Optimising yield
through novel canopy architecture in intensive cocoa-growing systems

Thesis by

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Declaration of authenticity

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Abstract

The increasing demand for cocoa and the challenges associated with boosting production have prompted the exploration of intensive production techniques. The potential of hedgerow systems to increase cocoa yield by maximizing canopy light interception was investigated in two factorial experiments: Biomass 1, with trellis or conventional tree architecture, 1-4 branches, and four densities (625 - 1111 trees.ha^{-1}); and Biomass 2, with three densities (2000 - 5000 trees.ha^{-1}), East-West or North-South orientation, and two clones (M01 and 45).

Pruning treatments significantly increase the light-saturated photosynthetic rate by 15-54%. Middle-aged leaves exhibit the highest photosynthetic rate, indicating that frequent pruning can improve tree productivity (17-67% higher than younger leaves and 27-61% higher than older leaves). However, optimal pruning levels require further investigation.

The study revealed that clone 45 grown at density of 2000 trees.ha^{-1} resulted in higher bean and pod harvest index values over three production years, due to reduced competition for resources compared to other densities. The yield efficiency of clones 45 and M01 varied between 0.02-0.05, with 38-46% pod biomass observed in this study. These findings suggest that planting density and genetic variation are essential factors to consider in cocoa cultivation and can contribute to improved productivity.

Clone 45 trees aged 26 and 38 months had the highest yields at 4.46 and 4.6 tonnes.ha^{-1}, but productivity declined in the third year due to limited assimilation conditions. Clones 45 and M01 were unsuitable for high-density planting on the trellis system due to excessive vegetative biomass.

The results show that although clone 45 can improve cocoa yield in a trellis-based system at a moderate density, it was not the best option. Five crosses were identified with high yield efficiency, high yield, and low trunk growth increment. These findings highlight the potential of suitable clones to improve productivity in intensive cacao growing systems.

Table of Contents

List of Figures and Tables

Chapter 1. General Introduction

1.1 Introduction to *Theobroma cacao*

Cacao (*Theobroma cacao L.*) is one of the most important cash crops grown in the humid tropics and is utilised primarily in chocolate manufacturing. It was formerly assigned to the Sterculiaceae (Cuatrecasas, 1964) but was later reclassified to the Malvaceae (Alverson et al., 1999).

Theobroma cacao is a diploid tree species native to the rainforests of South America. It was first domesticated approximately 5,300 years ago in the upper Amazon region (Zarrillo et al., 2018). The Criollo type was first domesticated in Central America more than 2000 years ago, and a Lower Amazon Forastero variety (Amelonado type) was domesticated in Brazil (Solorzano et al., 2012) in the 18th century.

Cacao is now grown in tropical areas in West Africa, Central and South America, and Asia (Marita et al., 2001). Under natural wild conditions, the tree can reach a height of 20 to 25 m (Lachenaud et al., 1997). In contrast, under cultivation it is usually maintained at a height of between 3 to 5 m (Almeida & Valle, 2007).

Most cacao varieties can be classified into three broad categories based on morphological descriptors and geographic origin: Criollo, Forastero, and Trinitario (Cheesman, 1944; Enriquez & Soria, 1967; Engels, 1981; Bartley, 2005). Cacao Criollo produce fruits (pods) with thick, white or pinkish seeds (or "beans”) that are more flavoured and used for fine flavour chocolates (Marita et al., 2001). Varieties of the Forastero group are widely cultivated due to their higher yields and better disease resistance. They contribute about 80% of the world's output of cacao seeds (Marita et al., 2001). The Trinitario group was created from the natural hybridisation between Criollo and Forastero (Pound, 1932; Motamayor et al., 2003). This group is thought to have originated in Trinidad and was favoured due to its higher productivity, greater disease resistance and desirable Criollo flavour.

A new classification of cacao germplasm into ten major genetics clusters was developed by Motamayor et al. (2008). These are defined as Marañon, Curaray, Criollo, Iquitos, Nanay, Contamana, Amelonado, Purús, Nacional and Guiana. This new classification reflects more accurately the genetic diversity available for breeders rather than the traditional classification of Criollo, Forastero or Trinitario.

1.2 Economic importance of cacao

Cacao is the world's third most economically important agricultural export commodity and the second most important cash crop in the tropics (Galarza, 2012; Blare & Useche., 2013). The high global demand for cacao has grown steeply over the last 15 years, mainly from consuming countries in Western Europe, North America and Asia (ICCO, 2021). Approximately 5.1 million tonnes of cacao were produced in 2020/2021 (ICCO, 2021). Demand for cocoa grew by nearly 4.3% year on year in 2021, after a 1.6 % year-on-year decline in 2020 fuelled by reduced EU consumption (ICCO, 2021). It has been suggested that the supply may not continue to meet the growing demand as the market is predicted to continue to increase (WCF, 2014).

The largest producers of cocoa beans are in West Africa, which was responsible for 77% of global production in 2019 (ICCO, 2020). The top two producing countries are Côte d’Ivoire and Ghana; other significant producers in West Africa include Nigeria and Cameroon. Cocoa is also produced in the Americas (18%) (Ecuador and Brazil being the top two producers) and South-East Asia (5%) (Indonesia and Papua New Guinea being the top two producers) (ICCO, 2021).

It is estimated that more than 80% of cacao is produced by 7–8 million small family-managed cacao farms worldwide (CEPAL, 2015; Díaz-Montenegro et al., 2018). Many of these small-holder farmers grow cacao on about 2 hectares of land. The majority (95%) of cocoa farms in Indonesia are cultivated by 1.6 million smallholder farmers covering an area of 1.7 million ha. Two-thirds of the cocoa is produced in Sulawesi and 22% in Sumatra (UTZ, 2016). Larger cocoa farms can be found in various cocoa-growing countries, including Trinidad, Indonesia, Brazil and Ecuador.

1.3 Current cacao farming situation in Indonesia

Cacao is a perennial crop that is harvested all year round with two peak seasons in many parts of Indonesia. There have been various estimates of on-farm yields across Indonesia. The Indonesia Central Bureau of Statistics/BPS (2021) reported that the average cacao yield in Indonesia ranges from 371 to 728 kg.ha^{-1}, whereas FAOSTAT (2020) reported average yields between 476-490 kg ha^{-1} over the period 2018 to 2019. However, yield potential has been estimated as between 1 and 1.5 tonnes.ha^{-1} (Yasa, 2003; USAID, 2006). Yields also vary greatly between farms; for example, Daymond et al. (2020) observed that the mean average yields sampled across 120 farms ranged between 39 and 3586 kg.ha^{-1} between 2014-2017.

From 2016 to 2020, the land allocated for cocoa production in Indonesia decreased between 2.6-3.9% year on year (BPS, 2021). The gross production of cocoa has decreased because of declining yields per hectare that started in 2012 (FAOSTAT, 2020). Productivity per hectare will need to increase to meet future cocoa demand because of the limited availability of land.

In comparison with other cocoa-producing countries in the Africa region, the average yields obtained by farmers were reported to range from 300–600 kg ha^{-1} by Wessel and Quist-Wessel (2015). FAOSTAT (2020) reported average yields between 456-467 kg ha^{-1} in Ghana and 535-549 kg ha^{-1} in Cote d'Ivoire from 2018 to 2019. According to Abbott (2018), in Colombia calculated yields (national average) were between 332 kg.ha^{-1} to 441.8 kg.ha^{-1} in 2000-2015. In Panama, cacao production is low on many cacao farms, which is often a reflection of poor management. It was observed that very few farmers sell only cacao and rely on the sale of other crops to supplement their incomes (Griffith, 2013).

Indonesian government policy has exclusively focused on increasing cacao production. However, it has not paid attention to improving the price of cacao that farmers receive (Emelda et al., 2014). Consequently, many farmers have changed the crop they grow on their farms to another commodity, such as oil palm (Anggraini & Grundmann, 2013). Furthermore, they are often not interested in developing the quality of their cacao due to limited premiums for quality cacao.

Indonesian cocoa is usually traded as an unfermented, fat, bulk bean and volume-based in the worldwide market. Fermentation of cocoa beans is not commonly carried out in Sulawesi and some other regions. Nevertheless, some farmers ferment their cocoa beans before selling them in regions such as Java, Sumatera and West Papua. There has been an effort to encourage smallholder farmers in Sulawesi to expand the production of fermented beans. However, commercial incentives for farmers are limited (Yasa, 2003; Zainuri et al., 2021).

Pioneer planters experience a boom period after planting new cacao farms on former virgin forests. However, without continuous fertiliser application, soil fertility declines, which, combined with pests and disease infestations, results in a reduction in production (Abbate, 2007). This boom-and-bust cycle of cacao production is well-known and described by Ruf (1995) and Ruf and Yoddang (1999). In addition, cacao production at the expense of large-scale deforestation (Raschio et al., 2017) contributes to climate change (Schroth et al., 2016).

After initial forest conversion to a cacao farm, sustaining production is difficult due to dwindling yields as trees age, lower soil fertility, and pest and disease incidence increases (Flores & Sarandon, 2004; Clough et al., 2009). The typical productive life of a traditional plantation is around 15-20 years (Rice & Greenberg, 2000). Previous studies have observed that shaded cocoa trees produce lower but stable yield and thus continue to produce for more years. A considerably higher investment is needed for unshaded plantations since an increased fertiliser requirement is required (Zuidema et al., 2005).

The failure to revitalise plantations often leads to a shift of cacao production to other regions. Therefore, crop improvement through breeding, integrated pest management and good agricultural practice is needed to maintain gains in cocoa production per unit area in step with the pace of consumer demand. Adoption of more intensive growing systems developed in other crops, such as apples (Ju et al., 2006; Dadashpur et al., 2010) and peach (Hutton & McFadyen, 1987), represents a route to increase yield but has received limited attention in cocoa (De Araujo et al., 2017).

1.4 Environmental effects on cacao growth

1.4.1 Temperature

The optimal temperature for photosynthesis in cacao has been reported by Balasimha (1991) to be between 31°C-33°C, 33°C-35°C by Yapp (1992) and 35°C by Guers and Mousseau (1979). The maximum monthly temperature, at which cacao can be grown without reducing yield, has been quoted as between 30 and 32°C, and a minimum temperature of about 15°C (Wood, 1985). However, cocoa is cultivated at a higher temperature than this in some areas/ times of the year, such as during the dry season in West Africa (Acheampong et al., 2013).

Base temperatures at which vegetative growth ceases in cacao have been calculated to vary between 18.6 and 20.8°C (Daymond & Hadley, 2004). Base temperatures for pod growth were observed to vary between 7.5 and 12.9°C between various cacao genotypes (Daymond & Hadley, 2008).

1.4.2 Rainfall

Rainfall is considered the most critical environmental factor influencing cacao yields (Wood, 1985). Rainfall of between 1400–2000 mm year^{-1} is sufficient to support the growth of cacao. It has been claimed that less than 1200 mm year^{-1} results in soil water deficits and reduces growth and yield (Alvim, 1977). However, the annual distribution of rainfall can often be more critical than the yearly total. Cacao trees can tolerate a short dry period (where evapotranspiration is more than rainfall) of about three months. During this time, precipitation can be less than 100 mm month^{-1} (Wood, 1985). Soil water deficits on cacao farms can also be affected by different soil types with different water retention properties.

An extended period of dry weather can have substantial negative impacts on cacao tree growth. Irrigation may be required during these periods, but systems are not currently in place in most cacao-growing areas. However, there are exceptions, such as in southern India, where the dry period can last between three to six months; here, drip irrigation is advised for cacao growers (Carr & Lockwood, 2011).

1.4.3 Shade trees

In its natural habitat, cacao trees grow in the understory of a closed tree canopy (Wood, 1985). Cacao is traditionally cultivated under the shade of large forest trees, tall herbaceous plants, including bananas, plantains, and coconut, and trees planted to provide shade, such as *Gliricidia* (Hebbar, 2011).

Dense shade is necessary for establishing young cacao plants, as it prevents excessive evapotranspiration and can protect against wind damage (Alvim, 1977; Wood, 1985). However, older plants can be grown in full sunlight (Alvim, 1977). Removal of shade trees has been shown to result in higher yields in the short term. Increases in yield of up to 100% were obtained from cacao trees in a trial in Ghana grown without shading for 20 years. However, this significantly reduced the crop's lifespan by about 10-15 years (Ahenkorah, 1987).

Yield tends to decline after 25–30 years of growing cacao in full sun, and there can be a higher prevalence of pests, particularly mirids (Johns, 1999; Avelino et al., 2011). Traditional shaded farms have been converted into unshaded farms in many cacao-growing regions for short-term yield improvements (Franzen & Mulder, 2007).

Most of the cacao farms in Sulawesi are shade-grown cacao plantations. Trees such as *Gliricidia sepium* L. provide overhead shade (Groeneveld et al., 2010). Only a tiny number are grown in the full-sun system, although it is being promoted to increase production in the short term (Armengot et al., 2016). Traditional, shade-grown cacao has tended to be well-integrated with local agricultural practices. Studies in Central America have shown that shade systems are compatible with biodiversity conservation (Beer, 1988; Young, 1994).

1.5 Cacao biology

1.5.1 Leaf flushing

New leaves in cacao are produced in quick succession, a process known as flushing. A young tree produces a new flush approximately every two months in the field (Greathouse et al., 1971). However, under controlled environmental conditions, the flush leaves from seedling cacao plants have been reported to be nearly continuous, with an inter-flush period of about 27 days (Abo-Hamed et al., 1983).

The stages of leaf development have been described by Greathouse et al. (1971) and Orchard et al. (1980) as follows: F-1: bud swelling; the stipules around the bud spread apart, and the bud swells. F-2: leaf expansion; the expanding leaves are fragile and are usually light green or red. In this phase, all the leaves of the flush expand rapidly, and the stem elongates. I-1: leaf expansion completes, all leaves become green and an apical bud forms. Cuticle thickness increases throughout leaf development and reaches a maximum at this stage. I-2: all leaves are fully expanded and have developed a dark green colour. Young leaves have a dense covering of trichomes which declines as the foliage grows, and by full expansion, very few hairs remain. Stomatal development occurs throughout stages F-2 to I-1 (Abo-Hamed, 1983).

As the leaf develops, photosynthetic capacity and chlorophyll content also increase (Baker & Hardwick, 1973), but maximum chlorophyll synthesis and chloroplast development do not occur until the leaf expansion is completed (Baker & Hardwick, 1975). This pattern of chlorophyll development is uncommon in temperate species where chlorophyll content usually reaches a maximum before the leaf has expanded fully. The absence of green colour in very young leaves is because the chloroplasts are initially small and few rather than due to a delay in chloroplast development.

Leaves that develop late in the flush are usually smaller than those formed at the beginning. This is due to the depletion of available carbohydrates as the flush progresses. Once carbohydrate stocks have been replenished in the inter-flush period, a new flush of leaves emerges (Machado & Hardwick, 1988). The flush cycle appears to be controlled by both endogenous and environmental factors. More intense flushing has been observed in trees that have been subject to water deficit followed by rainfall (Alvim, 1977).

Soil moisture conditions also influence leaf area expansion. According to Almeida & Valle (1987), leaf water potential rather than soil moisture levels induces flushing after a dry period.

Joly and Hahn (1989) showed that the lag phase before rapid leaf expansion is extended under water-deficit conditions, reducing the time available for expansion and thereby delaying leaf area development. The final leaf area achieved in droughted trees is also reduced due to water deficit (Joly & Hahn, 1989). Temperature also impacts flushing. Sale (1968) found that the inter-flush period declined when growth temperatures were increased from 23.3 to 30°C, and de Almeida and Valle (2007) also noted a reduction in the flush period above 23°C.

1.5.2 Flowering and pod development

In cacao, flowers grow on the main trunk and branches of the plant in a process called cauliflory (Bartolome, 1951). The flowers take one month from initiation to emergence and are borne on long pedicels, but then the flower only remains viable for about two days before falling off (Rajamony, 1991; Smith, 1992).

The fully developed flowers consist of five sepals, five petals, ten stamens (five fertile and five non-fertile staminodes), and an ovary of five united carpels (Smith, 1992). The petals have a narrow shape at the base but expand into a cup-shaped pouch and end in a broad tip or ligule. The ovary has five parts holding many ovules set around a central axis, it contains 30 to 60 ovules which is a highly heritable cacao characteristic. Fruit setting and ovular fertility depend upon pollination and nutritional conditions (Lachenaud, 1995).

The colours of the petals are usually pink and white; the precise colouration and pattern may vary slightly and can indicate a given genotype. The cacao floral organisation is conserved across the genus *Theobroma* and its sister group *Herrania*. Nevertheless, it is variable in the subfamily *Byttnerioideae* (Alverson et al., 1999).

Flower development can begin at different tree ages depending on the variety and environmental conditions in which the tree is grown. In most cases, flowering occurs when trees are about 3-4 years old (Alvim, 1977). However, this is sooner for grafted materials. Low pod numbers are produced in the early years, increasing each year as the tree matures (Almeida & Valle, 2007).

A unique form of late-acting/ovarian self-incompatibility has been described in cacao. In this system, instead of incompatible pollen being rejected at the style or stigma, pollen tubes develop and grow towards the ovary, but they do not fuse; it is followed by the rejection of the entire ovary by floral abscission (Toxopeus, 1985; Ford & Wilkinson, 2012). A large number of flowers are produced, but only 0.5-5% are pollinated and successfully developed into pods (Almeida & Valle, 2007).

The degree of incompatibility diverges between different populations in cocoa. Self-compatible genotypes are found in Lower Amazon Forastero, Criollo and Trinitario. In contrast, the Upper Amazon Forasteros are generally self-incompatible (Eskes & Lanaud, 2001). Trinitario cultivars have a high proportion of self-incompatible trees, which will not cross with other self-incompatible trees, requiring pollen from self-compatible trees for successful pollination. The Amelonado population is entirely self-compatible (Malhotra & Apshara, 2017).

A factor that can reduce final pod numbers, besides low fruit set, is the occurrence of cherelle wilt, which is similar to the phenomenon of fruit thinning in temperate fruits such as apples. If too many pods develop on a tree or if assimilation is constrained, it can strain resources. Consequently, some pods are lost through the process of cherelle wilt (Valle et al., 1990). Mckelvie (1956) reported two development stages of the pods' wilting process: the first wilt occurred seven weeks after pollination and was marked by undeveloped cell walls; the second wilt occurred ten weeks after pollination in response to increased pod metabolism.

Ripe cocoa pods vary significantly between genotypes in length from 10-32 cm and also in shape, surface texture, and colour. The pod shape ranges from nearly sphere-shaped to cylindrical. The surface varies from warty and deeply crumpled to almost smooth. The number of beans per pod can range between 30 to more than 40 seeds (Wood, 1985).

1.6 Critical cacao pests and diseases in Indonesia

Cacao is prone to a range of pests and diseases. These have been estimated to be responsible for up to 38% annual losses in global production, or 1.7 million metric tonnes (ICCO, 2017). Even though some diseases are endemic, new pathogens have been encountered as cacao was dissipated from the Amazon rainforest to new plantation sites (Marelli et al., 2019).

In Indonesia, the primary cacao diseases and pests are the cocoa pod borer (*Conopomorpha cramerella*), black pod rot (*Phytophthora palmivora*), vascular streak dieback (VSD, *Ceratobasidium theobromae*) (Keane & Putter, 1992; Wardojo, 1992; McMahon et al., 2009), and various mirids of the genus *Helopeltis*. Genotypes that are partially resistant to VSD and black pod rot have been identified, but delivering the appropriate genetic material to farmers’ fields remains a significant challenge (McMahon et al., 2009; Susilo, 2009).

(a) (b) (c) (d) (e)

Figure 1.1 Picture of cacao pods (a) healthy (b) epidermis with scars due to feeding by *Helopeltis theobromae* (c) bisected pods with feeding damage by *Conopomorpha cramerella* (d) Lesions on pods caused by *Phytophthora palmivora* (e) Tree affected by VSD showing leaf yellowing, necrosis and dieback (Source: Marelli et al., 2019)

Mirids, predominantly of the species *Helopeltis theobromae* (*Hemiptera: Miridae*), feed on pods of all ages and young shoots of cacao (Giesberger, 1983). The surface of injured pods is covered with scars and a thick sclerotic layer (Figure 1.1b). This may encourage the abscission of young fruits (cherelles) (Muhamad & Way, 1995).

The cocoa pod borer *Conopomorpha cramerella* Snellen (*Lepidoptera: Gracillariidae*) is the most significant cacao pest in Southeast Asia, causing crop losses of up to 50% (Day, 1989). The larvae pit into medium-sized pods, impacting yield by impeding the separation of husk and pod contents (Figure 1.1c).

Black pod rot, stem canker and leaf and nursery blights, caused by species of *Phytophthora*, are responsible for more significant losses than any other disease of cacao. The dominant species in South-East Asia is *P. palmivora*. The disease can result in annual global yield losses of up to 20 to 30% and tree deaths of up to 10%. However, individual farms in wetter cacao growing areas may suffer more (Drenth & Guest, 2013). At the beginning of the contamination cycle, infection occurs from the primary inoculum present in soil and plant parts (ten Hoopen et al., 2010). Pods rot entirely within weeks and mummify in the canopy (Figure 1.1d). The rooted pods will then act as a possible inoculum source for years, and new epidemics commence in the rainy seasons (Surujdeo-Maharaj et al., 2016). *Phytophthora* can also infect the stem and branches, causing cankers, often killing the tree. Likewise, the root infections may not be economically impactful but are an inoculum source (Opoku et al., 2002; Akrofi et al., 2015). The number of interplanted non-cacao trees will increase the possibility of black pod incidence (Gidoin et al., 2014). Long-distance disease spreading occurs through the movement of flood and river water, contaminated soil, pruning tools, and infected pods (ten Hoopen et al., 2010; Djeumekop et al., 2017).

Vascular-streak dieback (VSD) was initially observed by the epidemic death of mature trees and seedlings in Papua New Guinea (PNG) in the 1960s. The disease was shown to be caused by a basidiomycete originally named *Oncobasidium theobromae* (Talbot & Keane, 1971), now known as *Ceratobasidium theobromae* (Samuels et al., 2012). The most susceptible genotypes had been infected with VSD by the 1970s. VSD can cause up to 80% local yield losses and regional losses of 14%. Around 60,000 tonnes annually were estimated to be lost globally (Bowers et al., 2001).

Together with cocoa pod borer (*Conopomorpha cramerella*), VSD contributed to the decline of large commercial plantations in West Malaysia and Sabah. It is widely spread in Indonesia, including in the cacao plantations in East and West Java and the extensive newer cacao plantings in Sulawesi. At lower elevations, the symptoms appear to be most severe (McMahon & Purwantara, 2016).

The most common initial symptom of VSD is chlorosis, which progressively develops in adjacent leaves up and down the stem, causing dieback (Guest & Keane, 2007; McMahon & Purwantara, 2016) (Figure 1.1e). The fungus may spread through colonised xylem vessels to other branches, and if it reaches the main stem will kill the tree. Consequently, the disease is most damaging in seedlings. Only the most susceptible genotypes are killed by infections beginning in the outer branches of older trees.

Aeration and sunlight penetration through shade and canopy management can aid the control of VSD, as moist conditions are required for sporulation and infection. These actions are best integrated with other management practices designed to control *P. palmivora* and significant pests and diseases (Ndubuaku & Asogwa, 2006; Famuwagun et al., 2018; Bukola et al., 2021).

1.7 Yield determinants in cacao

1.7.1 Photosynthetic rate

Photosynthesis can be considered to be the driving force behind the growth of all plants. Moreover, both genetic and environmental factors impact the photosynthetic rate. Studies of various clones grown under similar circumstances indicate that some genetic variability in photosynthetic potential exists (Yapp & Hadley, 1994; Galyuon et al., 1996). For example, light-saturated photosynthetic rates of between 1–4 μmol m^2 s^{-1} (Baligar et al., 2008) and 3.4 to 5.7 μmol m^2 s^{-1} (Daymond et al., 2011) have been reported in different genotypes of cacao.

Bastide and Jimmy (2003) stated that photosynthetic rates for cacao seedlings aged 3 months to more than 12 years ranged from 0.46–11.40 μmol m^2 s^{-1}.

Environmental parameters, including temperature, CO_2 concentration, solar radiation and soil fertility, can impact the photosynthetic rate. Balasimha et al. (1990) studied the influence of light, temperature and vapour pressure deficit (VPD) on the photosynthetic rate of cacao trees. The study observed that the highest photosynthetic rate occurred during low evaporative demand (low VPD) periods, which correlated with the stomata opening (Raja Harun & Hardwick, 1988). This has also been observed in other species (Jarvis, 1980).

The optimum temperature for photosynthesis has been reported as being between 31ºC-33ºC (Balasimha et al., 1991) and 33ºC-35ºC (Yapp, 1992).

Hutcheon (1977) and Lim (1980) observed that the light-saturated photosynthetic rate of leaves of cacao trees grown under shade was lower than those without overhead shade. Furthermore, studies of leaf photosynthesis in cacao typically show that higher rates have been measured under particularly fertile conditions (Yapp, 1992).

The stage of plant development can impact the photosynthetic rate in cacao. Photosynthetic rates of individual leaves are the highest for the first 4-5 months after leaf initiation and decline as the leaf ages (Baker & Hardwick, 1973; Miyaji et al., 1997). In juvenile cacao, the leaf photosynthetic rate can be correlated with vigour (Yapp & Hadley, 1994). However, the relationship between leaf photosynthesis and the growth of mature trees is less clear. This is partly because growth rate and yield are determined by the balance of assimilate partitioning between vegetative and reproductive components of the tree. Furthermore, canopy photosynthesis depends not only on the photosynthetic capacity of individual leaves but also on the amount of light intercepted, the proportion of the canopy illuminated and the leaf area index. These features are influenced by crop characteristics (genetics, stage of development), environment (light, temperature, water relations, carbon dioxide concentration), and cultural practices (nutrition, irrigation, pest management).

1.7.2 Genotypic variation in canopy characteristics

Genetic variability within a crop in terms of leaf area index, canopy architecture and consequently the proportion of incident radiation that the plant intercepts can represent an opportunity for exploitation in yield improvement programmes (Evans, 1975; Williams, 2000) but can also be utilised to optimise growing systems.

Some studies have demonstrated variation in these canopy traits amongst cacao clones. A previous study by Yapp & Hadley (1994) in Malaysia demonstrated genotypic variation in the proportion of incident photosynthetically active radiation intercepted by the canopy and the attenuation of solar radiation through the canopy.

Daymond et al. (2002a) showed a high degree of variability in canopy characteristics, such as fractional light interception, light distribution within the canopy (extinction coefficient), and leaf area index between ten different cacao genotypes grown in Bahia, Brazil. The leaf area index varied from 2.8 to 4.5 between clones in nine-year-old cacao trees (Daymond et al., 2002a). Differences in light extinction coefficients were also observed (mean values ranged from 0.63 for the clone TSH-565 to 0.82 for CC-10) (Daymond et al., 2002a).

1.7.3 Biomass partitioning in cacao trees

During the process of crop domestication, the yield has increased mainly by increasing the proportion of assimilates partitioned to the harvested parts of the plants and much less (or not at all) by increasing total biomass production (Evans, 1976).

Thong and Ng (1980) reported that a cacao farm with a yield of 2 tonnes ha^{-1} year^{-1} allocated 20% dry matter to cacao pods and 10% dry matter to cacao beans. Another farm which was reported to have a yield of 4.4 tonnes yield ha^{-1} year^{-1} resulted from trees grown without overhead shade on volcanic soil in Borneo (Lim, 1980). Using these data, combined with vegetative growth data from Thong and Ng (1980), a pod harvest index of 30% was estimated by Corley (1980).

Higher harvest indices have been calculated in other fruit-producing tree species, such as 15-84% of dry matter into fruits in apples (Maggs, 1963; McCormick et al., 2021; Tustin et al., 2022) and 50-58% for citrus (Lenz, 1979; Fan et al., 2020), although these fruits have a lower lipid content and so require less energy to produce compared with cacao pods.

The yield efficiency is a parameter that can be used as a non-destructive approximation of partitioning between vegetative and reproductive components and is a particularly useful measure for perennial tree crops. Yield efficiency is defined as the ratio of cumulative yield to the increase in trunk cross-sectional area over the same period. Daymond et al. (2002b) reported that the cacao yield efficiency ranged from 0.008 kg·cm^{-2} for clone CP-82 to 0.08 kg·cm^{-2} for clone EEG 29. The study of Daymond et al. (2002) demonstrated that two components (the proportion of reproductive to vegetative growth and the proportion of beans to husk) contribute to the variability in yield in cacao. Hence, selectively breeding for more efficient partitioning to the yield component should be an essential component of crop improvement in cacao.

1.8 Example of apple-orchard planting system

A holistic approach to improve cacao farm yields involves breeding, integrated pest management, and good agricultural practice. There is a potential to apply intensive cropping systems used in perennial crops like apples, grapes or pears to cacao.

The biomass distribution of apple and grape cultivars has significantly been improved through the development of customised agronomic practices, as seen in Figure 1.2. The optimal ratio of leaves and branches per kilogram of fruit is known in apples and other fruit crops, among many other vital parameters (Jackson, 2014).

Figure 1.2 Differences in biomass distribution in apple. Left: An apple tree in its natural state with a highly undesirable vegetative/reproductive biomass ratio. Right: A modern, high-density management system with an elite apple cultivar grafted onto a dwarfing rootstock and trained to conduit poles and trellis wires. The modern system produces a far better biomass distribution than trees in their natural state (Source: Omafra, 2012)

Tree canopy development has a seasonal and lifetime developmental pattern in perennial crops. For example, a single shoot tree apple would progress into a tall, umbrella-shaped tree. However, there are many drawbacks from shape, such as difficulty to prune, spray and manual/hand-harvest; delayed cropping due to low early life of optimum leaf area index, light interception, and canopy-covered fraction (Robinson et al., 1991a).

To tackle the challenges and limitations of vigorous trees in apples, many attempts have been made to increase planting density, accelerate yield development, reduce tree size, and improve the canopy form (Robinson et al., 1991a). The planting density in orchards 50 years ago ranged from 70 to 100 trees.ha^{-1}, however, today it could range from 1000 to 6000 trees.ha^{-1} or even higher up to 10,000 trees.ha^{-1} (Sansavini, 1996; Weber, 2000; Robinson et al., 2004). These planting density increases were possible due to the development of dwarfing rootstocks for apples. Both dwarfing rootstocks and high planting density factors have improved and accelerated tree productivity. In a modern high-density system, higher yields are expected in the third year, and mature yields are expected by year 5 or 6. In contrast, traditional low-density systems on vigorous rootstocks begin production around year 6 or 7; and do not reach mature yields until year 15 (Robinson, 2009).

In terms of reducing tree size and canopy improvement, many attempts have also been achieved through tree training, modifications in rootstocks and scions, and more extensive pruning. These four factors could make various tree forms, planting arrangements, tree heights, widths, and geometric structures (spheric, rectangular, conic V, T, and A-shaped). Their effects on the production efficiency increase would determine the success of the canopy modifications.

Posnette (1982) argued that successful high-density apple tree plantings also depend on controlling growth by using dwarfing rootstocks and new chemical growth suppressants to replace pruning. Failure of a high-density plantation is usually due to excessive competition for light and water, causing small, low-quality fruit and declining yields.

Most of the early attempts at the high-density plantation were unsuccessful because the trees were too vigorous (Walker, 1980). Previous research also stated the importance of using dwarfing rootstocks in the intensive system in apples, plums, and cherry trees (Webster, 1993). Early high yields are vital to pay for the high capital investment associated with such systems. Furthermore, the density and pattern of planting must also ensure regular cropping throughout the orchard’s life, while economic management demands some mechanisation.

The popularity of the hedgerow system depends on its accessibility to machinery. Because access to tractors is essential to reduce labour costs, various compromises have been tried, i.e. double rows and different hedge heights and orientations (north to south, or east to west) (Palmer & Jackson, 1977; Preston, 1978). A uniform distribution (square or triangular) gives the most negligible mutual shading and root competition). In contrast, a hedge with trees close together in widely spaced rows is the least efficient arrangement (Jackson, 1980).

1.9 Light interception and utilisation in apple orchard systems

Dry matter production (DM) in apples (and other crops) is a function of four key factors (Hall et al., 1985): DM = (RAD x %INT x PH) – RESP, where RAD is the amount of incident photosynthetically active radiation (PAR), %INT is the percentage of light intercepted by the crop, PH is the photosynthetic conversion of light energy into biomass and RESP is the respiratory carbon loss (Table 1.1).

Table 1.1 Reductive pathway of yearly total solar radiation (100%) and limiting factors of the efficiency of light conversion into fruit yield (<0.5%)

Limitations on the overall utilisation of solar energy		**The relative importance of factors limiting the efficiency of light conversion into fruit yield**	
50%	of total radiation is PAR	**50%**	latitude, cloud cover
75%	of the growing season (9 months) is used	**37.5%**	region (light energy input, long season)
40%	is the typical light interception by orchard systems	**15%**	orchard design, leaf area
5%	is photosynthetic conversion efficiency	**0.75%**	photosynthesis
15%	is whole tree respiration loss	**0.64%**	climate (temperatures)
60%	is typical harvest index	**0.38%**	partitioning

Source : (Wünsche and Lakso, 2000a)

A range of studies has demonstrated that fruit yields of orchards are related to the total amount of sunlight intercepted, and this generally is attributable to improved cultural practices that affect leaf growth, leaf duration and canopy width (Palmer & Jackson, 1977; Monteith, 1977; Gifford & Jenkins, 1982; Palmer, 1988; Robinson et al., 1991b; Wagenmakers & Callesen, 1995).

The total quantity of light intercepted by an apple orchard system depends primarily on orchard design aspects such as planting system, tree spacing, tree shape, tree height, alley width, row orientation, leaf area index and the span of the growing season. These various factors have been well studied over the past 40 years (Jackson, 1980; Palmer, 1989; Wagenmakers, 1990; Lakso, 1994).

The relationship between yield and solar radiation interception is typically curvilinear (Wünsche & Lakso, 2000a) (Figure 1.3), reflecting the curvilinear relationship between yield and leaf area (Palmer, 1988; Wünsche et al., 1996). As leaf area increases excessively, light interception or potential yield reaches a plateau or else declines due to increases in self-shading among the leaves and internal shading of fruiting positions. Suitable light distribution within the tree canopy is also essential to secure high fruit quality since low light causes a reduction in fruit weight and colour intensity, dry matter and soluble solids, whilst fruit firmness is increased (Jackson, 1980; Robinson et al., 1983; Lakso, 1994).

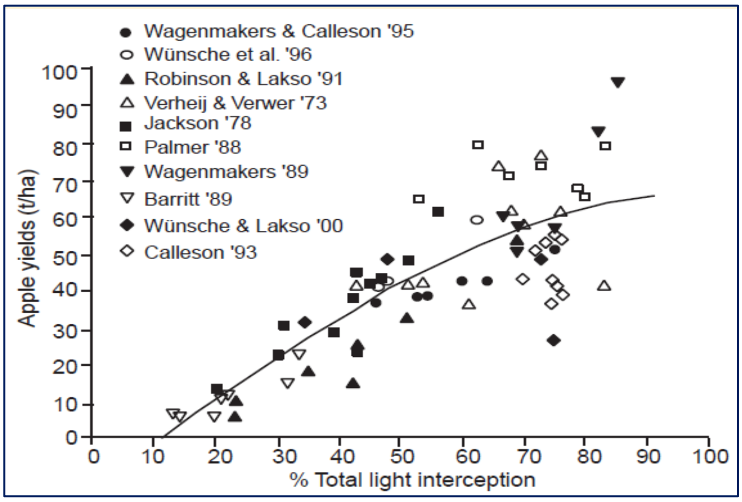


Figure 1.3 Summarized relationship between apple fruit yield and mid-season percent total orchard light interception from several reports in the literature (Source: Wünsche & Lakso, 2000a)

Apple yield and fruit quality result from cultural practices, environmental inputs, and physiological processes (leaf area development, light interception, photosynthesis, respiration). Nevertheless, there are only two possible ways to advance crop performance: increasing total dry matter yield and expanding the scale of dry matter partitioning toward the fruits (Wünsche & Lakso, 2000b).

Experiences from apples can be applied to cacao in terms of orchard and tree management. A better understanding of the role of light interception or distribution in yield and fruit quality variation is needed to optimise orchard, tree design, and canopy management. Suitable techniques to maximise sunlight exposure and support optimal fruit development will allow the cacao grower to increase yields.

Some recent developments in tree fruit production are relevant to cacao, but others are not because the constraint differs. Some sunburn issues have been observed on cacao pods; thus, fruit quality problems also apply to cacao. In comparison, pests and diseases that are paramount in cacao have few counterparts in temperate fruit trees.

1.10 Cacao intensive growing system

Important criteria of intensive growing systems for cacao are to achieve early cropping (i.e. within 2 or 3 years of farming), intercept light efficiently throughout their lifespan to maintain high and regular annual yields, and allow economic management at maturity.

A key feature of yield potential in cocoa genotypes is the interaction between yield potential, vigour, and planting density. The full yield potential of a genotype will only be expressed at a particular density peculiar to the genotype such that the optimum density of low-vigour genotypes will be high and more moderate for high-vigour genotypes. Therefore, any study of yield variation amongst cocoa genotypes needs to be carried out at a range of planting densities. Low vegetative vigour and compact growth are needed to establish a high-density orchard with low production costs and facilitate continuous harvesting (Connor et al., 2014).

The importance of involving and optimising planting density in clonal selection was highlighted by previous experiments in Sabah, Malaysia, on ninety-five cocoa clones at planting densities of 1,096 and 3,333 trees.ha^{-1} (Lockwood & Yin, 1996). The experiment explored the relationship between yield, density and genotype. The yield analysis results showed that the vigorous cacao clones were suited to low planting density and vice versa. However, some intermediate vigour clones showed similar performance at both low and high planting densities. An interaction between planting density and genotypes for several clones grown at 748; 1,495, and 2,990 trees ha^{-1} in Trinidad was observed by Mooleedhar and Lauckner (1990).

Some clones have been observed to be adapted to both conventional and high-density planting (Lockwood & Yin, 1996), while others have been shown to perform better at one particular density. For example, clone PA 121 was low-yielding at both densities (1,096 and 3,333 trees.ha^{-1}; 2.2 tonnes.ha^{-1} yield). Meanwhile, clone UF 676 (which has great vegetative vigour) showed better performance (3 tonnes.ha^{-1} yield) at 1,096 trees.ha^{-1} planting density. Clones PA 13 and PA 300 (generally have smaller vegetative vigour) produced a high yield (5.5 tonnes.ha^{-1} yield) at 3,333 trees.ha^{-1} with little change in yield at normal planting density. Clones IMC85 and 10P (moderately vigorous) exhibited plasticity to planting density (5.5 tonnes.ha^{-1} yield).

Attaining the optimal planting density is a critical component of a clone evaluation programme. Table 1.2 summarises various studies on optimal density with selected clones to produce the optimal yield.

Table 1.2 A summary table of studies on cacao planting density

	Optimum Density (trees.ha^{-1})	**Clones**	**Optimum Yield (tonnes.ha^{-1})**	**Field experiment (country)**
Mooleedhar & Lauckner, 1990	2990	TSH 919	N/A	Trinidad
Lockwood & Yin, 1996	1096	BR 25	9.86	Malaysia
Maharaj et al., 2003	3000	TSH 919	1.8	Trinidad
Souza et al., 2009	1059	N/A	N/A	Brazil
Olufemi et al., 2020	1111	TC	4.650.71-2.07	Nigeria
Zakariyya et al., 2022	1450	MCC02/45	0.71-2.072.96	Indonesia

Higher tree density and improved light interception efficiency per unit surface area have other favourable effects. For example, tree density correlates with a better response to fertiliser when the same dose is applied per tree (Lachenaud et al., 1998). Moreover, a higher tree density means less fertiliser is lost through leaching, and a more significant portion is absorbed.

Although studies have been conducted on cacao to produce systems that maximise light interception through optimising planting density, no research has been reported on trellis systems in cacao, which is the focus of this research.

1.11 Impact of pruning on cacao yield

Numerous factors could cause low cacao yields, for example, high pest and diseases incidence (Akrofi et al., 2015), ageing trees (Nalley et al., 2014), low yield potential of planting material (Edwin & Masters, 2005), loss of soil fertility due to soil nutrient management problems (Baah et al., 2011), inappropriate planting density(Asante et al., 2021), and inadequate good agricultural practices (Anim-Kwapong & Frimpong, 2004).

Pruning, which has been defined as cutting away portions of the plant (Ferree & Schupp, 2003), is one crucial low-cost technique associated with increasing productivity in cacao, as tree energy is routed to fruit production (Vos et al., 2003; Obeng Adomaa et al., 2022). Removing unwanted plant parts through pruning has been practised in other tree crops, for instance, apples (Elfving, 1990) and macadamia (Huett, 2004). In cacao, agronomists and researchers have identified several types of pruning corresponding to its purpose: formation pruning, structural pruning, and sanitation/maintenance pruning (Obeng Adoma et al., 2022). The objectives of formation pruning are to alter the first jorquette height and to create a proper shape during initial canopy formation. Structural pruning is conducted to shape the canopy of mature cacao trees to a desirable architectural shape and size. Sanitation pruning is intended to remove the unnecessary branches, chupons, epiphytes, mistletoes, mummified pods and the diseased part of trees (Opoku-Ameyaw et al., 2010; David, 2011).

In cacao, light capture and efficient nutrient use could be improved by correct pruning practice (Opoku-Ameyaw et al., 2010; Asare et al., 2018). It is also advised to control pests and diseases, reduce pesticide use (Opoku-Ameyaw et al., 2010), and as a method to get balanced growth and yield (Govindaraj & Jancirani, 2017).

Previous studies showed impacts of pruning on crop yield (Bahaudin et al., 1986; Balasimha, 2007), positive impacts only for specific medium or light pruning intensity (Govindaraj & Jancirani, 2017; Leiva-Rojas et al., 2019), neutral (Ampofo, 1986), or even adverse effects (Thomas & Balasimha, 1992). Nevertheless, another two long-term studies showed that the effect depended on crop development. For example, a study from Bonaparte (1996) observed a small positive effect on yield in juvenile trees; however, it became negative after 10 years. Meanwhile a study by KAU (1992) showed a negative effect on yield for the first four years, and then the effect was neutral to positive in the following two years.

1.12 Research aims and hypothesis

Research into an intensive growing system to maximise light interception in cacao is essential as a route towards meeting the increasing global demand for cocoa in the future without encroaching into new lands. Previous studies have proved that trellis systems can increase production in other crops, such as apples (Robinson et al., 1991b), but this has not yet been established for cocoa. Trellis systems are used for cane support to erect trees/cultivars (UNL, 2022) in order to maximize the light interception.

In the apple orchard, the large conventional tree has a poor light distribution throughout the canopy; the juvenile crop has a low light interception, leaf area index, and fraction of land covered by a canopy, leading to delayed cropping. These disadvantages have resulted in tree size reduction, tree density increase, canopy and yield development acceleration, and canopy form improvement to overcome the limitations of the large tree. Therefore, proper management of the plant architecture in an intensive cropping system in cacao would be needed to maximise the light interception and optimise the leaf area index, thereby maximising yield per unit area. Furthermore, it is necessary to determine clones suitable for cultivation in intensive cropping systems that partition a high proportion of dry matter into the fruits. The correlations between treatments involved within this trial to yield determinants and yield are shown in Figure 1.4. The components shaded in brown are those which are being researched in the thesis.

The aims of this thesis are as follows:

- To study the impact of pruning in an intensive growing system on yield determinants and yields
- To study the impact of maximising light interception using trellises in an intensive growing system on yields
- To study the impacts of different planting densities in a trellis system
- To examine yield efficiency within an intensive cropping system and whether this differs between two clones investigated
- To explore the range of yield efficiency amongst germplasm in breeding trials and identify candidate genotypes for use in the intensive cropping system

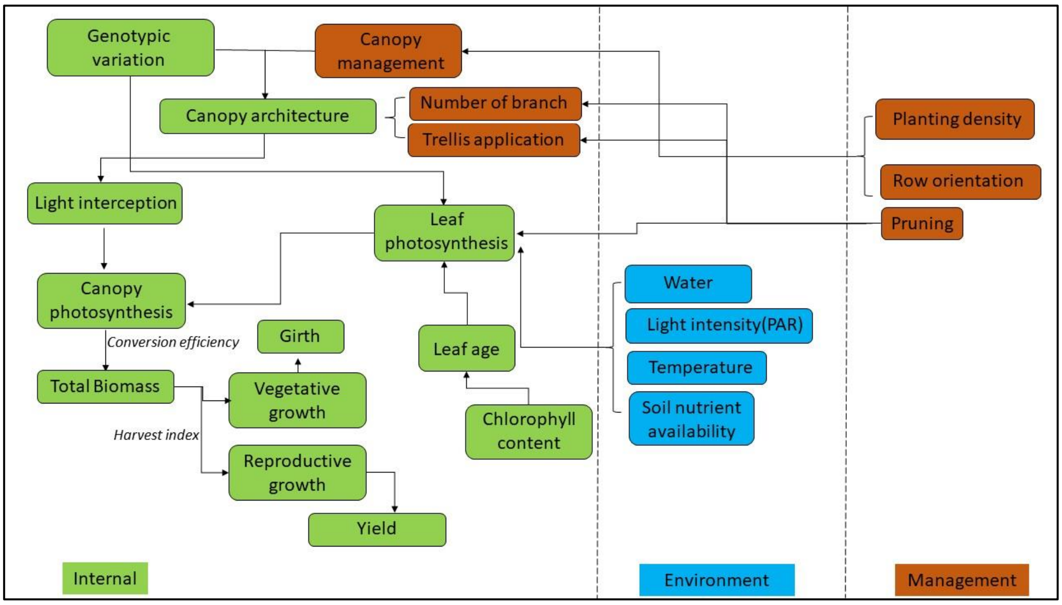


Figure 1.4 A schematic representation of yield components in cocoa

Chapter 2. Effect of Canopy Architecture Treatments on Yield Determinants and Yield Through Analysis of Cacao Grown in A Trellis and Conventional Growing System

2.1 Introduction

Several studies have tried to discern the most important parameters that determine yield in cacao (Zuidema et al., 2005; Almeida & Valle, 2007). Almeida & Valle (2007) suggested that the most critical yield determinants are light interception, photosynthesis, biomass partitioning, maintenance respiration, pod morphology, and seed (“bean”) fermentation.

The modelling study of Zuidema et al. (2005) highlighted light interception, photosynthesis, and maintenance respiration as key physiological yield determinants. They also found that post-harvest processes can impact final yield, specifically the bean fermentation process (i.e. the fraction of bean weight present after fermentation). However, published research addressing yield determinants has only been conducted under traditional production systems characterised by low inputs and minimal crop management. It is also necessary to understand the determinants of yield in a highly intensive production system. This will enable agronomic practices required to produce high yields to be identified.

Yield is the result of assimilate production and partitioning to the reproductive components of the tree. Assimilate production, in turn, is the product of the photosynthetic efficiency of the leaves (photosynthetic capacity) and the quantity of solar radiation (light) intercepted by the leaf canopy. The amount of light intercepted by the leaf canopy depends on its leaf area index (leaf area per unit ground area) and its architecture (the arrangement of branches and leaves, leaf size and leaf angle).

Although the relationship between light interception and yield has been studied only to a limited extent in cacao, yield appears to be closely related to light interception. Koko et al. (2013) demonstrated in a cacao mono-crop and inter-cropped with avocado and orange that yield was closely related to the amount of incident solar radiation intercepted by the cocoa. In the same study, yield and incident solar radiation could be described as a positive logistic function of the planting distance from the shade tree (Koko et al., 2013).

In juvenile cocoa, early vegetative growth is associated with leaf photosynthetic rate (Yapp & Hadley, 1994). However, in mature trees, where there is more self-shading within the canopy,

canopy photosynthesis will depend on the properties of the canopy as well as the leaf photosynthetic rate. Furthermore, the relationship between photosynthesis and the yield of mature trees is less clear because yield is also determined by the balance of assimilate partitioning between vegetative and reproductive components of the tree. At one extreme, a tree that partitions most of its assimilates towards vegetative growth will tend to grow faster but have a low yield, whereas trees that partition towards reproductive growth tend to be less vigorous but will have high rates of pod production relative to vegetative growth.

2.2 Research aims and hypothesis

The chapter aims to determine whether pruning trees to maintain different branch numbers impacts on yield. Several training systems with different numbers of branches and with/without trellis applications were tested to generate the desired canopy ideotypes. The aim was to develop a canopy ideotype for the high-yielding clone selected for this project (clone M01), as determined for other fruit crops (Smart et al., 1990).

Several studies have demonstrated a higher photosynthetic capacity in younger cocoa leaves (PF1: Previous Flush 1, young leaves) versus older leaves (PF2, PF3, middle age and mature leaves) (Machado & Hardwick, 1988). The abundance of photosynthetically inefficient older leaves results in a poor bean weight/overall biomass ratio (Bastide & Jimmy, 2003). Therefore, pruning is essential to stimulate new leaf growth with a higher photosynthetic rate.

In a monoclonal system, a set of pruning/training practices must be established to maximise the leaves' exposure to sunlight and drive more assimilates to bean production.

Preliminary analyses on cacao (Thomas & Balasimha, 1992) have shown that although the amount of Photosynthetically Active Radiation (PAR) intercepted by the canopy was increased through pruning, higher yields were observed in the non-pruned treatments due to an increase in assimilating allocation to leaf flushing, rather than to reproductive components of the plant in the pruning treatment. The authors of this study concluded: “From the preceding results, it is clear that the three consequences of pruning, increased transmittance of light, flushing and reduction of canopy area had an adverse effect on the productivity of cocoa.” However, there is limited research on pruning in cacao compared with other fruit crops for which pruning parameters are well established. It is important to note that most pruning studies on cacao were implemented on hybrid seedlings (segregating populations), and only a few studies on pruning have been published on clonal cacao. Sleigh et al. (1984) study on young seedlings demonstrated that removing new leaves and modifying sink ratios increased root growth. However, no data is available on the effects of pruning on partitioning between vegetative and reproductive components.

2.3 Material and methods

2.3.1 Experiment planning

The design of the experiment (number of tree replicates, density treatment and block design) was discussed in April 2011, whilst the planting materials were prepared in August 2011 and planted in the field in February 2012. The first objective of this experiment was to assess the tree performance at different planting densities (625, 816, 833 and 1111 trees.ha^{-1}). The trial was formerly planted in a conventional way (with four branches from the main trunk and growing without a trellis system). However, in April 2014, the trees were shaped to give a defined canopy architecture.

The author worked in the research station in April 2013 and joined the discussion and experiment set-up since then. She trained and managed another four team members on how to do the pruning and data collection protocol from April 2014. The intensive research started formally in April 2016.

2.3.2 Study site

The experimental site comprised plots of grafted clonal cacao trees and was located at the Mars Cocoa Research Station, Tarengge, South Sulawesi, Indonesia (Latitude 2º33’42.98” S and Longitude 120º49’16.25” E, elevation 27 m above sea level).

The site was planted with the M01 cacao clone, growing in rows in an East to West direction. The raising of material for planting in the field began in August 2011.

Variety M01 has been proven in the farmers' fields to produce strong, robust seedlings, which are relatively resistant to disease, fast-growing and widely available. It, therefore, serves as an excellent rootstock for experiment plants. Using seedling rootstock from more robust varieties is a common practice in most cacao-growing regions (Sodre, 2019; Isele et al., 2020), which makes for more efficient propagation and long-term health of trees in the field. A previous investigation of clone M01 (Asman et al., 2021) provides evidence that rootstocks derived from cocoa genotypes classified as resistant, moderately susceptible, or susceptible to Vascular Streak Dieback (VSD) exert negligible influence on cocoa scion resistance to VSD. In this regard, cocoa scion genotypes emerge as critical determinants of VSD resistance.

The source of seeds came from the farmers' superior trees (M01), listed as the main parental tree, with biclonal hand-pollination. The seeds were then washed to remove most of the pulp and soaked with a dilute fungicide solution (difenoconazole) at a concentration of 1g.L^{-1} for 15 minutes before germination in a wet jute bag for 1-2 days. The jute bag was watered two times a day to maintain the humidity.

After initial germination, seeds were sown in 20 cm wide and 25 cm high polybags with sandy clay loam medium and placed in the nursery at the Mars Research Station, Tarengge, South Sulawesi. The seedlings were placed in the nursery for six months: three months for the rootstock to grow enough for grafting; and three months after grafting. The nursery was constructed using a wooden frame with UV-transmitting plastic polyethylene film for the roof and polypropylene netting for the side of the nursery. On average, the light interception by the nursery netting and roof was 72±3%.

The seedlings were watered twice daily and fertilised with 31.2 grams of chicken manure per pot. Fungicide treatments were applied as required to control fungal pathogens (cuprous oxide and difenoconazole were applied according to the manufacturer's recommendations). In November 2011, cacao seedlings were top-grafted with scion material from the clone M01 (Figure 2.1). Three months later, on 18 February 2012, the grafted seedlings were planted in the trial.

Figure 2.1 Grafting process for plant materials preparation in the nursery

The trial was initially designed to examine planting density, although several tree architecture treatments were imposed at a later stage. The experiment consisted of 16 plots planted at four densities (625, 816, 833, and 1111 trees ha^{-1}), with each density replicated in four plots in a modified Latin square design (4 columns x 4 rows). Each density plot was randomised across columns and rows. Within a plot, there were seven rows, and each row was used for different canopy architecture treatments, as seen in Figure 2.2. Each canopy architecture treatment had five tree replicates; border trees were excluded from the analysis. No permanent shade plants were used in the experiment.

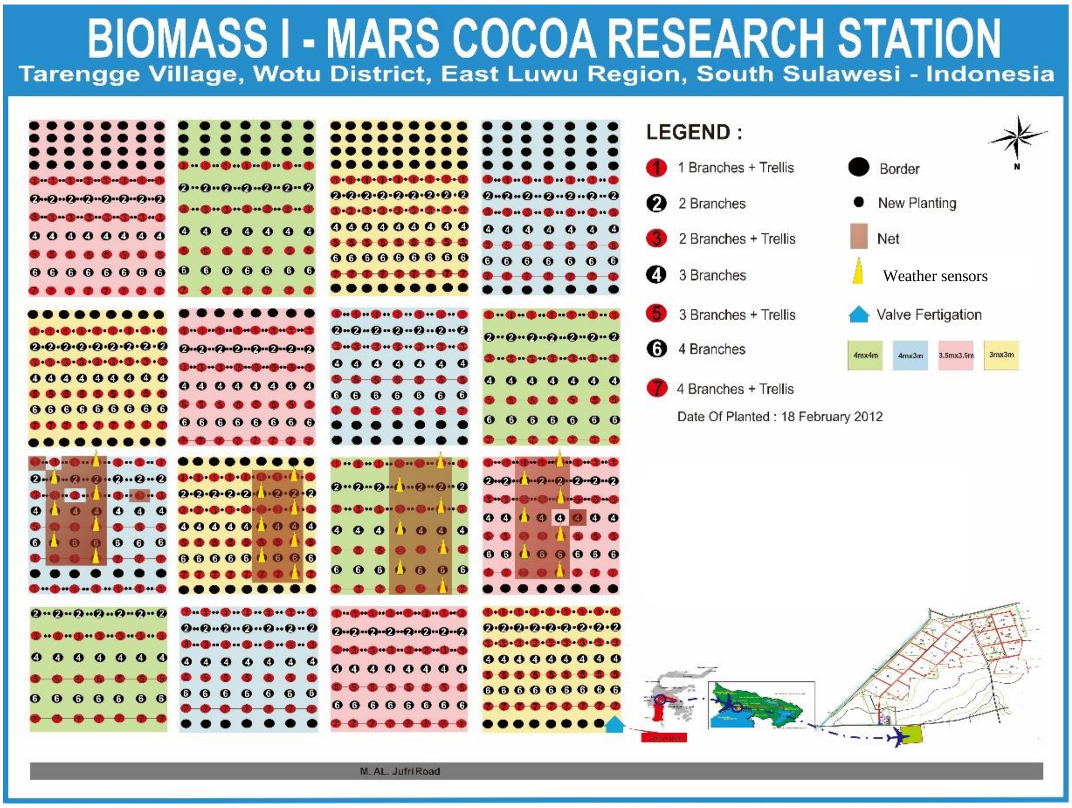


Figure 2.2 Diagram of the experimental layout and treatments

In April 2014, the trees were shaped to give a defined canopy architecture. Two designs were tested. Firstly, trees were arranged on a single plane on a trellis system. Trees on the trellis system were pruned such that they had one, two, three or four main branches. Secondly, trees were managed in a conventional way per regular farmer practice and pruned to give a range of main branches (two to four). The two canopy architectures (trellis and conventional) and different branch numbers were compared to identify the most effective agronomic systems. In total, there were seven types of canopy architecture treatments in each plot in the trial: 2, 3, and 4 branches for the conventional shape trees (2B, 3B, and 4B) and 1, 2, 3, and 4 branches grown on the trellis (1B+T, 2B+T, 3B+T, and 4B+T). Maintaining a cacao tree with a branch growing upright without a trellis was difficult. Therefore, only 2, 3, and 4 branches were imposed for the conventionally shaped trees, as shown in Figure 2.3.

The trellis consisted of a wired framework to support the trees; the distance between each 3m-high vertical trellis depended on its planting density, ranging from 8-14 m. The vertical distance between wires was 30 cm along each row except for the two uppermost trellis wires, where the gap was 60 cm. Upright branches of trees were attached to the trellis using rubber ties.

Figure 2.3 Canopy architecture treatments in the experiment. The picture was taken in 2017

A: 1B+T (1 branch on trellis) **B**: 2B (two branches) **C**: 2B+T (two branches on trellis) **D**: 3B (three branches) **E**: 3B+T (three branches on trellis) **F**: 4B (four branches) **G**: 4B+T (four branches on trellis)

For comparing the performances of the different treatments, trees were standardised based on total branch length (as shown in Table 2.1) and canopy height (2.5 meters maximum) every time pruning took place. Trees with three and four branches were allowed to expand on both the trellis and conventional systems until they met their neighbours. This was to equalise the amount of photosynthetic resources between replicate trees.

Since the objective of the treatment was to maximize light interception received by the sample trees, maintenance pruning was conducted every 6 weeks to remove unwanted plant parts, for example, any vertical branches that grew above 2.5 m and intertwined horizontal branches. It occurred six weeks after each leaf flushing period (January, April, July, and October). If necessary, minor corrections were carried out to maintain tree architecture. This 6-week pruning frequency is more frequent than what is usually done in everyday practice. Typically, cacao is pruned once or twice a year (Ramirez-Argueta et al., 2022).

Table 2.1 Tree canopy architecture pruning parameters

Number of main branches	**Trellis**		**Conventional-shaped**	
	Total branch length (m)	**Canopy width restriction (m)**	**Total branch length (m)**	**Canopy width restriction (m)**
1	8	1.5 m (0.75 on each side)		
2	11	1.5 m (0.75 on each side)	15	0.75 m around the trunk
3	13	-	20	-
4	16	-	25	-

2.3.3 Planting material

2.3.3.1 Clone characteristics

The clone M01 (formally named MCC01) was selected by the farmer Pak Muchtar on his farm in Lara Village in Baebunta District, North Luwu Regency, South Sulawesi, Indonesia, in 2000. Although its parentage is unknown, M01 originates from a Malaysian plantation (Ng et al., 2012; Forbes et al., 2019). It has been registered as a local variety authorised by the Government Of North Luwu District, South Sulawesi, Indonesia (Susilo & Sari, 2015).

The clone is highly productive and has reported yields of up to 3.6 tonnes ha^{-1} (Susilo et al., 2015). It also produces flowers within 6-7 months of grafting. On average, the clone has a pod index of 15-20 pods per kg of dry beans and has a large bean size (70-80 beans per 100 grams). The pod index is the average number of pods required to achieve 1 kg of dry cacao beans.

The beans of this clone have a relatively low-fat concentration. It is moderately susceptible to black pod disease and cocoa pod borer (CPB), although it exhibits moderate resistance to vascular streak dieback (VSD) (Susilo et al., 2015).

2.3.3.2 Estimation of off-type trees

For identification of any off-type tree, DNA from leaf samples from all trees were extracted, dried, and quantified in a DNA laboratory at Mars Makassar, Indonesia, in December 2015 using the Fast Spin© DNA extraction kit from MP Biomedicals (Ohio, USA), as described in Schnell (2005). The samples were then sent to the Mars-USDA Miami laboratory. The SNP (Single Nucleotide Polymorphism) method with Fluidigm™ technology was used for fingerprint identification, using 24 markers provided by Dapeng Zhang, USDA Miami (Fang et al., 2014).

A total of 48 off-types were found from the 560 samples (8.6% off-types). As of December 2018, 30 replanted trees and 15 dead trees were identified within the trial as a whole. These off-types, replanted and dead trees (93 trees in total), were excluded from the analyses.

2.3.4 Nutrient fertigation regime

The optimal irrigation regime required for cacao to maximise yield is unknown, as are the optimal water and fertiliser delivery systems. Each year on average, 45 kg of N, 13 kg of P_2O_5, 65 kg of K_2O, 10 kg of CaO, and 13 kg of MgO are removed from the traditional production system with each tonne of cacao beans exported (Koko et al., 2013). However, requirements vary among varieties, and no information is available for the most critical cacao clones other than CCN 51 (Espinosa et al., 2006).

Nutrient concentrations and balances are known to have significant effects on multiple traits and processes that are critical to the optimisation of growth and biomass partitioning; for example, Costa et al. (2001) observed a significant increase in chlorophyll levels of mature and shaded leaves at higher N doses in growing cacao seedlings under different light levels and N doses. For more advanced crops such as rice, agronomic and irrigation practices have been developed according to the physiological needs of specific cultivated varieties. This practice is already used for certain fruit crops, such as grapes (Medrano et al., 2003). In grapes, the effect of water and soil nutrition on fruit quality has already been studied in detail (Leeuwen et al., 2009; Ozden et al., 2010).

A fertigation (fertiliser and irrigation) system, supplied by Netafim (Tel Aviv, Israel) with drip irrigation, was installed on the field in November 2014 and put into operation in February 2015 as the experiment was established. Vegetative growth was supported by a high nitrogen regime, whereas higher potassium and phosphorus concentrations stimulated flowering.

The regime was arranged based on the fact that there are two peak harvest periods per year. Depending on the season, one dripper was installed for each tree, with 3-6 litres supplied per day as single irrigation. A diagram of the nutritional regime provided and cacao phenology is shown in Figure 2.3 and Table 2.2. The red-coloured arrow in Figure 2.4 represents the pruning time, while the black-coloured arrow represents the peak harvest time. At peak harvest time from March until July and September, the K_2O level was increased, whilst an increase of P_2O_5 and N was imposed one and two months before the peak harvest occurred.

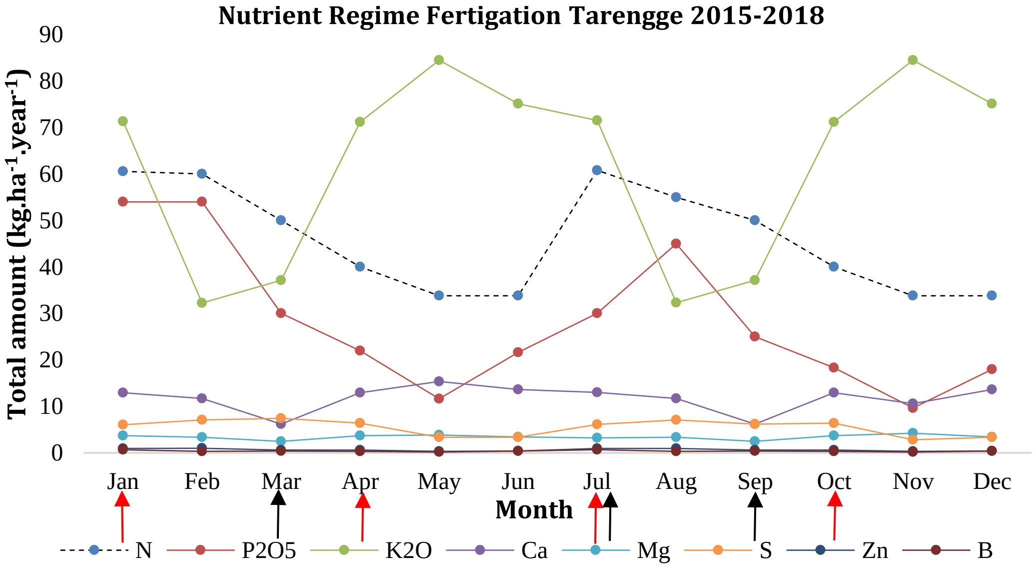


Figure 2.4 The nutrient regime for Biomass 1 fertigation 2015-2018 period. The red-coloured arrow represents the pruning time, while the black-coloured arrow represents the peak harvest time

Table 2.2 The nutrient regime for Biomass 1 fertigation 2015-2018 period. Amounts are in kg ha^{-1}.

(adjustable)	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Total amount (kg.ha^{-1}.year^{-1})**
N	60.6	60	50	40	33.8	33.8	60.8	55	50	40	33.8	33.8	552
P_2O_5	54	54	30	22	11.6	21.6	30	45	25	18.3	9.7	18	339
K_2O	71.3	32.3	37.2	71.2	84.5	75.1	71.5	32.3	37.1	71.2	84.5	75.1	743
Ca	13	11.7	6.2	12.9	15.4	13.7	13	11.7	6.2	12.9	10.6	13.7	141
Mg	3.7	3.3	2.5	3.7	3.8	3.4	3.3	3.3	2.5	3.7	4.2	3.4	41
S	6.1	7.1	7.4	6.4	3.4	3.4	6.1	7.1	6.2	6.4	2.8	3.4	66
Zn	1	1	0.6	0.6	0.3	0.4	1	0.9	0.6	0.6	0.3	0.4	8
B	0.7	0.4	0.4	0.3	0.2	0.4	0.7	0.4	0.4	0.3	0.2	0.4	5

Three categories of data were measured: growth, phenology, and yield. Measurement parameters and the annual measurement sequence schedule are shown in Figure 2.5.

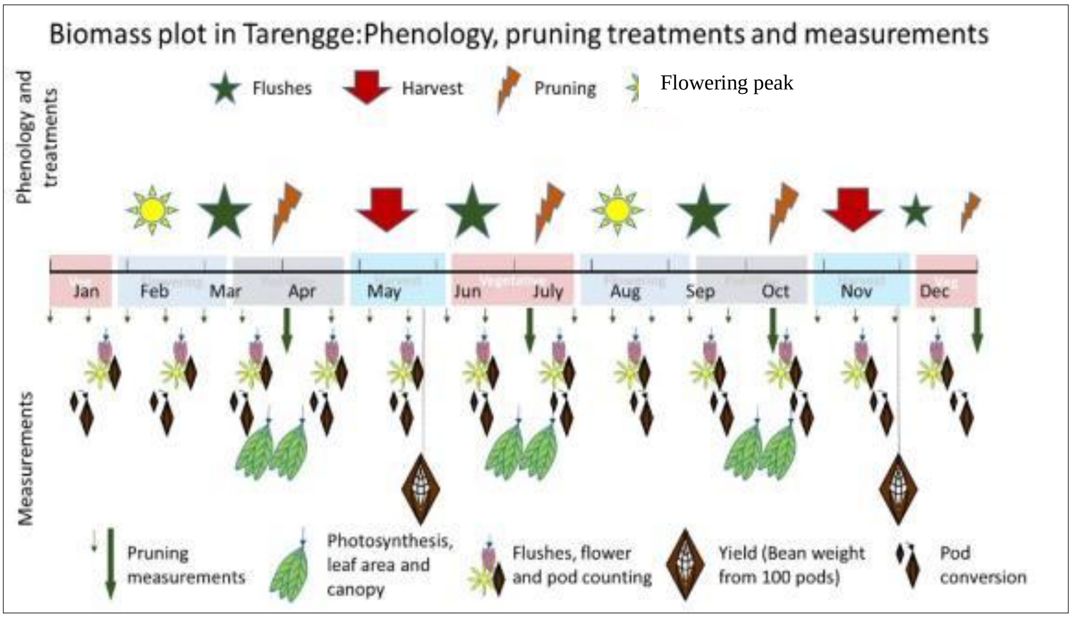


Figure 2.5 Annual programs of measurement parameters in Biomass 1 plot

2.3.5 Vegetative growth assessment

The fresh weight of pruned branches and leaves per-tree basis was measured at each pruning. The sampled trees for these measurements were from 4 planting densities with seven canopy architecture treatments and three replicate trees in each plot (84 trees in total). Subsequently, sub-samples of approximately 1,500 grams of the separated branch and 500 grams of leaf samples from each treatment were weighed and then dried for 2-3 days using a ventilated oven (Memmert UN30, Germany) set to 70°C and then re-weighed to obtain a dry matter conversion value.

The first pruning was conducted in April 2014 to shape tree branches into a trellis form, and the quantity of pruned material was then measured on 8 December 2014. Subsequently, pruning was conducted 16 times (once in 2014, four times in 2015, four times in 2016, three times in 2017, and four times in 2018).

2.3.6 Leaf area index measurement

Leaf area index (LAI) was measured using a plant canopy imager (CI-110, CID, USA) which employed the gap fraction method to measure LAI (Welles & Cohen, 1996; Weiss et al., 2004). Leaf area index was measured nine days before and nine days after pruning in planting densities 625, 816, 833, and 1111 trees.ha^{-1} (in row 2; columns 1,2,3,4) and seven canopy architecture treatments in January 2016, October 2016, and July 2018. Three readings were taken below the canopy for each marked tree, 10 cm distant from the tree trunk and at two different positions (north and south facing-side) at three canopy heights: 0.5, 1, and 1.5 m (Figure 2.6).

Figure 2.6 Left to right: (a) Old CI-110 plant canopy imager (January and October 2016) (b) Updated CI-110 canopy imager (July 2018) (c) Transmitted radiation (R_T) and LAI measurement

2.3.7 Yield assessment

Many treatment and tree replicates were involved in this experiment, resulting in large sample numbers. However, limited facilities in the research station made harvesting and drying all cocoa beans from the sample trees not possible logistically. Therefore yield estimates were made using the pod index. Furthermore, since the yield data was calculated using pod index value, the final yield data presented the potential yield (estimated yield; diseased pods included).

Pod Index is a value that defines the number of pods needed to produce 1 kg of dry cacao beans (Pound, 1932; Bekele & Butler, 2000). The higher the number, the smaller the bean mass per pod is. A new sample of 50 pods per treatment was used every six months to estimate pod index by drying and weighing beans from these pods. The pod index value oscillates slightly during the crop cycle, so it is essential to measure this periodically to maximise the precision of yield estimates.

The seed samples were dried using direct solar drying (Figure 2.7), a traditional method with a wooden platform and plastic roof with UV protection, for around 7 days (or until 7% moisture content) (Fagunwa et al., 2009).

Figure 2.7 Direct solar greenhouse for drying wet cocoa beans in Mars Cocoa Research Station Tarengge, South Sulawesi, Indonesia

This greenhouse drying has several advantages, such as being environmentally friendly (Manoj & Manivannan, 2013), low construction and running cost (Dzelagha et al., 2020), relatively low contamination, drying faster and giving a better quality product than the open-air method (Nidhi, 2015; Puello-Mendez et al., 2017). However, this method depends merely on natural solar intensity; therefore, it takes longer drying time and cannot give a uniform product compared to the controlled oven drying method (Dzelagha et al., 2020); also, there are risks of external moulding, insects infestation (Bonaparte et al., 1998), and ochratoxin A contamination (Dzelagha et al., 2020).

In this experiment, the harvested seeds were dried without initial fermentation. A previous study by Obenza et al. (2022) recorded that fermented bean samples will experience 38% weight loss due to drying, whilst unfermented beans have 54% weight loss. Typical Indonesian cocoa beans are not fermented; therefore, solar drying is relevant to Indonesian conditions.

2.3.7.1 Dry weight of cacao beans

All ripe pods, including diseased pods, from all trees were harvested and opened, pods were counted every 14 days from December 2014 until November 2018 (four production years).

Pods were harvested from all four replicate plots for each planting density. The potential yield (which would be achieved in the absence of disease) was then calculated. For the yield calculation, any off-types identified, replanted trees and trees that had died were excluded from the sample. Only the five internal replicate trees for each plot treatment were assessed to avoid edge effects.

The annual potential yield was calculated according to the following calculation:

$$\text{Annual number of pods per tree (pods. tree}^{-1}\text{)} = \frac{\text{The annual number of pods harvested (pods)}}{\text{Number of trees (trees)}}$$

$$\text{Annual potential yield per tree (kg. tree}^{-1}\text{)} = \frac{\text{Annual number pods per tree (pods. tree}^{-1}\text{)}}{\text{Pod index (pods. kg}^{-1}\text{)}}$$

$$\text{Annual potential yield (tonne. ha}^{-1}\text{)} = \frac{\text{Annual yield per tree (kg.tree}^{-1}\text{) X planting density (trees.ha}^{-1}\text{)}}{\text{1000 kg}}\text{. tonne}$$

The following equation was used to estimate pod index:

$$\text{Pod index (Pods. kg}^{-1}\text{)} = \frac{\text{1000 (g. kg}^{-1}\text{)}}{\text{(Total weight of beans) (g)}}\text{ x Number of pods harvested (pods)}$$

The plant harvest index, the ratio of the potential yield component to total plant weight, reflects the partitioning of photo-assimilates between the yield and the vegetative components of the plant (Corley, 1983; Sinclair, 1998).

In the calculation below, the harvest index term is differentiated between the pods harvest index (calculating of pods dry weight to the total plant dry weight), and the beans harvest index (calculating o dry bean weight to the total plant dry weight). The following equations were used to estimate the harvest index

$$\text{Pods harvest index} = \frac{\text{Dry weight of pods (kg. tree}^{-1}\text{)}}{\text{Dry weight of total pruned vegetative materials, beans, pod husks (kg. tree}^{-1}\text{)}}$$

$$\text{Beans harvest index} = \frac{\text{Dry weight of beans (kg. tree}^{-1}\text{)}}{\text{Dry weight of total pruned vegetative materials, beans, pod husks (kg. tree}^{-1}\text{)}}$$

2.3.7.2 Dry matter of cacao husk

At each pruning time, samples of cacao husks were taken from seven pods from each canopy architecture treatment to obtain a dry matter conversion value. The sample was weighed and then dried using a ventilated oven (Memmert UN30, Germany) for 48 h at 70°C and re-weighed.

2.3.8 Phenology

2.3.8.1 Flushing intensity index

Flushing intensity index (the proportion of the canopy that is flushing) was recorded from January 2017-December 2018, using a 1-4 scale as follows: N (index 1) = Nil (No flushing); L (index 2) = 1-35% of the canopy in flush; M (index 3) = 36-75% of the canopy in flush; H (index 4) = >75% of the canopy in flush. All of the leaf stages from "leaf emergence" (bursting bud), "leaf expansion", and "leaf expanded but not hardened" (as shown in Figure 2.8) were categorised as flushing.

Figure 2.8 Leaf flushing stage category. From left to right: leaf emergence, leaf expansion, leaf expanded but not hardened, leaf mature

Three replicate trees were sampled from each of four planting densities (625, 816, 833, 1111 trees.ha^{-1}) on seven canopy architecture treatments per replicate plot within the trial.

2.3.8.2 Number of open flowers

The number of open flowers (from the ground up to 2 m on all branches) was counted every 28 days and twice a week during the peak flowering times from January 2016 until December 2016. Three replicate trees were sampled from each of the seven canopy architecture treatments from the planting density (1111 trees.ha^{-1}) for each replicate plot. The flowering intensity was only measured in the standard recommended planting density since the primary objective of the experiment was only to see the effect of canopy treatments on the flowering intensity and not to compare the different density effects.

2.3.9 Leaf gas exchange Light-saturated photosynthetic rate (A), transpiration rate (E), and stomatal conductance (gs) were measured using a Portable Infra-Red Gas Analyser (LC-Pro-SD, ADC Bio scientific, Hoddesdon, UK) with an artificial light source, as shown in Figure 2.9.

Figure 2.9 Infra-Red Gas Analyser equipment LC-SD Pro from ADC Bioscientific

Instantaneous water use efficiency was calculated using the equation:

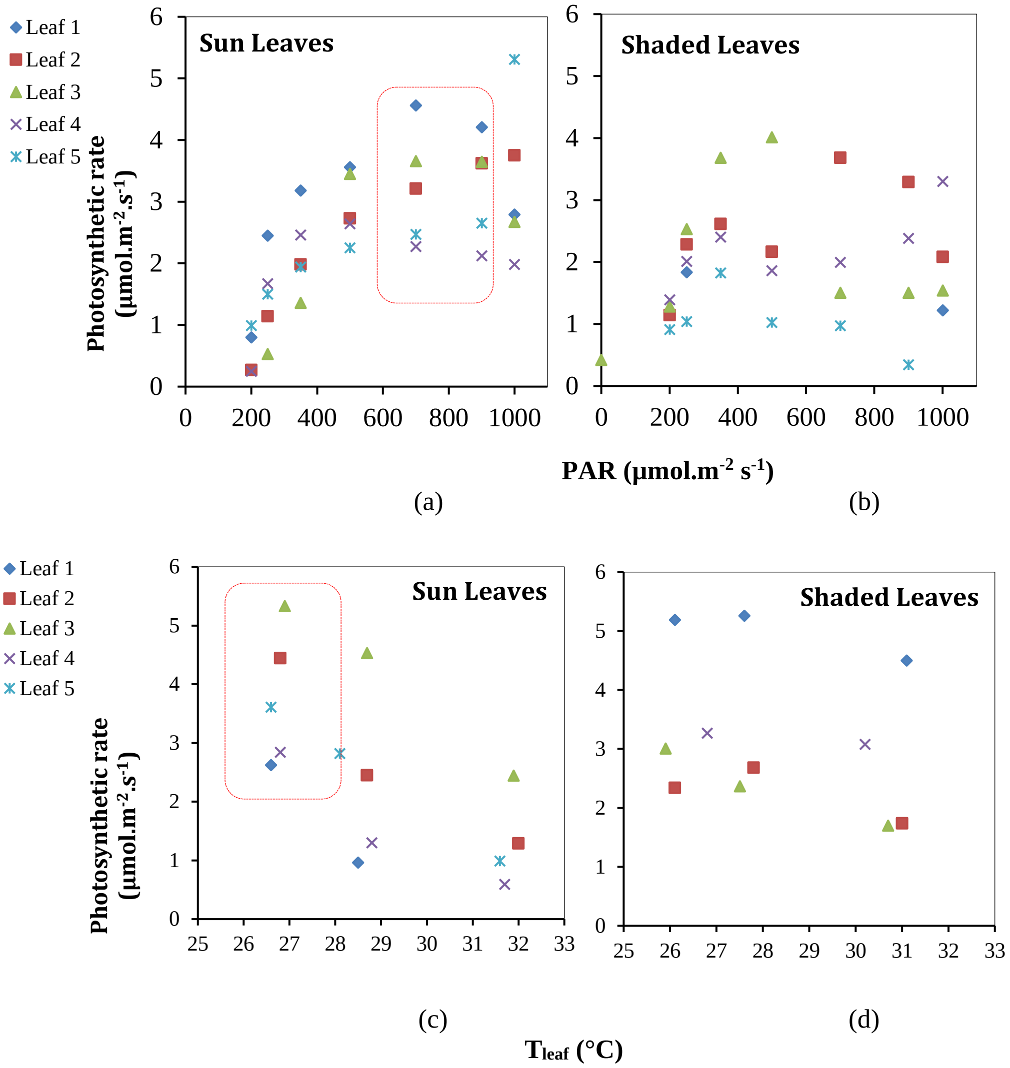
$$WUE = \frac{Photosynthetic\ rate\ (A)}{Tranpiration\ rate\ (E)}$$ (Comstock & Ehleringer, 1993)

Initial training in using the equipment was conducted on 4-15 August 2014 at the University of Reading, UK. A preliminary trial was conducted from 6-7 September 2014 in Tarengge, Indonesia, to determine the equipment settings.

To obtain a photosynthesis light response curve, the photosynthetic rate was measured on a leaf exposed to the sun and a shaded leaf from each of five different trees at a constant temperature inside the cuvette of 27°C and seven PAR settings (200, 250, 350, 500, 700, 900, and 1000 µmol.m^{-2} s^{-1}) (Figure 2.10a and 2.10b).

In order to get a photosynthesis temperature response curve, the photosynthetic rate was measured on a sun leaf and a shaded leaf from each of five different trees at a constant PAR of 900 µmol.m^{-2} and seven temperature settings (19, 20, 21, 23, 26, 28, 31°C) (Figure 2.10c and 2.10d).

Both responses indicated that the optimum photosynthetic rate for shade and the full-sun leaf was operated at a chamber temperature of 27°C, and the light attachment was set to provide photosynthetically active radiation of 900 µmol.m^{-2}s^{-1}, which is saturating for cacao (Salazar et al., 2018). Based on the above responses, the optimum chamber conditions were set at a temperature of 27°C and photosynthetically active radiation of 900 µmol.m^{-2}s^{-1} for measuring the maximum photosynthetic rate (P_{max}).



(a) (b)

(c) (d)

Figure 2.10 (a) Light response curve for sun leaves and (b) shaded leaves (c) Temperature response curve for the sun leaves and (d) shaded leaves from clone M01 trees in the Biomass 1 trial. The data was collected in September 2014

Measurements were made from January 2015 until October 2017. Sampled trees were from each of the seven canopy architecture treatments, and there were four replicate trees from each treatment from one block.

For each pruning event, a set of measurements was made 1-9 days before pruning was conducted and then repeated 1-9 days after pruning. Measurements were made between 6.30-10.30 am; the third, fourth or fifth healthy sun-exposed leaves from a given branch were chosen for the measurements. In total, ten sets (before and after pruning) of photosynthetic measurements were carried out.

The light-saturated photosynthesis was measured during the experiments to avoid artefacts caused by light intensity changing from treatment-to-treatment movement.

2.3.10 Statistical analysis

Two trees died in 2017 (Tree 1 (1 branch + trellis architecture treatment) in each planting density 625 and 833 trees.ha^{-1}) and were excluded from the analysis. The effects of canopy architecture, planting density, and their interactions on measurement parameters were tested using a two-way analysis of variance (ANOVA) with the Latin square for the dry weight of cacao beans (mean value among tree replicates). For leaf area index, leaf gas exchange parameters, vegetative pruned materials and phenology, density treatment was treated as replicates; and the split-plot design was applied. GenStat 19th edition software (VSN International Ltd., Hemel Hempstead, UK) was used for statistical analysis. The normal distribution test, histogram, residual plots and Least Significant Difference (LSD) were calculated using GenStat. The standard error of the mean was calculated by using Microsoft Excel. The standard error value was presented in the results, indicating the sample variability.

The main objective of the LSD is to compute the most negligible significant difference between two means as if these means are the only means to be compared; and to declare significant any difference larger than the LSD (Williams & Abdi, 2010). The LSD method has more power than other comparison methods because the α level for each comparison is not corrected for multiple comparisons (Williams & Abdi, 2010).

A basic assumption of an analysis of variance is that the underlying data should be normally distributed, and the variance should be the same for all treatment combinations (often referred to as homoscedastic). Therefore, for example, different treatments should not change the variability of the yield, only the average yield (Mead & Curnow, 1983). This often requires growth data to be transformed before carrying out an analysis of variance to ensure that the data are homoscedastic (typically using a log e transformation). A normality test was carried out using the Shapiro-Wilk test in Genstat. A square root transformation is often effective in normalizing data when interest measurement is a count or an area. Whilst an arc-sine transformation applies for observations that are proportions (Mead & Curnow, 1983).

2.3.11 Soil analysis

For nutrient analysis, soil samples (1500 g) from each plot were sent to the Oil Palm Research Center Medan (PPKS), North Sumatra, Indonesia. The samples were analyzed to measure soil nutrient contents and properties, including soil texture (percentage of sand, silt, and clay), macronutrients (carbon, nitrogen, phosphorus, potassium, calcium, magnesium and sulphur), micronutrients (zinc, aluminium), and other parameters (pH, saturated base cation, cation exchange capacity, saturated base).

The soil sample was a composite sample from nine different points using diagonal sampling within the plot (Figure 2.11). Each sample was taken close to a cacao tree in 20cm-depth soil (Carter & Gregorich, 2008).

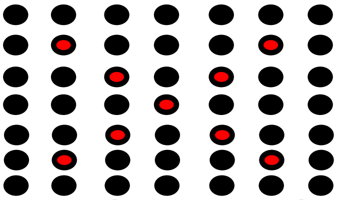


Figure 2.11 Soil composite sampling layout in each plot

The PPKS Lab used the analysis protocols of Sulaeman et al. (2005). Repeated samples from four different plots were taken to check the repeatability of the analysis result. The result of the soil sample analysis is shown in Appendix A.

The soil analysis results underpinned the Latin square design of this experiment, due to the presence of soil texture gradient differences, among different rows and columns. The soil texture influences its ability to store water and nutrients. The column in the middle of the plot had a higher content of silt and lower sand than the border. Thus, the area in the middle has a better moisture-holding capacity, while sandy soils at the edge have good drainage.

2.4 Results

2.4.1 The impact of planting density and canopy architecture on productivity per tree and area in clone M01

2.4.1.1 Potential yield per tree and per unit area

The overall cropping pattern across all treatments from 2014 to 2018 is shown in Figure 2.12. There was a significant decrease in potential yield per tree in year 3 compared with year 2 (54%), with a 54% increase in year 2 compared to year 1; whilst the potential yield per tree in year 4 increased by 18% compared to year 3 ($P<0.001$; Figure 2.12).

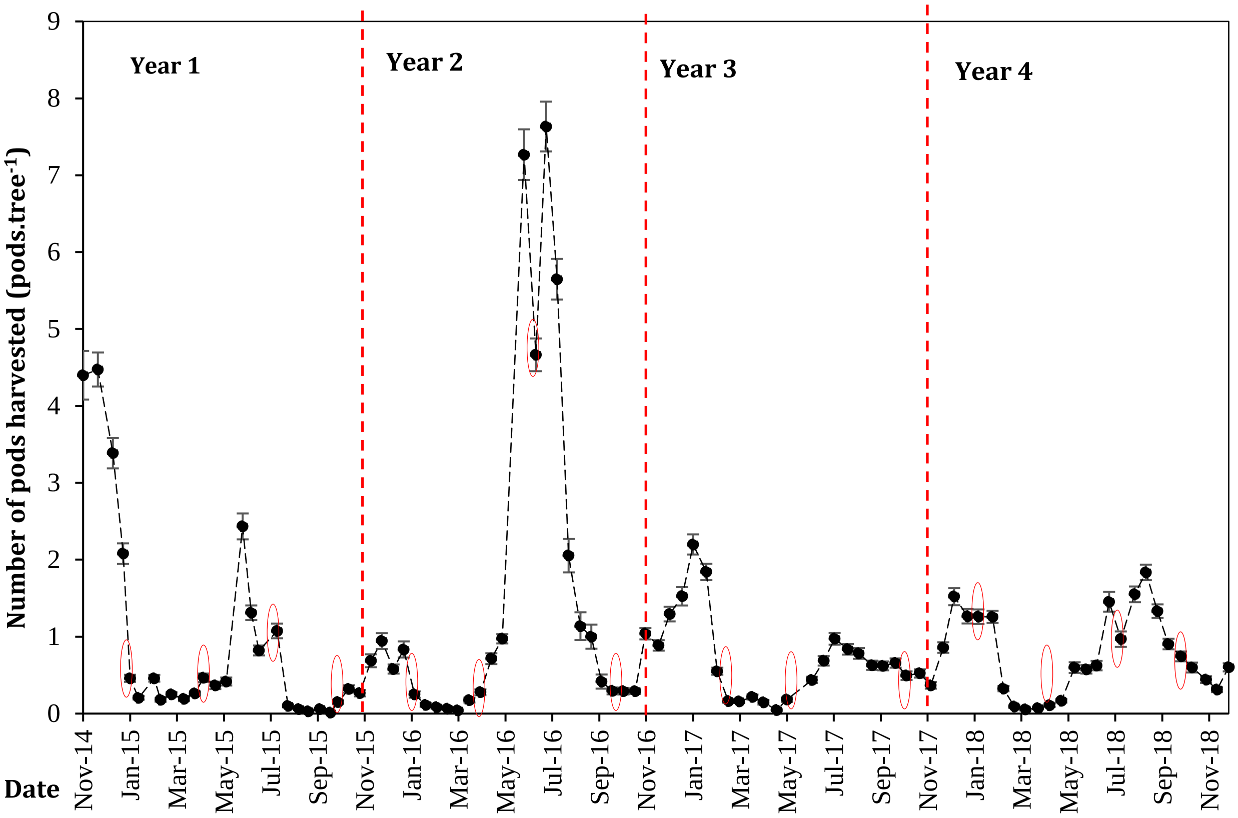


Figure 2.12 Average number of pods harvested per tree every two weeks in the Biomass 1 trial, measured from November 2014 until December 2018. Values are means across four planting densities, seven canopy architecture treatments, four plot replicates, and five trees replicate (+/- standard error of the mean). Red circles represent pruning times

The productivity per tree decreased with increasing planting density in year 2 (P=0.025), year 3 (P=0.021), and year 4 (P=0.002) (Figure 2.13). Yield per hectare generally increased with density in year 1 (P=0.016); a similar trend was also apparent in year 2. However, there was no effect of planting density on yield per hectare in years 3 and 4 (Figure 2.13).

No interaction between planting density and canopy architecture was observed on the yield per tree and area basis.

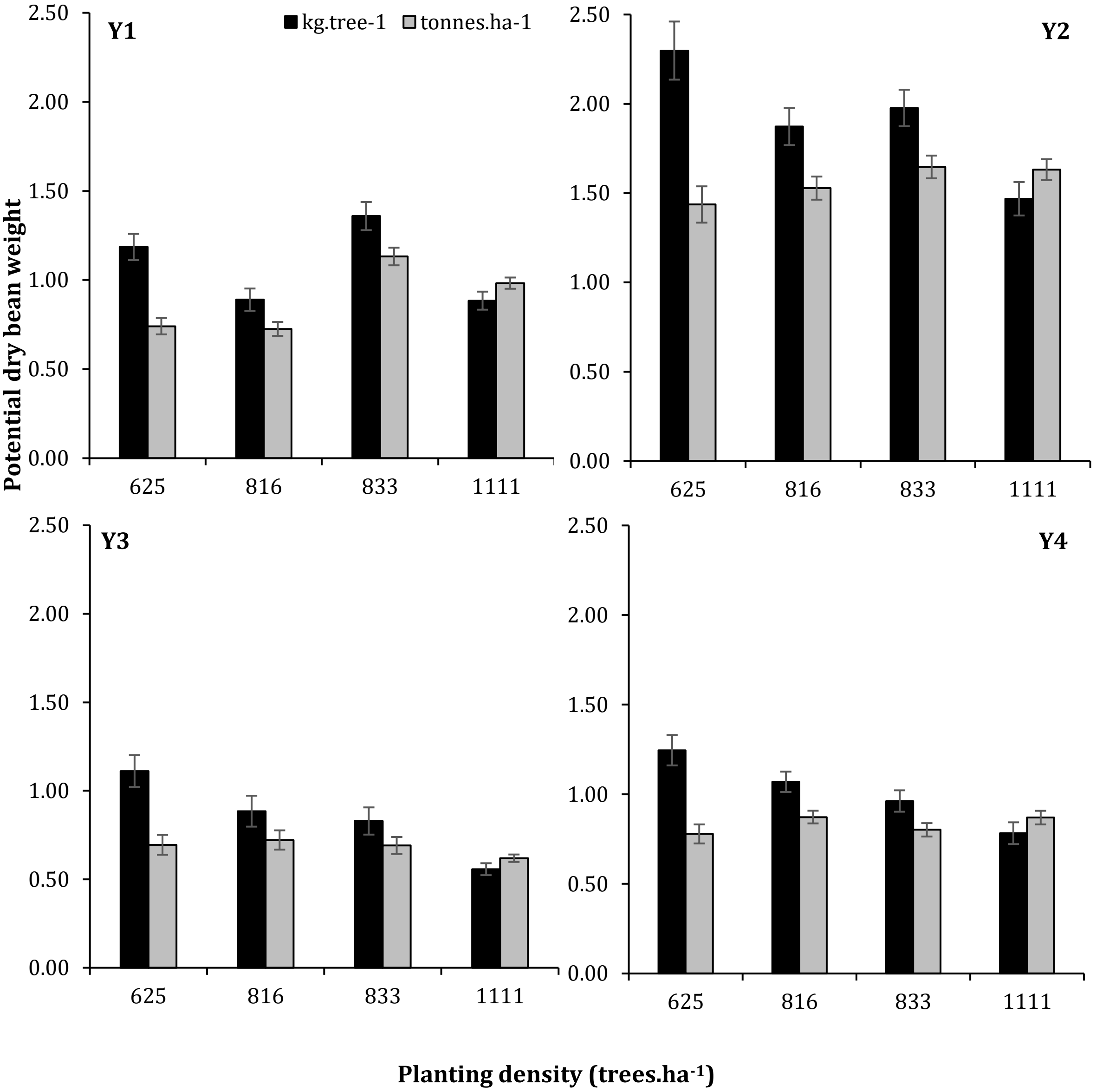


Figure 2.13 Yield per tree and per hectare in the Biomass 1 trial between different planting densities (625, 816, 833, 1111 trees.ha^{-1}), measured in Year 1 (December 2014-November 2015), Year 2 (December 2015-November 2016), Year 3 (December 2016-November 2017), and Year 4 (December 2017-November 2018). Values are means across seven canopy architecture treatments, four plots replicate, and five sample trees (+/- standard error of the mean)

The relationship between total branch length and a cumulative yield in all years (December 2014-November 2018) was examined for each density treatment (Figure 2.14). There was a positive relationship between yield and total branch length for density 833 trees.ha^{-1}.

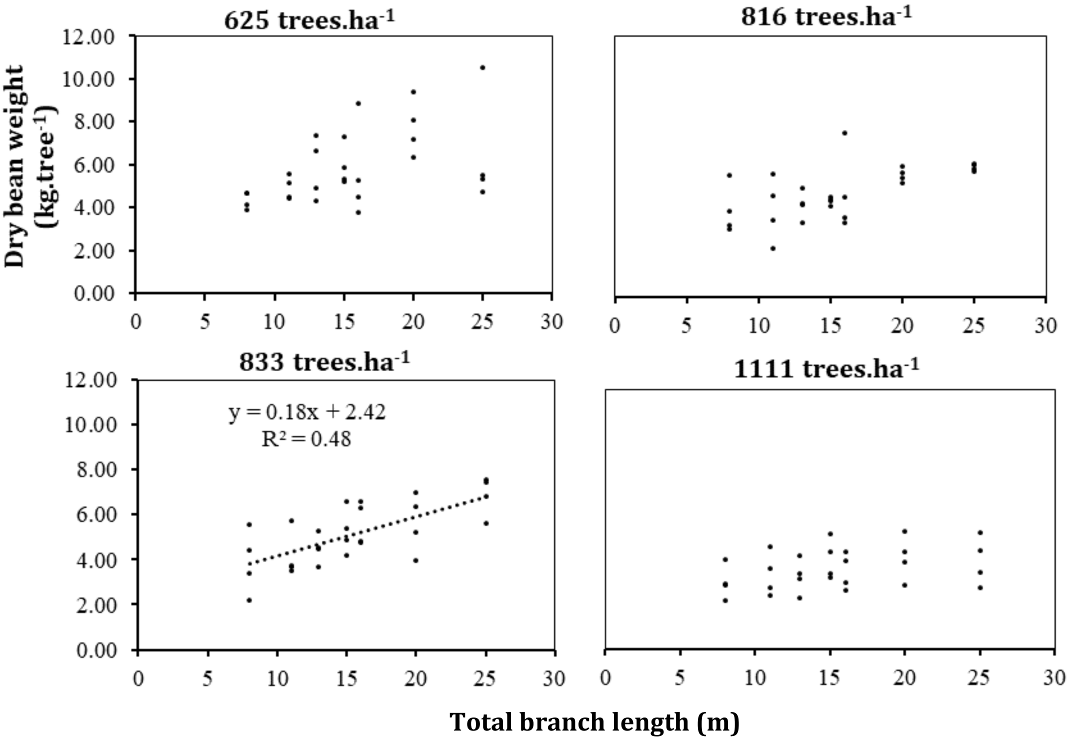


Figure 2.14 Relationship between branch length and cumulative dry bean weight over four production years of yield per tree in four planting densities (625, 816, 833, and 1111 trees.ha^{-1}) in Biomass 1 trial. Values are means across four plot replicates, seven canopy architecture treatments, and five sample trees.

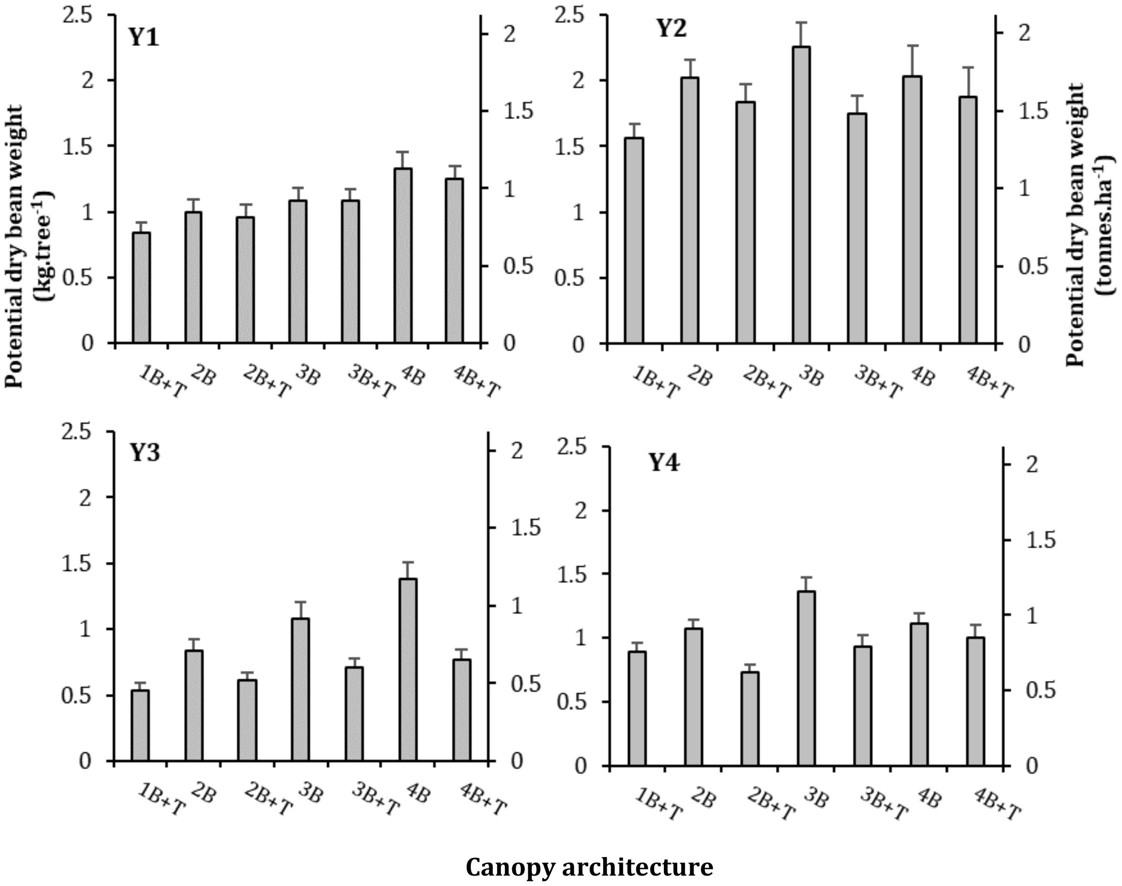


Figure 2.15 Yield (primary y-axis: per tree; secondary y-axis: per hectare) in the Biomass 1 trial, measured in Year 1 (December 2014- November 2015), Year 2 (December 2015-November 2016), Year 3 (December 2016-

November 2017), and Year 4 (December 2017-November 2018), between seven different canopy architecture treatments (1,2,3,4 branches- “B”; with and without trellis/conventional- “T”). Values are means across four planting densities, four plot replicates, and five sample trees (+/- standard error of the mean)

The potential yield from conventional trees at all branch numbers outyielded the trellis plants in years 2 ($P=0.011$), 3 ($P<0.001$), and 4 ($P<0.001$). A similar trend is observed in year 1 for the 2 and 4 branches treatments ($P=0.048$; Figure 2.15).

2.4.1.2 Pod Index

The average pod index varied over time ($P<0.001$), as shown in Figure 2.16.

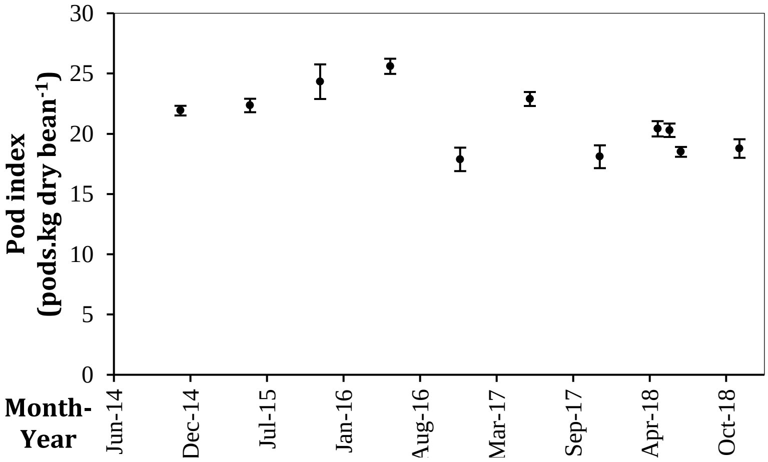


Figure 2.16 Pod index for Biomass 1 trial, measured from December 2014 until December 2018. Values are means of the seven canopy architecture treatments (+/- standard error of the mean)

2.4.2 The impact of planting density and canopy architecture on tree phenology in clone M01

Since there was no effect of planting density observed on the flushing index, planting density treatments were treated as replicates. The number of open flowers was only measured in the planting density 1111 trees.ha^{-1}.

2.4.2.1 Flushing index

The flushing index was observed from January 2017-December 2018 (2 years), as shown in Figure 2.17. In 2017, the highest flushing index was observed in January and December; and in April, July, and October in 2018.

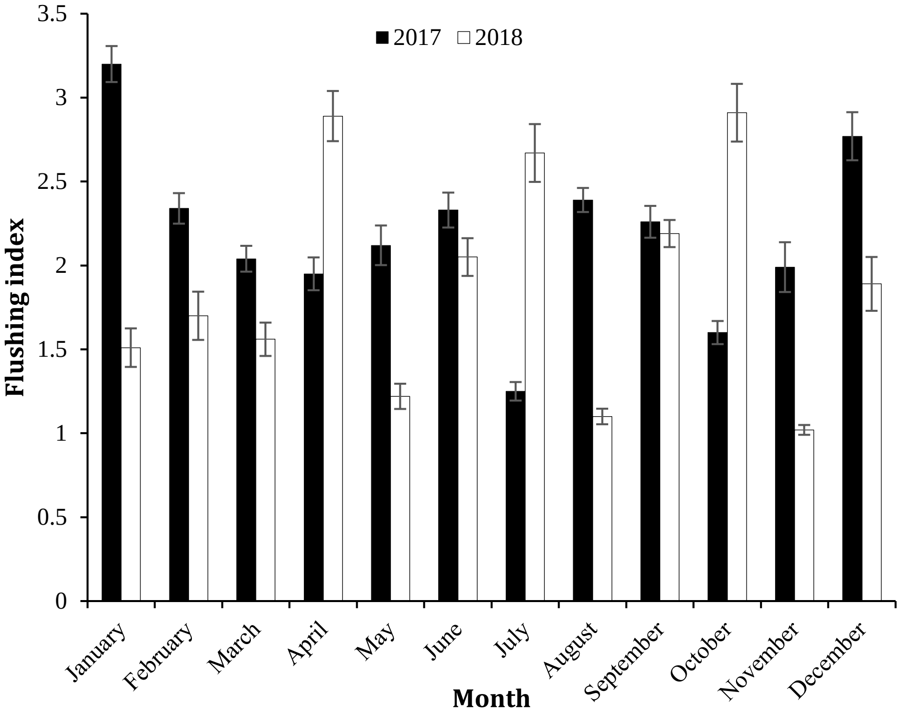


Figure 2.17 Average flushing index per tree in the Biomass 1 trial planted with the clone M01, measured between January 2017 and December 2018. Values are means across four planting densities, seven canopy architecture treatments, and three tree replicates (+/- standard error of the mean)

The flushing index in conventional treatments was marginally (5%) higher compared to the trellis treatments (P=0.049; Figure 2.18).

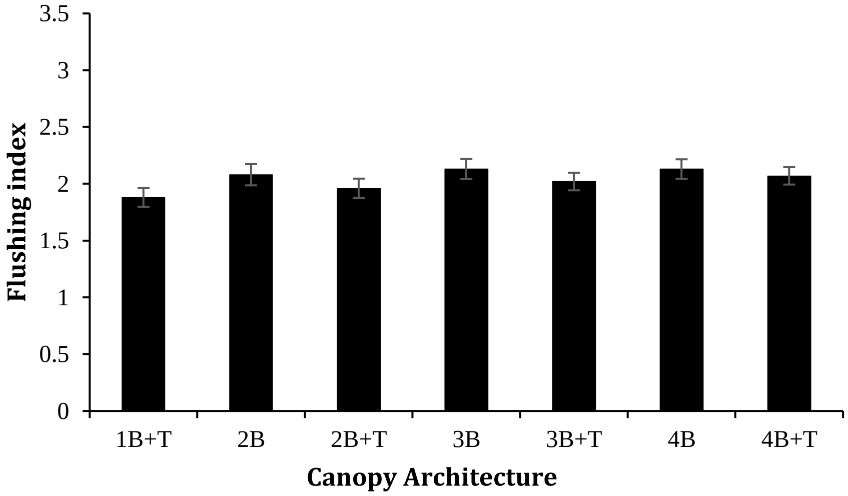


Figure 2.18 Average flushing index per tree in the Biomass 1 trial planted with the clone M01, between seven different canopy architecture treatments (1,2,3,4 branches; with and without trellis), measured between January 2017 and December 2018. Values are means across two year-measurement periods, four planting densities, and three tree replicates (+/- standard error of the mean)

2.4.2.2 Number of open flowers

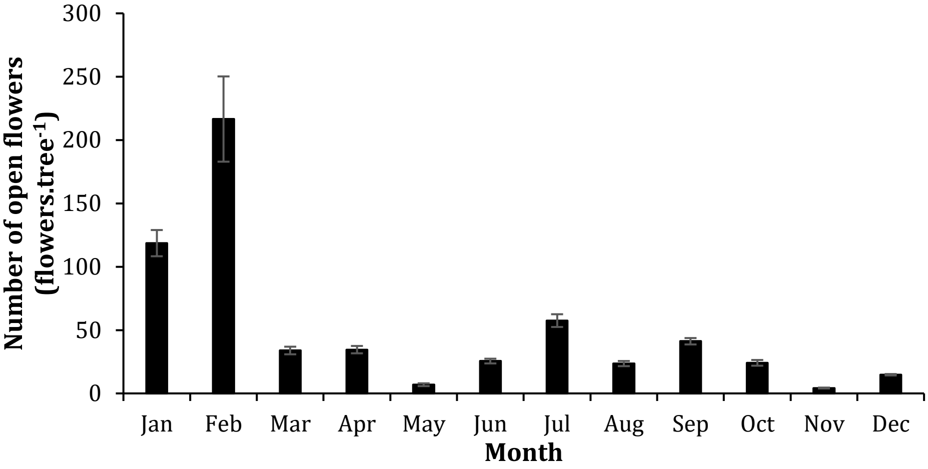


Figure 2.19 Average open flower numbers per tree in the Biomass 1 trial planted with the clone M01, measured between January 2016 and December 2016 in density 1111 trees.ha^{-1}. Values are means across seven canopy architectures, three plots and three trees as replicates (+/- standard error of the mean)

The number of open flowers was only measured in 2016. The peak period for flower intensity occurred in February 2016 (the average in February 2016 was 216 flowers tree^{-1}) and declined thereafter, as shown in Figure 2.19.

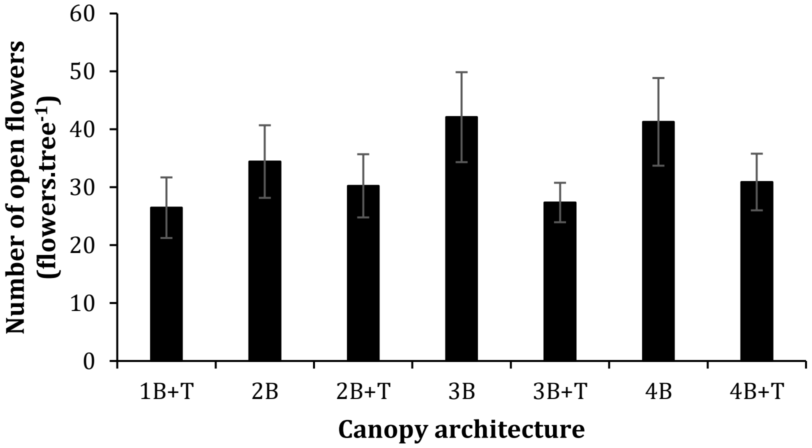


Figure 2.20 Average open flower numbers per tree per observation in the Biomass 1 trial planted with the clone M01, measured between January 2016 and December 2016 between seven different canopy architecture treatments in density 1111 trees.ha^{-1}. Values are means across three plots and three trees as replicates (+/- standard error of the mean)

The average flowering intensity was 33% higher in conventionally managed trees (2B, 3B, 4B) compared with the trellis system treatments (average number of flowers in conventionally managed trees was 39 flowers tree^{-1} per observation compared with 30 flowers tree^{-1} in trellis treatments; P=0.049) (Figure 2.20). A trend of increasing numbers of open flowers with increasing branch numbers for conventional systems (comparing 2B to 3B and 4B), respectively, was also observed, but not for the trellis system.

2.4.3 The impact of planting density and canopy architecture on vegetative growth in clone M01

The highest pruned branch dry weight per tree and per unit area was observed in the production year 2 (P<0.001), 92% and 52% higher compared to years 1 and 3, respectively. Pruned branch dry weight per tree decreased with increasing planting density in production year 3 (Figure 2.21; P<0.001). This pattern was also observed to a lesser extent in year 2, but pruned branch weight in year 1 was generally similar at all densities.

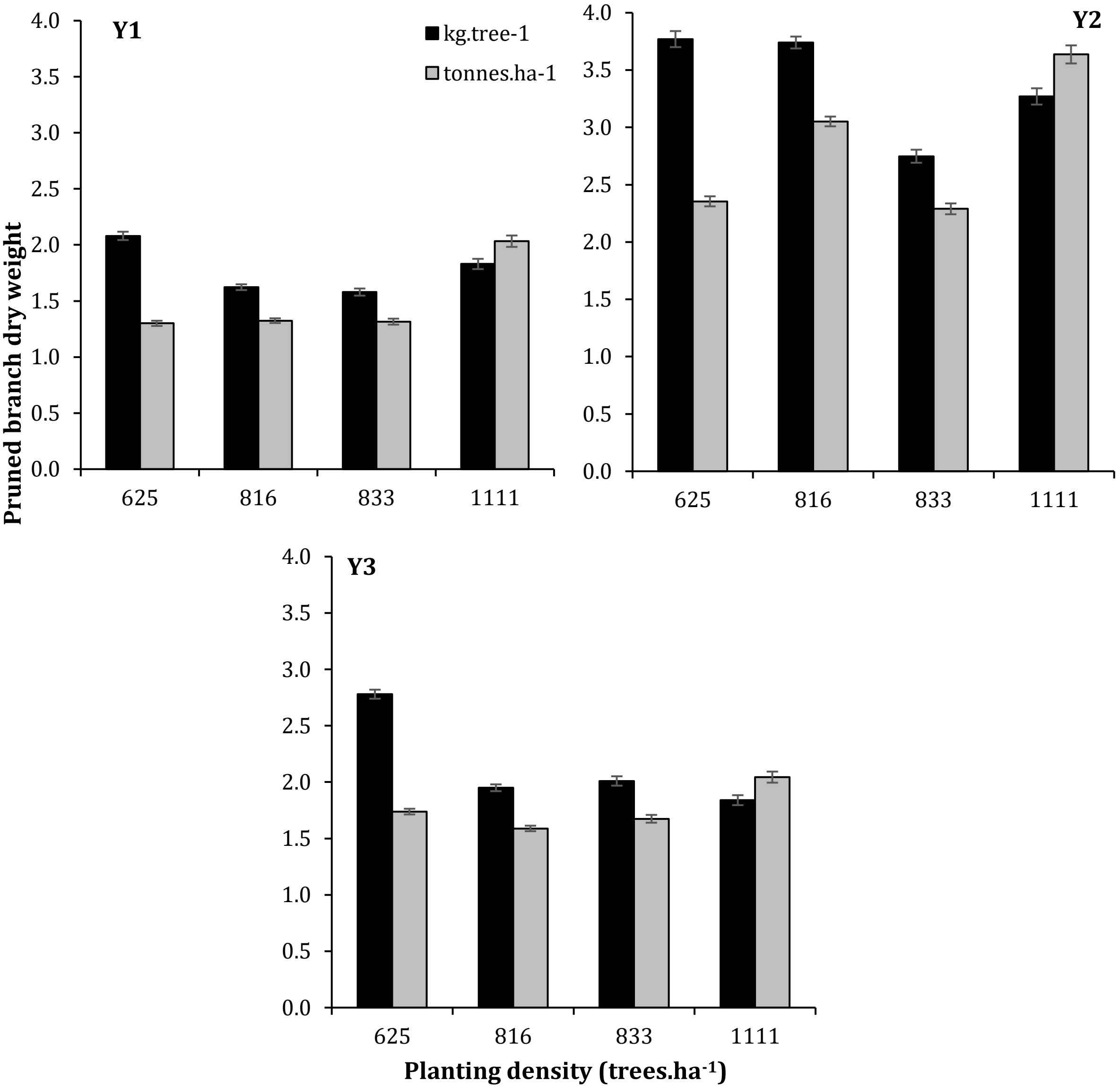


Figure 2.21 Pruned branch dry weight per tree and per hectare in the Biomass 1 trial, measured in Year 1 (December 2014-November 2015), Year 2 (December 2015-November 2016), Year 3 (December 2016-November 2017), between different planting densities (625, 816, 833, 1 111 trees.ha^{-1}). Values are means across seven canopy architectures and five sample trees (+/- standard error of the mean)

However, on an area basis, pruned branch weight increased with planting density in production year 1 (P=0.002) and year 2 (P=0.003), and a similar but smaller trend was also observed in year 3 (P=0.0028).

The conventional canopy architecture treatments produced a higher pruned branch dry weight per tree at the higher number of branches per tree in year 1 (P=0.009) and year 3 (P<0.001). There was also a similar trend in year 2 (Figure 2.22).

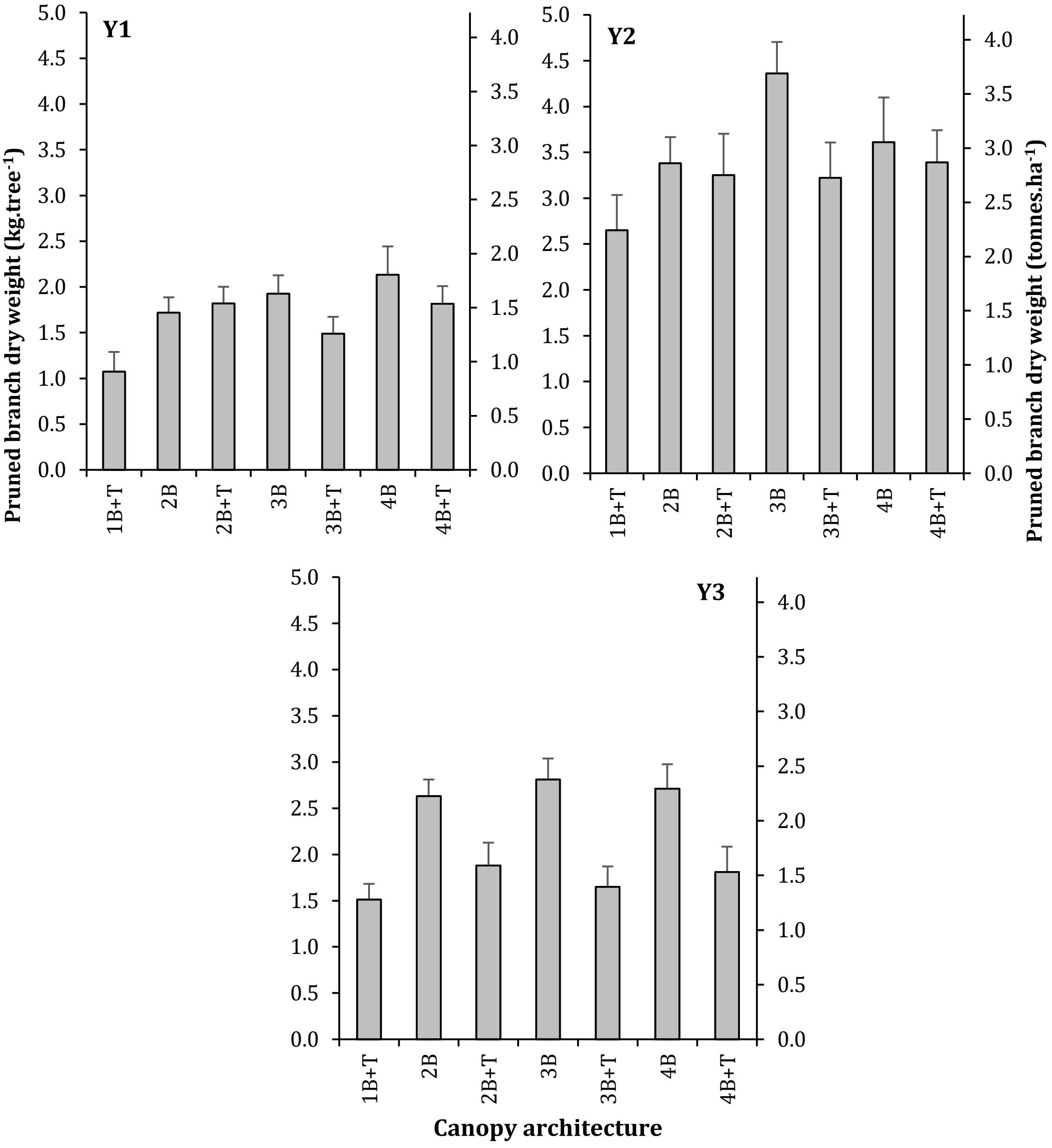


Figure 2.22 Pruned branch dry weight (primary y-axis: per tree; secondary y-axis: per hectare) in the Biomass 1 trial, measured in Year 1 (December 2014-November 2015), Year 2 (December 2015-November 2016), Year 3 (December 2016-November 2017), between seven different canopy architecture treatments (1,2,3,4 branches; with and without trellis). Values are means across four planting densities and five sample trees (+/- standard error of the mean)

Pruned leaf dry weight per tree decreased with increasing planting density in year 1 (P=0.019), year 2 (P=0.008) and year 3 (P<0.001), as shown in Figure 2.23. However, the opposite pattern was observed for pruned leaf dry weight per unit area. (year 1 (P=0.045), year 2 (P<0.001) and year 3 (P=0.020)).

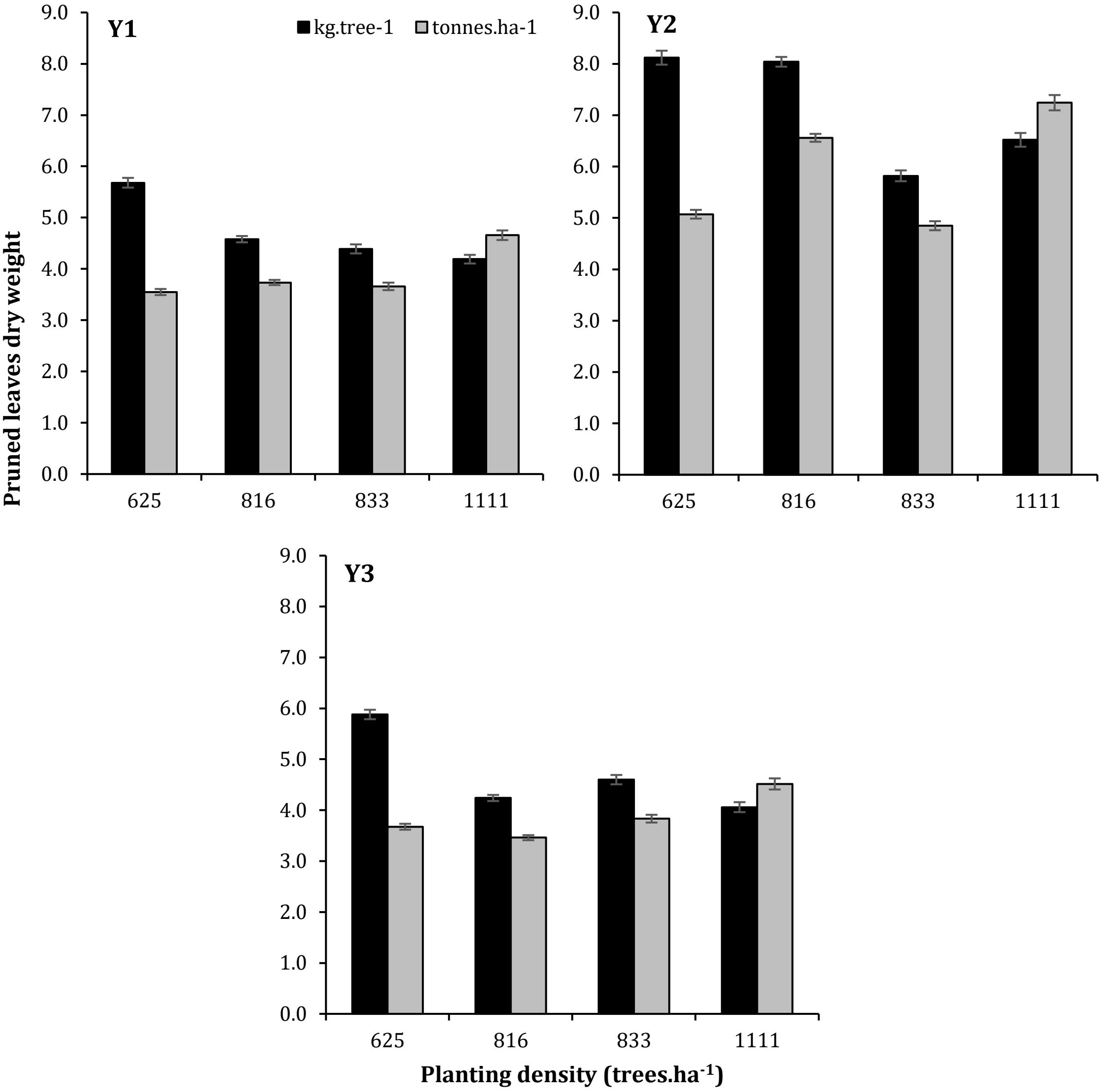


Figure 2.23 Pruned leaves dry weight per tree and per hectare in the Biomass 1 trial, measured in Year 1 (December 2014-November 2015), Year 2 (December 2015-November 2016), Year 3 (December 2016-November 2017), between different planting densities (625, 816, 833, 1 111 trees.ha^{-1}). Values are means across seven canopy architectures and five sample trees (+/- standard error of the mean)

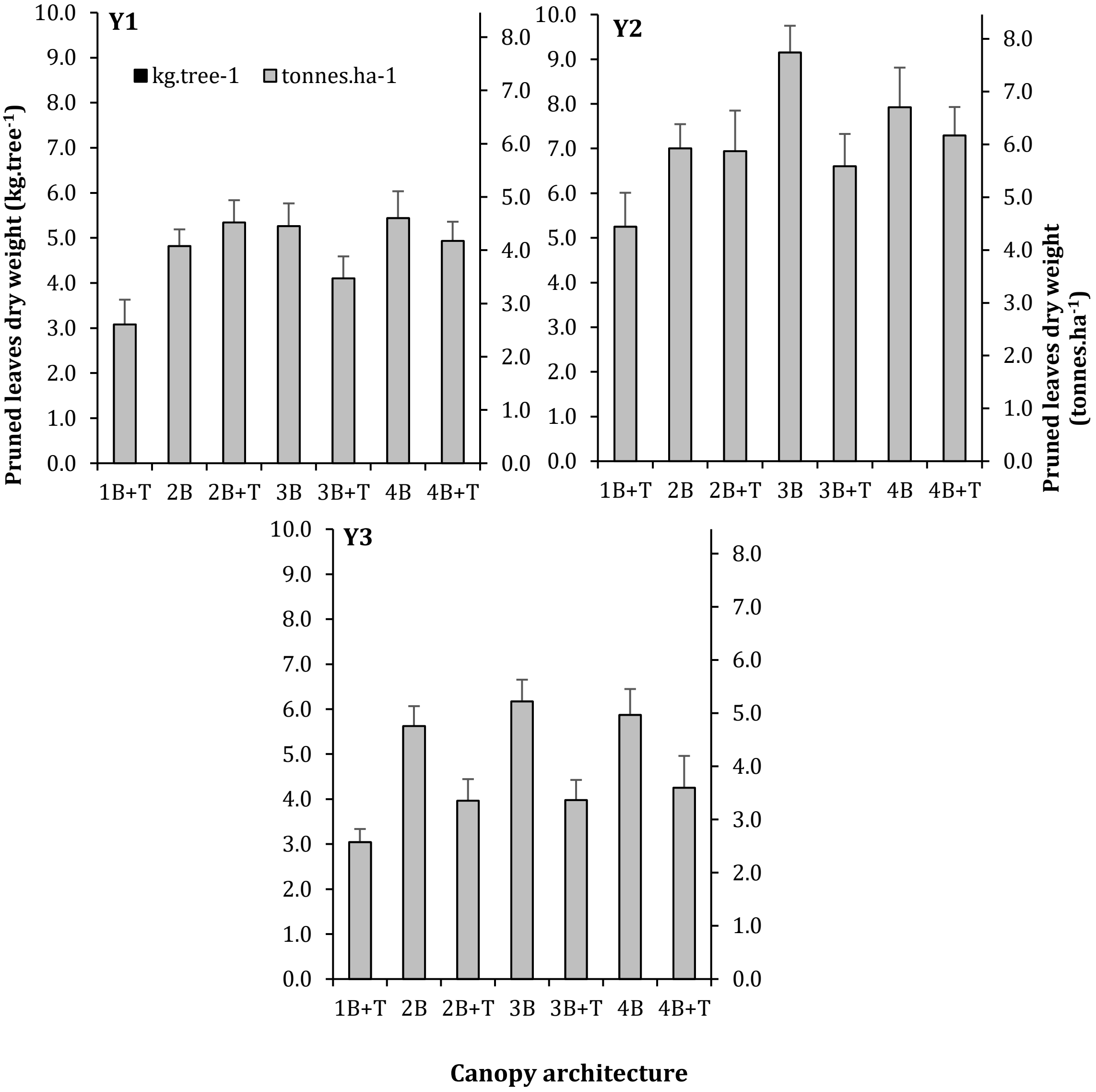


Figure 2.24 Pruned leaves dry weight (primary y-axis: per tree; secondary y-axis: per hectare) in the Biomass 1 trial, measured in Year 1 (December 2014-November 2015), Year 2 (December 2015-November 2016), Year 3 (December 2016-November 2017), between seven different canopy architecture treatments (1,2,3,4 branches; with and without trellis). Values are means across four planting densities and five sample trees (+/- standard error of the mean)

In year 1, pruned leaf weight was not significantly different between the trellis or conventional treatment. However, in year 2 (P=0.047) and year 3 (P<0.001), the pruned dry leaf weight from conventional treatments was higher than the trellis treatments (Figure 2.24).

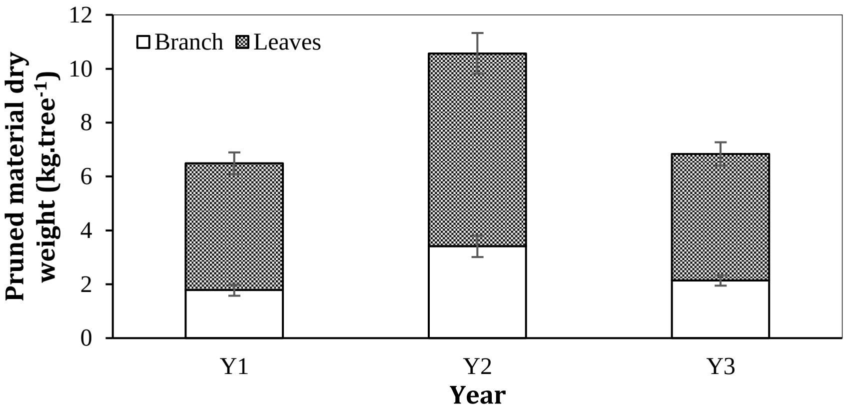


Figure 2.25 Pruned branches and leaves dry weight per tree in the Biomass 1 trial, measured in Year 1 (December 2014-November 2015), Year 2 (December 2015-November 2016), and Year 3 (December 2016-November 2017). Values are means across four planting densities, seven canopy architectures and five sample trees (+/- standard error of the mean)

When comparing years, the total weight of pruned material per tree averaged across all treatments in year 2 was 63% and 35% higher compared to year 1 and year 3, respectively (P<0.001 Figure 2.25). Pruned leaves weight was higher than branches in all years, with a ratio between 2.10 (year 2) - 2.65 (year 1).

When comparing across densities (Figure 2.26), the total weight of pruned material per tree decreased with increased planting density in year 2 (P=0.011), year 3 (P<0.001), and to a small extent in year 1 (P=0.041). The opposite pattern was observed for the weight per unit area and density (year 1 (P=0.030), year 2 (P=0.003) and year 3 (P=0.021).

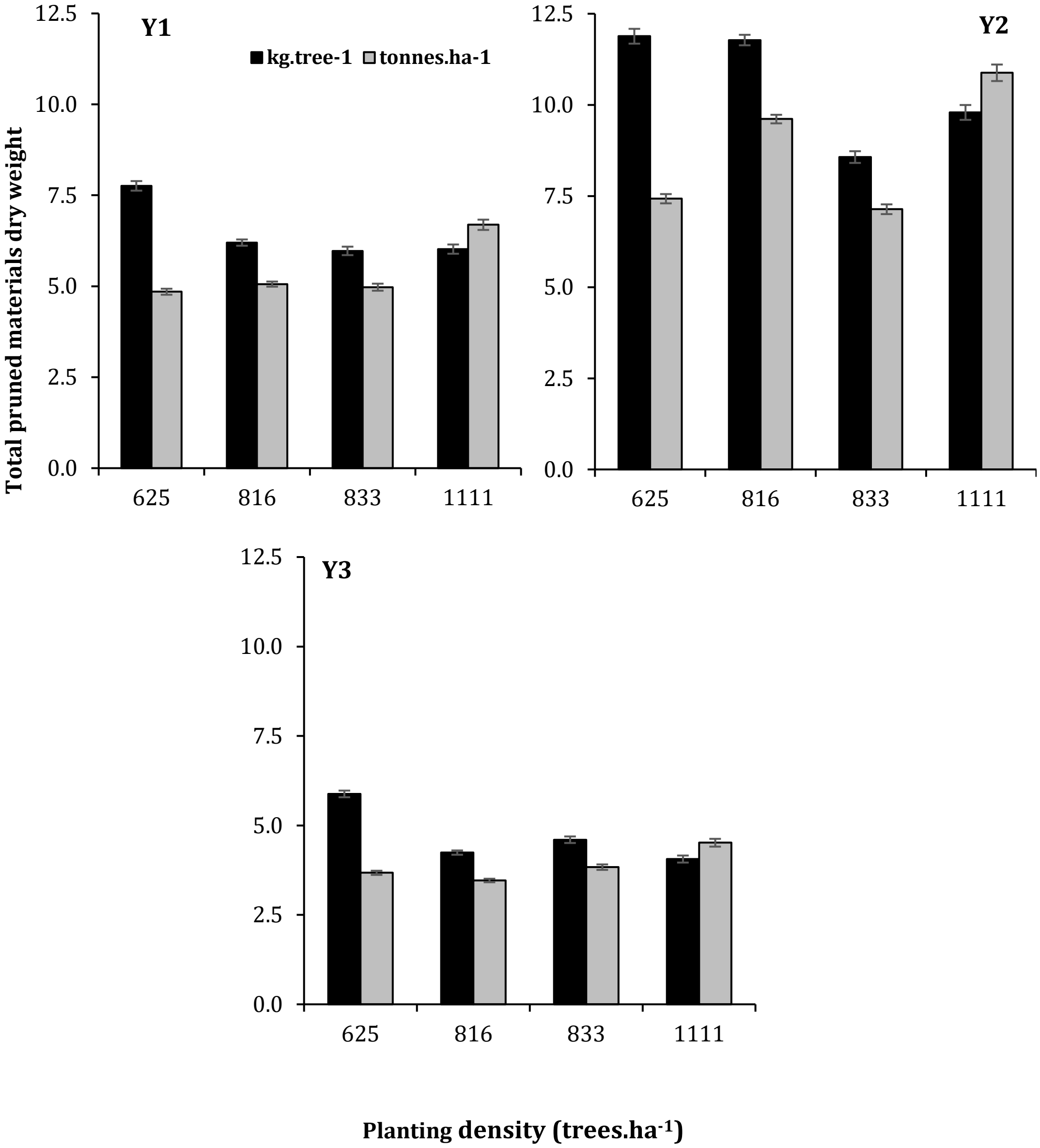


Figure 2.26 Total pruned materials weight per tree and per hectare in the Biomass 1 trial, measured in Year 1 (December 2014-November 2015), Year 2 (December 2015-November 2016), Year 3 (December 2016-November 2017), between different planting densities (625, 816, 833, 1 111 trees.ha^{-1}). Values are means across seven canopy architectures and five sample trees (+/- standard error of the mean)

When comparing across canopy architectures (Figure 2.27), generally, the weight of pruned material per tree and per unit area was higher for the conventionally grown trees than the trellis-grown trees in all three years (year 1 ($P=0.009$), year 2 ($P=0.025$), and year 3 ($P<0.001$)). In general, the total weight of pruned material per tree from conventional treatments was 12 % higher than the trellis treatment (Comparison of 2, 3, and 4 branch treatments).

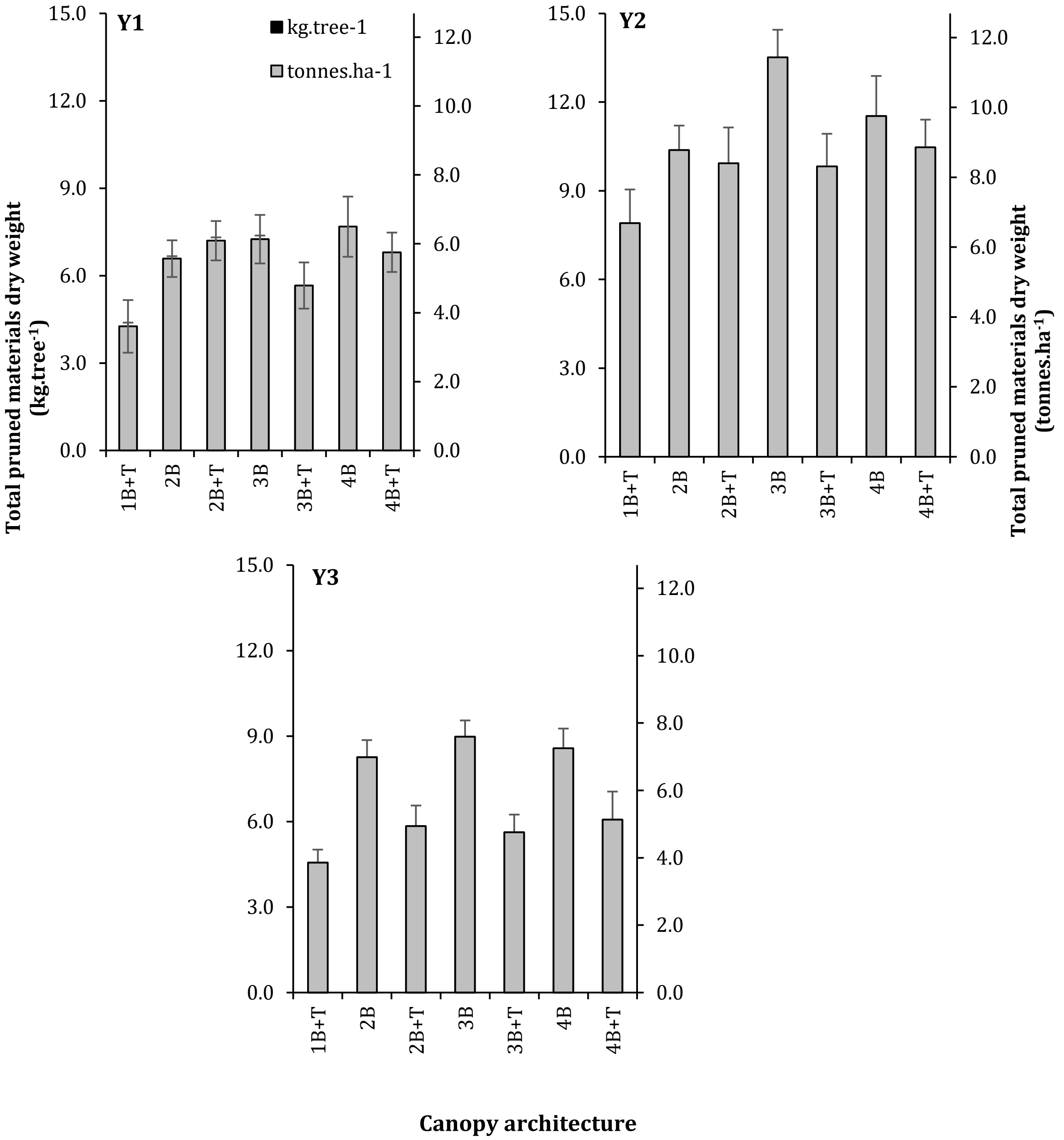


Figure 2.27 Total pruned materials weight (primary y-axis: per tree; secondary y-axis: per hectare) in the Biomass 1 trial, measured in Year 1 (December 2014-November 2015), Year 2 (December 2015-November 2016), Year 3 (December 2016-November 2017), between seven different canopy architecture treatments (1,2,3,4 branches; with and without trellis). Values are means across four planting densities and five sample trees (+/- standard error of the mean)

2.4.4 Harvest index in relation to pruned materials in clone M01

Figure 2.28 shows the dry weight of pruned branches and leaves, harvested beans, and husks for clone M01 trees across the different planting densities in years 1-3. In general, vegetative material weight increases as planting density decreases in years 1, 2 and 3 ($P<0.001$), likewise the reproductive material (bean and husk) weight. The weight of the pruned leaves dominated the total vegetative material (pruned branch, pruned leaves, and bean husk) with a ratio between 0.59-0.68 across planting densities (Figure 2.28) and canopy architectures (Figure 2.29) in the three production years; whilst, the ratio of the dry weight of cacao husk to the total pod weight ranged from 0.38-0.46.

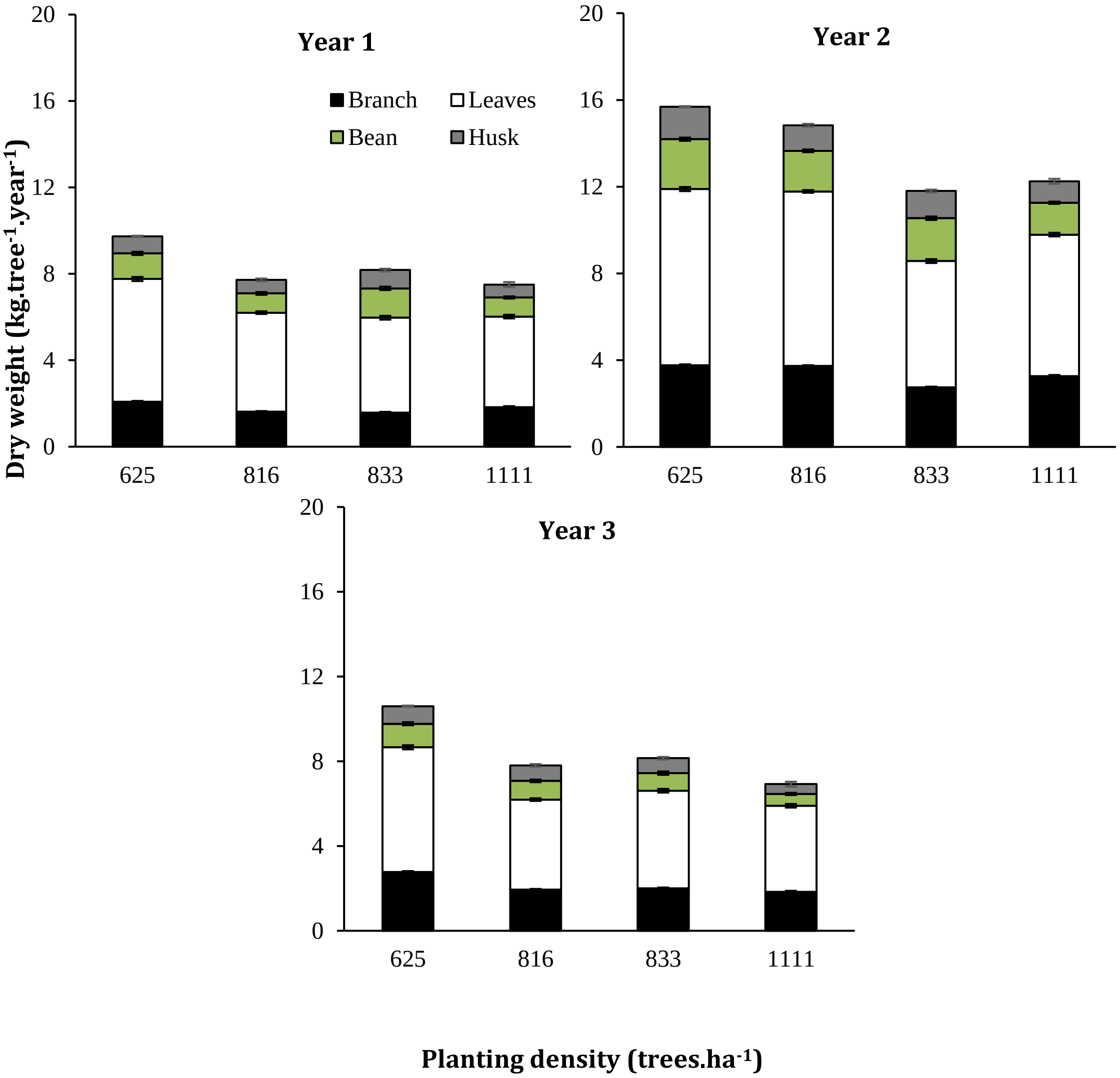


Figure 2.28 The dry weight of the pruned branch, pruned leaves and harvested beans, and husks per tree in three consecutive years in the four planting densities (625, 816, 833, 1111 trees.ha^{-1}) in the Biomass 1 trial. Values are means across four plots, three tree replicates, and seven canopy architecture treatments for Year 1 (December 2014-November 2015), Year 2 (December 2015-November 2016) and Year 3 (December 2016-November 2017) (+/- standard error of the mean)

Bean harvest index was low across all planting density treatments (Table 2.3), ranging from 7.4% to 18.3%, whilst pod harvest index varied from 12.5% to 29.9% (Table 2.3).

The bean harvest index declined as planting density increased in year 2 ($P<0.001$). A relatively similar trend was observed in year 1 but not in year 3.

A decline in pods harvest index as planting density increased was observed in year 2 ($P<0.001$) but not in years 1 and 3. The highest beans and pods harvest index was observed in planting density 833 trees.ha^{-1} in all three production years.

Table 2.3 Pods and Beans Harvest Index from clone M01 trees in four planting density treatments in three consecutive years Year 1 (December 2014-November 2015), Year 2 (December 2015-November 2016), and Year 3 (December 2016-November 2017)

	Pods Harvest Index				
	625	**816**	**833**	**1111**	**trees.ha^{-1}**
Year 1	21.47	20.67	29.98	23.19	
Year 2	25.66	21.49	29.91	12.49	
Year 3	18.41	20.52	21.54	18.55	
	Bean Harvest Index				
	625	**816**	**833**	**1111**	**trees.ha^{-1}**
Year 1	13.03	12.09	18.33	13.74	
Year 2	15.5	13.07	18.11	7.44	
Year 3	10.57	11.2	11.96	10.18	

The dry weight of pruned branches and leaves, harvested beans, and husks for clone M01 trees for the seven different canopy architecture treatments in years 1-3 are shown in Figure 2.29. Conventionally grown trees had a higher total weight of vegetative material than trees with trellis treatment in years 1, 2 and 3 ($P<0.001$); likewise, the reproductive material (bean and husk) weight. The weight of reproductive material decreased over the three production years (9-31% in year 2 and 28-66% in year 3).

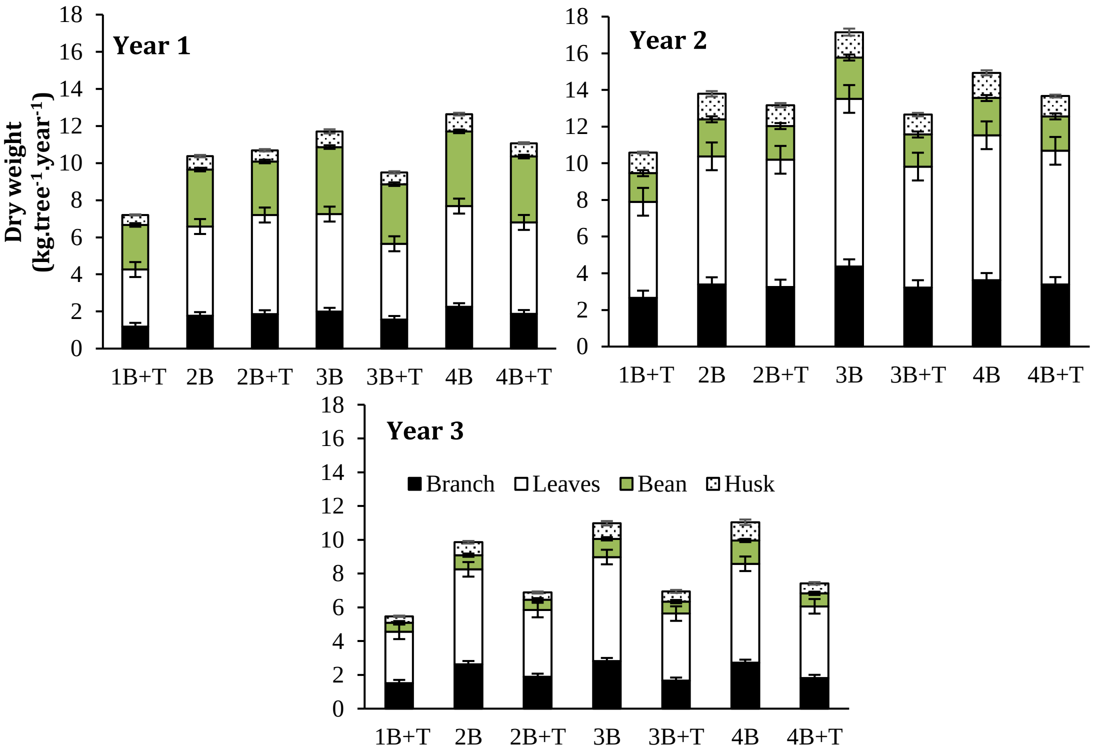


Figure 2.29 The dry weight of pruned branch, pruned leaves and harvested beans, and husks per tree in three consecutive years in the seven canopy architecture treatments in the Biomass 1 trial. Values are means across four plots, three tree replicates, and four planting densities for Year 1 (December 2014-November 2015), Year 2 (December 2015-November 2016) and Year 3 (December 2016-November 2017) (+/- standard error of the mean)

The beans harvest index of the clone M01 was low across all canopy architecture treatments (Table 2.4), ranging from 8.66% to 17.37%. Bean harvest index in year 2 decreased by 5% compared to year 1, and in year 3 decreased by 19% compared to year 2 ($P<0.001$).

In year 1 ($P=0.034$), the trellis treatment had a lower bean harvest index than conventional trees with sample trees with fewer branches (2 branches); however, the opposite trend occurred in the sample trees with more branches (3 and 4 branches). A slightly similar trend that trellis treatment trees have a higher bean harvest index was observed in year 3 but not in year 2.

Meanwhile, the pod harvest index varied from 18.01% to 28.76% (Table 2.4). Bean harvest index in year 2 decreased by 6% compared to year 1, and in year 3 decreased by 12% compared to year 2 ($P<0.001$). In year 1, the sample trees with trellis have a slightly lower pod harvest index than the conventional treatment in fewer branches ($P=0.041$), but not in years 2 and 3.

Table 2.4 Pods and Beans Harvest Index from clone M01 trees in seven canopy architecture treatments in three consecutive years Year 1 (December 2014-November 2015), Year 2 (December 2015-November 2016), and Year 3 (December 2016-November 2017)

	Pods Harvest Index						
	1B+T	**2B**	**2B+T**	**3B**	**3B+T**	**4B**	**4B+T**
Year 1	28.76	22.23	18.01	22.45	26.33	25.03	23.99
Year 2	24.75	23.43	22.18	19.88	21.55	24.09	20.84
Year 3	19.47	16.68	16.25	18.22	20.62	24.18	22.84
	Beans Harvest Index						
	1B+T	**2B**	**2B+T**	**3B**	**3B+T**	**4B**	**4B+T**
Year 1	17.37	12.93	10.85	12.6	16.41	14.75	15.19
Year 2	14.45	13.84	13.45	12.32	13.15	14.49	13.01
Year 3	11.51	8.66	9.56	9.82	11.12	13.48	12.72

2.4.5 The impact of pruning, planting density, and canopy architecture on leaf area index in clone M01

Leaf area index was measured before and after pruning in January 2016, October 2016 and July 2018. In January and October 2016, leaf area index (LAI) after pruning was, on average, 24% lower than before pruning (P<0.001; Figure 2.30). However, in July 2018 leaf area index after pruning was 51% lower than before (P<0.001). The differences between canopy architecture treatments were much more significant for before pruning compared with after pruning (Figure 2.30; P<0.001). In January 2016, leaf area index increased with an increasing number of branches and with the trellis treatment (except for the four branches treatment) (P<0.001; Figure 2.30), but this pattern was less clear in October 2016 and July 2018.

Leaf area index generally increased with increasing planting density in all measurement periods (P<0.001; Figure 2.31). No interaction between planting density and canopy architecture treatment was observed.

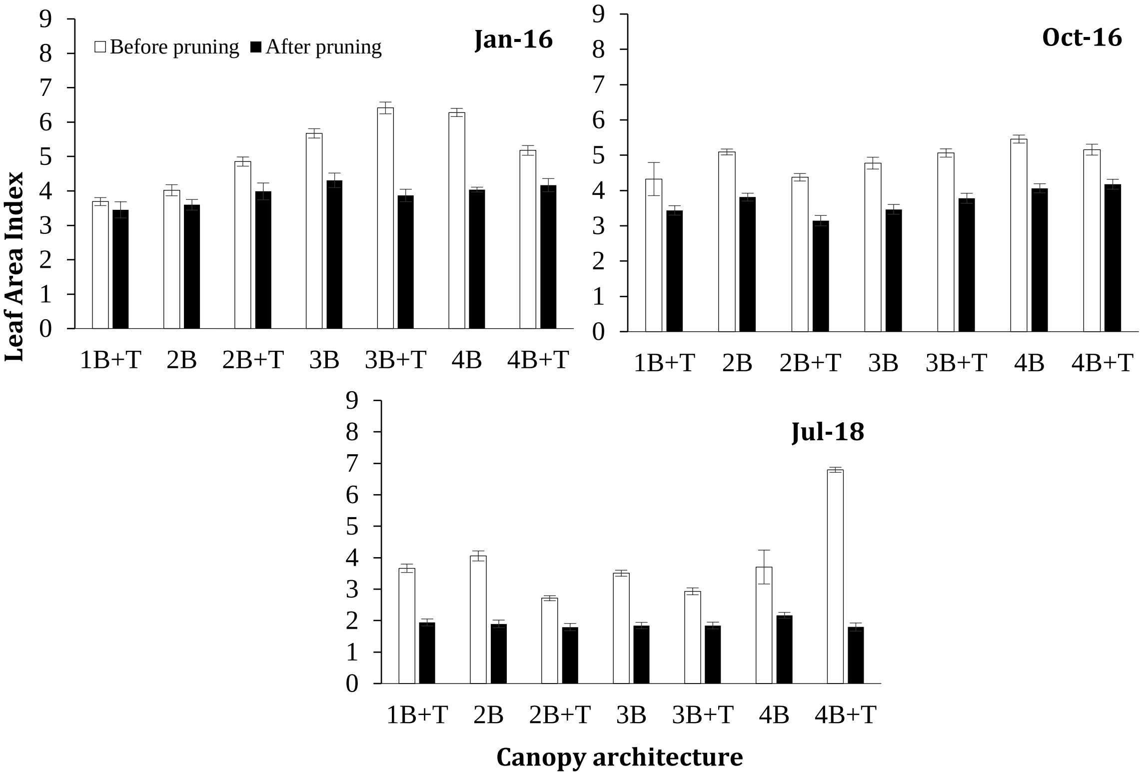


Figure 2.30 Average leaf area index in the Biomass 1 trial planted with the clone M01, measured on January 2016, October 2016 and July 2018, compared before and after pruning, also between seven different canopy architectures. Values are means across four planting densities, two different directions, three canopy heights and three trees as replicates (+/- standard error of the mean)

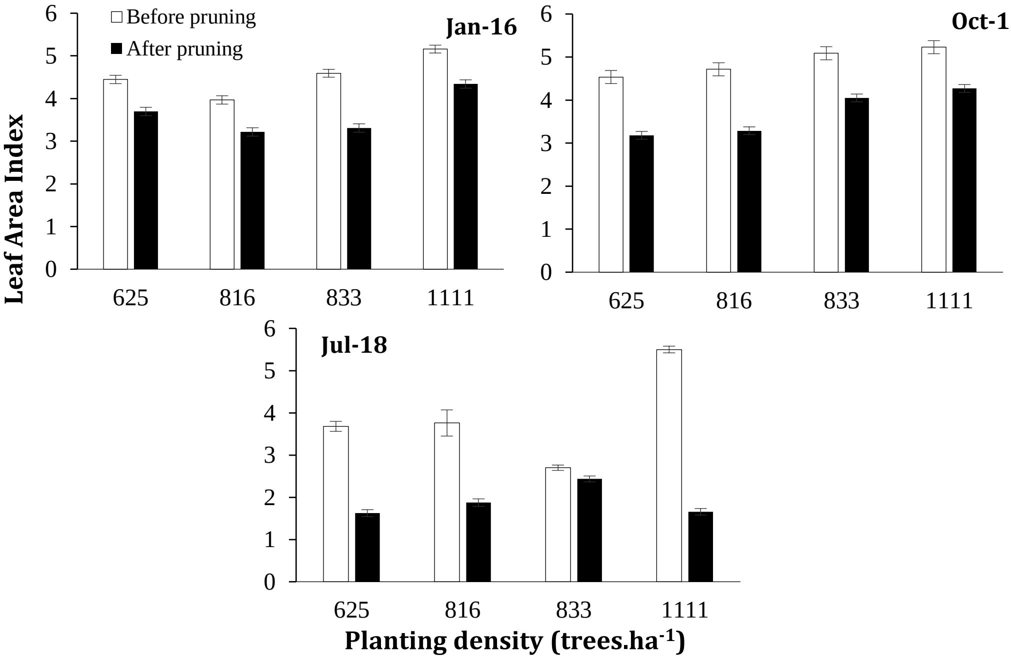


Figure 2.31 Average leaf area index in the Biomass 1 trial planted with the clone M01, measured on January 2016, October 2016, and July 2018, compared before and after pruning, also between four different planting densities. Values are means across seven canopy architectures, two different directions, three canopy heights and three trees as replicates (+/- standard error of the mean)

2.4.6 The effect of maintenance pruning on the photosynthetic activity in different planting density and canopy architecture treatment

A. Light-saturated photosynthetic rate (A)

Light-saturated leaf photosynthetic rate per unit area was significantly higher after pruning events on several occasions (January 2015, 44% increase, P=0.003; April 2015, 42% increase, P<0.001; October 2016, 19% increase, P<0.001; January 2017, 20% increase, P<0.001; April 2017, 36% increase, P<0.001; and October 2017, 17% increase, P<0.001), as shown in Figure 2.32. No significant differences in the photosynthetic rate before and after pruning was observed on the other occasions.

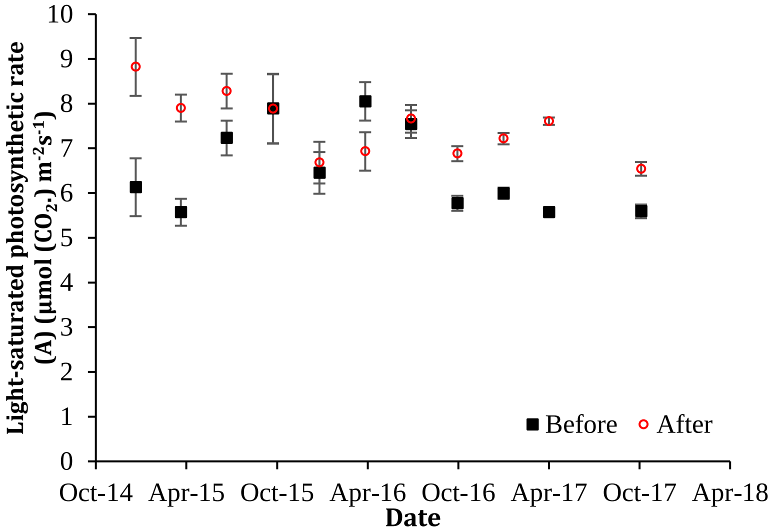


Figure 2.32 Light-saturated photosynthetic rate (A) of clone M01 trees in the Biomass 1 trial before and after pruning, measured between January 2015 and October 2017. Values are means across four different planting densities, seven different canopy architecture treatments and three measurement replicates (+/- standard error of the mean)

There was no significant effect of the different canopy architecture treatments or plant density on the photosynthetic rate. Also, no interaction was observed between canopy architecture and planting density before and after pruning conditions.

B. Transpiration rate (E)

A general decline in transpiration rate per unit leaf area was observed over the measurement period (P<0.001). Transpiration rates were generally higher after pruning in July 2015 (13% increase; P=0.021), January 2016 (20% increase; P=0.013), July 2016 (20% increase; P=0.002), October 2016 (41% increase; P<0.001), January 2017 (24% decrease; P=0.041), April 2017 (30% increase; P=0.033), October 2017 (80% increase; P<0.001), as shown in Figure 2.33. However, there were no differences in the transpiration rate before and after pruning in January 2015, April 2015, October 2015, and April 2016.

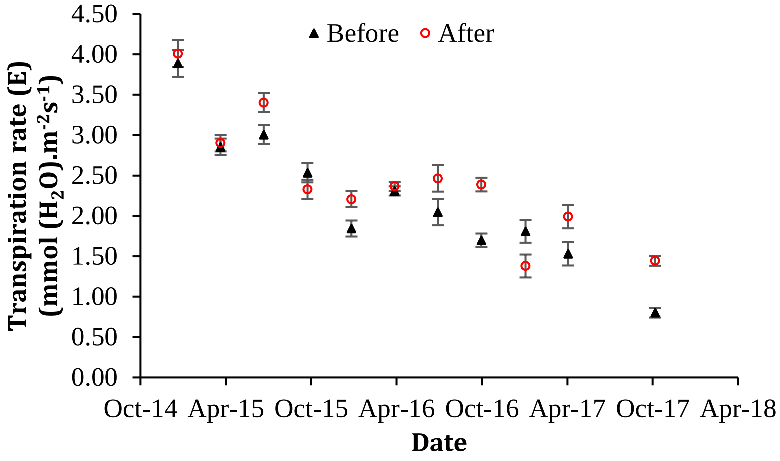


Figure 2.33 Transpiration rate (E) of clone M01 trees in the Biomass 1 trial before and after pruning, measured between January 2015 and October 2017. Values are means across four different planting densities, seven different canopy architectures and three measurement replicates (+/- standard error of the mean)

In general, there were no differences in transpiration rate between canopy architecture treatments or plant densities on all measurement occasions. There was also no interaction between canopy architecture and planting density observed before and after pruning conditions.

C. Water Use Efficiency (WUE)

A general increase in WUE was observed over the measurement period primarily as a result of the decline in transpiration rate over the same period ($P<0.001$). WUE was higher after pruning in January 2015 (40% increase; $P=0.021$), April 2015 (40% increase; $P=0.006$), and January 2017 (58% increase; $P<0.001$), as shown in Figure 2.34. The opposite trend was observed in January 2016 (13% decrease; $P=0.022$), April 2016 (16% decrease; $P=0.018$), July 2016 (16% decrease; $P=0.020$), October 2016 (15% decrease; $P=0.012$), and October 2017 (35% decrease; $P<0.001$). No effect of pruning was observed on WUE in July 2015, October 2015, and April 2017.

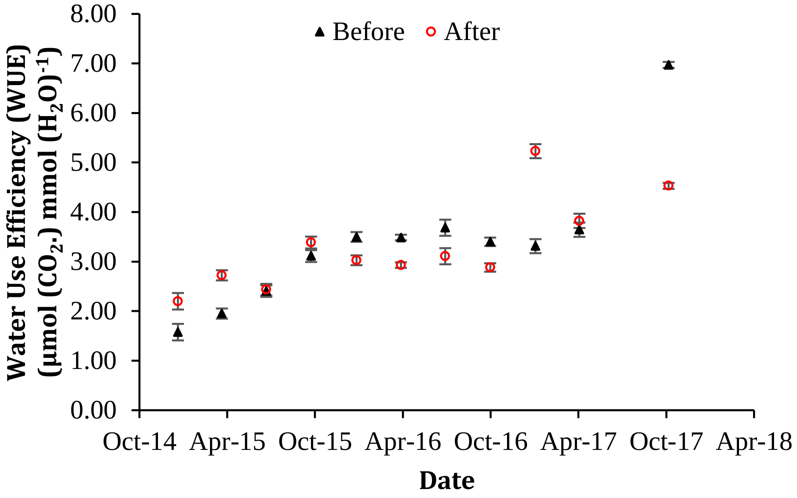


Figure 2.34 Water use efficiency (WUE) of clone M01 trees in the Biomass 1 trial before and after pruning, measured between January 2015 and October 2017. Values are means across four different planting densities, seven different canopy architectures, and three measurement replicates (+/- standard error of the mean)

No canopy architecture or planting density effect was observed on WUE. There was also no interaction between canopy architecture and planting density observed before and after pruning conditions.

D. Stomatal conductance (gs)

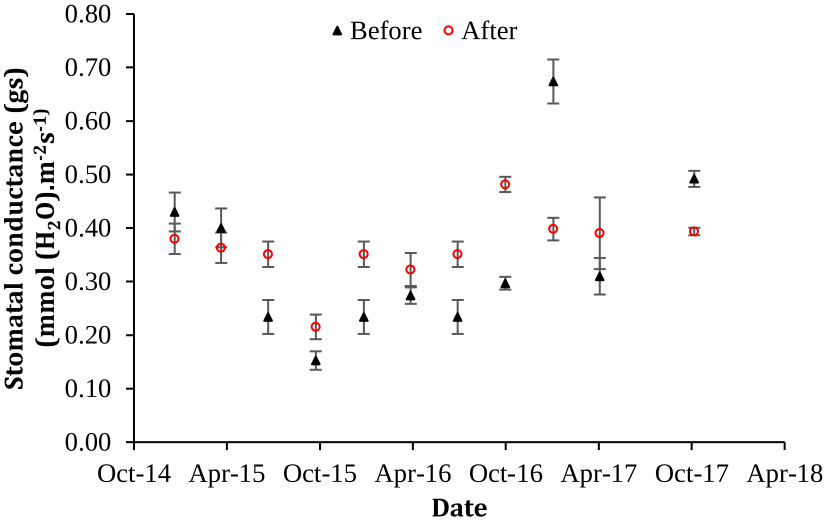


Figure 2.35 Stomatal conductance (gs) of clone M01 trees in the Biomass 1 trial before and after pruning, measured between January 2015 and October 2017. Values are means across four different planting densities, seven different canopy architectures and three measurement replicates (+/- standard error of the mean)

Stomatal conductance was generally higher after pruning (July 2015 (50% increase; $P<0.001$), October 2015 (41% increase; $P=0.006$), January 2016 (50% increase; $P<0.001$), July 2016 (50% increase; $P<0.001$), October 2016 (50% increase; $P<0.001$)), as shown in Figure 2.35. However, the opposite pattern was observed in January 2017 ($P<0.001$) and October 2017

($P<0.001$), and there were no differences in stomatal conductance after pruning in January 2015, April 2015, April 2016, and April 2017.

No effect of planting density, canopy architecture, or any interaction between factors was observed on the stomatal conductance over the measurement period.

Table 2.5 Results summary of Biomass 1 trial

	Productivity		**Harvest Index**		**Phenology**		**Vegetative growth**		
	Per tree	**Per hectare**	**Pods**	**Bean**	**Flowering intensity**	**Flushing index**	**Pruned branches weight per tree**	**Pruned leaves weight per tree**	**Pruned materials weight per tree**
Years	Year 2 54% higher than year 1 Year 3 54% lower than year 2 Year 4 18% higher than year 3		Range: 12.5%-29.9%	Range: 7.4% - 18.3%. Year 2 6% lower than year 1 Year 3 12% lower than year 2	Peak season in 2016: February	Peak season in 2017: January and December. Peak season in 2018: April, July, and October	The highest: Year 2 92% & 52% higher than year 1 and year 3		Year 2 63% & 35% higher than year 1 and year 3. Ratio pruned leaves weight to pruned branches: 2.10 (year 2) - 2.65 (year 1). Pruned materials in conventional treatment was higher than trellis for years 1, 2 and 3
Density	Decreased with increasing density in years 2, 3, 4. A positive relationship between yield and total branch length for density 833 trees.ha^{-1}	Increased with density in years 1 and 2		Declined as density increased in year 2. The highest beans and pods harvest index was observed in density 833 trees.ha^{-1} in all three production years			Decreased with increasing density in year 3	Decreased with increasing density in years 1, 2 and 3. The opposite pattern was observed for pruned leaf dry weight per unit area in years 1, 2 and 3	Decreased with increased density in years 2 and 3. The opposite pattern was observed for the weight per unit area and density (years 1, 2, and 3)

Canopy architecture	Yield in conventional treatment is higher than trellis in years 2, 3, and 4		Trellis treatment trees have a slightly lower pod harvest index than the conventional treatment in fewer branches, but not in years 2 and 3	In year 1, the trellis treatment had a lower bean harvest index than conventional trees with fewer branches (2 branches). The opposite trend occurred in the sample trees with more branches (3 and 4 branches).	Flowering in conventional is 33% higher than trellis	Flushing in conventional is 5% higher than trellis	Higher in the conventional treatments at the higher number of branches per tree in years 1 and 3	In years 2 and 3, the pruned dry leaf weight from conventional treatments was higher than the trellis treatments	Weight per tree and per unit area was higher for the conventional treatments (12%) all three years (years 1, 2, and 3).

*Year 1 (December 2014-November 2015), Year 2 (December 2015-November 2016), Year 3 (December 2016-November 2017), and Year 4 (December 2017-November 2018)

	Gas exchange parameters				**Canopy characteristics**
	Light-saturated photosynthetic rate (A)	**Transpiration rate (E)**	**Stomatal conductance (gs)**	**Water Use Efficiency (WUE)**	**Leaf Area Index (LAI)**
Experiment period (time)		A general decline		A general increase	
Density					Generally increased with increasing planting density
Canopy treatment					Difference among canopy architecture treatments were more significant for before pruning compared with after pruning.
Maintenance pruning	Significantly higher after pruning events in: -January 2015, 44% -April 2015, 42% -October 2016, 19%	Generally higher after pruning in: -July 2015, 13% -January 2016, 20% -July 2016, 20%	Generally higher after pruning in: -July 2015, 50% -October 2015, 41% -January 2016, 50%	Higher for after pruning in: -January 2015, 40% -April 2015, 40% -January 2017, 58%	In January and October 2016, (LAI) after pruning was 24% lower than before pruning. in July 2018 (LAI) after pruning

	-January 2017, 20% -April 2017, 36% -October 2017, 17%	-October 2016, 41% -January 2017, 24% -April 2017, 30% -October 2017, 80%	-July 2016, 50% -October 2016, 50% The opposite pattern was observed in January 2017 and October 2017	The opposite trend (decrease) was observed in -January 2016, 13% -April 2016, 16% -July 2016, 16% -October 2016, 15% -October 2017, 35%	was 51% lower than before.

2.5 Discussion

2.5.1 The impact of planting density and canopy architecture on productivity per tree and area in clone M01

The productivity of cacao beans in the Biomass 1 trial showed an overall increase over time, reaching its peak in 54 months old trees in year 2. However, a significant decline in the total number of pods harvested was observed in year 3 (66 months old trees), which was attributed to a general degradation of the trees and symptoms of *Phytophthora* disease. Previous research has suggested that the optimal age for cocoa tree production is between 5-10 years, with a 20% drop in yields reported in cocoa orchards over 30 years of age. Various studies indicate that yield decline occurs in Ghana and Nigeria after 15-20 years of age (Ofori-Bah & Asafu-Adjaye, 2011; Vernon & Morris, 2015; Aneani et al., 2017).

The highest productivity in the previous study by Thong and Ng (1980) was reported for 39 months old trees and declined until the end of the experiments when the tree reached 87 months old. The study concluded that the yield decrease at the end of the experiment was due to excessive leaf area index on the sample trees (around 8.7-10.3 for 50-87 months old cacao trees). The yield decline in year 3 suggests that the trees were prematurely under severe source limitations. The inadequate supply of assimilates observed in this study, caused by excessive pruning likely led to yield reduction.

A lower planting density (625 trees.ha^{-1}) results in higher productivity per tree due to reduced competition, leading to a more efficient light interception. This is because lower planting densities mitigate competition for nutrition and light, resulting in increased tree yields.

The cultivation of low vigour cocoa clones with fewer branches at high density may enable an intensive growing system to be more efficient and more easily mechanized. Consequently, a trial of intensive growing systems in a trellis/hedgerow system with different clones (high and low vigour) and planting densities was the basis for the Biomass 2 Trial described in Chapter three.

An incomplete light interception in the trellis system may have caused the yield differences between conventional and trellis. Incomplete light interception refers to a situation where plants do not fully capture all the available light. This can occur for several reasons, including insufficient plant density, inadequate leaf area, or limited light availability due to shading by surrounding vegetation or other factors. Incomplete light interception can negatively impact plant growth and development, reducing the amount of energy plants can convert through photosynthesis. As a result, incomplete light interception can lead to lower yields, reduced crop quality, and other adverse effects on plant growth and productivity.

Menzel and Lagadec (2017) observed that yield of mango trees that grew in high density started to decline due to overcrowding and shading earlier than in the traditional orchard; the lower shoot started to die, the productivity fell, and the trees became susceptible to pests and diseases. The study also noted that the implication of light pruning was better for production than severe pruning. A similar pattern was also observed in the previous study on mango by Sing et al. (2010) and Ram et al. (2001), which found that yield per tree in year 14 decreased with planting density, whereas yield per area increased. This result was correlated with a decrease in the canopy growth as planting density increased. Therefore, in a high-density system, defining optimum planting density and canopy management are essential to optimize the light distribution through the canopy and enhance leaves and shoot regrowth. It is also dependent on the use of dwarfing cultivars or rootstocks.

2.5.2 The effect of pruning on the light-saturated photosynthetic rate in clone M01

This study showed that the light-saturated photosynthetic rate was significantly higher on a number of occasions after pruning. Pruning stimulates assimilate demand, and photosynthesis stimulates new vegetative growth. New cacao leaves are initially photosynthetic sinks (Zuidema et al., 2005; Almeida & Valle, 2007); therefore, the source-sink ratio is altered, stimulating an increased photosynthetic rate. The result implies that cacao trees can increase the leaf assimilation rate in response to increased carbohydrate demand from pruning.

A similar response of higher photosynthetic rate after pruning has been found in other perennial crops, such as in urban tree species (*Acer pseudoplatanus L.*) (Fini et al., 2015), London plane (Hipps et al., 2014), Eucalyptus (Pinkard et al., 1998), leguminous agroforestry tree *Erythrina poeppigiana* (Walpers) (Nygren et al., 1996), other woody plants (Ovaska et al., 1993; Turnbull et al., 2007; Maurin and DesRochers, 2013;

Pinkard and Beadle, 2016), various apple cultivars (Mika and Antoszewski, 1972; Porpiglia and Barden, 1980; Tustin et al., 1992; Wünsche et al., 1996; Li et al., 2003; Grappadelli 2009), coffee (Morais et al., 2012), grapevines (Hunter and Visser, 1988; Jackson, 2014), mango (Ravishankar, 2014), and olive (Albarracín et al., 2017).

A general decline in transpiration rate was also observed throughout the measurement period. This decline might relate to the tree's declining condition, which impacted its lower vigour. As vigour becomes lower, water flow from the soil to plants becomes increasingly restricted, gradually reducing the transpiration rate. Plants close their stomata to prevent water loss from their tissues, thereby inhibiting transpiration (Flore et al., 1984).

Li et al. (2003) studied the effect of summer pruning on apples. The study indicated that light availability in the inner and middle regions of the canopy significantly increased by commercial summer pruning, and, as a result, transpiration rates were also higher after pruning (Li et al., 2003). Similarly, after pruning, leaf-to-air vapour pressure deficit (VPD) increases as the light exposure increases, and since VPD is the driving force for the transpiration rate in apple leaves (Landsberg et al., 1975), this leads to an increase in transpiration rate. These reasons explain the similar pattern of transpiration rate increasing after pruning observed in this study on cacao leaves.

A general increase in WUE was observed over the measurement period primarily as a result of the decline in transpiration rate over the same period.

Stomata are sensitive to changes in environmental factors, such as light, temperature, the internal and external concentration of carbon dioxide, plant water conditions, and atmospheric humidity. Hicklenton et al. (2000) observed that the stomatal conductance of lowbush blueberry (*Vaccinium angustifolium* Ait.) in the pruning season was doubled compared to the unpruned/fruiting season. The study concluded that stomatal conductance (g_s) is one of several factors influencing seasonal assimilates capacity, and the linkage between g_s and leaf water potential is still unclear.

Previous studies, i.e. in apples (Jones et al. 1983) and conifers (Jarvis, 1980), observed that there was a tendency for stomatal closure as leaf water potential declines, but other studies observed that moderate water deficit has no direct effect on g_s in various plants (Ehlig and Gardner, 1964).

However, previous research on cacao has shown diurnal water potential changes and stomatal conductance show a clear relationship (Sena Gomes et al., 1987; Raja Harun and Hardwick, 1988). This cacao study also observed increased stomatal conductance following pruning, which could correlate with the increasing photosynthetic rate.

2.5.3 The impact of pruning, planting density, and canopy architecture on leaf area index in clone M01

The leaf area index (LAI) in this study ranged between 3.5-7 for 54-87 months old trees, lower than a previous study conducted in Malaysia reported by Thong and Ng (1980), which were 8.7-10.3 for clonal cacao trees of the same age.

However, the LAIs recorded in this study were similar to other studies' findings, e.g. a study by Jaimez et al. (2013) which observed LAI between 1-5 for 24-month-old trees in Venezuela; studies in Bahia, Brazil, observed LAI between 2.8-4.5 for six year old trees (Daymond (2002a), 3.9 for eight-year-old trees by Miyaji et al. (1997), a range of 3.7 to 5.7 (Alvim (1977)); and 3.1-4.5 for 6-year-old trees in Central Sulawesi, Indonesia (Moser et al. (2010)).

In this study, the leaf area index is proportional to the number of branches and planting density. Furthermore, throughout the measurement period, the leaf area index declined significantly. This decline might relate to the impact of pruning which reduced the vigour of the trees.

2.5.4 The impact of planting density and canopy architecture on tree phenology in clone M01

The flushing and flower numbers in conventional trees were slightly higher than in the trellis. This could have been caused by the increasing assimilation allocation in conventional treatment trees compared to the trellis. There is a possibility that lower flushing and flower numbers in trees with the trellis system were due to the restrained canopy growth.

Meanwhile, in this experiment on cacao, the highest flower numbers were observed in February 2016 and the lowest in November 2016. This condition is aligned with the highest harvest six months after the peak flowering.

2.5.5 The impact of planting density and canopy architecture on vegetative growth in clone M01

Overall, there was an increase in the total dry weight of pruned material in production year 2, compared to year 1, as the bean production was also increasing. A similar higher vegetative growth increase in response to pruning was observed in other fruit tree species (Génard et al., 1998; Stephan et al., 2007; Bussi et al., 2011; Pasa and Einhorn, 2014; Albarracin et al., 2017). However, there was a decrease in pruned materials weight in the third production year compared to year 2.

A study by Thong and Ng (1980) showed that the cacao growth pattern was sigmoidal, such that the growth of cocoa plants is very rapid during the first three to four years in the field, after which a steady growth was reached. Nevertheless, the heavy pruning required to maintain the treatments would have resulted in source limitation and a consequent decline in biomass production in year 3. The significant decline in year 3 was caused by the impact of heavy pruning, which reduced the vigour of the trees. A similar declining pattern was also observed in other fruit trees and peaches (Mika 1986; Génard et al., 2008), which may have occurred due to changes in carbon partitioning or long-term hormone level changes related to branch apices removal due to pruning.

In general, planting density did not significantly affect vigour regarding the total dry weight of pruned materials since the density difference was not significant (625-1111 trees.ha^{-1}), even though a declined value by increasing planting density pattern was observed in pruned leaves weight. Cacao trees grown on the trellis system generally showed a reduction in the dry weight of pruned branches and leaves compared to the conventionally grown trees in three subsequent production years.

2.5.6 Harvest index in relation to pruned materials

The bean harvest index for clone M01 estimated here was around 7-18% for 52-76 months old trees, and the pod harvest index varied between 13-30%. This is a slightly higher value range for the bean harvest index and a relatively similar pod harvest index noted in previous literature (Thong and Ng, 1980; Corley, 1983). Thong and Ng (1980) reported that the bean harvest index was between 1-10%, and the pod harvest index varied from 3 to 28% for 5-87 months old trees in Malaysia. Meanwhile, Corley (1983) reported cacao pod harvest index is around 30-40%. The yield quoted by Corley (1983) is for trees grown without overhead shade on volcanic soil in Borneo (Lim, 1980).

The bean and pods harvest index declined as the density increased in the early production years in this study might be caused by light competition for assimilates production.

The weight of the pruned leaves dominated the total vegetative materials (pruned branch, pruned leaves, and bean husk) with a ratio between 0.6-0.7, showing the vigour characteristics of the clone M01. Based on this result, it can be concluded that the high vigour exhibited by the clone M01 made it unsuitable for use in a trellis system. Therefore, there is a need to reduce the balance of the vegetative biomass growth to reproductive growth. When the source becomes limited by heavy pruning, tree degradation occurs, as shown by subsequent declining bean and pod harvest index in production years 2 and 3.

2.5.7 Conclusion

Canopy management is essential for a high-density orchard, and pruning is vital to control tree growth. However, in Biomass 1 trial, the impact of heavy pruning reduced the vigour of the trees, shown by the reduced bean production and pruned material weight. Moreover, the high vigour exhibited by the clone M01 makes it unsuitable for use in a trellis in an intensive growing system. Therefore, the bean harvest index observed in this study is relatively lower than the study aims for, and there were not many differences between the trellis and conventional systems.

This study suggests better canopy management with optimum pruning intensity and less vigorous clones for the following research. The different canopy architecture treatments in this study were imposed 2.5 years after the experiment was established. Therefore, there was the need to conduct an experiment whereby the trellis system was imposed from an early stage.

Chapter 3. The Performance of Two Sulawesi Cacao Clones Maintained at Various Planting Densities in A Trellis Growing System

3.1 Introduction

One route towards increasing productivity is increasing density, as has been achieved in previous studies in cocoa density trials (Mooleedhar & Lauckner, 1990; Lockwood & Yin, 1996; Maharaj et al., 2003; Zakariyya et al., 2022). The Biomass 1 experiment, as has already been described (Chapter 2), sought to accomplish this by manipulating established trees by training them on a trellis and using different pruning regimes to increase the efficiency of canopy illumination. In this chapter, two cacao clones were trained onto a trellis from the establishment point and maintained at significantly higher densities than Biomass 1.

Moreover, an intensive growing system requires varieties with less vegetative vigour. Therefore, there is a need to establish optimal densities within trellis systems for clones with different vegetative vigour characteristics. This chapter will highlight the main processes of tree canopy light interception/distribution and carbon partitioning in limiting cacao yield in two high-yielding clones from Sulawesi (45 and M01), which have different vegetative vigour.

There are two possible ways of improving crop performance in an orchard system. The first is to increase total dry matter yield, and the second is to increase the partitioning of dry matter production towards the fruits (Wünsche and Lakso, 2000). The considerable variation between cocoa genotypes in vigour and canopy architecture presents an opportunity to increase yield per hectare through the optimal matching of clones with planting density.

The partitioning of assimilates in annual crops is frequently measured in terms of harvest index (Cannell, 1985), i.e. the proportion of the mass of the harvested component to the plant's total weight. In perennial tree crops, yield efficiency is a more easily measured parameter, i.e. the ratio of yield to vegetative growth over a defined period. For temperate plants, such as apples, trunk cross-sectional area (inferred from trunk circumference) is often used as a crude measure of vegetative growth (Cannell, 1985). Daymond et al. (2002a) demonstrated a ten-fold variation in yield efficiency (i.e. the proportion of the yield to trunk cross-sectional growth over 18 months) among seven clones and five bi-parental crosses grown under similar conditions in Bahia, Brazil. Yield efficiency is valuable for tree crop breeders and most fruit tree agronomists (Larsen and Fritts, 1982) and can be used to quantify the variation amongst breeding materials in the ratio of vegetative to reproductive growth.

Several studies have noted significant variations in physiological traits among cacao clones. For example, Yapp and Hadley (1994) demonstrated considerable variability in canopy characteristics across various cocoa clones grown at high planting density at the former BAL Plantations in Sabah, Malaysia, which indicates the potential for more appropriate matching of germplasm with planting density to achieve high productivity per area.

Measurements of canopy architecture in trials that consider the interaction between genotype and planting density, e.g., Osei-Bonsu et al. (2002), are crucial to evaluating the role of light competition in optimising planting density. Consequently, assessing a given genotype's growth characteristics can be used to match a particular genotype with planting density.

In maize (Tollenaar, 2006; Li et al., 2015), higher plant density improves light interception efficiency per unit area and negatively affects the higher harvest index after the optimum planting density. Increased planting density enhances stress for plants for resource capture and resource utilization of the individual plants within the crop canopy. Resource capture includes the solar irradiance absorption by the crop canopy and water and nutrients absorption by the roots. Resource utilization includes conversion of absorbed solar irradiance into dry matter and partitioning dry matter to economically plant components (Tollenaar, 2006).

The total yield potential of a cultivar will only be expressed at a specific density for that cultivar so that the optimal planting density of low-vigour varieties will tend to be high and vice versa for high-vigour varieties. Thus, one aim here is to identify optimal plant densities for the clones used in this field experiment.

There are relatively few studies on the use of intensive growing systems for growing cocoa. The MC-Intensa TBroma S.A. company previously set up a trial with high planting density, called the “T-project”, featuring high-density planting of 6,000 trees.hectare^{-1} with a specific shape and training of trees on a trellised infrastructure (Cyprich, 2016). However, there is limited published information about this trial. Another intensive growing system in Brazil uses a planting density of 1,600 trees.hectare^{-1} (Sodré and Leite, 2018) and uses an experimental technique of pruning called “candlestick”, which kept the plant in the shape of a candlestick with independent and separate lateral branches. In the fourth year of cultivation, this system produced 2,130 kg.ha^{-1}.year^{-1} with clone BN34.

The maximum planting density employed in the Biomass 1 trial is 1111 trees.ha^{-1}, which aligns with the prevailing optimal density utilized by farmers at 3x3m spacing. In contrast, the Biomass 2 trial implements a planting density of 2000 trees.ha^{-1}, representing a twofold increase from the density used in the Biomass 1 trial and is achieved through tighter spacing at 1.6x1.6m.

Row orientation can impact the amount of light intercepted (Jackson and Palmer,1972). They observed that the estimated percentage of interception by east-west hedgerows varied over the season in a temperate location. However, it was reasonably constant for north-south hedgerows. Hedgerows oriented from east to west would have distinctive advantages on a south-facing slope where hedgerows, possibly with more vertical northern sides, would combine even illumination on their southern sides with a high degree of light interception (Jackson and Palmer, 1972). Consequently, the row orientation is a factor that has also been incorporated into the design of this experiment.

3.2 Research Aims

This chapter aimed to examine the potential for maximising the yield of cocoa grown at high densities in a trellis system. Two clones were included, which differed in vigour and were planted in two different row orientations and three different planting densities. The key research questions were:

- What is the impact of planting density on yield in a trellis system?
- Are there differences between two clones in vigour on yield in a trellis system?
- What was the effect of row orientation on yield in a trellis system?

3.3 Material and Methods

3.3.1 Experiment planning

Biomass 2 trials presented a prototype of how a trellis implemented an intensive growing system in cacao. The experiment's design, including cacao variety selection, the number of tree replicates, density treatment, and block design, was initially discussed in April 2014. The preliminary results from Biomass 1 until March 2014 also influenced the decision of how the experiment was designed. Therefore, the non-trellis system was not included in the experiment design.

Subsequently, the planting materials were prepared in July 2014 and planted in the field in October 2014, and the first pod was harvested in August 2016.

The author of the study joined the research station in April 2013 and contributed to the discussion and experiment set-up from that point onward. Whilst the intensive research formally commenced in April 2016. She trained and supervised a team of four members on pruning and data collection protocols starting in August 2016. In total, eight people were trained to support data collection in the field, with four assigned to each trial.

3.3.2 Study site

The experimental site is located at the Mars Cocoa Research Station, Tarengge, South Sulawesi, Indonesia (Latitude 2º33’42.98” S and Longitude 120º49’16.25” E, elevation 27 m above sea level). The experiment was established on a trellis system with the following treatments: two row-orientations (east-west and north-south), three planting densities (2000 trees.ha^{-1}- single row; 3300 trees.ha^{-1}- single row; and 5000 trees.ha^{-1} in a double row) and two clones (M01 and 45).

The trial was set up as a split-plot randomised block design, with each treatment replicated three times as a consequence of the soil texture condition distribution within the plot (see Appendix B). Each plot (treatment combination) consisted of 48 trees distributed in six rows with the same clone, with eight trees planted in each row. The position of both clones was randomised within each density. The total area for the Biomass 2 trial was 0.99 ha.

The nursery for the propagation of planting material was located close to the trial in Bosso Batu village Burau East Luwu, South Sulawesi. The nursery area was constructed using UV-transmitting polyethylene film supported on a wooden roof with polypropylene netting for the side of the nursery, as shown in Figure 3.1(a). On average, light transmission inside of the nursery was 78±4%.

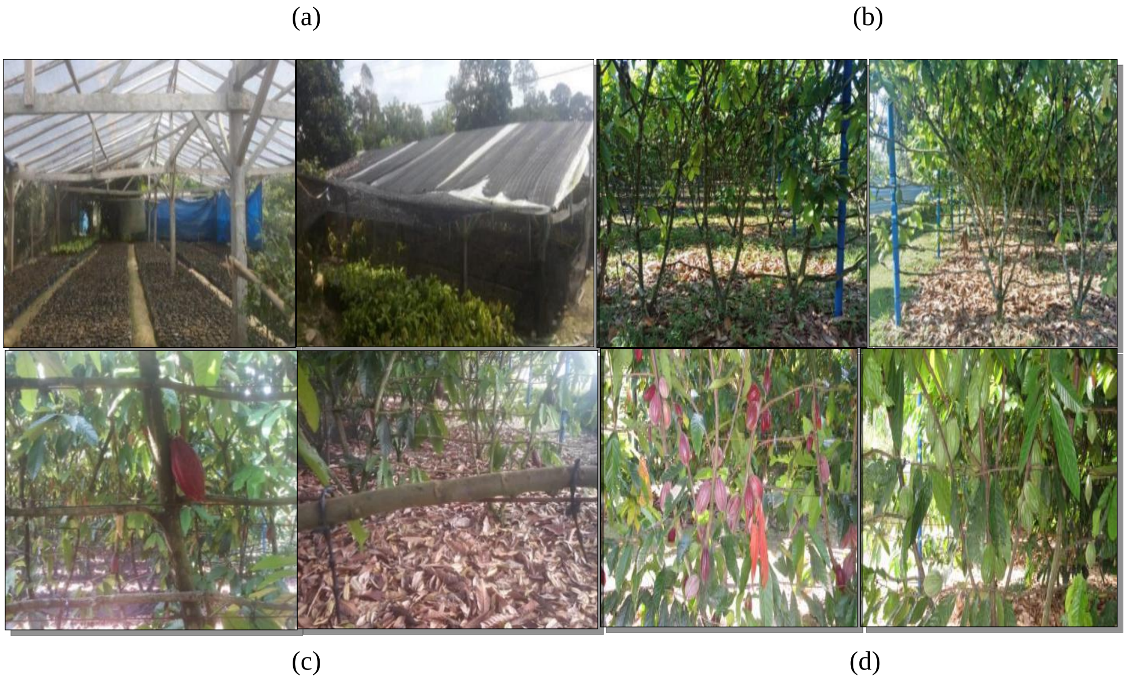


Figure 3.1 (a) Views of the nursery where planting material used in Biomass 2 was raised (b) View of trellis system, planted at a density of 3300 trees.ha^{-1} with two branches per tree (left) and 2000 trees.ha^{-1} with three branches per tree (right) (c) Close up picture of vertical and lateral branch arrangement onto the trellis (d) Cacao trees maintained on the trellis for clones 45 (left) and M01 (right)

All processes carried out in the nursery, including germination, fertiliser, fungicide, and watering frequency were described in section 2.3.1.

In July 2014, cacao seedlings were grafted using a top-grafting method; clone 45 and M01 trees were grafted to clone 45 rootstocks. Variety 45 exhibits greater pest and disease resistance while displaying lower vegetative vigour traits relative to variety M01, making it suitable for rootstock materials.

The grafted cacao plants were planted in the trial in October 2014, three months after grafting. In February 2015, the plants were pruned to suit the specific density they were to be planted. Also, they were trained for growth on a trellis system by letting two vertical branches grow for planting density 5000 trees ha^{-1} and 3300 trees ha^{-1}, and three vertical branches for density 2000 trees ha^{-1} (Figure 3.1(b)).

Vertical and lateral branches of all the trees were trained onto the trellis system to maximise radiation interception, and the canopy height was maintained at 2.75 m, with regular pruning. The first pruning of mature trees was performed in August 2016 and subsequently every three months (January, April, July, and October).

There were eight horizontal trellis wires along each row, 30 cm apart, except for the two uppermost trellis wires, which were 60 cm apart. Vertical and lateral branches of trees were fixed to the trellis using rubber ties. Vertical branches were trained onto the trellis to give a shape similar to a hedgerow. Likewise, the lateral branches also were trained onto the trellis, avoiding any intertwined branches, to give a form identical to an espalier training system. Finally, any inter-twined lateral branches were removed to leave more substantial lateral branches (Figure 3.1(c)).

Figure 3.1(d) shows the pods production from clone 45 (red-coloured pods) and M01 (green coloured pods) on the first production year.

The planting distance between each tree in 5000 and 3300 trees.ha^{-1} density plots were 1 m and 1.6 m in the 2000 trees.ha^{-1}. The trees at 3300 and 2000 trees.ha^{-1} were on a single trellis system, and the 5000 trees per hectare were planted on a double trellis system. The distance between rows for all densities was 3 m, while the gap between the two rows of the double trellis system (5000 trees.ha^{-1}) was 0.82 m. The trial was fertigated with the same method as the Biomass 1 trial (see section 2.3.10).

3.3.3 Planting material

3.3.3.1 Clone characteristic

The experiment used two clones: M01 and 45. The characteristics of M01 were described in chapter 2.3.2.1. The second clone in the trial was clone 45 (formally named MCC02). This clone was selected in Sulawesi from a farmer's field. This genotype was identified by Pak Nasir in Tigkara Village in West Malangke District, North Luwu Regency, South Sulawesi, Indonesia, in 2007. The parentage of 45 is unknown. It produces flowers within four months of grafting. Tolerance to CPB, black pod and VSD are moderate. On average, clone 45 has a pod index of 15-20 pods per kg of dry beans and a large bean size (70 beans per 100 grams) (Susilo et al., 2015).

Clones M01 and 45 are self-incompatible clones. Self-incompatibility is a genetic mechanism that prevents self-fertilization in some plant species, including cacao. In cacao, self-incompatibility is thought to promote outcrossing, which can increase genetic diversity and potentially lead to higher yields.

While some studies have suggested that self-incompatibility can improve yield by promoting genetic diversity and reducing inbreeding depression (Narayanapur et al., 2018), other studies have found that self-incompatible have a lower yield than self-compatible cacao cultivars (Royaert et al., 2011; Anita-Sari et al., 2017). The relationship between self-incompatibility and yield in cacao is complex and depends on several factors, such as the specific cultivar, growing conditions, and management practices.

However, clone M01 and 45 are cross-compatible with each other. M01 has an I/O allele combination, and 45 has I/33, both of which make them self-incompatible clones. The X/Y allele combination associates the linked egg and sperm fusion phase to two-microsatellite (SSR) markers. “I” represents one pair of alleles, “O” represents another and “33” is a third allele set. O is the most dominant of the incompatible alleles. Hence if a plant with an O allele is crossed (or selfed) with another plant with an O allele, it will not fuse and form a pod. M01, which has one O allele and one I allele (I/O) when selfed would be I/O x I/O, the O alleles are dominant over I, so both mother and father act like O and will not cross (O cannot cross with another O) (Livingstone, unpublished data).

Clone 45 is more complicated because the I allele will cross if it is with another I, but only if both of those trees are homozygous for I. For example, I/I will intersect with another I/I, but I/I will not cross I/x, and I/x will not cross with I/y. Clone 45 has an I/33 allele combination. The I alleles are dominant and should not fuse. If M01 (I/O) is crossed with 45 (I/33), then the O is dominant in M01, and the I is predominant in 45, so it behaves like an O x I cross which is compatible. The compatibility characteristics of clones M01 and 45 were elucidated from experiments performed at USDA-Mars Miami (Livingstone, unpublished data).

3.3.2.2 Estimation of off-type trees

The identification process to identify off-type trees was similar to that already described in section 2.3.2.2. Seven off-type trees were identified, which were then excluded from the analysis.

Similar to the previous experiment, four categories of data were measured: growth, physiological characteristics, phenology, and yield. Measurement parameters and the annual measurement sequence schedule are shown in Figure 2.4.

3.3.4 Vegetative growth assessment

3.3.4.1 Biomass of pruned materials

From August 2016-July 2019, pruned material (pruned branches and leaves) from sampled trees for each treatment was weighed at each pruning. The sample trees were in rows 3 and 4 from each plot for all treatments. Thus, every row consisted of 6 trees. In total, twelve measurements of pruned material were carried out throughout the trial. In addition, dry biomass and the percentage of dry matter from pruned materials were measured using the same procedure described in section 2.3.3.1.

3.3.3.2 Weight of senesced leaves

A net was installed around four trees (third, fourth, fifth, and sixth tree) in rows 3 and 4 for each plot/replication (Figure 3.2). The leaves were collected weekly from January 2017 until July 2019. Dry biomass and the percentage of dry matter from pruned materials were measured using the same method described in section 2.3.3.2.

Figure 3.2 Net for capturing fallen material in the Biomass 2 trial

3.3.3.3 Trunk and branch diameter

The trunk circumference of all experimental trees was measured every six months, from January 2017, using a tape measure 20 cm above the soil surface, while the main branches were measured 10 cm from the branching point. Trees were marked with paint for circumference measurements for consistency of results.

3.3.5 Yield assessments

3.3.5.1 Dry matter of cacao husk

In October 2018, samples of cacao husks were taken from three pods from each clone. Weighed sub-samples of 500 g were cut and dried in a freeze drier (FreeZone Console 12L Labconco™ freeze drier, USA) using an 8.4 g hr^{-1} drying speed for three days. The drying process was continued in a ventilated oven (Memmert UN30, Germany) set at a temperature of 70°C for one day until reaching a constant weight. This was faster compared to using oven drying alone, which could take seven days for cacao husks. A dry matter conversion value was obtained through a comparison of husk wet and dry weight.

3.3.4.2 Dry weight of cacao beans

All ripe pods from all experimental trees were harvested, opened, and seeds counted every 14 days from August 2016 until July 2019 (three production years). Healthy-pods were separated from diseased/infested pods. The number of pods harvested was recorded according to branch location (vertical and horizontal) and whether or not the pods showed “sun damage”. The pod's colour is atypically dark when the pods experience “sun damage” symptoms (Figure 3.3).

Figure 3.3 Example of “sun damage” symptom on cacao pods in clones 45 (left) and M01 (right) in Biomass 2 trial

For the calculation of the yield parameters, off-types and border trees were not sampled, and only the internal 24 trees were assessed in each plot (the blue dots in Figure 3.4).

Thus, across the three replicate plots, 72 trees were therefore sampled per treatment (minus any off-types). The potential yield was defined as yield achieved in the absence of pests and diseases and was calculated from the average pod production per tree (healthy and diseased pods) to estimate the dry bean weight per pod.

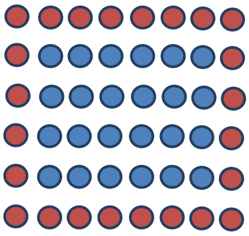


Figure 3.4 Map of sample trees (blue-coloured) in each treatment plot in Biomass 2

The same method was used to obtain the value of pod index, annual yield per tree and potential yield per hectare as described in section 2.3.4.3.

3.3.6 Phenology assessment

3.3.6.1 Flushing index

The flushing intensity index was measured every 28 days, from April 2016 until August 2019, using a scale of 1 to 4. This index is described in section 2.3.5.1. Sampled trees for flushing index assessment were the same as those used for the measurement of pruned material.

3.3.6.2 Flowering intensity index

The flowering intensity index was evaluated from April 2016 until August 2019 using the same method described in section 2.3.5.2. The trees on which flowering data were collected were the same as those used for flushing evaluations.

3.3.7 Trunk Cross-Sectional Area (TCSA), Yield efficiency (YE), and Harvest Index (HI)

Yield Efficiency (YE) combines yield with vegetative growth (Daymond et al., 2002; Padi et al., 2016). The Yield Efficiency is calculated by dividing the accumulated yield per tree by the corresponding Trunk Cross-Sectional Area (TCSA) (Hrotkó et al., 2002; Perez-Perez et al., 2005; Sotiropoulos, 2006; Zegbe and Behboudian, 2008; Ikinci et al., 2014).

$$\text{Yield Efficiency per tree } (\text{kg. cm}^{-2}) = \frac{\text{Accumulative fruit yield per tree } (\text{kg. tree}^{-1})}{\text{TCSA } (\text{cm}^{2})}$$

The Trunk Cross-sectional Area (TCSA) was calculated using the formula below (Westwood et al., 1963).

$$\mathrm{TCSA(cm^2)} = \pi\left(\frac{\text{Trunk diameter (cm)}}{2}\right)^2$$

The Harvest Index (HI) was calculated with the same method described in section 2.3.6.

3.3.8 Bean quality assessment

Beans from sun-damaged and normal pods were assessed for quality analysis (moisture, fat, and flavanol content) in the Bean and Chemical Laboratory of PT Mars Indonesia, Makassar, South Sulawesi, Indonesia.

Before the analysis, the beans were de-pulped, placed in liquid nitrogen and dried using a freeze drier (FreeZone 1Lt benchtop model, Labconco™, Missouri, USA). A sample of 150 g of beans was roasted in an oven (Memmert BE400, Germany) at 140°C for 30 minutes, and the nibs (cotyledons) and shells were separated. The nibs were then pulverised using a planetary ball mill (PM 400, Retsch™, Haan, Germany) for 45 minutes until they reached a particle size of 150 microns. Before analysis, the slurry was maintained in an incubator (Binder FD 56 E3.1, Germany) at 50°C.

The bean fat and flavanol content analyses were conducted using NIRS (Near Infrared Spectroscopy) (InfraXact™ Lab/Pro from Foss (Hilleroed, Denmark) equipped with Win ISI II software from Infrasoft International LLC. According to internal laboratory quality standards, NIR calibration was conducted every three months between Mars Makassar, Indonesia and Mars Veghel, Netherlands.

3.3.9 Canopy characteristics and radiation interception

Incident solar radiation (R_I) above the canopy was measured using a PAR sensor (PAR quantum sensor SKP215, Skye Instruments, UK) connected to a Skye solar monitor (Figure 3.5a). The sensor was attached to a PVC tube and lifted above the cacao canopy, as shown in Figure 3.5b. A mean of three readings was recorded above the centre of a tree. A mean of three readings below the same tree was recorded at the same time using a plant canopy imager (CI-110, CID, USA) to measure transmitted radiation (R_T) and also leaf area index (LAI) as described in section 2.3.7.

The two sensors had been previously calibrated by comparing 175 simultaneous readings over a range of light conditions. A calibration factor of 1.140 was used to compare the Skye sensor (Figure 3.5a) with the original CI-110 canopy imager (Figure 2.10a). While for the updated CI-110 canopy imager (Figure 2.10b), a calibration factor of 0.60 was used to compare the Skye sensor with the updated CI-110. Fractional radiation (I) interception was calculated according to the equation: $I = (R_I - R_T)/R_I$ (where R_I is incident light, and R_T is transmitted light).

Leaf area index was always measured simultaneously with the transmitted radiation measurement, using the gap fraction analysis method (Martens, Ustin and Rousseau, 1993; Weiss et al., 2004). For each marked tree, three readings were taken below the canopy, 10 cm and 50 cm from the tree trunk and each side of the tree (front and back) at three canopy heights (1 m, 1.5 m, and 2 m from the ground), using a CI-110 canopy imager.

Light attenuation was calculated using the extinction coefficient (k) (Monsi & Saeki, 1953), calculated from the equation: $k = -\ln(1-I)/LAI$. Where LAI is the leaf area index.

Figure 3.5 Left to right: (a) Skye PAR quantum sensor and monitor display (b) R_I measurement by PAR sensors

Pruning was conducted in all plots every three months (January, April, July, and October) to maximise solar interception. Incident radiation (R_I), transmitted radiation (R_T), and leaf area index (LAI) data were measured within nine days before and nine days after pruning, from October 2016 until April 2019.

Canopy and photosynthetic measurement (described in section 3.3.9) were divided into three periods:

1. Period 1: February 2017, May 2017, and October 2017 on sample trees of clones 45 and M01 grown east to west row-orientation, and planting density 2000

trees.ha^{-1}. Objective: Effect of clone, leaf age, and pruning treatment on canopy and gas exchange characteristics.

2. Period 2: January 2018 and April 2018 on sample trees of clone 45 grown east to west row-orientation with planting density 2000, 3300 and 5000 trees.ha^{-1}. Objective: Effect of planting density, leaf age, and pruning treatment on canopy and gas exchange characteristics.
3. Period 3: October 2018 and January 2019 on sample trees of clones 45 and M01, grown east to west and north to south row-orientations with planting density 2000, 3300, and 5000 trees.ha^{-1}. Objective: Effect of clone, row-orientation, leaf age, and pruning treatment on canopy and gas exchange characteristics.

3.3.10 Photosynthetic measurements

Photosynthetic and transpiration rates were measured using a portable leaf photosynthesis analyser (LC-Pro-SD, ADC Bio scientific, Hoddesdon, UK), as described in section 2.3.8.

Two trees were chosen randomly from each plot for photosynthetic measurements (six trees at each planting density and each row-orientation). Leaf gas exchange on each tree was measured at three different heights: 1 meter, 1.5 meters, and 2 meters. For each height, leaves of three different ages were sampled: “young”, “middle-aged”, and “old” leaves.

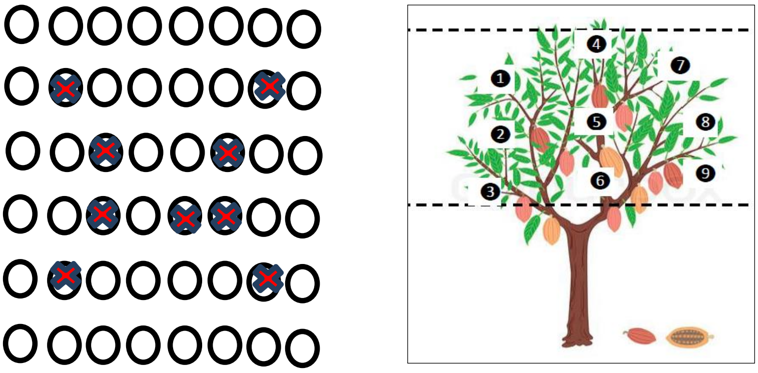
The leaf-age difference was determined by its chlorophyll content measured using a *SPAD 502 Plus* (Minolta, Japan) chlorophyll content meter. The values in SPAD units are estimated based on the amount of light transmitted by the leaf in two wavelength regions; the red area (600-700nm) and Near Infra-Red (850-1050nm). Young leaves had a SPAD unit range between 25 and 44, while middle-age leaves were between 45 and 55, and old leaves were between 60 and 90. Older leaves tend to have a higher chlorophyll content.

3.3.11 Soil and foliar analysis

The soil in the experimental area was sampled in April 2018 and March 2019 and subsequently analysed by the Oil Palm Research Center PPKS Lab (Medan, North Sumatra) as described in section 2.3.11. In 2019, lime was added to Biomass 2 soil to adjust the pH condition.

Figure 3.6 Leaf sample preparation for foliar analysis in Oil Palm Research Center Lab Medan North Sumatera

One thousand five hundred grams of leaves were sampled in January 2018 and January 2019. These were taken from nine different trees in a diagonal position within each replicate plot (Figure 3.7a) and nine different areas within each tree (Figure 3.7b) to ensure the homogeneity of the composite sample.



(a) (b)

Figure 3.7 (a) Diagonal pattern of sample trees (b) Area sampling within each tree for foliar analysis

Foliar analysis to determine leaf nitrogen content was conducted at (PPKS) using the Kjeldahl method described by Sulaeman et al. (2005) (Figure 3.6). The PPKS Lab is one of the soil laboratories in Indonesia that is certified by WEPAL (Wageningen Evaluating Programs for Analytical Laboratories).

3.3.12 Statistical analysis

The significance of clone, planting density, row orientation, and interactions between these factors on the measured parameters were tested by analysis of variance (ANOVA) using GenStat 19th edition software (VSN International Ltd., Hemel Hempstead, UK) as a split-plot randomised block design. Significant differences between means were determined using a Least Significant Difference (LSD significance level 5%).

3.4 Results

3.4.1 The effect of clone selection, planting density and row-orientation on yield and assimilate partitioning

3.4.1.1 Yield

A. Yield (number of pods harvested per tree)

All ripe pods from all trees were harvested, opened, and seeds counted every 14 days from August 2016 until July 2019 (i.e. three years).

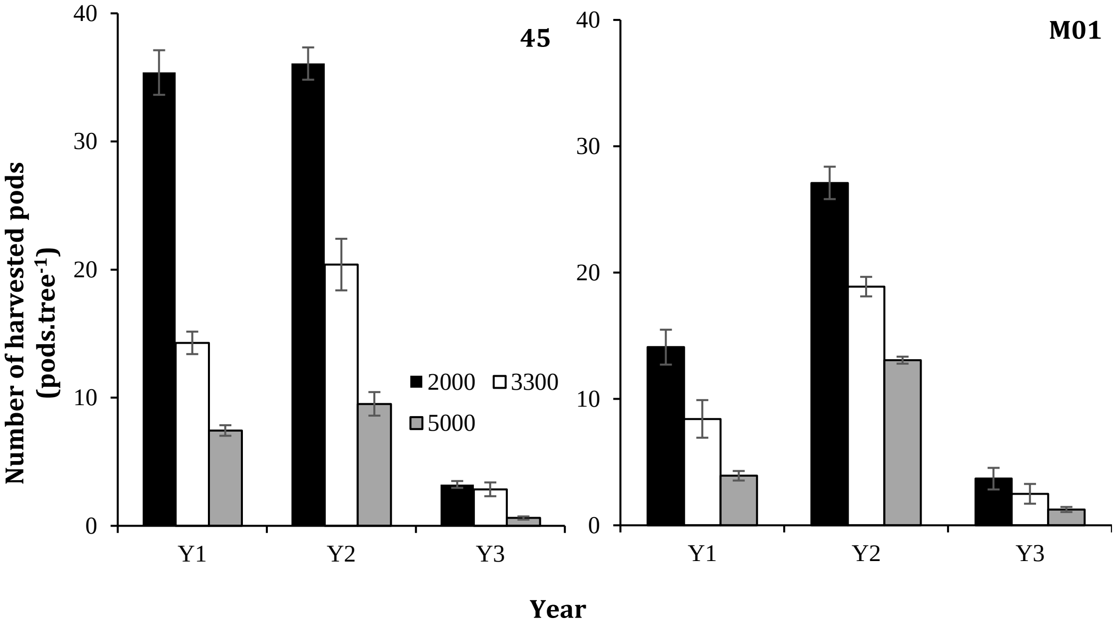
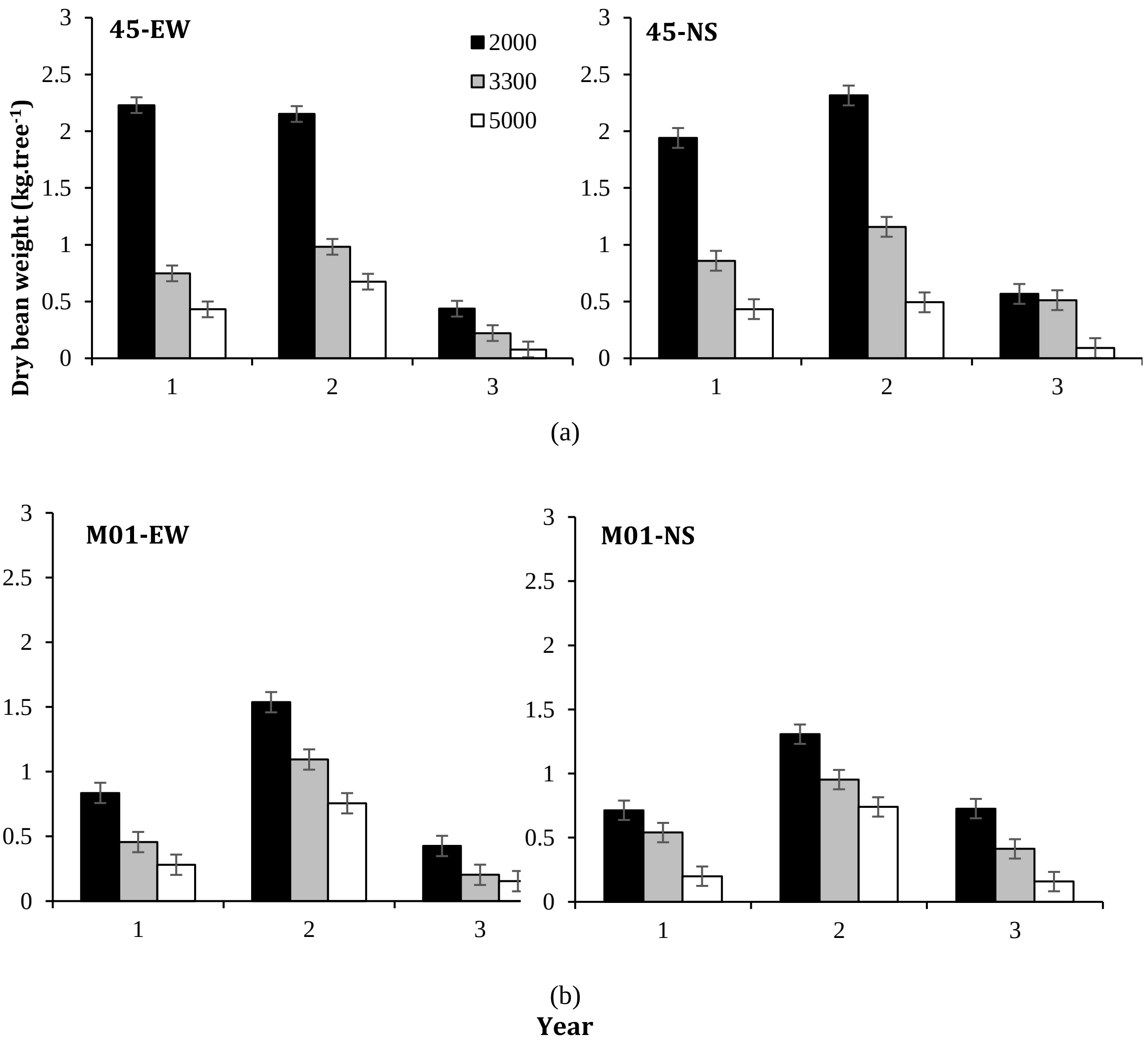


Figure 3.8 The number of pods harvested per tree from clones 45 (left) and M01 (right) grown at three different planting densities (2000, 3300, and 5000 trees.ha^{-1}), measured in year 1 (Aug 16-Jul 17), year 2 (Aug 17-Jul 18), year 3 (Aug 18-Jul 19). Values are means across both orientations and three plots replicate (each plot consists of 24 trees, excluding the off-types) (+/- standard errors)

The number of harvested pods per tree was similar for both orientations; therefore, data for both orientations were combined (Figure 3.8). Overall, the number of harvested pods was higher for clone 45 than clone M01 at all densities in year 1 ($P<0.001$) in both row-orientations. However, the number of pods harvested was relatively similar for both clones in years 2 and 3, except for the lowest density in year 2 when the yield for clone 45 was significantly higher than clone M01, whilst the opposite pattern was observed in planting density 5000 trees.ha^{-1} ($P<0.001$). In general, there was a decrease in the number of pods.tree^{-1} with an increase in density in all three years ($P<0.001$). There was a significant decline in pod production for both clones in year 3 at both row-orientations (P<0.001).

B. Yield (dry bean weight) expressed on an individual tree basis



(a)

(b)

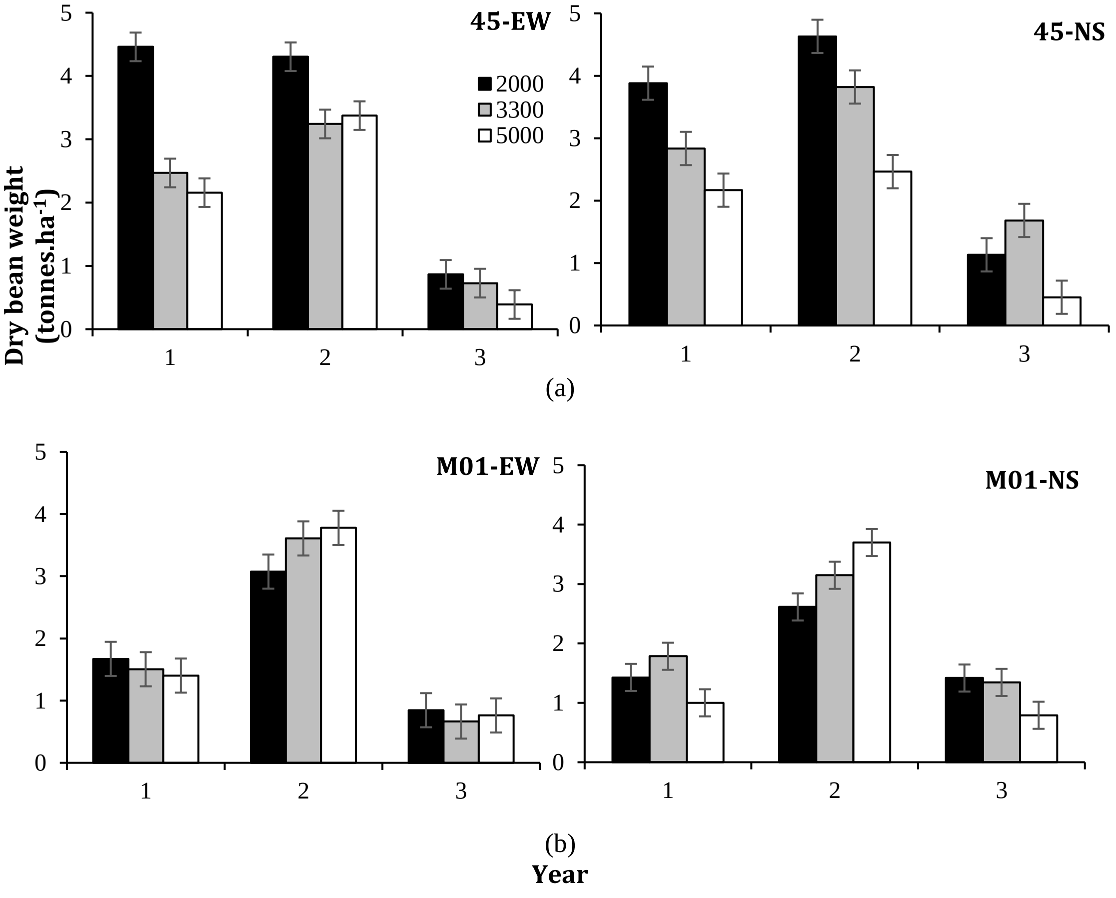
Figure 3.9 The potential yield per tree from clones (a) 45 and (b) M01, grown at three different planting densities (2000, 3300, and 5000 trees.ha^{-1}), from east-west (left) and north-south (right) row orientations, measured in Year 1 (Aug 16-Jul 17), Year 2 (Aug 17-Jul 18), and Year 3 (Aug 18-Jul 19). Values are means across three plot replicates (each plot consists of 24 trees, excluding the off-types) (+/- standard errors)

The yield per tree for clone 45 was higher than clone M01 in years 1 and 2 at all densities for both row-orientations (P<0.001; Figure 3.9). However, the yield of both clones was relatively similar in year 3. Yield per tree declined with increased density in all three years (P<0.001). The yield was also significantly lower in year 3 compared to years 1 and 2 (P<0.001). Similar to the number of pods harvested in year 2 with planting density 2000 trees.ha^{-1}, yield per tree from clone 45 at 2000 trees.ha^{-1} was higher compared to clone M01. In contrast, the yield at a planting density 5000 trees.ha^{-1} was higher in clone M01 than in clone 45 ($P<0.001$).

There was no effect of row-orientations on yield per tree, except in year 3 when the yield per tree in NS orientation in year 3 was higher than in EW ($P<0.001$).

C. Yield (dry bean weight) expressed on an area basis

The yield per hectare was higher for clone 45 than for clone M01 in year 1 ($P<0.001$; Figure 3.10). However, yields per hectare were higher for clone 45 at a density of 2000 and 3000 plants per hectare than clone M01, but yields were similar for both clones at 5000 plants per hectare. Yield per hectare was similar at both orientations in years 1 and 2. However, in year 3, the NS orientation yield was higher than EW orientation ($P<0.001$).



(a)

(b)

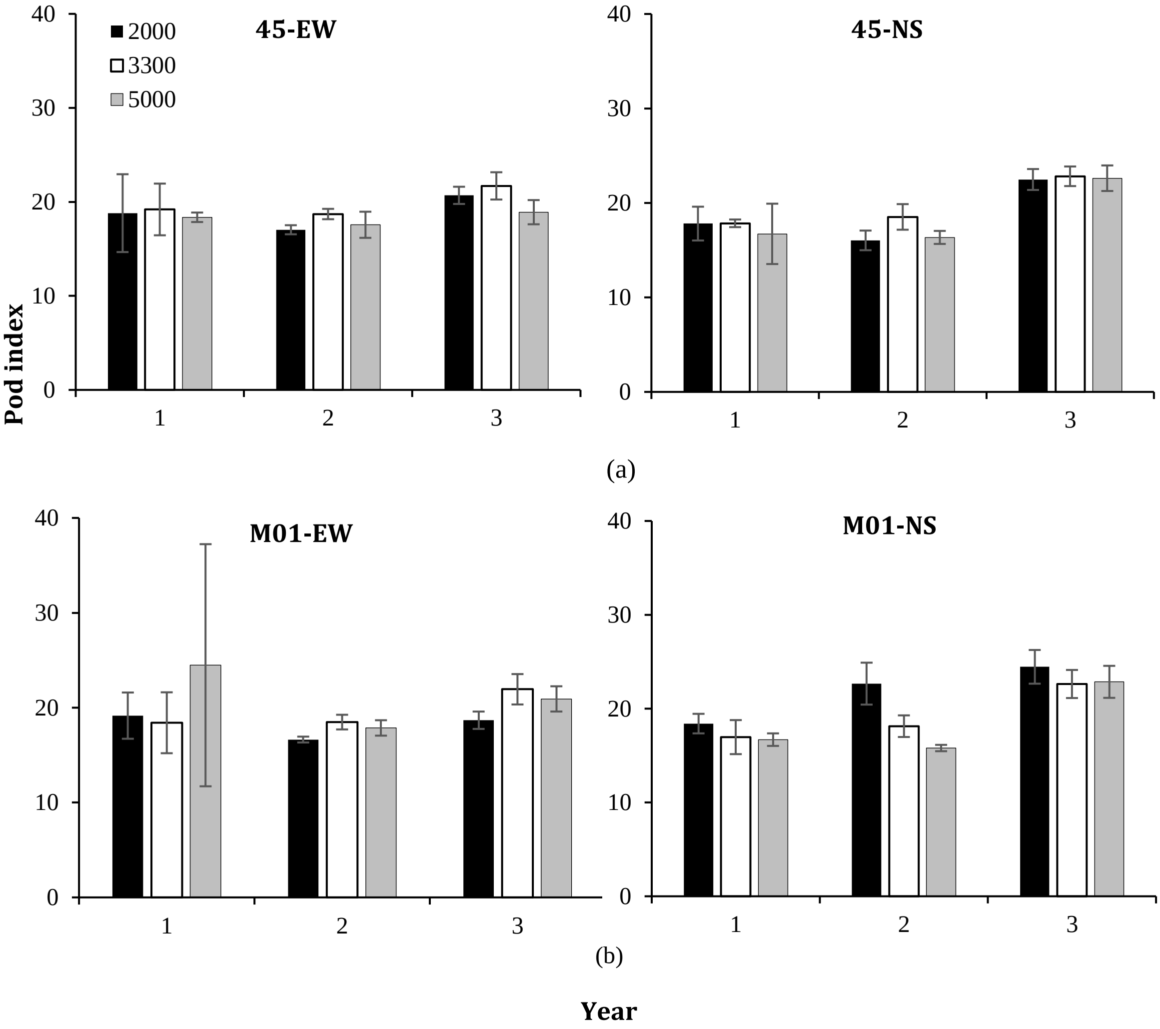
Figure 3.10 The potential yield per hectare from clones (a) 45 and (b) M01 grown at three different planting densities (2000, 3300, and 5000 trees.ha^{-1}), from east-west (left) and north-south (right) row orientations, measured in Year 1 (Aug 16-Jul 17), Year 2 (Aug 17-Jul 18), and Year 3 (Aug 18-Jul 19). Values are means across three plot replicates (each plot consists of 24 trees, excluding the off-types) (+/- standard errors).

Yield per hectare declined with an increase in density in year 1 ($P=0.002$), year 2, and year 3 ($P<0.001$) for both clones except for M01 in year 2 when yields increased with increasing density. Yields increased for both clones in year 2 compared with year 1 (markedly for clone M01) but declined significantly in year 3 compared with years 1 and 2 ($P<0.001$).

D. Pod index

Pod index was measured from December 2016 until June 2019. Pod index ranged from 13-37 for clones 45 and M01, respectively. In the EW row orientation in all three years and NS row orientation in the first 2 years, the pod index was similar for both genotypes across all three planting densities. In the NS orientation, the pod index was significantly higher for clones M01 ($P=0.040$) compared to 45 at all three densities in year 3. The pod index in NS year 3 was higher than in years 1 and 2 ($P=0.017$; Figure 3.11).

The high variance in pod index measured for clone M01 at a density of 5000 plants per hectare in EW row-orientation in year 1 was caused by a low sample number.



(a)

(b)

Figure 3.11 Pod index value of clones (a) 45 and (b) M01 grown at three different planting densities (2000, 3300, and 5000 trees.ha^{-1}), from east-west (left) and north-south (right) row orientations, measured from August 2016-July 2017 as year 1, August 2017-July 2018 as year 2, and August 2018-July 2019 as year 3. Values are means across three plot replicates (+/- standard errors)

E. Ratio of yield from horizontal and vertical branches

The number of pods harvested was measured separately for horizontal and vertical branches and compared across the measurement period from February 2017 until August 2019. A significantly higher ratio of pods was harvested from vertical branches than from horizontal branches; therefore, the maximum value for the ratio of pods harvested from horizontal branches to total pods (vertical and horizontal) does not exceed 50%, as shown in Figure 3.12.

In general, the highest ratio of pods harvested from horizontal branches was observed in clone M01 compared to clone 45 (13.3% compared to 11.4%; P<0.001) from October 2017-January 2019. In the first year of measurement (February 2017-February 2018), the ratio of horizontal pods in the planting density 3300 trees.ha^{-1} (14.6%) was higher than the density 2000 (11.7%) and 5000 trees.ha^{-1} (10.8%) (P<0.001; Figure 3.12).

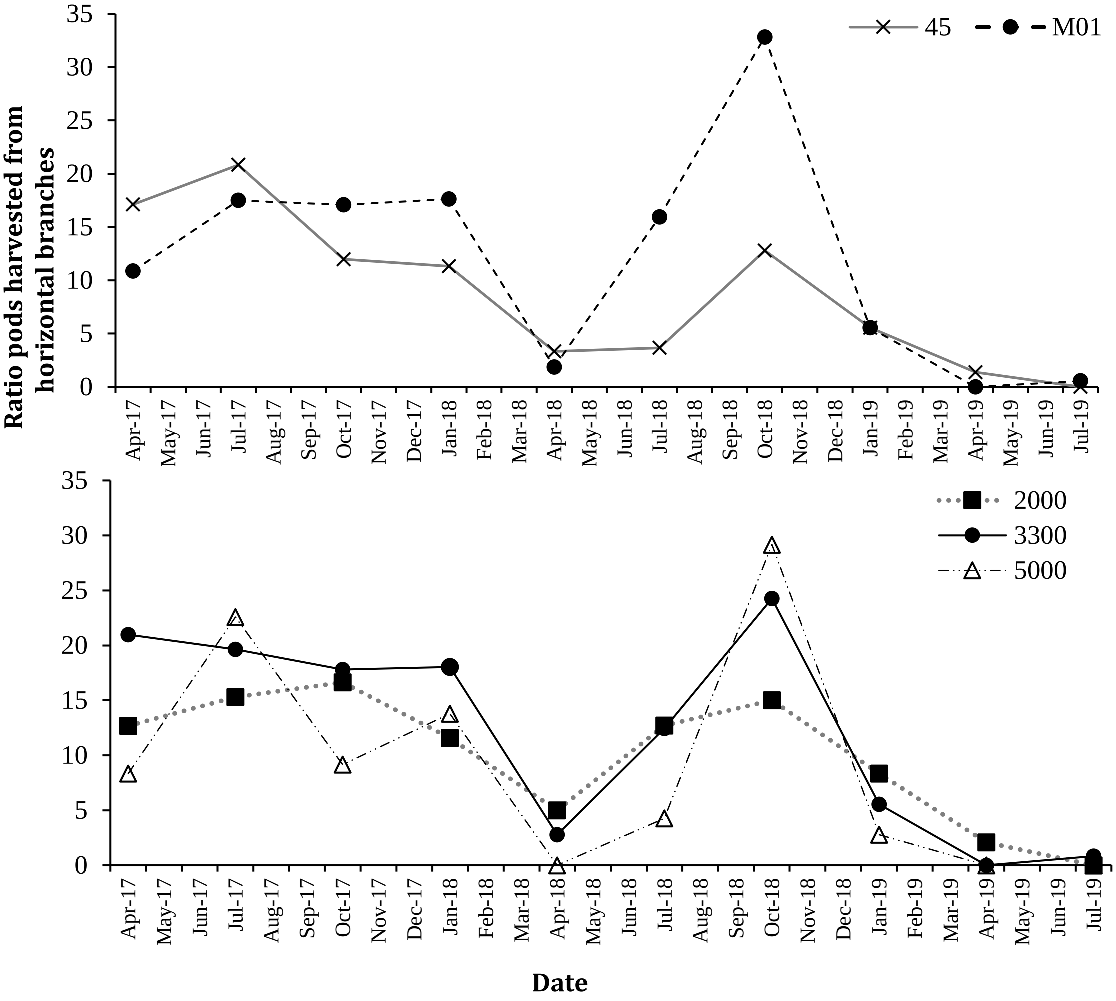


Figure 3.12 Ratio of pods harvested from vertical to horizontal branches collected from different clones (45, M01) (top) and different planting densities (2000, 3300, 5000 trees.ha^{-1}) (bottom) in the Biomass 2 trial, measured from February 2017 until August 2019. Values are means across three plots as replicates and two orientations (LSD clones=1.51; LSD density=1.85).

E. Effect of pruning on the percentage of "sun damaged-pods"

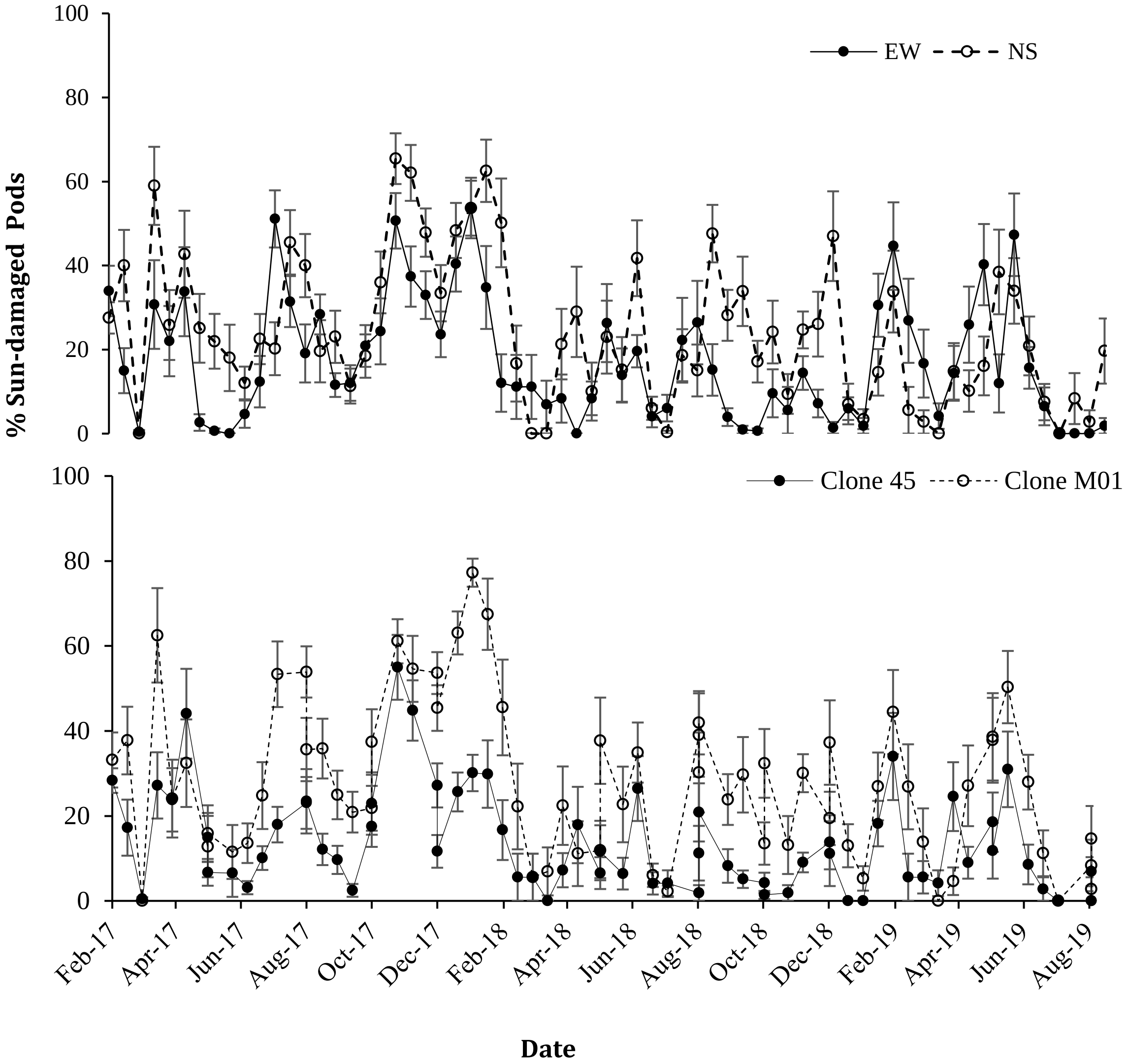


Figure 3.13 The percentage of burnt to whole pods harvested from trees grown in row-orientation EW and NS (top) with clones 45 and M01(bottom). Values are means across three plots as replicates, and three planting densities (+/- standard errors)

Biweekly measurements were carried out from February 2017 until August 2019 to assess the effect of clone and row-orientation on burning effect (sun-damaged case) on pods. Pods from clone M01 were more prone to have a burning effect compared to clone 45 (27.88% burnt compared to 13.69% burnt, respectively ($P<0.001$; Figure 3.13).

EW row orientation had the lowest % burnt pods compared to the NS orientation ($P<0.001$). The % burnt pods in EW orientation was 17.28% compared to 24.28% in NS. However, no effect of density was observed on the burning effect.

Similarly, no effect of planting density, clone or row-orientation was observed on the bean quality (moisture, fat, and flavanol content) throughout the measurement period.

3.4.1.2 Assimilate Partitioning

A. Harvest Index

The harvest index uses dry pod weight (pod harvest index) and dry bean weight (bean harvest index) (as the reproductive components) to total dry weight (dry weight of pruned vegetative materials and dry weight of beans and pod husks). The harvest index was calculated using the same method described in section 2.3.6. The harvest index term will be differentiated between harvest index pods (calculating between pods dry weight to the total dry weight) and harvest index beans (calculating between bean dry weight to the total dry weight).

In year 1, clone 45 had a higher pod harvest index than clone M01 (P<0.001). The pod harvest index for clone 45 was 32.6% compared to 19.6% for clone M01. However, an opposite pattern was observed in years 2 (P=0.033) and 3 (P<0.001), as seen in Table 3.1.

In year 3, a significant decline in productivity was observed in all treatments compared to years 1 and 2. On average, the pod harvest index in year 3 was 11.72%, compared to 26.07% in year 1 and 25.90% in year 2.

Harvest index fell consistently with increased density in both clones in all three years (P<0.001). Overall, the pod harvest index at planting density 2000 was 25.87%, compared to 23.98% at density 3300 and 13.85% at density 5000 trees.ha^{-1}.

The effect of row orientation on harvest index was observed in year 3 (P<0.001) but not in years 1 and 2. In year 3, the pod harvest index pods were higher in NS orientation (15.02%) than EW (8.42%).

Table 3.1 Pod harvest index (pod dry weight divided by total dry weight) for clones 45 and M01, grown at different planting densities (2000, 3300, 5000 trees.ha^{-1}) and different row orientations (east to west (EW) and north to south (NS)) measured from August 2016 until July 2017 as Year 1, August 2017 until July 2018 as Year 2, August 2018 until July 2019 as Year 3, and from August 2016 until July 2019 as accumulative Y1Y2Y3. Values are means across three plots as replicates

Orientation	Clone	Density	HI (%) Year 1	HI (%) Year 2	HI (%) Year 3	HI (%) Y1Y2Y3
EW	45	2000	46.67	28.31	8.95	27.98
EW	45	3300	32.6	24.04	8.65	21.76
EW	45	5000	19.33	17.37	3.54	13.41
EW	M01	2000	21.15	23.39	10.56	18.37
EW	M01	3300	17.49	27.94	9.2	18.21
EW	M01	5000	14.14	28.14	9.64	17.31

NS	45	2000	41.66	31.36	13.71	28.91
NS	45	3300	36.80	29.83	18.14	28.26
NS	45	5000	18.44	11.53	3.71	11.23
NS	M01	2000	26.14	32.96	25.59	28.23
NS	M01	3300	28.75	34.11	20.23	27.70
NS	M01	5000	9.72	21.87	8.73	13.44

Likewise, for the bean harvest index, which represents the fraction between bean and total dry weight (vegetative biomass and pod), clone 45 had a higher bean harvest index (17.45%) than clone M01 (10.03%) in year 1 ($P<0.001$), but there was no difference in bean harvest index between clones in year 2. Nevertheless, the opposite pattern was observed in year 3 ($P<0.001$; Table 3.2). In year 3, the bean harvest index for clone M01 was higher (7.1%) than for clone 45 (4.8%).

Table 3.2 Bean harvest index (bean dry weight divided by total dry weight) for clones 45 and M01, grown at different planting densities (2000, 3300, 5000 trees.ha^{-1}) and different row orientations (east to west (EW) and north to south (NS)) measured from August 2016 until July 2017 as Year 1, August 2017 until July 2018 as Year 2, August 2018 until July 2019 as Year 3, and from August 2016 until July 2019 as accumulative Y1Y2Y3. Values are means across three plots as replicates

Orientation	**Clone**	**Density**	**HI (%) Year 1**	**HI (%) Year 2**	**HI (%) Year 3**	**HI (%) Y1Y2Y3**
EW	45	2000	25.56	18.74	4.84	16.38
EW	45	3300	16.74	15.19	4.30	12.08
EW	45	5000	10.80	12.21	1.73	8.25
EW	M01	2000	10.54	15.19	5.67	10.47
EW	M01	3300	9.33	17.84	4.80	10.66
EW	M01	5000	7.86	18.20	4.81	10.29
NS	45	2000	22.57	21.55	6.61	16.91
NS	45	3300	19.07	19.08	9.52	15.89
NS	45	5000	9.95	7.95	1.79	6.56
NS	M01	2000	12.56	14.86	5.07	10.83
NS	M01	3300	19.59	21.51	14.24	18.45
NS	M01	5000	12.99	9.82	4.26	9.02

The bean harvest index declined with increased planting density in year 1 ($P<0.001$; except for clone M01, NS row-orientation); year 2 ($P=0.002$; except clone M01), and year 3 only for clone 45 NS row-orientation ($P=0.029$). The highest bean harvest index was observed in planting density 2000 (13.7%) trees.ha^{-1} in all three years, compared to density 3300 (12.6%) and 5000 trees.ha^{-1} (7.3%).

Row orientation did not affect the bean harvest index in 2, but an effect was observed in years 1 and 3 ($P<0.001$). In years 1 and 3, the bean harvest index in NS orientation was higher (16.1 and 6.9%) compared to EW orientation (13.5 and 4.4%).

The allocation of vegetative biomass (pruned material) to yield (bean and husk) between clone 45 and clone M01 across the three accumulative years is shown in Figure 3.29. These graphs demonstrate that only a tiny portion of the assimilated product goes into yield (dry bean weight). In general, vegetative material weight increases as planting density decreases in years 1, 2 and 3 ($P<0.001$), likewise the reproductive material (bean and husk) weight. The weight of the pruned materials (branches and leaves) dominated the total biomass (pruned materials, beans and husk) with a ratio between 0.69-0.88 for clone 45 and 0.72-0.85 for clone M01 across planting densities (Figure 3.14); whilst the ratio of the dry weight of cacao husk to the total pod weight ranged from 0.38-0.44 for clone 45 and from 0.39-0.46 for clone M01.

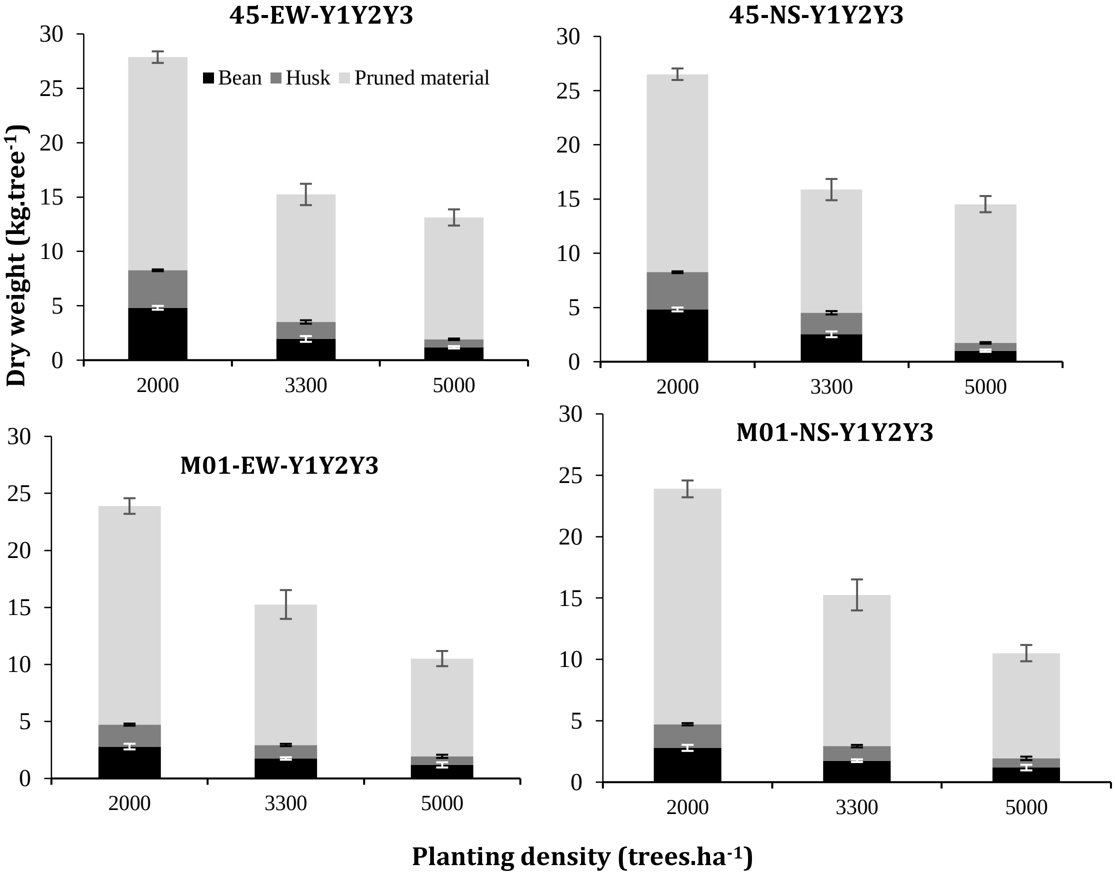


Figure 3.14 The dry weight of vegetative biomass and yield produced in accumulative three production years, from August 2016 until July 2019, of trees grown at Biomass 2 trial with two clones (45 and M01), two row-orientations (EW and NS), and three planting densities (2000,3300,5000 trees.ha^{-1}). Values are means across three plots as replicates (+/- standard errors)

B. Increase in Trunk Cross-Sectional Area (TCSA) and Yield Efficiency (YE)

The increase in Trunk Cross-Sectional Area (TCSA) was calculated over two consecutive years (January 2017-January 2019). Yield efficiency (YE) was calculated by dividing the cumulative yield by Trunk Cross-Sectional Area (TCSA) at the end of each year.

There was no difference in the increase in TCSA between different row orientations in either year, so data were combined for both orientations (Figure 3.15). Clone 45 had a higher TCSA increase than clone M01 in both years 1 and 2 at all densities ($P<0.001$). However, the increase in TCSA in year 2 was significantly lower compared to year 1 ($P<0.001$).

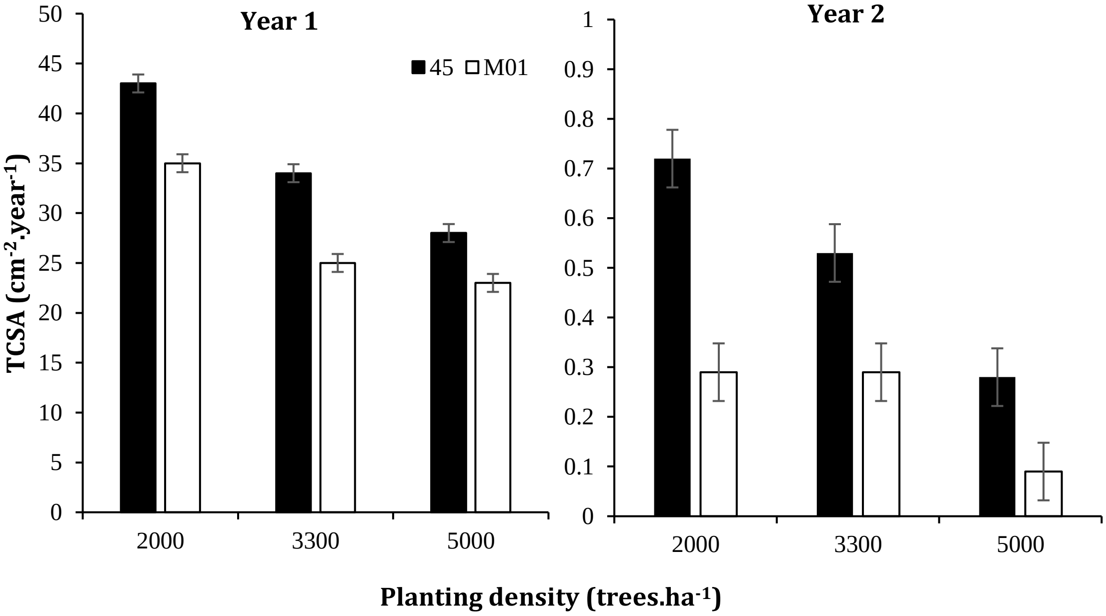
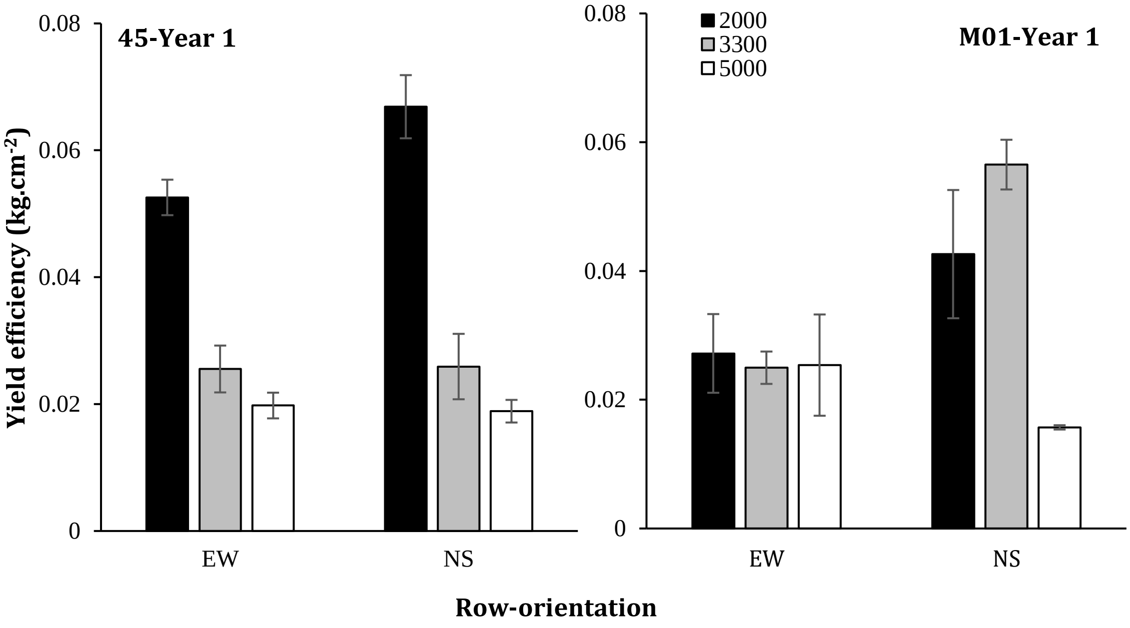


Figure 3.15 Trunk cross-sectional area (TCSA) for clones 45 and M01 grown at different planting densities (2000, 3300, and 5000 trees.ha^{-1}), measured between January 2017 and January 2018 as year 1 (left) and between January 2018 and January 2019 as year 2 (right). Values are means across two orientations and three plots as replicates (+/- standard errors). Note: year 1 y-axis scale is significantly enlarged than year 2.

The increase in TCSA declined with increasing planting density for both clones in both years ($P<0.001$).



(a) (b)

Figure 3.16 Yield efficiency (YE) for clones (a) 45 and (b) M01 grown at different row orientations, east to west (EW) and north to south (NS), measured between January 2017 and January 2018 as Year 1. Values are means across three plots as replicates (+/- standard errors)

Since the increase in trunk cross-sectional area in year 2 was significantly lower, yield efficiency was only calculated for year 1 (Figure 3.16).

Yield efficiency (YE) was generally similar for both clones at the higher density, but at a density of 2000 trees.ha^{-1}, YE from clone 45 was significantly higher than clone M01 (P<0.001). Yield efficiency generally declined with increasing density for clone 45, but this was less clear for clone M01 (P=0.002). Overall, yield efficiency was similar in both row orientations for clone 45 but was significantly higher in the two lower densities in the NS orientation than in the EW orientation for clone M01 (P=0.024; Figure 3.16).

3.4.2 The effect of pruning and intensive growing system on phenology of two Sulawesi cacao clones

3.4.2.1 Flowering intensity

Clone M01 had a higher flowering index compared to clone 45 during the first phase of the measurement (May 16-Oct 16), when the age of the tree was around 1-1.5 years old (P<0.001). Clone M01 had a higher flowering index compared to clone 45 in year 1 (P<0.001), whilst the opposite pattern was observed in year 3 (P<0.001). However, no difference between clones was observed in years 2 and 4 (Figure 3.17). The flowering index also generally declined with increasing density in both clones in each of the four years in both orientations (P<0.001).

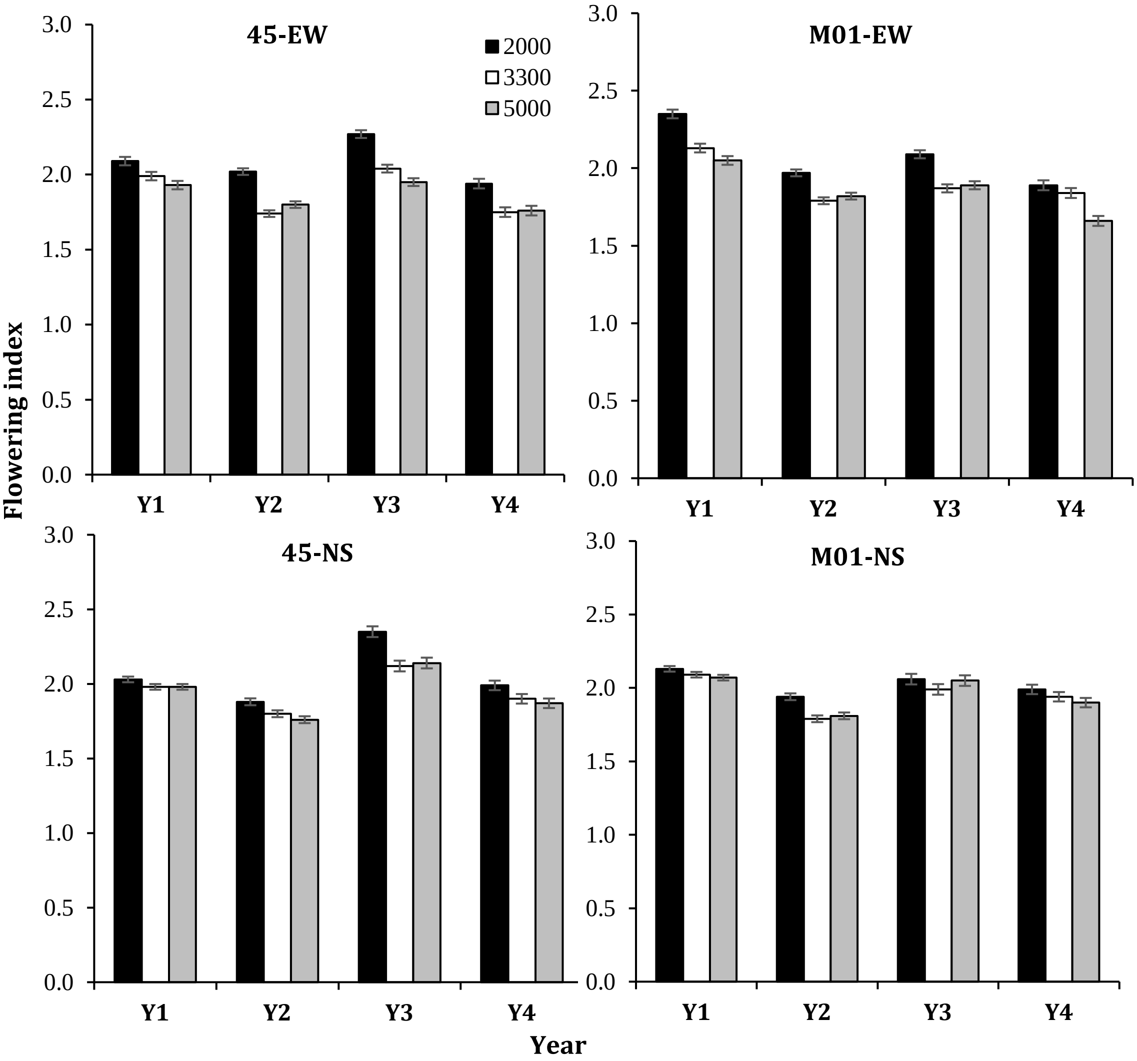


Figure 3.17 Average flowering intensity index from cacao trees with clones 45 (left) and M01 (right) in the Biomass 2 trial grown at different row-orientation: east to west (EW) and north to south (NS), measured in year 1: April 2016-March 2017, year 2: April 2017-March 2018, year 3: April 2018-March 2019, year 4: April 2019-August 2019 (5mo). Values are means across three densities and three plots as replicates (+/- standard errors)

A higher flowering index was observed in the north-south orientation than the east-west in years 3 and 4 (P<0.001). However, the opposite pattern was observed in years 1 and 2 (P<0.001).

3.4.3.2 Flushing intensity

Figure 3.18 shows the cycle of flushing intensity in sample trees in Biomass 2 during pruning time between January 2017 and August 2019. No effect of clone or density was observed on the flushing index across the three subsequent years; moreover, no interactions from factors were observed in either row orientation. The east-west orientation had a higher flushing index than the north-south in year 3 (P<0.001), but not in the previous years.

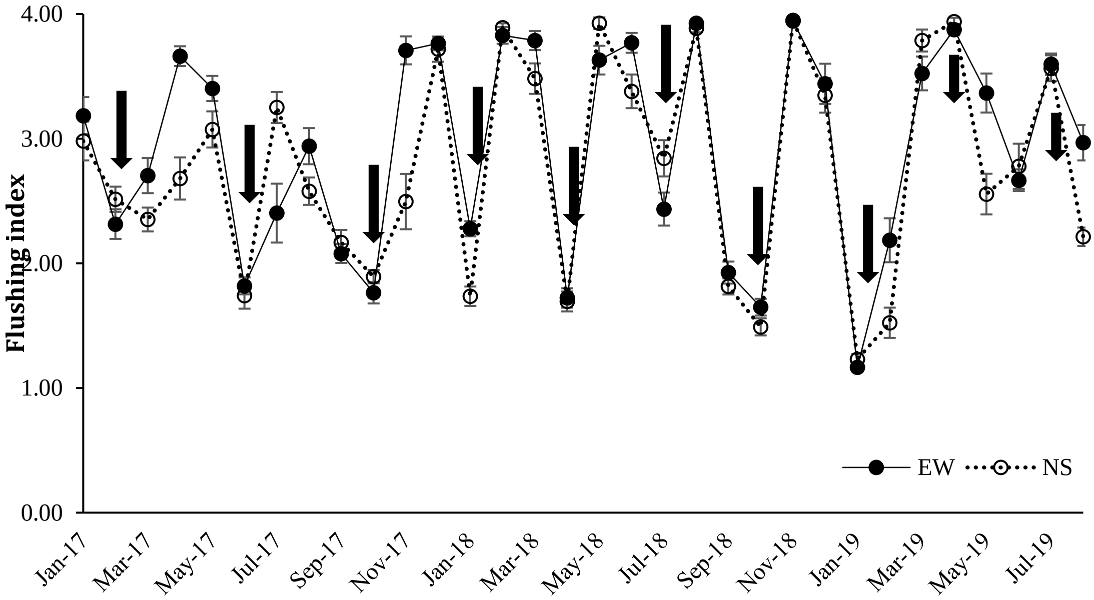


Figure 3.18 Average flushing index per tree for trees grown at two different orientations (EW, NS) in the Biomass 2 trial, measured in January 2017 until August 2019 (April 2017-March 2018 as year 1, April 2018-March 2019 as year 2, April 2019-August 2019 as year 3). Values are means across three plots as replicates, three planting densities, and two clones (+/- standard errors). The black-coloured arrows represent pruning times.

3.4.3 Canopy characteristics of two clones grown at different planting densities and orientations

3.4.3.1 Preliminary trial: The effect of equipment position from the tree trunk and canopy height on canopy characteristic parameters

A preliminary experiment was conducted in July 2018 to test the effect of canopy height and equipment position from the tree trunk on the estimation of leaf area index (LAI), fractional light interception (I), and extinction coefficient (k). The objective of the experiment is to define the optimum position to measure LAI, I and k.

The trial was conducted at three different canopy heights (0.5;1;1.5 m from the ground), five different distances from the tree trunk (10, 20, 30, 40, 50 cm), and two different measurement directions (north and south-facing side for EW orientation). Clone 45 trees from the Biomass 2 trial were chosen, and two tree replicates for each planting density 2000 and 3300 trees.ha^{-1} were used.

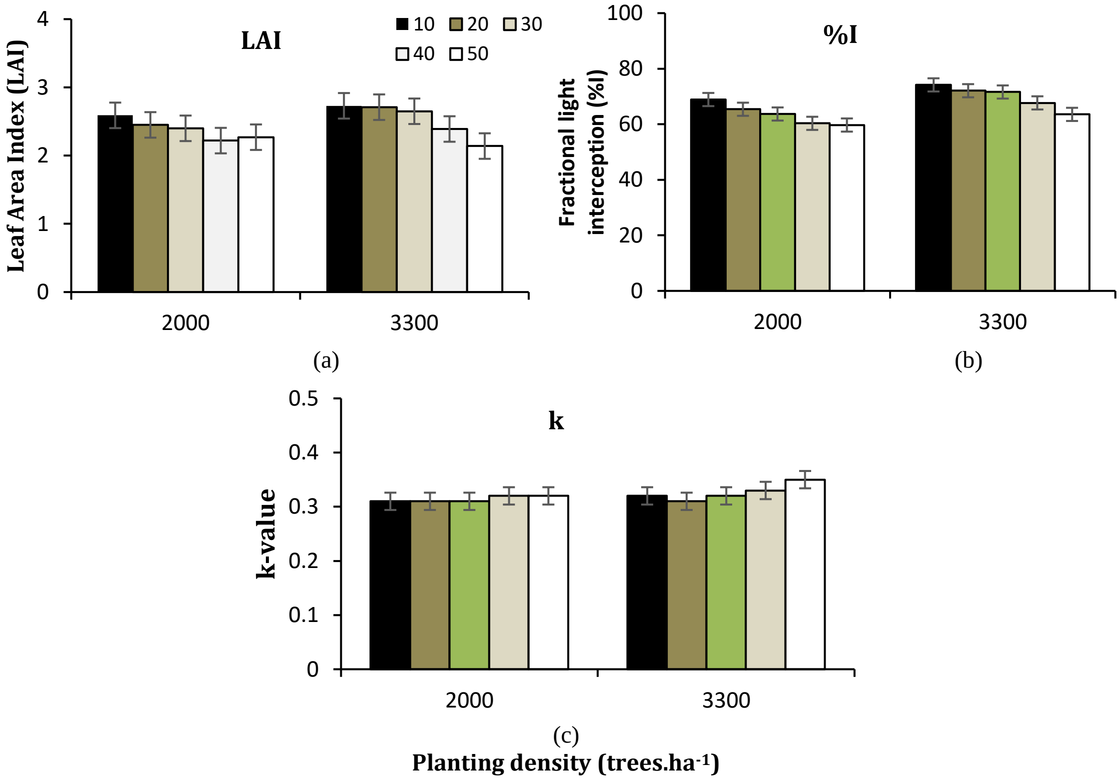
1. Effect of direction on LAI, I, and k

In both planting densities, measurement direction significantly affected the estimation of LAI and I ($P<0.001$). LAI and fractional light interception (I) measured from the north-facing side of the tree at both 2000 and 3300 trees.ha^{-1} were higher compared to those measured on the south-facing side. Similarly, the extinction coefficient (k) measured from the north-facing side was higher at both densities.

2. Effect of measurement position from the tree trunk on the estimation of LAI, I, and k

LAI and fractional light interception (%I) measured closer to the trunk had a higher estimated value compared to LAI, and %I measured further away from the trunk in both planting densities ($P<0.001$; Figure 3.19a,b).

However, the k-value measured closer to the trunk at a planting density of 3300 trees.ha^{-1} ($P<0.001$) had a lower value than the k-value measured further away from the trunk, but this effect was not significant at a density of 2000 trees.ha^{-1}.



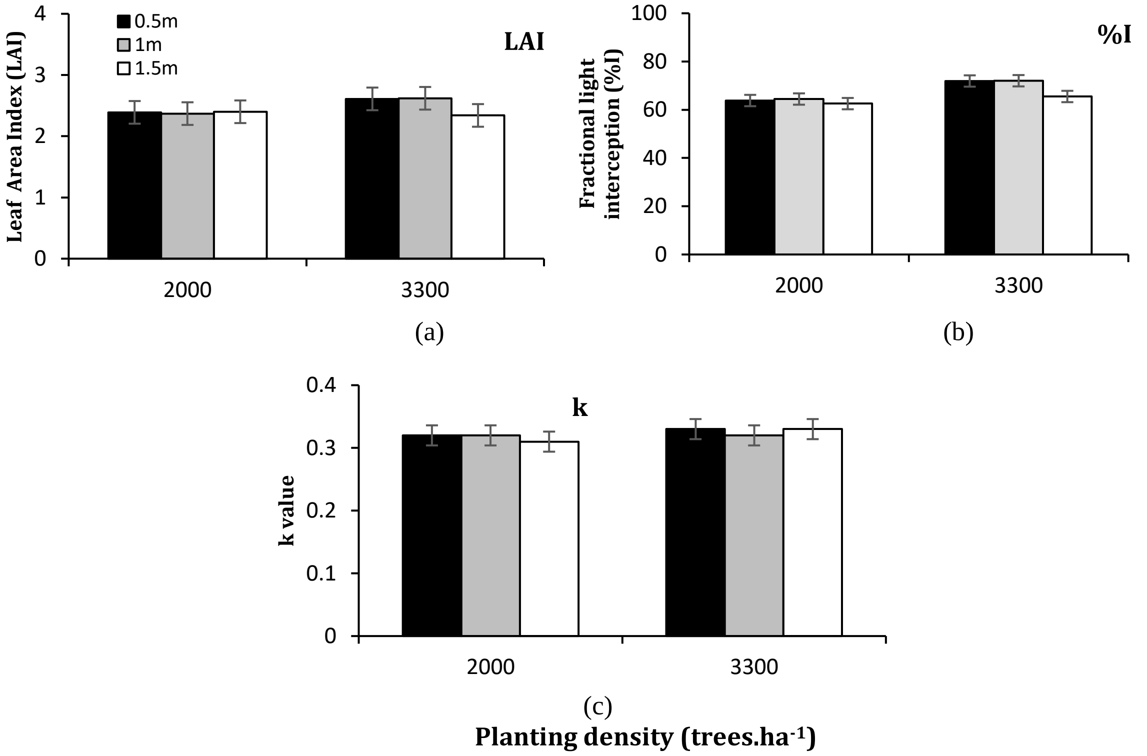
(a) (b)

(c)

Figure 3.19 (a) Leaf area index, (b) fractional light interception (I), and (c) k-value from cacao trees grown at two planting densities (2000, 3300 trees.ha^{-1}) measured at 10, 20, 30, 40, and 50 cm distance away from the tree trunk in Biomass 2 trial, in the year 2018. Values are means across two measurement directions, three canopy heights, and two trees as replicates (+/- standard errors)

3. Effect of canopy height on LAI, I, and k

Canopy height did not significantly affect the estimation of LAI, fractional light interception or light extinction coefficient at either density except for a measurement height of 1.5 m, leading to an underestimate of LAI and I at 3300 trees.ha^{-1}. From the logistic ease point of view, a canopy height of 1 m was considered the best measurement point.



(a) (b) (c)

Figure 3.20 (a) Leaf area index (LAI), (b) fractional light interception (I), and (c) extinction coefficient (k) from cacao trees grown at two planting densities (2000, 3300 trees.ha^{-1}) measured at canopy height 0.5, 1, 1.5m in Biomass 2 trial, in the year 2018. Values are means across two measurement directions, five distances, and two trees as replicates (+/- standard errors)

These preliminary trial results concluded that the next experiment would use a 10 cm position relative to the tree trunk at 1 m canopy height from the north-facing side direction for the subsequent measurement analysis.

3.4.3.2 The effect of clone, planting density, and row orientation on canopy characteristics

No effect of row-orientations nor its interaction was observed in the canopy characteristics.

1. Changes in LAI, fractional light interception (%I), and extinction coefficient (k) during the measurement period.

Leaf area index declined from 3 at the beginning of the measurement period to 2 in February 2017 before increasing to over 6 by May 2018. Subsequently, LAI declined progressively during the last measurement period to an LAI of 1 by February 2019 (P=0.015; Figure 3.21). Pruning generally reduced LAI by 0.4-2.6.

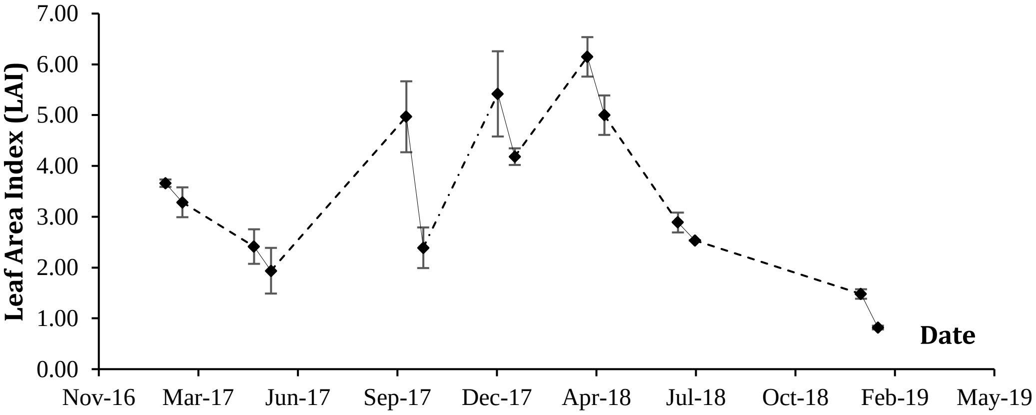


Figure 3.21 Leaf area index (LAI) measured from February 2017-January 2019 from clone 45 grown at planting density 2000 trees.ha^{-1} and EW row-orientation. Values are means across three plots and two trees as replicates (+/- standard errors). Note: the solid line connects before and after conditions on each pruning; the dashed line connects pruning events.

Fractional light interception rose from the beginning of the measurement period to reach a maximum between September and December 2017. It then gradually declined through to the end of the measurement period in February 2019. Pruning resulted in a decrease in fractional light interception ranging from 6-44% (P<0.001; Figure 3.22).

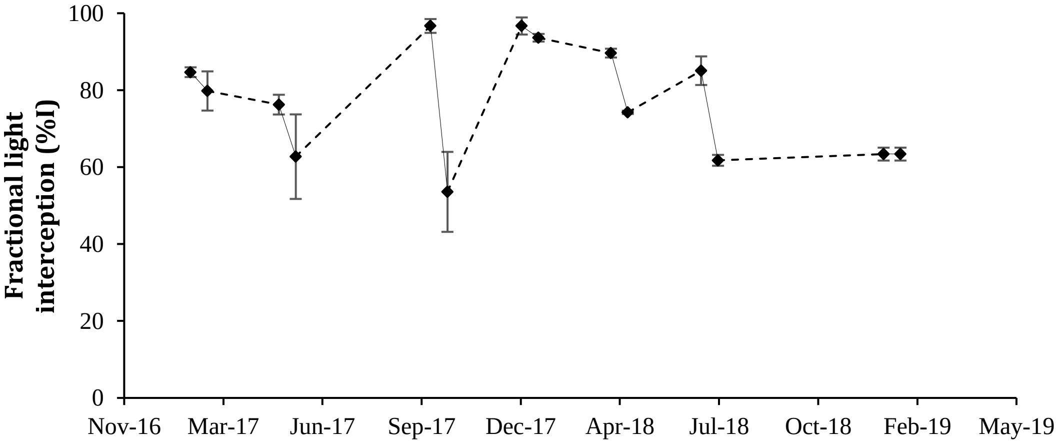


Figure 3.22 Fractional light interception (%I) measured from February 2017-January 2019 from clone 45 grown at planting density 2000 trees.ha^{-1} and EW row-orientation. Values are means across three plots and two trees as replicates (+/- standard errors). Note: the solid line connects before and after conditions on each pruning; the dashed line connects pruning events.

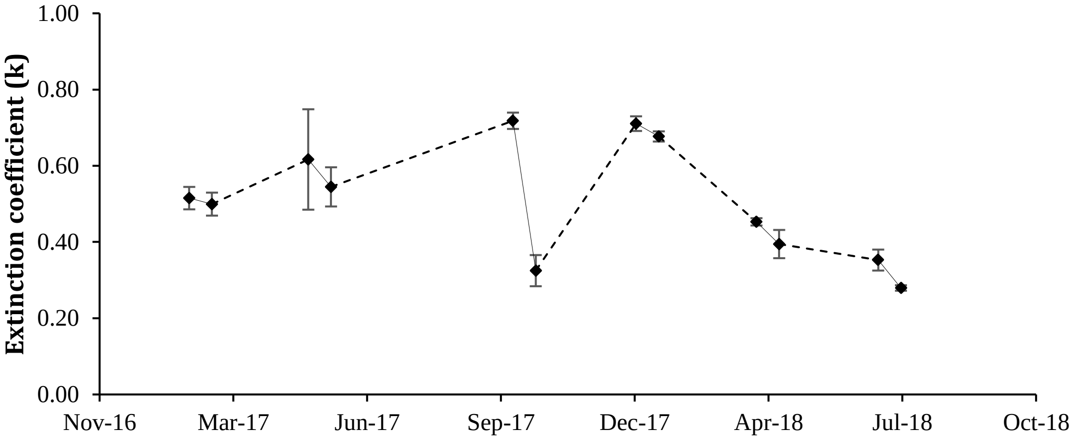


Figure 3.23 Light extinction coefficient (k) measured from February 2017-July 2018 from clone 45 grown at planting density 2000 trees.ha^{-1} and EW row-orientation. Values are means across three plots and two trees as replicates (+/- standard errors). Note: the solid line connects before and after conditions on each pruning; the dashed line connects pruning events.

The light extinction coefficient showed a similar pattern to fractional light interception, increasing from the beginning of the measurement period to reach a maximum between September and December 2017 and then falling to the end of the measurement period. The light extinction coefficient generally declined between 0.02-0.07 after each pruning except for the pruning carried out in September 2017, when the light extinction coefficient fell from 0.7 to 0.3 (Figure 3.23).

2. LAI, fractional light interception (%I), and extinction coefficient (k) difference among clones 45 and M01

Leaf area index (LAI) of clones 45 and M01 was measured in parallel only in period 1 (February 2017, May 2017, and October 2017) and period 3 (July 2018 and January 2019) in EW orientation and density 2000 trees.ha^{-1}, as described in section 3.3.8.

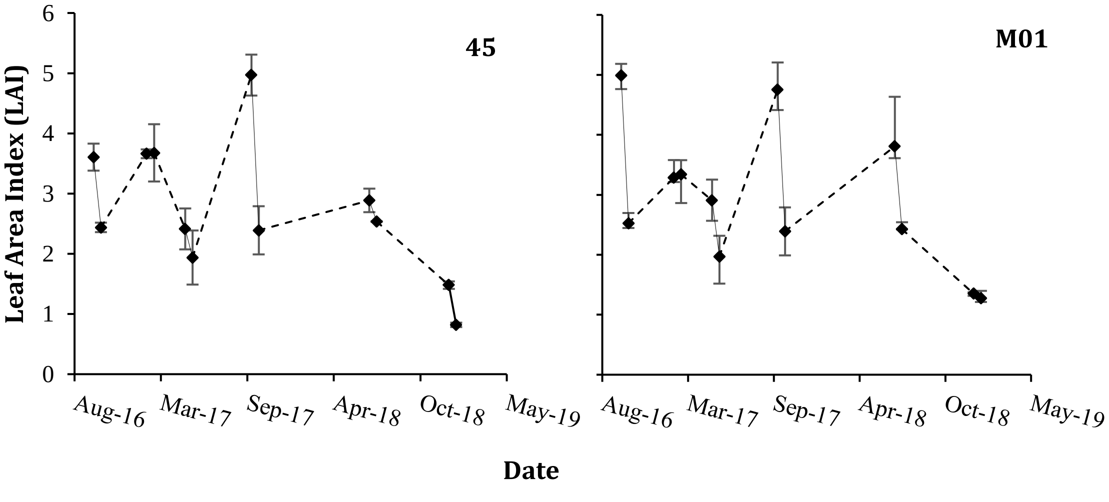


Figure 3.24 Leaf area index (LAI) from clones 45 (left) and M01 (right) measured from October 2016-January 2019, grown at planting density 2000 trees.ha^{-1} and EW row-orientation. Values are means across

three plots and two trees as replicates (+/- standard errors). Note: the solid line connects before and after conditions on each pruning; the dashed line connects pruning events.

Any LAI difference among clones was only observed in the initial period in October 2016 between clones M01 and 45, which has LAI around 5 and. However, no significant differences were observed in other periods (Figure 3.24). At the end of the measurement in January 2019, a 59-73% decline in LAI was measured in both clones for before pruning treatment compared to the initial period, and 49-66% decline for after pruning treatment. No interaction between clone and pruning time was observed during the measurement.

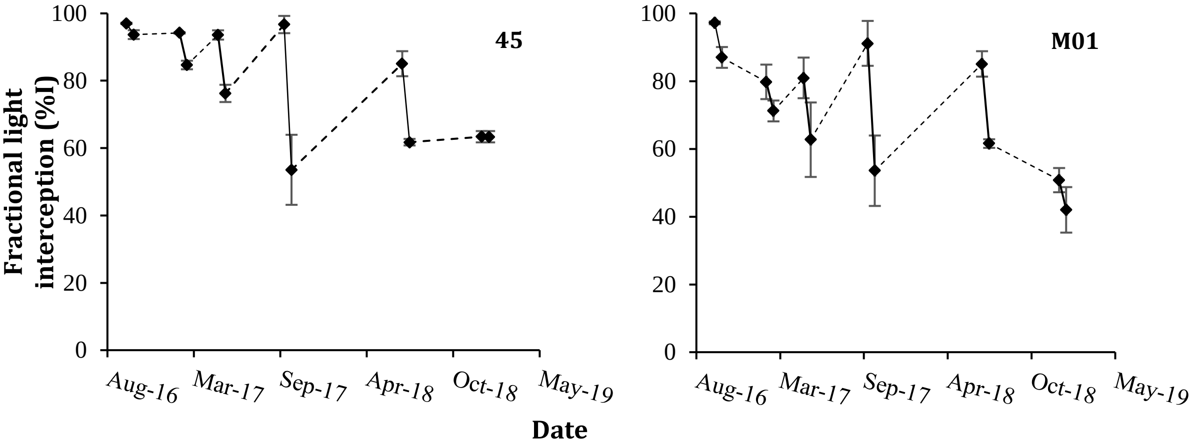


Figure 3.25 Fractional light interception (%I) from clones 45 (left) and M01 (right) measured from October 2016-January 2019, grown at planting density 2000 trees.ha^{-1} and EW row-orientation. Values are means across three plots and two trees as replicates (+/- standard errors). Note: the solid line connects before and after conditions on each pruning; the dashed line connects pruning events.

The fractional light interception was similar between similar throughout all measurements, as seen in Figure 3.25. No interaction between clone and pruning time was observed. At the end of the measurement period in January 2019, a 32-42% decline in %I was observed compared to August 2016 for before pruning treatment and 33-56% decline for after pruning.

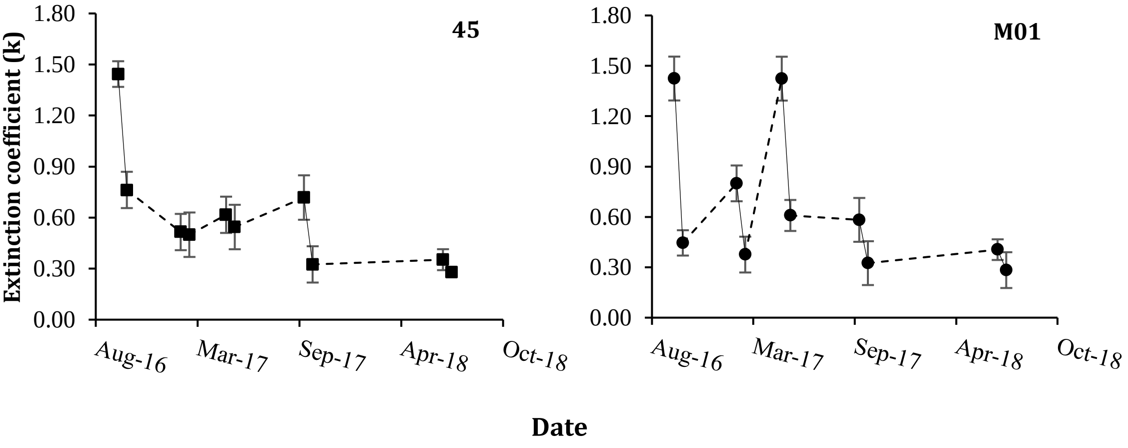


Figure 3.26 Light extinction coefficient (k) from clones 45 (left) and M01 (right) in Biomass 2 trial, measured from October 2016-July 2018, grown at planting density 2000 trees.ha^{-1} and EW row-

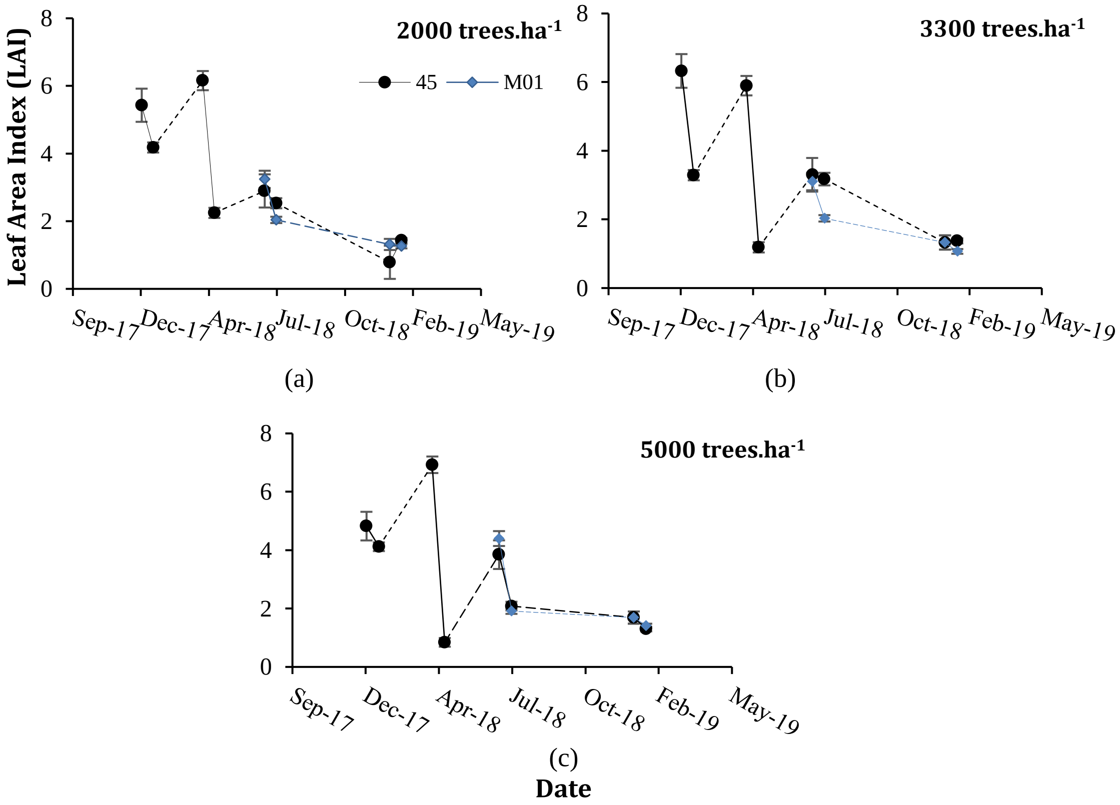
orientation. Values are means across three plots and two trees as replicates (+/- standard errors). Note: the solid line connects before and after conditions on each pruning; the dashed line connects pruning events.

No significant differences in extinction coefficient were observed between clones throughout the measurement period (Figure 3.26). No interaction between clone and pruning time was observed. At the end of the measurement period in January 2019, a 76-80% decline in k value was observed compared to August 2016 in both clones for before pruning treatment and 9-70% decline for after pruning.

3. LAI, fractional light interception (%), and extinction coefficient (k) differences between planting densities

Leaf area index (LAI) at densities of 2000, 3300, and 5000 trees.ha^{-1} was measured in parallel only in cycle 2 (January 2018 and April 2018) in EW orientation with clone 45; and cycle 3 (July 2018 and January 2019) in EW and NS orientations with clone 45 and M01, as described in the section 3.3.8.

LAI was highest at the planting density 5000 compared to 2000 and 3300 trees.ha^{-1} both before and after pruning for clone 45(P<0.001; Figure 3.27); for clone M01, this difference was not observed after pruning condition. No interaction between density and pruning time was observed during the measurement.



(a)

(b)

(c)

Figure 3.27 Leaf area index (LAI) from clones 45 and M01, grown in EW row-orientation at planting density (a) 2000, (b) 3300, and (c) 5000 trees.ha^{-1}, between January 2018-January 2019. Values are means across three plots and two trees as replicates (+/- standard errors). Note: the solid line connects before and after conditions on each pruning, and the dashed line connects pruning events.

No density or interaction effect was observed during the measurement on fractional light interception (I) and extinction coefficient (k).

4. Relationship between leaf area index and fractional light interception

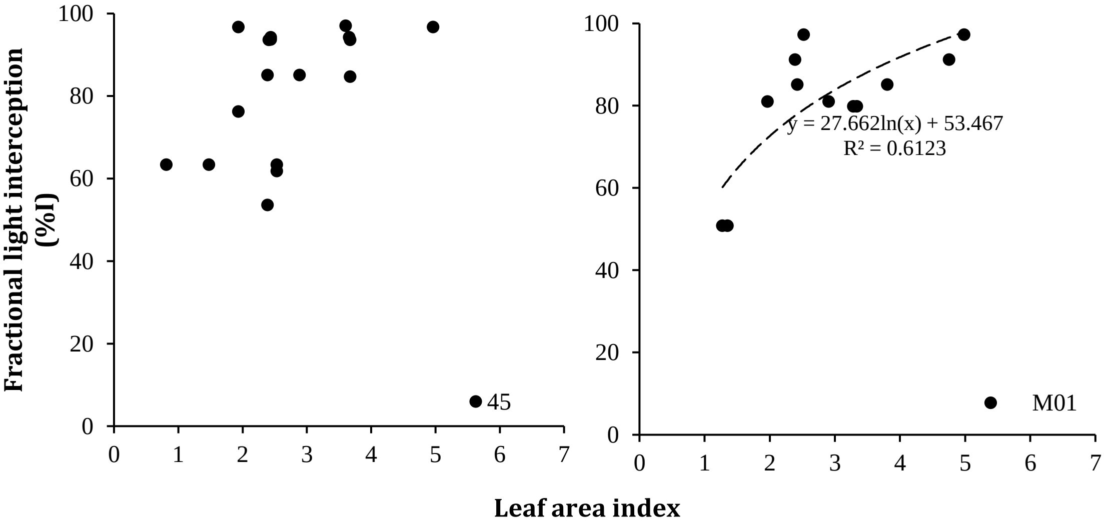


Figure 3.28 The relationship between leaf area index and fractional light intercepted from cacao trees with clones 45 (left) and M01 (right) in the east to west row-orientation.

Figure 3.28 shows the relation between leaf area index (LAI) and fractional light interception (I). In clone M01, fractional light interception positively correlated with leaf area index.

5. Relationship between leaf area index and potential yield per tree

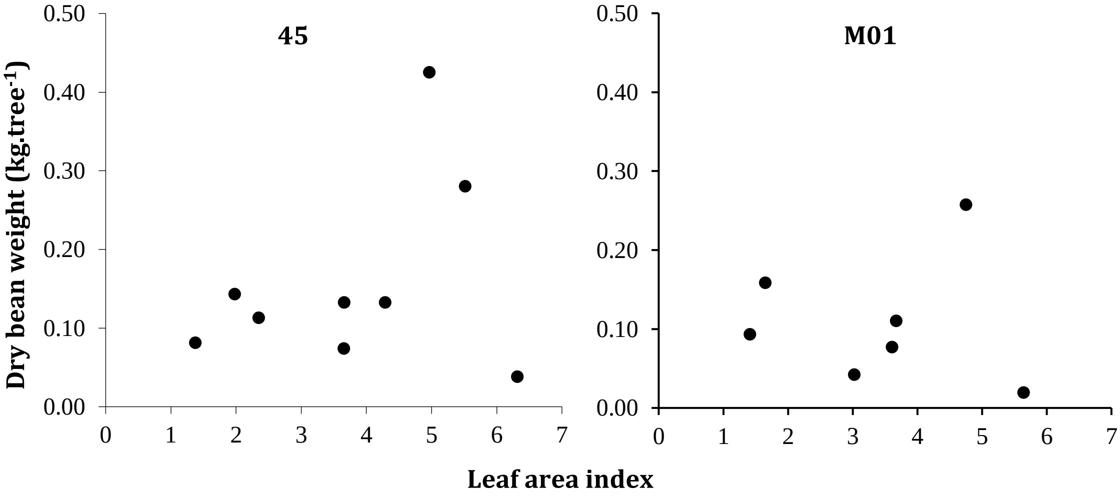


Figure 3.29 The relationship between leaf area index and potential yield per tree from cacao trees with clones 45 (left) and M01 (right). The sample trees were grown at different planting densities (2000, 3300, 5000 trees.ha^{-1}) and different row orientations (east to west and north to south)

Figure 3.29 shows the relationship between leaf area index (LAI) and potential yield per tree. However, there was no correlation between LAI and potential yield per tree observed in both clones.

6. Relationship between fractional light interception (%I) and potential yield per tree

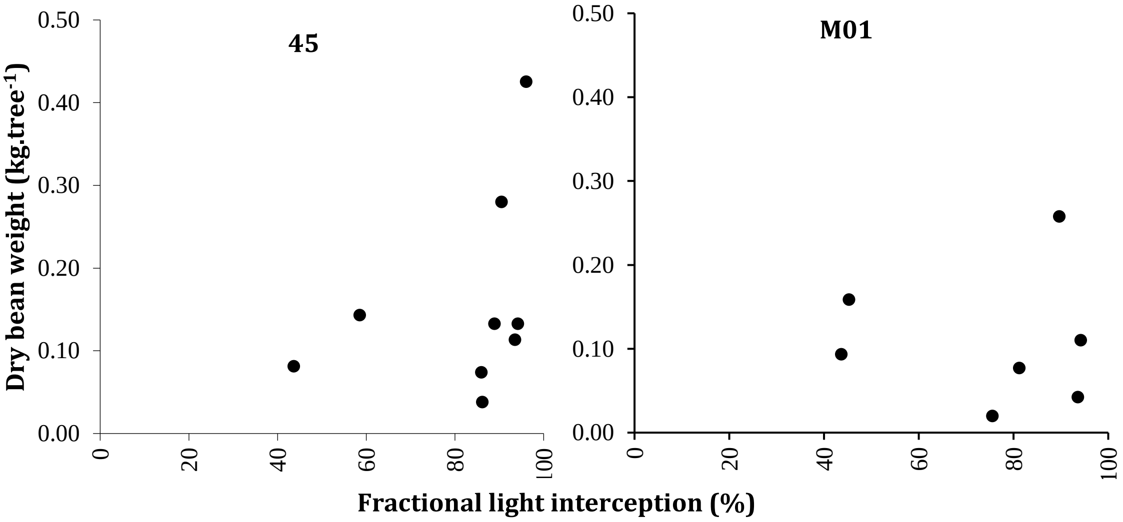


Figure 3.30 The relationship between fractional light interception and potential yield per tree from cacao trees with clones 45 (left) and M01 (right). The sample trees were grown at different planting densities (2000, 3300, 5000 trees.ha^{-1}) and different row orientations (east to west and north to south)

Figure 3.30 shows the relationship between fractional light interception (%I) and potential yield per tree. However, no correlation was observed for both clones.

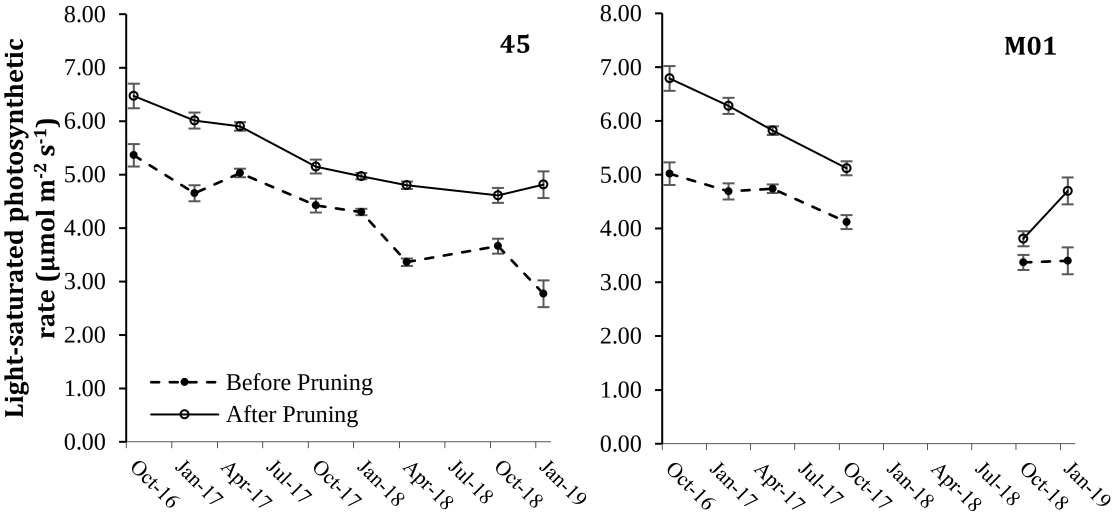
3.4.4 The effect of pruning and leaf age on the photosynthetic rate

Single leaf light-saturated net photosynthetic rate was measured before and after pruning on eight occasions from October 2016 until January 2019.

There were generally no two or three-way interactions between treatments on the single leaf light-saturated net photosynthetic rate, so only main effects are shown. A significant decline in the net photosynthetic rate for both clones was observed throughout the measurement period (P<0.001).

a. Effect of pruning

Light saturated leaf photosynthetic rate per unit area for both clones was significantly higher after each pruning (P<0.001) on each measurement occasion (Figure 3.31). In the cycle 2 measurement (January 2018 and April 2018), only clone 45 in east-west row-orientation at planting density 2000 trees.ha^{-1} was measured.

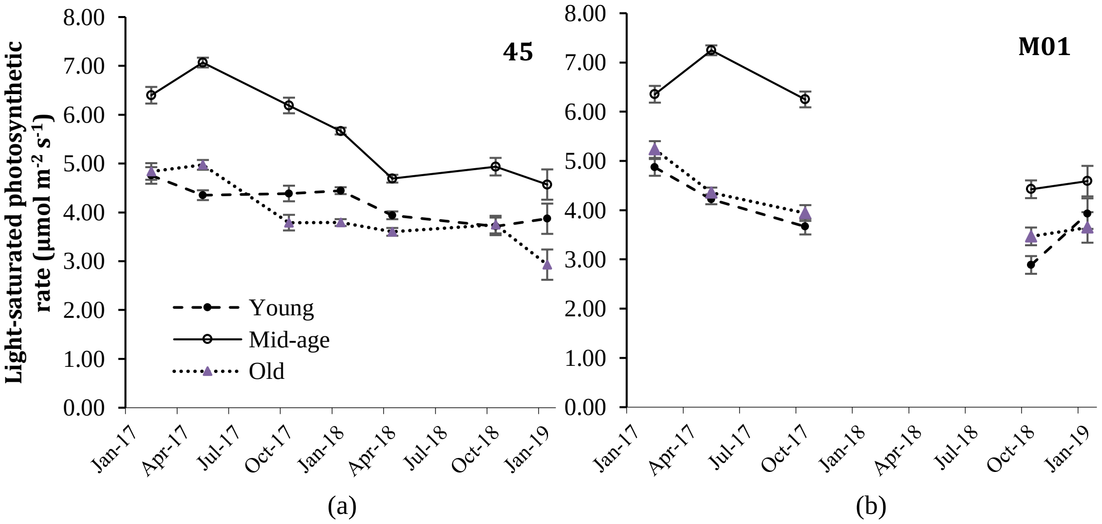


(a) (b)

Figure 3.31 Light-saturated photosynthetic rate measured before and after pruning, measured from October 2016-January 2019 in clones (a) 45 and (b) M01 grown from east to west row-orientation and at planting density 2000 trees.ha^{-1}. Values are means across three leaf-age samples, three sample trees as replicates, and three canopy heights (+/- standard errors)

b. Effect of leaf age

Light saturated leaf photosynthetic rate per unit area of middle-aged leaves was significantly higher than the older and younger leaves (P<0.001; Figure 3.32).

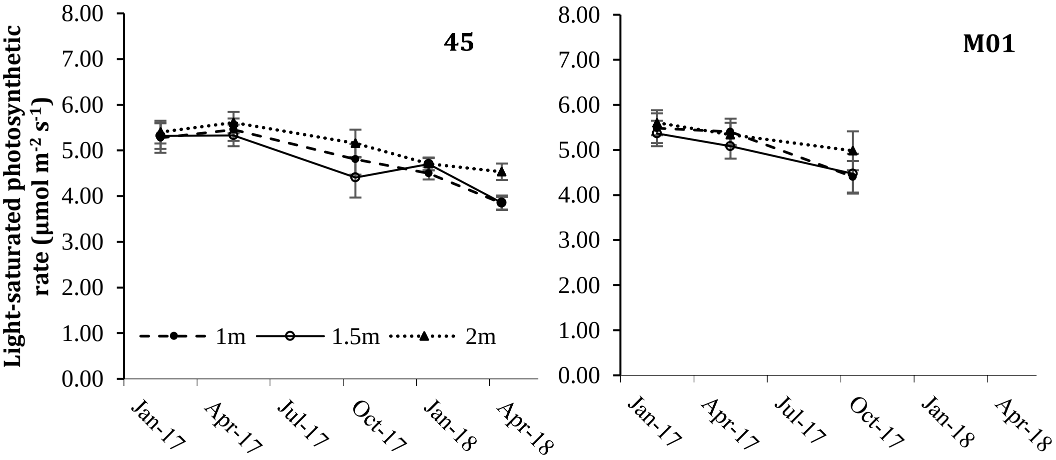


(a) (b)

Figure 3.32 Light-saturated photosynthetic rate of three different leaf ages (Young, Middle, Old) measured from February 2017-January 2019 in clones (a) 45 and (b) M01 grown from east to west row-orientation and at planting density 2000 trees.ha^{-1}. Values are means across three sample trees as replicates, two different pruning treatments, and three different canopy heights (+/- standard errors)

c. Effect of canopy height

Leaves at the highest level in the canopy had the highest light-saturated photosynthetic rate per unit area in (May 2017 (P=0.022), October 2017 (P=0.001), and April 2018 (P<0.001); Figure 3.33).



(a) (b)

Figure 3.33 Light-saturated photosynthetic rate of sample cacao trees in clones (a) 45 and (b) M01 from three different canopy heights (1m, 1.5m, and 2m) grown from east to west row-orientation and at planting density 2000 trees.ha^{-1}, measured from February 2017- April 2018. Values are means across two different pruning treatments, three different leaf ages, and three sample trees as replicates

d. Effect of planting density and row orientation

The photosynthetic rate of clone 45 grown at different planting densities was measured in January and April 2018. In April 2018, small differences in photosynthetic rate were observed. The photosynthetic rate was lowest in density 2000 (3.68 µmol m^{-2} s^{-1}) compared to trees in density 3300 (4.37 µmol m^{-2} s^{-1}) or density 5000 trees.ha^{-1} (4.18µmol m^{-2} s^{-1}; P=0.003).

The photosynthetic rate of clones 45 and M01 grown at different row orientations were measured in October 2018 and January 2019. No differences in photosynthetic rate among row-orientations were recorded in October 2018 and January 2019. However, in October 2018, trees with clone M01 had a lower photosynthetic rate (P=0.030) compared to clone 45 ^{1} (3.82 µmol m^{-2} s^{-1} compared to ^{1} 4.15 µmol m^{-2} s^{-1}).

3.4.5 Effect of different planting density, clones, and row-orientation on vegetative growth

3.4.5.1 Trunk diameter

Annual girth increment was measured during the fruit-bearing phase, between January 2017 and December 2018 (two years).

Trunk diameter increment was not affected by the row-orientation in both years, so the data for both orientations were combined (Figure 3.34). In general, trunk diameter increment for clone 45 was higher than for clone M01 at each density in years 1 (P<0.001) and 2 (P<0.001).

Trunk diameter increment declined with increasing density in both clones in year 1 in both row-orientations (P<0.001) and also in year 2 (P=0.007).

A significant decline in average trunk diameter increment was observed in year 2 compared to year 1 in both clones in each row-orientation (P<0.001).

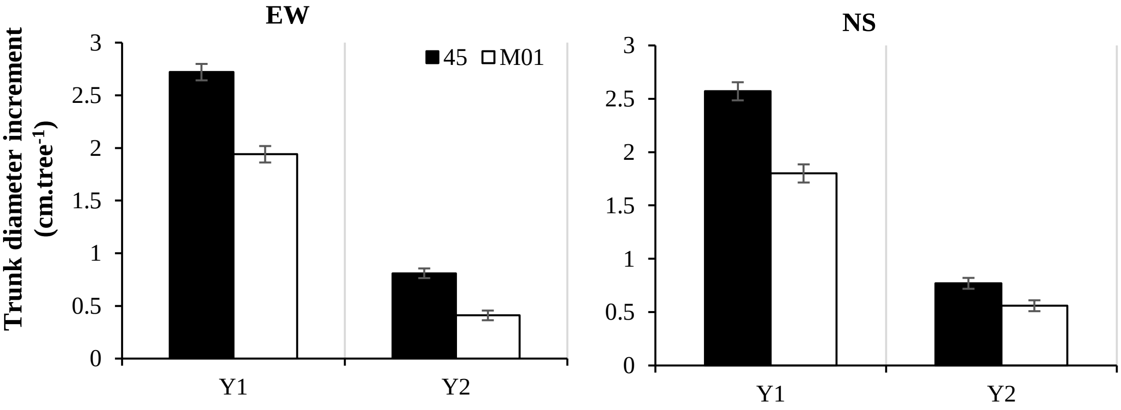
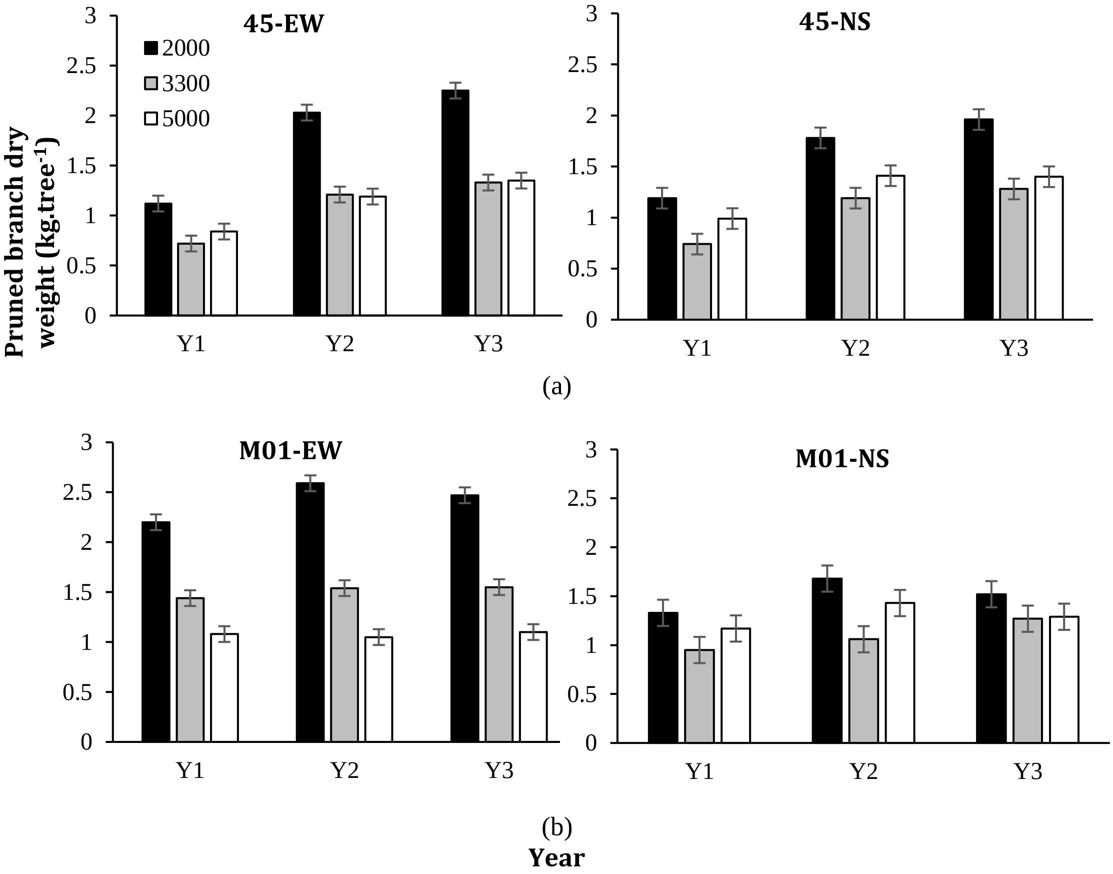


Figure 3.34 Trunk diameter increment per tree for clones 45 (left) and M01 (right), measured between January and December 2017 (year 1) and between January and December 2018 (year 2). Values are

3.4.5.2 Weight of pruned branches



(a)

(b)

Figure 3.35 Pruned branch dry weight per tree from clones (a) 45 and (b) M01 grown at three planting densities (2000, 3300, and 5000 trees.ha^{-1}) from east to west (left) and north to south (right) row-orientation, measured in years 1, 2, and 3, corresponding to August 2016 to July 2017, August 2017 to July 2018, August 2018 to July 2019. Values are means across three replicate plots (+/- standard errors)

Clone 45 produced less pruned branch weight per tree compared to clone M01 in the east-west (EW) row-orientation in year 1 ($P<0.001$) and year 2 ($P=0.007$; Figure 3.37). However, no clone effect was observed in year 3 in the EW row-orientation. Clonal differences were also not observed in the north-south (NS) row-orientation in three consecutive years.

Pruned branch weight was higher for planting density 2000 compared to 3300 and 5000 trees ha^{-1} in each of the three years (year 1 ($P<0.001$), year 2 ($P<0.001$) and year 3 ($P<0.001$)) in EW and also NS row-orientations in year 1 ($P=0.007$), year 2 ($P<0.001$), and year 3 ($P<0.001$).

Pruned branch weight per tree increased significantly over the three years (P<0.001) in both orientations at each density for clone 45; but only slightly increased for clone M01, especially in year 2.

Pruned branch weight in the NS orientation was lower than EW orientation in year 1 (P=0.006), year 2 (P=0.013), and year 3 (P<0.001). Also, in EW row-orientation, in both clones, the highest weight of pruned material per tree was observed for density 2000 trees.ha^{-1} in year 1 (P<0.001), year 2 (P=0.008), and year 3 (P=0.022).

3.4.5.3 Weight of pruned leaves

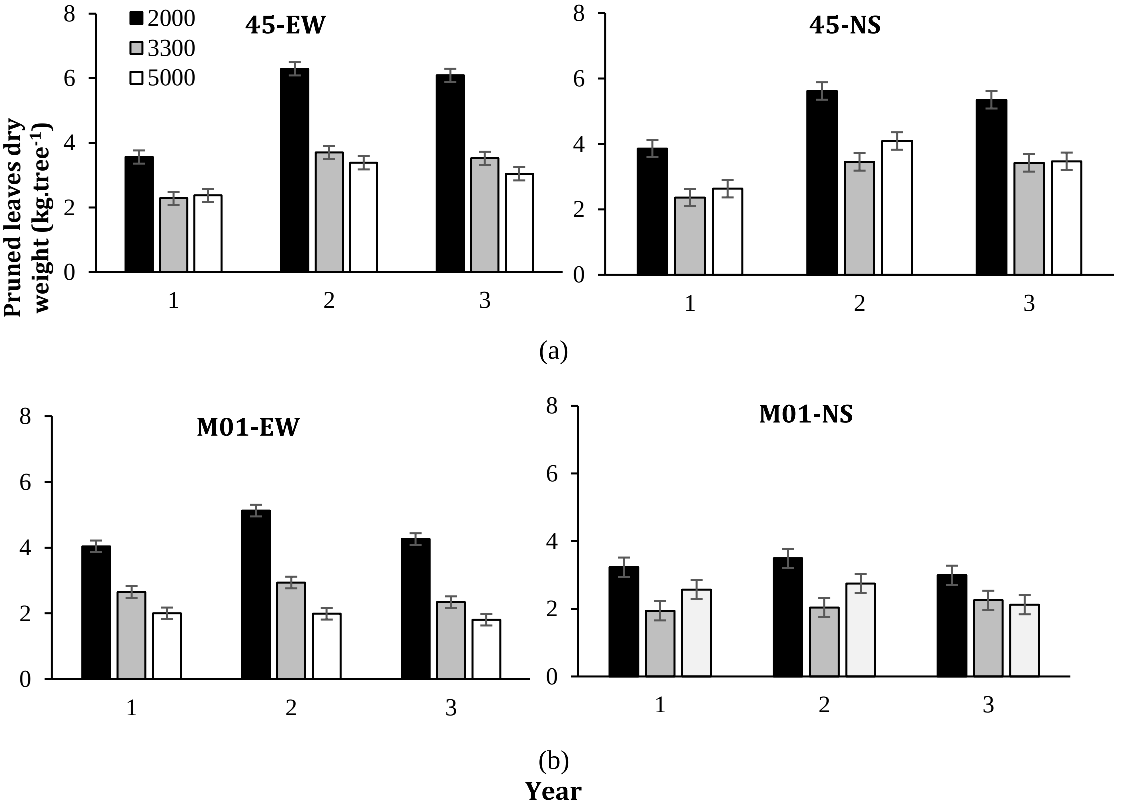


Figure 3.36 Pruned leaves dry weight per tree from clones (a) 45 and (b) M01 grown at three planting densities (2000, 3300, and 5000 trees.ha^{-1}) from east to west (left) and north to south (right) row-orientation, measured in years 1, 2, and 3, corresponding to August 2016 to July 2017, August 2017 to July 2018, August 2018 to July 2019. Values are means across three plots as replicates (+/- standard errors)

Clone 45 generally produced a higher weight of pruned leaves per tree compared to clone M01 except in year 1 for both row-orientations (P<0.001) (Figure 3.38).

Pruned leaves weight per tree at a density of 2000 trees.ha^{-1} was higher than at the other densities for both orientations (P<0.001). However, in EW orientation, pruned leaves weight was higher in year 2 than in years 1 and 3 (P<0.001), but not in NS.

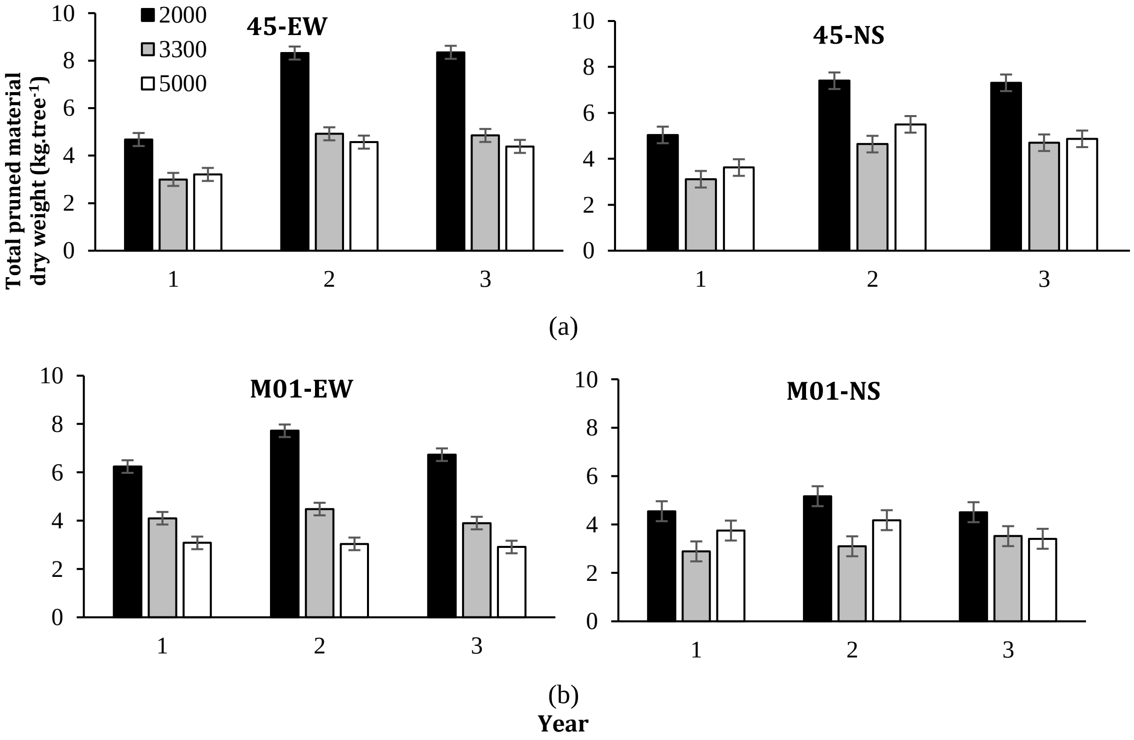
The average pruned leaves weight per tree for both clones was 38% and 24% higher in years 2 and 3, respectively, compared to year 1 in EW orientation. However, in the NS orientation, years 2 and 3 were 28% and 18% higher than year 1. Thus, in general, pruned leaves weight in EW is 7% higher overall than in NS (P=0.013).

Pruned leaves weight per tree of clone 45 did not show a significant difference between row-orientations. However, pruned leaves weight per tree of clone M01 in the EW orientation was 16% higher than in the NS orientation (P=0.013).

In the EW orientation, pruned leaves weight per tree generally declined, with tree density increasing (P<0.001) in all three years. However, this pattern was not as apparent in the NS orientation.

3.4.5.4 Total pruned materials (branches and leaves) weight

The total dry weight of pruned materials was calculated as an accumulation from the pruned branch and pruned leaves weight (as already described in sections 3.4.2.1.2 A and B.



(a)

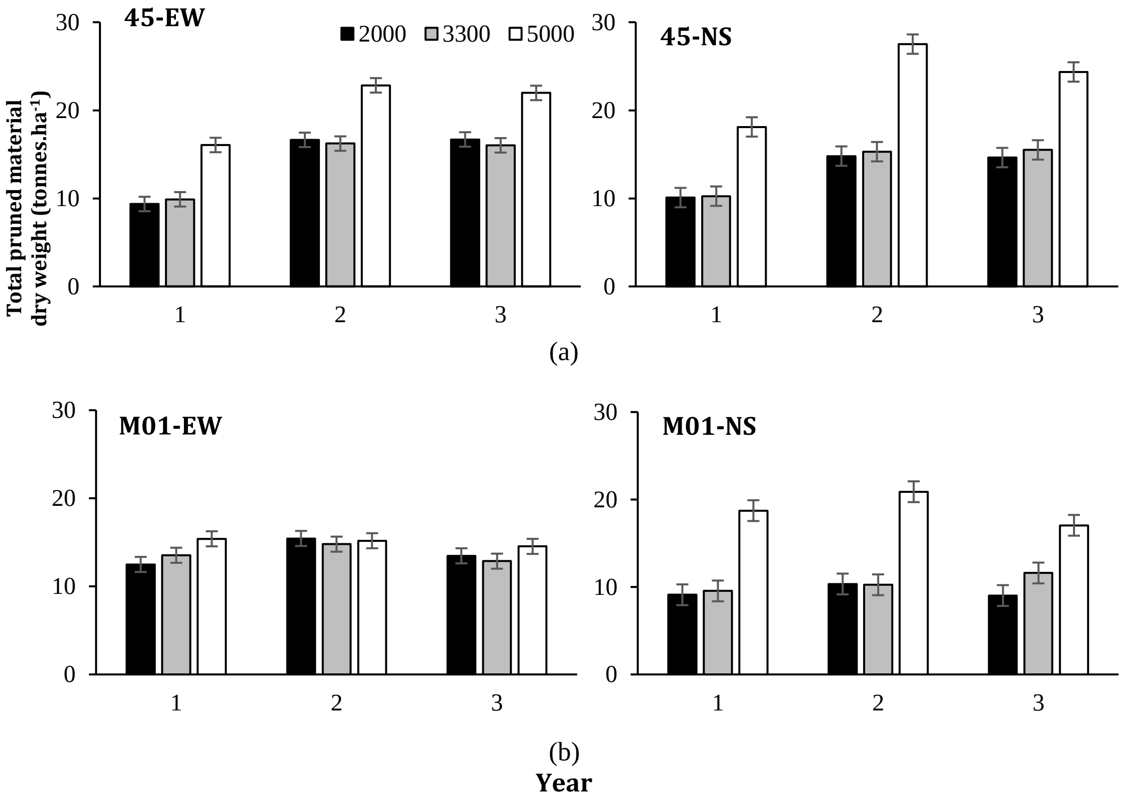
(b)

Figure 3.37 Total pruned material dry weight per tree from clones (a) 45 and (b) M01 grown at three planting densities (2000, 3300, and 5000 trees.ha^{-1}) from east to west (left) and north to south (right) row-orientation, measured in year 1, 2, and 3, corresponding to August 2016 to July 2017, August 2017 to July 2018, August 2018 to July 2019. Values are means across three plots as replicates (+/- standard errors)

The total pruned material (branch and leaves) weight per tree was significantly higher in clone 45 than in clone M01 in years 2 (P=0.002) and 3 (P<0.001), as seen in Figure

3.39. In year 1 in the EW orientation, the total pruned material weight from clone 45 was lower than clone M01 (P<0.001), but this did not occur in NS orientation.

On a hectare basis, the highest total pruned material weight was at the highest density (P<0.001), except in years 2 and 3 for clone M01 grown from east-west, as seen in Figure 3.40. In general, except in year 1, the total pruned materials weight in clone 45 was higher than in clone M01 (P<0.001). In year 1, sample trees grown from east-west, the total weight of pruned material from clone M01 was higher than 45 (P=0.002). The highest pruned material weight was observed from clone 45, with planting density 5000 trees.ha^{-1} grow from east-west in production year 2 (22.83 tonnes.ha^{-1} year^{-1}). The lowest weight was observed from the sample trees with clone 45 grown in planting density 2000 trees.ha^{-1} from east-west in production year 1 (9.36 tonnes.ha^{-1} year^{-1}).



(a)

(b)

Figure 3.38 Total pruned material dry weight per hectare from clones (a) 45 and (b) M01 grown at three planting densities (2000, 3300, and 5000 trees.ha^{-1}) from east to west (left) and north to south (right) row-orientation, measured in year 1,2, and 3, corresponding to August 2016 to July 2017, August 2017 to July 2018, August 2018 to July 2019. Values are means across three plots as replicates (+/- standard errors).

3.4.5.5 Weight of senescence leaves

There was no significant difference in senescent leaves weight per tree between the NS and EW orientations in either year. Therefore, data for each orientation have been combined (Figure 3.41). The senescent leaves weight per tree from clone M01 was higher than for clone 45 for years 1 and 2 ($P<0.001$). Also, senescent leaves weight per tree for clones 45 ($P<0.001$) and M01 ($P=0.003$) was lower in year 2 than in year 1.

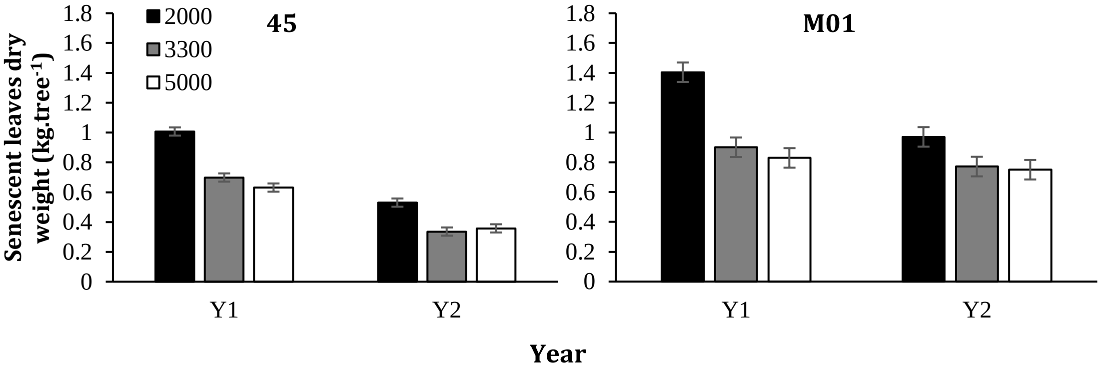


Figure 3.39 Senescent leaves dry weight per tree grown at three different planting densities (2000, 3300, and 5000 trees.ha^{-1}) in clones 45 (left) and M01 (right) for year 1 (January-December 2017) and year 2 (January-December 2018). Values are means across the two orientations and three replicate plots (+/- standard errors)

In year 1, senescent leaves weight per tree declined with increasing density ($P<0.001$). Similarly, in year 2, senescent leaves weight per tree was highest at a density of 2000 trees.ha^{-1} than at 5000 trees.ha^{-1} and 3300 trees.ha^{-1} ($P<0.001$).

Figure 3.42 shows senescent leaves in years 1 and 2 relatives to pruning time. The weight of senescent leaves was the lowest following each pruning, but this increased in the period prior to the next pruning time.

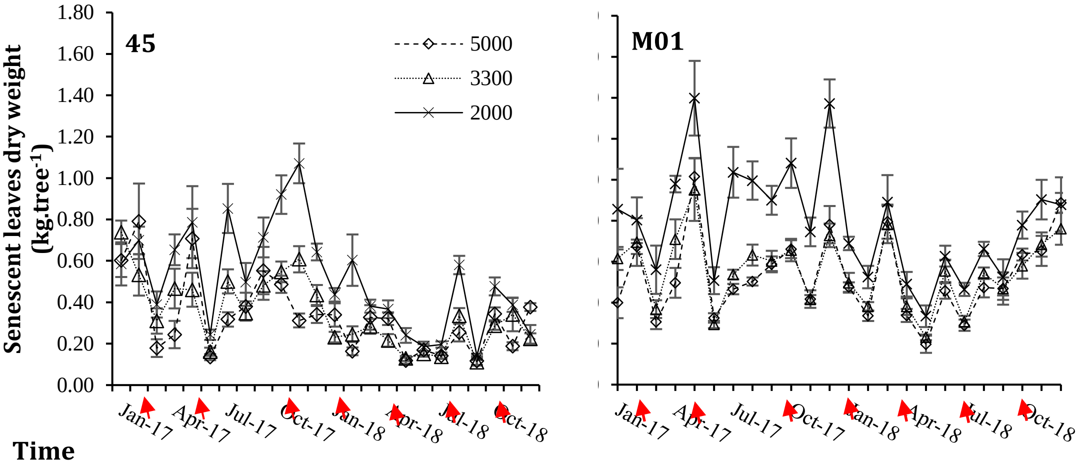


Figure 3.40 Senescent leaves dry weight per tree grown at three different planting densities (2000, 3300, and 5000 trees.ha^{-1}) in clones 45 (left) and M01 (right) in relation to the pruning times represented by the red-coloured arrow. Values are means across three plots as replicates and two different row-orientations (+/- standard errors)

Similar to the results on a per tree basis, the weight of senescent leaves per area basis (Figure 3.43) from clone M01 was higher than for clone 45 for years 1 and 2 (P<0.001). The weight of senescent leaves per ha for both clones was lower in year 2 than in year 1 (P<0.001). No row-orientation effect was observed, and a positive correlation between the senescent leaves weight and planting density was observed. The highest senescent leaves weight was observed in clone M01 with planting density 5000 trees.ha^{-1} in year 1 (2.05 tonnes.ha^{-1}). The lowest was seen in clone 45 with planting density 2000 trees.ha^{-1} in year 2 (1.05 tonnes.ha^{-1}).

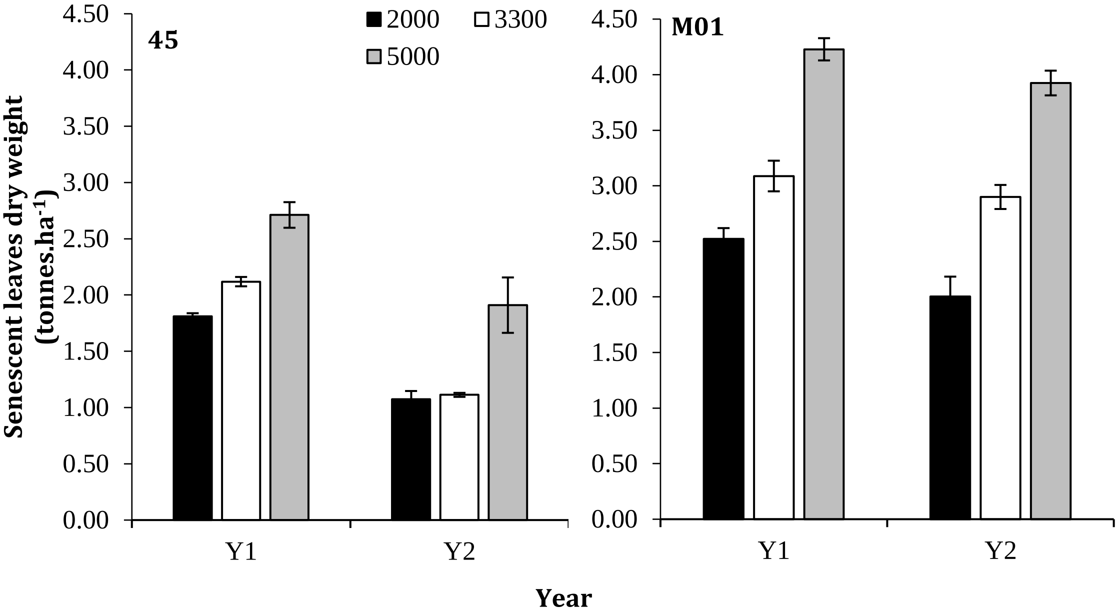


Figure 3.41 Senescent leaves dry weight per hectare grown at three different planting densities (2000, 3300, and 5000 trees.ha^{-1}) in clones 45 (left) and M01 (right) for year 1 (January-December 2017) and year 2 (January-December 2018). Values are means across the two orientations and three replicate plots (+/- standard errors)

3.4.6 Relationship between factors

3.4.6.1 Relation of yield and trunk diameter increment

For clone 45, the annual yield was linearly and positively related to trunk diameter increment for year 1 (P<0.001; R^2=0.72) and year 2 (P<0.001; R^2=0.59), as shown in Figure 3.44. However, there was no correlation between the annual yield and trunk diameter increment in clone M01 in both years.

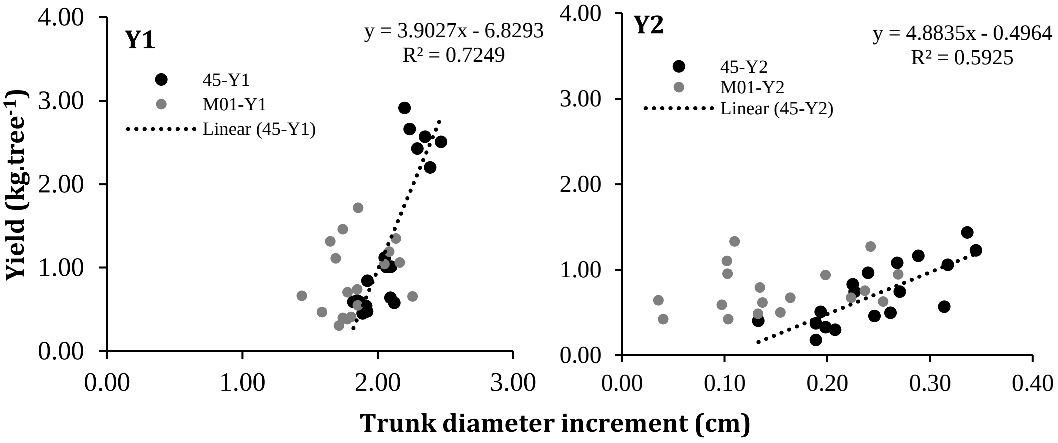
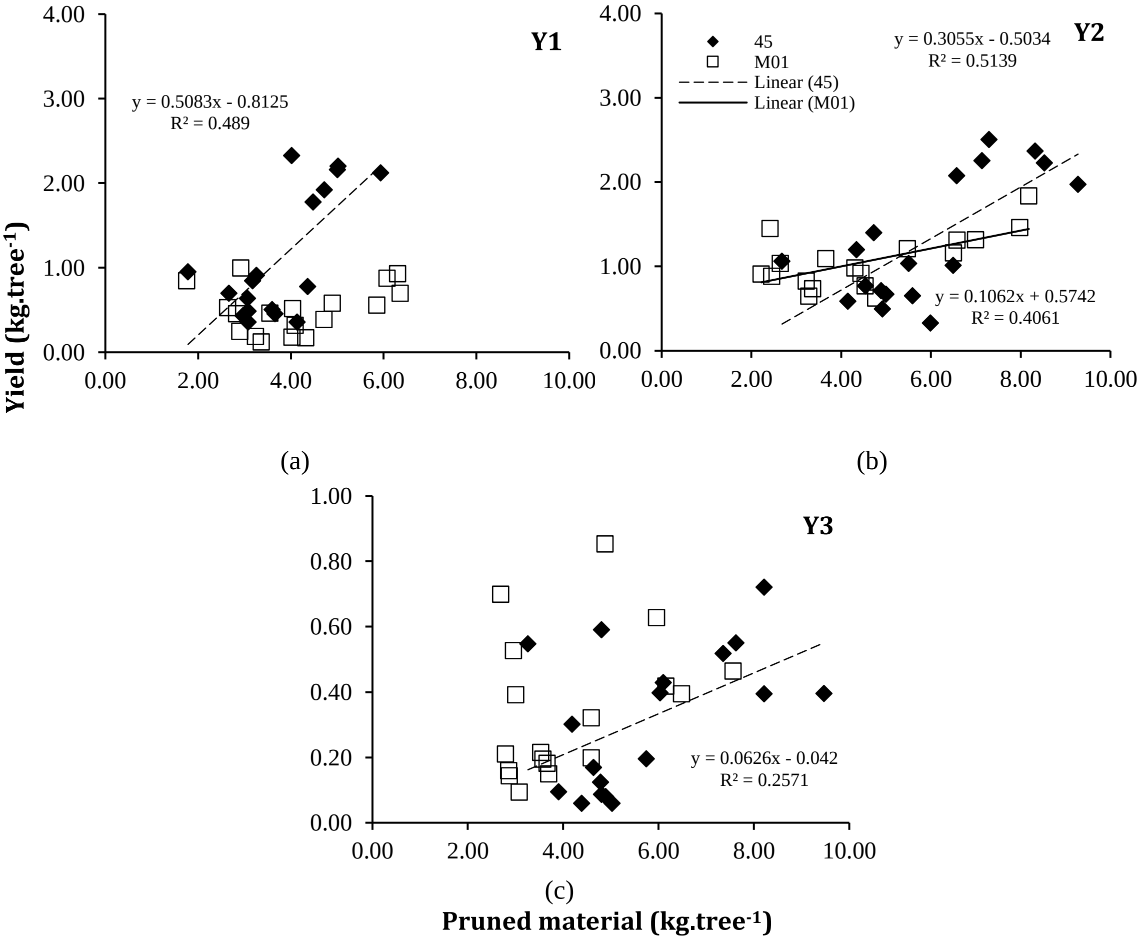


Figure 3.42 Relationship between yield and trunk diameter increment per tree in year 1: January 2017-January 2018 (left) and year 2: January 2018-January 2019 (right), from trees grown at three different densities and two row-orientations in the Biomass 2 trial with clones 45 and M01. Values are means across three plots as replicates. Note: x-axis scale for year 1 was enlarged compared to year 2.

3.4.6.2 Relationship between yield and pruned material weight



(a)

(b)

(c)

Figure 3.43 Relationship between yield and pruned material weight per tree in (a) year 1: August 2016-July 2017, (b) year 2: August 2017-July 2018, and (c) year 3: August 2018-July 2019 from trees grown at three different densities and two row-orientations. Values are means across three plots as replicates. Note: y-axis scale for years 1 and 2 was enlarged compared to year 3

As seen in Figure 3.45, in years 1 and 2, clone 45 has a higher yield to pruned material ratio than clone M01. However, both clones behaved similarly in year 3. There was a positive linear relationship between pruned material and yield for clones 45 in year 1 ($P=0.001$; $R^2=0.49$), year 2 ($P<0.001$; $R^2=0.51$), and year 3 ($P=0.031$; $R^2=0.26$); whilst the correlation was seen in clone M01 only in year 2 ($P=0.004$; $R^2=0.41$).

3.5 Discussion

3.5.1 The performance of different clones on yield and assimilate partitioning

a. Yield

This study aimed to compare the performance of cacao clones 45 and M01 in terms of pod production and yield in different planting densities and row orientations. Results showed no significant difference in cacao pod size between the two clones. However, pod production in clone 45 was more evenly spread throughout years 1 and 2, and its potential yield per tree was higher than clone M01, mainly in planting density 2000 trees.ha^{-1}. In contrast, clone M01 exhibited higher tolerance to high planting density, with less reduction in yield observed in higher density conditions of 3300 and 5000 trees.ha^{-1}. In planting density 2000 trees.ha^{-1}, compared to clone M01, the potential yield per tree from clone 45 was 167% higher between August 2016-July 2017 (year 1) and 40% higher between August 2017-July 2018 (year 2). In contrast, in year 3 (August 2018-July 2019), trees from clone 45 had a slightly 2.4% higher potential yield per tree than M01.

Regarding yield, traditional cacao farming uses a planting density of 625 trees.ha^{-1} with clone 45 could produce around 1.56 tonnes.ha^{-1} year^{-1} dry bean, while clone M01 could yield up to 2.3 tonnes.ha^{-1} year^{-1}. Meanwhile, using the trellis system in the Biomass 2 trial with planting density ranging from 2000 to 5000 trees.ha^{-1} resulted in a higher potential yield for clone 45, particularly in years 1 and 2, compared to clone M01. The potential yield for year 1 ranged from 2.16 to 4.46 tonnes.ha^{-1} for clone 45 and 1.4 to 1.67 tonnes.ha^{-1} for M01. In year 2, the annual yield increased to 3.37-4.3 tonnes.ha^{-1} for 45 and 3.07-3.78 tonnes.ha^{-1} for M01. Whilst in year 3, the yield for 45 was 0.05-0.13 tonnes.ha^{-1} and 0.02-0.16 tonnes.ha^{-1} for M01. Clone 45 has a greater potential to grow on the high-density system with trellis up to year 2 production compared to clone

M01 due to less vigorous characteristics. However, both clones exhibited declining yield in year 3 due to source limitation caused by heavy pruning.

Corley (1983) estimated that the potential annual above-ground biomass productivity for cocoa could reach up to 56 tonnes ha^{-1}, with a maximum seed yield of 11 tonnes ha^{-1}. However, the best-recorded yields without shade are only 4.4 tonnes ha^{-1}, and commercial yields range from 1.5 to 2.5 tonnes ha^{-1}. Factors that limit yield include water stress, pests and diseases, shade trees, high and low temperatures, and foliage susceptible to wind damage.

Furthermore, row orientation was found to affect pod quality, with the East-to-West (EW) orientation having the lowest ratio of sun-damaged pods compared to the North-to-South (NS) orientation. In NS, during the day, trees receive a longer duration of light exposure than EW. The fact that there were fewer sun-damaged pods in clone 45 may be attributed to the higher anthocyanin content in clone 45, preventing colour formation from light exposure (Kim et al., 2011). Similar sun-damaged symptoms were also observed in apples (Severino et al., 2021), with polyphenols and β-carotenoids synthesis being the protective mechanism against photo-oxidative stress which is caused by high solar incident radiation and high air temperature (Schrader et al., 2003; Piskolczi et al., 2004; Wünsche et al., 2005; Felicetti et al., 2008; Yuri et al., 2010). Other factors, such as tree vigour, orchard characteristics, presence of windbreak, row direction, and sensible heat of the fruit, may also contribute to sun-damaged symptoms (Yuri et al., 2000; Torres et al., 2016a; Torres et al., 2016b; Severino et al., 2020; Szabo et al., 2021).

Lastly, the percentage of pod production from lateral branches decreased over time from January 2017 to January 2019. This was likely due to the reduced amount of light received by these branches, resulting in changes in growth substances and carbohydrate distribution. Therefore, the horizontal branches grew less vigorously and became less robust.

b. Assimilate partitioning

The study combined pruning weight measurements with yield measurements to determine yield-to-pruning weight ratios. This method indirectly measures tree productivity, as it represents the balance between vegetative and reproductive growth (Smart and Robinson, 1991).

Clones 45 and M01 differed in biomass partitioning only in the first year of production, consistent with previous research that demonstrated variation in yield efficiency and harvest index among cacao clones (Yapp & Hadley, 1994; Daymond, 2002b). These findings suggest that yield improvement programs could further exploit such variability.

The planting density of 2000 trees ha^{-1} resulted in higher bean and pod harvest index values in all three production years due to reduced competition for resources, compared to other densities.

Overall, the observed yield efficiency and harvest index values for cacao clones 45 and M01 were relatively low compared to other tree species (Corley, 1983). The clone used in this study has excessive vegetative biomass in comparison to generative biomass, which made it unsuitable for use at high planting density on the trellis system. Previous research efforts have been focused on increasing harvest index and yield efficiency in cocoa by using dwarfing rootstocks, but limited studies have reported the value range of these measurements.

Daymond et al. (2002a; 2002b) demonstrated that selectively breeding for efficient biomass partitioning to the yield component can enhance cocoa yields. In a field experiment conducted in Bahia, Brazil, a seven-fold difference in dry bean yield was observed among 12 genotypes over 18 months, with yield efficiencies ranging from 0.008 kg cm^{-2} to 0.08 kg cm^{-2}, with beans accounting for 32% to 45% of pod biomass among the seven clones compared.

In this study, it was observed that the yield efficiency of clones 45 and M01 varied between 0.02-0.05, with 38-46% pod biomass.

3.5.2 The effect of pruning and intensive growing system on phenology of two Sulawesi cacao clones

Although variation in flowering rate pattern was observed at certain times, pruning did not inhibit flower formation noticeably since the flowering process, once initiated, is not suppressed quickly. On the contrary, pruning encourages more flow of nutrients and water to the remaining shoots that flowers develop to form fruits (Dhillon and Thakur, 2014). Optimal pruning will ensure a balance between loss of potential bearing surface and the nutrient supply.

The califerous nature of cacao trees does not significantly impact their flowering surface area compared to other tree crops. Cacao trees' distinctive flowering and fruiting patterns could pose challenges in cultivation and harvesting compared to other crops. The flowers grow directly on the trunk and older branches, necessitating skilled labour for proper pruning and maintenance and a more labour-intensive and time-consuming harvest as the fruit grows directly on the trunk and branches.

The planting density of 2000 trees ha^{-1} resulted in the highest flowering index, partly due to reduced competition and improved access to vital resources, such as light and nutrients, compared to other planting densities. A similar pattern of flowering intensity increasing by density declining was also observed in the previous studies on bean bush *(Phaseolus vulgaris*) (Doust, 1992), paprika pepper (*Capsicum annum* L.) (Jovicich et al., 2003; Ara et al., 2007: Aminifard et al., 2010), and tomato (*Lycospersicon esculentum)* (Law-Ogbomo and Egharevba, 2009).

3.5.3 Canopy characteristics of two clones grown at different planting densities and orientation

In this study on intensive growing systems grown on the trellis, the leaf area index ranged from 1.01 to 5.69 over the three-year measurement period. Other studies (Daymond et al., 2002a) indicate that the leaf area index from cocoa grown conventionally in Bahia, Brazil, ranged from 2.8 to 4.5. The results of Daymond et al. (2002a) studies revealed the breeding potential for more photosynthetically efficient cacao canopies. A previous study has recommended that cacao's optimal leaf area index grown in the conventional system is around 4.0 (Yapp & Hadley, 1994). Based on these previous findings, it is difficult to maintain the condition of these vigorous clones in this study (clones 45 and M01) at these unusually high densities with frequent heavy pruning.

The leaf area index for trees at a density of 5000 trees.ha^{-1} was 10-52% higher than trees at a density of 2000 and 3300 trees.ha^{-1}reflecting the greater number of trees per unit area. In particular, the overlapping canopy volume in the double row at the density 5000 trees.ha^{-1} caused its leaf area index to be more extensive.

In general, the leaf area index declined throughout the period between October 2016 and April 2019. This decline was correlated with the limited plant source to produce vegetative biomass. Thus, differences in the leaf area index could explain overtime corresponding changes in the fractional light interception.

Fractional light interception rose from the beginning of the measurement and then gradually declined to the end of the measurement period. The fractional light interception observed was generally higher than the values reported by Daymond et al. (2002a), who reported values ranging from 75%-88% for different clones grown at lower densities and conventional cacao growing system. These trellis/conventional system and planting densities difference would make the value difference of fractional light interception measured. Previous studies were also observed fractional light interception difference between plant canopy grown at different densities, such as in sunflowers (Ferreira and Abreu, 2001) and apples (Tustin et al., 2022); also between different systems in apples (narrow-row and planar cordon) (Tustin et al., 2022).

A linear relationship between leaf area index and a fractional light interception for various crops have been observed for many species, e.g. for potato (Milthorpe & Moorby, 1974; Scott & Wilcockson, 1978), apple (Monteith, 1977), and Indian mustard (Kumar et al., 1997). A curvilinear relationship has also been observed in others, such as potato (Firman and Allen, 1989), soybean (Wells, 1991), and maize (Maddonni and Otegui, 1996). This study indicated a linear relationship for clone 45 (Figure 3.44), similar to the relationship observed by Daymond et al., 2002a.

A range of extinction coefficients from 0.20 to 0.83 was lower than previous studies noted. For example, Daymond et al.(2002a) observed the extinction coefficient from cacao in Bahia, Brazil, from 0.63 to 0.82 for different clones; whilst Yapp (1992) observed values from 0.61 to 0.96 for different genotypes and (Zuidema et al., 2003) quoted values between 0.4-0.7. Previous data collected in Bahia on the `Catongo' variety have suggested mean extinction values of 0.62 (Alvim, 1977) and 0.61 (Miyaji et al., 1997). On the other hand, the relatively lower values recorded in this study may be related to the vertical and horizontal branches trained on the trellis in this study, thus allowing more solar radiation to penetrate through the canopy.

The extinction coefficient for cocoa trees at a density of 5000 trees.ha^{-1} was observed to be higher than at other densities, which was likely related to a large proportion of self-shading, a higher leaf area index and lower fractional light intercepted. However, in previous research, some cacao clones perform better at high densities (Lockwood and Yin, 1996).

In clone 45, yield per tree basis positively correlates with leaf area index and fractional light interception (%I) that it was curvilinear, so there was a benefit of an increase in LAI only up to a particular point. During vegetative growth in environments with optimal water and mineral nutrients supplies, the crop growth rate is a linear function of the amount of radiation intercepted (Milthorpe and Moorby, 1974). Similar results were also observed in the yield of sugar beet and potatoes (Scott and Wilcockson, 1978) and also in cereals and apples (Monteith, 1977).

3.5.4 The effect of pruning and leaf age on the photosynthetic rate in Biomass 2 trial

The light-saturated photosynthetic rate of leaves increased by 15-54% after pruning. A possible explanation for this increase in photosynthetic rate is that pruning stimulates new leaf growth, altering the source-sink ratio. The results suggest that cacao might increase its assimilation rate in response to the demand for carbohydrates through the stimulation of leaf growth. Increasing photosynthetic rate after pruning has also been observed in other crops, such as apples (Li, 2001) and grapes (Hunter and Visser, 1988).

The highest photosynthetic rate was observed in middle-aged leaves; 17-67% higher than younger, and 27-61% higher than older ones. Previous studies on cocoa have demonstrated higher photosynthetic capacity of younger leaves vs older leaves (Baker and Hardwick, 1973; Machado & Hardwick, 1988). This higher capacity in the mid-aged leaves is because the photosynthetic capacity and chlorophyll content increase parallel with leaf development (Baker and Hardwick, 1973). This study observed that the chlorophyll content of middle-aged leaves was higher than that of younger leaves, although the difference was not always statistically significant.

Maximum chlorophyll synthesis and maximum chloroplast development do not occur until leaf expansion is completed. Stomata do not become fully functional until about day 17 of the flush cycle (Baker and Hardwick, 1975). This phenomenon explains the lower photosynthetic rate observed here in young leaves since they were not fully expanded, and therefore, chloroplast development is likely not to have been completed.

Similar effects of leaf age on photosynthesis rate have also been found in other tree species, for example, maple and oak (Reich et al., 1991).

No differences in photosynthetic rate were observed between clones M01 and 45. However, differences in photosynthetic rates have been observed previously between other cacao clones. Photosynthetic rate variation was observed among eight contrasting cacao genotypes at the International Cocoa Quarantine Centre at The University of Reading. The net photosynthetic rate ranged from 3.4 µmol (CO_2) $m^{-1}.s^{-1}$ for the genotype IMC 47 to 5.7 µmol (CO_2) $m^{-1}.s^{-1}$ for SCA 6, which was related to variance in stomatal conductance and leaf nitrogen per unit area (Daymond et al., 2011).

On some occasions, leaves at the highest level in the canopy had the highest light saturated-leaf photosynthetic rate. The vertical profile in leaf photosynthetic capacity was also observed in different crops from previous studies (Thomas, 1996; Carswell et al., 2000; Yasuoka et al., 2018).

3.5.5 The effect of pruning and intensive growing system on vegetative growth of two Sulawesi cacao clones

Pruning weights, which are comparatively easy to collect, were utilized to characterise plant vigour. Different vegetation characteristics of pruned branch weight per tree were observed during the initial experimental period between clones M01 and 45. In general, the total weight of pruned branches and leaves rose in year 2 compared to year 1 before declining in year 3. A similar pruned material weight increase was observed in other studies (Cown, 1973; Albarracín et al., 2017). The weight declined in year 3 due to severe pruning conditions. Throughout the duration of the experiment, the frequency of pruning remained consistent, with a higher level of intensity than that of typical maintenance pruning, in order to optimize light interception throughout the canopy.

A higher dry weight of pruned branches, leaves, and trunk diameter increment was recorded at 2000 trees.ha^{-1} compared with the other planting densities. This was caused by less competition for resources generally, particularly light, among individual plants. Several previous studies have concentrated on plant competition (Fetene, 2003; Hunt et al., 2006; Berger et al., 2008; Manning et al., 2009) because it is a crucial process affecting plant populations and communities (Berger et al., 2008).

Clone 45 had a 33-59% higher weight of prunings than clone M01 in years 2 and 3, implying that it was much more vigorous during this period. On a unit area basis, in years 2 and 3, clone 45 had the potential to produce 9-23 tonnes.ha^{-1}.year^{-1} dry weight of pruned material, depending on the planting density; whilst, clone M01 could provide 13-15 tonnes.ha^{-1}.year^{-1} of pruned material. The weight of the total pruning material is approximately correlated with the new vegetative growth of the sample trees.

Annual trunk girth increment was measured for both clones (M01 and 45) in years 2 and 3. Year 2 was a productive fruit-bearing period, whilst, a significant yield decline was observed in year 3. The decrease in girth increment in year 3 mirrored the sharp decline in yield. As with the decline in pruning weight, the significant decline in trunk diameter increment in year 3 was likely caused by severe pruning, leading to declining bean production. Previous studies on many species have observed pruning as the main factor affecting the diameter compared to height growth (Moller, 1955; Sutton, 1973; Takeuchi & Hatiya, 1977). The depression in growth increases as pruning severity increases. In *Cryptomeria japónica*, the reduction in stem volume increment increased exponentially with the percentage of leaves removed (Fujimori, 1972). The higher increase in annual trunk growth observed in clone 45 compared with M01 is a further indication of the clone's high vigour characteristics.

3.5.6 Conclusion

The Biomass 2 trial suggested that productivity per area could increase at suitable planting density. On yield, the effect of row orientation was not observed; meanwhile, the interaction between genetic variation and planting density was seen. The productivity per tree was significantly higher for clone 45, only at the 2000 trees.ha^{-1} density. The highest productivity per area observed on the treatment was 4.46 tonnes.ha^{-1} for 26 month-old trees and 4.6 tonnes.ha^{-1} for 38 month-old trees. The results to date have shown the potential of the trellis-based system using low vigour clone (clone 45) in specific planting density for yield improvement. Clone 45 is more efficient in biomass partitioning compared to M01, nevertheless not the best one.

Similar to what has already occurred in Biomass 1, tree degradation condition in Biomass 2 was observed in year 3. This was caused by the heavy pruning, which reduced the vigour of the trees, as shown by the declined beans production and total pruned material weight.

Chapter 4. Examining the Yield Efficiency of Diverse Cocoa Germplasm in Sulawesi

4.1 Introduction

Increased and stable yield is a significant objective in nearly all breeding programmes. Productivity indicators have been developed to provide a reliable basis for tree and orchard productivity comparison. One of the critical factors influencing yield is the amount of dry matter partitioned to the yield component of the crop (Corley, 1983; Cannell, 1985; Daymond et al., 2002b). The discrepancy between the crops' total harvested and economical products must be conducted when assessing yield data. However, only limited studies have observed the dry matter production data in cacao.

Cocoa exhibits significant genotypic variability in both morphological and physiological traits linked to yield, as reported by Yapp and Hadley (1994). This implies that the genetic makeup determines the potential vigour of the cocoa tree, allowing for the breeding of cocoa trees with varying sizes. However, such studies in cacao are limited in the amount of germplasm that has been characterized.

A non-destructive index of vegetative growth is needed to measure dry matter increase and partitioning in cacao since a destructive analysis is often not practical for a perennial tree crop. One of the examples is “yield efficiency”, which has been defined by several tree crop studies as the ratio of cumulative yield to cross-sectional trunk area (Pearce, 1952; Larsen & Fritts, 1982, 1987; Hill et al., 1987; Larsen et al., 1992; Webster, 1995). The argument for the importance of this measure is that the cross-sectional trunk area is linearly correlated with above-ground biomass (Westwoods & Roberts, 1970) so that later, yield efficiency would reflect methods on tree management inputs, i.e. minor pruning required. Previous studies on apples have highlighted that yields based on a unit of trunk circumference were more meaningful than the total weight of fruit per tree (Waring, 1920; Sudds & Anthony, 1928; Wilcox, 1940) since it provides a measure of yield in relation to vegetative growth. Yield efficiency has proven to be a reasonable basis for breeding yield improvement in apples (Holland, 1968; Robinson & Lakso, 1991).

A similar concept has been employed in cacao, in which yield efficiency was defined as yield over a discrete period in relation to trunk growth over the same period. In cacao, beans are the only portion of the harvested pods with a primary economic value. Consequently, yield in cacao can be expressed as the total weight of the pods, including the husks that represent another component of biomass, or more typically as the total weight of the beans (Adomako, 1999; Daymond et al., 2002b; Sitepu et al., 2005; Pang, 2006; Lachenaud et al., 2007; Pang & Lockwood, 2008; Padi et al., 2012; Ofori et al., 2016; Mustiga et al., 2018). A study by Daymond et al. (2002b) employed the concept of yield efficiency in cacao in Brazil, demonstrating the potential for yield improvement by selectively breeding for more efficient partitioning of the yield component.

High-yield efficiency needs to be an objective for researchers in breeding programmes in cacao-producing countries. To increase reproductive growth in cacao, varieties for commercial planting are expected to have significant vegetative growth reduction, thus maximising yield efficiency. It has been suggested that progenies, which continue to display significant vegetative growth increases when the trees come into production, are expected to be low-yielding (Glendinning, 1966). However, in a previous Ofori et al. (2016) study, some high-yielding progenies also showed high vegetative vigour. Therefore, it is also essential to consider yield and yield efficiency in parallel.

The results of the previous chapters highlighted that the intensive growing systems require lower vigour varieties that can be grown on a trellis. The two clones (clones 45 and M01) used had a high vigour and needed a lot of pruning; furthermore, they had a low partitioning of assimilates to the pod (i.e. low harvest indices and yield efficiencies). Therefore, there is a need to examine a broader range of cacao germplasm available for potential utility in a trellis system.

This chapter investigates the potential of prospective cacao clones for use in an intensive growing system by examining the genotypic variation in yield and yield efficiency of cacao in Sulawesi, Indonesia. Data from trunk diameter and potential yield were analysed from 2015-2016 (referred to as "year 1”), 2016-2017 (referred to as "year 2") and 2017-2018 (referred to as "year 3”).

4.2 Materials and methods

4.2.1 Study site

The experimental site comprised plots of grafted clonal cacao plants and was located at the Mars Cocoa Research Station Tarengge, South Sulawesi, Indonesia (Latitude 2º33'42.98" S and Longitude 120º49'16.25" E, elevation 27 m above sea level).

4.2.2 Experimental design and planting materials

The breeding trial consisted of 62 crosses (Table 4.1) from 16 parental clones (Table 4.2) and was planted in three stages in 2012 and 2013. There were 400 trees for each cross. Parental trees for the crosses were located on the farm of local farmers in South Sulawesi, Indonesia. The seeds were produced by hand pollination in January 2012, with pods being ripe from June 2012 onwards.

The author worked in the research station in April 2013 and was been involved in the data collection since then. She trained another 15 team members in measurement protocol and organized the data management. However, the intensive yield and trunk diameter data analysis were started in April 2016, when the research began.

The propagation plant material nursery was located at Mars Research Station Tarengge, South Sulawesi. The nursery area was constructed using UV-transmitting polyethylene film supported on a wooden roof with polypropylene netting for the side of the nursery. All processes carried out in the nursery, including germination, fertiliser, fungicide, and watering frequency were described in section 2.3.1.

In 2012 and 2013, cacao seedlings were germinated and grown in the nursery for 6 months before the young seedlings were planted in the field. The trial was planted at a density of 3000 trees ha^{-1}, covered with coconut leaves as their temporary shade for 3 months after planting, and no permanent shade was used after that.

Each cross was planted in four replicate plots. Each plot consisted of 100 trees arranged in a randomised block design. However, trees on the plot's border were treated as guards, resulting in 64 trees per plot. In the trial, cacao trees were planted at close spacing, 1.5 m from each other (planting density 3000 trees.ha^{-1}; conventional system), so there were high levels of competition between trees. Trees that died and those for which a negative girth increment was calculated were excluded from the analyses.

Table 4.1 List of crosses in the breeding trial, Mars Research Station Sulawesi, Indonesia

No	Cross	No	Cross	No	Cross	No	Cross
1	45 x BB01	21	M01 x THR	41	Sulawesi1 x 45	61	THR x M07
2	45 x M01	22	M01 x TR01	42	Sulawesi1 x Aryadi02	62	THR x TR01
3	45 x M04	23	M04 x 45	43	Sulawesi1 x “CCN51”		
4	45 x M07	24	M04 x Aryadi02	44	Sulawesi1 x M01		
5	45 x Sulawesi1	25	M04 x BB01	45	Sulawesi1 x M04		
6	45 x TR01	26	M04 x “CCN51”	46	Sulawesi1 x M07		
7	AP x 45	27	M04 x HP	47	Sulawesi1 x MT		
8	AP x M07	28	M04 x M05	48	Sulawesi1 x Sulawesi2		
9	AP x Sulawesi1	29	M04 x MT	49	Sulawesi1 x THR		
10	BB01 x Sulawesi2	30	M04 x Sulawesi1	50	Sulawesi1 x TR01		
11	“CCN51”* x 45	31	M04 x Sulawesi2	51	Sulawesi2 x BB01		
12	“CCN51” x M07	32	M06 x “CCN51”	52	Sulawesi2 x M01		
13	M01 x 45	33	M06 x M01	53	Sulawesi2 x M04		
14	M01 x Aryadi02	34	MT x BB01	54	Sulawesi2 x M07		
15	M01 x “CCN51”	35	MT x “CCN51”	55	Sulawesi2 x Sulawesi1		
16	M01 x HP	36	MT x M01	56	THR x 45		
17	M01 x M05	37	MT x M04	57	THR x BB01		
18	M01 x MT	38	MT x M07	58	THR x “CCN51”		
19	M01 x Sulawesi1	39	MT x Sulawesi1	59	THR x M01		
20	M01 x Sulawesi2	40	MT x TR01	60	THR x M04		

*molecular fingerprinting showed that this was not the true CCN 51

Table 4.2 List of parental clones in the breeding trial Mars Research Station Sulawesi, Indonesia, with their characteristics

No	Parental clones	Characteristics
1	45/MCC02	High yield, susceptible to CPB, large bean size
2	BB01	Black-pod resistance
3	M01/MCC01	High yield, large bean size
4	M04	High yield
5	M07	VSD resistance
6	Sulawesi1/PBC123	High yield, small bean size, has many beans
7	TR01	High-fat content
8	AP	Big pod size
9	Sulawesi2/BR25	Moderate pest disease resistant, high yield
10	“CCN51”	High yield
11	Aryadi02	CPB resistance
12	HP	Black pod resistance
13	M05	Small bean size
14	MT	High yield
15	HR	High yield, high flowering rate
16	M06	Moderate disease resistance, small bean size

4.2.3 Measurement methods

Potential yield evaluations were conducted every two weeks, while trunk diameter/girth measurements were made every six months, using the same measurement protocols as in sections 2.3.6 and 3.3.3.3. Trunk cross-sectional area (TCSA) and yield efficiency were calculated for each replicate of each cross as outlined in section 3.3.6. The potential yield was used, rather than the actual yield, due to limitations in the station facilities including space for bean drying of samples from whole plots. Furthermore, potential yield is a commonly utilized parameter for calculating the partitioning between vegetative and reproductive components (Gifford & Evans, 1981).

The periods during which girth increment and yield were measured from 2015 to 2018 are demonstrated in Figure 4.1. The cumulative yield for production year 1 was compared with TCSA for the same year. Then the cumulative yield for the following year was also compared with the TCSA increase that year, and so on.

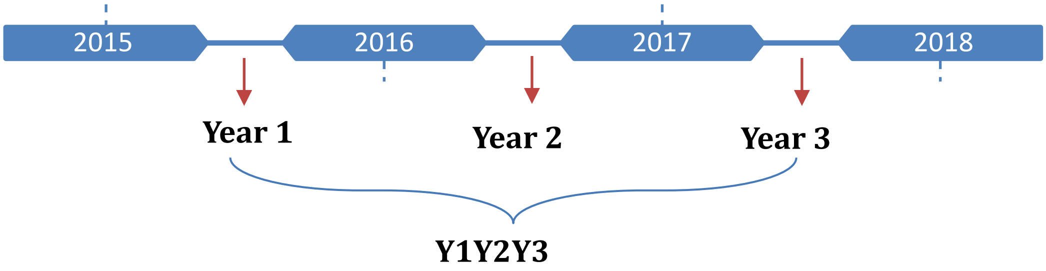


Figure 4.1 Calculation for yield and girth increment in the breeding trial

4.2.4 Statistical analysis

Trunk cross-sectional (TCSA) increment, yield, and yield efficiency data were analysed using one-way analysis of variance (ANOVA) with genotype as the factor and individual trees as replicates. The potential effect of blocks was included in the analysis. Regression analysis was utilised to test any relationship between trunk diameter growth and yield. All analyses were carried out using Genstat 19th edition software (VSN International Ltd., Hemel Hempstead, UK). The normal distribution test, histogram, residual plots and Least Significant Difference (LSD) were calculated using GenStat. The standard error of the mean was calculated using Microsoft Excel. The standard error value was presented in the results, indicating the sample variability between individual trees.

The reciprocal or parental effect on the performance of the cacao crosses was not analysed in this study.

4.3 Results

4.3.1 Trunk cross-sectional area (TCSA) increment

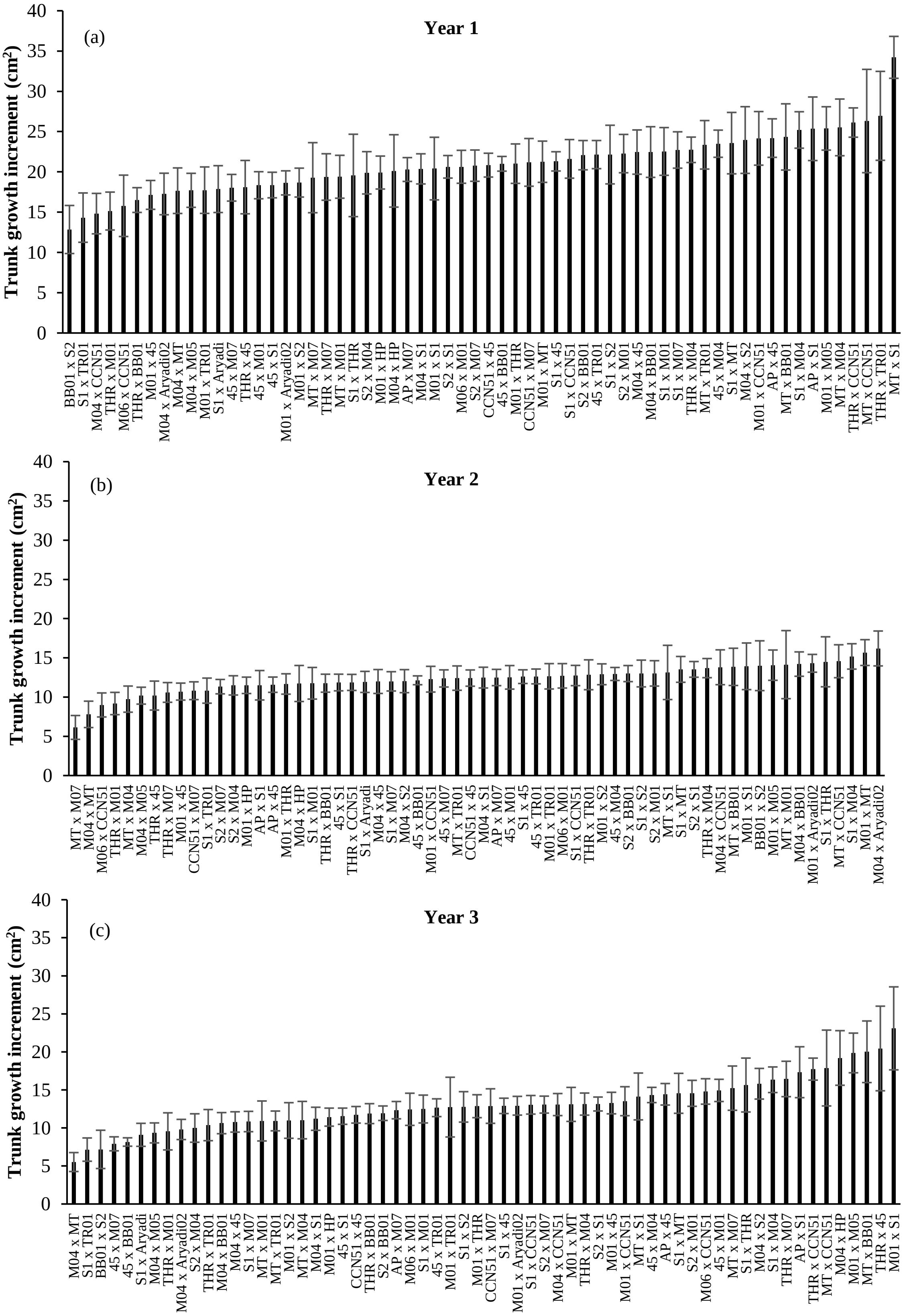


Figure 4.2 Trunk cross-sectional area increment (increased in cross-sectional area) per tree (cm^2) among 62 crosses in (a) Y1 first production year (2015-2016) (b) Y2 second production year (2016-2017) and (c) Y3 third production year (2017-2018). Values are means between four plot replicates and 64 sample tree replicates per plot (+/- standard error of the mean)

Significant variation was observed between crosses in trunk cross-sectional area increment between 2015-2016 as the first production year and 2017-2018 as the third production year. The range was from 12.85 cm^2 for **BB01xS2** to 34.22 cm^2 for **MTxS1** in year 1 (Figure 4.2a; P=0.002), 6.13 cm^2 for **MTxM07** to 16.19 cm^2 for **M04xAryadi02** in year 2 (Figure 4.2b; P=0.875), 5.52 cm^2 for **M04xMT** to 23.09 cm^2 for **M01xS1** in year 3 (Figure 4.2c; P<0.001), and 30.98 cm^2 for **M04xMT** to 61.50 cm^2 for **MTxS1** across the accumulated three years (Figure 4.3; P<0.001).

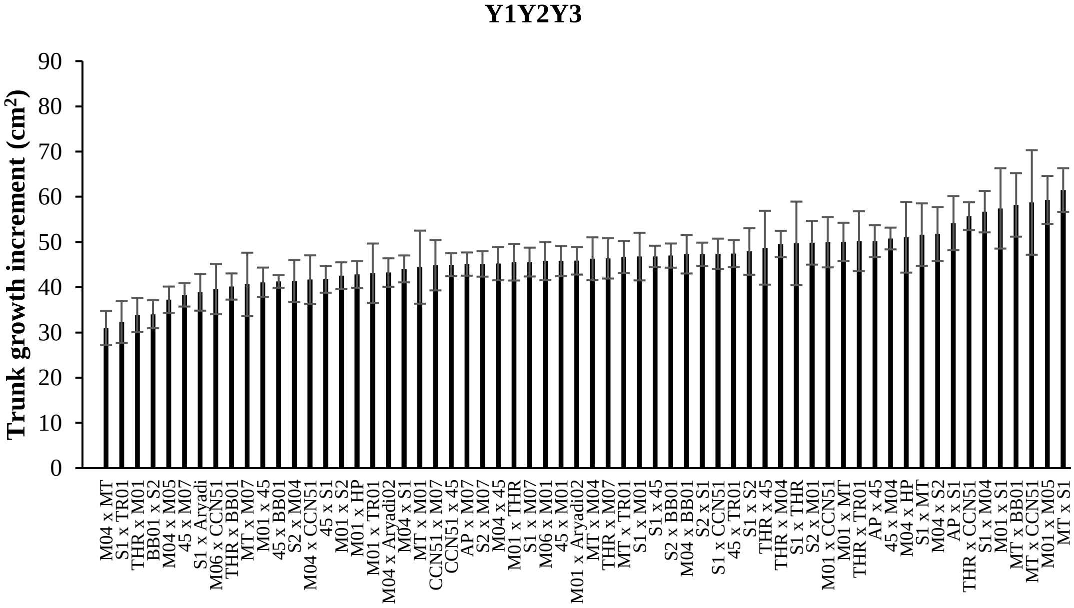


Figure 4.3 Cumulative trunk growth increment per tree (cm^2) among 62 crosses for the production years 2015-2018. Values are means between three plot replicates and 64 sample tree replicates per plot (+/- standard error of the mean)

4.3.2 Yield

During the first production year, potential yield varied 4-fold between crosses, with dry bean weight ranging from 0.11 kg.tree^{-1} for **MTxS1** to 0.41 kg.tree^{-1} for **MTxM01**. Based on a planting density of 3000 trees.ha^{-1}, this is equivalent to 0.33 tonnes.ha^{-1} for **MTxS1** to 1.23 tonnes.ha^{-1} for **MTxM01**. The differences, however, were not statistically significant (Figure 4.4a). In the second production year, potential yield varied 2-fold between crosses, with dry bean weight ranging from 0.17 kg.tree^{-1} for **MTxM07** to 0.40 kg.tree^{-1} for **THRxM04**. Based on a planting density of 3000 trees.ha^{-1}, this is equivalent to 0.51 tonnes.ha^{-1} for **MTxM07** to 1.20 tonnes.ha^{-1} for **THRxM04**.

The differences, however, were not statistically significant (Figure 4.4b). In the third production year, potential yield varied 6-fold between crosses, with dry bean weight ranging from 0.08 kg.tree^{-1} for **MTxM01** to 0.46 kg.tree^{-1} for **S1xAryadi**. Based on a planting density of 3000 trees.ha^{-1}, this is equivalent to 0.24 tonnes.ha^{-1} for **MTxM01** to 1.38 tonnes.ha^{-1} for **S1xAryadi** (Figure 4.4c; P<0.001).

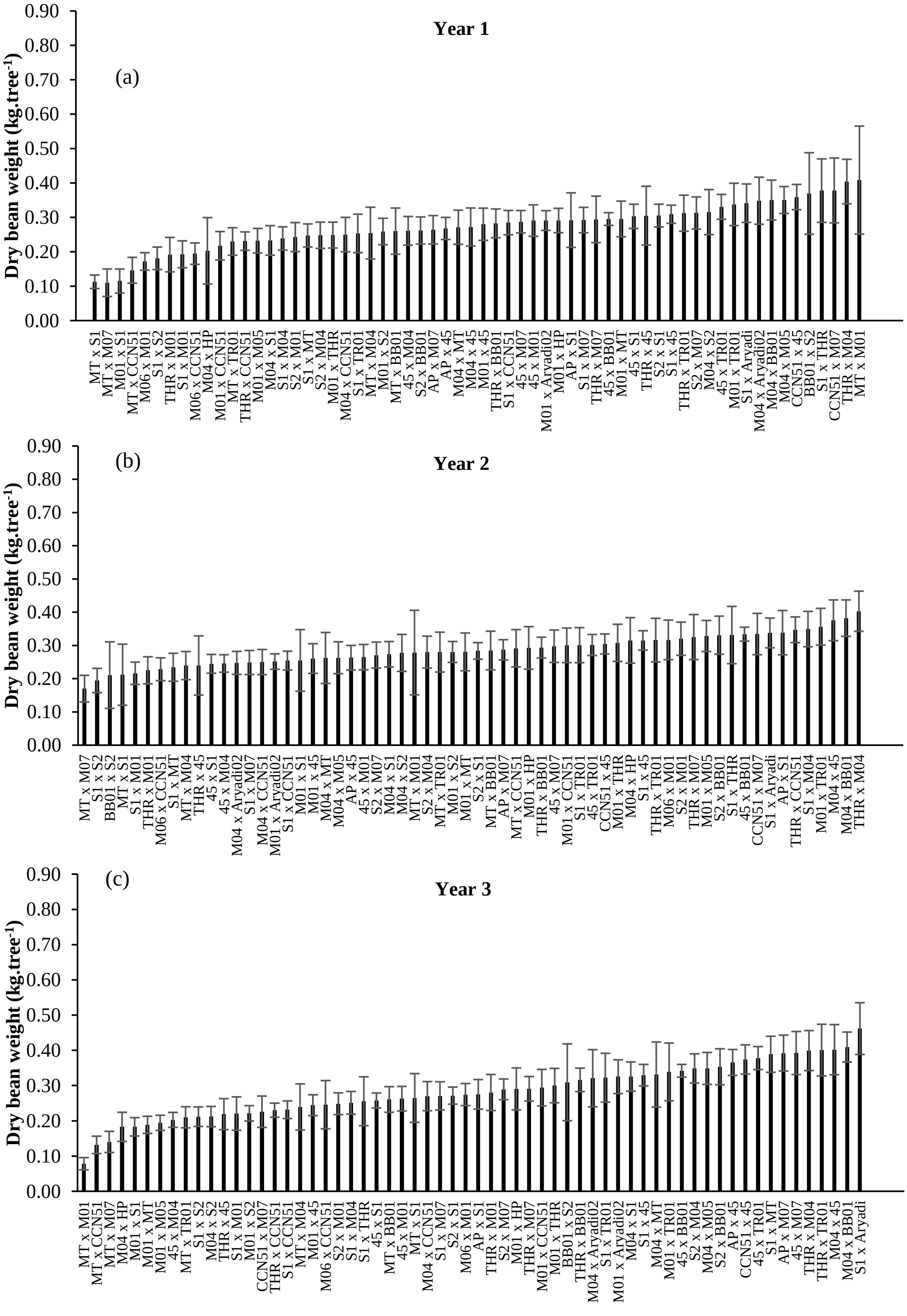


Figure 4.4 Annual potential yield per tree among 62 crosses in the (a) Y1 first production year (2015-2016), (b) Y2 second production year (2016-2017) and (c) Y3 third production year (2017-2018). Values are means between four plot replicates and 64 sample tree replicates per plot (+/- standard error of the mean)

Potential yield over the three years (2015-2018) varied 2-fold between crosses, with dry bean weight ranging from 0.42 kg.tree^{-1} for **MTxM07** to 1.21kg.tree^{-1} for **THRxM04**. Based on a planting density of 3000 trees.ha^{-1}, this is equivalent to 1.26 tonnes.ha^{-1} (0.42 tonnes.ha^{-1}.yr^{-1}) for **MTxM07** to 3.63 tonnes.ha^{-1} (1.21 tonnes.ha^{-1}.yr^{-1}) for **THRxM04** (Figure 4.5; P=0.002).

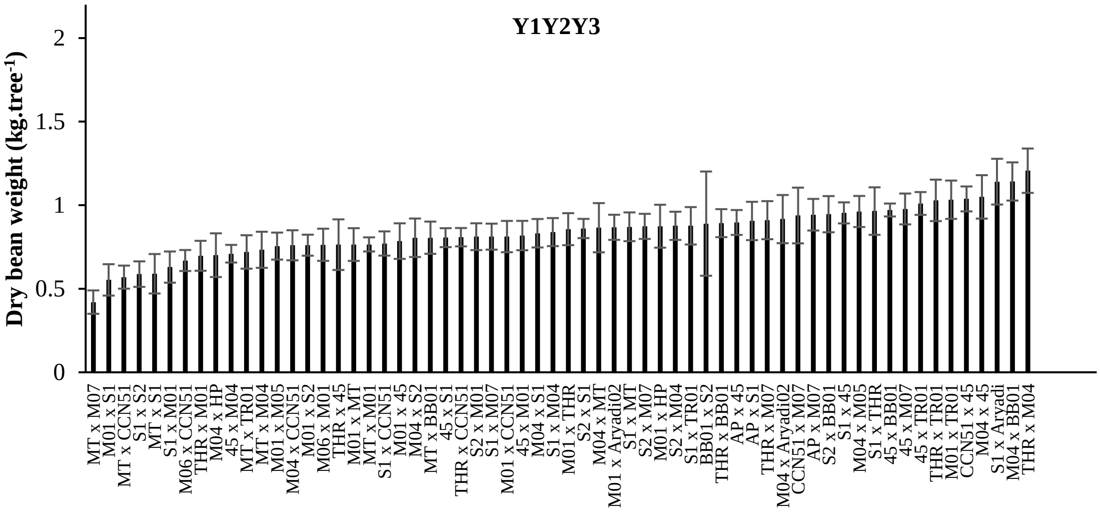


Figure 4.5 Cumulative potential yield per tree among 62 crosses in the three production years (2015-2018). Values are means between four plot replicates and 64 sample tree replicates per plot (+/- standard error of the mean)

4.3.3 Yield efficiency

During the first production year, yield efficiency varied 14-fold between crosses, ranging from 0.003 kg.cm^{-2} for **MTxS1** to 0.043 kg.cm^{-2} for **S1xAryadi** (Figure 4.6a; P=0.017). In the second production year, yield efficiency varied 3-fold between crosses, ranging from 0.015 kg.cm^{-2} for **BB01xS2** to 0.052 kg.cm^{-2} for **45xBB01.** However, the differences were not significant (Figure 4.6b). In the third production year, yield efficiency varied 10-fold between crosses, ranging from 0.009 kg.cm-2 for MTxM01 to 0.098 kg.cm-2 for 45xBB01 (Figure 4.6c; P <0.001).

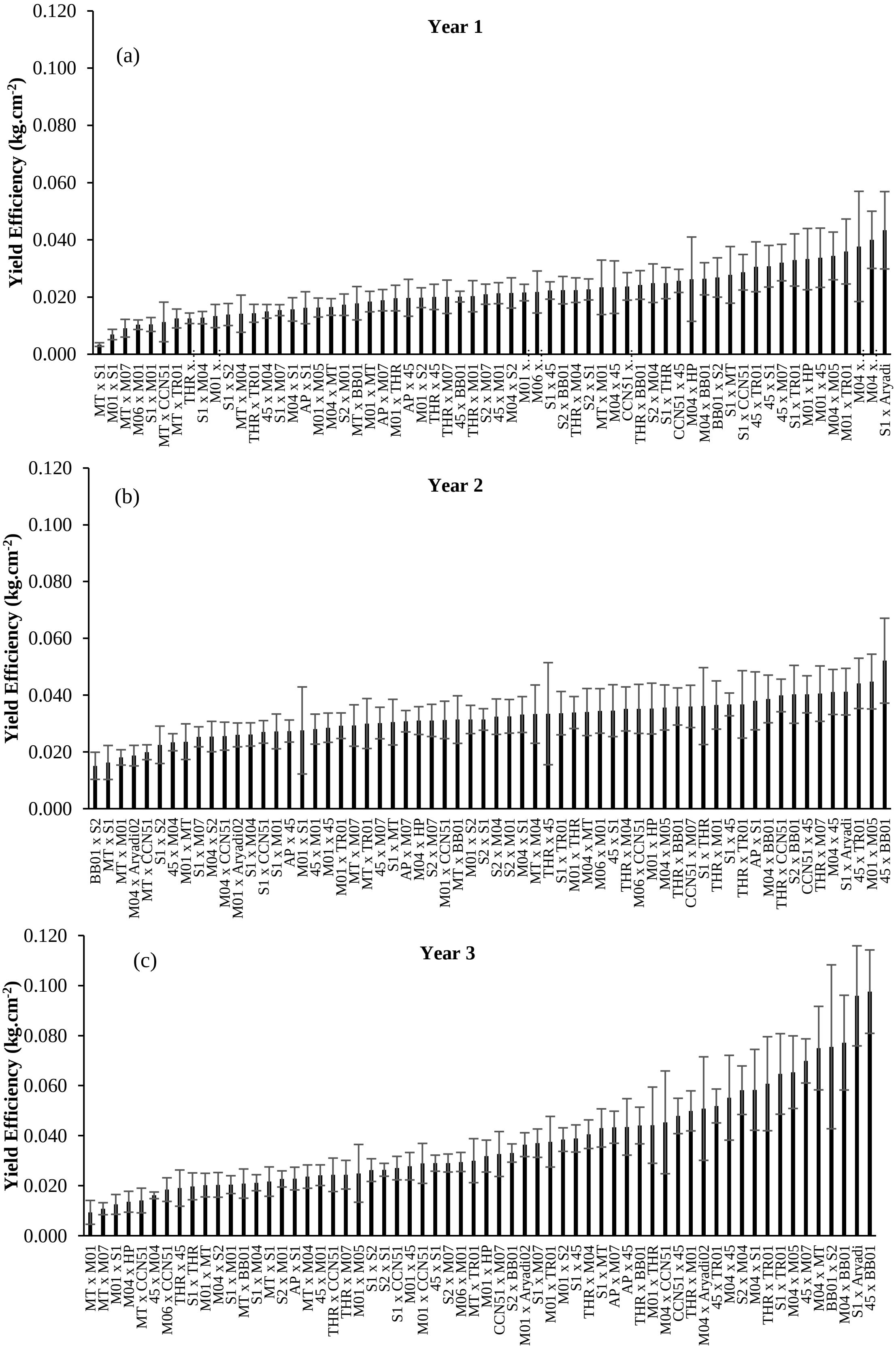


Figure 4.6 Annual yield efficiency per tree among 62 crosses in the (a) Y1 first production year (2015-2016), (b) Y2 second production year (2016-2017) and (c) Y3 third production year (2017-2018). Values are means between four plot replicates and 64 sample tree replicates per plot (+/- standard error of the mean)

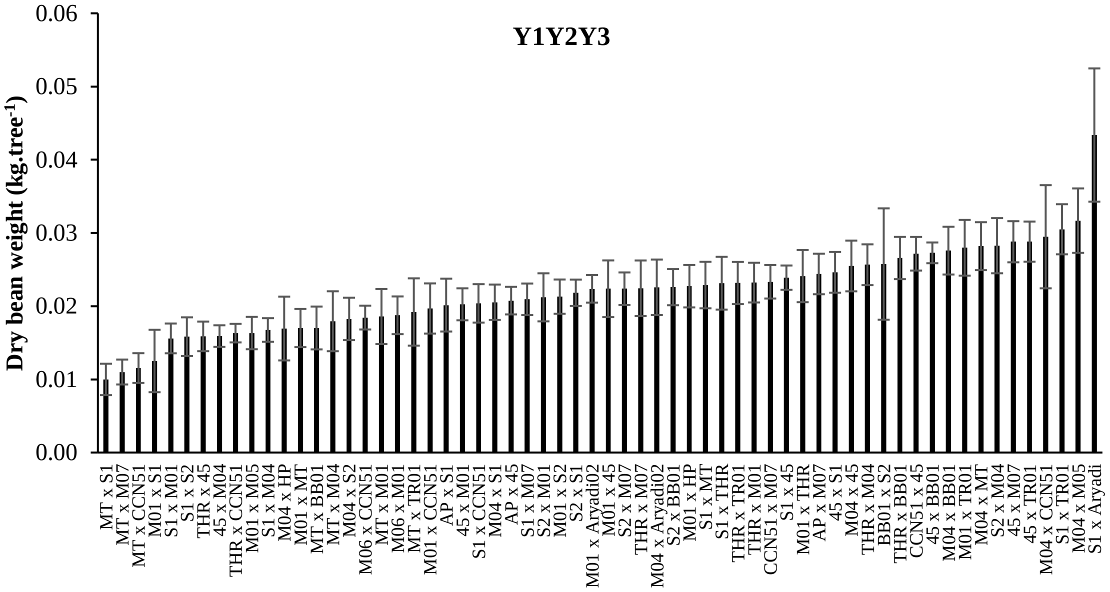


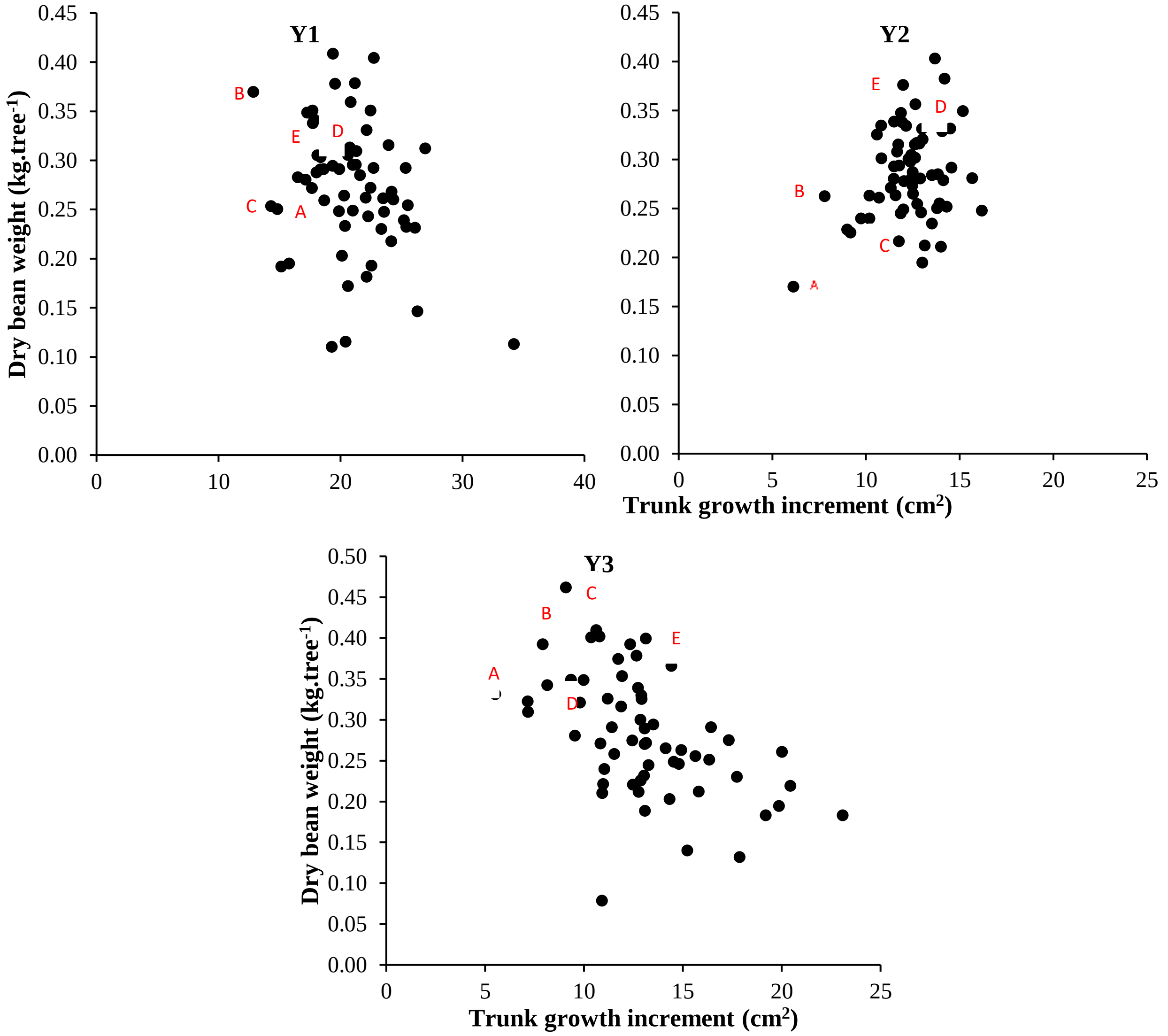
Figure 4.7 Cumulative yield efficiency per tree among 62 crosses across the three production years (2015-2018). Values are means between four plot replicates and 64 sample tree replicates per plot (+/- standard error of the mean)

Yield efficiency over the three years (2015-2018) varied 4-fold between crosses, with values ranging from 0.010kg.cm^{-2} for **MTxS1** to 0.043 kg.cm^{-2} for **S1xAryadi** (Figure 4.7; $P<0.001$).

4.3.4 Relationships between yield and trunk growth increment

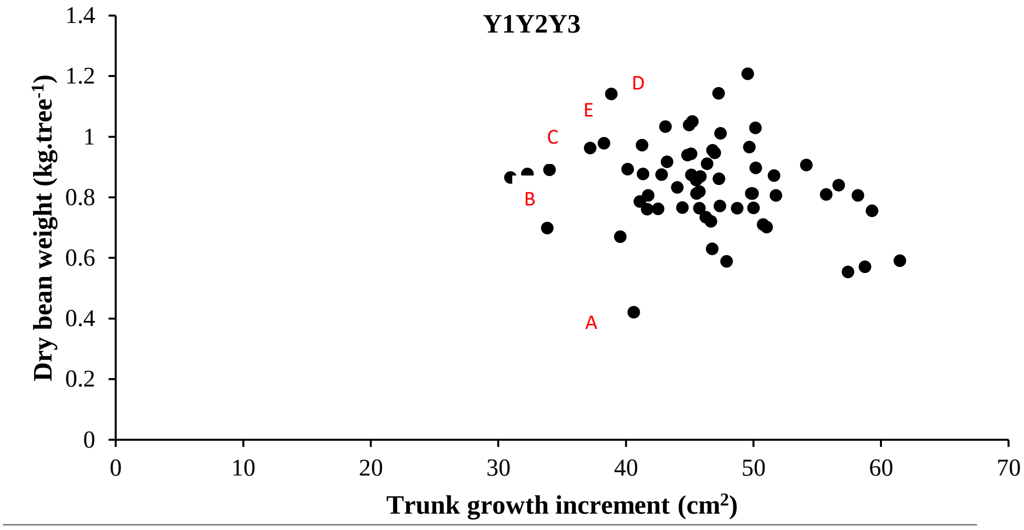
The relationship between trunk growth and yield for each production year is shown in Figure 4.8 and the three production years combined in Figure 4.9. The low R-square value suggested a weak relationship between trunk growth increment and dry bean weight.

In order to achieve a high yield in an intensive growing system with a high planting density, prospective clones should have high yield, high yield efficiency and, therefore, a relatively low trunk growth increment. The highlighted clones represent those that combine a high yield with a high yield efficiency. The clones with the lowest trunk growth increment and high yield are indicated by letters A, B, C, and other clones with high yield efficiency and medium trunk growth increment are indicated by letters D and E. Some clones that showed consistently high yield and high yield efficiencies between years were **M04xMT**, **S1xTR01**, **BB01xS2**, **S1xAryadi**, and **45xTR01**.



Cross	Yield Efficiency (kg.cm^{-2})					
	Year 1		Year 2		Year 3	
M04 x CCN51	**A**	0.040				
BB01 x S2	**B**	0.027				
S1 x TR01	**C**	0.033				
S1 x Aryadi	**D**	0.043				
M01 x TR01	**E**	0.036				
MTxM07			**A**	0.029		
M04xMT			**B**	0.034		
45 x S1			**C**	0.035		
45 x BB01			**D**	0.052		
S1 x Aryadi			**E**	11.93		
M04xMT					**A**	0.034
45xM07					**B**	0.030
S1xAryadi					**C**	0.041
45 x BB01					**D**	0.052
45 x TR01					**E**	0.044

Figure 4.8 Relationship between yield per tree and trunk growth increment among 62 crosses in the Y1 first production year (2015-2016), Y2 second production year (2016-2017), and Y3 third production year (2017-2018). Values are means of four plot replicates and 64 sample tree replicates per plot. Note the difference in the x-axis scale for Y1 compared to Y2 and Y3. The clones highlighted with letters represent those that combine a high yield with a high-yield efficiency



No	Cross	Yield Efficiency Y1Y2Y3 ($kg.cm^{-2}$)
A	**M04 x MT**	0.028
B	**S1xTR01**	0.031
C	**BB01xS2**	0.026
D	**S1 x Aryadi**	0.043
E	**45 x TR01**	0.029

Figure 4.9 Relationship between trunk growth increment and yield per tree and among 62 crosses over the three production years (2015-2018). Values are means between four plot replicates and 64 sample tree replicates per plot. Highlighted clones are those which combine a high yield efficiency and yield.

4.4 Discussion

The values for yield from the crosses studied here planted at high density were lower than a previous study of genotypic yield variation in cacao (Lockwood & Yin, 1996) conducted in Malaysia. It was observed that there was a two-fold difference in accumulated potential yield over three years between the lowest and highest-yielding crosses. The Tarengge field was well-fertigated and maintained. However, not all progenies performed well under high planting density conditions (3000 trees.ha^{-1}). Cacao trees grown at close spacing admit little light to the ground through the canopy, and the roots of the trees intermesh, thus restricting light and nutrients available to the trees. Nevertheless, yields as high as the equivalent of 1.21 tonnes.ha^{-}1.year^{-1} were observed for the best cross after three years of cropping.

Yield efficiency is an index that relates yield with vegetative growth (Adomako et al., 1999a; Daymond et al., 2002b; Pang, 2006) and is an indicator to be considered alongside yield, particularly for high planting density in intensive growing systems. The highest yield efficiency value observed in this study was 0.096-0.098 kg.cm^{-2}.year^{-1} for S1xAryadi and 45xBB01 in production years three, respectively. These values were notably higher than the highest yield efficiencies observed for clones 45 and M01, grown at a planting density 3300 trees.ha^{-1} in the Biomass 2 trial (see Chapter 3), which ranged from 0.026-0.057 kg.cm^{-2}.year^{-1}.

The highest yield efficiency observed in this study was also higher than in other studies of germplasm in Brazil and Ghana (Daymond et al., 2002b; Ofori et al., 2016; Padi et al., 2017). Padi et al. (2017) measured yield efficiency over 24 months on 58 cacao progenies in Ghana and obtained the highest values for the progenies T63/971 × SCA 9 (0.0398 kg.cm^{-2}.year^{-1}) and T85/799 × A1/154 (0.0366 kg.cm^{-2}.year^{-1}).

A range of yield efficiencies has been observed in other tree crops planted in high densities planting. A study on Honeycrisp apples by Xu et al. (2021) observed a yield efficiency range between 0.65 kg.cm^{-2} for rootstock G.41 to 1.07 kg.cm^{-2} for G.214 among ten-year-old trees grafted on eight different rootstocks, grown at planting density 2250 trees.ha^{-1} in Canada. Furthermore, Williamson and Coston (1990) noted a range of yield efficiency between 0.13-0.33 kg.cm^{-2} in peaches grown at density 5000 trees.ha^{-1} in Clemson, USA. A study by Camposeo et al. (2021) observed a yield efficiency value of about 0.5 kg.cm^{-2} among three-year olive trees planted at density 1200 trees.ha^{-1} in Foggia, Italy.

This study demonstrates that several cacao crosses have a high yield potential, a high yield efficiency, and a small trunk growth increment. These properties could gain interest in making them suitable clones for future experiments to grow cacao under high planting density using trellises. Based on this study, clones derived from the following crosses should be considered for high intensity growing systems: 45xS1, M04xTR01, S1xAryadi, and M04xMT.

Propagation through seedlings can lead to a significant plant variability in crop yield and quality. To avoid such issues, the selection of clones from within these crosses should be conducted.

Chapter 5. General Discussion

5.1 Introduction

The 2020 pandemic caused a decline in demand for chocolate and cocoa beans in Europe, but the global demand decreased slightly. This suggests that if the market returns to pre-pandemic conditions, the supply of cocoa beans may not meet the growing demand, as the market is expansive and constantly expanding.

Therefore, a holistic approach is needed to improve cacao farm yields, involving breeding, integrated pest management, and good agricultural practices. Adopting more intensive production techniques on existing farms, rather than expanding the cultivated area, can increase cocoa production, which benefits farmers' income and reduces deforestation linked to agricultural expansion.

Intensive production techniques in hedgerow systems have been successfully applied to other crops, such as apples and grapes, and their use on cacao is promising. By increasing planting density, using various trellis systems, and optimizing tree canopy shape, height, row spacing configurations, and orientation, yields of modern apple and grape cultivars have significantly improved. Cacao growers can increase their yields by using suitable techniques to maximize sunlight exposure and support optimal fruit development. Such learnings can be applied to cacao in terms of orchard and tree management, and a better understanding of the relationship between light interception or distribution and yield is needed to optimize orchard design and canopy management. Exploiting genetic variability within crops in yield efficiency represents an opportunity for management systems.

This study examined whether hedgerow systems designed to optimise light interception by the canopy can increase yield. This was achieved by: firstly, investigating whether it was possible to adapt an existing experiment to improve light interception and yield; secondly, by exploring whether it is possible to maximise yield in a trellis system through changing the planting density and growing orientation and thirdly, by exploring genetic variation in biomass partitioning to optimise planting materials used in trellis systems.

5.2 How sink stimulation through pruning impacts photosynthetic rate

Frequent pruning is a necessary aspect of cacao maintenance on a trellis system. The experiment data has revealed that the light-saturated photosynthetic rate was significantly higher on multiple occasions after pruning. A plausible explanation for this enhanced photosynthetic rate is stimulating new growth production following pruning. The new cacao leaves initially act as photosynthetic sinks, altering the source-sink ratio during leaf flushing. As heavily pruned trees demand more sugars for leaf growth, an increased activity of photosystems accelerates linear photosynthetic electron flow. These findings align with carbon allocation models based on the sink-source theory (Bellingham & Sparrow, 2000).

A previous study indicated that optimal pruning may enhance stomatal conductance in residual leaves, as observed in poplar (*Populus tremuloides*) by Hart et al. (2000). Similar to these results, this study on cacao also revealed an increase in stomatal conductance following pruning.

However, despite the observed increase in leaf-level photosynthesis, it was not enough to counteract the negative impacts of heavy pruning on yield decline over time. There remains to be more clarity regarding the optimal pruning intensity for cacao cultivation in trellis systems. Further research is required to determine the ideal pruning intensity for achieving maximum yields in this context.

5.3 Phenology changes between two clones within an intensive cropping system

Intensive growing systems designed to maximise light interception can potentially impact the tree's phenology, consequently impacting yield. In the Biomass 1 trial, leaf flushing was impacted by canopy manipulation. The conventional treatment exhibited a slightly higher flushing index than the trellis treatments, indicating higher vegetative vigour and more significant pruning requirements. This implies that the clones employed in the experiment were not well-suited for growth in the trellis system.

Differences in flushing associated with planting orientation in Biomass 2 did not affect yield. The study site's proximity to the equator renders the planting orientation as insignificant treatment.

For flowering intensity, conventionally managed trees in Biomass 1 exhibited higher flowering intensity than those in the trellis system treatments. Studies on olives have shown that restraining canopy growth in a trellis system can alter the source/sink ratio, reducing carbohydrate requests from the shoot and potentially interfering with fruit growth and flower bud differentiation (Smith & Samach, 2013). Thus, the fewer flowers in trees with the trellis system may be due to restrained canopy growth. Further research should investigate the effect of early or late trellis establishment on tree flowering.

Moderate pruning is the most effective way to produce many flowers and pods in a tree (Sitohang et al., 2019), while heavy pruning can lead to a premature drop of flowers and fruitless due to inadequate assimilate supply. Consequently, the fewer flowers in trees with a trellis system could also have resulted from excessive pruning.

The Biomass 2 trial demonstrated light intensity's importance on cacao flowering. The North-South row orientation showed a slightly higher flowering index in years 3 and 4, likely due to less external shading from nearby trees. Additionally, the flowering index generally decreased with increasing density for both clones, with the highest flowering intensity observed at 2000 trees ha^{-1}.

5.4 The impact of manipulating canopy architecture, row orientation, and planting densities in an intensive growing system on yields

The use of intensive growing systems in cacao requires higher productivity in the first years of the growing system to achieve similar break-even times than more conventional systems. The accumulation of dry matter in the fruit is a product of resource partitioning defined by the interaction between the capacity of growth (genetics), resource availability (environment), and inter-organ competition driven by source-sink relationships. The purpose of a trellis system is to maximise assimilation through increased light interception.

The trellis system in Biomass 1 did not improve yields compared to the conventional system. The clone M01 used in the experiment had high vigour, and it was shaped onto a trellis system when the trees were already 2.5 years old. The excessive pruning caused tree degradation, which led to productivity declining in general.

The highest production in the Biomass 2 trial was achieved with a planting density of 2000 trees.ha^{-1} in the East-West row orientation with clone 45. A yield of around 4.5

tons.ha^{-1} in production years 1 and 2 was achieved, which is towards the top end of what has been recorded for cocoa. The highest estimated annual cacao yield on-farm in Indonesia that has been reported was 3586 kg.ha^{-1} (Daymond et al., 2020).

Although yields of these magnitudes were only obtained for two years, the Biomass 2 trial was considerably more successful than Biomass 1. The fact that yields were lower in the planting densities of 5000 trees.ha^{-1} and 3333 trees.ha^{-1} implies more light competition between trees. There was no effect of row-orientations on yield per tree observed in production years 1 and 2. Being close to the equator, both row-orientations in the trial received about the same amount of solar radiation over the course of the day.

Canopy density and architecture variability between clonally propagated genotypes provide opportunities for exploiting high-intensity growing systems. For instance, canopy characteristics have been shown to vary considerably between genotypes, highlighting the potential for genetic variability in assimilate production in cacao.

5.5 Yield efficiency between two clones within an intensive cropping system

Using less vigorous clones with high yield efficiency is beneficial in intensive growing systems to reduce the need for frequent heavy pruning. Yield efficiency is a valuable parameter for describing the partitioning ratio between vegetative and generative processes in perennial crops.

In the Biomass 1 experiment, there was some evidence that canopy manipulation had an impact on biomass partitioning. The bean harvest index of the clone M01 was low across all treatments, ranging from 7.4% to 18.3%, whilst the pod harvest index varied from 12.5% to 29.9%, a similar value observed in the previous studies. The value implies that the bean husk proportion to the whole pod is very high. This result demonstrated that clone M01 had a high vegetative vigour and a high husk to bean ratio, making it unsuitable to use in the intensive growing system.

In the Biomass 2 experiment, the pod harvest index and productivity per tree for clone 45 was higher than M01 (32.6% compared with 19.6%) in year 1 at all densities for both row orientations. However, the yield of both clones behaved relatively similarly in years 2 and 3. Thus, it was observed that in the short term (until production year 2), clone 45 was a better clone to grow in the intensive growing system. Nevertheless, both clones had a low ability to buffer stress induced by repeated pruning. Since cacao needs to fulfil the high demand for vegetative biomass, the carbohydrates available to pods are reduced over time. A significant decline in yield in year 3 was a likely response to this excessive pruning resulting in a depletion of carbohydrate reserves.

The yield efficiency of cacao clones 45 and M01 was relatively moderate, with a high percentage of pod biomass compared to other cacao variations studied previously (Daymond, 2002b). This observation, combined with the large amount of material that needed to be pruned, suggests that the clones used in this study may not be suitable for a high planting density trellis system.

5.6 Genotypic variation in yield efficiency

The results from the survey of a broader range of genetic material highlighted that several crosses have a high yield combined with a high yield efficiency value and a small trunk growth increment. These properties could gain interest in making them suitable clones for future experiments to grow cacao with high planting density in an intensive growing system. Based on this study, clones derived from the following crosses should be considered for high intensity growing systems are **45xS1**, **M04xTR01**, **S1xAryadi**, and **M04xMT**.

5.7 Conclusions and future work

The present study investigates the effects of pruning and the trellis system on cacao productivity. The experimental results suggest that cacao grown in the intensive trellis system can significantly improve yields in the short term. This finding provides a sound basis for confidently stating that the trellis system can effectively enhance cacao productivity.

However, since both clones used are vigorous, heavy pruning made them unsuitable for growing on such an intensive system. Future research should focus on how genetic variation in cacao can be exploited and whether clones with high yield, high yield efficiency, and smaller trunk growth perform better in high-density trellis systems. Techniques used to control tree growth, such as rootstocks development, should be exploited further. The impact of trellis instalment at the establishment or later stage on the trees yield was also need to investigate further. Other than that, further investigation should be conducted on other types of high-density orchards in various tree forms, planting arrangements, tree heights, width, and geometric structures (spheric, rectangular, conic V, T, and A-shaped). One of the examples is only keeping vertical branches on the trellis with the bottom part of the canopy growing more, with less pruning intensity and frequency.

Installation and analysis of environmental data monitoring (temperature, soil moisture, Photosynthetic Active Radiation (PAR), leaf wetness) are essential research tools. Connecting phenology with the environmental parameters is needed in the future. Improvements in the field support system, such as irrigation scheduling, fertigation and mechanisation of pruning and harvesting, are crucial in improving yields. It is also important to do cost-benefit analyses of intensive systems to identify ways to make them more cost-efficient.

In conclusion, this study provides valuable insights into the potential benefits of the trellis system in cacao farming, but more research is required to evaluate its long-term sustainability and economic viability.

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Appendix

A. Basic soil condition of Biomass 1 trial

Table A.1 Biomass 1 soil analysis results of samples taken in April 2017, based on different planting densities. Values are means across four plots as replicates in four randomised row blocks

	625 trees.ha^{-1}		**816 trees.ha^{-1}**		**833 trees.ha^{-1}**		**1111 trees.ha^{-1}**	
	Mean	**s.e**	**Mean**	**s.e**	**Mean**	**s.e**	**Mean**	**s.e**
%Sand	43.50	3.07	43.50	3.10	42.75	2.21	43.13	4.33
%Silt	33.75	2.59	34.00	2.94	34.25	2.17	33.50	3.86
%Clay	22.75	0.48	22.50	0.50	23.00	0.58	23.50	0.50
pH	4.46	0.10	4.28	0.09	4.56	0.05	4.56	0.07
C Org (%)	1.13	0.05	1.03	0.03	1.20	0.03	1.02	0.06
N (%)	0.14	0.00	0.14	0.01	0.15	0.00	0.13	0.01
C/N	8.00	0.00	7.75	0.25	7.88	0.31	7.75	0.25
N Org (%)	0.13	0.00	0.13	0.01	0.14	0.00	0.12	0.01
P (ppm)	20.83	4.17	13.94	3.60	23.79	0.94	18.15	3.56
K (m.e/100g)	0.34	0.05	0.36	0.03	0.38	0.05	0.36	0.04
Ca (m.e/100g)	1.40	0.32	0.83	0.12	1.91	0.24	1.32	0.23
Na (m.e/100g)	0.04	0.01	0.04	0.01	0.07	0.01	0.06	0.02
Mg (m.e/100g)	0.32	0.04	0.22	0.06	0.47	0.07	0.33	0.05
Saturated base cation (m.e/100g)	2.09	0.41	1.44	0.20	2.82	0.31	2.07	0.32
Cation exchange capacity (m.e/100g)	10.44	0.45	9.72	0.84	10.92	0.25	10.01	1.08
Saturated base (%)	20.00	4.33	15.25	2.50	25.75	2.66	20.38	1.46
Al-dd (m.e/100g)	0.74	0.16	0.73	0.09	0.66	0.08	0.60	0.09

The soil texture influences its ability to store water and nutrients. The column in the middle of the plot had a higher content of silt and lower sand than the border. Thus, the area in the middle has a better moisture-holding capacity, while sandy soils at the edge have good drainage.

B. Basic condition of Biomass 2 trial

B.1 Soil analysis

Based on the soil analysis carried out in 2018 and 2019, higher clay content was observed in the NS row orientation than in EW row orientation plots. Soil texture, which relates to its percentage content of sand (2-0.02 mm diameter), silt (0.02-0.002 mm diameter), and clay (<0.002 mm diameter), would determine the capacity to hold and exchange the nutrients, also for water drainage and availability. After being saturated, water in the soil will drain from the most prominent pores, similar to sponge characteristics. However, clay soil contains many tiny pores and a few large pores; therefore, it will drain less water. Conversely, sand soil has a high percentage of large pores; thus, a more significant amount of water will be drained quickly due to gravity (Milthorpe & Moorby, 1974; Munroe, 2018).

Due to lime application in 2019, soil pH and calcium content 2019 was higher than in 2018. Recent studies have observed that reduced growth of many plants under low soil pH conditions is associated with increased solubility of manganese and aluminium and decreased availability of calcium and phosphorus (Gilbert & Pember, 1935; Adams & Lund, 1966). Moreover, Baligar & Fageria, 2005 and previous studies (Wessel, 1971; Santana and Cabala-Rosand, 1984; Cabala-Rosand P et al., 1989) have shown that the main constraints for the low yield of cacao are aluminium toxicity and nutrient deficiencies which are mainly found in acid soils.

The chemical properties in the topsoil are most important for cocoa since the tree has a large number of roots in the topsoil. Since the pH was higher in 2019 than in 2018, the improved absorbance of nutrients was reflected in the concentration of both leaf macronutrients (N, K, Ca, Cu, Fe, and Mn) and micronutrients (Cu and Fe) which were found to be higher in 2019 compared to 2018.

The soil analysis results are summarised in Table B.1. No significant difference was observed for all parameters between the repeated samples sent in the year 2018 and the year 2019, except for the 2019 pH, which was higher than the pH in 2018 (P<0.001), also Ca and K content (P<0.001) were both higher in 2019 than in 2018. This difference was caused by the effect of lime added to the soil at the beginning of 2019. In contrast, %C (P<0.001), %N (P=0.019) and % organic N (P=0.006) were higher in 2018 than in 2019.

Table B.1 Biomass 2 soil analysis results, comparing between year 2018 and year 2019 data. Values are means across two clones, three plots as replicates, three planting densities, two row-orientations, and two sample replicates

	2018	**s.e**	**2019**	**s.e**
Soil texture				
Sand (%)	40.39	1.927	40.30	3.870
Silt (%)	31.22	2.039	30.44	1.840
Clay (%)	28.39	1.278	29.22	1.130
pH	4.39	0.096	5.02	0.085
Macronutrients				
C (%)	0.76	0.077	0.60	0.069
N (%)	0.11	0.006	0.09	0.007
C/N	6.92	0.689	6.28	0.868
Organic N content (%)	0.10	0.007	0.09	0.007
P (mg.kg^{-1})	5.66	3.184	6.37	3.930
K (me.100g^{-1})	0.43	0.064	2.63	0.304
Ca (me.100g^{-1})	0.94	0.262	1.50	0.298
Mg (me.100g^{-1})	0.23	0.057	0.19	0.033
Micronutrients				
Na (me.100g^{-1})	0.09	0.016	0.08	0.010
Zn (ppm)	0.18	0.058	0.23	0.075

The percentage of sand and silt in east-west orientation plots (EW) was higher than in north-south orientation plots (NS) in 2019 (P<0.001; Table B.2), while % clay was higher in NS than EW in both years (P<0.001) and %silt in 2018 (P<0.001; Table B.3).

Table B.2 Biomass 2 soil analysis results in year 2019, comparing east to west (EW) and north to south (NS) data. Values are means across two clones, three plots as replicates, three planting densities, and two sample replicates

	EW	**s.e**	**NS**	**s.e**
Soil texture				
Sand (%)	47.00	1.58	34.00	1.58
Silt (%)	38.00	0.75	24.40	0.75
Clay (%)	25.00	0.46	41.60	0.46
pH	4.90	0.03	5.10	0.03
Macronutrients				
C (%)	0.89	0.03	0.63	0.03
N (%)	0.09	0.003	0.11	0.003
C/N	6.67	0.36	5.89	0.36
Organic N content (%)	0.08	0.002	0.09	0.002
P (mg.kg^{-1})	3.20	1.60	9.50	1.60
K (me.100g^{-1})	2.29	0.12	2.97	0.12
Ca (me.100g^{-1})	0.96	0.12	2.03	0.12
Mg (me.100g^{-1})	0.14	0.013	0.25	0.013
Micronutrients				
Na (me.100g^{-1})	0.07	0.004	0.09	0.004
Zn (ppm)	0.162	0.03	0.299	0.03

Table B.3 Biomass 2 soil analysis results in year 2018, comparing east to west (EW) and north to south (NS) data. Values are means across two clones, three plots as replicates, three planting densities, and two sample replicates

	EW	**s.e**	**NS**	**s.e**
Soil texture				
Sand (%)	48.89	0.787	31.89	0.787
Silt (%)	24.44	0.832	38.00	0.832
Clay (%)	26.67	0.522	30.11	0.522
pH	4.28	0.039	4.51	0.039
Macronutrients				
C (%)	0.631	0.032	0.891	0.032
N (%)	0.093	0.003	0.127	0.003
C/N	6.83	0.281	7.00	0.281
Organic N content (%)	0.085	0.003	0.117	0.003
P (mg.kg^{-1})	6.91	1.300	4.41	1.300
K (me.100g^{-1})	0.323	0.026	0.539	0.026
Ca (me.100g^{-1})	0.577	0.107	1.294	0.107
Mg (me.100g^{-1})	0.216	0.023	0.244	0.023
Micronutrients				
Na (me.100g^{-1})	0.086	0.006	0.091	0.006
Zn (ppm)	0.140	0.024	0.212	0.024

Differences in soil nutrients among different planting densities were relatively small compared to row-orientation differences, as seen in Table B.4 for 2018 and Table B.5 for 2019.

Table B.4 Biomass 2 soil analysis results in year 2018, comparing planting density 2000, 3300 and 5000 trees.ha^{-1} data. Values are means across two clones, three plots as replicates, two row-orientations, and two sample replicates

	2000	**s.e**	**3300**	**s.e**	**5000**	**s.e**
Soil texture						
Sand (%)	41.67	0.964	38.33	0.964	41.17	0.964
Silt (%)	29.50	1.020	32.67	1.020	31.50	1.020
Clay (%)	28.83	0.639	29.00	0.639	27.33	0.639
pH	4.392	0.048	4.408	0.048	4.383	0.048
Macronutrients						
C (%)	0.714	0.038	0.727	0.038	0.843	0.038
N (%)	0.105	0.003	0.108	0.003	0.118	0.003
C/N	6.92	0.345	6.67	0.345	7.17	0.345
Organic N content (%)	0.098	0.003	0.098	0.003	0.108	0.003
P (mg.kg^{-1})	3.66	1.592	4.08	1.592	9.25	1.592
K (me.100g^{-1})	0.305	0.032	0.458	0.032	0.530	0,032
Ca (me.100g^{-1})	0.901	0.131	1.021	0.131	0.886	0.131
Mg (me.100g^{-1})	0.225	0.028	0.248	0.028	0.218	0.028
Micronutrients						
Na (me.100g^{-1})	0.089	0.008	0.088	0.008	0.089	0.008
Zn (ppm)	0.158	0.029	0.159	0.029	0.212	0.029

Table B.5 Biomass 2 soil analysis results in year 2019, comparing planting density 2000, 3300 and 5000 trees.ha^{-1} data. Values are means across two clones, three plots as replicates, two row-orientations, and two sample replicates

	2000	**s.e**	**3300**	**s.e**	**5000**	**s.e**
Soil texture						
Sand (%)	39.3	1.93	38.3	1.93	43.3	1.93
Silt (%)	29.5	0.92	32.7	0.92	31.5	0.92
Clay (%)	30.3	0.56	27.7	0.56	29.7	0.56
pH	5.05	0.04	5.08	0.04	4.95	0.04
Macronutrients						
C (%)	0.714	0.03	0.727	0.03	0.843	0.03
N (%)	0.106	0.003	0.095	0.003	0.096	0.003
C/N	5.67	0.434	6.92	0.434	6.25	0.434
Organic N content (%)	0.094	0.003	0.085	0.003	0.085	0.003
P (mg.kg^{-1})	4.1	1.97	4.3	1.97	10.7	1.97
K (me.100g^{-1})	2.57	0.15	2.40	0.15	2.94	0.15
Ca (me.100g^{-1})	1.29	0.14	1.71	0.14	1.50	0.14
Mg (me.100g^{-1})	0.14	0.02	0.21	0.02	0.22	0.02

Micronutrients						
Na (me.100g^{-1})	0.090	0.005	0.077	0.005	0.075	0.005
Zn (ppm)	0.168	0.038	0.221	0.038	0.303	0.038

B.2 Leaf nutrient analysis

Leaf nutrients measured across all plots in 2018 and 2019 are shown in Table B.6. There was a higher variability among replicates for micronutrients compared to macronutrients. A difference in leaf nutrients between 2018 and 2019 and different row-orientations was observed, although it was small.

The concentration of N (P<0.001), Ca (P<0.001), Cu (P<0.001), Fe (P<0.001), and Mn (P<0.001) were higher in 2019 compared to 2018. However, decreases in the level of P (P<0.001), Mg (P=0.026), S (P=0.007), and Zn (P<0.001) were observed in 2019 compared to 2018.

Table B.6 Macro and micronutrient concentration of leaves from Biomass 2 trial in the year 2018 and year 2019. Values are means across three replicate plots, two clones, three planting densities, two row-orientations, and two sample replicates (+/- standard errors)

	2018	**s.e**	**2019**	**s.e**		**2018**	**s.e**	**2019**	**s.e**
Macronutrients					**Micronutrients**				
N (%)	2.51	0.02	2.70	0.02	B (mg.kg^{-1})	71.7	1.62	71.5	1.62
P (%)	0.17	0.001	0.16	0.001	Cu (mg.kg^{-1})	2.88	0.12	5.62	0.12
K (%)	1.89	0.05	1.95	0.05	Zn (mg.kg^{-1})	59.29	1.14	17.61	1.14
Ca (%)	1.17	0.03	1.80	0.03	Fe (mg.kg^{-1})	22.4	1.98	94.8	1.98
Mg (%)	0.41	0.01	0.37	0.01	Mn (mg.kg^{-1})	294	16.6	408	16.6
S (%)	0.16	0.003	0.15	0.003					

Leaf N concentration was higher in the EW row-orientation than in the NS orientation in 2018 (P=0.001) and 2019 (P=0.025), whereas leaf K in 2018 (P<0.001) and 2019 (P<0.001), Mg in 2018 (P<0.001) and 2019 (P=0.049), and Cu in 2018 (P=0.002) and 2019 (P<0.001) were higher in NS compared to EW (Table B.7 and B.8). Meanwhile, B concentration in EW was higher compared to NS in 2018 (P<0.001) and 2019 (P<0.001).

Table B.7 Macro and micronutrient concentration of leaves from Biomass 2 trial in 2018 comparing east-west (EW) and north-south (NS) row orientation. Values are means across three replicate plots, two clones, three planting densities, and two sample replicates (+/- standard errors)

	EW	**s.e**	**NS**	**s.e**		**EW**	**s.e**	**NS**	**s.e**
Macronutrients					**Micronutrients**				

N (%)	2.57	0.02	2.45	0.02	B (mg.kg^{-1})	63.7	2.89	79.6	2.89
P (%)	0.17	0.001	0.17	0.001	Cu (mg.kg^{-1})	2.42	0.20	3.33	0.20
K (%)	1.75	0.06	2.05	0.06	Zn (mg.kg^{-1})	62.9	1.65	55.7	1.65
Ca (%)	1.05	0.04	1.29	0.04	Fe (mg.kg^{-1})	23.02	1.03	21.73	1.03
Mg (%)	0.29	0.02	0.52	0.02	Mn (mg.kg^{-1})	298	30.2	290	30.2
S (%)	0.161	0.003	0.164	0.003					

Table B.8 Macro and micronutrient concentration of leaves from Biomass 2 trial in 2019 comparing east-west (EW) and north-south (NS) row orientation. Values are means across three replicate plots, two clones, three planting densities, and two sample replicates (+/- standard errors)

	EW	**s.e**	**NS**	**s.e**		**EW**	**s.e**	**NS**	**s.e**
Macronutrients					**Micronutrients**				
N (%)	2.75	0.03	2.66	0.03	B (mg.kg^{-1})	77.1	1.80	65.9	1.80
P (%)	0.155	0.003	0.163	0.003	Cu (mg.kg^{-1})	4.60	0.19	6.63	0.19
K (%)	1.33	0.08	2.58	0.08	Zn (mg.kg^{-1})	15.8	1.73	20.0	1.73
Ca (%)	1.81	0.05	1.78	0.05	Fe (mg.kg^{-1})	100.0	5.24	89.6	5.24
Mg (%)	0.35	0.02	0.40	0.02	Mn (mg.kg^{-1})	448	22	368	22
S (%)	0.17	0.006	0.13	0.006					

There were no other significant differences in leaf nutrients between clones. However, differences in leaf nutrients were observed between different planting densities in 2018 and 2019 (Table B.9 and B.10). Planting density 5000 trees.ha^{-1} had the highest concentration of P in 2018 ($P<0.001$) and 2019 ($P=0.004$), K in 2018 ($P<0.001$) and 2019 ($P=0.019$), S in 2018 ($P<0.001$) and 2019 ($P<0.001$), and Fe in 2018 ($P<0.001$) and 2019 ($P=0.013$).

Table B.9 Macro and micronutrient concentration of leaves from Biomass 2 trial in the year 2018 comparing different planting densities. Values are means across three replicate plots, two clones, two row-orientations, and two sample replicates (+/- standard errors)

	2000	**s.e**	**3300**	**s.e**	**5000**	**s.e**
Macronutrients						
N (%)	2.59	0.03	2.46	0.03	2.49	0.03
P (%)	0.17	0.002	0.17	0.002	0.18	0.002
K (%)	1.68	0.07	1.84	0.07	2.17	0.07
Ca (%)	1.29	0.05	1.02	0.05	1.19	0.05
Mg (%)	0.44	0.02	0.34	0.02	0.44	0.02
S (%)	0.16	0.003	0.15	0.003	0.18	0.003
Micronutrients						
B (mg.kg^{-1})	70.3	3.54	75.4	3.54	69.2	3.54
Cu (mg.kg^{-1})	2.88	0.25	2.51	0.25	3.24	0.25
Zn (mg.kg^{-1})	55.8	2.02	62.5	2.02	59.6	2.02
Fe (mg.kg^{-1})	15.56	1.26	23.61	1.26	27.94	1.26
Mn (mg.kg^{-1})	259	36.9	218	36.9	404	36.9

Table B.10 Macro and micronutrient concentration of leaves from Biomass 2 trial in 2019 comparing different planting densities. Values are means across three replicate plots, two clones, two row-orientations, and two sample replicates (+/- standard errors)

	2000	**s.e**	**3300**	**s.e**	**5000**	**s.e**
Macronutrients						
N (%)	2.57	0.03	2.72	0.03	2.82	0.03
P (%)	0.15	0.003	0.16	0.003	0.17	0.003
K (%)	1.80	0.09	1.92	0.09	2.14	0.09
Ca (%)	1.74	0.06	1.81	0.06	1.84	0.06
Mg (%)	0.38	0.015	0.38	0.015	0.35	0.015
S (%)	0.15	1.83	0.13	1.83	0.17	1.83
Micronutrients						
B (mg.kg^{-1})	68.6	1.83	68.9	1.83	77.0	1.83
Cu (mg.kg^{-1})	6.59	0.15	5.22	0.15	5.04	0.15
Zn (mg.kg^{-1})	13.9	1.88	18.9	1.88	20.0	1.88
Fe (mg.kg^{-1})	92.9	4.46	86.3	4.46	105.2	4.46
Mn (mg.kg^{-1})	541.7	16.43	319.7	16.43	361.8	16.43