



Branching responses to pruning in young cocoa trees

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ABSTRACT

The branching pattern of a tree determines the efficiency of light interception and carbon assimilation. Pruning can modify the branching pattern, because of changes in physiological and environmental conditions, and ultimately pruning can have major effects on yield. For one of the major tropical tree crops, cocoa (*Theobroma cacao*), very little is known about branching response to pruning. To address this knowledge gap, we performed a pruning experiment on young cocoa trees in Côte d'Ivoire.

We applied five treatments to two parcels planted with different genetic materials: two heading treatments (the removal of the terminal apex or 66% of a primary branch) and two thinning treatments (the removal of 1 or 2 primary branches) and one unpruned control. The secondary-branching pattern of the primary branches was described by the number, position, and length of secondary branches right after pruning, and the same observations were repeated after a cycle of leaf production. The probability of branching and the length of secondary branches along a primary branch, in pruned and unpruned conditions, was analyzed using generalized and linear mixed effect models, respectively.

In unpruned conditions, the probability of secondary-branch presence was higher towards the middle of the primary branches and lower at the base and the tip. Secondary-branch length decreased from the base to the tip of a primary branch. After one cycle of leaf production, secondary branch emergence differed between the two parcels. In one parcel more branches emerged on the distal section of a primary branch, while in the other they emerged preferentially in the more proximal section. Pruning increased the probability of branch emergence mostly towards the tip of a primary branch, with heavy heading having the strongest effect. By contrast, heavy thinning increased branch emergence also toward the base of the primary branch.

Our results can be applied to improve formation pruning, as this may trigger branching in different parts of the crown, depending on the form of pruning. Our study also assists the development of three-dimensional tree models that could further our understanding of the impact of pruning on cocoa growth and productivity.

1. Introduction

One of the most important determinants of tree architecture is the branching pattern of the tree. The appearance, location, and size of the branches determine the distribution of the foliage, influencing the efficiency of light interception and thus whole-plant photosynthesis (Ninemets, 2010). The branching pattern is the result of the interaction between genes, environmental conditions and perturbations (McSteen and Leyser, 2005; Sachs and Novoplansky, 1995). Interspecific differences in branching patterns also reflect divergent adaptations to different habitats (Poorter et al., 2006).

Branching is regulated by correlative inhibition. This comprises various forms of suppressive signaling among organs. One of the best-

known forms of correlative inhibition is apical dominance whereby an apical bud exerts a suppressive signal on axillary buds in basipetal direction. The presence of other organs, such as branches or leaves, also contributes to the inhibition of bud break and branch growth. This inhibition can be exerted by organs on the same branch (within-branch inhibition) or by nearby branches (between-branch correlative inhibition) (Wubs et al., 2013; Zieslin and Halevy, 1976).

Pruning is an agricultural or horticultural practice mostly used to modify the architecture of woody perennials, such as tree crops and ornamental trees, to control tree size, increase light capture efficiency, increase yield and/or fruit quality, facilitate harvesting and spraying activities or for esthetic purposes (Ferree and Schupp, 2003). Pruning interventions typically consist of a combination of heading and thinning

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cuts. A heading cut removes a portion of a branch including the apex. This releases the apical dominance stimulating bud outgrowth and branch vigor in the remaining branch section (Wilson, 2000). In contrast, a thinning cut removes an entire branch, reducing between-branch correlative inhibition. This can result in the outgrowth of some buds on the remaining branches, but compared to a heading cut, the response triggered by a thinning cut is generally found to be weaker (Ferree and Schupp, 2003).

Pruning-induced branching and increased branch vigor, or a combination of the two, are possible strategies of compensatory responses with which trees mitigate the negative effect of biomass removal (Anten et al., 2003). Up to a certain level, compensatory responses become stronger as biomass removal increases. For instance, in apple the size of the new branches increases with the intensity of the heading cut (Mika, 1986), while in mango trees, more intense heading cuts increase the number but not the size of new lateral shoots (Persello et al., 2019).

The influence of pruning on branching has been studied in several perennial crops in temperate (Fumey et al., 2011; Li et al., 2010; Marini and Sherif, 2020; Wubs et al., 2013) and tropical climates (Persello et al., 2019). However, to date, very limited information on branching pattern and branching responses to pruning is available for cocoa (*Theobroma cacao*), one of the most important tropical tree crops (Asante et al., 2022; Fairtrade Foundation, 2016). Pruning of cocoa is considered an important yield-enhancing practice, while it may also help with tree management (e.g., disease control) and, as such, is recommended to farmers. Different types of pruning are performed in cocoa: formation pruning to establish the structure of the crown after the tree has developed the first whorl of branches; and structural and maintenance pruning, that are performed one or more times per year throughout the tree life cycle to reduce excessive self-shading and constrain tree dimensions (IITA, 2020). Scientific evidence on the effect of formation pruning is scarce (KAU, 1988, 1989, 1991) and evidence of the effect of maintenance pruning on yield is mixed (Tosto et al., 2022). Additionally, pruning recommendations tend to be very general. This has resulted in low adoption of the practice (Obeng Adomaa et al., 2022). Therefore, understanding the branching response of young cocoa to pruning may contribute to the development of effective formation pruning practices, that would result in efficient and productive tree structures.

We analyzed branching patterns and biomass allocation in unpruned and pruned trees. Since cocoa is originally a shade-adapted species (Lachenaud et al., 2005), we hypothesize that cocoa will exhibit a branching pattern that minimizes leaf overlap (hypothesis 1). We expect therefore that trees will have more and longer secondary branches in the distal sections of the primary branches than in the more proximal sections. When pruning is applied, we hypothesize that heading cuts will strongly stimulate branching (hypothesis 2), especially in the distal sections of the pruned primary branch due to the removal of apical dominance. Additionally, we hypothesize that thinning cuts will induce a weak branching response (hypothesis 3), mostly concentrated at the base of the remaining branching due to a decrease in correlative inhibition. Finally, we hypothesize that more intensive pruning (i.e., removing more biomass) will result in increased biomass allocation to new and existing secondary branches as a result of compensatory growth mechanisms (hypothesis 4).

To test our hypotheses, we applied two levels of heading and thinning cuts to the primary plagiotropic branches of two-year-old field-grown cocoa trees in an experimental plantation in Côte d'Ivoire. The branching pattern of pruned cocoa trees was quantified and compared with unpruned trees and related to the intensity and type of pruning.

2. Materials and methods

2.1. Study species

Cocoa is originally an understory species of the Amazon forest

(Lachenaud et al., 2005) and its architecture follows the Nozeran's model (Hallé et al., 1978; Fig. 1a). This model consists of the determinate growth of a vertical (orthotropic) shoot which terminal apex stops growing after 1–2 years. At this stage, a whorl of 3–6 horizontally-spreading (plagiotropic) branches develop at the end of the orthotropic shoot forming the "jorquette". Those jorquette branches will hereafter be referred to as primary branches. Axillary buds of these primary branches produce lateral plagiotropic branches. In this study the analysis of lateral plagiotropic branches was limited to second order branches (hereafter referred as secondary branches), as higher order branches were not observed at this stage. A new orthotropic shoot, referred as sucker, develops from below the jorquette and forms a second jorquette. The reiteration of this process allows for the tree vertical growth. Under cultivated conditions, however, vertical growth is limited to one or two jorquettes (Niemenak et al., 2009). In this study, we only allowed for the development of the first jorquette, by timely removing all developing suckers.

Hallé et al. (1978) reported that primary cocoa branches are proleptic, meaning that they develop from a terminal apex of the orthotropic shoots after a rest period when the buds are dormant. Higher order plagiotropic branches are instead described as sylleptic, meaning that they develop from a lateral meristem formed by a terminal meristem without undergoing a period of dormancy. Finally, cocoa exhibits a rhythmic production of leaves and shoots followed by a period of dormancy, commonly known as flushing (Greathouse et al., 1971). Cocoa trees typically produce several flushes per year. In this study we followed secondary branch formation during one flushing cycle.

2.2. Study sites

This study was carried out at a research center located in the municipality of Tiassalé (5.913,338 N, 4.867,181 W), Côte d'Ivoire. Throughout the study period (2018–2021) the average daily temperature ranged from 22.1 to 31.0 °C and the mean annual cumulative precipitation was 1431 mm, with a dry season (<100 mm of precipitation per month) spanning approximately from December to March. Climate data were obtained from a weather station located ≈1 km from the study site. The soil there has a clay-loam texture and a pH at planting of 5.4.

2.3. Field planting design and field maintenance

Two adjacent parcels, A (0.41 ha) and B (1.08 ha), were established in a former young rubber plantation. In December 2017, we planted plantains (*Musa* sp.) and framire (*Terminalia ivorensis*), a fast-growing tree species commonly used as shade trees in cocoa agroforestry systems, to create sufficient shade for the establishment of cocoa seedlings (see Figure S 1 for planting design). In June 2018, six-month-old cocoa seedlings were planted at a distance of 3 m by 3 m (density of 1111 plants per ha, Figure S 1). In parcel A, a mix of Upper Amazonian hybrids was used, and in parcel B cocoa hybrids F1 and F2 (from *center National de Recherche Agronomique*, CNRA). Initially, only parcel A was intended to be used for the current study. However, due to plant mortality and slow development, too few suitable plants remained in parcel A in 2020. To increase sample size we therefore choose to include F1 plants from parcel B. In statistical analyses, parcel identity is explicitly accounted for.

At the moment of planting, each cocoa seedling received 50 g of Triple superphosphate, 150 g of N-P-K (15-15-15) fertilizer and 10 liter of organic matter (mix of well-decomposed sawdust, rice husks, and chicken dung). Fertilization continued during the course of the experiment at a rate of 100 g/tree/year of N-P-K (15–15–15) applied twice a year (in May and in Oct).

Both parcels were equipped with an irrigation system with a dripping line along each cocoa-plantain-framire row. Supplementary irrigation was provided daily during establishment phase (June 2018- August

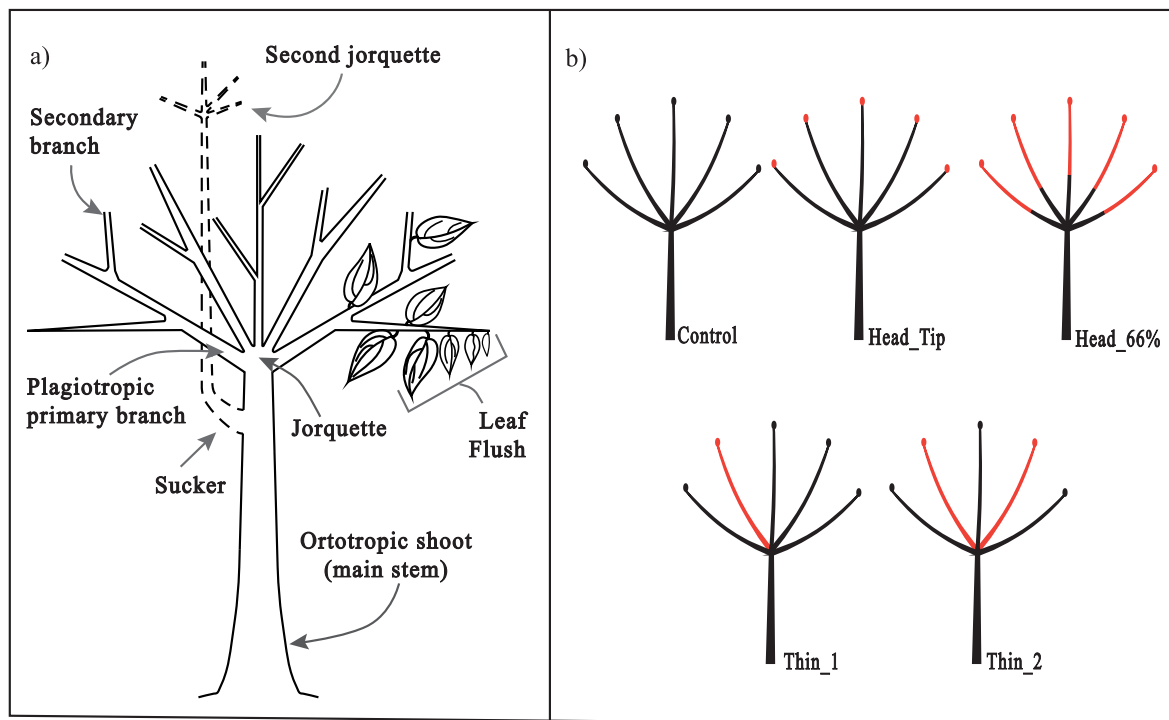


Fig. 1. a) Schematic illustration of cocoa architecture. The sucker is represented with a dashed line to indicate that suckers were removed in this experiment. b) Schematic representation of pruning treatments. In red are sections removed by the treatments. Heading cuts: the removal of terminal apex (Head_tip) of all primary branches; removal of primary branch section corresponding 66% of buds (Head_66%). Thinning cuts: removal of one (Thin_1) or two (Thin_2) primary branches. Note that in panel b secondary branches are not represented for clarity.

2018) and in the following years during drier months (December-March) to avoid water stress. Regular maintenance of the parcels (weeding, pesticide application) was carried out during the full duration of the experiment in both parcels. All suckers developing on the orthotropic stems of cocoa plants were removed, once per month. Once per year, lateral suckers of plantains were removed leaving only two pseudostems per plant to maintain shade to a stable level. In parcel B, shade levels were monitored throughout the study period with three Onset HOBO MX2202 light sensors (placed half a meter from a cocoa seedling). A reference sensor was placed in the proximity of the field in full sun. During the study period, the plantain-framire layer provided an average shade level of about 25% ($SD=21\%$). We can reasonably assume a similar level of shading in parcel A since the planting design and planting time of the shade canopy was the same as in Parcel B. The shade cast by plantain and framire was relatively similar as, at this stage, they had similar sizes.

2.4. Treatments description and allocation

Our experiment had one control and four pruning treatments: two heading treatments and two thinning treatments. Heading treatments entailed the removal of either the apical bud (Head_tip) or 2/3 of the internodes (Head_66%) from all primary branches. Thinning treatments consisted of the complete removal of one (Thin_1) or two (Thin_2) primary branches. Control plants (Control) were left unpruned (Fig. 1b).

Treatments were randomly assigned to plants in parcels A and B that had developed the first whorl of plagiotropic branches (referred hereafter as jorquette) and possessed mostly branches of >30 cm length. Those branches were the result of one or more flushing cycles. However, the number of flushes that formed each branch was not recorded. Furthermore, Control, Thin_1 and Thin_2 treatments were only assigned to plants of which the majority of the apical buds of the primary branches were intact, and treatments Thin_1 and Thin_2 were only assigned to plants with at least four primary branches. This assignment

procedure did result in trees in Thin_2 having on average more branches prior to the treatment than trees in the Head_66% treatment and the Control. However, these differences in branch number did not translate into initial differences in tree vigor: stem diameter and primary branch length did not differ among treatments (Table S 1).

Treatment allocation, initial baseline measurements, and pruning treatments were carried out at the same time when plants were in the dormant phase of the flushing cycle. This meant that leaves from previous flushes were fully hardened and terminal buds were not swollen or producing new leaves. As flushing was not synchronized across all plants, treatment allocation and pruning intervention were carried out in multiple batches from September 2020 to December 2020 in parcel A and from November 2020 to January 2021 in parcel B. Within each batch, trees were assigned to the various treatments including control.

2.5. Tree architecture description

We measured the diameter of the main stem below the jorquette and the number of primary branches of each selected plant. We then counted the number of internodes (corresponding to the number of axillary buds) and measured the length (cm) of each primary branch of a plant. Finally, we measured the length and noted the position (rank) of all secondary branches on each primary branch, starting from the top of the primary branches. In the Head_66% treatment, we counted and measured secondary branches only on the remaining section of the primary branches, and for Thin_1 and Thin_2 treatments only on the remaining primary branches.

After pruning (or after the initial measurements in the case of Control plants) flushing activity was monitored every two weeks to determine when a plant had completed one full flushing cycle. A flush event was considered complete when all the newly formed leaves were fully expanded and dark green in color.

After the completion of one flushing cycle, we conducted the same measurements as described in the previous paragraph. For Control,

Thin_1, and Thin_2 plants, we also recorded whether the apical bud of each primary branch was still present (as apical buds can be lost due to insect or physical damage, thus invalidating the treatments).

2.6. Data analysis

2.6.1. Calculation of variables

In our statistical analyses, we excluded 36%, 33% and 21% of primary branches of the Control, Thin_1 and Thin_2 treatments respectively, because those branches lost their apex during the experiment (Table S 2).

Based on the architectural descriptions at the moment of treatment application and after one flushing cycle, we created two binary variables, with values for all internodes: one for the presence of secondary branches at the end of the flush (that includes existing secondary branches and newly emerged ones) and a second variable for secondary branch emergence during the flush (Figure S 2).

We calculated branch length increment of each secondary branch by subtracting the initial branch length from the final branch length. To calculate total branch-length increment at primary branch level we then summed the increment of all secondary branches in a primary branch plus the terminal length increment of the primary branch itself for Control, Thin_1 and Thin_2, where the apical bud was retained and produced a new section of primary branch during the flushing cycle. Finally, we summed the total increment of all primary branches of each plant to obtain total branch length increment at plant level. The last variable was calculated only for those plants for which all primary branches were included in the analysis (i.e., no loss of terminal apexes due to e.g. insect damage). Sample sizes for this analysis are given in parenthesis in Table S 2.

2.6.2. Statistical analysis

For unpruned trees only, we applied linear generalized mixed effect models (GLMMs, binomial distribution) to explain the variation in the probability of secondary branch presence. Branch presence was described as a quadratic function of branch rank, with parcel identity as an additional fixed factor to control for possible effects of differences in local conditions and/or genotype between parcels. In addition, we also included the date of treatment application to account for possible differences in weather conditions experienced by the plants in the different batches. To explain the variation of branch length increment and final branch length we applied linear mixed effect models (LMM) as a function of rank, parcel and date of treatment application.

For all trees, pruned and unpruned, we applied GLMM (binomial distribution) to explain the variation in the probability in secondary branch emergence as a quadratic function of rank. For branch emergence we also tested the effect of pruning treatments (Control, Head_tip, Head_66%, Thin_1 and Thin_2), number of secondary branches already present at pruning (N. old branches), parcel identity and date of treatment application. Finally, branch length increment at secondary and primary branch level and at plant level was tested with LMMs as a function of pruning treatment, parcel and date of pruning application. All two-way interactions were included in each of the full models. In all analyses, we included random intercepts for primary branches nested in plants to account for the nesting of secondary branches on the primary branch.

To determine the best-fitting model we tested all possible combinations of fixed effects and their two-way interactions, including an intercept-only model. Model selection was based on Akaike Information Criteria (AIC), which provides an approximation of model predictive accuracy, as measured by out-of-sample deviance (McElreath, 2018). We selected the model with the lowest AIC and in the case multiple models had Δ AIC smaller than 2, the simplest model was selected. Confidence intervals (CI) at 95% of each parameter were calculated as ± 1.96 times its standard error. Parameters were considered significant if the CI did not overlap with zero.

If the selected model contained an interaction between pruning treatments and a discrete variable we estimated the slope of the relation for each pruning treatment and tested whether the estimated slopes were significantly different from control.

All analyses were performed using R 4.2.1 (R Core Team 2022). For both GLMMs and LMMs we used the glmmTMB function from the glmmTMB packages (Brooks et al., 2017). For the post-hoc test we used the emmeans (Lenth, 2022) and multcomp packages (Hothorn et al., 2008). To calculate pseudo R^2 for mixed effect models we use the r.squaredGLMM function from the MuMIn package (Bartoń, 2022).

3. Results

3.1. Branching pattern of unpruned trees

3.1.1. Branch emergence

In unpruned trees, 86% of new secondary branches of trees in Parcel A developed on the first eight internodes of the old branch section (section "Flush n" in Fig. 2), while in Parcel B, 37% of new secondary branches were found in this section. Those eight internodes correspond roughly to the section of the primary branch produced in the penultimate flushing episode, if we 1) consider that on average eight new internodes were produced per primary branch during the last flush and 2) assume that the number of internodes produced during a flush remains constant during consecutive flushing. In Parcel A, 9% of new branches emerged on the older section of the branch (top-down rank > 8) and the last 5% were unaccounted for, due to errors in rank assignment. In Parcel B instead, 63% of new branches developed on the older section of the branch.

3.1.2. Distribution of secondary branches along a primary branch

To test whether cocoa trees exhibit a branching pattern that minimizes leaf overlap (hypothesis 1), we first looked at the distribution of secondary branches (existing branches plus newly emerged ones) along the primary branches of unpruned trees. The probability of observing a branch (branch presence) was high for trees in parcel A compared to parcel B (Fig. 3). In both parcels, from the tip to the base of a primary branch, branch presence first slightly increased (reaching an estimated maximum of 0.34 at rank 11 for trees in parcel A and 0.17 at rank 33 for trees in parcel B) and then decreased slowly. This pattern was best described by a concave parabola (Fig. 3). However, the observed variation explained by the fixed factors was very low, while the variation explained jointly by the fixed and random factor was higher, indicating that a substantial part of the variation was given by differences among primary branches and among plants. The date of treatment application was not retained in the best fitting model for this response variable nor for any of the other response variables described below.

3.1.3. Secondary branch length increment and final branch length

To further test our first hypothesis, we evaluated how secondary-branch increment and length of secondary branches changed along a primary branch in unpruned condition. The length increment did not change significantly with rank (slope=0.03, p-value=0.48), implying that during a flushing episode, the length of secondary branches increased at a rate which was unrelated to their position along the primary branch (Fig. 4a). The length of secondary branches increased going from the tip to the base of a primary branch (slope=1.71, p-value<0.001; Fig. 4b). The fixed terms in this linear model explained a substantial part (38%) of the observed variation. When including the random effect of primary branch and tree identity, total explained variation was 61%. We found no difference in secondary branch length increase and branch length between the two parcels.

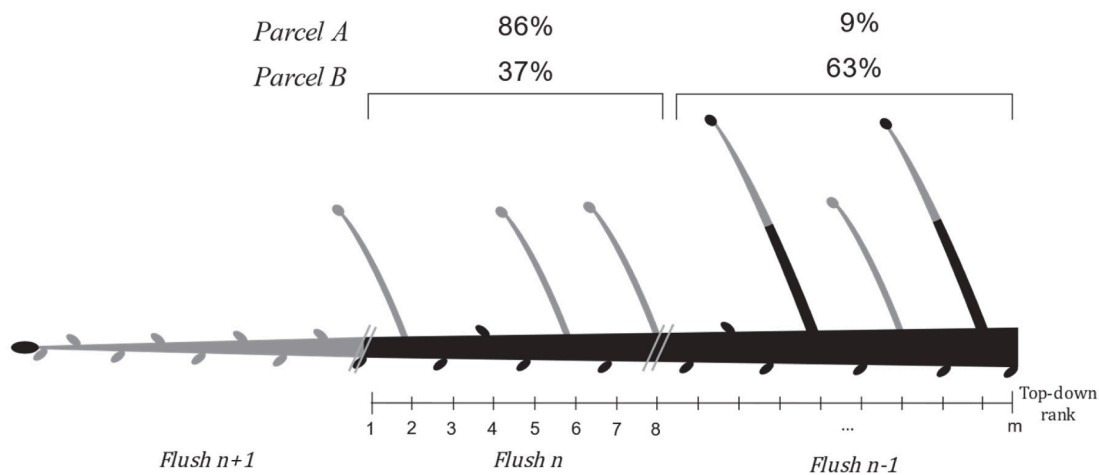


Fig. 2. Visualization of emergence of new branches in various sections of a primary branch in unpruned condition. Average percentage of branches emerged in each section in this study are given for Parcel A and Parcel B separately. Branch sections produced in the last flush ($n + 1$) are shown in gray.

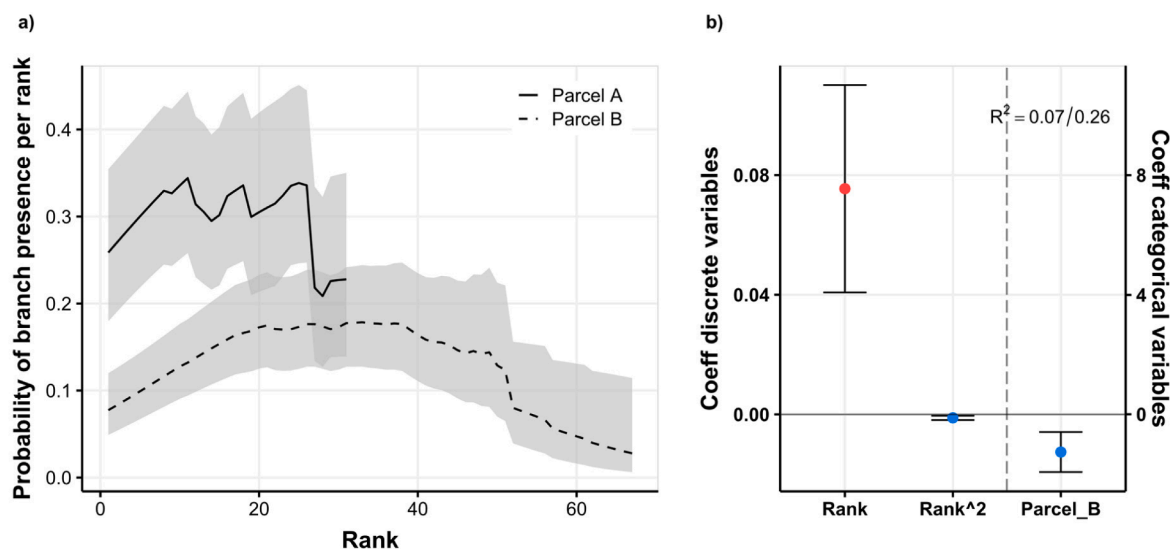


Fig. 3. Branching patterns of unpruned cocoa plants. a) Estimated probability of presence of secondary branches along a primary branch in unpruned trees for parcel A (continuous) and parcel B (dashed) using the mixed effect logistic regression. Gray areas indicates 95% confidence interval. b) Coefficients of the mixed effect logistic regression for discrete (left y axis) and categorical (right y axis) variables. Positive significant coefficient shown in red, negative significant coefficient shown in blue. Vertical error bars indicate 95% confidence intervals. Marginal and conditional R^2 are shown. Rank indicates the position of a secondary branch along a primary branch, starting from the top of the primary branch.

3.2. Pruning effect on branching pattern

3.2.1. Pruning effect on branch emergence

We then tested whether the two types of pruning treatments modified the pattern of branch emergence compared to unpruned trees (hypothesis 2 and 3). In all treatments, our model showed that the probability of branch emergence was higher in the distal part of the branch (excluding section $n + 1$), and non-linearly decreased toward the base of the primary branch (Fig. 5a). In addition, we found that the probability of branch emergence decreased with increasing number of secondary branches already present on the primary branch. The probability of branch emergence was overall lower in parcel B than in parcel A.

All pruning treatments increased the overall probability of branch emergence with Head_66% having the strongest effect (Fig. 5b). However, the effect of the removal of the apical bud (Head_Tip) was not significantly different from Control (Fig. 5b). Except for Thin_2 (the removal of two primary branches), all other pruning treatments had a

stronger negative relation with rank than the control (slope= -0.05 ± 0.01), with Head_66% (slope= -0.22 ± 0.03) showing the steepest relation followed by Head_Tip (slope= -0.11 ± 0.01) and Thin_1 (slope= -0.10 ± 0.01) (Table S 3 and Table S 4). Thus, most pruning treatments concentrated branch emergence more towards the tip of a primary branch compared to Control. In Thin_2 instead, the weaker relation with rank and the main effect of the treatment on branch probability resulted in an increase of probability of branch emergence also at higher ranks compared to control (Fig. 5a). An increase in probability of branch emergence at the base of the primary branches was also observed in Control trees of Parcel B. However, for trees in the Thin_2 and Control treatments in Parcel B, this pattern was highly uncertain due to the lower number of branches with a high number of internodes (Fig. 5a).

3.2.2. Effect of pruning on branch length increment

Finally, we tested whether the type and intensity of the pruning treatments affected biomass allocation to secondary branches

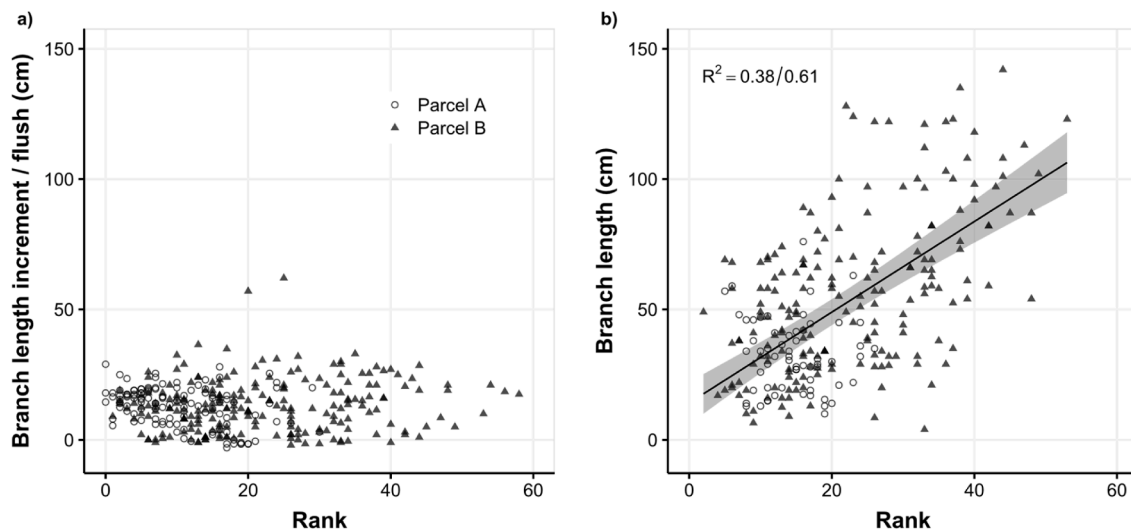


Fig. 4. Branch growth and length in unpruned cocoa plants. a) Branch length increase of secondary branches and b) final length of secondary branches. Circles indicate branches of plants from parcel A and triangles plants from parcel B. Model prediction are shown (continuous line indicates a significant relation). 95% confidence interval of model predictions are shown in gray. “Rank” indicates the position of a secondary branch along a primary branch, starting from the top of the primary branch. Marginal and conditional R^2 are given.

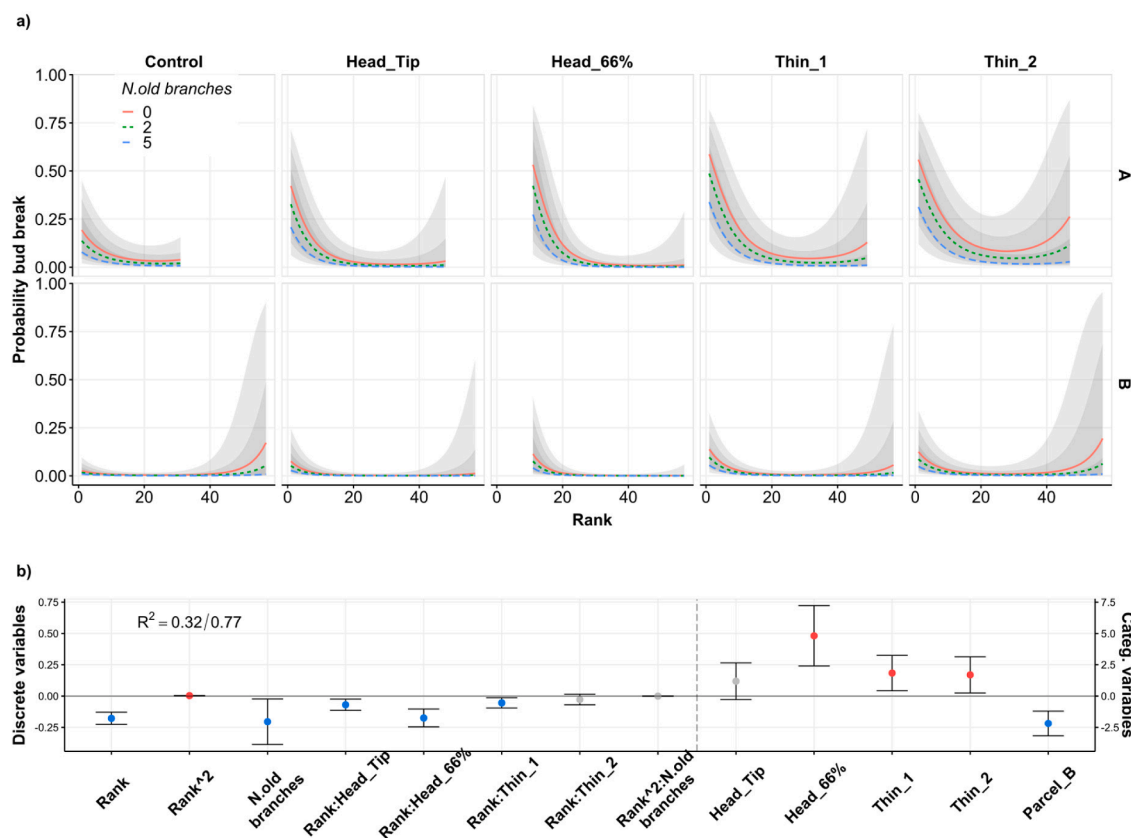


Fig. 5. Effect of different pruning treatments on branch emergence in cocoa plants. a) Probability of branch emergence vs branch rank for primary branches with 0 (red continuous line), 2 (green dashed) and 5 (blue dashed) branches, for parcel A (upper panels) and B (lower panels). Gray areas indicates 95% confidence interval. b) Coefficients of the mixed effect logistic model for continuous (left axis) and categorical (right axis) variables. Positive significant coefficients shown in red, negative significant coefficient shown in blue, and not significant coefficient in gray. Vertical error bars indicate 95% confidence intervals. Marginal and conditional R^2 are given. ‘Control’ is no pruning, ‘Head_tip’ and ‘Head_66%’ are the removal of the tip and 2/3 of all primary branches, and Thin_1 and Thin_2 are the removal of one or two primary branches, respectively. “Rank” indicates the position of a secondary branch along a primary branch, starting from the top of the primary branch.

(hypothesis 4). In line with the observations on control trees, the length increment of secondary branches after one flushing episode was not influenced by rank and it was also not influenced by pruning treatments. However, contrary to what we observed in control trees, mean branch length increase was larger in parcel A than in parcel B ($A = 17.29$, $B = 11.8$, $p\text{-value} < 0.001$, Fig. 6a).

In the Head_{66%} treatment, total branch length increment at primary branches level (the sum of the length increments of all secondary branches plus the terminal increment of primary branch length) was significantly lower than that in control (Coeff = -34 ± 11.9 , $p\text{-value} < 0.01$, Fig. 6b), while other treatments did not differ significantly from control (Table S 5). The total length increment per primary branch was significantly higher in parcel A than in parcel B ($A = 104.5$, $B = 71.998$, $p\text{-values} < 0.001$).

Finally, the total branch length increment per tree was also high for parcel A compared to parcel B ($A = 390.7$, $B = 216.6$, $p\text{-values} < 0.001$, Fig. 6c). The variation explained by the fixed terms in the three models was low, while a substantial part of variation was explained by differences among primary branches and among trees (i.e., the random variables in the model).

4. Discussion

4.1. Cocoa branching pattern

In unpruned trees, the primary plagiotropic branches showed a certain level of secondary branch outgrowth. This can be classified as an “intermediate” level of apical dominance (sensu Cline 1997) meaning that the inhibition imposed by the terminal apex is partial and some axillary buds can grow into a branch when the terminal apex is present.

Considering that cocoa is a shade-tolerant species, we expected a branching pattern that allowed horizontal spreading and minimized leaf overlap, for example with more and longer secondary branches in the distal section of a primary branch (Niinemets, 2010). This expectation was mostly confirmed by our results. In agreement with our expectations, branching probability was lower toward the base of primary branches, the emergence of new branches was concentrated in the distal section of a primary branch, and branching was inhibited if more secondary branches were present. Yet, in contrast to our expectation, we found no preferential allocation to more distal secondary branches, and basal secondary branches were (therefore) longer than more distal ones.

Secondary branches emerged in the sections of the primary branch formed in the flushes before the last flush, but not on newly formed

sections of a branch. This pattern of emergence suggests that axillary buds formed in the last flush need to undergo a short period of dormancy of at least one flushing cycle, before being able to outgrow into a branch. Therefore, secondary plagiotropic branching is to a certain degree proleptic. This finding contrasts those reported by Hallé et al. (1978), who classified lateral plagiotropic branches of cocoa as sylleptic.

In Parcel A the majority of new branches emerged in the penultimate flush section, a section that at the beginning of the last flushing episode was unbranched. Fewer branches emerged further away from the growing tip, where some secondary branches were already present. In Parcel B instead, more secondary branches developed in the more proximal part of the primary branches. Such difference could be ascribed to genetic differences between the planting material in the two parcels.

Our results suggest that both within and between branches correlative inhibition plays a role in regulating branching pattern, a pattern that is also observed in roses (Wubs et al., 2013; Zieslin and Halevy, 1976). The emergence of new branches in fact was strongly limited by the presence of other secondary branches, that in addition to exerting an inhibitory signal, possibly also divert assimilates from the nearby buds (Costes and Guédon, 2002). Finally, the presence of secondary branches decreases slightly at the base of a primary branch, suggesting an inhibitory effect exerted by the other primary branches.

Light quality and quantity are also known to influence branching, and buds in different positions of the crown may have experienced different light conditions (Schneider et al., 2019). However, as internal and environmental signaling are deeply interconnected in the regulation of branch outgrowth disentangling those various factors is difficult (McSteen and Leyser, 2005) and would require dedicated experiments in controlled environment (Wubs et al., 2013), possibly in combination with modeling of the interaction between architecture and light environment (Evers et al., 2011).

A possible implication of this branching pattern is that, as the primary branch grows, the secondary branch structure of a section is almost completely determined in the following flushing episode. In later flushes, this structure seems not to change much, except for some occasional branching events. This process creates an age gradient among secondary branches, with older branches at the base and newer branches toward the tip of the branch. Given that we found no difference in secondary branch growth (i.e., rates of length increment) along a primary branch, the branch ages explain the observed gradient in branch length.

Trees in parcel B, all belonging to a single hybrid, showed an overall

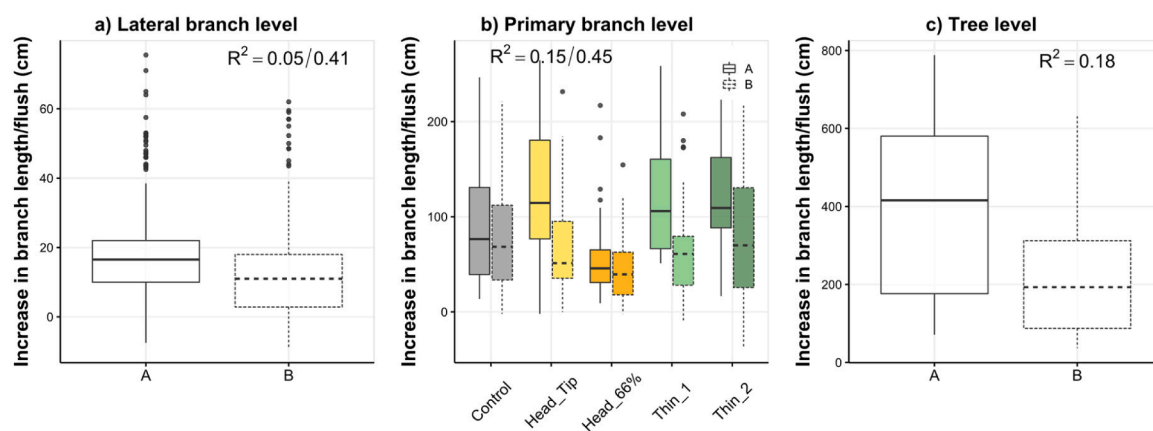


Fig. 6. Effects of different pruning treatments on branch growth in cocoa plants. a) Increment in branch length at secondary branch level in parcel A and B. b) Total increment in branch length at primary branch level for each treatment in parcel A (continuous line) and B (dashed line). Colors indicates different treatments. c) Total branch increment at plant level in parcel A and B. Boxplots show the range (rectangle from 25th to 75th percentile), the median (middle line), the calculated minimum (25th percent. - 1.5 * the range) and calculated maximum value (75th percent. + 1.5 * the range, whiskers), and outliers (dots). ‘Control’ is no pruning, ‘Head_tip’ and ‘Head_66%’ are the removal of the tip and 2/3 of all primary branches, and Thin_1 and Thin_2 are the removal of one or two primary branches, respectively. Marginal and condition R^2 are given.

low level of branching compared to the trees in parcel A, a mix of several hybrids. Our experimental setup did not allow us to disentangle a possible genotypic effect from a location effect. However, we consider it to be unlikely that the location of the parcels would have induced these differences, given that the parcels are less than 150 m from each other and were managed in the same way. Follow-up experiments to characterize the branching pattern of different cocoa hybrids and to test their performance in different shade levels are needed to provide plant material recommendations for different cocoa cropping systems (i.e., full sun vs agroforestry). As branching structure is only one of several factors that determine the light interception efficiency of a tree crown, such experiments should also take into account characteristics such as branch and leaf angle. In addition, since allocation and branching pattern have been shown to vary with branch order and light availability in several tree species (Suzuki, 2022; Suzuki and Suzuki, 2009), dynamic processes such as branch loss and change in branching pattern in older tree crowns should be included.

4.2. Branching responses to heading and thinning cuts

We hypothesized that heading cuts would induce a strong branching response towards the end of the primary branches, while thinning cut would induce a less strong response, towards the base of the primary branches. The response to pruning varied with pruning treatments and was overall in line with our expectations, except for the fact that thinning cuts induced a stronger response than expected and similar to what observed for the heading cuts.

Heading cuts stimulated the production of new branches in the proximity of the cut. This was more evident when we removed approximately two-thirds of the branch than when we only removed the terminal apex. Similar results were reported for mango trees, in which the number of lateral branches increased with pruning intensity (Perrullo et al., 2019). Our results suggest that both apical dominance and within-branch correlative inhibition limit branching, as the removal of the terminal apex alone did not suffice to induce a strong branching response. Yet, our results differ from those in apple trees, where close to 100% of buds in very close proximity of the pruning cut develop into a branch after applying heading cuts (Fumey et al., 2011). In our experiment, the probability of a branch emerging on the first rank below the cut did not exceed 53% in parcel A and 11% in parcel B, indicating that in cocoa, branching responses to heading are less predictable than in other fruit crops.

Both intensities of thinning cuts stimulated the production of new branches in the distal and proximal parts of the remaining primary branches. This was more evident when we removed two primary branches than when only one primary branch was removed. The stimulation of branch emergence on the remaining primary branches suggests a release of the between-branches correlative inhibition as also observed in roses (Wubs et al., 2013). The removal of a number of primary branches may have also improved the light conditions of the remaining branches and triggered bud break deeper in the crown. The probability of secondary branching in the middle section of the primary branch was instead lower, likely due to the inhibition exerted by the presence of more secondary branches in this section of the primary branch. Additional experimental and architectural models (Evers et al., 2011) are needed to disentangle and quantify those effects.

In our experiment, the two types of pruning cuts did not trigger very contrasting responses. This contradicts with results for other tree crop, such as apple trees, where heading cuts trigger a strong branching response while thinning cuts mostly enhance branch vigor without inducing much branching (Ferree and Schupp, 2003; Fumey et al., 2011). We hypothesize that in cocoa the responses to the two types of pruning cuts may converge as a result of constraints induced by the shade-tolerant nature of the crop. This convergence may result from strong inhibitory signaling between organs. Understory trees need to balance between a modest branching response after the apex loss to

avoid leaf overcrowding, on the one hand, while still producing new branches after branch loss to recover total light capture. In tropical forest understory conditions, both leaf overcrowding and a limited capacity of intercepting light reduce growth and survival (Niinemets, 2010).

4.3. Lack of compensatory growth responses

As large amounts of biomass were removed in three of the four pruning treatments, we expected plants in these treatments to show a compensatory response (Anten et al., 2003), in terms of an increase in branch growth per flush, a proxy measurement for vegetative production. However we found no clear evidence for such a compensatory response after one flushing episode.

At secondary branch level, the length of a single flush was rather stable and insensitive to perturbances such as pruning. The average number of internodes produced (eight) in a single flush was similar to what we observed in fully developed trees of similar hybrids (A. Tosto, personal observation) and to that reported by a previous study on cocoa flushing (Greathouse et al., 1971). Removal of 2/3 of the branches showed a much lower total branch length increase, a possible consequence of strong reduction in total bud number after this intensive pruning. The increase in branch length in the other pruning treatments was slightly – and non-significantly – higher than in the control treatment.

The lack of a clear compensatory response contrasts with previous research on vegetative responses to pruning in adult cocoa plants, which reported an increase in vegetative production following pruning (Leiva-Rojas et al., 2019; Tosto et al., 2022). This may be due to the large variability and relatively small sample size of our dataset, and to tree age: adult trees have larger amounts of stored reserves, allowing for more compensatory growth. A larger sample size and longer periods of monitoring biomass and leaf area are likely needed to evaluate evidence for compensatory growth after pruning.

4.4. Research outlook and implications

To our knowledge, this study was the first to quantitatively describe branching patterns of plagiotropic branches in cocoa and to quantitatively assess how pruning intervention changes the pattern of branch emergence. As such, it contributes important inputs to develop effective pruning strategies in cocoa and more generally, adds to the limited knowledge of tree architectural development and responses to (partial) branch loss.

Our study provides some practical insights into the manipulation of cocoa crowns. For young cocoa trees, our results can be applied to inform formation pruning interventions. For example, in a shaded system, where a strong branching response may result in leaf overcrowding, light heading cuts should be preferred over heavy heading cuts. On the other hand, in full-sun high-density systems, where the goal is to have small compact crowns, heavy heading is preferred. Thinning cuts, that stimulate branching along the entire primary branch, could be used to create open crowns with a more uniform distribution of leaves, which may result in higher light interception efficiency. However, we found that in contrast with other fruit trees, pruning responses in cocoa are less determinate. This generates difficulties for practitioners to finely manipulate crown structure through pruning. Additionally, our results can serve as a basis for the development of three-dimensional models of cocoa architecture (Louarn and Song, 2020) that can further our understanding of the performance of cocoa in contrasting shade levels, as well as allowing for exploration of direct and long-term effects of pruning intervention on cocoa functioning.

As pruning interventions are often a combination of different pruning cuts of different intensities, the interactive effect of various pruning cuts should be assessed in follow-up studies to allow for a more complete understanding of pruning responses at the crown level. In addition, since

a decrease in branching probability might be expected in mature trees due to a reduction in vigor (Mencuccini et al., 2005), and since light availability can influence branch production and growth (Leduc et al., 2014), pruning responses should also be investigated in mature cocoa crowns, while accounting for the vertical light distribution gradients and for different shade levels. Finally, to assess whether manipulations of the crown structure in young cocoa trees have a positive impact on cocoa yield, long-term pruning experiments that extend into the reproductive phase are also needed.

CRedit authorship contribution statement

Ambra Tosto: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft. **Jochem B. Evers:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Niels P.R. Anten:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition. **Pieter A. Zuidema:** Conceptualization, Methodology, Data curation, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data used in this study are available on the DANS-EASY database: <https://doi.org/10.17026/dans-x86-6cf3>.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.scienta.2023.112439](https://doi.org/10.1016/j.scienta.2023.112439).

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