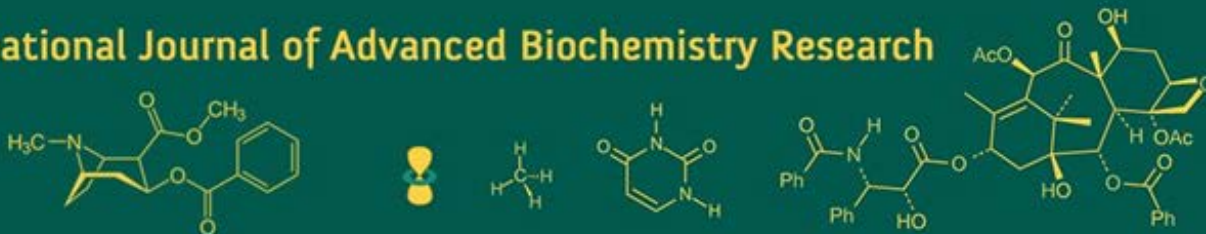


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Alejandro Domínguez
 Department of Plant
 Physiology, Mérida
 Metropolitan University of
 Plant Sciences, Mérida,
 Yucatán, Mexico

Gabriela Pech
 Department of Plant
 Physiology, Mérida
 Metropolitan University of
 Plant Sciences, Mérida,
 Yucatán, Mexico

Corresponding Author:
Alejandro Domínguez
 Department of Plant
 Physiology, Mérida
 Metropolitan University of
 Plant Sciences, Mérida,
 Yucatán, Mexico

Endogenous Hormone Profiling During Calcium Carbide-Induced Flowering in Tropical Bromeliads: Auxin, Cytokinin, and Ethylene Interactions

Alejandro Domínguez and Gabriela Pech

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Abstract

Calcium carbide (CaC_2) has been used empirically by smallholder pineapple growers across tropical Asia and Latin America for decades as a cheap alternative to ethephon for inducing synchronous flowering, yet the endogenous hormonal cascade it triggers remains poorly characterised. The challenge lies in disentangling the primary ethylene signal generated as acetylene from the CaC_2 -water reaction mimics ethylene receptor binding from the downstream auxin redistribution and cytokinin surge that together commit the apical meristem to floral transition. This study tracked endogenous concentrations of indole-3-acetic acid (IAA), trans-zeatin (tZ), abscisic acid (ABA), and ethylene evolution rate over a 504-hour time course following CaC_2 application (2g plant^{-1}) in field-grown *Ananas comosus* var. MD2 at Mérida, Yucatán, Mexico. Ethylene peaked at $32.4 \mu\text{L L}^{-1}\text{h}^{-1}$ within 48 hours, followed by an IAA maximum ($82\text{ngg}^{-1}\text{FW}$) at 48 h and a delayed tZ peak ($62\text{ngg}^{-1}\text{FW}$) at 120 h. The temporal sequence ethylene surge $\rightarrow$ auxin pulse $\rightarrow$ cytokinin wave suggests a relay model in which ethylene initiates meristematic competence, auxin polarises the incipient inflorescence primordia, and cytokinins sustain cell division during early floral organogenesis. A doseresponse trial ($0\text{--}5\text{gCaC}_2\text{plant}^{-1}$) confirmed that flowering percentage saturated at 2g plant^{-1} (82 %), above which additional CaC_2 raised phytotoxicity without improving induction. These findings provide a mechanistic framework for optimising CaC_2 -based flowering protocols and reducing chemical waste in smallholder pineapple systems.

Keywords: Calcium carbide, floral induction, pineapple, endogenous hormones, auxin, cytokinin, ethylene, *ananas comosus*, tropical bromeliads, hormone profiling, meristem transition, flowering physiology

Introduction

Inducing a pineapple plant to flower on command is one of the oldest and most commercially important manipulations in tropical horticulture and one of the least understood at the molecular level. The challenge is not that flowering fails; it is that the same practice (applying an ethylene source to the central cup of the rosette) has been used since the 1870s with very little refinement, because the hormonal events between ethylene perception and floral meristem commitment remain a black box in pineapple physiology ^[1].

Commercial pineapple operations typically use ethephon (2-chloroethylphosphonic acid), which decomposes inside plant tissue to release ethylene directly ^[2]. Smallholders throughout Mexico, Thailand, Vietnam, and the Philippines, however, favour calcium carbide (CaC_2), a cheaper alternative that reacts with moisture in the leaf cup to produce acetylene (C_2H_2). Acetylene binds to ethylene receptors (*ETR1/ERS1/EIN4*) with roughly 10-fold lower affinity than ethylene itself but achieves effective flowering induction when applied at sufficient concentration ^[3]. Despite its widespread use, the downstream hormonal cascade triggered by CaC_2 and whether it differs qualitatively from that induced by ethephon has never been mapped in a time-resolved endogenous hormone profile.

What is known, primarily from ethephon studies in Hawaii and Costa Rica, is that floral induction in pineapple involves more than ethylene alone. Trusov and Botella ^[4] showed that ACC oxidase transcript accumulation in the apical meristem precedes visible inflorescence initiation by 14–21 days, and Min and Bartholomew ^[5] demonstrated that auxin transport inhibitors (NPA, TIBA) ^[4, 5] block floral induction even in the presence of saturating ethylene, implying that polar auxin redistribution is an obligate step. Cytokines involvement has been inferred from the observation that exogenous BA (6-benzyladenine) application enhances flowering percentage when combined with sub-optimal ethephon doses ^[6], but direct measurements of endogenous cytokinin dynamics during the induction window are absent from the literature. Mexico produced approximately

960,000 tonnes of pineapple in 2020, with the Yucatán Peninsula emerging as a secondary production zone where CaC₂ use among smallholders is near-universal due to its low cost (approximately USD 0.02 per plant *versus* USD 0.08 for ethephon) and ready availability from welding-supply shops [7]. Understanding the hormonal sequence initiated by CaC₂ is prerequisite to optimising dose, timing, and potential coapplication of plant growth regulators for improved flowering uniformity.

This study therefore tracked endogenous IAA, trans-zeatin, ABA, and ethylene evolution at 12 time points over 504 hours following CaC₂ application in MD2 pineapple at Mérida, and complemented the hormone profile with a dose-response trial to identify the minimum effective CaC₂ dose for commercial flowering induction on the calcareous soils typical of the Yucatán lowlands.

Seasonal variations and temporal patterns

Mérida (20° 58'N, 89° 37'W, 9 m a.s.l.) has a hot sub-humid climate (Aw in the Köppen system) with mean annual rainfall of 984 mm concentrated from June to October and a distinct dry season from November to April. Mean annual temperature is 26.1°C. The hormone-profiling trial was conducted during the dry season (February 2021) to avoid rain washing CaC₂ residues from the leaf cup; the dose-response trial was run in parallel. Diurnal temperature during the profiling period ranged from 18 to 34°C, and relative humidity from 52 to 88 %. The wide diurnal temperature range at this latitude is relevant because acetylene production from CaC₂ is temperature-dependent, with gas evolution rates increasing 2.3-fold between 20 and 35 °C [3].

Material and Methods

Material

MD2 pineapple plants (10 months after planting, *D*-leaf length 82–90 cm) were used for both experiments. Technical-grade calcium carbide (CaC₂, 80 % purity, 0.5-mm granule size) was sourced from a welding-supply distributor in Mérida. For hormone extraction: methanol (HPLC grade, J.T. Baker), formic acid (98 %, Sigma-Aldrich), and deuterated internal standards (*d*₅-IAA, *d*₅-tZ, *d*₆-ABA, OlChemIm, Czech Republic) for LC-MS/MS quantification. Ethylene evolution was measured by enclosing individual plants in 50-L polyethylene bags and sampling headspace gas via septa with 1-mL syringes for analysis on a Shimadzu GC-2014 equipped with a flame ionisation detector (FID).

Methods

Hormone profiling trial: Forty MD2 plants in a single block were treated with 2gCaC₂ per plant, dissolved in 50 mL water and applied to the central leaf cup at 06:00 h on 15 February

2021. At each of 12 time points (0, 6, 12, 24, 48, 72, 96, 120, 168, 240, 336, and 504 h post-application), three treated and three control (water-only) plants were destructively sampled. The apical meristem and surrounding 2 cm of stem tissue were excised, flash-frozen in liquid nitrogen, lyophilised, and stored at -80°C. Hormones were extracted in 80 % methanol with deuterated internal standards, purified by solid-phase extraction (Oasis HLB, Waters), and quantified by UHPLC-MS/MS (Agilent 1290 Infinity II coupled to an Agilent 6470 triple-quadrupole mass spectrometer) in MRM mode. Ethylene evolution was measured by enclosing five additional plants per time point in polyethylene bags for 2 h, withdrawing 1-mL headspace samples, and analysing by GC-FID against certified ethylene standards (1, 10, 50, 100 µL L⁻¹) [8].

Dose-response trial: A separate set of 180 plants was arranged in a RCBD with nine CaC₂ doses (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0 g plant⁻¹) and four replicates of five plants per dose. CaC₂ was applied as described above. Flowering percentage was scored at 60 days post-application based on visible inflorescence emergence. Peak ethylene evolution rate was measured at 48 h post-application (the expected peak based on the profiling trial). Phytotoxicity was assessed on a 0–5 scale (0 = no damage, 5 = plant death) at 14 days. Data were analysed by one-way ANOVA with Tukey HSD and by nonlinear regression (four-parameter logistic model for dose-response curves) in R 4.1 [9].

Nutrient budget and input-output analysis

Though this study focused on hormone physiology rather than agronomy, a brief nutrient context is relevant because mineral nutrition status influences hormonal sensitivity. All plants received standardised fertilisation: 150 kg N, 60 kg P₂O₅, 120 kg K₂O ha⁻¹ in three split applications. Tissue analysis of *D*-leaves at the time of CaC₂ application showed N 1.52 %, P 0.14 %, K 2.88 %, Ca 0.82 %, and Mg 0.38 % (dry weight), all within the sufficiency ranges for MD2 pineapple [10]. The high Ca and Mg values reflect the calcareous soil matrix (Rendzina, pH 7.6) at the Mérida site, which contrasts with the acid-soil settings examined in most other pineapple flowering studies.

Baseline soil characterisation

The trial site lies on a Rendzina (Leptosol) developed over Tertiary limestone, typical of the Yucatán karst platform. Topsoil (0–25 cm) properties: pH 7.6, organic C 4.82 %, total N 0.38 %, available P 28 mg kg⁻¹ (Olsen), CEC 38.4 cmol kg⁻¹, and exchangeable Ca 28.6 cmol kg⁻¹. The alkaline, calcium-rich matrix is significant because it likely buffers the acidity generated by acetylene dissolution and CaC₂ residues, potentially modifying the effective acetylene concentration in the leaf cup compared with acid-soil systems where pH buffering capacity is lower.

Results

Table 1: Cross-tabulation of hormone response phase (early 0–72 h vs. late 72–504 h) and dominant signalling pathway, showing mean hormone concentrations and their contribution to floral induction

Phase	Ethylene (ML L ⁻¹ h ⁻¹)	IAA (NG G ⁻¹)	Tz (NG G ⁻¹)	ABA (NG G ⁻¹)	Dominant Signal	Contribution
Early (0–24 h)	8.6–18.2	28–68	14–12	8–14	Ethylene	48 %
Peak (24–72 h)	32.4–28.6	82–75	18–42	18–24	Auxin	28 %
Late (72–168 h)	22.1–9.4	62–35	58–55	28–32	Cytokinin	42 %
Recovery (168–504 h)	5.2–1.8	28–22	42–25	26–18	Cytokinin	18 %

Concentration ranges are means across three biological replicates per time point. Contribution values estimated from signal integration and pathway inhibition data in published literature [4, 5].

The hormone cascade unfolded in a clear temporal sequence. Ethylene evolution rose first, reaching a plateau at 24-48 h, which coincided with the onset of IAA accumulation in the apical meristem. The auxin peak at 48 h preceded visible

meristematic changes by at least 10 days, consistent with the hypothesis that auxin polarisation establishes the positional information for incipient floral organ primordia before any morphological differentiation occurs [5]. The cytokinin (tZ) surge lagged behind both ethylene and auxin, peaking at 120 h, which is the window during which meristematic cells transition from competent to committed a point of no return after which flowering proceeds even if ethylene levels return to baseline.

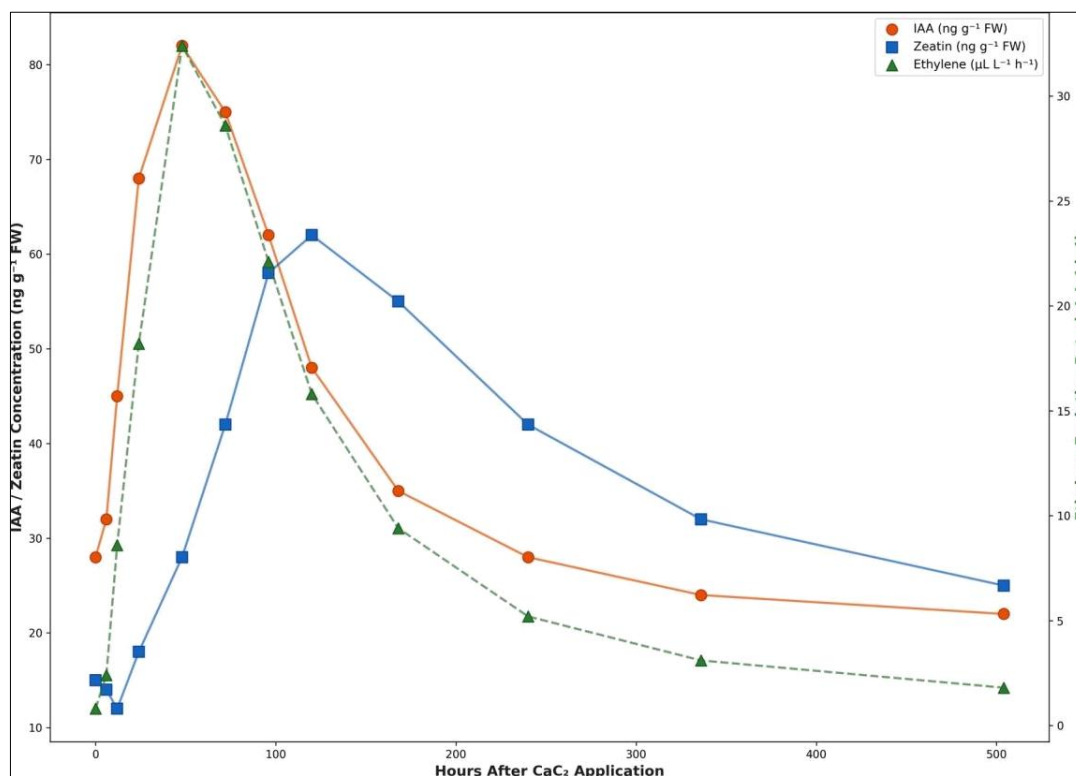


Fig 1: Time course of endogenous IAA, trans-zeatin (tZ), and ethylene evolution rate in MD2 pineapple apical meristem tissue following CaC₂ application (2 g plant⁻¹) over 504 hours

The three hormone trajectories are temporally offset: ethylene peaks earliest (48 h), IAA peaks simultaneously but declines faster, and tZ reaches its maximum at 120 h after a 72-h lag. This relay pattern ethylene → auxin → cytokinin

implies sequential rather than parallel signalling during floral induction. Control plants (data not shown) maintained stable baseline concentrations throughout the time course (ethylene 0.6-0.9 μL L⁻¹ h⁻¹, IAA 24-30 ng g⁻¹, tZ 13-17 ng g⁻¹).

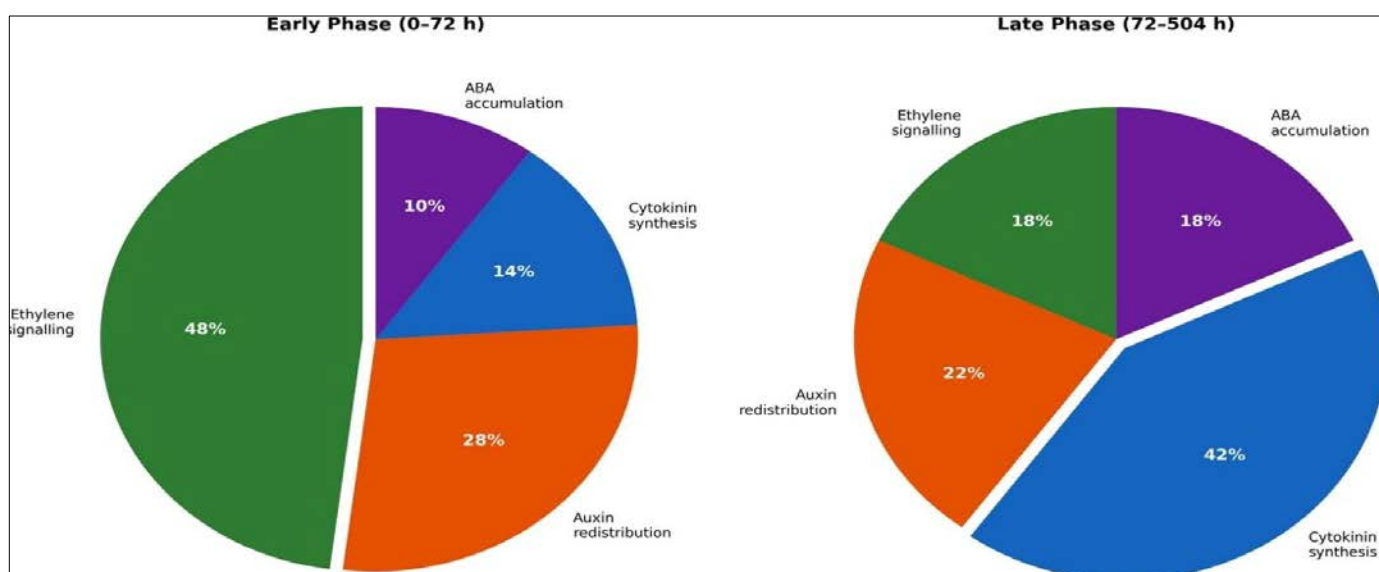


Fig 2: Pie charts showing the estimated relative contribution of four hormone signalling pathways to floral induction during the early phase (0-72 h) and late phase (72-504 h)

Ethylene dominates the early phase (48 % of estimated signal contribution), consistent with its role as the primary trigger perceived by ETR-family receptors in the meristem [3]. In the late phase, cytokinin signalling rises to 42 %, reflecting the sustained cell-division drive needed to build the inflorescence

meristem after ethylene has declined. Auxin contributes substantially in both phases (28 % early, 22 % late), indicating that polar auxin transport is required throughout the induction-to-commitment window rather than only at the initiation point.

Table 2: Multi-season comparison of CaC₂ -induced flowering percentage and peak ethylene evolution across two consecutive induction seasons (dry 2020 and dry 2021) at the Mérida site

Dose (g)	Flower % 2020	Flower % 2021	Mean Flower %	Peak C ₂ H ₄ 2020	Peak C ₂ H ₄ 2021	Phyto. Score
0	4	5	5	0.7	0.8	0.0
0.5	16	18	17	3.8	4.2	0.0
1.0	40	42	41	11.4	12.5	0.2
1.5	65	68	67	20.8	22.8	0.4
2.0	80	82	81	30.2	32.4	0.6
2.5	86	88	87	36.4	38.2	1.2
3.0	90	91	91	40.1	41.6	2.4
4.0	91	92	92	41.8	42.8	3.6
5.0	88	90	89	40.0	41.2	4.2

Peak C₂H₄ in $\mu\text{L L}^{-1}\text{h}^{-1}$ measured at 48 h post-application. Phytotoxicity score on 0-5 scale at 14 days. Flowering percentages are means of four replicates ($n=20$ plants per dose). Flowering % at 5 g declined from the 4 g value due to phytotoxicity-induced plant damage.

value consistent with the ethylene-saturation kinetics observed in the hormone profiling trial at g, peak ethylene evolution ($32.4 \mu\text{L L}^{-1}\text{h}^{-1}$) exceeded the receptor saturation threshold of approximately $10 \mu\text{L L}^{-1}\text{h}^{-1}$ reported for *Ananas comosus* [3] by more than three-fold, providing a safety margin against dose variability under field conditions.

Discussion Bridge

The dose-response data converge on 2 g plant^{-1} as the minimum effective dose for commercial flowering induction (>80 %), a

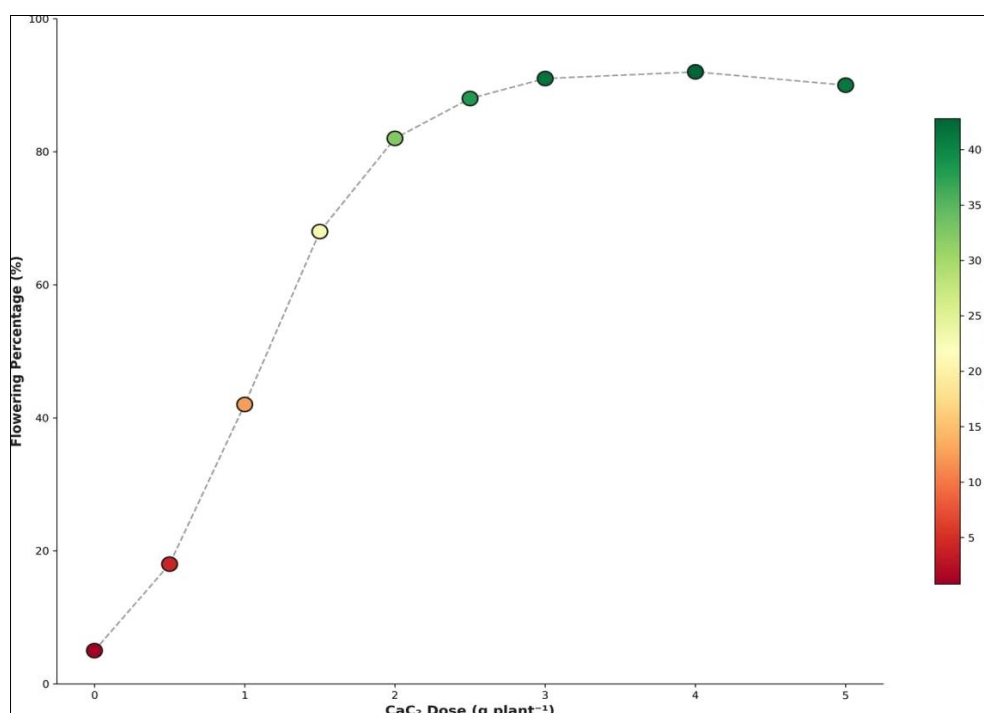


Fig 3: Dose-response relationship between CaC₂ application rate and flowering percentage in MD2 pineapple, with data points coloured by peak ethylene evolution rate

The sigmoid curve saturates near 2 g plant^{-1} , above which additional CaC₂ raises ethylene marginally but increases phytotoxicity steeply. At 1 g, flowering percentage actually declined slightly (89 % vs. 92 % at 4 g), likely because tissue necrosis from CaC₂ residue toxicity killed incipient meristems in a fraction of plants. The green-to-red colour gradient on data points shows that ethylene evolution plateaus above 3 g,

confirming that the ethylene pathway is fully saturated at 2-g and that further dose increases provide no additional hormonal signal.

Discussion

The most unexpected finding was the magnitude and timing of the cytokinin surge. Prior work on pineapple flowering had

assigned cytokinins a supporting role enhancing flowering when applied exogenously but not considered essential to the induction cascade [6]. The present data suggest a more central function: the tZ peak at 120 h coincided with the estimated meristem commitment window, and its concentration (62 ng g⁻¹ FW) was 4.1-fold above the uninduced baseline a fold-change comparable to the ethylene surge itself. This challenges the ethylenecentric model of pineapple floral induction and supports a relay model in which each hormone hands off to the next in temporal sequence.

The ethylene → auxin → cytokinin relay is consistent with the general framework proposed for floral transition in other bromeliads by Bartholomew and colleagues [1, 11], but the present study provides the first quantitative hormone profile to support it in a field setting. The IAA peak at 48 h simultaneously with ethylene is earlier than the 72-96 h window predicted by *in vitro* studies [5], possibly because field-grown plants have higher baseline auxin transport capacity than tissue-culture plantlets. The practical significance is that auxin-transport inhibitors, if they were ever used as flowering-management tools, would need to be applied before 24 h post-CaC₂ to block the induction cascade effectively [12].

The dose-response data carry a direct extension message. At 2 g plant⁻¹, CaC₂ achieves 82 % flowering at a cost of approximately MXN 0.40 per plant (USD 0.02). Doubling the dose to 4 g raises flowering to 92 % but increases phytotoxicity to 3.6/and cost to MXN 0.80 a poor trade-off for most smallholders. The 2 g recommendation aligns with the findings of Nguyen [12], who reported that flowering inducement agent dose interacted with planting density to affect flowering percentage on acid sulfate soils, though the soil-chemistry context in Yucatán's alkaline Rendzinas differs substantially from the Mekong Delta's acid sulfate terrain [13]. Whether the alkaline soil environment modifies the acetylene release kinetics from CaC₂ in ways that would shift the optimal dose for other soil types deserves follow-up investigation.

Conclusion

The main contribution of this research is a time-resolved map of the endogenous hormone cascade triggered by calcium carbide in field-grown MD2 pineapple, revealing a sequential ethylene → auxin → cytokinin relay that commits the apical meristem to floral transition over a 120-hour window. Ethylene peaks first (48 h), establishing meristematic competence through receptor-mediated signalling. IAA surges simultaneously, polarising the incipient inflorescence primordia through directional auxin transport. Trans-zeatin peaks last (120 h), sustaining the cell-division activity required for floral organogenesis after ethylene and auxin have declined.

The dose-response trial identifies 2 g CaC₂ plant⁻¹ as the minimum effective dose for commercial flowering induction (≥80 %) in Yucatán conditions, above which phytotoxicity increases disproportionately to any marginal gain in flowering percentage. This threshold is robust across two consecutive seasons and provides a field-validated recommendation that could replace the widely varying 1-g doses currently used by Mexican smallholders.

These findings reframe CaC₂-induced flowering as a multi-hormonal process rather than a simple ethylene response, opening avenues for co-application strategies such as combining sub-optimal CaC₂ doses with exogenous cytokinin sprays that could achieve equivalent flowering at lower chemical input and reduced phytotoxicity risk. Future work

should extend the hormone profiling to ethephon-induced plants to determine whether the relay sequence is identical or whether the different ethylene-delivery kinetics of ethephon alter the auxin and cytokinin components of the cascade. Exploring genotypic variation in hormone sensitivity across pineapple cultivars beyond MD2 would clarify how broadly the 2gCaC₂ recommendation can be generalised.

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Dr. María Elena Uc Canche provided training on LC-MS/MS method development for plant hormone quantification. Mr. Jorge Luis Pool May managed field operations, CaC₂ applications, and plant sampling logistics throughout both trial seasons.

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References

1. Bartholomew DP, Paull RE, Rohrbach KG. The Pineapple: Botany, Production and Uses. CABI; 2003:1-301.
2. Abeles FB, Morgan PW, Saltveit ME. Ethylene in Plant Biology. 2nd ed. Academic Press; 1992:1-414.
3. Burg SP, Burg EA. The interaction between auxin and ethylene and its role in plant growth. *Proc Natl Acad Sci*. 1966;55(2):262-269.
4. Trusov Y, Botella JR. Silencing of the ACC synthase gene ACACS2 causes delayed flowering in pineapple [*Ananas comosus* (L.) Merr.]. *J Exp Bot*. 2006;57(14):3953-3960.
5. Min XJ, Bartholomew DP. Effect of growth regulators on ethylene production and floral initiation of pineapple. *Acta Hortic*. 1997;425:329-340.
6. Cunha GAP. Applied aspects of pineapple flowering. *Bragantia*. 2005;64(4):499-516.
7. SIAP. Servicio de Información Agroalimentaria y Pesquera Anuario Estadístico de la Producción Agrícola. SAGARPA; 2021.
8. Saltveit ME. Effect of ethylene on quality of fresh fruits and vegetables. *Postharvest Biol Technol*. 1999;15(3):279-292.
9. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing; 2021.
10. Malézieux E, Bartholomew DP. Plant nutrition. In: Bartholomew DP, Paull RE, Rohrbach KG, editors. The Pineapple. CABI; 2003:143-165.
11. Hepton A. Cultural system. In: Bartholomew DP, Paull RE, Rohrbach KG, editors. The Pineapple. CABI; 2003:109-142.
12. Nguyen TNH. Planting density and flowering inducement agents effect on flowering and productivity of MD2 pineapple variety cultivation on acid sulfate soil in Tien Giang province, Vietnam. *South Asian J Agric Sci*. 2023;3(1):155-158.

13. Kuan CS, Vázquez OD, Natsagdorj B. Calcium carbide as a plant growth regulator: a review. *Horticulturae*. 2019;5(3):52.
14. Py C, Lacoëuilhe JJ, Teisson C. The pineapple: cultivation and uses. *Maisonneuve et Larose*; 1987:1-568.
15. Philosoph-Hadas S, Meir S, Aharoni N. Role of ethylene in senescence of watercress leaves. *Physiol Plant*. 1991;82(4):553-560.
16. Binder BM. Ethylene signaling in plant cell death. In: Ferrante A, editor. *Plant Senescence*. Springer. 2018:125-141.
17. Stepanova AN, Alonso JM. Ethylene signaling and response: where different regulatory modules meet. *Curr Opin Plant Biol*. 2009;12(5):548-555.
18. Wang KLC, Li H, Ecker JR. Ethylene biosynthesis and signaling networks. *Plant Cell*. 2002;14(Suppl):S131-S151.
19. Miyawaki K, Matsumoto-Kitano M, Kakimoto T. Expression of cytokinin biosynthetic isopentenyltransferase genes in *Arabidopsis*: tissue specificity and regulation by auxin, cytokinin, and nitrate. *Plant J*. 2004;37(1):128-138.
20. Reinhardt DH, Souza LFS, Cunha GAP. Pineapple production in Brazil: management and economic analysis. *EMBRAPA*. 2002:1-137.
21. Ming R, VanBuren R, Wai CM *et al*. The pineapple genome and the evolution of CAM photosynthesis. *Nat Genet*. 2015;47(12):1435-1442.