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Shoot manipulations improve flushing and flowering of mandarin citrus in Indonesia

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Abstract

Mechanical shoot manipulations through bending and defoliation were applied on mandarin citrus cv. Borneo Prima in order to stimulate flushing and flowering during the rainy season, Oct. 2016-July 2017 in the tropical lowland of Indonesia. Four-year-old citrus trees were treated with bending, defoliation and its combination; and were replicated ten times with single tree as an experimental unit. All manipulated shoots exhibited rapid flushing, particularly of those with defoliation. Unfortunately, no flower was produced on trees treated with defoliation or its combination. Bending stimulated a larger number of flowering and fruiting trees than control. Flower drop was lower in bending, resulted in higher number of fruitset and fruitlets than control. At the generative stage, mandarin showed high C/N ratio due to low nitrogen content, irrespective of treatment. The failure to produce flower on defoliation and its combination could be ascertained by steady C/N ratio. Overall, bending could be applied to improve flowering and support sustainable mandarin production in tropical condition, especially at lowland production fields in Indonesia.

Key words: *Citrus reticulate* Blanco, bending, defoliation, sustainable fruit production, canopy architecture

Introduction

Mandarin citrus (*Citrus reticulate* Blanco) is native to southeastern part of China and southern part of Japan, and also being cultivated in tropical region, including Indonesia (Irsyam, 2015). Among the 18 citrus species identified in Indonesia, mandarin is popular but seasonal, therefore it is highly competitive and fetches a good price in the local market (Budiyati 2014). Development of mandarin citrus in Indonesia faces distinct obstacles compared to region of their origin due to the lack of low temperature to trigger flowering induction. However, water stress could be used for flowering induction under tropical climate (Srivastava *et al., 2*000; Poerwanto and Susila 2014). Water stress is absent due to extended wet season especially during La Nina, so it reduces flower and fruit production by about 60 % and 40-60 %, respectively (Sutopo *et al., 2*016).

The government of Indonesia has dedicated an area for mandarin citrus production at lowland, lower than 600 m above sea level. Therefore Borneo Prima, a well-adapted mandarin citrus on lowland, has been introduced since 2007 (Apriyantono, 2007). The fruit has an orange peel that suits local market demand (Nafisah *et al., 2*014). However, it had dense and vertical oriented canopy (Azizu *et al., 2*016). The dense canopy was less effective for horticulture management (Poerwanto and Susila, 2014) and more sensitive to pest attack (Morales and Davies, 2000; Fake, 2012). In addition, the variety expressed low flower production especially in absence of water stress condition (Sutopo *et al.,* 2016). Developing a low-cost method to improve flowering in such cultivar is important to improve local mandarin production. Therefore, canopy manipulation was proposed in this research as an alternative method.

Canopy manipulation through bending, pruning and defoliation has been adopted in horticulture production to regulate flowering and fruit production (Poerwanto and Susila, 2014) such as in acid lime (Ingle *et al.,* 2001), apple (Mika *et al.,* 2016), guava (Singh *et al.,* 1999), grapefruit (Sharma *et al.,* 2007), lemon (Joubert *et al.,* 2002), orange (Yuan *et al.,* 2005), peach (Singh and Saini, 2013) and pear (Jana, 2016). Pruning also eases harvesting, saves labour cost (Fake, 2012) and stabilizes fruit production through overcoming the alternate bearing problem on citrus (Joubert *et al.,* 2000). Bending is reported to improve flowering response on apple (Poerwanto and Susila, 2014) and cut-flower roses (Kim and Lieth, 2004). Defoliation is commonly practiced in apple and guava (Poerwanto and Susila, 2014). Defoliation significantly improves fruit size of citrus (Morales and Davies, 2000) and mango (Gopu *et al.,* 2014); and also inhibits the spread of pest and disease (Sharma *et al.,* 2007). Defoliation followed by bending in apple stimulates offseason flowering (Notodimedjo, 1994). However, scientific evidence of shoot manipulation on local mandarin was limited. In the present experiment, bending, defoliation and its combination were evaluated to stimulate flowering in mandarin *cv*. Borneo Prima during the extent of rainy season in tropical lowland of Indonesia.

Materials and methods

Study site: Field experiment was conducted from October 2016 to July 2017 at Sindangbarang experimental field of Bogor Agricultural University, Indonesia (6°35'25.02"S, 106°46'9.60"E and 239 m above sea levels). The soil had clay texture and classified as latosol. The water table was around three meters below soil surface. Soil moisture of mandarin trees depends on surface water especially rainfall. Irrigation was not applied during the experiment. The monthly rainfall, daily temperature and relative humidity ranged from 370-525 mm (average 423 mm), 23-31 °C (average 25.8 °C), 82-88 % (average 85.9 %), respectively during the course experiment.

Plant materials: Four-year-old mandarin trees of *cv.* Borneo Prima grafted onto Rough Lemon rootstock were evaluated (Table 1). Trees were planted in October 2013 with spacing about 4 x 4 m and never bear flowers. Trees had ±5-7 primary branch and ±15-20 primary branch. One month prior to the experiment, 10 kg organic fertilizer and 0.2 kg dolomite ($CaCO_3$) were applied for each tree. N, P and K fertilizers at rate 50 g N, 30 g P_2O_5 and 30 g K_2O, respectively, were applied through soil drench. Additional micronutrient (Nutriboron) consisted of 46 % B_2O_3, 1 % Zn and 0.5 % MgO was applied through foliar application.

The experiment was arranged in randomized completely block design with four mechanical shoot manipulation treatments, i.e., control without treatment, shoot bending, defoliation and combination of shoot bending and defoliation. A total of 40 trees were evaluated with a set of ten trees for each treatment in the present experiment.

Shoot manipulation: In bending treatment, all secondary branches were bent down by an angle of 70-80^o from the vertical axis. Control plant had branching angle less than 45^o. The branch position was held with rope and fixed to the bamboo on the ground (Fig. 1). Defoliation was conducted manually by removing all leaves and green immature branches. In combination treatment, branches were bent prior to being defoliated. Shoot manipulation was done manually by two people and time required to set a treatment was recorded (Table 1).

Table 1. Mandarin tree characteristics at initial stage of experiment and time devoted to set a shoot manipulation treatments

Treatment	Plant height (cm)	Canopy width (cm)	The number of leaves	Completion time (minutes)
Control (C)	259.8a	129.8c	2150.0a	0.0d
Defoliation (D)	209.4b	96.1d	0.0b	21.6b
Bending (B)	250.2a	256.0a	2022.2a	14.2c
Combination (B+D)	195.0b	177.2b	0.0b	36.8a
CV (%)	8.17	13.19	20.50	15.58

Means followed by different alphabets in the same column are significantly different based on DMRT at α 5 %; DAT-days after treatment, CV-coefficient of variant.

Observations: Flushing, canopy characteristics, and flowering rate were evaluated. New shoots including leaves (flush) were observed up to sixth month after treatment. New flush length was measured from base to upper growing point. Flush distribution in a tree and a branch were also illustrated. One meter long secondary branch was selected and then imaginary partitioned every 10 cm, starting from base to top branch. The number of flushes growth in every branch segment was noted. Canopy characteristics such canopy shape, number of leaves and branches were noted. Number of negative and dead branches were compared to total branches and showed as percentage level. Any branch that was not exposed to sunlight directly was deemed as negative branch (Poerwanto and Susila, 2014).

Flowering was observed daily, including flower drop, fruit set, and fruit number. Level of nitrogen (N) and carbon (C) was evaluated from fully expanded leaves at the third to fourth position from the dormant growing tips by using Kjeldahl and Gravimetric method, respectively. Number of branches having flower, flush or dormant status was compared to total branches and used to evaluate distribution of those branches on the entire mandarin canopy.

Statistical analysis: Analysis of variance was performed by using Statistical Analysis Software (SAS) version 9.4. For any significant differences between treatments was evaluated by Duncan Multiple Range Test (DMRT) at P=0.05.

Results

New flush growth: The number of flushes, leaves number per flush and the length of flush were affected by treatments (Table 2). The control tree had the lowest flushing performances than others, indicated by significantly few and small flushes with limited number of leaves. On the other hand, bended trees produced the longest flush and the highest number of leaves per flush, although the number of flushes was significantly lower than defoliation and

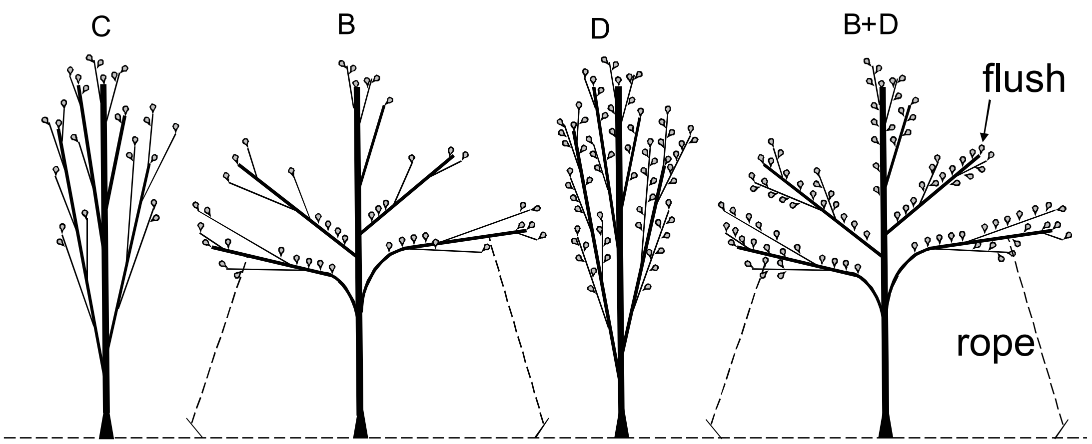

Fig. 1. Illustration of new flush distribution in tree (1.1) and secondary branch (1.2) under different shoot manipulation treatments at 40 DAT. Drawing the canopy without leaves aimed for simplification. The segment is measured from the branch base to top. C-control, B-bending, D-defoliation, B+D-combination of bending and defoliation treatment.

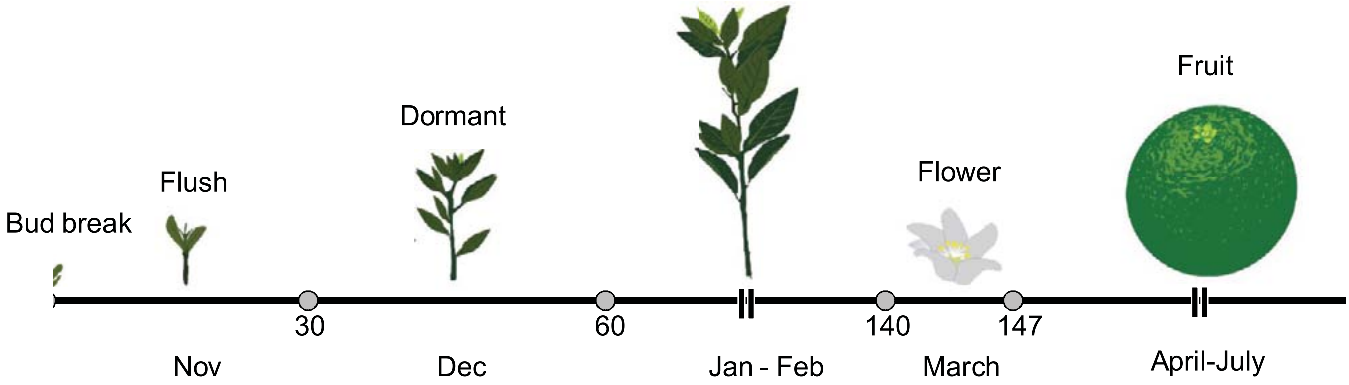

Fig. 2. Illustration of vegetative and generative growth of a new flush in both control and bending treatments.

combination treatments. In the present experiment, the highest number of flush was induced in combination treatment.

Flush distribution along the branches was different among treatments (Fig. 1). Most of new flush emerged at outer canopy in control tree. Bended tree predominantly produced new flush at inside the opened canopy; although few number of flushes were found at top of the canopy, as commonly found in mandarin. Interestingly, new flush distributed evenly at inner and outer canopies as trees received defoliation and combination treatment.

Shoot growth in mandarin was indicated by flush and dormant periods. After dormant stage, shoot tip started to grow. First flush started emerging at 7 DAT and was followed by rapid flushing during the first month after application (Fig. 2). Dormancy usually occurred after a flush produced fully developed leaves. First dormancy lasted for a month, and then emerged the second flush within the third month.

Table 2. Flushing response of mandarin under different shoot manipulation treatments at 30 DAT

Treatment	Number of flushes	Flush length (cm)	Number of leaves per flush
Control (C)	17.6d	14.0c	7.7c
Defoliation (D)	131.5b	15.5c	11.3b
Bending (B)	55.7c	34.7a	20.3a
Combination (B+D)	184.3a	18.1b	12.3b
CV (%)	9.91	15.62	24.93

Means followed by different alphabets in the same column are significantly different based on DMRT at α 5 %; DAT-days after treatment, CV-coefficient of variant.

Table 3. Canopy characteristics of mandarin under different shoot manipulation treatments at the ± 260 DAT

Treatment	Total of branches	Negative branches (%)	Dead branches (%)	Number of leaves	Leaf area (cm^2)	Canopy shape
Control (C)	127b	25a	12a	1984b	20.6a	ellipsoid
Defoliation (D)	105c	11c	6b	826d	8.4b	ellipsoid
Bending (B)	161a	18b	9b	2507a	21.3a	obloid
Combination (B+D)	114c	12c	7b	1020c	11.0b	obloid
CV (%)	7.42	15.22	25.47	11.14	8.43	

Means followed by different alphabets in the same column are significantly different based on DMRT at α 5 %; DAT-days after treatment, CV-coefficient of variant, canopy shape refers to IPGRI (1999).

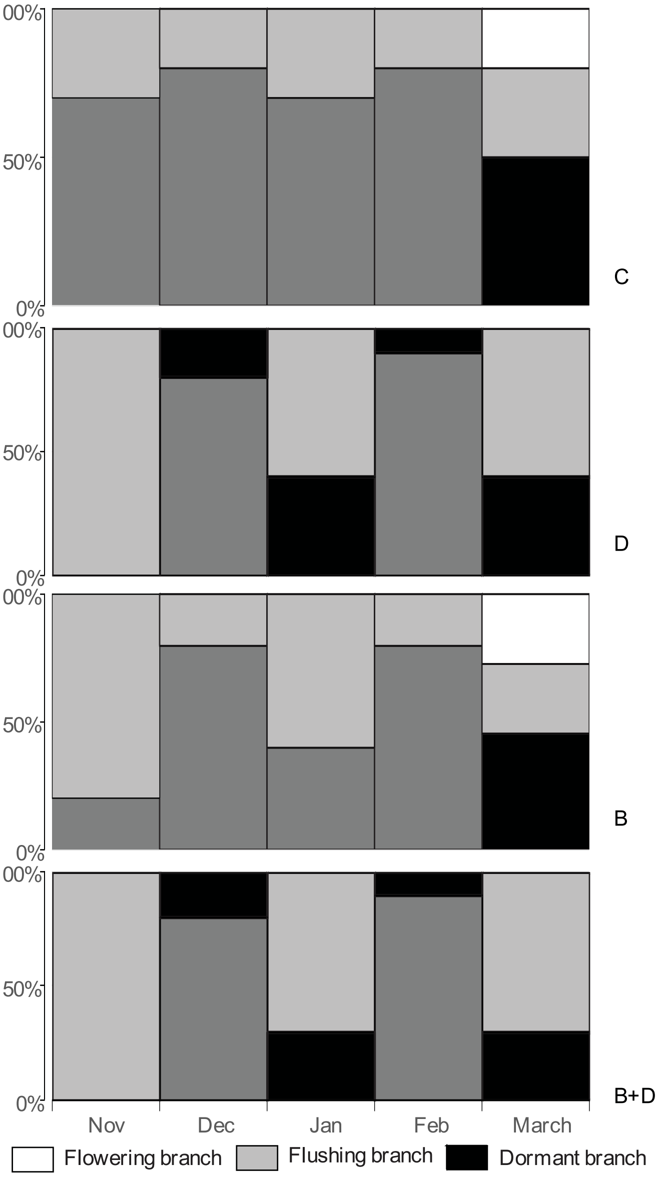

Fig. 3. Percentage of dormant, flushing and flowering branches of mandarin under different shoot manipulation treatments.C-control, B-bending, D-defoliation, B+D-combination of bending and defoliation treatment.

Alternate flush – dormant period seemed common in mandarin branches of all treatments (Fig. 3). However, such flush and dormant cycle within and among branches were likely affected by treatments. In control, more than 50 % branches underwent dormancy during the experiment. While, bending treatment

Table 4. Flowering and fruiting characterictics response of mandarin under different shoot manipulation treatments

Treatment	Flowering trees (%)	Number of flowers	Flower retention (days)	Flower drop (%)	Fruit set (%)	Number of fruit
Control (C)	40	24.3a	4.00b	61.93a	38.07b	8.75b
Defoliation (D)	0	0.0b	0.00c	0.00c	0.00c	0.00c
Bending (B)	80	42.5a	4.80a	34.74b	65.26a	28.50a
Combination (B+D)	0	0.0b	0.00c	0.00c	0.00c	0.00c
CV (%)	-	33.43	26.53	18.32*	18.75*	23.19*

Means followed by different alphabets in the same column are significantly different based on DMRT at α 5 %; flowering tree and flowers per tree were noted at 143 DAT; flower drop, fruitset and fruitlets were noted at 155 DAT; CV-coefficient of variation, cv*-transformation data with $= \sqrt{\bar{x} + 1}$

Table 5. Total carbon, nitrogen and their ratio of mandarin leavesunder different shoot manipulation treatment at 30 and 155 DAT

Treatment	30 DAT			155 DAT		
	C (%)	N (%)	C/N	C (%)	N (%)	C/N
Control (C)	52.49b	3.35a	15.70a	50.64a	2.22b	22.96b
Defoliation (D)	54.08a	3.24a	16.71a	50.47a	2.94a	17.21c
Bending (B)	54.43a	3.85a	14.30a	51.26a	1.98b	26.10a
Combination (B+D)	54.30a	3.32a	16.36a	50.39a	2.90a	17.31c
CV (%)	0.38	8.75	7.83	0.89	5.86	7.50

Means in the same column followed by different alphabets are significantly different based on DMRT at α 5 %; CV-coefficient of variation; DAT-days after treatment; C/N-carbon-nitrogen ratio; C-organic carbon; N-total nitrogen.

was able to reduce dormant branches to less than 50 %, with exception during December and February. Interestingly, all secondary branches produced flush during November in trees treated with defoliation and its combination. In general, defoliated trees showed similar flush-dormant cycle pattern, except the absence of flowering and kept on intermittent flushing during that period.

Canopy characteristics: At the end of the experiment, total number of branches was significantly determined by shoot manipulation treatment (Table 3). As compared to control, bending treatment significantly improved total number of branches by about 27 %, while defoliation alone and its combination with bending reduced total number of branches by about 17 and 10 %, respectively. Among treatments, control trees had a high percentage of negative and dead branches. Both defoliation and its combination reduced the number of leaves and leaf area. On the contrary, bent canopy produced more leaves, although the leaf area was not statistically different than control. The unbent tree had an ellipsoid canopy, while bent ones tended to have obloid shape, irrespective of the presence of defoliation (Table 3).

Flowering and fruiting: Flowering response varied under different shoot manipulations. After twice flushing and dormant growth alternately, flower on certain treatments emerged in the middle of March and subsequently followed by fruiting (Fig. 2). Several control and bent trees underwent flowering at 140 DAT, while all defoliated tree, with or without bending, failed to produce flower and maintained vegetative stage. Massive flowers mostly emerged at outer canopy during the first week after initial flower were observed, *i.e.*, 140-147 DAT. Eight of ten bending trees underwent flowering and fruiting; accounted about two folds larger than flowering trees of control (Table 4). Bended tree produced more flowers than control. Flower retention on bended tree was significantly longer than control. Flower retention time varied from 3-5 days allowing flower to swell, bloom and then drop. Flower drop mostly occurred after anthesis. Trees subjected to bending treatment had lower flower drop and higher fruitset by about 27 % than control trees. As a result, fruit number in bent trees was significantly greater than control trees.

CN ratio: At 30 DAT, the status of C content was significantly higher in manipulated trees than control, while all plant exhibited similar status of C at 155 DAT (Table 5). On the other hand, N level was similar among treatments at 30 DAT and varied at 155 DAT depending on treatments. Combination and defoliation treatment had higher N level than control and bending. There was no significant difference on leaf C/N ratio and nitrogen content at 30 DAT across treatments. Leaf C/N ratio obtained from vegetative stage mandarin ranged from 14.30-16.71 at 30 DAT, while control and bent tree underwent generative stage at 155 DAT have C/N ratio about 22.96-26.10. Flush from defoliation and combintion treatments failed to flower up to 155 DAT and had lower C/N ratio than flowering trees; it seemed close to vegetative ones.

Discussion

Flush stimulation: Defoliation, with or without bending, produced more flush (Table 2) and distributed evenly surrounding the pruned canopy (Fig. 1). Bending also stimulated flush, however, the flush mostly emerged at proximal and distal ends of a branch. It is likely that flush growth in defoliation and bending treatment occoured through different mechanisms that could not be attributed solely to sink-source balance. Defoliation seemed to reduce apical dominance in a branch as a whole, resulting in the growth of flush from the entire canopy. Removing apical dominance, chemically and manually was effective to stimulate flushing in citrus species (Singh *et al.*, 2016; Aliyah *et al.,* 2015; Sharma *et al.*, 2007) and other fruit trees (Yeshitela *et al.*, 2003; Mika *et al.*, 2016; Kumar *et al.*, 2015; Singh *et al.*, 1999). In bending treatment, the bending process probably caused micro-damage along the shoot that reduced apical dominance effect. Treatment to damaged shoot bark such as strangulation, ringing, pinching were previously reported to stimulate flush in mandarin and other citrus species (Aliyah *et al.* 2015; Darmawan *et al.*, 2014; Thamrin *et al.*, 2009; Rivas *et al.*, 2006).

However, flush morphology was different among treatments. All defoliated trees produced shorter flush and fewer leaves than bending and control, as the consequence of resources-limited situation after leaf removal. Yuan *et al.* (2005) confirmed the reduction of leaf size due to defoliation on oranges. Bended trees produced longer flush since the existing foliage serve additional assimilates for better growth than defoliated trees (Table 2). Moreover, the length of flush positively correlated to nitrogen level (coef. =

0.97, p-value = 0.03). It was likely that the existing foliage was important to allow re-translocation of nutrient, as reported by Yeshitela *et al.* (2003).

Flowering dependent leaves: Flower bud mostly emerged from new flush, in both control and bending treatments. Since more new flush was stimulated by bending treatment, it is understood why bending produced more flower bud than control. Unlike flushing, flowering response depended on the presence of mature leaves (Table 4). The presence of mature leaves on existing canopy most likely served as pre-requisite for mandarin citrus flowering as evident from control and bending treatments. Bended trees have better canopy performances such as more foliage, less negative and dead branches than control (Table 3) used to gain more assimilates; therefore bending showed better flower and fruiting response than control. Similar argument have been proposed by Yeshitela *et al.* (2003) and Martínez-alcántara *et al.* (2015). Bending treatment exposed leaves to more red light in the morning which had flower inducing effects (Poerwanto and Susila 2014).

Low number of leaves on post defoliated canopy (Table 3) might be associated with unsuccessful flower promotion; thus flowering in mandarin was sensitive to leaf distribution. We assumed that defoliated tree need longer than 155 DAT to recover its canopy. Eissenstat and Duncan (1992) reported that pruned citrus tree need 9-11 months to regain leaves biomass and fine roots. In this experiment, full defoliation might have induced young mandarin growth. Ingle *et al.* (2001) recommended moderates pruning on acid lime instead of heavy ones to attain more fruits as a consequence of more vegetative-generative growth which led to better shoot/root ratio and greater sunlight exposure.

Mandarin flowering is supposed to be stimulated by pytohormone, such as gibberellin. Low endogenous gibberellin was important for citrus flowering (Darmawan *et al.,* 2014). Bended branches contained less gibberellin than upright ones in relation to the distinct gravity for mobilization of that substance (Mullins, 1967).

The transition from vegetative to generative stage of mandarin could be evaluated by changing of leaf C/N ratio (Darmawan *et al.,* 2014). Higher C/N ratio was required for citrus flowering (Barnier *et al.,* 1981; Poerwanto and Susila, 2014). In present study, the flowering was confirmed by high C/N ratio on bending and control. The high C/N ratio was caused by reduction of N level. Defoliation and combination treatment resulted in vegetative stages; associated with low C/N ratio and high nitrogen levels. Fujita *et al.* (1994) reported improvement of nitrogen levels and nitrogen assimilation rates because of defoliation. Trees tend to produce more vegetative shoot when it is high in nitrogen and low in carbohydrate reserves (Phillips, 1978).

Canopy architecture and its orientation: Mandarin citrus cv Borneo Prima naturally had vertical oriented or ellipsoid canopy with dense branching and acute branching angle (Fig. 1). Such canopy orientation was associated with slower flowering performance compared to tangerine (Mulyanto, 2016). In present experiment, four-year-old mandarin had dense foliage with more than 2000 leaves in a tree (Table 1). In such canopy shape, we estimated more than quarter of total leaves stood under shade, there by reducing its flushing and flowering capacity.

Bended trees had wider canopy, more positive branches and less dead branches than control (Table 3). Thus, bending facilitated better air circulation (Acquaah, 2005), sunlight penetration (Azizu *et al.,* 2016) and subsequently greater carbon assimilation (Yuan *et al.,* 2005), especially for previously shaded leaves with low photosynthesis rate (Septirosya, 2016) and carbohydrate levels (Garcia-luis *et al.,* 1995). The presence of bending treatment altered canopy shape from ellipsoid to obloid by expanding canopy width.

Canopy manipulation had been introduced to control plant growth (Gilman and Black, 2011), including canopy rejuvenation. Defoliated trees underwent rapid flushing to rejuvenate canopy. In such canopy, new leaves were much productive than old or shaded ones (Septirosya, 2016; Goldschmidt, 1999). The more productive foliage, the much carbon assimilation (Phillips, 1978). The number of dead and negative branches were low in defoliated trees. Unfortunately, there was significant reduction of leaf number and size in all defoliated trees as compared to control (Table 3). Nevertheless, there was similar canopy shape on trees treated with control and defoliation.

Implication for sustainable mandarin production: Sustainable mandarin production in Indonesia was challenging especially at low altitude region. In local farmers' field, flowering of mandarin citrus mostly occurred in October to November or at the beginning of rainy season. Mandarin production took about eight months from flowering to harvest. Mandarin fruit was available in the local market in June to July as on-season, while October to November as off-season. Water stress during dry season around July to September was assumed to induce flowering. Monthly rainfall less than 100 mm was a prerequisite for the success of flowering on mandarin citrus (Srivastava *et al., 2*000).

Many farmers sprayed chemicals such as paclobutrazol to enforce flowering during offseason. However, such effort was less effective during heavy rains (Darmawan *et al.*, 2014). Here we showed the success of bending treatment to stimulate flowering in mandarin growing in lowland. As the best treatment, bending promoted flowering in less than 30 % of the total branches, while the rest of the branches kept on intermittent flushing (Fig. 3). However, present study showed fewer flowers and fruit numbers (Table 4) compared to previous report (Thamrin *et at.,* 2009; Darmawan *et al.,* 2014; Yuan *et al.,* 2005; Azizu *et al.,* 2016). In addition, bending technique was cost-effective than defoliation or its combination since it required less time to set up (Table 1). In present experiment, harvesting time supposed to occur in November, which is off-season for most mandarin farmers.

In short, shoot manipulation such as bending, defoliation and its combination enhanced flushing of mandarin. Defoliation stimulated flushing more markedly than others. However, no flowers emerged on defoliation and its combination. Bending solely exhibited better canopy architecture and significantly had larger number of flower and fruitset than control. Flowering mandarin exhibited high leaf C/N ratio due to significantly low N level, and vice versa. Bending technique has potential to enhance sustainable mandarin production during rainy season in Indonesia. Further research is required to evaluate long-term effect of shoot manipulation on fruit production stability.

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