

SABRINA ANGELA CASSOL

***Ceratocystis fimbriata* - *Actinidia* spp. PATHOSYSTEM: MECHANICAL DISPERSAL,
DISEASE CONTROL AND PATHOGENICITY OF ISOLATES
FROM DIFFERENT HOSTS**

Dissertation submitted to the Plant Pathology
Graduate Program of the Universidade Federal de
Viçosa in partial fulfillment of the requirements
for the degree of *Magister Scientiae*.

Adviser: Rafael Ferreira Alfenas

**VIÇOSA - MINAS GERAIS
2023**

**Ficha catalográfica elaborada pela Biblioteca Central da Universidade
Federal de Viçosa - Campus Viçosa**

T

C345c
2023
Cassol, Sabrina Angela, 1995-
Ceratocystis fimbriata - *Actinidia* spp. pathosystem:
mechanical dispersal, disease control and pathogenicity of
isolates from different hosts / Sabrina Angela Cassol. – Viçosa,
MG, 2023.

1 dissertação eletrônica (69 f.): il. (algumas color.).

Texto em inglês.

Orientador: Rafael Ferreira Alfenas.

Dissertação (mestrado) - Universidade Federal de Viçosa,
Departamento de Fitopatologia, 2023.

Inclui bibliografia.

DOI: <https://doi.org/10.47328/ufvbbt.2023.251>

Modo de acesso: World Wide Web.

1. Fungicidas - Testes. 2. Quiuí - Doenças e pragas. 3.
Ceratocystis fimbriata. I. Alfenas, Rafael Ferreira, 1983-.
II. Universidade Federal de Viçosa. Departamento de
Fitopatologia. Programa de Pós-Graduação em Fitopatologia.
III. Título.

CDD 22. ed. 632.952


SABRINA ANGELA CASSOL

***Ceratocystis fimbriata* - *Actinidia* spp. PATHOSYSTEM: MECHANICAL DISPERSAL,
DISEASE CONTROL AND PATHOGENICITY OF ISOLATES
FROM DIFFERENT HOSTS**


Dissertation submitted to the Plant Pathology
Graduate Program of the Universidade Federal de
Viçosa in partial fulfillment of the requirements
for the degree of *Magister Scientiae*.

APPROVED: February 09, 2023.

Assent:

Documento assinado digitalmente
 SABRINA ANGELA CASSOL
Data: 08/05/2023 17:28:36-0300
Verifique em <https://validar.iti.gov.br>

Sabrina Angela Cassol
Author

Documento assinado digitalmente
 RAFAEL FERREIRA ALFENAS
Data: 08/05/2023 17:56:54-0300
Verifique em <https://validar.iti.gov.br>

Rafael Ferreira Alfenas
Adviser

*To my little brothers, Amanda and
Ezequiel, as a reminder to always
follow their dreams.*

ACKNOWLEDGMENTS

To my mom and dad, thank you for all the support you have given me. You have been a part of every achievement in my life.

To all my family, thank you for your love and encouragement.

I would like to express my deepest gratitude to my advisor Professor Rafael Ferreira Alfenas for his guidance and teaching during these two years of my Master's degree. Thank you for your trust, the exchange of knowledge, and most importantly, for your support and patience during my decision-making process.

To my co-adviser Professor Acelino Couto Alfenas, thank you for inspiring me to learn more every day. Your shared knowledge, advice, and confidence in my work have been invaluable. I will be forever grateful.

I also want to thank my undergraduate advisor Volmir Kist and all the other excellent professors and advisors who have significantly impacted my academic life.

To Sara, thank you for your availability and help in resolving administrative issues related to the Graduate Program.

To the following Brazilian research agencies that supported in part this study: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

To the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) to granting the scholarship.

I would like to thank the company Clonar Resistência a Doenças Florestais for providing the plants, space, and labor necessary to conduct the experiments. The entire Clonar team's essential collaboration, receptivity, and friendship were also crucial.

To Zespri International Limited for the financial support to the development of this work.

To my colleagues of the Forest Pathology laboratory for the friendship and help.

To all my longtime friends, especially Andressa, Rhayana, Maurício, Mariana, Amanda, Kimberly, and Ana Maria, thank you for your unwavering support in pursuing my goals.

To my friend Gerson, thank you for the unforgettable moments we shared and for bringing me to Viçosa.

To the family I found in Viçosa, including Flávia, Fernando, Gustavo, Vitor, Priscila, João, Eugene, Polly, Edmundo, and José, thank you for all the emotional support and shared moments of happiness. You were essential during my Master's degree.

Last but not least, to my partner Letícia, thank you for being by my side during the most challenging moments of this journey and bringing lightness and happiness to my life.

I am grateful to everyone who directly or indirectly contributed to this work and to my personal and professional development.

ABSTRACT

CASSOL, Sabrina Angela, M.Sc., Universidade Federal de Viçosa, February, 2023. ***Ceratocystis fimbriata* - *Actinidia* spp. pathosystem: mechanical dispersal, disease control and pathogenicity of isolates from different hosts.** Adviser: Rafael Ferreira Alfenas.

Ceratocystis wilt, caused by *Ceratocystis fimbriata*, is a vascular disease that causes wilt, root rot and canker in various agricultural and forestry crops worldwide. It is currently one of the main limiting factors for kiwifruit cultivation and expansion of the crop in Brazil and constitutes a serious threat to the world's largest kiwifruit producers, where the disease has not yet been reported. Studies developed since the occurrence of Ceratocystis wilt in kiwifruit have provided important information about the pathosystem, however, there are some questions to be answered for the development of disease management strategies. There are few studies on the control of *C. fimbriata* in kiwifruit and there is no information on the effectiveness of fungicides against the pathogen, one of the objectives of this Dissertation. Thus, in Chapter 1, among four fungicides tested (metalaxyl + mancozeb, boscalide + cresoxim-methyl, pyraclostrobin, copper hydroxide) against the *in vitro* mycelial growth and spore germination of the fungus, metalaxyl + mancozeb and pyraclostrobin were the most effective, but they were not effective as protectors of pruning wounds against *C. fimbriata* infection, evaluated in two kiwifruit cultivars. In Chapter 2, we found that *C. fimbriata* can spread from diseased to healthy kiwifruit vines by pruning tools and that its immersion in hot water at 80 °C for 15 seconds is an effective method for eradication of the pathogen. In chapter 3, we determined that isolates of the fungus from different hosts can infect kiwifruit vines, with different levels of aggressiveness, including *C. lukuohia* from ohia. The results of these studies contribute to increase knowledge about the *C. fimbriata*-kiwi pathosystem and provide useful information for the disease management.

Keywords: Ceratocystis wilt. Chemical control. Host range. Kiwifruit. Pruning tools.

RESUMO

CASSOL, Sabrina Angela, M.Sc., Universidade Federal de Viçosa, fevereiro de 2023. ***Ceratocystis fimbriata* - *Actinidia* spp. patossistema: dispersão mecânica, controle da doença e patogenicidade de isolados de diferentes hospedeiros.** Orientador: Rafael Ferreira Alfenas.

A murcha-de-ceratocystis, causada por *Ceratocystis fimbriata*, é uma doença vascular que causa murcha, podridão radicular e cancro em várias culturas agrícolas e florestais em todo o mundo. É, atualmente, um dos principais fatores limitantes para o cultivo de kiwi e a expansão da cultura no Brasil e constitui uma séria ameaça aos maiores produtores mundiais de kiwi, onde a doença ainda não foi relatada na cultura. Estudos desenvolvidos desde a ocorrência da murcha-de-ceratocystis em kiwi têm fornecido informações importantes sobre o patossistema, no entanto, existem algumas questões a serem respondidas para o desenvolvimento de estratégias de manejo da doença. Há poucos estudos sobre o controle de *C. fimbriata* em kiwi e não existem informações sobre a eficiência de fungicidas para o controle da doença, um dos objetos desta Dissertação. Assim, no Capítulo 1, dentre quatro fungicidas testados (metalaxyl + mancozeb, boscalide + cresoxim-methyl, pyraclostrobin, copper hydroxide) contra o crescimento micelial e germinação de esporos *in vitro* do fungo, metalaxyl + mancozeb e pyraclostrobin foram os mais eficazes, mas não foram eficazes como protetores de ferimentos de poda contra infecção de *C. fimbriata*, avaliada em duas cultivares de kiwi. No Capítulo 2, constatamos que *C. fimbriata* pode se dispersar de plantas doentes para plantas saudáveis por meio de ferramentas de poda, cuja imersão em água quente a 80 °C por 15 segundos é eficaz para erradicação do patógeno, dentre os métodos testados incluindo imersão em calda fungicida e sanitizantes. No capítulo 3, determinamos que isolados do fungo de diferentes hospedeiros podem infectar plantas de kiwi, com diferentes níveis de agressividade, incluindo *C. lukuohia*. Os resultados desses estudos contribuem para aumentar o conhecimento sobre o patossistema *C. fimbriata*-kiwi e fornecem informações úteis para o manejo da doença.

Palavras-chave: Controle químico. Gama de hospedeiros. Kiwi. Murcha-de-ceratocystis. Ferramentas de poda.

SUMMARY

GENERAL INTRODUCTION	9
REFERENCES	12
CHAPTER 1 – The effectiveness of fungicides as protectors of pruning wounds from <i>Ceratocystis fimbriata</i> infection in kiwifruit cultivars	15
ABSTRACT	15
INTRODUCTION	16
MATERIAL AND METHODS	17
RESULTS	20
DISCUSSION	22
CONCLUSIONS	24
ACKNOWLEDGMENTS	24
REFERENCES	24
FIGURES AND TABLES	27
CHAPTER 2 – Mechanical dispersal of <i>Ceratocystis fimbriata</i> and disinfestation strategies	32
ABSTRACT	32
INTRODUCTION	33
MATERIAL AND METHODS	34
RESULTS	37
DISCUSSION	39
ACKNOWLEDGMENTS	42
REFERENCES	42
FIGURES AND TABLES	45
CHAPTER 3 – Pathogenicity of <i>Ceratocystis</i> spp. isolates from different hosts and geographic regions on kiwifruit cultivars	52
ABSTRACT	52
INTRODUCTION	53
MATERIAL AND METHODS	54
RESULTS	56
DISCUSSION	57
ACKNOWLEDGMENTS	59
REFERENCES	60
FIGURES AND TABLES	63

GENERAL INTRODUCTION

Ceratocystis wilt, caused by *Ceratocystis fimbriata* Ellis & Halst., is a vascular disease that causes wilt, root rot and canker in several agricultural and forestry crops around the world (HARRINGTON et al., 2011; OLIVEIRA et al., 2015). The wide host range of *C. fimbriata* (s.l.) includes economically important species, such as *Ipomea batatas* Lam. (WEBER and STAHMANN, 1964), *Eucalyptus* spp. (OLIVEIRA et al., 2016), *Coffea arabica* Linn. (MARIN et al., 2003), *Mangifera indica* Linn. (OLIVEIRA et al., 2015), *Theobroma cacao* Linn. (ENGELBRECHT et al., 2007), *Acacia mangium* Willd. (TARIGAN et al., 2011), *Ficus carica* Linn. (HARRINGTON et al. 2011), *Tectona grandis* Linn. f. (FIRMINO et al., 2012), *Metrosideros polymorpha* Gaudish. (FORTINI et al., 2019), *Caryocar brasiliensis* Cambess. (SILVA et al., 2017), among others.

Although *C. fimbriata* (s.l.) has been reported causing diseases in several host plants for more than 75 years in Brazil, only in 2010 the Ceratocystis wilt was first reported in kiwifruit vines, in the state of Rio Grande do Sul, Brazil (SONEGO et al., 2010). In kiwifruit vines the symptoms include wilting, dark-brown lesions in the xylem, reduced fruit length and death of infected plants (PIVETA et al., 2016; FERREIRA et al., 2017). In some kiwifruit cultivars, the pathogen can cause mortality of up to 30% of the plants in the orchard (PIVETA et al., 2016). Ceratocystis wilt is a serious disease affecting kiwifruit crop in Brazil, where it is currently one of the main limiting factors for the cultivation and expansion of kiwifruit crop in the country (PIVETA et al., 2016, FERREIRA et al., 2017). The disease also poses a threat to other major kiwifruit producing countries such as China, Italy, Iran, Greece, Chile, and New Zealand, where the disease has not yet been reported (OLIVEIRA et al., 2016; FERREIRA et al., 2017; FAOSTAT, 2022).

The high intraspecific genetic diversity found among isolates of *C. fimbriata* (s.s.) in a single kiwifruit farm, located in the state of Rio Grande do Sul, Brazil, suggests that this is an indigenous soilborne population and was spread to other farms through the use of infected grafting material (FERREIRA et al., 2017). This rapid dissemination of the disease has led to its presence in major kiwifruit growing regions in Brazil (PIVETA et al. 2016). Observations in kiwifruit vines have shown that the most infections occur first through the roots, due the presence of the *C. fimbriata* inoculum in the soil (PIVETA et al., 2016) and seems to spread the disease in kiwifruit vines during pruning activities of the vines (Gervásio Silvestrin, personal information, 2022), similarly to other hosts that need pruning, such as mango

(OLIVEIRA et al., 2016), acacia (SYAZWAN et al., 2021), teak (SILVA et al., 2021) and eucalypt for lumber (ALFENAS et al., 2009). Thus, the disinfection of equipment and pruning tools, can be an important strategy to control *Ceratocystis* wilt in kiwifruit (OLIVEIRA et al., 2016; ROSADO et al. 2010; CHI et al., 2019). However, there is a limited number of published research related to the control of *C. fimbriata* on kiwifruit, and there is no information on fungicide efficacy against the pathogen in the crop.

Planting of resistant genotypes has been an effective control strategy against *Ceratocystis* wilt in several crops (ROSADO et al., 2010; ARRIEL et al., 2016). Although, the kiwifruit cultivars planted in Brazil are susceptible to *Ceratocystis* wilt (PIVETA et al., 2016; OLIVEIRA et al., 2020), the genetic variability for resistance to *Ceratocystis* wilt in kiwifruit has already been reported (OLIVEIRA et al., 2020). Furthermore, the resistance pattern and different levels of susceptibility found among kiwifruit cultivars, suggest that the resistance in kiwifruit against *C. fimbriata* is quantitative, as observed in other hosts (GUIMARÃES et al., 2010; ROSADO et al., 2010; ARRIEL et al., 2016; OLIVEIRA et al., 2020). Quantitative resistance is polygenic and occurs after effector-triggered resistance has been overcome by the pathogen (GUIMARÃES et al., 2010; COWGER AND BROWN, 2019; LECLERC et al., 2019), and some level of specificity according to pathogen genotype is expected (COWGER AND BROWN, 2019; LECLERC et al., 2019). Because of this, the variability of the pathogen population is an important factor to consider when assessing disease control viability based on quantitative resistance (LECLERC et al., 2019; FOURIE et al., 2019). The complex *Ceratocystis fimbriata* sensu lato (s.l.) is formed by a group of species with a high genetic variability, with some species or isolates related to single hosts or geographic regions, while others infect a wide range of hosts (THORPE et al., 2005; TYSON et al., 2020). Furthermore, the *C. fimbriata* intraspecific variability is related to aggressiveness variation and specific interaction may occur with particular cultivar or clone (COWGER et al., 2000; OLIVEIRA et al., 2020). Thus, in addition to the variability of *C. fimbriata* in kiwifruit, it is important to consider the genetic variability of *C. fimbriata* (s.l.) and its interaction with cultivars for the selection of disease-resistant genotypes.

Studies developed since *Ceratocystis* wilt was reported in kiwifruit have provided important information about the pathosystem, however, there are still knowledge gaps to development of effective disease management strategies. Therefore, our work aims to answer some research questions related to this pathosystem and is presented here in three chapters: (i) The effectiveness of fungicides as protectors of pruning wounds from *Ceratocystis fimbriata* infection in kiwifruit cultivars (ii) Mechanical dispersal of *Ceratocystis* wilt by pruning tools

and disinfestation strategies and (iii) Pathogenicity of *Ceratocystis* spp. isolates from different hosts and geographic regions on kiwifruit cultivars.

REFERENCES

- ALFENAS, A.C.; ZAUZA, E.A.V.; MAFIA, R.; ASSIS, T.F. **Clonagem e doenças do eucalipto**. 2. ed. Viçosa-MG: Editora UFV, 2009. 500 p.
- ARRIEL, D.A.A.; GUIMARÃES, L.M.S.; RESENDEC, M.D.V.; NETOD, F.P.L.; SILVA, D.F.S.H.S.; SIQUEIRA, D.L.; ALFENAS, A.C. **Genetic control of resistance on *Mangifera indica* to *Ceratocystis* wilt**. *Scientia Horticulturae*, v. 211, p. 312–318, 2016.
- CHI, N.M.; THU, P.Q.; MOHAMMED C. **Screening disease resistance of *Acacia auriculiformis* clones against *Ceratocystis manginecans* by artificial and natural inoculation methods**. *Australasian Plant Pathology*, v. 10, p. 1-8, 2019.
- COWGER, C.; BROWN, J.K.M. **Durability of quantitative resistance in crops: greater than we know?** *Annual Review of Phytopathology*, v. 57, n. 12, p. 1-12, 2019.
- COWGER, C.; HOFFER, M.E.; MUNDT, C.C. **Specific adaptation by *Mycosphaerella graminicola* to a resistant wheat cultivar**. *Plant Pathology*, v. 49, p. 445–451, 2000.
- ENGELBRECHT, C.J.B.; HARRINGTON, T.C.; ALFENAS, A.C.; SUAREZ, C. **Genetic variation of populations of the cacao wilt pathogen, *Ceratocystis cacaofunesta***. *Plant Pathology*, v. 56, p. 923- 933, 2007.
- FAOSTAT (2022) **Production of kiwi: top ten producers**. <<https://www.fao.org/faostat/en/#search/kiwi>> Accessed: 25 jan. 2023.
- FERREIRA, M.A.; HARRINGTON, T.C.; PIVETA, G.; ALFENAS, A.C. **Genetic variability suggests that three populations of *Ceratocystis fimbriata* are responsible for the *Ceratocystis* wilt epidemic on kiwifruit in Brazil**. *Tropical Plant Pathology*, v. 42, p. 86–95, 2017.
- FIRMINO, A.C.; TOZZE, H.J.; FURTADO, E.L. **First report of *Ceratocystis fimbriata* causing wilt in *Tectona grandis* in Brazil**. *New Disease Reports*, v. 25, p. 24-24, 2012.
- FORTINI, L.B.; KAISER, L.R.; KEITH, L.M.; PRICE, J.; HUGHES, R.F.; JACOBI, J.D.; FRIDAY, J.B. **The evolving threat of Rapid ‘Ōhi‘a Death (ROD) to Hawai‘i’s native ecosystems and rare plant species**. *Forest Ecology and Management*, v. 448, p. 376-385, 2019.
- FOURIE, A.; VAN, D.E.R.; NEST, M.A.; DE VOS, L.; WINGFIELD, M.J.; WINGFIELD, B.D.; BARNES, I. **QTL mapping of mycelial growth and aggressiveness to distinct hosts in *Ceratocystis* pathogens**. *Fungus Genetic and Biology*, v. 131, p. 103242, 2019.
- GUIMARÃES, L.M.S.; TITON, M.; LAU, D.; ROSSE, L.N.; OLIVEIRA, L.S.S.; ROSADO, C.C.G.; CHRISTO, G.G.O.; ALFENAS, A.C. ***Eucalyptus pellita* as a source of resistance to rust, *Ceratocystis* wilt and leaf blight**. *Crop Breeding and Applied Biotechnology*, v. 10, p. 124–131, 2010.
- HARRINGTON, T.C.; THORPE, D.J.; ALFENAS, A.C. **Genetic Variation and Variation in Aggressiveness to Native and Exotic Hosts Among Brazilian Populations of *Ceratocystis fimbriata***. *Phytopathology*, v. 101, n. 5, p. 555-566, 2011.

LECLERC, M.; CLÉMENT, J.A.J.; ADRIVON, D.; HAMELIN, M. **Assessing the effects of quantitative host resistance on the life-history traits of sporulating parasites with growing lesions.** Proceedings of the Royal Society B, v. 286, p. 20191244, 2019.

MARIN, M.; CASTRO, B.; GAITAN, A.; PREISIG, O.; WINGFIELD, B.D.; WINGFIELD, M.J. **Relationships of *Ceratocystis fimbriata* isolates from Colombian coffee-growing regions based on molecular data and pathogenicity.** Journal of Phytopathology 151:395–405, 2003.

OLIVEIRA, L.S.S.; DAMACENA, M.B.; GUIMARÃES, L.M.S.; SIQUEIRA, D.L.; ALFENAS, A.C. ***Ceratocystis fimbriata* isolates on *Mangifera indica* have different levels of aggressiveness.** European Journal of Plant Pathology, v. 145, p. 847–856, 2016.

OLIVEIRA, L.S.S.; HARRINGTON, T.C.; FERREIRA, M.A.; MICHELLE, B.D.; AL-SADI, A.M.; AL-MAHMOOLI, I.H.S.; et al. **Species or Genotypes? Reassessment of four recently described species of the *Ceratocystis* wilt Pathogen, *Ceratocystis fimbriata*, on *Mangifera indica*.** Phytopathology, v. 105, p. 1229-1244, 2015.

OLIVEIRA, L.S.S.; PIMENTA, L.V.A.; GUIMARÃES, L.M.S.; de SOUZA, P.V.D.; BHERING, L.L.; ALFENAS, A.C. **Resistance of kiwifruit cultivars to *Ceratocystis* wilt: an approach considering the genetic diversity and variation in aggressiveness of the pathogen.** Plant Pathology, v. 70, p. 349-357, 2020.

PIVETA, G.; FERREIRA, M.A.; MUNIZ, M.; VALDETARO, D.; VALDEBENITO-SANHUEZA, R.; HARRINGTON, T.; et al. ***Ceratocystis fimbriata* on kiwifruit (*Actinidia* spp.) in Brazil.** New Zealand Journal of Crop and Horticultural Science, v. 44, p. 13–24, 2016.

ROSADO, C.C.G.; GUIMARÃES, L.M.D.S.; TITON, M.; LAU, D.; ROSSE, L.; RESENDE, M.D.V.; ALFENAS, A.C. **Resistance to *Ceratocystis* wilt (*Ceratocystis fimbriata*) in parents and progenies of *Eucalyptus grandis* x *E. urophylla*.** Silvae Genetica, v. 59, p. 99-106, 2010.

SILVA, J.E.C.; CRUZ, A.M.; PEREIRA, B.L.C.; OLIVEIRA, A.C.; HOFFMANN, W.R. **Properties of teak wood infected by *Ceratocystis fimbriata*.** Floresta, v. 51, n. 4, p.1037-1045, 2021.

SILVA, A.C.; CANDIDO, T.S.; SALES, N.L.P.; HARRINGTON, T.C.; ALFENAS, A.C. **First Report of *Ceratocystis* Wilt Caused by *Ceratocystis fimbriata* on *Caryocar brasiliense* Trees in Brazil.** Plant Disease, v. 101, n. 10, p. 1822-1822, 2017.

SONEGO, O.R.; FERREIRA, M.A.; SANHUEZA, R.M.; GAVA, R.; GARRIDO, L.R.; ALFENAS, A.C. **Primeiro relato da murcha-de-ceratocystis em kiwi.** Tropical Plant Pathology, v. 35, p. S233, 2010.

SYAZWAN, A.; MOHD-FARID, A.; WAN-MUHD-AZRUL, W.; SYAHMI H.M.; ZAKI, A.M.; ONG, S.P.; MOHAMED, R. **Survey, Identification, and Pathogenicity of *Ceratocystis fimbriata* Complex Associated with Wilt Disease on *Acacia mangium* in Malaysia Samsuddin.** Forests, v. 12, p. 1782, 2021.

TARIGAN, M.; ROUX, J.; WYK, M.V.; TJAHOJONO, B.; WINGFIELD, M.J. **A new wilt and die-back disease of *Acacia mangium* associated with *Ceratocystis manginecans* and**

***C. acaciivora* sp. nov. in Indonesia.** South African Journal of Botany, v. 77, p. 292–304, 2011.

THORPE, D.J.; HARRINGTON, T.C.; UCHIDA, J.Y. **Pathogenicity, internal transcribed spacer-rDNA variation, and human dispersal of *Ceratocystis fimbriata* on the family Araceae.** Phytopathology, v. 95, p. 316-323, 2005.

TYSON, J.L.; MANNING, M.A.; WRIGHT, P.J. **Pathogenicity of *Ceratocystis fimbriata* from New Zealand kumara on kiwifruit cultivars.** New Zealand Plant Protection, v. 73, p. 6–12, 2020.

WEBER, D.J.; STAHMANN, M.A. ***Ceratocystis* infection in sweet potato: its effect on proteins, isozymes, and acquired immunity.** Science, v. 146, n. 3646, p. 929-31, 1964.

CHAPTER 1 – The effectiveness of fungicides as protectors of pruning wounds from *Ceratocystis fimbriata* infection in kiwifruit cultivars

Sabrina A. Cassol,^a Samuel A. Santos,^{a*} Marston H. D. Franceschini,^a Flavia S. Alexandre,^a Davi M. Macedo,^a Chandan Pal,^b Acelino C. Alfenas,^a and Rafael F. Alfenas.^{a#}

^aLaboratory of Forest Pathology, Department of Plant Pathology/Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO), Universidade Federal de Viçosa, Viçosa, MG, 36570-900, MG, Brazil.

^b Innovation Manager -Biosecurity at Zespri International Limited Postal address: Zespri International Limited, PO Box 4043, Mount Maunganui 3149. Physical address: 400 Maunganui Road, Mount Maunganui, New Zealand.

Address correspondence to Rafael F. Alfenas, rafael.alfenas@ufv.br.

*Present address: Samuel A. Santos, Plant Health Program, Fiber R&D Asia Pacific Resources International Ltd (APRIL), Pangkalan Kerinci 28654, Riau, Sumatra, Indonesia.

To be published at: New Zealand Plant Protection.

ABSTRACT

Ceratocystis wilt, caused by *Ceratocystis fimbriata*, is the most important disease in kiwifruit and is limiting factor for the cultivation and expansion of the crop in Brazil. It also poses a threat to the world's largest kiwifruit producing countries, where the disease was not reported yet. In kiwifruit vines, the infection starts mainly through the roots, but also by pruning wounds. There is, currently, a lack of proven effective disease control strategies and there is no information on the effectiveness of fungicides against the pathogen. In the present study, among four fungicides tested (metalaxyl + mancozeb, boscalide + cresoxim-methyl, pyraclostrobin, copper hydroxide), we found that metalaxyl + mancozeb and pyraclostrobin were the most effectiveness against *in vitro* mycelial growth and spore germination of *C.*

fimbriata isolates, but these had no statistically significant effect (Tukey HSD test with p -value ≤ 0.05) as wound pruning protectors when evaluated in two kiwifruit cultivars.

Keywords: *Actinidia* spp., Ceratocystis wilt, chemical control, crop management, mycelial growth, spore germination.

INTRODUCTION

Ceratocystis wilt, caused by *Ceratocystis fimbriata* (s.l.), is a vascular disease that causes wilt, root rot and canker on a wide range of economically important forest and agricultural hosts around the world (Weber and Stahmann 1964; Engelbrecht et al. 2007; Harrington et al. 2011; Tarigan et al. 2011; Firmino et al. 2012; Oliveira et al. 2015; Silva et al. 2017; Fortini et al. 2019; Nasution et al. 2019). In 2010, *C. fimbriata* was first reported causing disease in kiwifruit vines, in the state of Rio Grande do Sul, Brazil (Sonego et al. 2010). The disease symptoms include wilt, dark-brown lesions in the xylem, reduced fruit size and eventual death of infected vines (Piveta et al. 2016; Ferreira et al. 2017). In some kiwifruit cultivars, the mortality can reach up to 30% in the orchards (Piveta et al. 2016). Currently, Ceratocystis wilt occurs in the main kiwifruit producing areas in Brazil and it is considered the most severe disease and is a limiting factor for the cultivation of the plant and expansion of the crop in the country (Piveta et al. 2016; Ferreira et al. 2017). Additionally, the disease is a threat to the world's largest kiwifruit producing countries, such as China, Italy, Iran, Greece, Chile and New Zealand, where the disease has not been reported yet on kiwifruit (Oliveira et al. 2016; Ferreira et al. 2017; FAOSTAT, 2022).

Observations in kiwifruit orchards, showed that most infections start mainly through the roots, due the presence of the *C. fimbriata* in the soil (Piveta et al. 2016), but it wounds, made by human activities in orchards, are also a relevant infection points for penetration of the pathogen in hosts (Harrington 2013; Nasution et al. 2019). Pruning in commercial kiwifruit vines is a common practice, done twice a year, to improve yield and fruit quality (KVH 2019). Because of this, like in others *C. fimbriata* hosts that need pruning, such as mango (Oliveira et al. 2016), acacia (Syazwan et al. 2021), teak (Silva et al. 2021) and eucalypt for lumber (Alfenas et al. 2009), fresh pruning wounds are in practice an important infection site for the pathogen in kiwifruit vines (Gervásio Silvestrin, personal information 2022). In these cases, disinfection of pruning tools and equipment becomes important, since

the pathogen can spread from diseased plants to healthy plants by infested pruning tools (Harrington 2013). Furthermore, *C. fimbriata* spores can also be dispersed by wind, rain and insects in orchards (Ferreira et al. 2011; Harrington 2013; Nasution et al. 2019). For this reason, protection of fresh pruning wounds with the application of fungicides or other protective barriers (Brown et al. 2021; Ayres et al. 2022), can potentially be an effective control strategy against *C. fimbriata* if incorporated into the integrated disease management. However, there is a limited number of studies related to chemical control of *C. fimbriata* on kiwifruit vines, and no fungicides have been registered for this pathosystem so far.

In this context, the aims of this study were: (i) to evaluate the *in vitro* effectiveness of fungicides against mycelial growth and spore germination of *C. fimbriata* isolates from kiwifruit plants; and (ii) to determine the effectiveness of the most effective fungicides identified in the *in vitro* assays as pruning wound protectors against Ceratocystis wilt on kiwifruit cultivars.

MATERIAL AND METHODS

***Ceratocystis fimbriata* isolates**

The *C. fimbriata* isolates (LPF1443, 1673, 1688, 1697 and 1701) used in this study were obtained from infected kiwifruit plants in commercial orchards of Rio Grande do Sul and Santa Catarina States, between 2010 and 2015, and have previously been characterized as aggressive on kiwifruit (Piveta et al. 2016; Oliveira et al. 2020). Pure cultures of these isolates were replicated and stored in an ultra-low temperature (ULT) freezer at -80°C.

Plant material

Two kiwifruit cultivars, Monty (*Actinidia deliciosa*) from Brazil and Gold3 (*Actinidia chinensis*) from New Zealand, were used in the present study. Rooted cuttings were grown in 15 × 20 cm polyethylene bags, containing Carolina[®] substrate supplemented with 70 g of Osmocote[®] Plus (NPK: 19:06:10) and 150 g of single super phosphate per 8 kg of substrate, and then kept for 120 days under greenhouse conditions (25 °C ± 5 °C), with regular irrigation, until inoculation.

***In vitro* spore germination**

The efficacy of four commercial fungicides four fungicides, metalaxyl 4% + mancozeb 64% (Ridomil Gold MZ[®]), boscalide 20% + cresoxim-methyl 10% (Collis[®]), pyraclostrobin 25% (Comet[®]) and copper hydroxide 69.1% (Tutor[®]), was evaluated against the spore germination of five *C. fimbriata* kiwifruit isolates (LPF1443, 1673, 1688, 1697 and 1701). The fungus isolates were grown in potato dextrose broth (PDB) culture medium, for 48 h at 25 °C under shaking (150 rpm). The fungal spores were collected by filtering, using a double layer of sterile gauze and resuspended in PDB. The spore concentration was determined by using a hemocytometer and adjusted to 1×10^5 spore mL. Aliquots of 100 μ L of stock fungicide solutions or distilled autoclaved water (control) were added into a 2 mL microcentrifuge tube containing 1.9 mL of the spore suspension of each *C. fimbriata* isolate to obtain final doses of 0 (control), 10, 50, 250, 500, 750, and 1,000 μ g active ingredient (a.i.) mL⁻¹. For each treatment (*C. fimbriata* isolate \times fungicide \times dose), four replicates (2 mL tubes) were used. The tubes were gently agitated and maintained at 25 °C for 6 h. After incubation, 50 μ L of the suspension were pipetted to single-well glass slides and the spore germination (presence of a germ tube greater than the spore diameter) was microscopically (20 \times) evaluated. In each replicate, 100 spores were counted and used to calculate the percentage of spore inhibition, as described in Equation 1.

$$\text{Spore Germination Inhib.} = \frac{(N.\text{of spores germinated}_{\text{control}} - N.\text{of spores germinated}_{\text{treatment}})}{N.\text{of spores germinated}_{\text{control}}} \times 100 \quad (1)$$

Additionally, for those fungicides that inhibited 100 % of the spore germination, the fungistatic or fungicidal effect was further evaluated by placing a droplet of 10 μ L of the spore and fungicide suspension in the center of a Petri plate containing potato dextrose agar (PDA) and incubated at 25 °C and 12 h photoperiod for seven days. After incubation, the mycelial growth was evaluated, and for the isolates that did not grow in PDA it was considered that the treatment had a fungicidal effect, for the isolates that grew it was considered that the effect of the treatment on the pathogen was fungistatic.

***In vitro* mycelial growth**

Besides spore germination, the same four fungicides were tested for mycelial growth inhibition for the same five *C. fimbriata* kiwifruit isolates. Final fungicide concentrations of 0 (control), 50, 100, 500, and 1000 μ g a.i. mL⁻¹ were used. Commercial products were suspended in autoclaved distilled water to obtain stock solutions (2000 μ g a.i. mL) and the

appropriate volume was added to PDA, to obtain the different concentrations. The *C. fimbriata* isolates were grown in PDA medium, at 28°C and 12 h photoperiod, for twelve days. After incubation, mycelial plugs (6 mm diameter) were taken from the margin of the colonies, for each *C. fimbriata* isolate and placed in the center of Petri dishes (60 mm diameter) containing PDA amended with the fungicides. Mycelial growth was evaluated after approximately seven days of incubation, at 25 °C and 12 h photoperiod, until one of the colonies reached the border of the plate. The colony diameter was measured in two perpendicular directions and the mycelial growth was calculated by discounting the plug diameter and subsequently used to determine the percentage of inhibition of mycelial growth, as described in Equation 2.

$$\text{Mycelial Growth Inhib.} = \frac{(\text{Radial mycelial growth}_{\text{Control}} - \text{Radial mycelial growth}_{\text{Treatment}})}{\text{Radial mycelial growth}_{\text{Control}}} \times 100 \quad (2)$$

For each treatment (*C. fimbriata* isolate × fungicide × dose), four replicates (plates) were used, and the experiment was repeated once. Additionally, for those fungicides that inhibited 100% the mycelial growth, the fungistatic or fungicide effect was evaluated by transferring the mycelial plugs to a new plate, containing PDA, and incubated at 25 °C and 12 h photoperiod for seven days. After incubation, the mycelial growth was evaluated, and for the isolates that did not grow in pure PDA, a fungicidal effect was considered and for the isolates that grew the effect was fungistatic.

Fungicide effectiveness as pruning wound protector against *Ceratocystis fimbriata* on kiwifruit vines

The most effective *in vitro* fungicides (metalaxyl + mancozeb and pyraclostrobin) were tested as wound protectors against *C. fimbriata* infection on Gold3 and Monty kiwifruit cultivars. For this, the stem apex of the kiwifruit vines was pruned and each fungicide solution at 250 mg active ingredient (a.i.). L⁻¹ was sprayed, in volume sufficient to obtain thorough vine coverage, using a manual pressure regulated sprayer. After 1 h, 500 µL of inoculum suspension of *C. fimbriata* (LPF1443) at 1.0 x 10⁷ spores/mL was applied on pruned stem apex of each vine. A swab moistened cotton was placed in the inoculation site and the treated plants were incubated in a greenhouse (28 ± 5 °C), with regular irrigation. Two references were adopted for comparison: 1) *non-inoculated control*: pruned vines

sprayed with distilled autoclaved water; and *inoculation control*: pruned vines sprayed with distilled autoclaved water and inoculated. A completely randomized design with five replicate plants per treatment and controls was used. At 45 days of inoculation, the disease incidence (number of infected vines divided by the number of inoculated vines and expressed as percentage) and lesion length (cm), in the inner stem tissues, were measured. Disease severity was determined by dividing lesion length by vine height, and it was expressed in percentage as well. The infection by *C. fimbriata* was confirmed using carrot baits, prepared with stem tissue samples from the evaluated plants. The experiment was repeated once.

Statistical analyses

The *in vitro* assays, performed to evaluate the inhibition of mycelial growth and spore germination according to treatment, comprised more factors in the experimental design than those of interest in the study. Besides the treatments and doses (main or fixed effects), experimental replicates and pathogen isolates also integrated the experimental factors, which presented some degree of significance. Therefore, a framework based on linear mixed effects (LME) model was adopted to compare the marginal mean inhibition resulting from the different treatments and doses. In this analysis, replicates and isolates were included as random effects for model intercept and slope, respectively. For the *in vivo* trial, treatments, kiwifruit cultivars and experimental replicates comprised the experimental factors. Similar to the *in vitro* assays, LME model was used to represent the relationship between disease severity and the different experimental factors. While treatments and cultivars were considered as main or fixed effects, replicates were included in the model as random intercept. All analysis were implemented in R environment v. 4.1.3 (R Core Team 2022) using mainly the packages ‘lme4’ (Bates et al. 2015) and ‘emmeans’ (Length 2022), with a significance level of 0.05 (p-value) in ANOVA and Tukey’s HSD tests, approaches used to evaluate the significance of the experimental factors included in the model and for marginal means comparison, respectively. It is worth noting that since inhibition and severity values were given in percentage, they were logit transformed before analysis, to map these values to real numbers interval and to potentially improve residuals distribution normality.

RESULTS

***In vitro* spore germination**

The majority of *C. fimbriata* spores dispersed in autoclaved distilled water (control) germinated very well and presented a long germs tube after 6 h of incubation. In contrast, the number of germinated spores decreased when treated with fungicides (Fig. 1a). All fungicides inhibited 100% spore germination at 750 and 1000 $\mu\text{g a.i. mL}^{-1}$. Metalaxyl + mancozeb and pyraclostrobin were the most effective and copper hydroxide was the only fungicide that did not inhibit 100% of spore germination at 500 $\mu\text{g a.i. mL}^{-1}$. Inhibition of spore germination increased according to the increasing of fungicide concentration (Fig 1a). Metalaxyl + mancozeb was the only fungicide capable of killing fungus spores. The others were only fungistatic.

***In vitro* mycelial growth**

The inhibition of mycelial growth varied according to the fungicides and concentrations (Fig 1b). Metalaxyl + mancozeb was the most effective (Fig 2) and capable of inhibiting 100% of mycelial growth for all *C. fimbriata* isolates, at the concentrations of 250, 750 and 1000 $\mu\text{g a.i. mL}$ (Fig. 2 and 3). Among the tested fungicides, metalaxyl + mancozeb (50, 250, 750 and 1000 $\mu\text{g a.i. mL}$), pyraclostrobin (250, 750 and 1000 $\mu\text{g a.i. mL}$) and copper hydroxide (750 and 1000 $\mu\text{g a.i. mL}$) resulted in inhibition of mycelial growth greater than 50% when compared to the control. These results demonstrate that metalaxyl + mancozeb and pyraclostrobin were the most effective fungicides against the *C. fimbriata* isolates tested. However, pyraclostrobin was only fungistatic.

Fungicide effectiveness as pruning wound protector against *Ceratocystis fimbriata* on kiwifruit

There were no statistically significant differences ($p\text{-value} \leq 0.05$) in disease incidence and disease severity between inoculated kiwifruit vines previously treated with metalaxyl + mancozeb and pyraclostrobin in comparison to vines only treated with distilled autoclaved water (inoculation control), for both kiwifruit cultivars evaluated (Table 1). All inoculated plants exhibited disease symptoms, including an external necrotic dark lesion in the stem tissue located at the inoculation point (Fig. 4). Infection by *C. fimbriata* was confirmed in all vines that expressed disease symptoms by the development of pathogen structures in carrot baits (Fig. 5), which demonstrates that none of the commercial products had a fungicidal action on the pathogen, under tested conditions. Conversely, none of the

non-inoculated vines developed disease symptoms, and the absence of the pathogen was also confirmed using carrot baits (Fig. 4 and 5). The Linear Mixed Effects (LME) model adopted during the statistical analysis included cultivar (2 factors) and treatments (5 factors, 2 controls and 3 fungicides) as fixed experimental factors (i.e., main factors of interest). The same lack of statistical significance observed between inoculated treatments occurred for the effectiveness of treatments between the evaluated kiwifruit cultivars (p-value of 0.243 in ANOVA). Additionally, analysis of the model residuals indicated only slightly deviation from normality (results not shown), ensuring that the model structure adopted represented well the dataset and model assumptions were respected, therefore allowing reliable evaluation of statistical significance for the different experimental factors through ANOVA and Tukey's HSD test. These results showed that the fungicides evaluated were ineffective, in the dosage evaluated in the present work, as pruning wound protectors against *C. fimbriata* in kiwifruit vines.

DISCUSSION

This study demonstrated that spore germination and mycelium growth of *C. fimbriata* isolates in kiwifruit were inhibited by four different fungicides (metalaxyl + mancozeb, boscalide + cresoxim-methyl, pyraclostrobin, copper hydroxide). The most effective fungicides in the *in vitro* assays were subsequently evaluated as pruning wound protectors against Ceratocystis wilt in two kiwifruit cultivars.

In vitro assays revealed that the spores of *C. fimbriata* are more sensitive than mycelium to fungicides. Metalaxyl + mancozeb (Ridomil Gold MZ[®]) was highly effective against mycelial growth of *C. fimbriata* isolates. This result corroborates the work of Khan et al. (2017), in which both active ingredients, mancozeb (Dithane M-45) and metalaxyl (Ridomil 75 WP), significantly inhibited *in vitro* mycelial growth of *C. fimbriata* isolate from pomegranate. Somu et al. (2018) also demonstrated that among seven non-systemic fungicides, cymoxanil + mancozeb, mancozeb and captan were the most effective in inhibiting *in vitro* mycelial growth of *C. fimbriata*. Tran et al. (2018) tested fungicides for the control of *Ceratocystis manginecans* (sin *C. fimbriata*), on *Acacia mangium*, and indicated that metalaxyl + mancozeb effectively inhibited *in vitro* mycelial growth of the fungus. Metalaxyl + mancozeb has systemic and residual action, the active ingredient metalaxyl is quickly distributed through the plant green parts. In addition, mancozeb is non-systemic and provides a protective film on the surfaces of the plants, which acts inhibiting

spore germination (Somu et al. 2018). In the present study, we could not demonstrate the individual effect of each active ingredient that comprises the Ridomil Gold MZ[®] formulation, and the concentrations tested were too high for estimating the EC50.

Pyraclostrobin, the ingredient active of the systemic fungicide Comet[®], are effective in inhibiting sporulation, spore germination, and mycelial growth of the target fungal pathogens and has demonstrated efficacy in other pathosystems as pruning wound protector (Sosnowski et al. 2013; Liang et al. 2015; Ayres et al. 2022). This fungicide act in the mitochondrial respiration chain of the pathogen, blocking the transport of electrons in the quinol-oxidizing site of the cytochrome bc1 complex (complex III) and resulting in the interruption of the synthesis of adenosine triphosphate (Liang et al. 2015). Brown et al. (2021) evaluated pruning wound protectors in *Vitis vinifera* vineyard against trunk pathogens, and showed that pyraclostrobin + boscalid was highly effective against *Botryosphaeria-dieback*, *Neofusicoccum parvum*, and *Phomopsis-dieback*, *Diaporthe ampelina*, suggesting that the spray application of these protectants could minimize infection by these pathogens through pruning wounds in vineyards. Ayres et al. (2022) reported that the fungicides tebuconazole, fluazinam and pyraclostrobin can protect grapevine pruning wounds against *Eutypa lata* and *Neofusicoccum luteum*. In Brazil, Comet[®] is registered for kiwifruit crop to control *Colletotrichum gloeosporioides* and *Oidium mangiferae* (Amaral et al. 2021). In our study, pyraclostrobin was effective in inhibiting spore germination and mycelial growth of *C. fimbriata* isolates. However, the fungicides metalaxyl + mancozeb and pyraclostrobin did not prevent the infection by *C. fimbriata* through pruning wounds in kiwifruit vines, in the methods tested in this work. These results show that *in vivo* assays are necessary to confirm the effectiveness of the fungicides in controlling the targeted fungus in a living organism.

Although all inoculated kiwifruit vines became infected, plants pulverized with metalaxyl + mancozeb and pyraclostrobin solutions had generally smaller lesions compared to plants only pulverized with water. Even though this reduction in disease severity in plants treated with fungicides was not statically significant compared to the control, it is interesting to consider that the inoculum rate used in the experiment was higher than what naturally occurs in the field (Ayres et al. 2017), and ideal conditions were used for the infection by the pathogen to occur. Furthermore, the products may have toxic effects on the pathogen by retarding disease progress or inhibiting the sporulation, which can contribute to the reduction of infection in the field. However, more research is needed to fully understand this effect.

The present study showed the effectiveness of fungicides in inhibiting spore germination and mycelial growth of *C. fimbriata* kiwifruit isolates. Therefore, it can serve as a guide for further studies related to chemical control strategies for the disease. Additionally, we showed that the selected fungicides are not effective in protecting kiwifruit pruning wounds against Ceratocystis wilt, however, it is known that the mode of application and dosage can affect the effectiveness of the fungicide and therefore testing other methods and fungicides becomes interesting. To our knowledge, this is the first evaluation of chemical control of *C. fimbriata*- kiwifruit pathosystem.

CONCLUSIONS

In the present study, among four fungicides tested (metalaxyl + mancozeb, boscalide + cresoxim-methyl, pyraclostrobin, copper hydroxide), we found that metalaxyl + mancozeb and pyraclostrobin were the most effective fungicides against the *in vitro* mycelial growth and spore germination of *C. fimbriata* isolates from kiwifruit vines, but they had no effectiveness as pruning wound protectors when evaluated in two kiwifruit cultivars.

ACKNOWLEDGMENTS

This work was supported by: ‘Conselho Nacional de Desenvolvimento Científico e Tecnológico’ (CNPq); ‘Coordenação de Aperfeiçoamento de Pessoal de Nível Superior’ (CAPES). The authors are grateful for the assistance of Maik Leão dos Santos, Monalisa de Cól, Carolina Pessanha Alonso, and to Zespri Group Limited and ‘Clonar Resistência a Doenças Florestais’ for providing plant material used in this study.

REFERENCES

- Alfenas AC, Zauza EAV, Mafia R, Assis TF (2009) Clonagem e doenças do eucalipto. 2. ed. Viçosa-MG: Editora UFV, 500p.
- Amaral AL, Cagliari C, Souza EB, Pereira FMD, Santos RSS, Garrido LR (2021) Grade de agrotóxicos do kiwifruit. Bento Golçalves: Embrapa Uva e Vinho, 2021. 19 p. (Embrapa Uva e Vinho) INSS 188-680
- Ayres MR, Wicks TJ, Scott ES, Sosnowski MR (2017) Developing pruning wound protection strategies for managing Eutypa dieback. Aust J Grape Wine Res 23:103–111
- Ayres MR, Billones-Baaijens R, Savocchia S, Scott ES, Sosnowski MR (2022) Critical timing of fungicide application for pruning wound protection to control grapevine

- trunk diseases. *Aust J Grape Wine Res* 28:70–74
- Bates D, Maechler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67(1), 1-48.
- Brown AA, Travadon R, Lawrence DP, Torres G, Zhuang G, Baumgartner K (2021) Pruning-wound protectants for trunk-disease management in California table grapes. *J Crop Prot* 141:105490
- Engelbrecht CJB, Harrington TC, Alfenas AC, Suarez C (2007) Genetic variation of populations of the cacao wilt pathogen, *Ceratocystis cacaofunesta*. *Plant Pathol J* 56: 923- 933
- FAOSTAT (2022) Production of kiwi: top ten producers. Available at: <https://www.fao.org/faostat/en/#search/kiwi>. Accessed on January 25, 2023
- Ferreira A, Harrington TC, Alfenas AC, Mizubuti ESG (2011) Movement of Genotypes of *Ceratocystis fimbriata* Within and Among Eucalyptus Plantations in Brazil. *Ecol and Epidemiology* 101(8):1005-1012
- Ferreira MA, Harrington TC, Piveta G, Alfenas AC (2017) Genetic variability suggests that three populations of *Ceratocystis fimbriata* are responsible for the Ceratocystis wilt epidemic on kiwifruit in Brazil. *Trop Plant Pathol* 42:86–95
- Firmino AC, Tozze HJ, Furtado EL (2012) First report of *Ceratocystis fimbriata* causing wilt in *Tectona grandis* in Brazil. *New Dis Rep* 25:24-24
- Fisher DJ, Hayes AL (1982) Mode of action of the systemic fungicides furalaxyl, metalaxyl and ofurace. *Pest Manag Scie* 13:330-339
- Fortini LB, Kaiser LR, Keith LM, Price J, Hughes RF, Jacobi JD, Friday JB (2019) The evolving threat of rapid ‘ohi’a death (ROD) to Hawaii's native ecosystems and rare plant species. *For Ecol. Manage* 448:376-385
- Harrington TC (2013) Ceratocystis Diseases. *Infectious Forest Diseases* (ed) P Gonthier and G Nicolotti, Wallingford, UK, pp 230-255
- Harrington TC, Thorpe DJ, Alfenas AC (2011) Genetic Variation and Variation in Aggressiveness to Native and Exotic Hosts Among Brazilian Populations of *Ceratocystis fimbriata*. *Annu Rev Phytopathol* 101(5):555-566
- Jenkins C (2011) Putting the KVH Winter Management Programme into Practice. *NZ Kiwifruit J* 8-10
- Khan HSI, Ravindra H, Ekbote S, Narayaswamy H, Narayaswamy P, Pradeep S (2017) Efficacy of Fungicides and Bio Agents against *Ceratocystis fimbriata* Ell. and Halst. Causing Wilt Disease of Pomegranate. *Int J Curr Microbiol App Sci* 6(6):2902-2907
- Length RV (2022) emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.8.2. <https://CRAN.R-project.org/package=emmeans>. Accessed 12 January 2023
- Liang H-J, Di Y-L, Li J-L, You H, Zhu F (2015) Baseline Sensitivity of Pyraclostrobin and Toxicity of SHAM to *Sclerotinia sclerotiorum*. *Plant Dis* 99:267-273
- KVH (2019) Readiness plan - *Ceratocystis fimbriata*. 40 p.
- Nasution A, Glen M, Beadle C, Mohammed C (2019) Ceratocystis wilt and canker - A disease that compromises the growing of commercial Acacia-based plantations in the tropics. *Aust For.* 82:80–93
- Oliveira LSS, Harrington TC, Ferreira MA, Michelle BD, Al-Sadi, AM, Al-Mahmooli IHS et al (2015) Species or Genotypes? Reassessment of four recently described species of the Ceratocystis wilt Pathogen, *Ceratocystis fimbriata*, on *Mangifera indica*. *Phytopathol* 105:1229-1244
- Oliveira LSS, Damacena MB, Guimarães LMS, Siqueira DL, Alfenas AC (2016) Ceratocystis fimbriata isolates on *Mangifera indica* have different levels of

- aggressiveness. *Eur J Plant Pathol* 145:847–856
- Oliveira LSS, Pimenta LVA, Guimarães LMS, de Souza PVD, Bhering LL, Alfenas AC (2020) Resistance of kiwifruit cultivars to *Ceratocystis* wilt: an approach considering the genetic diversity and variation in aggressiveness of the pathogen. *Plant Pathol J* 70:349-357
- Piveta G, Ferreira MA, Muniz M, Valdetaro D, Valdebenito-Sanhueza R, Harrington T et al (2016) *Ceratocystis fimbriata* on kiwifruit (*Actinidia* spp.) in Brazil. *New Zeal J Crop Horticult Sci* 44:13–24
- R Core Team (2022) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>. Accessed 24 January 2023.
- Silva AC, Cândido TS, Sales NLP, Harrington TC, Alfenas AC (2017) First Report of *Ceratocystis* Wilt Caused by *Ceratocystis fimbriata* on *Caryocar brasiliense* Trees in Brazil. *Plant Dis* 101(10):1822-1822
- Silva JEC, Cruz AM, Pereira BLC, Oliveira AC, Hoffmann WR (2021) Properties of teak wood infected by *Ceratocystis fimbriata*. *Floresta* 51(4):1037-1045
- Somu R, Mesta RK, Kiran KKC (2018) Effect of non systemic, systemic fungicides and bio control agents against wilt of pomegranate caused by *Ceratocystis fimbriata*. *Int J of Chem Stud* 6(2):397-402
- Sonego OR, Ferreira MA, Sanhueza RM, Gava R, Garrido LR, Alfenas AC (2010) Primeiro relato da murcha-de-ceratocystis em kiwifruit. *Trop Plant Pathol* 35:S233
- Sosnowski MR, Loschiavo AP, Wicks TJ and Scott ES (2013) Evaluating treatments and spray application for the protection of grapevine pruning wounds from infection by *Eutypa lata*. *Plant Dis* 97:1599–1604
- Syazwan A, Mohd-Farid A, Wan-Muhd-Azrul W, Syahmi HM, Zaki AM, Ong SP, Mohamed R (2021) Survey, Identification, and Pathogenicity of *Ceratocystis fimbriata* Complex Associated with Wilt Disease on *Acacia mangium* in Malaysia Samsuddin. *Forests* 12:1782
- Tarigan M, Roux J, Wyk MV, Tjahjono B, Wingfield MJ (2011) A new wilt and die-back disease of *Acacia mangium* associated with *Ceratocystis manginecans* and *C. acaciivora* sp. nov. in Indonesia. *S Afri J of Bot* 77:292–304
- Tran TTT, Pham TQ, Barber PA, Nguyen CM (2018) Control of *Ceratocystis manginecans* causing wilt disease on *Acacia mangium* seedlings. *Aust Plant Pathol* 47(6):579-586
- Weber DJ and Stahmann MA (1964) *Ceratocystis* infection in sweet potato: its effect on proteins, isozymes, and acquired immunity. *Science* 146(3646):929-31

FIGURES AND TABLES

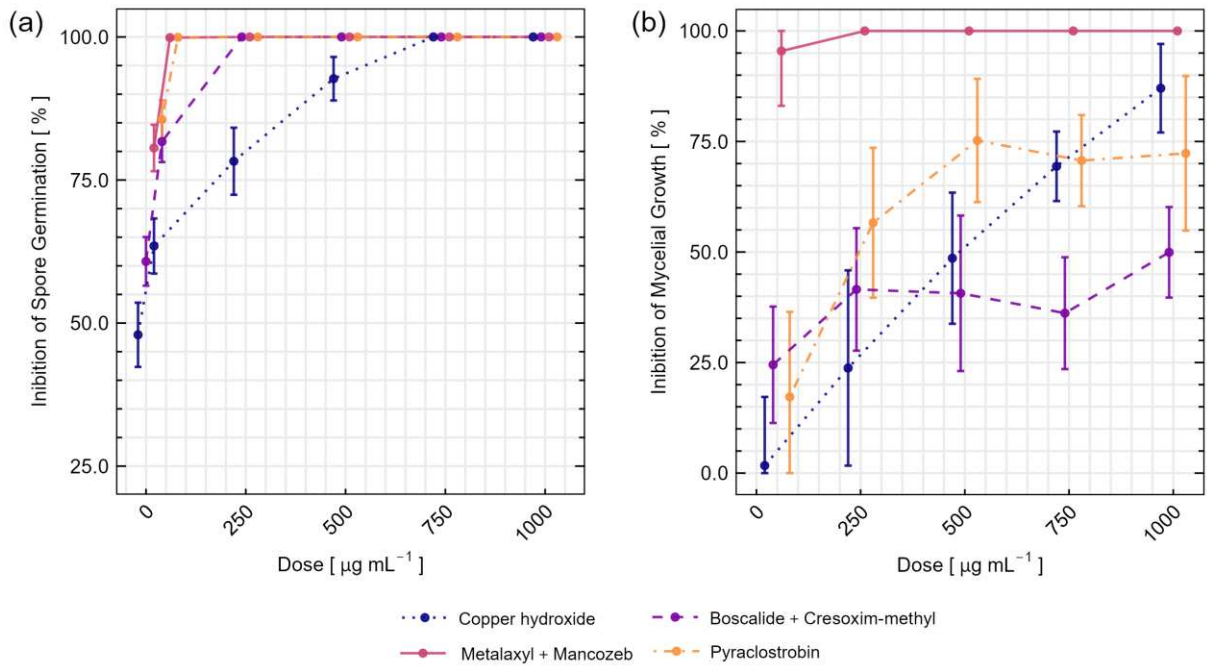


Fig. 1 Sensitivity *in vitro* of: (a) spore germination and (b) mycelial growth of *Ceratocystis fimbriata* to fungicides metalaxyl 4% + mancozeb 64% (Ridomil Gold MZ[®]), boscalide 20% + cresoxim-methyl 10% (Collis[®]), pyraclostrobin 25% (Comet[®]), and copper hydroxide 69.1% (Tutor[®]). Error bars indicate the standard deviation derived from the dataset in each case. Points for each dose are represented slightly shifted when necessary, according to treatment, to avoid overlap.

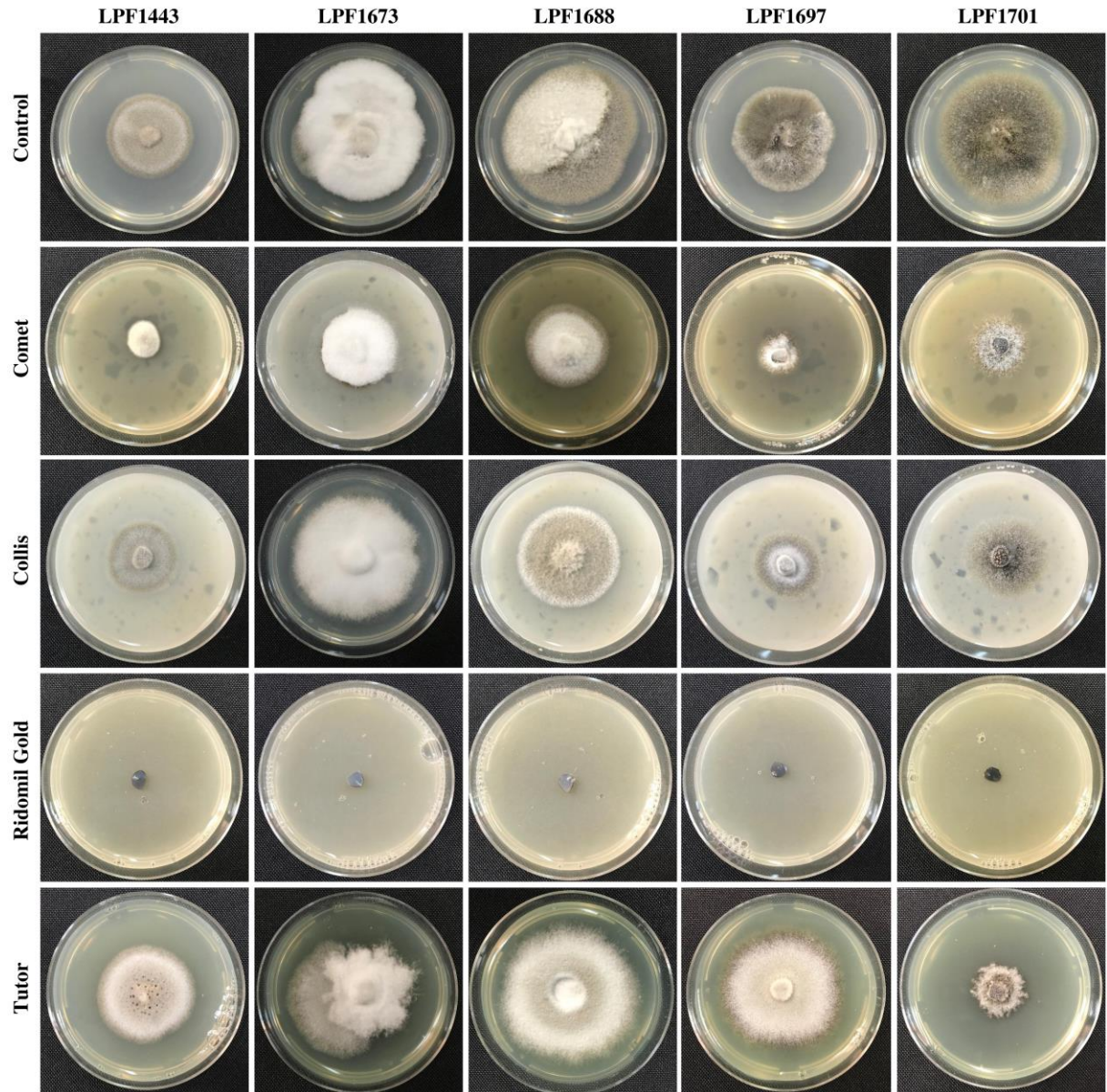


Fig. 2 Colonies of *Ceratocystis fimbriata* isolates (LPF1443, LPF1673, LPF1688, LPF1697, and LPF1701) non-treated (Control) and exposed to different fungicides [metalaxyl 4% + mancozeb 64% (Ridomil Gold MZ[®]), boscalide 20% + cresoxim-methyl 10% (Collis[®]), pyraclostrobin 25% (Comet[®]), and copper hydroxide 69.1% (Tutor[®])] at 250 $\mu\text{g a.i. mL}^{-1}$.

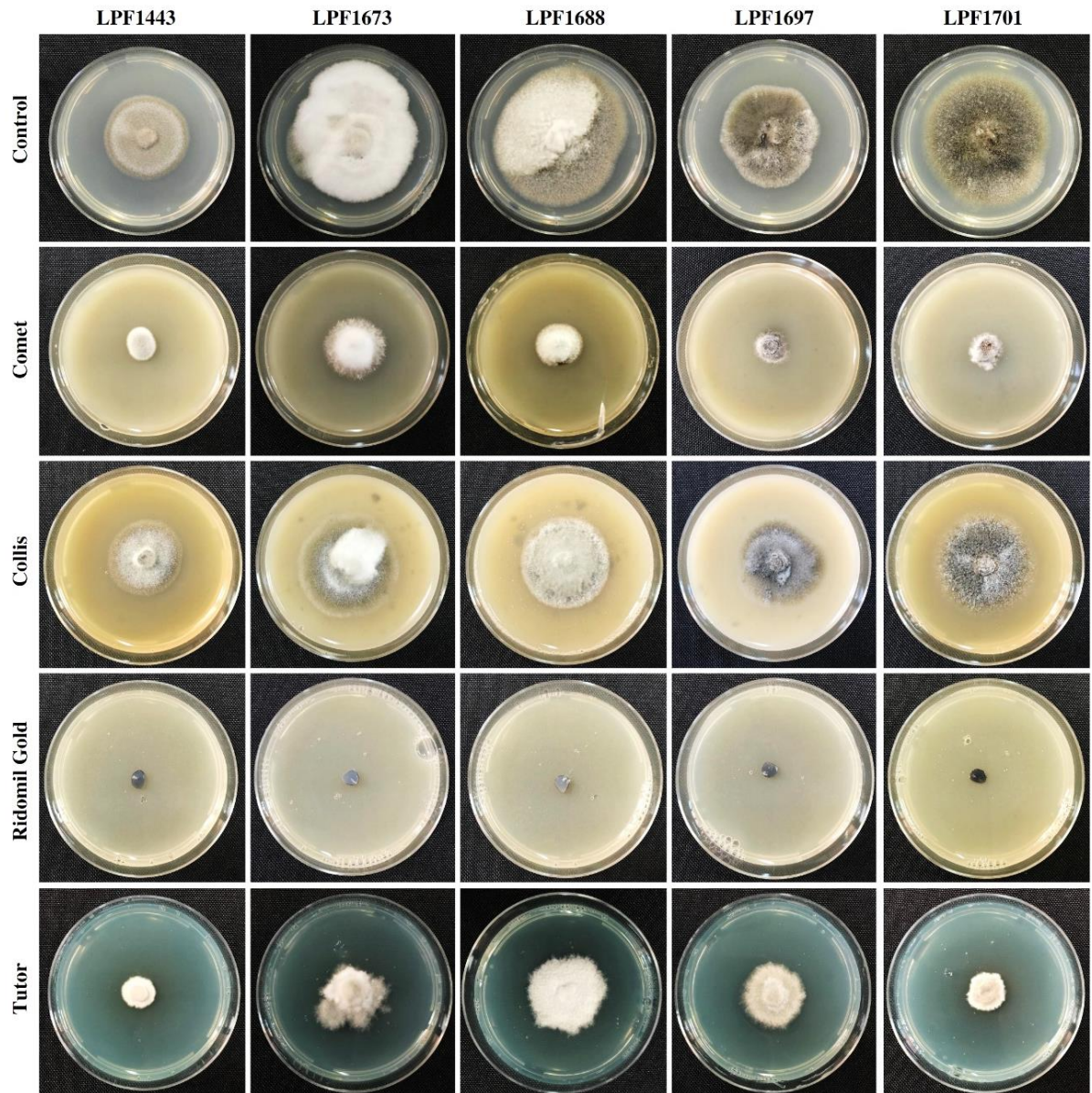


Fig. 3 Colonies of *Ceratocystis fimbriata* isolates (LPF1443, LPF1673, LPF1688, LPF1697, and LPF1701) non-treated (Control) and exposed to different fungicides [metalaxyl 4% + mancozeb 64% (Ridomil Gold MZ[®]), boscalide 20% + cresoxim-methyl 10% (Collis[®]), pyraclostrobin 25% (Comet[®]), and copper hydroxide 69.1% (Tutor[®])] at 500 $\mu\text{g a.i. mL}^{-1}$.

Table 1 Wound protector efficacy of fungicides at 250 µg active ingredient mL⁻¹, sprayed after pruning and followed by inoculation with *Ceratocystis fimbriata* in two kiwifruit cultivars

Treatment	Incidence (%)		Severity (%) ¹		Carrot bait ²	
	“Gold3”	“Monty”	“Gold3”	“Monty”	“Gold3”	“Monty”
non-inoculated control	0	0	0a	0a	-	-
inoculation control	100	100	35,22b	45,83b	+	+
pyraclostrobin 25%	100	100	26,83b	48,6b	+	+
metalaxyl 4% + mancozeb 64%	100	100	28b	30,2b	+	+

¹Means within a column followed by the same letters are not significantly different from each other according to Tukey’s HSD test (p-value<0.05).

²+ : Presence of pathogen structures in carrot bait. - : Absence of pathogen structures in carrot bait.



Fig. 4 Kiwifruit vines from the experiment done to evaluate the effectiveness of metalaxyl 4% + mancozeb 64% (Ridomil Gold MZ[®]) and pyraclostrobin 25% (Comet[®]) as pruning wound protectors against *Ceratocystis fimbriata*, after 45 days from the inoculation date: **A** – Pruned vines sprayed with autoclaved distilled water and non-inoculated (control); **B** – Pruned apex of one vine that received the control treatment; **C** – Absence of internal lesion in the stem of control-treated vines; **D** – Pruned vines sprayed with metalaxyl 4% + mancozeb 64% and inoculated with *C. fimbriata*; **E** – External lesion of pruned vine sprayed with metalaxyl 4% + mancozeb 64% and inoculated; **F** – Internal lesion in the stem of vine sprayed with metalaxyl 4% + mancozeb 64% and inoculated.

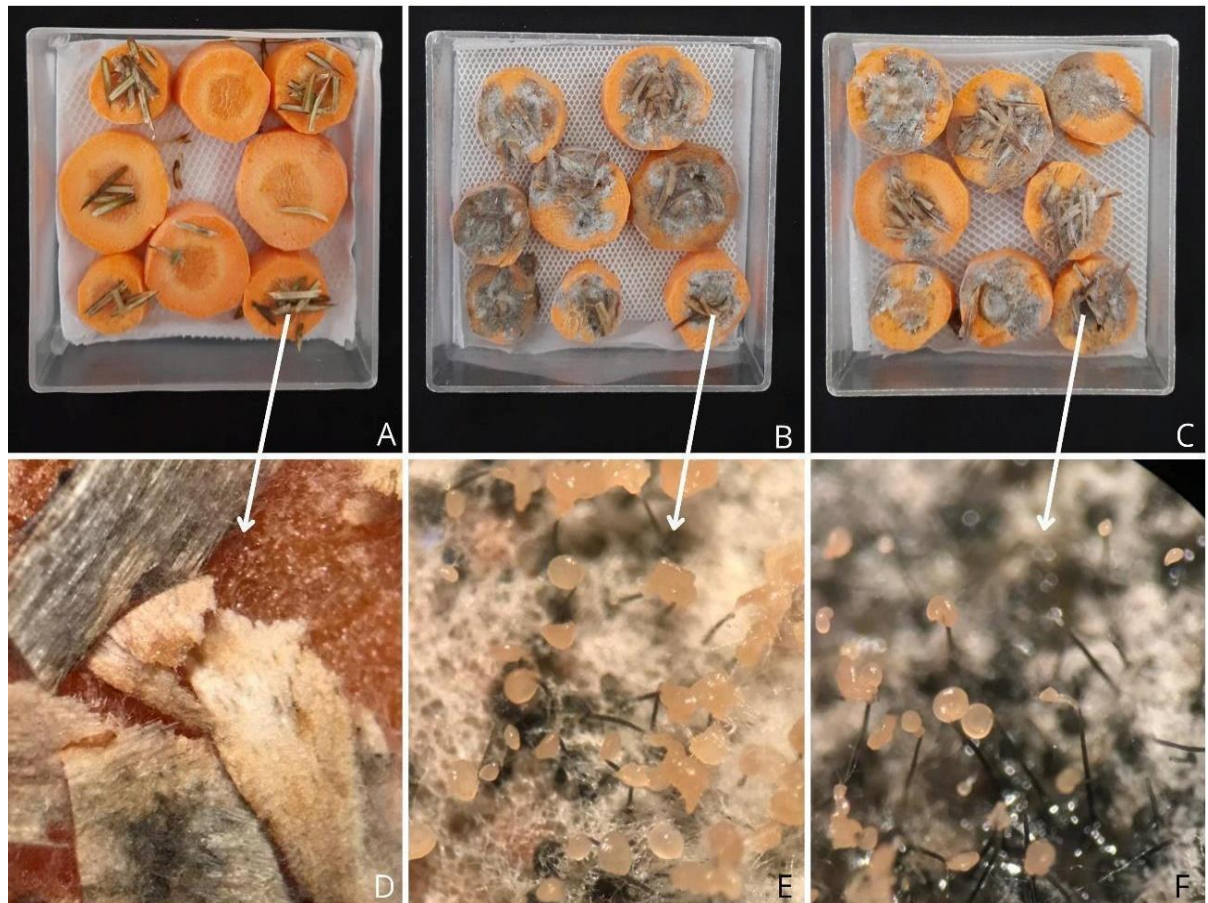


Fig. 5 Carrot baits made with stem samples of kiwifruit vines used to detect *Ceratocystis fimbriata*: **A** – Carrot baits made with stem samples from non-inoculated pruned plants treated with autoclaved distilled water (control); **B** – Carrot baits made with stem samples from pruned vines treated with metalaxyl 4% + mancozeb 64% (Ridomil Gold MZ[®]) and inoculated; **C** – Carrot baits made with stem samples from pruned plants treated with pyraclostrobin 25% (Comet[®]) and inoculated; **D** – No fungus growth on stem samples from control plants; **E** – *C. fimbriata* structures (mycelium and perithecia) detected in a carrot bait made with stem samples of vines treated with metalaxyl 4% + mancozeb 64%; **F** – *C. fimbriata* structures (mycelium and perithecia) detected in a carrot bait made with stem samples of vines treated with pyraclostrobin 25%.

CHAPTER 2 – Mechanical dispersal of *Ceratocystis fimbriata* and disinfestation strategies

Sabrina A. Cassol,^a Marston H. D. Franceschini,^a Davi M. Macedo,^a Chandan Pal,^b Acelino C. Alfenas,^a and Rafael F. Alfenas.^{a#}

^aLaboratory of Forest Pathology, Department of Plant Pathology/Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO), Universidade Federal de Viçosa, Viçosa, MG, 36570-900, MG, Brazil.

^bInnovation Manager -Biosecurity at Zespri International Limited Postal address: Zespri International Limited, PO Box 4043, Mount Maunganui 3149. Physical address: 400 Maunganui Road, Mount Maunganui, New Zealand.

Address correspondence to Rafael F. Alfenas, rafael.alfenas@ufv.br.

To be submitted to: Plant Pathology.

ABSTRACT

Ceratocystis wilt is the most important disease and is has been the most limiting factor to the cultivation of kiwifruit in Brazil. In several pathosystems, the disease dissemination and pathogen dispersal occur mainly by insects, infected rootstock of grafted vines and infested pruning tools and equipment. In this work, we demonstrated that *Ceratocystis* wilt can be mechanical disseminated from diseased kiwifruit vines to healthy kiwifruit vines by pruning tools. Additionally, we evaluated the *in vitro* effectiveness of different sanitizers and hot water (80°C) against mycelial growth of five *Ceratocystis fimbriata* isolates and *in vivo* the effectiveness of ammonium chloride (Vet+20), hot water and metalaxyl + mancozeb (Ridomil Gold MZ) in the disinfestation of pruning tools against the pathogen in two kiwifruit cultivars. For this, two inoculation methods were performed in independent bioassays: 1) apical pruning with pruning shears, and 2) stem wound with scalpel. The tools infested with spore suspension of *C. fimbriata* were immersed in a solution of each product or in hot water for 15 seconds, and subsequently used to prune or wound kiwifruit vines. At 45 days after inoculation, disease incidence and disease severity were determined. *In vitro*, ammonium chloride and hot water were the most effective treatments to inhibit mycelial

growth of *C. fimbriata* isolates, but only hot water (80°C) effectively killed *C. fimbriata* in tools. Kiwifruit vines pruned or wounded with infested tools immersed in metalaxyl + mancozeb and ammonium chloride solution reduced disease severity, but all vines were infected. We conclude that the disinfestation of pruning tools by immersion, for 15 seconds, in hot water (80 °C) is an effective alternative to prevent the mechanical spread of *C. fimbriata* on kiwifruit vines.

Keywords: *Actinidia* spp., Cultural management. Ceratocystis wilt. Disinfestant. Kiwifruit. Pruning shears.

INTRODUCTION

Kiwifruit (*Actinidia* spp.) is native to China, but was domesticated and first successfully cultivated commercially in New Zealand (Saquet & Brackmann 1995, Ferguson 2004, Testolin et al. 2016). After domestication, the commercial cultivation of kiwifruit has spread throughout the world (Ferguson 2004), leading to the emergence of important diseases that can cause significant losses in the crop (Huang, 2016). Among them, Ceratocystis wilt, caused by *Ceratocystis fimbriata* Ellis & Halst., is one of the most severe diseases that affects the crop. In 2010, the disease was reported in kiwifruit orchards in Rio Grande do Sul, state of Brazil (Sonego et al. 2010) and since then, it has rapidly spread to other kiwifruit producing regions of the country (Piveta et al. 2016). The disease symptoms include wilt, reduced fruit size and death of infected plants, with an annual mortality ranged from 25 to 30% (Piveta et al. 2016, Ferreira et al. 2017). Currently, Ceratocystis wilt is the main limiting factor to the cultivation of kiwifruit in Brazil and it is a serious threat to the other kiwifruit producing countries where the disease has not been reported yet on the crop.

Dissemination of Ceratocystis wilt may occur by insects (Hinds 1972), infected rootstock of grafted plants, and infested pruning tools and equipment (Harrington 2013). Observations in kiwifruit vines indicate that most infections start at the roots, due the presence of *C. fimbriata* inoculum in the soil (Piveta et al. 2016). Furthermore, according to Brazilian kiwifruit growers in Farroupilha (RS), Ceratocystis wilt appears to be disseminated during pruning activities (Gervásio Silvestrin, personal information, 2022). Considering that pruning in kiwifruit vines is generally done twice a year (winter and summer) to improve yield and fruit quality (KVH 2019a, 2019b), investigating whether the fungus can be spread

by pruning tools is important for the development of effective strategies for disinfestation and for the proper management of the disease in kiwifruit orchards.

Given the importance of understanding the spread of *C. fimbriata* in kiwifruit and the development of control strategies, the present work aims to evaluate: (i) The mechanical dispersal of *C. fimbriata* from diseased vines to healthy vines by pruning tools; (ii) *in vitro* effectiveness of sanitizers and hot water (80°C) against mycelial growth of different *C. fimbriata* isolates; and (iii) effectiveness of different tool disinfestation strategies to prevent Ceratocystis wilt in kiwifruit cultivars.

MATERIAL AND METHODS

***Ceratocystis fimbriata* isolates**

Ceratocystis fimbriata isolates (LPF1443, 1673, 1688, 1697 and 1701) used in this study were obtained from infected kiwifruit vines in commercial orchards of Rio Grande do Sul and Santa Catarina States, between 2010 and 2015, and previously characterized as aggressive on kiwifruit (Piveta et al. 2016, Oliveira et al. 2020). Pure cultures of these isolates are routinely replicated and subsequently stored in an ultra-low temperature (ULT) freezer at -80°C, as part of the pure culture collection of the ‘Laboratory of Forest Pathology/Bioagro’ (LPF) of the ‘Universidade Federal de Viçosa’ (UFV), Viçosa, Minas Gerais, Brazil.

Plant material

Two kiwifruit cultivars, cultivar Monty (*Actinidia deliciosa*) from Brazil and cultivar Gold3 (*Actinidia chinensis*) from New Zealand, were used in the present assay. Rooted cuttings were grown in 15 × 20 cm polyethylene bags, containing Carolina substrate supplemented with 70 g of Osmocote Plus (NPK: 19:06:10) and 150 g of single super phosphate per 8 kg of substrate, and then kept for 120 days under greenhouse conditions (25 °C ± 5 °C), with regular irrigation, until inoculation.

Effectiveness of sanitizers and hot water for inhibiting mycelial growth of *Ceratocystis fimbriata*

Four sanitizers, ethanol (70%), Vet+20 (25.7% ammonium chloride), household bleach (2 – 2,5% sodium hypochlorite), sodium hypochlorite (2%) and hot water (80°C) were

tested for their capacity to inhibit mycelial growth of five *C. fimbriata* kiwifruit isolates (LPF1443, 1673, 1688, 1697 and 1701). For this, the fungus isolates were grown in Petri dishes (90 mm diameter) containing potato-dextrose-agar medium (PDA) at 25°C and 12 h photoperiod, for 15 days. For each *C. fimbriata* isolate, 6 mm diameter mycelial plugs taken from pure fungus colonies were immersed in the sanitizer solutions or hot water for 15, 30, 45, or 60 s and immediately placed in the center of a Petri dish (60 mm diameter), containing PDA. Non-treated mycelial plugs, taken from fungus colonies and placed directly in the plates with PDA, were used as control. For each treatment (*C. fimbriata* isolate × sanitizer × time), four replicates (plates) were included in the experiment. After the treatment, plates were incubated at 25°C and 12 h photoperiod for approximately one week, until one of the fungus colonies reached the border of the plate. Then, each colony diameter was measured in two perpendicular directions and mycelial growth was calculated by discounting the plug diameter and used to determine the inhibition of mycelial growth, as described in the following equation:

$$\% \text{ Mycelial Growth Inhib.} = \frac{(\text{Radial mycelial growth}_{\text{Control}} - \text{Radial mycelial growth}_{\text{Treatment}})}{\text{Radial mycelial growth}_{\text{Control}}} \times 100$$

Mechanical dissemination of *Ceratocystis* wilt by pruning tools

For evaluating the spread of *C. fimbriata* from diseased to healthy kiwifruit vines through infested pruning shears, 60 days before running the experiment, five rotted cuttings of “Monty” were inoculated with 500 µL of *C. fimbriata* (isolate LPF 1443) spore suspension (1.0×10^7 spores/mL), to be used as inoculum source (diseased plant). After 60 days of incubation, tissues of disease vines expressing wilting and xylem darkening were cut with pruning shears, and subsequently, the same pruning shears were used for pruning the apex of healthy kiwifruit vines (pruning shears treatment) of two kiwifruit cultivars, Gold3 and Monty. This procedure was repeated for each clonal replicate of each cultivar. In addition, two controls were adopted: apical pruning of healthy vine followed by inoculation with *C. fimbriata* spore suspension in the pruning wound (positive control), and apical pruning of healthy vine followed by application of 500 µL of autoclaved distilled water in the pruning wound (negative control). Five rooted cuttings of each cultivar were used for each treatment and control. At 60 days of incubation, the disease incidence (number of infected vines divided by the number of inoculated vines, expressed in percentage) was evaluated considering the infected vines from each treatment, subsequently the lesion length (cm) was measured in

these vines and disease severity (lesion length divided by plant height and expressed in percentage) was calculated.

Disinfestation of pruning tools against *Ceratocystis fimbriata*

The effectiveness of three treatments, including hot water (80°C), ammonium chloride 25.7% (Vet+20) and metalaxyl 4% + mancozeb 64% (Ridomil Gold MZ), were evaluated for disinfestation of pruning tools against *C. fimbriata* in two kiwifruit cultivars, Gold3 and Monty. Treatment solutions were prepared with autoclaved distilled water. The active ingredient solution concentrations were: 1.28 % for ammonium chloride and 250 µg a.i. mL⁻¹ for metalaxyl + mancozeb. Hot water temperature, 80 °C ± 5 °C, was monitored throughout the treatment implementation with the use of a mercury thermometer. Two inoculation methods were adopted in independent bioassays: 1) *pruning inoculation*: pruning of the stem apex and two petioles using pruning shears; and 2) *wound inoculation*: wounding (three superficial cuts) of the stem base and two petioles using a scalpel. First, the tools (scalpel or pruning shears) were immersed in a *C. fimbriata* spore suspension (1x10⁷ spores mL, kiwifruit isolate LPF1443). Subsequently, the infested tools were immersed separately in the respective product solutions or in hot water (80 °C), for 15 seconds, and used to prune or wound kiwifruit vines, according to the inoculation method. The tools infestation and immersion in treatment solutions were carried out independently for each replicate. Three controls were performed, for each inoculation method: 1) *pathogen control*: vines pruned or wounded with infested tools, without disinfestation; 2) *water control*: vines pruned or wounded with infested tools, immersed in autoclaved distilled water for 15 seconds, and 3) *spore suspension*: vines inoculated at the stem base with 500 µL of spore suspension (Alfenas and Mafia, 2016). A completely randomized design with five replicates (plants) per treatment or control was used. After 45 days of inoculation (dai), disease incidence (number of infected plants divided by the number of inoculated plants and expressed as percentage) was determined through visual assessment for symptoms and confirmed with carrot baits. In addition, we also measured the lesion length (cm) and calculated the disease severity (lesion length divided by plant height, expressed in percentage).

Statistical analyses

The effectiveness of the different sanitizers according to exposure time was assessed by comparing means of mycelial growth inhibition corresponding to the different treatments. Besides the main or fixed factors, i.e., sanitizer and duration of the contact with the product, experimental replicates (4 in total) and different *C. fimbriata* isolates (5 different isolates, as indicated in Table 1) were also part of the experimental design. Therefore, in an analysis framework based on Linear Mixed Effects (LME) model, replicates and isolates were included as random effects for intercept and slope, respectively, since these were not the main factors of interest in the study but still were potentially significant sources of variability. Also, for the *in vivo* assays, a LME model was used to represent the relationship between disease severity, and the different experimental factors. While treatment and cultivar were considered as main or fixed effects, replicates were included as random intercept in the model. All analysis were implemented in environment R v. 4.1.3 (R Core Team, 2022) using mainly the packages ‘lme4’ (Bates et al. 2015) and ‘emmeans’ (Length 2022), with a significance level of 0.05 (p-value) in ANOVA and Tukey’s HSD tests, methods used to evaluate the significance of the experimental factors included in the model and for marginal means comparison, respectively. It is worth noting that inhibition and disease severity values were given in percentage and therefore they were logit transformed before analysis, to map these values to real numbers interval and potentially improve residuals distribution normality.

RESULTS

Effectiveness of different sanitizers and hot water against mycelial growth of *Ceratocystis fimbriata*

The most effective treatments in the inhibition of mycelial growth were Vet+20 and hot water (80°C), considering the means for all *C. fimbriata* isolates evaluated (Fig. 1). Hot water (80°C) showed highly effectiveness, inhibiting 100% the mycelial growth regardless of the *C. fimbriata* isolate in all times tested. The effectiveness of Vet+20 increased with increasing exposure time, and with 45 and 60 s of exposure the product solution inhibited 100% of the fungus growth. Household bleach and 70 % ethanol were the least effective treatments in inhibiting mycelium growth of all *C. fimbriata* isolates tested. The different isolates were included as random effects for intercept and slope, since these were not the main factors of interest in the study but still were potentially significant sources of variability,

as is possible observed in the error bars indicate the standard deviation derived from the dataset in each case (Fig. 1).

Dissemination of *Ceratocystis* wilt by tools

Significant differences were found between treatments for both kiwifruit cultivars (Table 2). Vines apically pruned with pruning shears, which were previously used to cut infected tissues of diseased vines (pruning shears treatment), resulted in 20% of disease incidence, and all vines apically pruned and inoculated with spore suspension were infected (100% incidence). Disease severity in vines from the pruning shears treatment was generally lower than severity observed in vines from the control treatment, for both cultivars evaluated (Table 1). The lesion length in plants pruned and inoculated with spore suspension varied from 12 to 16 cm in “Gold3” and from 7 to 25 cm in “Monty”. In the pruning shears treatment, only one of the five replicates showed disease symptoms for both cultivars, reaching 8 cm in “Gold3” and 6.5 cm in “Monty”. Control plants, pruned and treated with distilled water, did not show disease symptoms or internal lesions in the stem. The infection by *C. fimbriata* was confirmed in all plants that expressed disease symptoms by the presence of fungus structures in carrot baits made with samples of plant stems.

Disinfestation of tools against *Ceratocystis fimbriata*

Significant differences were found between the disinfestation treatments evaluated (Table 2). Results of ANOVA performed after fitting a Linear Mixed Effects (LME) model to the data indicated that the main fixed effect considered, i.e. treatments, was highly significant, which was subsequently evaluated in the pairwise comparison described in Table 3 (Tukey’s HSD test). Also, analysis of residuals for the LME model indicated only small deviation from normality (results not shown), ensuring that the model structure represented well the dataset and model assumptions were in most part respected, allowing reliable evaluation of statistical significance for the different experimental factors. Hot water (80°C/15 sec) was the only treatment effective in the disinfestation of pruning tools contaminated with *C. fimbriata*. None of the kiwifruit vines inoculated with infested tools, after these tools have been immersed in hot water, developed symptoms of *Ceratocystis* wilt (Fig. 2E-F and 3E-F), and the carrot bait test was negative for this treatment. In the wound inoculation method, two of the five vines of “Monty” inoculated with an infested scalpel

immersed in ammonium chloride solution wilted, while all five “Gold3” vines became infected (Table 2). However, vines inoculated with infested tools immersed in ammonium chloride solution showed lower disease severity when compared to controls (Table 2). On the other hand, disinfestation of tools with metalaxyl + mancozeb solution resulted in a higher disease incidence, in comparison to ammonium chloride solution (Table 2). All control plants inoculated with infested tools immersed in autoclaved distilled water presented infection symptoms (Fig. 4E-F and 5E-F), showing that the solvent used to prepare the disinfestation solutions had no disinfestation effect. Infection by *C. fimbriata* was confirmed in all vines that expressed disease symptoms by identifying the presence of the pathogen structures in carrot baits. The different inoculation methods resulted in distinct patterns of disease symptoms and disease severity (Fig. 2-5). In vines wounded with an infested scalpel (Fig. 2 and 4), it was possible to observe canker formation, at the stem base, and a high number of vines died until the evaluation (45 dai). On the other hand, vines apically pruned with infested pruning shears showed external necrotic dark lesions in the stem tissues from the inoculation point (Fig. 3 and 5), and no vines died within the evaluation period. In the wound inoculation method, in addition to the wound at the stem base, two petioles were wounded with infested scalpel and, at 45 dai, these petioles were present only in vines wounded with infested scalpel immersed in hot water (Fig. 6B). In vines of the other treatments and controls, the wounded petioles dropped or became necrotic (Fig. 6A). These results demonstrate that disinfestation of pruning tools by immersion in hot water (80 °C), for 15 seconds, is effectiveness against *C. fimbriata* infection in kiwifruit plants.

DISCUSSION

Pruning in commercial kiwifruit orchards is a common management practice done in winter and summer to improve yield and fruit quality (KVH 2019a). However, pruning may become an important entrance gateway for pathogens such as *Pseudomonas syringae* pv. *Actinidiae* – Psa (Woodcock 2016). According to Brazilian kiwifruit growers in Farroupilha (RS), *Ceratocystis* wilt appears to be disseminated by pruning the vines (Gervásio Silvestrin, personal information, 2022), which was proved in the present work.

We showed that kiwifruit vines pruned and subsequently inoculated with spore suspension resulted in higher disease incidence and severity, when compared to plants pruned by shears previously used to prune diseased plants. A similar study conducted with *Calosphaeria pulchella*, causal agent of *Calosphaeria* canker in sweet cherry, showed that

pruning healthy plants with infested pruning shears can spread the pathogen, but with lower percentage of disease incidence and severity significantly than that by inoculation with mycelial plugs (Berbegal and Armengol 2018). It is known that inoculum source has great influence on the incidence and intensity of diseases (Stahr and Quesada-Ocampo 2021), which can be observed in areas infested by Psa (Woodcock 2016). In our study, the inoculum concentration in the infested pruning shears is considerably lower than that in the artificial inoculation with spore suspension, which may explain the difference observed in the percentage of disease incidence and severity. However, we showed that the pathogen can be spread from diseased to healthy plants by pruning shears in kiwifruit vines. From these results, it can be concluded that disinfestation of pruning tools during pruning operations in orchards is an important disease management practice.

Our results showed that only hot water (80 °C) is effective for disinfestation of pruning tools against *C. fimbriata* on kiwifruit vines. The exposure of the inoculum to high temperature causes protein denaturation or membrane disruption in the fungus (Ahnoff et al. 2015). The effectiveness of the technique depends directly on the combination of temperature and exposure time, based on the lethal temperature of the target organism (Fallik 2004, Ghatrehsamani et al. 2019), that is between 45 to 60 °C for most plant pathogens (Ghatrehsamani et al. 2019). Additionally, hot water is a safe and environmentally friendly disinfestation strategy (Fallik 2004). It has been effectively used against several plant pathogens (Fallik 2004), as to prevent rot in fruit and vegetables (Ndlela et al. 2022), inactivation of root-infecting pathogens in water from soilless crops (Runia et al. 1988), soil disinfestation to prevent soil borne diseases (Uematsu et al. 2003), and others (Nafussi et al. 2001, Olesen et al. 2004, Brash et al. 2010, Ghatrehsamani et al. 2019). The main challenge for the use of hot water for the disinfestation of pruning tools is the availability of adequate equipment that could be transported to the field to be used during pruning operations (Ghatrehsamani et al. 2019, Rocha et al. 2020). For this reason, it is necessary to test other alternatives that can expose the pruning shears to high temperatures, more practical and more easily adopted in orchards, such as the use of fire to flame the tools.

Quaternary ammonium compounds are used to control many pathogenic species, including fungi, bacteria and virus (Schubert and Sun 2003, Nguyen et al. 2017). We showed that a quaternary ammonium-based product (Vet+20) is effective in the inhibition of *C. fimbriata* mycelial growth *in vitro*. However, the product solution was not effective in the disinfestation of pruning tools in the immersion time tested and that is why we intend to test a longer time in future trials.

Although fungicides are not the best alternative for disinfestation of pruning tools because of their relatively high cost and toxicity, we tested the fungicide metalaxyl + mancozeb (Ridomil Gold MZ), due to the lack of better alternatives for effectively disinfesting pruning tools against *C. fimbriata* and because of its *in vitro* effectiveness against *Ceratocystis* spp. from pomegranate (Somu et al. 2018) and mango (Tran et al. 2018). Despite the results from previous studies, the fungicide was not effective in disinfesting pruning tools against the *C. fimbriata*, similarly to Vet+20. However, these products reduced disease severity when compared to controls. In the *in vivo* assay, we used a high concentration of inoculum to infest the pruning tools, to favor infection. Nevertheless, such a high inoculum concentration probably does not occur naturally in the orchards when pruning an infected plant, as demonstrated in the dissemination assay. It is known that the inoculum source can have great influence on disease severity (Stahr and Quesada-Ocampo 2021, Woodcock 2016). Therefore, it is possible that these products were toxic to the inoculum of the pruning tools, which may have impaired the capacity of the pathogen colonization and/or reduced the inoculum concentration in the pruning tools, probably resulting lower disease severity by affecting disease progression. However, a longer incubation time also leading to plant mortality.

Kiwifruit vines wounded with scalpel infested with *C. fimbriata* showed high disease incidence and severity. This result demonstrates that, in addition to pruning with infested pruning shears, the spread of the pathogen can also occur from wounds caused by other tools and equipment used during management operations in kiwifruit vines, such as tractors, brush cutters, and scalpels used during the propagation of plants by grafting. Furthermore, the severity of the disease was higher in kiwifruit vines inoculated by wounding the stem base than those inoculated by apical pruning. These results show that disinfestation of all equipment and tools used in the management practices of kiwifruit vines is an important strategy to avoid dissemination of *Ceratocystis* wilt and that the disease appears to progress more quickly in an upward direction than in a downward direction in kiwifruit vines.

Our results showed that tools, such pruning shears and scalpels, can mechanically disperse *C. fimbriata* from infected kiwifruit vines to healthy kiwifruit vines during routine management activities in the crop. Since high temperature (hot water at 80°C/15 sec) was highly effective against *C. fimbriata*, it is necessary to adapt an equipment to keep the desired temperature or use fire for flambe the pruning tools, since it is a non-chemical, safe and environmentally friendly disinfestation method to prevent *Ceratocystis* wilt dissemination.

ACKNOWLEDGMENTS

This work was supported by: ‘Conselho Nacional de Desenvolvimento Científico e Tecnológico’ (CNPq); ‘Coordenação de Aperfeiçoamento de Pessoal de Nível Superior’ (CAPES); and ‘Financiadora de Estudos e Projetos’ (Finep). The authors are grateful for the assistance of Flavia Sampaio Alexandre, Monalisa de Cól, Carolina Pessanha Alonso, and to Zespri Group Limited and ‘Clonar Resistência a Doenças Florestais’ for providing plant material used in this study.

REFERENCES

- Ahnoff M, Cazares LH, Skold K (2015) Thermal inactivation of enzymes and pathogens in biosamples for MS analysis. *Bioanalysis* 7(15):1885–1899
- Alfenas AC, Mafia RG (2016) *Métodos em Fitopatologia*. 2ª Ed. Editora UFV, Viçosa, MG. 516p.
- Bates D, Maechler M, Bolker B, Walker S (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67(1):1-48
- Berbegal M, Armengol J (2018) Pruning practices influence infection and dissemination of *Calosphaeria pulchella*, the cause of Calosphaeria canker of sweet cherry. *Phytopathologia Mediterranea* 57(1)3-7
- Brash DW, Woolf A, Olsson S (2010) Hot water drenches for control of soft rots on washed kumara (Sweet Potato) Postharvest Pacifica 455-462
- Fallik E (2004) Prestorage hot water treatments (immersion, rinsing and brushing). *Postharvest Biology and Technology* 32:125–134
- Ferguson AR (2004) 1904 – the year that kiwifruit (*Actinidia deliciosa*) came to New Zealand, *New Zealand Journal of Crop and Horticultural Science* 32(1):3-27
- Ferreira MA, Harrington TC, Piveta G, Alfenas AC (2017) Genetic variability suggests that three populations of *Ceratocystis fimbriata* are responsible for the Ceratocystis wilt epidemic on kiwifruit in Brazil. *Tropical Plant Pathology* 42:86–95
- Ghatrehsamani S, Abdulridha J, Balafoutis A, Zhang X, Ehsani R, Ampatzidis Y (2019) Development and evaluation of a mobile thermotherapy technology for in-field treatment of Huanglongbing (HLB) affected trees. *Biosystems Engineering* 182:1-15
- Harrington TC (2013) Ceratocystis Diseases. *Infectious Forest Diseases* (eds P. Gonthier and G. Nicolotti) 230-255
- Hinds TE (1972) Ceratocystis canker of aspen. *Phytopathology* 62:213-220
- Huang H (2016) *Kiwifruit: The genus Actinidia*. 1st Ed. Beijing, China. Elsevier Academic Press.
- KVH (2019a) Readiness plan – *Ceratocystis fimbriata*. 40 p.

KVH (2019b) Readiness and Response Plan for *Ceratocystis fimbriata* affecting kiwifruit and kiwifruitberries. 45 p.

Length, R. V. 2022. Emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.8.2. Available at: <https://CRAN.R-project.org/package=emmeans>. Accessed on January 14, 2023

Nafussi B, Ben-Yehoshua B, Rodov V, Peretz, J, Ozer BK, D'Hallewin G (2001) Mode of action of hot-water dip in reducing decay of lemon fruit. *Journal of Agricultural and Food Chemistry* 49:107– 113

Ndlela S, Mwando NL, Mohamed S (2022) Advances in Postharvest Disinfestation of Fruits and Vegetables Using Hot Water Treatment Technology-Updates from Africa. In (Ed.), *Postharvest Technology – Recent Advances, New Perspectives and Applications*.

Nguyen KA, Forster H, Adaskaveg JE (2017) Quaternary Ammonium Compounds as New Sanitizers for Reducing the Spread of the Olive Knot Pathogen on Orchard Equipment. *Plant Disease* 101(7):1188-1193

Olesen T, Nacey L, Wiltshire N, O'Brien S (2004) Hot water treatments for the control of rots on harvested litchi (*Litchi chinensis* Sonn.) fruit. *Postharvest Biology and Technology* 32(2):135-146

Oliveira LSS, Pimenta LVA, Guimarães LMS, de Souza PVD, Bhering LL, Alfenas AC (2020) Resistance of kiwifruit cultivars to *Ceratocystis* wilt: an approach considering the genetic diversity and variation in aggressiveness of the pathogen. *Plant Pathology* 70: 349-357

Piveta G, Ferreira MA, Muniz MdFB, Valdetaro D, Valdebenito-Sanhueza R, Harrington TC, Alfenas AC (2016) *Ceratocystis fimbriata* on kiwifruit (*Actinidia* spp.) in Brazil. *New Zealand Journal of Crop and Horticultural Science* 44: 31-24

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: URL <https://www.R-project.org/>. Accessed on January 14, 2023

Rocha AC, Mendonça SP, Taketaro T, Watanabe G (2020) Analysis of different tracking intervals for Parabolic Trough Collectors for water disinfestation in agricultural applications. *Semina: Ciências Agrárias* 41: 7-32

Runia WT, Os EAV, Bollen GJ (1988) Disinfection of drainwater from soilless cultures by heat treatment. *Netherlands Journal of Agricultural Science* 36: 231-238

Saquet AA, Brackmann A (1995) A cultura do kiwi. *Ciência Rural* 25:177-182

Schubert TS, Sun X (2003) Bacterial citrus canker. *Plant Pathology Circular* 377:1-6

Somu R, Mesta RK, Kiran KKC (2018) Effect of nonsystemic, systemic fungicides and bio control agents against wilt of pomegranate caused by *Ceratocystis fimbriata*. *International Journal of Chemical Studies* 6(2):397-402

Sonego OR, Ferreira MA, Sanhueza RM, Gava R, Garrido LR, Alfenas AC (2010) Primeiro relato da murcha-de-ceratocystis em kiwifruit. *Tropical Plant Pathology* 35:S233

Stahr MN, Quesada-Ocampo (2021) Effects of Water Temperature, Inoculum Concentration

and Age, and Sanitizers on Infection of *Ceratocystis fimbriata*, Causal Agent of Black Rot in Sweetpotato. *Plant Disease* 105:1365-1372

Testolin R, Huang HW, Ferguson AR (2016) *The Kiwifruit Genome*. 1st Ed. Suíça. Springer Nature.

Tran TTT, Pham TQ, Barber PA, Nguyen CM (2018) Control of *Ceratocystis manginecans* causing wilt disease on *Acacia mangium* seedlings. *Australasian Plant Pathology* 47(6): 579-586

Uematsu S, Nishi K, Kita N (2003) Hot water soil sterilization begins in Japan. *Farming Japan* 37(2):35-41

Woodcock SD (2016) A review of research and development undertaken on Psa. Kiwifruit vine health. 49p.

FIGURES AND TABLES

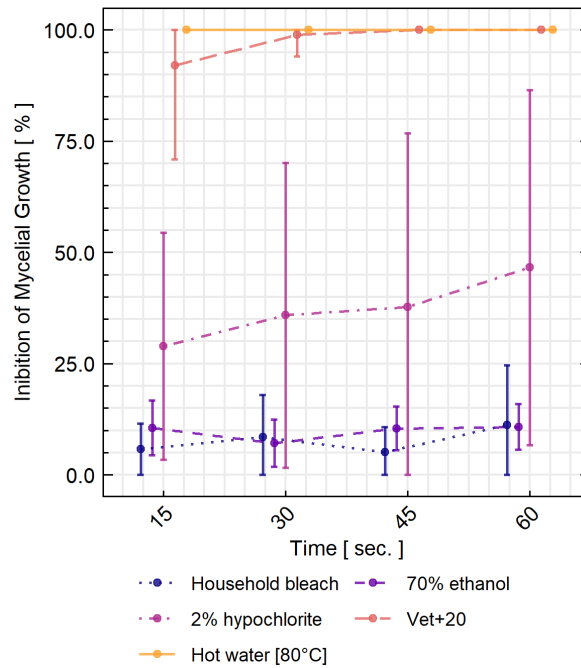


Fig. 1 Sensitivity *in vitro* of *Ceratocystis fimbriata* mycelial growth to sanitizers and hot water (80°C). Error bars indicate the standard deviation derived from the dataset in each case. Points for each dose are represented slightly shifted when necessary, according to treatment, to avoid overlap.

Table 1 Means of incidence (%) and severity (%) of *Ceratocystis fimbriata* infection on kiwifruit vines pruned with pruning shears previously used to cut tissues of diseased vines (pruning shears treatment), pruned and inoculated with spore suspension (positive control) and pruned and treated with distilled water (negative control). Two cultivars were included in the experiment (i.e., Gold3 and Monty)

Treatment	Incidence (%)		Severity (%) ¹	
	“Gold3”	“Monty”	“Gold3”	“Monty”
Negative control	0	0	0a	0a
Positive control	100	100	50.5c	45c
Pruning shears	20	20	13.3b	10.8b

¹Means within a column followed by the same letters are not significantly different from each other according to Tukey’s HSD test (p-value<0.05).

Table 2 Means of disease incidence (%) and severity (%), on Gold3 and Monty kiwifruit cultivars, evaluating the disinfestation efficacy against *Ceratocystis fimbriata* of pruning shears treated with quaternary ammonium (Vet+20), hot water at 80°C and Ridomil Gold MZ, through two inoculation methods (pruning inoculation and wound inoculation)

Treatment	Pruning inoculation				Wound inoculation			
	Incidence (%)		Severity (%) ¹		Incidence (%)		Severity (%) ¹	
	“Gold3”	“Monty”	“Gold3”	“Monty”	“Gold3”	“Monty”	“Gold3”	“Monty”
Spore suspension	100	100	63.08c	44.18c	100	100	63.08c	44.18c
Pathogen control	100	100	43.86c	11.75bc	100	100	85.45c	37.2c
Water control	100	100	17.05bc	13.52bc	100	100	71.49c	15bc
Ridomil Gold MZ	100	100	7.71b	7.33bc	100	100	46.11bc	6.3b
Vet+20	100	100	12.16b	5.02b	100	40	34.05b	11b
Hot water	0	0	0a	0a	0	0	0a	0a

¹Means of disease severity among treatments of each column followed by the same letter are not significantly different from each other using Tukey’s HSD test (p-value<0.05).



Fig. 2 Kiwifruit vines inoculated with *Ceratocystis fimbriata* for evaluating the effectiveness of disinfection of pruning tools with quaternary ammonia (Vet+20), hot water at 80°C and Ridomil Gold: **A** – “Gold3 NZ” vines inoculated with infested scalpel immersed in Ridomil Gold solution; **B** – “Monty BR” vines inoculated with infested scalpel immersed in Ridomil Gold solution; **C** – “Gold3 NZ” vines inoculated with infested scalpel immersed in Vet+20 solution; **D** – “Monty BR” vines inoculated with infested scalpel immersed in Vet+20 solution; **E** – “Gold3 NZ” vines inoculated with infested scalpel immersed in hot water (80

°C); and **F** – “Monty BR” vines inoculated with infested scalpel immersed in hot water (80°C).

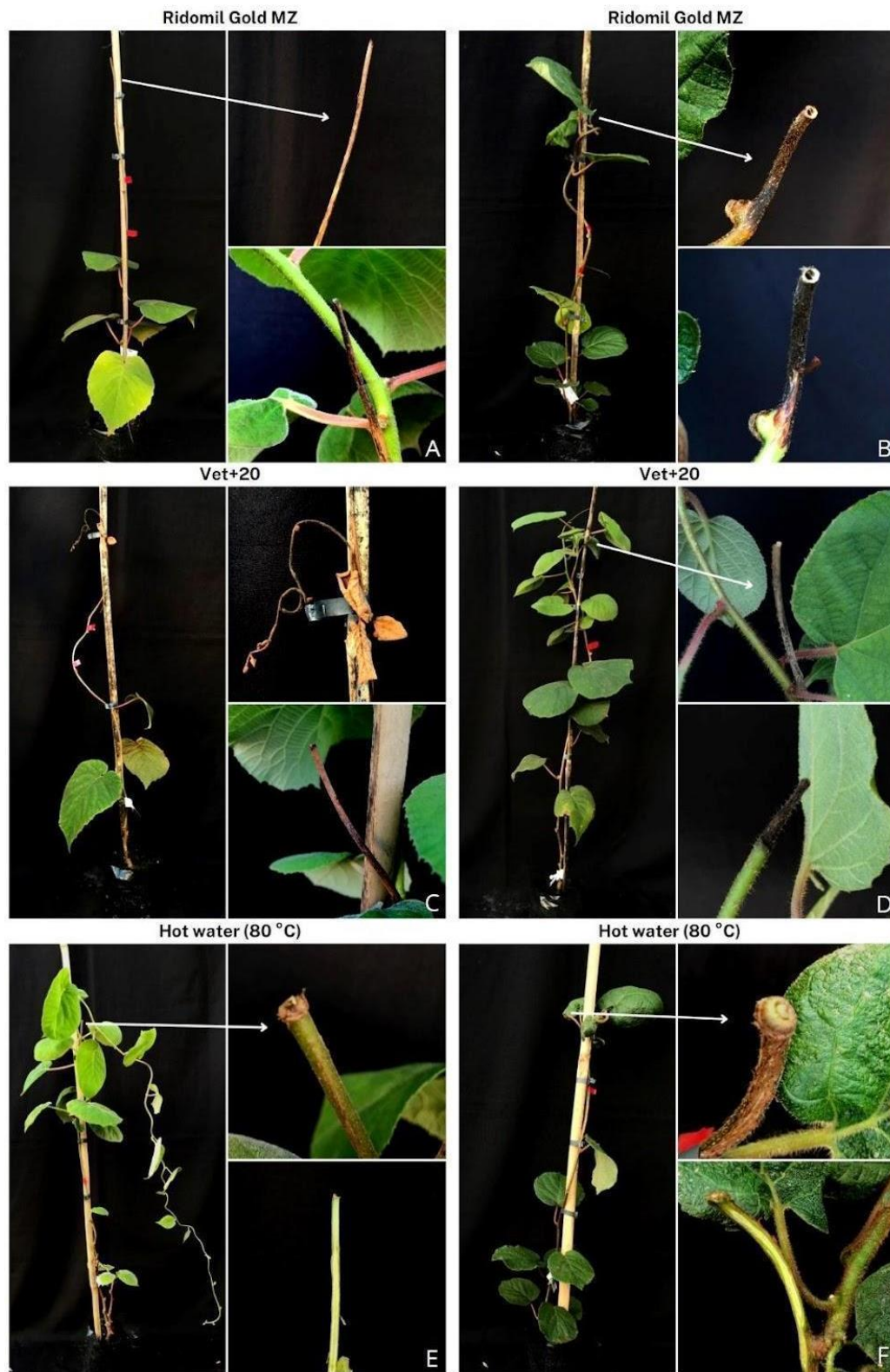


Fig. 3 Kiwifruit vines inoculated with *Ceratocystis fimbriata* for evaluating the effectiveness of disinfestation of pruning tools with quaternary ammonia (Vet+20), hot water at 80°C and Ridomil Gold: **A** – “Gold3 NZ” vines inoculated with infested pruning shears immersed in Ridomil Gold solution; **B** – “Monty BR” vines inoculated with infested pruning shears

immersed in Ridomil Gold solution; **C** – “Gold3 NZ” vines inoculated with infested pruning shears immersed in Vet+20 solution; **D** – “Monty BR” vines inoculated with infested pruning shears immersed in Vet+20 solution; **E** – “Gold3 NZ” vines inoculated with infested pruning shears immersed in hot water (80 °C); and **F** – “Monty BR” vines inoculated with infested scalpel immersed in hot water (80°C).



Fig. 4 Kiwifruit vines inoculated with *Ceratocystis fimbriata* for evaluating the effectiveness of disinfestation of pruning tools with quaternary ammonia (Vet+20), hot water at 80°C or

Ridomil Gold: **A** – “Gold3 NZ” vines inoculated with spore suspension in the stem base; **B** – “Monty BR” vines inoculated with spore suspension in the stem base; **C** – “Gold3 NZ” vines inoculated with infested scalpel; **D** – “Monty BR” plants inoculated with infested scalpel; **E** – “Gold3 NZ” vines inoculated with infested scalpel immersed in autoclaved distilled water; and **F** – “Monty BR” vines inoculated with infested scalpel immersed in autoclaved distilled water.



Fig. 5 Kiwifruit vines inoculated with *Ceratocystis fimbriata* for evaluating the effectiveness of disinfection of pruning tools with quaternary ammonia (Vet+20), hot water at 80°C and Ridomil Gold: **A** – “Gold3 NZ” vines inoculated with spore suspension in the stem base; **B** – “Monty BR” vines inoculated with spore suspension in the stem base; **C** – “Gold3 NZ” vines inoculated with infested pruning shears; **D** – “Monty BR” vines inoculated with infested pruning shears; **E** – “Gold3 NZ” vines inoculated with infested pruning shears immersed in autoclaved distilled water; and **F** – “Monty BR” vines inoculated with infested pruning shears immersed in autoclaved distilled water.

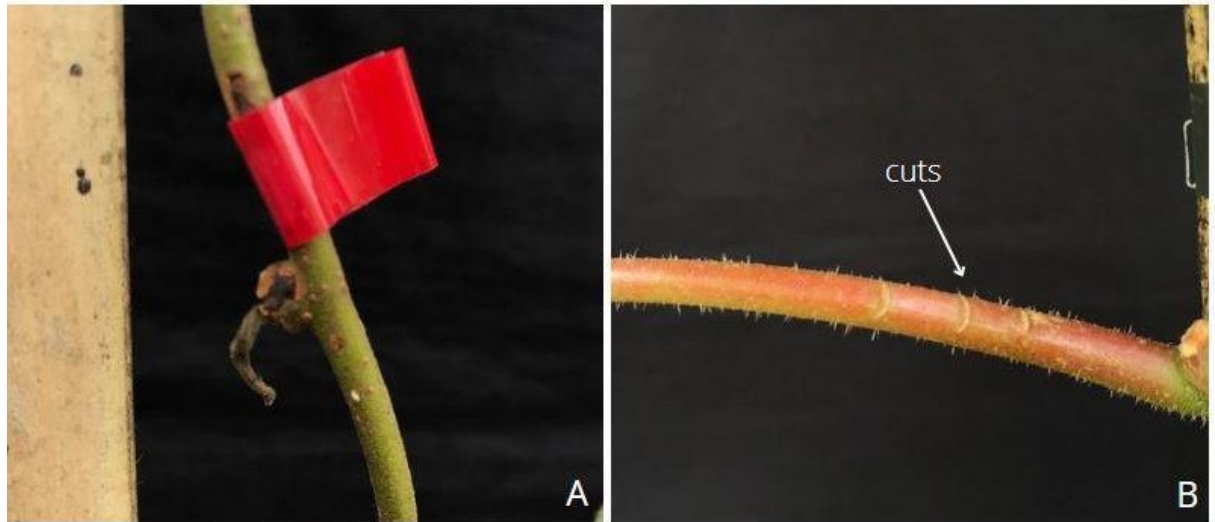


Fig. 6 Kiwifruits vines: **A** – Petiole inoculated with scalpel infested by *C. fimbriata*; and **B** – Petiole inoculated with scalpel infested by *C. fimbriata* and immersed in hot water (80 °C).

CHAPTER 3 – Pathogenicity of *Ceratocystis* spp. isolates from different hosts and geographic regions on kiwifruit cultivars

Sabrina A. Cassol,^a Chandan Pal,^b Lisa M. Keith,^c Acelino C. Alfenas,^a and Rafael F. Alfenas.^{a#}

^aLaboratory of Forest Pathology, Department of Plant Pathology/Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO), Universidade Federal de Viçosa, Viçosa, MG, 36570-900, MG, Brazil.

^b Innovation Manager -Biosecurity at Zespri International Limited Postal address: Zespri International Limited, PO Box 4043, Mount Maunganui 3149. Physical address: 400 Maunganui Road, Mount Maunganui, New Zealand (chandan.pal@zespri.com).

^c Research Plant Pathologist, USDA Agric. Research Serv., Daniel K. Inouye U.S. Pacific Basin Agric. Research Center, Hilo, Hawai‘I (lisa.keith@usda.gov).

^d Department of Plant Pathology, Iowa State University, Ames 50011 (tcharrin@iastate.edu).

Address correspondence to Rafael F. Alfenas, rafael.alfenas@ufv.br.

To be published at: Plant Pathology.

ABSTRACT

This study investigates the pathogenicity of ten *Ceratocystis* spp. isolates from different hosts and geographic regions in two kiwifruit cultivars, Monty (*Actinidia deliciosa*) and Gold3 (*Actinidia chinensis*). For this, the kiwifruit vines were inoculated with mycelial plugs from each isolate. Vines inoculated with medium plug without the fungus were used as control. At 45 days of inoculation, disease incidence and disease severity (xylem lesion length divided by plant height) were evaluated and infection by *C. fimbriata* was confirmed using carrot baits. Significant differences were found among the *Ceratocystis* spp. isolates and, eventually, between cultivars for the same isolate. The *C. huliobia* and the sweet potato isolates were not pathogenic in both kiwifruit cultivars, whereas the mango isolate was not pathogenic only in “Monty”. The disease severity was lower in “Monty” compared to “Gold3” for all pathogenic isolates tested. However, only for teak and mango isolates that

this difference was statistically significant ($p\text{-value} \leq 0.05$). Kiwifruit and teak isolates were the most aggressive in “Gold3”, with 100% of plant mortality in both cases. Whereas, in “Monty”, the kiwifruit and *C.lukuohia* isolates were the most aggressive, but with lower number (60%) of dead vines in the 45 days of incubation. These results showed that *Ceratocystis* spp. isolates from different hosts can infect kiwifruit vines, and that these isolates vary in aggressiveness and species host range. Additionally, this is the first report of the pathogenicity of *C. lukuohia* in kiwifruit vines.

Keywords: *Actinidia deliciosa*. *Actinidia chinensis*. Host range. *Ceratocystis fimbriata*. *Ceratocystis lukuohia*. Pathogenicity.

INTRODUCTION

Ceratocystis fimbriata Ellis & Halst., which causes Ceratocystis wilt, is an important pathogen that causes significant losses in many agricultural and forestry crops worldwide (Harrington et al. 2011, Oliveira et al. 2015). The complex *Ceratocystis fimbriata* sensu lato (s.l.) is formed by a group of very similar fungi and with a high number of isolates widely distributed geographically, some species are related to single hosts or geographic regions, while others infect a range of hosts (Thorpe et al. 2004, Tyson et al. 2020). The wide host range of *C. fimbriata* (s.l.) includes economically important species, such as *Ipomea batatas* (Weber and Stahmann 1964), *Eucalyptus* spp. (Oliveira et al. 2016), *Coffea arabica* (Marin et al. 2003), *Mangifera indica* (Oliveira et al. 2015), *Theobroma cacao* (Engelbrecht et al. 2007), *Acacia mangium* (Tarigan et al. 2011), *Ficus carica* (Harrington et al. 2011), *Tectona grandis* (Firmino et al. 2012), *Metrosideros polymorpha* (Fortini et al. 2019), among others.

Although *C. fimbriata* (s.l.) has been reported causing diseases in several hosts for more than 75 years in Brazil, only in 2010 the pathogen was first reported in kiwifruit vines, in the state of Rio Grande do Sul, Brazil (Sonego et al. 2010), causing wilting, dark-brown lesions in the xylem, reduced fruit length and death of infected plants (Piveta et al. 2016, Ferreira et al. 2017). Currently, the Ceratocystis wilt is also a serious threat to the world's largest kiwifruit producing countries, such as China, Italy, Iran, Greece, Chile and New Zealand, where the pathogen has not been reported yet causing infecting kiwifruit vines (Oliveira et al. 2016, Ferreira et al. 2017, FAOSTAT 2022).

The kiwifruit cultivars planted in Brazil are susceptible to *Ceratocystis* wilt (Piveta et al. 2016, Oliveira et al. 2020). Furthermore, the resistance pattern and different levels of susceptibility found among kiwifruit cultivars suggest that the resistance in kiwifruit vines against *C. fimbriata* is quantitative, such as observed in other hosts (Guimarães et al. 2010, Rosado et al. 2010, Arriel et al. 2016, Oliveira et al. 2020). The quantitative resistance is polygenic, occurs after effector-triggered resistance has been overcome by the pathogen, and each of the genes involved has a contribution to plant resistance (Guimarães et al. 2010, Cowger and Brown 2019, Leclerc et al. 2019). In this way, some level of specificity with certain pathogen genotypes is expected (Cowger and Brown, 2019, Leclerc et al. 2019). Thus, it is important that the *C. fimbriata* genetic variability be considered in the selection of resistant genotypes of kiwifruit.

For species of the *C. fimbriata* species complex (s. l.), the interspecific and intraspecific variability is related to host speciation, aggressiveness variation and specific interactions with certain cultivars or clones (Cowger et al. 2000, Oliveira et al. 2020). There are species of the *Ceratocystis* related to single hosts or geographic regions, while others infect a range of hosts, including many economically important crops (Thorpe et al. 2004, Tyson et al. 2020). Therefore, knowing the pathogenicity of different isolates of the *Ceratocystis* is important for the development of effective disease control tactics based on host genetic resistance (Leclerc et al. 2019, Fourie et al. 2019). In this context, the aim of this work was to evaluate the pathogenicity of *Ceratocystis* spp. isolates from different hosts and geographic regions in kiwifruit cultivars.

MATERIAL AND METHODS

Fungus isolates

Ten *Ceratocystis fimbriata* (s.l.) isolates from different hosts and geographic regions were used in this study (Table 1). The importation and use in inoculation assay of isolates from other countries were authorized and supervised by the Ministério da Agricultura e Pecuária. These fungus isolates were maintained in an ultra-low temperature (ULT) freezer at -80 °C, as part of the pure culture collection of the 'Laboratory of Forest Pathology/Bioagro' (LPF) of the 'Universidade Federal de Viçosa' (UFV), Viçosa, Minas Gerais, Brazil. For the inoculation, the *Ceratocystis* spp. isolates were grown in PDA (Potato Dextrose Agar) medium, at 28°C and 12 h photoperiod, for twelve days.

Plant material

Two kiwifruit cultivars, “Monty” (*Actinidia deliciosa*) from Brazil and “Gold3” (*Actinidia chinensis*) from New Zealand, were used in the assay. Rooted cuttings were grown in 15 × 20 cm polyethylene bags, containing Carolina substrate supplemented with 70 g of Osmocote Plus (NPK: 19:06:10) and 150 g of single super phosphate per 8 kg of substrate, and then kept for 120 days under greenhouse conditions (25 °C ± 5 °C) until the inoculation.

Pathogenicity of *Ceratocystis* spp. isolates on kiwifruit cultivars

The pathogenicity of ten *Ceratocystis* spp. isolates from different hosts and geographic regions (Table 1) were evaluated in two kiwifruit cultivars. For this, 4 months old vines, of “Gold3” and “Monty” were inoculated with mycelial plugs of the *Ceratocystis* spp. isolates. For this, a 5 mm disk of the plant’s bark was removed at the stem base and replaced by a mycelial plug of the same size. A moistened cotton swab was placed just below the inoculation point and covered with PVC plastic to prevent contamination and dehydration. Vines that received PDA plugs, without fungus, served as control. Five vines of each cultivar were used for each isolate and control. The inoculated vines were incubated in greenhouse (28 ± 5 °C) and at 45 days of inoculation, the disease incidence (number of infected plants divided by the number of inoculated plants and expressed as percentage) and xylem lesion length (cm), in the inner stem tissues, were measured and infection by *Ceratocystis* spp. confirmed with carrot baits, prepared with stem tissue samples of the evaluated plants. In addition, the xylem lesion length was used to derive disease severity (lesion length divided by plant height and expressed as percentage), and for the inoculated vines that died during the 45 days of incubation, a disease severity of 100% was considered.

Statistical Analysis

Pathogenicity of the *C. fimbriata* different isolates was evaluated by comparing marginal means of disease severity for the two cultivars. The experimental design comprised pathogen isolates and kiwifruit cultivars as main or fixed effects and experimental replicates, which was represented as random intercept in a Linear Mixed Effects model (LME) based analysis framework. Disease severity was given in percentage of tissue affected and before comparing means these values were logit transformed to map it to real numbers interval and potentially improved residuals distribution normality. The analysis was implemented in R v.

4.1.3 (R Core Team, 2022) using the packages ‘lme4’ (Bates et al. 2015) and ‘emmeans’ (Length 2022), with a significance level of 0.05 (p-value) in ANOVA and Tukey’s HSD tests, used to evaluate the significance of the experimental factors included in the model and for marginal means comparison, respectively.

RESULTS

Significative differences were observed between the isolates tested for each kiwifruit cultivar and eventually between cultivars for the same isolate (Table 2). Results of ANOVA for the Linear Mixed Effects (LME) model indicated that the fixed factors, i.e. isolates and cultivar, were highly significant (p-value ≤ 0.001). This aspect was further investigated in the pairwise comparison described in Table 2 (Tukey’s HSD test). Also, analysis of residuals for the LME model indicated only small deviation from normality (results not shown). This way, the model structure represented well the dataset and model assumptions were mostly fulfilled, allowing reliable evaluation of statistical significance for the different experimental factors. Considering each kiwifruit cultivar separately, isolates that did not cause disease symptoms in all inoculated plants (0% disease incidence) (Table 2), and that had no fungus growth observed on carrot baits (Fig. 3), were considered non-pathogenic. The *C. huliokia* isolate from ohia and *C. fimbriata* isolate from sweet potato were not pathogenic, in both kiwifruit cultivars (Table 2, Fig. 1P and 2O). Whereas the *C. fimbriata* isolate from mango was nonpathogenic only in “Monty” (Table 2, Fig. 2G-H). For the inoculated vines that expressed disease symptoms (Fig. 1 and 2), the infection by *Ceratocystis* spp. was confirmed by the presence of the pathogen structures in carrot baits (Fig. 3). Conversely, none of the non-inoculated vines (control plants) developed disease symptoms (Fig. 1A-C and 2A-C), and again in this case the absence of the pathogen was confirmed with carrot baits (Fig. 3A-B). Significant disease severity differences were observed between *Ceratocystis* spp. isolates, for both cultivars evaluated (Table 2). Considering the pathogenic isolates for both cultivars, the disease severity was significantly lower in “Monty” than in “Gold3”, for the *C. fimbriata* isolate from teak. The *C. fimbriata* isolates from kiwifruit and teak were highly aggressive in “Gold3” (Table 2 and Fig. 1I, 1D-F), causing death of all the inoculated vines during the incubation period (45 days). Additionally, *C. fimbriata* isolates from mango and eucalyptus and *C. lukuohia* isolate from ohia also caused death of “Gold3” vines (Table 2, Fig. 1K, 1N-O and 1Q). For “Monty”, the most aggressive isolates were *C. fimbriata* isolate from

kiwifruit and *C. lukuohia* isolate from ohia, which were the only isolates that caused mortality for vines of this cultivar during the incubation period (Table 2 and Fig. 2J-L).

DISCUSSION

Our findings revealed that *Ceratocystis* spp. isolates from different hosts are pathogenic in kiwifruit vines, with different levels of aggressiveness. It has been recognized that isolates of *Ceratocystis* are generally not host specialized, and that can have different levels of aggressiveness to different hosts or even cultivars of the same host (Thorpe et al. 2004, Piveta et al. 2016).

All *Ceratocystis* isolates capable of infecting kiwifruit vines in the present work belong to the Latin American Clade (LAC) (Ferreira et al. 2011, Harrington et al. 2011, Oliveira et al. 2015). Baker et al. (2003) studying the host specificity in *Ceratocystis* isolates of the Latin American clade showed that American hosts, like cacao and sweet potato, were resistant to the isolates from other hosts, and exotic hosts have been found to be susceptible to *Ceratocystis* spp. isolates belonging to the Latin American clade, even when the isolates are derived from different hosts. Because of this, if some genotypes of the pathogen are propagated to other regions, the exotic hosts can be seriously affected. That which is in accordance with the high aggressiveness of *Ceratocystis* wilt in kiwifruit. Considering that it is an exotic host in the American region (Saquet and Brackmann 1995) and *Ceratocystis* spp. isolates from other hosts can infect kiwifruit vines.

The *C. fimbriata* kiwifruit isolate was one the most aggressive isolates for both kiwifruit cultivars evaluated. This corroborates with other studies that demonstrate that isolates from their own host are generally more aggressive than isolates from other hosts (Baker et al. 2003, Muslim et al. 2022). However, different levels of aggressiveness can also be observed between kiwifruit *C. fimbriata* isolates on kiwifruit vines (Piveta et al. 2016, Oliveira et al. 2020). In the present study, one *C. fimbriata* kiwifruit isolate previously related as highly aggressive in “Monty” was used (Oliveira et al. 2020). Therefore, the isolates from other hosts that did not differ in aggressiveness in comparison to the kiwifruit isolate, *C. fimbriata* isolate from teak in “Gold3” and *C. lukuohia* in “Monty”, can also be considered highly aggressive in these kiwifruit cultivars.

Recently, high tree mortality was related in native ohia (*Metrosideros polymorpha*) forests in Hawaii, with symptoms similar to *Ceratocystis* wilt, including dark-brown to black radial discoloration in woody xylem (Harrington 2013, Keith et al. 2015, Hughes et al. 2020).

Actually, the disease is referred to as rapid ohia death (ROD), and is associated with two new *Ceratocystis* spp., *C. lukuohia* and *C. huliohia*. The *C. lukuohia* is the more aggressive of the two pathogens, causing rapid wilt and mortality, whereas *C. huliohia* is associated with smaller mortality events and sometimes associated with wilt of individual limbs (Barnes et al. 2018, Fortini et al. 2019, Hughes et al. 2020). The disease is a serious threat to ohia forests in Hawaii, as well to other continents and islands that have ohia forests, such as New Zealand (Hughes et al. 2020). Additionally, this work showed that *C. lukuohia* can infect kiwifruit vines, with high aggressiveness, to our knowledge this is the first study demonstrating that *C. lukuohia* is not host specific. Considering this, the implementation of biosecurity measures to prevent the spread of *C. lukuohia* in Hawaii and also to the largest kiwifruit producing countries becomes important. Furthermore, it is important to carry out studies evaluating the pathogenicity of *C. lukuohia* in other commercial crops.

The *C. fimbriata* isolate from sweet potato was not pathogenic, in both kiwifruit cultivars evaluated. This supports Tyson et al. (2020), who demonstrated that *C. fimbriata* isolated from sweet potato is not pathogenic in five kiwifruit cultivars. Similarly, Baker et al. (2003) showed that sweet potato isolates were not pathogenic to mango (*Mangifera indica*), yam (*Dioscoera* spp.), cacao (*Theobroma cacao*) and sycamore (*Platanus* spp.). These inoculation assays indicate that *C. fimbriata* isolates from sweet potato are highly host specialized (Steimel et al. 2004, Engelbrecht and Harrington 2005). This could be one of the explanations of why *C. fimbriata* occurs in important kiwifruit producing countries, like New Zealand (Tyson et al. 2020) and China (Li et Al. 2016), causing disease in sweet potato, but there are no reports of the disease occurring in kiwifruit vines.

Pomegranate wilt disease, caused by *C. fimbriata*, is currently one of the important diseases affecting pomegranate (Abhishek et al. 2022). The disease was first reported in 1999, in India (Somasekhara 1999), and recently, was reported in China (Xu et al. 2011) and Pakistan (Alam et al. 2016). Studies suggest that the *C. fimbriata* population that causes pomegranate wilt in China originated from Brazil and was introduced into the country through cuttings of eucalyptus (Li et al. 2016). In present study, we showed that a *C. fimbriata* isolate from pomegranate plants in China can infect kiwifruit vines. Considering, that China is the largest kiwifruit producing country (FAOSTAT, 2022) and that the pathogen is present in the country, the knowledge of the symptoms of the disease by kiwifruit producers can be a good strategy, so that, should the disease occur, strategic management measures can be taken quickly. This is also a serious threat, to other kiwifruit producer countries, such Iran,

that is one of the world's biggest producers of pomegranate and kiwifruit, and borders Pakistan.

Interaction between isolate and cultivar was observed for *C. fimbriata* isolates from mango and teak. These isolates were highly aggressive in “Gold3”, while in “Monty” the teak isolate was significantly less aggressive and the mango isolate was not pathogenic. The fact that “Gold3” is not cultivated in Brazil, and that the *C. fimbriata* isolate from mango is not pathogenic in “Monty”, can be related to the fact that Ceratocystis wilt was just recently reported in kiwifruit vines, while occurring in mango since 1930 in the country (Carvalho 1938). Furthermore, considering that “Gold3” is widely cultivated in New Zealand, the entry of mango or teak *C. fimbriata* isolates into the country is a serious threat to kiwifruit vines.

Ceratocystis wilt is currently the most important disease in kiwifruit in Brazil and a serious threat for the world's largest kiwifruit producing countries, where the pathogen has not been reported yet on kiwifruit vines (Oliveira et al. 2016, Ferreira et al. 2017, FAOSTAT, 2022). The selection of resistant genotypes is the best strategy to control Ceratocystis wilt, as observed for other host crops (Zauza et al. 2004, Rosado et al. 2010, Arriel et al. 2016). However, the use of highly aggressive isolates is important to assess host genetic resistance (Fourie et al. 2019, Oliveira et al. 2020). In the present work, we showed that *Ceratocystis* isolates from other hosts are highly aggressive in kiwifruit vines. Therefore, it is interesting that they are considered during the selection of kiwifruit resistant cultivars. Furthermore, we showed that *C. fimbriata* isolates from other hosts and *C. lukuohia*, which was present in other countries, are pathogenic in kiwifruit vines. In view of this, it is extremely important that measures to prevent the entry and promote rapid detection of these pathogens are taken by the main kiwifruit producing countries.

ACKNOWLEDGMENTS

This work was supported by: ‘Conselho Nacional de Desenvolvimento Científico e Tecnológico’ (CNPq); ‘Coordenação de Aperfeiçoamento de Pessoal de Nível Superior’ (CAPES). The authors are grateful for the assistance of Carolina Pessanha Alonso, Fernando Montezano Fernandes and Flávia Alexandre Sampaio, to Department of Agriculture, Livestock and Food Supply (MAPA) for the import and inoculation guidelines, and to Zespri Group Limited and ‘Clonar Resistência a Doenças Florestais’ for providing plant material and structure used in this study.

REFERENCES

- Abhishek1 G, Manjunatha G, Mesta RK, Gunnaiah R, Suma, R (2022) Identification of Sources of Resistance to Wilt caused by *Ceratocystis fimbriata* in Pomegranate. *Biological Forum – An International Journal* 14(1):1193-1198
- Alam MW, Gleason ML, Mehboob S, Riaz K, Rehman A (2016) First report of *Ceratocystis fimbriata* causing pomegranate wilt in Pakistan. *Plant Disease* 101:251
- Arriel DAA, Guimarães LMS, Resende MDV, Neto FPL, Silva DFSHS, Siqueira DL et al. (2016) Genetic control of resistance on *Mangifera indica* to Ceratocystis wilt. *Scientia Horticulturae* 211:312-318
- Baker CJ, Harrington TC, Krauss U, Alfenas AC (2003) Genetic Variability and Host Specialization in the Latin American Clade of *Ceratocystis fimbriata*. *Phytopathology* 93(10):1274-1283
- Barnes I, Fourie A, Wingfield MJ, Harrington TC, McNew DL, Sugiyama LS, Luiz BC, Heller WP, Keith LM (2018) New Ceratocystis species associated with rapid death of *Metrosideros polymorpha* in Hawaii. *Persoonia* 40:154-181
- Bates D, Maechler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67(1):1-48.
- Carvalho MB (1938) Sobre dois insetos nocivos à Mangueira. *Boletim da Secretaria da 20 Agricultura, Indústria e Comércio, Pernambuco*, 3(2):130-132
- Cowger C, Brown JKM (2019) Durability of quantitative resistance in crops: Greater than we know? *Annual Review of Phytopathology* 25(57):253-277
- Cowger C, Hoffer ME, Mundt CC (2000) Specific adaptation by *Mycosphaerella graminicola* to a resistant wheat cultivar. *Plant Pathology* 49:445–451
- Engelbrecht CJB, Harrington TC (2005) Intersterility, Morphology and Taxonomy of *Ceratocystis fimbriata* on Sweet Potato, Cacao and Sycamore. *Mycologia* 97:57-69
- Engelbrecht CJB, Harrington TC, Alfenas AC, Suarez C (2007) Genetic variation of populations of the cacao wilt pathogen, *Ceratocystis cacaofunesta*. *Plant Pathology* 56:923-933
- FAOSTAT (2022) Production of kiwi: top ten producers. Available at: <https://www.fao.org/faostat/en/#search/kiwi>. Accessed on January 25, 2023
- Ferreira A, Harrington TC, Alfenas AC, Mizubuti ESG (2011) Movement of Genotypes of *Ceratocystis fimbriata* Within and Among Eucalyptus Plantations in Brazil. *Ecology and Epidemiology* 101(8):1005-1012
- Ferreira MA, Harrington TC, Piveta G, Alfenas AC (2017) Genetic variability suggests that three populations of *Ceratocystis fimbriata* are responsible for the Ceratocystis wilt epidemic on kiwifruit in Brazil. *Tropical Plant Pathology* 42:86–95
- Firmino AC, Tozze HJ, Furtado EL (2012) First report of *Ceratocystis fimbriata* causing wilt in *Tectona grandis* in Brazil. *New Disease Reports* 25:24-24

- Fortini LB, Kaiser LR, Keith LM, Price J, Hughes RF, Jacobi JD, Friday JB (2019) The evolving threat of Rapid 'Ōhi'a Death (ROD) to Hawai'i's native ecosystems and rare plant species. *Forest Ecology and Management* 448:376-385
- Fourie A, Nest MA, Vos L, Wingfield MJ, Wingfield BD, Barnes I (2019) QTL mapping of mycelial growth and aggressiveness to distinct hosts in *Ceratocystis* pathogens. *Fungus Genetics and Biology* 131:103242
- Guimarães LMS, Titon M, Lau D, Rosse LN, Oliveira LSS, Rosado CCG, Christo GGO, Alfenas AC (2010) *Eucalyptus pellita* as a source of resistance to rust, *Ceratocystis* wilt and leaf blight. *Crop Breeding and Applied Biotechnology* 10:124–131
- Harrington TC (2013) *Ceratocystis* Diseases. *Infectious Forest Diseases* (eds P. Gonthier and G. Nicolotti) 230-255
- Harrington TC, Thorpe DJ, Alfenas AC (2011) Genetic Variation and Variation in Aggressiveness to Native and Exotic Hosts Among Brazilian Populations of *Ceratocystis fimbriata*. *Phytopathology* 101(5):555-566
- Hughes MA, Juzwik J, Harrington TC, Keith LM (2020) Pathogenicity, Symptom Development, and Colonization of *Metrosideros polymorpha* by *Ceratocystis lukuohia*. *Plant Disease* 104:2233-2241
- Keith LM, Hughes RF, Sugiyama LS, Heller WP, Bushe BC, Friday JB (2015) First report of *Ceratocystis* wilt on Ohia (*Metrosideros polymorpha*). *Plant Disease* 99:1276
- Leclerc M, Clément JAJ, Adrison D, Hamelin M (2019) Assessing the effects of quantitative host resistance on the life-history traits of sporulating parasites with growing lesions. *Proceedings of the Royal Society B* 286:20191244
- Length, R. V. 2022. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.8.2. Available in: <https://CRAN.R-project.org/package=emmeans>. Accessed on December 14, 2022
- Li Q, Harrington TC, McNew D, Li J, Huang Q, Somasekhara YM, Alfenas AC (2016) Genetic bottlenecks for two populations of *Ceratocystis fimbriata* on sweet potato and pomegranate in China. *Plant Disease* 100(11):2266-2274
- Marin M, Castro B, Gaitan A, Preisig O, Wingfield BD, Wingfield MJ (2003) Relationships of *Ceratocystis fimbriata* isolates from Colombian coffee-growing regions based on molecular data and pathogenicity. *Journal of Phytopathology* 151:395–405
- Muslim A, Pratama R, Suwandi S, Hamidson H (2022) Diseases Severity, Genetic Variation, and Pathogenicity of *Ceratocystis* Wilt on *Lansium domesticum* in South Sumatra, Indonesia. *Plant Pathology Journal* 38(2):131-145
- Oliveira LSS, Damacena MB, Guimarães LMS, Siqueira DL, Alfenas AC (2016) *Ceratocystis fimbriata* isolates on *Mangifera indica* have different levels of aggressiveness. *European Journal of Plant Pathology* 145:847–856
- Oliveira LSS, Harrington TC, Ferreira MA, Michelle BD, Al-Sadi, AM, Al-Mahmooli HIS, et al. (2015) Species or Genotypes? Reassessment of four recently described species of the *Ceratocystis* wilt Pathogen, *Ceratocystis fimbriata*, on *Mangifera indica*. *Phytopathology* 105:1229-1244.

Oliveira LSS, Pimenta LVA, Guimarães LMS, de Souza PVD, Bhering LL, Alfenas AC (2020) Resistance of kiwifruit cultivars to *Ceratocystis* wilt: an approach considering the genetic diversity and variation in aggressiveness of the pathogen. *Plant Pathology* 70:349-357

Piveta G, Ferreira MA, Muniz MFB, Valdetaro D, Valdebenito-Sanhueza R, Harrington TC, Alfenas AC (2016) *Ceratocystis fimbriata* on kiwifruit (*Actinidia* spp.) in Brazil. *New Zealand Journal of Crop and Horticultural Science* 44:31-24

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available in: URL <https://www.R-project.org/>. Accessed on December 20, 2022

Rosado CCG, Guimarães LMS, Titon M, Douglas L, Resende MDV, Alfenas AC (2010) Resistance to ceratocystis wilt (*Ceratocystis fimbriata*) in parents and progenies of *Eucalyptus grandis* X *E. urophylla*. *Silvae Genetica* 3:99-106

Saquet AA, Brackmann A (1995) A cultura do kiwifruit. *Ciência Rural* 25:177-182

Somasekhara YM (1999) New Record of *Ceratocystis fimbriata* Causing Wilt of Pomegranate in India. *Plant Disease* 83(4):400

Sonego OR, Ferreira MA, Sanhueza RM, Gava R, Garrido LR, Alfenas AC (2010) Primeiro relato da murcha-de-ceratocystis em kiwifruit. *Tropical Plant Pathology* 35: S233

Steimel J, Engelbrecht CJB, Harrington TC (2004) Development and characterization of microsatellite markers for the fungus *Ceratocystis fimbriata*. *Molecular Ecology Resources* 4: 215–218

Tarigan M, Roux J, Wyk MV, Tjahjono B, Wingfield MJ (2011) A new wilt and die-back disease of *Acacia mangium* associated with *Ceratocystis manginecans* and *C. acaciivora* sp. nov. in Indonesia. *South African Journal of Botany* 77:292–304

Thorpe DJ, Harrington TC, Uchida JY (2004) Pathogenicity, Internal Transcribed Spacer-rDNA Variation, and Human Dispersal of *Ceratocystis fimbriata* on the Family Araceae. *Phytopathology* 95: 316-323

Tyson JL, Manning MA, Wright PJ (2020) Pathogenicity of *Ceratocystis fimbriata* from New Zealand kūmara on kiwifruit cultivars. *New Zealand Plant Protection* 73:6-12

Weber DJ and Stahmann MA (1964) *Ceratocystis* infection in sweet potato: its effect on proteins, isozymes, and acquired immunity. *Science* 146(3646):929-31

Xu B, Zheng XH, Guo WX, Zhou XP, He P (2011) First Report of Pomegranate Wilt Caused by *Ceratocystis fimbriata* in Sichuan Province. *Plant Disease* 95(6):776

Zauza EAV, Alfenas AC, Harrington TC, Mizubuti ESG, Silva JF (2004) Resistance of *Eucalyptus* clones to *Ceratocystis fimbriata*. *Plant Disease* 88:758–760.

FIGURES AND TABLES

Table 1 *Ceratocystis* spp. isolates of different hosts which had their pathogenicity evaluated in two kiwifruit cultivars

Isolate code ¹	Species	Host	Origin
LPF1443	<i>Ceratocystis fimbriata</i>	Kiwifruit (<i>Actinidia deliciosa</i>)	Brazil
LPF1912	<i>Ceratocystis fimbriata</i>	Eucalyptus (<i>Eucalyptus</i> spp.)	Brazil
LPF1156	<i>Ceratocystis fimbriata</i>	Mango (<i>Mangifera indica</i>)	Brazil
LPF2199	<i>Ceratocystis fimbriata</i>	Teak (<i>Tectona grandis</i>)	Brazil
LPF2463	<i>Ceratocystis fimbriata</i>	Acacia (<i>Acacia magium</i>)	Indonesia
LPF2464	<i>Ceratocystis fimbriata</i>	Sweet potato (<i>Ipomea batatas</i>)	USA
LPF2465	<i>Ceratocystis fimbriata</i>	Pomegranate (<i>Punica granatum</i>)	China
LPF2293	<i>Ceratocystis huliohia</i>	Ohia (<i>Metrosideros polymorpha</i>)	Hawaii
LPF2257	<i>Ceratocystis lukuohia</i>	Ohia (<i>Metrosideros polymorpha</i>)	Hawaii
LPF1336	<i>Ceratocystis fimbriata</i>	Ficus (<i>Ficus carica</i>)	Brazil

¹Isolate code of culture collection of the Laboratory of Forest Pathology of the Universidade Federal de Vicosa, Brazil.

Table 2 Incidence, mortality and severity of kiwifruit vines, cultivars Gold3 and Monty, inoculated with *Ceratocystis* spp. isolates from different hosts

Code isol.	Host	Incidence (%)		Mortality (%)		Severity (%) ¹		Carrot bait ²	
		"Gold3"	"Monty"	"Gold3"	"Monty"	"Gold3"	"Monty"	"Gold3"	"Monty"
LPF2463	Acacia	100	100	0	0	8.63bcA	6.72bcA	+	+
LPF1912	Eucalyptus	100	100	20	0	3,18bcA	3.72bA	+	+
LPF1336	Ficus	100	100	0	0	3,18bA	4.72bcA	+	+
LPF1443	Kiwifruit	100	100	100	60	36,54eA	36,54dA	+	+
LPF1156	Mango	100	0	60	0	5,96bcA	0aB	+	-
LPF2257	Ohia	100	100	20	60	12,27cdA	13,09cdA	+	+
LPF2293	Ohia	0	0	0	0	0aA	0aA	-	-
LPF2465	Pomegranate	100	100	0	0	9.81bcA	10.36bcA	+	+
LPF2464	Sweet potato	0	0	0	0	0aA	0aA	-	-
LPF2199	Teak	100	100	100	0	27.54deA	7.72bcB	+	+
Control	PD*	0	0	0	0	0aA	0aA	-	-

¹Means within a column followed by the same letters are not significantly different from each other using Tukey's HSD test (p-value<0.05).

²+: Presence of pathogen structures in carrot bait. -: Absence of pathogen structures in carrot bait.



Fig. 1 Kiwifruit vine of Gold3 cultivar after 45 days of inoculation with *Ceratocystis* spp. isolates from different hosts: **A, B** – Vines inoculated with a disk of autoclaved agar; **C** – Xylem of the vine inoculated with a disk of autoclaved agar; **D** – Vines inoculated with *Ceratocystis fimbriata* isolate from teak; **E** – Dead vine inoculated with *C. fimbriata* isolate from teak; **F** – Xylem lesion of the vine inoculated with *C. fimbriata* isolate from teak; **G** – Vines inoculated with *C. fimbriata* isolate from pomegranate; **H** – Xylem lesion of the vine inoculated with *C. fimbriata* isolate from pomegranate; **I** – Dead vine inoculated with *C. fimbriata* isolate from kiwifruit. (Continue)

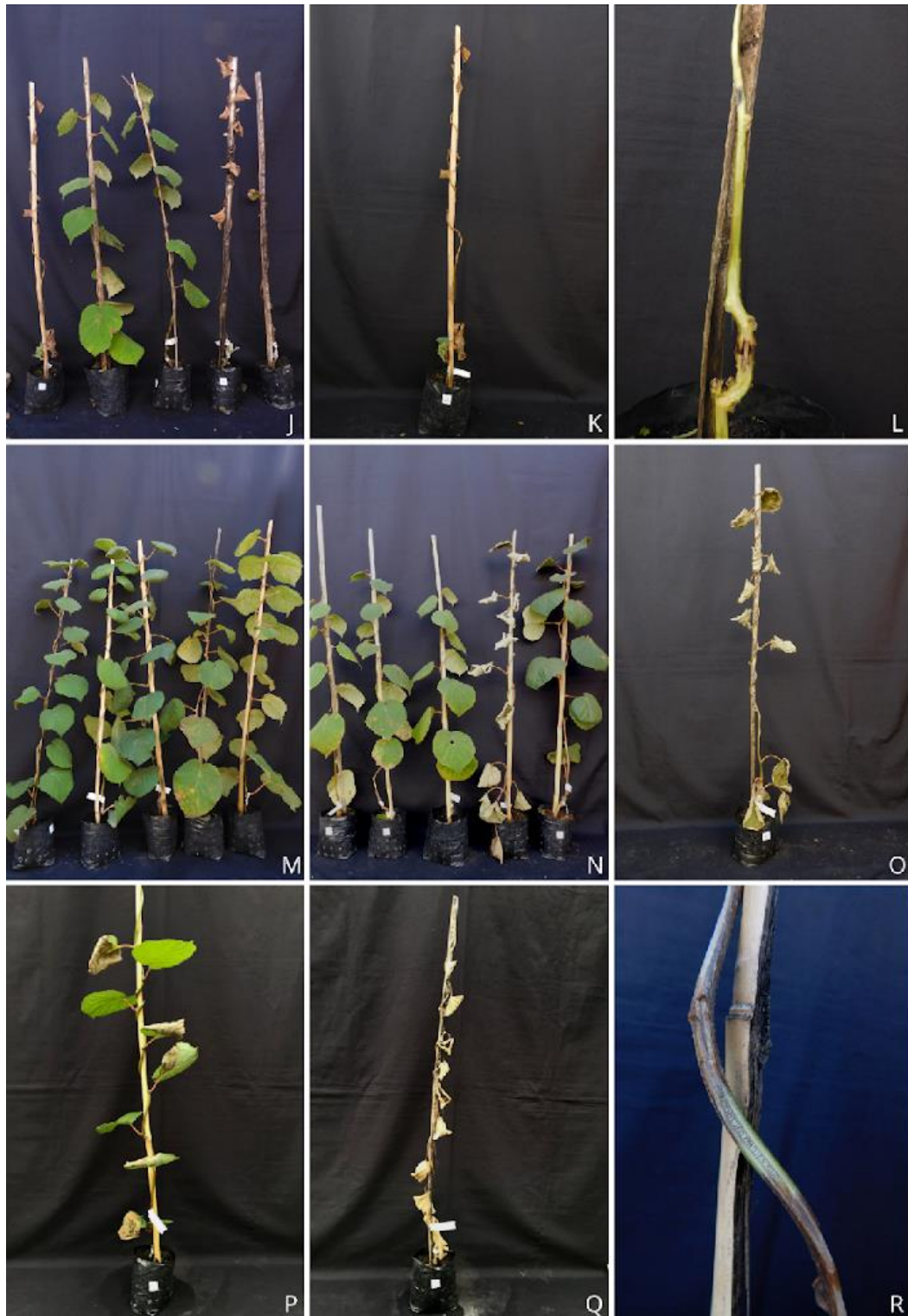


Fig. 1 (Continuation) Kiwifruit vine of Gold3 cultivar after 45 days of inoculation with *Ceratocystis* spp. isolates from different hosts: **J** – Vines inoculated with *C. fimbriata* isolate from mango; **K** – Dead vine inoculated with *C. fimbriata* isolate from mango; **L** – Xylem lesion of the vine inoculated with *C. fimbriata* isolate from mango; **M** – Vines inoculated with *C. fimbriata* isolate from ficus; **N** – Vines inoculated with *C. fimbriata* isolate from eucalyptus; **O** – Dead plant inoculated with *C. fimbriata* isolate from eucalyptus; **P** – Plant inoculated with *Ceratocystis uluohia* isolate from ohia; **Q** – Dead plant inoculated with *Ceratocystis lukuohia* isolate from ohia; **R** – Xylem lesion of the plant inoculated with *C. lukuohia* isolate from ohia.

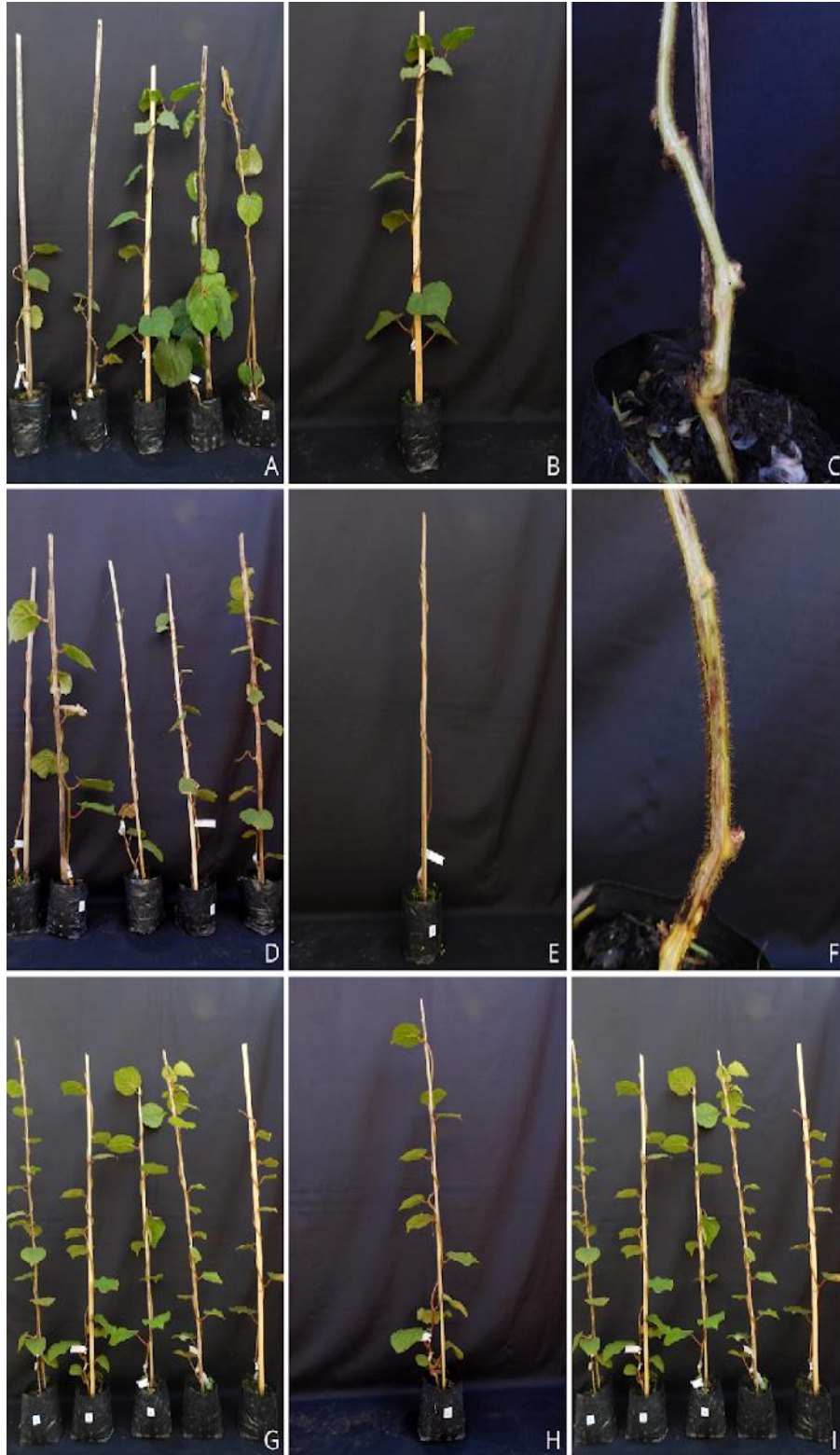


Fig. 2 Kiwifruit vine of Monty cultivar after 45 days of inoculation with *Ceratocystis* spp. isolates from different hosts: **A, B** – Vines inoculated with a disk of autoclaved agar; **C** – Xylem of the vine inoculated with a disk of autoclaved agar; **D** – Vines inoculated with *Ceratocystis fimbriata* isolate from kiwifruit; **E** – Dead vine inoculated with *C. fimbriata* isolate from kiwifruit; **F** – Xylem lesion of the plant inoculated with *C. fimbriata* isolate from kiwifruit; **G, H** – Vines inoculated with *C. fimbriata* isolate from mango; **I** – Vines inoculated with *C. fimbriata* isolate from eucalyptus. (*Continue*)

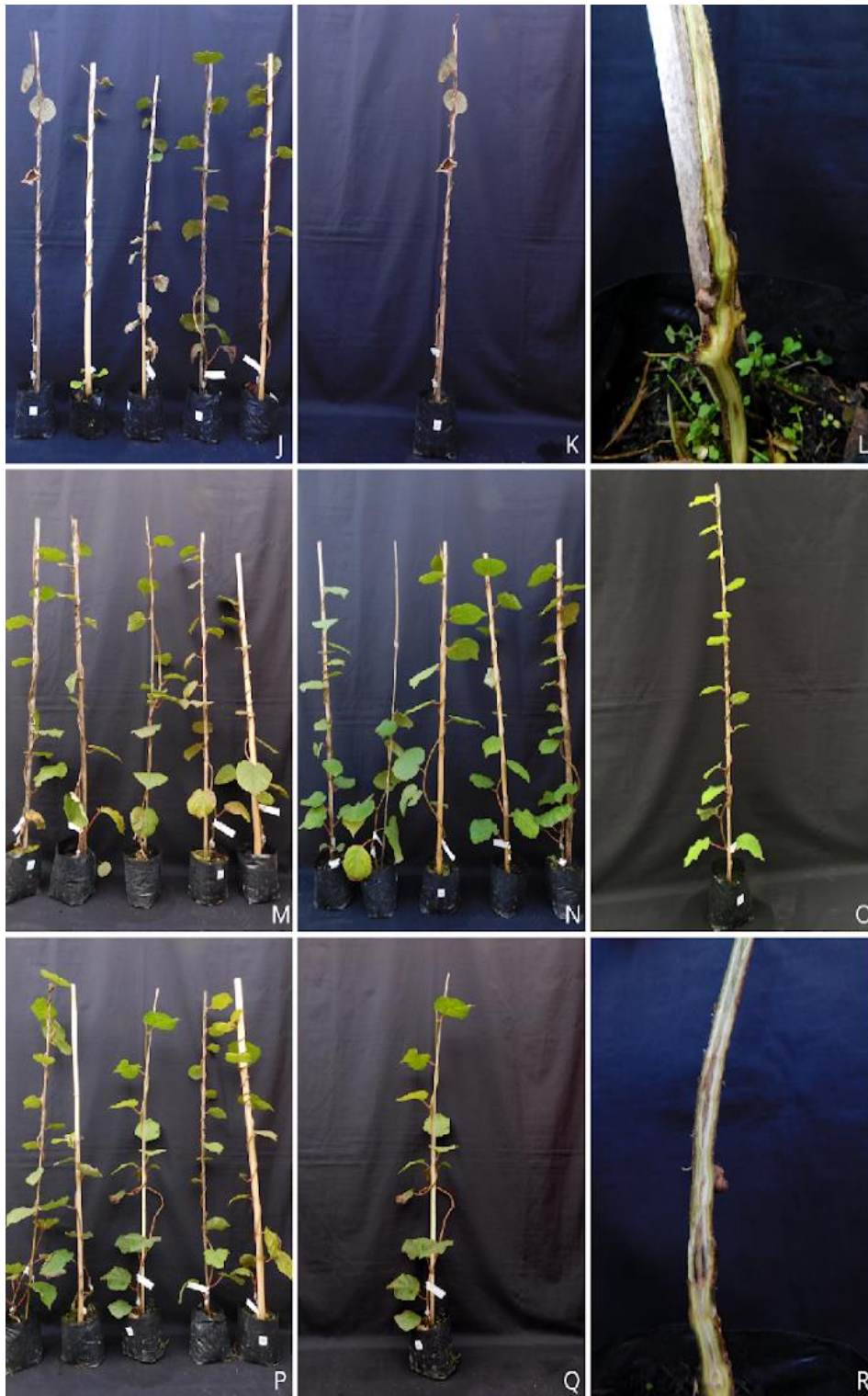


Fig. 2 (Continuation) Kiwifruit vine of Monty cultivar after 45 days of inoculation with *Ceratocystis* spp. isolates from different hosts: **J** – Vines inoculated with *Ceratocystis lukuohia* isolate from ohia; **K** – Dead vine inoculated with *C. lukuohia* isolate from ohia; **L** – Xylem lesion of the vine inoculated with *C. lukuohia* isolate from ohia; **M** – Vines inoculated with *C. fimbriata* isolate from ficus; **N** – Vines inoculated with *C. fimbriata* isolate from acacia; **O** – Vine inoculated with *C. uluohia* isolate from ohia; **P, Q** – Vines inoculated with *C. fimbriata* isolate from teak; **R** – Xylem lesion of the vine inoculated with *C. fimbriata* isolate from teak.

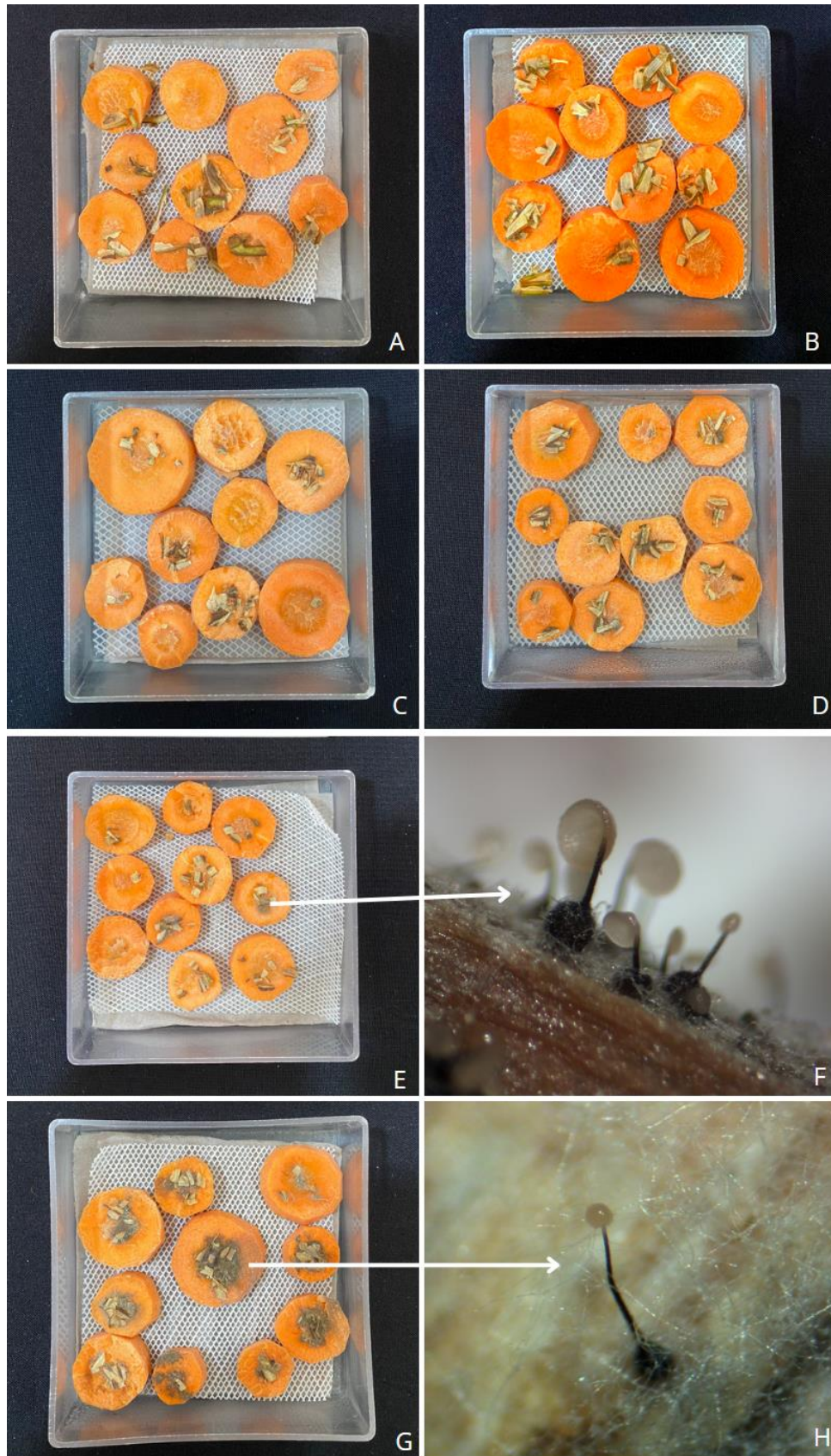


Fig. 3 Carrot baits used to detect *Ceratocystis* spp.: **A** – No fungus growth on carrot baits made with stem samples from non-inoculated kiwifruit vines, cultivar Monty; **B** – No fungus growth on carrot baits made with stem samples from non-inoculated kiwifruit vines, cultivar Gold3; **C** – No fungus growth on carrot baits made with stem samples from inoculated vines,

cultivar Gold3, with *Ceratocystis uluohia* isolate from ohia; **D** – No fungus growth on carrot baits made with stem samples from inoculated vines, cultivar Gold3, with *Ceratocystis fimbriata* isolate from mango; **E** – Presence of fungus growth on carrot baits made with stem samples from inoculated vines, cultivar Gold3, with *C. fimbriata* isolate from mango; **F** – *C. fimbriata* structures detected in a carrot bait made with stem samples of inoculated vines, cultivar Gold3; **E** – Presence of fungus growth on carrot baits made with stem samples from inoculated vines, cultivar Gold3, with *Ceratocystis lukuohia* isolate from ohia; **F** – *C. lukuohia* structures detected in a carrot bait made with stem samples of inoculated vines, cultivar Gold3.