# The Determination of Harmful Hymenoptera and Lepidoptera Species in Forest Nurseries in the Western Black Sea Region of Türkiye

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#### **Abstract**

*Aim of the study*: The aim of this study was to determine the harmful Hymenoptera and Lepidoptera species in some forest nurseries between 2021 and 2022.

*Area of study*: The study areas were selected from forest nurseries located in the Western Black Sea Region of Türkiye.

*Material and methods*: Visual inspections were performed on plants, then larvae and gall samples were collected by hand and photographed on host plants. Gall wasps were reared with the galls and examined by scanning electron microscopy. Collected caterpillars and sawfly larvae samples were placed with the host plant in an insect rearing cage for adult emergence under constant conditions. The oak gall wasp was also identified by molecular methods.

Main results: In this study, four species of Hymenoptera and six species of Lepidoptera were determined. These are Dryocosmus kuriphilus, Diprion pini, Craesus septentrionalis, Hyphantria cunea, Euproctis chrysorrhoea, Calliteara pudibunda, Dioryctria simplicella, Phalera bucephala and Acronicta rumicis. The oak gall wasp was identified as Cynips quercusfolii with 97.87% identity.

Research highlights: Amog these species, C. septentrionalis was new record for the insect fauna of the Western Black Sea Region. In addition, C. pudibunda and P. bucephala were reported for the first time from Kastamonu province.

Keywords: Forest Nurseries, Hymenoptera, Lepidoptera, Pests, Türkiye

## Türkiye'nin Batı Karadeniz Bölgesi Orman Fidanlıklarında Zararlı Hymenoptera ve Lepidoptera Türlerinin Belirlenmesi

Öz

*Çalışmanın amacı*: Bu çalışmanın amacı, 2021 ve 2022 yılları arasında bazı orman fidanlıklarında zararlı Hymenoptera ve Lepidoptera türlerinin belirlenmesidir.

Çalışma alanı: Çalışma alanı Türkiye'nin Batı Karadeniz Bölgesi'nden seçilen orman fidanlıklarıdır.

Materyal ve yöntem: Bitkiler üzerinde görsel incelemeler yapılmış, ardından larva ve gal örneklerinin konukçu bitkiler üzerinde fotoğrafları çekilerek elle toplanmış ve plastik bir kaba konulmuştur. Gal arıları gallerden çıkarılmış ve taramalı elektron mikroskobu ile incelenmiştir. Toplanan tırtıllar ve testere sinekleri larvaları sabit koşullar altında erginleşmeleri için konukçu bitkiler ile birlikte bir böcek yetiştirme kafesine yerleştirilmiştir. Meşe gal arısı moleküler yöntemler kullanılarak da teşhis edilmiştir.

Temel sonuçlar: Çalışmanın sonucunda Hymenoptera takımına ait 4 tür ve Lepidoptera takımına ait 6 tür belirlenmiştir. Bunlar, Dryocosmus kuriphilus, Diprion pini, Craesus septentrionalis, Hyphantria cunea, Euproctis chrysorrhoea, Calliteara pudibunda, Dioryctria simplicella, Phalera bucephala ve Acronicta rumicis'tir. Meşe gal arısı %97.87 benzerlik ile Cynips quercusfolii olarak tanımlanmıştır.

Araştırma vurguları: Bu türlerden C. septentrionalis Batı Karadeniz Bölgesi böcek faunası için yeni kayıt olmuştur. Ayrıca C. pudibunda ve P. bucephala Kastamonu ilinden ilk kez rapor edilmiştir.

Anahtar Kelimeler: Hymenoptera, Lepidoptera, Orman Fidanlığı, Türkiye, Zararlı Böcekler



#### Introduction

Forest ecosystems play a critical role to maintain biodiversity and provide diverse ecosystem services essential for the life (Brockerhoff et al., 2017; Jenkins & Schaap, 2018). It is well known that climate change disturbs the balance of forest ecosystems and natural habitats and affects the sustainable use of biodiversity (Sintayehu, 2018; Weiskopf et al., 2020; Zhou et al., 2021) and accelerates species extinction rates (Verma, 2021). In addition, the reproductive potential, development, survival and dispersal capacity of pest insects are directly impacted by climate change (Skendžić et al., 2021). In this context, afforestation studies are particularly important to ensure sustainability of forest areas under climate change. The quality of tree seedlings greatly influences the success of afforestation (Duan & Abduwali, 2021). This success depends on the development of high quality seedlings. Providing healthy seedlings is a critical requirement for successful implementing of forest nursery techniques and to protect the seedlings against various pests and diseases (Uslu, 1995).

Insect pests are one of the main causes of the severe decrease in productivity of plant species and cause great economic losses (Oliveira et al., 2014). These pests can negatively affect the production of high quality and quantity of seedlings in nurseries (Sutherland et al., 1989). Therefore, effective and periodic monitoring of these pests will help to detect problems early.

Lepidoptera and Hymenoptera are among the most diverse insect orders having more than 255 000 and 153 000 species in the world respectively (Peters et al., 2017; Gunathunga et al., 2022). Some Lepidoptera and Hymenoptera species are known as important pests and insect defoliators in forest ecosystems (Rasplus et al., 2010; Sial et al., 2017; Wagner et al., 2008).

Insect pests seriously affect the growth, production and survival of plant species in forest nurseries (Sutherland et al., 1989; Jacob et al., 2018). To manage insect damages it is necessary to know which species attacking and causing damages to plants in forest nurseries.

A few studies have been conducted about the damage of insect species in nurseries around the Türkiye (Kahraman, 1993; Uslu, 1995; Çüşen, 2007; Aslan, 2018). The Lepidoptera and Hymenoptera fauna of forest nurseries in the Western Black Sea Region have been studied poorly. Therefore, the aim of this study was to contribute to the knowledge of the harmful Hymenoptera and Lepidoptera fauna of forest nurseries in the Western Black Sea Region of Türkiye.

#### **Materials And Methods**

Study Area

The study was carried out in six forest nurseries in Düzce, Bolu, Kastamonu and Sinop provinces located in the Western Black Sea Region of Türkiye. In these studied nurseries, some native forest tree species saplings and some exotic ornamental plants used grown and especially reforestation activities for both the region and other parts of Türkiye. Cedrus libani (A. Rich.), Cornus mas (L.), Corylus colurna (L.), Malus sylvestris ((L.) Mill.), Mespilus germanica (L.), Picea abies ((L.) H. Karst.), Picea pungens (Engelm), Pinus nigra (Arnold.), P. nigra ssp. pallasiana var. seneriana, Pinus sylvestris (L.), P. sylvestris var. compacta, Platycladus orientalis ((L.) Franco), Pyrus elaeagnifolia (Pall.), Rosa canina (L.), Tilia cordata (Mill.), and Thuja occidentalis (L.) are produced in Bolu forest nursery. In Pinar forest nursery; Acer negundo (L.), Castanea sativa (Mill.), Cupressus arizonica (Greene), Cupressus sempervirens (L.), Fagus orientalis (Lipsky), Hypericum perforatum (L.), Juglans regia (L.), Laurus nobilis (L.), Lavandula sp. (L.), Ligustrum vulgare (L.), Matricarya recutita (L.), Melisa officinalis (L.), Origanum onites (L.), P. nigra, Robinia pseudoacacia (L.), and Tilia sp. (L.) are produced. A. pseudoplatanus (L.), Betula pendula (Roth.), C. colurna, Echinacea purpurea (L.), Fraxinus excelsior (L.), Picea orientalis ((L.) Link), P. abies, P. nigra, P. sylvestris (L.), Salvia officinalis (L.), and T. cordata are produced in Muzaffer Büyükterzi forest nursery. In Daday forest nursery; A. pseudoplatanus, Abies sp. (L.), A. negundo, C. colurna, C. libani, E. purpurea, F. excelsior, J. regia, P. abies, Picea orientalis,

P. nigra, P. sylvestris, Populus nigra (L.), Prunus cerasifera (Ehrh.), P. elaeagnifolia, Prunus dulcis (Mill.), Quercus robur (L.), R. pseudoacacia, S. officinalis, and T. cordata are produced. In Gölköy forest nursery; C. libani, J. regia, Lavandula sp. (L.), Malus sp. (Mill.), P. pungens, P. orientalis, P. cerasifera, Q. robur, R. pseudoacacia, Thuja orientalis (L.), and T. cordata are produced. Finaly, in Sinop forest nursery, Arbutus unedo (L.), Cerasus avium ((L.) Moench.) C.

sativa, C. mas, C. arizonica, C. sempervirens, Elaeagnus angustifolia (L.), Eucalyptus camaldulensis (Dehnh.), L. nobilis, Ligustrum vulgare (L.), Hibiscus syriacus (L.), J. regia, Morus alba (L.), Q. robur, P. abies, Pinus pinaster (Aiton), P. dulcis, Prunus laurocerasus (L.), and Vaccinium myrtillus (L.) are produced.

The selected nurseries and their geographical location were shown in Table 1.

Table 1. Geographic coordinates of the study locations

Provinces	Name of Nurseries	Coordination				
		Latitude	Longitude	Altitude (m)		
Düzce	Pınar Forest Nursery	40°48'54.72"	31°13'58.70"	199		
Bolu	Bolu Forest Nursery	40°42'10.12"	31°34'58.38"	718		
	Daday Forest Nursery	41°28'37.70"	33°31'10.44"	844		
Kastamonu	Gölköy Forest Nursery	41°27'0.44"	33°45'53.64"	732		
	Muzaffer Büyükterzi Forest Nursery	41°24'17.58"	34°22'29.03"	1170		
Sinop	Sinop Forest Nursery	41°56'29.30"	34°59'35.89"	81		

In field studies, plant materials were examined visually along approximately 100 - 120 m long nursery beds in forest nurseries, and photographed on host plants (Figure 1). Photographed caterpillars and sawfly larvae

specimens were collected by hand and put into a labeled plastic container individually. Sampling works were performed between 9.00 am and 3.00 pm monthly between May and September during 2021-2022.



Figure 1. Insect damage to (A) Corylus colurna in Taşköprü district of Kastamonu. (B) Tilia cordata in Daday district of Kastamonu. (C) Platanus orientalis in Düzce. (D) Cornus mas in Sinop. (E) Malus sp. in Gölköy village of Kastamonu. (F) Tilia cordata in Taşköprü district of Kastamonu

In addition, galls were detected on parts of plants such as branches and leaves in forest nurseries in Düzce and Sinop provinces (Figure 2). In Tasköprü, 10 *Pinus* 

*sylvestris* L. were randomly selected in September and damaged cones were collected from the selected pines.



Figure 2. (A) Oak galls on leaves in Sinop. (B) Chestnut galls on branches in Düzce provinces. (C) Damaged pine cones in Taşköprü

#### Laboratory Studies

The caterpillars and sawfly larvae were respectively placed in an insect rearing cage (60x60x70) together with the plant parts. In order to obtain the adult gall wasp, plant leaves and buds were brought into the laboratory. The cynipids reared on galls at room temperature (20-25°C) in jars covered by tulle. The specimens were observed using an Olympus SZ51 microscope and identified by using related literatures (Aslan, 2018; Azmaz, 2015; Cetin et al., 2014; Dar et al., 2019; EPPO, 2005; İlçin, 2022; İpekdal & Avcı, 2023; Karaca & Katılmış, 2020; Katılmış, 2010; Kondur & Şimşek, 2018; Morimoto & Pietras, 2020; Sarıkaya et al., 2019; Sourakov & Paris, 2011; Şimşek & Kondur, 2016; Tuncer et al., 2020). Adult gall wasps were examined with a FEI Quanta FEG 250 scanning electron microscope (SEM) set at 20 kV.

#### Molecular Studies

Identification based on morphology can be difficult and insufficient to distinguish some Cynipini species (Tavakoli et al., 2019). Therefore, both adult morphology and molecular methods were used for the accurate identification of species (Liu et al., 2017; Tavakoli et al., 2019). The molecular analysis were carried out in the BM Labosis laboratory, Ankara. Before DNA extraction. specimens were identified morphologically using the literatures (Melika & Abrahamson, 2002; Mete, 2009). For DNA isolation, the EurX GeneMATRIX Tissue & Bacterial DNA isolation kit (Poland) was used. Spectrophotometric measurement was performed on the Thermo Scientific Nanodrop 2000 device (USA) to assess concentration and purity of DNA. Then, the 18S rRNA genes were amplified. 5'-

GGTCAACAAATCATAAAGATATTGG-3' (LCO-F) and TAAACTTCAGGGTGACCAAAAAATCA -3' (HCO-R) primers were used to replicate the target region for species identification. One-step PCR was performed to amplify the target region of approximately 1000 bases. The reaction was performed with Solis BioDvne (Estonia) FIREPol® DNA Polymerase Tag polymerase enzyme. PCR conditions were listed in Table 2.

Table 2. PCR protocol

Stock Concentration	Final Concentration
10X	1X
25mM	1.5 mM
20 mM	0.2 mM
10 μΜ	0.3 μΜ
10 μM	0.3 μΜ
5U/ μl	$2\dot{\mathrm{U}}$
3 μ	μl
	10X 25mM 20 mM 10 μM 10 μM 5U/ μl

Completed to 35 µl with PCR grade water

PCR reaction conditions were 5 minutes initial denaturation at 95°C, 40 cycles (45 seconds denaturation at 95°C, annealing at 57°C for 45 seconds, extension at 72°C for 60 seconds), and final extension at 72°C for 5 minutes. The temperature was reduced to 4°C and the PCR was completed. The MAGBIO "HighPrep™ PCR Clean-up System" (AC- 60005) kit was used for the purification stage of the PCR product. The ABI 3730XL Sanger sequencing instrument BigDye Terminator and v3.1 Cycle Sequencing Kit were performed in the Macrogen Netherlands laboratory for Sanger sequencing. Reads obtained with the LCO-F - HCO-R primers were contiguous to form a consensus sequence. For this purpose, the CAP contig assembly algorithm was used in BioEdit software. The comparison of similarity between sequences was performed based on Nucleotide BLAST (basic local alignment search tool) program of the NCBI database

(https://blast.ncbi.nlm.nih.gov/Blast.cgi?PRO GRAM=blastn&PAGE\_TYPE=BlastSearch &LINK\_LOC=blasthome). The maximum likelihood method in Mega11 (Molecular Evolutionary Genetics Analysis Version 11) software was used for phylogenetic analyses (Tamura et al., 2021).

#### **Results and Discussion**

In this study, 10 harmful insect species belonging to 7 families and 2 orders were obtained from different nurseries. These species were given in Table 3.

Table 3. Harmful Hymenoptera and Lepidoptera species found in forest nurseries in the Western Black Sea Region of Türkiye

Species	Host Species	M./Y.*	Study Area**					
Species			T	D	GL	S	DΖ	В
Cynips quercusfolii	Quercus robur	Sep./2022				+		
Dryocosmus kuriphilus	Castanea sativa	May/2022					+	
Diprion pini	Pinus sylvestris	Sep./2022	+					
Craesus septentrionalis	Corylus colurna	Jun, Aug., Sep./2022	+	+				
Hyphantria cunea	Acer negundo/Platanus orientalis	Jun, July, Aug./2021, 2022					+	
Euproctis chrysorrhoea	Cornus mas	Sep./2021Jun/2022				+		
Calliteara pudibunda	Tilia cordata	Sep./2022	+					
Acronicta rumicis	Picea orientalis/Malus sp.	Jun, Sep./2022			+			+
Phalera bucephala	Tilia cordota	July, Aug., Sep./2022	+	+				
Dioryctria simplicella	Pinus sylvestris	Sep./2022	+					

Abbreviation: \*M: Month, Y: Year, Sep: September, Aug: August, \*\*T: MUZAFFER BÜYÜKTERZİ FOREST NURSERY (F.N.) (TAŞKÖPRÜ), D: DADAY F.N., GL: GÖLKÖY F.N., S: SİNOP F.N., DZ: PINAR F.N. (DÜZCE), B: BOLU F.N.

In May 2022, chestnut gall wasps (*Dryocosmus kuriphilus* Yasumatsu, Hymenoptera: Cynipidae) were collected

Pınar forest nursery (Düzce). SEM images of *D. kuriphilus* were given in Figure 3.

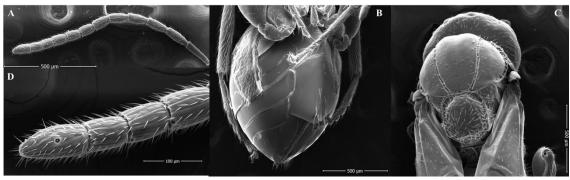


Figure 3. *Dryocosmus kuriphilus* Yasumatsu, 1951. (A-D) Detailed and general view of the antenna showing the flagellum. (B) Metasoma in lateral view. (C) Mesosoma in dorsal view

In September 2022, oak gall wasps were collected in Sinop forest nursery and

observed using SEM (Figure 4).

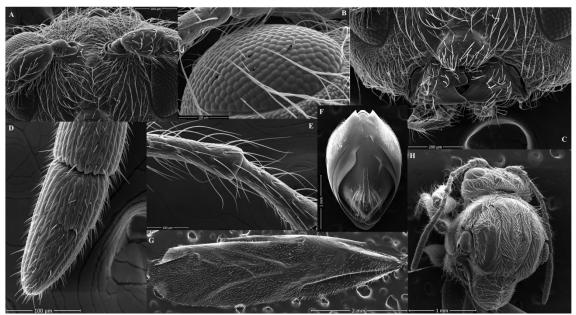


Figure 4. *Cynips quercusfolii* Linnaeus, 1758. (A-C) Head, anterior view. (B) External morphology of compound eyes. Arrows indicate interfacetal hairs. (D-E) Detailed view of the antenna showing the flagellum. (F) Metasoma in ventral view. (G) Fore wing. (H) Mesosoma in dorsal view

According to the molecular study results, the oak gall wasp was identified as *Cynips quercusfolii* Linnaeus, 1758 (Hymenoptera: Cynipidae) with a total of 677 bases, 97.87%

identity. The phylogenetic trees generated by MEGA11 based on the maximum likelihood method showed 93% similarity to *Cynips quercusfolii* (Figure 5).

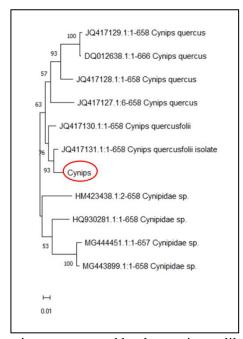


Figure 5. Phylogenetic tree generated by the maximum likelihood method

Craesus septentrionalis Linnaeus, 1758 (Hymenoptera: Tenthredinidae) larvae were collected from Daday forest nursery (Daday-Kastamonu) in June 2022. This species was also collected from the Muzaffer Büyükterzi forest nursery (Taşköprü-Kastamonu) in August and September 2022. Hyphantria cunea Dry. (Lepidoptera: Erebidae) were collected in Pınar forest nursery from June to August in 2021 and 2022 (Figure 6). In Türkiye, the larvae caused damage to some hazelnut orchards in Marmara region (Sakarya Kocaali, Karasu etc.) between 2015 and 2016. It has been reported that the plants may remain leafless as a result of the larvae feeding of C. septentrionalis on hazelnut leaves (Tuncer et al., 2020).

Euproctis chrysorrhoea Linnaeus, 1758 (Lepidoptera: Erebidae) were collected from

Sinop forest nursery in September and June in 2021 and 2022. Acronicta rumicis Linnaeus, 1758 (Lepidoptera: Noctuidae) were collected from Bolu forest nursery in June 2022 and from Gölköy forest nursery (Gölköy-Kastamonu) in September 2022. Diprion pini Linnaeus, 1758 (Hymenoptera: Diprionidae) and Calliteara pudibunda Linnaeus, 1758 (Lepidoptera: Erebidae) were collected from Taşköprü in September 2022. Dioryctria simplicella Heinemann, 1863 (Lepidoptera: Pyralidae) and Phalera bucephala (Linnaeus, 1758) (Lepidoptera: Notodontidae) were collected from Taşköprü in September 2022. In addition, P. bucephala larvae were collected from Daday in July, August and September 2022.

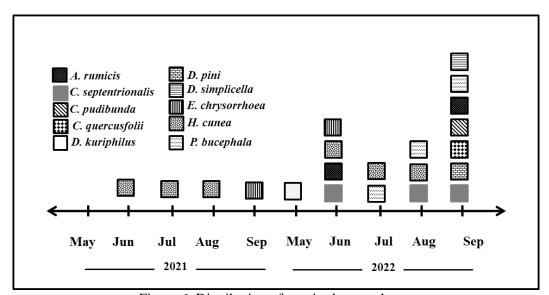


Figure 6. Distribution of species by months

Among the harmful insects found in this study, the Asian chestnut gall wasp, *Dryocosmus kuriphilus* is an alien species to Europe and attacks species of the genus *Castanea* worldwide (EFSA, 2010; Castedo-Dorado et al., 2023). In Türkiye, *D. kuriphilus* was detected for the first time in Yalova in 2014 (Çetin et al., 2014) then it has been expanded its geographical ranges and reported from different provinces of Yalova, Bursa, İstanbul, Sakarya, Kocaeli, Balıkesir, Bilecik, Düzce, Giresun, Bartın, Zonguldak, Sinop and İzmir (Mıcık & İpekdal, 2021; Yıldız et al., 2020). It has

been reported that heavy gall wasp infestations can reduce chestnut production by up to 80% (Battisti et al., 2014; Castedo-Dorado et al., 2023).

The fall webworm, *Hyphantria cunea* is native to North America but has rapidly spread to central Europe and eastern Asia (Edosa et al., 2018; Ning et al., 2022). In 1975, it was recorded for the first time in Edirne, İstanbul (Çatalca, Silivri) and Tekirdağ in Türkiye (Baş, 1982). The larvae of *H. cunea* can feed on more than 600 host plant species and cause significant economic losses (Lu et al., 2017; Moon et al., 2022;

Ning et al., 2022). In Türkiye, *H. cunea* is considered as an economically important pest in hazelnut plantations, orchards and broadleaved forests (Işık & Yanılmaz, 1992).

The brown-tail moth, *Euproctis chrysorrhoea* causes great damage to agricultural and forest areas in the world (İlçin, 2022). It is native to Europe and has spread almost everywhere in Türkiye (Hussain et al., 2019; İlçin, 2022). This pest caused 50-90% yield losses in villages of Ladakh in India (Hussain et al., 2019).

Another important species, the pale tussock moth, Calliteara pudibunda is native to the Northwestern Palearctic region. It has been detected in the provinces of Artvin, Balıkesir, Bursa, Düzce, Giresun, Hatay, Kütahya, Malatya, İstanbul. Sakarya, Samsun, and Yalova in Türkiye (Ipekdal & Avcı, 2023). It caused damage on alder and birch trees in 2005 in Türkiye (Göktürk & Aksu, 2005). C. pudibunda is a defoliating species that consumes leaves or needles (Ipekdal, 2022; Ipekdal & Avcı, 2023). Sarıkaya et al. (2019) reported the outbreak occurred in oriental beech (Fagus orientalis Lipsky) forests of İnegöl (Bursa), Türkiye between 2018 and 2019. In addition, it has been estimated that the potential distribution areas of C. pudibunda has been gradually increasing.

Pine sawflies (Hymenoptera: Diprionidae) are one of the serious pests causing economic damages in conifer forests (Blomqvist et al., 2022). In Finland, the economic impact of *Diprion pini* was reported to be €288 per hectare after one-year outbreak (Lyytikäinen-Saarenmaa & Tomppo, 2002). Kulman (1971) reported 2-3% tree mortality following a single-year outbreak of *D. pini*. However, mortality rate could reach 60-75% after outbreaks in two consecutive years (Långström et al., 2001).

Dioryctria Zeller (Lepidoptera: Pyralidae) species cause a significant damage to cones and seeds (Aslan, 2018; Bracalini et al., 2013; Leal-Sáenz et al., 2021). Damage caused to tree cones by *Dioryctria* spp. often leads to serious economic losses (Roe et al., 2006). In Türkiye, *D. abietella* Denis & Schiffermüller, 1775 caused 80-100% damage to cones of *Picea orientalis* (L.) Link. stands in Meryem Ana-Trabzon in

September 1975 (Çanakçıoğlu & Mol, 2000). Aytar (2001) reported that the size of damaged area by *Dioryctria splendidella* Herrich-Shaeffer reached 390.0 ha in 1997, 1707.0 ha in 2000, and 1704.0 ha in 2001 in *P. brutia* forests of Adana and Mersin Forest Regional Directorate. In addition, Aslan (2018) reported that the larvae of *D. simplicella* caused damage to *P. sylvestris* in Kastamonu in Turkey. In northern Arizona, *Dioryctria auranticella* Grote destroyed 38-81% of *Pinus ponderosa* Doug. ex Laws. cones in 1984 (Blake et al., 1989).

#### Conclusion

In this study, harmful Lepidoptera and Hymenoptera species in forest nurseries in the Western Black Sea Region of Türkiye were investigated. At the end of the study, four species of Hymenoptera and six species of Lepidoptera were determined. Craesus septentrionalis was a new record for the insect fauna of the Western Black Sea Region. In Türkiye, the first record of C. septentrionalis was made by Benson (1968) in Trabzon. Later, this species was reported from Edirne, İstanbul, Ankara and Erzurum (Çanakçıoğlu & Mol, 1998; Çalmaşur & Özbek, 2004). Calliteara pudibunda and Phalera bucephala were reported for the first time from Kastamonu province.

In the current study, the highest number of insect species was recorded in Muzaffer Büyükterzi forest nursery located in Taşköprü province of Kastamonu. The number of species collected was highest in September 2022 (Figure 6).

To produce high quality seedlings for afforestation activities, it is necessary to protect tree seedlings from harmful insects in forest nurseries. It is crucial to monitor insect populations regularly and to implement control techniques against harmful insects. The results of this study may contribute to concerned persons and governmental institutions.

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# Ethics Committee Approval N/A

## Peer-review

Externally peer-reviewed.

#### **Author Contributions**

Conceptualization: B.Y., N.Ö.; Investigation: N.Ö., İ.B.; Material and Methodology: N.Ö., B.Y., İ.B., S.A.; Supervision: B.Y., S.A.; Visualization: N.Ö.; Writing-Original Draft: N.Ö.; Writingreview & Editing: N.Ö., B.Y., İ.B., S.A.; Other: All authors have read and agreed to the published version of manuscript.

#### **Conflict of Interest**

The authors have no conflicts of interest to declare.

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