

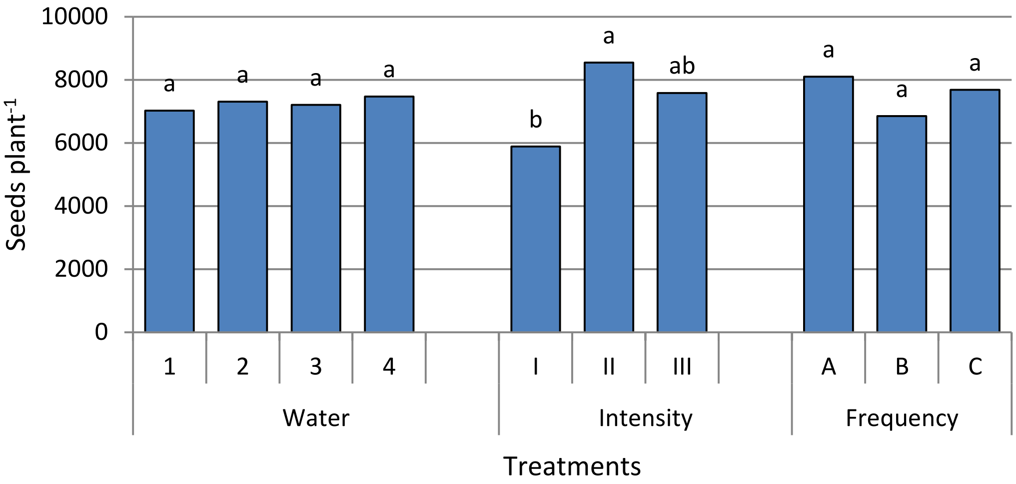

Figure 10.10: Number of seeds plant^{-1} for *Pentzia incana* at the different water and defoliation treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve monthly or control. Different letters show significant differences ($P < 0.001$) within treatments.

In comparing the number of seeds per plant with the number of flowers per plant, it can be argued that the plants compensated for lower flower numbers at the harsher treatments, by increasing the number of seed per flower which resulted in more or less similar number of seeds per plants between treatments.

10.4.4 Germination of seeds – *P. incana*

Germination of *P. incana* seed was fairly low at 3.6% for the 2007 germination with fresh seed from the current year. The germination three years later (2010) was even lower at only 1.3%. Although some variation in germination data occurred between treatments, none of it tested significant ($P > 0.05$). The lower germination percentage of the most severely water stressed treatment (W4) is notable, indicating that severe water stress does influence the production of viable seed. The slightly higher germination percentage of frequency A (most frequently defoliated) could be part of the compensatory behaviour of *P. incana*. By contrast, frequency A had a very low germination percentage at the 2010 germination.

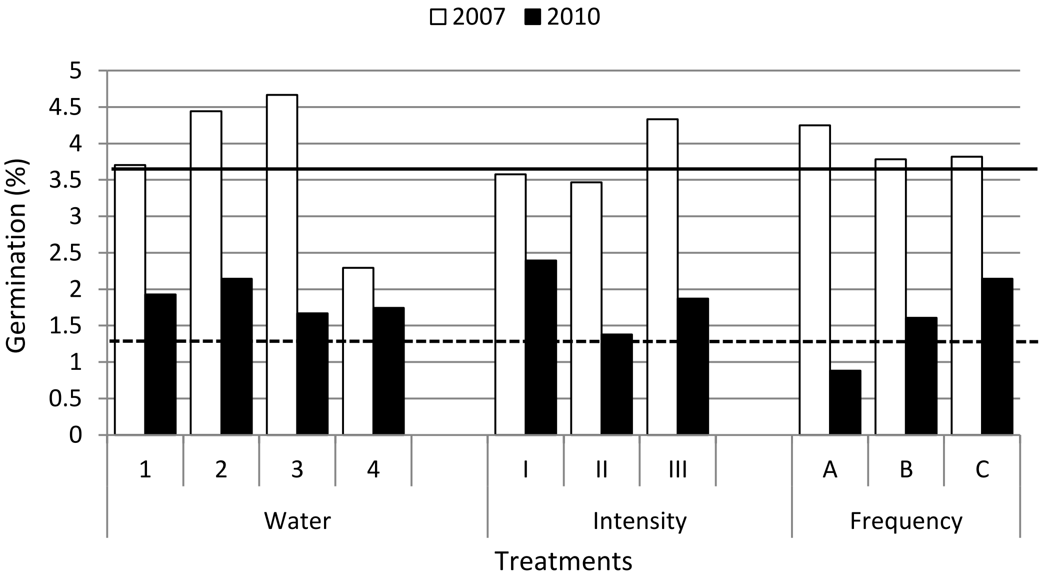

Figure 10.12: Germination (%) of *Pentzia incana* seeds at the different water and defoliation treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve monthly or control. The solid dark black horizontal line indicates the average for the 2007 germination while the dark black dotted line indicate the average for the 2010 germination.

The germination percentage for each treatment was used to determine the number of viable seeds (seeds with the ability to germinate) per plant. The average number of viable seeds per plant over all treatments was 260. The treatments that produced the lowest number of viable seeds were W4 (most severely water stressed) and defoliation intensity I (most intensely defoliated). It is interesting to note that defoliation frequency A (three-monthly defoliation) produced the highest number of viable seeds.

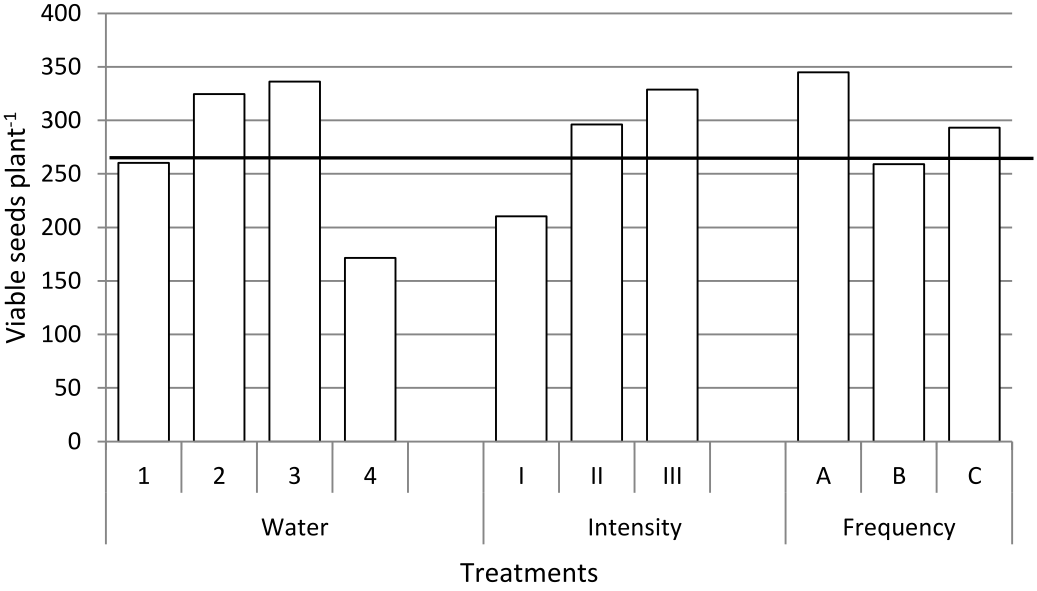

Figure 10.13: Number of viable seeds plant^{-1} for *Pentzia incana* at the different water and defoliation treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Defoliation intensities are: I = defoliation to a height of 50 mm, II = 125 mm and III = 200 mm. Defoliation frequency: A = three-monthly, B = six-monthly and C = twelve monthly or control.

10.5 Discussion

From the phenological data for both species, it can be argued that water availability has a smaller influence on flowering than defoliation intensity and frequency. These results correspond well with findings of Milton (1992, 1994) that argued that defoliation could have a significant impact on flowering of Karoo shrubs. Similar results were reported by Keya (1997) for *Indigofera spinosa* from northern Kenya. Miranda and Jorquera (2014) reported that water stress had no influence on the flowering of a semi-arid Mediterranean shrub, which supports the data from this study.

Pentzia incana is well known for its ability of multiplying vegetatively by branches anchoring to the soil via roots which then forms new plants in time (le Roux et al. 1994). Surprisingly, the results of this study indicated that *P. incana* also has the ability to produce a fairly large number of viable seed, especially the frequently defoliated plants. The more frequently defoliated plants are most likely also the plants that will not get the opportunity to spread through anchoring of branches. The least frequently defoliated plants (defoliation frequencies B and C) produced fewer viable seed, but have a better chance to multiply through vegetative anchoring.

The most frequently and intensely defoliated plants produced more viable seeds than water stressed plants. This might be an indication that plants adapt to the more unfavourable conditions of frequent and intense defoliation and chooses sexual reproduction in favour of vegetative reproduction to ensure survival. This same argument was raised by Miranda and Jorquera (2014) for a semi-arid shrub from a Mediterranean ecosystem.

The germination percentages were extremely low. Germination trials done by Henrici (1939) on *P. incana* showed higher germination percentages of 18% at 20°C and 6% at 30°C, compared to the results of this trial of only 3.6% at 25°C. In future different temperatures could be used for similar germination trials, but it could be wise to imitate temperature from the natural environment. Some seeds may also contain germination suppressors that may have to be broken down by temperature, moisture, scarification or time (maturation). It seems that germination could be better during the cooler spring and autumn months, compared to warm summer months. Although the germination percentages of *P. incana* were very low, the number of viable seed is acceptable due to the extremely high seed production. This tendency is therefore a way to ensure their survival.

10.6 Conclusion

It is often thought that drought or water stress has the biggest influence on vegetation dynamics in most vegetation types. This study, however, proved that both defoliation frequency and intensity had a bigger influence on seed production and germination ability, than water stress on its own. Indiscreet rangeland management could thus be more detrimental to the survival of Karoo shrubs than droughts. Structural damage, caused by overgrazing, reduces the flower bearing area of these shrubs. *Pentzia incana*, however, showed the ability to compensate for excessive defoliation by an increase in the number of viable seed when more frequently defoliated. This information must be implemented in future management systems to ensure sustainable animal production. Furthermore, better understanding of the reproductive ability of Karoo shrubs could contribute to improvement of rangeland restoration actions.

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CHAPTER 11

MORPHOLOGICAL STUDY OF THE LEAVES OF *Pentzia incana* AND *Nenax microphylla*

11.1 Introduction

The arid (west) to semi-arid (east) Nama-karoo Biome of South Africa is dominated by Karoo shrubs (bushes), which are utilized in extensive sheep farming. In these dry areas water is one of the most important and limited environmental factors influencing plant production. Unfortunately there is a lack of knowledge on water use of Karoo shrubs and their drought tolerance. In the previous chapters some light was shed on some of these aspects. One additional aspect under investigation was the leaf morphology and micromorphology of these plants over a soil water gradient. Information regarding micromorphological adaptations of these plants might reveal some reasons for their adaptability to the harsh climatic condition of the arid Nama-karoo Biome. This investigation will focus on some of the xerophytic morphological and micromorphological traits of these plants.

11.2 Materials and methods

Plant material was collected from plants in the glasshouse for investigation under an electron microscope. Leaf bearing stem portions were harvested from plants of each of the four water treatments, for both *N. microphylla* and *P. incana*. One stem portion was randomly harvested from each of three plants per watering treatment. This was done at 12h00, and prior to watering to ensure the plants were water stressed at harvesting. From these stem portions, older leaves, at three nodes below the growth point, were selected for microscopic investigation. For *P. incana* a younger leaf was also included to investigate the trichomes. The four watering treatments were: W1 = 0-25% depletion (non-stressed) of plant available water (PAW), W2 = 25-50% depletion (mildly stressed) of PAW, W3 = 50-75% depletion (moderately stressed) of PAW and W4 = 75-100% depletion (severely stressed) of PAW. Leaf samples were prepared according to the method of Glauert (1974) before the samples were placed in the scanning microscope. The JEOL JSM6400 Scanning Electron Microscope was used to study the leaf micromorphology. Micrographs were taken with the JEOL microscope after which stomatal density was determined from the micrographs. For each water treatment, four micrographs were used to calculate stomatal density. Stomatal openings (pores) were also measured for leaves from the different watering treatments. A series of photographs were also taken for both species to illustrate the recovery of a severely water stressed leaf after watering.

Data were analysed using the statistical program GenStat® (Payne et al. 2012). The generalized linear mixed model analysis was used to test for differences between four water levels using the Poisson distribution. Means were compared using Fishers protected LSD test at the 5% level.

11.3 Results and discussion

The aim was to observe and describe differences in stomata influenced by the four watering treatments. Unfortunately the process of preparation of plant material involved submersion of the samples in some liquids which could have rehydrated the water stressed leaves to some extent. None the less, the leaf morphology and micromorphology could still be described in detail.

Both species have relatively small leaves which are typical for many of the Karoo shrubs. Small leaves are regarded as a xerophytic trait of plants (Gordon and Solbrig 1977). The two Karoo shrubs showed big differences regarding their adaptations as xerophytes. Therefore the two species will be discussed separately.

11.3.1 Leaf morphology and micromorphology of *Nenax microphylla*

The leaves of *N. microphylla* have a shiny appearance (Figure 11.1) which usually is a xerophytic trait to reflect solar radiation and to keep the plants cool during hot summer days (Chaves et al. 2002). Stomata are only found on the abaxial side (lower surface) of the leaves and the leaves are therefore hypostomatic. The stems are covered by papillae, which are also found on the leaf edges and here and there on the lower surface, close to the main vein of the leaves. Signs of wax were also observed on both leaf surfaces which probably contribute to the shiny appearance of the leaves of *N. microphylla* (Figure 11.1).

Figure 11.1: Leaves, stems and flowers of *Nenax microphylla*.

The stems of *N. microphylla* were completely covered with papillae (Figure 11.2) with some also visible closer to the main vein on the leaves (Figure 11.3). Metcalfe and Chalk (1979) describe these papillae as epidermal outgrowths which could have the function of inhibiting the effect of sunlight on the plants.

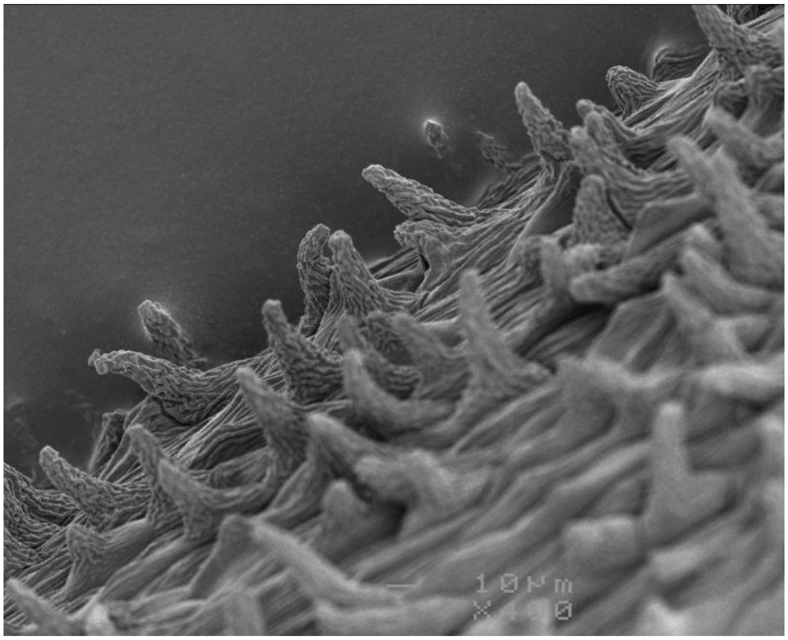

Figure 11.2: Papillae on the stem of *Nenax microphylla*. Magnification: x400.

Stomata of *N. microphylla* were not arranged in any specific order (Figure 11.3), as is usually the case for graminoids (grass-like plants) where stoma are evenly spaced and orientated (Metcalfe and Chalk 1979). Stomata appeared more sunken in the water stressed treatments. All visible stomata appeared open over all four water treatments. This could either be attributed to the preparation technique used for the microscope viewing, or the plants were not stressed enough for the stomata to close.

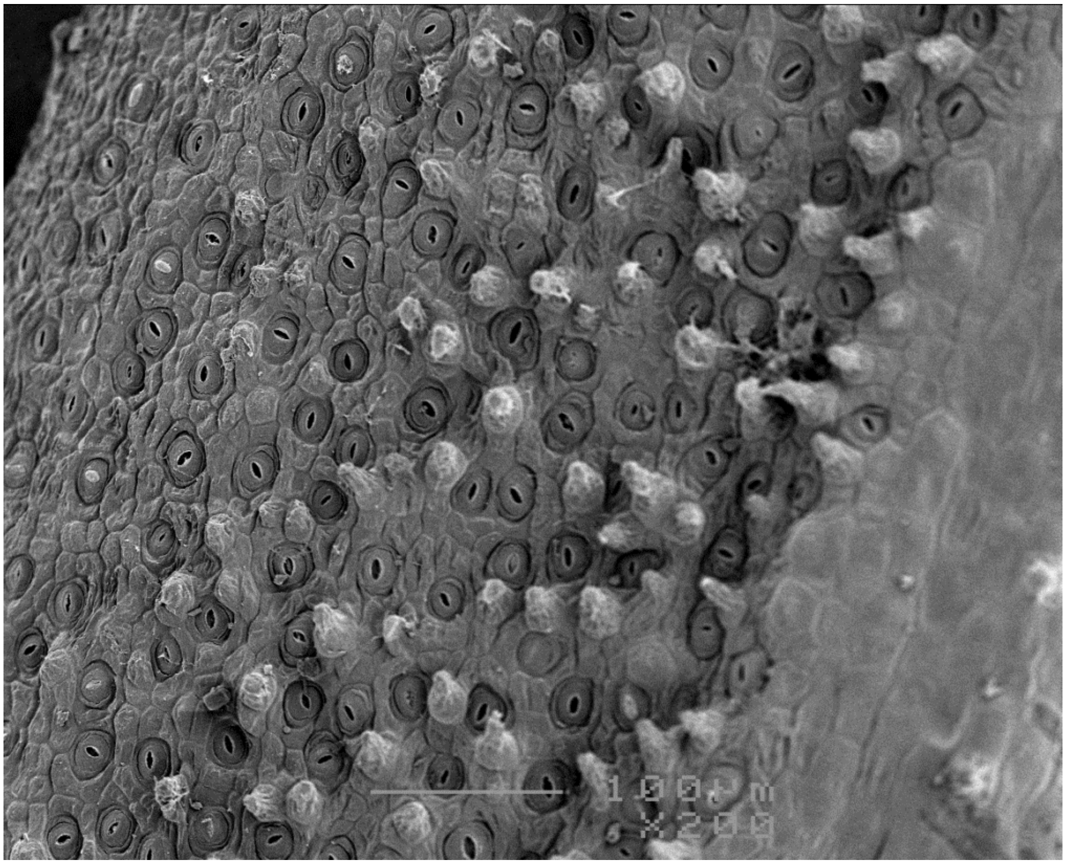

Figure 11.3: Abaxial side of a leaf of *Nenax microphylla*, indicating the stomata and papillae. Magnification: x200.

A single stoma of *N. microphylla* is indicated in Figure 11.4. This stoma is partially sunken and the pore of this stoma is wide open.

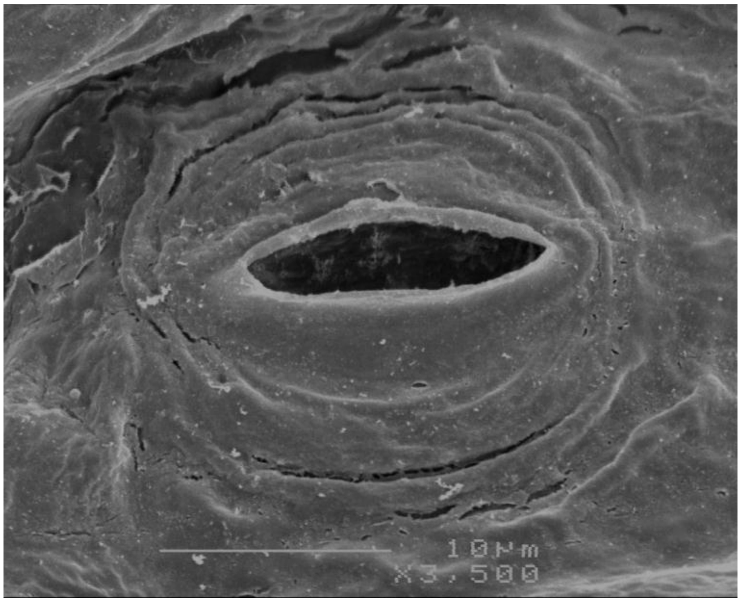

Figure 11.4: Single stoma of *Nenax microphylla*. Magnification: x3500.

The average stomatal density for the four watering treatments was 485 stomata mm^{-2}. Some variation in stomata density did occur between watering treatments but it was insignificant ($P > 0.05$) and also showed no clear trend (Figure 11.5).

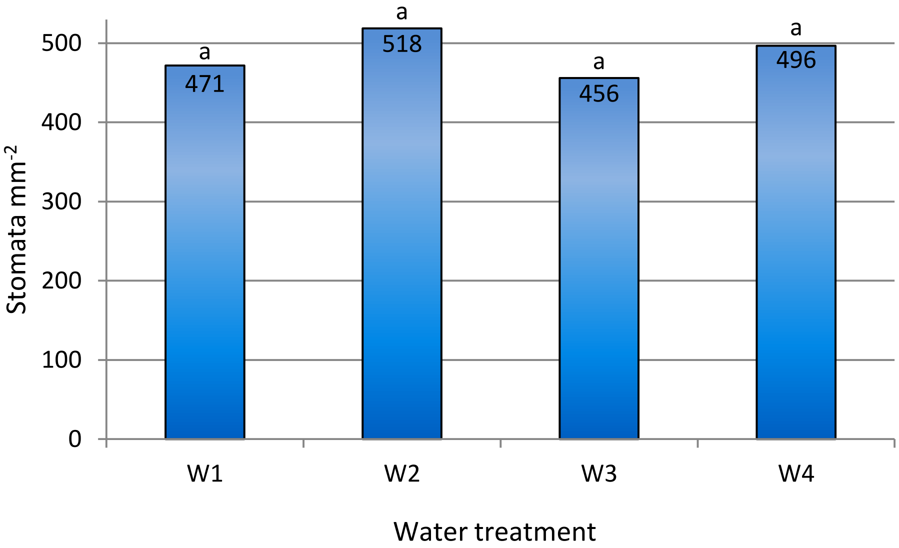

Figure 11.5: Stomatal density (stomata mm^{-2}) of *Nenax microphylla* over four watering treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Different letters show significant differences ($P < 0.001$) within treatments.

The length of the stoma opening (pore) was 9 µm on average over the four watering treatments (Figure 11.6). Although the pore opening was slightly smaller at W4 (most severely stressed), its variation from the other treatments was insignificant (P > 0.05). The pore width was, however, significantly (P < 0.001) smaller for W3 and W4 than for W1 and W2 (Figure 11.6). The indication is that the stomata gap was slightly smaller for the more water stressed plants than for unstressed plants.

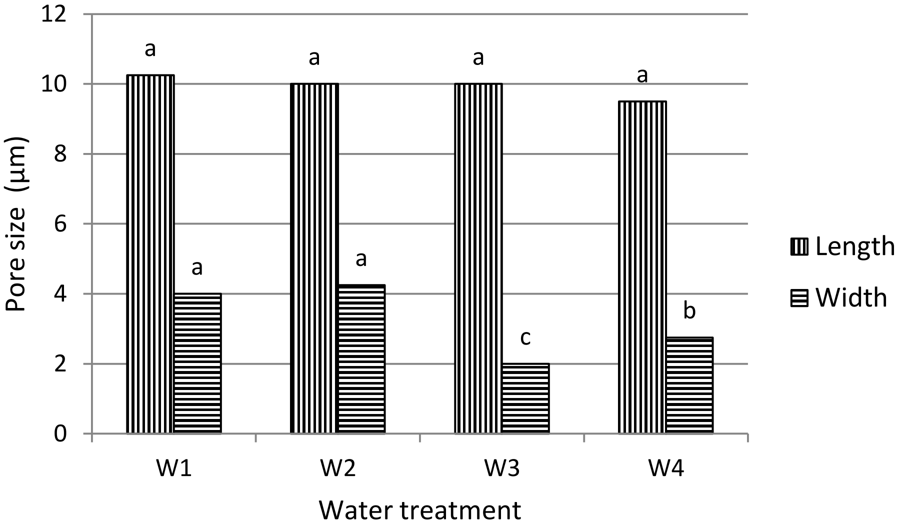

Figure 11.6: Pore sizes (µm) of stomata for *Nenax microphyll*a at four watering treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Different letters show significant differences (P < 0.001) within measurements, between watering treatments.

A series of photographs were taken to illustrate how a severely water stressed plant recovered directly after watering (Figure 11.7). The plant in a severely stressed state is presented at A in the picture. The leaves are folded and bend toward the stem of the plant. The abaxial surface (where stomata are situated) of the leaf is turned towards the stem and away from the sun, probably to reduce transpiration. Eight hours later (picture B) the leaves already started folding open and away from the stem and 24 hours later (picture C), the leaves are back in their normal position and fully extended.

Figure 11.7: Recovery of a water stressed *Nenax microphylla* shoot with leaves over a 24 hour period after watering. A = severely stressed (W4 = 75 – 100% depletion), B = 8 hours after watering, C = 24 hours after watering.

11.3.2 Leaf morphology and micromorphology of *Pentzia incana*

Stomata were visible on both sides of the leaves of *Pentzia incana*, with 9% more on the adaxial side. Leaves with stomata on both sides are called amphistomatic (Metcalfe and Chalk 1979). Both sides of the leaves were covered with many broad, flat shaped macro hairs or trichomes, which give the plants a silver-grey appearance (Figures 11.8 and 11.9). The high density of these trichomes is an adaptation to protect the plants from water loss by partially shading the stomata (Lovegrove 2003). Another function of these silver-grey trichomes could be to reflect solar radiation to keep the plant cool during hot summer days (Gordon and Solbrig 1977). Glands were also visible on the leaves. Stomata appeared more sunken in the higher stressed water treatments.

The leaves of *P. incana* are lobed and almost look like a small hand (Figure 11.9). This reduction in leaf surface area minimizes transpiration (Metcalfe and Chalk 1979).

In nature, some plants have green stems which also have the ability to photosynthesize (Metcalfe and Chalk 1979). When plants like *P. incana* shed leaves as a drought avoidance mechanism (Esler et al. 2006), the stems still have the ability to photosynthesize (Lovegrove 2003) to keep the plant alive during prolonged dry periods.

Figure 11.8: Leaf and stem of *Pentzia incana*.

The leaves of *P. incana* are covered with unbranched trichomes (Figure 11.9). These trichomes are more prominent on the younger leaves than on older leaves. The leaves usually have a silver appearance (Figure 11.8) which is due to the colour of these trichomes (Figure 11.16), which reflects the radiation to cool the leaves down.

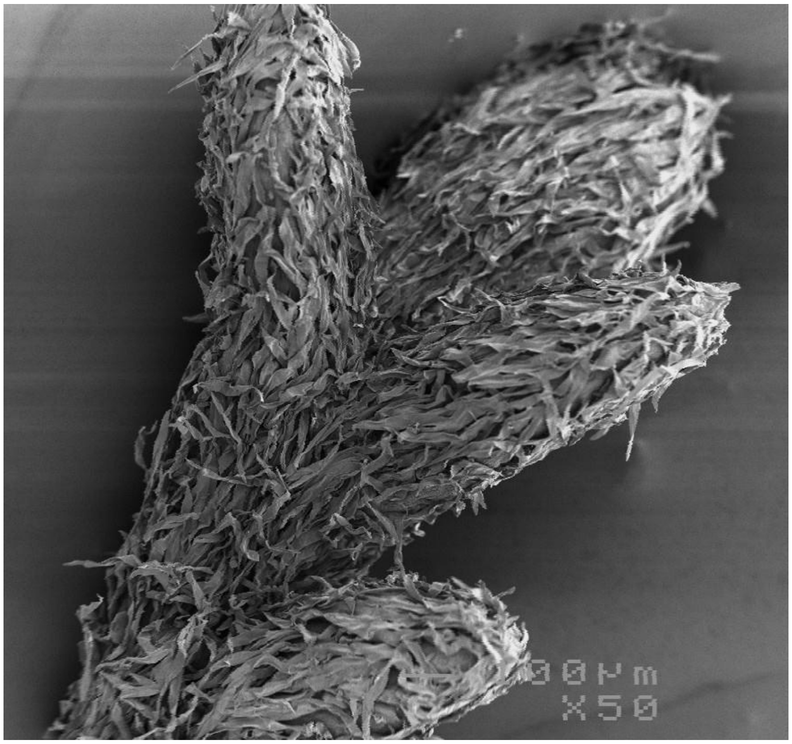

Figure 11.9: A young leaf of *Pentzia incana* completely covered by trichomes. Magnification: x50.

The trichomes of *P. incana* seem to be hollow (Figure 11.10). If they are hollow it would increase the effectiveness of the trichomes to protect the leaves from heat.

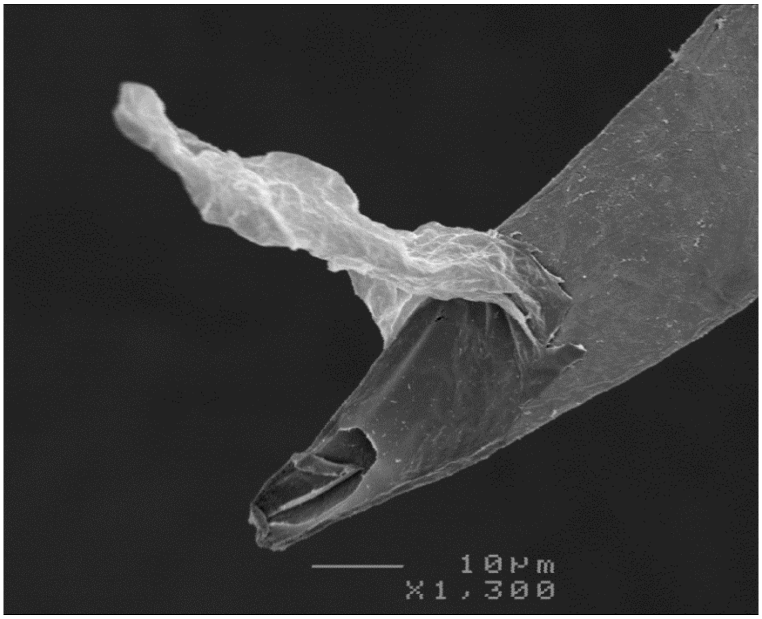

Figure 11.10: The tip of a trichome found on a *Pentzia incana* leaf. Magnification: x1300.

Stomata of *P. incana* are randomly spaced on both sides of the leaf (Figure 11.11). Glands are also visible on the leaves and are most likely causing the very strong aromatic smell when *P. incana* leaves are rubbed (Figure 11.11).

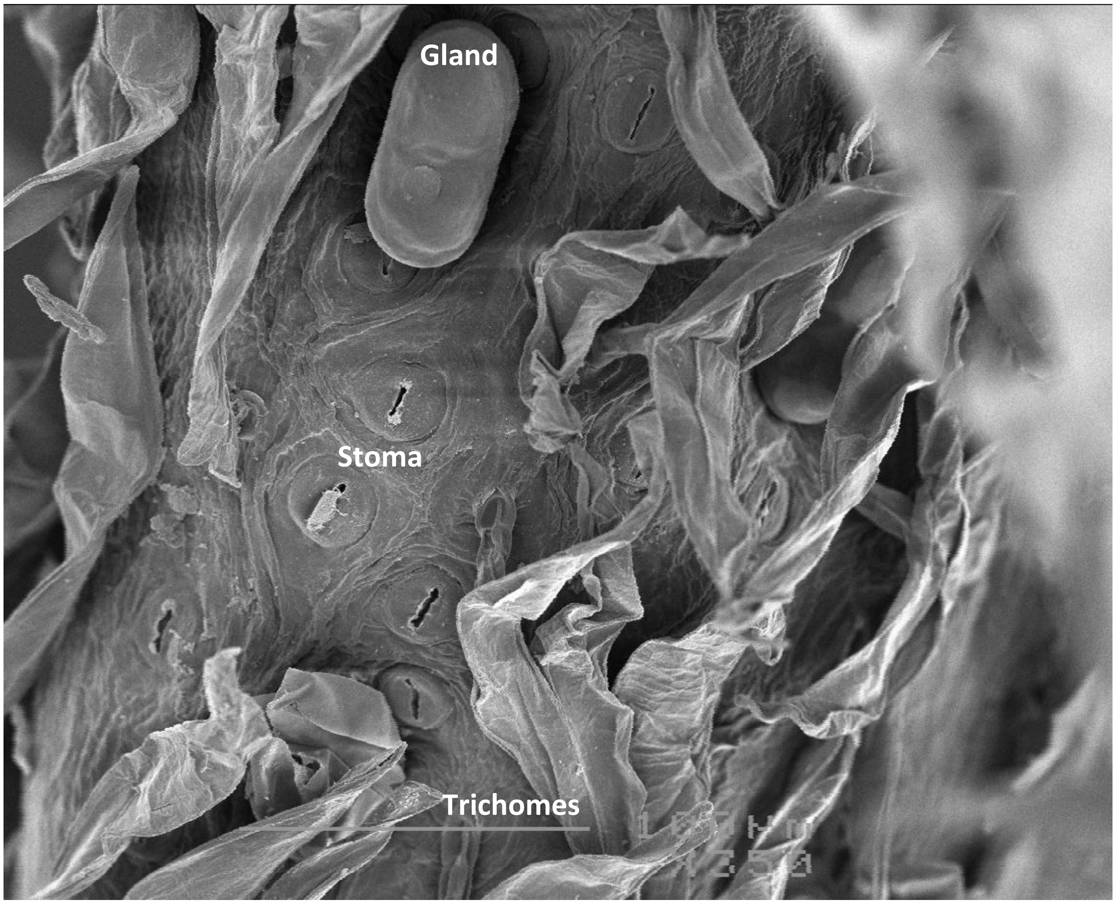

Figure 11.11: Adaxial leave surface of Pentzia incana, indicating stomata, trichomes and a gland hair. Magnification: x350.

Figure 11.12 indicates a single stoma of *P. incana*. The kidney shaped guard cells are clearly visible.

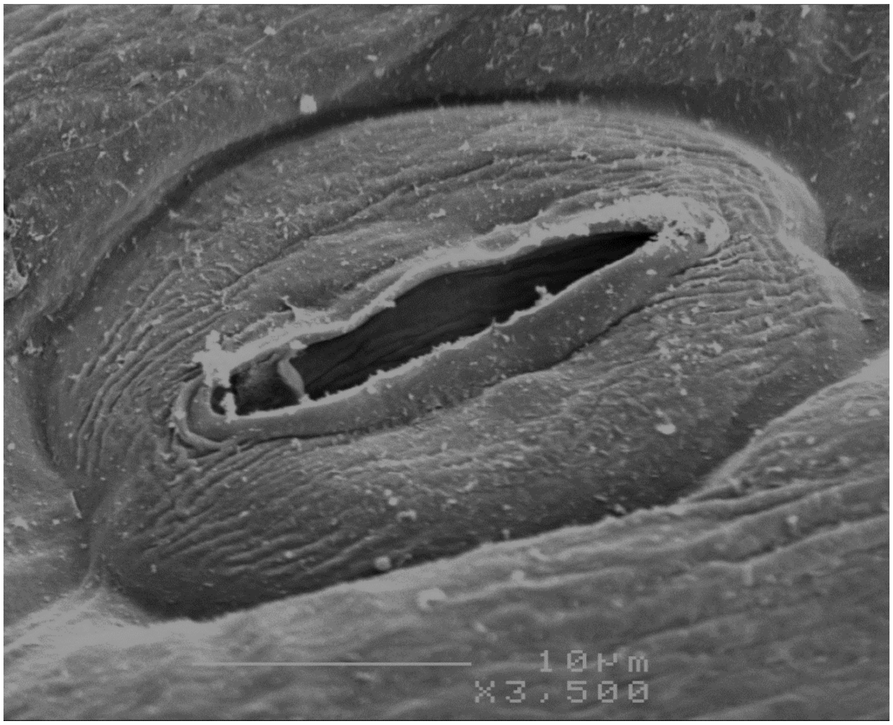

Figure 11.12: Single stoma of *Pentzia incana*. Magnification: x3500.

A micrograph of a *P. incana* pollen grain is illustrated in Figure 11.13. This is a typical shape of plants from the Asteraceae family.

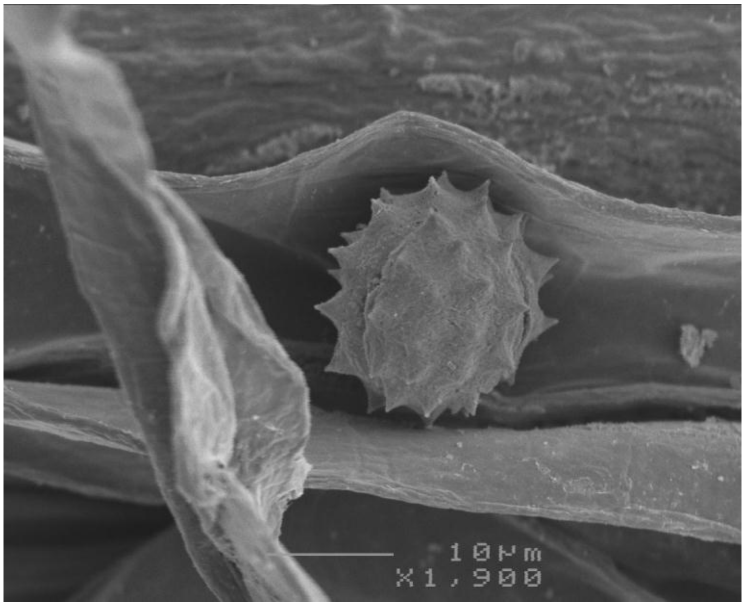

Figure 11.13: Pollen grain of *Pentzia incana*. Magnification: x1900.

The average stomatal density was 219 per mm^{-2} in the adaxial and 200 per mm^{-2} on the abaxial side of the leaf. The stomatal density at W4 (most severely water stressed) was significantly higher ($P < 0.05$) than that of the other water treatments. This could probably be attributed to the leaf being wilted and shrinked, causing smaller inter stoma distances.

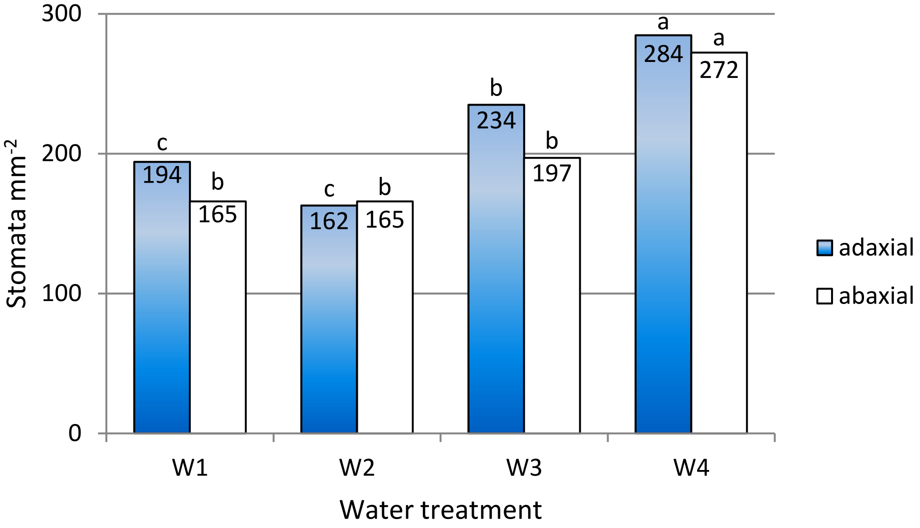

Figure 11.14: Stomata density (stomata mm^{-2}) of *Pentzia incana* over four watering treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Different letters show significant differences ($P < 0.001$) within treatments.

The pore openings were closed at W4 (most severely water stressed) and, by contrast, open at the other watering treatments (Figure 11.15). The pore length was 10 µm compared to the 15 µm of the other treatments, while the pore was also closed, compared to the pore width of 2 µm of the other water treatments. The smaller, closed stomatal pore under water stress is a typical way for plants to reduce transpiration when water gets extremely limited (Chaves et al. 2002). Midgley and Moll (1993) reported that stomatal conductance of *P. incana* became conservative at very low soil water contents, which usually results from stomatal closure as found in this trial.

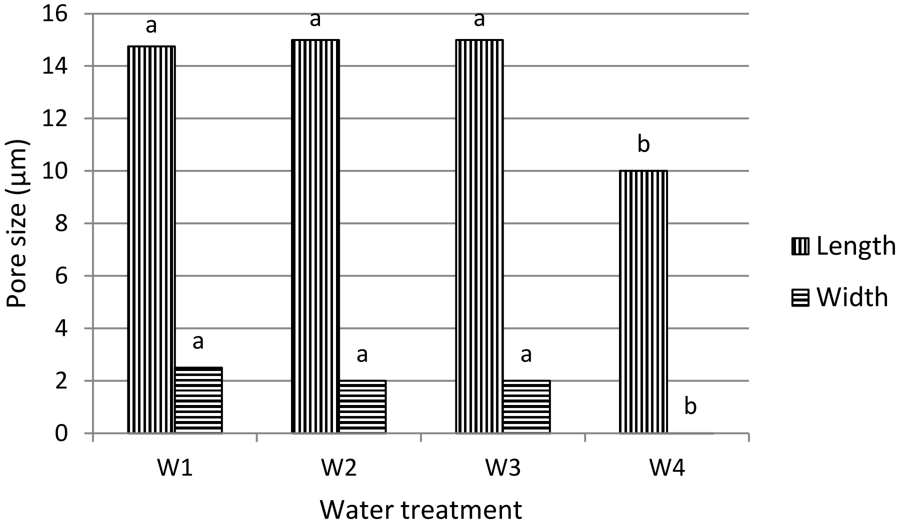

Figure 11.15: Pore sizes (µm) of stomata for *Pentzia incana* at four watering treatments. Water treatments are: W1 = 0 - 25% depletion (non-stressed), W2 = 25 – 50% depletion (mildly stressed), W3 = 50 – 75% depletion (moderately stressed) and W4 = 75 – 100% depletion (severely stressed). Different letters show significant differences (P < 0.001) within treatments.

A series of photographs were taken to illustrate how a most severely water stressed plant recovered directly after watering (Figure 11.16). The plant in a severely stressed state is presented at A in Figure 11.16. The leaves are folded and bend toward the stem of the plant, with the hand shaped leaves almost hugging the stem. The adaxial surface of the leaf is turned towards the stem and away from the sun, probably to reduce transpiration. It is interesting to note that the direction in which the leaves bend when wilted is opposite than that of *N. microphylla*. Eight hours later (picture B) the leaves already started folding open, away from the stem and 24 hours later (picture C), the leaves are back in their normal position and straightened out. When looking at A in Figure 11.16, the leaf indicated by the arrow, is an almost white to silver colour due to increased trichome surface of the wilted leaf. The trichomes are protecting the already stressed leaf from further exposure to solar radiation and high temperatures. When the same leaf is not stressed (Figure 11.16 – C), the green colour of the leaf surface gets more prominent as the leaf is not wilted anymore and fully expanded. The trichomes are less prominent and normal transpiration can also take place.

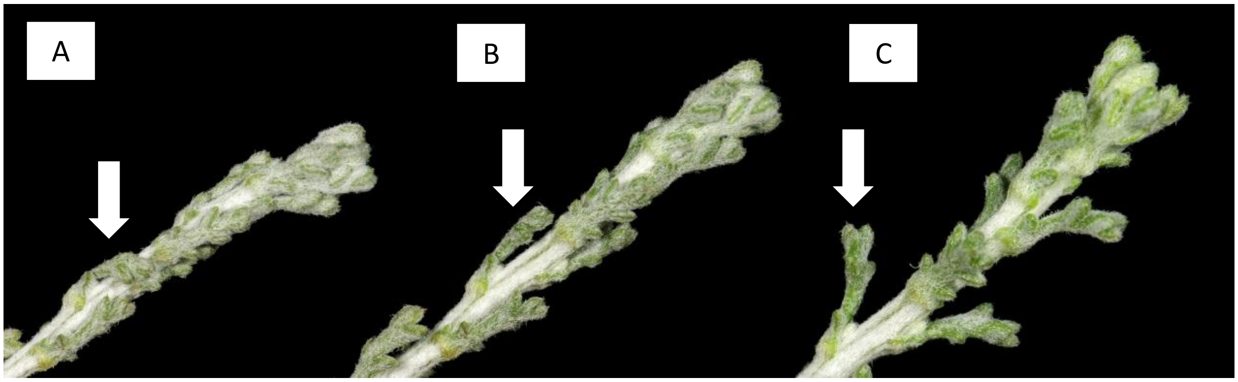

Figure 11.16: Recovery of a water stressed *Pentzia incana* shoot with leaves over a 24 hour period. A = severely stressed (W4 = 75 – 100% depletion), B = 8 hours after watering, C = 24 hours after watering.

11.4 Discussion

Both *P. incana* and *N. microphylla* have relatively small leaves which are an important adaptation of plants from arid regions. Xerophytic leaves have the ability to photosynthesize at greater water stress levels than mesophytic leaves. On the contrary, at lower water stress levels the xerophytic leaves photosynthesize at a lower rate than that of mesophytic leaves (Gordon and Solbrig 1977).

Xerophytic plants generally have high stomatal densities (Metcalfe and Chalk 1979). Desert shrubs were recorded to have stomatal densities from 150 – 300 stomata per mm^{-2} (Larcher 2001). The stomatal densities of *P. incana* also ranged between 150 and 300 per mm^{-2} and stomata occurred on both the adaxial and abaxial sides, while that of *N. microphylla* was higher at around 500 per mm^{-2}, but occurs only on the abaxial side. Stomatal densities of grasses are generally lower and range between 50 and 100 per mm^{-2} (Larcher 2001) or could also be slightly higher at 200 to 300 per mm^{-2} (Letts et al. 2010). According to Gordon and Solbrig (1977) high stomatal densities enable plants to grow faster (in this study probably compensatory) through higher photosynthetic rates when adequate soil water is available. The higher stomatal density also enables the plant to extract water when soils are getting drier. Xu and Zhou (2008) highlighted that high stomatal densities correlated positively with water-use efficiency which could explain the higher WUE of *P. incana* under water stressed conditions. Furthermore higher stomatal densities also have a cooling effect in the course of transpiration, when gas is exchanged through the stomata (Lovegrove 2003). Water deficit caused an increase in stomata density at *P. incana*, which could probably be attributed to the leaf being wilted and shrinked, causing smaller inter stomatal distances. On the contrary, no clear effects of water stress on stomatal densities were recorded for *N. microphylla*. Marais (2005) registered similar varying stomatal densities in relation to water availability for various grass species.

The stomatal pore lengths of 10 to 15 µm as measured in this study is exactly the same as that mentioned by Larcher (2003). Data for pore widths are limited, but this can range from 1 to 5 µm (Larcher 2003), which is similar to the results of this study where pore widths were measured as lower than 5 µm. Decrease in stomatal pore size with increased water stress, as was the case with *P. incana*, was also reported by Xu and Zhou (2008). In general *P. incana* had slightly lower stomatal numbers than *N. microphylla*, but they were, however, slightly larger than that of *N. microphylla*. Similar observations were reported by Marais (2005) for different grass species.

Stomatal response is therefore closely related to soil-water content and also responds to chemical signals from dehydrating roots (Midgley and Moll 1993, Chaves 2002, Xu and Zhou 2008). Letts et al. (2010) discovered that shrubs like *Rhus trilobata* and *Artemisia cana*, from semi-arid regions, exhibited stomatal control mechanisms which favoured WUE during extreme droughts. In this study, as reported in Chapter 8, *P. incana* also showed higher WUE under water stressed conditions. The observation of some stomata situated in depressions, sunken below the cuticle surface, usually has a marked effect on the rate of water loss (Lovegrove 2003).

Some plants have a dense trichome layer for increased reflectance as a protective measure (Chaves et al. 2002, Letts et al. 2010) of which *P. incana* is a very good example. Such reflectance effectively reduces leaf temperatures (Downs and Black 1999). Trichome densities may also play a role in affecting rates of photosynthesis and transpiration. Plants could have steep leaf angels as a protective measure against radiation (Chaves et al. 2002, Lovegrove 2003) of which *N. microphylla* is a good example. Furthermore the presence of wax deposits that were observed on the leaf surface of *N. microphylla* has the function to improve water proofing to retard water loss (Lovegrove 2003, Museum and Dimmitt 2013).

Both shrub species folded their leaves during water stress periods (Figures 11.7 and 11.16) which reduce the impact of direct solar radiation and hence water loss through evaporation (Lovegrove 2003).

11.5 Conclusion

From this study it can be concluded that both shrub species are morphologically well adapted (equipped) to droughts which is more the reality than the exception in the Nama-karoo Biome. *Pentzia incana* has a high density of reflective trichomes that protects the plant very well against heat and solar radiation. It also has a high stomatal density which allows increased photosynthetic rates when growth conditions are favourable. Furthermore the stomata appeared to be slightly sunken and its pores can close when severely water stress conditions appears.

Leaves of *N. microphylla* have a shiny appearance which enables it to reflect solar radiation to reduce leaf temperatures. Furthermore it has a very high stomatal density which could contribute to its compensatory growth ability when adequate water is available. The stomata occur only on the abaxial side of the leaf which protects them from direct sunlight and heat.

These xerophytic traits allow both species to not only survive the harsh climatic condition of the Nama-karoo, but also enable them to increase phytomass production under favourable growth conditions.

11.6 References

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CHAPTER 12

GENERAL CONCLUSIONS AND RECOMMENDATIONS

12.1 Rationale and research questions

This study was planned and executed to explore the influence of water availability and defoliation on the whole-plant productivity and functioning of two keystone shrubs from the Nama-karoo Biome. The main objective of any rangeland management system is to maximize plant productivity and to ensure sustainable animal performance by improving vegetation utilization without natural resource degradation. Environmental plant stress plays an important role in this process of sustainable rangeland utilization. It was, therefore, the aim of this study to quantify the impact of some of these environmental stresses on individual plants, to better understand the functioning of the Nama-karoo ecosystem. With global warming a reality the findings of this study can largely contribute to improved scientific rangeland management approaches in especially the arid and semi-arid rangelands.

The general theoretical literature on the aspect of rangeland management systems in arid and semi-arid areas is plentiful, but lacks information on the more specific species' impact on the functioning of the ecosystem and on several research questions that are vital to ensure sustainable animal production (Figure 12.1). This study, therefore sought to answer the following five questions:

How efficiently can Karoo shrubs utilize **water** following a soil-water gradient in terms of productivity (above- and belowground phytomass), WUE and nutritive value of Karoo shrubs?

Does intensity and frequency of **defoliation** influence the productivity (above- and belowground phytomass), WUE and nutritive value of Karoo shrubs?

How are Karoo shrubs adapted to the **interaction** between different combinations of defoliation and water availability?

Can water stress and defoliation influence the **reproduction** of Karoo shrubs?

How does leaf **morphology** of Karoo shrubs influence the adaptability of these plants to water stress?

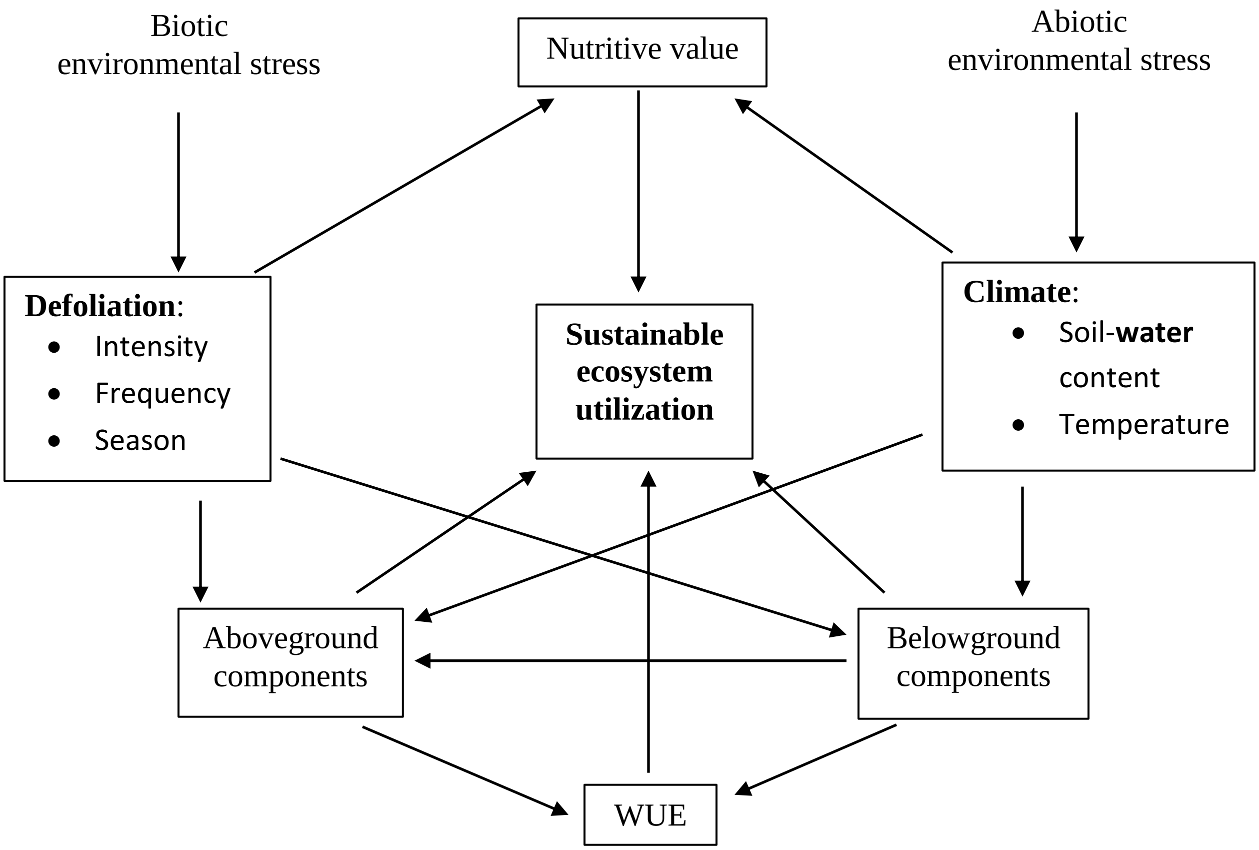

Figure 12.1: Interaction between abiotic and biotic parameters determining sustainable utilization of the rangeland ecosystem.

12.2 Empirical findings

The empirical findings are discussed below in accordance to the five research questions.

How efficiently can Karoo shrubs utilize water following a soil-water gradient in terms of productivity (above- and belowground phytomass), WUE and nutritive value of Karoo shrubs?

Water availability proved to be the single most important factor influencing both above- and belowground **productivity** of arid and semi-arid rangelands (Figure 12.1). Water accounted by far for most of the variation in phytomass production data, much more than the defoliation treatments. This finding supports the fact that variation in annual rainfall has a higher impact on rangeland productivity than defoliation. Water deficit also reduced shoot length and leaf size of both shrub species. Increased water availability, by contrast, resulted in exponentially higher productivity which could be ascribed to the expression of a compensatory growth ability by both *N. microphylla* and *P. incana.* Increased water deficit also resulted in increased root:shoot ratios. This is one of the few studies known where root:shoot ratios for specific shrubs was calculated. Although water deficit decreased root growth, that decrease was less than that observed for the aboveground phytomass production.

This is also one of the few studies where root growth was also included in calculating WUE (Figure 12.1). Furthermore, the expression of WUE in terms of transpiration as was done in this study, is more sensitive for describing ecosystem functioning than evapotranspiration, as was done in most studies in the past. Water use tends to be uneconomical when water is abundantly available resulting in a low **WUE**. The plants expressed rapid phytomass accumulation when water was not limited, almost as if they were making use of the opportunity to increase carbon assimilation. As soil water becomes limited, the plants are expressing awareness to the water deficit by increasing their WUE for improved survival rather than improved phytomass production. Inclusion of belowground phytomass production in WUE calculations increased the WUE by 30%. The different efficiencies at which, for example *P.*

incana, uses water is very interesting. It can arguably use a low WUE at high water availability to possibly outcompete neighboring plants, through rapid growth with uneconomical water use. By contrast, it can increase its WUE when water gets limited, to increase its survival potential. This indicates how these shrubs can adapt by changing their WUE under varying water availabilities, to always ensure the optimal position for surviving any unfavorable climatic conditions.

Bringing the **nutritive value** results in context with the phytomass production, a better explanation of the feed value of these shrubs in terms of animal production is possible (Figure 12.1). Higher edible phytomass productions have lower CP percentages, but higher CP production values (per plant or area) than those with the lower phytomass production, but with higher CP percentages. By contrast, the higher edible phytomass productions resulted in higher NDF values which caused lower digestibility. From this study the question can be raised whether the increased CP content with increased water stress might contribute to the compensatory growth and increased WUE. In the literature strong evidence was found that this could indeed be the case. It therefore seems that the increased CP content of water and defoliation stressed plants could partially be seen as a compensatory ability to increase growth and WUE the moment more favorable growth conditions return.

Does intensity and frequency of defoliation influence the productivity (above- and belowground phytomass), WUE and nutritive value of Karoo shrubs?

Defoliation intensity had the lowest impact on **productivity** for both species, while the impact of defoliation frequency was markedly higher on both above- and belowground phytomass production. Although defoliation intensity accounted for the least variation in data, it might, however, still impact on the physiological functioning of the plant. Infrequent defoliation may result in shrubs being less sensitive to defoliation intensity, while a light defoliation may result in a lower sensitivity to frequent defoliation. The results emphasised the extremely negative impact of a high frequency of defoliation in combination with high intensity of defoliation, which is extremely detrimental in terms of phytomass production of Karoo shrubs (Figure 12.1).

Both *N. microphylla* and *P. incana* showed increased **WUE** under increased defoliation stress, regardless of the watering treatment. For *P. incana*, there was a distinct shift in WUE, with decreased defoliation frequency. At the three-monthly defoliation frequency, defoliation intensity played a bigger role than water availability, while water availability had the biggest effect on WUE when plants were defoliated every 12 months. This indicates how the plant defends itself, by increased WUE, against the factor that is causing the most harm to its survival at a given time. The WUE of *N. microphylla* was less affected by defoliation frequency, than that of *P. incana*, indicating that a variation in WUE exists between different Karoo shrub species.

The indication from this study is that the more frequent and intense the plants were defoliated, the higher the **nutritive value** of the produced edible phytomass. It must be borne in mind that the quantity of this produced phytomass is unfortunately low. The nutritive values of both *P. incana* and *N. microphylla*, however, met the standards for optimal animal production when leniently defoliated. The danger exists that these already overgrazed plants will be over utilized recurrently due to the high nutritive value and high palatability of the regrowth of severely defoliated plants.

How are Karoo shrubs adapted to the interaction between different combinations of defoliation and water availability?

For *P. incana* productivity was mostly dominated by water treatment regardless of the defoliation treatment applied. *Pentzia incana* expressed the ability to improve its WUE under severe water stress in combination with grazing (defoliation) pressure. This higher WUE was accompanied with the lowest productivity which implies improved WUE to ensure survival ability and not to increase productivity. Although unlimited water availability in combination with low grazing pressure resulted in very poor WUE, it was the interactions that produced the most phytomass. Although not significant, the harsher defoliation in combination with the highest water deficit resulted in the lowest phytomass production. It could therefore be argued that *P. incana* possesses the ability to adapt to a wide range of water availability and defoliation interactions. This can simultaneously be done by reducing growth and increasing WUE under grazing pressure and water stress, to increased productivity with low WUE when under no pressure. These adaptations increase the survival ability of *P. incana.*

For *N. microphylla* productivity was also mostly dominated by water treatment regardless of the defoliation applied. Water-use efficiency was more affected by defoliation treatments than by water availability. Plants under both grazing pressure and water stress showed low water-use efficiencies, while plants that were exposed to high grazing pressure, without water stress, had the highest WUE. Although it seems that *N. microphylla* is less adapted to various water and defoliation treatment combinations than *P. incana*, both its mean phytomass production and WUE were slightly higher than that of *P. incana*, indicating that it is well adapted to the range of applied treatment combinations.

Can water stress and defoliation influence the reproductive ability of Karoo shrubs?

Surprisingly, water stress had no marked influence on the reproductive ability of the investigated shrubs. It was, however, proved that both defoliation frequency and intensity had a bigger influence on seed production and germination ability, than water stress. Indiscreet rangeland management could thus be more detrimental to the long term persistence of Karoo shrubs than droughts. The structural damage, due to defoliation, reduces the flower bearing area of the plant. *Pentzia incana* showed the ability to produce a fairly large number of viable seed, especially when frequently defoliated.

How does leaf morphology of Karoo shrubs influence the adaptability of these plants to water stress?

Both shrub species are morphologically well equipped to drought conditions, which is more the reality than the exception for plants in the Nama-karoo Biome. *Pentzia incana* has a high density of reflective trichomes that provides protection against heat and solar radiation. It also has a high stomatal density which allows increased photosynthetic rates when growth conditions are favourable. Furthermore the stomata appeared to be slightly sunken and the pores can close under severe water stress conditions.

The stomata of *N. microphylla* occur only on the abaxial (lower) side of the leaf which protects them from direct sunlight and heat. It has a very high stomatal density which could contribute to its compensatory growth ability when adequate water is available. Furthermore the leaves have a shiny appearance which enables it to reflect solar radiation to reduce leaf temperatures.

12.3 Practical implication

It is clear that water has the greatest impact on phytomass production of both *N. microphylla* and *P. incana*. Unfortunately land users do not have control over the rainfall, and therefore the water available to Karoo shrubs, like *N. microphylla* and *P. incana* (Figure 12.1). On the contrary, two of the most manageable variables that influence plant response to grazing are frequency and intensity of grazing, which the land manager have fully control over. Rainfall interacts strongly with impact of defoliation and it is therefore more detrimental to graze Karoo shrubs when soil-water conditions are unfavourable.

The possible compensatory ability could be exploited with strategic resting periods of certain areas directly after good rains, which occurs mostly during spring and autumn in the Nama-karoo Biome. The knowledge that *N. microphylla* and *P. incana* do show compensatory growth after defoliation under favourable soil-water conditions can be used to increase recovery by strategically withdrawing camps from grazing directly after good rains during especially spring, summer and autumn months.

Furthermore the land user should aim to adopt a more lenient defoliation intensity and frequency regime to get a higher phytomass production even though the nutritive value could be a bit lower. Frequently grazed plants have a higher nutritive value but the danger exists that these overgrazed plants will be over utilized recurrently due to the high nutritive value and high palatability of the regrowth of such plants. This emphasizes that the most sustainable defoliation would be an intermediate intensity and frequency which will benefit both the animal and concurrently also the plant.

12.4 Policy implication

Signs of global warming in combination with rangeland degradation pose a major threat to sustainable animal production in the Nama-karoo Biome. This study highlighted the important role of water availability in whole plant productivity and functioning (Figure 12.1). The land user, however, has no control over rainfall. The only aspect the land user has control over is defoliation intensity and frequency, through grazing management and animal numbers. Karoo shrubs are well known for their resilience, but this trait cannot be exploited sustainably. Rangeland management policies, focusing on animal numbers and management systems, should therefore be developed and implemented to sustain rangeland productivity and to ensure future existence of valuable Karoo-shrubs.

12.5 Recommendation for future research

Most Karoo shrubs are easy to propagate from seed as few have dormancy seed. Many Asteraceae are also easy to propagate from cuttings. However, results known to nurseries have probably not been quantified or published. Future research on the same topic should include a wider range of Karoo shrubs. The two shrubs that were investigated are not a representative sample of the various growth forms available in the large pool of available Karoo plant species.

It might however be a challenge to propagate enough plant material from other species for glasshouse trials. Trials could be planned simpler, with fewer interactions, by separating WUE and defoliation into different trials. The 36 treatment combinations applied in this study complicated some aspects, like the statistical analyses and also created too many interactions. Shoot growth should be measured by cutting and weighing individual shoots rather than measuring shoot length. More plants per species should be included to have plants available for the measurement of seasonal root growth. In this study roots that died back after each defoliation were not measured and were therefore not included in total root production and total WUE. A study that measures root depth in the veld could also be very valuable to bring some clarity to the speculation of how deep roots of Karoo shrubs actually penetrate into the soil. Furthermore studies on propagation of Karoo shrubs through stem cuttings as well on seed production could contribute to improved production of plant material and seed for use in restoration actions.

12.6 Closing remarks

The Nama-karoo Biome remains a sensitive and diverse ecosystem. The resilience of this rangeland type was misused by land users for many years, mainly because of ignorance. Care should be taken in the future to sensibly balance grazing management with rainfall occurrence to ensure sustainable animal production without harming the vulnerable vegetation resource. A farmer that takes care of his vegetation invests in the future of his family.