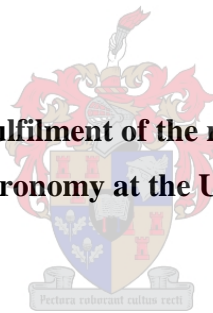


**THE EFFECT OF TIMING OF STRIPPING ON HOP PRODUCTION
UNDER SOUTH AFRICAN CONDITIONS.**

by

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**Thesis presented in partial fulfilment of the requirements for the degree of
Master of Science in Agronomy at the University of Stellenbosch**



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Declaration

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ABSTRACT

Hops is a small, but important agricultural commodity in South Africa. The hop cones contain secondary metabolites responsible for the flavour, aroma and bitterness in beer. The George and Waboomskraal area are the best suited for growing hops due to climate and geographical location. The optimum latitude for hops growing is 45° - 54° north and south of the equator, while these areas are at 34° S. International hop varieties are not adapted to the South African climate. Winters are too warm and summer days too short, thus all the varieties grown in South Africa have been bred for these specific conditions.

The Hop breeding and research program strives to develop internationally competitive varieties with higher yields, desirable brewing characteristics, reducing input costs and increased productivity. To evaluate the current agricultural practices and to determine whether some of these practices negatively affect the plant health and yield, a study was conducted to investigate the effect of the time of stripping. Stripping refers to the practice of removing basal growth of the lower laterals and untrained bines. The study was done in two different microclimates, namely George and Waboomskraal. The aim was to determine the effect of the time of stripping on the vegetative growth, light interception, biomass, soft resins (alpha and beta acids), yield, rootstock weights and carbohydrate concentrations. The effect of stripping was also evaluated on different planting systems and plant maturity, namely Tram lines and conventional planting and 3 years old versus 5 year old plants.

The effect of early stripping in terms of dry weight on mature plants is considerably less than the effect on younger plants. Young plants delivered lower dry weights, but accumulated higher carbohydrate reserves in the early stripping treatment. On young plants early stripping showed a significant difference favouring yield on tramlines. There is a general trend across all sites of higher crop efficiencies in the early stripped treatments. Growers could significantly increase yields without negatively affecting the quality (alpha and beta acids) by applying early stripping, especially in the George area. The effect of early stripping becomes more apparent in consecutive years as the time of defoliation affects foliage, carbohydrate concentration, canopy microclimate and light interception resulting in increased yields, especially on tramlines.

From this study it can be concluded that early stripping appeared to have an influence on the dry root weight, carbohydrate concentration, light interception, crop efficiencies, yield and biomass. This practice is not only environmentally friendly by using less herbicide, it also delivers an economic gain. The effect of the time of stripping in consecutive years and different varieties deserves further study.

UITTREKSEL

Hops is 'n klein, maar belangrike landbou kommoditeit in Suid-Afrika. Die hopkeëls bevat sekondêre metaboliëte wat verantwoordelik is vir die smaak, aroma en bitterheid van bier. Die George en Waboomskraal gebied is die beste geskik vir die kweek van hops, a.g.v. die klimaat en geografiese ligging. Die optimum breedtegraad vir hops verbouing is tussen 45° - 54° noord en suid van die ewenaar, waar hierdie gebiede by 34° S lê. Internasionale hops varieteite is nie aangepas by die Suid-Afrikaanse klimaat nie, want die winters is te warm en die daglengte in die somer is te kort. Al die varieteite in Suid Afrika is geteel vir dié spesifieke klimaatstoestande.

Die Hopsteling en -navorsingprogram streef daarna om internasionaal mededingende varieteite met hoër opbrengste, verminderde insetkoste, verhoogde produktiwiteit en wenslike broueierskappe te ontwikkel. 'n Studie is gedoen om die effek van stropingstyd te evalueer en te identifiseer of hierdie praktyk negatiewe invloede op die gesondheid van plante en opbrengste het. Stroping verwys na die praktyk van die verwydering van basale groei van die laer laterale en onopgeleide ranke. Die studie is gedoen in twee verskillende mikroklimata, naamlik George en Waboomskraal. Die doel was om te bepaal wat die effek van stropingstyd op die vegetatiewe groei, lig onderskepping, biomassa, alfasure en betasure, opbrengs, wortelmasse en koolhidrate konsentrasies is. Die effek van stropingstyd was ook geëvalueer op verskillende plantsisteme en plant volwassenheid, naamlik Tramlyne en Konvensionele - plantsisteem, en 3 jaar oue teenoor 5 jaar oue plante.

Die effek van vroeë stroping in terme van droë gewig op volwasse plante is aansienlik minder as die effek op jonger plante. Jong plante het laer droë gewig, maar hoër koolhidraat reserwes in die vroeë stroping behandeling gelewer. Vroeë stroping van jong plante het 'n beduidende verskil getoon ten opsigte van opbrengste op die Tramlyn-plantsisteem. Daar is 'n algemene tendens op alle lokaliteite van hoër opbrengs doeltreffendheid in die vroeë stropings behandelings. Produsente, veral in die George area, kan opbrengste aansienlik verhoog sonder om die kwaliteit (alfa en beta sure) negatief te beïnvloed, deur die toepassing van vroeë stroping. Die effek van vroeë stroping word meer duidelik in agtereenvolgende jare, omdat die stropingstyd die koolhidrate konsentrasie, mikroklimaat binne in die hopranke en

lig onderskepping wat lei tot verhoogde opbrengste, veral op die Tramlyn- plantsisteem, beïnvloed.

Uit hierdie studie kan afgelei word dat vroeë stroping 'n invloed op die ligonderskepping, plantgewas doeltreffendheid, biomassa, opbrengs, droë wortelgewig en koolhidraatkonsentrasie het. Hierdie praktyk is nie net omgewingsvriendelik nie, maar gebruik minder onkruidododer en lewer ekonomiese voordele vir die produsent. Die effek van stropingstyd op agtereenvolgende jare en verskillende varieteite verdien verdere studie.

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Chapter 1

1.1 General introduction

In 2013, 1.97 billion litres of beer was produced globally (Meier 2014), compared to 1.47 billion litres 10 years ago (Barth 2004), while South Africa had a 25% increase in beer production over the last 10 years (Meier 2014). Water, malt, hops and yeast are the basic ingredients of beer. Hops are a highly priced commodity because of their unique flavour, aroma and preservative properties (De Keukeleire 2000). The preservative and organoleptic properties of hops derive from secondary metabolites that accumulate in the plant cones (Michener et al. 1948; Hough et al. 1957; Neve 1991). The increasing demand for differentiated hops with improved agronomical performance motivates the development of new hop varieties and innovation in hop cultivation.

Hops are an essential ingredient in the brewing process, not only imparting a pleasant bitterness to beer, but also acting as a preservative (De Keukeleire 2000). The antimicrobial effects of hops sterilize most microorganisms, making it a natural preservative (Michener et al. 1948; Hough et al. 1957; Neve 1991). The secondary metabolites found in hops are unique to *Humulus lupulus*, and there is no natural alternative to hops as a flavouring and preservative ingredient in beer (Verzele 1986; De Keukeleire 2000). While hops are used almost exclusively in the production of beer, alternative usages outside the brewing industry are growing in popularity. Hop acids are being used as naturally occurring antimicrobials for a range of purposes (Larson et al. 1996; Dumas et al. 2009; Leite et al. 2013), 8-prenylnaringenin, as a phytoestrogen (Milligan et al. 2002), while xanthohumol, has anti-cancer properties (Miranda et al. 1999), and sedative effects contributing to the treatment of sleep disturbances and anxieties (Chadwick et al. 2006; Zanolli and Zavatti 2008). Besides these properties, anti-mycotic, anti-oxidative, anti-proliferative and anti-bacterial effects have been reported in numerous studies (Stevens and Page 2004; Chadwick et al. 2006; Zanolli and Zavatti 2008). Hops were crowned “Medicinal plant of the year 2007” due to its versatility (Biendl 2008).

Hop cultivars are unique in their bittering, flavouring and aroma potentials (Sharpe and Laws 1981; Čeh et al. 2007). Beer can therefore be differentiated based on the specific hop

cultivars used in brewing. Hops are widely cultivated throughout the temperate zones of the world between latitudes 40°- 60° north and south of the equator (Verzele and De Keukeleire 1991). It's commercially grown in Europe, America, South Africa, Australia, and New Zealand. The largest hop growing areas are in Germany (36.4%), USA (30.8%), Czech Republic (9.3%), and China (6.19 %). South Africa makes up 0.89% of the world hop production (Meier 2014).

Growing hops at 34°S in George, South Africa has limitations due to the non-ideal latitude. Shorter day lengths and warm winters necessitated a breeding program to develop varieties adapted to the local climate, as international varieties do not produce commercially viable yields (Brits 2008). Higher input costs and lower yields force growers to evaluate the efficiency of agricultural practices and to improve hop crop productivity under non ideal climatic conditions. Agricultural practices such as the timing of stripping is very important in determining current and future yields (Sirrione et al. 2010). Food reserves which are used for growth and development the following season are stored in the roots and basal stem portions of the hop plant. The total reserves at the end of a season will be largely dependent on the time during which the plant carried its full leaf system and the effective size of the leaf system. Stripping influences both the above, by reducing bine and root development for the remainder of the season, and reducing the rate of carbohydrate accumulation. This is especially true in young plants, as in the first three to four years the plants are in the establishing phase of their rootstocks and building up carbohydrate reserves, increasing annually until maturity (Williams 1961). Stripping should be kept to a minimum in young plants so that some leaves are left on the plant after harvest, which will allow for additional photosynthesis and build-up of carbohydrates, and in turn influence subsequent yields (Neve 1991). Differences exist in the timing of stripping between local and international practices. No information is available on the effect of the timing of stripping on the quality and yield under South African conditions.

General objectives: A study was carried out during the 2009-2010 hop growing season in George, South Africa to compare the effects of timing of stripping (early stripping as done internationally with late stripping as presently done in South Africa) on the relative growth, light interception and carbohydrate concentration, as well as the effect on the quality (alpha and beta acids) and yield.

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Chapter 2

Literature review

2.1 Introduction to hops

Hops, (*Humulus lupulus* L) which is a member of the Cannabaceae family that comprises of two genera *Humulus* and *Cannabis*, can be described as a dioecious, anemophilous, clockwise twining, herbaceous vine (Small 1978; Neve 1991; Mahaffee and Pethybridge 2009; Serrine et al. 2010).

The commercial interest of hops lies in the lupulin glands, situated in the cones, which contains resins in the glands, bracts, bracteoles and perianth. This is used in the brewing industry as a bittering, preserving and flavouring agent in beer (Neve 1991; De Keukeleire 2000; Nesvadba et al. 2013). Burgess (1964) described hops as a cold-hardy plant, indigenous to northern temperate regions of 45°-54° latitude. In the dormant state the underground plant is able to withstand several degrees below freezing point without injury. However, aboveground plant parts are sensitive to freezing particularly during early shoot emergence in spring. Depending on climate and photoperiod, the hop plant has an overwintering rootstock, which can produce many new shoots the following year. These die down in autumn when the subterranean stem and roots undergo secondary thickening and extension of the overwintering underground rootstock. Flowers are produced along the bine on lateral side shoots ("laterals"). As the number of laterals and flowers induced are dependent on bine size, it is critical to ensure that the bines reach the top trellis wire before flowering. Up to 25% of the dry weight of the female inflorescence may be resinous material, especially in some of the newer cultivars that are being bred for maximum alpha-acid production (Thomas and Schwabe 1969).

Hops require a warm, moist climate, preferably with summer rainfall, and low winter temperatures that encourage full dormancy. Hop plants have a strict day length requirement, which restricts production to between 40° and 60° latitude in either hemisphere. Hops are commercially grown in Europe and the United Kingdom (43° - 54°), Asia (35° - 44°), North America (38°- 51°), Australia (37°- 43 °S), New Zealand (41° - 42 °S) and South Africa at 34°S (Verzele and De Keukeleire 1991).

Table 1. World hop production, area planted (ha) and production (metric tons) in 2009 and 2010 (Meier 2011).

| | 2009 | | 2010 | |
|-------------------|-----------------|--------------------|-----------------|--------------------|
| | Acreage (ha) | Production (mt) | Acreage (ha) | Production (mt) |
| Germany | 18472 | 31344 | 18386 | 34234 |
| Czech Republic | 5307 | 6616 | 5210 | 7772 |
| European Union * | 30687 | 48789 | 30110 | 51192 |
| Rest of Europe ** | 2103 | 2154 | 2082 | 1404 |
| America | 16278 | 43268 | 12906 | 29969 |
| Asia | 6283 | 16487 | 5739 | 14510 |
| South Africa | 481 | 798 | 492 | 913 |
| Australia | 514 | 1343 | 448 | 1099 |
| New Zealand | 401 | 832 | 379 | 793 |
| World | 56747 | 113669 | 52156 | 99879 |

* Poland, Slovenia, England, France, Spain, Romania, Austria, Slovakia, Belgium, Bulgaria, Hungary, Portugal.

** Ukraine, Russia, Turkey, Belarus, Serbia, Switzerland, Croatia.

2.2 Hop growing in South Africa

The South African Hop Industry, managed by the The South African Breweries Hop Farms (Pty) Limited, is a wholly-owned subsidiary of SABMiller PLC. It is the only hop industry in the world to be successful at low latitudes (Brits 2008). The industry, located in George in the Southern Cape, initially set up as a strategic local source of hops, succeeded against significant (low latitude, mild winters and short days) odds in becoming a world-class hop producer. Established in the late 1930's, the industry grew through various trials and tribulations of poor variety performance, to the point where it is now recognised as a successful hop supplier. This area is best suited for growing hops because it has the longest days in the country (Brits 2008), but George still has 2-3 hours less sunlight than is ideal. The weather conditions experienced in the northern hemisphere, where the hop plant originated, are well known for long cold winters during the dormant periods and long daylight hours during the growing season.

In addition, rainfall of at least 700 mm per annum usually occurs. The shorter days in the George district results in early onset of flowering, which shortens the relative vegetative growth period and therefore restricts the proliferation of cone bearing laterals, which results in lower yields. To overcome this, all the varieties grown commercially, have been bred for South African conditions i.e. warm winters and short days in summer as international varieties are not adapted for local conditions (Brits 2008). There are five local commercial varieties grown, namely Southern Star, Southern Promise, Southern Dawn, Southern Passion and Southern Aroma. Well known international varieties fail to produce commercial yields at 34°S as they don't reach the top wire, forming a terminal burr and produce approximately 50% of what would be a commercial yield (Brits 2008).

The total area planted in 2014 was 412 ha of which 76.7% is located in Waboomskraal (-33.86954°22.34939) (Figure 2.1), 16.6% in Herold (-33.859333°22.451763), and 6.5% in George (-33.52355°22.2143.8). The entire crop is sold to The South African Breweries, microbreweries and other small enterprises. The major fungal pathogens affecting hop growing worldwide include downy mildew (*Pseudoperonospora humuli*), powdery mildew (*Podosphaera macularis*) and Verticillium wilt caused by several *Verticillium* species (Seigner et al. 2005; Johnson et al. 2009; Mahaffee et al. 2009; Radišek 2009). The South African hop industry is fortunate in that these pathogens are not yet found on hops in South Africa. Strict quarantine efforts to prevent its introduction are enforced (Johnson et al. 2009; Mahaffee et al. 2009). The only two pest of economic importance are American bollworm (*Helicoverpa armigera*), and Two spotted spider mite (*Tetranychus urticae*). American bollworm is controlled by integrated pest management strategies of regular scouting, adhering to thresholds and chemical sprays. Two spotted spider mite is controlled by predatory mites (*Phytoseiulus persimilis*) and occasional chemical sprays (Linsley-Noakes 2013.)



Figure 2.1. Hop fields in Waboomskraal area, George, Western Cape, during the harvest period (Geoff Spyby).

2.3 Hop Botany

The size and extent of the hop root system is largely determined by soil type, moisture, temperature, nutrition, aeration and ease of penetration. Hop root systems consist of two clearly defined types, namely fibrous roots (horizontal) and fleshy roots (vertical). The proportion of each type of root is governed by soil conditions (Beard 1943). A fully developed hop root system may be large, growing to a depth of more than 4 m deep with a horizontal spread of up to 5m from the crown in suitable soils (Burgess 1964; Beatson et al. 2009). The underground perennial overwintering hop plant is commonly known as the "rootstock". It consists of the extensive storage and foraging root system and the thickened underground stem section. This extensive storage and foraging root system is primarily responsible for uptake and storage of nutrients and water needed for rapid vegetative growth in spring and summer (Burgess 1964; Beatson et al. 2009). The underground stem section increases in size every year and undergoes secondary thickening with annual rings, developing into a tough, woody stump. All shoots (bines) arise from buds on this perennial stem and not from the thickened roots. During autumn, in temperate climates, the bines are killed by the first frost and die back to soil level. The subterranean stem section and its root systems are then able to withstand freezing temperatures. Like many temperate perennials, the hop rootstock has a chilling requirement of about 4 - 5 weeks at temperatures below 5 °C for successful shoot emergence the following spring (Thomas and Schwabe 1969; Thomas 1982). The carbohydrate reserves in the hop plant accumulate in the rootstock, but when a hop bine or any portion thereof is placed in the dark it will become a storage organ. When the

basis of the hop bine is covered with soil, it will also accumulate reserves (Neve 1991). In a study by Thomas (1967) the percentage of starch in old rootstocks (mature plants) during winter was considerably lower than those in young plants, and this could be explained due to the increased fibrous material in mature plants.

During spring, shoots (bines) emerge from the underground terminal buds. Timing of emergence is dependent on the severity of the winter and day length (photoperiod) (Pavlovic et al. 2010), and can reach up to 7 m or more (Hampton et al. 2001; Mahaffee and Pethybridge 2009). These shoots (bines) are clockwise twining, with the aid of hooked hairs (trichomes) as opposed to vines that climb with the aid of tendrils (Probasco 1997; Mahaffee and Pethybridge 2009). Lateral branches develop horizontally from buds on the main bine, and the amount of lateral development is critical in determining hop yield (Neve 1991). These laterals are the main sites of hop flower and cone formation, and the amount of lateral development per hectare will largely determine the yield. Once flowering commences, the growing point on a lateral changes to a terminal flower and no further vegetative development occurs (Neve 1991), female flowers develops into cones and mature (Thomas and Schwabe 1969; Pavlovic et al. 2010).

Hops are a dioecious species with male and female flowers borne on separate, but morphologically similar plants. Monoecious plants are occasionally found (Neve 1991; Parker and Clark 1991; Shephard et al. 2000). Only female plants are grown commercially, with males planted only when seed is required for breeding purposes. Small pistillate flowers, each consisting of a cup-like perianth and a single pistil with two elongate stigmas, are borne in the axils of bracts and bracteoles on a condensed axis, forming the "cone", commonly referred to as a "burr" before the stigmas have abscised. The burr appearance is due to the stigmas being prominently exposed during the time they are receptive to pollen. Whether pollinated or not, the stigmas die and the bracts and strigs begin to elongate, producing the pine-cone shaped strobili (Burgess 1964).

2.3.1 Annual cycle of the hop plant

Hops can be divided into five different growth phases. The slow growth phase, rapid extensive phase, reproductive phase (burr, cone formation, cone maturation), onset of dormancy and winter dormancy (Figure 2.2). The sprouting (slow growth) of the hop plant is the most important growth phase because this is where photosynthesis begins in green bines and leaves (Rybaček 1991). Bines are normally difficult to train and tend to fall of their strings. The plants seem to exhibit a type of dormancy where shoots are characterised by dark, green, leathery leaves with short internodes (Thomas and Schwabe 1969). Yields can be severely affected by temperatures above 24° (Thomas and Goldwin 1980) and weed competition. During the rapid extensive phase the plant consumes a lot of root reserve carbohydrates (Haunold 1980). Rapid extension growth requires a minimum day length of approximately 13h, and is also dependent on temperature and variety (Thomas and Schwabe 1969; Neve 1987). During this period the primary shoots grow extensively to reach the top wire. Hops become responsive to flowering stimuli once the photoperiod has exceeded 16h and have differentiated a minimum amount of 20-25 nodes (Thomas and Schwabe 1969; Neve 1987; Haunold 1980; Pavlovic et al. 2010). This is characterised by a decrease in the diameter of the apical meristem in the terminal bud and initiating the development of lateral branches (Burgess 1964).

The plant physiology changes during the onset of flowering. The developing cones and the rootstock develop increasingly stronger sinks, relative to the shoots and leaves. This creates the stimulus for the onset of leaf and shoots maturation and eventually top senescence before winter dormancy. The plant will not make new growth even in favourable conditions. The change is initiated by shorter day lengths (Haunold 1980; Mahaffee and Pethybridge 2009), death of the shoots and finer root system, the transfer and accumulation of food reserves in the storage roots and the development of large buds on the rootstock. Temperature and day length are the prime environmental parameters that regulate this process (Williams and Weston 1959; Thomas and Schwabe 1969; Pavlovic et al. 2010). Cone maturation follows cone formation when the cone dry matter accumulation plateaus. During the maturation and senescence process, the roots accumulate carbohydrates and reserves which are required for the shoot growth the following season (Williams and Weston 1959). “The rate of accumulation or depletion of reserves will depend on the balance of the photosynthates

produced by the effective leaf area, and the current utilisation of carbohydrates that is required for development, growth and reproduction” (Williams 1961).



Figure 2.2. Annual hop growth cycle in the southern hemisphere with different agricultural practices (Nel et al. 2012).

2.4 Hop cultivars and breeding program

Hop plants are usually diploid ($n=20$) (Neve 1991), however New Zealand successfully produce triploid varieties (Beatson and Alspach 2007). Hop varieties can be divided into two types based on their bittering and aroma potential. Bittering hops contain high levels of alpha acids, are high in isobutyric acid and consequently have a lower humulone to cohumulone ratio. The main emphasis of the South African breeding programme is producing high quality, agronomically suitable, disease resistant and internationally competitive varieties. Cultivars are selected based on cone yield, soft resin concentration in particular alpha acid concentration, essential oil concentration, resistance to major fungal diseases, time of ripening, biological features (cone type, lateral formation, bearing length, root development, housiness, cone: leaf ratio), pellet storability and brewing qualities (Brits 2008). Five commercial varieties are cultivated under South African conditions namely: Southern Star (bittering), Southern Promise (bittering), Southern Dawn (bittering), Southern Passion (aroma) and Southern Aroma (aroma). Aroma hops have a low alpha acid concentration, alpha: beta ratio of 1:1, and high essential oil composition (Brits 2008). The South African

Hop breeding program also selects for new aroma “flavour” hops with very high essential oil composition. Craft brewing is on the increase in South Africa and brewers have been waiting in anticipation of these new and exciting research varieties, with all the new and exciting aromas and flavours that they will bring to the local market (Taylor 2013).

2.5 Hop Chemistry

The cone is a greatly condensed inflorescence, readily differentiable into nodes and internodes (Davis 1957). The axis condensation is a varietal characteristic with pollination and seed development having the greatest influence. Bracts and bracteoles are arranged alternately on an axis known as the strig. The oval-shaped bracteoles bear the female flowers and seed at the base. Multicellular, balloon-like lupulin glands are found in great numbers on the bracts and bracteoles. These glands contain lupulin, a yellow resinous substance, giving hops its distinctive aroma and principal brewing value (Fore and Sather 1947; O’Rourke 2003). The hop secondary metabolite profile consists of three chemical groups: hop acids (alpha and beta acids), essential oils and polyphenols (Benitez et al. 1997). Thirty percent of the hop cones are dominated by alpha and beta acids (Murphey and Probasco 1996; Benitez et al. 1997; De Keukeleire et al. 2003; Van Cleemput et al. 2009; Clark et al. 2013), three to six percent by polyphenols and tannins, and essential oils range between 0.5 – 5 ml 100g⁻¹ (Benitez et al. 1997; Eri et al. 2000; Van Cleemput et al. 2009). Soft resins are the most important in brewing, soluble in hexane and consist predominantly of alpha- and beta-acids. Alpha acids are the precursors of iso-alpha acids which are responsible for beer bittering (O’Rourke 2003). During wort boiling alpha acids are isomerised into iso-alpha acids which are soluble in water. The soft resins account for 90% to 95% of the total resin complex (O’Rourke 2003).

Alpha-acids constitute about 90% of the strength of bitters of hop. They are a mixture of analogues humulone, cohumulone and adhumulone. Alpha acids are primarily responsible for the bittering, aroma and taste of beer, as well as the formation and retention of foam (Henning and Townsend 2005). In addition, they have anti-microbial functions which prevent fermentation and act as preservatives (Shimwell 1937; Michener et al. 1948; Hough et al. 1957; Neve 1991). Beta acids are largely unchanged during wort boiling. However, only about 1/9 of the total beta acids contribute to bittering after oxidation, as isomerized beta-acids have no bittering potential. Thus during storage, oxidised beta-acids increasingly

contribute to the bittering potential of a hop accompanying the loss in alpha-acids (Verzele and De Keukeleire 1991). Hard resins are the oxidation product of the soft resins. The organoleptic properties and functionality of the hard resin fraction is not well understood (Yoshimasa et al. 2014). Essential oils are responsible for flavour and aroma (O'Rourke 2003). They consist of hydrocarbons, oxygenated fraction and sulphur containing compounds. The hydrocarbon fraction is the most volatile and few survive the wort boiling process. The main hydrocarbons are monoterpenes (C₁₀), Myrcene and sesquiterpenes (C₁₅) caryophyllene, humulene and farnesene (Benitez et al. 1997, Eri et al. 2000, Van Cleemput et al. 2009). Polyphenol compounds have potential pharmaceutical applications, predominantly 8-prenylnaringenin as a phytoestrogen (Milligan et al. 2002) and xanthohumol as a cancer chemo preventative agent (Miranda et al. 1999).

Alpha acids correspond strictly with the growing conditions during the crop year (Srećec et al. 2008; Pavlovic 2012). Weather conditions will promote an increase in the biosynthesis of alpha acids in the plant, while water stress will lead to a reduction in alpha acid levels due to the poor condition of hop plants (De Keukeleire et al. 2007). In studies by Zattler and Jehl (1962), Thomas (1980); Srećec et al. (2008); Kucera and Krofta (2009) and Možny et al. (2009), data was not consistent as to which weather-related parameter had the maximum impact on alpha acid production or precisely during which vegetative period. In a study done by Pavlovic et al. (2013) in Slovenia, it was found that temperature strongly affects the alpha acid content from the start of growth until August (mid-summer), and has a significant influence from intensive vegetative growth until the end of flowering. Rainfall has the highest correlation with alpha acids and this association declines as the plant begins to flower and cones are formed (Pavlovic et al. 2013). Burgess (1964) also found that there was an association between alpha acid concentration and the number of sunshine hours, harvest time and weather patterns. Environmental factors responsible for alpha acid levels are, however, extremely complex.

2.6 Hop cultivation

Hops require a warm, moist climate, preferably with summer rainfall. The sites for hop growing should be deep, nutrient rich, well-drained soil to promote optimal growth (Burgess 1964). Yield is influenced by a number of environmental factors, nutrient availability, water supply (Svoboda et al. 2008; Srećec et al. 2008; Kučera and Krofta 2009), temperature

(Thomas and Goldwin 1980; Srečec et al. 2008; Kučera and Krofta 2009), irradiance (Srečec et al. 2008; Kučera and Krofta 2009), agricultural practice (Kořen 2007) and pests and diseases (Pethybridge et al. 2002; Krofta and Nesvadba 2003, Weihrauch 2005). Low winter temperatures encourage full dormancy, provided that the area is frost-free from mid-October. Crown buds require temperatures below 5 °C for at least 4-5 weeks for optimum spring growth (Thomas 1982).

Hops can be propagated by several means: softwood cuttings, strap cuttings, sets and meristem culture. Soft wood cutting involves the cutting of bines into one node sections with two leaves approximately five to eight cm in length, placed in a peat/sand mixture and allowed to root in a mist bed (Williams and Sykes 1959). Strap cuttings are the most commercially practiced method in South Africa, producing clones of the parent variety. It involves covering the base of the hop bines with ± 150 mm of soil late in the season (December), which stimulates the development of perennial buds. The new buds are removed during the following year in the dormancy period (Neve 1991). Meristem culture is only used for the rapid multiplication of promising breeding lines (Brits 2008). Hop plants have been known to survive for up to 100 years (Haunold 1981), but replanting every 10 to 20 years is common and this is dictated by market demands or diminishing yields (Beatson et al. 2009).

Hop production methods differ according to region and country. Plant row spacing, which influences canopy density, is one of the most important agricultural practices that have an influence on yield. This differs for different countries. Plant row spacing, in some of the main hop growing areas in the world is for example in Bavarian Hallertauer (Germany), 3.2m between rows; in Elbe- Saale (East Germany); Czech Republic 3.0 m and in South Africa a mixture of 2.4 and 3.6m. The general trend in Europe is to use wider rows with smaller distances between plants (Kořen 2007) on trellis heights of 7 m or more (Hampton et al. 2001; Mahaffee and Pethybridge 2009). In South Africa 14000 - 15000 plants (Linsley-Noakes 2013) are planted per ha versus 2000 - 2500 plants per hectare internationally (Sirrinc et al. 2010). In early spring, two strings are placed between the crown and the top of the trellis (Figure 2.3). The strings are tied to the top wires of the trellis and then fastened to ground-wires. String densities vary between 9000 –14000/ ha depending on variety and plant age. The less vigorous varieties and weaker fields require higher stringing densities in order to provide acceptable yields (Linsley-Noakes 2013). In spring three to four shoots (bines) are

trained on each string. Shoots (bines) are trained in a clockwise direction with the aid of tiny hooked hairs on the leaves and main stems (Probasco 1997; Mahaffee and Pethybridge 2009). The timing of training is important. In a study in Steknik (Czech Republic) it was found that late training (on 01 June) reduced fresh hop yields by 38.5%, while early training (on 4th of May) reduced yield by 10.3% (Rybaček 1991). In the southern hemisphere training of the biner commences in late October. If trained too early the biner will develop about 20 - 32 nodes (>1.5m) before day lengths are sufficiently long to prevent premature flowering. The biner reach the top wires of the trellis by middle December and continue to elongate until their weight causes them to sag over the top wire. At this time lateral shoot growth commences and continues until mid-January, by which time the plants are in full flower (Linsley-Noakes 2013).

Perennial and annual weeds are controlled by herbicides and bush cutting throughout the season. In arid regions, irrigation is generally required from mid-spring until shortly before harvest (Beatson et al. 2009). The soil moisture is monitored by continuous loggers and neutron probes, and irrigation is applied predominantly by overhead sprinklers, micro jets and drip irrigation (Linsley-Noakes 2013). The typical hops water requirement in South Africa is 10000m³ ha⁻¹ per year (Nel. et al. 2012). Fertiliser (180 - 250 kg N ha⁻¹; 0 - 60 kg P ha⁻¹; 0 - 150 kg K ha⁻¹) is divided into 2-3 applications, as recommended. Routine micro elements of Manganese, Copper, Zinc and Boron are applied as deficiencies are identified through leaf analysis (Linsley-Noakes 2013).

If too much nitrogen is applied, growth as well as shading due to increased foliage will have an adverse effect on yield (Neve 1991). In other hop growing countries, when hops have grown 0.4 - 0.6 m, surplus basal growth of the lower laterals and untrained biner are removed to control hop maturation timing and yields, and to reduce disease incidence (Sirriner et al. 2010). Basal shoots are allowed to grow down the rows in Australia and is controlled between rows by regular mowing, or later in the season by grazing sheep (Inglis 2001). In America and United Kingdom mechanical leave stripping or desiccant herbicide sprays are used to limit fungal diseases and reduce canopy humidity (Gent 2009). Hops are stripped manually, chemically or with livestock (Figure 2.4) up to 1m when the plants have grown +- 1.8 m to encourage airflow and limiting the spread of diseases (Sirriner et al. 2010). In South Africa hops are stripped when biner have reached 5m (during the onset of dormancy) up to

1m from ground level, to control weeds and to make harvesting easier, by removing excess shoots (Linsley-Noakes 2013). The timing of stripping is very important in determining current and future yields (Sirrione et al. 2010).



Figure 2.3. Hop stringing on the South African Breweries Hop Research farm, George, Western Cape (Gerrie Brits).



Figure 2.4. Stripping of basal hop growth after 3-4 bines have been trained by means of grazing sheep in Tasmania.

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Chapter 3

Materials and Methods

3.1 Experimental site

The research was done during the growing season of 2009-2010 at two localities in the hop growing area in South Africa, differing in soil and climatic conditions. George locality: Rob Roy Research Farm near George (-33.52355°, 22.2143.8, altitude 223 m). Waboomskraal locality: Heidekruijn Farm in Waboomskraal (-33.86954°, 22.34939, altitude 601 m). The Rob Roy Farm research area consisted of sandy-loam sands with clay content of 10-15%, with good physical conditions, optimal stock of nutrients, and good drainage. The Rob Roy Farm area covered by this study included two soil forms, Escourt and Sterkspruit. Heidekruijn research sites were both Vilafontes soil forms (Farm B Tram and Farm B Conv) and the site was deep, loamy (category 30-40%), arable land with a low content of sand. The chemical composition of the soils and pH range of 5.3 – 6.0 at the start of the study is shown in Table 3.1.in the different sites.

Table 3.1. Soil chemical properties at the beginning of the 2009 -2010 hop growing season on the three different sites in George: Farm A Tram line system and Waboomskraal: Farm B Tram line system, Farm B Conventional planting system (SGS 2010).

| | pH | Ca | Mg | K | Na | T value | P | Cu | Zn | Mn | B | C |
|-------------|-----|-----------|-----------|---------|---------|-----------|---------|---------|---------|---------|---------|-----|
| | KCl | (cmol/kg) | (cmol/kg) | (mg/kg) | (mg/kg) | (cmol/kg) | (mg/kg) | (mg/kg) | (mg/kg) | (mg/kg) | (mg/kg) | (%) |
| Farm A Tram | 5.3 | 3.8 | 1.2 | 224.0 | 40.0 | 7.2 | 102.0 | 1.0 | 12.4 | 9.3 | 0.4 | 1.8 |
| Farm B Tram | 6.0 | 7.5 | 2.7 | 202.0 | 38.0 | 10.5 | 138.0 | 1.2 | 6.7 | 10.5 | 0.0 | 2.4 |
| Farm B Conv | 5.9 | 6.4 | 1.5 | 193.0 | 41.0 | 8.4 | 54.0 | 0.7 | 6.0 | 13.4 | 0.0 | 2.0 |

3.2 Climate

The hop producing area in the Southern Cape of South Africa is characterized by all year rainfall, with the driest months experienced in winter and rainfall peaks in autumn and spring. George is one of the highest rainfall regions in South Africa, where winter rainfall is associated with frontal bands, while summer rain is associated with tropical temperate rainfall bands (Nel et al. 2012). There were measurable reductions in rainfall, groundwater level, and streamflow (Holloway et al. 2012) which were necessary for dam filling in recent commercial hop production. The drought experienced in the 2009 growing season in George and

Waboomskraal was the worst (Meier 2010) in 50 years, and 30 to 40% of producers ran out of water 2 months before the harvest season (Conway 2010).

The average temperature for George during July 2009 was slightly above the long term average. This dormancy period of 5 weeks during July is critical for successful emergence of hop shoots in spring. The total rainfall recorded for the George locality (Rob Roy Research Farm- Farm A Tram) in 2009 (Fig 3.1) was 603.8 mm which was 15.2 % lower than the long term average (1960-2009). The total rainfall recorded for Waboomskraal locality (Heidekruijn Farm- Farm B Tram & Conv) in the same period (Fig 3.2) was 669 mm, which was 17 % lower than the long term average. The rainfall in the Waboomskraal locality was especially low at the start of the hop growing season in September. This trend was also seen in November when rapid shoot growth was prominent. The continued drought experienced in November, January and March in both localities necessitated growers to pump from boreholes as this is the critical period for active vegetative growth, lateral formation, flowering and cone formation period. Heavy rainfall was experienced in the George locality in the beginning of February as harvest was approaching. Below average temperatures in Waboomskraal during October and November and during November in George resulted in a prolonged vegetative growth as the start to the season was not rapid. The above average temperatures experienced in Waboomskraal (February and March) and the continued water scarcity in both localities resulted in the hops becoming over mature. This was also noted in the overall crop percentage dry matter (Pretorius 2010) which was above the long term average. Hop growth was hampered by the continued drought situation experienced in November to March 2010.

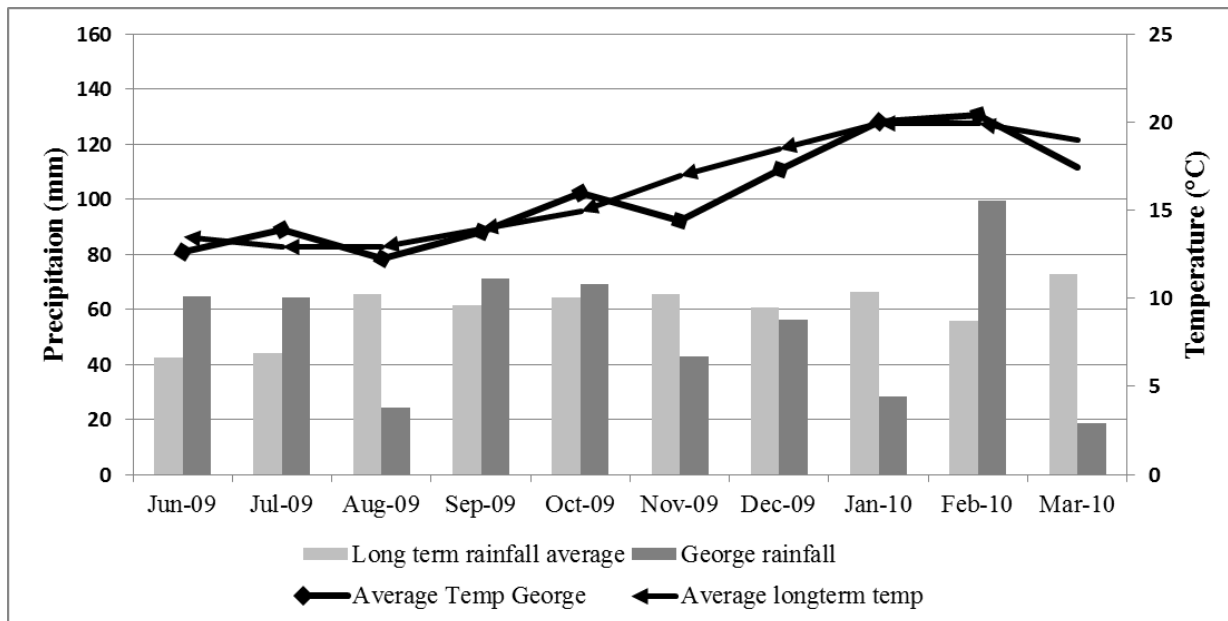


Figure 3.1. The George microclimate long term rainfall and average long term monthly temperature data (SAWS 2010) compared to rainfall and average monthly temperature for the June 2009 – March 2010 period (Dept.of Agric 2010).

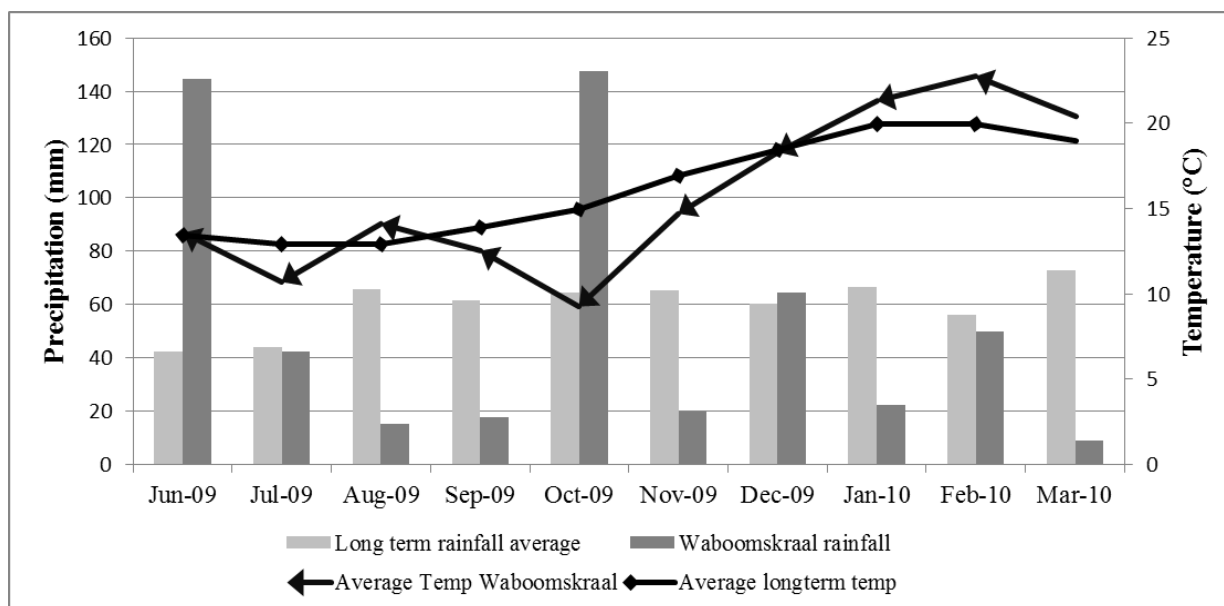


Figure 3.2. The Waboomskraal microclimate long term rainfall and average long term monthly temperature data compared to the rainfall and average monthly temperature for the June 2009 – March 2010 period (Hortec 2010).

3.3 Management of experimental blocks

The management of the blocks in terms of irrigation, fertilisation, weed and pest control and harvest was in accordance with the best operating practices of the SA Hops Growers Association (Linsley-Noakes 2013).

Plots were laid out with overhead sprinklers and micro jet irrigation. Irrigation scheduling on trial sites was conducted by using soil moisture measurements with a neutron moisture meter. Measurements were performed twice weekly in October and three times per week during the active growing season. Nitrogen was applied at a rate of 200 – 250 kg ha⁻¹, as recommended. The nitrogen applications on Farm A Tram (200 kg ha⁻¹), and Farm B Conv. (200 kg ha⁻¹) were divided into three applications, namely 40 % during the active growing phase (mid October), 30% when the hops were 2.5m on the trellis system (mid-November), and 30 % before flowering (end December). The nitrogen application on Farm B Tram (270 kg ha⁻¹) was divided into four 25 % applications, one during the active growing season, when the plants were 2.5 m on trellis system, when the hops reached the top wire, and the last application before flowering. Potassium was applied at the following rates: Farm B Tram (120 kg ha⁻¹), Farm B Conv. (100 kg ha⁻¹) and Farm A Tram (75 kg ha⁻¹) divided into two applications, 50 % before flowering (end- December) and 50% during cone formation (mid-January). Phosphorus was only applied on Farm B Conv. at a rate of 60 kg ha⁻¹. Routine micro elements (Table 3.2) were applied twice in the season on all three sites. Copper, zinc, manganese and boron were applied when the hops were 2.5 m on the trellis system (400l ha⁻¹) and before flowering (1000l ha⁻¹). Urea was applied at a rate of 2 kg ha⁻¹ (1st application) and 5 kg ha⁻¹ (2nd application).

Table 3.2. Routine micro element application (ml ha⁻¹) on the research sites during the hop growing season of 2009-2010

| | 1 st application | 2 nd application |
|----------------|-----------------------------|-----------------------------|
| Copper (ml) | 800 | 1600 |
| Boron (ml) | 375 | 750 |
| Zinc (ml) | 600 | 1500 |
| Manganese (ml) | 400 | 1000 |

All weeds in the trial and control plots were manually removed. Pest control was carried out on all sites throughout the season when necessary. Methomex ® (Methomyl) and Acarstin ® (Cyhexatin) was used to control American bollworm and red spider mite.

3.4 Experimental layout and treatments

The effect of early chemical stripping of hop bines during the active growing phase (mid-November) was evaluated against the routine late stripping of hop bines before harvest (mid-January) (Figure 3.4) and the subsequent effect on the growth, yield and quality. After four bines have been trained (mid- November) in a clockwise direction, 400 mm of the basal growth was defoliated (Figure 3.6) with Gramoxone at a rate of 11 ha⁻¹ with a Nobili spraycart to promote apical dominance. One third of the normal rate has been applied as bines were still young. The routine late stripping treatment serves as the control (Figure 3.5) and was defoliated up to 1 m during onset of dormancy (mid-January) at a rate of 3 litres Gramoxone ha⁻¹, with a Nobili spray cart, as this is the current practice.

The experimental design was a randomized complete block design with 5 replications (blocks) for each of the two treatments namely early chemical stripping (treatment) and late chemical stripping (control). The Southern Star variety was used for this trial.

The trial was repeated on three different sites namely: The Rob Roy Research Farm (Farm A Tram), Heidekruijn Farm (Tramline planting system – Farm B Tram)(Figure 3.4), and the Heidekruijn Farm (conventional planting system –Farm B Conv) (Figure 3.3).Farm A Tram and Farm B Tram were both three years old and Farm B Conv was a five year old hop field.



Figure 3.3. Conventional planting system with between row spacing of 2.4m used in this study (Farm B Conv).



Figure 3.4. Tram line planting system on raised soil surface with between row spacing of 3.6m (Farm A and Farm B Tram).



Figure 3.5. Chemical stripping during cone maturation as in practice (Control).



Figure 3.6. Chemical removal of basal hop growth during the active hop growing phase (Early stripping treatment)

3.5 Data collection

3.5.1 Light interception

Photosynthetically active radiation (PAR- $\mu\text{mol m}^{-2} \text{sec}^{-1}$) measurements were taken for each replication of the control and treatment (early stripping) blocks from the start of the growing season, in October, when active growth was present, using the Sun Fleck PAR ceptometer. Readings were taken between 1200h and 1400h, on plant rows at the following fixed heights: soil level, 1 meter, 3 meters and 5 meters (above the highest point of the canopy). This was repeated weekly for all levels, irrespective of growth stage. The percentage of light intercepted was calculated as follows: $(\text{Intensity reading at 5m} - \text{intensity reading at specific height (soil level, 1m, 2m or 3m)}) / (\text{intensity reading at 5m}) * 100$.

3.5.2 Vegetative growth

Three plants were randomly selected at the start of the season and tagged on each control and treatment (early stripping) block. Plant height measurements were recorded weekly, by measuring the distance from the ground level to the highest point on the plant until it reached the highest point in the canopy at 5 meters. Relative growth % was calculated weekly for 15 to 18 weeks by using the following formula: $((x - y)/y) * 100$ where x = the bine length at the measurement date at end of current week and y = the bine length of measurement date of end of previous week.

3.5.3 Harvest

To determine harvest readiness, a composite ripening sample was picked in field to determine the total dry matter (TDM) concentration. For the total dry matter calculation the dry matter (%) and moisture (%) is needed. Dry matter (DM) determination: One hundred (100) grams of Fresh hop cones was weighed out (Fresh hops), microwaved for 10-15 min and reweighed (Dry Hops) (Analytica-EBC, 2000). Dry matter (%) was calculated using the following formula: $\text{Dry matter} = (\text{Dry hops}/\text{Fresh hops}) * 100$ (Pretorius 2008). Moisture determination: Dry hops from DM determination was milled and weighed to 4g (sample weight), placed in an oven proof metal dish, dried in a convection oven for 1h at 104°C, and reweighed (Sample & Dish Dry). Sample was allowed to cool in a desiccator and weighed again (Analytica-EBC, 2000). Moisture content (%) was calculated using the following formula: $(\text{Sample Weight} - (\text{Sample \& Dish Dry} - \text{Dish})) / \text{Sample Weight} * 100$ (Pretorius 2008).

To calculate the total dry matter the formula used was: $\text{Total Dry matter} = (\text{Dry Hop} - [(\text{Dry Hop} / \text{Sample weight}) * (\text{Sample Weight} - \{\text{Sample \& Dish Dry} - \text{Dish}\})]) / \text{Fresh Hop} * 97.5$ (Pretorius 2008). Harvest was commenced when the total dry matter of the hop field reached 22%. All the bines in the research blocks were cut \pm 1-1.5 metre above the ground. The cut bines were pulled from the top wire, placed onto shading nets, kept separate, and transported to the static Rob Roy Farm hop picker. At the picking machines the bines were offloaded at the feeding mechanism of the picker (bine track) and fed base first through the primary pickers, here the bines were stripped and cones, leaves and some laterals are removed. At a second stage of picking, laterals torn off in the primary picker are picked. Cones were collected at the final product belt and weighed (kg green weight). Bines and leaves were collected at the waste belt and weighed.

3.5.4 Biomass production

Cones were dried in mini kilns at SAB Hop Farms Research facilities for 9h at 65°C, dry weight recorded (dry hop mass). These mini kilns simulate commercial hop drying. The bines were cut into smaller pieces, together with the leaves dried at $\pm 80^\circ\text{C}$ for 48h, and weight recorded. The following formula was used to determine biomass (kg ha^{-1}): (dry hop mass (kg)/Area (ha)).

3.5.5 Yield

Hop cones were dried to 92 % dry matter (or 8% moisture - standard practice in the industry). The following calculation was used to determine the Yield: [(Total dry matter (%)/ (92 / green weight (kg))]/Area (ha) (Pretorius 2008).

3.5.6 Alpha acids determination

Hop bitter acids (alpha and beta acids) were analysed by HPLC method according to EBC 7.7 (Analytica-EBC 2000). Three composite samples was analysed, after been kilned dried for 9h at 65°C. Dried hop cones were milled, 10 g was weighed out into a 250ml glass bottle and a mixture of diethyl ether (100 ml, Merck) and methanol (20 ml, Merck) was added. The sample was then shaken for 30 min to extract it and then for another 10 min after adding 40 ml of 0.1 M HCl solution (Merck). The extract was allowed to stand for 15 min for it to settle and for the ether phase to separate. A grade pipettes were used to transfer 4 ml of the filtrate to a 50 ml (A grade) volumetric flask and made up to volume with HPLC grade methanol. The extracts were fractioned by HPLC (Agilent 1100) using a Nucleodur 100_5 C18, 5 μ ODS RP18, 250 mm \times 4 mm column (Marcherey-Nagel, Düren, Germany). The injection sling was 10 μ l. Determination was carried out by UV/VIS detector, with external calibration at 314 nm. The mobile phase used for separation was solvent A (methanol-water-phosphoric acid = 750:240:10). Solvent B (methanol-water = 1:1) was used for cleaning of the column after each run. Water was prepared according to ISO 3696: 1998, second grade, and phosphoric acid was 85%, purchased from Merck. Solvents A and B were filtered through a membrane filter ($\phi = 47$ mm; 0.2 μ m) before use. The flow rate was 1.5 ml/min. Peaks were identified by comparison of the retention times with those of standard reference compounds, as well as by inspection of the respective UV spectra. An external calibration standard was prepared to quantify α - and β -acid. Fifty (50) mg ICE-3: International Calibration Extract 3;

(13.88% cohumulone, 30.76% humulone + adhumulone, 13.44% colupulone, 10.84% lupulone + adlupulone), Versuchsstation Schweizerische Brauerei, Zürich, Switzerland) was dissolved in 100 ml acidic methanol (0.25ml 85% phosphoric acid in 500 ml HPLC methanol). The mixture was left for 60 min and a 10%, 20%, 30% and 50% dilution made for a multi-level calibration. These were dispensed into HPLC vials, capped and kept at 0°C.

3.5.7 Total carbohydrates: Sample collection and preparation

Three random plants were tagged in each of the five replicate treatment and control blocks. These plants were removed from the field on 06th of April 2010 after harvest. After washing out the root system it was separated into storage roots (which included the basal perennial portion of the stem, where new shoots arose, referred to as the rootstock). The rootstock was cut into a representative sample (10 roots measured at 10cm), weighed and dried for 48h at 70°C. It was allowed to overnight in the desiccator, grounded with a hammer mill, and stored at room temperature until chemical analysis.

The total carbohydrate was estimated by the anthrone method (Hedge and Hofreiter 1962). Carbohydrates are first hydrolyzed into simple sugars using dilute hydrochloric acid. In a hot acidic medium, glucose is dehydrated to hydroxyl methyl furfural. This compound forms a green coloured product with anthrone with an absorption maximum at 630 nm. The anthrone reagent was made up by dissolving 200 mg anthrone in 100 ml of ice-cold 95% H₂SO₄. Standard glucose stock was made up by dissolving 100mg in 100ml distilled water. Ten millilitres of the stock solution was diluted to 100 ml distilled water to make up the working standard. One hundred (100) mg of the milled sample was weighed and placed into a glass boiling tube in a boiling water bath. This was hydrolyzed by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N HCl and then cooled to room temperature. The solution was neutralized with solid sodium carbonate until the effervescence ceased and made to 100 ml. The supernatant was collected and 0.5 and 1 ml aliquots were taken for analysis. A Standard solution was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard. The 0ml served as a blank. The volume was made to 1 ml in all the tubes including the sample tubes by adding distilled water. Then 4 ml of anthrone reagent was added and heated for eight minutes in a boiling water bath and then cooled. Green to dark green colour was recorded at 630 nm. A standard graph was drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. The amount of carbohydrate present

in the tube was calculated from the graph as follow: Amount of carbohydrate present in 100 mg of the sample = (mg of glucose / volume of test sample *100)

3.5.8 Statistical Analysis

SAS/STAT software version 12 (SAS 2014) was used to do a Two way Analysis of Variance (ANOVA). The normal probability plot was performed to identify normality of residuals, and least significant difference. The General linear model procedure was used to calculate the least square means. Repeated measures of ANOVA were performed to test the equality of the means.

3.6 References

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Chapter 4

The effect of early stripping on the vegetative growth, light interception and biomass of hops

4.1 Introduction

Photosynthesis is the fundamental physiological process of plant growth (Wullschleger and Oosterhuis 1990; Yu et al. 2001). The annual hop cycle of hops consists of a period of dormancy followed by vegetative growth. The main ontogenetic phases in hops are linear growth of bines, a period of establishment, shoot formation and shooting, period of butonization and inflorescence, and cone formation (Rybacek 1991). Hops grow up to 8m in height during a growing season leading to leaves of different ages on the hop plant. Photosynthetic maturity is reached much earlier in leaves at the bottom of the canopy, than those at the top. They thus reach senescence earlier (Ananieva et al. 2008).

The leaf angle, leaf area distribution and leaf area index plays a very important role in light interception, canopy photosynthesis, and consequently yield (Hirose et al. 1997). All the above ground organs of the hop plant are modified for photosynthesis except the fruit (cones). The photosynthetic process starts as soon as the plants begin sprouting and increases gradually. The balance between photosynthesis and respiration facilitates the thickening of each organ and elongation growth (Rybacek et al. 1980). The intensity of photosynthesis is increased as the plant transitions from vegetative to regenerative growth, because of the shift in assimilate distribution and significant changes in enzyme activity. Very young leaves have a smaller leaf area compared to fully grown leaves, resulting in capturing smaller amounts of radiation and contain less chlorophyll. High photosynthetic capacity is experienced during the hop generative period (flowering and cone formation) (Rybacek et al. 1980; Pokorny et al. 2011). Older leaves have a lower photosynthetic efficiency (Kenny 2005), compared to younger leaves.

Stripping is a common practice in hop growing regions around the world whereby all excess shoots, leaves and laterals are chemically or mechanically removed from the lowest part of the bines (Sirrinc et al. 2010), when plants have grown ± 1.8 m. The timing and frequency has a significant influence on the carbohydrate concentration which will affect the vegetative

growth phase the following year. In South Africa this practice is carried out just before harvest, whereby all basal growth is chemically removed. By removing all basal leaves before harvest, photosynthesis and carbohydrate accumulation before dormancy is restricted (Madden and Darby 2012). This study was conducted to evaluate whether the timing of stripping in South Africa negatively affects the vegetative growth, biomass and light interception.

4.2 Materials and Methods

4.2.1 Locality

The trial was repeated on three different localities, during the growing season of 2009-2010, namely: The Rob Roy Research Farm (Farm A Tram) near George (-33.52355°22.2143.8°, altitude 223 m) where a tramline planting system was used and Heidekruin Farm (Tramline planting system – Farm B Tram) and the Heidekruin Farm (conventional planting system – Farm B Conv), in Waboomskraal valley (-33.86954°22.34939°, altitude 601 m). Farm A Tram and Farm B Tram fields were both three years old and Farm B Conv was a five year old hop field. The experimental design was a randomized complete block design with 5 replications (blocks) for each of the two treatments namely: early chemical stripping (early stripping) and late chemical stripping (control).

4.2.2 Cultivation practices and treatments

Early chemical stripping was done during the active growing phase (mid-November) by defoliating leaves and killing basal growth on the vines up to a height of 0.4 meter with Gramoxone at a rate of 1 l ha⁻¹ during mid-November, while the control blocks were only defoliated and basal growth killed up to 1.0 meter height during cone formation (mid-January). The Southern Star variety was used for this trial. The management of the blocks in terms of irrigation, fertilisation, weed and pest control and harvest was in accordance with the best operating practices of the SA Hops Growers Association (Linsley-Noakes 2013), as per standard operating procedures described in Chapter 3.

4.2.3 Data collected

4.2.3.1 Climate

The drought experienced in the 2009 growing season in George and Waboomskraal was the worst (Meier 2010) in 50 years and 30 to 40% of producers ran out of water 2 months before the harvest season (Conway 2010). The average temperature for George during July was slightly above the long term average, resulting in a lower dormancy level. This dormancy period of 5 weeks during July is critical for successful emergence of hop shoots in spring. The total rainfall recorded for the George locality (Rob Roy Research Farm- Farm A Tram) in 2009 was 603.8 mm which was 15.2 % lower than the long term average (1960-2009). The total rainfall recorded for Waboomskraal locality (Heidekruin Farm- Farm B Tram & Conv) in the same period was 669 mm, which was 17 % lower than the long term average. The rainfall in the Waboomskraal locality was especially low at the start of the hop growing season in September. This trend was also seen in November when rapid shoot growth was prominent. Continued drought conditions were experienced in November, January and March in both localities. This is the critical period of active vegetative growth, lateral formation, flowering and cone formation. Heavy rainfall was experienced in the George locality in the beginning of February as harvest was approaching. Below average temperatures in Waboomskraal during October and November and during November in George resulted in a prolonged vegetative growth as the start to the season was not rapid. The above average temperatures experienced in Waboomskraal (February and March) and the continued water scarcity in both localities resulted in the hops becoming over mature. This was also noted in the overall crop percentage dry matter which was above the long term average. Hop growth was hampered by the continued drought situation experienced in November to March.

4.2.3.2 Vegetative growth

Three plants were randomly selected at the start of the season and tagged on each early stripping and control block. Plant height measurements were recorded weekly, by measuring the distance from the ground level to the highest point on the plant until it reached the highest point in the canopy at 5 meters.

Relative growth % was calculated by using the following formula: $((x - y)/y) * 100$ where x = the bine length at the measurement date at end of current week and y = the bine length of measurement date of end of previous week, for 15-18 weeks.

4.2.3.3 Photosynthetically active radiation

Photosynthetically active radiation (PAR- $\mu\text{mol m}^{-2} \text{sec}^{-1}$) measurements were taken for each replication of the early stripping and control blocks from the start of the growing season in October, when active growth was present, using the Sun Fleck PAR ceptometer. Readings were taken between 1200h and 1400h, on plant rows at the following fixed heights: soil level, 1 meter, 3 meters and 5 meters. This was repeated weekly for all levels, irrespective of growth stage. The percentage of light intercepted was calculated as follows: (Intensity reading at 5m – intensity reading at specific height (average of soil level, 1m or 3m)/ (intensity reading at 5m)*100.

4.2.3.4 Total biomass

At harvest all plants in the early stripping and control blocks were harvested, cutting the bines at 1 – 1.5 meters above ground level. Plants were transported to the Rob Roy Research facility where it was picked with a static Wolf hop picking machine. The fresh weight of the cones was recorded together with the leaf, stem and bine weight. Cones were dried in mini kilns at SAB Hop Farms Research facilities for 9h at 65°C, dry weight recorded (dry hop mass). These mini kilns simulate commercial hop drying. The bines were cut into smaller pieces, together with the leaves dried at $\pm 80^\circ\text{C}$ for 48h, and weight recorded. The following formula was used to determine biomass (kg ha^{-1}): (dry hop mass (kg)/Area (ha)).

4.2.3.5 Statistical analysis

Repeated measures of ANOVA were performed to test the equality of the means, on weekly measurements. A two way Analysis of variance was performed, using SAS/STAT software version 12 (SAS 2014). The normal probability plot was performed to identify normality of residuals, and least significant difference. The General linear model procedure was used to calculate the least square means.

4.3 Results and Discussion

4.3.1 Vegetative growth

No significant farm and treatment interaction during the 2009-2010 hop growing season ($P=0.44248$) was recorded. Relative growth (%) across the different farms did not differ significantly ($P=0.55770$) (Figure 4.1), however relative growth tends to be higher during week 3 on Farm A Tram (George microclimate). The rainfall in the Waboomskraal locality (Farm B Tram and Farm B Conv) was especially low at the start of the hop growing season during September (Week 2-4), and below average temperatures was experienced during October which is the critical period for vegetative growth. This can explain why Farm A Tram yielded higher relative growth during week 3 compared to Farm B Tram and Farm B Conv. Relative growth during the rest of the growing season was constant across the different sites, but a general trend was observed that the relative growth on Farm A tram took at least 3 weeks longer to reach the top wire, this phenomenon can be attributed to climatic differences between the two localities i.e. insufficient winter chilling and drought conditions that prevailed during the course of the study.

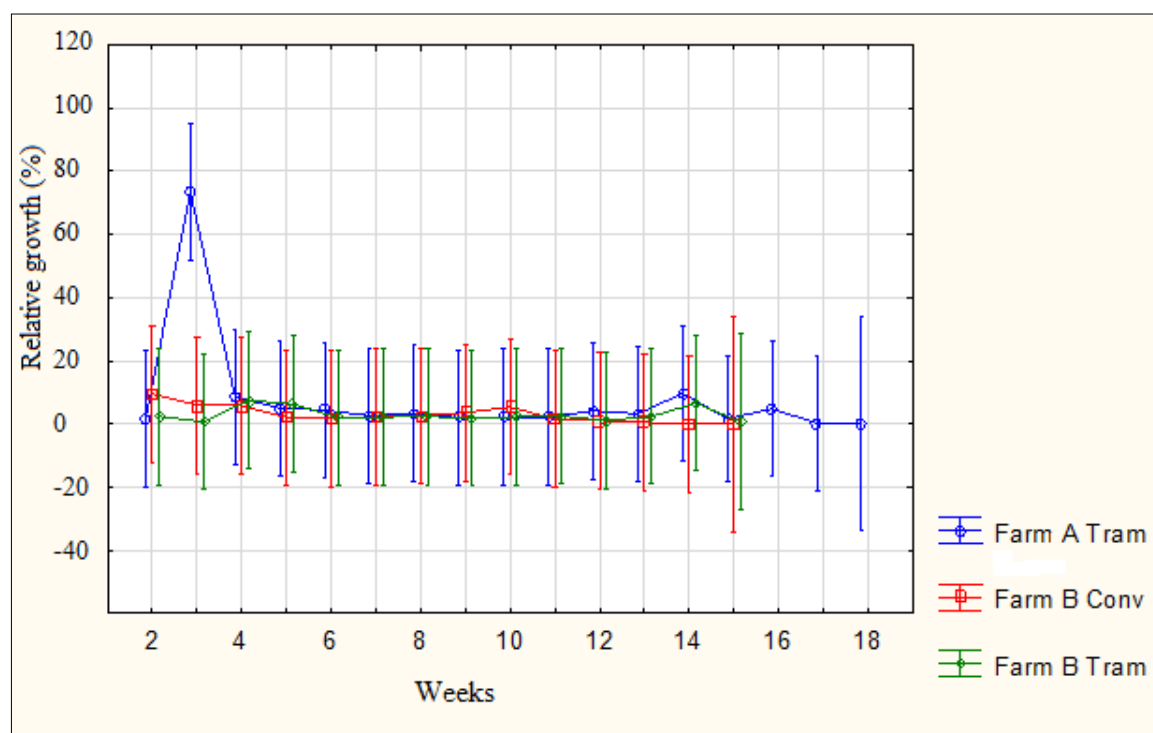


Figure 4.1. Mean percentage relative growth of on three different sites during the 2009-2010 hop growing season ($P=0.55770$). Vertical bars denote 95% confidence intervals.

The percentage relative growth for both the early stripping and control plants followed the same trend across the different localities during the growing season (Figure 4.2), except for week 3 where the control plants were growing quicker than the early stripping plants. This could be due to plant stress as the early stripping plants were chemically burnt off the previous week. Little difference was observed between the treatments, in both the active vegetative growth phase (week 4-11), and final growth (up to week 14). A general trend was observed from week 15 to week 18 where the early stripping plants tend to grow quicker than the control plants. This phenomenon can be due to increased competition in the control blocks, because more basal shoots are attempting to climb onto the hop strings, whereas in the case of the early stripping treatment the four main bines exhibited apical dominance.

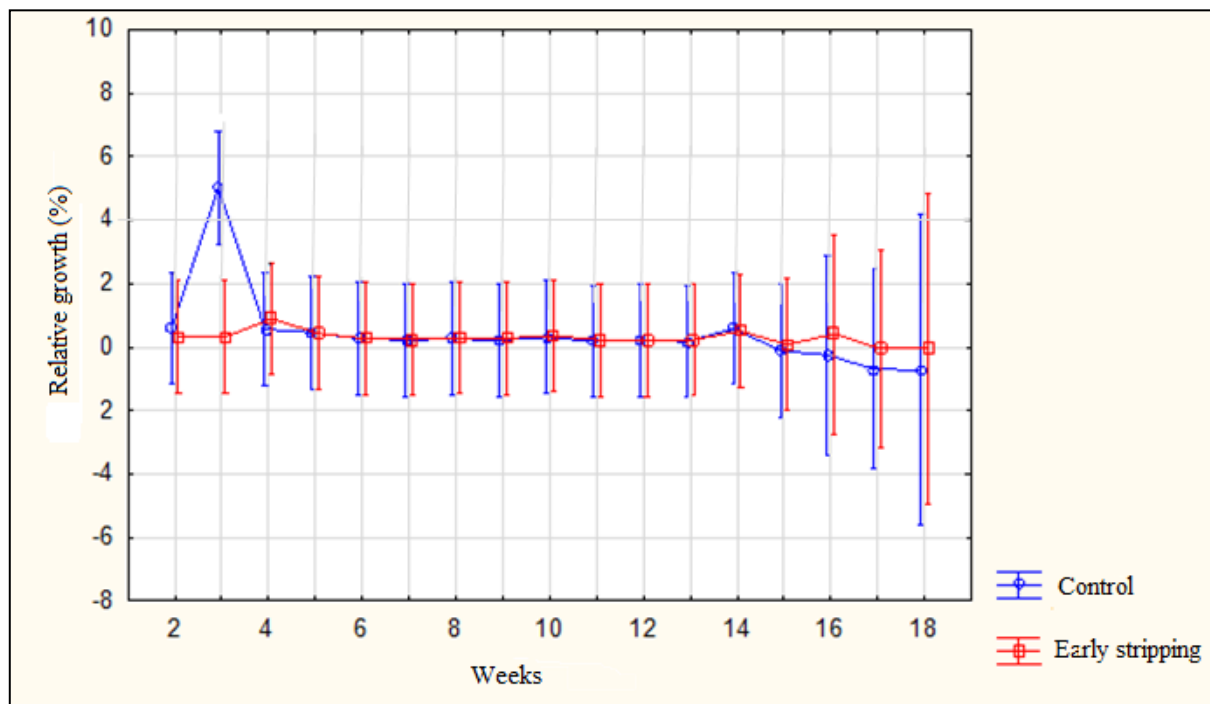


Figure 4.2. Mean percentage relative growth of the treatments on three different sites during the 2009-2010 hop growing season ($P=0.50547$). Vertical bars denote 95% confidence intervals.

4.3.2 Percentage light interception and light intensity

4.3.2.1 Percentage light interception

The structure of the canopy and spatial distribution of leaves is important in determining light interception and productivity (Keller 2010), as the light intercepted in the canopy reflects crop growth (Monteith 1969). Knowledge of canopy light distribution and absorption is fundamental for understanding many aspects of crop growth and productivity (Whisler et al. 1986). Leaf area development is an important factor determining light interception and as a result yield and photosynthetic potential (Milthorpe and Moorby 1974). Denser crop canopies results in higher interception values, thus higher potential photosynthetic areas. Significant differences were observed between the two treatments on the different localities ($P=0.00001$). Early stripping across all sites trended lower than the control treatments; this can be attributed to the less dense canopies due to the removal of excess shoots early in the season, as light interception is influenced by canopy density. In general (Figure 4.3) the mean light interception (soil level, 1m and 3m) in both the control and early stripping treatment on Farm A Tram (George locality) were lower than that for the same treatments on the Farm B Tram and especially Farm B Conv (Waboomskraal localities). Farm B Conv trended higher than both Farm A Tram and Farm B Tram on both the control and early stripping treatment.

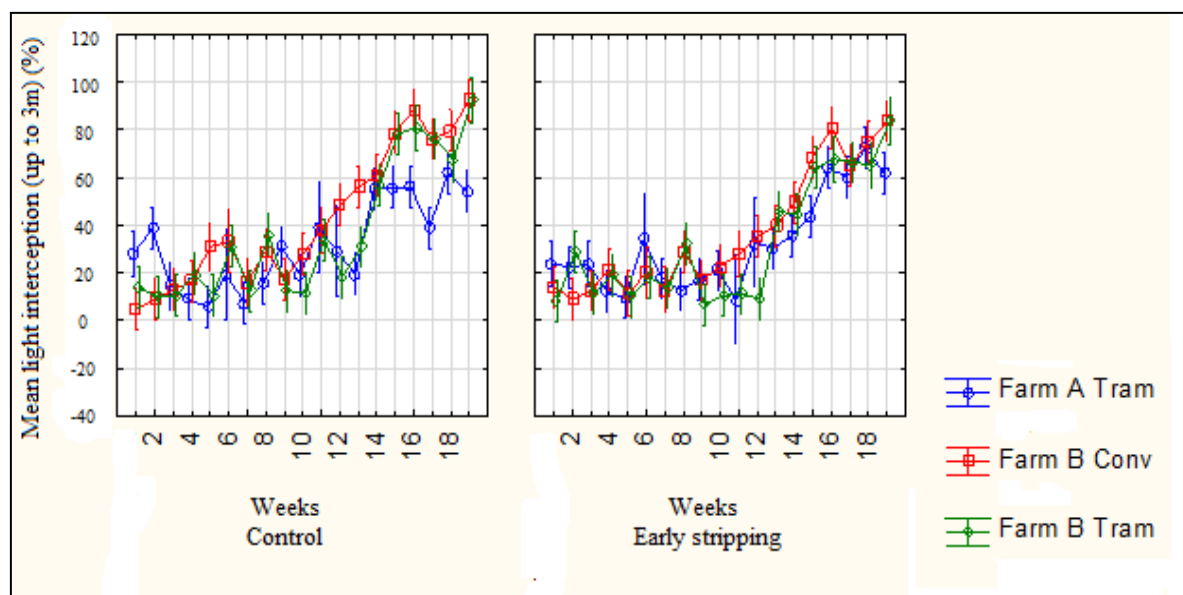


Figure 4.3. Mean percentage light interception of soil level, 1m and 3m of the early stripping and control treatments on three different farms during the 2009-2010 hop growing season ($P=0.00001$). Vertical bars denote 95% confidence intervals.

Planting method on the conventional rows (Farm B Conv) was more favourable for light interception compared to the tramlines, this can be ascribed to the spacing between rows.

4.3.2.2 Light intensity

Light intensity refers to the amount of light that plants receive. The quality is as important as the quantity. Light is an absolute requirement for plant growth and development. Plants have different optimum requirements and both deficient and excessive light intensities are detrimental to the plant. Subject to physiological limits, an increase in the intensity of light will result in an increase in the rate of photosynthesis (Manaker 1981). In general the results showed a significant treatment x farm interaction ($P= 0.04074$). The time of stripping did not have the same effect on light intensity on the different farms. Compared to the control, early stripping tended to result in higher light intensity on Farm A Tram and Farm B Conv, but this tendency was not observed on Farm B Tram. Significantly lower light intensity levels were experienced in both control and early stripping treatments on Farm A Tram (George microclimate) compared to Farm B Tram and Farm B Conv.

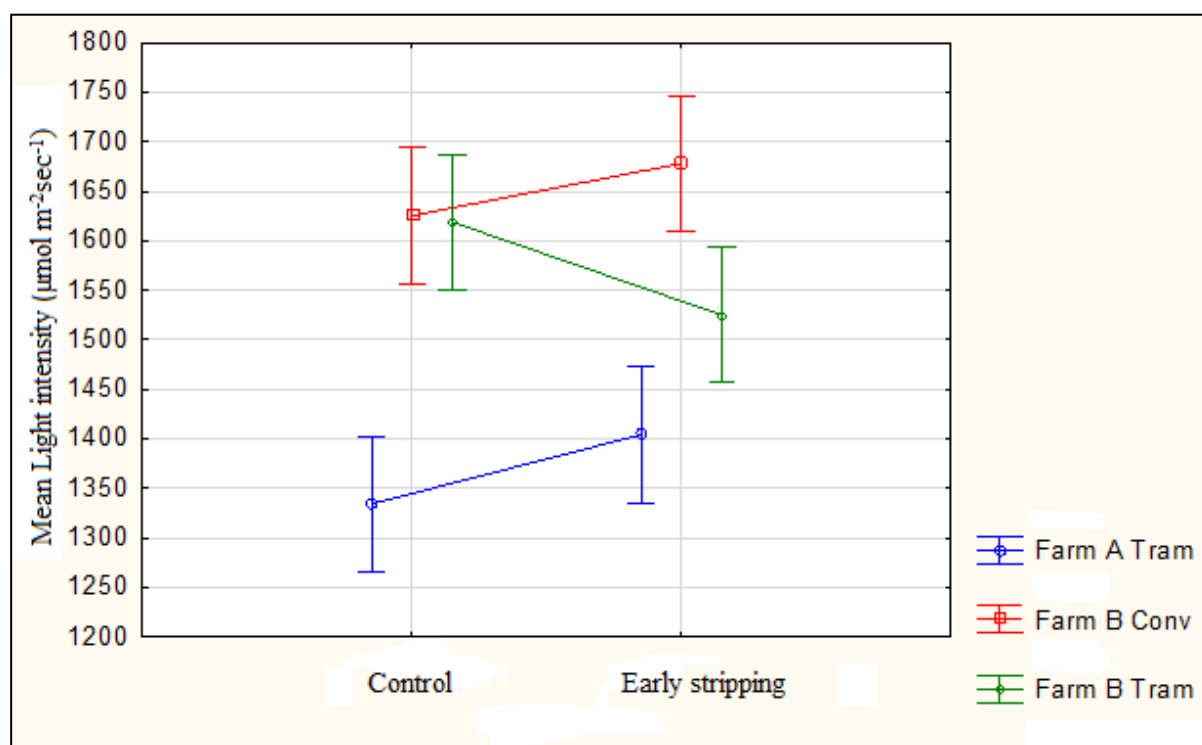


Figure 4.4. Mean light intensity levels of the treatment farm interaction on three different farms during the 2009-2010 hop growing season ($P=0.04074$). Vertical bars denote 95% confidence intervals.

4.3.3 Total biomass

The time of stripping had a significant effect on the biomass produced in this study, but the effect differed for different localities (microclimates) (Figure 4.5). Both Farm B Tram and Farm B Conv (Waboomskraal microclimate) showed a decrease in biomass production for the early stripping treatments when compared to the control, but such a response was not shown for Farm A (George microclimate). Differences in the microclimate and drought conditions that prevailed during the course of the study could also attribute to the differences observed in the total biomass production, between the two localities. The higher values for the control plots on Farm B can be attributed to the fact that only four bines were allowed to grow on the twine of the early stripping treatments, while there was a spike in the relative growth in the control treatment on Farm A (Figure 4.2), but this trend was not observed throughout the season. More bines competing in control treatments on Farm A tram resulted in very few bines climbing onto the strings and reaching maturity, this can also be seen in the longer vegetative growth period as illustrated in Figure 4.1. In the control there was no prevention of additional bines training themselves on the twine, thus resulting in more bines per string, and greater biomass production.

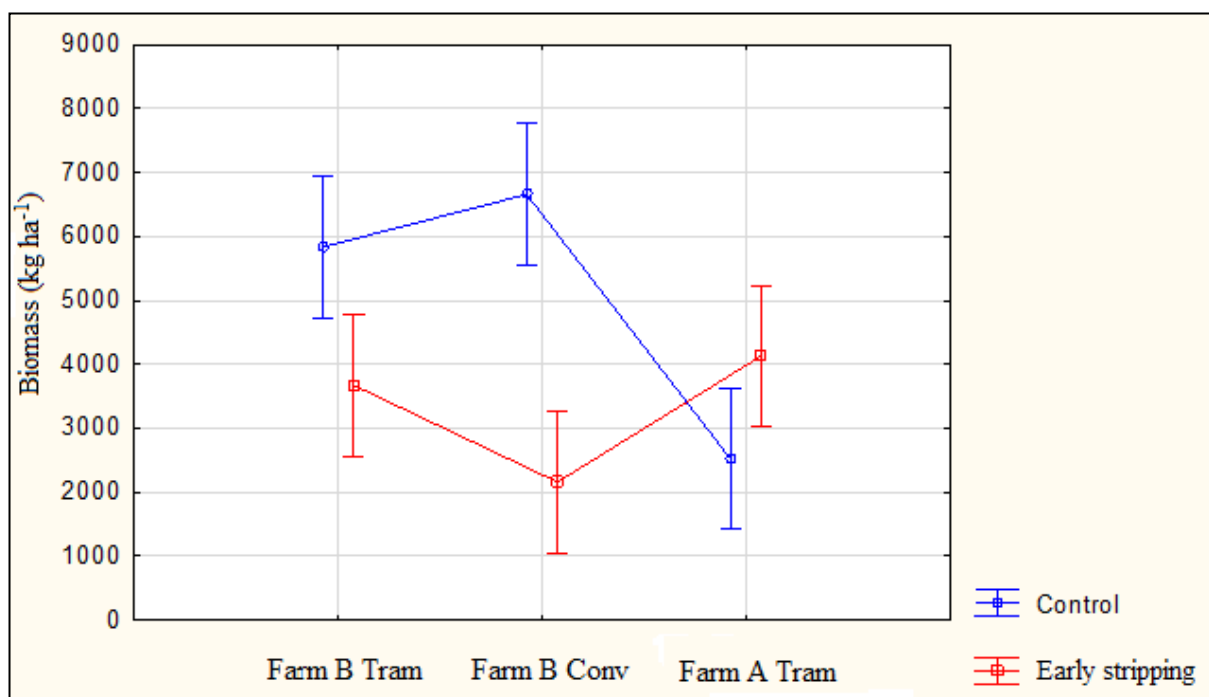


Figure 4.5. Plant biomass production (kg ha^{-1}) as influenced by the timing of stripping on the three different sites, Farm A Tram (George microclimate), Farm B Tram and Farm B Conv. (Waboomskraal microclimate) during the 2009-2010 hop growing season ($P=0.00003$). Vertical bars denote 95% confidence intervals.

On average vegetative growth in the George microclimate (Farm A Tram) tend to be lower when compared to the Waboomskraal microclimate for the same production system (Farm B Tram) and early stripping tend to result in a higher biomass production with apical dominance while poorer plant growth in the control treatment lead to premature flowering before bines reached the top wire.

4.4 Conclusion

A general trend was observed that relative growth on Farm A Tram (George locality) took at least 3 weeks longer to reach full growth potential than relative growth on Farm B Tram and Farm B Conv (Waboomskraal locality), which could result in bines not fully mature at onset of dormancy. This phenomenon can be attributed to climatic differences between the two localities and drought conditions that prevailed during the course of the study. Control treatments across all sites resulted in denser canopies compared to early stripping treatments as seen in the mean percentage light interception. This phenomenon can be ascribed to the controlled amount of bines in the early stripping treatment and no late sucker growth as in the case of the control treatments. The timing of stripping did not have the same effect on light intensity across the different farms. Significant differences were observed in total biomass production, this is due to different ages of the plants, different planting method used and different microclimates.

The control treatments yielded more biomass due to more bines being allowed to grow, as the early stripped treatments were chemically stressed in the beginning of the season resulting in limited bine growth. Early stripping favoured biomass production on Farm A, this could be the result of bines exhibiting apical dominance and more bines competing in control treatments resulted in very few reaching full growth maturity. Microclimate had a big influence on the vegetative growth and light interception, which influences the plants responsiveness to increasing day lengths and the growth rate, but is unfortunately not controllable. Early stripping does seem to be advantageous in the Waboomskraal microclimate in terms of total biomass production.

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Chapter 5

Reaction of soft resins (alpha & beta acids) content and yield to time of stripping

5.1 Introduction

The alpha-acids content of hops is an important commercial and varietal characteristic, both to the hop buyer and the hop grower (Pavlovic and Pavlovic 2011). The genetic make-up of a particular variety establishes the limits within which the alpha-acids content of that variety will fluctuate from year to year and even, within one year, from district to district and from farm to farm (Pavlovic et al. 2013).

The hop secondary metabolite profile consists of three chemical groups: hop acids (alpha and beta acids), polyphenols and essential oils (Benitez et al. 1997). Thirty percent of the hop cones are dominated by alpha and beta acids (Murphey and Probasco 1996; Benitez et al. 1997; De Keukeleire et al. 2003; Van Cleemput et al. 2009; Clark et al. 2013), three to six percent by polyphenols and tannins, and essential oils range between 0.5 – 5 ml 100g⁻¹ (Benitez et al. 1997; Eri et al. 2000; Van Cleemput et al. 2009). These resins are enclosed in the lupulin glands in each strobilus (Oliveira and Pais 1990; Sugiyama et al. 2006). Alpha acids are responsible mainly for the bitter taste in beer (Neve 1991). Beta acids are responsible for bitterness, preservative qualities, and antimicrobial activities (Shimwell 1937; Michener et al. 1948; Hough et al. 1957; Neve 1991), whilst the fractions in the essential oils are responsible for flavour and aroma. Polyphenol compounds have been found to have potential pharmaceutical applications, predominantly 8-prenylnaringenin as a phytoestrogen (Milligan et al. 2002) and xanthohumol as a cancer chemo preventative agent (Miranda et al. 1999). The ratio between alpha and beta acids is fairly consistent from year to year, and is genetically determined (Likens et al. 1978). Secondary metabolite profiles are variety specific, with unique bittering and flavouring potential (Sharpe and Laws 1981; Čeh et al. 2007). Cone maturation and environmental conditions have an influence on the chemical profile of varieties (Murphy and Probasco 1996; De Keukeleire et al. 2003).

Alpha acids strictly correspond with the growing conditions during the crop year (Srecec et al. 2008; Pavlovic 2012). Only ten percent of alpha acids are formed two weeks before

harvest. The rest have already formed by that stage (Hecht et al. 2004). Certain weather conditions can promote an increase in the biosynthesis of alpha acids in the plant, while water stress will lead to a reduction in alpha acid levels due to the poor condition of hop plants (De Keukeleire et al. 2007). In studies by Zattler and Jehl (1962); Thomas (1980); Srecec et al. (2008); Kucera and Krofta (2009); and Mozny et al. (2009), data was not consistent as to which weather-related parameter had the maximum impact on alpha acid production or precisely during which vegetative period. In a study done by Pavlovic et al. (2013) in Slovenia, it was found that temperature strongly affects the alpha acid content from the start of growth until August (mid-summer), and has a significant influence from intensive vegetative growth until the end of flowering. Rainfall has the highest correlation with alpha acids and this association declines as the plant begins to flower and cones are formed (Pavlovic et al. 2013). Burgess (1964) also found that there was an association between alpha acid concentration and the number of sunshine hours, harvest time and weather patterns. The environmental factors responsible for alpha acid levels are extremely complex. Cone weight, resin content and resin gland sizes are all influenced by environmental factors during flower initiation. Temperatures below or above a threshold during flowering, result in lower alpha acid concentrations. Warm weather prior to harvest increases alpha acid ranges (Burgess 1964). In a study in Australia it was found that there might be two temperature thresholds, one during late January and again the first half of February that might independently exert a depressing influence on alpha acid biosynthesis. Their critical period was from 18th -23rd January until 7th-12th of February with average minimum and maximum temperatures of 10.4°C and 23.1°C (Versluys 1981). It was suggested that mean air temperatures between time of flowering and harvest has considerable influence on alpha-acids content, and this relationship is linear on both sides of the optimum temperature which may vary with hop variety. Alpha-acids content decreases above or below these optimum temperatures (Smith 1970).

Agricultural practices do not seem to have an effect on alpha acid content (Versluys 1981). Variations in the quantities of applied N-P-K fertilizers had a negligible effect on the alpha-acids contents of Fuggle and Cluster hops. High nitrogen could reduce the alpha acid levels (Keller and Magee 1954; Brits and Linsley-Noakes 1994). A combination of low wirework and close spacing, resulting in heavier shading, has no appreciable effect on the alpha-acids contents (Thompson and Jary 1967). Plants that are grown in the field under 50 per cent

shade have the same alpha-acids content as comparable plots grown normally (Thomas 1968). Farrar et al. (1970) and Connaughton (1977), also shared the view that the weather parameter most closely associated with alpha-acids content in English and Irish hops is the mean air temperatures during the pre-harvest period.

Zattler and Jehl (1962) concluded from a correlation of annual mean soft resin contents of hops grown in the German Hallertau area with weather conditions over a period of 35 years, that hot dry summers with much sunlight during cone formation produce hops with low “preservative values”, while moist summers with low temperatures and normal amount of sunshine give very good preservative values.

One of the breeding program aims is to increase the yield by means of directly increasing alpha acids in hop cones, or increasing the amount of flowers and subsequent cones (Brits 2009). Yield is very complex, and hops are no different to other cultivated plant species (Keller and Likens 1955; Roberts et al. 1980; Henning and Townsend 2005). It is influenced by genetics, and integrated physiological and biochemical processes (Blum 1988; Heslop-Harrison and Schwarzacher 2011). Yield may also be influenced by a number of environmental factors, such as day length (Thomas and Schwabe 1969; Pavlovic et al. 2010), nutrient availability, water supply (Svoboda et al 2008; Srečec et al 2008; Kučera and Krofta 2009), temperature, irradiance (Srečec et al 2008; Kučera and Krofta 2009), agricultural practice (Kořen 2007) and pests and diseases (Pethybridge et al. 2002; Krofta and Nesvadba 2003; Weihrauch 2005).

High operating and fixed costs necessitates research to develop a better understanding of increasing hop crop productivity under “non- ideal” climatic conditions, without negatively affecting quality of the product. From literature it became clear that both the yield and soft resin content are largely affected by genetic properties but may also be affected by growing conditions. However, little is known about the response of South African hops to such conditions. The stripping treatment might influence the micro climate within the canopy and this effect may differ for different production conditions. For this reason this study was done in an effort to determine the effect of early stripping on the yield and quality parameters of hops in two hop producing areas.

5.2 Materials & Methods

5.2.1 Locality

The research was done during the growing season of 2009 - 2010 at three sites in the hop growing area in South Africa. One site was in the George area on the Rob Roy Research Farm near George (-33.52355°22.2143.8°, altitude 223 m) and will be referred to as Farm A Tram). The other two sites were in the Waboomskraal area: on the Heidekruin Farm (-33.86954°22.34939°, altitude 601 m) and will be referred to as Farm B Tram and Farm B Conv). Hop field on Farm A Tram and Farm B Tram sites were planted on tramlines during the growing season of 2006, while Farm B Conv. was planted in 2004 on conventional rows (See Chapter 3 for details).

5.2.2 Cultivation practices and treatments

The experimental design at each site was a randomized complete block design with 5 replications (blocks) for each of the two treatments, namely early chemical stripping (Early stripping) and late chemical stripping (control) which is the current practice used by local hop producers. Early chemical stripping was done during the active growing phase (mid-November) by defoliating leaves and killing basal growth on the bines up to a height of 0.4 meter with Gramoxone at a rate of 11 ha⁻¹ during mid-November , while the control blocks were only defoliated and basal growth killed up to 1.0 meter height during cone formation (mid-January). See Chapter 3 for the detailed description of treatments. The Southern Star variety was used for this trial. The management of the blocks in terms of irrigation, fertilisation, weed and pest control and harvest was in accordance with the best operating practices of the SA Hops Growers Association (Linsley-Noakes 2013), as per standard operating procedures described in Chapter 3.

5.2.3 Data collected

5.2.3.1 Climate

The average temperature for George during July was slightly above the long term average. This dormancy period of 5 weeks during July is critical for successful emergence of hop shoots in spring. The total rainfall recorded for the George locality (Rob Roy Research Farm- Farm A Tram) in 2009 was 603.8 mm which was 15.2 % lower than the long term average (1960-2009). The total rainfall recorded for Waboomskraal locality (Heidekruin Farm- Farm

B Tram & Conv) in the same period was 669 mm, which was 17 % lower than the long term average. The rainfall in the Waboomskraal locality was especially low at the start of the hop growing season in September. This trend was also seen in November when rapid shoot growth was prominent. Heavy rainfall was experienced in the George locality in the beginning of February as harvest was approaching. Below average temperatures in Waboomskraal during October and November and during November in George resulted in a prolonged vegetative growth as the start to the season was not rapid. The above average temperatures experienced in Waboomskraal (February and March) and the continued water scarcity in both localities resulted in the hops becoming over mature. This was also noted in the overall crop percentage dry matter which was above the long term average. Hop growth was hampered by the continued drought situation experienced in November to March.

5.2.3.2 Yield

At harvest all plants in the early stripping and control blocks were harvested, cutting the bines at 1 – 1.5 meters above ground level. The cut bines were pulled from the top wire, placed onto shading nets and kept separate. These plants were transported to the Rob Roy Research facility where it was picked with a static Wolf hop picking machine. The fresh weight of the cones (green weight) was recorded together with the leaf, stem and bine weight (biomass). Three composite samples of the fresh cones were analysed in the SAB Hop Farms laboratory to determine the moisture, dry matter, and total dry matter content of the hops, as per standard operating procedures described in Chapter 3. The following calculation was used to determine the Yield: $[(\text{Total dry matter (\%)} / (92 / \text{green weight (kg)}))] / \text{Area (ha)}$ (Pretorius 2008).

5.2.3.3 Biomass production

Cones were dried in mini kilns at SAB Hop Farms Research facilities for 9h at 65°C, dry weight recorded (dry hop mass). These mini kilns simulate commercial hop drying. The bines were cut into smaller pieces, together with the leaves dried at $\pm 80^\circ\text{C}$ for 48h, and weight recorded. The following formula was used to determine biomass (kg ha^{-1}): $(\text{dry hop mass (kg)} / \text{Area (ha)})$.

The Harvest Index was determined using the following equation: $\text{Harvest Index} = (\text{Total Hop Yield in } \text{kg ha}^{-1}) / (\text{Total Biomass produced in } \text{kg ha}^{-1})$.

5.2.3.4 Alpha and beta acid determination

Hop bitter acids (alpha and beta acids) were analysed by HPLC method according to EBC 7.7 /Analytica-EBC (2000). The five replicates (blocks) were harvested at the Research Farm picking facility and samples were taken to the SAB Hop Farms laboratory and kiln dried for 9 hours at 65 °C. Dried hop cones were milled, 10 g was weighed out into a 250ml glass bottle and a mixture of diethyl ether (100 ml, Merck) and methanol (20 ml, Merck) was added. The sample was then shaken for 30 min to extract it and then for another 10 min after adding 40 ml of 0.1 M HCl solution (Merck). The extract was allowed to stand for 15 min for it to settle and for the ether phase to separate. A grade pipettes were used to transfer 4 ml of the filtrate to a 50 ml (A grade) volumetric flask and made up to volume with HPLC grade methanol. The extracts were fractionated by HPLC (Agilent 1100) using a Nucleodur 100_5 C18, 5 μ ODS RP18, 250 mm \times 4 mm column (Marcherey-Nagel, Düren, Germany). The injection volume was 10 μ l. Determination was carried out by UV/VIS detector, with external calibration at 314 nm. The mobile phase used for separation was solvent A (methanol-water-phosphoric acid = 750:240:10). Solvent B (methanol-water = 1:1) was used for cleaning of the column after each run. Water was prepared according to ISO 3696: 1998, second grade, and phosphoric acid was 85%, purchased from Merck. Solvents A and B were filtered through a membrane filter (ϕ = 47 mm; 0.2 μ m) before use. The flow rate was 1.5 ml/min. Peaks were identified by comparison of the retention times with those of standard reference compounds. An external calibration standard was prepared to quantify α - and β -acid. Fifty (50) mg ICE-3: International Calibration Extract 3; (13.88% cohumulone, 30.76% humulone + adhumulone, 13.44% colupulone, 10.84% lupulone + adlupulone), Versuchsstation Schweizerische Brauerei, Zürich, Switzerland) was dissolved in 100 ml acidic methanol (0.25ml 85% phosphoric acid in 500 ml HPLC methanol). The mixture was left for 60 min and a 10%, 20%, 30% and 50% dilution made for a multi-level calibration. These were dispensed into HPLC vials, capped and kept at 0°C.

5.2.3.5 Statistical analysis

Analysis of variance was performed, using SAS/STAT software version 12 (SAS 2014). The normal probability plot was performed to identify normality of residuals, and least significant difference.

5.3 Results and discussion

5.3.1 Yield

Hop yield is influenced by the number of bines that reach the top wire (Kořen 2007). Plant row spacing is one of the most important agricultural practices that have an influence on yield, as this influences canopy density. This differs for different countries. Plant row spacing, in some of the main hop growing areas in the world are for example in Bavarian Hallertauer (Germany), 3.2 m between rows, in Elbe- Saale (East Germany) and Czech Republic, 3.0 m, in South Africa a mixture of 2.4 and 3.6 m. The general trend in Europe is to use wider rows and smaller distances between plants (Kořen 2007). In this study two different row spacing's were used, Tramlines with double rows and between row spacing of 3.6 m, and conventional planting system of 2.4 m (See Chapter 3).

The difference in yield between the control and early stripping differed significantly between farms and production systems (Figure 5.1). In particular, very little difference between the control and treatment was observed on Farm B (Waboomskraal) irrespective of the planting system (Tram or Conventional planting systems), whereas Farm A Tram does result in differences in treatments ($P= 0.00966$), with significantly higher yields as a result of the early stripping treatment. By early defoliation on Farm A Tram it was ensured that only four bines reached the top wire with no competition from additional shoots. This resulted in increased yield. Bines on the control areas flowered prematurely before it reached the top wire, resulting in yield loss and this can also be seen in the reduced biomass produced (Figure 4.11). The amount of bines that reaches the top wire is critical in increasing yields as described by Kořen (2007). Yield of control plots on Farm A were significantly lower when compared to Farm B, irrespective of the planting system (Figure 5.1). This difference can be attributed to the differences observed in the microclimate and temperatures during the growing season (Figure 3.1 and 3.2). Temperature and water supply (rainfall/irrigation) are key environmental factors that influence yield. The temperatures experienced during the

dormancy period in the George microclimate (Farm A Tram) were slightly above the long term average, and relatively higher than the Waboomskraal microclimate (Figure 3.1 and 3.2), which resulted in a slow start to the growing season. Lower temperatures in the George microclimate compared to Waboomskraal microclimate were experienced during flowering, cone formation and harvest, which also explains the lower yields in the George microclimate (Farm A Tram) (Figure 3.1 and 3.2).

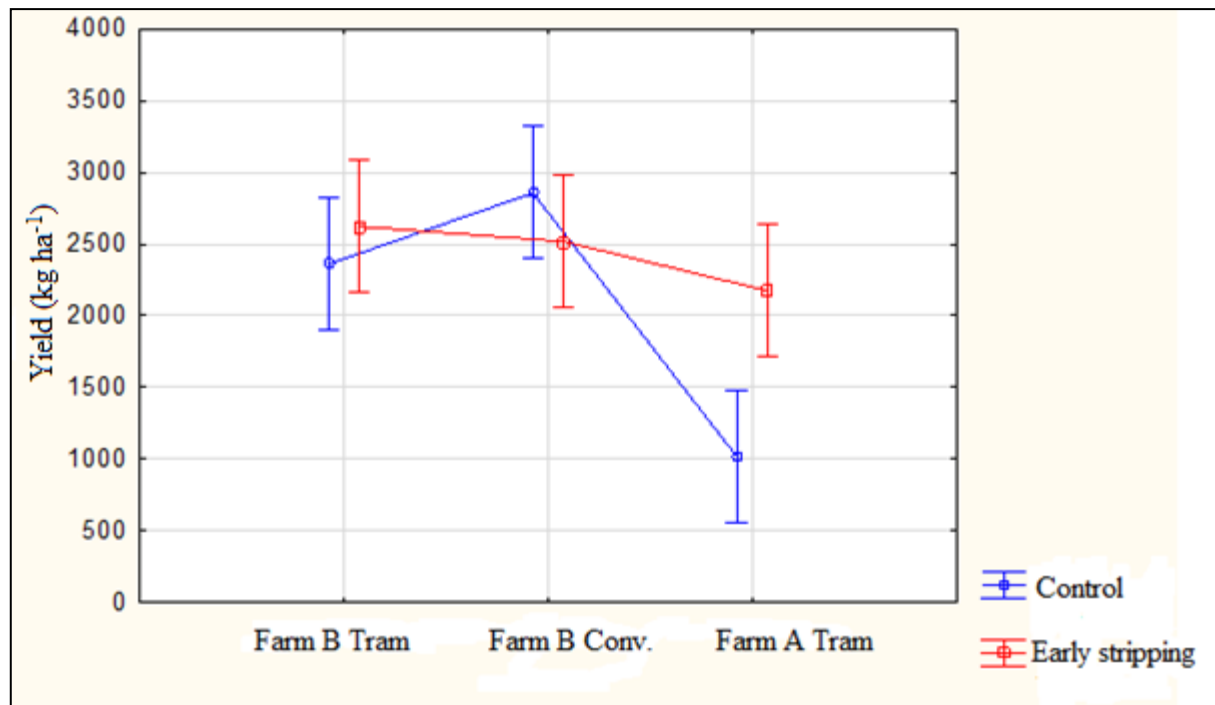


Figure 5.1. Average yield (kg ha⁻¹) as influenced by timing of defoliation (stripping) on the three different locations Farm A Tram (George microclimate), Farm B Tram and Farm B Conv. (Waboomskraal microclimate) during the 2009-2010 hop growing season (P=0.00966). Vertical bars denote 95% confidence intervals.

There was no prevention of additional vines training themselves on the twine in control treatments. This could also be responsible for significant differences between the control and early stripped treatment on Farm A Tram. Climatic stress (drought and lower temperatures) and unlimited shoots on the control treatment could be the cause of reduced yields.

5.3.2 Harvest index

The Harvest Index (HI) is the ratio of yield to total plant biomass and an indicator of the crop's efficiency to convert biomass production into economic yield and thus productivity (Sinclair 1998). In general harvest index tend to be higher in the early stripping treatments compared to the controls, but differences were only statistically significant at the Farm B Conv. site (Figure 5.2). Harvest index on the control plots did not show any difference between sites (Farms), but harvest index of the early stripping treatment was significantly higher at Farm B conv. compared to the tram line production systems on both Farm A and Farm B. These results indicate that although early stripping did not result in higher yields at all sites, hop production was more efficient and this was especially true in the conventional production systems.

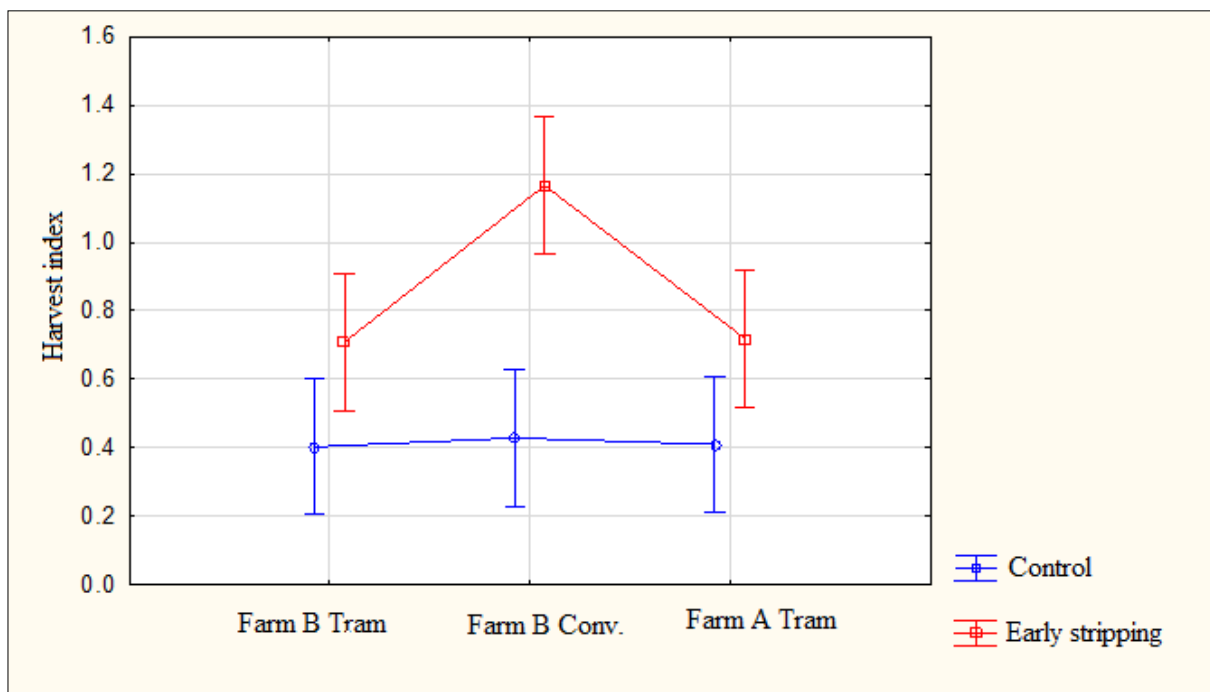


Figure 5.2. Harvest Index (HI) of hops as influenced by timing of defoliation on the different locations: Farm A Tram (George microclimate), Farm B Tram and Farm B Conv. (Waboomskraal microclimate) for the 2009-2010 growing season ($P= 0.0096$). Vertical bars denote 95% confidence intervals.

5.3.3 Alpha acid concentration

Mean alpha acid concentrations did not differ significantly due to the farm and treatment interaction ($P=0.33511$). Higher concentrations of alpha acids were however, observed on both Farm B Conv and Farm B Tram (Waboomskraal locality) when compared to Farm A Tram (George microclimate) (Figure 5.3).

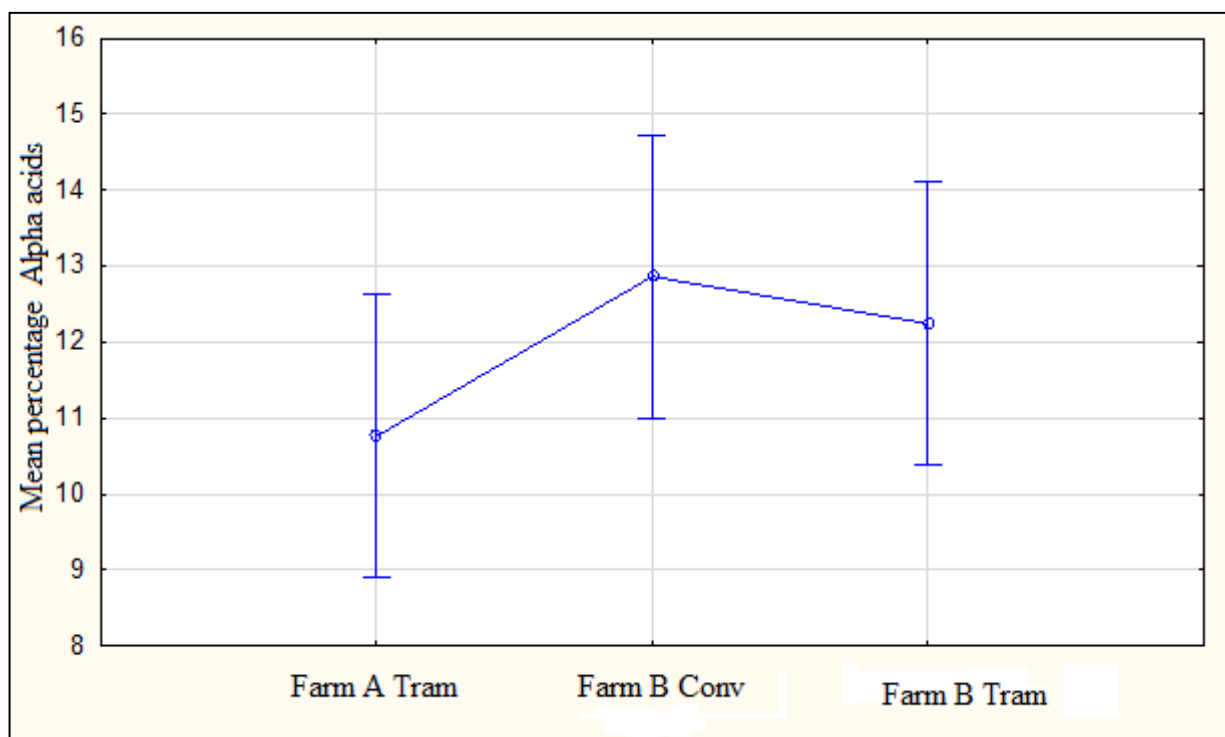


Figure 5.3. Mean percentage alpha acids for the 2009-2010 growing season for the different sites in two microclimates (Farm A Tram – George, Farm B Tram and Farm B Conv.- Waboomskraal) $P= 0.25829$. Vertical bars denote 95% confidence intervals.

Low rainfall in the Waboomskraal locality (Farm B Tram and Farm B Conv) was experienced during November when rapid shoot growth was prominent. Continued drought conditions were experienced in November, January and March in both localities. The mean air temperature during January and February 2010 in the George locality (Farm A Tram) was in line with the 20 °C long term average, whilst the Waboomskraal locality (Farm B Tram and Farm B Conv) experienced mean temperatures of 1.31 °C – 2.78 °C higher than the long term average of 20 °C for that region. Heavy rainfall was experienced in the George locality at the beginning of February as harvest was approaching. This period is critical for lupulin gland filling with resin content, and this can explain the lower concentration of alpha acids on

Farm A Tram. No significant difference was observed between the same planting systems in the two different microclimates. The control treatments tend to result in higher concentrations of alpha acids (Figure 5.4). Large variations were observed between the different samples and this re-emphasises the variation in the hop samples. The trend of reduced alpha acids in the early stripping when comparing the two microclimates also supports this variation. The differences observed were large enough to investigate further. The alpha acid concentration of the variety was generally lower than the long term average, which could indicate plant stress during the growing period, as temperature greatly influences alpha acid production according to literature. The temperature differences between the microclimates during the flowering period and just prior (December - February) to harvest differed slightly in both the George and Waboomskraal microclimate (Figure 3.1-3.2) this could account for the alpha acid differences, but it is difficult to be conclusive with one year's data.

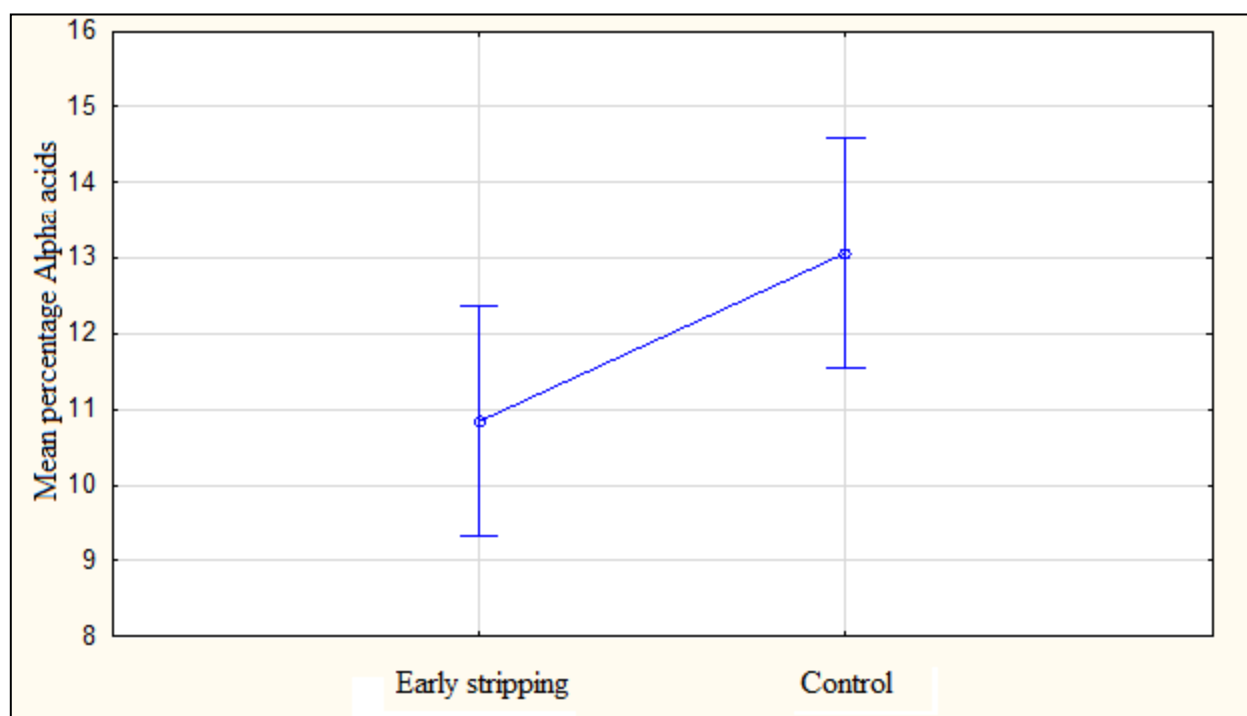


Figure 5.4. Mean percentage alpha acids for the 2009-2010 growing season for the different sites in two microclimates (Farm A Tram – George, Farm B Tram and Farm B Conv.-Waboomskraal) $P= 0.04310$. Vertical bars denote 95% confidence intervals.

5.3.4 Beta acid concentration

Mean beta acid concentrations showed no significant interactions between the farm and treatment ($P=0.337671$). The same trend for Beta acids was observed as in the alpha acid concentration. Higher concentrations of beta acids was observed on both Farm B Conv and Farm B Tram (Waboomskraal locality), compared to Farm A Tram (George locality) (Figure 5.5).

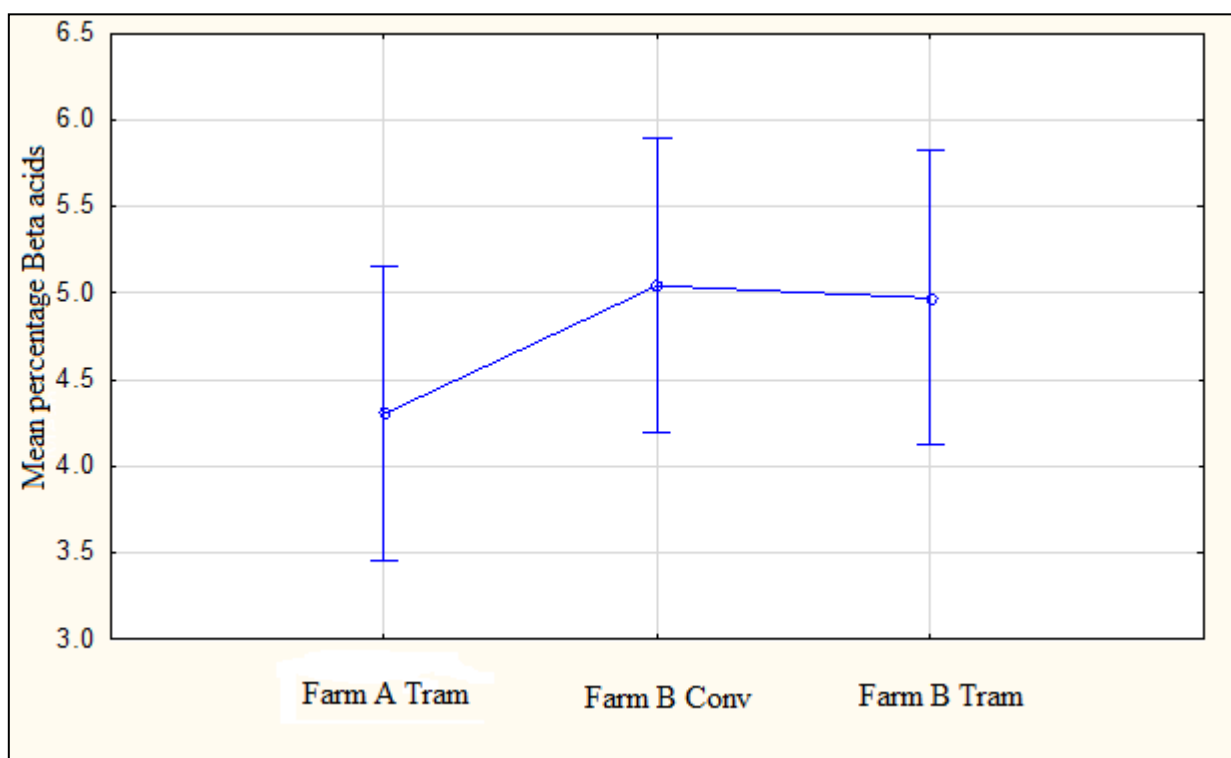


Figure 5.5. Mean percentage beta acids for the 2009-2010 growing season for the different sites in two microclimates (Farm A Tram – George, Farm B Tram and Farm B Conv.-Waboomskraal) $P= 0.38775$. Vertical bars denote 95% confidence intervals.

The mean air temperature during January and February 2010 in the George locality (Farm A Tram) was in line with the 20 °C long term average, whilst the Waboomskraal locality (Farm B Tram and Farm B Conv) experienced mean temperatures of 1.31 °C – 2.78 °C higher than the long term average of 20 °C for that region. Heavy rainfall was experienced in the George locality at the beginning of February as harvest was approaching (Figure 3.1-3.2). This period is critical for of lupulin gland filling with resin content, and this can explain the lower concentration of beta acids on Farm A Tram. Early stripping did not significantly affect the beta acid concentration ($P= 0.14223$), the same trend was observed as with the alpha acid

concentration. Whereby the control treatments tended to deliver higher concentration compared to the early stripping treatment (Figure 5.6).

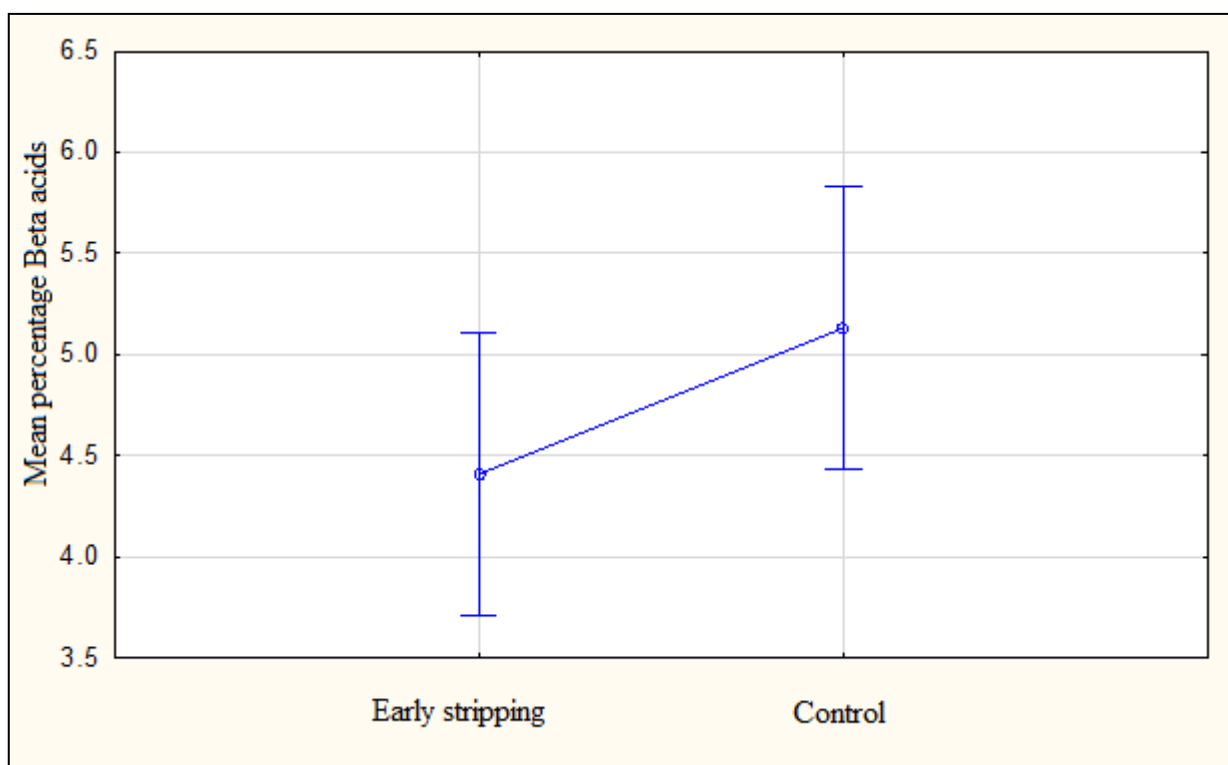


Figure 5.6. Mean percentage beta acids for the 2009-2010 growing season for the different sites in two microclimates (Farm A Tram – George, Farm B Tram and Farm B Conv.-Waboomskraal) $P= 0.14223$. Vertical bars denote 95% confidence intervals.

5.4 Conclusion

This study represents one year's crop data showing significant differences in yields between early stripping and control treatments in the George microclimate. There is a general trend across all sites of higher crop efficiencies in the early stripped treatments. These results indicate that although early stripping did not result in higher yields at all sites, hop production was more efficient and this was especially true in the conventional production systems. On young plants early stripping had a significant difference favouring yield on tramlines. This research delivered promising results indicating that early stripping in the George microclimate could significantly increase yields without negatively affecting the quality (alpha and beta acids). Yield on the same tramline planting system was significantly lower in George microclimate compared to Waboomskraal microclimate. These differences are due to climatic differences between the two regions. Temperatures experienced during the dormancy

period in the George microclimate were slightly above the long term average, and relatively higher than the Waboomskraal microclimate.

Agricultural practices such as timing of stripping did not seem to have a significant effect on the alpha and beta acids across the different farms, however a significant difference was observed between the different treatments favouring the control. Alpha and beta acids are genetically determined, and mean air temperatures during the critical periods of lupulin gland filling seemed to influence the alpha acid concentration. The effect of early stripping becomes more apparent in consecutive years as the timing of defoliation affects foliage and canopy microclimate resulting in increased yields.

5.5 References

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Chapter 6

The effect of early stripping on hop rootstock weights and carbohydrate concentration.

6.1 Introduction

Mature hops have a perennial root system that can grow up to 4 m deep and up to 5 m laterally (Burgess 1964; Beatson et al. 2009). Hop plants have been known to survive for up to 100 years (Haunold 1981), but replanting every 10 to 20 years is common and this is dictated by market demands or diminishing yields (Beatson et al. 2009).

This extensive storage and foraging root system is primarily responsible for uptake and storage of nutrients and water that is needed for rapid vegetative growth in spring and summer (Burgess 1964; Beatson et al. 2009). The relationship between carbohydrate accumulation and depletion is closely related to the annual growth cycle of the hop plant (Williams and Weston 1959; Williams 1960; Williams et al. 1961). High levels of starch, soluble sugars and fibrous material are found in the rootstocks during winter. Starch is converted to sugars during winter months where it is used early in spring for the rapid growth period. Rapid decreases are found in the starch content from end of winter to early spring, when the decrease slows down. The starch is used to sustain the plant during the dormant phase when no photosynthesis is possible and to grow rapidly in the growing phase to produce strong above ground growth. During the rapid growth phase, hops grow out long shoots with little leaf area. The absence of leaves mean that most of the shoot growth comes from the starch and sugars in the rootstock and not photosynthesis (Buchanan 1978). Starch levels were found to be reduced to very low levels during mid-summer, but these levels are replenished during cone formation, cone growth, cone ripening and post ripening. The lower foliage of the hop vines and on laterals is very important in starch production (Buchanan 1978). Any process or treatment during the growing season that minimises the photosynthetic capacity will influence the performance of the plant in the current year as well as dormancy and growth in the succeeding year (Buchanan 1978).

Early season hop sucker growth must be suppressed to stimulate apical dominance and to eliminate foliage that favours the development of downy mildew in affected countries. Late season sucker growth is desirable because it produces carbohydrates that replenish hop root reserves after harvest and before frost kills the aerial growth (Ogg and Zimmermann 1976; Madden and Darby 2012). Carbohydrates are an essential source of reserve energy in perennial plants. They can be mobilised for metabolism or translocated to other plant organs. Factors such as temperature, light, time of planting and moisture influences the concentration and localization of carbohydrates (Daie 1985). The lower main bine leaves are often removed during a process called “stripping”, which is performed mechanically or chemically (Beatson et al. 2009). Stripping is done on the bottom meter of stem to increase air-flow needed to minimize the development of downy and powdery mildew in affected countries (Beatson et al. 2009). This practice reduces humidity and downy mildew inoculum density (Beatson et al. 2009; Johnson et al. 2009) around the base of the hop plant. Williams (1962) found that in the first three to four years the plant is building up carbohydrate reserves in the initial small rootstock. The plant increases its rootstock size, as well as the carbohydrate reserves it contains during this establishment phase until it reaches maturity. Williams (1962) also concluded in his study that the primary function of leaves in mature fields is to replenish reserves that have been used up in a season, whereas younger hop plants are still establishing their rootstock. Mature plant’s rootstocks are less affected by stripping or early cutting because of their bigger rootstocks, but repeated annual stripping may have a cumulative weakening effect which may in the end reduce both production and the lifespan of the hop plant (Buchanan 1978).

Stripping timing and frequency may have a significant effect on the carbohydrate reserves, and excessive stripping can have detrimental effects on early maturing varieties or infected hop root systems (Beatson et al. 2009). In other hop production areas hops are stripped up to 1m from the ground level when the plants have grown ± 1.8 m (Sirrinc et al. 2010). In South Africa hops are stripped up to 1m from the ground level, when bines have reached 5 m (during the onset of dormancy). This is to control weeds and to make harvesting easier, by removing excess shoots (Linsley-Noakes 2013). This study was conducted to determine whether the timing of stripping under South African conditions was optimal, and if the current practice negatively influences the carbohydrate reserves.

6.2 Materials & Methods

6.2.1 Localities

The trial was repeated on three different localities namely: The Rob Roy Research Farm (Farm A Tram) near George (-33.52355°22.2143.8°, altitude 223 m), Heidekruin Farm (Tramline planting system – Farm B Tram) and the Heidekruin Farm (conventional planting system – Farm B Conv), in Waboomskraal valley (-33.86954°22.34939°, altitude 601 m), during the growing season of 2009-2010. Farm A Tram and Farm B Tram fields were both three years old and Farm B Conv was a five year old hop field. The experimental design was a randomized complete block design with 5 replications (blocks) for each of the two treatments namely: Early chemical stripping (early stripping) and late chemical stripping (control).

6.2.2 Cultivation practices

The Southern Star variety was used for this trial. The management of the blocks in terms of irrigation, fertilisation, weed and pest control and harvest was in accordance with the best operating practices of the SA Hops Growers Association (Linsley-Noakes 2013), as per standard operating procedures described in Chapter 3.

6.2.3 Treatments

Early chemical stripping was done during the active growing phase (mid-November) by defoliating leaves and killing basal growth on the bines up to a height of 0.4 meter with Gramoxone at a rate of 1 l ha⁻¹ during mid-November, while the control blocks were only defoliated and basal growth killed up to 1.0 meter height during cone formation (mid-January), using Gramoxone at a rate of 3.0 l ha⁻¹ as currently done by most hop producers.

6.2.4 Data collected

6.2.4.1 Sample collection and preparation

Three plants were randomly tagged in each of the five treatments and control replicates (blocks). These plants were removed from the field on 06th of April 2010 after harvest, because starch levels are reduced to very low levels during mid-summer, but these levels are replenished during cone formation, cone growth, cone ripening and post ripening (Buchanan 1978). After washing the root system to remove soil, it was separated into storage roots

(which included the basal perennial portion of the stem, where new shoots arose, referred to as the rootstock), and fibrous roots. The rootstock was cut into 10 pieces which measured 10 cm each (composite sample). The composite sample was weighed and dried for 48h at 70°C. It was allowed to cool overnight in the desiccator, ground with a hammer mill, sealed in foil bags and stored at room temperature until chemical analysis.

6.2.4.2 Total Carbohydrates analysis

The total carbohydrate was estimated by the anthrone method (Hedge and Hofreiter 1962). Carbohydrates are first hydrolyzed into simple sugars using dilute hydrochloric acid. In a hot acidic medium, glucose is dehydrated to hydroxyl methyl furfural. This compound forms a green coloured product with anthrone with an absorption maximum at 630 nm. The anthrone reagent was made up by dissolving 200 mg anthrone in 100 ml of ice-cold 95% H₂SO₄. Standard glucose stock was made up by dissolving 100 mg in 100 ml of distilled water. Ten millilitres of the stock solution was diluted to 100 ml distilled water to make up the working standard. One hundred (100) mg of the milled sample was weighed and placed into a glass boiling tube in a boiling water bath. The sample was hydrolyzed by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N HCl and then cooled to room temperature. The solution was neutralized with solid sodium carbonate until the effervescence ceased and made up to 100 ml. The supernatant was collected and 0.5 and 1 ml aliquots were taken for analysis. A standard solution was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard. The 0 ml (zero) served as a blank. The volume was made to 1 ml in all the tubes including the sample tubes by adding distilled water. Then 4 ml of anthrone reagent was added and heated for eight minutes in a boiling water bath and then cooled. Green to dark green colour was recorded at 630 nm. A standard graph was drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. The amount of carbohydrate present in the tube was calculated from the graph as follow: Amount of carbohydrate present in 100 mg of the sample = (mg of glucose / volume of test sample *100)

6.2.4.3 Statistical analysis

SAS/STAT software version 12 (SAS 2014) was used to do an Analysis of Variance (ANOVA). The normal probability plot was performed to identify normality of residuals, and least significant difference. The general linear model procedure was used to calculate the least square means.

6.3 Results and discussion

6.3.1 Dry Rootstock weights

The Rootstock dry weight (g) was not significantly affected by the timing of stripping when evaluating the farm treatment interaction ($P= 0.67217$). The rootstock dry weight differed significantly across the different farms ($P=0.0120$). The effect of stripping off the leaves in terms of dry weight on mature plants (Farm B Conv) tend to be significantly less than the stripping on younger plants (Farm B Tram) (Figure 6.1). The three year old blocks (Farm Tram A and Farm Tram B) are still in the initial phase of establishing their perennial rootstock and building up carbohydrate reserves. Farm B Conv which is in the same microclimate as Farm B Tram, tended to deliver significantly higher dry weights. This can be ascribed to the fact that Farm B Conv hop plants have already reach maturity (5 years old) (Figure 6.1). The effect of stripping off the leaves on mature plants is considerably less than the stripping on younger plants.

In general early stripping did not negatively affect the root development and perennating rootstock (Figure 6.2). The size of the rootstock increases in proportion to the rate of carbohydrate accumulation. Early stripped plants new basal laterals continue to accumulate reserves whereas the control treatment accumulation is restricted by late stripping. The leaves on fruiting laterals are responsible for the development of the current crop. Food reserves and primarily starch accumulates in the roots and basal parts of the hop plant. The quantity of reserves is dependent on the length of time that the plant carried its full leaf system. Early stripping removed relatively small amounts of leaves compared to the conventional late stripping.

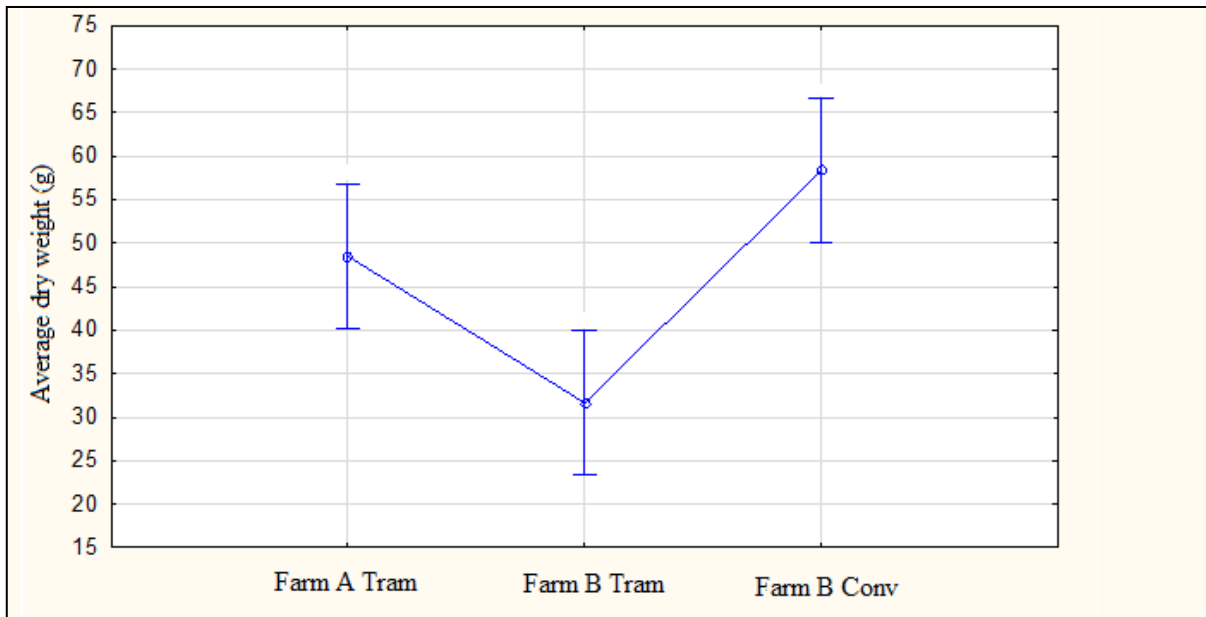


Figure 6.1. Average root dry weight (g) on different farms in two microclimates, Farm A Tram (George microclimate), Farm B Conv and Farm B Tram (Waboomskraal microclimate) during the 2009-2010 hop growing season ($P= 0.0120$). Vertical bars denote 95% confidence intervals.

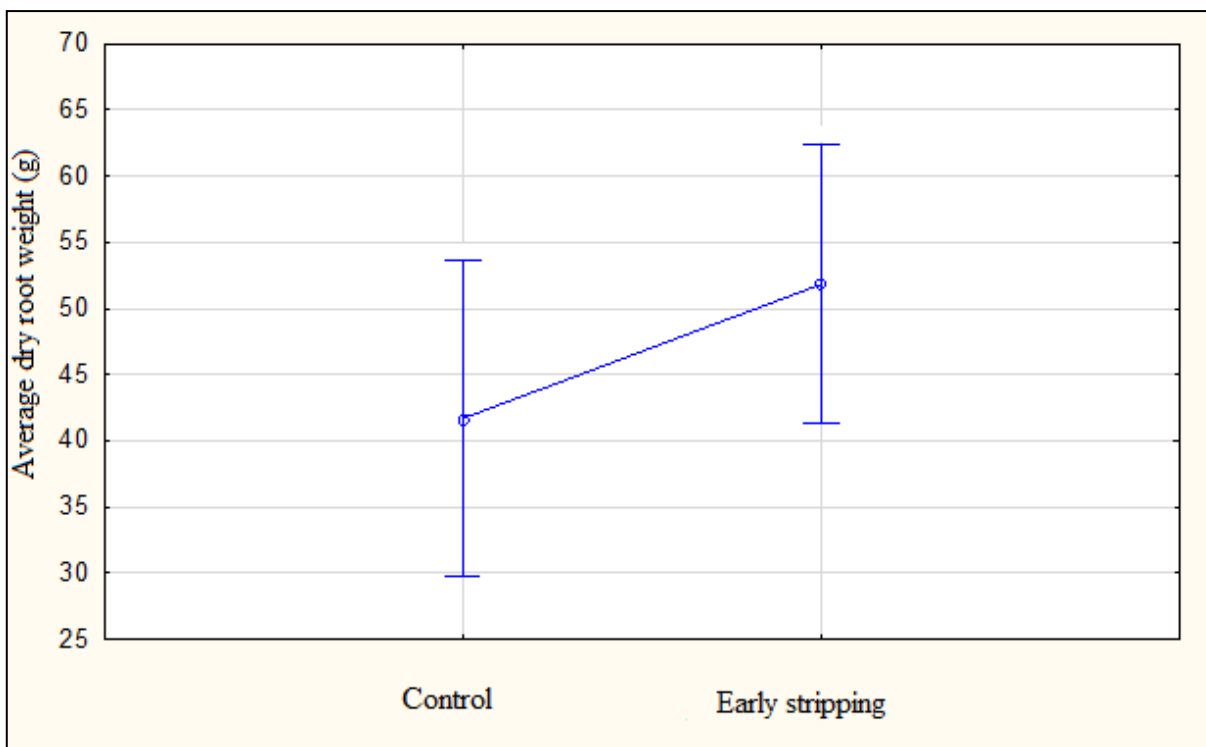


Figure 6.2. Average root dry weight (g) of the treatment interaction during the 2009-2010 hop growing season during the 2009-2010 hop growing season ($P= 0.16521$). Vertical bars denote 95% confidence intervals.

6.3.2 Carbohydrate concentration

Total carbohydrate concentrations did not show a significant farm x treatment interaction ($P=0.42749$). Farm A Tram and Farm B Tram tended to yield higher concentration carbohydrates compared to Farm B Conv (Figure 6.3).

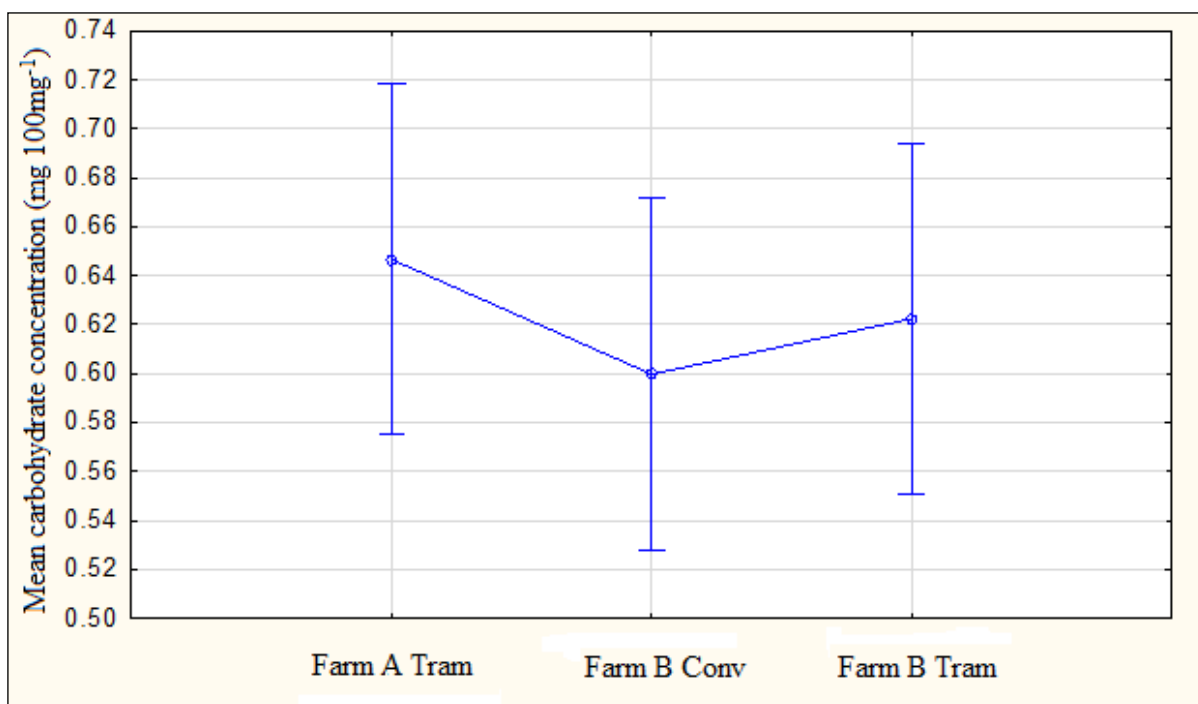


Figure 6.3. Mean carbohydrate concentration (mg 100mg⁻¹) of the site treatment interaction on three different farms during the 2009-2010 hop growing season ($P=0.63930$). Vertical bars denote 95% confidence intervals.

Both Farm A Tram and Farm B Tram are three years old and are still in the phase of building up carbohydrate reserves in the initial small rootstock, until it reaches maturity, whilst the primary function of The Farm B Conv is to replenish reserves that have been used up in the season. Temperature tended to also influence the concentration and localization of carbohydrates. Farm B Tram (Waboomskraal locality, 3 year old) yielded less carbohydrates compared to Farm A Tram (George locality, 3 year old), utilizing the same planting method. Below average temperatures during October and November were experienced in Waboomskraal, and during November in the George microclimate. This resulted in a prolonged vegetative growth as the start to the season was not rapid. Continued drought conditions were experienced in November, January and March in both localities.

These are the critical periods for active vegetative growth, lateral formation, flowering and cone formation. A relatively small amount of leaves were removed on the early stripping (400mm) compared to the late stripping (1.5m, control). Early stripping tended to yield more carbohydrates than the control during the study ($P= 0.05381$) (Figure 6.4). This trend can be ascribed to the late season sucker growth in the early stripping treatment that produce carbohydrates which replenish hop root reserves after harvest. By late defoliation in the control treatments, the carbohydrate accumulation is disturbed and this could lead to the carbohydrate reduction.

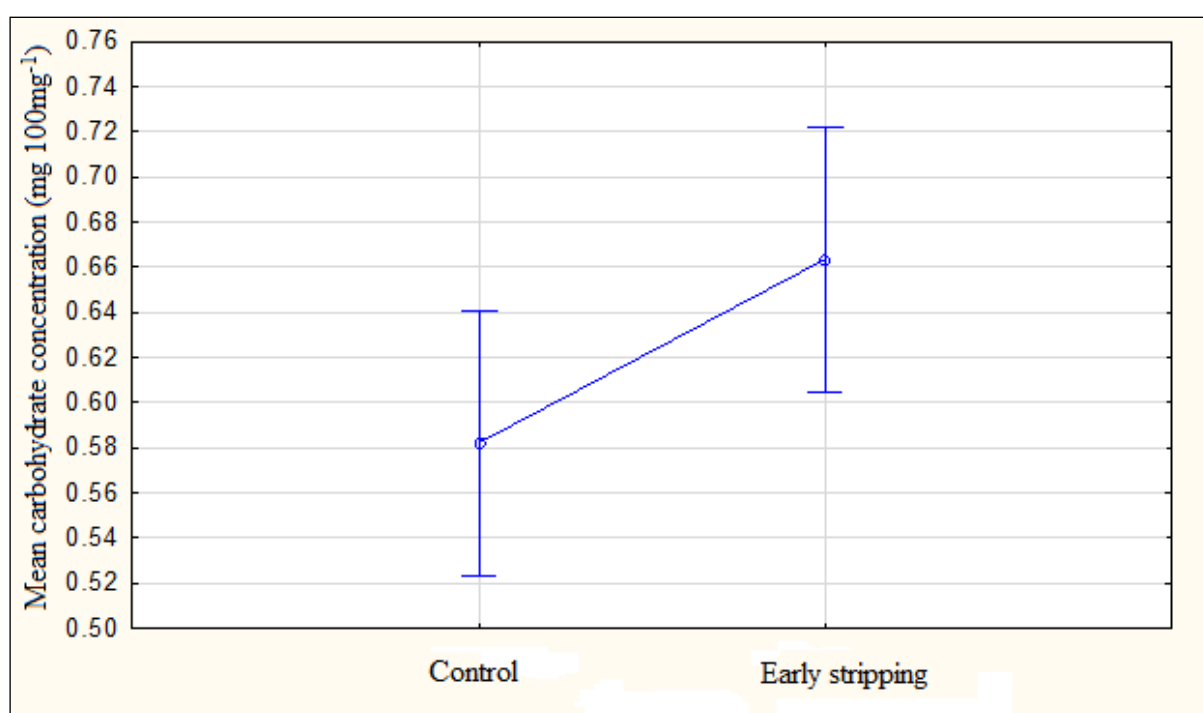


Figure 6.4. Mean carbohydrate concentration (mg 100mg⁻¹) of the treatment interaction on three different farms during the 2009-2010 hop growing season ($P= 0.05381$). Vertical bars denote 95% confidence intervals.

6.4 Conclusion

The effect of stripping off the leaves in terms of dry weight on mature plants is considerably less than the stripping on younger plants. The three year old blocks (Farm Tram A and Farm Tram B) were still in the initial phase of establishing their perennial rootstock. Young plants on tramline planting systems (Farm A Tram and Farm B Tram) delivered lower dry weights, but accumulated higher carbohydrate reserves compared to mature plants on conventional

planting systems (Farm B Conv) The size of the rootstock increases in proportion to the rate of carbohydrate accumulation. In early stripped plants new basal laterals continue to accumulate reserves whereas in the control treatment accumulation is restricted by late stripping thus delivering less dry weight.

Early stripping tended to yield more carbohydrates than the control during the study. This trend can be ascribed to the late season sucker growth in the early stripping treatment that produce carbohydrates which replenish hop root reserves after harvest. By late defoliation in the control treatments, the carbohydrate accumulation is disturbed and this could lead to the carbohydrate reduction. Dry weight and carbohydrate accumulation showed the same trend, with early stripping being higher in both instances. Agricultural practices that influence the dry weight and carbohydrate concentration of roots can also influence future vegetative growth and yield.

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Chapter 7

Conclusion

The microclimate data in this study shows that the Waboomskraal hop growing area is more favourable for hops growing. This can be seen in the crop density, biomass and higher yields. Whilst the microclimate is favourable, growers can manipulate the timing of stripping, which in effect can manage the carbohydrate accumulation, canopy density, light interception and increase the yields of future years. Microclimate had a big influence on the vegetative growth and light interception, which influences the plants responsiveness to increasing day lengths and the growth rate, but is unfortunately not controllable. A general trend showed that light interception was lower in George microclimate compared to Waboomskraal microclimate. Early stripping resulted in a trend of higher light intensity, due to the controlled amount of bines on the strings. Relative growth showed differences between the treatments favouring the control treatment in week 2 of the study and favouring early stripping in week 15 to 18. During the active growing phase little difference was observed between treatments, with Farm A Tram reaching full growth potential only three weeks later, which can affect the potential yield.

The effect of stripping off the leaves in terms of dry weight on mature plants is considerably less than the stripping on younger plants. The three year old blocks (Farm Tram A and Farm Tram B) were still in the initial phase of establishing their perennial rootstock. Young plants on tramline planting systems (Farm A Tram and Farm B Tram) delivered lower dry weights, but accumulated higher carbohydrate reserves compared to mature plants on conventional planting systems (Farm B Conv). Early stripped plants new basal laterals continue to accumulate reserves whereas the control treatment accumulation is restricted by late stripping thus delivering less dry weight. The size of the rootstock increases in proportion to the rate of carbohydrate accumulation. Early stripping tended to yield more carbohydrates than the control during the study. This trend can be ascribed to the late season sucker growth in the early stripping treatment that produce carbohydrates which replenish hop root reserves after harvest. By late defoliation in the control treatments, the carbohydrate accumulation is disturbed and this could lead to the carbohydrate reduction. Dry weight and carbohydrate accumulation showed the same trend, with early stripping being higher in both instances.

Agricultural practices that influence the dry weight and carbohydrate concentration of roots can also influence future vegetative growth and yield.

On young plants early stripping had a significant difference favouring yield on tramlines. The commercial average yield for the George microclimate is 1750 kg ha⁻¹ compared to the 2100 kg ha⁻¹ commercial yield in the Waboomskraal microclimate. There is a general trend across all sites of higher crop efficiencies in the early stripping treatments. These results indicate that although early stripping did not result in higher yields at all sites, hop production was more efficient and this was especially true in the conventional production systems. This research delivered promising results indicating that early stripping in the George microclimate could significantly increase yields without negatively affecting the quality (alpha and beta acids). The effect of early stripping becomes more apparent in consecutive years as the timing of stripping affects foliage, canopy microclimate, light interception and carbohydrate concentration resulting in increased yields especially on tramlines. Growers in both microclimates can benefit from this agricultural practice by adopting a change in timing of defoliation.

From this study it can be concluded that early stripping appeared to have an influence on the light interception, crop efficiencies, biomass production, yield, dry root weight and carbohydrate concentration. This practice is not only sustainable but also an environmental friendly one, using less herbicide. It for this reason also delivers an economic gain. Future research is needed to confirm this on consecutive years and different varieties as this study was done for a limited period and climatic conditions were not optimal.