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Research Article

Effect of watering on incidence of self-pruning in lime (*Citrus × aurantiifolia* (Christm.) Swingle)

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Abstract

The process of self-pruning represents a form of self-destruction among shaded or diseased branches, which has the potential to deplete the resources of the tree. This phenomenon is also observed in natural ecosystems. The self-pruning mechanism can be exploited to reduce the cost of pruning in cultivated plants, such as lime trees. The objective of this study was to ascertain the mechanisms that occur during self-pruning in response to drought and flooding induction. The experiment was arranged in a randomized complete block design (RCBD) with three replications. Each replication contained three plants, giving a total of 45 plants. Samples were taken from three plants per treatment. The treatments comprised irrigation at field capacity, irrigation every day, irrigation every three days, irrigation every five days, and irrigation every seven days, and the data were analyzed using the Duncan Multiple Range Test at the 95% level. The secondary, tertiary, and quaternary branches that experienced self-pruning due to drought and flooding treatments had average lengths ranging from 3.06 to 9.53 cm and diameters between 0.50 and 1.84 mm. The physiological responses, including the photosynthetic rate, showed that both drought and inundation treatments led to a decrease in stomatal conductance and transpiration. Furthermore, the concentrations of ethylene, abscisic acid, and proline increased markedly after seven months of treatment-induced self-pruning.

Keywords: abscisic acid; ethylene; proline

Introduction

Lime citrus (*Citrus x aurantiifolia* (Christm.) Swingle) production is strongly influenced by environmental factors, genetics and cultivation techniques, one of which is maintenance. Citrus maintenance includes pest and disease control, weeding and pruning. Pruning is basically divided into shape pruning, which is done when the plant is 1-3 years old, and maintenance pruning for plants that have produced. Light is an important factor in the growth and production of citrus, especially in inducing flowering, blossoming and fruit formation (Abobatta, 2018). Citrus pruning management consists of 3 principles according to the growth phase of the plant, namely induction of branching in young trees, maintenance of branches to form tree crowns and pruning to reduce shade (Matias et al., 2023). The reduction of branches forms a more regular plant architecture, so that each part of the plant receives more optimal sunlight. Pruned citrus plants had 85% flower number, 76% fruit set, and 105.3 g higher fruit flesh weight than without pruning (Sugiyatno et al., 2019).

Additionally, citrus farmers undertake two rounds of pruning per year. This presents a challenge for farmers who wish to increase production, as the associated costs are high. In order to address this issue, a technique for cultivating citrus trees that facilitates the process of pruning must be developed. One method for facilitating pruning is to engineer the process of branch and leaf abscission, whereby the plant itself initiates the shedding of excess growth, a phenomenon known as self-pruning (Paisey et al., 2025; Zou et al., 2020). The occurrence of self-pruning can be induced by both genetic and environmental factors (Paisey et al., 2023). The phenomenon of self-pruning has been observed in various plant species, including citrus, cocoa, tomato, and fruit trees, where the form of natural abscission occurs at the apex of the plant (McGarry & Ayre, 2021; Nagamine & Ezura, 2024; Paisey et al., 2022; Zhang et al., 2014).

The environment's growth conditions, specifically those of drought, have a significant impact on pruning. The response of the citrus plant to drought stress is the production of secondary metabolites, including chloride, proline, and phytohormones such as salicylic acid, indole-3-acetic acid, abscisic acid, and jasmonic acid (Vives-Peris et al., 2017). The concentration of proline was observed to increase in the shoots when the temperature was elevated, while a decrease was noted in the roots. In contrast, the content of phytohormones, namely abscisic acid, salicylic acid, jasmonic acid and auxin, differs between roots and leaves (Vives-Peris et al., 2017). In young mandarin orange plants that were irrigated effectively, the level of abscisic acid (ABA) was found to be significantly lower, at 0.2 nmol g^{-1} dry weight. However, the concentration of ABA exhibited a continuous increase, reaching 4.9 nmol g^{-1} dry weight (24 times higher) during a 24-hour period of drought. Following this, the plants were transferred to containers containing water (Gómez-Cadenas et al., 2000). In conditions of flooding, hormonal responses become particularly significant, with a progressive increase in leaf abscisic acid levels that correlate with different levels of tolerance among citrus genotypes, emphasising the role of this hormone in plant stress responses. The increase in the final concentration of 1-aminocyclopropane-1-carboxylic acid, in conjunction with severe leaf damage, indicates that ethylene plays a role in the ageing of leaves in citrus plants (Polko & Kieber, 2019).

In order to enhance the competitiveness of fruit commodities, it is possible to reduce production costs without compromising fruit quality. One method is self-pruning. If self-pruning is developed, it is expected to reduce the cost of pruning. Self-pruning is a physiological process of cell death that can occur in plant tissues such as leaves, flowers, fruits, and branches. Additionally, self-pruning can be induced exogenously through the interval or timing of water application. The objective of this study was to evaluate the impact of drought and flooding on the occurrence of self-pruning in citrus plants.

Materials and methods

The study was conducted from August 2020 to April 2023 at the Leuwikopo Experimental Field, the Seed Center Laboratory of the Department of Agronomy and Horticulture, the Postharvest Laboratory of the Department of Agronomy and Horticulture, and the Seed Microtechnique Laboratory of the Department of Agronomy and Horticulture, IPB University, Dramaga, Bogor, Indonesia.

The experiment was arranged in a randomized complete block (RCB) design with five irrigation treatments: continuous waterlogging for 7 days (SJ), daily irrigation (S1), irrigation every 3 days (S3), irrigation every 5 days (S5), and irrigation every 7 days (S7). Each treatment was replicated three times, resulting in a total of 15 experimental units. Each experimental unit consisted of three plants, giving a total of 45 plants used in the study. Plant materials were selected from six-month-old plants with relatively uniform growth characteristics, as indicated by similar plant height and number of leaves before treatment application.

The seedlings were transferred to 45 L planter bags containing a growing medium composed of a 3:2:1 mixture of soil, manure, and rice husk. Nutrient supplementation was provided through nitrogen (N), phosphorus (P_2O_5), and potassium (K_2O) fertilisers, applied in two doses following the recommended N:P_2O_5:K_2O ratio of 10:5:5 g per plant, as outlined by Balitjetro. To protect the plants from adverse weather conditions such as precipitation, a transparent plastic canopy was installed at a height of 2–3 m above the plants.

Irrigation treatments commenced after the plants had reached two months of age following transplantation into the planter bags. The irrigation regimes consisted of S1 (watering once per day), S3 (watering once every three days), S5 (watering once every five days), S7 (watering once every seven days), and SJ (seven days of flooding followed by seven days of drying). applications over a seven-month period, and irrigation was applied until the soil reached water saturation. Each irrigation was performed until the soil moisture content reached field capacity (100%) using a free-drainage method, typically within 48–72 hours. Soil moisture was monitored using a tensiometer placed at a depth of 15 cm (Easton et al. 2016). Soil moisture was monitored using a tensiometer, which measures soil water potential. Field capacity was defined as a soil water potential range of approximately 10–30 kPa. Irrigation was applied when the measured values approached the upper limit of this range to maintain soil moisture near field capacity and to prevent both water deficit and excess water conditions. Additionally, the water-saturation treatment involved maintaining a water level of approximately 4 cm above the soil surface for one-week intervals over the course of seven months (García-Sánchez et al., 2007).

Observation

1. The percentage of secondary branches that underwent abscission is calculated by comparing the number of such branches to the total number of branches at the end of the observation period.
2. The percentage of tertiary branches that have fallen prey to mortality is calculated by dividing the number of fallen tertiary branches by the total number of tertiary branches at the conclusion of the observation period.
3. The percentage of quaternary branches that were lost or died was calculated by dividing the number of dead or lost quaternary branches by the total number of quaternary branches at the end of the observation period.

4. The diameter of the secondary, tertiary, and quaternary branches that died was measured in millimetres from the point of death.
5. The endogenous ethylene content of the observed plant tissue was that of leaves that had undergone the process of yellowing but remained attached to the branch. The leaves were collected at 3, 5, and 7 months during the treatment period (Yan et al., 2011).
6. The endogenous ABA content was analysed using samples of leaves that had turned yellow but remained attached to the branches. These samples were taken at 3, 5 and 7 months during the treatment period. The analytical procedure is provided in the supplementary materials.
7. Proline analysis was conducted on leaves and roots. The samples were observed at the conclusion of the experiment (7 months after treatment).
8. The measurement of photosynthetic rate, transpiration and stomatal conductance was conducted on adult leaves at three months post-treatment (García-Sánchez et al., 2007) using the LI-COR 6400 instrument.
9. The measurement of soil water potential was conducted using a tensiometer affixed to the plants throughout the treatment period (for a duration of three months).
10. The determination of leaf chlorophyll content was performed in the third month of the treatment period (Kamble et al., 2015), with the analytical procedure outlined in the supplementary materials. Fully expanded mature leaves located at the fifth position from the shoot apex were selected for the measurements.

Results and discussion

The results of further analysis on the branch size of lime plants undergoing self-pruning, particularly the length of secondary branches, indicated that the daily irrigation treatment (S1) produced an average branch length of 5.00 cm. This value was lower than that observed under the S7 treatment (irrigation once every seven days) but was not significantly different from the S3 and S5 treatments. In contrast, the lengths of tertiary and quaternary branches that experienced self-pruning did not differ significantly among treatments (Table 1). Overall, the lengths of secondary, tertiary, and quaternary branches that underwent self-pruning were below 9.53 cm. This suggests that plants may suppress branch growth as a strategy to minimize energy expenditure. This finding is consistent with the statement of Yu (2023), who reported that self-pruning functions as a selection mechanism among twigs with varying efficiencies, whereby inefficient branches and twigs are eliminated.

Table 1. Branching length of self-prunigised lime plants at 7 months after treatment

Watering treatment	Branch length (cm)		
	Secondary	Tertiary	Quaternary
S1 (Watering once per day)	5.00±1.00b	5.22±2.01	3.06±0.63
S3 (Watering once every 3 days)	7.67±3.18ab	5.33±2.65	4.56±2.50
S5 (Watering once every 5 days)	7.88±0.98ab	6.92±2.67	5.67±0.67
S7 (Watering once every 7 days)	9.53±0.34a	5.86±3.65	5.67±1.15
SJ (7 days flooding and 7 days drying)	6.83±1.88ab	3.22±2.91	4.33±2.08

Note: Numbers with different letters in the same column indicate significant differences based on the DMRT test at the 5% level.

The results of further analysis of branch diameter in lime plants undergoing self-pruning (Table 2) indicated no significant differences among secondary, tertiary, and quaternary branches. The mean diameters of these branches were comparable. This similarity may be attributed to the fact that the branches were still at an early developmental stage. In the case of secondary branches, the negative angle of branching may have contributed to structural weakness, predisposing them to self-pruning. Overall, the diameters of the branches that underwent self-pruning were below 1.86 mm.

Table 2. Diameter of branch junctions in lime plants that have undergone self-pruning at 7 months after treatment

Watering treatment	Branch diameter (mm)		
	Secondary	Tertiary	Quaternary
S1 (Watering once per day)	1.14±0.32	1.00±0.44	0.50±0.17
S3 (Watering once every 3 days)	1.50±0.44	1.11±0.54	0.97±0.71
S5 (Watering once every 5 days)	1.53±0.20	1.42±0.44	1.11±0.42
S7 (Watering once every 7 days)	1.84±0.06	1.32±0.72	1.16±0.57
SJ (7 days flooding and 7 days drying)	1.50±0.50	0.67±0.60	0.61±0.10

Ethylene content (Table 3) measured at 3, 5, and 7 months after treatment showed that the S7 treatment resulted in higher ethylene levels in lime leaves compared to the other treatments. The concentration of ethylene increased with the duration of the treatment, reflecting the adaptive response of lime plants to water deficit. Ethylene plays a central role in plant adaptation to environmental stress by regulating growth and developmental processes, including leaf senescence and growth inhibition. These responses reduce leaf area and transpiring surface, thereby limiting water loss and enhancing plant survival under adverse conditions (Khan et al., 2024). According to Jin et al. (2015), leaf abscission is influenced by the interaction between auxin and ethylene. While ethylene directly regulates leaf abscission, its effect is modulated by auxin levels in the leaves. The antagonistic relationship between ethylene and auxin becomes evident in the abscission zone, where auxin levels determine cellular sensitivity to ethylene. Thus, the balance between auxin and ethylene is a critical factor in regulating leaf abscission. During senescence, leaves produce higher amounts of ethylene, further contributing to the abscission process.

Table 3. Ethylene content at 3, 5, and 7 months after treatment in lime plants

Water treatment	3 months (ppm)	5 months (ppm)	7 months (ppm)
S1 (Watering once per day)	0.67±0.48b	6.19±0.46e	21.55±0.86d
S3 (Watering once every 3 days)	0.97±0.08b	13.64±1.38d	22.78±1.17d
S5 (Watering once every 5 days)	0.95±0.07b	26.12±0.05b	55.39±2.26b
S7 (Watering once every 7 days)	6.05±0.35a	37.03±3.45a	63.77±2.86a
SJ (7 days flooding and 7 days drying)	1.16±0.06b	20.87±1.68c	43.24±2.35c

Note: Numbers with different letters in the same column indicate significant differences based on the DMRT test at the 5% level.

Abscisic acid (ABA) is a plant hormone highly sensitive to water deficit, and it modulates ethylene concentrations, which act as a driving force in the hormonal regulation of leaf abscission (Chen et al., 2019). Based on the results of further analyses, the content of ABA in lime leaves (Table 4) at three months after treatment showed significant differences, with the S5 treatment (12.23 ppm) exhibiting higher ABA levels compared to the other treatments. A similar trend was observed at five months after treatment. At seven months, the ABA concentration in the S5 treatment (12.74 ppm) remained higher than in the other treatments, although it did not differ significantly from S7 (12.01 ppm) and SJ (11.17 ppm). These findings are consistent with reports by De Ollas et al. (2013) and Santana-Vieira et al. (2016), who demonstrated that ABA concentrations in leaves and roots increased under severe drought stress in *Valencia orange* (VO) and *Tahiti acid lime* (TAL) grafted onto two lime rootstocks, *Rangpur lime* (RL) and *Sunki Maravilha mandarin* (SM). Furthermore, flooding has also been shown to elevate ABA levels, as reported by Martínez-Cuenca et al. (2021) that ABA concentrations decreased in the roots of most hybrids under waterlogging, while leaf ABA levels increased in *Carrizo citrange (CC)* and hybrids 05019 and 050110. This suggests that ABA accumulation in leaves may contribute to stomatal closure and water-loss prevention as part of the plant's adaptive mechanism to flooding stress.

Table 4. Abscisic acid content at 3, 5, and 7 months after treatment in lime plants

Watering treatment	Abscisic acid (ppm)		
	3 month	5 month	7 month
S1 (Watering once per day)	4.25±0.70d	4.22±0.59d	4.08±1.42b
S3 (Watering once every 3 days)	8.35±1.29c	7.19±0.24c	5.38±0.84b
S5 (Watering once every 5 days)	12.23±0.73a	12.57±0.46a	12.74±0.65a
S7 (Watering once every 7 days)	8.77±0.92bc	11.00±0.83b	12.01±0.18a
SJ (7 days flooding and 7 days drying)	10.44±0.56b	10.91±0.29b	11.17±0.25a

Note: Numbers with different letters in the same column indicate significant differences based on the DMRT test at the 5% level.

The content of proline (Table 5) reduces the osmotic potential of the cell in order to maintain turgor by balancing the soil water potential. The synthesis of proline in plants is contingent upon the intensity and duration of illumination. The longer the stress is applied, the greater the accumulation of proline (Argamasilla et al., 2014). The highest concentration of proline in roots was observed in treatment S7 (0.44 ppm), in comparison to the other treatments. Similarly, the highest concentration of proline in leaves was observed in treatment S7 (1.16 ppm), in comparison to the other treatments. In higher plants, proline is synthesised via the glutamine and ornithine pathways. The glutamine pathway represents the primary route for proline biosynthesis in conditions of drought stress. The content of proline is a consequence of drought stress (Garcıa-Sanchez et al., 2007; Zandalinas et al., 2016). The results of the study indicate that proline accumulation occurs at a potential water pressure of -22.85 kPa (Table 5). This finding is consistent with the results of the study by Girardi et al. (2018), which demonstrated that proline accumulation occurs at a potential water pressure of -25 kPa.

Table 5. Proline content at 7 months after treatment in lime plants

Watering treatment	Proline (ppm)	
	Roots	Leaves
S1 (Watering once per day)	0.11±0.02c	0.49±0.02d
S3 (Watering once every 3 days)	0.17±0.02c	0.68±0.03c
S5 (Watering once every 5 days)	0.27±0.01b	0.89±0.04b
S7 (Watering once every 7 days)	0.44±0.07a	1.16±0.05a
SJ (7 days flooding and 7 days drying)	0.13±0.03c	0.36±0.04e

Note: Numbers with different letters in the same column indicate significant differences based on the DMRT test at the 5% level.

The subsequent analysis of chlorophyll a yielded that the S5 treatment exhibited a lower chlorophyll a concentration (1.03 mg g^{-1}) compared to the other treatments. However, this was not statistically different from the S7 treatment (1.58 mg g^{-1}). A similar trend was observed for chlorophyll b and total chlorophyll, which also did not differ significantly from the S7 treatment (Table 6). Changes in chlorophyll content may be attributed to various factors, including water, soil, temperature, and pressure, which indirectly influence leaf area, morphology, thickness, and chloroplast distribution (Gogoi & Basumatary, 2018). Drought can reduce chlorophyll content, but the extent of this reduction depends on the physiological response of the species in question and their capacity to withstand environmental stress (Zhou et al., 2017).

Table 6. Content of chlorophyll a, b, and total at 3 months after treatment in lime plants

Watering treatment	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total chlorophyll (mg g^{-1})
S1 (Watering once per day)	1.83±0.14a	0.64±0.06a	2.48±0.21a
S3 (Watering once every 3 days)	1.68±0.26a	0.57±0.09a	2.25±0.35a
S5 (Watering once every 5 days)	1.03±0.56b	0.33±0.19b	1.35±0.76b
S7 (Watering once every 7 days)	1.58±0.26ab	0.53±0.09ab	2.11±0.35ab
SJ (7 days flooding and 7 days drying)	1.75±0.34a	0.61±0.13a	2.36±0.47a

Note: Numbers with different letters in the same column indicate significant differences based on the DMRT test at the 5% level.

Table 7 illustrates that the highest rate of lime fruit photosynthesis was observed in the daily irrigation treatment (S1), with a CO_2 concentration of 26.89 $m^{-2}s^{-1}$, in comparison to the other treatments. However, this rate was not significantly different from that observed in the seven-day irrigation treatment (26.05 $m^{-2} s^{-1}$). The lowest rate of photosynthesis, at 24.55 CO_2 $m^{-2} s^{-1}$, was observed in the treatment involving seven-day irrigation (S7). Furthermore, the lowest stomatal conductance was also caused by treatment S7, which differed from the other treatments. There was no significant difference in transpiration rate between the treatments. The reduction in photosynthetic rate and stomatal conductance is a consequence of the plants experiencing drought conditions. This finding aligns with the results of the study by García-Sánchez et al. (2007) on Citrange orange and Cleo mandarin oranges, which were subjected to both submergence and drought treatments, and exhibited a decline in photosynthetic rate and stomatal conductance. The lowest potential of groundwater was observed in the S7 irrigation treatment, which was found to have a value of -22.85 kPa. This extremely low potential of groundwater has been identified as a factor that can influence the physiological processes of lime plants. The absence of soil water potential readings under the 7-day flooding followed by 7-day drying treatment may be attributed to the operational limitations of the tensiometer. Following the flooding period, the subsequent drying phase likely reduced soil water potential beyond the measurement range of the instrument (approximately −80 kPa). Under such conditions, cavitation may occur within the tensiometer, disrupting the water column and preventing accurate measurements of soil matric potential.

Table 7. Physiology of lime plants at 3 months after watering treatment

Watering treatment	Photosynthetic rate (μmol CO_2 $m^{-2}s^{-1}$)	Stomatal conductance (mol H_2O $m^{-2} s^{-1}$)	Transpiration (mmol H_2O $m^{-2} s^{-1}$)	Ground water potential (kPa)
S1 (Watering once per day)	26.89±0.30a	1.46±0.26a	1.21±0.01a	8.63±0.16a
S3 (Watering once every 3 days)	25.06±0.49bc	1.27±0.29a	1.21±0.00a	-15.,93±2.43b

S5 (Watering once every 5 days)	24.96±0.84bc	1.81±0.76a	1.29±0.16a	-20.15±0.50c
S7 (Watering once every 7 days)	24.55±0.76c	0.38±0.13b	1.21±0.00a	-22.85±0.23d
SJ (7 days flooding and 7 days drying)	26.05±0.64ab	1.75±0.42a	1.21±0.01a	-

Note: Numbers with different letters in the same column indicate significant differences based on the DMRT test at the 5% level.

Figure 1 depicts the appearance of lemon trees seven months after treatment. It can be observed that the plants irrigated once a day (S1), three times a day (S3), and allowed to stand in water for a week (S7) exhibited satisfactory growth. The positive outcome was observed in the case of the lime plants that were irrigated every five days (S5) and every seven days (S7), which resulted in transient wilting. This phenomenon, commonly referred to as transient wilting, was reversible upon replenishment of the plants' water requirements, leading to a return to normal condition.

The occurrence of self-pruning in secondary branches is predominantly influenced by the number of secondary branches. Secondary branches undergoing self-pruning represent negative branches that are connected to the primary branches. The highest incidence of self-pruning on tertiary branches was observed in the treatment with five-day irrigation (S5), at 3.64%. However, this was not significantly different from the treatments S7 and S3. The highest incidence of self-pruning on quarters was observed in the seven-day irrigation treatment (S7), at 5.11%. However, this was not significantly different from the S5 and S3 treatments. The occurrence of self-pruning in lime trees is caused by leaf drop on the branches, which is a result of reduced transpiration due to drought conditions.

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

Figure 1. The lime tree in various applications of irrigation treatments: (a) S1 (watering once per day), (b) S3 (watering once every three days), (c) S5 (watering once every five days), (d) S7 (watering once every seven days), and (e) SJ (seven days of flooding followed by seven days of drying)

The relationship between drought and self-pruning is a significant one. When plants experience drought stress, the element potassium (K) plays a role in closing the stomata, thereby reducing the conductivity of the stomata and enabling the plant to reduce transpiration. Conversely, when the level of potassium is low, the plant will produce the hormone ethylene, which causes the leaf to drop. This results in the branch no longer receiving assimilates, leading to its death (self-pruning). This phenomenon has been elucidated by Saleem et al. (2024), who demonstrated that K nutrition significantly impacts the closure of sunflower stomata. In Experiment 3, the five-day and seven-day irrigation intervals exhibited the highest levels of abscisic acid and ethylene, which may have been intended to induce leaf abscission to reduce transpiration. Consequently, the production of assimilates is restricted, which impairs the development of the branching structure. The lack of assimilates in the branches ultimately results in their death, leading to self-pruning.

The presence of waterlogging also impairs the absorption of nutrients and oxygen from the soil. The correlation between the condition of the plants that are submerged and the occurrence of self-pruning is significant. When the plants are subjected to a state of inundation, they synthesize hormones such as abscisic acid and ethylene at elevated levels. This is a mechanism employed by the plants to accelerate the life cycle of their organs. The results of the experiment, which involved providing water at specific intervals and submerging the lime plants for a week, indicated that the plants exhibited higher levels of abscisic acid and ethylene compared to the control group. This phenomenon is believed to be an adaptive mechanism, resulting in the yellowing and subsequent drop of the leaves. The acceleration of the leaf cycle results in the inefficient production of assimilates by the branches, ultimately leading to the death of the lime tree's branching structure.

Conclusions

The secondary, tertiary and quarter branches that underwent self-pruning as a result of drought and flooding treatments exhibited an average length of 3.06-9.53 cm and an average diameter of 0.50-1.84 mm. The application of drought and inundation treatments resulted in a reduction in stomatal conductance and transpiration. Additionally, the levels of ethylene, abscisic acid, and proline increased significantly after seven months of self-pruning, which was induced by the treatments.

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