Impacts and trapping of ambrosia beetles *Euwallacea fornicatus* and *E. similis* in *Acacia* plantations in Vietnam

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There are over two million hectares of *Acacia* plantations in Vietnam and loss from infestations of ambrosia beetles is of increasing concern. In this study, we first determined the key taxa and the extent of damage, and then identified effective trap and lure combinations for trapping adult beetles, and finally quantified the seasonality of flight of *Euwallacea* for informing pest management decisions. Based on analysis of the mitochondrial cytochrome c oxidase subunit I gene, the greatest threats were confirmed as *Euwallacea fornicatus* and *E. similis* (Coleoptera: Curculionidae), which are native to Southeast Asia but known as invasive species in South Africa and elsewhere. The damage incidence of *Euwallacea* ranged from 16.7% to 34.9% in *Acacia* plantations throughout Vietnam. There were no significant differences in the numbers of beetles captured between the three trap types (plastic bottles, funnels and panels). Ethanol and quercivorol were more effective as lures than cubeb oil, α-pinene or a distilled water control. Trapping from June 2020 to May 2021 revealed a bimodal frequency of *E. fornicatus* and *E. similis* in *A. mangium* and *A. hybrid* plantations, with captures peaking in April (spring) and October (autumn). These findings can be applied to develop early detection and trapping control programmes for *Euwallacea* in *Acacia* plantations in Vietnam and elsewhere.

Keywords: *Acacia* hybrid, *Acacia mangium*, COI gene, flight season, invasive species, polyphagous shot-hole borer, traps and lures, Xyleborini

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Introduction

There are more than two million hectares of *Acacia* in Vietnam (ACIAR 2020; Thu et al. 2021), making up nearly half of the country’s total forest plantation estate (FAO 2020; MARD 2020). *Acacia* plantations provide large-scale material for the processing and timber industries for domestic and export markets. The export of all wood products was worth US$13.2 billion in 2020, largely from *Acacia* plantations (Socialist Republic of Vietnam 2021). However, *Acacia* plantations in Vietnam are vulnerable to damage from insect pests and fungal pathogens (Thu et al. 2021). *Euwallacea fornicatus* (polyphagous shot-hole borer) was recorded in *Acacia* hybrid plantations in 2013 (Stouthamer et al. 2017) and since has spread into *A. auriculiformis* and *A. mangium* plantations throughout Vietnam (Thu et al. 2021). More recently, *Euwallacea similis* was found on *Ricinus communis* (Stouthamer et al. 2017) and captured in *A. mangium* plantations (Smith et al. 2018) in Vietnam. While this pest has attacked *A. crassicarpa* in Indonesia (Lynn et al. 2021), so far it has not been reported to damage *Acacia* in Vietnam.

A number of ambrosia beetles and their associated fungal symbionts (Hulcr and Stelinski 2017) have become biosecurity threats globally (Grousset et al. 2020). One of these species is *E. fornicatus*, which has spread widely outside its native range in Southeast Asia. It infests a wide range of hosts (Gomez et al. 2019), including amenity trees (Paap et al. 2018; EPPO 2019), native forests, plantation forests and fruit trees (Mendel et al. 2012; Eskalen et al. 2013; Freeman et al. 2013). *Euwallacea similis* is native in Asia and some Oceania countries, such as Australia, Papua New Guinea and Solomon Islands (Wood and Bright 1992), and it has dispersed to parts of Africa (notably South Africa), the United States and South America (Rabaglia et al. 2006; Wood 2007).

A range of traps and lures have been employed to capture ambrosia beetles for the purposes of early detection and pest monitoring (Reding et al. 2011; Kendra et al. 2020). In particular, ethanol, quercivorol, α-copaene and α-pinene have been used as lures with Lindgren and cross-vane traps to monitor ambrosia beetles (Miller and Rabaglia 2009; Dodge et al. 2017; Kendra et al. 2017; Byers et al. 2020). However, the effectiveness of the lure type can vary with the ambrosia beetle species. Ethanol was most effective for xylosebinic ambrosia beetles (Oliver and Mannion 2001; Miller and Rabaglia 2009) and kairomones were effective in the capture of *E. perbrevis* (Clade TSHBa) in avocado orchards in Florida (Byers et al. 2017). In addition, in a study in Israel, the trapping efficiency of *E. fornicatus* increased with quercivorol or a quercivorol + ethanol combination when compared with ethanol (Carrillo et al. 2015).

Thu et al. (2010) conducted trapping using cross-vane traps and an α-pinene ultra-high-release lure (Synergy Semiochemicals Corporation, British Columbia, Canada)
and captured more than 500 bark beetles (Dryocoetes villosus) in 2 months in Pinus plantations in Vietnam. Smith et al. (2018) used funnel traps with ethanol, quercivorol, α-copaene or cubeb oil to capture xyleborine beetles, mostly species of Euwallacea et al. (2018) used funnel traps with ethanol, quercivorol, Dryocoetes lure trapping has not been undertaken for long-term monitoring of Euwallacea in Acacia plantations in Vietnam.

To determine which ambrosia beetle species are causing severe borer damage to Acacia plantations in Vietnam, we undertook research to identify the most-efficient lures and traps to employ. Furthermore, as the seasonal flight of ambrosia beetles varies greatly between geographical regions, we also determined the seasonal flight activity of the main species present over a year of trapping. For these objectives, we first identified the main ambrosia beetle pest species present in Acacia plantations throughout Vietnam and then assessed the extent of damage. The findings will assist in developing a plan for ambrosia beetle management in Acacia plantations in Vietnam.

Materials and methods

Identification of Euwallacea species
To determine which Euwallacea species are causing severe borer damage to Acacia plantations in Vietnam, 14 infested Acacia plantations, ranging from 3.8 to 6.2 years in age, were selected from 12 provinces having the largest area of Acacia plantations in Vietnam (Figure 1). Next, 10 severely damaged trees (> 30 entry holes per 1 000 cm² surface area of the bole) were randomly chosen in each plantation and then felled using a hand saw, in June to August 2019. The boles were cut into 0.5 m length logs and moved for dissection at the laboratory of the Forest Protection Research Centre (FPRC) in Hanoi. Insects located within the wood were preserved in 90% ethanol.

Adult beetles were sorted and examined using a Leica M165C (Leica Corporation, Japan) light microscope and Nikon V1.18 software (Nikon Corporation, Japan). As only species of Euwallacea were found, the taxa were tentatively identified using morphological features of the body, pronotum and head in the Xyleborini ambrosia beetles key (Hulcr and Smith 2010) and the descriptions of Euwallacea species (Gomez et al. 2018).

Twenty-six specimens collected from dissected boles in separate geographical locations were used for phylogenetic analysis to confirm the identity of the taxa (Table 1). DNA was extracted from the legs using the prepGEM™ Universal DNA extraction kit (Zygem, International (Pty) Ltd), following the manufacturer’s protocol. The extracted DNA was subjected to PCR amplification of the mitochondrial cytochrome oxidase c subunit I gene (COI), using the forward primer LCO1490 (5'-GGTCAACAAAATCATAAAGATTATGG-3') and reverse primer HCO2198 (5'-TAAACTTCAAGGGT GACCAAAAATCA-3') (Folmer et al. 1994). Each PCR reaction mixture consisted of 12.5 µl of GoTaq® Green Mastermix (Pomega, USA), 7.5 µl of sterile DNase-free water, 1 µl of 10 nM stock of both forward and reverse primers, and 4 µl of DNA template, for a 25 µl total reaction mixture. PCR amplification was carried out on a C1000 Touch™ thermal cycler (Bio-Rad, Hercules, California) with a program following the method of Stouthamer et al. (2017). Amplification was confirmed by 2 µl of PCR product and releasing the fragments on a 1% agarose gel stained with 2 µl of GelRed™ nucleic acid gel (Biotium, Hayward, California), followed by visualisation under UV light.

The PCR products were purified using ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific, Massachusetts) and sent to the First BASE Laboratory Sdn Bhd (Selangor, Malaysia) for Sanger sequencing. All nucleotide sequences obtained were viewed and manually edited using Geneious R 7.1.8 for non-commercial use (Biomatters Ltd, Auckland, New Zealand). All the sequences in this study were submitted under GenBank numbers OL704761–OL704786. The detail of sequences obtained in this study and the existing GenBank accession numbers are given in Figure 2 and Supplementary Table S1. The sequences were aligned using the multiple alignment sequence program MAFFT version 7 (Kato et al. 2019), and then evaluated using MEGA 7 software (Kumar et al. 2016). A phylogenetic tree was constructed using maximum-likelihood (ML) based on the selected Hasegawa–Kishino–Yano (HYK) model, invariant sites (I) (Hasegawa et al. 1985) with bootstrapping analyses of 1 000 random replications in MEGA 7 software. The resulting tree was viewed using MEGA 7 and edited using PowerPoint. COI sequences of Ambrosiophilus atratus (HM064120 and MK993013) were used as the outgroup.

Assessing damage caused by Euwallacea
The 14 plantations described in the previous section were used to quantify damage caused by Euwallacea. Ten field plots, each 500 m² (20 × 25 m), were laid out randomly in each plantation, in June 2019 (Locations 1–8) or August 2019 (Locations 9–14). Each plot contained 70–80 trees that had been planted at a density of 1 660 trees ha⁻¹, with 3 m spacing between rows and 2 m spacing within rows; the plots were at least 30 m from plantation edges and were at least 30 m from each other. We recorded data from all trees in the plots, including tree status (healthy, weak, or dead) and the density of active holes (resin exudation, fresh frass, or no outer bark cover). The active holes on stems were recorded from 0.5 to 1.5 m above the ground using glazed paper (232 cm² total area) and a marking pen, following the method of Coleman et al. (2019).

The stem circumference was measured using a measuring tape at 0.5 m and at 1.5 m above the ground. The bark area was calculated as a truncated cone using the formula:

\[ S = \pi \times (R_1 + R_2) \times h \]

where \( S \) is the surface of bark area; \( R_1 \) is tree radius at 0.5 m; \( R_2 \) is tree radius at 1.5 m; and \( h \) is tree slant height. For assessing the damage severity at different levels (nil, low, medium, high) with the uniform factor of tree diameter, the density of active holes was calculated per 1 000 cm² of bark area. The damage index (DI) was then determined in four categories as follows: 0 = tree with no active holes; 1 = tree with 1–10 active holes per 1 000 cm²; 2 = tree...
**Figure 1**: Location of Acacia plantation study sites in Vietnam (province names are given in Table 1)

**Table 1**: Specimens of *Euwallacea fornicatus* and *E. similis* from Acacia plantations in Vietnam that were used for phylogenetic analysis

| Host          | Location: district (province) | Map reference (Figure 1) | Specimen E. fornicatus | GenBank accession | E. similis | GenBank accession |
|---------------|------------------------------|--------------------------|------------------------|-------------------|------------|-------------------|
| *Acacia auriculiformis* | Cam Lo (Quang Tri) | 9 | VN45 | OL704781 | – | – |
|                | Song May (Dong Nai)        | 13 | VN67 | OL704782 | – | – |
| *Acacia mangium*     | Yen Binh (Yen Bai)       | 2 | VN84 | OL704784 | VN12 | OL704763 |
|                        |                            | VN18 | OL704769 |           |            |        |
|                        |                            | VN19 | OL704772 |           |            |        |
|                        |                            | VN28 | OL704770 |           |            |        |
|                        | Tuyen Quang city (Tuyen Quang) | 1 | VN38 | OL704786 | – | – |
|                        | Dong Hy (Thai Nguyen)      | 3 | VN100 | OL704783 | – | – |
|                        |                            | VN216 | OL704780 |           |            |        |
|                        | Doan Hung (Phu Tho)        | 4 | VN206 | OL704785 | VN212 | OL704768 |
| *Acacia hybrid*       | Yen The (Bac Giang)        | 6 | VN213 | OL704776 | – | – |
|                        | Thanh Ba (Phu Tho)         | 5 | VN214 | OL704778 | VN200 | OL704765 |
|                        | Tan Lac (Hoa Binh)         | 7 | VN27 | OL704773 | VN202 | OL704766 |
|                        | Do Luong (Nghe An)         | 8 | VN31 | OL704771 | VN85 | OL704762 |
|                        | Huong Tra (Thu Thien Hue)  | 10 | VN215 | OL704777 | – | – |
|                        | M’Drak (Dak Lak)           | 11 | VN211 | OL704775 | VN210 | OL704767 |
|                        | Xuan Loc (Dong Nai)        | 12 | VN115 | OL704779 | VN13 | OL704764 |
|                        | U Minh (Ca Mau)            | 14 | VN95 | OL704774 | VN32 | OL704761 |

* These are natural hybrids that have been commercialised. Plantations at map references 5, 6, 7, 8, 10 and 11 were *Acacia mangium* × *A. auriculiformis* hybrids (Clones BV10 and BV16), and at 12 and 14 were *A. auriculiformis* × *A. mangium* hybrids (Clone AH7).

*E. fornicatus* = *Euwallacea fornicatus*  
*E. similis* = *Euwallacea similis*
with 11–30 holes per 1 000 cm²; 3 = tree with > 30 holes per 1 000 cm². Since the trees were not dissected we did not know what species of *Euwallacea* were present in the boles, and hence the data are reported at the genus level.

From the recorded number of trees attacked by beetles, the average damage incidence for 10 plots in each plantation was calculated as:

\[
P\% = \left( \frac{n}{N} \right) \times 100
\]

(2)

where \( n \) = the number of trees infected at damage index \( i \); \( v_i \) = the damage index at level \( i \); and \( N \) = total number of plants assessed. The data of \( P\% \) and DI in each plantation were calculated using MS Excel version 2007.

**Trap and lure combinations for monitoring Euwallacea species**

To identify effective lures and traps for monitoring *Euwallacea* species in Acacia plantations, two trapping experiments were undertaken. A randomised block split-plot design was used in which five lure treatments were employed in sub-plots randomised within main plots for the three trap types. The main plots were randomised within each of four replicate blocks (Supplementary Figure S1). The blocks were separated by 20 m buffers, the main plots were separated by 15 m buffers, and the sub-plots were separated by 10 m buffers. One trap plus
lure was placed in the centre of each 24 m² (6 × 4 m) plot. The three trap treatments were a self-made plastic bottle trap, Lindgren funnel trap (Bio Quip, California) and cross-vane panel trap (Bio Quip, California). There were five lure treatments: 90% ethanol, quercivorol (1S,4R)-p-menth-2-en-1-ol (Synergy Semiochemicals Corporation, British Columbia, Canada), cubeb oil (Synergy Semiochemicals Corporation), α-pinene (Synergy Semiochemicals Corporation) and a distilled water control. The experiments were undertaken from March to May in 2020. This trapping season is spring in Vietnam, which is recommended as the most suitable time for capture of *Euwallacea* spp. (Byers et al. 2017). One experiment was in a 4-year-old *A. mangium* plantation in Phu Tho province (5.5 ha, location 4: Figure 1), and the other was in an 8-year-old *Acacia* hybrid plantation (6.2 ha, location 5: Figure 1). The stands selected had 25–30% infested trees with active holes. The collection cups in each trap were filled with propylene glycol and water (50:50 by volume) to produce an agglutination mix to hold the beetles in the container. The mix was replaced every 10 days and the old mix with trapped insects was taken to the laboratory for processing. The lures were placed inside unsealed plastic bags. The bags were attached underneath near the top of the bottle and panel traps, and to the middle funnel of the Lindgren funnel traps. All lures, including the distilled water control, were replaced monthly.

The data of beetle trapping for the trap and lure combination were log-transformed before conducting an analysis of variance (ANOVA) of the split-plot design in R version 4.2.1 (R Core Team 2022). Significant effects of trap and lure in each trapping site were analysed with two-way ANOVA, followed by Duncan’s multiple-range test for comparisons of means. The ANOVA showed that lure was the only factor affecting the number of captured beetles in each trapping site. Hence, further analysis was conducted to assess various factors, including trapping site on the trapping number.

The influence of factors (traps, lures, trapping sites) on the effectiveness of beetle capture was determined by fitting generalised linear models using the *glm* function in R version 4.2.1 (R Core Team 2022). The response variable used for model construction was discrete data for the number of captured beetles over three months. The candidate explanatory variables of the model were traps (plastic bottle trap, Lindgren funnel trap, cross-vane panel trap), lures (ethanol, quercivorol, cubeb oil, α-pinene, distilled water), and trapping sites (*A. mangium* or *A. hybrid plantations*). The interaction terms among trap, lure and trapping site were included in the model predicting the number of captured beetles. As the response variable followed a Poisson distribution, we set the link function of the model as a log link. A set of candidate models was constructed with combinations of explanatory variables, and the best-fit model was chosen with the lowest Akaike information criterion (AIC).

**Seasonal flight pattern of *Euwallacea* species**

To identify the temporal pattern of *Euwallacea* flights, a 12-month trapping experiment was established in each of the two plantations used for the trap × lure experiments described above. We used Lindgren funnel traps and one lure (quercivorol) for this study because of their efficacy in earlier trials. Each experiment comprised eight randomised plots, each 24 m² (6 × 4 m), with the plot boundaries 15 m apart and > 20 m from plantation edges (Supplementary Figure S2). One lure trap was placed in the centre of each plot at a height of 1.5 m above the ground, in June 2020. The understory surrounding the traps was cleared to make an optimal flight route to the traps. Every 10 days the lure was replaced and the beetles in the collection cups were taken to the PPRC laboratory to be sorted under a microscope. The *Euwallacea* species were separated out, counted and stored in 90% ethanol. The remaining taxa comprised low numbers of unidentified species of *Ambrosiodmus, Hypothenemus, Xyleborus* and *Xylosandrus*, and were deposited as voucher specimens in the insect collection of PPRC to be used in future research.

The trapping data were log-transformed before analysis using R version 4.2.1 (R Core Team 2022). Significant effects of trapping time per month were analysed with ANOVA, followed by Duncan’s multiple-range test for comparisons of means.

**Results**

**Species identification**

The specimens of *Euwallacea* collected in *Acacia* plantations (Figure 4e, f) were morphologically like *Euwallacea fornicatus* or *E. similis* as described by Gomez et al. (2018) and Smith et al. (2019). Phylogenetic analysis using the COI gene region revealed that *E. fornicatus* specimens in Vietnam belong to the unique haplotype in the polyphagous shot-hole borer (PSHB) cluster with a reliable bootstrapping of 84% (Figure 2). The sequences of *E. fornicatus* collected in *Acacia* plantations varied by a maximum of five nucleotides. The sequences of *E. fornicatus* in the present study were identical to KU727021, KU727022, KU727012 (Stouthamer et al. 2017) and MN268681 (Smith et al. 2019) collected in a previous study in Vietnam, but they had 0.005 genetic distance from KU727011 (Stouthamer et al. 2017). Furthermore, the *E. fornicatus* sequences for specimens collected in *Acacia* hybrid plantations (OL704769, OL704772, OL704770 and OL704771) were close to *E. fornicatus* specimens from Taiwan and Japan (genetic distance of 0.01) (Stouthamer et al. 2017). Sequences of a second ambrosia beetle species were identical to *E. similis* sequences from Thailand (KU727035) (Stouthamer et al. 2017), with a strong bootstrapping of 100%. The occurrence of *E. fornicatus* was found from all dissected boles in all study sites, whereas *E. similis* was collected from a minority of dissected boles in eight locations (Table 1).

**Assessment of damage**

A total of 14 *Acacia* plantations in 12 provinces of Vietnam were assessed for ambrosia beetle pest damage based on visible damage from *Euwallacea* to the boles via the density of active exit holes (Figure 4a, c). The mean damage incidence (P%) ranged from 16.7% to 34.9%, and the mean damage index (DI) ranged from 0.23 to 0.58. The greatest damage occurred in *A. mangium* plantations in...
Phu Tho (P% = 32.7, DI = 0.52) and Yen Bai (P% = 34.9, DI = 0.58). The P% values were slightly lower for the *Acacia* hybrid (18.1–30.7%) and *A. auriculiformis* (16.7–17.1%) plantations (Table 2).

**Effective trap and lure combinations**

In the trap and lure experiment, the number of captures was not significantly different between the types of traps \((p = 0.195–0.777)\) and there was no interaction between traps and lures \((p = 0.433–0.980)\) with split-plot ANOVA using Duncan’s multiple-range test (Supplementary Table S2). These findings agreed with the GLM analysis in which the explanatory variable of trap was not included in the best-fit model of the predicted number of captured beetles (Figure 3). Although, the \(\alpha\)-pinene lure showed a significant difference in the number of *E. fornicatus* captures between the *Acacia* hybrid and *A. mangium* plantations, this lure had no significant effect on the trapping number of *E. similis* in the two trapping sites (Table 3). Trapping success was similar across the three trap types for both *E. fornicatus* and *E. similis* (Supplementary Figure S3). However, there was a significant effect of lure \((p < 0.001)\) on the capture number of *Euwallacea* adults. Quercivorol and ethanol lures were the most effective, whereas cubeb oil and \(\alpha\)-pinene were weakly effective or ineffective (Table 4). In *A. mangium* plantations, the number of *E. fornicatus* beetles captured with ethanol and quercivorol lures reached 30.3 and 45.2 beetles per trap, respectively. Similarly, in the *Acacia* hybrid plantations these lures, respectively, gave the highest number of captured beetles with 24.1 and 33.2 beetles per trap. In addition, the number of *E. similis* captures (9.2–11.6 beetles per trap) was significantly lower than the *E. fornicatus* captures. The numbers of

**Table 2: Damage incidence of *Euwallacea* in *Acacia* plantations**

| Host               | Location: district (province)           | P% ± SE  | DI ± SE  |
|--------------------|-----------------------------------------|----------|----------|
| *Acacia auriculiformis* | Cam Lo (Quang Tri)                        | 17.1 ± 0.4 | 0.25 ± 0.01 |
|                    | Song May (Dong Nai)                       | 16.7 ± 0.3 | 0.23 ± 0.01 |
| *Acacia mangium*   | Yen Binh (Yen Bai)                       | 34.9 ± 1.1 | 0.61 ± 0.02 |
|                    | Tuyen Quang city (Tuyen Quang)           | 29.4 ± 0.8 | 0.47 ± 0.02 |
|                    | Dong Hy (Thai Nguyen)                     | 26.4 ± 1.2 | 0.44 ± 0.01 |
|                    | Doan Hung (Phu Tho)                       | 32.7 ± 1.6 | 0.55 ± 0.03 |
| *Acacia hybrid*    | Yen The (Bac Giang)                       | 24.4 ± 0.6 | 0.36 ± 0.02 |
|                    | Thanh Ba (Phu Tho)                        | 28.8 ± 0.8 | 0.46 ± 0.03 |
|                    | Tan Lac (Hoa Binh)                        | 30.7 ± 1.1 | 0.44 ± 0.04 |
|                    | Do Luong (Nghe An)                        | 18.1 ± 0.5 | 0.27 ± 0.01 |
|                    | Huong Tra (Thua Thien Hue)                | 33.3 ± 1.3 | 0.57 ± 0.05 |
|                    | M’Drak (Dak Lak)                          | 26.4 ± 0.6 | 0.43 ± 0.01 |
|                    | Xuan Loc (Dong Nai)                       | 28.1 ± 0.5 | 0.43 ± 0.02 |
|                    | U Minh (Ca Mau)                           | 22.7 ± 0.4 | 0.32 ± 0.01 |

P% = mean of damage incidence (%)  
DI = mean of damage index  
values are means \((n = 10)\) ± SE

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**Figure 3:** Predicted counts of beetle numbers obtained from GLM analysis. The two explanatory variables influencing the number of captured beetles were lure (E: ethanol; P: quercivorol, C: cubeb oil, \(\alpha\): \(\alpha\)-pinene, W: distilled water) and trapping site (AM: *Acacia mangium* plantation, AH: *Acacia hybrid* plantation). EF: *Euwallacea fornicatus*, ES: *E. similis*
E. similis captures were low for the cubeb oil and α-pinene lures (1.0–7.4 beetles per trap). In addition to Euwallacea, other genera were collected in the lure traps, mainly Xylosandrus, Hypothenemus and Xyleborus (Table 4), but the number of captures of these genera was low across all the lure treatments, ranging from 0.3 to 3.6 beetles per trap in the A. mangium plantations and 0.2 to 4.8 beetles per trap in the Acacia hybrid plantations. Of the total number of trapped beetles in the Acacia hybrid and A. mangium plantations, E. fomicatus accounted for 69.7% and 72.0%, respectively, and E. similis accounted for 22.0% and 20.6%, respectively.

Seasonal flight of Euwallacea species

Trapping from June 2020 to May 2021 revealed a bimodal trapping pattern of Euwallacea species in both A. mangium and Acacia hybrid plantations (Figure 5a, b). Seasonal flight peaked in April and October, during the spring and autumn seasons in Vietnam, when the mean temperature was in the range 21–28 °C and rainfall was < 150 mm per month (Figure 5c). The highest monthly captures of E. fomicatus were 150 in April and 116 in October. Peak captures for E. similis were 90 in April and 68 in October. The number of E. similis captures fell by almost 50% in May and November, to 46 and 38 beetles per month, respectively.

### Discussion

**Euwallacea in Acacia plantations in Vietnam**

Euwallacea fomicatus was present in all Acacia plantations surveyed in Vietnam, whereas E. similis was recorded in 57% of the surveyed locations (8/14 plantations) and was not found in A. auriculiformis. Phylogenetic analysis using the COI gene region confirmed that the E. fomicatus specimens that were sequenced all clustered with the haplotype of polyphagous shot-hole borer (PSHB) in the E. fomicatus complex. In previous studies, E. fomicatus specimens from plantations (KU727011, KU727012, KU727021, KU727022) and natural forests (MN266861) in Vietnam, as well as from Japan (KU727027) and Taiwan (KU727028), were determined to belong to the PSHB clade (Stouthamer et al. 2017; Smith et al. 2019). So far, the Kuroshio shot-hole borer (KSHB) and tea shot-hole borer (TSHB) haplotypes in the E. fomicatus complex have not been found in Vietnam. In addition, a second Euwallacea species was confirmed as E. similis. This species was previously shown to occur in infested trees in natural forest (Cognato et al. 2020) and was also trapped in forest plantations in Vietnam (Smith et al. 2018).

Euwallacea fomicatus occurred in all dissected boles of Acacia species in all collection sites and it dominated the beetles captured in field traps (at ~70%). By contrast, E. similis was found in some Acacia hybrid and A. mangium
planted with a frequency of 6/8 and 2/4 plantations, respectively, and it comprised only ~20% of the total beetle captures. The present study shows that Euwallacea has now reached most of the geographical areas where Acacia plantations are being grown in Vietnam.

Extent of damage from Euwallacea

Previously, E. fornicatus and E. similis were captured in trapping surveys in Acacia hybrid and A. mangium plantations but estimates of damage were not reported (Thu et al. 2010; Smith et al. 2018). Eventually, Coleman et al. (2019) reported a damage incidence of 2.9% for E. fornicatus in A. mangium in Vietnam. There is now a relatively high impact on tree health and the two Euwallacea species are of great concern to plantation owners. E. similis is considered by some to be a secondary species (Prior 1986; Balasundaran and Sankaran 1991; Lynn et al. 2021), and we have observed E. similis in Acacia trees already damaged by E. fornicatus. Overall, E. fornicatus was the main species damaging Acacia plantations in the present study. Euwallacea fornicatus is strongly polyphagous (Gomez et al. 2019), and further monitoring is required to determine whether it can become a pest of other forest plantations in Vietnam.

Effectiveness of traps and lures

In the trapping experiment, E. fornicatus was the most-abundant ambrosia beetle, followed by E. similis. A small number of unidentified Xylosandrus, Hypothenemus and Xyleborus species were trapped yet they were not found in the dissected trees. Hence, in the trapping results we focused on Euwallacea. We found no difference in the efficacy of the three types of traps ($p = 0.195–0.777$) (Supplementary Table S2). In avocado orchards, 3-vane interception traps were more efficient than Lindgren funnel traps (Kendra et al. 2020). Our finding that the ethanol and quercivorol lures were the most-effective in capturing E. fornicatus is similar to the results of trapping trials for Euwallacea spp. in avocado orchards in southern Florida (Burbano et al. 2012; Carrillo et al. 2015b; Dodge et al. 2017). Smith et al. (2018) also found that quercivorol and ethanol were effective lures for trapping E. fornicatus. We found that cubeb oil and α-pinene lures were inefficient, as the number of captured beetles was very low (1.0–7.4 beetles per trap). Previously, Monterrosa et al. (2021) showed that ethanol lures can remain stable for up to eight weeks in the field. We replaced the lures at monthly intervals to ensure there was continuity in effectiveness of the pheromone (quercivorol) and the generalist lures. Ethanol lures attracted many bark and ambrosia beetles, including Euwallacea nr. fornicatus (Carrillo et al. 2015; Kendra et al. 2020). However, quercivorol lures gave higher trap success than a combination of high-release ethanol and quercivorol lures (Dodge et al. 2017). Also, interactions between ethanol and quercivorol lures led to a decrease in catches of E. fornicatus and E. kuroshio (Monterrosa et al. 2021). Because about half of the plantation area in Vietnam is owned and managed by individual growers (MARD 2020), the use of ethanol lures in combination with self-made plastic bottle traps is advantageous owing to the low cost, availability of materials, and relatively simple deployment.

Seasonality of capture of Euwallacea adults

The adult beetles captured were most abundant in two periods (March–May and September–November), with clear peaks of flight activity in April and October. When the maximum temperature rose above 32 °C and rainfall exceeded 150 mm per month in the interval from June to August, the number of Euwallacea captures declined. Umeda and Paine (2019) showed that E. fornicatus can develop in the temperature range 13–33 °C, with an optimum
Facilitate more-effective monitoring and assist in formulating the seasonality of flight of the major pest species should trap and lure combination together with information on throughout Vietnam. The identification of a cost-effective Euwallacea is now a major threat to Acacia plantations in Vietnam. Furthermore, to help control the pests, growers will need to employ applications of pesticides.

Conclusions

Euwallacea is now a major threat to Acacia plantations throughout Vietnam. The identification of a cost-effective trap and lure combination together with information on the seasonality of flight of the major pest species should facilitate more-effective monitoring and assist in formulating a management strategy for ambrosia beetles in Acacia plantations in Vietnam.

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References

ACIAR (Australian Centre for International Agricultural Research). 2020. Acacia breeding in Vietnam. Canberra, Australia: ACIAR.
north of Mexico, with an illustrated key. *Annals of the Entomological Society of America* 99: 1034–1056. https://doi.org/10.1603/0013-8746(2006)99[1034:ROAXCC]2.0.CO;2

Reding ME, Schultz PB, Ranger CM, Oliver JB. 2011. Optimizing ethanol-baited traps for monitoring damaging ambrosia beetles (Coleoptera: Curculionidae, Scolytinae) in ornamental nurseries. *Journal of Economic Entomology* 104: 2017–2024. https://doi.org/10.1603/EC11119

Smith SM, Gomez DF, Beaver RA, Hulcr J, Cognato AI. 2019. Reassessment of the species in the *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) complex after the rediscovery of the ‘lost’ type specimen. *Insects* 10: article 261. https://doi.org/10.3390/insects10090261

Smith SM, Rabaglia RJ, Beaver RA, Thu PQ, Cognato AI. 2018. Attraction of ambrosia beetles (Coleoptera: Curculionidae: Scolytinae: Xyleborini) to semiochemicals in Vietnam, with new records and a new species. *The Coleopterists Bulletin* 72: 838–844. https://doi.org/10.1649/0010-065X-72.4.838

Socialist Republic of Vietnam. 2021. Vietnam Decision No. 523/QD-TTg Dated 1/4/2021 of the Prime Minister of the Socialist Republic of Vietnam on Approving the Vietnam Forestry Development Strategy for the Period of 2021–2030, with a Vision to 2050. Hanoi: The Socialist Republic of Vietnam.

Stouthamer R, Rugman-Jones P, Thu PQ, Eskalen A, Thibault T, Hulcr J et al. 2017. Tracing the origin of a cryptic invader: phylogeography of the *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) species complex. *Agricultural and Forest Entomology* 19: 366–375. https://doi.org/10.1111/afe.12215

Thu PQ, Quang D, Chi N, Hung T, Binh L, Dell B. 2021. New and emerging insect pest and disease threats to forest plantations in Vietnam. *Forests* 12: 1301.

Thu PQ, Quang D, Dinh V, Tiep B. 2010. Insect list (Coleoptera and Hemiptera) collected from insect trap program in Dai Lai, Vinh Phuc. *Vietnam Journal of Forest Science* 3: 1363–1369.

Umeda C, Paine T. 2019. Temperature can limit the invasion range of the ambrosia beetle *Euwallacea nr. fornicatus*. *Agricultural and Forest Entomology* 21: 1–7. https://doi.org/10.1111/afe.12297

Wood S. 2007. *Bark and ambrosia beetles of South America*. Provo: Monte L. Bean Science Museum.

Wood S, Bright D. 1992. *A catalog of Scolytidae and Platypodidae (Coleoptera)*, Part 2: taxonomic index. Provo: Great Basin Nature Memoirs.