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**Sunitha ND**

Professor, Department of  
Agricultural Entomology,  
College of Agriculture,  
Vijayapura, Karnataka, India

**Prashanth G**

Research Scholar, Department of  
Agricultural Entomology,  
College of Agriculture,  
Vijayapura, Karnataka, India

**Remedia PD**

Research Scholar, Department of  
Agricultural Entomology,  
College of Agriculture,  
Vijayapura, Karnataka, India

## *Zeuzera leuconotum* (Butler) (Lepidoptera: Cossidae): A new threat to *Vitis vinifera* (L.)

Sunitha ND, Prashanth G and Remedia PD

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**Abstract**

Grape (*Vitis* sp.) belonging to Family Vitaceae is a commercially important fruit crop of India. It is a temperate crop which has got adapted to sub-tropical climate of peninsular India. Viticulture is a billion dollar agricultural sector in India, where new unidentified insect pest species can disrupt management practices. We report herein on a new pest associated with vineyards, the carpenter moth *Zeuzera leuconotum* (Butler). It appears to be a newly recognized wood-boring pest of *V. vinifera* (L.) in regions of northern parts of Karnataka, India, where its initial occurrence has not been reported in India. The regions of occurrence of this new wood borer, larva and symptoms of damage are illustrated. *Z. leuconotum* distribution and symptoms of damage on different hosts, other cossids of genus *Zeuzera* and other genera found on *V. vinifera* are updated including published records and new data. Notes on possible routes of entry of this new wood borer into vine orchards and possible monitoring and management options on grapes are also discussed. Tools for the Integrated Pest Management of *Z. leuconotum* should include the quick and reliable identification of the insect and its nature of damage, thorough understanding of its bioecology, the application of monitoring methods and eco-friendly management strategies.

**Keywords:** Grape vine, carpenter moth, new wood borer, Karnataka, India

**Introduction**

Wood boring beetles and moths can damage cultivated crops, particularly fruit and ornamental trees. Among moths, species in the Cossidae, Hepialidae, Sesiidae and Xyloryctidae are widely recognized as having larvae that are wood boring pests on tree crops, where they feed upon both the vascular and/or structural tissues of the plant. The trophic activity of these larvae can cause a reduction in structural stability of the host plant, and direct plant-stress, and may promote the establishment of phytopathogens that further damage plant growth or crop yield (Scaccini *et al.*, 2021) <sup>[1]</sup>.

Worldwide, grape value exceeds \$68 billion (USD) with about 7.1 million ha in production (Alston and Sambucci, 2019) <sup>[2]</sup>. Grape is one of the major fruit crops grown in India and it accounts for about 2.5 per cent of total fruit production. It is grown on 1, 48,000 ha in India, with a production of 31, 86,000 MT and a productivity of 21.37 MT/ha, with exports of 246134.80 MT. After Maharashtra, Karnataka is India's second largest producer, with a total area of 26,615 ha, a production of 5, 25,200 MT and a productivity of 19.90 MT/ha. Vijayapura district in Karnataka stands first in grape cultivation, with a total area of 10,650 hectares with a production of 211.65 MT (Anon, 2020) <sup>[3]</sup>. Manik Chaman, Thompson seedless, Sonaka, Sharad seedless, Tas-A-Ganesh and Flame seedless are some of the most popular varieties grown in this area (Anon, 2019) <sup>[4]</sup>.

Monitoring of insect pests and their natural enemies in *V. vinifera* L. orchards is a regular programme since two decades for the staff and research students of department of Agricultural Entomology, College of Agriculture, Vijayapura (16° 50' N, 75° 47' E.), Karnataka (India). Cerambycid wood borer *Celosterna scabrator* Fab. has emerged as major pest (Sunitha, 2018) <sup>[5]</sup>. *Stromatium barbatum* Fab causing extensive damage to grape orchards in Maharashtra state is reported by Salini and Yadav (2011) <sup>[6]</sup>. Later *S. barbatum* for the first time from grape orchards of Vijayapura, Karnataka was put on records (Sunitha and Chavan, 2020) <sup>[7]</sup>. The carpenter moth *Dervishiya cadambae* (Moore, 1865) (Lepidoptera: Cossidae) causing extensive damage to grape vines was reported by Yadav *et al.* (2020) <sup>[8]</sup> is a recent addition to the existing wood borers in Maharashtra.

**Corresponding Author:****Sunitha ND**

Professor, Department of  
Agricultural Entomology,  
College of Agriculture,  
Vijayapura, Karnataka, India

The same authors also reported that farmers at Vijayapura in the Karnataka state of India also reported similar infestation symptoms and presence of red coloured wood borer larvae during April, 2016. The wood borer was later identified as *D. cadambae*. Ongoing through this report, extensive surveys were conducted to find out the occurrence of this cossid pest and its extent of damage in major grape growing localities of Vijayapura. The larvae extracted from affected grapevines were found morphologically different from *D. cadambae* which made us to go for molecular studies for quick and correct identification of this new lepidopteran wood borer on grapevines. The aim of this paper is to report on a new wood boring carpenter moth infestation on grape vines and its distribution in major grape growing regions of Northern parts State Karnataka, India and to present its nature and diagnostic symptoms of damage.

## Materials and methods

### A) Distribution and nature of damage

Extensive roving surveys were conducted during 2020-2021 in Vijayapura district where in 50 randomly selected vine orchards from major grape growing villages in five talukas

(Fig 1) were visited and from each orchard 50 vines were randomly selected covering the entire orchard and were examined for wood borer incidence. Grape vines showing the symptoms of wood borers damage were categorized as Cerambycid wood borers (Sunitha 2018<sup>[5]</sup>; Salini and Yadav 2011<sup>[6]</sup>, Sunitha and Chavan 2020)<sup>[7]</sup> and Lepidopteran wood borer (Yadav *et al.*, 2020)<sup>[8]</sup>.

Care was taken to select the orchards with different locations within a village. Geographical locations of all the 250 orchards surveyed were also recorded. Other parameters associated with the orchards like age of the orchard, variety of grape, spacing and trees located around the orchard were also recorded. Grape vines with symptoms of lepidopteran wood borer (Yadav *et al.*, 2020)<sup>[8]</sup> were tagged and all the visible symptoms of damage were recorded. Later vines were cut opened to collect the larvae. The collected larvae were well preserved and shifted to Bar code Bio sciences (BBS), an ISO certified company, located at Bengaluru, Karnataka state, India for DNA bar-coding for quick and reliable identification. The infested grape cordons were brought into the laboratory and kept for adult emergence.



**Fig 1:** Study areas in Vijayapura district



Fig 2: Emergence of *Z. leuconotum* adult in the laboratory.

### B) DNA bar-coding

DNA was isolated from the larval sample. Its quality was evaluated on 1.0 % agarose gel, a single band of high-molecular weight DNA has been observed. Fragment of COI gene was amplified by LCO (LCO1490 GGTCACAAATCATAAAGATATTGG) and HCO (HCO2198 TAAACTTCAGGGTGACCAAAAATCA) primers. A single discrete PCR amplicon band of 700 bp was observed when resolved on agarose gel. The PCR amplicon

was purified to remove contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with LCO and HCO primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of COI gene was generated from forward and reverse sequence data using aligner software. The COI gene sequence was used to carry out BLAST with the 'nr' database of NCBI GenBank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 6.

## Results and discussion

### A) Pest Identification

The wood borer larvae named as sample 1 which was collected from within the grape vine stem and sent for molecular characterization was found to be *Zeuzera leuconotum* (Butler) which showed high similarity based on nucleotide homology and phylogenetic analysis. Sample 1 showed highest per cent match with *Zeuzera sp.* SSG-2014 isolate M17 and *Zeuzera leuconotum* voucher ZLyn1 (99.83% and 97.27% respectively) (Table1 and Fig. 3).

Table 1: Sequences producing significant alignments of *Zeuzera leuconotum* (Butler)

Description	Max Score (No)	Total Score (No)	Query Cover (%)	E value	Per. Ident (%)	Accession
<i>Zeuzera leuconotum</i> voucher ZLyn1	1116	1116	98	0.00	97.27	KC791485.1
<i>Zeuzera sp.</i> PK01 voucher NIBGE MOT-01535	1105	1105	98	0.00	96.97	KX861709.1
<i>Zeuzera sp.</i> LTOLB937-11 voucher AYK-04-0819-03	1099	1099	98	0.00	96.81	JN287266.1
<i>Zeuzera sp.</i> BOLD:AAV2990 voucher AYK-04-0779-04	1094	1094	98	0.00	96.66	KF492184.1
<i>Zeuzera sp.</i> SSG-2014 isolate M17	1090	1090	88	0.00	99.83	KJ380851.1
<i>Zeuzera sp.</i> PK02 voucher NIBGE MOT-02930	1088	1088	98	0.00	96.51	KX860327.1
<i>Zeuzera sp.</i> PK02 voucher NIBGE MOT-02929	1077	1077	98	0.00	96.21	KX860870.1
<i>Zeuzera quieta</i> voucher 10ANIC-09448	939	939	98	0.00	92.41	HQ952097.1
<i>Zeuzera coffeae</i> COX 1 gene	937	937	85	0.00	96.01	AB935215.1
<i>Mixodetis sp.</i> ANIC1 voucher 10ANIC-05236	928	928	98	0.00	92.12	HQ948791.1

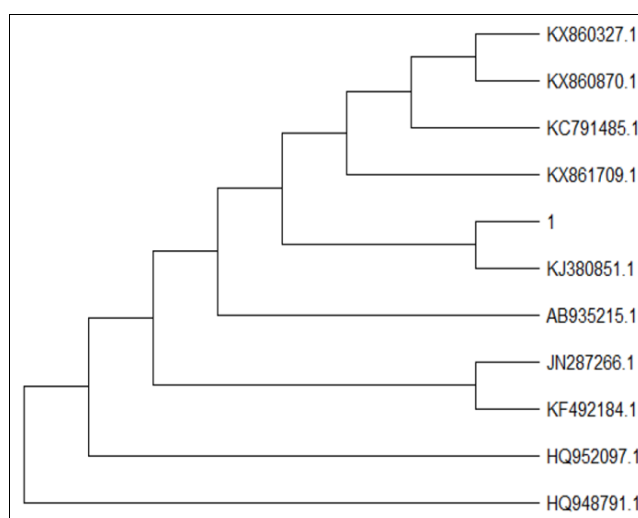


Fig 3: Molecular phylogenetic analysis by maximum likelihood method for *Zeuzera leuconotum* (Butler)

### B) Distribution and Symptoms of damage

Among the 150 orchards visited, the new lepidopteran wood borer was recorded from three orchards, two orchards located at Tikota and one orchard at Babanagar village on "Thompson seedless" variety. Percent incidence ranged between 2-4 with 2% at Babanagar and 4% at Tikota village.

The vines affected by new wood borer *Z. leuconotum* showed

ring excavated on the trunk nearer to which was found the circular entry hole (Fig.4) when the trunks were slit open longitudinally, pinkish red larva with head and thoracic part and tip of the abdomen showing yellow color was observed. Larva was found feeding on inside of the trunk by making small galleries and tunnels in which packets of excretory pellets we found (Fig 5) The presence of caterpillars can be

detected by the pallets of frass on the ground below the infested branches. Complete drying of leaves followed by withering with no berry formation were the other visible

symptoms of damage by this new wood borer *Z. leuconotum*. The grape vines presented burnt appearance (Fig 6).



**Fig 4:** *Z. leuconotum* larva in its excavated tunnels. Arrow indicated entry hole. Larva imaged after removing from the tunnel is at the right side.



**Fig 5:** *Z. leuconotum* larva within the tunnel. Excretory pellets marked with red arrow. Entry hole marked with black arrow



**Fig 6:** Wilting of grapevine due to *Z. leuconotum*. Part of grape vine is removed to extract the larva

Though the new cossid wood borer *Z. leuconotum* is not reported on grape to our knowledge, the other moth wood borers belonging to same taxonomic position are reported by many authors. Chang (1984) [9] reported the Cossid coffee borer, *Zeuzera coffeae* Nietner as one of the most important

pests of grape vine in Taiwan.

Reports of carpenter moth larvae damage to grapevines most commonly refer to the cossid *Cossus Cossus* (Linnaeus) (Feron *et al.* 1996) [10], which has a long history as a polyphagous pest in southern Europe vineyards and orchards and often requires the application of cultural or chemical management practices (Lieutier *et al.*, 2004) [11]. Solaiman and Ali (2016) [12] recorded the Leopard moth, *Zeuzera pyrina* (L.) (Lepidoptera: Cossidae) as an economic importance on grape vine. Other known wood boring cossids include *Paropta paradoxus* (Herrich-Schäffer) that was recognized as grapevine pest in Israel and, more recently, in Turkey (Atay *et al.*, 2019) [13] and *Polyphagozerra coffeae* (Nietner, 1861), a polyphagous pest, feeding on more than 50 plant species, and reported to cause severe damage to grapevines in Taiwan when high population densities were left unchecked (Tavares *et al.*, 2020) [14]. In the 1980s, the cossid *Coryphodema tristis* (Drury) was documented to feed on and damage mature (>1-yr-old) grapevine wood in South Africa and was also associated with rot fungi that further damaged the vine (Hoppner and Ferreira, 1990) [15]. More recently, the carpenter moth *Dervishiya cadambae* (Moore, 1865) (Lepidoptera: Cossidae) was found boring in both the sapwood and heartwood of grapevine trunks in India, reducing vine vitality and productivity (Yadav *et al.* 2020) [8]. *Giviraethela* (Neumoegen and Dyar, 1893) a new California grape pest, belonging to the family of carpenter moths joined

the list of grapevine wood boring pests (Scaccini *et al.*, 2021)<sup>[1]</sup>.

Whereas the same wood borer, *Z. leuconotum* presently reported on *Tamarix chinensis*(Lour) which is a foundation plant species of salt marshes in northern China by Ning *et al.*, (2020)<sup>[16]</sup>. The authors described in detail on nature and symptoms of damage by the pest and its biology. The symptoms produced on grape vine by *Z. leuconotum* are similar to that produced on *T.chinensis*.

The caterpillar of the wood-borer makes a hole and feeds inside plant shoots and roots that heavily damage the xylem tissue of the. The caterpillar feeds inside of the stems and roots of the host plant (*T.chinensis*) throughout the larva stage, and excavates a tunnel network for their movement. The stem with yellow leaves indicates that the health of the plant is heavily infested by the wood-borer. Further the authors also detailed biology of *Z. leuconotum*.

The larva approximately has five instars, and exuviates at night, pupates at fifth instar old larvae, pupation time needs about 10–14 days. During its pupation, the fifth instar caterpillar forms a pupal chamber inside of the tunnel using its excreta and sawdust, and bites an oval emergence hole, while only leaves a thin layer of bark membrane as the cover of the hole. When the pupal development finishes, the adult emerges and flies out from the emergence hole with the assist of sea wind to some extent. During the adult stage, the females and males usually mate at night. Then, the fertilized females oviposit eggs in the crack of the plant bark. Generally, the life span of adults is approximately 7-10 days. This insect has one generation per year. The hatched larvae from eggs oviposited by adult females infect the stem, which cause the death of plants; the caterpillar moves to the roots of plant for overwintering when they reached the final stage and these caterpillars reactivate to feeding on xylem tissues from roots to another shoots of plants until the larvae were fully developed, and pupated inside of the plant stem until they developed into the adult.

The wood-borer insect is a native to China that feeds on a relative wide range of host plants. Previous observations have reported that this species could feed on the stem of many woody plants in terrestrial ecosystems, such as *Fraxinus chinensis* Roxb., *Robinia pseudoacacia* Linn., *Salix babylonica* Linn. (Liu *et al.* 2010)<sup>[17]</sup>.

In the present investigations the trees found in common around the 250 orchards visited were *Annona* sp, *Tamarindus* sp., *Phyllanthus* sp., *Terminalia* sp., *Psidium* sp., *Ficus* sp., *Citrus* sp., *Ziziphus* sp., *Prosopis* sp., *Azadirachta* sp., *Bauhinia* sp., *Cocos* sp. and *Manilkara* sp., any of the trees might harbored *Z. leuconotum*.

The present work reports *Z.leuconotum* as a new wood boring pest of grapevines in Karnataka state of India and updates its distribution in the area. Pest identification comprising molecular features helps in rapid and reliable identification of the pest and is a key tool for proper pest management. Nature and symptoms of damage are also presented for the further diagnosis of the new pest in a much easier way.

Management strategies should include all possible strategies, prior to which understanding its distribution on *V. vinifera* and other hosts if any is needed. Better monitoring tools like light traps and baited traps need to be adopted. Many of the cossids are known to attracted towards black lights, mercury vapor lights, and ultraviolet (UV) light traps. Traps baited with sex pheromones, a possible tool that could be developed, as mating disruption with sex pheromones has been used

against other carpenter moth species. Mass trapping has also been tested for the cossids *Zeuzera pyrina* (Linnaeus, 1761) and *C. Cossus*. Another case in the use of sex pheromones is for the cossid *Corypha dematristis* (Drury) on *Eucalyptus nitens* (H. Deane and Maiden) Maiden in South Africa, where the large-scale mass trapping suppressed this pest. Notably, the use of sticky UV-light traps in combination with sex pheromone traps successfully attracted *Z. pyrina* adults, where the light is attractive for both males and females. However, UV light traps are a wide-spectrum attractants in respect to moths, and they may have considerable effects on non-target insects and even vertebrates. For these reasons, monitoring methods should include highly specific traps in order to minimize their impact on non-target species (Scaccini *et al.*, 2021)<sup>[1]</sup>.

In a crop like grape, unlike field crops, 2-4% vine damage leads to huge economic losses. Hence there is an urgent need to understand possible routes of entry of this new wood borer into grape orchards and its bioecology.

**Disclaimer:** The authors declare no conflicts of interest.

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