



Response to the detection of *Fusarium* dieback associated with ambrosia beetles on *Acer negundo* in New South Wales

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Received: 13 February 2024 / Accepted: 18 July 2024 / Published online: 13 August 2024
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Abstract

Symptoms consistent with polyphagous shot hole borer (*Euwallacea fornicatus*) and *Fusarium* dieback (*Fusarium euwallaceae*), recognised as high priority environmental pests for Australia, were observed on a single box elder (*Acer negundo*) tree in Sydney, New South Wales, in March 2022 during routine forest biosecurity surveillance. Delimiting surveys were undertaken around the Sydney basin, extending radially from the original box elder, resulting in the detection of a further three box elder and one tuckeroo (*Cupaniopsis anacardioides*) with suspicious symptoms. Samples were taken from the five trees and associated beetles diagnosed using morphological and molecular methods. Associated *Fusarium*-like fungi were identified according to phylogenetic inference using regions of the internal transcribed spacer, as well as the translation elongation factor 1- α and RNA polymerase II genes. The tea shot hole borer, *Euwallacea perbrevis*, was identified along with its known fungal mutualist, *Fusarium obliquiseptatum*. This pair have been reported in Queensland on avocado (*Persea americana*) and tuckeroo, and there are unpublished records of *E. perbrevis* from northern NSW, but this is the first report of both species occurring simultaneously in NSW. The biosecurity response following the initial detection, including the establishment of an Incident Management Team, surveillance, diagnostics, and stakeholder engagement, is discussed. This paper highlights the importance of surveillance for early detection of invasive pests and biosecurity systems and processes for enabling a timely response.

Keywords *Euwallacea fornicatus* · *Fusarium obliquiseptatum* · Surveillance · Biosecurity · Invasive

The shot-hole borer beetle species complex, *Euwallacea fornicatus sensu lato* (Coleoptera: Curculionidae: Scolytinae: Xyleborini), is a group of plant pests originating in Asia with a highly successful record of invasion around the world (Smith et al. 2019). The complex includes four cryptic

species which can only be separated using molecular and phylogenetic inference (Smith et al. 2019). Their invasion success is in part due to their extensive host range; collectively, species within the complex have been recorded from more than 412 plant species across 75 families (Gomez et al. 2019).

Euwallacea species have symbiotic relationships with a range of fungi carried within specialised organs known as mycangia (Batra 1963) and cultivated as food sources within galleries in host tree xylem. Many of these fungal symbionts are in the genus *Fusarium*, some of which can be pathogenic and cause wilting in successfully colonised trees, contributing to dieback and death. Whilst tree mortality is rarely observed in the native range of *Euwallacea*-symbiont complexes, it is common in naïve hosts, posing a serious threat to horticulture, urban amenity, and native environments of invaded territories (Hulcr and Dunn 2011; O'Donnell et al. 2016). Furthermore, tree hosts can be categorised as reproductive and non-reproductive. Reproductive

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hosts are the most concerning from a biosecurity standpoint, supporting recurring generations of beetles (Mendel et al. 2021).

Although in their native ranges *Euwallacea* species partner with multiple fungal symbionts, in invaded territories they are often found with only one or two (Mendel et al. 2021). The Ambrosia *Fusarium* clade (AFC), within *Fusarium solani sensu lato*, is one of the primary fungal symbiont groups of *Euwallacea* ambrosia beetles (Kasson et al. 2013; O'Donnell et al. 2015). The AFC includes at least 19 phylogenetically unique species; 12 are formally named, with the remaining taxa denoted by “AF” numbers (Kasson et al. 2013; Lynn et al. 2021).

The most invasive and damaging *Euwallacea* species include *E. fornicatus* (Eichhoff), the polyphagous shot hole borer (PSHB), *E. perbrevis* (Schedl), the tea shot hole borer (TSHB), and *E. kuroshio*, the Kuroshio shot hole borer (KSHB). PSHB is notorious for the substantial damage caused to agricultural production and amenity trees in Israel, United States and South Africa in recent decades (Eskalen et al. 2012; Mendel et al. 2012; Freeman et al. 2013; Paap et al. 2018; Van Rooyen et al. 2021). TSHB, coupled with fungal mutualists *F. ambrosium* and *F. reka-num*, is an invasive pest on avocado (*Persea americana*) trees in Florida (United States) (Carrillo et al. 2016). It has also been reported in Hawaii, Costa Rica, and Panama (Smith et al. 2019), and, coupled with *F. obliquiseptatum*, also known from Queensland, Australia (O'Donnell et al. 2015; Aoki et al. 2019). KSHB has been reported on various woody hosts including avocado, California sycamore (*Platanus racemose*) and other woody hosts in the United States, Taiwan and elsewhere (Na et al. 2018; Smith et al. 2019; Jiang et al. 2021). However, ascertaining the true spread of individual *Euwallacea* species is challenging

due to the taxonomic confusion that existed until recently (Smith et al. 2019; Rugman-Jones et al. 2020), and because they are rarely noticed until they become a problem (Lynn et al. 2021).

Fusarium euwallaceae and its ambrosia beetle vector, PSHB, are considered high-risk biosecurity threats because of the high likelihood of their entry, establishment and potential for substantial economic and environmental impact in new regions (Gomez and Johnson 2019). *Euwallacea fornicatus* was identified as a risk of establishing in Australia based on trait similarity analysis (Nahrung and Carnegie 2022), and the *F. euwallaceae*-PSHB complex is on Australia's National Priority List of Exotic Environmental Pests, Weeds and Diseases (DAFF, 2020) and a high priority threat in the Acacia Biosecurity Plan (Plant Health Australia unpublished).

PSHB and *Fusarium* dieback were detected in Perth, Western Australia, in August 2021 (Cook and Broughton 2023). The Western Australia Department of Primary Industries and Regional Development (DPIRD) mounted a response which included delimiting surveillance, implementation of containment strategies, and stakeholder engagement, with the aim of eradicating this invasive pest (DPIRD 2023). Following this detection, surveillance of key hosts began in Sydney, New South Wales (NSW) in late 2021; an expansion on the annual forest biosecurity surveillance program (Carnegie et al. 2018). This primarily focused on box elder (*Acer negundo*), a recognised reproductive ‘magnifier’ host in Perth and overseas (Mendel et al. 2017, 2021), but also *Platanus* and other known key hosts.

On 14 March 2022 symptoms of PSHB, including upper-branch dieback with insect frass (Fig. 1), were detected on a single box elder tree in Artarmon, Sydney. Closer examination revealed galleries typical of ambrosia beetles and



Fig. 1 Symptoms of tea shot hole borer (*Euwallaceae perbrevis*) and fusarium dieback (*Fusarium obliquiseptatum*) on box elder (*Acer negundo*) in Sydney, New South Wales

identical to those of PSHB (Fig. 1). The NSW Chief Plant Protection Officer was notified within 24 h, and an Incident Management Team was established on 16 March. Initial discussions were held with Willoughby Local Council staff on potential tree removal and the location of other box elder trees in the area. Delimiting surveys were conducted in the Sydney basin over a three-week period (Fig. 2), extending radially from the original tree and covering more than 1,100 trees, including approximately 800 box elders and other known hosts of PSHB/TSBH such as tuckeroo (*Cupaniopsis anacardioides*), London plane (*Platanus x acerifolia*), and oak (*Quercus*) trees (Fig. 2). A further three box elder and a single tuckeroo were found with suspicious symptoms and subsequently sampled.

Identification of adult beetles was undertaken at Biossecurity Collections Unit (BCU) (Orange, NSW). Using morphological characters and the USDA Lucid key to SE Asian Ambrosia beetles (<https://idtools.org/tools/1065/index.cfm>) (Smith et al. 2022) the specimens were verified as *Euwallacea*. The ability to reliably identify *Euwallacea* species morphologically is limited (Gomez et al. 2018; Smith et al. 2019), therefore molecular analysis of seven

beetle specimens was conducted using excised legs for species identification. The 5' region of the mitochondrial cytochrome c oxidase I (COI) gene was targeted for PCR amplification using primer pairs LCO1490 with HCO2198 (Folmer et al. 1994). Amplification yielded 658 bp fragments from each of the seven specimens which identified 99.8 to 100% to *E. perbrevis* when queried to NCBI & BOLD identification databases. Sequences obtained were deposited at BOLD (Accession numbers OAIBC060-23 to OAIBC066-23).

As a precaution, and due to previously ambiguous nature of the identity of *E. fornicatus*/*E. perbrevis*, we additionally asked colleagues at DPIRD (Western Australia) to compare our sequences with theirs: they eliminated our sequence as *E. fornicatus* and concluded the identity of our sequences as *E. perbrevis* (M. Kehoe pers. comm.). Furthermore, our sequences matched those of *E. perbrevis* from south-east Queensland (Nahrung et al. in prep.). The sequenced beetle specimens are vouchered in BCU (ASCT00237535, ASCT00237536, ASCT00238073, ASCT00238094, ASCT00238095, ASCT00240756, ASCT00178610).

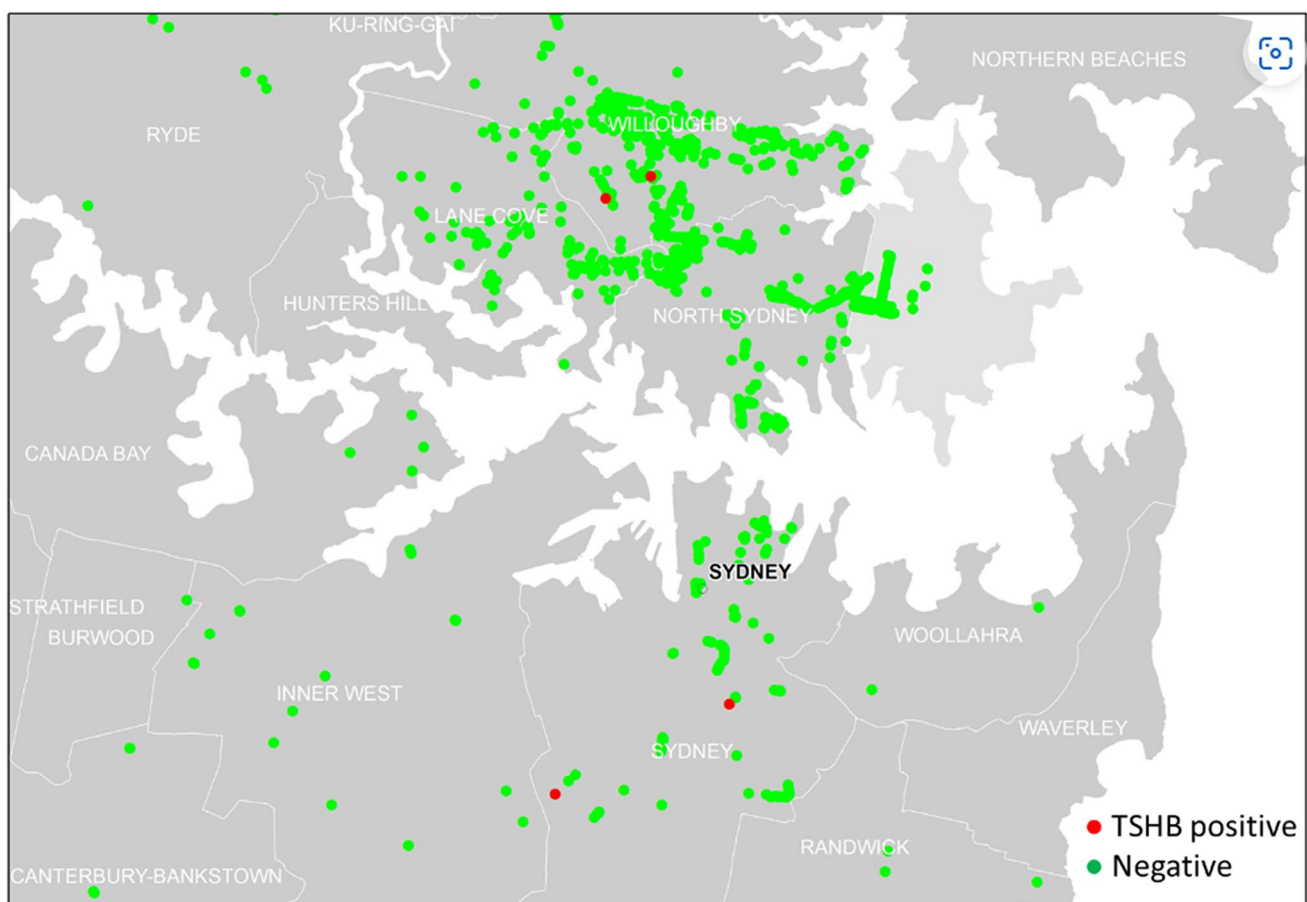


Fig. 2 Surveillance for tea shot hole borer and fusarium dieback in Sydney, New South Wales. Green (host tree, negative), red (positive *Acer negundo*)

Identification of fungi was undertaken by NSW DPI at the Elizabeth Macarthur Agricultural Institute. Potential symbiont and pathogenic fungi were recovered by plating different materials from the branch samples including the wood adjacent to beetle galleries, fungal hyphae found growing within the tunnels, and beetles and larvae. Wood pieces from within or adjacent to tunnels were excised, cut into pieces of approximately 3 mm². Fungal mycelium was scraped out with sterile needles and dotted directly onto plates. Beetles and larvae were also plated directly or sometimes cut in half before plating. All materials were plated onto ¼ strength potato dextrose agar (Difco™) amended with novobiocin. Plates were grown in darkness at 23 °C and checked daily from three days after plating. *Fusarium*-like growth was targeted due to the known association of *Euwallacea* species with *Fusarium* species and the occurrence of fusarium dieback. *Fusarium*-like growth was subcultured and species showing morphological resemblance to *F. solani* (Leslie and Summerell 2006) were selected for molecular and phylogenetic identification.

Isolates underwent PCR and sequencing of a region of the translation elongation factor 1- α (EF-1 α) gene using the primers set EF1/EF2, the internal transcribed spacer (ITS) using ITS5/ITS4, and RNA polymerase II gene (RPB2) using the primer pairs 5F2/7CR (RPB2-1) and 7CF/11AR (RPB2-2) and the PCR conditions described in White et al. (1990), Carbone and Kohn (1999), and Moretti et al. (2018), respectively. PCR amplicons were purified and sent to the Australian Genome Sequencing Facility (AGRF, Sydney) for sequencing. Isolate identity was confirmed through phylogenetic analysis using Geneious Prime Version 2021.2.2. Reverse and forward sequences were aligned to form consensus sequences using de novo assembly with default settings. The alignments were edited manually with reference to the chromatograms. Reference sequences were downloaded from relevant studies (Jiang et al. 2021) and these were included in the analyses. All sequences were aligned using the MAFFT sequence alignment function (Katoh et al. 2002; Katoh and Standley 2013) and gaps were stripped from the alignment. Initially, single-locus trees based on the EF-1 α sequences were generated using Randomized Accelerated Maximum Likelihood (RAxML) model (Stamatakis 2014), and a Neighbour-Joining model using the Geneious tree builder function and the Tamura-Nei genetic distance model (Tamura and Nei 1993) with 1000 bootstrap replicates. The EF-1 α , ITS, and RPB2 datasets were concatenated, aligned using MAFFT, had any gaps stripped, and a RAxML multi-loci tree was generated with 1000 bootstrap replicates. Following identification, cultures obtained in this study were submitted to the NSW mycology herbarium under DAR85933, 85,934, 85,939, and 85,947. Sequences

were submitted to Genbank under PP765794-96 and PP758983.

Two species of *Fusarium* were identified from samples. *Fusarium obliquiseptatum* (AF7) was identified (Fig. 3) from three out of four of the box elder trees, recovered from dark stained wood adjacent to beetle galleries, from a beetle and from hyphae growing within tunnels. *Fusarium metavorans*, which is in the FSSC but not the AFC, was recovered from two of the four box elder trees as well as the tuckeroo. It was recovered from white hyphae growing in tunnels, black pencil-like lines in bark, larvae and a beetle in the galleries and wedge-shaped discoloration in the wood. The tea shot hole borer, *E. perbrevis*, was recovered from three of the four box elder trees, but not from the tuckeroo. We considered the four box elder trees positive for fusarium dieback even though of these four, one tree (Erskineville) had *F. obliquiseptatum* and no TSHB and one (Naremburn) had TSHB and no *F. obliquiseptatum* because *E. perbrevis* and *F. obliquiseptatum* are known symbionts, and because symptoms were consistent across all four box elder trees. The tuckeroo was deemed negative because the tunnels were not consistent (too large) with *Euwallacea* colonisation and no *Euwallacea* were found.

Within one week of the first detection, the *E. perbrevis* and *F. obliquiseptatum* were identified to species level and the results were reported to the national Consultative Committee on Emergency Plant Pests (CCEPP). Initial plans were developed to remove and destroy the infested trees under biosecure procedures, including deep burial, but these were not progressed. The CCEPP notified relevant industry parties, informing them of the detection and that *E. perbrevis* and *F. obliquiseptatum* are not listed as Emergency Plant Pests under schedule 13 of the Emergency Plant Pest Response Deed and are not listed as a High Priority Pests in any Industry Biosecurity Plan. The notification stated that given this was an extension of geographic and host range of a pest already recorded as present in Australia and not under official control, eradication was unnecessary and unfeasible. Further, the likely pathway of the pest into Sydney was unknown and therefore uncontrollable. Industry parties were given the chance to “propose an alternative plan” but did not. Surveillance and monitoring will continue.

This is the first report of *E. perbrevis* and *F. obliquiseptatum* occurring simultaneously in NSW, Australia. This pair has been reported previously in Queensland (O'Donnell et al. 2015; Aoki et al. 2019), where they were causing limited damage to avocados but more serious damage to a limited number of tuckeroo trees (Nahrung et al. in prep.), and there are unpublished records of *E. perbrevis* from northern NSW. So far, *E. perbrevis* has been found to partner with at least eight members of the AFC (*F. ambrosium* (AF-1), AF-6, *F. obliquiseptatum* (AF-7), AF-8, AF-13, AF-14, AF-17,



Fig. 3 Phylogenetic tree (based on concatenated EF-1α, ITS, and RPB2 sequence regions) showing placement of *Fusarium obliquiseptatum* isolates (bold) from *Acer negundo* in Sydney, New South Wales

AF-18) causing different levels of damage to a variety of hosts (Carrillo et al. 2019; Lynn et al. 2020; Jiang et al. 2021; Kendra et al. 2023). However, due to taxonomic uncertainty prior to the molecular characterisation of the species (Smith et al. 2019), it is likely there are more pairings than those recorded here. *Fusarium obliqueseptatum* has only previously been reported from Queensland (O'Donnell et al. 2015; Aoki et al. 2019).

A second *Fusarium* species occurred in some samples. *Fusarium metavorans* (FSSC-6) is in clade three of the *F. solani* species complex and was formally named and described in 2018 (Al-Hatmi et al. 2018). During the surveillance for PSHB/TSHB, this species was recovered from the tuckeroo with larger tunnels, as well as some of the box elders including trees that were also positive for TSHB and *F. obliqueseptatum*. *Fusarium metavorans* is generally described as a cosmopolitan saprobe which has been recovered from a diversity of substrates including humans, plants and soil, however, it has also been recovered from the guts and larvae of the wood-boring cerambycid *Anoplophora glabripennis* (Geib et al. 2012; Scully et al. 2012; Herr et al. 2016). Its significance here is unknown and might be worth investigating given its reoccurrence across samples and its known ability to breakdown lignocellulose (Al-Hatmi et al. 2018).

Although TSHB does not appear to be causing significant damage to box elder in Sydney, its presence is not without risks and implications. Firstly, in Queensland, TSHB and *F. obliqueseptatum* have been associated with some serious damage to a small number of tuckeroo (Nahrung et al. in prep.). Also, due to the taxonomic uncertainty surrounding the *Euwallacea* group, the true host range of TSHB is unknown, and thus its potential to cause economic and ecological damage in Australia is undefined. In addition, recent research on members of the *E. fornicatus* complex has showed that the relationship between the beetle species and the ambrosia fungi they cultivate is likely more diverse and promiscuous than previously described (O'Donnell et al. 2015; Carrillo et al. 2019, 2020). This suggests there is the potential for TSHB to partner with more aggressive native or introduced fungi, which could result in greater damage to host species. For example, TSHB partnered with different AFC species causes damage to avocado (Carrillo et al. 2016; Kendra et al. 2023) and macadamia (*Macadamia integrifolia*) (Mitchell and Maddox 2010; Rugman-Jones et al. 2020) overseas. These are two economically important crop species for Australia. Invasive populations of *Euwallacea* have been found to partner with fungi they are not typically found within their native areas (e.g., Rugman-Jones et al. 2020), making it nigh on impossible to predict potential new pairings and host shifts.

The risk of PSHB arriving from Perth, or from overseas, into eastern Australia is ongoing and thus continuing surveillance and extension for both TSHB and PSHB is critical (given the symptoms they cause are indistinguishable). Careful diagnostics of infected samples is also important given the co-infection of multiple *Euwallacea* species and mutualist fungi in single trees is not uncommon (Stouthamer et al. 2017; Rugman-Jones et al. 2020). Current extension work includes awareness workshops for local councils and arborists alerting them to the symptoms of PSHB, as well as the production of fact sheets (<https://www.dpi.nsw.gov.au/forestry/science/forest-health/protect-what-we-love>). New fact sheets and information sources should include mention of TSHB.

Since the detection in March 2022, TSHB has been detected on three other occasions in Sydney: caught in a single sticky trap with a *Euwallacea* species lure in Centennial Park, Randwick (February 2023) during DPI surveillance, and from dieback on *Ficus macrophylla* (August 2023) and *Acer paxii* (April 2024) reported by garden staff in the Royal Botanic Garden, Sydney. Infested trees from the original detections have shown no significant increase in damage.

This case illustrates the benefits of routine surveillance resulting in early detections and rapid biosecurity responses.

Acknowledgements We would like to thank the Plant Health Diagnostic Service team at Elizabeth Macarthur Agriculture Institute for performing diagnostic tests on samples. Also thanks to Helen Nahrung and Geoff Pegg for reviewing the manuscript. Thanks to Conrad Trolip for help with handling sequence data.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions.

Declarations

Ethical approval Not applicable.

Conflict of interest The authors declare no conflict of interest.

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