UNIVERSIDADE ESTADUAL DO MARANHÃO - UEMA
CENTRO DE CIENCIAS AGRÁRIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM AGROECOLOGIA

DIOGO HERISON SILVA SARDINHA

HELICONIA PSITTACORUM: INFLUÊNCIA DE FITOESTIMULANTES NO CONTROLE DE MANCHAS FOLIARES E NA QUALIDADE DE FLORES PÓS-COLHEITA

São Luís
2018

DIOGO HERISON SILVA SARDINHA

Engenheiro Agrônomo

HELICONIA PSITTACORUM: INFLUÊNCIA DE FITOESTIMULANTES NO CONTROLE DE MANCHAS FOLIARES E NA QUALIDADE DE FLORES PÓS-COLHEITA

Tese apresentada ao Programa de Pós-Graduação em Agroecologia da Universidade Estadual do Maranhão, para obtenção do título de Doutor em Agroecologia.

Orientadora: Dra. Antonia Alice Costa Rodrigues

São Luís
2018

Sardinha, Diogo Herison Silva.
Heliconia psittacorum: influência de fitoestimulantes no controle de manchas foliares e na qualidade de flores pós-colheita / Diogo Herison Silva Sardinha. – São Luís, 2018.

83 f

Tese (Doutorado) – Curso de Agroecologia, Universidade Estadual do Maranhão, 2018.

Orientador: Profa. Dra. Antônia Alice Costa Rodrigues.

1.Indução de resistência. 2.Espécies reativas de oxigênio. 3.Flores tropicais. 4.Vida de vaso. 5.Complexo fúngico. I.Título

CDU: 632.9

Elaborado por Giselle Frazão Tavares- CRB 13/665

HELICONIA PSITTACORUM: INFLUÊNCIA DE FITOESTIMULANTES NO CONTROLE DE MANCHAS FOLIARES E NA QUALIDADE DE FLORES PÓS-COLHEITA

Diogo Herison Silva Sardinha

Aprovado em: / /

BANCA EXAMINADORA:

_____
Dra. Antônia Alice Costa Rodrigues (Orientadora)
Universidade Estadual do Maranhão - UEMA

_____
Dr. Fabricio de Oliveira Reis (1° examinador)
Universidade Estadual do Maranhão - UEMA

_____
Dra. Anna Christina Sanazario de Oliveira (2° examinador)
Universidade Estadual do Maranhão - UEMA

_____
Dra. Sandra Maria Cruz Nascimento (3° examinador)
Instituto Federal Ciência e Tecnologia do Maranhão – IFMA

_____
Dra. Ivaneide de Oliveira Nascimento (4° examinador)
Universidade estadual da Região Tocantina do Maranhão- UEMASUL

A Deus fonte de todo conhecimento, por me amparar nos momentos de aflição.

AGRADEÇO

A minha família que me apoiou em todos os momentos. Minha fonte de inspiração e força são vocês, mãe, pai, irmã, tias, avós.

A minha esposa Werdna e a minha filha Ana Catarina, por estarem comigo na reta final desta jornada.

DEDICO

AGRADECIMENTOS

A Deus por ser meu guia em todos os momentos.

Aos meus pais, Ana e João, pelo amor, orações, carinho, compreensão e apoio em minha vida, e a minha irmã “meu inteiro” pelo carinho, apoio e amizade.

Aos minha avó Delzina e ao meu avô Raimundo (*in memoriam*), por todo amor, carinho e apoio em todos os momentos da minha vida.

A minha madrinha Regina, a minha tia Alda e minha tia Ilza, por estarem presentes e contribuírem com meu desenvolvimento pessoal.

Aos meus tios e tias paternos, em especial tio Ribamar, tia Joselina, pelo apoio durante toda jornada acadêmica.

A Universidade Estadual do Maranhão e ao Programa de Pós-Graduação em Agroecologia pela oportunidade de realização do curso de doutorado.

Ao Instituto Federal Ciência e Tecnologia do Maranhão – IFMA, instituição ao qual faço parte, pela liberação para conclusão do curso de doutorado.

A Profaº. Draº. Antônia Alice Costa Rodrigues pela orientação, por todo o conhecimento transmitido, pelas contribuições para meu crescimento profissional e pessoal, pela paciência e amizade, não apenas no doutorado, mas em toda minha vida acadêmica.

Aos amigos de laboratório, pelo auxílio nos momentos difíceis, amizade, e pelo companheirismo ao longo de todo o período de estudos.

Aos amigos da limpeza, em especial a Neto, pela ajuda e contribuição nas atividades de campo.

A todos aqueles que direta ou indiretamente contribuíram para a realização deste trabalho.

SUMÁRIO

	RESUMO GERAL	viii
	GENERAL ABSTRACT	ix
1	**CAPÍTULO I – Referencial Teórico**	01
1.1	Mercado de flores e plantas ornamentais no Brasil e no mundo	02
1.2	O Segmento das Flores Tropicais	03
1.3	Família Heliconiaceae	04
1.4	Aspectos Fitossanitários de Plantas Ornamentais Tropicais	05
1.5	Ativação de defesa em plantas	07
1.6	Aspectos pós-colheita de flores tropicais	09
	Referências	11
2	**CAPITULO II – Inducing Resistance in *Heliconia psittacorum* cv. Golden Torch to Naturally Occurring Leaf Diseases**	16
	Abstract	17
	Introduction	17
	Material and Methods	18
	Results	19
	Discussion	23
	Considerations	24
	References	24
3	**CAPITULO III – Phytostimulants Influence The Vase Life Of *Heliconia psittacorum* cv. Golden Torch**	27
	Abstract	28
	Introduction	29
	Material and Methods	30
	Results	35
	Discussion	45
	Conclusion	50
	References	50
4	**CONSIDERAÇÕES FINAIS**	55
	ANEXOS	57

RESUMO GERAL

Um dos entraves à produção de helicônias são as doenças foliares que podem reduzir a fotossíntese, danificar as brácteas e inviabilizar as flores para comercialização. A utilização de fitoestimulantes pode reduzir a incidência das manchas foliares e influenciar a qualidade das flores pós-colheita. O objetivo deste trabalho foi identificar fitoestimulantes capazes de induzir resistência em *H. psittacorum* cv. Golden Torch e influenciar positivamente a pós-colheita desta espécie. Os fitoestimulantes Agro-Mos® (10 ml L^{-1}), Bion® (0,5 g L^{-1}), Quartz® (40 ml L^{-1}), K - Fosfitotal® (3 g L^{-1}) e Ca - Fosfitotal® (3 g L^{-1}) foram aplicados com auxílio de pulverizador costal e cada parcela foi protegida durante a aplicação eliminando a deriva dos produtos. A indução foi avaliada durante o cultivo experimental com a quantificação da severidade causada pelo complexo fúngico, da taxa fotossintética das plantas e da coleta de material vegetal para análise da atividade enzimática de peroxidase, polifenoloxidase, β-1,3 gluconase. A influência pós-colheita foi mensurada em flores de *H. psittacorum* cv. Golden Torch colhidas no período da manhã considerando os parâmetros: aspecto visual, perda de massa fresca, extravasamento de eletrólitos, peroxidação lipídica, além da quantificação de carboidratos solúveis e das enzimas peroxidase, polifenoloxidase, superóxido dismutase e compostos fenólicos solúveis totais. Os resultados indicaram que os fitoestimulantes Bion® e os fosfitos aplicados, reduziram a severidade do complexo fúngico, aumentaram a atividade de peroxidase, polifenoloxidase, β-1,3 gluconase e não promoveram alterações na taxa de fotossíntese liquida das plantas. O valor encontrado para $\beta > 1$, sugere que a estimativa visual da severidade do complexo fúngico é um bom indicador visual do efeito dos fitopatógenos na taxa fotossintética do hospedeiro. Já a influência dos fitoestimulantes na qualidade das hastes demonstraram ser positivas, preservando a qualidade das hastes florais colhidas. Nos parâmetros avaliados os fosfitos, sobretudo o K - Fosfitotal®, obtiveram as melhores notas para o aspecto visual, apresentando reduzido extravasamento de eletrólitos e menor peroxidação lipídica. Os resultados deste trabalho, são respostas concretas a produtores do setor, sobre alternativas de manejo das doenças associadas ao cultivo de helicônias.

Palavras-chave: Indução de resistência, espécies reativas de oxigênio, flores tropicais, vida de vaso, complexo fúngico.

GENERAL ABSTRACT

Obstructions to the production of heliconia are foliar diseases that can reduce photosynthesis, damage as broccoli and become unfeasible as flowers for commercialization. The use of phytostimulants can reduce leaf spot and influence the quality of post-harvest flowers. The objective of this work was to identify resistance forms in H. psittacorum cv. Golden Torch and positively influence the post-harvest of this species. Agro-Mos® phytostimulants (10 ml L-1), Bion® (0.5 g L-1), Quartz® (40 ml L-1), K - Fosfitotal® (3 g L-1) and Ca - Fosfitotal® (3 g L-1) was the auxiliary commissioner of costal spraying and each plot was protected during an application that eliminated the drift of the products. The induction was evaluated during the experimental cultivation with the quantification of the growth rate of the fungal complex, the photosynthetic of the plants and the collection of vegetal material for the enzymatic analysis of the enzyme peroxidase, polyphenoloxidase, β-1,3 gluconase. Post-harvest color was measured on flowers of H. psittacorum cv. Golden Torch. At the moment of the analysis, the visual effects, fresh mass loss, electrolyte extravasation, lipid peroxidation, besides the quantification of soluble carbohydrates and enzymes peroxidase, polyphenoloxidase, superoxide dismutase and total soluble phenolic compounds. The results indicate that Bion® phytostimulants and applied phosphites reduced fungal complex severity, increased peroxidase, polyphenoloxidase, β-1,3 gluconase activity, and did not promote changes in the net photosynthesis rate of plants. The value found for β> 1, which is visual visual of the severity of the fungal complex, is a good visual indicator of the effect of phytopathogens on the photosynthetic rate of the host. The quality of the vitamins demonstrated is positive, preserving the quality of the plants harvested. The parameters were the phosphites, especially K - Fosfitotal ®, obtained the best notes for the visual aspect, revealed the extravasation of electrolytes and lower lipid peroxidation. The results of this work are concrete answers to the producers of the sector, on the alternatives of management of the diseases associated with the cultivation of heliconia.

Keywords: Induction resistance, reactive oxygen species, tropical flowers, pot life, fungal complex.

Capitulo I - Referencial Teórico

1 INTRODUÇÃO

1.1 Mercado de flores e plantas ornamentais no Brasil e no mundo

A comercialização mundial de flores e plantas ornamentais movimentou em 2013 cerca de 21 bilhões de dólares. Historicamente, a Holanda é o principal país produtor e comercializador de flores, acompanhado da China, Estados Unidos e Japão (NEVES; PINTO, 2015). Nos anos de 2014 a 2015 o volume das exportações brasileiras chegou a apenas 21,9 mil dólares segundo o Ministério do Desenvolvimento Indústria e Comércio Exterior, valores muito abaixo dos apresentados entre os anos de 2004 a 2005, que somaram em torno 12,7 milhões de dólares (BRASIL, 2017), no entanto vale destacar que o principal consumidor das flores e plantas ornamentais produzidas no Brasil é o próprio mercado interno brasileiro com mais de 96 % do total (SEBRAE, 2015).

No Brasil, todo o setor de flores e plantas ornamentais obteve faturamento, no ano de 2015, de mais de R$ 6 bilhões (ALENCAR; GALERA, 2016), o que mostra o seu tamanho e importância na economia nacional. Apresentando no mesmo período uma área plantada de aproximadamente 15.000 hectares. Esse número é resultado de um aumento recorrente da área destinada a essa atividade no país, já que em 2012 a área foi estimada em torno de 11.800 hectares e em 2013 de aproximadamente 14.000 hectares (NEVES; PINTO, 2015).

Em nível nacional, o estado de São Paulo é o maior produtor de flores e plantas ornamentais do Brasil, concentrando 45 % da área de produção, e quase 30 % dos produtores que se dedicam à atividade e o varejo tem tido uma atuação relevante, em termos de volume comercializado, com uma movimentação que atingiu em 2014 cerca de R$ 1,98 bilhão (NEVES; PINTO, 2015). No entanto, essa produção vem se expandindo para outras áreas ou regiões do país, de acordo com o Instituto Brasileiro de Floricultura – IBRAFLOR (2014), a expansão do cultivo de flores e plantas ornamentais no Brasil deriva, dentre outros fatores, da própria biodiversidade e a amplitude de climas e solos do País.

1.2. O Segmento das Flores Tropicais

Entre os vários segmentos do mercado e dentre as diversas espécies de flores, destaca-se a floricultura tropical, que há algum tempo é uma atividade em ascensão no Brasil e segundo Loges et al., (2005), deve-se a características peculiares, tais como durabilidade, beleza e diversidade de cores e formatos, que propiciam grande aceitação pelo mercado consumidor e elevado potencial de crescimento no mercado nacional e internacional.

No Brasil o cultivo de flores tropicais, propriamente dito, é realizado há vários anos e principalmente nos estados de Pernambuco, Alagoas, Ceará, Bahia, Sergipe, Pará, Amazonas, Rio de Janeiro, São Paulo e no Distrito Federal (JUNQUEIRA; PEETZ, 2007), não existindo dados de produção oficiais para o seguimento. Para Villela (1999) o Brasil possui uma ampla variedade de solos e condições climáticas que favorecem o cultivo de uma grande diversidade de flores ornamentais tropicais, sendo que o Nordeste desponta como grande produtor, destacando-se os Estados de Pernambuco, Alagoas e Ceará, onde as condições de clima permitem o cultivo durante todo o ano, sem a necessidade de investimentos em insumos mais caros, resultando em custos que possibilitem a região competir com vantagem no mercado mundial.

Não existem estudos recentes sobre a cadeia produtiva no estado do Maranhão, sendo a produção local, de caráter não empresarial e focada essencialmente no abastecimento de São Luís, é concentrada na exploração de flores e folhagens tropicais de corte, palmeiras, bromélias, samambaias, mini rosas e crótons, entre outros produtos. (SEBRAE, 2015).

O último estudo sobre a cadeia produtiva de flores ornamentais tropicais no Maranhão foi realizado a mais de uma década (SEBRAE, 2003) e quantificou 41 produtores, sendo 14 em Paço do Lumiar, 14 em São José de Ribamar e 12 em São Luís, ocupando, ao todo, aproximadamente 35 ha. Na sua maioria, produtores de pequeno porte, que utilizavam mão-de-obra familiar, empregando, em média, de três a quatro pessoas por unidade de produção, estas quase sempre inferiores a 01 ha (um hectare) (SEBRAE, 2015).

Segundo estudos conduzidos pela Universidade Estadual do Maranhão, apenas nove dos produtores que participam da produção de flores e plantas ornamentais, tem como produto principal de suas atividades, o cultivo de espécies consideradas tropicais (SARDINHA, 2008). Não existem dados oficiais publicados recentemente, porém informações coletadas durante está pesquisa sugerem manutenção do número de produtores. As espécies tradicionalmente cultivadas no Estado do Maranhão são das famílias Musaceae, Heliconiaceae, Zingiberaceae, Marantaceae (SARDINHA et al., 2012), dentre estas famílias, as espécies de maior aceitação no mercado são as helicônias (*Heliconia* spp., bastão do imperador (*Etlingera elatior* (Jack) R. M. Smith) e alpínias (*Alpinia purpurata* (Vieill.) K. Schum).

1.3 Família Heliconiaceae

Originalmente as helicônias pertenciam à família Musaceae, o gênero Heliconia, em função de suas características próprias de individualização, passou a constituir a família Heliconiaceae como único representante (CASTRO et al., 2011). São plantas de origem neotropical que aparecem naturalmente em clareiras, bordas de florestas e matas ciliares, na América Central e América do Sul.

Existem aproximadamente 250 espécies de helicônias e algumas dessas espécies são muito utilizadas como plantas de jardim e flores de corte (MOSCA et al., 2004). No Brasil ainda não há um consenso, mas devem existir entre 40 e 65 espécies nativas de helicônias, dentre as quais: *H. episcopalis, H. bihai, H. stricta, H. spathocircinata, H. lourteigiae, H. farinosa, H. kautzkiana, H. rivularis, H. sampaioana, H. velloziana, H. chartaceae, H. juruana, H. pendula, H. acuminata, H. angusta, H. psittacorum, H. richardiana, H. aemygdiana, H. pseudoaemygdiana, H. densiflora, H. lasiorachis, H. metallica, H. subulata, H. apparicioi, H. hirsuta, H. marginata, H. latispatha, H. x rauliniana, H. julianii, H. rostrata, H. standley, H. tenebrosa, H. timothei e H. velutina* (CASTRO et al., 2011).

As helicônias são plantas de porte herbáceo, com diferentes tamanhos, podendo chegar até 12 m de altura. Propagam-se por meio de rizomas subterrâneos, que emitem brotações à superfície, podendo ser solitários ou agregados. Cada planta é composta por pseudocaule, folhas e uma única inflorescência (MOSCA et al., 2004).

1.4 Aspectos Fitossanitários de Plantas Ornamentais Tropicais

Os prejuízos relacionados com a produção e qualidade de flores tropicais podem ser significativos caso não exista um controle eficiente sobre fitopatógenos presentes nas áreas de cultivo. Os agentes causais de doenças em flores tropicais podem estar associados ao rizoma, às raízes das plantas e às folhas, o que pode ser agravado pela importação de mudas não certificadas, em decorrência da expansão do cultivo, contribuindo para o aumento da incidência e severidade dos problemas fitossanitários. Em alguns casos, as espécies tropicais se tornam veículo de disseminação de doenças para outras espécies cultivadas. Gasparotto et al. (2005) relata a sigatoka-negra, causada pelo fungo *Mycosphaerella fijiensis* Morelet patogênica a *Heliconia psittacorum*, e afirma que *H. psittacorum* pode atuar como veículo de disseminação desse fungo a longas distâncias, principalmente quando suas flores são exportadas para regiões do País onde não ocorre a doença.

Há algum tempo estudos avaliam a presença de fitopatógenos associados a espécies de flores tropicais, dentre os quais Lins; Coelho (2004) que em levantamento na zona da mata pernambucana relataram doenças causadas por fungos, sendo assinaladas a antracnose (*Colletotrichum gloeosporioides* Penz) em *Heliconia* spp., *Etlingera elatior*, *Tapeinochilos ananassae*, causando lesões em folhas e inflorescências; manchas foliares (*Bipolaris* spp., *Cercospora* sp., *Curvularia lunata* (Walker) Boedijn, *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk, *Guignardia* sp. e *Deightoniella torulosa* (Syd.) M.B. Ellis em *Heliconia* spp., *Calathea burle* marx e *Musa coccinea*; podridão de rizomas e raízes (*Rhizoctonia solani* Kuhn e *Fusarium oxysporum* f. sp. *cubense* (E.F. Sm.) W.C. Snyder & H.N. Hansen em *E. elatior* e *Heliconia chartacea* cv. Sex Pink.

Coutinho (2006) abordou uma série de doenças fúngicas que atacam plantas ornamentais e seu controle. Em helicônias, de acordo com o processo fisiológico afetado as doenças foram classificadas em: doenças do rizoma e raiz (*Calonectria spathiphylli* El-Gholl, J.Y. Uchida, Alfenas, T.S. Schub., Alfieri & A.R. Chase, *Phytophthora nicotianae* Breda de Haan e *Pythium* sp.) causando podridão em raízes e rizomas de plantas suscetíveis; e, doenças foliares: *C. spathiphylli* causando amarelecimento e secamento das margens da folha, bainha e queimadura dos pecíolos, *Bipolaris* spp. e *B. incurvata*, cujos sintomas nas folhas iniciam-se com pequenas pontuações, que aumentam de tamanho e número, passando a manchas ovais ou irregulares de coloração marrom

claro com bordos escuros e halo amarelado ao redor, atacando também pecíolo, bainha, brácteas e flores, e *Exserohilum rostratum* (Drechsler) Leonard; Suggs, que causa lesões semelhantes ao *Bipolaris* spp.. E como manejo dessas doenças cita a qualidade sanitária do material de propagação, o controle da umidade, a remoção de plantas velhas e sem função, o controle de plantas daninhas, retirada e queima de folhas ou partes da planta atacadas e restos de cultura.

Em outro estudo Coelho; Lins (2002) e Coelho; Warumby (2002), relatam que no Estado de Pernambuco, as doenças em plantas tropicais comumente encontradas são: manchas foliares causadas por *Bipolaris* sp. *Cercospora* sp. e *Curvularia lunata* em helicônia, podridão de rizomas e raízes, associadas aos fungos *Rhizoctonia solani* e *Fusarium oxysporum* (S.F.Smith) em bastão do imperador, murcha bacteriana (*Ralstonia solanacearum* (Smith) Yabuuchi, Kosako, Yano, Hotta & Nishiuchi em helicônias, fitonematoses causadas por *Meloidogyne* sp., *Radopholus* sp. *Helicotylenchus* sp. e *Pratylenchus*, principalmente em alpínias, bastão do imperador, musas e helicônias. A única virose detectada foi observada em *Tapeinochilo ananassae* causando nanismo, clorose das folhas e variegação das inflorescências.

Em estudos detalhados sobre os patógenos causadores de doenças em helicônias no Havaí, Sewake; Uchida (1995) observaram que os fungos encontram-se em maior diversidade, causando lesões nas folhas, flores, podridão de rizoma e raízes, destacando-se *C. spathiphylli*, *Bipolaris* sp., *Exserohilum rostratum*, *Pyriculariopsis* sp., *Cercospora* sp., *Colletotrichum* spp., *Pestalotiopsis* sp., *Phomopsis* sp., *Fusarium* spp., *Pythium* sp. e *P. nicotianae*. Entre as bactérias, apenas *R. solanacearum* foi detectada causando murcha, porém, os fitonematóides associados ao sistema radicular foram mais diversificados, destacando-se *Radopholus* sp. *Meloidogyne* sp., *Rotylenchus reniformis* e *Helicotylenchus* sp.. No bastão do imperador, um dos problemas mais sérios está relacionado com a podridão de rizoma e raízes, provocada por *Rhizoctonia solani*, associadas em cultivos instalados em locais de drenagem inadequada (LAMAS, 1999; COELHO; LINS, 2002).

No Maranhão são escassos os diagnósticos, entretanto, Sardinha et al. (2012) verificou a ocorrência de 16 agentes causais de doenças em flores tropicais na Ilha de São Luís, destacando-se as doenças causadas por fungos. Os agentes causais de doenças fúngicas com maior frequência em espécies da família Heliconiaceae foram *Curvularia*

eragrostides (Henn.), *Pestalotiopsis* sp., *Colletotrichum gloeosporioides* (Penz) e *Curvularia lunata* (Wakker) Boedijn.

1.5 Ativação de defesa a doenças em plantas

Os vegetais produzem grande variedade de compostos orgânicos, conhecidos como metabólitos secundários. Atualmente sabe-se que muitos desses produtos do metabolismo secundário têm funções ecológicas importantes, como atuar na competição planta-planta e na simbiose plantas-microrganismos. Também podem agir como atrativos (odor, cor, sabor) para animais polinizadores e dispersores de sementes. Ou ainda, protegem as plantas contra herbívoros e contra infecção por microrganismos patogênicos (TAIZ; ZEIGER, 2013).

Atualmente, não existe uma solução única para o controle de pragas e doenças na floricultura, o melhor enfoque baseia-se na integração de diferentes estratégias de manejo, incluindo medidas de controle químico, cultural, físico e biológico. Destes, o controle químico ainda é o mais utilizado e eficiente, entretanto é o mais danoso ao meio ambiente. Entre as alternativas de controle atualmente estudadas estão os métodos físicos como a solarização, inseticidas naturais extraídos de plantas, utilização de indutores de resistência e o controle biológico propriamente dito.

A resistência sistêmica adquirida (RSA) ou resistência sistêmica induzida (RSI) são as duas formas conhecidas para explicar o fenômeno da indução de resistência, sendo o processo de indução efetivo, contra amplo espectro de patógenos, associado à produção de proteínas relacionadas à patogênese (PRPs), neste caso desencadeando a RSA (PIETERSE et al., 2014). As várias PRPs produzidas são reconhecidas há algum tempo, muitas possuem atividade microbiana e funcionam como sinalizadoras de indução de resistência (HAMMERSCHMIDT; DANN, 1997). Já a RSI tem como mediadores na sinalização, o ácido jasmônico e o etileno e não ocorre o acumulo de PRPs (VAN LOON, 2006; PIETERSE, et al. 2014). Entre as várias enzimas envolvidas na indução de resistência destacam-se as β-1,3- glucanases, peroxidades, polifenoloxidases e a fenilalanina amônia-liase, que quando ocorre à indução, após contato com agente indutor, aumentam sua atividade na planta (LEON-KLOOSTERZIEL et al., 2005).

Podemos citar como produtos utilizados com indutores o Bion® ou Acibenzolar-S-Methyl (ASM) que é um análogo de ácido salicílico e atua no metabolismo vegetal induzindo processos fisiológicos e bioquímicos (LIMA et al., 2017), regulando rotas metabólicas secundárias ou síntese de compostos de defesa estruturais (GLAZEBROOK, 2005), o que é favorecido pela sua rápida absorção pelos tecidos foliares (FURTADO et al., 2010) e consequente atuação na ativação e acumulo das PR proteínas (LOON; REP; PIETERSE, 2006). O Bion® já vem demonstrando resultados interessantes em diferentes patosistema controlando fitopatógenos, podemos citar o controle da ferrugem e cercosporiose em cafeeiro (FERNANDES et al., 2013), controle de Sigatoka negra em banana (UCHÔA et al., 2014), além de induzir resistência a *Xanthomonas axonopodis* pv. *passiflorae* em maracujazeiro (BORO, 2011). O acibenzolar-S-metil também é utilizado em associação com fungicidas já tendo efeito positivo no combate a ferrugem da soja (CARVALHO, 2013)

O Agro-Mos®, constituído à base de um mananoligossacarídeo fosforilado proveniente da parede celular de *Saccharomyces cerevisiae* Meyen e tem sido utilizado objetivando o controle de doenças pós-colheita (COSTA et.al., 2010; MELO et al., 2016). Resultados de pesquisas ao longo dos anos demonstram que o Agro-Mos® pode controlar fitopatógenos dentre os quais podemos citar *Colletotrichum gloeosporioides* e *Uncinula necator* em videira (GOMES et al., 2007), *Colletotrichum gloeosporioides* e *Fusarium* spp. em mamão (DANTAS et al., 2004), *Fusarium guttiforme* em abacaxi (MELO et al., 2016).

O Quartz® um produto biológico a base de *Bacillus methylotrophicus*, o gênero Bacillus tem sido estudado visando à qualidade nutricional de plantas, promoção de crescimento e controle de doenças (MOREIRA et al, 2013; HARSHAVADHAN et al, 2016). Yánez-Mendizábal; Falconí (2018) relatam que o gênero Bacillus é eficaz para controlar a infecção por antracnose em sementes de tremoço, além de induzir a ativação de enzimas de defesa peroxidase e catalase.

Os fosfitos são uma forma reduzida de fosfatos, estão surgindo como bioestimulador na horticultura e podem atuar no metabolismo vegetal (GOMES-MERINO, 2015). A ação dos fosfitos sobre fitopatógenos já é estudada a algum tempo e algumas publicações evidenciam sua eficácia em reduzir a severidade de doenças em

plantas (MITCHELL; WALTERS, 2004; MANN; KETTLEWELL, 2004; DIANESE et al., 2008; BUFFARA et al., 2013).

Os produtos citados podem ser classificados como fitoestimulantes ou elicitores de natureza biótica ou abiótica, estes podem eficientemente controlar doenças em plantas e frutos de forma direta ou indireta, através da indução de resistência. A eficiência destes elicitores no controle de doenças em plantas tem sido reafirmada em diversos trabalhos (DANTAS; COELHO, 2006; GOMES et al., 2007; GOMES et al., 2011).

1.6 Aspectos pós-colheita de flores tropicais

As flores de corte são um produto extremamente perecível e perdem suas características ótimas para a comercialização de forma muito rápida, principalmente pelo aumento da atividade respiratória, resultando em um maior consumo das reservas energéticas (PROMYOU et al., 2012) consequentemente acelerando os efeitos da senescência. Outra consequência do efeito da senescência, é o aumento das espécies reativas de oxigênio (ERO), que são moléculas danosas a membrana celular (MILLER et al., 2008), entretanto, são produzidas normalmente, mas, quando submetidas a algum tipo de estresse tem seus níveis aumentados, resultando em extravasamento de conteúdo celular e morte (CAO et al., 2010).

As ERO`s não são específicas e reagem com muitas moléculas biológicas como os lipídeos de lipoproteínas e membranas celulares iniciando o que denominamos de peroxidação lipídica. A peroxidação lipídica se inicia quando os lipídeos poli-insaturados das membranas biológicas são atacados por radicais livres e sofrem uma série de reações autocatalíticas, produzindo uma ampla variedade de intermediários e outros subprodutos altamente tóxicos (SINGH et al., 2015). O malondialdeído é um dos subprodutos mais abundantes, servindo como um sinalizador da peroxidação lipídica, a qual implica na perda da integridade da membrana celular, acarretando danos graves, como redução da fluidez e perda da seletividade.

As plantas desenvolveram mecanismos antioxidantes, enzimáticos e não enzimáticos, com o objetivo de combater os efeitos maléficos das ERO's. O mecanismo antioxidande enzimático é constituído por proteínas que catalisam reações químicas e mediam grande variedade de reações bioquímicas que constituem a vida, portanto, são

essenciais para a manutenção adequada de qualquer organismo. As enzimas antioxidantes estão presentes em diferentes compartimentos celulares e contribuem para o controle das ROS em plantas, o que confere um estádio de homeostase redox no sistema. Destacam-se entre as enzimas antioxidantes a superóxido dismutase (SOD, EC: 1.15.1.1), peroxidases (POD, EC 1.11.1.7), catalase (CAT, EC 1.11.1.6) e polifenoloxidase (PPO, EC 1.14.18.1) (BARBOSA et al., 2014).

Cada uma destas enzimas possui um papel específico dentro do sistema antioxidante. Após a colheita das flores, alterações na atividade destas enzimas, podem interferir nos processos fisiológicos, por exemplo a reação responsável pelo escurecimento do tecido vegetal ocorre por meio da ação da polifenoloxidase (PPO) e da peroxidase (POD) na presença de oxigênio. O rompimento das membranas celulares, devido ao envelhecimento ou qualquer tipo de injuria, pode causar uma desestruturação das células unindo essas enzimas e seus substratos fenólicos que se acumulam em diferentes compartimentos celulares (AZEVEDO, 2004). Em tecidos intactos, os compostos fenólicos e as enzimas são separados em diferentes organelas, limitando o escurecimento enzimático. Enquanto os compostos fenólicos estão presentes no vacúolo, a PPO é encontrada nos plastídeos (KOU et al.2015).

A SOD é a primeira enzima na linha de defesa contra EROs e catalisa a dismutação de radicais O_2^- a H_2O_2 e O_2. Em vegetais, três maiores categorias de SOD são descritas e cada uma definida pela utilização de um metal particular como grupo prostético delas (Mn, Fe e Cu/Zn) (VAN CAMP et al., 1996). As enzimas SOD são localizadas em diferentes compartimentos celulares: a isoforma Cu/Zn-SOD é encontrada no cloroplasto, no citosol e nos peroxissomos; a isoforma Fe-SOD, no cloroplasto; enquanto a Mn-SOD é localizada primariamente na mitocôndria (HALLIWELL, 1987; BARBOSA et al., 2014; BROETTO et al., 2014).

Acredita-se que os diferentes produtos avaliados neste trabalho possam interferir nos processos fisiológicos de *Heliconia psittacorum* proporcionando efeitos positivos no controle de manchas foliares, favorecendo a tolerância da planta ao ataque de fitopatógenos. Além disso, os diferentes produtos poderiam reduzir os efeitos negativos das espécies reativas de oxigênio em pós-colheita, aumentando assim a vida útil das hastes florais *Heliconia psittacorum.*

REFERÊNCIA

ALENCAR, B. de; GALERA V. Mercado de flores atinge faturamento esperado para este ano. **Revista Globo Rural**, Junho, 2016. Disponível em: <http://revistagloborural.globo.com/Noticias/Agricultura/noticia/2016/06/mercado-de-flores-atinge-expectativa-de-faturamento-para-o-ano.html> Acesso em: 16 de novembro de 2017.

AZEREDO, H.M.C. **Fundamentos de estabilidade de alimentos.** Fortaleza: Embrapa Agroindústria Tropical, 2004. 195p

BARBOSA, M. R., SILVA, M. M. DE A., WILLADINO, L., ULISSES, C., CAMARA, T. R. Geração e desintoxicação enzimática de espécies reativas de oxigênio em plantas. **Ciência Rural**, v. 44, p.453 - 460, 2014.

BORO, M. C.; BERIAM, L. O. S.; GUZZO, S. D. Induced resistance against xanthomonas axonopodis pv. passiforae in passion fruit plants . **Tropical Plant Pathology**, v. 36, n. 2, p. 74–80, 2011.

BRASIL. Sistema de Análise das Informações de Comércio Exterior, 2017. Disponível em: <http://aliceweb.mdic.gov.br/> Acesso em: 12/10/2017

BROETTO, F. **Métodos de trabalho em bioquímica vegetal e tecnologia de enzimas.** Botucatu: Editora Cultura Acadêmica, 2014. 92f.

BUFARRA, C.R.S; ANGELOTTI, F; TESSMANN, D.J.; *et al*. atividade de fosfito de potassio na pré e pós-infecção de Phakopsora euvitis em folhas de videira. *In*: **Semana: Ciências Agrarias**. Londrina: Semana: Ciências Agrarias, 2013, v. 34, p. 3333–3340.

CAO, S.; HU, Z.; ZHENG, Y.; LU, B. Synergistic effect of heat treatment and salicylic acid on alleviating internal browning in cold-stored peach fruit. Postharvest Biol. Technol. v. 58, p. 93–97, 2010.

CARVALHO, B. O. et al. Action of defense activator and foliar fungicide on the control of Asiatic rust and on yield and quality of soybean seeds. **Journal of Seed Science**, v. 35, n. 2, p. 198–206, 2013.

CASTRO, C. E. F. de, GONÇALVES, C., MOREIRA, S. R., FARIA, O. A. Helicônias brasileiras: características, ocorrência e usos. **Revista Brasileira de Horticultura Ornamental**. V. 17, Nº.1, p. 5-24, 2011.

COELHO, R.S.B.; LINS, S.R.O. Levantamento de doenças em plantas ornamentais tropicais no Estado de Pernambuco. **Fitopatologia Brasileira.** Brasília: v.27, p.181, 2002 (Suplemento).

COELHO, R.S.B.; WARUMBY, J. Doenças em plantas ornamentais tropicais detectadas na Zona da Mata de Pernambuco. **Floricultura em Pernambuco**, Recife, v.1, p.67-69, 2002.

COSTA, J.C.B.; RESENDE, M.L.V.; RIBEIRO JÚNIOR, P.M.; CAMILO, F.R.; MONTEIRO, A.C.A.; PEREIRA, R.B. Indução de resistência em mudas de cacaueiro contra *Moniliophthora perniciosa* por produto à base de mananoligossacarídeo fosforilado. **Tropical Plant Pathology**. Brasília: v.35, n.5, 2010.

COUTINHO, L. N. Aspectos de fungos fitopatogênicos em plantas ornamentais e seu controle. **In**: **Reunião Itinerante de Fitossanidade do Instituto Biológico. Plantas Ornamentais**, 14. **Anais eletrônicos.** Pariquera-Açu-SP, 2006. p. 13-20 Disponível em: <http://www.biologico.sp.gov.br/rifib/XIVRifib/coutinho.PDF >. Acesso em: 20 ago. 2011.

DANTAS, S.A.F.; COELHO, R.S.B. Controle alternativo com indução de resistência. In: OLIVEIRA, S.M.A.; TERAO, D.; DANTAS, S.A.F.; TAVARES, S.C.C.H (Eds.) **Patologia pós-colheita:** frutas olerícolas e ornamentais tropicais. Brasília: Embrapa Informação Tecnológica, 2006. P. 290-350.

DANTAS, S.A.F.; OLIVEIRA, S.M.A.; BEZERRA NETO, E.; COELHO, R.S.B.; SILVA, R..L.X. indutores de resistencia na proteção do mamão contra podridões pós-colheita. **Summa Phytopathologica**, Botucatu, v. 30, n.3, p. 314-319, 2004.

DIANESE, A.C.; BLUM, L.E.B.; DUTRA,J.B.; LOPES, L.F.; SENA, M.C.; FREITAS, L.F. Avaliação do efeito de fosfitos na redução da varíola (*Asperisporium caricae*) do mamoeiro (*Carica papaya*). **Revista Brasileira de Fruticultura**, Jaboticabal, v. 30, n.3, p 834-837, 2008.

FERNANDES, L.H.; RESENDE, M.L.V.; PEREIRA, R.B.; COSTA, B.H.G.; MONTEIRO, A.C.A.; RIBEIRO JUNIOR, P.M. Acibenzolar-s-metil no controle da ferrugem e da cercosporiose do cafeeiro em condições de campo. **Coffe Science**, Lavras, v.8, n.1, p.24-32, 2013.

FURTADO, L.M.; RODRIGUES, A.A.C.; ARAÚJO, V.S.; SILVA, L.L.S.; CATARINO, A.M. Utilização de Ecolife® e Acibenzolar-s-metil (ASM) no controle da antracnose da banana em pós-colheita. **Summa Phytopathologica**, Botucatu, v.36, n.3, p.237-239, 2010.

GASPAROTTO, L.; PEREIRA, J.C.R.; URBEN, A.F.; HANADA, R.E.; PEREIRA, M.C.N. *Heliconia psittacorum*: hospedeira de *Mycosphaerella fijiensis*, agente causal da sigatoka-negra da bananeira. **Fitopatologia brasileira**, Brasília, v.30, p.423-425, 2005.

GLAZEBROOK, J. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. **Annual Review of Phytopathology**. Palo Alto, v.43, p.205-227, 2005.

GOMES, E.C.S.; LEITE, R.P.; SILVA, F.J.A.; CAVALCANTI, L.S.; NASCIMENTO, L.C.; SILVA, S.M. Manejo do míldio e ferrugem em videira com indutores de resistência: produtividade e qualidade pós-colheita. **Tropical Plant Pathology**, Brasilia, v.36, p.332-335, 2011.

GOMES, E.C.S.; PEREZ, J.O.; BARBOSA, J.; NASCIMENTO, E.F.; AGUIAR, I.F. Efeitos de indutores de resistencia na proteção de uva "Italia" e uva de vinho "Cabernet Sauvignon" contra o oídio e míldio no Vale do São Francisco. II Congresso de Pesquisa e Inovação da Rede Norte Nordeste de Educação Tecnologica, 2007. João Pessoa – PB.

GOMES-MERINO, F. C.; TREJO-TELLEZ, L. I. Biostimulant activity of phosphite in horticulture. **Scientia Horticulturae**. v. 196, p. 82–90, 2015.

HALLIWELL, B. Oxidative damage, lipid peroxidation and antioxidant protection in chloroplasts. **Chemistry and Physics of Lipids**, v. 44, p. 327-340, 1987.

HAMMERSCHMIDT, H.; DANN, E. K. Induced resistance to disease. In RECHCIGL, N. A.; RECHCIGL, J. E. (Eds.). **Environmentally safe approaches to crop disease control**. Boca Raton: CRC, 1997. p. 177-199.

HARSHAVADHAN, M. KUMAR, D. P., YATHINDRA, H.A., RAJESH A. M., HONGAL, S. Effect of integrated soil health, nutriente uptake, flower quality and yield of carnation (*Dianthus caryophyllus* L.). **Environment & Ecology**. v. 34, p. 1862-1867, 2016.

IBRAFLOR-INSTITUTO BRASILEIRO DE FLORICULTURA. **Mercado Interno 12.2014**. Holambra, SP: IBRAFLOR, 2015. Disponível em: <http://www.ibraflor.com/publicacoes/vw.php?cod=234>. Acesso em: 15/10/2015.

JUNQUEIRA, A. H.; PEETZ, M. S. Las exportaciones brasileñas de flores y plantas ornamentales crecen más del 124% entre 2001 y 2006. **Horticultura Internacional**, Espanha: v.56, p.76-78, 2007.

KOU, X.; WU, M.; LI, L.; WANG, S.; XUE, Z.; LIU, B.; FEI, Y. Effects of CaCl2 dipping and pullulan coating on the development of brown spot on 'Huangguan'pears during cold storage. **Postharvest Biology and Technology**, v.99, p.63-72, 2015.

LAMAS, A.M. **Plantas ornamentais tropicais e floricultura tropical** [curso de técnicas de cultivo]. Fortaleza: Frutal/Sindifruta, 1999. 58p.

LEON-KLOOSTERZIEL, K.M., VERHAGEN, B.W.M., KEURENTJES, J.J.B., VANPELT, J.A., REP, M., VAN LOON, L.C., PIETERSE, C.M.J. Colonization of the *Arabidopsis rhizosphere* by fluorescente *Pseudomonas* spp. activates a root-specific, ethylene responsive PR-5gene in the vascular bundle. **Plant Molecular Biology**, Dordrecht, v. 57, p. 731-748, 2005

LIMA, M. A. G., PEIXOTO, A. R., BORGES, I. V., SILVA, M. S., BARBOSA, M. A. G., CAVALCANTI, L. S. Indução de Resistência em Mudas de Videira A *Xanthomonas campestris* pv. *Vitícola*. **Rev. Brasileira de Fruticultura**. v. 39, p. 669-679, 2017.

LINS, S. R. O.; COELHO, R. S. B. Ocorrência de doenças em plantas ornamentais tropicais no Estado de Pernambuco. **Fitopatologia Brasileira**, v. 29, n. 3, p. 332–335, 2004.

LINS, S.R.O.; COELHO, R.S.B. Ocorrência de doenças de plantas ornamentais tropicais no Estado de Pernambuco. **Fitopatologia Brasileira**, Brasília, v.29, p.332, 2004.

LOGES, V; TEIXEIRA, M. do C. F.; CASTRO, A. C. R.; *et al*. Colheita, pós-colheita e embalagem de flores tropicais em Pernambuco. **Horticultura brasileira**, v. 23, n. 3, p. 699–702, 2005.

MANN, R.L.; KETTLEWELL, P.S.; JENKINSON, R. Effect of foliar-applied potassium chloride on septoria leaf blotch of winter wheat. **Plant Pathology**, Singapore, v.53, n.5, p.653-659, 2004.

MELO, L. G. DE L., SILVA, E. K. C. E, CAMPOS NETO, J. R. M., LINS, S. R. DE O., RODRIGUES, A. A. C., & OLIVEIRA, S. M. A. DE. Indutores de resistência abióticos no controle da fusariose do abacaxi. **Pesq. agropec. bras.**, Brasília, v. 51, n. 10, p. 1703-1709, 2016.

MILLER, G.; VLADIMIR SHULAEV, V.; MITTLER, R. Reactive oxygen signaling and abiotic stress. **Physiol. Plant**. 133, p. 481 – 489, 2008.

MITCHELL, A.F.; WALTERS, D.R. Potassium phosphate induces systemic protection in barley to powdery mildew infection. **Pest Management Science**, Sussex, v.60, n.2, p.126-134, 2004.

MOREIRA, A. L. de L.; ARAUJO, F. F.de. Bioprospecção de Isolados de *Bacillus* spp. como Potenciais Promotores de Crescimento de *Eucalyptus urograndis*. **Revista Árvore**. v. 37, p.933-943, 2013.

MOSCA, J.L.; QUEIROZ, M.B.; ALMEIDA, A.S.; et al. **Helicônia: Descrição, Colheita e Pós-Colheita.** Fortaleza: Embrapa, Documentos n. 91, 2004. 33p.

NEVES, M. F. PINTO, M. J. A. **Mapeamento e Quantificação da Cadeia de Flores e Plantas Ornamentais do Brasil**. São Paulo: OCESP, 2015. Disponivel em: <http://ocesp.org.br/download/Livro_Mapeamento_e_Quantificacao_Cadeia_de_Flores_FINAL.pdf> Acesso em: 10/10/2017

PIETERSE, Corné M.J.; ZAMIOUDIS, Christos; BERENDSEN, Roeland L.; et al. Induced Systemic Resistance by Beneficial Microbes. **Annual Review of Phytopathology**, v. 52, n. 1, p. 347–375, 2014. Disponível em: <http://www.annualreviews.org/doi/10.1146/annurev-phyto-082712-102340>.

PROMYOU, Surassawadee; KETSA, Saichol; VAN DOORN, Wouter G. Salicylic acid alleviates chilling injury in anthurium (*Anthurium andraeanum* L.) flowers. **Postharvest Biology and Technology**, v. 64, n. 1, p. 104–110, 2012.

SARDINHA, D.H.S, RODRIGUES, A. A. C., DINIZ, N. B., LEMOS, R. N. S. de; SILVA, G. S. da. Fungos e nematóides fitopatogênicos associados ao cultivo de flores tropicais em São Luís - MA. **Summa phytopathol.**, Botucatu , v. 38, n. 2, p. 159-162, 2012.

SARDINHA, D.H.S. **Flores tropicais**: ocorrência de doenças e nível de resistência a fitopatógenos detectados na Ilha de São Luís, Maranhão. Monografia (Graduação em Agronomia) São Luís: UEMA, 2008.

SEBRAE. Cadeia produtiva da floricultura na grande São Luís: SEBRAE/MA, 2003. 61p.

SEBRAE. Flores e Plantas Ornamentais do Brasil – Serie Estudos Mercadológicos, 2015. Disponível em: <http://www.bibliotecas.sebrae.com.br/chronus/ARQUIVOS_CHRONUS/bds/bds.nsf/7ed114f4eace9ea970dadf63bc8baa29/$File/5518.pdf> Acesso em: 14/10/2017

SEWAKE, K.T.; UCHIDA, J.Y. **Diseases in heliconia in Hawaii**. Honolulu: University of Hawaii-Research Extension, 1995. 18p.

SINGH, M.; KAPOOR, A.; BHATNAGAR, A. Oxidative and reductive metabolism of lipid-peroxidation derived carbonyls. **Chemico Biological Interactions**, v. 234, p. 261-273, 2015.

TAIZ, L.; ZEIGER, E. Metabólitos Secundários e Defesa Vegetal. In: ____. Fisiologia Vegetal. 5ed. p.369-400. Porto Alegre: Artmed, 2013

UCHÔA, C.N.; POZZA, E.A,; UCHÔA, K.S.A.; RIBEIRO JUNIOR, P.M.; TOYOTA, M.; MORAES, W. S.; FREITAS, M. L. O.; SILVA, B.M. Acibenzolar-S-metil e silício como indutores de resistência à Sigatoka-negra em bananeira cultivar Grand Naine (AAA). **Revista Agrarian**. Dourados, v.7, n.24, p.189-196, 2014.

VAN CAMP, W.; CAPIAU, K.; MONTAGU, M.V.; INZE, D.; SLOOTEN, L. Enhancement of oxidative stress tolerance in transgenic tobacco plants over producing Fe-superoxide dismutase in chloroplasts. **Plant Physiology**, v. 12, p. 1703-1714, 1996.

VAN LOON, L.C.; REP, M.; PIETERSE, C.M.J. Significance of Inducible Defense-related Proteins in Infected Plants. **Annual Review of Phytopathology**. Palo Alto. v. 44, n. 1, p. 135–162, 2006. Disponível em: <http://www.annualreviews.org/doi/10.1146/annurev.phyto.44.070505.143425>.

VILLELA, G. Plantas tropicais: flores que encontram o mundo. **Panorama Rural**. São Paulo: PC&Baldan v.1, p. 42-48, 1999.

YÁNEZ-MENDIZÁBAL, V.; FALCONÍ, C. E. Efficacy of Bacillus spp. to biocontrol of anthracnose and enhance plant growth on Andean lupin seeds by lipopeptide production. **Biological Control**, v. 122, p. 67–75, 2018.

Capitulo II - Inducing Resistance in Heliconia psittacorum cv. Golden Torch to Naturally Occurring Leaf Diseases

Journal of Agricultural Science; Vol. 10, No. 12; 2018
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Inducing Resistance in *Heliconia psittacorum* cv. Golden Torch to Naturally Occurring Leaf Diseases

D. H. S. Sardinha[1,2], A. A. C. Rodrigues[2], J. R. M. Campos Neto[1], L. G. L. Melo[3], L. J. M. G. Oliveira[2], F. A. S. Diniz[2] & D. S. Paz[2]

[1] Instituto Federal Ciência e Tecnologia do Maranhão, Caxias, Maranhão, Brazil

[2] Universidade Estadual do Maranhão, São Luis, Maranhão, Brazil

[3] Verde Planta Ltda, Brazil

Correspondence: D. H. S. Sardinha, Instituto Federal de Educação, Ciência e Tecnologia do Maranhão, 65600-505, Caxias, Maranhão, Brazil. Tel: 559-8981-268-217. E-mail: diogosardinha@ifma.edu.br

Received: August 17, 2018 Accepted: September 29, 2018 Online Published: November 15, 2018

doi:10.5539/jas.v10n12p385 URL: https://doi.org/10.5539/jas.v10n12p385

Abstract

One of the bottlenecks to heliconia production is leaf diseases, which have the main characteristic of forming necrotic spots, that can reduce photosynthesis, damage the heliconia bracts and make the flowers unsuitable for commercialisation. The objective of the present study was to identify inducers capable of inducing resistance in *H. psittacorum* cv. Golden Torch, assessing by enzymatic action the reduction in the severity of the fungus complex associated to the cultivation of this species and verifying the action of the severity in relation to the net photosynthesis rate of the plants. The inducers were applied to the plants using a back spray and during application the plots were protected to prevent the products from drifting. The concentrations applied were Agro-Mos® (10 ml L^{-1}), Bion® (0.5 g L^{-1}), Quartz® (40 ml L^{-1}), potassium phosphite (3 g L^{-1}) and calcium phosphite (3 g L^{-1}). During the experimental cultivation, the severity caused by the fungus complex and the photosynthetic rate of the plants were assessed and plant material was collected to analyse the enzymatic activity. The results indicated that the inducers Bion® and the phosphites applied reduced the fungus complex severity, increased the activity of peroxidase, polifenoloxidase and β-1.3 gluconase but did not cause alterations in the net photosynthesis rate of the plants. The value found for $\beta > 1$ suggested that the visual estimation of the fungus complex severity is a good visual indicator of the effect of the plant pathogens on the host photosynthetic rate. The results are concrete responses to producers in the sector on management alternatives for diseases associated to heliconia cultivation.

Keywords: tropical flowers, disease management, enzymatic action, photosynthetic rate

1. Introduction

Heliconia are tropical herbaceous plants, belonging to the family Heliconiaceae. They are popularly known and appreciated because the blossoms have a wide range of colours and shapes (Taniguchi, Castro, T. F. Silva, E. B. da Silva, & Martins, 2016). One of the bottlenecks to heliconia production is the occurrence of leaf diseases, which have the main characteristic of forming dark necrotic spots but can also damage the heliconia bracts and prevent their commercialisation. The leaf spots can also reduce photosynthesis because, according to Xavier et al. (2015), the main visual effect of leaf spot causing pathogens is reduced healthy, photosynthesising leaf area.

In the state of Maranhão, Brazil, diagnosis by Sardinha et al. (2012) indicated the occurrence of a fungus complex, causer of leaf spots, associated to species of Heliconia, including the fungal diseases caused by *Curvularia eragrostides* (Henn.), *Curvularia lunata* (Wakker) Boediin, *Pestalotiopsis* sp. and *Colletotrichum gloeosporioides* (Penz) frequently found in species of the family Heliconiaceae. Currently there is no single solution to control pests and diseases in floriculture and the best approach is based on integrating different management strategies, including chemical, crop, physical and biological control measures.

Management alternatives include the use of inducers that can induce plant resistance to plant pathogens. These inducers include Bion® or Acibenzolar-S-Methyl (ASM) that is a salicylic acid analog and acts on the plant metabolism inducing physiological and biochemical processes (Lima et al., 2017). Agro-Mos®, constituted on the base of a phosphorylated mannanoligosaccaride from the cell wall of *Saccharomyces cerevisiae* Meyen, has

been used to control post harvest diseases (Costa et al., 2010; Melo et al., 2016). Quartz® is a biological product based on *Bacillus methylotrophicus*, the genus Bacillus has been studied for plant nutritional quality, growth promotion and disease control (Harshavadhan, Kumar, Yathindra, Rajesh, & Hongal, 2016; Moreira & Araújo, 2013). Lastly the phosphites are a reduced form of phosphates and are suggested as biostimulators in horticulture that act on the plant metabolism (Gómez-Merino & Trejo-Téllez, 2015). These products may influence the activity of some enzymes that are in directly associated to plant defence processes, including the enzymes β-1.3-glucanases, peroxidases and polyphenol oxidases.

In this context the objective of the present study was to identify inducers capable of inducing resistance in *H. psittacorum* cv. Golden Torch, assessed by enzymatic action and reduced severity of the fungus complex associated to the cultivation of this species, and to verify the action of the severity on the net photosynthesis rate of the plant.

2. **Material and Methods**

2.1 Experimental Field

The experiments were carried out at the Nucleus of Agronomic Biotechnology at the State University of Maranhão, Brazil (2°30′ S and 44°18′ W), where *H. psittacorum* cv. Golden Torch plants were cultivated in soil classified as dystrophic sandy red yellow Argissolo (EMBRAPA, 2013), corrected to pH 6 by applying limestone and fertilized monthly with cattle manure (0.2 kg/m^2). The cultivation was standardized by drastic pruning in all the area planted with *H. psittacorum* cv. Golden Torch and 30 days later the experiment was started by applying inducers to control the naturally occurring leaf plant pathogens associated to the culture. The inducer applications were repeated every 20 days, totaling five applications during the experiment. The inducers were applied using a back spray and during application the treatment plots were protected to prevent the products from drifting. The concentrations applied were: Agro-Mos® (10 ml L^{-1}), Bion® (0.5 g L^{-1}), Quartz® (40 ml L^{-1}), potassium phosphite (3 g L^{-1}) and calcium phosphite (3 g L^{-1}).

During the experiment, the severity of the leaf spots nd the net photosynthetic rate of the plants were assessed and plant material was collected to analyse the enzymatic activity.

2.2 Identification of Naturally Occurring Plant Pathogens in the Experimental Area

Leaves with disease symptoms were collected in the experimental area throughout assessment period. The collected material was isolated and identified according to Sardinha et al. (2012). Due to the similar symptomology of the various diseases associated to *H. psittacorum* cv. Golden Torch cultivation the term fungus complex was used for the group of plant pathogens identified in experimental area.

2.3 Determining Leaf Spot Severity and Net Photosynthesis

Leaf spot severity and net photosynthesis were assessed at 30, 60 and 120 days after the start of the experiment. All treatments were compared to each other to demonstrate the results. To quantify the leaf spot, four leaves with similar physiological age were randomly collected with various infection levels. All the material was scanned and the lesions quantified using the software WinDias-Image Analysis System.

The gas exchanges of the *H. pisitacorum* cv. Golden Torch leaves were measured using the Li-6400 XT infrared gas analyser (LI-Cor, Lincoln, NE, USA), in response to irradiance of 2000 μmol photons m^{-2} s^{-1} and 400 μmol mol^{-1} CO_2. Measurements were only taken between 8 a.m. and 10:30 a.m. and on uniformly sunny days to minimize the sources of diurnal heterogeneity.

2.4 Severity X Leaf Photosynthesis

The relation between the fungus complex severity and the relative net photosynthetic rates (ratio between the net photosynthesis of diseased leaves and the mean net photosynthetic rate of healthy leaves, P_x/P_o) was determined by the model by Bastiaans (1991). The β values were estimated by the equation $P_x/P_o = (1 - x)^{\beta}$. The T test was carried out to verify whether the β values differed from the unit.

2.5 Enzymatic Analyses

Plant material for the enzymatic analysis was collected 0, 24, 48, 96 and 120 hours after applying the products, 50 days after the drastic pruning. Immediately after collection, the samples were frozen using liquid nitrogen, identified and kept in an ultrafreezer at -80 °C until the assessments.

2.5.1 Obtaining the Extract for Enzyme Quantification

The extraction was made according to the methodology adapted by Simões et al. (2015). Liquid nitrogen was used to homogenize 1 g fresh leaf tissue in 13 mL potassium phosphate buffer 0.2 mol (pH 6.0) previously kept

at 4 ºC. The extract was centrifuged at 13.000 × *g* for 21 minutes at 4 ºC. The extract was stored in an ultrafreezer at -80 °C until the analyses were made.

2.5.2 Peroxidase (POD, EC: 1.11.1.7) and Polyphenol Oxidase (PPO, EC: 1.10.3.1) Activity

The POD trial was determined by adding 300 µL of the supernatant to the reaction medium containing 1000 µL phosphate buffer 0.2 mol (pH 6.0), 100 µL guaiacol (0.5%) and 100 µL hydrogen peroxide (0.08%). The readings were made on a spectrophotometer at 470 nm and 25 ºC, for three minutes. The peroxidase activity was calculated based on the molar extinction coefficient of 26.6 mM cm^{-1} for guaicol, and expressed in µmol g^{-1} min^{-1}. (MF)

The PPO trial was determined by adding 50 µL of the supernatant to reaction medium containing 1650 µL phosphate buffer 0.2 mol (pH 6.0) and 1300 µL catechol (0.2 mol). The readings were made in a spectrophotometer at 425 nm and 25 ºC, for two minutes. The PPO activity was calculated based on the molecular extinction coefficient of 34 mmol cm^{-1} for catechol and expressed in µmol g^{-1} min^{-1} (MF).

2.5.3 β-1.3-Gluconase (EC: 3.2.1.39) Activity

β-1.3-gluconase was determined by the dosage of glucose released with lamarine hydrolysis (Tuzun, Rao, Vogeli, Schardl, & Kuc, n.d.). The following were transferred to two test tubes: 25 µL enzymatic extract, 200 µL potassium phosphate buffer (0.2 mol and pH 6.0) and 200 µL laminarine (5 mg mL^{-1}). This material was incubated at 37 ºC for 30 minutes and then 1 mL Somogyi reagent (Smogyi, 1952) and 5 ml deionised distilled water were added and shaken for 10 minutes. After shaking, the material was heated to 100 ºC for 15 minutes and chilled in a ice bath. Then 1 mL Nelson reagent (Smogyi, 1952) and 15 ml deionised distilled water were added and shaken for 15 minutes. The spectrophotometric readings at 760 nm of the samples were compared with glucose standards. The standard glucose curve was prepared by adding the standard, in the same way as the samples, substituting the laminarine with glucose solutions ranging from 0 to 800 mg L^{-1}.

2.6 Statistical Analysis

The experimental field measured 22 × 14 meters and the experimental plots 2 × 2 m, totaling 4 m² and 1 m² central useful area per plot. A completely randomised design was used, placed in random blocks, with four replications. The data found for severity and relative net photosynthesis were correlated and submitted individually to analysis of variance in a factorial scheme (6 products × 3 periods), while the enzymatic activity was also submitted to analysis of variance, but analyzed separately in each period. The means of the parameters, when significant, were compared by the Tukey test ($p < 0.05$), using the software STATISTICA (Stat-Soft, Tulsa, EUA).

3. Results

3.1 Identification of Naturally Occurring Plant Pathogens in the Experimental Area

The following naturally occurring plant pathogens were identified in the experiment: *Curvularia eragrostides* (Henn.) J. A. Mey, *Pestalotiopsis* sp., *Colletotrichum gloeosporioides* (Penz), *Curvularia lunata* (Wakker) Boedijn and *Alternaria* sp. The plant pathogens cited formed the fungus complex associated with *H. psittacorum* cv. Golden Torch cultivation, present in the lesions, that tended to coalesce causing complete drying of the leaves and served as inoculum source for the bract infection.

3.2 Severity

The statistical analysis was significant for the measured factors. Generally the fungus complex severity, associated with *H. psittacorum* cv. Golden Torch cultivation, increased during the three assessment periods. However, the fungus complex was shown to expand its colonisation differentially when plants treated with inducers were compared with the control treatment (Figure 1).

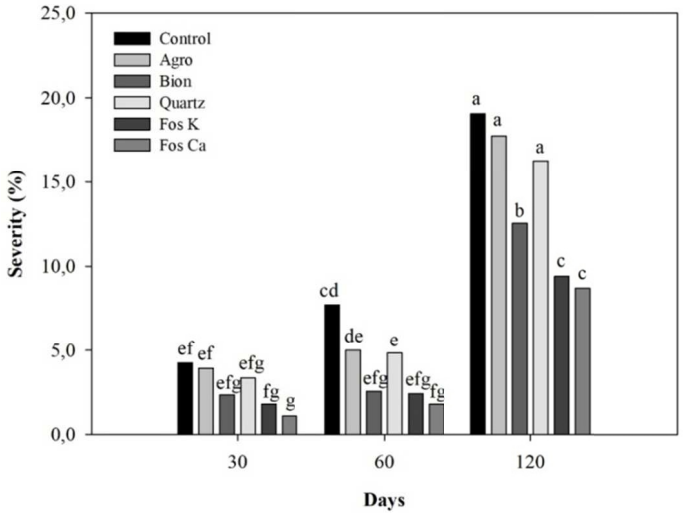


Figure 1. Severity of leaf diseases associated with *Heliconia psittacorum* cv. Golden Torch treated with Agro-Mos® (Agro), Bion®, Quartz®, potassium phosphite (Fos K) and calcium phosphite (Fos Ca). The same letters indicate equality among the treatments by the Tukey test (5%)

Among the inducers used, calcium phosphite differed statistically from the control treatment throughout the assessment period. Starting at the second assessment, potassium phosphite and Bion® were also different from the control. Notably, the plants treated with calcium phosphite, potassium phosphite and Bion® presented reduced severity at the end of the assessments, compared to the other treatments.

3.3 Leaf Photosynthesis

The statistical analysis was significant only for the assessment period. Generally a decrease was observed in the photosynthetic rate, especially in the 60-120 day interval in the assessments. However, the inducers assessed did not cause significant alterations in the photosynthetic rate compared to the control (Figure 2).

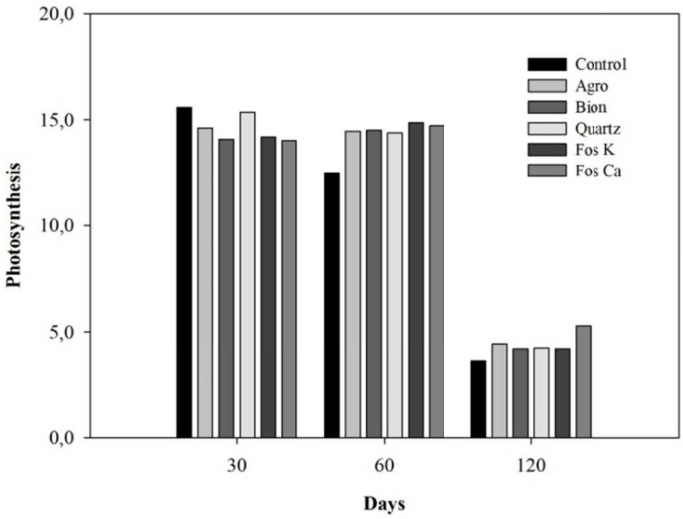


Figure 2. Photosynthesis measured in *Heliconia psittacorum* cv. Golden Torch plants treated with Agro-Mos® (Agro), Bion®, Quartz®, potassium phosphite (Fos K) and calcium phosphite (Fos Ca)

3.4 Severity X Leaf Photosynthesis

Photosynthesis and severity were strongly and negatively correlated (r = -0.8527) and generally leaves with fungus lesions had a lower photosynthetic rate compared to healthy leaves (Figure 3).

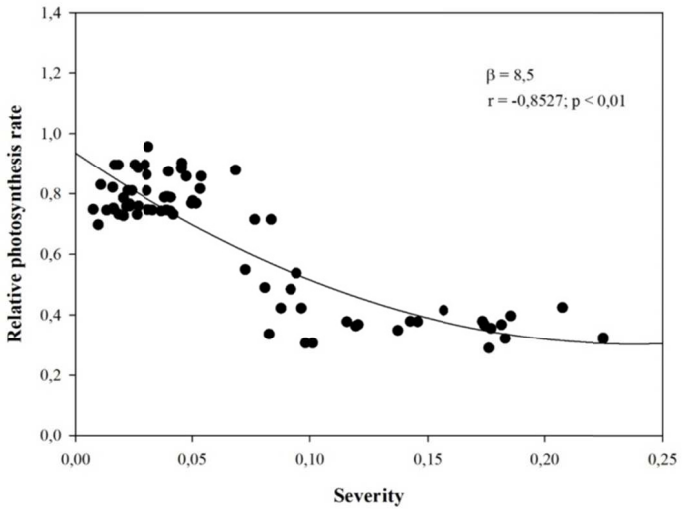


Figure 3. Relative net photosynthetic rate (Px/Po) of *Heliconia psittacorum* Golden Torch leaves infected naturally, in function of the magnitude of the fungus complex severity

The maximum severity observed in the *H. psittacorum* cv. Golden Torch leaves was 19.05 %, the mean value observed in the plants of the control treatment (Table 1).

Table 1. Observations, maximum severity, β coefficients and confidence interval, for the fungus complex associated with *Heliconia psittacorum* cv. Golden Torch

Observations	Maximum severity (%)	β Coefficients	Confidence interval of the β coefficient	
			Inferior	Superior
72	19.05	8.5	5.93	11.06

The estimated β (±standard error) parameter was 8.5 (±0.78) ($p < 0.01$) for leaves attacked by the fungus complex. The β value obtained for the fungus complex associated to *H. psittacorum* cv. Golden Torch was statistically bigger than 1 ($p < 0.01$).

3.5 Peroxidase (POD, EC: 1.11.1.7) and Polyphenol Oxidase (PPO, EC: 1.10.3.1) Activity

It was usually possible to characterize increase in enzymatic activity for the enzymes peroxidase (POD) and polyphenol oxidase (PPO) in inducer treated plants. The POD enzyme performance was characterised by increase in activity after 24 hours, when the potassium phosphite stood out, but the activity peak of this enzyme in plants treated with inducers occurred 48 hours after the applications, for most of the treatments, highlighting Bion®, calcium phosphite and potassium phosphite, in this order, that presented bigger enzymatic activity, differing statistically from the control treatment and other inducers (Figure 4). The other inducers, Quartz® and Agro-Mos®, presented POD activity peak 96 hours after application, and differed statistically from the control treatment, but were not different compared to Bion®, calcium phosphite and potassium phosphite.

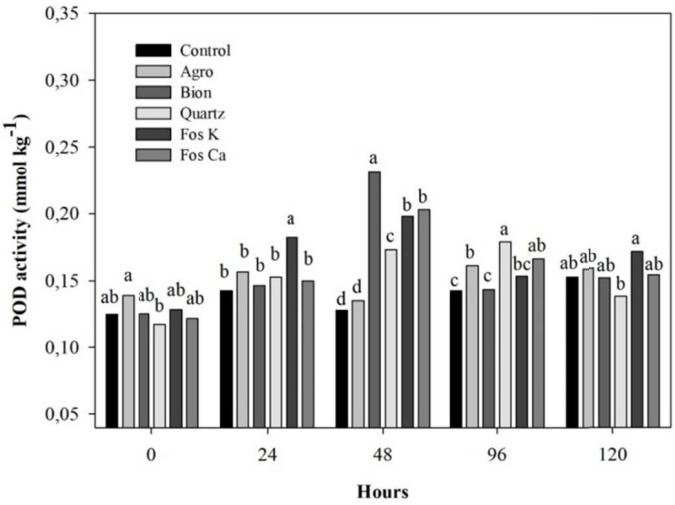


Figure 4. Peroxidase activity associated to *Heliconia psittacorum* cv. Golden Torch treated with Agro-Mos® (Agro), Bion®, Quartz®, potassium phosphite (Fos K) and calcium phosphite (Fos Ca)

The PPO enzyme presented pronounced activity peak 48 hours after applying inducers. Plants treated with calcium phosphite, Bion® and potassium phosphite, in this order, differed from the other treatments, including the control. The inducers Quartz® and Agro-Mos® presented PPO activity peak 24 hours after application and were different statistically from the control treatment, but did not differ compared to the other inducers, calcium phosphite, Bion® and potassium phosphite (Figure 5).

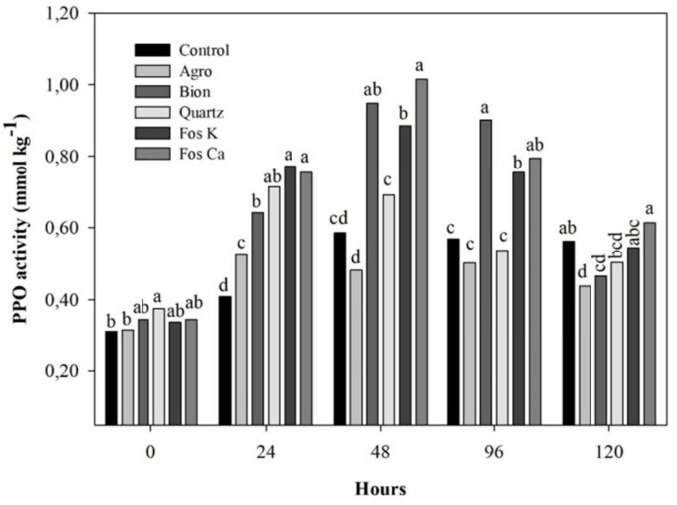


Figure 5. Polyphenol oxidase activity associated with *Heliconia psittacorum* cv. Golden Torch treated with Agro-Mos® (Agro), Bion®, Quartz®, potassium phosphite (Fos K) and calcium phosphite (Fos Ca)

3.6 β-1.3-Glucanase Activity

Generally, β-1,3-glucanase activity increased during the period assessed, but an activity peak could not be characterized for all the inducers tested. Only Bion® presented an activity peak statistically bigger than the control treatment and only 96 hours after application (Figure 6).

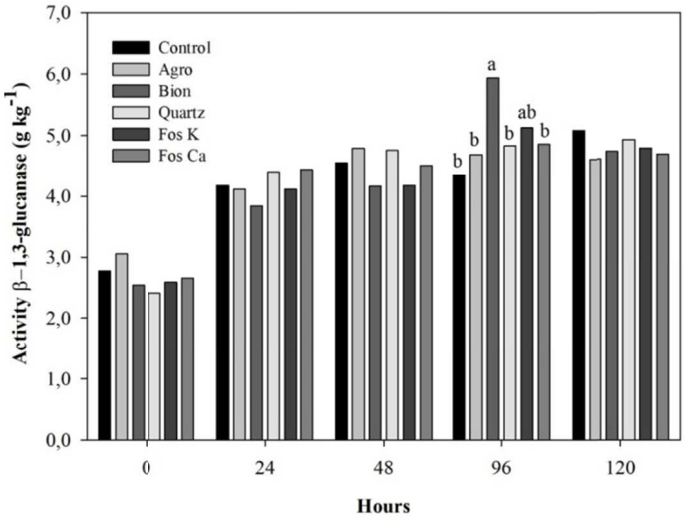


Figure 6. β-1,3-glucanase activity associated to *Heliconia psittacorum* cv. Golden Torch treated with Agro-Mos® (Agro), Bion®, Quartz®, potassium phosphite (Fos K) and calcium phosphite (Fos Ca)

4. Discussion

The naturally occurring plant pathogens identified during the experiment had already been reported in tropical flower cultivation on the island of São Luís, Brazil, as causal agents of leaf diseases in heliconia species (Sardinha et al., 2012) and can be considered a fungus complex causer of diseases in *H. psittacorum* cv. Golden Torch.

According to the results found, the inducers calcium phosphite, potassium phosphite and Bion® were able to reduce damage caused by the fungus complex, possibly ensuring better conditions for the plant to produce higher quality flowers. The reduction in severity resulting from the phosphites probably involved direct control of the fungus complex because the fungitoxic action of phosphite was studied previously in several pathogen systems over the years (Araújo, Valdebenito-Sanhueza, & Stadnik, 2010; Dianese et al., 2008; Sobrinho et al., 2016; Spolti, Valdebenito-Sanhueza, Campos, & Del Ponte, 2015), including the genus Colletotrichum (Oliveira, Pinto Viana, & Valentim Martins, 2016) that is frequently found in the fungus complex associated to *H. psittacorum* cv. Golden Torch cultivation.

In addition to direct control, it can be suggested that the phosphites induced resistance in *H. psittacorum* cv. Golden Torch plants because peroxidase and polyphenol oxidase activity increased compared to the control treatment. The use of inducers and plant stress can cause increase peroxidase activity and consequently cell protection against oxidative reactions (Anterola & Lewis, 2002). Increases in the percentages of peroxidase activity were also been detected in peachtree plants with early death symptoms, consequence of the situation of physiological stress caused by the disease (Marafon, Herter, Bacarin, & Hawerroth, 2009), since the infectious action caused by the pathogen can result in hydrogen peroxide synthesis (Furstenberg-Hagg, Zagrobelny, & Bak, 2013). Peroxidase and phenol oxidase are integrated in various physiological processes in the plant, such as lignification, suberization, cell wall component formation and reticulation and senescence (Nascimento & Barrigossi, 2014).

According to Lobato et al. (2011) potassium phosphite leaf applications induced increase in peroxidase and polyphenol oxidase activity in potato tubers, and these alterations were part of the defence mechanism induced by phosphites. Ramezani et al. (2017) suggested that potassium phosphite prepared plants for a rapid, intense response to infection, involving the activation of various defence responses, including the expression of defence genes via the phenylpropanoid route.

The reduction in severity caused by Bion® was not associated to direct control because it showed no or low fungitoxic activity (Barros, Fonseca, Balbi-Peña, Pascholati, & Peitl, 2015; Oliveira et al., 2016), but its rapid absorption by the plant stimulated resistance by interfering in physiological and biochemical processes (Debona et al., 2009). It is also frequently associated to the salicylic acid metabolic paths because it activates genes referent to resistance signalling (Vitti, 2009) and consequently the action of proteins relative to the pathogenesis (PRP`s) (Felipini & Piero, 2013). In the present study, the enzymes assessed, peroxidase, polyphenol oxidase and

β-1,3-gluconase, increased their activity and the plants treated with this inducer characterized the process of resistance induction.

The other inducers, Agro-Mos® and Quartz®, although they acteds efficaciously in other pathogen systems as resistance inducers or even plant pathogen antagonists (Melo et al., 2016; Yánez-Mendizábal & Falconí, 2018), did not reduce the severity of the fungus complex in *H. psittacorum* cv. Golden Torch cultivation.

None of the inducers assessed interfered directly in the net photosynthesis rate of *H. psittacorum* cv. Golden Torch, but the severity and net photosynthesis rate were strongly and negatively correlated, suggesting that fungus infection, if it is not controlled, may reduce net photosynthesis in the plant.

The β value estimated at 8.5 (±0.78) indicated that the plants infected by the fungus complex had photosynthesis damage not only in the lesion area, but also in the apparently healthy region of the leaf. Consequently, the calculated severity is a good indicative of the effect of the fungus complex on photosynthesis in *H. psittacorum* cv. Golden Torch plants. Although the net photosynthetic rate measurements demonstrated that the fungus complex reduced photosynthesis in the remaining green tissue, these measurements did not elucidate the mechanism responsible for the reduction or indicate the localization of this effect. Johnson (1987) considered that pathogen presence in diseased tissue may influence the crop development by reducing the solar radiation interception (RI) by the green matter or by interference in the radiation use efficiency (RUE). This experiment demonstrated that the spots caused by the fungus complex is an example where both effects occur. References were not found for this parameter in this pathogen system. However, this effect has been observed in pathogen systems such as Phaeosphaeria maydis in corn (Godoy, Amorim, & Bergamin Filho, 2001) and Corynespora cassiicola in soybean (Xavier et al., 2015).

These results suggested that when promoting reduction in the fungus complex severity associated to *H. psittacorum* cv. Golden Torch the phosphites, indirectly, permitted the plant to maintain its net photosynthesis rate close to that found in healthy plants, that would probably permit better quality flower production. These results have been confirmed in research already carried out by the authors (data in publication) when the effects were observed of the inducers, potassium phosphite and calcium phosphite, applied in the field, on the quality of *H. psittacorum* cv. Golden Torch flowers post harvest.

5. Considerations

The inducer Bion® and the phosphites applied in the field were efficacious in reducing the fungus complex severity associated to *H. psittacorum* cv. Golden Torch

The net photosynthesis rate was not affected by the inducers assessed, but the fungus complex can reduce the photosynthesis rate as severity increases.

The value found for β bigger than 1, suggests that the visual estimate of the fungus complex severity is a good in visual indicator of the effect of the plant pathogens on the photosynthetic rate of the host.

The results presented here contribute to the understanding of a little studied pathogen system and give concrete responses to tropical flower producers on management alternatives for the diseases associated to heliconia cultivation.

Acknowledgements

We thank the Foundation for Research Support and Scientific and Technological Development of the State of Maranhão (Fundação de Amparo à Pesquisa e Desenvolvimento Científico e Tecnológico do Estado do Maranhão (FAPEMA) for supporting this research.

References

Anterola, A. M., & Lewis, N. G. (2002). Trends in lignin modification: A comprehensive analysis of the effects of genetic manipulations/mutations on lignification and vascular integrity. *Phytochemistry, 61*(3), 221-294. https://doi.org/10.1016/S0031-9422(02)00211-X

Araújo, L., Valdebenito-Sanhueza, R. M., & Stadnik, M. J. (2010). Evaluation of potassium phosphite formulations against *Colletotrichum gloeosporioides in vitro* and for post-infection control of Glomerella leaf spot in apple. *Tropical Plant Pathology, 35*(1), 54-59. https://doi.org/http://dx.doi.org/10.1590/S1982-56762010000100010

Barros, D. C. M., Fonseca, I. C. de B., Balbi-Peña, M. I., Pascholati, S. F., & Peitl, D. C. (2015). Biocontrol of *Sclerotinia sclerotiorum* and white mold of soybean using saprobic fungi from semi-arid areas of Northeastern Brazil. *Summa Phytopathologica, 41*(22), 251-255. https://doi.org/10.1590/0100-5405/2086

Bastiaans, L. (1991). Ratio Between Virtual and Visual Lesion Size as a Measure to Describe Reduction in Leaf Photosynthesis of Rice Due to Leaf Blast. *Phytopathology, 81*(6), 611. https://doi.org/10.1094/Phyto-81-611

Costa, J. D. B., de Resende, M. L. V, Ribeiro, P. M., Camilo, F. R., Monteiro, A. C. A., & Pereira, R. B. (2010). Induction of resistance in cacao seedlings against *Moniliophthora perniciosa* by a phosphorilated mannanoligosaccharide based product. *Tropical Plant Pathology, 35*(5), 285-294.

Debona, D., Figueiró, G. G., Corte, G. D., Navarini, L., Domingues, L. da S., & Balardin, R. S. (2009). Effect of seed treatment with fungicides and acibenzolar-S-methyl in soybean cultivars on Asian rust control and seedlings growth. *Summa Phytopathologica, 35*(1), 26-31. https://doi.org/10.1590/S0100-54052009000100004

Dianese, A. D. C., Blum, L. E. B., Dutra, J. B., Lopes, L. F., Sena, M. C., & De Freitas, L. F. (2008). Evaluation of phosphite applications for severity of papaya black spot (*Asperisporium caricae*). *Revista Brasileira De Fruticultura, 30*(3), 834-837. https://doi.org/10.1590/S0100-29452008000300047

EMBRAPA. (2013). In H. G. dos Santos, P. K. T. Jacomine, L. H. C. dos Anjos, V. Á. de Oliveira, J. F. Lumbreras, M. R. Coelho, … J. B. de Oliveira (Eds.), *Sistema Brasileiro de Classificação de Solos* (3rd ed.). Brasilia: EMBRAPA. Retrieved from http://livimagens.sct.embrapa.br/amostras/00053080.pdf

Felipini, R., & Piero, R. Di. (2013). PR-protein activities in table beet against *Cercospora beticola* after spraying chitosan or acibenzolar-S-methyl. *Tropical Plant Pathology, 38*(December), 534-538. https://doi.org/10.1590/S1982-56762013000600009

Furstenberg-Hagg, J., Zagrobelny, M., & Bak, S. (2013). Plant Defense against Insect Herbivores. *International Journal of Molecular Sciences, 14*(5), 10242-10297. https://doi.org/10.3390/ijms140510242

Godoy, C. V., Amorim, L., & Bergamin Filho, A. (2001). Changes in photosynthesis and transpiration of corn leaves infected by *Phaeosphaeria maydis*. *Fitopatologia Brasileira, 26*(2), 209-215. https://doi.org/10.1590/S0100-41582001000200017

Gómez-Merino, F. C., & Trejo-Téllez, L. I. (2015). Biostimulant activity of phosphite in horticulture. *Scientia Horticulturae*. https://doi.org/10.1016/j.scienta.2015.09.035

Harshavadhan, M., Kumar, D. P., Yathindra, H. A., Rajesh, A. M., & Hongal, S. (2016). Effect of integrated nutrient management on soil health, nutrient uptake, flower quality and yield of carnation (*Dianthus caryophyllus* L.). *Environment & Ecology, 34*, 1862-1867.

Johnson, K. B. (1987). Defoliation, Disease, and Growth: A Reply. *The American Phytopathological Society, 77*(11), 1495-1497.

Lima, M. A. G., Peixoto, A. R., Borges, I. V., Silva, M. S., Barbosa, M. A. G., & Cavalcanti, L. S. (2017). Induction of Resistance to *Xanthomonas campestris* pv. Viticola in Grapevine Plants. *Sociedade Brasileira de Fruticultura, 39*(4). https://doi.org/10.1590/0100-29452017669

Lobato, M. C., Machinandiarena, M. F., Tambascio, C., Dosio, G. A. A., Caldiz, D. O., Daleo, G. R., … Olivieri, F. P. (2011). Effect of foliar applications of phosphite on post-harvest potato tubers. *European Journal of Plant Pathology, 130*(2), 155-163. https://doi.org/10.1007/s10658-011-9741-2

Marafon, A. C., Herter, F. G., Bacarin, M. A., & Hawerroth, F. J. (2009). Atividade da peroxidase durante o período hibernal de plantas de pessegueiro (*Prunus persic*a (L.) Batsch.) cv. jubileu com e sem sintomas da morte precoce. *Revista Brasileira de Fruticultura, 31*(4), 938-942. https://doi.org/10.1590/S0100-29452009000400004

Melo, L. G. de L., Silva, E. K. C. e, Campos Neto, J. R. M., Lins, S. R. de O., Rodrigues, A. A. C., & Oliveira, S. M. A. de. (2016). Abiotic resistance inducers for control of pineapple fusariosis. *Pesquisa Agropecuária Brasileira, 51*(10), 1703-1709. https://doi.org/10.1590/s0100-204x2016001000001

Moreira, A. L. de L., & Araújo, F. F. de. (2013). Bioprospectionof *Bacillus* spp. as potential growth promoters in *Eucalyptus urograndis*. *Revista Árvore, 37*(5), 933-943. https://doi.org/10.1590/S0100-67622013000500016

Nascimento, J. B., & Barrigossi, J. A. F. (2014). The Role of Antioxidant Enzymes in Plant Defense Against Herbivorous Insects and Phytopathogens. *Agrarian Academy, 1*(1), 235.

Oliveira, E. de, Pinto Viana, F. M., & Valentim Martins, M. (2016). Alternatives to fungicides in the control of banana anthracnose. *Summa Phytopathologica, 42*(4), 340-350. https://doi.org/10.1590/0100-5405/2000

Ramezani, M., Rahmani, F., & Dehestani, A. (2017). The effect of potassium phosphite on PR genes expression

and the phenylpropanoid pathway in cucumber (*Cucumis sativus*) plants inoculated with *Pseudoperonospora cubensis*. *Scientia Horticulturae, 225*, 366-372. https://doi.org/10.1016/j.scienta.2017.07.022

Sardinha, D. H. S., Rodrigues, A. A. C., Diniz, N. B., Lemos, R. N. S. de, & Silva, G. S. da. (2012). Fungi and phytopathogenic nematodes associated with tropical flower cultivation in São Luís, Maranhão State, Brazil. *Summa Phytopathologica, 38*(2), 159-162. https://doi.org/10.1590/S0100-54052012000200010

Simões, A. D. N., Moreira, S. I., Mosquim, P. R., Soares, N. D. F. F., & Puschmann, R. (2015). The effects of storage temperature on the quality and phenolic metabolism of whole and minimally processed kale leaves. *Acta Scientiarum. Agronomy, 37*(1), 101. https://doi.org/10.4025/actasciagron.v37i1.18123

Smogyi, M. (1952). Notes on sugar determination. *J Biol Chem, 195*(1), 19-23.

Sobrinho, G. G. R., Rodrigues, G. B., Santos, A., Jesus Junior, W. C. de, Novaes, Q., & De, S. (2016). Effect of potassium phosphite on the mycelial growth and density of *Fusarium solani* in passion flower vine. *Summa Phytopathologica, 42*(2), 180-182. https://doi.org/http://dx.doi.org/10.1590/0100-5405/2139

Spolti, P., Valdebenito-Sanhueza, R. M., Campos, Â. D., & Del Ponte, E. M. (2015). Modo de ação de fosfitos de potássio no controle da podridão olho de boi em maçã. *Summa Phytopathologica,* (9), 42-48. https://doi.org/10.1590/0100-5405/1982

Taniguchi, C. A. K., Castro, A. C. R. de, Silva, T. F., Silva, E. B. da, & Martins, T. da S. (2016). Growth, nutrient accumulation and export by heliconia 'Red Opal.' *Ornamental Horticulture, 22*(3). https://doi.org/10.14295/oh.v22i3.954

Tuzun, S., Rao, M. N., Vogeli, U., Schardl, C. L., & Kuc, J. (n.d.). Induced systemic resistance to blue mold: Early induction and accumulation of beta-1,3-glucanases, chitinases, and other pathogenesis-related proteins (b-proteins) in immunized tobacco. *Phytopathology (USA), 79*.

Vitti, A. J. (2009). *Soybean seed treatment with abamectin, thiabendazole and acibenzolar-S-methyl for nematodes management*. Retrieved from http://repositorio.bc.ufg.br/tede/handle/tde/446

Xavier, S. A., Mello, F. E. de, Canteri, M. G., & Godoy, C. V. (2015). Fotossíntese de folhas de soja infectadas por *Corynespora cassiicola* e *Erysiphe diffusa*. *Summa Phytopathologica, 12*, 156-159. https://doi.org/10.1590/0100-5405/1923

Yánez-Mendizábal, V., & Falconí, C. E. (2018). Efficacy of *Bacillus* spp. to biocontrol of anthracnose and enhance plant growth on Andean lupin seeds by lipopeptide production. *Biological Control, 122*, 67-75. https://doi.org/10.1016/j.biocontrol.2018.04.004

Capitulo III - Phytostimulants Influence the Vase Life of *Heliconia psittacorum* cv. Golden Torch

PHYTOSTIMULANTS INFLUENCE THE VASE LIFE OF *HELICONIA PSITTACORUM* CV. GOLDEN TORCH

ABSTRACT

Many factors influence post-harvesting of tropical flowers, including management of the plant still in the field and oxidative stress, which can be considered the main factor for determining quality during vase life, as it involves complex chemical and physiological changes in the plant. In this context the present study aimed to assess the influence of phytostimulants applied to *Heliconia psittacorum* cv. Golden Torch in quality maintenance during the vase life of this species. The products applied in the field were Bion®, Agro-Mos®, Quartz®, Ca - Fosfitotal® (calcium phosphite) and K - Fosfitotal® (potassium phosphite). The flower stems used during the experiment were harvested in the morning to assess the visual aspect, fresh matter loss, electrolyte extravasation, lipid peroxidation and the soluble carbohydrates and the enzymes peroxidase, polyphenoloxidase, superoxide dismutase and total soluble phenolic compounds were quantified. The results showed a positive influence of the Fosfitotal® in the maintenance of stem quality of *Heliconia psittacorum* cv. Golden Torch during vase life. In the parameters assessed, the Fosfitotal®, especially K - Fosfitotal®, obtained the best scores for the visual aspect, presenting reduced electrolyte extravasation and less lipid peroxidation. These results were confirmed with the quantifications of the enzymes peroxidase, polyphenoloxidase, superoxide dismutase and total soluble phenolic compounds. Treatments in the field, with phytostimulants, are interesting to maintain the stem quality *Heliconia psittacorum* cv. Golden Torch during vase life.

Keywords: Tropical flowers, Oxidative stress, Electrolyte extravasation, Lipid peroxidation, Enzymatic defense.

1. INTRODUCTION

Heliconias are tropical herbaceous plants, belonging to the family Heliconiaceae and are popularly known due to their flowers in a wide range of colors and shapes (Taniguchi et al., 2016). Inflorescences are perishable products and after harvest can have their quality reduced quickly, making it impossible to commercialize them. Quality can be lost naturally through senescence, chlorosis, stem bending, excessive desiccation and transpiration (Folha et al., 2016). Furthermore, plant species interact constantly with external factors, that constantly alter and are potentially harmful (Ncube et al., 2012).

Reactive oxygen species (ROS) are inevitably formed by aerobic organisms (Sáenz et al. 2015). ROS are toxic, but to prevent ROS accumulation in the cells, plants have developed enzymatic and non-enzymatic antioxidant defense systems. The enzymatic defense system consists of several enzymes including superoxide dismutase (SOD), peroxidase (POD) and polyphenoloxidase (PPO) (Barbosa et al., 2014). The first plays a key role in the antioxidant defense system by O_2^- dismutation in H_2O_2 and O_2 and the other to catalyze reactions using H_2O_2 as oxidant and phenolic compounds as electron donors (Barbosa et al., 2014; Blokhina et al., 2003; Scandalios, 2005). The imbalance between ROS production and elimination results in oxidative stress which is partly responsible for the loss of quality during vase life and involves complex chemical and physiological changes, resulting in overproduction and accumulation of reactive oxygen species and consequently, activation of stress or cell death (Sáenz et al. 2015).

Applying phytostimulants to heliconia flowers may influence enzymatic activity in the bract tissue, the percentage of absolute cell membrane integrity and stem weight maintenance in the shelf period (Mangave et al., 2013). Products such as Bion®, Agro-Mos®, Quartz®, Ca - Fosfitotal® (calcium phosphite) and K - Fosfitotal® (potassium phosphite) are used in health management in several plant species and their use while still in the field is a factor that may

influence cut flower post-harvest quality. Bion® or Acibenzolar-S-Methyl (ASM) is an analogue of salicylic acid and acts on the plant metabolism inducing physiological and biochemical processes (Lima et al., 2017). Agro-Mos® is constituted on the base of phosphoryl Mannan oligossacaride derived from the *Saccharomyces cerevisiae* Meyen cell wall and has been used to control postharvest diseases (Costa et al., 2010; Melo et al., 2016). Quartz® is a biological product based on *Bacillus methylotrophicus*, the genus *Bacillus* has been studied for plant nutritional quality, growth promotion and disease control (Harshavadhan et al., 2016; Moreira and Araújo, 2013). The phosphites are a reduced form of phosphates and are used as bioestimulants in horticulture and may act on the plant metabolism (Gómez-Merino and Trejo-Téllez, 2015).

Thus, the present study aimed to assess the influence of the products Agro-Mos®, Bion®, Quartz®, Ca - Fosfitotal® (calcium phosphite) and K - Fosfitotal® (potassium phosphite), applied in the field, on the postharvest quality of *Heliconia psittacorum* cv. Golden Torch flowers during vase life.

2. MATERIAL AND METHODS

Heliconia psittacorum plants were grown in an experimental field at the State University of Maranhão, Brazil, (2°30' S and 44°18' W) in soil classified as Red-yellow dystrophic argissol, sandy texture (EMBRAPA, 2013), corrected to pH 6 by applying lime, fertilized with cattle manure (0.2 kg $m^{2\,-1}$) and sprinkler irrigation daily. During cultivation six groups of plants were sprayed every 20 days for six months with sterile distilled water, Agro-Mos® (10 mL L^{-1}), Bion® (0.5 g L^{-1}), Quartz® (40 mL L^{-1}), Ca - Fosfitotal® (3 g L^{-1}) and K - Fosfitotal® (3 g L^{-1}). Phytostimulants were applied with a backpack sprayer and 2 L syrup volume in all treatments. Concentrations were used according to the manufacturers' recommendations.

The flower stems were harvested at approximately 07:30 AM, 48 h after the last spraying and hydrated for 12 h, when they were standardized to 40 cm length and kept in pots with sucrose *pulsing* at 20 % for 48 h. After this period the solution was replaced with 0,5 L distilled water, renewed every three days. During the experiment the temperature, humidity and photoperiod were maintained constant at 22 ± 2 ºC, RH 70 ± 5 % and 12 h, respectively.

The parameters assessed were the visual aspect, fresh matter loss, electrolyte extravasation and the enzymes peroxidase, polyphenoloxidase, total soluble phenols, superoxide dismutase, soluble carbohydrate content and associated lipid peroxidation. Samples of the bracts for enzyme quantification were collected in five periods throughout the storage period and kept in ultra-freezer at -80 °C. All the data were expressed on a fresh weight basis.

2.1. Visual Aspect

The visual aspect was quantified until the twelfth day of vase life. A subjective grading scale ranged from 1 to 5 (Figure 1). Each grade represented: score 1 - necrosis and a marked darkening on the stem and/or bract; improper for commercialization; score 2 - flower with dark marks on at least two bracts, marked darkening and turgidity loss, improper for commercialization; score 3 - turgidity loss, dryness at the bract tips and coloring loss, minimum quality threshold for commercialization; score 4 - flower with no darkening, start of coloring loss, still improper commercialization; score 5 - up to 3 bracts open and without apparent damage; turgid stem and bracts, bright coloring and perfect flower for commercialization (adapted from de Souza et al., 2008). The final grade represented the average scores assigned by five independent evaluators.

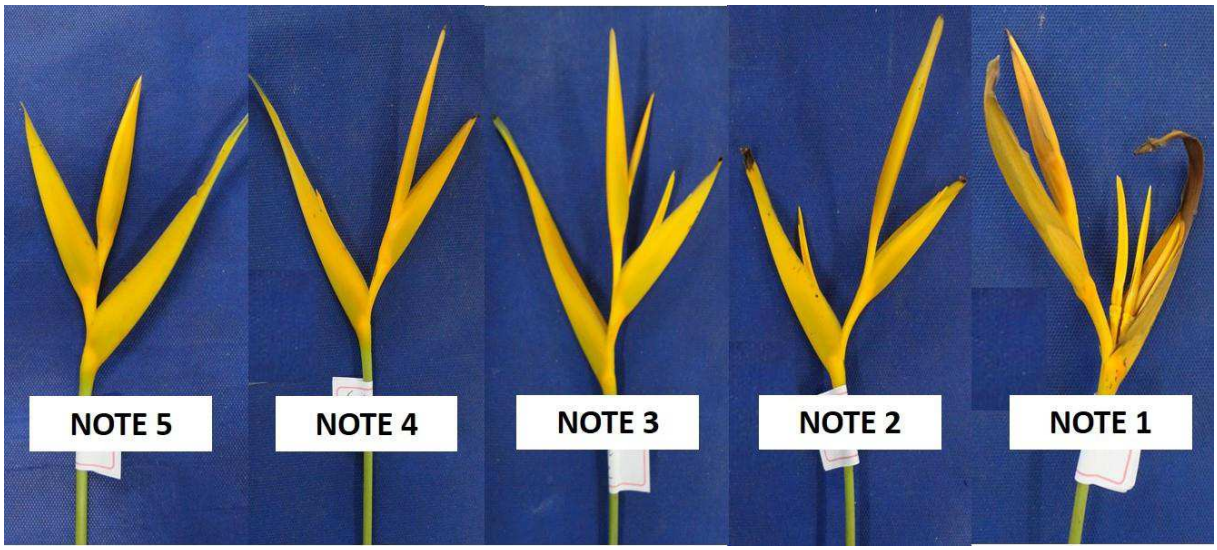


Figure 1. Visual aspect for score assignment.

2.2. Fresh matter loss

The stems were weighed on semi-analytical scales. The dry matter values of the stems were obtained by the percentage difference from the initial dry matter and the dry matter on the day of analysis. The percentage loss of dry matter was determined by the following formula, where: DML: dry matter loss (%), Idm: initial dry matter, Fdm: final dry matter, on the day of analysis.

$$DML: [(Idm – Fdm)/Idma] \times 100$$

2.3.Electrolyte extravasation

Electrolyte extravasation was determined, as described by Shanahan et al. (1990), by weighing 0.3 g of 4 cm discs from the bracts that were immersed in 10 mL deionized distilled water in closed test tubes and incubated for 24 h (Mangave et al., 2013), obtaining the extract called C1 at 25 ºC. Next, the electric conductivity was measured using a conductivity meter. The same tubes were incubated at 100 °C for one hour in a water bath and then kept at ambient temperature until they reached 25 ºC and the C2 extract was obtained, in which the electric

conductivity was again that measured. The electrolyte extravasation was estimated by the ratio below and the result was expressed in percentage.

$$EE = (C1/C2) \times 100$$

2.4.Peroxidase (POD, EC:1.11.1.7) and Polyphenoloxidase (PPO, EC:1.10.3.1) extraction and activity trial

Extraction was made following the methodology adopted by Simões et al. (2015). Homogenization in liquid nitrogen was performed on 0.1 g fresh tissue in 1.3 mL phosphate buffer 0.2 M (pH 6.0) kept previously at 4 ºC. The extract was centrifuged for 21 min at 13.000 x *g* and 4 ºC.

The POD trial was determined by adding 0.3 mL supernatant to the reaction medium containing 1 mL phosphate buffer 0.2 M (pH 6.0), 0.1 mL guaiacol (0.5 %) and 0.1 mL hydrogen peroxide (0.08 %). The readings were made in a spectrophotometer at 470 nm, at 25 ºC, for three minutes. The POD activity was calculated based on the coefficient of molar extinction of 26.6 $mmol^{-1}$ cm^{-1} for guaiacol and expressed in mmol kg^{-1}.

The PPO trial was determined by adding 0.05 mL supernatant to the reaction medium containing 1.65 mL phosphate buffer 0.2 M (pH 6.0) and 1.3 mL catechol (0.2 M). The readings were made in a spectrophotometer at 425 nm, at 25 ºC, for two minutes. The PPO activity was calculated based on the coefficient of molar extinction of 2.47 $mmol^{-1}$ cm^{-1} for catechol and expressed in mmol kg^{-1}.

2.5.Total soluble phenols

The total soluble phenols were quantified following the method by Folin and Ciocalteu (1927). The extraction was made from 0.1 g tissue squashed in a pestle and mortar containing

1.3 mL methanol. The samples then rested for 20 h in the dark at 4 °C. After this period the methanol extract was centrifuged for 21 min at 13.000 x g and 4 ºC. The trial was carried out using 0.15 mL supernatant, 2.4 mL distilled water, 0.15 mL Folin Cioucauteu reagent. The mixture was shaken for three minutes and 0.3 mL sodium carbonate were added. The tubes were kept in the dark at 25 ºC for two hours. The readings were made in a spectrophotometer at 725 nm and the results expressed in g kg^{-1}.

2.6.Superoxide dismutase activity extraction and trial (SOD, EC:1.15.1.1).

Extraction was made using liquid nitrogen and homogenizing 0.15 g tissue in 2 mL phosphate buffer 0.1 M (pH 7.0). The extract was centrifuged for 21 min at 13.000 x g and 4 ºC. The SOD was determined as described by Giannopolitis and Ries (1977). Alequots of 0.1 mL supernatant were added to 1.66 mL phosphate buffer 0.05 M (pH 7.8) containing (0.01 M EDTA and 0.013 M methionine), 0.04 mL riboflavin 0.002 M and 0.2 mL Nitro Blue Tetrazolium chloride (NBT) at 0.75 M.

The reaction medium was kept under light incidence (25 W fluorescent lamp) for six minutes and later read in a spectrophotometric tower at 560 nm. The activity was determined based on the inhibition of NBT reduction, defined as unit of the activity, the positive enzyme necessary to inhibit 50 % photoreduction (Beauchamp and Fridovich, 1971). The activity was expressed in U kg^{-1}.

2.7.Lipid peroxidation – TBARS

Extraction was made using liquid nitrogen and homogenizing 0.5 g tissue in 2 mL trichloroacetic acid - TCA (1 %). The extract was centrifuged for 21 min at 13.000 x g and 4 ºC. To determine the lipid peroxidation, 0.5 mL aliquots of the sample and 2 mL thiobarbituric acid – TBA (20 %) were added to test tubes with buffer. The tubes were kept in a water bath at

100 °C for one hour and were then placed in an ice bath for five minutes. Supernatant absorbance was measured at 532 nm. The ideal for the non-specific absorption at 600 nm was subtracted. The TBARS concentration was calculated using the absorption coefficient of 155 $mmol^{-1}$ cm^{-1} and the results was expressed in mmol kg^{-1}.

2.8.Soluble carbohydrate extraction and determination

The soluble carbohydrates were extracted and determined according to Dubois et al. (1956), using liquid nitrogen and homogenizing 0.05 g of the tissue in 1.3 mL distilled water. The extract was centrifuged for 21 min at 13.000 x g and 4 ºC.

After separating 0.025 mL supernatant, 0.475 mL distilled water were added and placed in test tubes, together with 0.5 mL phenol (5 %) and 2.5 mL sulfuric acid PA. The extract rested for 10 min, and then the test tubes were shaken and kept on a tray containing water at 25 °C for 20 min. The readings were made in a spectrophotometer at 490 nm and the results was expressed in g kg^{-1}.

2.9.Statistical analysis

A randomized complete block design with treatments arranged in split plots was used, where products were allocated to plots and time to sub-plots. The plot useful area comprised twenty-five flower stems and the subplot five flower stems, stored in vases filled with distilled water. The results were submitted to analysis of variance and the means compared by the Tukey test at the 5 % level of probability using the STATISTICA software (Stat-Soft, Tulsa, USA).

3. RESULTS

3.1.Visual Aspects

The scores attributed to the *Heliconia psittacorum* flower stems decreased during the vase life. The flower stems markedly lost points, considering the score scale, accumulating

undesirable characteristics over time. However, in general, the stems treated with phosphite obtained higher visual scores, especially when compared to the control treatment. At the start of vase life, all the treatments received score 5, maximum for quality but starting on the third day the flower stems treated with the K - Fosfitotal® and Ca - Fosfitotal® phosphites already stood out by obtaining higher scores compared to the control, Agro-Mos®, Bion® and Quartz® treatments, that continued throughout the vase life (Figure 2). The other stems treated with Agro-Mos®, Bion® and Quartz® obtained intermediate scores during the assessment, compared to the control and the phosphites, especially the treatments with Bion® and Quartz®, that scored higher than the control, starting on the sixth and third day, respectively. On the 12th day, only the flower stems treated with K - Fosfitotal® obtained score 3, the minimum necessary for commercialization. The scores attributed to the other treatments on the 12th day, considered the stems with dark marks on at least two bracts, darkening and accentuated turgidity loss, making them improper for commercialization according to the cut grade, below 3 used for this in the experiment.

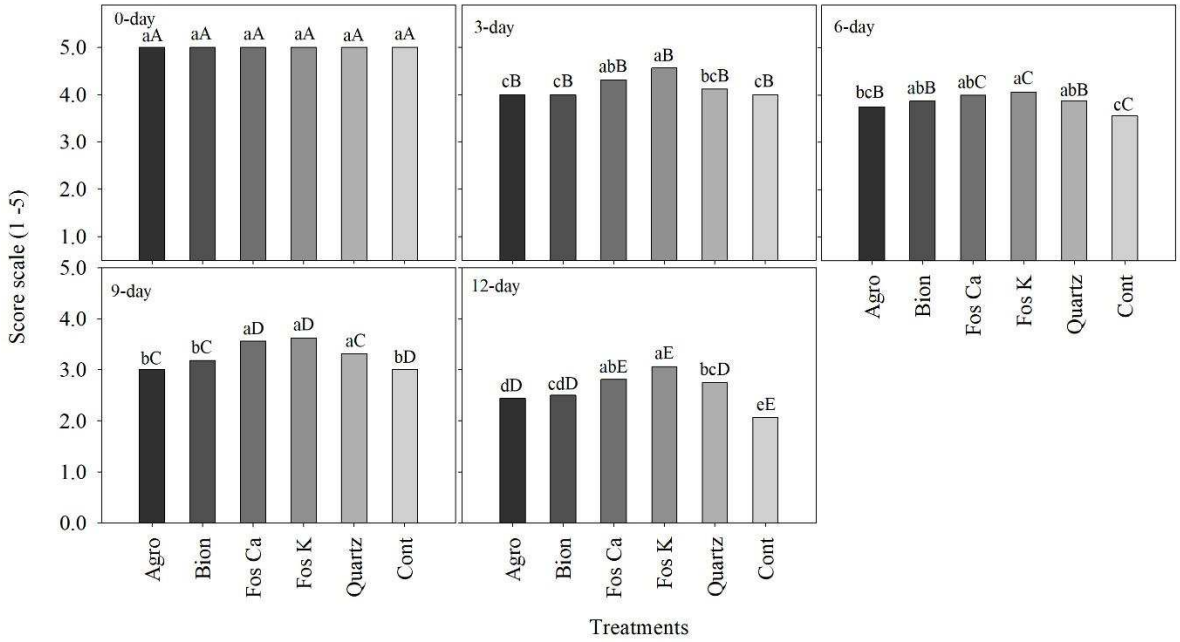


Figure 2. Score scale (scores from 1 to 5) for *Heliconia psitacorum* stems, treated with Agro-Mos® (Agro), Bion®, Quartz®, K - Fosfitotal® (Fos K), Ca - Fosfitotal® (Fos Ca) and distilled water (Cont). The means of the treatments were compared by the Tukey test ($P < 0.05$). Lowercase letters compare the treatments on the day of the evaluation and uppercase letters compare the treatment over the vase life.

3.2.Electrolyte extravasation

Statistical analysis identified a significant interaction between treatments (plot) and time (subplot) for the parameter evaluated. The results showed increased electrolyte extravasation over the assessment period, that indicated bigger deterioration of the bract tissue. In each evaluated period, the stems treated with potassium and calcium Fosfitotal® maintained low electrolyte extravasation levels compared to the control (Figure 3). At the start of vase life Fosfitotal®- treated stems presented reduced electrolyte extravasation and among these, the Ca - Fosfitotal® was best. On the third and sixth vase life days, stems treated with Quartz® and Bion® also maintained electrolyte extravasation levels compared to the control and up to the

12th vase life day it was possible to characterize the Fosfitotal® as the best treatments for this parameter. The stems treated with Agro-Mos® did not show reduced electrolyte extravasation compared to the control except in an isolated form on the 6th vase life day.

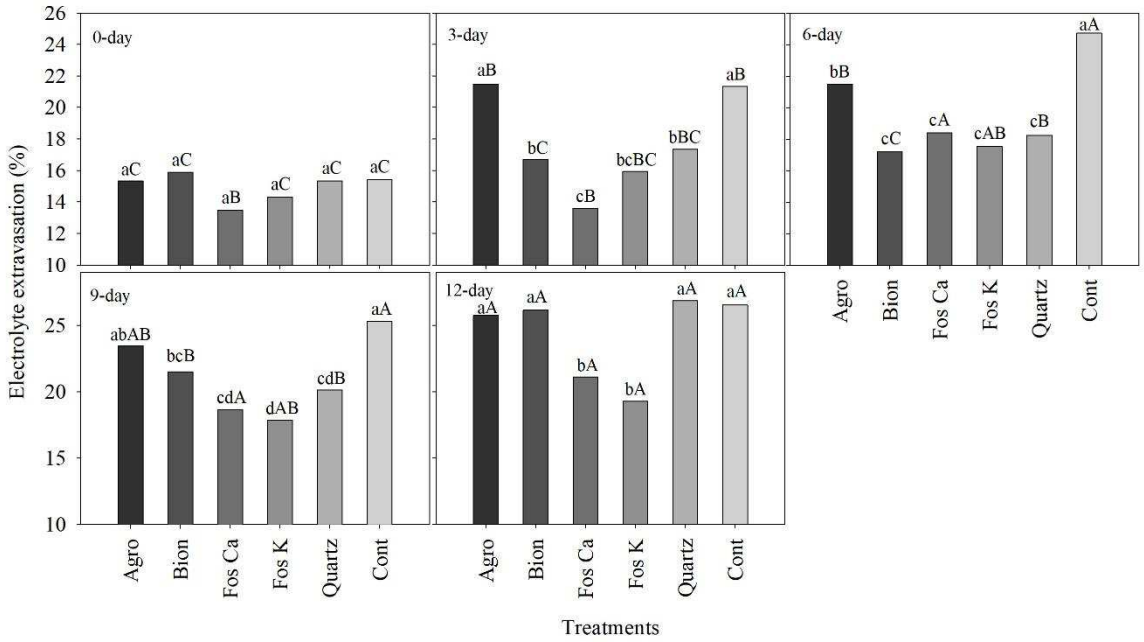


Figure 3. Electrolyte extravasation (%) in *Heliconia psitacorum* stems, treated with Agro-Mos® (Agro), Bion®, Quartz®, K - Fosfitotal® (Fos K), Ca - Fosfitotal® (Fos Ca) and distilled water (Cont). The means of the treatments were compared by the Tukey test ($P < 0.05$). Lowercase letters compare the treatments on the day of the evaluation and uppercase letters compare the treatment over the vase life.

3.3.Fresh matter loss

Statistical analysis did not identify significant interaction between treatments (plot) and time (subplot) for the parameter evaluated. Only time significantly influenced the treatments. Fresh matter loss in the *H. psittacorum* stems increased throughout the assessment period, but no statistical difference was observed between the treatments for this parameter (Figure 4).

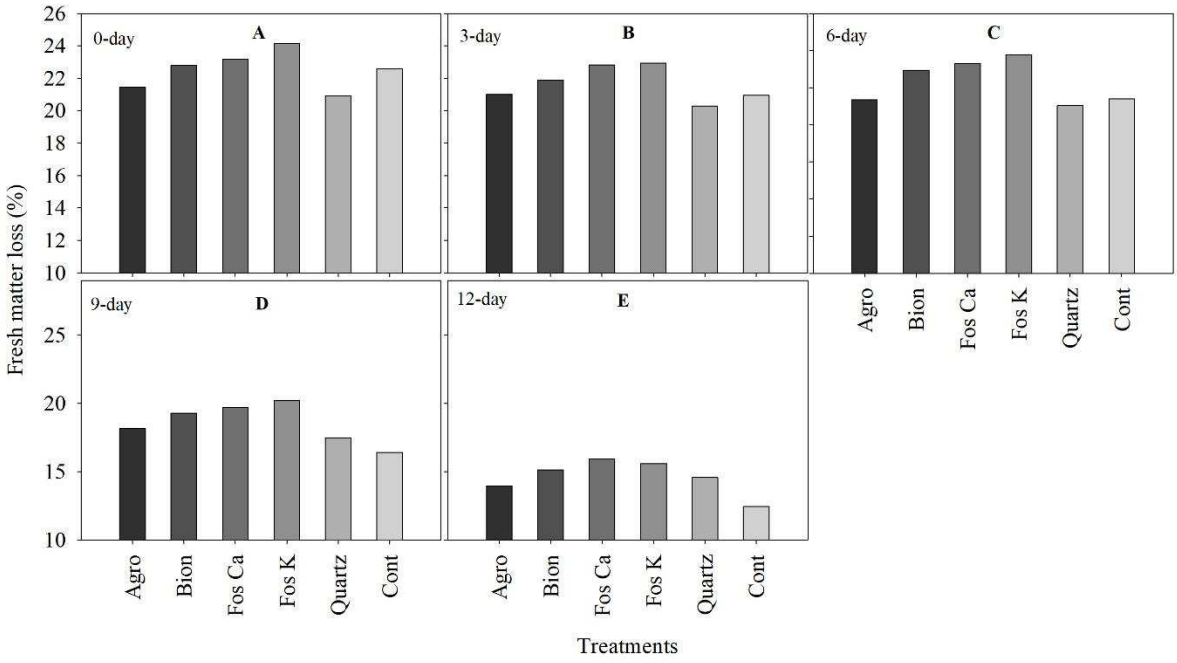


Figure 4. Fresh matter loss in *Heliconia psitacorum* stems, treated with Agro-Mos® (Agro), Bion®, Quartz®, K - Fosfitotal® (Fos K), Ca - Fosfitotal® (Fos Ca) and distilled water (Cont). The means of the treatments were compared by the Tukey test ($P < 0.05$). The letters compare the different periods of evaluation throughout the vase life.

3.4.Determining soluble carbohydrates

Statistical analysis identified a significant interaction between treatments (plot) and time (subplot) for the parameter evaluated. The soluble carbohydrate content in the *H. psittacorum* flower stems decreased throughout the vase life, that indicated reserve consumption, to maintain the physiological functions.

Figure 5 shows that at the start of the experiments the Fosfitotal® - treated stems already presented a higher carbohydrate concentration than the other treatments, including the control. In the following periods, the treatments with Fosfitotal® continued to present higher levels of

carbohydrates in comparison with the other treatments but were equal to them only in the last evaluated period.

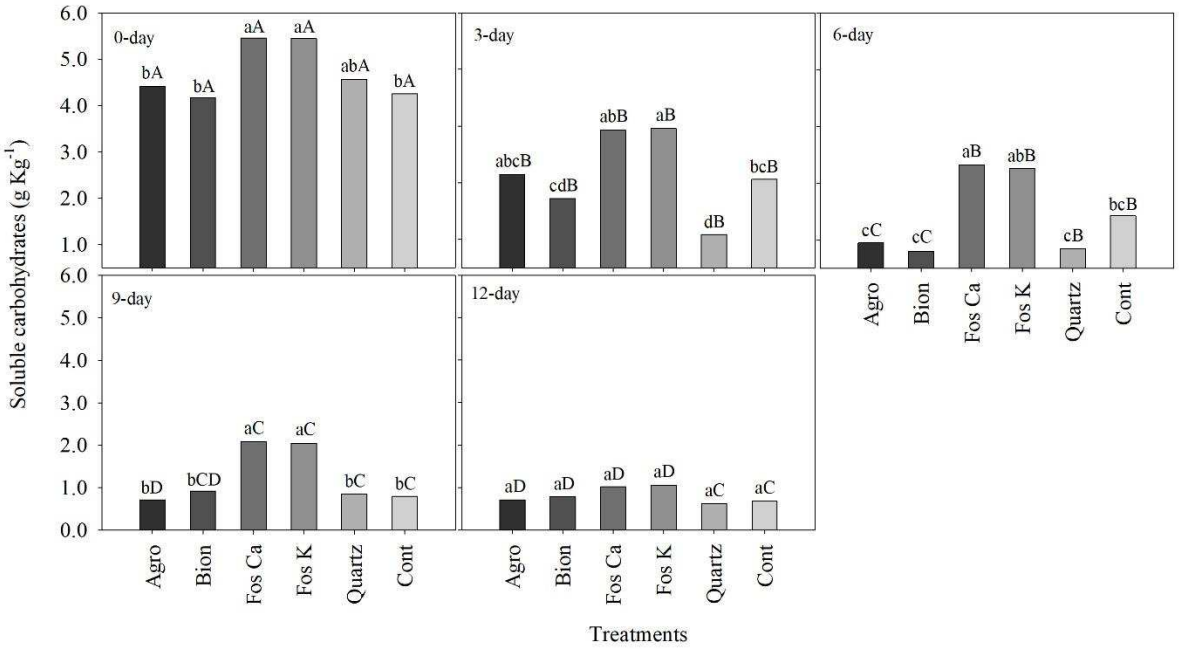


Figure 5. Soluble carbohydrate in *Heliconia psitacorum* stems, treated with Agro-Mos® (Agro), Bion®, Quartz®, K - Fosfitotal® (Fos K), Ca - Fosfitotal® (Fos Ca) and distilled water (Cont). The means of the treatments were compared by the Tukey test ($P < 0.05$). Lowercase letters compare the treatments on the day of the evaluation and uppercase letters compare the treatment over the vase life.

3.5.Peroxidase (POD, EC:1.11.1.7) and Polyphenoloxidase (PPO, EC:1.10.3.1) activity

The statistical analysis identified a significant interaction between treatments (plot) and time (subplot) for POD and PPO enzymes. POD activity in *H. psittacorum* flower stems increased during the period assessed and peaked between the sixth and ninth days but decreased on the 12th day in the vase for treatments with Fosfitotal® and Quartz® (Figure 6). The treatments evaluated differed only on the 12th day in the vase, when Ca - Fosfitotal® and K - Fosfitotal® presented lower activity of this enzyme in relation to the control treatments.

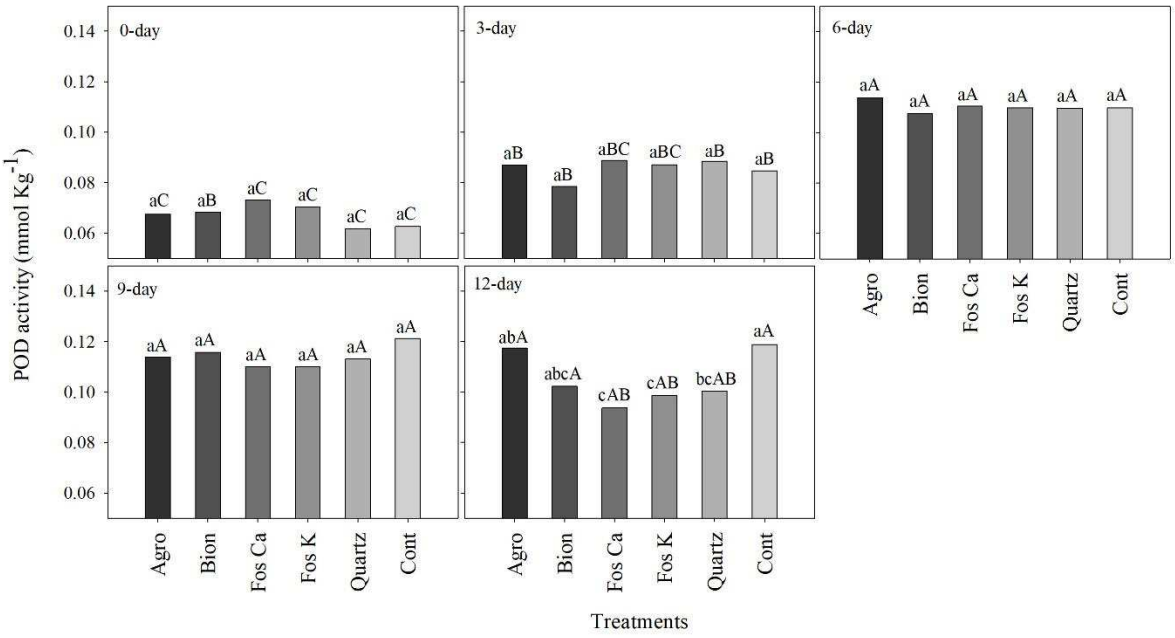


Figure 6. Peroxidase (POD) activity in *Heliconia psitacorum* stems, treated with Agro-Mos® (Agro), Bion®, Quartz®, K - Fosfitotal® (Fos K), Ca - Fosfitotal® (Fos Ca) and distilled water (Cont). The means of the treatments were compared by the Tukey test ($P < 0.05$). Lowercase letters compare the treatments on the day of the evaluation and uppercase letters compare the treatment over the vase life.

Activity peaked between the sixth and ninth days and decreased on the last day in the vase, similar to the POD enzyme performance. On the first vase life day there was no significant difference between the treatments assessed but during the assessments the stems treated with potassium and calcium Fosfitotal® maintained PPO levels lower than the control treatment. Only on the sixth day of vase life the stems treated with Agro-Mos® and Bion® presented lower PPO levels compared to the control (Figure 7).

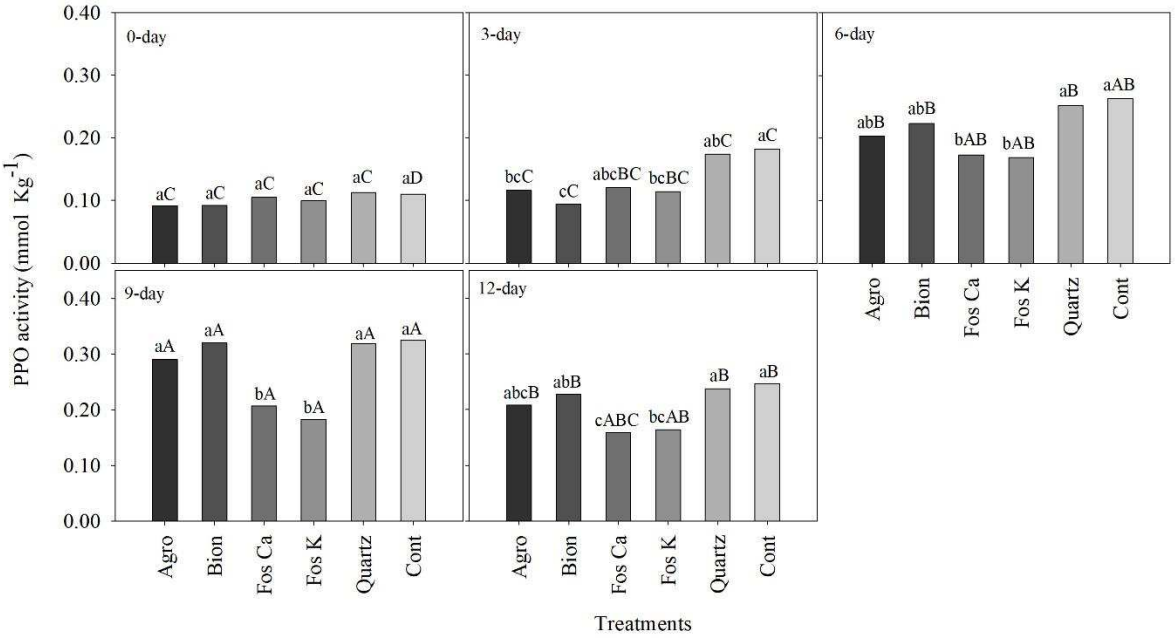


Figure 7. Polyphenoloxidase (PPO) activity in *Heliconia psitacorum* stems, treated with Agro-Mos® (Agro), Bion®, Quartz®, K - Fosfitotal® (Fos K), Ca - Fosfitotal® (Fos Ca) and distilled water (Cont). The means of the treatments were compared by the Tukey test ($P < 0.05$). Lowercase letters compare the treatments on the day of the evaluation and uppercase letters compare the treatment over the vase life.

3.6. Superoxide dismutase (SOD, EC:1.15.1.1) activity

Statistical analysis identified a significant interaction between treatments (plot) and time (subplot) for the parameter evaluated. SOD activity in *H. psittacorum* flower stems tended to increase over the vase life, with occasional reductions on the third vase life, for the stems in the control, Agro-Mos® and Quartz® treatments and on the 12th day for the stems treated with Bion®. However, it was observed that, from the third day of evaluation, the stems treated with the Fosfitotal® and Bion® always presented higher SOD activity in relation to the control (Figure 8).

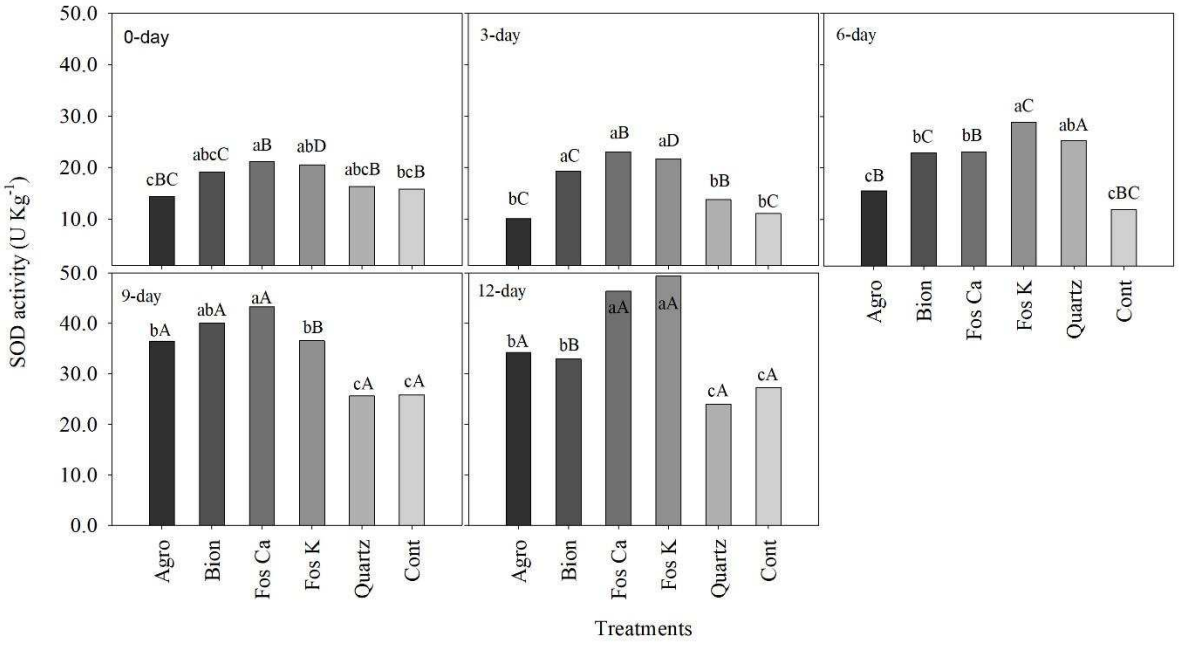


Figure 8. Superoxide dismutase (SOD) activity in *Heliconia psitacorum* stems, treated with Agro-Mos® (Agro), Bion®, Quartz®, K - Fosfitotal® (Fos K), Ca - Fosfitotal® (Fos Ca) and distilled water (Cont). The means of the treatments were compared by the Tukey test ($P < 0.05$). Lowercase letters compare the treatments on the day of the evaluation and uppercase letters compare the treatment over the vase life.

3.7. Total soluble phenols

It was not possible to characterize peaks for total soluble phenolic contents in floral stems of *H. psittacorum*. The applied statistics showed that there was a significant interaction between treatments (plot) and time (subplot). However, the treatments influenced this parameter from the sixth day of vase life (Figure 9).

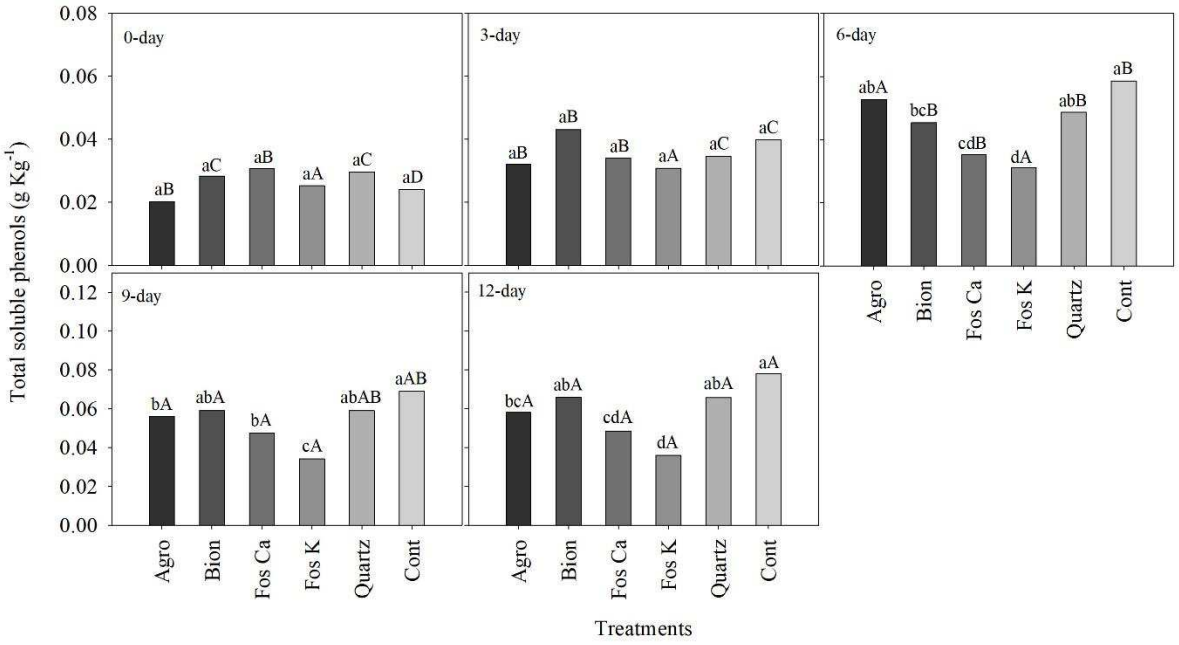


Figure 9. Total soluble phenols in *Heliconia psitacorum* stems, treated with Agro-Mos® (Agro), Bion®, Quartz®, K - Fosfitotal® (Fos K), Ca - Fosfitotal® (Fos Ca) and distilled water (Cont). The means of the treatments were compared by the Tukey test ($P < 0.05$). Lowercase letters compare the treatments on the day of the evaluation and uppercase letters compare the treatment over the vase life.

Starting on the sixth vase life the stems treated with the potassium and calcium Fosfitotal® differed from the control, presenting lower phenol compound levels that did not alter until the end of the assessment period.

3.8.Lipid peroxidation – TBARS

The statistical analysis identified a significant interaction between treatments (plot) and time (subplot) for the parameter evaluated. At the beginning of the experiment lipid peroxidation increased but over time remained stable for all treatments. From the third day of vase life, a difference was observed between the treatments, which was shown in favor of the

Fosfitotal®, as compared to the control, from the sixth day in the vase (Figure 10). The results observed on the 9th and 12th days in the vase showed that the Fosfitotal® treatments had the lowest lipid peroxidation values, especially when compared to the control treatment.

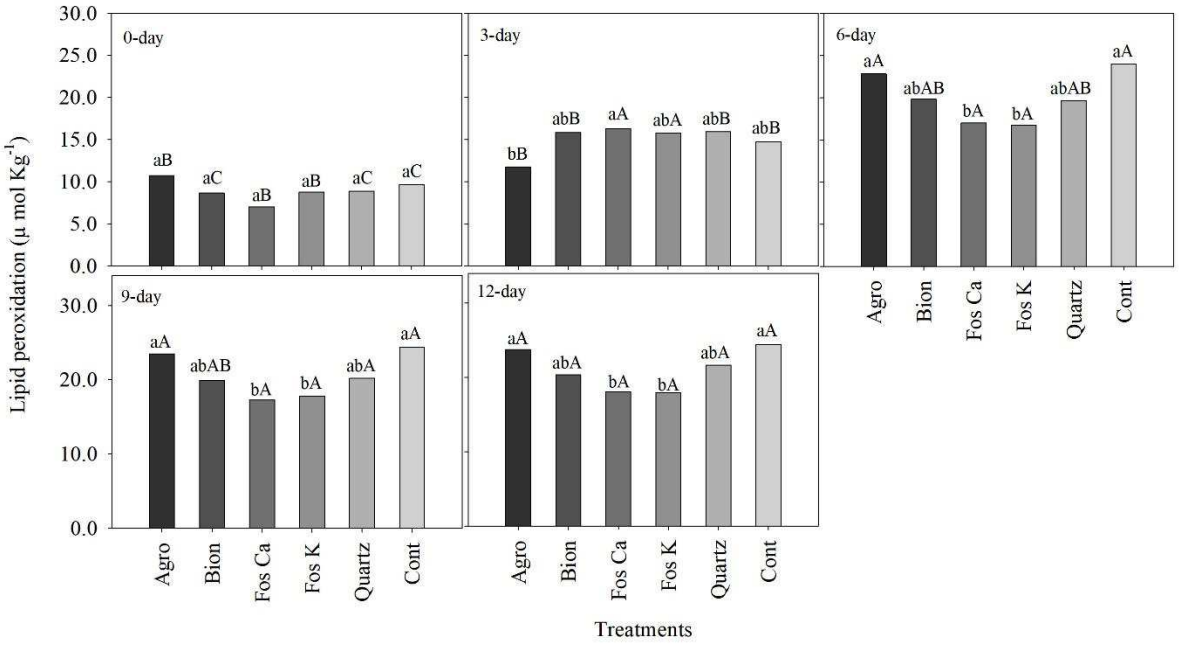


Figure 10 - Lipid peroxidation in *Heliconia psitacorum* stems, treated with Agro-Mos® (Agro), Bion®, Quartz®, K - Fosfitotal® (Fos K), Ca - Fosfitotal® (Fos Ca) and distilled water (Cont). The means of the treatments were compared by the Tukey test ($P < 0.05$). Lowercase letters compare the treatments on the day of the evaluation and uppercase letters compare the treatment over the vase life.

4. DISCUSSION

The visual scores attributed to the treatments demonstrated that the Fosfitotal®, especially K - Fosfitotal®, enabled a greater preservation of stem quality during vase life, possibly by acting on the plant metabolism. Maganve et al. (2013) reported that *H. psittacorum* plants not treated in post-harvesting had their vase life limited to 9 days and these results were

also observed in the present study but the stems treated with Fosfitotal® presented higher quality on the 12th day.

The reduction in visual scores during vase life is acceptable, because the flowers are in the process of senescence. Costa et al. (2011) stated that the visual quality of *H. bihai* flowers diminished during the useful vase life, due to damage or senescence. The results expressed by electrolyte extravasation corroborate with those observed in the visual analysis and indicate the better quality of Fosfitotal® - treated stems. The treatments with calcium and potassium Fosfitotal® may have increased tolerance to water loss and as a consequence prolonged the flower stem quality. According to Demidchik et al. (2014) ROS have been shown to activate gens GORK, SKOR, and annexins. ROS-activated K^+ efflux through GORK channels results in dramatic K^+ loss from plant cells, which stimulates proteases and endonucleases, and promotes programmed cell death. This mechanism is likely to trigger plant programmed cell death under severe stress. However, in moderate stress conditions, K^+ efflux could play an essential role as a metabolic switch in anabolic reactions, stimulating catabolic processes and saving 'metabolic' energy for adaptation and repair needs. From this assumption we can suggest that the Fosfitotal® supplying K^+ and Ca^+ cations interfere in these cellular mechanisms, since there is as yet no consensus that phosphite alone is metabolizable by plants (Gómez-Merino and Trejo-Téllez, 2015). Burra et al. (2014) reported that phosphite interfere in processes associated to the primary and secondary metabolisms, inducing an increase in gene expression related to abiotic stress, including the early response to dehydration.

Fresh matter maintenance is usually reflected in higher visual scores and stems with bigger fresh matter content are of better quality. However, in the present experiment, the treatments did not show reduction in fresh matter. According Mangave et al. (2013) fresh matter loss is a process expected during the vase life and can vary according to specific post-harvest treatments, such as spray application. In addition, fresh matter loss probably occurrs due to the

senescence process caused by physiological changes in the plant, such as water loss (Costa et al., 2011).

The soluble carbohydrate content was bigger in the stems treated with Fosfitotal® until the last storage day, when all the treatments were equal for soluble carbohydrates. These results suggest an association between carbohydrate content and bract quality because the Fosfitotal® - treated stems obtained better visual scores compared to the other treatments used. We can raise two hypotheses from these results, the first linked to the natural senescence process, that may have accelerated in the control, Agro-Mos®, Bion® and Quartz® treatments or been delayed in the treatments with Fosfitotal®. According to Woltering (2017), decreasing carbohydrate levels may trigger the senescence process. The second hypothesis is that increased lipid peroxidation leads to greater membrane damage. According to Bañuelos-Hernández et al. (2017), the bigger the damage, the bigger will be the release of non-selective ions and soluble sugars and amino acid loss. In the present case, it is possible to associate the bigger electrolyte extravasation with the bigger the soluble carbohydrate degradation and consequent reduction in the physiological functions necessary to maintain the flower stem quality.

The results expressed for POD when associated to the visual scores showed that plants with less POD activity obtained better scores and may represent an indication of less oxidative stress, for the stems treated with Fosfitotal® and Bion®. Rapid Bion® absorption by the plant stimulated resistance by interfering in physiological and biochemical processes (Debona et al., 2009). It is also frequently associated to the salicylic acid metabolic paths because it activates genes referent to resistance signaling (Thakur and Sohal, 2013). According to Bañuelos-Hernández et al. (2016), *H. psittacorum* stems with higher POD levels show greater damage and stress at cell level and this damage is represented by dark marks and visible desiccation on the bract, that was confirmed by the lowest scores attributed to the control and Agro-Mos® treatments.

The PPO activity in the treatments with the Fosfitotal® was lower compared to the control treatment and was probably related to the smaller membrane damage associated to the treatment, and there was also less tissue darkening and better visual scores at the end of vase life for the Fosfitotal® - treated flower stems. Tissue darkening may be a consequence of cell disorganization, caused by cell membrane rupture, because, according to Taranto et al. (2017) the PPO´s are oxidation reaction and cell disorganization catalyzers, caused by membrane rupture that places PPO´s in contact with phenol compounds, resulting in tissue darkening, a fact observed more often in the control treatment. The results found for PPO and POD activity contradict other studies that indicated an increase in the activity of these enzymes in plants under stress caused by *Pythium ultimum* in cucumber plants (Mofidnakhaei et al., 2016), but our results were obtained in the absence of biotic factors, that is, absence of phytopathogens. In this sense the phosphites may have acted differently in the metabolism of *H. psittacorum* in response to the pre-harvest treatment, including stimulating other antioxidant mechanisms.

Regarding the total soluble phenols, it can be inferred that low visual scores are associated to increase in total soluble phenol production and consequent raise in the PPO and POD enzyme activity. Based on these data is it can be suggested that the control treatment showed more damage, possibly caused by ROS, especially in the second half of the vase life when there were statistical differences between the treatments. Considerations by Araujo et al. (2015) support our views by stating that plants respond to physical lesions, infections by pathogens, abiotic and biotic elicitors or different types of stress by increasing the pre-existing total soluble phenol concentration or producing ones, using various metabolic paths. In this case, the heliconia stems with bigger associated damage increased production of these compounds to reduce damage caused by ROS, but without success.

The results expressed for SOD show that the Fosfitotal® - treated stems presented higher activity of this enzyme, compared to the control, throughout the vase life. These results,

associated to the visual scores attributed to the Fosfitotal® - treated flower stems, indicate that the high SOD activity may be associated to longer vase life of bract quality post-harvest. The SOD activity probably resulted in greater protection against reactive oxygen species and higher quality of Fosfitotal® - treated stems, because the SODs catalyze dismutation of two O_2^- radicals generating H_2O_2 and O_2 (Barbosa et al., 2014). These results suggest that the Fosfitotal® can induce antioxidant defense mechanisms because the stems were under similar stress conditions. However, further studies are needed on the effect of this product post-harvest, especially on tropical flowers. According to Gómez-Merino and Trejo-Téllez (2015) there are few studies on phosphites relating their effects to abiotic stress tolerance. In spite of the scarcity of these studies, Mofidnakhaei et al. (2016) reported increase in SOD activity in cucumber plants treated with potassium phosphite and considered that phosphite ions activated the defense system of the plant against biotic and abiotic stresses, that partly confirmed our (suspicions) hypotheses.

The results expressed by lipid peroxidation showed that the Fosfitotal® guarantee greater cell membrane integrity and consequently reduced electrolyte extravasation. It can also be stated, based on the SOD enzyme activity, that the treatments with phosphites maintained a better balance between ROS production and elimination, possibly reducing these deleterious effects when compared to the control treatment, that resulted in higher visual scores for the heliconia flower stems grown with this phytostimulant. Mangave et al. (2013) reported in a study on heliconia that increase in lipid peroxidation was accompanied by reduced membrane integrity and increase in electrolyte extravasation, as observed in the present experiment. These associated factors would confer higher quality to flowers treated with Fosfitotal®.

The products Agro-Mos®, Bion® and Quartz®, phytostimulants recognized as biochemical mechanism elicitors (Araujo et al., 2015; Gomes et al., 2016; Melo et al., 2016), in the present experiment did not stimulate sufficiently positive responses to the protective

enzyme activity in *H. psittacorum* treated flower stems, compared to the potassium and calcium Fosfitotal®.

5. CONCLUSION

Applications of phosphite during cultivation resulted in better conservation in post-harvest with quality gain to the *Heliconia psittacorum* stems and may have activated as yet unknown physiological and/or biochemical mechanisms. The results demonstrated that the plants treated with phosphites had reduced damage associated to oxidative stress, increased superoxide dismutase activity, a key enzyme in the protection process against reactive oxygen species and delayed lipid peroxidation.

6. REFERENCES

1. Araujo, L., Bispo, W., Rios, V., Fernandes, S., Rodrigues, F.A., 2015. Induction of the Phenylpropanoid Pathway by Acibenzolar-S-Methyl and Potassium Phosphite Increases Mango Resistance to Ceratocystis fimbriata infection. Plant Dis. 141029130041000. https://doi.org/10.1094/PDIS-08-14-0788-RE

2. Bañuelos-Hernández, K.P., García-Nava, J.R., Leyva-Ovalle, O.R., Peña-Valdivia, C.B., Trejo, C., Ybarra-Moncada, M.C., 2017. Chitosan coating effect on vase life of flowering stems of Heliconia bihai (L.) L. cv. Halloween. Postharvest Biol. Technol. 132, 179–187. https://doi.org/10.1016/j.postharvbio.2017.05.009

3. Bañuelos-Hernández, K.P., García-Nava, J.R., Leyva-Ovalle, O.R., Peña-Valdivia, C.B., Ybarra-Moncada, M.C., 2016. Flowering stem storage of Heliconia psittacorumL. f. cv. Trópica. Postharvest Biol. Technol. 112, 159–169. https://doi.org/10.1016/j.postharvbio.2015.10.006

4. Barbosa, M.R., Silva, M.M.A., Willadino, L., Ulisses, C., Camara, T.R., 2014. Plant generation and enzymatic detoxification of reactive oxygen species. Cienc. Rural 44. https://doi.org/10.1590/S0103-84782014000300011

5. Beauchamp, C., Fridovich, I., 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44, 276–287. https://doi.org/10.1016/0003-2697(71)90370-8

6. Blokhina, O., Virolainen, E., Fagerstedt, K. V., 2003. Antioxidants, oxidative damage and oxygen deprivation stress: A review. Ann. Bot. https://doi.org/10.1093/aob/mcf118

7. Burra, D.D., Berkowitz, O., Hedley, P.E., Morris, J., Resjö, S., Levander, F., Liljeroth, E., Andreasson, E., Alexandersson, E., 2014. Phosphite-induced changes of the transcriptome and secretome in Solanum tuberosum leading to resistance against Phytophthora infestans. BMC Plant Biol. 14. https://doi.org/10.1186/s12870-014-0254-y

8. Costa, A.S., Nogueira, L.C., Santos, V.F. dos, Camara, T.R., Loges, V., Willadino, L., 2011. Storage of cut Heliconia bihai (L.) cv. Lobster Claw flowers at low temperatures. Rev. Bras. Eng. Agrícola e Ambient. 15, 966–972. https://doi.org/10.1590/S1415-43662011000900013

9. Costa, J.D.B., de Resende, M.L. V, Ribeiro, P.M., Camilo, F.R., Monteiro, A.C.A., Pereira, R.B., 2010. Induction of resistance in cacao seedlings against Moniliophthora perniciosa by a phosphorilated mannanoligosaccharide based product. Trop. Plant Pathol. 35, 285–294. https://doi.org/10.1590/S1982-56762010000500003

10. Debona, D., Figueiró, G.G., Corte, G.D., Navarini, L., Domingues, L. da S., Balardin, R.S., 2009. Effect of seed treatment with fungicides and acibenzolar-S-methyl in soybean cultivars on Asian rust control and seedlings growth. Summa Phytopathol. 35, 26–31. https://doi.org/10.1590/S0100-54052009000100004

11. Demidchik, V., Straltsova, D., Sokolik, S.S.M.G.A.P.A., Yurin, V., 2014. Stress-induced electrolyte leakage: the role of K+-permeable channels and involvement in programmed cell death and metabolic adjustment. J. Exp. Bot. 65, 1259–1270. https://doi.org/10.1093/jxb/eru004

12. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric Method for Determination of Sugars and Related Substances. Anal. Chem. 28, 350–356. https://doi.org/10.1021/ac60111a017

13. EMBRAPA, 2013. Sistema Brasileiro de Classificação de Solos, 3 ed. ed. EMBRAPA, Brasilia.

14. Folha, W.R., de Souza, R.R., Amaral, G.C., Silva, A.A., de Carvalho, J.N., Cavalcante, M.Z.B., 2016. Heliconia "Golden Torch" postharvest: Stem ends cutting and renewing vase water benefits. Ornam. Hortic. 22. https://doi.org/https://doi.org/10.14295/oh.v22i2.908

15. Folin, O., Ciocalteu, V., 1927. Tyrosine and tryptophan determinations proteins. J. Biol. Chem. 73, 627–650. https://doi.org/DOI: 10.1021/ac60128a025

16. Giannopolitis, C.N., Ries, S.K., 1977. Superoxide Dismutases: I. Occurrence in Higher Plants. PLANT Physiol. 59, 309–314. https://doi.org/10.1104/pp.59.2.309

17. Gomes, R.S.S., Demartelaere, A.C.F., do Nascimento, L.C., Maciel, W.O., Wanderley, D.B.N.S., 2016. Bioactivity ofresistance inductors in the management of guava (Psidium guajava L.) anthracnose. Summa Phytopathol. 42. https://doi.org/10.1590/0100-5405/2103

18. Gómez-Merino, F.C., Trejo-Téllez, L.I., 2015. Biostimulant activity of phosphite in horticulture. Sci. Hortic. (Amsterdam). https://doi.org/10.1016/j.scienta.2015.09.035

19. Harshavadhan, M., Kumar, D.P., Yathindra, H.A., Rajesh, A.M., Hongal, S., 2016. Effect of integrated nutrient management on soil health, nutrient uptake, flower quality and yield of carnation (Dianthus caryophyllus L.). Environ. Ecol. 34, 1862–1867.

20. Lima, M.A.G., Peixoto, A.R., Borges, I.V., Silva, M.S., Barbosa, M.A.G., Cavalcanti, L.S., 2017. INDUCTION OF RESISTANCE TO Xanthomonas campestris pv. viticola IN GRAPEVINE PLANTS. Soc. Bras. Frutic. 39. https://doi.org/10.1590/0100-29452017669

21. Mangave, B.D., Singh, A., Mahatma, M.K., 2013. Effects of different plant growth regulators and chemicals spray on post harvest physiology and vase life of heliconia inflorescence cv. Golden Torch. Plant Growth Regul. 69, 259–264. https://doi.org/10.1007/s10725-012-9768-1

22. Melo, L.G. de L., Silva, E.K.C. e, Campos Neto, J.R.M., Lins, S.R. de O., Rodrigues, A.A.C., Oliveira, S.M.A. de, 2016. Abiotic resistance inducers for control of pineapple fusariosis. Pesqui. Agropecuária Bras. 51, 1703–1709. https://doi.org/10.1590/s0100-204x2016001000001

23. Mofidnakhaei, M., Abdossi, V., Dehestani, A., Pirdashti, H., Babaeizad, V., 2016. Potassium phosphite affects growth, antioxidant enzymes activity and alleviates disease damage in cucumber plants inoculated with Pythium ultimum. Arch. Phytopathol. Plant Prot. 49, 207–221. https://doi.org/10.1080/03235408.2016.1180924

24. Moreira, A.L. de L., Araújo, F.F. de, 2013. Bioprospectionof Bacillus spp. as potential growth promoters in Eucalyptus urograndis. Rev. Árvore 37, 933–943. https://doi.org/10.1590/S0100-67622013000500016

25. Ncube, B., Finnie, J.F., Van Staden, J., 2012. Quality from the field: The impact of environmental factors as quality determinants in medicinal plants. South African J. Bot. https://doi.org/10.1016/j.sajb.2012.05.009

26. Scandalios, J.G., 2005. Oxidative stress: Molecular perception and transduction of signals triggering antioxidant gene defenses. Brazilian J. Med. Biol. Res. https://doi.org/10.1590/S0100-879X2005000700003

27. Shanahan, J.F., Edwards, I.B., Quick, J.S., Fenwick, J.R., 1990. Membrane Thermostability and Heat Tolerance of Spring Wheat. Crop Sci. 30, 247. https://doi.org/10.2135/cropsci1990.0011183X003000020001x

28. Simões, A.D.N., Moreira, S.I., Mosquim, P.R., Soares, N.D.F.F., Puschmann, R., 2015. The effects of storage temperature on the quality and phenolic metabolism of whole and minimally processed kale leaves. Acta Sci. Agron. 37, 101. https://doi.org/10.4025/actasciagron.v37i1.18123

29. Souza, S.O., Lima, M.A.C., Santos, A.C.N., Costa, A.C.S., Oliveira, A.H., 2008. Post-harvest conservation of Golden Torch heliconia inflorescences in aminoethoxyvinylglycine pulsing solution [WWW Document]. EMBRAPA. URL https://www.alice.cnptia.embrapa.br/bitstream/doc/160515/1/OPB1596.pdf (accessed 5.8.18).

30. Taniguchi, C.A.K., Castro, A.C.R. de, Silva, T.F., Silva, E.B. da, Martins, T. da S., 2016. Growth, nutrient accumulation and export by heliconia 'Red Opal.' Ornam. Hortic. 22.

31. Taranto, F., Pasqualone, A., Mangini, G., Tripodi, P., Miazzi, M.M., Pavan, S., Montemurro, C., 2017. Polyphenol oxidases in crops: Biochemical, physiological and genetic aspects. Int. J. Mol. Sci. https://doi.org/10.3390/ijms18020377

32. Thakur, M., Sohal, B.S., 2013. Role of Elicitors in Inducing Resistance in Plants against Pathogen Infection: A Review. ISRN Biochem. 2013, 1–10. https://doi.org/10.1155/2013/762412

33. Woltering, E.J., 2017. Flower Senescence, in: Encyclopedia of Applied Plant Sciences. Elsevier, pp. 292–299. https://doi.org/10.1016/B978-0-12-394807-6.00010-1

Considerações finais

Considerações Finais

As flores são artigos de contemplação e quando adquiridos por consumidores precisam ser vistosas, coloridas e perfeitas. Durante a produção, o ataque de fitopatógenos pode danificar a flor, reduzindo as qualidades que atraem o consumidor.

Os resultados da tese propiciaram ao produtor soluções viáveis para o controle de fitopatógenos durante a produção, além de manutenção da qualidade durante o período de armazenamento da flor.

Outro aspecto importante desta tese é a contribuição científica em uma área pouco explorada, sobretudo no Maranhão, e que muitos produtores demandam informações técnicas que subsidiem uma produção com qualidade.

Espera-se que os resultados aqui apresentados possam incentivar pesquisadores a ingressar nesta área de pesquisa e contribuir para o desenvolvimento da cadeia produtiva de flores tropicais.

ANEXOS

Anexo I

Normas de submissão para a Postharvest Biology and Technology (Capítulo III)

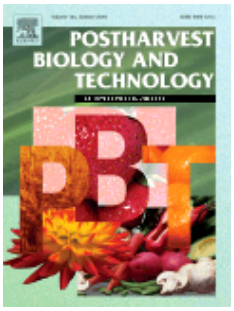
POSTHARVEST BIOLOGY AND TECHNOLOGY

An International Journal

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

- **Description** **p.1**
- **Audience** **p.1**
- **Impact Factor** **p.1**
- **Abstracting and Indexing** **p.2**
- **Editorial Board** **p.2**
- **Guide for Authors** **p.4**



ISSN: 0925-5214

DESCRIPTION

The journal is devoted exclusively to the publication of original papers, review articles and frontiers articles on **biological** and **technological postharvest research**. This includes the areas of postharvest storage, treatments and underpinning mechanisms, quality evaluation, packaging, handling and distribution of fresh horticultural crops including fruit, vegetables, flowers and nuts, but excluding grains and forages.

Articles on their postharvest treatment as affecting the eventual quality of processed product may be included, but articles on processing of such crops beyond refrigeration, packaging and minimal processing will not be considered. Papers reporting novel insights from fundamental and interdisciplinary research will be particularly encouraged. These disciplines include systems biology, bioinformatics, entomology, plant physiology, plant pathology, (bio)chemistry, engineering, modelling, and technologies for nondestructive testing.

Benefits to authors
We also provide many author benefits, such as free PDFs, a liberal copyright policy, special discounts on Elsevier publications and much more. Please click here for more information on our author services.

Please see our Guide for Authors for information on article submission. If you require any further information or help, please visit our Support Center

AUDIENCE

Scientists in the area of agriculture and horticulture, Agricultural Engineers, Crop Scientists, Entomologists, Plant Breeders, Plant Physiologists, Agricultural Economists, Applied Biologists.

IMPACT FACTOR

2017: 3.112 © Clarivate Analytics Journal Citation Reports 2018

ABSTRACTING AND INDEXING

Current Contents/Agriculture, Biology & Environmental Sciences
EMBiology
CAB Abstracts
Scopus
FSTA (Food Science and Technology Abstracts)

EDITORIAL BOARD

GUIDE FOR AUTHORS

Your Paper Your Way

We now differentiate between the requirements for new and revised submissions. You may choose to submit your manuscript as a single Word or PDF file to be used in the refereeing process. Only when your paper is at the revision stage, will you be requested to put your paper in to a 'correct format' for acceptance and provide the items required for the publication of your article.
To find out more, please visit the Preparation section below.

INTRODUCTION

Postharvest Biology and Technology

The journal is devoted exclusively to the publication of original papers, review articles and frontiers articles on biological and technological postharvest research. This includes the areas of postharvest storage, treatments and underpinning mechanisms, quality evaluation, packaging, handling and distribution of fresh horticultural crops including fruit, vegetables, flowers and nuts, but excluding grains and forages.

Articles on their postharvest treatment as affecting the eventual quality of processed product may be included, but articles on processing of such crops beyond refrigeration, packaging and minimal processing will not be considered. Papers reporting novel insights from fundamental and interdisciplinary research will be particularly encouraged. These disciplines include systems biology, bioinformatics, entomology, plant physiology, plant pathology, (bio)chemistry, engineering, modelling, and technologies for nondestructive testing.

Types of paper
1. Research Papers (regular papers)
2. Invited Review Articles
3. Frontiers Articles

Research Papers should report the results of original research. The material should not have been previously published elsewhere, except in a preliminary form without peer review. Preference is given to manuscripts that provide more scientific insight in the postharvest physiology of horticultural products or lead to novel postharvest technologies.

Invited Review articles should cover subjects falling within the scope of the journal which are of active current interest. They are on an invitation basis only and should focus on major results and future perspectives rather than exhaustively summarise the literature on the topic. Selected review articles on ground breaking research will be published as Kader reviews, named in recognition of the late postharvest scientist Adel Kader.

Frontiers Articles should address hot emerging topics for which there is not enough literature available yet to warrant a regular review. They are also on an invitation basis only.

For questions on submissions or scope please contact one of the Editors-in-Chief.

Submission checklist

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
- E-mail address
- Full postal address

All necessary files have been uploaded:
Manuscript:
- Include keywords
- All figures (include relevant captions)

• All tables (including titles, description, footnotes)
• Ensure all figure and table citations in the text match the files provided
• Indicate clearly if color should be used for any figures in print
Graphical Abstracts / Highlights files (where applicable)
Supplemental files (where applicable)

Further considerations
• Manuscript has been 'spell checked' and 'grammar checked'
• All references mentioned in the Reference List are cited in the text, and vice versa
• Permission has been obtained for use of copyrighted material from other sources (including the Internet)
• A competing interests statement is provided, even if the authors have no competing interests to declare
• Journal policies detailed in this guide have been reviewed
• Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center.

BEFORE YOU BEGIN

Ethics in publishing

Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

Declaration of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential competing interests include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Authors must disclose any interests in two places: 1. A summary declaration of interest statement in the title page file (if double-blind) or the manuscript file (if single-blind). If there are no interests to declare then please state this: 'Declarations of interest: none'. This summary statement will be ultimately published if the article is accepted. 2. Detailed disclosures as part of a separate Declaration of Interest form, which forms part of the journal's official records. It is important for potential interests to be declared in both places and that the information matches. More information.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection service Crossref Similarity Check.

Preprints

Please note that preprints can be shared anywhere at any time, in line with Elsevier's sharing policy. Sharing your preprints e.g. on a preprint server will not count as prior publication (see 'Multiple, redundant or concurrent publication' for more information).

Changes to authorship

Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.

Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors **after** the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Article transfer service
This journal is part of our Article Transfer Service. This means that if the Editor feels your article is more suitable in one of our other participating journals, then you may be asked to consider transferring the article to one of those. If you agree, your article will be transferred automatically on your behalf with no need to reformat. Please note that your article will be reviewed again by the new journal. More information.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (see more information on this). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases.

For gold open access articles: Upon acceptance of an article, authors will be asked to complete an 'Exclusive License Agreement' (more information). Permitted third party reuse of gold open access articles is determined by the author's choice of user license.

Author rights

As an author you (or your employer or institution) have certain rights to reuse your work. More information.

Elsevier supports responsible sharing
Find out how you can share your research published in Elsevier journals.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

Funding body agreements and policies
Elsevier has established a number of agreements with funding bodies which allow authors to comply with their funder's open access policies. Some funding bodies will reimburse the author for the gold open access publication fee. Details of existing agreements are available online.

Open access

This journal offers authors a choice in publishing their research:

Subscription

• Articles are made available to subscribers as well as developing countries and patient groups through our universal access programs.
• No open access publication fee payable by authors.
• The Author is entitled to post the accepted manuscript in their institution's repository and make this public after an embargo period (known as green Open Access). The published journal article cannot be shared publicly, for example on ResearchGate or Academia.edu, to ensure the sustainability of peer-reviewed research in journal publications. The embargo period for this journal can be found below.

Gold open access

• Articles are freely available to both subscribers and the wider public with permitted reuse.
• A gold open access publication fee is payable by authors or on their behalf, e.g. by their research funder or institution.

Regardless of how you choose to publish your article, the journal will apply the same peer review criteria and acceptance standards.

For gold open access articles, permitted third party (re)use is defined by the following Creative Commons user licenses:

Creative Commons Attribution (CC BY)
Lets others distribute and copy the article, create extracts, abstracts, and other revised versions, adaptations or derivative works of or from an article (such as a translation), include in a collective work (such as an anthology), text or data mine the article, even for commercial purposes, as long as they credit the author(s), do not represent the author as endorsing their adaptation of the article, and do not modify the article in such a way as to damage the author's honor or reputation.

Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)
For non-commercial purposes, lets others distribute and copy the article, and to include in a collective work (such as an anthology), as long as they credit the author(s) and provided they do not alter or modify the article.

The gold open access publication fee for this journal is **USD 3300**, excluding taxes. Learn more about Elsevier's pricing policy: https://www.elsevier.com/openaccesspricing.

Green open access
Authors can share their research in a variety of different ways and Elsevier has a number of green open access options available. We recommend authors see our green open access page for further information. Authors can also self-archive their manuscripts immediately and enable public access from their institution's repository after an embargo period. This is the version that has been accepted for publication and which typically includes author-incorporated changes suggested during submission, peer review and in editor-author communications. Embargo period: For subscription articles, an appropriate amount of time is needed for journals to deliver value to subscribing customers before an article becomes freely available to the public. This is the embargo period and it begins from the date the article is formally published online in its final and fully citable form. Find out more.

This journal has an embargo period of 24 months.

Elsevier Researcher Academy
Researcher Academy is a free e-learning platform designed to support early and mid-career researchers throughout their research journey. The "Learn" environment at Researcher Academy offers several interactive modules, webinars, downloadable guides and resources to guide you through the process of writing for research and going through peer review. Feel free to use these free resources to improve your submission and navigate the publication process with ease.

Language and language services
Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who require information about language editing and copyediting services pre- and post-submission please visit http://www.elsevier.com/languageediting or our customer support site at service.elsevier.com for more information. Papers with English that do not meet our standards will be returned to authors for improvement.

Submission
Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. Please submit the manuscripts, tables, and graphics in native word processor or graphics files. Figures should not be embedded within the word processing document. The system automatically converts source files to a single PDF file of the article, which is used in the peer-review process. Do not submit original or revised manuscripts as PDF files. Please note that even though manuscript source files are converted to PDF files at submission for the review process, these source files are needed for further processing after acceptance. All correspondence, including notification of the Editor's decision and requests for revision, takes place by e-mail removing the need for a paper trail.

Submit your article
Please submit your article via http://ees.elsevier.com/postec/

Referees
Please submit, with the manuscript, the names, addresses and e-mail addresses of up to 4 potential referees, who are not affiliated with any of the authors' organizations. Note that the editors retain the sole right to decide whether or not the suggested reviewers are used.

PREPARATION

NEW SUBMISSIONS

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts your files to a single PDF file, which is used in the peer-review process.

As part of the Your Paper Your Way service, you may choose to submit your manuscript as a single file to be used in the refereeing process. This can be a PDF file or a Word document, in any format or lay-out that can be used by referees to evaluate your manuscript. It should contain high enough quality figures for refereeing. If you prefer to do so, you may still provide all or some of the source files at the initial submission. Please note that individual figure files larger than 10 MB must be uploaded separately.

Cover Letter

Submission of a manuscript must be accompanied by a cover letter that includes the following statements or acknowledgements:

The work is an original research carried out by the authors. All authors agree with the contents of the manuscript and its submission to the journal. All Authors listed have contributed significantly to the work and agree to be in the author list. No part of the research has been published in any form elsewhere, unless it is fully acknowledged in the manuscript. Authors should disclose how the research featured in the manuscript relates to any other manuscript of a similar nature that they have published, in press, submitted or will soon submit to Postharvest Biology and Technology or elsewhere.

References

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct.

Formatting requirements

There are no strict formatting requirements but all manuscripts must contain the essential elements needed to convey your manuscript, for example Abstract, Keywords, Introduction, Materials and Methods, Results, Conclusions, Artwork and Tables with Captions.

If your article includes any Videos and/or other Supplementary material, this should be included in your initial submission for peer review purposes.

Divide the article into clearly defined sections.

Please ensure the text of your paper is double-spaced and has consecutive line numbering - this is an essential peer review requirement.

Figures and tables embedded in text

Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file. The corresponding caption should be placed directly below the figure or table.

Peer review

This journal operates a single blind review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. More information on types of peer review.

REVISED SUBMISSIONS

Use of word processing software

Regardless of the file format of the original submission, at revision you must provide us with an editable file of the entire article. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). See also the section on Electronic artwork.

To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Subdivision - numbered sections
Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction
State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods
Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

Results
Results should be clear and concise statements of the results as documented by the data collected and their subsequent analysis.

Discussion
This section should confront the results of the work to the state of the art, explore their significance but not contain a restatement of them. It is advised to not combine the Results and Discussion section. Avoid extensive citations and discussion of published literature.

Conclusions
Authors are encouraged to succinctly state conclusions relative to the objectives of the study. The main conclusions may be presented in a short Conclusions section, which may stand alone or form a subsection within the Discussion section.

Appendices
If there is more than one appendix, each should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on.

Essential title page information

• ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
• ***Author names and affiliations.*** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any future queries about Methodology and Materials. **Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**
• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Optional graphical abstract
A Graphical abstract is optional and should summarize the contents of the paper in a concise, pictorial form designed to capture the attention of a wide readership online. Authors must provide images that clearly represent the work described in the paper. Graphical abstracts should be submitted

with a caption. Supply captions separately, not attached to the graphical abstract. A caption should comprise a brief title (**not** on the graphical abstract itself). Graphical abstracts should be submitted as a separate file in the online submission system. Maximum image size: 400 × 600 pixels (h × w, recommended size 200 × 500 pixels). Preferred file types: TIFF, EPS, PDF or MS Office files. See http://www.elsevier.com/graphicalabstracts for examples.

Highlights
Highlights are mandatory for this journal. They consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). You can view example Highlights on our information site.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations
Define abbreviations that are not standard in this field in the text where they are first presented. Such abbreviations that are unavoidable in the abstract must be defined at their first mention. Ensure consistency of abbreviations throughout the article.

Acknowledgements
Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Formatting of funding sources
List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, please include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Nomenclature and Units
The SI system should be used for all scientific and laboratory data. The reference for all SI unit matters can be found at http://www.bipm.org/utils/common/pdf/si_brochure_8_en.pdf. Common corrections required before the manuscript may enter the review process:

• Only SI base units or derived units are permitted, possibly preceded by a prefix, as well as non-SI units that are accepted by the International Committee for Weights and Measures (CIPM, see aforementioned reference). Also acceptable are CFU (colony forming units) and U (enzyme activity). If relevant non SI units may be mentioned between brackets, but this practice is disencouraged.

• This journal requires that orders of magnitude are aggregated into the numerator's prefix (e.g., 5.3 mg L^{-1} instead of 5.3 µg mL^{-1}) unless the numerator has no units (e.g., as in 4.2 cm^{-1}) or the numerator is a non-SI unit (e.g., as in CFU mL^{-1}) for which there is no SI alternative.

• SI does not permit the use of intervening or modifying words among the terms in units; e.g., FW for fresh weight is not permitted, or g / 100 g. Explain when first needed in your materials and methods section that results are expressed on a fresh weight basis, and use for example g kg^{-1} or mol kg^{-1} in the remainder of your manuscript

• The base unit second (s) is the preferred unit of time. Other units (i.e., minute, min; hour, h; day, d) are acceptable

• SI requires that numerals be followed by proper SI units; e.g. 12 d, not 12 days. Likewise words should not be followed by unit abbreviations; e.g., twelve days, not twelve d.

• SI requires units to be preceded by a space. Note that this includes requiring a space before "C" and "%".

• 'ppm' or 'ppb' are ambigous units; numbers should be expressed on a mass or molar basis. An exception is chemical shift in NMR spectroscopy.

• This journal uses a space between terms in units and does not use dots.

• This journal requires that respiration rate and other measurements of evolved gases be reported in mass or mole units; e.g., nmol kg^{-1} s^{-1}.

• The units of TSS or SSC are %, not Brix, Bx or %Brix

• The units of (penetrometer) firmness are N, not kg, kgf or kg cm^{-2}

• This journal requires to use "L" as the symbol for liter when used by itself or as part of a compound unit such as mL.

• Centrifugation rates should be expressed in terms of g; rpm is not allowed

• The plural of fruit is fruit

Authors and Editor(s) are, by general agreement, obliged to accept the rules governing biological nomenclature, as laid down in the International Code of Botanical Nomenclature, the International Code of Nomenclature of Bacteria, and the International Code of Zoological Nomenclature.

Math formulae
In principle, variables are to be presented in italics and preferably consist of a single letter symbol with subscripts to clarify their meaning. For example, if P is used as the symbol of pressure, P_{eth} would denote the pressure of ethylene. Do not use the underscore to indicate a subscript; P_eth is to be avoided. Transcendental functions such as sin, exp or log are written in upright font. Brackets and numbers are in upright font as well.
Formulas should preferably be embedded in your manuscript as an equation object.
Give the meaning of all symbols immediately after the equation in which they are first used. For simple fractions use the solidus (/) instead of a horizontal line.
Equations should be numbered serially at the right-hand side in parentheses. In general only equations explicitly referred to in the text need be numbered.
The use of fractional powers instead of root signs is recommended. Also powers of e are often more conveniently denoted by exp.
Levels of statistical significance which can be mentioned without further explanation are: $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.
In chemical formulae, valence of ions should be given as, e.g., Ca^{2+}, not as Ca^{++}. Isotope numbers should precede the symbols, e.g., ^{18}O.

Footnotes
Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article.

Electronic artwork
General points
• Make sure you use uniform lettering and sizing of your original artwork.
• Preferred fonts: Arial (or Helvetica), Times New Roman (or Times), Symbol, Courier.
• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Indicate per figure if it is a single, 1.5 or 2-column fitting image.
• For Word submissions only, you are requested to provide figures and their number labels and captions, and tables within a single file at the revision stage.
A detailed guide on electronic artwork is available.
You are urged to visit this site; some excerpts from the detailed information are given here.
Formats
Regardless of the application used, when your electronic artwork is finalized, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):
EPS (or PDF): Vector drawings. Embed the font or save the text as 'graphics'.
TIFF (or JPG): Color or grayscale photographs (halftones): always use a minimum of 300 dpi.
TIFF (or JPG): Bitmapped line drawings: use a minimum of 1000 dpi.
TIFF (or JPG): Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.
Please do not:
• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low.
• Supply files that are too low in resolution.
• Submit graphics that are disproportionately large for the content.

Color artwork
Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article**. Please indicate your preference for color: in print or online only. Further information on the preparation of electronic artwork.

Figure captions
Ensure that each illustration has a caption. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used. Provide error bars when possible and explain their meaning.

Tables
Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules. Provide error measures and explain their meaning.

References
Citation in text
Please ensure that every reference cited in the text is also present in the reference list (and vice versa). References should be listed in the text chronologically, from earliest to most recent. Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included

in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Reference links
Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is highly encouraged.

A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: VanDecar J.C., Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. Journal of Geophysical Research, https://doi.org/10.1029/2001JB000884. Please note the format of such citations should be in the same style as all other references in the paper.

Web references
As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

Data references
This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

References in a special issue
Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference management software
Most Elsevier journals have their reference template available in many of the most popular reference management software products. These include all products that support Citation Style Language styles, such as Mendeley and Zotero, as well as EndNote. Using the word processor plug-ins from these products, authors only need to select the appropriate journal template when preparing their article, after which citations and bibliographies will be automatically formatted in the journal's style. If no template is yet available for this journal, please follow the format of the sample references and citations as shown in this Guide. If you use reference management software, please ensure that you remove all field codes before submitting the electronic manuscript. More information on how to remove field codes.

Users of Mendeley Desktop can easily install the reference style for this journal by clicking the following link:
http://open.mendeley.com/use-citation-style/postharvest-biology-and-technology
When preparing your manuscript, you will then be able to select this style using the Mendeley plug-ins for Microsoft Word or LibreOffice.

Reference formatting
There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

Reference style
Text: All citations in the text should refer to:

1. *Single author:* the author's name (without initials, unless there is ambiguity) and the year of publication;
2. *Two authors:* both authors' names and the year of publication;
3. *Three or more authors:* first author's name followed by 'et al.' and the year of publication.
Citations may be made directly (or parenthetically). Groups of references can be listed either first alphabetically, then chronologically, or vice versa.
Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999)…. Or, as demonstrated (Jones, 1999; Allan, 2000)… Kramer et al. (2010) have recently shown …'
List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.
Examples:
Reference to a journal publication:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59. https://doi.org/10.1016/j.Sc.2010.00372.
Reference to a journal publication with an article number:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2018. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.
Reference to a book:
Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.
Reference to a chapter in an edited book:
Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.
Reference to a website:
Cancer Research UK, 1975. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/ (accessed 13 March 2003).
Reference to a dataset:
[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. https://doi.org/10.17632/xwj98nb39r.1.

Journal abbreviations source
Journal names should be abbreviated according to the List of Title Word Abbreviations.

Video
Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. . In order to ensure that your video or animation material is directly usable, please provide the file in one of our recommended file formats with a preferred maximum size of 150 MB per file, 1 GB in total. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

AudioSlides
The journal encourages authors to create an AudioSlides presentation with their published article. AudioSlides are brief, webinar-style presentations that are shown next to the online article on ScienceDirect. This gives authors the opportunity to summarize their research in their own words and to help readers understand what the paper is about. More information and examples are available. Authors of this journal will automatically receive an invitation e-mail to create an AudioSlides presentation after acceptance of their paper.

Data visualization
Include interactive data visualizations in your publication and let your readers interact and engage more closely with your research. Follow the instructions here to find out about available data visualization options and how to include them with your article.

Supplementary material

Supplementary information (extra tables, figures, video, etc.) may be added if necessary. Formulae and equations in supplementary information should be given separate numbering: Eq. (S.1), Eq. (S.2), etc.

Research data

This journal encourages and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings. To facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. If you are sharing data in one of these ways, you are encouraged to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

Data linking

If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on ScienceDirect with relevant repositories, giving readers access to underlying data that gives them a better understanding of the research described.

There are different ways to link your datasets to your article. When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the database linking page.

For supported data repositories a repository banner will automatically appear next to your published article on ScienceDirect.

In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

Mendeley Data

This journal supports Mendeley Data, enabling you to deposit any research data (including raw and processed data, video, code, software, algorithms, protocols, and methods) associated with your manuscript in a free-to-use, open access repository. During the submission process, after uploading your manuscript, you will have the opportunity to upload your relevant datasets directly to *Mendeley Data*. The datasets will be listed and directly accessible to readers next to your published article online.

For more information, visit the Mendeley Data for journals page.

Data in Brief

You have the option of converting any or all parts of your supplementary or additional raw data into one or multiple data articles, a new kind of article that houses and describes your data. Data articles ensure that your data is actively reviewed, curated, formatted, indexed, given a DOI and publicly available to all upon publication. You are encouraged to submit your article for *Data in Brief* as an additional item directly alongside the revised version of your manuscript. If your research article is accepted, your data article will automatically be transferred over to *Data in Brief* where it will be editorially reviewed and published in the open access data journal, *Data in Brief*. Please note an open access fee of 500 USD is payable for publication in *Data in Brief*. Full details can be found on the Data in Brief website. Please use this template to write your Data in Brief.

MethodsX

You have the option of converting relevant protocols and methods into one or multiple MethodsX articles, a new kind of article that describes the details of customized research methods. Many researchers spend a significant amount of time on developing methods to fit their specific needs or setting, but often without getting credit for this part of their work. MethodsX, an open access journal, now publishes this information in order to make it searchable, peer reviewed, citable and reproducible. Authors are encouraged to submit their MethodsX article as an additional item directly alongside the revised version of their manuscript. If your research article is accepted, your methods article will

automatically be transferred over to MethodsX where it will be editorially reviewed. Please note an open access fee is payable for publication in MethodsX. Full details can be found on the MethodsX website. Please use this template to prepare your MethodsX article.

Data statement
To foster transparency, we encourage you to state the availability of your data in your submission. This may be a requirement of your funding body or institution. If your data is unavailable to access or unsuitable to post, you will have the opportunity to indicate why during the submission process, for example by stating that the research data is confidential. The statement will appear with your published article on ScienceDirect. For more information, visit the Data Statement page.

AFTER ACCEPTANCE

Proofs
One set of page proofs (as PDF files) will be sent by e-mail to the corresponding author (if we do not have an e-mail address then paper proofs will be sent by post) or, a link will be provided in the e-mail so that authors can download the files themselves. Elsevier now provides authors with PDF proofs that can be annotated; for this you will need to download Adobe Reader version 7 (or higher) available free from http://www.adobe.com/products/acrobat/readstep2.html. Instructions on how to annotate PDF files will accompany the proofs (also given online). The exact system requirements are given at the Adobe site: http://www.adobe.com/products/acrobat/acrrsystemreqs.html#70win.
If you do not wish to use the PDF annotations function, you may list the corrections (including replies to the Query Form) and return them to Elsevier in an e-mail. Please list your corrections quoting line number. If, for any reason, this is not possible, then mark the corrections and any other comments (including replies to the Query Form) on a printout of your proof and return by fax, or scan the pages and e-mail, or by post. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. We will do everything possible to get your article published quickly and accurately. Therefore, it is important to ensure that all of your corrections are sent back to us in one communication: please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility. Note that Elsevier may proceed with the publication of your article if no response is received.

Offprints
The corresponding author will, at no cost, receive a customized Share Link providing 50 days free access to the final published version of the article on ScienceDirect. The Share Link can be used for sharing the article via any communication channel, including email and social media. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's Webshop. Corresponding authors who have published their article gold open access do not receive a Share Link as their final published version of the article is available open access on ScienceDirect and can be shared through the article DOI link.

AUTHOR INQUIRIES

Visit the Elsevier Support Center to find the answers you need. Here you will find everything from Frequently Asked Questions to ways to get in touch.
You can also check the status of your submitted article or find out when your accepted article will be published.