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# Diagnostic damage symptoms and distribution of avocado stem borer (*Zeuzera* spp.) in the Western Ghats region of India

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ABSTRACT

Avocado (*Persea americana* Mill.), a high-value exotic fruit, is gaining popularity in India due to its nutritional, pharmaceutical, and industrial applications. Initially cultivated as a homestead or intercrop within coffee estates, its large-scale cultivation has recently expanded, leading to shifts in pest dynamics, the documentation of new pests and changes in host ranges commonly observed in horticultural crops. The present study was carried out during 2022–23 at the Central Horticultural Experiment Station (CHES), Chettalli, Kodagu, Karnataka to document the occurrence and distribution of the avocado stem borer, and to identify the pest species using molecular techniques along with detailed observations of damage symptoms. Morphological observations classified the pest under the genus *Zeuzera*. Molecular analysis of partial COI sequences of avocado stem borer isolates revealed a monophyletic clade with *Z. aeglospila*, *Z. caudata*, *Z. indica*, and *Zeuzera* spp. (BOLD: AAY7619), but did not cluster with *P. coffeae*, a common pest of coffee and forest trees, suggesting a different species infesting avocado other than *P. coffeae*. Surveys revealed stem borer incidence rates of 1.13 ± 0.46% in Kodagu (Karnataka), 8.16 ± 3.11% in Nilgiris and Dindugal (Tamil Nadu), and 12.46 ± 3.58% in Wayanad (Kerala), highlighting the distribution of *Zeuzera* spp. across South India. This is the first study to document the diagnostics damage symptoms and regional distribution of avocado stem borer, *Zeuzera* spp., in India. Ongoing research focuses on the species identification through morphological characterization and damage monitoring, to develop effective management strategies as avocado cultivation continues to expand in the Western Ghats.

**Keywords**: Distribution, *Persea americana,* Survey, Western Ghats, *Zeuzera* spp.

Avocado (*Persea americana* Mill.), a high-value fruit from the Lauraceae family, originated in Mexico and central America. It is widely cultivated in Mexico, South America, Australia, South Africa, and India, where it is gaining momentum (Knight 2002, Tripathi *et al.* 2014). Global avocado production reached 8.98 million tonnes in 2022, with Mexico as the lead producer (FAOSTAT 2024). Insect-pests pose a major challenge, with 22 species reported worldwide and 2–3 causing serious crop losses (Dennill and Erasmus 1992, Pena 2003). In India, avocado is mainly cultivated in Karnataka, Kerala, Tamil Nadu, Maharashtra, and parts of the northeast (Tripathi *et al*. 2014, Madhu *et al*. 2023). Although, initially grown as an intercrop with coffee, its nutritional, pharmaceutical, and industrial value is driving a shift toward monoculture systems, increasing the risk of pest and disease outbreaks similar to those seen in other countries including India (Erichsen and Schoeman 1992, Venkataravanappa *et al*. 2025a).

The genus *Zeuzera* (Latreille) (Lepidoptera: Cossidae), commonly known as leopard moths, cryptic wood borer moths, or carpenter moths is a significant group of wood borers in the subfamily Zeuzerinae (Hegazi *et al*. 2015). The genus *Zeuzera* is polyphagous, cosmopolitan, and widely distributed across the Holarctic, Indo-Australian, and New World regions, with a notable presence in the Oriental region (Hegazi *et al*. 2015, Sutrisno 2015). It affects over 150 plant species across 20 genera (Arora 1976, Kutinkova *et al.* 2006, Suheri *et al.* 2022). Globally, 15 Cossidae species have been reported as pests of forests and horticultural trees (Hoppner and Ferreira 1990, Yadav *et al.* 2020). In India, 25 species from six genera have been reported (Hampson 1892). Of the 52 known *Zeuzera* species worldwide, 14 occur in the Oriental region and five species with one subspecies have been reported in India (Arora 1976). Economically significant species include *Polyphagozerra coffeae* (Nietner) (Lepidoptera: Cossidae) which infests over 30 plants such as coffee, teak, and mahogany while, *Zeuzera multistrigata* Moore (Lepidoptera: Cossidae),

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attack cherry and sandalwood (Beeson 1941). Although *P. coffeae* is listed as a potential avocado pest (CABI 2021), detailed studies confirming its damage to avocado are lacking. Information on *Zeuzera* damage symptoms and their distribution in avocado plantations remains scarce, with no previous records from India or elsewhere. This study aimed to identify *Zeuzera* species affecting avocado using molecular tools, document damage symptoms, and assess their incidence across avocado-growing regions particularly in southern India. This is the first report of a stem-boring insect affecting avocados in India.

## MATERIALS AND METHODS

*Study area and insect collection*: The study was carried out during 2022–23 at the Central Horticultural Experiment Station, Chettalli, Kodagu (12°26'N, 75°47'E; at an elevation of 1050 m amsl), Karnataka. Pest monitoring relies on the visual inspection of larval activity (Hegazi *et al.* 2015). Borer incidence was recorded in healthy and Phytophthora wilt affected avocado blocks with 3–5 year-old trees. Trees showing external and internal stem borer damage symptoms were documented using Canon EOS 200D DSLR camera (Tokyo, Japan). Infested trees with active larvae were excised using wood chisels to collect undamaged larvae and the surrounding wood. The larvae were preserved in 95% ethanol for molecular identification using the *mtCOI* gene. Stems infested with larvae were brought to the Entomology laboratory at ICAR-Indian Institute of Horticultural Research, Central Horticultural Experiment Station (27±1°C, 70±10% RH, 16:8 h light: dark cycle) and monitored daily for adult emergence.

*Morphological and molecular identification*: The avocado stem borer, a cryptic wood-boring insect, completes most of its life cycle inside tree stems, making rearing larvae to adults challenging under laboratory conditions due to high mortality rates among larvae and pupae. The laboratory rearing of *Zeuzera* from infested avocado branches and stems is shown in Fig. 1. A single adult moth emerging from an infested avocado stem was sent to a taxonomist for species identification. This study describes the external morphology of both the larval and adult stages of the avocado stem borer and employs COI-based molecular identification using universal primers LCO1490 and HCO2198 (Folmer *et al.* 1994). Genomic DNA was extracted from larvae collected from infested avocado trees by using a modified CTAB protocol (Asokan *et al.* 2015). A partial region of the *mtCOI* gene was amplified by PCR under the following thermal conditions: initial denaturation at 94°C for 3 min, 35 cycles of 94°C for 45 s, 47°C for 40s, and 72°C for 1 min 30 s, followed by a final extension at 72°C for 7 min (Venkataravanappa *et al*. 2025). The PCR amplicons were separated on 0.8% agarose gels stained with ethidium bromide and visualized using a gel documentation system. Sanger sequencing was carried out at Eurofins India Pvt. Ltd., Bengaluru. Sequences were edited and assembled using BioEdit software v7.7.1 (Hall 1999) and compared using BLAST on the NCBI database for species identification. Phylogenetic analysis included sequences of 16 known *Zeuzera* species and two cossid outgroup species, *Dervishiya cadambae* (Moore) and *Givira ethela* (Neumoegen and Dyar) (Lepidoptera: Cossidae) (Supplementary Table 1). Sequences were aligned using the CLUSTALW programme, and a phylogenetic tree was constructed using the Neighbour-Joining method with the Tamura-Nei model and 1000 bootstrap replications in MEGA 11.0.13 (Tamura *et al.* 2021). Partial *COI* gene sequences of the four avocado stem borer isolates identified in this study have been submitted to the NCBI GenBank with accession numbers PP409487.1, PP998619.1, PQ350401, and PQ356894.

*Survey for stem borer incidence in Western Ghats region*: In 2023–24, a rapid roving survey was conducted across major avocado-growing regions in Karnataka (Kodagu), Tamil Nadu (Nilgiris and Dindigul), and Kerala (Wayanad) to document the incidence of the stem borer, *Zeuzera* spp., on avocados. Based on initial observations at the CHES, Chettalli, trees showing clear external symptoms of infestation (Fig. 2) were identified and recorded along with their GPS coordinates and cropping systems. Collecting insect specimens from infested trees is challenging and often requires tree felling. During the survey, the incidence of stem borers was recorded at each location, and the average percentage incidence for each state was calculated. The GPS coordinates of surveyed sites across districts were mapped using QGIS 3.34.8 software.

## RESULTS AND DISCUSSION

*Diagnostic damage symptoms of avocado stem borer*: Stem borer infestations in avocado were observed in trees

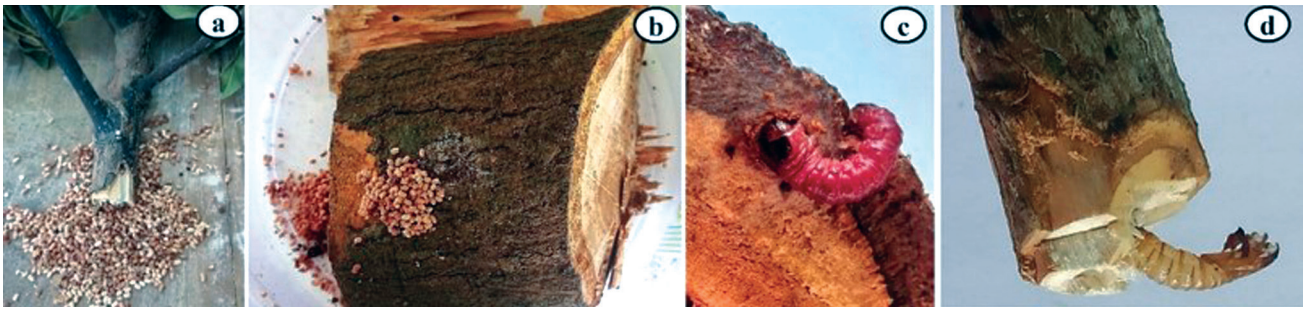


Fig. 1 Laboratory rearing of *Zeuzera* on infested branch (a) and stem (b) of Avocado brought from field (c) Larva feeding on the avocado stem piece in laboratory (d) Pupal case protruding from an exit hole in the avocado branch.

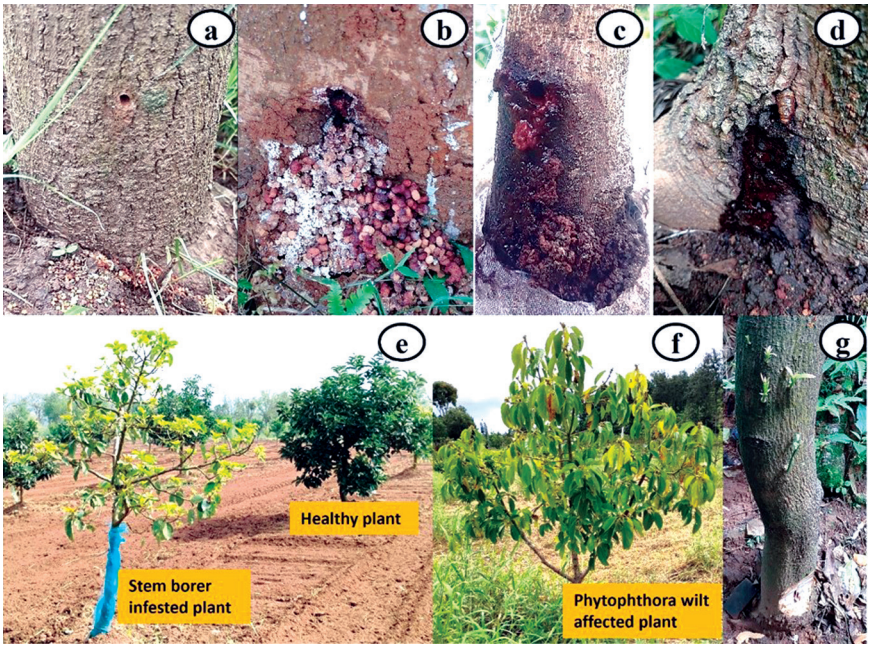


Fig. 2 External symptoms of *Zeuzera* infestation on avocado trees (a) Hole initiation, (b) Frass and wood exudate below the entry, (c) Hole widening with gummy exudation and chewed fibres at trunk base, (d) Brown gummy mucilage with pupal case protruding, (e) Comparison of healthy and infested plants, (f) Phytophthora wilt-affected plant, (g) New shoot emergence above the borer-damaged area.

over two-three years old, characterized by small entry holes (≥5 mm) on the trunk. The larvae typically bore into the stem at a height of ½ to 1 ft above ground level (Fig. 1), creating extensive tunnels that extend both upward and downward within the tree (Fig. 3). In some cases, larvae bore higher, approximately 3–4 feet above ground, forming horizontal tunnels that reach the central pith (Supplementary Fig. 1–3). Visible signs of infestation include frass deposits resembling fibrous fecal pellets or chewed-up fiber near entry holes or at the tree base [Fig. 2 (a-c), Supplementary Fig. 1–3] often accompanied by the oozing of gum-like substances (Fig. 2c-d). As infestation progressed, the entry hole enlarges to 10–15 mm in diameter (Fig. 2c). Fully mature larvae pupated within the larval gallery and created an exit hole before pupation. After adult emergence, the pupal case was visible, with its cephalic end protruding from the exit hole (Fig. 2d). This behaviourism consistent across various *Zeuzera* and Cossid species (Scaccini *et al.* 2021, Suheri *et al.* 2022, Thurman 2022). Severely stem borer-infested trees exhibited symptoms such as excessive flowering, yellowing, and sometimes wilting, resembling symptoms of *Phytophthora* infection (Fig. 2e-f). Occasionally, new shoots emerged above the bored holes (Fig. 2g). In cases where branches are affected, their leaves dry up, whereas the rest of the tree appears healthy. Few studies have reported that, *Phytophthora* wilt, dieback and stem borer affected avocado plants exhibit similar wilting symptoms (Beeson 1941, Madhu *et al.* 2024, 2025, 2025a, Ayyandurai *et al*. 2025).

Internal damage included wide, circular holes beneath the bark, where larvae reside throughout their immature stages (Fig. 3a-b, 3e). Cut sections revealed larval tunnels filled with chewed wood fibers and gummy brown mucilage (Fig. 3c-d). Extensive tunnelling in major branches and trunks disrupts water and nutrient flow, causing branch breakage and potentially killing young trees within months. Similar damage symptoms have been reported in other *Zeuzera* and Cossid species (Gebeyehu *et al.* 2005, Kingsley-Umana *et al.* 2022, Suheri *et al.* 2022).

*Morphological description of the avocado stem borer*: During the field assessment, no stem borer eggs were found, likely because they had already hatched and developed into later stages, such as larvae, pupae, or adults. Rearing larvae to the adult stage for accurate identification and characterization remains a significant challenge. The mature larvae of *Zeuzera* spp. measure about 50 mm in length and 1 cm in thickness, with a pinkish-red colour and 4–6 small black spots on each segment (4 on the dorsal and 2 on the lateral surface). The body of the larvae was covered with fine scattered hairs. Its head was light to dark brown, and the thoracic and anal plates were shiny black (Fig. 4a).

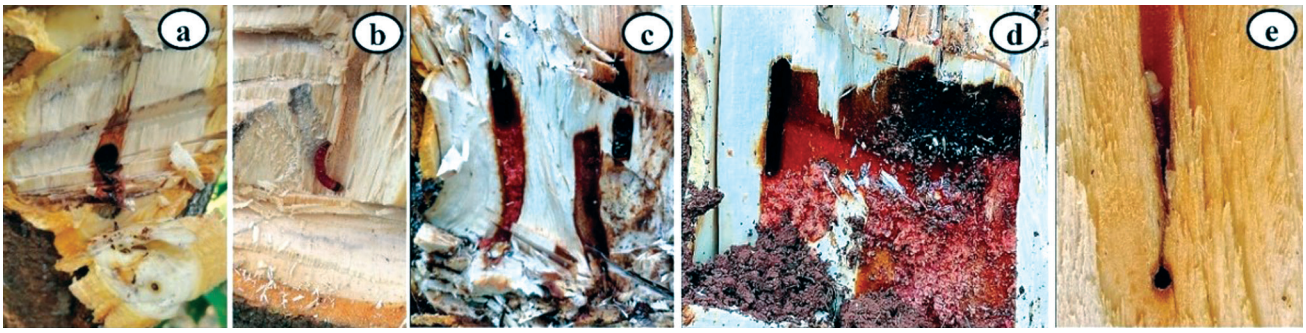


Fig. 3 Internal damage symptoms of *Zeuzera* infestation in avocado trees (a) Circular entrance hole under bark, (b) Larva tunnelling downward, (c) Internal feeding tunnels, (d) Chewed fibres with brown gummy mucilage, (e) Stem borer larva inside stem.

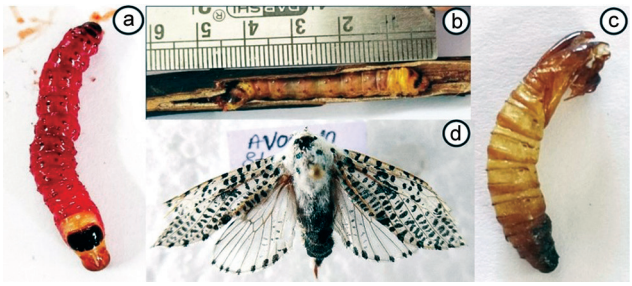


Fig. 4 Life stages of Avocado stem borer (a) Larvae of *Zeuzera* spp. (b) Larva (approx. 4–5 cm in length) just before pupation (c) Pupal case (d) *Zeuzera* spp. adult moth.

Immediately before pupation, fully mature larvae turned light to pale pink (Fig. 4b). The pupae were brownish and attached to the exit hole after adult emergence (Fig. 4c). The adult moth has whitish-graybody hairs and scales. The thorax was notably hairy with six black spots arranged in two parallel lines (Fig. 4d). The abdomen is black and relatively long. The wings were predominantly white or gray, with forewings having numerous black spots, particularly concentrated between the veins. The hindwings feature light black spots toward the anal margin. Both the forewings and hindwings had prominent black spots at the tips of each vein along the apical margin of the wing (Fig. 4d). The morphological description of the avocado stem borer moth aligned with the traits described by Holloway (1986) and partially matches the morphology of *Z. pyrina* (Linnaeus) (Boyes 2023).

*Molecular identification of avocado stem borer*: The partial mitochondrial cytochrome oxidase subunit I (*COI*) gene sequences obtained from the sequencing of avocado stem borer were analyzed using the NCBI-BLAST tool to determine nucleotide sequence similarity. Pairwise identity analysis revealed that the avocado stem borer sequence exhibited 93.59% nucleotide (nt) identity with *Zeuzera indica* Herrich-Schaffer (GenBank accession no. JN287264.1) and 93.56% nt identity with *Z. aeglospila* Turner (HQ952094.1). Additionally, the sequences showed 91.88% nt identity with *Z. caudata* Joicey and Talbot (AB935221.1) and 91.41% nt identity with *Zeuzera* spp. (BOLD:AAY7619; KF492183.1) (Supplementary Table 1). Representative type isolate sequences with high similarity were retrieved from the NCBI database for phylogenetic comparison.

Phylogenetic tree was developed using MEGA 11 software with the Neighbour-Joining (NJ) method and 1,000 bootstrap replicates. Outgroups from *Dervishiya* Yakovlev (Cossinae) and *Givira* (Hypoptinae) were included for tree rooting. The resulting phylogenetic tree resolved the nine avocado stem borer isolates from the present study into two major clades (Fig. 5). In the first clade, three isolates—AVCHES (PP409487.1), AVCHES1 (PP998619.1), and AVCHES3 (PQ356894) clustered together with strong bootstrap support (100%). These isolates further grouped with *Z. aeglospila* and *Z. caudata* with a bootstrap support value of 79%. In the second clade, the isolate AVCHES2 (PQ350401) formed a distinct cluster with *P. coffeae* (previously *Z. coffeae*), supported by a 97% bootstrap value, and exhibited 96.10% nucleotide identity with *P. coffeae* in the BLAST analysis (Fig. 5). Phylogenetic analysis of partial mitochondrial cytochrome oxidase I (*COI*) sequences revealed that the three avocado stem borer isolates form a distinct, well-supported monophyletic clade closely related to *Zeuzera aeglospila*, *Z. caudata*, *Z. indica*, and *Zeuzera* spp. (BOLD: AAY7619), but clearly separated from *P. coffeae*, a known pest of coffee and forest trees. Only one isolate (AVCHES2) was identified as *P. coffeae*, while the others represented a divergent lineage (Fig. 5). These findings suggest potential cryptic diversity within the *Zeuzera* complex infesting

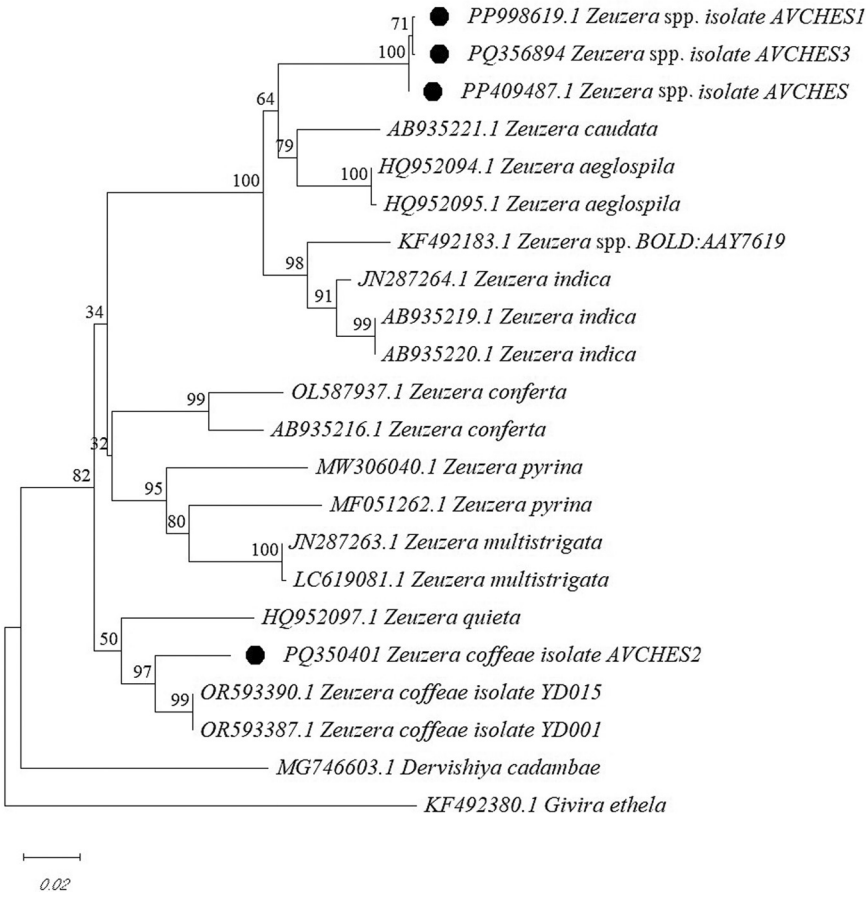


Fig. 5 Molecular phylogenetic analysis of *Zeuzera* species based on *COI* sequences using the Neighbour-Joining method (1000 bootstrap replicates). *Dervishiya cadambae* (Moore) and *Givira ethela* (Neumoegen and Dyar) used as outgroups. Black dot marks sequences from the present study.

avocado, possibly representing an uncharacterised species or an intraspecific variant related to *Z. aeglospila* or *Z. caudata*. Further morphological studies are necessary to validate its taxonomic identity and evolutionary placement.

Previous studies have shown the genetic diversity within the genus *Zeuzera*. Sutrisno (2015) identified distinct clades for *Z. aeglospila*, *Z. caudata*, and *Z. indica* among Indonesian *Zeuzera* species, whereas Dolati *et al.* (2017) reported regional genetic variation in *Z. pyrina* linked to climate. *COI* analysis has proven useful in detecting population differences by geography or host, as observed in *Spodoptera frugiperda* (Levy *et al.* 2002). In India, the red coffee borer (*Zeuzera* spp.) may shift or expand its host range owing to changing farming practices, host plant conditions and climate. Host shifts are often linked to speciation contributed either by reproductive isolation before or after the host shift or due to host-associated genetic differences (Piper 2009, Forbes *et al.* 2017). The establishment of Cossidae stem borers in new crops is influenced by factors related to insect biology, crop characteristics, and environmental conditions. Their polyphagous nature enables adaptation to various woody hosts (Choochuen and Foit 2025). This study's molecular analysis confirmed the avocado stem borer is a *Zeuzera* species, with species identity verified through morphological studies.

Choochuen and Foit (2025) reviewed the diversity, biology, and damage caused by cossid moths and their management on woody plants. Studies on host-pest interactions show that many cossid larvae prefer fast-growing trees, such as forest, fruit, and ornamental species, over slow-growing ones (Abdel-Moaty *et al.* 2019, Choochuen *et al.* 2024). This aligns with the growth-differentiation balance hypothesis, which suggests that rapid-growing trees invest more in growth than in defence, making them more susceptible to insect attack (De la Mata *et al.* 2017). Cossid moths often infest weak or pathogen-affected trees (Kaplan and Turanlı 2018). Notably, *Neurozerra conferta* infestation in mature *Aquilaria malaccensis* trees can enhance agarwood quality (Khakhlari and Sen 2023). In avocados, borer incidence and damage are mainly affected by climatic variations, biotic stresses such as fungal wilt, abiotic stresses like nutrient deficiencies and extreme weather, as well as tree age and location.

*Survey of avocado stem borer incidence in Western Ghats region*: A survey was conducted across four major avocado-growing districts in the Western Ghats i.e. Kodagu (Karnataka), Wayanad (Kerala), and Nilgiris and Dindigul (Tamil Nadu) to assess stem borer incidence (Supplementary Table 2). Infestations were identified through visible symptoms without damaging trees, following the diagnostic features described in Fig. 2 and 3 and Supplementary Fig. 1–3. Visual inspection remains the standard method for monitoring cossid pests (Kutinkova *et al.* 2006, Kingsley-Umana *et al.* 2022). In Kodagu, infestations appeared in 3–8 year-old trees within monocrops and coffee plantations, with incidence rates of 1.32–2.67%. In Wayanad, infestations occurred in all six surveyed coffee plantations and four of the nine homesteads, affecting healthy and Phytophthora-wilted trees, with rates ranging from 8.33–25.00% in plantations and 5.00–50.00% in homesteads. In Nilgiris and Dindigul, the incidence ranged from 6.45–40.00% across seven of 18 sites. (Supplementary Table 2). Average infestation rates were highest in Kerala (12.46 ± 3.58%), followed by Tamil Nadu (8.16 ± 3.11%), and the lowest in Karnataka (1.13 ± 0.46%) (Fig. 6). Stem borer incidence was higher in coffee plantations than in monocropping or homestead systems. Although the incidence was observed in avocado trees of all ages and conditions (young/old, and healthy/diseased/stressed), some studies suggested that infestation increases with tree age because older trunks are larger, richer in the phloem, and better suited for larval development or larval preference for older trunks (Mathew 1990, Scaccini *et al.* 2021).

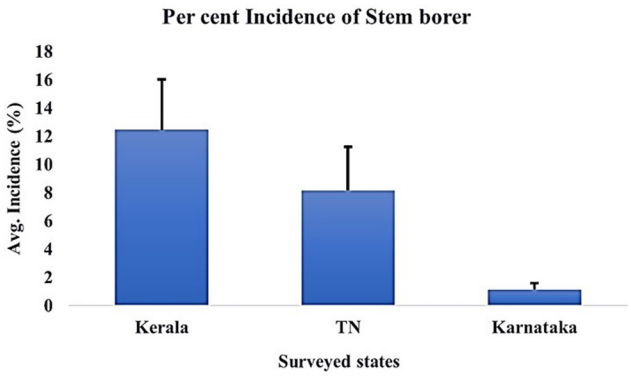


Fig. 6 Mean per cent incidence of Avocado stem borer, *Zeuzera* spp., in Kerala (n=16), Tamil Nadu (n=18) and Karnataka (n=5); n, Number of locations.

This study highlights the rising risk of stem borer incidence with expanding avocado cultivation, driven by changes in plant quality, climate, and reduced natural control. Similar trends include *Eulophonotus myrmeleon* Felder (Lepidoptera: Cossidae) becoming a major cocoa pest in Nigeria due to larval migration caused by climate change (Kingsley-Umana *et al.* 2022). *Zeuzera* species can cause significant damage to fruit crops, plantations, and forest trees. *Z. pyrina* has led to 37–42% olive yield losses in Egypt (Hegazi *et al.* 2015) and up to 70% apple orchard damage without protection (Kutinkova *et al.* 2006). *P. coffeae* affects coffee, tea, and other crops (Sutrisno 2015, Suheri *et al.* 2022), whereas *Z. leuconotum* Butler (Lepidoptera: Cossidae) was recently reported in grape vineyards in northern Karnataka, India (Sunitha *et al.* 2022).

Developing an integrated pest management (IPM) strategy for *Zeuzera* spp. involves accurate pest identification, understanding its distribution on avocado, and establishing effective monitoring tools (Daane *et al.* 2018). Many carpenter moths are attracted to light and baited traps, making these useful for monitoring. Various trap types such as black, mercury vapour, and UV-light traps (Infusino *et al.* 2017), as well as pheromone-baited sticky traps for mass trapping, mating disruption and to study population

dynamics of *Zeuzera* spp. (Hegazi *et al.* 2015, Hoshi *et al.* 2016, Noeth *et al.* 2020, Suheri *et al.* 2020, 2022) have been successfully used against other cossids. IPM strategies for cossids also include cultural, biological, mechanical, and chemical methods—such as pruning infested twigs, using parasitoids and entomopathogenic fungi, and applying insecticides judiciously (Ahmad 2017, Sabbour 2017, Yadav *et al.* 2020).

In conclusion, this study is the first to report *Zeuzera* infestation in avocado, detailing its diagnostic damage symptoms, incidence, and distribution across cropping systems in the Western Ghats of India. Both external and internal symptoms are crucial for detection without the need for felling trees. Molecular and phylogenetic analyses identified the pest as a *Zeuzera* species, indicating that it may be an uncharacterised species or an intraspecific variant related to *Z. aeglospila* or *Z. caudata* that is potentially distinct from *P. coffeae*. As avocado cultivation expands, this borer can become a serious threat. This study also provided valuable molecular data for rapid identification as the range expands. Ongoing research is focused on species confirmation, damage assessment, and understanding of its biology, distribution, and population dynamics to support sustainable IPM strategies.

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