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Bożena Denisow; University of Life Sciences in Lublin, Poland; https://orcid.org/0000-0001-6718-7496

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ORIGINAL RESEARCH PAPER

Self-pruning in lime (*Citrus aurantifolia* Swingle) after treatments with ichiphon, abscisic acid and nitrogen, phosphorus, potassium fertilizers

Elda Kristiani Paisey [1,2], Edi Santosa [2*], Deden Derajat Matra [2], Ani Kurniawati [2], Supijatno [2]

[1] Graduate School of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University, Bogor 16680, West Java, Indonesia
[2] Department Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University, Bogor 16680, West Java, Indonesia

[*] To whom correspondence should be addressed. Email: edisang@gmail.com

Abstract

Self-pruning can lower production costs, especially in the case of annual crops such as citrus. The study aimed to determine self-pruning of lime treated with growth regulators and a fertilizer. Self-pruning was applied on a one-year-old of *Citrus aurantifolia* from February 2020 to April 2022 in Bogor, Indonesia. The treatments used NPK at three levels: 22.5:7.5:2.5 g/tree, 32.5:17.5:12.5 g/tree, and 42.5:27.5:22.5 g/tree, and growth regulators: 500 ppm ichiphon + 100 µM ABA, 750 ppm ichiphon + 50 µM ABA, and 1000 ppm ichiphon. Initial symptoms of self-pruning, namely leaf fall, which correlated with the ethylene concentration in the leaves, occurred in all treatment applications. The combination of the NPK fertilizer 32.5:17.5:12.5 g/tree with 750 ppm ichiphon + 50 M ABA gave the highest ethylene concentration. The highest concentration of ABA was found on the first day after the treatment with the NPK fertilizer 42.5:27:5:22.5 g/tree and growth regulators 500 ppm ichiphon + 100 M ABA; however, it did not differ from the treatment with the NPK fertilizer 42.5:27.5:22.5 g/tree and the 1000 ppm ichiphon growth regulator on the fourth and twelfth days. The percentage of secondary, tertiary, and deciduous branches did not differ between the treatments. Self-pruning that occurs as a result of induction by ichiphon, abscisic acid, and NPK fertilizers, can be an alternative to mechanical pruning.

Keywords

abscission; branch death; chlorophyll; exogenous hormones; growth regulators

1. Introduction

One of the problems of citrus farmers, including lime farmers, is the high cost of maintenance, especially pruning, which reaches 10–30% of production costs (Goldental-Cohen et al., 2017). Based on the results reported by Martí and González (2010), manual pruning of citrus trees costs 979.67 EUR/ha. A research report by Martin-Gorriz et al. (2021) demonstrated that the manual pruning treatment required an average pruning time of 81 h/ha each year from 2016 to 2019, which was higher than in the mechanical pruning practice. Similarly, in terms of cost, manual pruning costs an average of 807 EUR/ha annually, higher than mechanical pruning. Pruning aims to optimize the radiation received by the plant, in addition to optimizing branch growth, so there are production pruning and maintenance pruning. Radiation is essential in citrus growth and production, especially for flower initiation, blooming, and fruit formation (Abobatta, 2018). Pruning branches make the plant canopy broader and more regular so that every part of the plant gets optimal light intensity. Pruned citrus plants have 85% more flowers, 76% more fruit, and 105.3 g more flesh than trees

without pruning (Sugiyatno et al., 2019). To overcome this problem, a cultivation technique that simplifies the process of pruning citrus plants is needed (Martin-Gorriz et al., 2021). Therefore, there are significant efforts to reduce the cost of pruning.

The abscission of plant organs such as leaves, fruits, and flowers occur naturally if they are mature or unnecessary for the plant. Self-pruning is a type of abscission that occurs at the tip of the shoot and the branch. As shown by Paisey et al. (2022), self-pruning naturally occurs in tropical fruit plants, including tamarind, avocado, kapulasan, longan, guava, orange, and lime. It has been observed in oranges, chestnuts, and tomatoes (Jiang et al., 2008; Plummer et al., 1991; Zhang et al., 2014). In tomatoes, the self-pruning gene affects the tomato growth habit at least in part by influencing auxin transport and responsiveness, which can be manipulated for controlling plant growth habit and improving productivity (Silva et al., 2018). Self-pruning is also found in grapevines; it can increase horticultural production efficiency if utilized effectively to reduce manual pruning (Zou et al., 2020). In citrus plants, self-pruning occurs in lateral shoots, which begin to collect nutrients for flower bud differentiation (Zhang et al., 2014). Branch shedding is usually connected with an adaptation to environmental stresses, such as shading and drought, mediated by a decreased hydraulic conductance (Bellani & Bottacci, 1995; Davis et al., 2002; Lauri et al., 2011; Minemba et al., 2019).

Self-pruning can occur if induced by genetic or environmental factors. The hormones ethylene and abscisic acid play an essential role in self-pruning. Ethylene compounds are affected by nutrients, as shown by Kaack and Pedersen (2014), who performed factor analysis of data from an experiment with fertilization of 'Spartan' apples using various levels of nitrogen, phosphorus, and potassium. Their interactions exhibited significance between ethylene and potassium, soluble solids and potassium, acidity and phosphorus, ethylene and phosphorus, and ethylene and nitrogen. Ethylene response is highly dependent on the availability of mineral nutrients, and potassium deficiency can increase ethylene production and reduce stomatal response (Benlloch-González et al., 2010). Nitrogen availability also affects photosynthesis, stomatal conduction, and ethylene production. In addition, the synthesis of ABA was influenced by N, P, and K fertilization, which was proved in *Camptotheca acuminata* plants, where fertilization with N, P, and K (50 g/plant with the N, P, and K ratio of 19:7:12) resulted in a significant increase in the amount of ABA (Liu et al., 1999). Self-pruning can be used as an alternative to pruning citrus plants to reduce production costs. Therefore, it is crucial to investigate the effect of fertilization and growth regulators on self-pruning in lime plants. Many trees prune themselves better than others. In the case of lime plants, self-pruning has not been studied; hence, it is essential to investigate this phenomenon. The study aimed to determine self-pruning incidence with a morphological and biochemical approach to lime plants treated with growth regulators and NPK fertilizers.

2. Materials and methods

The research was conducted at the Experimental Garden of IPB Leuwikopo Dramaga (Latitude: −6.564508, longitude: 106.725009), Bogor–Indonesia from February 2020 to April 2022. The study was conducted under a 50 m long, 5 m wide, and 3 m high shade house. The average daily climate conditions during the study (February 2020–April 2022) were as follows: temperature 26.13 °C, RH (Relative Humidity) 83.01%, light intensity radiation 349.08 μmol m^{-2} s^{-1}, duration of sunshine 13.67 h/d, 11.82 mm/d rainfall.

The study was conducted in a randomized block design. The first factor was the NPK dose (F) and the second factor was growth regulators (Z). Inorganic fertilizer was used at three doses: 22.5:7.5:2.5 g/tree (F1), 32.5:17.5:12.5 g/tree (F2), and 42.5:27.5:22.5 g/tree (F3). The variants of growth regulators were as follows: 500 ppm ichiphon + 100 M ABA (Z1), 750 ppm ichiphon + 50 M ABA (Z2), and 1000 ppm ichiphon (Z3). The treatment was repeated three times, each repetition consisting of 3 plants so that there were 81 individual plants.

The plant material included local lime seedlings obtained from propagation between a rootstock derived from Rough lemon (*Citrus jambhiri* Lush.) and a scion derived

from lime (*Citrus aurantifolia* Swingle). The plant material was planted in a mixture of wet rice husk, dry rice husk, and chicken manure with a ratio of 1:1:1, which was maintained for six months. Lime plant seeds were transferred into polybags when they were one year old. The media used was a mixture of clay soil, chicken manure, and rice husks with a ratio of 3:2:1 (37.5:25:12.5) in a 75-liter volume planter. ICHIPHON 480 SL (RI 01040120041974/petrosida gresik) and abscisic acid $C_{15}H_{20}O_4$ (PhytoTech labs HHY0102072A) were used as growth regulators. ABA purity was min 95% according to manufacture description. Ichiphon 480 SL contained 480 g/L ethephon ($C_2H_6ClO_3P$).

The fertilization treatment was conducted two times a year in February and September when the plants were 12 and 19 months old. The fertilizer was applied by creating circular arrays in the root area at a depth of 5 cm; then the fertilizer was spread in the array and re-covered with the planting medium. The ichiphon and ABA spraying treatments were applied only once on 1st July 2021 or when the lime plant was 18 months old. Ichiphon was sprayed on citrus plants and then covered with plastic to reduce the evaporation of ichiphon into the air, whereas abscisic acid was applied to the root area. Observations were made for 9 months after the treatment.

Lime plant maintenance included weeding and spraying with the sidamethrin insecticide against ladybugs and leaf caterpillars. The sidamethrin insecticide contains the active compound cypermethrin (R.S-alpha-cyano-3-phenoxybenzyl (1R.1S), trans-3 (2.2)-dimethylcyclopropanecarboxlyic acid) with the concentration of the active compound of 50 g/L, which is a systemic insecticide that works as a stomach poison. Sidamethrin was given 3 months before the treatment at a dose of 1 mL/L. Spraying was performed when pests attacked the plants. Watering was done every morning and evening when it did not rain. Watering was carried out until the soil moisture reached 80%, as measured by a soil pH-moisture meter (VT05) from China.

2.1. Percentage of self-pruning on secondary branches, tertiary branches, and leaves and morphology of branches

The percentage of self-pruning indicates branches and leaves that experience self-pruning. Dead and dry branches remaining on the tree are also known as self-pruning events. Morphological measurements of dead secondary and tertiary branches were conducted by measuring the stem diameter with an electronic digital caliper Sketmat Sigmat Vernier 0–150 mm (Nankai, China). Illustration of lime tree branching is presented in Figure 1. The length of the branches was measured from their base to the tip using a tape measure or a ruler. The number of dead secondary branches was observed and counted from the treatment until four months after the treatment by a hand counter (Kenko, Japan). The number of tertiary branches that died was observed and counted from the time of treatment until four months after the treatment. Self-pruning of leaves was counted every day after the plants were given the treatment. The calculations were conducted until the plants no longer dropped their leaves.

2.2. Analysis of ethylene levels

Ethylene analysis was conducted on samples of leaves that dropped on the first day to the 12th day after the treatment. It was conducted using gas chromatography. The sample was homogenized, and then 1 g was weighed and put into a 10-ml tube. The tube was closed with a rubber cover; then the gas was taken in a 1-ml tube. Acetylene gas was injected into the tube with the same volume, and then incubated for 1–2 h. The gas in the sample was injected into a Hitachi gas chromatograph type 263-50, with an initial temperature of 80 °C, final temperature of 100 °C, injection temperature of 150 °C, detector temperature of 200 °C, contents of silicon ov-17 column, a hydrogen and nitrogen carrier, and detector FID (Yu & Yang, 1979; McNair & Miller, 1998).

2.3. Analysis of abscisic acid levels

Abscisic acid was determined on the first day after the treatment and 12 days after the treatment with the procedure proposed by Yokota et al. (1994). A standard solution was prepared by weighing 0.0020 g of abscisic acid into a 5-ml volumetric flask and

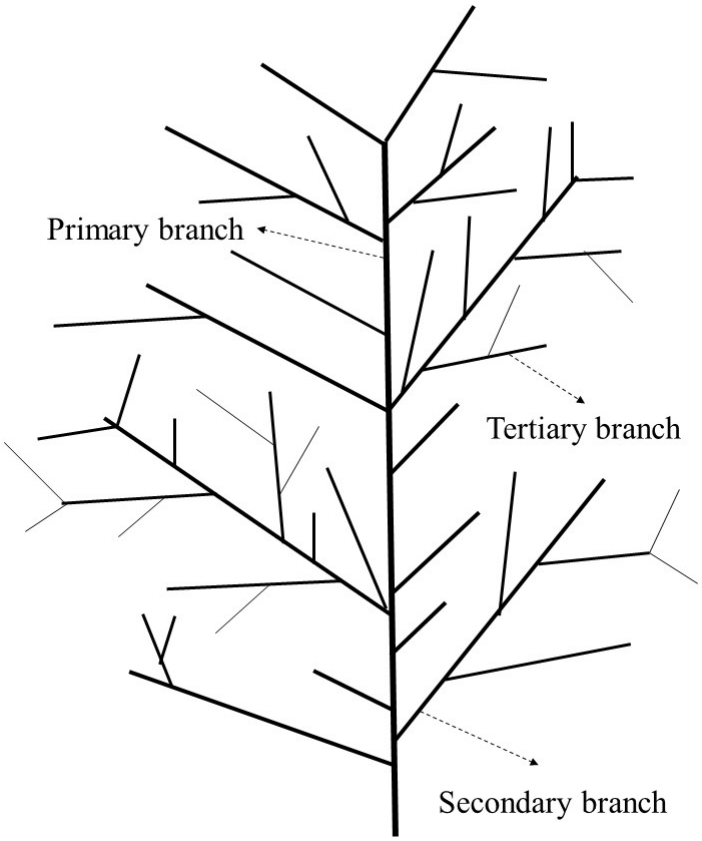

Figure 1 Lime tree branching pattern (*Citrus aurantifolia* Swingle).

adding the eluent to the mark. The stock standard was 40 ppm. That standard solution was made of a standard mixture of abscisic acid with a concentration of 12.5, 25, 50, 100, 250, and 1000 ppb. About 1 g of lime leaves was finely chopped, and 25 ml of 6.8 mH H_3PO_3 methanol was added. 25 mg BHT/500 was added to methanol and the pH was set to 8.5 by adding 1 M $NaHCO_3$. The solution was evaporated at a temperature of 35 °C so that the liquid phase remained. The solution phase was purified with a seppak column with C18, then added to 10 or 25 ml with the mobile phase, and filtered with 0.45 µM. The solution was ready to be injected into HPLC. The HPLC specifications were as follows: column C18 eluent H_3PO_4 68 mM:MeOH (50:50), flow 0.5 ml/min, PDA detector, and a wavelength of 260 nm. The samples were analyzed at 1 and 12 days after treatment.

2.4. Chlorophyll analysis

Chlorophyll content was analyzed 14 days after the treatment using the Warren (2008) method. Fresh leaf discs (0.56 cm^2) were ground in a mortar. Chlorophylls were extracted from the ground samples by adding 1.00 mL of methanol and shaking for two minutes at 30 Hz. The samples were centrifuged for 2 min at 16873 g (Micro centrifuge 5418, Eppendorf, Hamburg, Germany) and the supernatant was transferred to another micro centrifuge tube. The pellet was re-extracted from a second 1.00 mL aliquot of methanol by adding 1.00 mL of methanol to the pellet, shaking for another two minutes, centrifuging, and removing the supernatant. The pellet was discarded while the two supernatants were pooled and used for measurement of chlorophylls.

Micro plate measurements were generally made with the lid on the micro plate so as to reduce evaporation. Spectrophotometer measurements were made by transferring 200 µL of the sample (or blank) into a 1-cm path length quartz cell and reading absorbance in a spectrophotometer with 1 nm bandwidth (UV-2550, Shimadzu, Kyoto, Japan).

Figure 2 Performance of lime plants before (**A**) and after treatment (**B**).

The absorbance of 200 µL of the sample in a micro plate (A652 micro plate, A665 microplate) was converted into a 1-cm path length corrected absorbance using the measured path length:

A652, 1 cm = (A652, microplate − blank)/path length

A665, 1 cm = (A665, microplate − blank)/path length

The chlorophyll concentration was calculated from a 1-cm corrected path length:

Chl a (µg/mL) = −8.0962 A652, 1 cm + 16.5169 A665, 1 cm

Chl b (µg/mL) = 27.4405 A652, 1 cm − 12.1688 A665, 1 cm.

2.5. Statistical analysis

The data obtained were analyzed using ANOVA, followed by a significant difference test using the Duncan Multiple Range Test at 5% alpha. The data were analyzed using software-R.

3. Results

3.1. Self-pruning of leaves, and secondary and tertiary branches

Based on the results of the observations, the treatment caused self-pruning in secondary and tertiary branches. Thinning at the secondary and tertiary branches resulted in even distribution of sunshine on the plant canopy (Figure 2). The average percentage of the incidence of self-pruning on secondary branches of lime plants receiving ichiphon and abscisic acid and fertilization treatment was about 6.70%, which was higher than the self-pruning of tertiary branches (only 5.17%). Based on the results of the further DMRT test, the fertilization factors and regulatory substances applied to the lime plants significantly affected the mortality of tertiary branches (Table 1). Secondary and tertiary branches experiencing self-pruning were located at the bottom of the canopy; hence, they were shaded by the top branches. Due to this condition, the leaves were exposed to lower intensity of radiation to conduct photosynthesis, causing the branches to become weak.

Table 1 Percentage of self-pruning branches and leaves.

N:P:K fertilizers (g/tree)	Growth regulators	Branch (%)		Leaves (%)
		Secondary	Tertiary	
F1 (22.5:7.5:2.5)	Z1	2.75 ± 2.48a	1.95 ± 0.42ab	38.00 ± 4.72bcd
	Z2	3.08 ± 2.14a	1.63 ± 0.80b	49.33 ± 4.06ab
	Z3	4.11 ± 1.92a	3.99 ± 1.74ab	53.33 ± 2.46a
F2 (32.5:17.5:12.5)	Z1	2.79 ± 2.04a	2.64 ± 1.13ab	35.67 ± 5.34cd
	Z2	2.96 ± 0.83a	4.57 ± 3.15ab	44.33 ± 7.51abc
	Z3	3.67 ± 4.45a	5.17 ± 1.96a	50.00 ± 3.83ab
F3 (42.5:27.5:22.5)	Z1	3.27 ± 0.59a	2.37 ± 0.20ab	32.33 ± 11.85cd
	Z2	4.05 ± 1.59a	1.83 ± 1.94ab	30.67 ± 5.03d
	Z3	6.70 ± 4.19a	3.69 ± 1.52ab	53.00 ± 11.35a

Note: Values in a column followed by a similar letter are not significantly different using DMRT α 5%; Z1 = 500 ppm ichiphon + 100 µM ABA, Z2 = 750 ppm ichiphon + 50 µM ABA, Z3 = 1000 ppm ichiphon.

Table 2 Size of self-pruning branches.

N:P:K fertilizers (g/tree)	Growth regulators	Branch diameter (mm)		Branch length (cm)	
		Secondary	Tertiary	Secondary	Tertiary
F1 (22.5:7.5:2.5)	Z1	1.49 ± 1.30a	1.57 ± 0.12a	9.69 ± 8.41a	8.61 ± 1.55a
	Z2	1.27 ± 0.60a	1.36 ± 0.39a	9.11 ± 5.67a	7.94 ± 4.49a
	Z3	1.85 ± 0.69a	1.46 ± 0.34a	11.78 ± 4.35a	8.75 ± 2.13a
F2 (32.5:17.5:12.5)	Z1	1.62 ± 0.67a	1.25 ± 0.38a	7.32 ± 2.56a	5.26 ± 1.08a
	Z2	2.01 ± 0.84a	1.88 ± 0.44a	13.22 ± 2.17a	10.19 ± 1.66a
	Z3	1.03 ± 1.06a	1.68 ± 0.54a	6.02 ± 7.24a	9.43 ± 2.01a
F3 (42.5:27.5:22.5)	Z1	2.45 ± 0.99a	1.78 ± 0.41a	13.17 ± 5.22a	8.28 ± 2.18a
	Z2	1.76 ± 1.35a	1.00 ± 1.09a	8.24 ± 5.14a	4.83 ± 4.66a
	Z3	1.96 ± 0.46a	1.93 ± 0.20a	11.74 ± 2.70a	8.55 ± 4.11a

Note: Values in a column followed by a similar letter are not significantly different using DMRT α 5%; Z1 = 500 ppm ichiphon + 100 µM ABA, Z2 = 750 ppm ichiphon + 50 µM ABA, Z3 = 1000 ppm ichiphon.

As shown by the results of further tests on the percentage of self-pruning of leaves (Table 1) due to the fertilizer application and PGR, a higher percentage of leaves were found in the F1 and Z3 treatments, but it was not significantly different from the F2Z3 and F3Z3 treatments. These treatments included fertilization below the standard and ichiphon growth regulators of 1000 ppm without ABA.

3.2. Morphology of self-pruning secondary and tertiary branches

Based on the DMRT test results, there was no significant difference in the morphological characters of the diameter and length of secondary and tertiary branches after the treatment (Table 2). The results showed that, after the treatment with ichiphon and abscisic acid at various doses and the fertilizer, the secondary branches had on average a diameter of 1.72 mm and an average length of 10.03 cm to die. Smaller branches are unable to survive the loss of leaves as a source of photosynthesis due to their lesser size than the others, which causes these branches to die. This also occurred in tertiary branches with an average diameter of 1.55 mm and a length of 9.98 cm (Table 2). Death only occurred in the case of branches with a diameter of less than 2 mm; in addition, the stem was still juvenile. Self-pruning began with the fall of leaves on the branches the day after the treatment. The assimilation needed by the lime plant branches became unavailable. After four months, the branch died, which was indicated by a change in the color of the branch from green to blackish brown, and then the branch dried up and broke quickly. This caused secondary and tertiary branches to die slowly.

3.3. Endogenous ethylene and ABA hormones

Based on the DMRT test results on the concentration of ethylene hormone due to the fertilization treatment and the concentration of ichiphon and ABA (Table 3, Table 4), the highest average concentration was found in combination F2 (32.5:17.5:12.5 g/tree) and Z2 (750 ppm and 50 μM ABA) from the first to the 12th day after the treatment. The highest ethylene content was found in F2Z2 on the third day (126.44 ppm). In all treatments, there was an increase in the ethylene concentration on the second, third, and fourth days except for the F1Z1, F2Z2, F2Z3, F3Z2, and F3Z3 treatments, which exhibited a decrease in the ethylene concentration on the fourth day. The fertilization recommendation given by the Indonesian Subtropical Fruit and Citrus Research Institute (BALITJESTRO) was implemented in the F2 treatment, causing self-pruning events in addition to the administration of the growth regulators. Moreover, the lowest hormone concentrations were found in all treatments on the 12th day.

Based on the results of the further DMRT test, it was found that the application of the fertilizers and growth regulators produced a difference between the treatments: the highest ABA concentration was found in the F3Z1 (10.67 ppm) variant on the first day after the treatment. Meanwhile, the highest concentration of ABA was found in the F3Z3 treatment (5.94 ppm) on the fourth day, but it was not significantly different from that detected in the F3Z1 treatment (5.68 ppm). On day 12, there was a decrease in the ABA concentration in all treatments. The lowest ABA concentration was found in the F1Z3 treatment (0.58 ppm), but it was not significantly different from that in F2Z2 (0.75 ppm).

3.4. Stomatal conductance

There was an increase in stomatal conductance in all treatments compared to the controls (Figure 3B). Although there was no significant difference between the treatment with ethylene hormone, abscisic acid, and fertilization, the highest stomatal conductance was found in treatment F3Z2 (0.55 mol H_2O m^{-2} s^{-1}).

3.5. Chlorophyll a and b

The observation of chlorophyll a after the treatment showed its highest amount (166.08 mg/g) in the experiments using fertilizers above the recommendation from BALITJESTRO (F3) accompanied by 500 ppm ichiphon spray and 100 μM ABA, compared to the F2Z1 treatment (77.60 mg/g). However, the result was not significantly different from the other treatments (Table 5). In turn, the highest content of chlorophyll b was found in the F3Z1 treatment (96.99 mg/g), but it was not significantly different from the other variants. There was a significant difference in total chlorophyll caused by the F3Z1 (263.07 mg/g) and F2Z1 (124.18 mg/g) treatments. However, there was no difference between both treatments and the other treatments.

3.6. Rate of photosynthesis

The highest photosynthetic rate, i.e. 24.77 μmol CO_2 m^{-2} s^{-1}, was found in treatment F1Z2 (22.5:7.5:2.5) g/tree and 750 ppm ichiphon + 50 M ABA, and the lowest value was noted in F2Z3 (22 μmol CO_2 m^{-2} s^{-1}) (Figure 3A). Nonetheless, there was no significant difference in the average rate of photosynthesis among the treatments.

3.7. Transpiration

There was no difference in transpiration among the lime trees subjected to the treatments. The lowest transpiration of 4.79 mmol H_2O m^{-2} s^{-1} occurred in the F2Z2 treatment (32.5:17.5:12.5 g/tree and 750 ppm ichiphon + 50 μM ABA) and the F1Z1 treatment (22.5:7.5:2.5 g/tree and 500 ppm ichiphon + 100 μM ABA). Meanwhile, the highest transpiration rate of 5.51 mmol H_2O m^{-2} s^{-1} was recorded in the F1Z3 treatment (Figure 3C).

Table 3 Ethylene concentration (ppm) on the days after application.

N:P:K fertilizers (g/tree)	Growth regulators	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 9	Day 12
F1 (22.5:7.5:2.5)	Z1	32.34 ± 7.17d	47.06 ± 0.03d	57.99 ± 0.17d	37.59 ± 0.67g	20.62 ± 1.55f	8.54 ± 0.62g	1.97 ± 0.11f	0.70 ± 0.03a
	Z2	22.79 ± 0.36ef	30.78 ± 0.32g	39.65 ± 0.57g	45.84 ± 0.45e	24.95 ± 0.27cd	8.22 ± 0.25g	1.93 ± 0.15f	0.90 ± 0.02a
	Z3	19.17 ± 0.13fg	28.23 ± 0.29h	32.11 ± 0.43h	40.87 ± 1.39f	23.78 ± 0.32de	11.16 ± 0.25f	3.62 ± 0.25d	1.00 ± 0.02a
F2 (32.5:17.5:12.5)	Z1	14.86 ± 0.16g	19.76 ± 0.70i	33.00 ± 0.76h	47.81 ± 0.85d	25.81 ± 0.38c	17.07 ± 0.76d	5.24 ± 0.44c	1.11 ± 0.07a
	Z2	92.07 ± 0.05a	108.23 ± 0.29a	126.44 ± 0.56a	116.35 ± 0.85a	53.15 ± 0.32a	30.53 ± 0.83s	8.83 ± 0.10a	2.51 ± 0.47a
	Z3	44.55 ± 0.12c	54.99 ± 0.42c	63.29 ± 0.62c	44.85 ± 0.51e	22.95 ± 1.13e	15.18 ± 0.45e	2.63 ± 0.32e	1.52 ± 0.08a
F3 (42.5:27.5:22.5)	Z1	29.18 ± 0.14d	38.08 ± 0.13e	46.75 ± 0.38e	57.99 ± 0.04c	45.12 ± 1.07b	24.66 ± 0.47b	5.79 ± 0.30b	1.36 ± 0.50a
	Z2	27.56 ± 0.09de	33.84 ± 0.94f	44.33 ± 0.78f	26.70 ± 0.47h	13.64 ± 0.59g	5.90 ± 0.33h	1.39 ± 0.05g	1.08 ± 0.95a
	Z3	67.47 ± 0.11b	77.25 ± 0.46b	92.34 ± 0.09b	60.43 ± 0.13b	45.69 ± 0.53b	22.11 ± 0.12c	3.69 ± 0.13d	1.00 ± 0.82a

Note: Values in a column followed by a similar letter are not significantly different using DMRT α 5%; Z1 = 500 ppm ichiphon + 100 µM ABA, Z2 = 750 ppm ichiphon + 50 µM ABA, Z3 = 1000 ppm ichiphon.

Table 4 ABA concentration (ppm) on the days after application.

N:P:K fertilizers (g/tree)	Growth regulators	Day 1	Day 4	Day 12
F1 (22.5:7.5:2.5)	Z1	7.08 ± 0.11c	2.97 ± 0.06d	1.18 ± 0.06cd
	Z2	6.09 ± 0.19c	4.25 ± 0.73b	1.36 ± 0.02c
	Z3	3.10 ± 0.29d	2.66 ± 0.22d	0.58 ± 0.06e
F2 (32.5:17.5:12.5)	Z1	8.22 ± 022b	3.98 ± 0.66bc	1.72 ± 0.20b
	Z2	3.68 ± 0.07d	1.68 ± 0.17e	0.75 ± 0.10e
	Z3	6.50 ± 0.50c	3.56 ± 0.27bcd	1.15 ± 0.03d
F3 (42.5:27.5:22.5)	Z1	10.67 ± 0.93a	5.68 ± 0.70a	2.01 ± 0.22a
	Z2	9.06 ± 0.04b	3.11 ± 0.06d	1.11 ± 0.03d
	Z3	8.61 ± 0.73b	5.94 ± 0.16a	1.98 ± 0.01a

Note: Values in a column followed by a similar letter are not significantly different using DMRT α 5%; Z1 = 500 ppm ichiphon + 100 µM ABA, Z2 = 750 ppm ichiphon + 50 µM ABA, Z3 = 1000 ppm ichiphon.

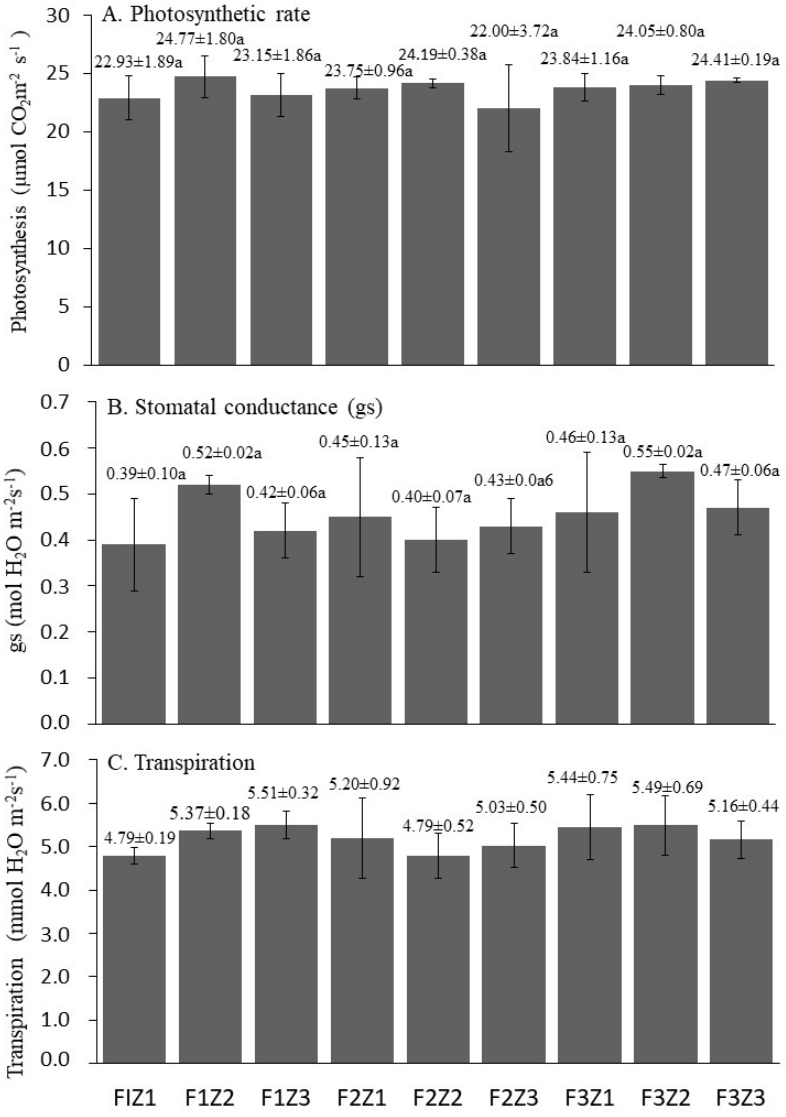

Figure 3 (**A**) Photosynthesis rate. (**B**) Stomatal conductance. (**C**) Transpiration 5 months after treatment. Fertilizer combinations (nitrogen, phosphorus, potassium): F1 (32.5:17.5:12.5) g/tree; F2 (22.5:7.5:2.5) g/tree; F3 (42.5:27.5:22.5) g/tree and growth regulators (Z): Z1 (500 ppm ichiphon + 100 µM ABA); Z2 (750 ppm ichiphon + 50 µM ABA), and Z3 (1000 ppm ichiphon).

Table 5 Chlorophyll a, b, and total in leaves at 7 days after application.

N:P:K fertilizers (g/tree)	Growth regulators	Chl a (mg/g)	Chl b (mg/g)	Total (mg/g)
F1 (22.5:7.5:2.5)	Z1	141.02 ± 33.20ab	87.08 ± 29.81a	228.10 ± 62.33ab
	Z2	96.78 ± 23.77ab	73.44 ± 7.75ab	170.22 ± 30.36ab
	Z3	106.65 ± 31.84ab	73.47 ± 19.93ab	180.12 ± 51.08ab
F2 (32.5:17.5:12.5)	Z1	77.60 ± 51.21b	46.58 ± 20.97b	124.18 ± 70.44b
	Z2	112.23 ± 30.23ab	66.70 ± 18.25ab	178.93 ± 48.30ab
	Z3	130.03 ± 63.64ab	81.30 ± 15.86a	211.33 ± 79.35ab
F3 (42.5:27.5:22.5)	Z1	166.08 ± 38.93a	96.99 ± 17.06a	263.07 ± 54.45a
	Z2	129.11 ± 45.24ab	80.61 ± 24.33ab	209.72 ± 69.43ab
	Z3	110.53 ± 35.59ab	67.06 ± 11.07ab	177.59 ± 45.90ab

Note: Values in a column followed by a similar letter are not significantly different using DMRT α 5%; Z1 = 500 ppm ichiphon + 100 μM ABA, Z2 = 750 ppm ichiphon + 50 μM ABA, Z3 = 1000 ppm ichiphon.

4. Discussion

Self-pruning occurs not only through the abscission zone. Branches in lime plants that experience self-pruning persist on the parent tree for months until external factors such as wind, rain, and animals make them fall. The ethephon and abscisic acid applied did not form abscission zones on the branches but only on the lime leaves. This is consistent with the findings of previous studies (Ferrante & Francini, 2006; Goldental-Cohen et al., 2017) showing that abscission is referred to as the process of natural separation of leaves from the parent plant, which is induced by the hormone ethylene and abscisic acid. It is currently known that ethylene and ABA act as activators in plant organ abscission (Li et al., 2019). In olives, leaf abscission was accelerated by ABA or ethylene treatment, and the combination of ABA and ethylene treatments showed an effective additive effect in leaf abscission. Abscisic acid can accelerate abscission directly (Estornell et al., 2013; Taylor & Whitelaw, 2001), while some studies suggest that ABA promoting organ abscission depends on interactions with ethylene and auxin (Roberts et al., 2002; Wilmowicz et al., 2016; Dimitrova & Nacheva, 2021). Moreover, ABA can promote abscission through ethylene (Gómez-Cadenas et al., 2000). Ethylene is probably the main hormonal activator of abscission (Gómez-Cadenas et al., 1996; Gómez-Cadenas et al., 2000).

Fallen lime leaves left the branches bare and caused them to lose the source of photosynthate needed for growth (Figure 2). This condition caused the branches to die and dry out, and then they were exposed to wind, rain, or animals and became broken. In other words, the branches were led to a slow death. The death that occurred is called cladoptosis (Hearn, 2016). Leaves that self-prune generated abscission areas, specifically on the petiole and leaf blades. This finding is in accordance with Estornell's report (2013) that abscission occurs at a specific position called the abscission zone (AZ). It can be divided into the following four main stages: AZ ontogeny, acquisition of competence of AZ cells to respond to abscission signals, activation of cell separation, and differentiation of the protective layer. Generally, abscission is a gradual process and is accompanied by cell wall degradation, which depends on the activity of certain cell wall hydrolases (Roberts et al., 2002). Homogalacturonan (HG), as the main pectin, is one of the main components of the primary cell wall and can be degraded by polygalacturonase (PG), which is important for organ abscission in many fruit plants (Atmodjo et al., 2013; Liljegren, 2012; Tan et al., 2013).

The fertilization treatments conducted twice during the experiment did not directly cause leaf fall, but the leaves were shed the day after the ethephon and abscisic acid treatments. Based on the field observations, the leaf fall was dominated by leaves located in the lower canopy shaded by the upper crown, and leaves that were young and small also experienced self-pruning. This is consistent with the findings reported by Raden (2009), i.e. if there are too many leaves, the lower leaves do not receive enough light for photosynthesis; hence, the leaves only function as sinks and do not function as sources. In addition, the self-pruning that occurred was due to external factors, such as soil fertility, as nutrients play an essential role in the formation of the

hormone ethylene and abscisic acid. As reported by Iqbal et al. (2011) and Kaack and Pedersen (2014), low doses of potassium can cause endogenous ABA concentrations to decrease, and the effect of ethylene will decrease when plants experience nitrogen deficiency. Moreover, nitrogen, phosphorus, and potassium are positively correlated with endogenous ethylene in apple plants, and these minerals play a significant role in several biochemical reactions that occur in apple plants; therefore, this compound is essential.

High light intensity supports the occurrence of stomatal conductance of CO_2, hence it has a considerable influence on the maximum photosynthetic rate. As indicated by Raden's research report (2009), at low light intensity, there is almost no CO_2 absorption because the rate of CO_2 absorption through photosynthesis is lower than the rate of CO_2 evolution from mitochondrial respiration. The plants given the treatment had high stomatal conductance, although it was not significantly different. According to Budiarto et al. (2019), stomatal conductance increases, and the concentration of CO_2 in the chloroplasts also increases. Stomatal conductance in plants is also influenced by ambient temperature. As shown by Raden (2009), an increasing environmental temperature increases the rate of photosynthesis due to an increase in enzyme activity, which enhances the capacity for CO_2 utilization. CO_2 fixation is a reaction controlled by enzymes. It increases with enzyme activity due to increasing temperature until it reaches a temperature that causes denaturation of the enzymes.

The higher dose of ethephon and abscisic acid caused the degradation of the chlorophyll concentration, which was caused by the increase in enzymatic activity increasing the incidence of self-pruning in the lime plants. The findings reported by An et al. (2021) revealed that that MdABI5 works with its positive or negative interaction partners to regulate ABA-mediated leaf senescence in apple, in which it acts as a core regulator. As explained by Edgerton and Blanpied (1968) and Hu et al. (2021), strigolacton (SL) and ethylene interactively regulated leaf senescence, mainly by controlling chlorophyll degradation induced by darkness in perennial ryegrass.

The enhancement in photosynthesis by the ichiphon, abscisic acid, and fertilizer application was mediated through ethylene-induced changes. It was also associated with a higher level of intercellular CO_2. As demonstrated by Edgerton and Blanpied (1968), ethephon is a direct source of ethylene release when applied to plants and elicits a response identical to that induced by ethylene gas. Ethephon application to plants grown with variable nitrogen, phosphor, and potassium levels induced stomatal and photosynthetic responses. In addition, there was an interaction between ethylene, N availability, and photosynthetic characteristics. Kumar et al. (2002) showed a strong positive correlation with 1-aminocyclopropane carboxylic acid synthase, a rate-limiting enzyme in ethylene biosynthesis. Nitrogen influences the quantity, structure, and composition of the photosynthetic apparatus. It also plays a crucial role in determining the plant's photosynthetic capacity in natural and agricultural environments.

The changes in the photosynthetic rate, transpiration, and stomatal conductance indicated a response of the lime plants to the treatments. The more leaves the plant produces, the higher the transpiration rate. The transpiration rate is closely related to leaf area. As shown by Akmal et al. (2018), plants with larger leaves have a higher transpiration rate, in turn, according to Dulbari et al. (2018), the relationship between stomatal conductance and the ability of photosynthesis and transpiration is important aspect in relation to environment responses. Thus, lime plants still need to be studied further with consideration of some influencing factors, such as the greenness of leaves/chlorophyll content, water availability, and solar energy factors to better understanding on self-pruning incidents.

5. Conclusions

Self-pruning events can occur in various treatment applications. The F1Z3 treatment resulted in the highest percentage of self-pruning in leaves (53.55%), which was not different from the other treatments except F3Z2 (30.67%), where the percentage was lower than in the other treatments. There was no significant difference in the self-pruning events of the secondary branches, whereas the F2Z3 treatment caused

the highest percentage of self-pruning events in the tertiary branches (5.17%), which was significantly different from the F1Z2 treatment (1.63%), which was lower than the others. The combined treatment variant F2Z2 was characterized by the highest ethylene content and the combined F3Z1 treatment resulted in the highest abscisic acid content one day after the treatment. The highest ABA concentration was recorded in the F3Z3 treatment (5.94 ppm) on the fourth day, but it was not significantly different from the F3Z1 treatment (5.68 ppm), likewise on the 12th day. This proves that self-pruning can occur due to the induction of compounds that play a role in abscission.

References

Abobatta, W. (2018). Improving Navel orange (*Citrus sinensis* L) productivity in Delta Region Egypt. *Advances in Agriculture and Environmental Science*, *1*, 36–38. https://doi.org/10.30881/aaeoa.00006

Akmal, A., Santosa, E., Poerwanto, R., Yunus, I., & Handayani, Rd. S. (2018). The relationship between transpiration and calcium fertilization on mangosteen (*Garcinia mangostana* L.) seedlings. *Journal Tropical Horticulture*, *1*(1), 15–19. https://doi.org/10.33089/jthort.v1i1.7

An, J.-P., Zhang, X.-W., Liu, Y.-J., Zhang, J.-C., Wang, X.-F., You, C.-X., & Hao, Y.-J. (2021). MdABI5 works with its interaction partners to regulateabscisic acid-mediated leaf senescence in apple. *The Plant Journal*, *105*, 1566–1581. https://doi.org/10.1111/tpj.15132

Atmodjo, M. A., Hao, Z., & Mohnen, D. (2013). Evolving views of pectin biosynthesis. *Annual Review of Plant Biology*, *64*, 747–779. https://doi.org/10.1146/annurev-arplant-042811-105534

Bellani, L. M., & Bottacci, A. (1995). Anatomical studies of branchlet abscission related to crown modification in *Quercus cerris* L. *Trees*, *10*(1), 20–23. https://doi.org/10.1007/BF00197775

Benlloch-González, M., Romera, J., Cristescu, S., Harren, F., Fournier, J. M., & Benlloch, M. (2010). K+ starvation inhibits water-stress-induced stomatal closure via ethylene synthesis in sunflower plants. *Journal of Experimental Botany*, *61*(4), 1139–1145. https://doi.org/10.1093/jxb/erp379

Budiarto, R., Poerwanto, R., Santosa, E., Efendi, D., & Agusta, A. (2019). Agronomical and physiological characters of kaffir lime (*Citrus hystrix* DC) seedling under artificial shading and pruning. *Emirates Journal of Food & Agriculture*, *31*(3), 222–230. https://doi.org/10.9755/ejfa.2019.v31.i3.1920

Davis, S. D., Ewers, F. W., Sperry, J. S., Portwood, K. A., Crocker, M. C., & Adams, G. C. (2002). Shoot dieback during prolonged drought *Ceanothus* (Rhamnaceae) chaparral of California: A possible case of hydraulic failure. *American Journal of Botany*, *89*(5), 820–828. https://doi.org/10.3732/ajb.89.5.820

Dimitrova, N., & Nacheva, L. (2021). An optimized micropropagation protocol by ex vitro rooting of pear rootstock OHF 333 (*Pyrus communis* L.). *Acta Agrobotanica*, *74*, Article 744. https://doi.org/10.5586/aa.744

Dulbari, Santosa, E., Koesmaryono, Y., & Sulistyono, E. (2018). Yield loss estimation on rice lodging due to strong wind and high rainfall incidents. *Indonesian Journal of Agronomy*, *46*(1), 17–23. https://doi.org/10.24831/jai.v46i1.14376

Edgerton, L., & Blanpied, G. (1968). Regulation of growth and fruit maturation with 2-chloroethanephosphonic acid. *Nature*, *219*, 1064–1065. https://doi.org/10.1038/2191064a0

Estornell, L. H., Agustí, J., Merelo, P., Talón, M., & Tadeo, F. R. (2013). Elucidating mechanisms underlying organ abscission. *Journal of Plant Science*, *199–200*, 48–60. https://doi.org/10.1016/j.plantsci.2012.10.008

Ferrante, A., & Francini, A. (2006). Ethylene and leaf senescence. In N. A. Khan (Ed.), *Ethylene action in plants* (pp. 51–67). Springer. https://doi.org/10.1007/978-3-540-32846-9_3

Goldental-Cohen, S., Burstein, C., Biton, I., Ben-Sasson, S., Sadeh, A., Many, Y., Doron-Faigenboim, A., Zemach, H., Mugira, Y., Schneider, D., Birger, R., Meir, S., Philosoph-Hadas, S., Irihomovitch, V., Lavee, S., Avidan, B., & Ben-Ari, G. (2017). Ethephon-induced oxidative stress in the olive leaf abscission zone enables the development of a selective abscission compound. *BMC Plant Biology*, *17*(1), Article 87. https://doi.org/10.1186/s12870-017-1035-1

Gómez-Cadenas, A., Tadeo, F. R., Talon, M., & Primo-Millo, E. (1996). Leaf abscission induced by ethylene in water-stressed intact seedlings of Cleopatra mandarin requires previous abscisic acid accumulation in roots. *Plant Physiology*, *112*(1), 401–408. https://doi.org/10.1104/pp.112.1.401

Gómez-Cadenas, A., Mehouachi, J., Tadeo, F. R., Primo-Millo, E., & Talon, M. (2000). Hormonal regulation of fruitlet abscission induced by carbohydrate shortage in citrus. *Planta*, *210*(4), 636–643. https://doi.org/10.1007/s004250050054

Hearn, D. J. (2016). Perennial growth, form and architecture of angiosperm trees. In A. Groover & Q. Cronk (Eds.), *Comparative and evolutionary genomics of angiosperm trees. Plant genetics and genomics: Crops and models* (Vol. 21, pp. 179–204). Springer. https://doi.org/10.1007/7397_2016_25

Hu, Q., Din, F., Li, M., Zhang, X., Zhang, S., & Huang, B. (2021). Strigolactone and ethylene inhibitor suppressing dark-induced leaf senescence in perennial ryegrass involving transcriptional down regulation of chlorophyll degradation. *Journal of the American Society for Horticultural Science*, *146*(2), 79–86. https://doi.org/10.21273/JASHS04933-20

Iqbal, N., Nazar, R., Syeed, S., Masood, A., & Khan, N. A. (2011). Exogenously-sourced ethylene increases stomatal conductance, photosynthesis, and growth under optimal and deficient nitrogen fertilization in mustard. *Journal of Experimental Botany*, *62*, 4955–4963. https://doi.org/10.1093/jxb/err204

Jiang, C. Z., Lu, F., Imsabai, W., Meir, S., & Reid, M. S. (2008). Silencing polygalacturonase expression inhibits tomato petiole abscission. *Journal of Experimental Botany*, *59*, 973–979. https://doi.org/10.1093/jxb/ern023

Kaack, K., & Pedersen, H. L. (2014). Effects of potassium, phosphorus, and nitrogen fertilization on endogenous ethylene and quality characteristics of apples (*Malus domestica* L.). *Journal of Plant Nutrition*, *37*(7), 1148–1155. https://doi.org/10.1080/01904167.2013.868484

Kumar, P. A., Parry, M. A. J., Mitchell, R. A. C., Ahmad, A., & Abrol, Y. P. (2002). Photosynthesis and nitrogen-use efficiency. In C. H. Foyer & G. Noctor (Eds.), *Photosynthetic nitrogen assimilation and associated carbon and respiratory metabolism* (pp. 23–34). Kluwer Academic Publishers.

Lauri, P. É., Gorza, O., Cochard, H., Martinez, S., Celton, J. M., Ripetti, V., Lartaud, M., Bry, X., Trottier, C., & Costes, E. (2011). Genetic determinism of anatomical and hydraulic traits within an apple progeny. *Plant, Cell and Environment*, *34*, 1276–1290. https://doi.org/10.1111/j.1365-3040.2011.02328.x

Li, C., Ma, X., Huang, X., Wang, H., Wu, H., Zhao, M., & Li, J. (2019). Involvement of HD-ZIP I transcription factors LcHB2 and LcHB3 in fruitlet abscission by promoting transcription of genes related to the biosynthesis of ethylene and ABA in litchi. *Tree Physiology*, *39*(9), 1600–1613. https://doi.org/10.1093/treephys/tpz071

Liljegren, S. J. (2012). Organ abscission: Exit strategies require signals and moving traffic. *Current Opinion in Plant Biology*, *15*(6), 670–676. https://doi.org/10.1016/j.pbi.2012.09.012

Liu, Z., Adams, J. C., Viator, H. P., Constantin, R. J., & Carpenter, S. B. (1999). Influence of soil fertilization, plant spacing, and coppicing on growth, stomatal conductance, abscisic acid, and camptothecin levels in *Camptotheca acuminata* seedlings. *Physiologia Plantarum*, *105*(3), 402–408. https://doi.org/10.1034/j.1399-3054.1999.105303.x

Martí, B. V., & González, E. F. (2010). The influence of mechanical pruning on cost reduction, production of fruit, and biomass waste in citrus orchards. *Applied Engineering in Agriculture*, *26*(4), 531–540. https://doi.org/10.13031/2013.32056

Martin-Gorriz, B., Martinez-Barba, C., & Torregrosa, A. (2021). Lemon trees respond to different long-term mechanical and manual pruning practices. *Scientia Horticulturae*, *275*, Article 109700. https://doi.org/10.1016/j.scienta.2020.109700

McNair, H. M., & Miller, J. M. (1998). *Basic gas chromatohography*. Wiley Inc.

Minemba, D., Gleeson, D. B., Veneklaas, E., & Ryan, M. H. (2019). Variation in morphological and physiological root traits and organic acid exudation of three sweet potato (*Ipomoea batatas*) cultivars under seven phosphorus levels. *Scientia Horticulturae*, *256*, Article 108572. https://doi.org/10.1016/j.scienta.2019.108572

Paisey, E. K., Santosa, E., Matra, D. D., Kurniawati, A., & Supijatno. (2022). Self-pruning assesment on several tropical fruit species. *Indonesian Journal of Agronomy*, *50*(3), 330–336. https://doi.org/10.24831/jai.v50i3.43211

Plummer, J. A., Mullins, M. G., & Vine, J. H. (1991). Seasonal changes in endogenous ABA and IAA and the influence of applied ABA and auxin in relation to shooting growth and abscission in Valencia orange (*Citrus sinensis* (L.) Osbeck). *Plant Growth Regulation*, *10*, 139–151. https://doi.org/10.1007/BF00024960

Raden, I. (2009). *Shoot architecture and its relation to photosynthesis, production, and seed oil content of physic nut (*Jatropha curcas *L.) [Disertation]*. Bogor Agricultural University. https://repository.ipb.ac.id/handle/123456789/40552

Roberts, J. A., Elliott, K. A., & Gonzalez-Carranza, Z. H. (2002). Abscission, dehiscence, and other cell separation process. *Annual Review of Plant Biology*, *53*, 131–158. https://doi.org/10.1146/annurev.arplant.53.092701.180236

Silva, B. W., Vicente, M. H., Robledo, J. M., Reartes, S. D., Ferrari, R. C., Bianchetti, R., Araújo, L. W., Freschi, L., Peres, L. E. P., & Zsögöna, A. (2018). Self-pruning acts synergistically with diageotropica to guide auxin responses and proper growth form. *Plant Physiology*, *176*(4), 2904–2918. https://doi.org/10.1104/pp.18.00038

Sugiyatno, A., Yenni, F. N., & Al Fanshuri, B. (2019). Effect of pruning canopy on productivity and fruit quality of Mandarin cv Pulung. *Journal Hortikultura*, *29*, 199–208.

Tan, L., Eberhard, S., Pattathil, S., Warder, C., Glushka, J., Yuan, C., Hao, Z., Zhu, X., Avci, U., Miller, J. S., Baldwin, D., Pham, C., Orlando, R., Darvill, A., Hahn, M. G., Kieliszewski, M. J., & Mohnen, D. (2013). An Arabidopsis cell wall proteoglycan consists of pectin and arabinoxylan covalently linked to an arabinogalactan protein. *Plant Cell*, *25*, 270–287. https://doi.org/10.1105/tpc.112.107334

Taylor, J. E., & Whitelaw, C. A. (2001). Signals in abscission. *New Phytologist*, *151*, 323–340. https://doi.org/10.1046/j.0028-646X.2001.00194.x

Warren, C. R. (2008). Rapid measurement of chlorophylls with a microplate reader. *Journal of Plant Nutrition*, *31*(7), 1321–1332. https://doi.org/10.1080/01904160802135092

Wilmowicz, E., Frankowski, K., Kućko, A., Świdziński, M., Alché, J. D., Nowakowska, A., & Kopcewicz, J. (2016). The influence of abscisic acid on the ethylene biosynthesis pathway in the functioning of the flower abscission zone in *Lupinus luteus*. *Journal of Plant Physiology*, *206*, 49–58. https://doi.org/10.1016/j.jplph.2016.08.018

Yu, Y.-B., & Yang, S. F. (1979). Auxin-induced ethylene production and its inhibition by aminoethoxyvinyiglycine and cobalt ion. *Plant Physiology*, *64*, 1074 –1077. https://doi.org/10.1104/pp.64.6.1074

Yokota, T., Nakayama, M., Harasawa, I., Sato, M., Katsuhara, M., & Kawabe, S. (1994). Polyamines, indole-3-acetic acid and abscisic acid in rice phloem sap. *Plant Growth Regulation*, *15*, 25–128.

Zhang, J.-Z., Zhao, K., Ai, X.-Y., & Hu, C.-G. (2014). Involvements of PCD and changes in gene expression profile during self-pruning of spring shoots in sweet orange (*Citrus sinensis*). *BMC Genomics*, *15*, Article 892. https://doi.org/10.1186/1471-2164-15-892

Zou, L., Liu, W., Zhang, Z., Edwards, E. J., Gathunga, E. K., Fan, P., Duan, W., Li, S., & Liang, Z. (2020). Gene body demethylation increases expression and is associated with self-pruning during grape genome duplication. *Horticulture Research*, *7*(1), Article 84. https://doi.org/10.1038/s41438-020-0303-7