**SERVIÇO PÚBLICO FEDERAL**
**UNIVERSIDADE FEDERAL DO PARÁ**
**CAMPUS UNIVERSITÁRIO DE ALTAMIRA**
**PROGRAMA DE PÓS-GRADUAÇÃO EM BIODIVERSIDADE E CONSERVAÇÃO**

Jospin Soglohoun

# RESPOSTA DE *DIPTERYX ODORATA* (AUBL.) FORSYTH F. À APLICAÇÃO DE 24-EPIBRASSINOLÍDEO : EVIDÊNCIAS DE ESTÍMULO E INIBIÇÃO DO CRESCIMENTO

ALTAMIRA- PA

JANEIRO - 2026

PPGBC
PROGRAMA DE PÓS-GRADUAÇÃO EM
BIODIVERSIDADE E CONSERVAÇÃO

Jospin Soglohoun

# RESPOSTA DE *DIPTERYX ODORATA* (AUBL.) FORSYTH F. À APLICAÇÃO DE 24-EPIBRASSINOLÍDEO : EVIDÊNCIAS DE ESTÍMULO E INIBIÇÃO DO CRESCIMENTO

Dissertação apresentada à Universidade Federal do Pará, como parte das exigências do Programa de Pós-graduação em Biodiversidade e Conservação para obtenção do título de Mestre em Biodiversidade e Conservação.

Orientador: Prof. Dr. Emil José Hernández Ruz
Coorientadora: Profª. Dra. Karin Elisabeth Von Schmalz

ALTAMIRA - PA

JANEIRO - 2026

Jospin Soglohoun

# RESPOSTA DE *DIPTERYX ODORATA* (AUBL.) FORSYTH F. À APLICAÇÃO DE 24-EPIBRASSINOLÍDEO : EVIDÊNCIAS DE ESTÍMULO E INIBIÇÃO DO CRESCIMENTO

Orientador: Prof. Dr. Emil José Hernández Ruz

Coorientadora: Profª. Dra. Karin Elisabeth Von Schmalz

Dissertação apresentada à Universidade Federal do Pará, como parte das exigências do Programa de Pós-graduação em Biodiversidade e Conservação para obtenção do título de Mestre em Biodiversidade e Conservação.

Profa. Dra. **Luma de Oliveira**

*1ª Examinadora* – UFMA

Prof. Dr. **Fabio Miranda Leão**

*2º Examinador* – UFPA/Altamira

Prof. Dr. **Marcos Vieira**

*3º Examinador* – UFPA/Altamira

Prof. Dr. **Rodolfo Salm**

*4º Examinador* – UFPA/Altamira

Prof. Dra. **Moirah Menezes**

*5º Examinador* – UFPA/Bragança

**Dados Internacionais de Catalogação na Publicação (CIP) de acordo com ISBD**

**Sistema de Bibliotecas da Universidade Federal do Pará**

**Gerada automaticamente pelo módulo Ficat, mediante os dados fornecidos pelo(a) autor(a)**

---

S682e Soglohoun, Jospin.

EFEITOS DA APLICAÇÃO DE 24-EPIBRASSINOLÍDEO NO DESENVOLVIMENTO DE DIPTERYX ODORATA:

ENTRE A ESTIMULAÇÃO DO CRESCIMENTO E OS

EFEITOS INIBIDORES / Jospin Soglohoun. — 2026.

45 f.: il. color.

Orientador(a): Prof. Dr. Emil José Hernández Ruz

Coorientação: Profª. Dra. Karin Elisabeth Von Schmalz

Dissertação (Mestrado) - Universidade Federal do Pará, Campus Universitário de Altamira, Programa de Pós-Graduação em Biodiversidade e Conservação, Altamira, 2026.

1. Brassinosteroides. 2. hormônios vegetais. 3. produção de mudas. 4. espécies amazônicas. 5. restauração ambiental. I. Título.

CDD 577.3

---

# DEDICATÓRIA

Dedico este trabalho primeiramente a Deus, por ter me dado a vida e a oportunidade de me aprofundar ainda mais na área de biodiversidade e conservação.

Dedico este trabalho à minha família, aos meus colegas técnicos agrícolas da 60ª turma do Centro Internacional de Agricultura Integrada “Songhai”, e a todos que me apoiaram ao longo do curso.

# AGRADECIMENTOS

A Deus por me proporcionar a oportunidade de ter saúde e motivação para realizar o curso de mestrado.

Agradeço à CAPES pela concessão da bolsa de estudos, sem a qual meus estudos teriam sido difíceis.

Ao FUNBIO, o projeto que deu o recurso para comprar os insumos para produzir as mudas.

Ao Instituto de Desenvolvimento Florestal e da Biodiversidade do Estado do Pará (IDEFLOR-BIO) pelo fornecimento das sementes.

Ao Instituto Nacional de Meteorologia (INMET), por disponibilizar o Banco de Dados Meteorológicos (BDMEP), úteis para a elaboração deste projeto.

Ao Professor Doutor Emil J. Hernández Ruz pelo grande apoio, no decorrer do desenvolvimento do projeto de mestrado.

A Professora Doutora Karin Elisabeth von Schmalz por todo apoio durante o desenvolvimento do projeto de mestrado.

# SUMÁRIO

# RESUMO GERAL

Foi avaliado o efeito do 24-epibrassinolídeo no crescimento e desenvolvimento de mudas jovens de *Dipteryx odorata*, uma espécie amazônica de importância ecológica e econômica. O experimento foi conduzido nos viveiros da Universidade Federal do Pará (Altamira, Brasil), sob 50% de sombreamento, em 115 mudas divididas em cinco tratamentos (0, 10, 20, 30 e 40 nM do hormonio). Foram medidas os parâmetros morfológicos (altura, diâmetro basal, e número de folhas) e fisicobioquímicos foliares (clorofila, nitrogênio, umidade, e temperatura foliar). Para explorar as associações das diferenças em cada indivíduo por tratamento e escolher as variáveis para modelagem, realizamos uma análise de componentes principais. Os efeitos dos tratamentos sobre estas variáveis foram avaliados por meio de modelos lineares de efeitos mistos com estimativa de máxima verossimilhança. As dosagens do hormônio influenciaram significativamente o crescimento das plantas, com um efeito positivo acentuado na dose de 30 nM, resultando em maiores ganhos em altura, diâmetro e número de folhas, bem como no aumento da produção de clorofila. A dose de 40 nM proporcionou a melhor relação altura/diâmetro, indicando melhor estabilidade e qualidade das plantas. A dose de 10 nM, embora positiva para tamanho, diâmetro e taxa de crescimento, deu resultados menos relevantes do que doses altas. A dose de 20 nM causou efeitos inibidores, caracterizados por redução do crescimento e diminuição da produção de clorofila. Esses resultados confirmaram a importância do 24-epibrassinolídeo como regulador de crescimento, capaz de melhorar o desempenho fisiológico e morfológico de *Dipteryx odorata* em condições semicontroladas.

**Palavras chaves**: Brassinosteroides, hormônios vegetais, produção de mudas, espécies amazônicas, restauração ambiental.

## ABSTRACT

We evaluated the effect of 24-epibrassinolide on the growth and development of young seedlings of *Dipteryx odorata*, the Tonka Bean tree, an Amazonian species of ecological and economic importance. The experiment was conducted in the nurseries of the Federal University of Pará (Altamira, Brazil), under 50% shade, on 115 seedlings divided into five treatments (0, 10, 20, 30, and 40 nM of the hormone). We measured morphological parameters (height, basal diameter, and number of leaves) and leaf physicochemical parameters (chlorophyll, nitrogen, moisture, and leaf temperature). To explore the associations of differences in everyone per treatment and choose the variables for modelling, we performed a principal component analysis. The effects of the treatments on these variables were evaluated using linear mixed-effects models with maximum likelihood estimation. Hormone dosages significantly influenced plant growth, with a marked positive effect at the 30 nM dose, resulting in greater gains in height, diameter, and number of leaves, as well as increased chlorophyll production. The 40 nM dose provided the best height/diameter ratio, indicating better plant stability and quality. The 10 nM dose, although positive for size, diameter, and growth rate, yielded less relevant results than higher doses. The 20 nM dose caused inhibitory effects, characterized by reduced growth and decreased chlorophyll production, suggesting a sensitivity window associated with high ethylene production. These results confirmed the importance of 24-epibrassinolide as a growth regulator capable of improving the physiological and morphological performance of *Dipteryx odorata* under semi-controlled conditions.

**Keywords** Brassinosteroids, phytohormones, seedling production, Amazonian species, environmental restoration.

# 1 INTRODUÇÃO GERAL

Em escala global, as florestas cobrem cerca de 31% das terras emersas e fornecem habitat para a maoir parte da biodiversidade terrestre (FAO,2020). Na América do Sul, a Floresta Amazônica, um imenso maciço florestal, ocupa aproximadamente 6,7 milhões de km², dos quais cerca de 60% estão localizados no Brasil, constituindo um ecossistema de importância mundial que fornece inúmeros serviços ecossistêmicos essenciais à humanidade, entre eles a regulação do clima, a conservação da biodiversidade e a estabilidade hidrológica (Leal Filho et al., 2025).

De acordo com Ter Steege et al., 2020, estima-se a existência de mais de 15.000 espécies arbóreas na Amazônia, sendo que 9.108 espécies de árvores estão registradas para o Brasil na base de dados NeoTropTree (NTT) (Castuera-Oliveira; Oliveira-Filho; Eisenlohr, 2020). Essa elevada diversidade florística fornece diversos serviços ecossistêmicos fundamentais, tais como a produção de biomassa arbórea, o armazenamento de carbono nos solos, a produção de frutos e o potencial para a produção de caça silvestre (Gamfeldt et al., 2013).

Do ponto de vista econômico, o setor florestal global empregava diretamente mais de 18,21 milhões de pessoas e sustentava mais de 45,15 milhões de postos de trabalho por meio de seus impactos diretos, indiretos e induzidos, contribuindo diretamente com mais de 539 bilhões de dólares e, no total, com mais de 1.298 bilhões de dólares para o Produto Interno Bruto (PIB) mundial (Li; Mei; Linhares-Juvenal, 2019).

No Brasil, as espécies indígenas (EI) são aquelas que ocorrem naturalmente em determinada região, mantendo populações dentro de sua área de distribuição geográfica natural e participando das interações ecológicas dos ecossistemas locais (ICMBio, 2025). Essas espécies oriundas da biodiversidade brasileira são utilizadas em onze setores industriais, incluindo os setores agroalimentar, de bebidas, têxtil, vestuário, couro, madeira, celulose e papel, biocombustíveis, produtos farmacêuticos, borracha e mobiliário. Essas espécies representam uma utilização estimada de aproximadamente 48% dos produtos e 73% das atividades industriais, gerando um potencial de exportações anuais da ordem de 50 bilhões de dólares americanos (Carvalho Ribeiro et al., 2024).

Entre essas espécies indígenas destaca-se *Dipteryx odorata* (Aubl.) Forsyth F. (Fabaceae), conhecida como “cumaru” (Jaquetti; Gonçalves, 2017), uma espécie amazônica nativa com elevado potencial econômico e farmacológico (Silva et al., 2019). *D. odorata* proporcionam benefícios aos sistemas produtivos tanto de natureza técnica quanto econômica e ambiental (Martínez; Costa; Silva, 2021). No âmbito ecológico, a espécie tem sido identificada como uma espécie-chave para o desenvolvimento sustentável, apresentando ampla adaptação climática na Amazônia Legal, o que a torna relevante para ações de conservação e para o planejamento ecológico (Martorano et al., 2025). Ademais, o estabelecimento de plantios de *D. odorata* tem se mostrado uma alternativa eficaz para a restauração florestal de áreas degradadas, ao promover melhorias nas propriedades físicas e biológicas do solo em pastagens abandonadas (Brasil Neto et al., 2021).

Sua madeira é utilizada na produção de ferramentas agrícolas, na construção naval, na fabricação de cabos de ferramentas, postes, dormentes, carroças, estacas, suportes, assoalhos, vigas, itens de marcenaria laminada e mancais para eixos de hélices de barcos (Barbosa; Pinto; Morellato, 2008; Silva et al., 2019). As sementes de cumaru são utilizadas na confecção de colares e pulseiras, além do famoso feijão cumaru, produto de grande comércio no século passado devido ao seu excelente aroma, o que permitiu sua utilização na aromatização de chocolates, cigarros, charutos, doces, alimentos e uísques, bem como na produção de perfumes, sabonetes e outros produtos da indústria cosmética (Do Nascimento; Cascaes; Cruz, 2022). Dos grãos, se extrai um óleo essencial (óleo de cumaru) utilizado em perfumaria, de grande importância para a economia e a indústria regional da Venezuela (Loureiro et al., 1979 *apud* (Silva et al., 2019).

Segundo o "Diagnosis of Native Timber in the Legal Amazon Region in 2024", foram comercializados na região da Amazônia Legal aproximadamente 217.297,22 m$^3$ de madeira de *D. odorata* em 2024, com uma taxa de crescimento anual de 3,31%. No que se refere ao comércio da cumarina, provenientes de suas sementes e destinadas principalmente à indústria da perfumaria e de aromatizantes não alimentares, de acordo com a *Grokipedia*, o Brasil registrou uma produção de 233 toneladas na safra 2020/2021, concentrada principalmente nos estados do Pará (167 toneladas) e do Amazonas (63 toneladas), gerando um valor estimado de aproximadamente 5,7 milhões de reais.

A espécie figura na lista de espécies prioritárias para a conservação no bioma amazônico (Vieira et al., 2005), uma vez que é vulnerável a impactos negativos decorrentes da sobre-exploração, especialmente no estado do Pará (Cruz, 2021). *D. odorata* apresenta dificuldades de crescimento em determinados sistemas de exploração silvicultural, em sistemas agroflorestais constituídos por uma associação de cumaru com laranjeiras, por exemplo, Godinho Da Silva et al., 2020 demonstraram, utilizando o índice de saliência, que a espécie apresenta baixa taxa de crescimento e que o grau de esbeltez indicava plantações instáveis, exigindo a adoção de práticas de desbaste. *D. odorata* também apresenta sensibilidade fisiológica, quando cultivada em viveiro, seu crescimento pode ser influenciado pelo sombreamento, associado ou não a condições de estresse hídrico (Uchida; Campos, 2000).

De acordo com a União Internacional para a Conservação da Natureza (UICN, 2022), há uma necessidade urgente de aprimorar e expandir a restauração de ecossistemas, promovendo práticas cientificamente consolidadas e criando condições adequadas para a condução eficaz dos processos de restauração, de modo a responder aos múltiplos desafios socioambientais. Nesse contexto, o uso de fitohormônios, como os brassinosteroides, constitui uma das estratégias mais eficazes para reduzir o tempo de produção de mudas, uma vez que esses compostos interagem com outros hormônios vegetais, como auxinas, giberelinas, etileno e ácido abscísico, influenciando todos os aspectos do crescimento e do desenvolvimento das plantas. Os brassinosteroides atuam na germinação das sementes, no crescimento vegetativo e reprodutivo, na eficiência fotossintética, na diferenciação vascular, no rendimento e na qualidade dos frutos, bem como na resiliência aos estresses bióticos e abióticos (Aryal; Alferez, 2025).

Em 1970, J. W. Mitchell anunciou a descoberta de um novo hormônio vegetal, o brassinolídeo, extraído do pólen de canola (*Brassica napus*), que passou a integrar o sexto e último grupo de hormônios vegetais identificados, os brassinosteroides. Esses hormônios regulam diversos processos do desenvolvimento e da fisiologia vegetal, incluindo a germinação de sementes, a fotomorfogênese de plântulas, a diferenciação estomática, a delimitação de órgãos, a floração, a fertilidade masculina e as respostas a estresses bióticos e abióticos (Wang; Bai; Wang, 2014).

Os brassinosteroides são compostos esteroides vegetais envolvidos em múltiplas funções relacionadas ao desenvolvimento, metabolismo, sinalização e defesa, atuando frente a uma ampla gama de estresses bióticos e abióticos (Coll et al., 2015). Além disso, são substâncias orgânicas endógenas que podem atuar no local de síntese ou ser transportadas para outras partes da planta, onde, mesmo em baixas concentrações, promovem, inibem ou regulam qualitativamente o crescimento e o desenvolvimento vegetal (Taiz; Zeiger, 2013). Os brassinosteroides são, portanto, hormônios essenciais para o controle do desenvolvimento das plantas (Aparecida Longatti, 2012; Shimada et al., 2003), sendo capazes de regular milhares de genes (39.829) em condições de estresse, fortalecendo significativamente os mecanismos de defesa das plantas (Anwar et al., 2018).

Os brassinosteroides também podem atuar de forma dependente da dose, induzindo forte inibição do crescimento das plântulas à medida que a concentração aumenta (Jiroutová et al., 2019). (Hu et al., 2016) demonstraram que baixas concentrações de brassinolídeo favorecem o alongamento radicular e o desenvolvimento de raízes laterais da batata (*Solanum tuberosum*) cultivada *in vitro*, enquanto concentrações elevadas promovem a inibição do alongamento das raízes.

Estudos realizados com espécies lenhosas, como o álamo (*Populus* spp.), evidenciaram que os brassinosteroides desempenham um papel fundamental na regulação do crescimento secundário, na formação da madeira, bem como no desenvolvimento e nas propriedades anatômicas e químicas do lenho (Du et al., 2020; Lu et al., 2023). Em *Leucaena leucocephala*, os brassinosteroides exerceram um papel crucial no desenvolvimento de plantas lenhosas, por meio da regulação da xilogênese e da modificação da composição química da parede celular (Pramod et al., 2022).

A literatura existente sobre *D. odorata* não aborda o impacto do uso de hormônios na espécie. Então nosso objetivo neste estudo foi avaliar os parâmetros morfológicos e metabólicos de plantas jovens de *D. odorata* verificando os efeitos da aplicação de 24-epibrassinolídeo (24-EBL) em várias concentrações (10, 20, 30, e 40 nM) sobre o crescimento e desenvolvimento de mudas da espécie sob 50% de sombreamento. A hipótese central desse experimento era que, em resposta à aplicação de 24-EBL, as plantas exibiriam uma resposta dose-dependente, com concentrações (10–30 nM) potencialmente maximizando o crescimento morfológico e promovendo atividades metabólicas, enquanto concentrações mais altas (40 nM) poderiam não proporcionar nenhum benefício adicional, ou mesmo se tornar menos eficazes.

## 2 OBJETIVOS GERAIS

### Objetivo Geral

Avaliar os efeitos da aplicação exógena de 24-epibrassinolídeo (24-EBL) no crescimento, desenvolvimento morfológico e parâmetros metabólicos de mudas de *D. odorata*.

### Objetivos específicos

1- Avaliar as respostas fisiológicas, metabólicas e de crescimento de mudas de *D. odorata* à aplicação de diferentes concentrações (10, 20, 30 e 40 nM) de 24-epibrassinolídeo (24-EBL), analisando parâmetros como teor de clorofila, e outros indicadores bioquímicos relacionados ao metabolismo da planta.

2- Identificar a dose do hormônio 24-epibrassinolideo que proporcionará o melhor desempenho no crescimento em altura, diâmetro, número de folhas e quantidade de clorofila das plantas de cumaru no viveiro.

3- Determinar a resposta dose-dependente e o potencial do 24-epibrassinolídeo como ferramenta fisiológica para melhorar a qualidade de mudas de *D. odorata*, identificando as concentrações que promovem efeitos positivos ou inibitórios, levando em consideração as limitações fisiológicas da espécie.

## 3 REFERÊNCIAS

ANWAR, Ali *et al.* The physiological and molecular mechanism of brassinosteroid in response to stress: a review. **Biological Research**, v. 51, n. 1, p. 46, dez. 2018.

APARECIDA LONGATTI, Claudia. **Efeito de brassinosteróide no crescimento, metabolismo e fixação de nitrogênio em plantas de *Cajanus cajan* noduladas e sob estresse**

**salino**. Mestra em Biologia Vegetal—Campinas, SP: Universidade Estadual de Campinas, 31 ago. 2012.

ARYAL, Divya; ALFEREZ, Fernando. Brassinosteroids: Biosynthesis, Signaling, and Hormonal Crosstalk as Related to Fruit Yield and Quality. **Plants**, v. 14, n. 12, p. 1865, 18 jun. 2025.

BARBOSA, Anténor Pereira; PINTO, Antonio Moçambite; MORELLATO, Lpc. **Phénologie reproductive de *Dipteryx odorata* (Aubl.) Willd (Fabaceae) dans deux zones forestières de l'Amazonie centrale**. v. 38, 2008.

BRASIL NETO, Alberto Bentes *et al.* The commercial tree species *Dipteryx odorata* improves soil physical and biological attributes in abandoned pastures. **Ecological Engineering**, v. 160, p. 106143, fev. 2021.

CARVALHO RIBEIRO, Sónia *et al.* Bioeconomic markets based on the use of native species (NS) in Brazil. **Ecological Economics**, v. 218, p. 108124, abr. 2024.

CASTUERA-OLIVEIRA, Luciene; OLIVEIRA-FILHO, Ary Teixeira De; EISENLOHR, Pedro V. Emerging hotspots of tree richness in Brazil. **Acta Botanica Brasilica**, v. 34, n. 1, p. 117–134, mar. 2020.

COLL, Yamilet *et al.* Brassinosteroids roles and applications: an up-date. **Biologia**, v. 70, n. 6, p. 726–732, jun. 2015.

CRUZ, Eniel David. Germinação de sementes de espécies amazônicas: cumaru [*Dipteryx odorata* (Aubl.) Willd.]. **Embrapa Amazônia Oriental**, n. 326, p. 11, 2021.

DO NASCIMENTO, Lidiane Diniz; CASCAES, Márcia Moraes; CRUZ, Eniel David. Espécies Nativas da Flora Brasileira de Valor Econômico Atual ou Potencial. **Plantas-para-o-Futuro-Norte**, v. 13, p. 616–628, 2022.

DU, Juan *et al.* Brassinosteroid regulation of wood formation in poplar. **New Phytologist**, v. 225, n. 4, p. 1516–1530, fev. 2020.

GAMFELDT, Lars *et al.* Higher levels of multiple ecosystem services are found in forests with more tree species. **Nature Communications**, v. 4, n. 1, p. 1340, 8 jan. 2013.

GODINHO DA SILVA, Jéssica Aline *et al.* Morfometria de Plantios de *Dipteryx odorata* Aubl Willd (Cumaru) no Oeste do Pará. **Advances in Forestry Science**, v. 7, n. 3, p. 1171–1180, 3 nov. 2020.

HU, Yueqing *et al.* Brassinolide Increases Potato Root Growth *In Vitro* in a Dose-Dependent Way and Alleviates Salinity Stress. **BioMed Research International**, v. 2016, p. 1–11, 2016.

JAQUETTI, Roberto K. ; GONÇALVES, José Francisco C. Carbon and nutrient stocks of three Fabaceae trees used for forest restoration and subjected to fertilization in Amazonia. **Anais da Academia Brasileira de Ciências**, v. 89, n. 3, p. 1761–1771, set. 2017.

JIROUTOVÁ, Petra *et al.* Brassinosteroids Induce Strong, Dose-Dependent Inhibition of Etiolated Pea Seedling Growth Correlated with Ethylene Production. **Biomolecules**, v. 9, n. 12, p. 849, 9 dez. 2019.

LEAL FILHO, Walter *et al.* Managing ecosystem services in the Brazilian Amazon: the influence of deforestation and forest degradation in the world's largest rain forest. **Geoscience Letters**, v. 12, n. 1, p. 24, 16 maio 2025.

LI, Yanshu; MEI, Bin; LINHARES-JUVENAL, Thaís. The economic contribution of the world’s forest sector. **Forest Policy and Economics**, v. 100, p. 236–253, mar. 2019.

LU, Han *et al.* Brassinosteroids affect wood development and properties of Fraxinus mandshurica. **Frontiers in Plant Science**, v. 14, p. 1167548, 17 jul. 2023.

MARTÍNEZ, Gladys Beatriz; COSTA, Jonas Da Silva; SILVA, Arystides Resende. Fenologia reprodutiva do cumarú (*Dipteryx odorata*) para uso em sistemas silvipastoris / Reproductive phenology of cumarú (*Dipteryx odorata*) for use in silvopastoral systems. **Brazilian Journal of Animal and Environmental Research**, v. 4, n. 3, p. 3708–3714, 11 ago. 2021.

MARTORANO, Lucietta Guerreiro *et al.* Topoclimatic Zoning of Three Native Amazonian Forest Species: Approach to Sustainable Silviculture. **Sustainability**, v. 17, n. 4, p. 1366, 7 fev. 2025.

PRAMOD, S. *et al.* Effect of Exogenously Applied 24-Epibrassinolide and Brassinazole on Xylogenesis and Microdistribution of Cell Wall Polymers in *Leucaena leucocephala* (Lam) De Wit. **Journal of Plant Growth Regulation**, v. 41, n. 1, p. 404–416, 1 jan. 2022.

SHIMADA, Yukihisa *et al.* Organ-Specific Expression of Brassinosteroid-Biosynthetic Genes and Distribution of Endogenous Brassinosteroids in *Arabidopsis*. **Plant Physiology**, v. 131, n. 1, p. 287–297, 1 jan. 2003.

SILVA, Paulo Marcos Ferreira Da *et al.* Anatomical and histochemical characterization of *Dipteryx odorata* and *Taralea oppositifolia*, two native Amazonian species. **Revista Brasileira de Farmacognosia**, v. 29, n. 4, p. 425–433, jul. 2019.

TAIZ, Lincoln; ZEIGER, Eduardo. **Fisiologia vegetal**. 5$^{e}$ édition ed. Artmed, 2013.

TER STEEGE, Hans *et al.* Biased-corrected richness estimates for the Amazonian tree flora. **Scientific Reports**, v. 10, n. 1, p. 10130, 23 jun. 2020.

UCHIDA, Toshihiro; CAMPOS, Moacir A. A. Influência do sombreamento no crescimento de mudas de cumaru (*Dipteryx odorata* (Aubl.) Willd. - Fabaceae), cultivadas em viveiro. **Acta Amazonica**, v. 30, n. 1, p. 107–107, mar. 2000.

VIEIRA, Simone *et al.* Slow growth rates of Amazonian trees: Consequences for carbon cycling. **Proceedings of the National Academy of Sciences**, v. 102, n. 51, p. 18502–18507, 20 dez. 2005.

WANG, Wenfei; BAI, Ming-Yi; WANG, Zhi-Yong. The brassinosteroid signaling network — a paradigm of signal integration. **Current Opinion in Plant Biology**, v. 21, p. 147–153, out. 2014.

Este capítulo está formatado nas normas da revista Journal of Plant Growth Regulation, disponível em: https://link.springer.com/journal/344
.

Artigo (submetido)

## CAPÍTULO I

# RESPOSTA DE *DIPTERYX ODORATA* (AUBL.) FORSYTH F. À APLICAÇÃO DE 24-EPIBRASSINOLÍDEO : EVIDÊNCIAS DE ESTÍMULO E INIBIÇÃO DO CRESCIMENTO

# Effects of 24-epibrassinolide application on the development of *Dipteryx odorata*: between growth stimulation and inhibitory effects

Jospin Soglohoun[1], Karin Elisabeth von Schmalz[2]*, Emil Hernández Ruz[1,2]

[1] Laboratório de Zoologia, Universidade Federal do Pará, Faculdade de Ciências Biológicas, Campus de Altamira. Rua Cel. José Porfírio, Esplanada do Xingu, Altamira Pará, Brazil.

[2] Pós-Graduação em Biodiversidade e Biotecnologia da Rede Bionorte, Universidade Federal do Pará, Faculdade de Ciências Biológicas, Campus de Altamira. Rua Cel. José Porfírio, Esplanada do Xingu. Altamira, Pará, Brazil

*Corresponding author: karin.schmalz@gmail.com

Authors' ORCID:
Jospin Soglohoun: 0009-0005-7612-1719
Karin Elisabeth von Schmalz: 0009-0006-8807-8567
Emil Hernández Ruz: 0000-0002-3593-3260

**Acknowledgements**

The authors thank the Institute for Forest and Biodiversity Development of the State of Pará (IDEFLOR-BIO) and the Federal University of Pará, Brazil, for the material support. KEvS thanks Alexandra Elbakyan, Ijad Madisch, Sören Hofmayer, and Horst Fickenscher for their contributions to science.

**Funding information**

This research was funded by the Coordination for the Improvement of Higher Education Personnel (Capes) of the Brazilian Ministry of Education under grant number 88887.146542/2025-00.

## Abstract

We evaluated the effect of 24-epibrassinolide on the growth and development of young seedlings of *Dipteryx odorata*, the Tonka Bean tree, an Amazonian species of ecological and economic importance. The experiment was conducted in the nurseries of the Federal University of Pará (Altamira, Brazil), under 50% shade, on 115 seedlings divided into five treatments (0, 10, 20, 30, and 40 nM of the hormone). We measured morphological parameters (height, basal diameter, and number of leaves) and leaf physicochemical parameters (chlorophyll, nitrogen, moisture, and leaf temperature). To explore the associations of differences in each individual per treatment and choose the variables for modelling, we performed a principal component analysis. The effects of the treatments on these variables were evaluated using linear mixed-effects models with maximum likelihood estimation. The hormone dosages significantly influenced plant growth, with a marked positive effect at the 30 nM dose, resulting in greater gains in height, diameter, and number of leaves, as well as increased chlorophyll production. The 40 nM dose provided the best height/diameter ratio, indicating better plant stability and quality. The 10 nM dose, although positive for size, diameter, and growth rate, yielded less relevant results than higher doses. The 20 nM dose caused inhibitory effects, characterized by reduced growth and decreased chlorophyll production, suggesting a sensitivity window associated with high ethylene production. These results confirmed the importance of 24-epibrassinolide as a growth regulator capable of improving the physiological and morphological performance of *Dipteryx odorata* under semi-controlled conditions.

**Keywords** Brassinosteroids, phytohormones, seedling production, Amazonian species, environmental restoration.

## Introduction

*Dipteryx odorata* (Aubl.) Forsyth F. (Fabaceae), known as Tonka bean tree or cumaru (Jaquetti and Gonçalves 2017), is a large tree species, reaching up to 30 meters in height in primary forests, or less when cultivated in secondary forests (Silva et al. 2019). *D. odorata* is present in approximately 30.41% of the entire territory of the state of Pará, the second largest federative unit of Brazil (Martins Santos et al. 2023). It is found in the phytogeographic domains of the Amazon, in Terra Firme, seasonal semi-deciduous, and ombrophilous forests (Pinto et al. 2008), but also colonizes floodplains (Coradin et al. 2022). The species has an erect, cylindrical trunk, 50 to 70 cm in diameter, with thin, rough bark that peels off in irregular plates (Lorenzi 2002). The tree has heavy, high-quality wood with a density of 0.75 to 1.10 g/cm³, and its timber is commonly called Brazilian Teak (Holm et al. 2014), used in the production of agricultural tools, shipbuilding, the manufacture of tool handles, posts, railroad ties, carts, stakes, supports, floors, beams, laminated woodwork items, and bearings for boat propeller shafts (Pinto et al. 2008; Silva et al. 2019). Its fruit is a dense, oval, woody legume with a late-dehiscent endocarp after decomposition of the mesocarp, measuring 5 cm to 6.5 cm in length and 3.5 cm in width, with one or two seeds (Cruz and Barros 2021). Tonka beans are used to make necklaces and bracelets, in addition to being widely traded since the 19th Century due to its excellent aroma, which is used to flavor chocolates, cigarettes, cigars, sweets, foods, and beverages, as well as in the production of perfumes, soaps, and other cosmetic products (Do Nascimento et al. 2022). The beans are aromatic due to the presence of coumarin, a bioactive molecule that influences various plant development processes, including germination, root growth, and stem elongation; in combination with brassinosteroids, coumarin is capable of inducing hypocotyl elongation, promoting plant growth (Van Praet et al. 2025). An essential oil (cumaru seed oil) is extracted from the beans and used in perfumery, which is of great importance to Venezuela's regional economy and industry (Silva et al. 2019). For Brazil, the promotion of cumaru oil not only strengthens its position as a leader in the production and export of natural products, but also its identity on the international stage, promoting sustainable development and environmental preservation (Bonfim et al. 2025).

*D. odorata* is a species with great potential for reforestation in the Eastern Amazon, as it offers important environmental, economic, and cultural resources (Do Nascimento et al. 2022). Cumaru cultivation is an economic alternative for family farmers in the western region of Pará, and the species is an effective alternative for forest restoration in degraded areas, reforestation, and soil recovery in abandoned pastures, as it was demonstrated in the municipality of Óbidos, in Eastern Amazon, Brazil (Mota et al. 2022). The species is vulnerable to negative impacts due to overexploitation, especially in the state of Pará (Cruz and Barros 2021). According to the International Union for Conservation of Nature (IUCN 2021), there is an urgent need to improve and expand ecosystem restoration, promoting scientifically proven practices and creating suitable conditions for restoration. To this end, the use of *Dipteryx odorata* is a promising strategy, as the species is recognized for its relevance in reforestation programs, productivity starting at four years of age (Pinto et al. 2008), and superior performance in growth and survival rate in four-year monospecific experimental plantations conducted under soil and climate conditions similar to those of the Brazilian Central Amazon (Machado et al. 2018). The use of phytohormones, such as brassinosteroids, is one of the most effective ways to reduce the production time of young plants to accelerate the reforestation process. Brassinosteroids regulate various processes in plant development and physiology, including seed germination, seedling photomorphogenesis, stomatal differentiation, organ boundary formation, flowering, male fertility, and responses to biotic and abiotic stresses (Wang et al. 2014). One of the advantages of using brassinosteroids is that they increase resistance to biotic and abiotic stresses, such as pathogens, extreme temperatures, saline soils, water scarcity, metal contamination, and nutritional deficiencies (Krishna et al. 2010; Bajguz et al. 2016).

Brassinosteroids are known to improve production yields in several species essential for human use. For example, they can increase seed productivity in common beans *Phaseolus vulgaris*, Kusha cultivar, COS16 genotype (Mohammadi et al. 2020). They can also play an important role in improving growth, biomass, productivity, yield, and reducing oxidative damage in wheat (*Triticum aestivum*) under drought conditions (Khan et al. 2021).

The existing literature on *D. odorata* does not address the impact of hormone use on the species. Therefore, our objective in this study was to evaluate the morphological and metabolic parameters of young *D. odorata* plants, verifying the effects of applying 24-epibrassinolide (24-EBL) at various concentrations (10, 20, 30, and 40 nM) on the growth and development of seedlings of the species under 50% shading. The central hypothesis of this experiment was that, in response to the application of 24-EBL, plants would exhibit a dose-dependent response, with lower concentrations (10–30 nM) potentially maximizing morphological growth and promoting metabolic activities, while higher concentrations (40 nM) would not provide any additional benefits or even become less effective.

## 2 Materials and Methods

### 2.1 Study area

The experiment was conducted at the Federal University of Pará (Altamira, 3°12'43"S 52°12'49"W). According to the Köppen's climate classification, the region has a hot and humid tropical climate of types *Am* and *Aw*, with an average minimum annual temperature of 22.1 °C, an average maximum annual temperature of 32.4 °C, and annual precipitation of 2100 mm (Alvares et al. 2013). The plants were kept under 50% shade.

### 2.2 Seedling production

The seedlings were produced from cumaru fruits supplied by the Institute for Forest and Biodiversity Development of the State of Pará (IDEFLOR-Bio), from which intact seeds were removed. The seeds, from four categories of fruits (Fig. 1), were placed in sand contained in tubes with a diameter of 4 x 15 cm for 30 days. The characteristics and germination rates of each seed category differed greatly (Table 1).

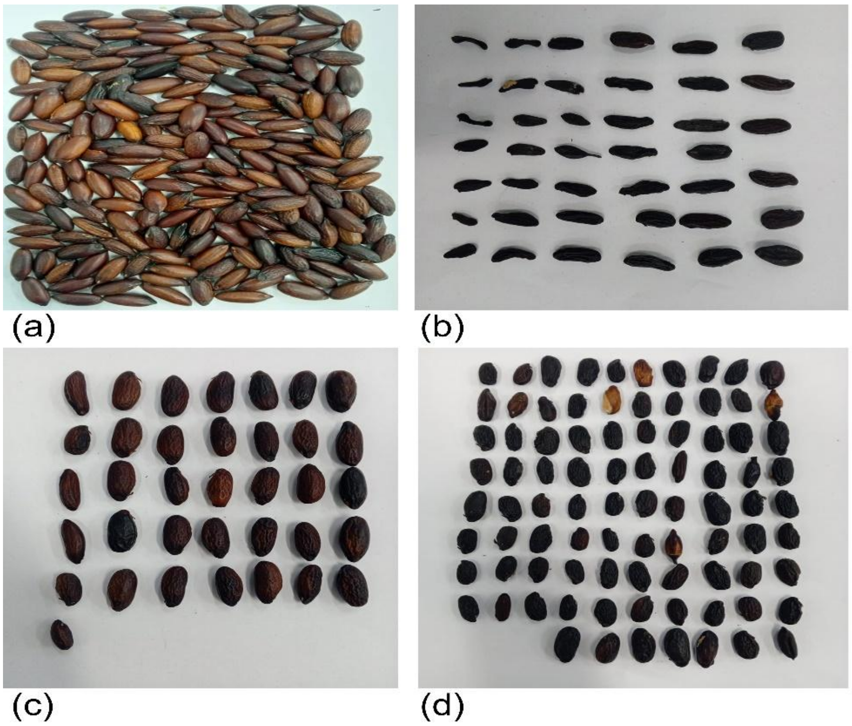

**Fig. 1** Photos of different categories of *Dipteryx odorata* fruit seeds grouped prior to germination for the 24-epibrassinolide application experiment in the greenhouse at the Federal University of Pará, Altamira Campus. (**a**) Large, healthy fruit seeds; (**b**) seeds from fruit eaten by insects; (**c**) light-colored seeds from healthy medium-sized fruit; (**d**) black seeds from healthy medium-sized fruit.

**Table 1** Categories of *Dipteryx odorata* seeds cultivated according to their quantities, total weight, minimum and. maximum weight, germination rate, and number of plants obtained for each category

| Seed category | Quantity | Total weight (g) | Minimum weight (g) | Maximum weight (g) | Average weight (g) | Quantity of seedlings | Germination rate (%) |
|---|---|---|---|---|---|---|---|
| a | 203 | 347.92 | 1.136 | 2.412 | 1.276 | 130 | 64 |
| b | 41 | 8.96 | 0.023 | 0.69 | 0.667 | 0 | 0 |
| c | 36 | 46.19 | 0.658 | 2.057 | 1.399 | 10 | 27.8 |
| d | 87 | 63.72 | 0.426 | 1.688 | 1.262 | 0 | 0 |
| Total | 367 | 466.79 | - | - | - | 140 | - |

A total of 140 plants were obtained from seeds in categories a and c; plants with inadequate shape and poor robustness were removed, and only 115 vigorous plants with the best morphological characteristics were selected for the experiment.

### 2.3 Soil preparation

The 40-day-old seedlings were placed in black polyethylene plastic bags measuring 15 x 25 cm in diameter and 20 µm in thickness, filled with a mixture of soil, fertilizer, organic matter, and Amafibra Golden Mix 47® commercial organic substrate. The mixture consisted of: 96 kg of coconut fiber, 12 kg of hillside soil, 1.5 kg of Yoorin® phosphate fertilizer, 1 kg of bone meal, 2 kg of NPK (4–14-8), 1.5 kg of Basacote® fertilizer (16-8-14), 3 kg of limestone, and 90 L of water. The technical report of the organic substrate's physical and chemical analysis can be found in the Supplementary File S1.

### 2.4 Experimental design

The experiment was conducted in a randomized block design, with five replicates and five groups of 23 *D. odorata* plants aged 70 days, totaling 115 plants. The plants measured 7.0-19.9 cm in height, with stem diameters of 2.53-4.17 mm, and two to four leaves at the beginning of the experiment. The research was conducted between December 2024 and May 2025.

We performed a single application of the treatment. The control group (t0) did not receive the phytohormone application. The experimental treatments, named t1, t2, t3, and t4, received a single dose of 24-EBL, respectively, in four treatments consisting of doses of 10, 20, 30, and 40 nM of the hormone.

For the application of 24-EBL, we separated each group of plants with black plastic bags at a height of approximately 1.5 m to avoid the drift effect of hormone application. The application was performed with a constant pressure backpack sprayer, equipped with a lance and fan nozzle, manufactured by Kawashima Company, model PEM-P20. After application, the plants remained protected from rain for 24 hours to prevent the loss of the hormone due to washing of the applied product.

Every 30 days we collected data, totaling six measurements over six consecutive months. One measurement (M0) was taken before treatment, and five others (M1-M5) were taken each month after application of the phytohormone.

## 2.5 Data collection

Starting in Month 0 (M0), plant height was measured using a 50-cm graduated ruler, the number of leaves was counted, and stem diameter was measured directly using a universal stainless-steel caliper (150 mm/6", accuracy ± 0.05 mm, Digimess®, Brazil). Starting in the second month (M1) and using a Goyojo® manual electronic chlorophyll meter, model GYJ-D, we measured leaf temperature, nitrogen, humidity, and chlorophyll indirectly using SPAD values. The values were collected in the morning between 7 a.m. and 8 a.m.

The collected data were grouped into morphological variables (height, basal diameter, and number of leaves), representing plant development, and leaf physicochemical variables (SPAD chlorophyll, leaf nitrogen, leaf temperature, and leaf humidity), representing plant metabolism. Plant height in millimeters was divided by the average diameter (the sum of the basal diameters collected twice and on two different sides divided by two) to calculate the growth index, or height/diameter ratio, commonly used to assess seedling health (Haase 2008).

## 2.6 Statistical analysis

We performed data analysis using the R v.4.5.1 language (R Core Team 2025) via RStudio v2025.05.1 Build 513 “Mariposa Orchid” (RStudio Team 2025). A first descriptive stage allowed us to characterize the distributions of the variables and assess their conformity with a normal distribution, using descriptive statistics, and visualizing the distributions with histograms and quantile-quantile plots. In the case of non-normal distribution, the variables were transformed by natural logarithm to approximate normality before use in parametric models.

For analyses that allowed grouping by fixed and random factors in order to correct for pseudo-replication of data, the database used contained repeated measurements ($n = 690$) from the 115 individuals. For analyses that do not accept pseudo-replicated data, an aggregate database per individual ($n = 115$) was created, where morphological and physicochemical leaf variables were calculated by subtracting the values collected at the beginning of the experiment from those collected at the end of the experiment (delta values, represented by $\Delta$).

To explore the associations of differences in each individual by treatment and choose the variables for modeling, a principal component analysis (PCA) was conducted with the $\Delta$ values using the R base package, with *post hoc* tests performed with the *factoextra* (Kassambara and Mundt 2020) and *emmeans* (Lenth 2024) packages.

The PCA was used to select variables representative of hormonal treatment effects, after verifying that they met the requirements for use in parametric statistical models: plant growth was represented by the height/diameter ratio, or growth index, after normalization by logarithmic transformation; SPAD chlorophyll, with normal distribution, represented photosynthetic capacity and nitrogen investment in the photosynthetic apparatus, indicating increasing potential for autotrophic carbon assimilation; and leaf temperature, with homogenous variance between groups verified  with the Levene’s test (Nordstokke et al. 2011), reflected the balance between absorbed radiation and cooling by transpiration, serving as an indicator of the water status of plants, their thermal load, and photosynthetic stress.

The effects of treatments on these variables were evaluated using linear mixed effects models (LME) with restricted estimation of maximum likelihood (REML) using the *lme4* package (Bates et al. 2015) on repeated measures, with time and treatments as fixed effects, and plant identity as a random effect to correct for pseudo-replication. The significance of the models was verified through analysis of variance (ANOVA), and pairwise mean comparisons were verified through Tukey's method (Tukey 1949), using *emmeans* and *lmertest* (Kuznetsova et al. 2017) packages. Akaike information criteria (AIC) and marginal and conditional $R^2$ values of the models were calculated using the *MuMIn* package (Barton 2025), and the explanatory power was calculated using the *performance* package (Lüdecke et al. 2021). The homoscedasticity of the adjusted values and residuals of the models were examined *post hoc* by Breusch-Pagan tests (Breusch and Pagan 1979). The *p*-values of hypothesis tests were adjusted by the Holm-Bonferroni method (Holm 1979).

Graphical representations were constructed using the *ggplot2* package (Wickham 2016), included in the *tidyverse* package (Wickham et al. 2019). Multiple graphs were constructed using the *patchwork* package (Pedersen 2024).

## 3 Results

### 3.1 Overall development of *Dipteryx odorata* plants in experimental cultivation

All 115 *Dipteryx odorata* plants grown experimentally survived throughout the study. The characteristics measured during the six-month study showed heterogeneous development when grouped by treatment (Fig. 2). There was no significant morphological variation in the treatments after the first 30 days. With regard to morphological characteristics such as height, number of leaves, and diameter (Fig. 2a-c), treatment t3 showed the best results from M2 until the end of the experiment. The plants were slightly taller on t3 than those on treatment t4; diameters were larger than the other treatments and, from M4 onwards, the number of leaves was larger than in the other treatments. Treatment t4 showed the best gains in height/diameter ratio, which is also affected by height (Fig. 2d).

Regarding phyto-physicochemical variables such as chlorophyll, leaf moisture, and nitrogen (Fig. 2e-g), treatment t3 showed better results until time M4, being then surpassed by t0 at time M5. Considering all variables, except leaf temperature, both morphological and phyto-physicochemical, treatment t2 showed marked limiting effects. From M2 (60 days after 24-EBL application), there was a marked effect of t2 on plant height, with high frequencies at the lowest values, indicating dwarfism, or a suppression in growth. At M4, t4 plants were substantially larger than t0 and t1 plants, but this difference disappeared after one month, leaving only treatment t2 with inhibitory effects. The number of leaves also showed an inhibitory effect of t2, when compared to the other treatments.

Leaf temperature developed differently from other leaf measurements (Fig. 2h), presenting an asymmetric distribution, with all treatments exhibiting similar variation and a drop in measurements in months

M3 and M4. This phenomenon does not appear to be related to the treatments, but rather to natural environmental conditions.

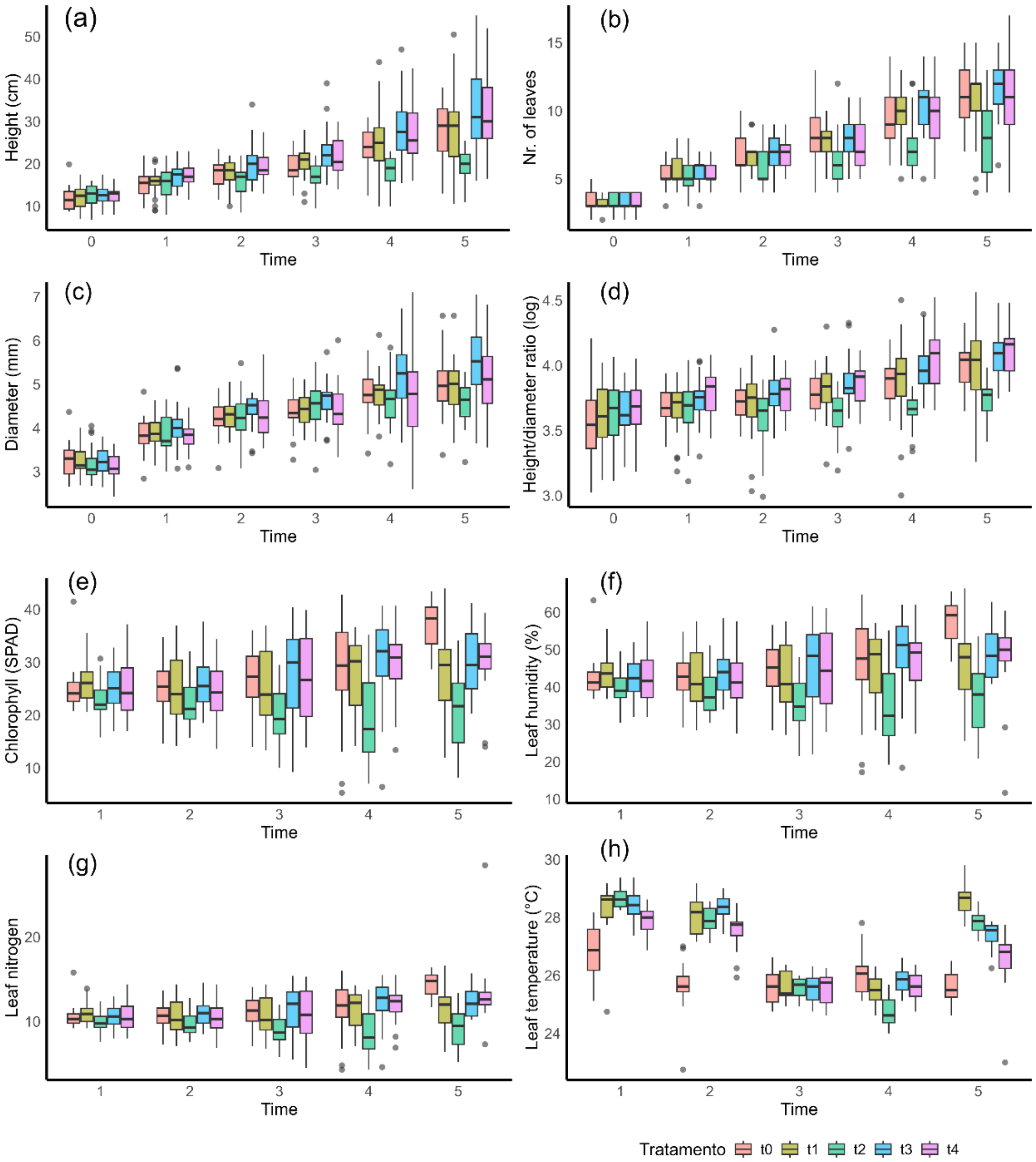

**Figure 2** Distribution of morphological and phyto-physicochemical variables, separated by treatment and time, from 115 *Dipteryx odorata* plants grown experimentally in the UFPA-Altamira nursery under 50% shade and different concentrations of the phytohormone 24-epibrassinolide for six months. (a) plant height in cm; (b) number of leaves; (c) average basal diameter in mm; (d) height/diameter ratio in mm; (e) SPAD leaf chlorophyll; (f) leaf humidity in %; (g) leaf nitrogen in mg/g; (h) leaf temperature in °C. Horizontal bars represent means, vertical bars (whiskers) represent data rage and gray dots represent outliers.

### 3.2 Principal component analysis (PCA)

The principal component analysis (PCA), corrected for repeated measurements of individuals, revealed clear multivariate patterns in the response of plants to different treatments. The first two components together explained 68.2% of the total variability (PC1 = 40.0%; PC2 = 28.2%) and indicated a clear separation between the experimental groups (Fig. 3a). PC1 was strongly associated with the height, diameter, and number of leaves of individual plants, while PC2 mainly reflected variations related to leaf nitrogen and chlorophyll (Table 2, Fig. 3b). Height, diameter, and growth were positively associated with leaf temperature, while leaf nitrogen, chlorophyll, and humidity were inversely related to this axis. Multivariate analysis of variance (MANOVA) indicated significant differences between treatments (MANOVA: Pillai = 0.582; $F_{(8,220)} = 11.30$; $p < 0.001$), and both PC1 and PC2 showed significant treatment effects ($p < 0.001$ and $p = 0.001$, respectively; Supplementary Table S1). Contrasts between groups were confirmed by Tukey's multiple comparison test (Supplementary Table S2).

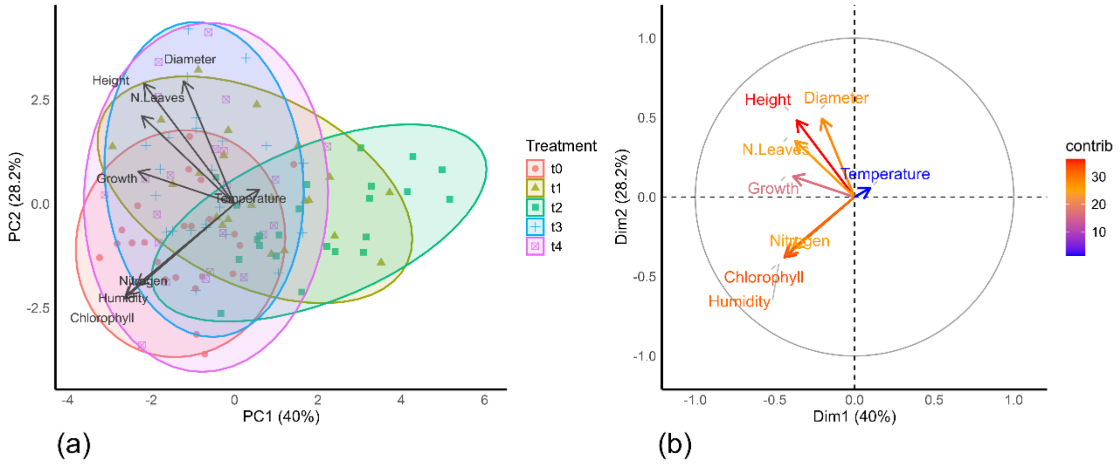

**Figure 3** Principal component analysis (PCA) of the relationships between morphological and biochemical variables from data collected from *Dipteryx odorata* plants grown experimentally under different concentrations of the phytohormone 24-epibrassinolide for six months. (a) Biplot of the distribution of individuals by data and treatment, organized in the first two components of the analysis; (b) loadings and contributions of the variables in the first two components of the analysis.

**Table 2** Variables used in principal component analysis (PCA), loadings of the two main components, and contributions, in percentage, of each variable, from data collected from *Dipteryx odorata* plants grown experimentally under different concentrations of the phytohormone 24-epibrassinolide for six months.

| **Variable** | **PC1** | **PC2** | **Contribution (%)** |
|---|---|---|---|
| Height (cm) | -0.3631 | 0.4819 | 18.21 |
| Diameter (mm) | -0.2036 | 0.4918 | 14.17 |
| Height/diameter ratio (mm) | -0.3853 | 0.1293 | 8.26 |
| Number of Leaves | -0.3712 | 0.3522 | 13.09 |
| Chlorophyll (SPAD) | -0.4399 | -0.3788 | 16.85 |
| Nitrogen (mg/g) | -0.3902 | -0.3256 | 12.91 |
| Temperature (°C) | 0.0986 | 0.05667 | 0.65 |
| Leaf humidity (%) | -0.4301 | -0.3639 | 15.87 |

There were three distinct groups of variables that behaved cohesively in the PCA: morphological variables (height, diameter, number of leaves, and height/diameter ratio, or growth index) had similar directions and high contributions to explaining variability between groups, totaling 53.73%. Three of the leaf biochemical characteristics (SPAD chlorophyll, nitrogen, and humidity) varied concomitantly, with very close loading values and a total contribution of 45.63%. Leaf temperature, on the other hand, with a low contribution to explaining the variability between groups (0.65%), showed an opposite direction to the other variables. Thus, to reduce the hypothesis testing dimensionality, the response variables chosen for the models – representing the three groups of variables – were growth index (height/diameter ratio), leaf chlorophyll, and leaf temperature. Chlorophyll showed normal distribution, the growth index approached normality after natural logarithm transformation, and leaf temperature showed homoscedasticity within treatments, allowing these three response variables to be used in parametric models.

### 3.3 Effect of 24-EBL treatments on the growth of *Dipteryx odorata* plants

A linear mixed effects model (LME/REML) revealed significant effects of treatments ($F_{4,110} = 6.41$; $p = 0.001$), time ($F_{5,550} = 129.27$; $p < 0.0001$), and their interaction ($F_{20,550} = 5.22$; $p < 0.0001$) on the height-diameter relationship, with effects controlled for each individual. The model explained a moderate proportion of the variance through fixed effects (marginal $R^2 = 35.8\%$) and a high proportion when random effects were included (conditional $R^2 = 76.1\%$). The model fit indices indicated good performance (AIC = −428.57; BIC = −284.82; log-likelihood = 246.29; Table 3). The complete values of random and fixed effects can be seen in Supplementary Table S3. The model residuals achieved homoscedasticity (Breusch-Pagan: BP = 2.72; $p = 0.099$), and the graph of adjusted values against residuals did not show clear patterns (Supplementary Figure S1).

**Table 3** Summary and adjustment measures of the LME/REML model of the effects of the experimental application of four concentrations of 24-EBL on the height/diameter ratio of 115 *Dipteryx odorata* plants monitored for six months. NumDf = degrees of freedom of the numerator; DenDf = degrees of freedom of the denominator.

| **LME/REML Model: Height/diameter ratio ~Treatment*Time (1\|ID)** | |
|---|---|
| Marginal $R^2$ | 0.358 |
| Conditional $R^2$ | 0.761 |
| AIC | -428.57 |
| BIC | -284.82 |
| LogLik | 246.29 |

**Model ANOVA**

| Term | **NumDf** | **DenDf** | **F** | ***p*** |
|---|---|---|---|---|
| (Intercept) | 1 | 550 | 55267.50 | <0.0001**** |
| Treatment | 4 | 110 | 6.41 | 0.001*** |
| Time | 5 | 550 | 129.27 | <0.0001**** |
| Treatment:Time | 20 | 550 | 5.22 | <0.0001**** |

Significance levels of p values * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001; **** ≤ 0.0001.

*Post hoc* paired comparisons were performed using estimated marginal means and Tukey's adjusted paired comparisons, and revealed no significant differences between treatments in the first two months of the experiment (M0 and M1; all $p > 0.40$). Differences between treatments began to emerge from the third month (M3), when plants under treatment t2 became significantly smaller than those under other treatments, and these differences increased in magnitude over the following months. In the last two months (M4 and M5), treatment t2 consistently showed significantly lower estimated marginal means than all other treatments ($p < 0.05$), while no significant differences were detected in the height/diameter ratio between treatments t0, t1, t3, and t4. These findings confirm that the divergence in treatment level depended on time and only became statistically distinguishable after the first measurement periods. The estimated marginal means, with standard errors and lower and upper confidence intervals (95% CI), can be seen in Supplementary Table S4 (Supplementary Figure S2). The difference between the first and last measurements (Δ) in each experimental group can be seen in Figure 4a. Paired contrasts over time (adjusted by Tukey) with significance levels can be seen in Supplementary Table S5 and are summarized in Figure 4b.

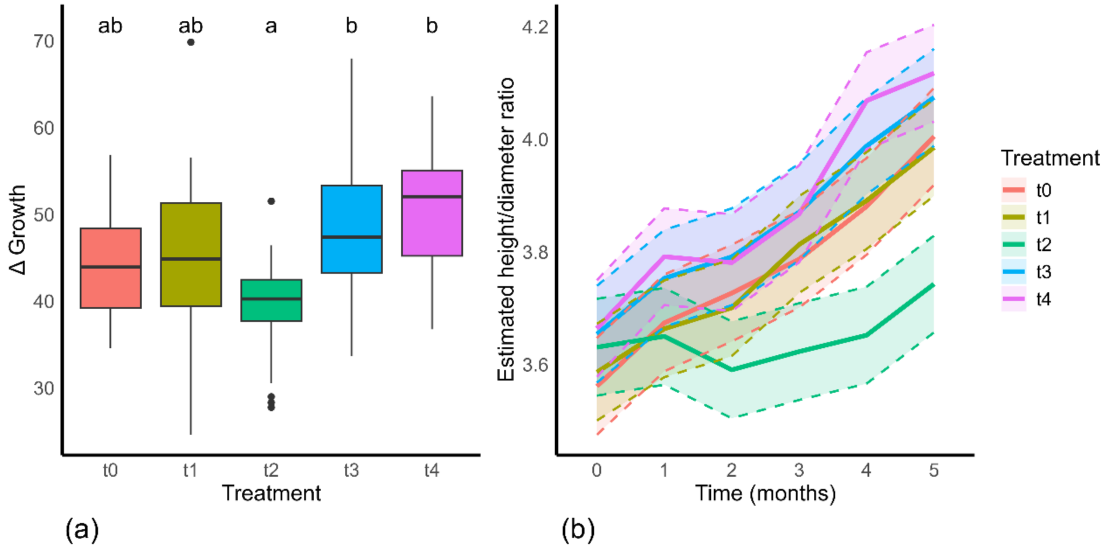

**Figure 4** (**a**) Boxplot of the difference between growth rates of *Dipteryx odorata* plants subjected to different concentrations of 24-EBL (t0 = control group) over six months of experimentation. Horizontal bars represent means; dots represent outliers. Letters result from paired comparisons adjusted by Tukey's test (α = 0.05). Treatments sharing letters belong to the same statistical group; the absence of common letters indicates significant differences. (**b**) Tukey-adjusted estimated measures of marginal means of treatments across months. Areas between dotted lines represent upper and lower limits of the 95% confidence interval.

### 3.4 Effect of 24-EBL treatments on the phyto-physicochemical characteristics of *Dipteryx odorata* plants

Two leaf physicochemical characteristics were tested with LME/REML: SPAD chlorophyll, which showed normal distribution, and leaf temperature, after verification of homoscedasticity (Levene's test: $F_{4,110} = 0.8351$; $p = 0.5057$) for experimental groups.

For SPAD chlorophyll, the linear mixed effects model was highly significant (constant: $F_{1,550} = 5150$; $p < 0.0001$), also with strong treatment effects ($F_{4,440} = 14.641$; $p < 0.0001$), time ($F_{4,440} = 14.947$; $p < 0.0001$), and the interaction between treatment and time ($F_{16,440} = 4.197$; $p < 0.0001$). As with the effects on plant growth, fixed effects moderately explained the variance (marginal $R^2 = 26.8\%$), and the explanatory power increased with the inclusion of random effects (conditional $R^2 = 43.7\%$), despite the lower values. However, the model had less power than the previous one, with higher adjustment values (AIC = 3676.871; BIC = 3793.238, log-likelihood = -1811.435; Table 4). The complete values of the random and fixed effects can be seen in Supplementary Table S6. Residuals showed homoscedasticity (Breusch-Pagan: BP = 0.229; $p = 0.6317$) and did not show visible patterns against adjusted values (Supplementary Figure S3).

**Table 4** Summary and model fit measures for the LME/REML model of the effects of the experimental application of four concentrations of 24-EBL on the leaf SPAD chlorophyll levels of 115 *Dipteryx odorata* plants monitored for five months. NumDf = degrees of freedom in the numerator; DenDf = degrees of freedom in the denominator.

| **LME/REML Model: Chlorophyll ~Treatment*Time (1\|ID)** | |
|---|---|
| Marginal $R^2$ | 0.268 |
| Conditional $R^2$ | 0.437 |
| AIC | 3676.871 |
| BIC | 3793.238 |
| LogLik | -1811.435 |

**Model ANOVA**

| Term | **NumDf** | **DenDf** | **F** | ***p*** |
|---|---|---|---|---|
| (Intercept) | 1 | 440 | 5159.072 | <0.0001**** |
| Treatment | 4 | 110 | 14.641 | <0.0001**** |
| Time | 5 | 440 | 14.947 | <0.0001**** |
| Treatment:Time | 16 | 440 | 4.197 | <0.0001**** |

Significance levels of *p* values * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001; **** ≤ 0.0001.

The application of 24-EBL influenced the physiological activity of treated plants during the period. *Post hoc* paired comparisons were performed using estimated marginal means and Tukey's adjusted paired comparisons, and revealed no significant differences between treatments in the first two months of the experiment (M1 and M2; all $p > 0.40$). The significant difference between t2 and the other treatments appears from M3 until the end of the experiment (Supplementary Table S8).

From M1 to M5, there was no variation in chlorophyll production between t3 and t4. From M1 to M4, there was no significant difference in chlorophyll production between t0 and treatments t1, t3, or t4. Chlorophyll increase reached a maximum of 44 in group t0 in the control treatment, which completely separated the distribution of the group's data. Chlorophyll production in t1 remained below t0 from M1 to M3, and t2 (having the lowest chlorophyll production) with an increasing inhibitory effect from M1 to M4. The contrast between t0 and all other treatments is significant (Supplementary Figure S4). The estimated marginal means, with standard errors and lower and upper confidence intervals (95% CI), can be seen in Supplementary Table S7 (Supplementary Figure S4). The control group (t0) showed the greatest differences in chlorophyll between the beginning and end of the experiment (Δ), while groups t1 and t2 showed the smallest differences (Fig. 5a). Over time, group t3 had higher levels of leaf chlorophyll, with a drop after the fourth month (M3) in its estimated values. More visible was the inhibitory effect of treatment t2, with much lower levels of chlorophyll estimated over time (Fig. 5b). Paired contrasts over time (adjusted by Tukey) with significance levels can be seen in Supplementary Table S8.

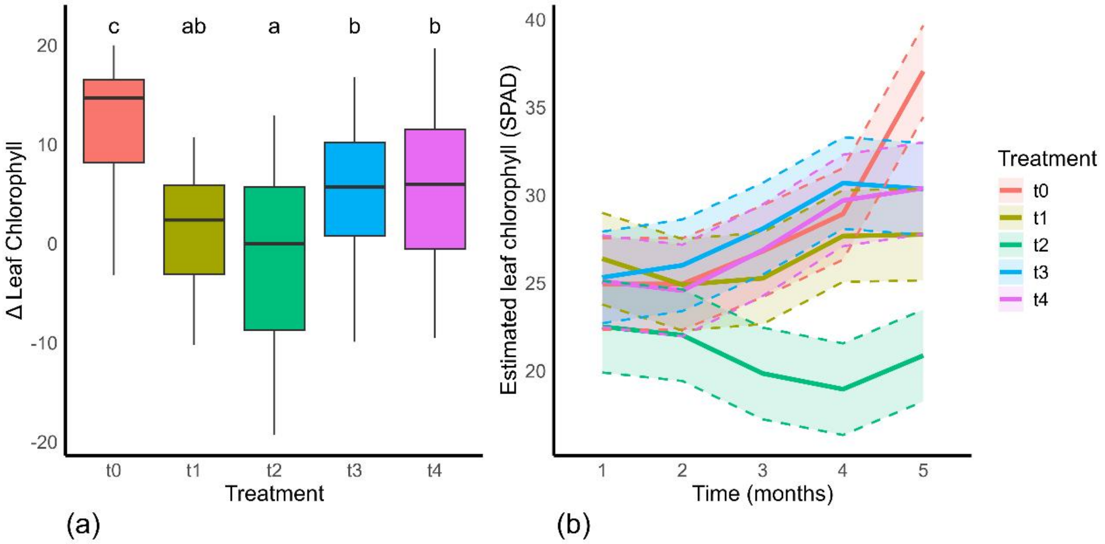

**Figure 5** (**a**) Boxplot of the difference between leaf SPAD chlorophyll levels of *Dipteryx odorata* plants subjected to different concentrations of 24-EBL (t0 = control group) over five months of experimentation. Horizontal bars represent means; dots represent outliers. Letters result from paired comparisons adjusted by Tukey's test (α = 0.05). Treatments sharing letters belong to the same statistical group; the absence of common letters indicates significant differences. (**b**) Tukey-adjusted estimated measures of marginal means of treatments across months. Areas between dotted lines represent upper and lower limits of the 95% confidence interval.

Regarding leaf temperature, which showed peculiar development in months M3 and M4, weather seemed to be a much more influential factor than the effect of treatments on the measured levels (Table 5, Supplementary Table S9), although plants in treatment t1 showed a clear difference in leaf temperature at the end of the experiment (Fig. 6a). The LMER/REML model indicated that most of the variation was explained by fixed effects (marginal $R^2$ = 0.825), with minimal contribution from random effects (conditional $R^2$ = 0.832). The main effect of time was highly significant, reflecting sharp reductions in leaf temperature in months 3 and 4, followed by recovery in month 5, as shown by marginal means estimates (Supplementary Table S10; Fig. 6b). The Treatment × Time interaction was also significant, indicating that differences between treatments varied across months; in particular, no differences between treatments were detected in M3 (Figure S6), while in the other months there were significant contrasts (Supplementary Table S11).

Estimates of coefficients, standard errors, and *p*-values are detailed in Table S9. Paired contrasts adjusted by Tukey's method are presented in the Supplementary Table S11. Figure 6a shows the differences (Δ) in leaf temperature throughout the experiment between treatments. Verification of the assumptions indicated adequate homoscedasticity (Breusch–Pagan: BP = 30.061; $p = 0.1827$), although the residual plot shows a slight pattern associated with months (Supplementary Figure S5).

**Table 5** Summary and adjustment measures of the LME/REML model of the effects of the experimental application of four concentrations of 24-EBL on leaf temperature (in °C) of 115 *Dipteryx odorata* plants monitored for five months. NumDf = degrees of freedom of the numerator; DenDf = degrees of freedom of the denominator.

| **LME/REML Model: Leaf temperature ~Treatment*Time (1\|ID)** | | | | |
|---|---|---|---|---|
| Marginal R² | 0.825 | | | |
| Conditional R² | 0.831 | | | |
| AIC | 1085.115 | | | |
| BIC | 1201.482 | | | |
| LogLik | -515.5573 | | | |
| **Model ANOVA** | | | | |
| **Term** | **NumDf** | **DenDf** | **F** | ***p*** |
| (Intercept) | 1 | 440 | 1076642.6 | <0,0001**** |
| Treatment | 4 | 110 | 78.8 | <0,0001**** |
| Time | 5 | 440 | 471.8 | <0,0001**** |
| Treatment:Time | 16 | 40 | 34.3 | <0,0001**** |

Significance levels of *p* values * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001; **** ≤ 0.0001.

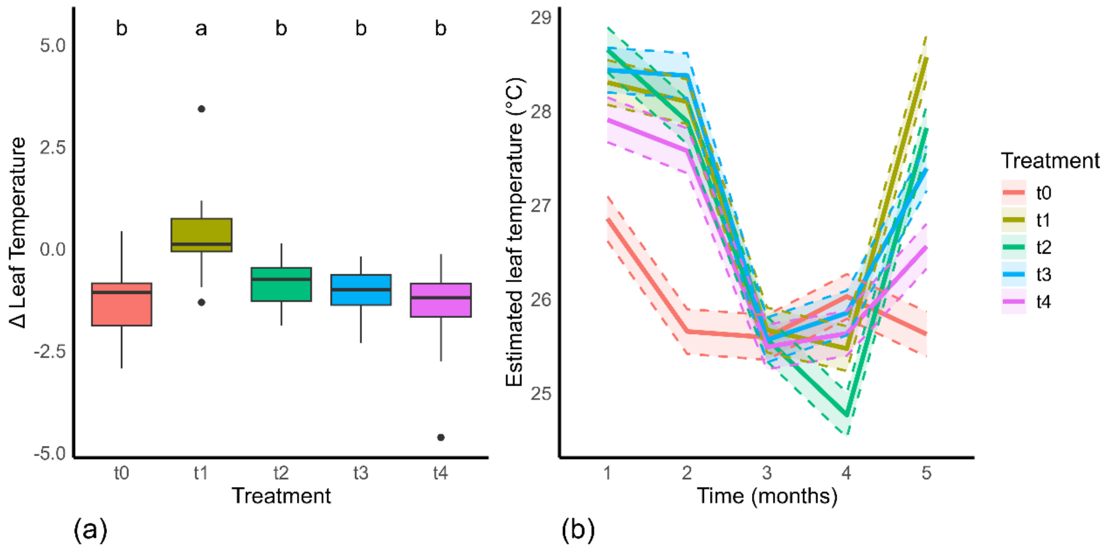

**Figure 6** (**a**) Boxplot of the difference between leaf temperature (in °C) of *Dipteryx odorata* plants subjected to different concentrations of 24-EBL (t0 = control group) over five months of experimentation. Horizontal bars represent means; dots represent outliers. Letters result from paired comparisons adjusted by Tukey's test (α = 0.05). Treatments sharing letters belong to the same statistical group; the absence of common letters indicates significant differences. (**b**) Tukey-adjusted estimated measures of marginal means of treatments across months. Areas between dotted lines represent upper and lower limits of the 95% confidence interval.

Regarding the decrease observed in leaf temperature, according to the Meteorological Database (BDMEP) of the Brazilian National Institute of Meteorology (INMET), rainfall in the region varied between 68.8 mm and 444.6 mm, while the mean environmental temperature varied between 20.9 °C and 32.5 °C. Precipitation in the region was 112.2 mm in M0, increasing to 444.6 mm in M1, with high values observed between M1 and M4, followed by a decrease to 68.8 mm in M5. As for the environmental temperature, the minimum values recorded were between M1 and M4. Monthly rain days in the city of Altamira from 1990 to 2019 averaged from the lowest of 17 days in December to 21 days in March. In the same historic period, the average hours of sunlight were the highest in December (7.4 h/day) and the lowest in April (4.8 h/day) due to cloud cover. The relevant data can be found in Supplementary Table S12.

## 4 Discussion

This study investigated the impact of different doses of 24-EBL on the growth and development of young *Dipteryx odorata* plants. Low germination rates were observed in seeds from insect-attacked fruits or in dark seeds from medium-sized fruits, whereas seeds from large, healthy fruits are essential for the production of seedlings with greater vigor and better morphological characteristics. It was demonstrated that, in this species, the morphology of seedlings from germination, especially collar diameter, is a reliable indicator of the physiological quality of seeds, being strongly correlated with root biomass, total biomass, and the overall vigor of seedlings, directly reflecting the germination performance of the species (Guimarães et al. 2024). The germination of *Dipteryx odorata* seeds results from physiological processes involving imbibition, metabolic activation, and hormonal regulation, dominated by the balance between abscisic acid and gibberellins, which control dormancy breaking, reserve mobilization, and radicle elongation, leading to seedling emergence (Bargah et al. 2025).

In this experiment, a much more noticeable effect was observed with high concentrations of 30 nM for the best gain in size, plant diameter, leaf production, and chlorophyll production; and 40 nM for the best plant growth rate. Limiting effects on growth and chlorophyll production were observed for this species, with a sensitivity window around 20 nM of 24-EBL. This was manifested by plants with less developed stem diameter, shorter heights, narrower leaves and chlorosis.

### 4.1 Effects of treatment on the morphological characteristics of *Dipteryx odorata* plants

In this study, significant morphological responses were observed in *D. odorata* plants 60 days after hormone application. This suggests slightly later significant morphological responses (between 30 and 45 days) in the species. Kumar et al. (2024) and Yuan et al. (2017), respectively, observed similar results when evaluating the physio-morphological differences in responses related to the application of 24-EBL after 7 to 28 days in corn (*Zea mays* L.) and 15 days in foxtail millet (*Setaria italica* L.). The impact of the hormone would therefore be conditioned by parameters such as dose, method of application (foliar, root, or injectable), and stage of plant development, which play an important role in the timing of morphological changes.

In *D. odorata*, increased size, leaf production, and diameter were observed in plants treated with 30 nM of the hormone. Yuan et al. (2017) showed that foliar application of 24-EBL can increase plant height. Plants treated with 40 nM showed the best growth rate. This concurs with the positive effect of 24-EBL on improving the morphological parameters of *D. odorata*. Similar studies conducted on *Ananas comosus* also showed that brassinosteroids increase the length, diameter, number of leaves, fresh and dry weight of the aerial parts of plants (Freitas 2010). In addition, brassinosteroids are known to increase resistance to biotic and abiotic stresses, such as pathogens, extreme temperatures, saline soils, water scarcity, metal action, and nutritional deficiencies (Krishna et al. 2010; Bajguz et al. 2016). These results highlight the importance of using 24-EBL to improve the biological parameters of young *D. odorata* plants for qualitative production with improved and beneficial growth yields, especially at optimal seasons to facilitate reforestation processes.

Plants treated with 20 nM of the hormone exhibited inhibitory effects characterized by slow growth in terms of diameter, size, and number of leaves. This highlights the existence of a sensitivity window around which 24-EBL would likely limit the morphological development parameters of *D. odorata*. Jiroutová et al. (2019) described a similar result with a species from the same family, *Pisum sativum*, where inhibitory effects of brassinosteroids on etiolated pea seedlings are mediated by an increase in ethylene production, and the minimum concentration of 24-EBL required to induce significant effects on ethylene production was approximately 20 nM.

Brassinosteroids do not act alone, but interact with other endogenous signaling molecules, especially the phytohormones auxins, cytokinins, gibberellins, abscisic acid, ethylene, jasmonates, salicylic acid and strigolactones, forming complex signaling networks that modulate plant growth and development (Guo et al. 2024). They influence auxin signaling by modulating its polar transport, thus impacting processes such as lateral root formation (Nemhauser et al. 2004). Studies also indicate that brassinosteroids and gibberellins interact in transcription factors, jointly influencing cell elongation and plant growth (Zhang et al. 2009). For *D. odorata*, the application of 30nM and 40 nM of the hormone appeared to have increased cell sensitivity to auxin and then promoted the effect of gibberellins. Therefore, these stimulated cell elongation and vascular differentiation, allowing the stem to grow in height and diameter. These hormones also seemed to have promoted phyllotaxy and then cell elongation, allowing for better-developed leaves.

Interactions between brassinosteroids and abscisic acid are also noteworthy, as abscisic acid can inhibit the brassinosteroid signaling pathway, suggesting cross-regulation between these hormones (Goda et al. 2008). Brassinosteroids can also regulate ethylene biosynthesis, affecting phenomena such as fruit ripening (Hansen et al. 2009). Arteca et al. (1983) and Yi et al. (1999) demonstrated that brassinosteroids induce ethylene production, either alone or in synergy with other phytohormones, in etiolated mung bean seedlings (*Vigna radiata*). In addition, ethylene is recognized as a cytokinin antagonist hormone (Stoynova-Bakalova et al. 2022), which aids in cell division and chloroplast formation, promoting the formation of young leaves and improving leaf surface area. This antagonistic effect was manifested in this experiment by slight chlorosis observed in the leaves of treated plants, being more severe at a dose of 20 nM when compared to the control, which would explain the inhibitory effect observed in those plants.

### 4.2 Effects of treatment on the metabolic characteristics of *Dipteryx odorata* plants

Chlorophyll and nitrogen were two highly correlated metabolic variables and also the most important ones identified in this study. In addition, a decrease in leaf temperature was observed during the third and fourth months. Since chlorophyll measurement directly assesses photosynthesis by a plant and chlorophyll is produced from nitrogen and water (humidity) in the same plant, it is likely that 24-EBL may contribute to this strong correlation (Yu et al. 2023). By measuring the chlorophyll produced, we found that different concentrations of 24-EBL applied influenced photosynthesis in different ways. Wang et al. (2015) showed that exogenous application of 24-EBL increased chlorophyll content in grapevine (*Vitis vinifera*) seedlings, as well as chlorophyll content, effective photochemical quantum yield of the Photosystem II (PSII) of chloroplast's thylakoid membranes, maximum photochemical efficiency of PSII, maximum fluorescence, and non-photochemical quenching coefficient under water stress at each concentration.

The 30 nM dose showed the best chlorophyll production in tonka beans seedlings, thus indirectly indicating the best photosynthesis achieved in the experiment, which would correspond to adequate nitrogen use. Akram et al. (2014) showed that *Jasminum sambac* plants treated with 3µM of 24-EBL showed better results for growth indices, such as plant height, number of branches per plant, fresh weight, and dry weight of the flower. Similar studies have shown that brassinosteroids promote growth and increase the nitrogen content of *Ananas comosus* seedling shoots. Yu et al. (2023) also showed that exogenous application of 24-EBL, by increasing photosynthesis, the activities of carbon (C) and nitrogen (N) assimilation enzymes, nitrate absorption and transport, and the synchronized optimization of C and N distribution in seedlings, playing a key role in improving nitrogen use efficiency in individuals. This explains the adequate growth observed in plants treated with 30 and 40 nM of hormone during this experiment.

In contrast, the 10 nM dose showed a slightly inhibitory effect after treatment, which was accentuated with the 20 nM dose by a considerable decrease in chlorophyll production, indicating possibly poor nitrogen utilization and metabolic stress. In pea seedlings, the minimum concentration of 24-EBL required to induce significant effects on ethylene production was approximately 20 nM (Jiroutová et al. 2019). Mechanistic evidence that brassinosteroids promote ethylene production was provided by Joo et al. (2006), who showed that 24-EBL induces expression of the auxin-responsive 1-aminocyclopropane-1-carboxylic acid (ACC) synthase gene AtACS4 in *Arabidopsis* sp. This suggests a significant ethylene production at hormone doses of 10 nM to 20 nM. Stoynova-Bakalova et al. (2022) showed that endogenous or applied ethylene represses the promoting influence of cytokinin on cell division and cotyledon expansion in etiolated seedlings of *Arabidopsis thaliana*, which affects plant growth. Thus, the effect of ethylene, as opposed to that of cytokinin on shoot emergence, leaf surface improvement, and chloroplast formation, reduces the plant's ability to develop photosynthesis, which explains the decrease in leaf chlorophyll.

In relation to the observed decrease in leaf temperature in all treatments, the highest rainfall occurred between months M1 and M4, and the lowest in months M0 and M5. For ambient temperature, the lowest averages were recorded between M1 and M3, while the highest occurred in M0 and M5. Perhaps more importantly, the historical average of daily sun hours is the lowest in March and April, corresponding to the experimental months M3 and M4, with March seeing the higher number of rainy days. The combination of heavy rainfall, broader cloud coverage and lower temperatures during M3 and M4 may explain the reduction in plant leaf temperature observed in this experiment.

### 4.3 Complementarity between morphological and metabolic variables in *Dipteryx odorata* plants

The use of 24-EBL to improve the growth and development of *D. odorata* plants influenced and then created strong inter-variable correlations in both categories of variables. As the experiment was conducted in a semi-controlled environment with 50% shading and regular irrigation, the effect of the treatment was freely demonstrated through the data collected and then by the results obtained after analysis.

The results showed that, at various doses, 24-EBL influences the biological parameters of *D. odorata* plants in different ways. Starting with temperature, which was not correlated with any other variable in the experiment but varied according to the environmental conditions, we found that it did not influence the growth of the different plant groups in the experiment. De Oliveira and Lameira (2017) showed that normal water conditions associated with 80% light stress affected stomatal conductance in the species, which can limit carbon dioxide ($CO_2$) absorption and reduce photosynthesis. Thus, the transpiration rates of *D. odorata* plants would be affected, which could reduce growth by affecting the plants' biological responses. Coll et al. (2015) found that brassinosteroids are plant steroid compounds involved in many functions related to plant development, metabolism, signaling, and defense against a wide range of biotic and abiotic stresses. Therefore, we suggest that the application of 24-EBL likely improves the defense of *D. odorata* plants against stress caused by environmental temperatures. Under 50% shade, combined with an appropriate dose of hormone, the stomata may open to capture as much light and CO2 as possible for photosynthesis, albeit generating a significant loss of water through transpiration due to the opening of the stomata which would require compensation through regular watering in order to ensure greater productivity.

This experiment suggested that applications of 30 and 40 nM of 24-EBL improved interactions between different endogenous hormones in *D. odorata* plants. The results may reflect that the combined action of auxin, cytokinin, and gibberellins could allow for better responses in morphological parameters, including faster growth, increased stem diameter, and a greater number of leaves. In addition, this combination could have contributed to the regulation of abscisic acid and ethylene, reducing physiological stress and resulting in improvements in metabolic parameters, such as greater nitrogen absorption and increased chlorophyll production. Aryal & Alferez (2025) showed that brassinosteroids interact with other hormones, such as auxins, gibberellins, ethylene, and abscisic acid, influencing all aspects of plant growth and development, acting on seed germination, vegetative and reproductive growth, photosynthetic efficiency, vascular differentiation, fruit yield and quality, as well as resilience to biotic and abiotic stresses. The 20 nM dose of 24-EBL showed an increased effect of morphological and metabolic stress, suggesting a possible hormonal imbalance associated with significant ethylene production.

*D. odorata* plants treated with different concentrations of 24-EBL under 50% shading showed adequate growth in height, diameter, number of leaves, and chlorophyll production with doses of 30 nM and 40 nM. The 40 nM dose provided the best growth rate. It was observed that high concentrations (40 nM and 30 nM) improved the morphological and metabolic parameters of *D. odorata* plants, while the 20 nM concentration triggered inhibitory effects that may characterize significant ethylene production.

This study showed the importance of using 24-EBL to improve the biological parameters of young *D. odorata* plants for the qualitative production of young plants with improved and beneficial growth yields, especially at better times to facilitate reforestation processes. Further research may elucidate the mechanisms behind inhibitory effects of 20 nM doses of 24-EBL in this species, and help to ascertain an optimal dosage for seedling production.

## Statements and Declarations

The authors certify that they have no affiliations or involvement with any organization or entity that has financial or non-financial interests in the subject matter or materials discussed in this manuscript.

## Author Contribution Statement

JS: Funding acquisition; Investigation; Resources; Writing – original draft.

KEvS: Data curation; Formal analysis; Methodology; Validation; Visualization; Writing – original draft; Writing – review & editing.

EHR: Funding acquisition; Project administration; Resources; Supervision.

## References

Akram A, Khan MA, Younis A, Ashfaq M (2014) Exogenous application of 24-epibrassinolide on morphophysiological, biochemical attributes and essential oil contents of *Jasminum sambac* L. Pak J Agric Res 51:881–886

Alvares CA, Stape JL, Sentelhas PC, et al (2013) Köppen's climate classification map for Brazil. Meteorol Z 22:711–728. https://doi.org/10.1127/0941-2948/2013/0507

Arteca RN, Tsai D, Schlagnhaufer C, Mandava NB (1983) The effect of brassinosteroid on auxin-induced ethylene production by etiolated mung bean segments. Physiol Plant 59:539–544. https://doi.org/10.1111/j.1399-3054.1983.tb06277.x

Aryal D, Alferez F (2025) Brassinosteroids: Biosynthesis, Signaling, and Hormonal Crosstalk as Related to Fruit Yield and Quality. Plants 14:1865. https://doi.org/10.3390/plants14121865

Bajguz I, Talarek M, Rajewska A (2016) Brassinosteroids and Response of Plants to Heavy Metals Action. Front Plant Sci 7: 629. https://doi.org/10.3389/fpls.2016.00629

Bargah AS, Sharma D, Kumar R, et al (2025) Enhancing Germination of Forest Tree Seeds in Chhattisgarh through PGR-Based Treatments: A Review. J Adv Biol Biotechnol 28:851–863. https://doi.org/10.9734/jabb/2025/v28i72603

Barton K (2025) *MuMIn*: Multi-Model Inference v1.48.11. https://CRAN.R-project.org/package=MuMIn. Accessed 25 June 2025

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using *lme4*. J Stat Softw 67:1–48. https://doi.org/10.18637/jss.v067.i01

Bonfim SA, Casabianca H, Oliveira AH (2025) Isotopic Characterization of Cumaru Essential Oil (*Dipteryx odorata*) for Quality and Authenticity Validation. Braz J Radiat Sci 13:e2837. https://doi.org/10.15392/2319-0612.2025.2837

Breusch TS, Pagan AR (1979) A Simple Test for Heteroscedasticity and Random Coefficient Variation. Econometrica 47:1287. https://doi.org/10.2307/1911963

Coll Y, Coll F, Amorós A, Pujol M (2015) Brassinosteroids roles and applications: an up-date. Biologia (Bratisl) 70:726–732. https://doi.org/10.1515/biolog-2015-0085

Coradin L, Camillo J, Vieira ICG (eds) (2022) Espécies Nativas da Flora Brasileira de Valor Econômico Atual ou Potencial - Plantas para o Futuro Região Norte. MMA - Ministério do Meio Ambiente, Brasília

Cruz, ED, Barros HSD (2021) Germinação de sementes de espécies amazônicas: cumaru [*Dipteryx odorata* (Aubl.) Willd.]. Embrapa, Belém

De Oliveira PC, Lameira HLN (2017) Physiological behavior of *Annona muricata*, *Dipteryx odorata* and *Copaifera langsdorffii* in response to water and light stress. J Med Plants Stud 5:193–199

Do Nascimento LD, Cascaes MM, Cruz ED, Andrade EH de A (2022) *Dipteryx odorata* - Cumaru. In: Espécies Nativas da Flora Brasileira de Valor Econômico Atual ou Potencial - Plantas para o Futuro - Região Norte. Brasília, pp 615–627

Freitas SDJ (2010) Brassinosteróides e Adubação no Desenvolvimento, Crescimento e Nutrição de Mudas de Abacaxizeiro. PhD Thesis, Universidade Estadual Do Norte Fluminense Darcy Ribeiro

Goda H, Sasaki E, Akiyama K, et al (2008) The AtGenExpress hormone- and chemical-treatment data set: Experimental design, data evaluation, model data analysis, and data access. Plant J 0:080414150319983. https://doi.org/10.1111/j.0960-7412.2008.03510.x

Guimarães Z, Da Silva D, Ferreira M (2024) Seedling quality and short-term field performance of three Amazonian forest species as affected by site conditions. IForest - Biogeosciences For 17:80–89. https://doi.org/10.3832/ifor4317-016

Guo F, Lv M, Zhang J, Li J (2024) Crosstalk between Brassinosteroids and Other Phytohormones during Plant Development and Stress Adaptation. Plant Cell Physiol 65:1530–1543. https://doi.org/10.1093/pcp/pcae047

Haase DL (2008) Understanding forest seedling quality: measurements and interpretation. Tree Plant Notes 52:24–30

Hansen M, Chae HS, Kieber JJ (2009) Regulation of ACS protein stability by cytokinin and brassinosteroid. Plant J 57:606–614. https://doi.org/10.1111/j.1365-313X.2008.03711.x

Holm JA, Chambers JQ, Collins WD, Higuchi N (2014) Forest response to increased disturbance in the central Amazon and comparison to western Amazonian forests. Biogeosciences 11:5773–5794. https://doi.org/10.5194/bg-11-5773-2014

Holm S (1979) A Simple Sequentially Rejective Multiple Test Procedure. Scand J Stat 6:65–70

IUCN (2021) Science-based ecosystem restoration for the 2020s and beyond. IUCN, Gland

Jaquetti RK, Gonçalves JFC (2017) Carbon and nutrient stocks of three Fabaceae trees used for forest restoration and subjected to fertilization in Amazonia. An Acad Bras Ciênc 89:1761–1771. https://doi.org/10.1590/0001-3765201720160734

Jiroutová P, Mikulík J, Novák O, et al (2019) Brassinosteroids Induce Strong, Dose-Dependent Inhibition of Etiolated Pea Seedling Growth Correlated with Ethylene Production. Biomolecules 9:849. https://doi.org/10.3390/biom9120849

Joo S, Seo YS, Kim SM, et al (2006) Brassinosteroid induction of AtACS4 encoding an auxin-responsive 1-aminocyclopropane-1-carboxylate synthase 4 in Arabidopsis seedlings. Physiol Plant 126:592–604. https://doi.org/10.1111/j.1399-3054.2005.00602.x

Kassambara A, Mundt F (2020) *factoextra*: Extract and Visualize the Results of Multivariate Data Analyses v 1.0.7. https://CRAN.R-project.org/package=factoextra. Accessed 24 June 2025

Khan I, Awan SA, Ikram R, et al (2021) Effects of 24-epibrassinolide on plant growth, antioxidants defense system, and endogenous hormones in two wheat varieties under drought stress. Physiol Plant 172:696–706. https://doi.org/10.1111/ppl.13237

Krishna UK, Rahman T, divi P (2010) Brassinosteroid-mediated stress tolerance in Arabidopsis shows interactions with abscisic acid, ethylene and salicylic acid pathways. BMC Plant Biol 10:151. https://doi.org/10.1186/1471-2229-10-151

Kumar B, Pal M, Yadava P, et al (2024) Physiological and biochemical effects of 24-Epibrassinolide on drought stress adaptation in maize (*Zea mays* L.). PeerJ 12:e17190. https://doi.org/10.7717/peerj.17190

Kuznetsova A, Brockhoff PB, Christensen RHB (2017) *lmerTest* Package: Tests in Linear Mixed Effects Models. J Stat Softw 82:1–26. https://doi.org/10.18637/jss.v082.i13

Lenth R (2024) *emmeans*: Estimated Marginal Means, aka Least-Squares Means v1.4.8. https://CRAN.R-project.org/package=emmeans. Accessed 29 August 2024

Lorenzi H (2002) Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil, 2a ed. Instituto Plantarum de Estudos da Flora, Nova Odessa

Lüdecke D, Ben-Shachar M, Patil I, et al (2021) *performance*: An R Package for Assessment, Comparison and Testing of Statistical Models. J Open Source Softw 6:3139. https://doi.org/10.21105/joss.03139

Machado MR, Camara R, Sampaio PDTB, et al (2018) Silvicultural performance of five forest species in the central Brazilian Amazon. Acta Amaz 48:10–17. https://doi.org/10.1590/1809-4392201700602

Martins Santos T, Shiela Silva Lisboa L, Souza Santos I, et al (2023) Áreas potenciais à silvicultura do Cumaru (Dipteryx odorata (Aubl.) Willd.) em polos bioeconômicos estratégicos no estado do Pará. Peer Rev 5:310–340. https://doi.org/10.53660/1062.prw2618

Mohammadi M, Tavakoli A, Pouryousef M, Mohseni Fard E (2020) Study the effect of 24-epibrassinolide application on the Cu/Zn-SOD expression and tolerance to drought stress in common bean. Physiol Mol Biol Plants Int J Funct Plant Biol 26:459–474. https://doi.org/10.1007/s12298-020-00757-7

Mota CGD, Pauletto D, Capucho HLV, et al (2022) O cultivo do cumaru como alternativa econômica para agricultores familiares: estudo de caso na região oeste do Pará. Res Soc Dev 11:e46511326732. https://doi.org/10.33448/rsd-v11i3.26732

Nemhauser JL, Mockler TC, Chory J (2004) Interdependency of Brassinosteroid and Auxin Signaling in *Arabidopsis*. PLoS Biol 2:e258. https://doi.org/10.1371/journal.pbio.0020258

Nordstokke DW, Zumbo BD, Cairns SL, Saklofske DH (2011) The operating characteristics of the nonparametric Levene test for equal variances with assessment and evaluation data. Pract Assess Res Eval 16:1–8. https://doi.org/10.7275/5t99-zv93

Pedersen TL (2024) *patchwork*: The Composer of Plots v1.2.0. https://CRAN.R-project.org/package=patchwork. Accessed 01 July 2024

Pinto AM, Morellato LPC, Barbosa AP (2008) Fenologia reprodutiva de *Dipteryx odorata* (Aubl.) Willd (Fabaceae) em duas áreas de floresta na Amazônia Central. Acta Amaz 38:643–649. https://doi.org/10.1590/S0044-59672008000400006

R Core Team (2025) R: A Language and Environment for Statistical Computing v4.5.1. https://www.r-project.org/. Accessed 4 October 2025

RStudio Team (2025) RStudio: Integrated Development Environment for R v2025.05.1-513.https://posit.co/products/open-source/rstudio/?sid=1. Accessed 4 October 2025

Silva PMFD, Silva EO, Rêgo MDSC, et al (2019) Anatomical and histochemical characterization of *Dipteryx odorata* and *Taralea oppositifolia*, two native Amazonian species. Rev Bras Farmacogn 29:425–433. https://doi.org/10.1016/j.bjp.2019.05.004

Stoynova-Bakalova E, Bakalov DV, Baskin TI (2022) Ethylene represses the promoting influence of cytokinin on cell division and expansion of cotyledons in etiolated *Arabidopsis thaliana* seedlings. PeerJ 10:e14315. https://doi.org/10.7717/peerj.14315

Tukey JW (1949) Comparing Individual Means in the Analysis of Variance. Biometrics 5:99. https://doi.org/10.2307/3001913

Van Praet S, Rizza A, Tang B, et al (2025) Coumarin Promotes Hypocotyl Elongation in Light-grown *Arabidopsis thaliana* Seedlings by Enhancing Brassinosteroid Signalling in an Auxin-Dependent Manner. J Plant Growth Regul 44:3290–3303. https://doi.org/10.1007/s00344-024-11617-z

Wang W, Bai M-Y, Wang Z-Y (2014) The brassinosteroid signaling network — a paradigm of signal integration. Curr Opin Plant Biol 21:147–153. https://doi.org/10.1016/j.pbi.2014.07.012

Wang Z, Zheng P, Meng J, Xi Z (2015) Effect of exogenous 24-epibrassinolide on chlorophyll fluorescence, leaf surface morphology and cellular ultrastructure of grape seedlings (*Vitis vinifera* L.) under water stress. Acta Physiol Plant 37:1729. https://doi.org/10.1007/s11738-014-1729-z

Wickham H (2016) *ggplot2*: Elegant Graphics for Data Analysis, 2nd edn. Springer International Publishing, New York

Wickham H, Averick M, Bryan J, et al (2019) Welcome to the *Tidyverse*. J Open Source Softw 4:1686. https://doi.org/10.21105/joss.01686

Yi HC, Joo S, Nam KH, et al (1999) Auxin and brassinosteroid differentially regulate the expression of three members of the 1-aminocyclopropane-1-carboxylate synthase gene family in mung bean (*Vigna radiata* L.). Plant Mol Biol 41:443–454. https://doi.org/10.1023/A:1006372612574

Yu B, Wang L, Guan Q, et al (2023) Exogenous 24-epibrassinolide promoted growth and nitrogen absorption and assimilation efficiency of apple seedlings under salt stress. Front Plant Sci 14:1178085. https://doi.org/10.3389/fpls.2023.1178085

Yuan X-Y, Zhang L-G, Huang L, et al (2017) Spraying Brassinolide improves Sigma Broad tolerance in foxtail millet (*Setaria italica* L.) through modulation of antioxidant activity and photosynthetic capacity. Sci Rep 7:11232. https://doi.org/10.1038/s41598-017-11867-w

Zhang S, Wei Y, Lu Y, Wang X (2009) Mechanisms of brassinosteroids interacting with multiple hormones. Plant Signal Behav 4:1117–1120. https://doi.org/10.4161/psb.4.12.9903

## CONCLUSÃO GERAL

Plantas de *D. odorata* tratadas com diferentes concentrações de 24-EBL sob sombreamento de 50% apresentaram bom crescimento em altura, diâmetro, número de folhas e produção da clorofila com a dose de 30 nM e 40 nM. A dose de 40 nM proporcionou a melhor taxa de crescimento. Foi observado que altas concentrações (40 e 30 nM) melhoraram os parâmetros morfológicos e metabólicos das plantas de *D. odorata,* enquanto a concentração de 20 nM desencadeou efeitos inibidores que podem caracterizar uma produção significativa de etileno.

Este estudo mostrou a importância da utilização do 24-EBL para a melhoria dos parâmetros biológicos de mudas jovens de *D. odorata*, visando à produção qualitativa de mudas com ganhos significativos no crescimento e efeitos benéficos, especialmente quando aplicado em períodos mais favoráveis, de modo a facilitar os processos de reflorestamento, garantindo a conservação da espécie e seu papel na manutenção do equilíbrio das interações entre espécies no âmbito da biodiversidade.

# Supplementary Materials: Effects of 24-epibrassinolide application on the development of *Dipteryx odorata*: between growth stimulation and inhibitory effects

Jospin Soglohoun[1], Karin Elisabeth von Schmalz[2]*, Emil Hernández Ruz[1,2]

[1]Laboratório de Zoologia, Universidade Federal do Pará, Faculdade de Ciências Biológicas, Campus de Altamira. Rua Cel. José Porfírio - Esplanada do Xingu, 68371-155, Altamira, Pará, Brasil.
[2] Pós-Graduação em Biodiversidade e Biotecnologia da Rede Bionorte, Universidade Federal do Pará, Faculdade de Ciências Biológicas, Campus de Altamira. Rua Cel. José Porfírio, 2515, Área 2, CEP 68372-040. Altamira, Pará, Brazil
*Corresponding author: karin.schmalz@gmail.com

## Supplementary Tables

**Table S1** Results of multivariate analysis of variance (MANOVA) with Pillai's test, and summary of analysis of variance (ANOVA) of the two main components of a principal component analysis (PCA) of 115 *Dipteryx odorata* plants subjected to five different treatments (t0 = control) with the phytohormone 24-EBL. Df = degrees of freedom; F = F statistic value; num Df = numerator degrees of freedom; den Df = denominator degrees of freedom; Sum Sq = sum of squares; Mean Sq = mean of squares.

**MANOVA Summary – Principal Component Analysis (PCA)**

| | Df | Pillai | *F* | num Df | den Df | *p* |
|---|---|---|---|---|---|---|
| Treatment | 4 | 0.5823 | 11.296 | 8 | 220 | <0.0001**** |
| Residuals | 110 | | | | | |

**ANOVA Summary – PC1**

| | Df | Sum Sq | Mean Sq | *F* | *p* |
|---|---|---|---|---|---|
| Treatment | 4 | 157.0 | 39.25 | 20.78 | <0.0001**** |
| Residuals | 110 | 207.8 | 1.89 | | |

**ANOVA Summary – PC2**

| | Df | Sum Sq | Mean Sq | *F* | *p* |
|---|---|---|---|---|---|
| Treatment | 4 | 39.14 | 9.786 | 4.927 | 0.0011*** |
| Residuals | 110 | 218.46 | 1.986 | | |

Significance levels of *p* values: * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001; **** ≤ 0.0001.

**Table S2** Multiple comparisons of paired means (Tukey HSD) from analyses of variance (ANOVA), with a 95% confidence interval, of the two main components of a principal component analysis (PCA) of 115 *Dipteryx odorata* plants subjected to five different treatments (t0 = control) with the phytohormone 24-EBL. Difference = difference between means; Lower CI = lower confidence interval limit; Upper CI = upper confidence interval limit; *p* (adj.) = adjusted *p* values.

**Tukey HSD of ANOVA – PC1**

| Pairs | Difference | Lower CI | Upper CI | *p* (adj.) |
|---|---|---|---|---|
| t1-t0 | 1.7309 | 0.6068 | 2.8550 | 0.0004*** |
| t2-t0 | 3.2762 | 2.1522 | 4.4003 | <0.0001**** |
| t3-t0 | 0.6242 | -0.4999 | 1.7483 | 0.5389 |
| t4-t0 | 0.5173 | -0.6068 | 1.6414 | 0.7062 |
| t2-t1 | 1.5453 | 0.4212 | 2.6694 | 0.0021** |
| t3-t1 | -1.1067 | -2.2308 | 0.0173 | 0.0558 |
| t4-t1 | -1.2137 | -2.3377 | -0.0896 | 0.0275* |
| t3-t2 | -2.6521 | -3.7761 | -1.5280 | <0.0001**** |
| t4-t2 | -2.7590 | -3.8830 | -1.6349 | <0.0001**** |
| t4-t3 | -0.1069 | -1.2310 | 1.0172 | 0.9989 |

**Tukey HSD of ANOVA – PC2**

| Pairs | Difference | Lower | Upper | *p* (adj.) |
|---|---|---|---|---|
| t1-t0 | 1.3589 | 0.2063 | 2.5115 | 0.0123* |
| t2-t0 | 0.5018 | -0.6508 | 1.6544 | 0.7471 |
| t3-t0 | 1.5744 | 0.4218 | 2.7270 | 0.0023** |
| t4-t0 | 1.1644 | 0.0118 | 2.3170 | 0.0464* |
| t2-t1 | -0.8571 | -2.0097 | 0.2955 | 0.2439 |
| t3-t1 | 0.2155 | -0.9371 | 1.3681 | 0.9853 |
| t4-t1 | -0.1945 | -1.3471 | 0.9581 | 0.9900 |
| t3-t2 | 1.0726 | -0.0800 | 2.2252 | 0.0809 |
| t4-t2 | 0.6626 | -0.4900 | 1.8152 | 0.5041 |

|  |  |  |  |  |
|---|---|---|---|---|
| t4-t3 | -0.4100 | -1.5626 | 0.7426 | 0.8608 |

Significance levels of *p* (adj.) values: * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001; **** ≤ 0.0001.

**Table S3** Random and fixed effects estimated from the linear mixed effects model (REML) testing the effects of five experimental treatments (t0 = control) with 24-EBL and its interaction with time on the logarithm-transformed growth rate (log height/diameter ratio), using repeated measurements of 115 *Dipteryx odorata* plants in six monthly observations (random intercepts by plant ID). SD = standard deviation; SE = standard error; DF = degrees of freedom.

<table>
<tr><th colspan="3">Random effects</th><th>Formula: ~1 | ID</th></tr>
<tr><td></td><td>(Intercept)</td><td>Residual</td><td></td></tr>
<tr><td>SD</td><td>0.1648</td><td>0.1269</td><td></td></tr>
</table>

**Fixed effects: log(height/diameter ratio) ~ Treatment * Time**

| Term | Value | SE | DF | t | *p* |
|---|---|---|---|---|---|
| (Intercept) | 3.5614 | 0.0434 | 550 | 82.1171 | <0.0001**** |
| Treatment t1 | 0.0252 | 0.0613 | 110 | 0.4103 | 0.6824 |
| Treatment t2 | 0.0695 | 0.0613 | 110 | 1.1336 | 0.2594 |
| Treatment t3 | 0.0925 | 0.0613 | 110 | 1.5089 | 0.1342 |
| Treatment t4 | 0.1027 | 0.0613 | 110 | 1.6742 | 0.0969 |
| Time M1 | 0.1128 | 0.0374 | 550 | 3.0126 | 0.0027** |
| Time M2 | 0.1656 | 0.0374 | 550 | 4.4238 | <0.0001**** |
| Time M3 | 0.2259 | 0.0374 | 550 | 6.0332 | <0.0001**** |
| Time M4 | 0.3196 | 0.0374 | 550 | 8.5387 | <0.0001**** |
| Time M5 | 0.4438 | 0.0374 | 550 | 11.8560 | <0.0001**** |
| Treatment t1: Time M1 | -0.0357 | 0.0529 | 550 | -0.6746 | 0.5002 |
| Treatment t2: Time M1 | -0.0934 | 0.0529 | 550 | -1.7647 | 0.0782 |
| Treatment t3: Time M1 | -0.0134 | 0.0529 | 550 | -0.2528 | 0.8005 |
| Treatment t4: Time M1 | 0.0148 | 0.0529 | 550 | 0.2805 | 0.7792 |
| Treatment t1: Time M2 | -0.0512 | 0.0529 | 550 | -0.9672 | 0.3339 |
| Treatment t2: Time M2 | -0.2058 | 0.0529 | 550 | -3.8865 | 0.0001**** |

| | | | | | |
|---|---|---|---|---|---|
| Treatment t3: Time M2 | -0.0280 | 0.0529 | 550 | -0.5294 | 0.5967 |
| Treatment t4: Time M2 | -0.0484 | 0.0529 | 550 | -0.9152 | 0.3605 |
| Treatment t1: Time M3 | 0.0010 | 0.0529 | 550 | 0.0180 | 0.9856 |
| Treatment t2: Time M3 | -0.2336 | 0.0529 | 550 | -4.4125 | <0.0001**** |
| Treatment t3: Time M3 | -0.0088 | 0.0529 | 550 | -0.1669 | 0.8675 |
| Treatment t4: Time M3 | -0.0223 | 0.0529 | 550 | -0.4220 | 0.6732 |
| Treatment t1: Time M4 | -0.0149 | 0.0529 | 550 | -0.2814 | 0.7785 |
| Treatment t2: Time M4 | -0.2983 | 0.0529 | 550 | -5.6351 | <0.0001**** |
| Treatment t3: Time M4 | 0.0147 | 0.0529 | 550 | 0.2786 | 0.7807 |
| Treatment t4: Time M4 | 0.0850 | 0.0529 | 550 | 1.6063 | 0.1088 |
| Treatment t1: Time M5 | -0.0446 | 0.0529 | 550 | -0.8419 | 0.4002 |
| Treatment t2: Time M5 | -0.3316 | 0.0529 | 550 | -6.2636 | <0.0001**** |
| Treatment t3: Time M5 | -0.0231 | 0.0529 | 550 | -0.4365 | 0.6626 |
| Treatment t4: Time M5 | 0.0097 | 0.0529 | 550 | 0.1834 | 0.8545 |

Significance levels of *p* values: * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001; **** ≤ 0.0001.

**Table S4** Estimated marginal means, with standard errors and lower and upper confidence intervals (95% CI) from the linear mixed effects model (REML) testing the effects of five experimental treatments (t0 = control) with 24-EBL over time on the logarithm-transformed growth rate (log height/diameter ratio), using repeated measurements of 115 *Dipteryx odorata* plants in six monthly observations (random intercepts by plant ID). EMM = estimated marginal mean; SE = standard error; DF = degrees of freedom; Lower CI = lower limit of confidence interval; Upper CI = upper limit of confidence interval.

| **Time** | **Treatment** | **EMM** | **SE** | **DF** | **Lower CI** | **Upper CI** |
|---|---|---|---|---|---|---|
| 0 | t0 | 3.56 | 0.0434 | 114 | 3.48 | 3.65 |
| 0 | t1 | 3.59 | 0.0434 | 110 | 3.50 | 3.67 |
| 0 | t2 | 3.63 | 0.0434 | 110 | 3.54 | 3.72 |
| 0 | t3 | 3.65 | 0.0434 | 110 | 3.57 | 3.74 |
| 0 | t4 | 3.66 | 0.0434 | 110 | 3.58 | 3.75 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | t0 | 3.67 | 0.0434 | 114 | 3.59 | 3.76 |
| 1 | t1 | 3.66 | 0.0434 | 110 | 3.58 | 3.75 |
| 1 | t2 | 3.65 | 0.0434 | 110 | 3.56 | 3.74 |
| 1 | t3 | 3.75 | 0.0434 | 110 | 3.67 | 3.84 |
| 1 | t4 | 3.79 | 0.0434 | 110 | 3.71 | 3.88 |
| 2 | t0 | 3.73 | 0.0434 | 114 | 3.64 | 3.81 |
| 2 | t1 | 3.70 | 0.0434 | 110 | 3.61 | 3.79 |
| 2 | t2 | 3.59 | 0.0434 | 110 | 3.50 | 3.68 |
| 2 | t3 | 3.79 | 0.0434 | 110 | 3.71 | 3.88 |
| 2 | t4 | 3.78 | 0.0434 | 110 | 3.70 | 3.87 |
| 3 | t0 | 3.79 | 0.0434 | 114 | 3.70 | 3.87 |
| 3 | t1 | 3.81 | 0.0434 | 110 | 3.73 | 3.90 |
| 3 | t2 | 3.62 | 0.0434 | 110 | 3.54 | 3.71 |
| 3 | t3 | 3.87 | 0.0434 | 110 | 3.78 | 3.96 |
| 3 | t4 | 3.87 | 0.0434 | 110 | 3.78 | 3.95 |
| 4 | t0 | 3.88 | 0.0434 | 114 | 3.80 | 3.97 |
| 4 | t1 | 3.89 | 0.0434 | 110 | 3.81 | 3.98 |
| 4 | t2 | 3.65 | 0.0434 | 110 | 3.57 | 3.74 |
| 4 | t3 | 3.99 | 0.0434 | 110 | 3.90 | 4.07 |
| 4 | t4 | 4.07 | 0.0434 | 110 | 3.98 | 4.15 |
| 5 | t0 | 4.01 | 0.0434 | 114 | 3.92 | 4.09 |
| 5 | t1 | 3.99 | 0.0434 | 110 | 3.90 | 4.07 |
| 5 | t2 | 3.74 | 0.0434 | 110 | 3.66 | 3.83 |
| 5 | t3 | 4.07 | 0.0434 | 110 | 3.99 | 4.16 |
| 5 | t4 | 4.12 | 0.0434 | 110 | 4.03 | 4.20 |

**Table S5** Paired contrasts across time (adjusted by Tukey) from the linear mixed effects model (REML) testing the effects of five experimental treatments (t0 = control) with 24-EBL on the logarithm-transformed growth rate (log height/diameter ratio), using repeated measurements of 115 *Dipteryx odorata* plants in six monthly observations (random intercepts by plant ID). Negative estimates indicate lower values for the first treatment in the comparison. For all contrasts, Standard error = 0.0613 and degrees of freedom = 110.

| **Time** | **Contrast** | **Estimate** | **t** | ***p* (adj.)** |
|---|---|---|---|---|
| M0 | t0 - t1 | -0.0252 | -0.410 | 0.9940 |
| M0 | t0 - t2 | -0.0695 | -1.134 | 0.7884 |
| M0 | t0 - t3 | -0.0925 | -1.509 | 0.5590 |
| M0 | t0 - t4 | -0.1027 | -1.674 | 0.4541 |
| M0 | t1 - t2 | -0.0444 | -0.723 | 0.9507 |
| M0 | t1 - t3 | -0.0674 | -1.099 | 0.8069 |
| M0 | t1 - t4 | -0.0775 | -1.264 | 0.7137 |
| M0 | t2 - t3 | -0.0230 | -0.375 | 0.9957 |
| M0 | t2 - t4 | -0.0332 | -0.541 | 0.9829 |
| M0 | t3 - t4 | -0.0101 | -0.165 | 0.9998 |
| M1 | t0 - t1 | 0.0106 | 0.172 | 0.9998 |
| M1 | t0 - t2 | 0.0239 | 0.390 | 0.9951 |
| M1 | t0 - t3 | -0.0792 | -1.291 | 0.6975 |
| M1 | t0 - t4 | -0.1175 | -1.916 | 0.3149 |
| M1 | t1 - t2 | 0.0134 | 0.218 | 0.9995 |
| M1 | t1 - t3 | -0.0897 | -1.463 | 0.5888 |
| M1 | t1 - t4 | -0.1281 | -2.088 | 0.2326 |
| M1 | t2 - t3 | -0.1031 | -1.680 | 0.4503 |
| M1 | t2 - t4 | -0.1414 | -2.306 | 0.1508 |
| M1 | t3 - t4 | -0.0384 | -0.626 | 0.9707 |
| M2 | t0 - t1 | 0.0260 | 0.425 | 0.9931 |

| | | | | |
|---|---|---|---|---|
| M2 | t0 - t2 | 0.1362 | 2.221 | 0.1797 |
| M2 | t0 - t3 | -0.0645 | -1.052 | 0.8304 |
| M2 | t0 - t4 | -0.0542 | -0.884 | 0.9022 |
| M2 | t1 - t2 | 0.1102 | 1.797 | 0.3808 |
| M2 | t1 - t3 | -0.0906 | -1.476 | 0.5799 |
| M2 | t1 - t4 | -0.0803 | -1.309 | 0.6864 |
| M2 | t2 - t3 | -0.2008 | -3.273 | 0.0122 |
| M2 | t2 - t4 | -0.1905 | -3.105 | 0.0200* |
| M2 | t3 - t4 | 0.0103 | 0.168 | 0.9998 |
| M3 | t0 - t1 | -0.0261 | -0.426 | 0.9930 |
| M3 | t0 - t2 | 0.1641 | 2.675 | 0.0643 |
| M3 | t0 - t3 | -0.0837 | -1.365 | 0.6513 |
| M3 | t0 - t4 | -0.0803 | -1.310 | 0.6857 |
| M3 | t1 - t2 | 0.1902 | 3.101 | 0.0203* |
| M3 | t1 - t3 | -0.0576 | -0.939 | 0.8811 |
| M3 | t1 - t4 | -0.0542 | -0.884 | 0.9022 |
| M3 | t2 - t3 | -0.2478 | -4.040 | 0.0009*** |
| M3 | t2 - t4 | -0.2444 | -3.985 | 0.0011** |
| M3 | t3 - t4 | 0.0034 | 0.055 | 1.0000 |
| M4 | t0 - t1 | -0.0103 | -0.167 | 0.9998 |
| M4 | t0 - t2 | 0.2288 | 3.730 | 0.0028** |
| M4 | t0 - t3 | -0.1073 | -1.749 | 0.4085 |
| M4 | t0 - t4 | -0.1877 | -3.061 | 0.0228* |
| M4 | t1 - t2 | 0.2391 | 3.898 | 0.0015** |
| M4 | t1 - t3 | -0.0970 | -1.582 | 0.5121 |
| M4 | t1 - t4 | -0.1775 | -2.893 | 0.0364* |
| M4 | t2 - t3 | -0.3361 | -5.480 | <0.0001**** |

| | | | | |
|---|---|---|---|---|
| M4 | t2 - t4 | -0.4165 | -6.791 | <0.0001**** |
| M4 | t3 - t4 | -0.0804 | -1.311 | 0.6848 |
| M5 | t0 - t1 | 0.0194 | 0.316 | 0.9978 |
| M5 | t0 - t2 | 0.2621 | 4.273 | 0.0004*** |
| M5 | t0 - t3 | -0.0694 | -1.132 | 0.7892 |
| M5 | t0 - t4 | -0.1124 | -1.832 | 0.3604 |
| M5 | t1 - t2 | 0.2427 | 3.956 | 0.0013* |
| M5 | t1 - t3 | -0.0888 | -1.449 | 0.5979 |
| M5 | t1 - t4 | -0.1318 | -2.149 | 0.2072 |
| M5 | t2 - t3 | -0.3315 | -5.405 | <0.0001**** |
| M5 | t2 - t4 | -0.3745 | -6.105 | <0.0001**** |
| M5 | t3 - t4 | -0.0430 | -0.700 | 0.9560 |

Significance levels of *p* values (adj.): * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001; **** ≤ 0.0001.

**Table S6** Random and fixed effects estimated from the linear mixed effects model (REML) testing the effects of five experimental treatments (t0 = control) with 24-EBL and its interaction with time on leaf SPAD chlorophyll level, using repeated measurements of 115 *Dipteryx odorata* plants in five monthly observations (random intercepts by plant ID). SD = standard deviation; SE = standard error; DF = degrees of freedom.

| **Random effects** | | | **Formula: ~1 \| ID** |
|---|---|---|---|
| | (Intercept) | Residual | |
| SD | 3.0360 | 5.5377 | |

**Fixed effects: Leaf chlorophyll (SPAD) ~ Treatment * Time**

| Term | Value | SE | DF | t | *p* |
|---|---|---|---|---|---|
| (Intercept) | 24.9478 | 1.3168 | 440 | 18.9453 | <0.0001**** |
| Treatment t1 | 1.4435 | 1.8623 | 110 | 0.7751 | 0.4399 |
| Treatment t2 | -2.4348 | 1.8623 | 110 | -1.3074 | 0.1938 |
| Treatment t3 | 0.3696 | 1.8623 | 110 | 0.1984 | 0.8431 |
| Treatment t4 | 0.1530 | 1.8623 | 110 | 0.0822 | 0.9347 |
| Time M2 | 0.0000 | 1.6330 | 440 | 0.0000 | 1.0000 |
| Time M3 | 1.8609 | 1.6330 | 440 | 1.1396 | 0.2551 |
| Time M4 | 3.9870 | 1.6330 | 440 | 2.4415 | 0.0150* |
| Time M5 | 12.1174 | 1.6330 | 440 | 7.4205 | <0.0001**** |
| Treatment t1: Time M2 | -1.4896 | 2.3094 | 440 | -0.6450 | 0.5193 |
| Treatment t2: Time M2 | -0.4826 | 2.3094 | 440 | -0.2090 | 0.8346 |
| Treatment t3: Time M2 | 0.6957 | 2.3094 | 440 | 0.3012 | 0.7634 |
| Treatment t4: Time M2 | -0.5313 | 2.3094 | 440 | -0.2301 | 0.8181 |
| Treatment t1: Time M3 | -2.9826 | 2.3094 | 440 | -1.2915 | 0.1972 |
| Treatment t2: Time M3 | -4.5261 | 2.3094 | 440 | -1.9599 | 0.0506 |
| Treatment t3: Time M3 | 0.9152 | 2.3094 | 440 | 0.3963 | 0.6921 |
| Treatment t4: Time M3 | -0.0878 | 2.3094 | 440 | -0.03803 | 0.9697 |

| | | | | | |
|---|---|---|---|---|---|
| Treatment t1: Time M4 | -2.7000 | 2.3094 | 440 | -1.1692 | 0.2430 |
| Treatment t2: Time M4 | -7.5478 | 2.3094 | 440 | -3.2684 | 0.0012** |
| Treatment t3: Time M4 | 1.3913 | 2.3094 | 440 | 0.6025 | 0.5472 |
| Treatment t4: Time M4 | 0.6165 | 2.3094 | 440 | 0.2670 | 0.7896 |
| Treatment t1: Time M5 | -10.7478 | 2.3094 | 440 | -4.6540 | <0.0001**** |
| Treatment t2: Time M5 | -13.7609 | 2.3094 | 440 | -5.9587 | <0.0001**** |
| Treatment t3: Time M5 | -7.0717 | 2.3094 | 440 | -3.0622 | 0.0023** |
| Treatment t4: Time M5 | -6.8139 | 2.3094 | 440 | -2.9506 | 0.0033** |

Significance levels of *p* values (adj.): * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001; **** ≤ 0.0001.

**Table S7** Estimated marginal means, with standard errors and lower and upper confidence intervals (95% CI) from the linear mixed effects model (REML) testing the effects of five experimental treatments (t0 = control) with 24-EBL over time on leaf SPAD chlorophyll level, using repeated measurements of 115 *Dipteryx odorata* plants in five monthly observations (random intercepts by plant ID). EMM = estimated marginal mean; SE = standard error; DF = degrees of freedom; Lower CI = lower limit of confidence interval; Upper CI = upper limit of confidence interval.

| Time | Treatment | EMM | SE | DF | Lower CI | Upper CI |
|---|---|---|---|---|---|---|
| M1 | t0 | 24.9 | 1.32 | 114 | 22.3 | 27.6 |
| M1 | t1 | 26.4 | 1.32 | 110 | 23.8 | 29.0 |
| M1 | t2 | 22.5 | 1.32 | 110 | 19.9 | 25.1 |
| M1 | t3 | 25.3 | 1.32 | 110 | 22.7 | 27.9 |
| M1 | t4 | 25.1 | 1.32 | 110 | 22.5 | 27.7 |
| M2 | t0 | 24.9 | 1.32 | 114 | 22.3 | 27.6 |
| M2 | t1 | 24.9 | 1.32 | 110 | 22.3 | 27.5 |
| M2 | t2 | 22.0 | 1.32 | 110 | 19.4 | 24.6 |
| M2 | t3 | 26.0 | 1.32 | 110 | 23.4 | 28.6 |
| M2 | t4 | 24.6 | 1.32 | 110 | 22.0 | 27.2 |
| M3 | t0 | 26.8 | 1.32 | 114 | 24.2 | 29.4 |

|  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|
| M3 | t1 | 25.3 | 1.32 | 110 | 22.7 | 27.9 |
| M3 | t2 | 19.8 | 1.32 | 110 | 17.2 | 22.5 |
| M3 | t3 | 28.1 | 1.32 | 110 | 25.5 | 30.7 |
| M3 | t4 | 26.9 | 1.32 | 110 | 24.3 | 29.5 |
| M4 | t0 | 28.9 | 1.32 | 114 | 26.3 | 31.5 |
| M4 | t1 | 27.7 | 1.32 | 110 | 25.1 | 30.3 |
| M4 | t2 | 19.0 | 1.32 | 110 | 16.3 | 21.6 |
| M4 | t3 | 30.7 | 1.32 | 110 | 28.1 | 33.3 |
| M4 | t4 | 29.7 | 1.32 | 110 | 27.1 | 32.3 |
| M5 | t0 | 37.1 | 1.32 | 114 | 34.5 | 39.7 |
| M5 | t1 | 27.8 | 1.32 | 110 | 25.2 | 30.4 |
| M5 | t2 | 20.9 | 1.32 | 110 | 18.3 | 23.5 |
| M5 | t3 | 30.4 | 1.32 | 110 | 27.8 | 33.0 |
| M5 | t4 | 30.4 | 1.32 | 110 | 27.8 | 33.0 |

**Table S8** Paired contrasts across time (adjusted by Tukey) from the linear mixed effects model (REML) testing the effects of five experimental treatments (t0 = control) with 24-EBL on leaf SPAD chlorophyll level, using repeated measurements of 115 *Dipteryx odorata* plants in five monthly observations (random intercepts by plant ID). Negative estimates indicate lower values for the first treatment in the comparison. For all contrasts, Standard error = 1.86 and degrees of freedom = 110.

| **Time** | **Contrast** | **Estimate** | **t** | ***p* (adj.)** |
|---|---|---|---|---|
| M1 | t0 - t1 | -1.4435 | -0.775 | 0.9373 |
| M1 | t0 - t2 | 2.4348 | 1.307 | 0.6872 |
| M1 | t0 - t3 | -0.3696 | -0.198 | 0.9996 |
| M1 | t0 - t4 | -0.1530 | -0.082 | 1.0000 |
| M1 | t1 - t2 | 3.8783 | 2.083 | 0.2350 |
| M1 | t1 - t3 | 1.0739 | 0.577 | 0.9782 |
| M1 | t1 - t4 | 1.2904 | 0.693 | 0.9576 |

|  |  |  |  |  |
|---|---|---|---|---|
| M1 | t2 - t3 | -2.8043 | -1.506 | 0.5609 |
| M1 | t2 - t4 | -2.5878 | -1.390 | 0.6356 |
| M1 | t3 - t4 | 0.2165 | 0.116 | 1.0000 |
| M2 | t0 - t1 | 0.0461 | 0.025 | 1.0000 |
| M2 | t0 - t2 | 2.9174 | 1.567 | 0.5219 |
| M2 | t0 - t3 | -1.0652 | -0.572 | 0.9789 |
| M2 | t0 - t4 | 0.3783 | 0.203 | 0.9996 |
| M2 | t1 - t2 | 2.8713 | 1.542 | 0.5378 |
| M2 | t1 - t3 | -1.1113 | -0.597 | 0.9753 |
| M2 | t1 - t4 | 0.3322 | 0.178 | 0.9998 |
| M2 | t2 - t3 | -3.9826 | -2.139 | 0.2114 |
| M2 | t2 - t4 | -2.5391 | -1.363 | 0.6522 |
| M2 | t3 - t4 | 1.4435 | 0.775 | 0.9373 |
| M3 | t0 - t1 | 1.5391 | 0.826 | 0.9219 |
| M3 | t0 - t2 | 6.9609 | 3.738 | 0.0027** |
| M3 | t0 - t3 | -1.2848 | -0.690 | 0.9583 |
| M3 | t0 - t4 | -0.0652 | -0.035 | 1.0000 |
| M3 | t1 - t2 | 5.4217 | 2.911 | 0.0346* |
| M3 | t1 - t3 | -2.8239 | -1.516 | 0.5542 |
| M3 | t1 - t4 | -1.6043 | -0.861 | 0.9102 |
| M3 | t2 - t3 | -8.2457 | -4.428 | 0.0002** |
| M3 | t2 - t4 | -7.0261 | -3.773 | 0.0024* |
| M3 | t3 - t4 | 1.2196 | 0.655 | 0.9654 |
| M4 | t0 - t1 | 1.2565 | 0.675 | 0.9615 |
| M4 | t0 - t2 | 9.9826 | 5.360 | <0.0001**** |
| M4 | t0 - t3 | -1.7609 | -0.946 | 0.8784 |
| M4 | t0 - t4 | -0.7696 | -0.413 | 0.9938 |

| | | | | |
|---|---|---|---|---|
| M4 | t1 - t2 | 8.7261 | 4.686 | 0.0001**** |
| M4 | t1 - t3 | -3.0174 | -1.620 | 0.4878 |
| M4 | t1 - t4 | -2.0261 | -1.088 | 0.8124 |
| M4 | t2 - t3 | -11.7435 | -6.306 | <0.0001**** |
| M4 | t2 - t4 | -10.7522 | -5.774 | <0.0001**** |
| M4 | t3 - t4 | 0.9913 | 0.532 | 0.9838 |
| M5 | t0 - t1 | 9.3043 | 4.996 | <0.0001**** |
| M5 | t0 - t2 | 16.1957 | 8.697 | <0.0001**** |
| M5 | t0 - t3 | 6.7022 | 3.599 | 0.0043* |
| M5 | t0 - t4 | 6.6609 | 3.577 | 0.0046* |
| M5 | t1 - t2 | 6.8913 | 3.700 | 0.0031* |
| M5 | t1 - t3 | -2.6022 | -1.397 | 0.6307 |
| M5 | t1 - t4 | -2.6435 | -1.419 | 0.6165 |
| M5 | t2 - t3 | -9.4935 | -5.098 | <0.0001**** |
| M5 | t2 - t4 | -9.5348 | -5.120 | <0.0001**** |
| M5 | t3 - t4 | -0.0413 | -0.022 | 1.0000 |

Significance levels of *p* values (adj.): * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001; **** ≤ 0.0001.

**Table S9:** Random and fixed effects estimated from the linear mixed effects model (REML) testing the effects of five experimental treatments (t0 = control) with 24-EBL and its interaction with time on leaf temperature (in °C), using repeated measurements of 115 *Dipteryx odorata* plants in five monthly observations (random intercepts by plant ID). SD = standard deviation; SE = standard error; DF = degrees of freedom.

| | | | | | |
|---|---|---|---|---|---|
| **Random effects** | | | **Formula: ~1 \| ID** | | |
| | (Intercept) | Residual | | | |
| SD | 0.1129 | 0.5649 | | | |
| **Fixed effects: Leaf temperature ~ Treatment * Time** | | | | | |
| Term | Value | SE | DF | t | *p* |

|  |  |  |  |  |  |
|---|---|---|---|---|---|
| (Intercept) | 26.857826 | 0.1201284 | 440 | 223.57604 | <0.0001**** |
| Treatment t1 | 1.448696 | 0.1698872 | 110 | 8.52740 | <0.0001**** |
| Treatment t2 | 1.796087 | 0.1698872 | 110 | 10.57223 | <0.0001**** |
| Treatment t3 | 1.579565 | 0.1698872 | 110 | 9.29773 | <0.0001**** |
| Treatment t4 | 1.051739 | 0.1698872 | 110 | 6.19081 | <0.0001**** |
| Time M2 | -1.200870 | 0.1665913 | 440 | -7.20848 | <0.0001**** |
| Time M3 | -1.263478 | 0.1665913 | 440 | -7.58430 | <0.0001**** |
| Time M4 | -0.828261 | 0.1665913 | 440 | -4.97181 | <0.0001**** |
| Time M5 | -1.230000 | 0.1665913 | 440 | -7.38334 | <0.0001**** |
| Treatment t1: Time M2 | 0.994348 | 0.2355957 | 440 | 4.22057 | <0.0001**** |
| Treatment t2: Time M2 | 0.436522 | 0.2355957 | 440 | 1.85284 | 0.0646 |
| Treatment t3: Time M2 | 1.142174 | 0.2355957 | 440 | 4.84802 | <0.0001**** |
| Treatment t4: Time M2 | 0.870000 | 0.2355957 | 440 | 3.69277 | 0.0002*** |
| Treatment t1: Time M3 | -1.370435 | 0.2355957 | 440 | -5.81689 | <0.0001**** |
| Treatment t2: Time M3 | -1.813478 | 0.2355957 | 440 | -7.69742 | <0.0001**** |
| Treatment t3: Time M3 | -1.606957 | 0.2355957 | 440 | -6.82082 | <0.0001**** |
| Treatment t4: Time M3 | -1.153478 | 0.2355957 | 440 | -4.89601 | <0.0001**** |
| Treatment t1: Time M4 | -2.003043 | 0.2355957 | 440 | -8.50204 | <0.0001**** |
| Treatment t2: Time M4 | -3.057391 | 0.2355957 | 440 | -12.97728 | <0.0001**** |
| Treatment t3: Time M4 | -1.755217 | 0.2355957 | 440 | -7.45012 | <0.0001**** |
| Treatment t4: Time M4 | -1.445652 | 0.2355957 | 440 | -6.13616 | <0.0001**** |
| Treatment t1: Time M5 | 1.499565 | 0.2355957 | 440 | 6.36499 | <0.0001**** |
| Treatment t2: Time M5 | 0.398696 | 0.2355957 | 440 | 1.69229 | 0.0913 |
| Treatment t3: Time M5 | 0.185217 | 0.2355957 | 440 | 0.78617 | 0.4322 |
| Treatment t4: Time M5 | -0.116522 | 0.2355957 | 440 | -0.49458 | 0.6211 |

Significance levels of *p* values (adj.): * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001; **** ≤ 0.0001.

**Table S10** Estimated marginal means, with standard errors and lower and upper confidence intervals (95% CI) from the linear mixed effects model (REML) testing the effects of five experimental treatments (t0 = control) with 24-EBL over time on leaf temperature, using repeated measurements of 115 *Dipteryx odorata* plants in five monthly observations (random intercepts by plant ID). EMM = estimated marginal mean; SE = standard error; DF = degrees of freedom; Lower CI = lower limit of confidence interval; Upper CI = upper limit of confidence interval.

| **Time** | **Treatment** | **EMM** | **SE** | **DF** | **Lower CI** | **Upper CI** |
|---|---|---|---|---|---|---|
| M1 | t0 | 26.9 | 0.12 | 114 | 26.6 | 27.1 |
| M1 | t1 | 28.3 | 0.12 | 110 | 28.1 | 28.5 |
| M1 | t2 | 28.7 | 0.12 | 110 | 28.4 | 28.9 |
| M1 | t3 | 28.4 | 0.12 | 110 | 28.2 | 28.7 |
| M1 | t4 | 27.9 | 0.12 | 110 | 27.7 | 28.1 |
| M2 | t0 | 25.7 | 0.12 | 114 | 25.4 | 25.9 |
| M2 | t1 | 28.1 | 0.12 | 110 | 27.9 | 28.3 |
| M2 | t2 | 27.9 | 0.12 | 110 | 27.7 | 28.1 |
| M2 | t3 | 28.4 | 0.12 | 110 | 28.1 | 28.6 |
| M2 | t4 | 27.6 | 0.12 | 110 | 27.3 | 27.8 |
| M3 | t0 | 25.6 | 0.12 | 114 | 25.4 | 25.8 |
| M3 | t1 | 25.7 | 0.12 | 110 | 25.4 | 25.9 |
| M3 | t2 | 25.6 | 0.12 | 110 | 25.3 | 25.8 |
| M3 | t3 | 25.6 | 0.12 | 110 | 25.3 | 25.8 |
| M3 | t4 | 25.5 | 0.12 | 110 | 25.3 | 25.7 |
| M4 | t0 | 26.0 | 0.12 | 114 | 25.8 | 26.3 |
| M4 | t1 | 25.5 | 0.12 | 110 | 25.2 | 25.7 |
| M4 | t2 | 24.8 | 0.12 | 110 | 24.5 | 25.0 |
| M4 | t3 | 25.9 | 0.12 | 110 | 25.6 | 26.1 |
| M4 | t4 | 25.6 | 0.12 | 110 | 25.4 | 25.9 |
| M5 | t0 | 25.6 | 0.12 | 114 | 25.4 | 25.9 |

| | | | | | | |
|---|---|---|---|---|---|---|
| M5 | t1 | 28.6 | 0.12 | 110 | 28.3 | 28.8 |
| M5 | t2 | 27.8 | 0.12 | 110 | 27.6 | 28.1 |
| M5 | t3 | 27.4 | 0.12 | 110 | 27.2 | 27.6 |
| M5 | t4 | 26.6 | 0.12 | 110 | 26.3 | 26.8 |

**Table S11** Paired contrasts across time (adjusted by Tukey) from the linear mixed effects model (REML) testing the effects of five experimental treatments (t0 = control) with 24-EBL on leaf temperature, using repeated measurements of 115 *Dipteryx odorata* plants in five monthly observations (random intercepts by plant ID). Negative estimates indicate lower values for the first treatment in the comparison. For all contrasts, Standard error = 0.17 and degrees of freedom = 110.

| **Time** | **Contrast** | **Estimate** | **t** | ***p* (adj.)** |
|---|---|---|---|---|
| M1 | t0 - t1 | -1.4487 | -8.527 | <0.0001**** |
| M1 | t0 - t2 | -1.7961 | -10.572 | <0.0001**** |
| M1 | t0 - t3 | -1.5796 | -9.298 | <0.0001**** |
| M1 | t0 - t4 | -1.0517 | -6.191 | <0.0001**** |
| M1 | t1 - t2 | -0.3474 | -2.045 | 0.2519 |
| M1 | t1 - t3 | -0.1309 | -0.770 | 0.9386 |
| M1 | t1 - t4 | 0.3970 | 2.337 | 0.1413 |
| M1 | t2 - t3 | 0.2165 | 1.275 | 0.7073 |
| M1 | t2 - t4 | 0.7443 | 4.381 | 0.0003*** |
| M1 | t3 - t4 | 0.5278 | 3.107 | 0.0199* |
| M2 | t0 - t1 | -2.4430 | -14.380 | <0.0001**** |
| M2 | t0 - t2 | -2.2326 | -13.142 | <0.0001**** |
| M2 | t0 - t3 | -2.7217 | -16.021 | <0.0001**** |
| M2 | t0 - t4 | -1.9217 | -11.312 | <0.0001**** |
| M2 | t1 - t2 | 0.2104 | 1.239 | 0.7288 |
| M2 | t1 - t3 | -0.2787 | -1.640 | 0.4751 |

|  |  |  |  |  |
|---|---|---|---|---|
| M2 | t1 - t4 | 0.5213 | 3.069 | 0.0223* |
| M2 | t2 - t3 | -0.4891 | -2.879 | 0.0378* |
| M2 | t2 - t4 | 0.3109 | 1.830 | 0.3619 |
| M2 | t3 - t4 | 0.8000 | 4.709 | 0.0001**** |
| M3 | t0 - t1 | -0.0783 | -0.461 | 0.9906 |
| M3 | t0 - t2 | 0.0174 | 0.102 | 1.0000 |
| M3 | t0 - t3 | 0.0274 | 0.161 | 0.9998 |
| M3 | t0 - t4 | 0.1017 | 0.599 | 0.9750 |
| M3 | t1 - t2 | 0.0957 | 0.563 | 0.9801 |
| M3 | t1 - t3 | 0.1057 | 0.622 | 0.9713 |
| M3 | t1 - t4 | 0.1800 | 1.060 | 0.8267 |
| M3 | t2 - t3 | 0.0100 | 0.059 | 1.0000 |
| M3 | t2 - t4 | 0.0843 | 0.496 | 0.9875 |
| M3 | t3 - t4 | 0.0743 | 0.438 | 0.9923 |
| M4 | t0 - t1 | 0.5543 | 3.263 | 0.0125* |
| M4 | t0 - t2 | 1.2613 | 7.424 | <0.0001**** |
| M4 | t0 - t3 | 0.1757 | 1.034 | 0.8391 |
| M4 | t0 - t4 | 0.3939 | 2.319 | 0.1468 |
| M4 | t1 - t2 | 0.7070 | 4.161 | 0.0006*** |
| M4 | t1 - t3 | -0.3787 | -2.229 | 0.1768 |
| M4 | t1 - t4 | -0.1604 | -0.944 | 0.8789 |
| M4 | t2 - t3 | -1.0857 | -6.390 | <0.0001**** |
| M4 | t2 - t4 | -0.8674 | -5.106 | <0.0001**** |
| M4 | t3 - t4 | 0.2183 | 1.285 | 0.7011 |
| M5 | t0 - t1 | -2.9483 | -17.354 | <0.0001**** |
| M5 | t0 - t2 | -2.1948 | -12.919 | <0.0001**** |
| M5 | t0 - t3 | -1.7648 | -10.388 | <0.0001**** |

| | | | | |
|---|---|---|---|---|
| M5 | t0 - t4 | -0.9352 | -5.505 | <0.0001**** |
| M5 | t1 - t2 | 0.7535 | 4.435 | 0.0002*** |
| M5 | t1 - t3 | 1.1835 | 6.966 | <0.0001**** |
| M5 | t1 - t4 | 2.0130 | 11.849 | <0.0001**** |
| M5 | t2 - t3 | 0.4300 | 2.531 | 0.0912 |
| M5 | t2 - t4 | 1.2596 | 7.414 | <0.0001**** |
| M5 | t3 - t4 | 0.8296 | 4.883 | <0.0001**** |

Significance levels of *p* values (adj.): * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001; **** ≤ 0.0001.

**Table S12** Meteorological data obtained from the Meteorological Database (BDMEP) of the Brazilian National Institute of Meteorology (INMET) during the experimental application period of four concentrations of 24-EBL on 115 Dipteryx odorata plants, monitored for six months, and historical averages (1990-2019) of rainy days and hours of sunshine for the corresponding months.

| **Month of experiment** | **Rainfall (mm)** | **Av. temp (°C)** | **Monthly average days of rain (1990-2019)** | **Monthly average daily sun hours (1990-2019)** |
|---|---|---|---|---|
| Dec/2024 (M0) | 122.2 | 26.28 | 17 | 7.4 |
| Jan/2025 (M1) | 444.6 | 25.14 | 20 | 5.9 |
| Feb/2025 (M2) | 411.0 | 25.27 | 19 | 5.2 |
| Mar/2025 (M3) | 367.8 | 25.47 | 21 | 5.0 |
| Apr/2025 (M4) | 333.2 | 25.80 | 20 | 4.8 |
| May/2025 (M5) | 68.8 | 26.31 | 20 | 5.4 |

**Document Link** https://figshare.com/s/080bf250a9f12f1958de

## Supplementary Figures

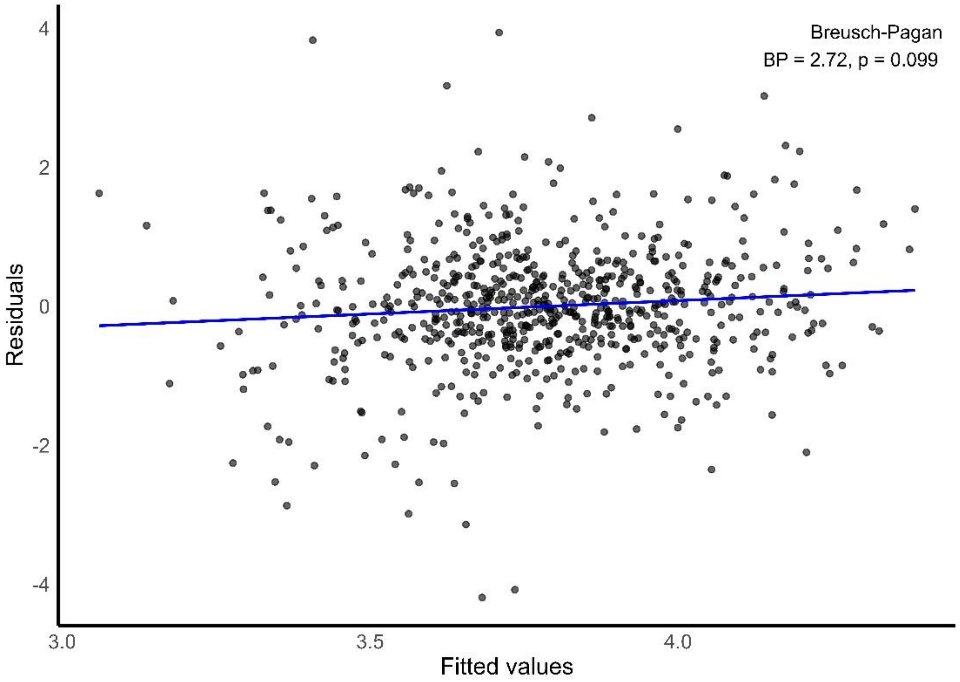

**Figure S1** Plot of residuals against adjusted values from the mixed effects linear model (REML) testing the effects of five experimental treatments with 24-EBL and its interaction with time on the logarithm-transformed growth rate (log height/diameter ratio), using repeated measurements of 115 *Dipteryx odorata* plants in six monthly observations. The Breusch-Pagan test of residuals confirms homoscedasticity, validating the parametric model.

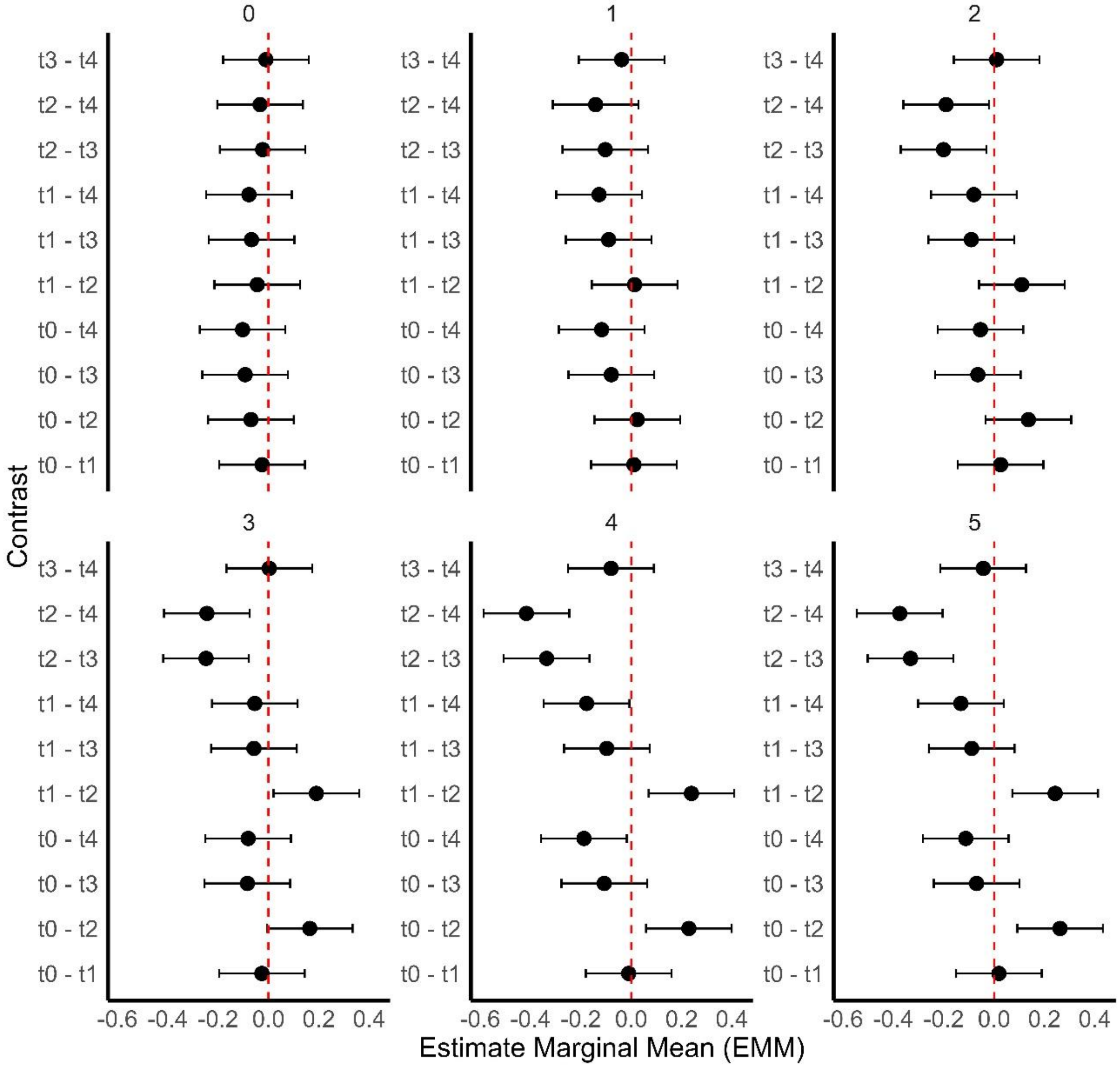

**Figure S2** Graphs of paired contrasts of estimated marginal means (adjusted by Tukey HSD) from the linear mixed effects model (REML) testing the effects of five experimental treatments with 24-EBL and their interaction with time on the logarithm-transformed growth rate (log height/diameter ratio), using repeated measurements of 115 *Dipteryx odorata* plants in six monthly observations.

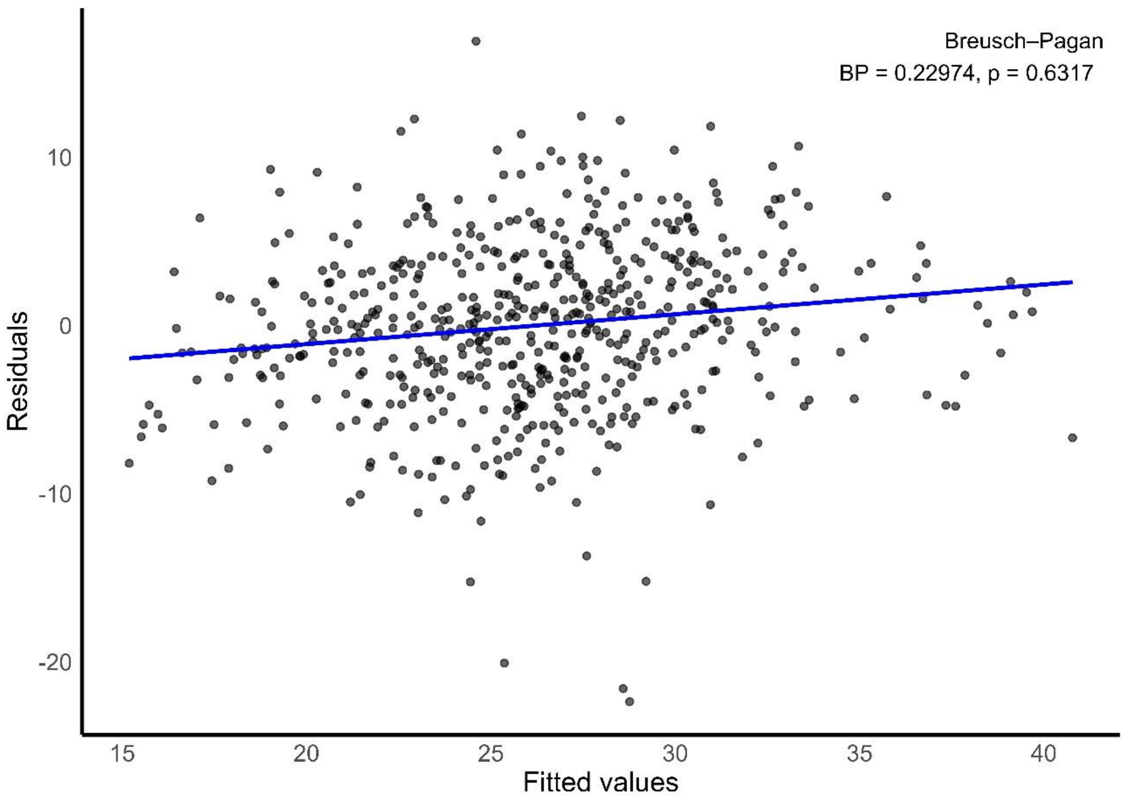

**Figure S3** Residuals against adjusted values plot from the mixed effects linear model (REML) testing the effects of five experimental treatments with 24-EBL and its interaction with leaf SPAD chlorophyll level, using repeated measurements of 115 *Dipteryx odorata* plants in five monthly observations (random intercepts by plant ID). The Breusch-Pagan test of residuals confirms homoscedasticity, validating the parametric model.

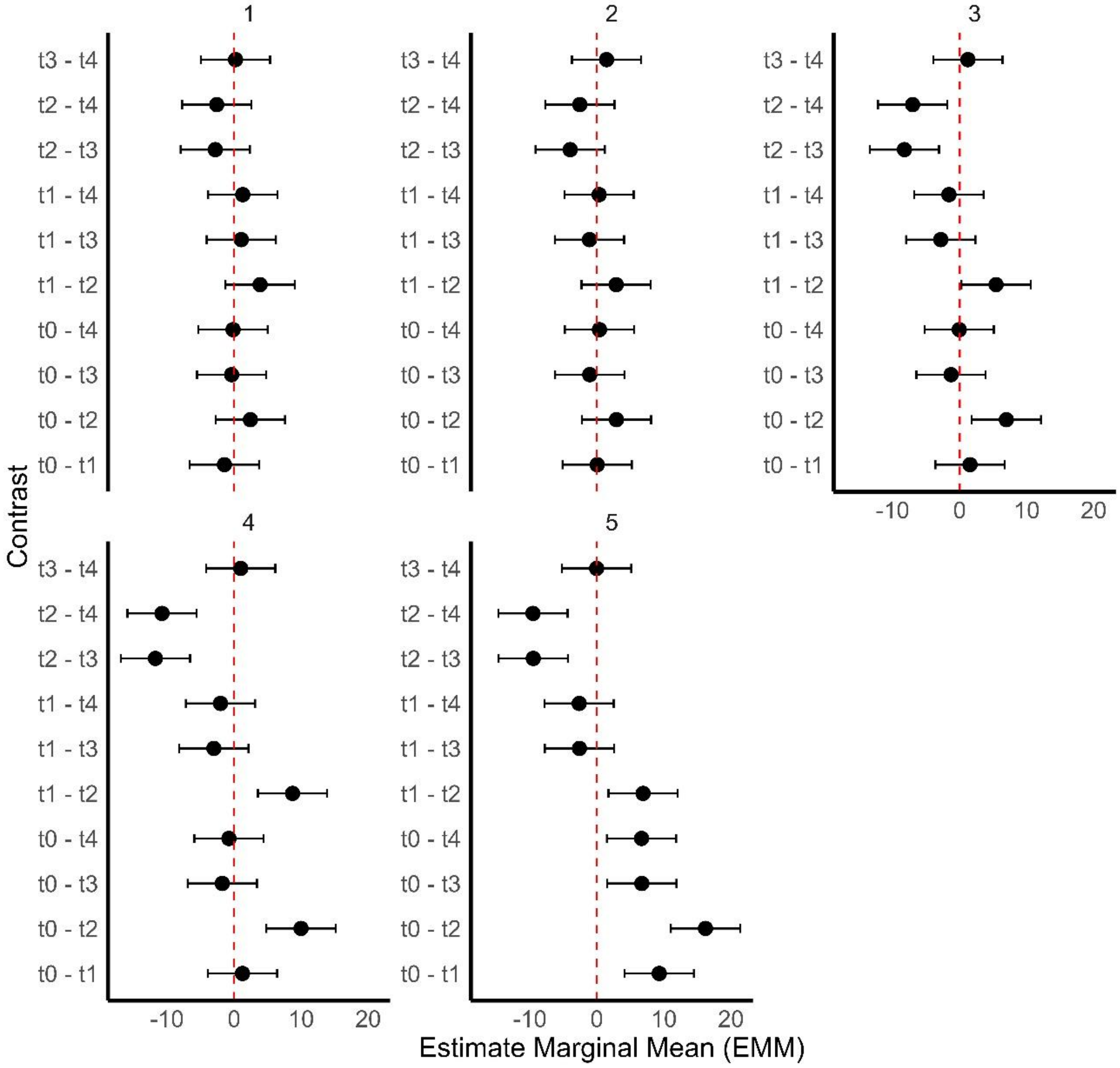

**Figure S4** Graphs showing paired contrasts of estimated marginal means (adjusted by Tukey HSD) from the linear mixed effects model (REML) testing the effects of five experimental treatments with 24-EBL and their interaction with time on leaf SPAD chlorophyll level, using repeated measurements of 115 *Dipteryx odorata* plants in five monthly observations.

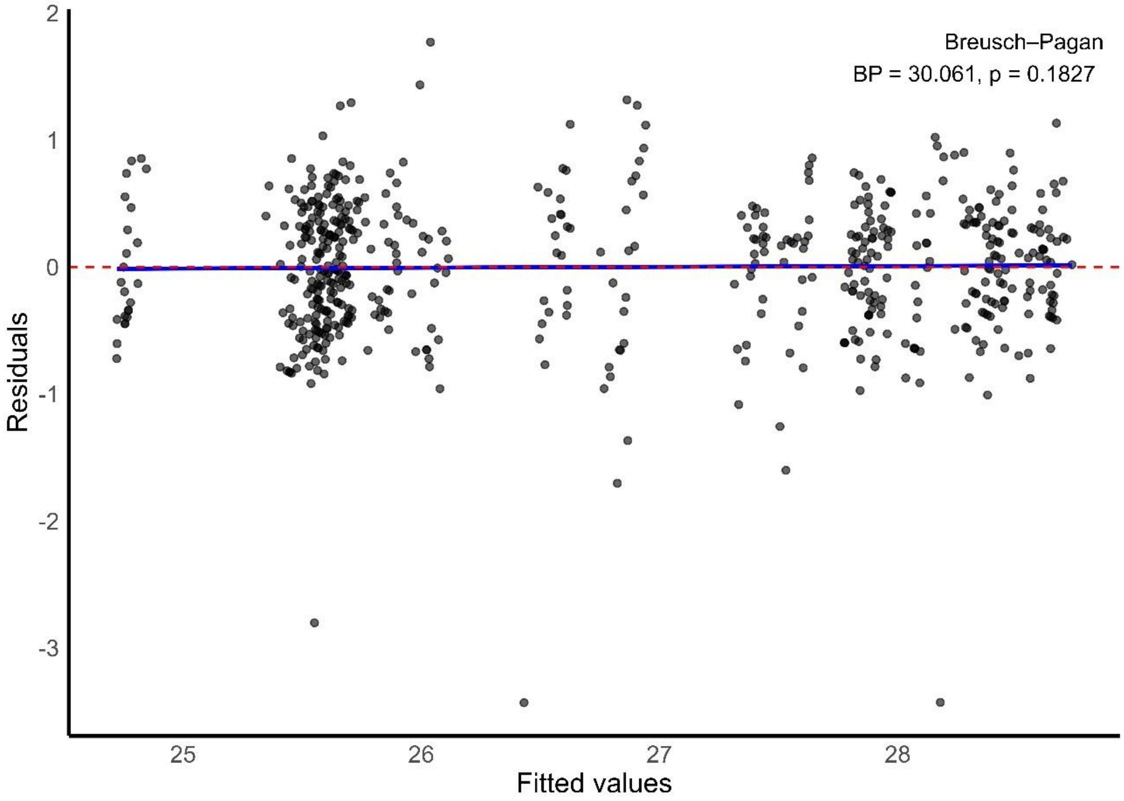

**Figure S5** Residual against adjusted values plot from the mixed effects linear model (REML) testing the effects of five experimental treatments with 24-EBL and its interaction with leaf temperature (in °C), using repeated measurements of 115 *Dipteryx odorata* plants in five monthly observations (random intercepts by plant ID). The Breusch-Pagan test of residuals confirms homoscedasticity, validating the parametric model.

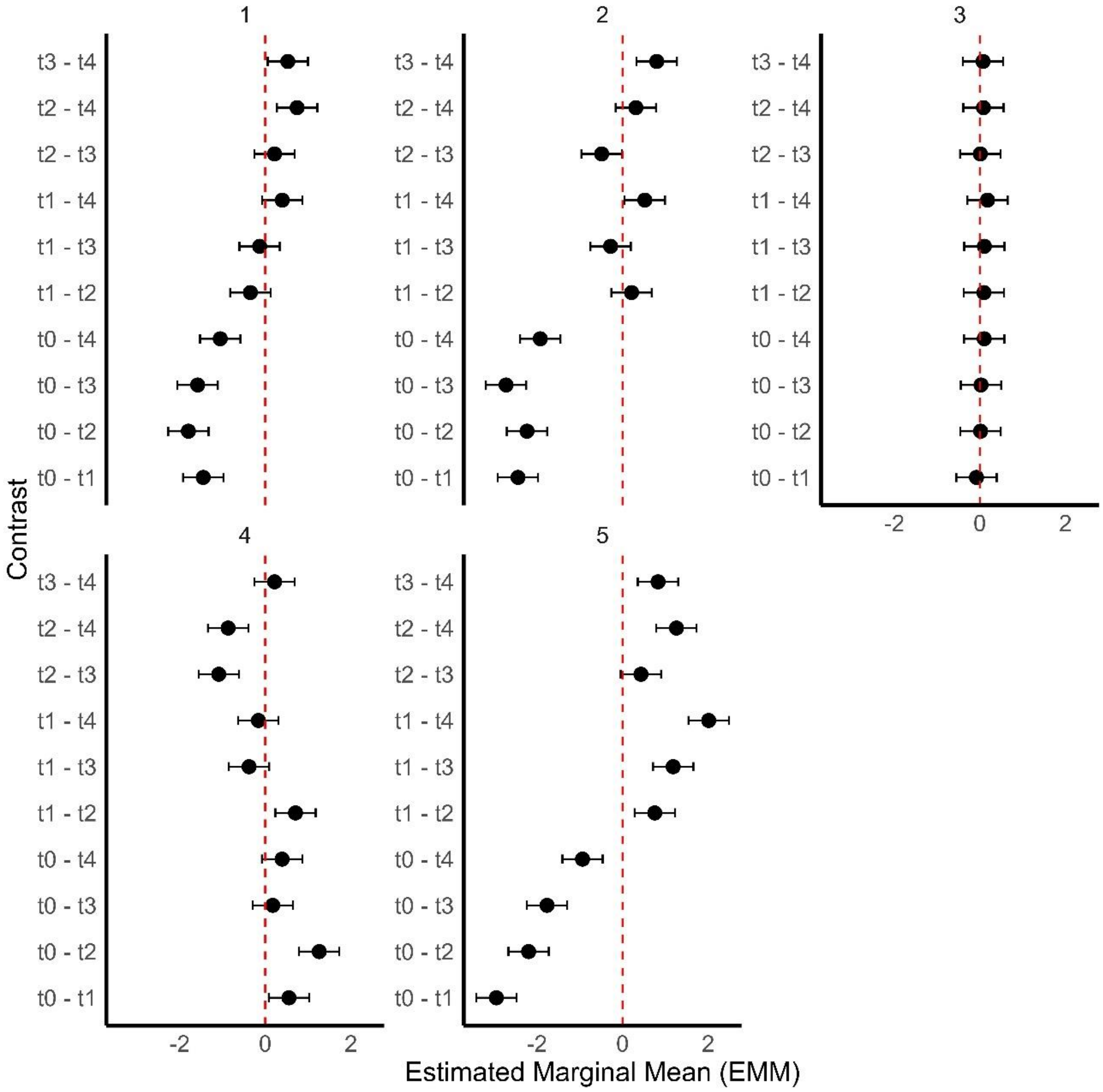

**Figure S6** Graphs showing paired contrasts of estimated marginal means (adjusted by Tukey HSD) from the linear mixed effects model (REML) testing the effects of five experimental treatments with 24-EBL and their interaction with leaf temperature (in °C) over time, using repeated measurements of 115 *Dipteryx odorata* plants in five monthly observations.

**Supplementary File**

**File S1** Technical report of the physicochemical analysis of the soil used as substrate for the experimental cultivation of 115 *Dipteryx odorata* plants, performed by Fullin Laboratory of Agronomical and Environmental Analysis, Linhares, Espírito santo, Brazil.

**Document Link** https://figshare.com/s/080bf250a9f12f1958de