Potential Threat of Ceratocystis lukuohia on kiwifruit crop

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Short Report

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Abstract

Ceratocystis wilt, caused by *Ceratocystis fimbriata*, is the most severe disease of the kiwifruit crop in Brazil and represents a threat to other kiwifruit-producing countries where the pathogen has not yet been reported infecting kiwifruit vines. *Ceratocystis* species have been reported in many countries, some are host specific, while others can infect a wide host range. Given the high impact of the disease on kiwifruit, evaluating whether *Ceratocystis* isolates from other hosts and countries can also infect kiwifruit is important for implementing biosecurity measures. In the present work, we evaluated if *C. lukuohia* and *C. huliohia*, isolated from ohia (*Metrosideros polymorpha*) in Hawaii are pathogenic to kiwifruit cultivars. Inoculations of both species in rooted cuttings of Monty and Gold3 cultivars showed that *C. lukuohia* can cause wilt in both kiwifruit cultivars evaluated, with high plant mortality. This is the first report that *C. lukuohia* can infect another host, demonstrating that it is not host-specific and extends its host range. In addition, considering that *C. lukuohia* is pathogenic and lethal in kiwifruit vines, kiwifruit-producing areas must implement biosecurity measures to prevent the introduction of this species.

Full Text

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). With the development of new varieties and increased demand for the fruit, the cultivation of kiwifruit became profitable and has spread throughout five continents of the world. With an annual production of approximately 4.5 million metric tons, China, New Zealand, and Italy stand as the largest kiwifruit-producing countries (FAOSTAT, 2022).

This increase in world production leads to the emergence of diseases that can cause significant losses in the crop, such as Bacterial canker, caused by *Pseudomonas syringae* pv. *actinidiae* (Vannest, 2017). Additionally, in 2010, Ceratocystis wilt, caused by *Ceratocystis fimbriata* Ellis & Halst., was reported in kiwifruit orchards in Rio Grande do Sul, state Brazil (Sonego et al. 2010), with symptoms of wilt, dark-brown lesions in the xylem, reduced fruit length, and death of infected vines (Piveta et al., 2016; Ferreira et al., 2017). With the rapid dissemination of the disease to other producing regions in the country and the extensive mortality caused, which can reach up to 30% (Piveta et al., 2016), Ceratocystis wilt emerged as the primary disease affecting kiwifruit cultivation in the country, leading to the abandonment or replacement of many kiwifruit orchards (Piveta et al., 2016).

The *Ceratocystis fimbriata* sensu lato (s.l.) complex is formed by a group of species containing high levels of genetic variation and widely distributed geographically. The intraspecific and interspecific variability in the *Ceratocystis* species is related to host speciation, aggressiveness variation, and specific interactions with certain cultivars or clones (Cowger et al., 2000; Oliveira et al., 2020). Some species and/or isolates are related to single hosts or geographic regions, while others infect a wide range of hosts (Thorpe et al., 2005; Tyson et al., 2020). Since the first report of Ceratocystis wilt on kiwifruit vines (Sonego et al., 2010), investigation of pathogenicity of *Ceratocystis* spp. found in other countries, mainly those occurring in kiwifruit-producing countries, has become important for implementing biosecurity measures to prevent the introduction of pathogenic isolates in kiwifruit orchards. Tyson et al. (2020) showed that New Zealand isolates of *C. fimbriata* from sweet potato (kumara) are not pathogenic to a range of kiwifruit cultivars (Hayward, Gold3, Gold14, Bounty). In addition, the sweet potato isolates were non-pathogenic on mango (*Mangifera indica*), yam (*Dioscoera* spp.), cacao (*Theobroma cacao*) and sycamore (*Platanus* spp.), which demonstrates a notable host specialization of *C. fimbriata* isolates from sweet potato (Steimel et al., 2004; Engelbrecht and Harrington, 2005). However, there are numerous species of *Ceratocystis* spread throughout the world (CAB International, 2023), and if these are pathogenic to kiwifruit, as well as the *C. fimbriata* isolates present in Brazil, they could pose a potential threat to kiwifruit cultivation in other regions of the world.

In 2015, Keith et al. described Ceratocystis wilt causing widespread mortality on ohia (*Metrosideros polymorpha*), the most ecologically and culturally significant native tree in Hawaii, USA. The phenomenon was called rapid ohia death (ROD), and symptoms including wilting of tree canopies and dark-brown lesions in woody xylem were first observed in the Puna district of Hawaii Island (Keith et al., 2015; Hughes et al., 2020). The fungal pathogens causing ROD were described as two new *Ceratocystis* species, *C. lukuohia* and *C. huliohia* (Barnes et al., 2018). *C. lukuohia* is the most aggressive, causing rapid wilt and widespread mortality, whereas *C. huliohia* is associated with less mortality, cankers, and is sometimes associated with wilt of individual limbs (Barnes et al., 2018; Fortini et al., 2019; Hughes et al., 2020). Microsatellite analyses showed two clonal lineages of these species, which were introduced into Hawaii (Barnes et al., 2018). Currently, Ceratocystis wilt caused by *C. lukuohia* occurs throughout Hawaii Island and Kauai and poses a serious threat to native Hawaiian forests. The *Metrosideros* forests in other continents and islands like New Zealand may also be in danger (Hughes et al., 2020; Luiz et al., 2021; 2022). Barnes et al. (2018) showed that *C. lukuohia* and *C. huliohia* are non-pathogenic to arrowhead vine (*Syngonium podophylum*), taro (*Colocasia esculenta*), and london planetree (*Platanus × acerifolia*). However, given that certain *Ceratocystis* spp. can infect a wide range of hosts, and the frequent occurrence of *Ceratocystis* species introduction into new countries (CAB International, 2023), it is crucial to evaluate the pathogenicity of these two new *Ceratocystis* species in additional crops, particularly in high-value crops such as kiwifruit, considering the high impact of the disease in this host.

Front of this, the present study aimed to evaluate if *C. lukuohia* and *C. huliohia,* isolated from infected *M. polymorpha* in Hawaii, can induce Ceratocystis wilt in kiwifruit cultivars. Firstly, the identity of the *Ceratocystis* species was confirmed by sequencing ITS and TEF-1 gene regions of DNA and subsequent comparison with other sequences of the respective species in GenBank (Oliveira et al., 2015). For the pathogenicity assay, the *Ceratocystis* isolates were grown on Potato Dextrose Agar (PDA) at 28°C with a 12 h photoperiod for twelve days. After incubation, PDA plugs containing the fungus were inoculated on rooted cuttings of 'Monty' (*Actinidia deliciosa*) from Brazil and 'Gold3' (*Actinidia chinensis var. chinensis 'Zesy002'*) from New Zealand following established protocols (Alfenas and Mafia, 2016). Kiwifruit vines inoculated with the *C. fimbriata* isolate from infected kiwifruit vines in Brazil and vines that received PDA-only plugs served as positive and negative controls, respectively. A completely randomized design with five replicate plants per treatment and controls was used. The inoculated kiwifruit vines were incubated in the greenhouse (28 ± 5 °C). Forty-five days post-inoculation, vines were evaluated and the number of wilted vines was recorded. Additionally, xylem lesion length (cm) in the inner stem tissue of infected vines was measured and used to evaluate the aggressiveness of the pathogenic isolates on the two kiwifruit cultivars, considering a disease severity of 100% for the wilted vines. Carrot baits prepared with stem samples of inoculated vines were used to confirm the infection was caused by *Ceratocystis* species, from re-isolation and molecular confirmation by sequencing ITS and TEF-1 gene regions of DNA. The experiment was repeated once in the time. The data analysis was performed considering isolate and cultivar as fixed effects while replicates were considered as random effects. Significance of individual and interaction effects was evaluated at a 5% probability level.

All kiwifruit vines inoculated with *C. lukuohia* and *C. fimbriata* became infected (Fig. 1C, 2D), resulting in a 60% mortality rate of vines during the incubation period for both isolates. The *Ceratocystis* infection was confirmed by the presence of pathogen structures (perithecia and ascospores) in carrot baits (Fig. 1E, 1F), and from a subsequent comparison of ITS and TEF-1 gene regions of DNA with other sequences of the respective species in GenBank. None of the vines that were inoculated with *C. huliohia* (Fig. 1B) or PDA-only plugs showed disease symptoms (Fig. 1A), and no *Ceratocystis* structures were observed on carrot baits. Therefore, *C. huliohia* is non-pathogenic in either of the kiwifruit cultivars evaluated.

Our data indicate that cultivar and experiment time did not significantly affect ($p>0.05$) lesion size and disease severity. Only isolate had a significant effect ($p<0.05$) on these variables. *C. fimbriata* caused significantly larger lesions and was more aggressive in kiwifruit than *C. lukuohia* (Fig. 2). However, it is important to consider that there is significant variation in aggressiveness among *Ceratocystis* isolates, and the *C. fimbriata* isolate used in the present study (LPF1443) has been identified as a highly aggressive in kiwifruit vines (Oliveira et al., 2020). Given the susceptibility of both kiwifruit cultivars to *C. lukuohia*, along with high plant mortality rates, there is a potential threat that this pathogen may pose not only to kiwifruit-producing areas in Hawaii but also to other countries with kiwifruit cultivation, such as New Zealand and Italy.

Although *C. lukuohia* and *C. huliohia* are associated with ROD, only *C. lukuohia* is considered a systemic vascular wilt pathogen on *M. polymorpha* (Hughes et al., 2020). Similarly, we showed that of the two ROD pathogens, only *C. lukuohia* causes wilt on kiwifruit vines, with high aggressiveness and mortality. This corroborates other studies that suggest that *C. lukuohia* causes Ceratocystis wilt on *M. polymorpha*, and this species is the main cause of the high mortality caused by ROD, and that the distinct symptoms caused by *C. huliohia* seem to be less important (Keith et al., 2015; Hughes et al., 2020).

This is the first report indicating that *C. lukuohia* is not host-specific, which suggests that it poses a threat to other agricultural and forestry crops growing in Hawaii and the Pacific Basin. Implementation of biosecurity measures, including rapid detection and eradication strategies of the pathogen are imperative to prevent local and long-distance spread. Furthermore, knowing that *C. lukuohia* can cause

Ceratocystis wilt on kiwifruit vines, it is essential to avoid the introduction of this pathogen to kiwifruit-producing countries, especially where native *Metrosideros* spp. co-exist.

Declarations

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AUTHOR CONTRIBUTIONS STATEMENT

Investigation and methodology were performed by Sabrina Angela Cassol. The data curation and formal analysis were performed by Sabrina Angela Cassol. The funding acquisition and supervision by Acelino Couto Alfenas, Chandan Pal, and Rafael Ferreira Alfenas. The original draft was written by Sabrina Angela Cassol and all authors reviewed and edited previous versions of the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY

The data will be made available upon request from the corresponding author of the article.

CONFLICT OF INTEREST

Chandan Pal is employed by Zespri International Limited. All other authors have no competing interests.

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Figures

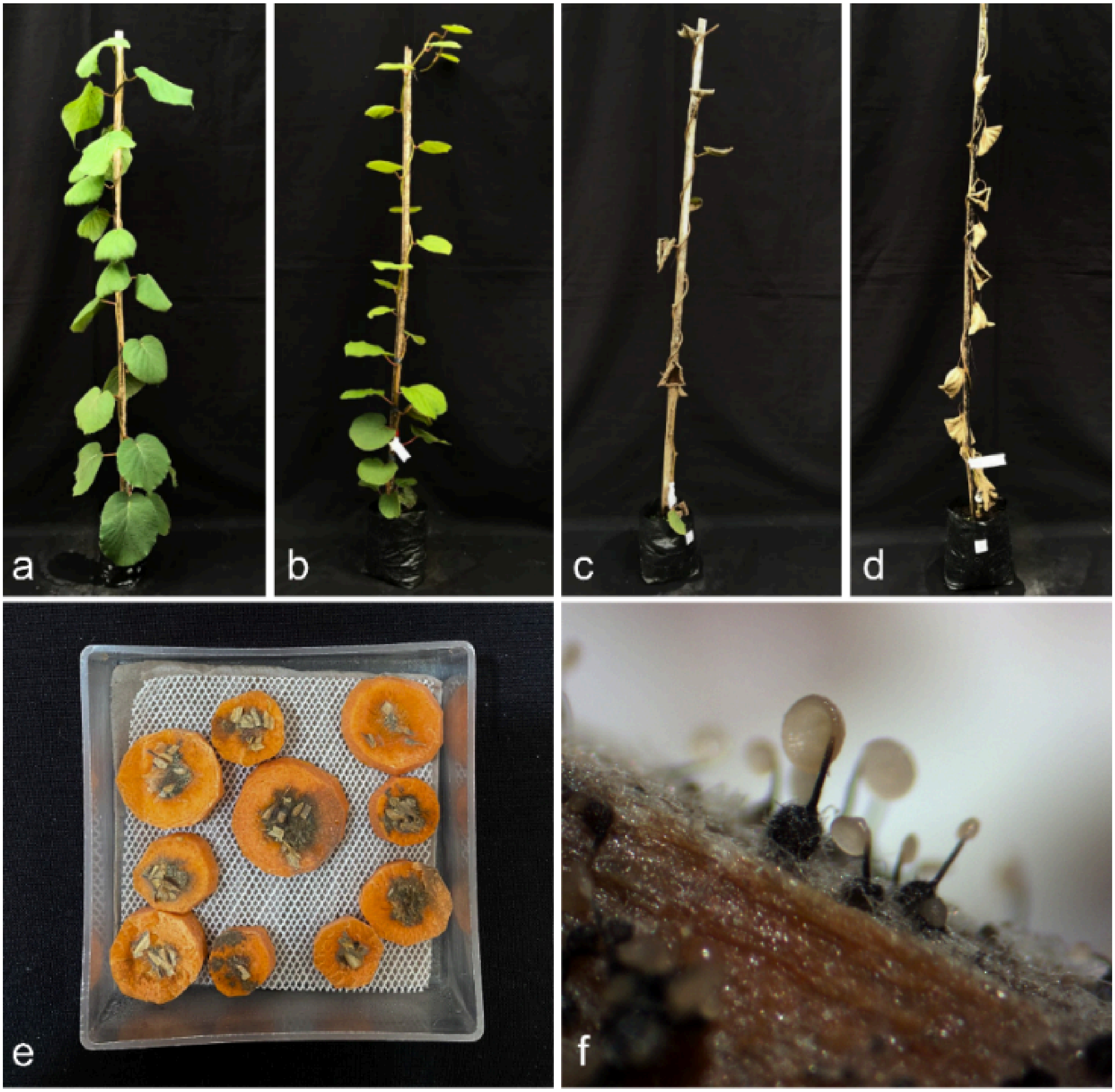

Figure 1

Kiwifruit vines, cultivar Gold3, 45 days post-inoculation with *Ceratocystis* spp. **A** Inoculated with PDA plug. **B** Inoculated with *Ceratocystis huliohia.* **C** Inoculated with *C. lukuohia*. **D** Inoculated with *C. fimbriata* from kiwifruit vine. **E**Carrot baits prepared with stem samples of kiwifruit vines inoculated with *C.* lukuohia **F –** *C. lukuohia*structures detected in carrot baits.

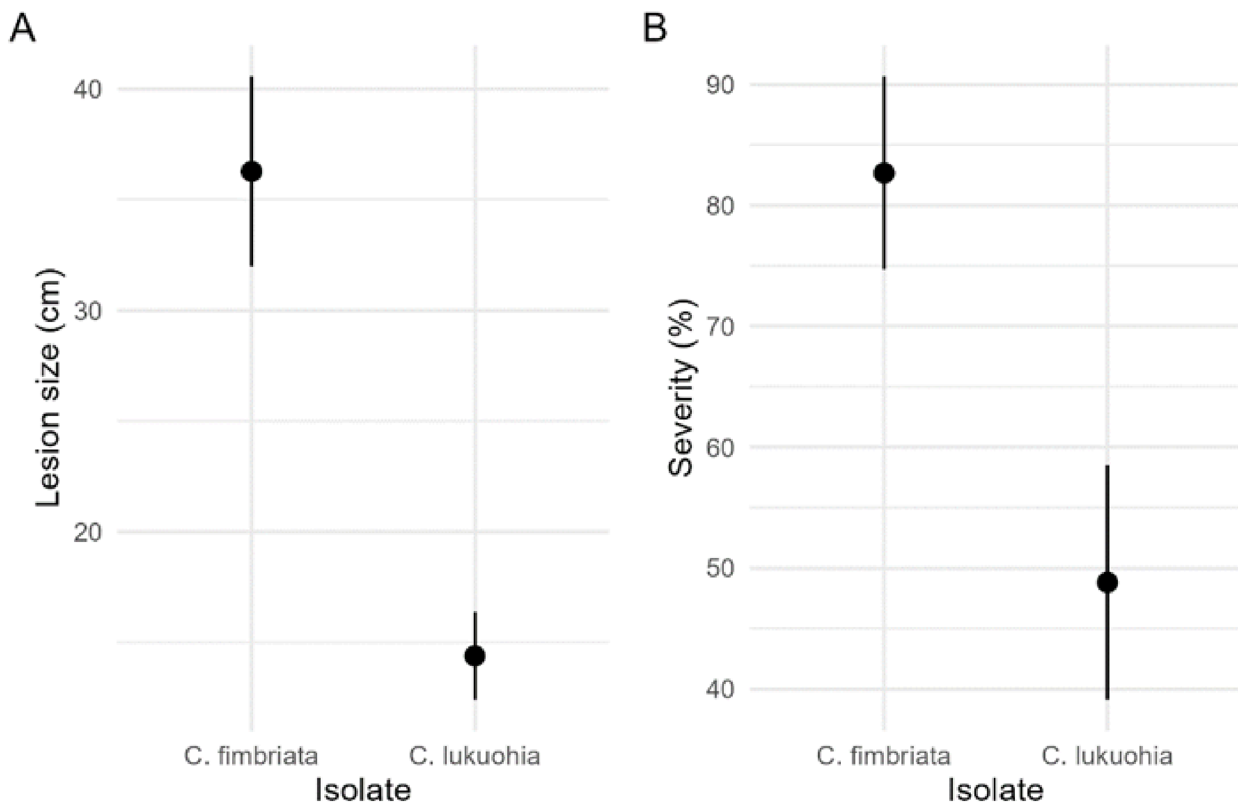

Figure 2

Lesion size and disease severity of kiwifruit vines inoculated with *Ceratocystis fimbriata* and *C. lukuohia*, after 45 days of incubation. **A** Average lesion size (cm) and **B** Average disease severity (%)