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Detection of *Fusarium euwallaceae* and its vector *Euwallacea fornicatus* on pear (*Pyrus communis*) and in deciduous fruit orchards in South Africa

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Abstract
We present the first report of *Fusarium euwallaceae* and its ambrosia beetle vector *Euwallacea fornicatus* in deciduous fruit tree orchards in South Africa. *Fusarium euwallaceae*, is shown to be pathogenic to pear (*Pyrus communis*) and the beetle can establish viable colonies in this host.

Keywords Pathogen · Pest · *Prunus* · *Pyrus* · Polyphagous shot hole borer

Fusarium euwallaceae, the mutualistic fungal nutritional associate of the polyphagous shot hole borer (PSHB; *Euwallacea fornicatus*) beetle (Coleoptera: Curculionidae: Scolytinae) is a pathogen of many woody hosts (Freeman et al. 2013). This fungus/beetle complex is native to Southeast Asia and has been unintentionally introduced to many countries (van Rooyen et al. 2021). In 2017, it was detected in South Africa in a botanical garden in the KwaZulu Natal province (Paap et al. 2018). Since then, it has been found in eight of South Africa's nine provinces. Adult female beetles construct galleries in the sapwood of trees and inoculate the vascular tissues with *F. euwallaceae* that are carried within mandibular mycangia (Stouthamer et al. 2017). The beetle can establish colonies in at least 100 tree species globally, many of which can die because of the boring activities of the beetle and the growth of the fungus into vascular tissues (Anon 2023). *Fusarium euwallaceae* can establish in at least another 300 species even after unsuccessful beetle colony formation. In terms of fruit trees, *F. euwallaceae* has been confirmed as pathogenic to avocado (Jones and Paine 2017), macadamia (Twiddy et al. 2021), almond (Moreno et al. 2018), apple (de Jager and Roets 2022a), nectarine and plum (de Jager and Roets 2023). Beetles can establish viable colonies on all of these (not yet confirmed on macadamia), but infestations with *F. euwallaceae* and PSHB have only been detected in commercial orchards of avocado (South Africa, USA, and Israel). All other reports to date are from urban settings.

In February 2022, a pear orchard (Packham's Triumph cultivar) in Somerset West, Cape Town, South Africa was observed showing symptoms typical of PSHB and *F. euwallaceae* infestations. Small holes of ca. 0.8 mm in diameter oozing plant sap were observed on trunks of some trees (Fig. 1). After bark removal, pinkish vascular streaking originating from galleries in the sapwood was observed, typical for wood colonised by *F. euwallaceae* (Fig. 1). *Fusarium euwallaceae* and PSHB have been isolated from other *Pyrus* species (*Pyrus calleryana*, *Pyrus kawakamii* and *Pyrus malus*), but no reports are known from common pear (*Pyrus communis*) (Mendel et al. 2021). The beetle can reportedly successfully establish colonies in *Pyrus calleryana* (Anon 2023). Determining the susceptibility of pear towards *F. euwallaceae* and PSHB is important since South Africa has a total pear production area of 12 676 ha with an export value of R3.1 billion (Johnson 2022; Department of Agriculture, Land reform and Rural Development 2020). Packham's Triumph accounts for 32% of this (Department of Agriculture, Land reform and Rural Development 2020).

Surveys of pear, apple, plum and grapevine plantings were conducted between February 2022 and June 2023 for possible colonization by PSHB and *F. euwallaceae*.

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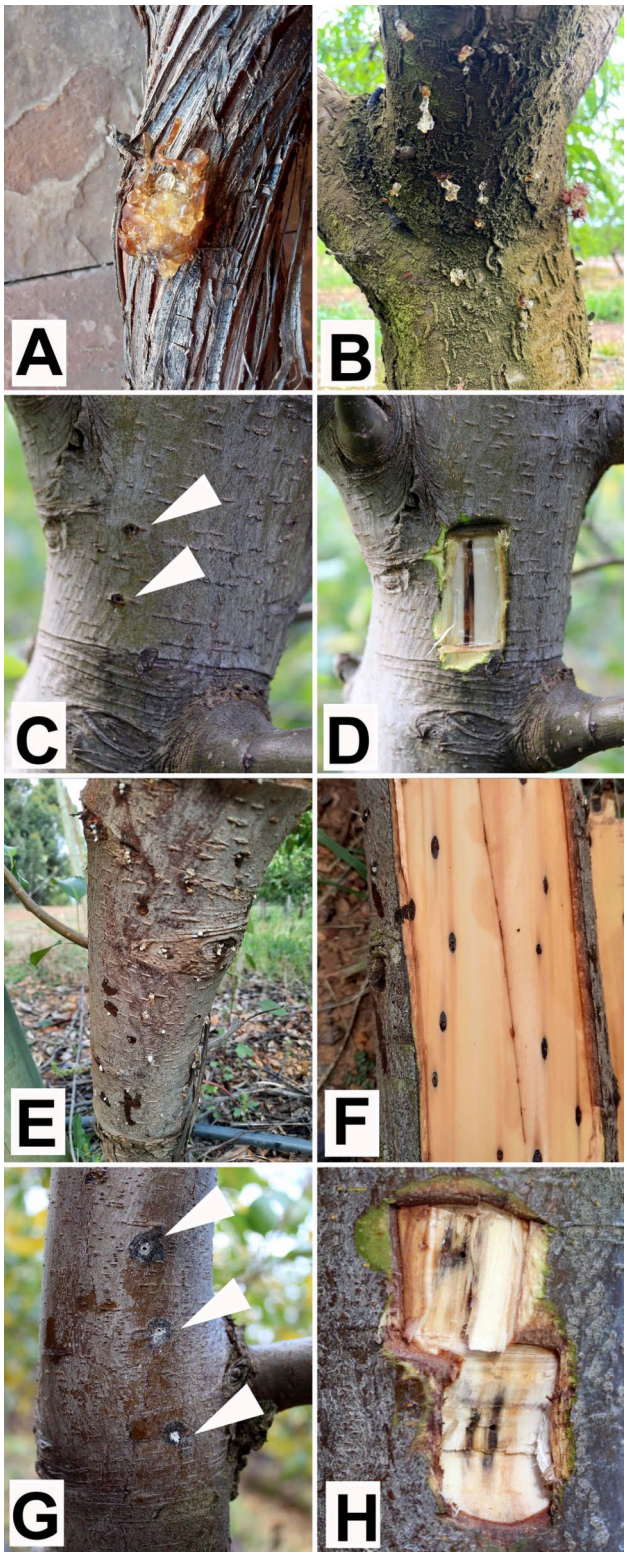

Fig. 1 Symptoms of infestations by the polyphagous shot hole borer beetle (PSHB, Euwallacea fornicatus) in vineyards and fruit orchards **A** Gumming typical of PSHB attack on grapevine (de Jager and Roets 2023). **B** Gumming typical of PSHB attacks on prune (de Jager and Roets 2023). **C** Two entrance wounds (arrows) typical of PSHB attack on apple (de Jager and Roets 2022a). **D** Same, but with bark removed to show the dark fungal streaking in the host woody tissues. **E** Fresh (ca. 2- to 3-month-old) entrance wounds typical of PSHB attack on pear. **F** Bark removed to show dead woody tissues (black spots) and reddish-brown fungal streaking in the host tissues. **G** Older (ca. 6- to 7-month-old) entrance wounds (arrows) of PSHB attack on pear evident by dark areas surrounding entrance holes (dead phloem and cambium tissues) and sugar-rich patches (white crystals). **G** Bark removed to indicate dark fungal streaking

Symptoms typical of infestations were observed in four pear orchards (two consisting of the Packham's Triumph cultivar and two consisting of the Forelle cultivar), one apple orchard (Golden delicious cultivar), two plum orchards and one grape orchard (Cabernet Sauvignon). Infestation levels in all but one orchard were relatively low with three of the pear orchards having less than 20 trees per orchard showing apparent beetle entrance holes (with clear and sugar-rich oozing plant sap), six apple trees showed symptoms (with dark grey oozing sap, de Jager and Roets 2022a), 12 grapevines showed symptoms (with amber-coloured gumming, de Jager and Roets 2022a) and ca. 30 plum trees (with amber-coloured gumming, de Jager and Roets 2023) per orchard showed symptoms (Fig. 1). One of the pear orchards (a seven-year-old Packham's Triumph orchard) displayed more substantial infestations and was the focus of more in-depth monitoring. The number of holes on each of the 8575 individual trees in this orchard was counted. A total of 392 (4%) of all trees were infested with 34 (9%) of these having more than 30 holes (heavy levels of attack), 62% had 10 or fewer holes (low levels of attack) and the remainder had intermediate numbers of holes. Galleries were excavated on one heavily infested pear tree and several living beetle individuals (including larvae) were collected from two gallery systems, but all other gallery systems were seemingly devoid of living individuals. Collected beetles were confirmed as *Euwallacea fornicatus* in morphological comparisons with individuals previously confirmed to be PSHB using DNA sequence data that were collected on the same property.

Samples of wood containing galleries and fungal staining were collected from attacked individuals of all host species and cultivars and taken to the laboratory for fungal identification. Samples of wood from branches on these same trees, but that showed no PSHB activity, were collected as controls. The presence of *F. euwallaceae* within fungus-stained samples from all hosts was confirmed using the rapid detection method of de Jager and Roets (2022b). The fungus was not detected in control samples. Fungal isolation from pear samples followed procedures outlined in de Jager and Roets (2022a). Three pure cultures of colonies resulting from isolations from fungus-stained pear wood (Packham's Triumph cultivar, one isolate per host tree) were subjected to DNA sequencing of the elongation factor 1-α region and the internally transcribed spacer region [(ITS 1 and 2 (partial

sequences), 5.8 S ribosomal RNA gene, and 28 S ribosomal RNA gene (partial sequence)] which were compared to that of the ex-holotype strain (NRRL 54,722) of *F. euwallaceae* (GenBank JQ038007 for the elongation factor 1-α and JQ038014.1 for ITS) following methods of de Jager and Roets (2022a); Freeman et al. (2013). All sequences (elongation factor 1-α GenBank OR545225 — OR545227; ITS GenBank OR704338-OR704340) were identical to the *F. euwallaceae* type isolate (NRRL 54,722) and reference isolates are kept at the Culture Collection of Innovation Africa, South Africa (CMW 58,755—CMW 58,757).

Three fungal isolates (CMWIA 6007—CMWIA 6009) obtained from infested pear trees were used in the pathogenicity testing of *F. euwallaceae* towards pear. Pathogenicity trials followed procedures outlined in de Jager and Roets (2023). Briefly, 10 individuals of each of pear cvs. 'Packham Triumph' and 'Forelle' on Lourensford Estate (-33°35′36″S, 18°55′53″E) were inoculated with the three *F. euwallaceae* isolates in winter (July 2022) using *F. euwallaceae*-colonised toothpicks. Uncolonized toothpicks served as controls. One branch on each tree individual received inoculum from each of the three fungal isolates and the control (following spacing and methods outlined in de Jager and Roets 2022a). Four weeks later, the outer layer of bark of inoculated branches was removed using a sterile knife to expose lesions. Lesion lengths (reddish-brown staining under the bark) were measured (mm) whereafter re-isolations were made to confirm *F. euwallaceae* as the causal organism.

Lesion length data were analysed using R software (version 3.6.3). Data were non-normally distributed (Shapiro–Wilk test, W = 0,94, p < 0.05) and therefore analysed using a generalized linear mixed-effects model fitted to a gamma distribution (*lme4* package, Bates et al. 2014). Treatment, cultivar, and their interaction were used as fixed effects and tree individual was used as a random effect in the full model. Significant main effects were separated using a Tukey test (*multicomp* package, Hothorn et al. 2008).

The full model had an AIC value of 685.2, a BIC value of 709.1 and a deviance of 665.2. The interaction between isolate and cultivar had no significant effect on the model ($Chi^2 = 4.2318$, $df = 3$, P = 0.237). There was no significant difference in lesion lengths between the different cultivars ($Chi^2 = 4.7617$, $df = 4$, P = 0.313). Treatment had a significant effect on lesion length ($Chi^2 = 58.966$, $df = 6$, P < 0.001). Lesions resulting from all isolates were significantly longer than those produced by the controls (Fig. 2). Lesions produced by CMW 58,756 were longer than those of CMW 58,757 but not of CMW 58,755 (Fig. 2). *Fusarium euwallaceae* was consistently reisolated from inoculated wounds but not controls.

We therefore confirm that *F. euwallaceae* is pathogenic to pear and previously also to plum and apple (de Jager and Roets 2022a; Jager and Roets 2023). Its vector beetle PSHB has invaded deciduous fruit orchards in South Africa and was able to transfer *F. euwallaceae* to vascular tissues in pear, apple, plum and grapevine. PSHB is also able to establish breeding colonies in pear, albeit at a seemingly low frequency. Although we have not seen any dieback symptoms on plants, we recommend continuous monitoring of these orchards to inform possible future impacts.

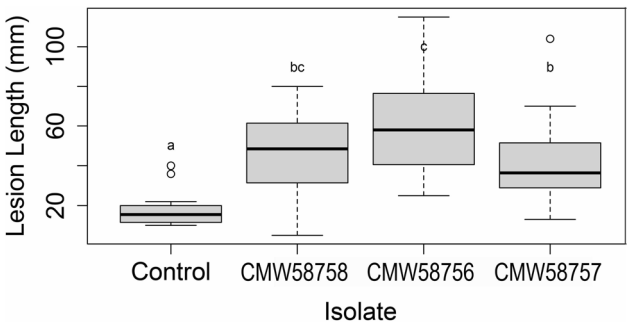

Fig. 2 Lesion length four weeks after incubation of three isolates of *Fusarium euwallaceae* inoculated into pear branches. Boxes indicate 25–75% data range, and whiskers indicate 1.5× the interquartile range. Different letters indicate significant differences in lesion lengths (P < 0.05)

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