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Leptoglossus occidentalis Heidemann, 1910 (Hemiptera: Coreidae) in Türkiye: Its distribution, life cycle, and fungal associations

Türkiye'de *Leptoglossus occidentalis* Heidemann, 1910 (Hemiptera: Coreidae): Yayılışı, biyolojisi ve funguslarla ilişkisi

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Abstract

In this study, the distribution, life cycle and associations with fungi of the alien invasive species *Leptoglossus occidentalis* Heidemann, 1910 (Hemiptera: Coreidae) in Türkiye were investigated. During the study, conducted between 2019 and 2021, the pest was found to have spread across 35 provinces in Türkiye. The biological data obtained in the study were divided into ecologically similar sub-regions (Ankara-Çankırı-Kırşehir, Artvin, Isparta-Burdur, Izmir-Aydın-Manisa-Muğla-Antalya) in order to reveal the life cycle of the species. Additionally, host plant and insect samples were examined for the presence of *Diplodia sapinea* (Fr.) Fuckel (pine shoot blight agent) and entomopathogenic fungi species through morphological and molecular identification methods. Consequently, it was found that *Leptoglossus occidentalis* has two generations in Türkiye. The timing of the first flight was in early May-mid June and the second generation was from mid-July to late August. Although *Diplodia sapinea* was detected in some of the sampled host plant tissues, it could not be isolated from *Leptoglossus occidentalis* individuals. Accordingly, it did not find any evidence that *Leptoglossus occidentalis* vectors *Diplodia sapinea* although the insect and the fungus cooccurs in sampling areas. Although it was detected 25 fungal species isolated from *Leptoglossus occidentalis* adults, none of them were entomopathogenic fungal species. It has been revealed that the pest has spread almost all over Türkiye and has become an important risk factor affecting seed yield and quality in coniferous species.

Keywords: Western conifer seed bug, alien invasive species, biology, *Diplodia sapinea*, Türkiye

Öz

Bu çalışmada yabancı istilacı bir tür olan *Leptoglossus occidentalis* Heidemann, 1910 (Hemiptera: Coreidae)'in Türkiye'deki yayılışı, biyolojisi ve funguslarla ilişkisi araştırılmıştır. 2019-2021 yılları arasında yürütülen bu çalışma boyunca 35 ilde zararlının yayılışı tespit edilmiştir. Çalışmada elde edilen biyolojik veriler, yoğun olarak örnekleme yapılan yerlerde ve ekolojik olarak benzer alt bölgelere (Ankara-Çankırı-Kırşehir, Artvin, Isparta-Burdur, İzmir-Aydın-Manisa-Muğla-Antalya) ayrılarak türün biyolojisi ortaya konulmuştur. Ayrıca, konukçu bitki ve böcek örnekleri *Diplodia sapinea* (Fr.) Fuckel (çam sürgün yanıklığı etmeni) ve entomopatojenik fungus türlerinin varlığı açısından morfolojik ve moleküler tanımlama yöntemleriyle incelenmiştir. Sonuç olarak Türkiye'de türün iki generasyona sahip olduğu belirlenmiştir. Birinci uçma zamanı mayıs başı-haziran ortası, ikinci generasyonun ise temmuz ortasından ağustos sonuna kadar olduğu tespit edilmiştir. Örneklenen bitki dokularının bazılarında *D. sapinea* tespit edilmiş olmakla birlikte, *Leptoglossus occidentalis*'ten izole edilememiştir. Buna göre, örnekleme alanlarında böcek ve fungus birlikte bulunmasına rağmen *Leptoglossus occidentalis*'in *Diplodia sapinea*'nın vektörü olduğuna dair herhangi bir kanıt bulunmamıştır. *Leptoglossus occidentalis*'ten izole edilen 25 mantar türü tespit edilmesine rağmen, tamamının entomopatojen fungus olmadığı belirlenmiştir. Zararlının Türkiye'nin hemen hemen tamamına yayıldığı ve ibreli türlerde tohum verimi ve kalitesi üzerinde önemli bir risk etmeni haline geldiği ortaya konulmuştur.

Anahtar Kelimeler: Çam kozalak emici böceği, yabancı istilacı tür, yaşam döngüsü, *Diplodia sapinea,* Türkiye

1. Introduction

The course of spread, life cycle, damage, and adaptation to new ecosystems of a newly introduced species may differ from the insect's native area. Favorable climatic parameters in the new ecosystem can lead to changes in the life cycle characteristics of the alien insect species, such as the number of generations and ovipositions, mortality because of warmer winters and/or lower natural enemy pressure. As a result, establishment success and damage level of the alien insect species can significantly increase in the new ecosystem (Richardson and Rejmánek, 2011; Liebhold et al., 2017).

Western conifer seed bug, *Leptoglossus occidentalis* Heidemann, 1910 (Hemiptera: Coreidae) is native to western North America. Its first record out of North America was from Italy in 1999 (Villa et al., 2001). It expanded its range in Europe rapidly (Barta, 2009; Petrakis, 2011; Putshkov et al., 2012). It was found in Asia in 2008 (Japan, Ishikawa and Kikuhara, 2009), Africa in 2011 (Tunisia, Ben Jaama et al., 2013) and South America in 2017 (Chile, Faúndez and Rocca, 2017). It was first recorded in Türkiye in September 2009 in İstanbul-Sarıyer (Arslangündoğdu and Hızal, 2010) and October 2009 in Edirne (Fent and Kment, 2011). It spread rapidly in Türkiye and has been found so far in Afyonkarahisar, Ankara, Antalya, Artvin, Balıkesir, Bilecik, Bolu, Burdur, Bursa, Çorum, Denizli, Düzce, Edirne, Elâzığ, Erzincan, Eskişehir, Giresun, Isparta, İzmir, Karabük, Kastamonu, Kayseri, Kırklareli, Kütahya, Manisa, Muğla, Ordu, Osmaniye, Sakarya, Samsun, Sinop, Tekirdağ, Tokat, Uşak, Yalova, Yozgat and Zonguldak (Hızal and İnan, 2012; Yıldırım et al., 2013; Çerçi and Koçak, 2016; Dursun, 2016; Özgen at al., 2017; Oğuzoğlu and Avcı, 2020; Çerçi et al., 2021; Kalkan et al., 2021).

Voltinism of *L. occidentalis* seems to be determined mainly by temperature (Tamburini et al., 2012). In North America, it has one generation per year; however, it can have up to three generations per year in Central America and Europe (İpekdal et al., 2019 and references therein). In Türkiye, it has two generations in the southwest (Oğuzoğlu and Avcı, 2020). However, İpekdal (2022) showed theoretically that *L. occidentalis* can potentially have one to five generations per year depending on the locality and altitude in Türkiye. The host plants of the species recorded so far in Türkiye are *Abies* sp., *A. concolor*, *Pinus brutia*, *P. nigra*, *P. pinea*, *P. radiata* and *P. sylvestris* (Arslangündoğdu and Hızal, 2010; Hızal and İnan, 2012; Hızal, 2012; Dursun, 2016; Özek and Avcı, 2017; Parlak, 2017; Özgen et al., 2017; Oğuzoğlu and Avcı, 2020).

The damage of the species has come to the agenda with the decrease in pine nut yield especially in Bergama, İzmir. Feeding on conifer seeds, it is not only an important economic pest due to pine nut loss, but it reduces the rate of natural regeneration potential of coniferous forests. It is also known to vector some fungal agents, especially the pine shoot blight agent, *Diplodia sapinea* (Luchi et al., 2012).

2. Material and Methods

2.1. Studies on the distribution and life cycle of *L. occidentalis*

In order to determine the distribution of the pest, we carried out field studies at a total of 366 points in 35 provinces in Aegean, Black Sea, Central Anatolia, Marmara, and Mediterranean Regions of Türkiye. Field visits were conducted in forest areas, urban parks and gardens between April and November from 2019 to 2021. We checked the cones on coniferous hosts for the presence of adults and nymphs, and needles for eggs. We recorded the location, date, coordinates (WGS84), elevation (m), and host tree species.

In order to determine the life cycle and voltinism of the pest, we carried out field observations in Aegean, Marmara, and Mediterranean Region (Antalya, Aydın, İzmir, Manisa, Muğla), Black Sea Region (Artvin), Central Anatolia Regions (Ankara, Çankırı, Kırşehir) and Inner Western Mediterranean Region (Burdur, Isparta). During regular field visits two to three times per month between April and November from 2019 to 2021, we recorded the number of eggs, nymphs, and adults along with the locality and date.

2.2. Studies on the fungal associations of *L. occidentalis*

In order to determine the role of *L. occidentalis* as a vector of *D. sapinea*, and to identify entomopathogenic fungal species associated with the pest insect, we carried out the following studies.

2.2.1. Plant and insect sampling

We carried out the samplings in the summer of 2020. The locations where the samples were collected and information about the samples are given in Table 1. Among the shoots with cones on which *L. occidentalis* was detected, at least four shoot samples per tree were collected from one to three different trees. We took care to collect healthy shoots with last year's needles and one or two old cones. If tip blight or desiccation was observed on

the shoots, cone samples (symptomatic samples) were collected from the shoot and the tree or from the ground in order to determine whether these desiccations were caused by *D. sapinea*.

For fungus isolation, three shoot samples and immature cones were separated per tree in the laboratory. For molecular studies, only one shoot and one immature cone were separated from each tree, placed in a ziplock bag and stored at -20 °C. Symptomatic specimens and opened cones were allowed to dry, then placed in paper bags and stored in a dry, moisture-free environment until examination.

2.2.2. Morphological detection of *D. sapinea* from plant samples

We carried out fungus isolation studies from shoot, cone and seed samples by using classical culturing method in a tannic acid medium through the following steps: surface sterilization, culturing in medium, and examination of isolated fungi.

Shoot samples were cut into 10-15 cm lengths and the needles were cleaned (Figure 1a). The surface was then subjected to sterilization (Figure 1b). During this process, the samples were firstly soaked in 4% NaOCl for 10 min, then washed twice for 5 min each in sterile distilled water and allowed to dry on blotting paper under aseptic conditions. After surface sterilization, approximately 1 cm pieces were cut from the shoots, vertically cut in half and placed in the medium. Buds were subjected to the same treatment. One- and two-year-old cones were first vertically cut in half and then cut into as small as possible slices and transferred to the medium (Figure 1c). Seeds were extracted from the cones under sterile conditions and placed on the medium (Figure 2). Mycelia emerging from seeds were subcultured to fresh PDA and cultures incubated at 22°C for morphological and molecular identifications. Isolates were examined under a compound microscope and grouped according to morphological characteristics of *Diplodia* species.

2.2.3. Molecular detection of *D. sapinea* from plant and insect samples

In order to detect *D. sapinea* in plant and insect samples by molecular methods, we carried out the following studies: surface sterilization of plants and insects, DNA isolation from plants and insects, and PCR.

Surface of *L. occidentalis* adults and healthy shoot samples on which the insects were found were sterilized following the methods described by Stanosz et al. (2001).

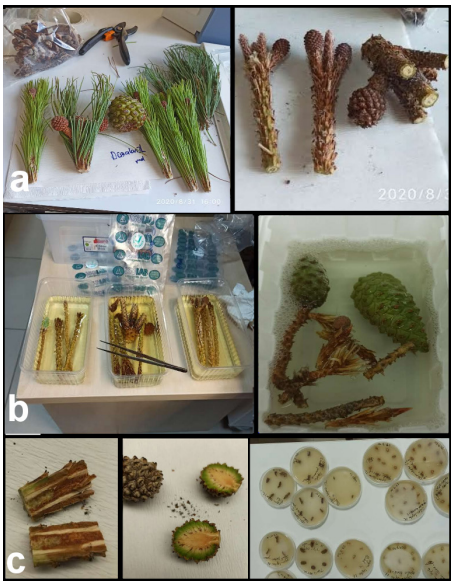

Figure 1. Preparation of shoot samples for surface sterilization (a), soaking the samples in NaOCl (b), dividing the shoots, buds and young cones into small pieces under aseptic conditions and culturing in media (c)
Şekil 1. Sürgün örneklerinin yüzey sterilizasyonuna hazırlanması (a), örneklerin yüzey sterilizasyonu için NaOCl'de bekletilmesi (b) ve sürgün, tomurcuk ve genç kozalakların aseptik koşullarda parçalanması ve besi ortamına ekimi (c)

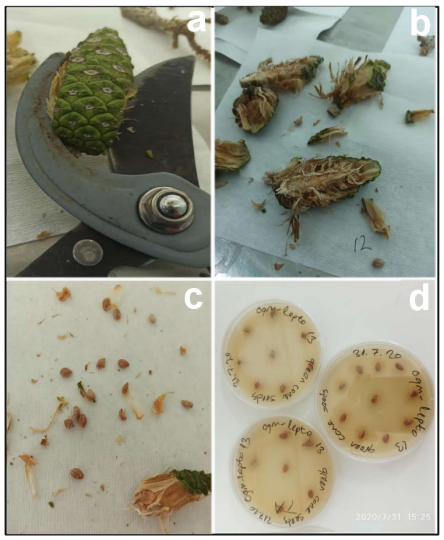

Figure 2. Extraction of seeds from surface sterilized cones under aseptic conditions and culturing in media
Şekil 2. Yüzey sterilizasyonuna tabi tutulan kızılçam kozalaklarından tohumların aseptik koşullarda çıkarılması ve besi yerine ekimi

DNA extraction was performed on the powdered branch and insect samples using the QIAamp DNA Stool Mini Kit (Qiagen, Germany). The DNA was cleaned with GeneJET Genomic DNA Purifica-

tion Kits (Thermo Fisher Scientific). The amount and purity of DNA obtained were measured at 260 nm wavelength in spectrophotometer. The average amount of DNA obtained from plants and insects was determined as 110 ng/µl.

Primers (DpF, DpR) and probes (DpP), and PCR conditions given by Luchi et al. (2005) were used. All PCRs were conducted by using a BioRad Mini Cycler and 300 nm from each primer, 200 nm fluorogenic probe, 12.5 µl TaqMan Master mix and 5µl DNA (totally 25 µl). For each DNA sample, three replicates were amplified.

DNA sample from *D. sapinea* TrPb01 isolate available in the laboratory was used in the standard curve. The standard DNA concentration was measured as 90 ng/µl at 260 nm. Using eight dilutions of this DNA, standard spots (100, 20, 4, 0.8, 0.16, 0.032, 0.0064, and 0.0013 ng) were generated. In RT-PCR cycles, the Ct value was defined as the point at which the reporter fluorescence signal was first recorded.

Table 1. Sampling localities where insects, pine shoots and cones were collected for the detection of *Diplodia sapinea* (n.a.: not available)
Tablo 1. *Diplodia sapinea*'nın tespiti amacıyla toplanan çam sürgün ve kozalak örneklerine ve örnekleme lokasyonlarına ait bilgiler (n.a.: bilgi yok)

Region	Province	Coordinates	*L. occidentalis* presence -density	Host species	Date	Number of sampled trees	Sample no
Aegean	İzmir	38°27'82" N 28°02'92" E	present – n.a.	*Pinus brutia*	25.06.2020	7	1-7
	İzmir, Foça, Kozbeyli	38°42'35" N 26°53'48" E	present - high	*Pinus brutia*	07.08.2020	3	17-19
	İzmir, Bergama, Kozak	39°15'24" N 27°08'42" E	present - n.a.	*Pinus pinea*	08.08.2020	4	20-23
Central Anatolia	Çankırı, Eldivan	40°30'51" N 33°26'06" E	present - low	*Pinus nigra*	08.09.2020	3	24-26
	Çankırı, Central	40°35'45" N 33°36'28" E	present - low	*Pinus nigra*	02.09.2020	2	27-28
Mediterranean	Isparta	37°53'47" N 30°23'03" E	present - medium	*Pinus nigra, Pinus brutia*	26.07.2020	5	8-12
	Antalya, Manavgat, Sarılar	36°47'54" N 31°24'46" E	present - n.a.	*Pinus brutia*	28.07.2020	1	13
	Antalya,Aksu, Kurşunlu	37°02'51" N 30°48'10" E	n.a.	*Pinus brutia*	28.07.2020	1	14
	Antalya, Aksu	37°06'13" N 30°48'33" E	n.a..	*Pinus brutia*	28.07.2020	1	15
	Antalya,Aksu, Yeşilkaraman	37°06'13" N 30°48'33" E	n.a.	*Pinus brutia*	28.07.2020	1	16

2.2.4. Molecular detection of entomopathogenic fungi from insect samples

Dead and unhealthy insects were brought to the laboratory in separate sample containers. In the laboratory, their surfaces were sterilized in 1% NaOCl for 5 min, then rinsed three times with distilled water and placed in Petri dishes containing moist filter paper. The insects were incubated at 25°C for one week and during the incubation period, the adults were checked daily for fungal growth.

Prior to DNA isolation, fungal isolates were grown on Malt extract agar medium with a cellophane membrane for one week at 24°C. The mycelia were scraped with a sterile scalpel and lysed with liquid nitrogen and DNA extraction was performed with a commercial kit (E.Z.N.A Plant Mini Kit Omega).

Twelve fungal DNA extracts were amplified for their ITS1-5.8S-ITS2 regions between 18S and 23S rRNA subunits by using 5'- TCCGTAGGTGAACC TGCGG-3' and 5'TCCTCCGCTTATTGATATATC G- 3' primers (White et al., 1990). The PCR reaction mixture for amplification was prepared as 200 µM of each dNTP, 100 pmol of each primer, 2.5 units of Taq-DNA-polymerase, 5 µl of 10X Taq DNA polymerase reaction buffer, 50 ng of genomic DNA and 50 µl of final volume of ddH2O. 5 µl of the products obtained as a result of the PCR reaction were electrophoresed in 1% agarose gel with 0.5 µg/ml ethidium bromide for 45 minutes at 90 V and visualized in BioRad gel imaging device.

3. Results and Discussion

3.1. Distribution

To determine the distribution of *L. occidentalis* in Türkiye, 366 observations were recorded, and 3114 individuals were counted in 35 provinces. With the present study, the current distribution of the pest includes 51 provinces in Türkiye (Figure 3). The highest number of individuals was recorded in Isparta (2036), İzmir (497) and Muğla (108) (Table 2). It was found at elevations ranging from sea level to 2016 m (Table 3). The highest number of individuals (30.8%) was found between 501 and 1000 m, followed by 1001-1500 m (28.7%) and 0-500 m (22.3%).

The host species on which *L. occidentalis* individuals were recorded were *Abies nordmanniana*, *Picea pungens*, *Pinus brutia*, *P. halepensis*, *P. mugo* and *P. nigra*.

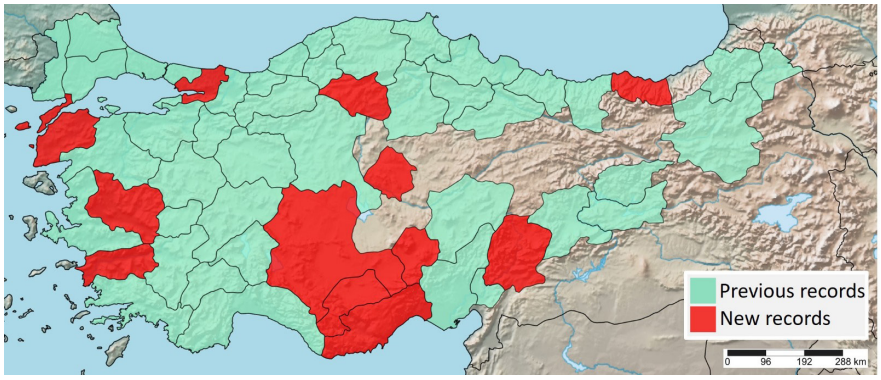

Figure 3. Turkish provinces where *Leptoglossus occidentalis* has been recorded so far
Şekil 3. *Leptoglossus occidentalis*'in Türkiye'de yayılış yaptığı tespit edilen iller

3.2. Life cycle

The beginning and end of overwintering of the adults were observed in Artvin, Bursa, Düzce, Isparta, İstanbul, and Kastamonu provinces (Table 4). Generally, the end of overwintering was in April and May 2019, and the beginning of overwintering was in October 2019 and November 2020. In all our observations, the overwintering individuals were adults. We found two main oviposition periods in the observed localities. Thus, we concluded that *L. occidentalis* has two generations per year in Türkiye (Figure 4). Depending on the altitude and latitude, the first oviposition period was between early May and mid-June, and the second one was between mid-July and late August. First instar nymphs were observed in the second week of May and second, third, and fourth instar nymphs were observed in mid-June. Fifth instar nymphs were observed in mid-July. There was no clear distinction between the nymphal stages, and they all had intertwined. Thus, we found all nymphal stages together both in the first and second generation. Nymphal stages of the second generation were completed at the end of October, and the adults moved to the overwintering sites.

The time-dependent changes of egg and nymph numbers in the Central Anatolia Region, Artvin, Isparta and Burdur provinces, Marmara Region, Izmir, Aydın, Manisa, Muğla and Antalya provinces are given in the graphs (Figure 5). When the biological periods of the species by regions are examined; it is seen that the egg period of the first generation starts in April in Artvin and Aegean and Mediterranean Regions, and in May in other regions. The egg period of the second generation was found to be from mid-July to early August in Artvin, throughout August and early September in the Inner West Mediterranean Region and in August in the Marmara, Aegean and Mediterranean Regions.

Leptoglossus occidentalis has one generation per year in its native range, western North America. However, it adapts to the climate in its introduced ranges. For example, it has three generations in Mexico (Koerber, 1963; Cibrian-Tovar et al., 1986; Mitchell, 2000), one to three generations in Italy and Spain (Bernardinelli et al., 2006; Maltese et al., 2009; Tamburini et al., 2012; Mas et al., 2013). In Türkiye, two generations were detected with the data collected from Afyonkarahisar, Bilecik, Burdur, Kastamonu and Muğla, and mostly Isparta (Oğuzoğlu and Avcı, 2020). Similar results were obtained in the present study. However, based on degree-day calculations, İpekdal (2022) showed that up to five generations are possible in southern Türkiye. Therefore, open field experiments are needed to solve this hypothesis which remains a topic of future studies.

We found that *L. occidentalis* is active between April and October which is compatible with Farinha et al. (2018) who determined the activity between May and November. Barta (2016) found the highest number of eggs in early June. We found two peaks of egg laying, in mid-May and late August.

Table 2. The number of *Leptoglossus occidentalis* eggs, nymphs, and adults according to provinces
Tablo 2. İllere göre *Leptoglossus occidentalis*'in yumurta, nimf ve ergin birey sayıları

Provinces	Number of observations	Eggs	Nymphs	Adults	Total
Isparta	167	154	1052	830	2036
İzmir	59	0	390	107	497
Muğla	13	0	53	55	108
Kırşehir	7	8	42	29	79
Artvin	19	21	23	24	68
Antalya	12	0	29	17	46
İstanbul	8	16	9	12	37
Bursa	8	0	2	31	33
Düzce	9	0	25	7	32
Denizli	2	0	0	31	31
Afyonkarahisar	2	0	18	4	22
Manisa	9	0	15	4	19
Burdur	8	0	9	8	17
Aydın	3	0	11	1	12
Çanakkale	4	0	8	3	11
Uşak	1	0	6	3	9
Bolu	2	0	3	5	8
Çankırı	6	0	0	8	8
Kırklareli	2	6	0	2	8
Ankara	5	0	1	3	4
Balıkesir	2	0	1	3	4
Mersin	2	0	0	4	4
Konya	1	0	2	1	3
Sakarya	1	0	0	3	3
Eskişehir	2	0	0	2	2
Kastamonu	2	0	0	2	2
Niğde	1	0	1	1	2
Tekirdağ	1	0	0	2	2
Çorum	1	0	0	1	1
Kahramanmaraş	1	0	0	1	1
Karaman	1	0	0	1	1
Kocaeli	1	0	0	1	1
Kütahya	1	0	0	1	1
Samsun	1	0	0	1	1
Trabzon	1	0	0	1	1
Total	366	205	1700	1209	3114

Table 3. Distribution of *Leptoglossus occidentalis* individuals in altitude groups
Tablo 3. *Leptoglossus occidentalis*'in yükselti basamaklarına göre yayılışı

Altitude groups (m)	Number of individuals	%
0-500	697	22.4
501-1000	957	30.7
1001-1500	892	28.6
1501-2000	565	18.1
>2000	3	0.1
Total	3114	100

Table 4. The beginning and end of overwintering of *Leptoglossus occidentalis* adults in Türkiye
Tablo 4. *Leptoglossus occidentalis*'in Türkiye'de kışlaktan çıkış ve kışlamaya giriş zamanları

Location	Altitude (m)	End of 2018-2019 overwintering	Beginning of 2019-2020 overwintering
İstanbul	120	06.04.2019	-
Artvin	180	15.04.2019	-
Artvin	550	22.04.2019	-
Düzce	150	27.04.2019	-
Bursa	1400	-	14.10.2019
Kastamonu	750	-	14.10.2019
İstanbul	124	-	30.10.2019
Isparta-Sütçüler	995	-	19.11.2020

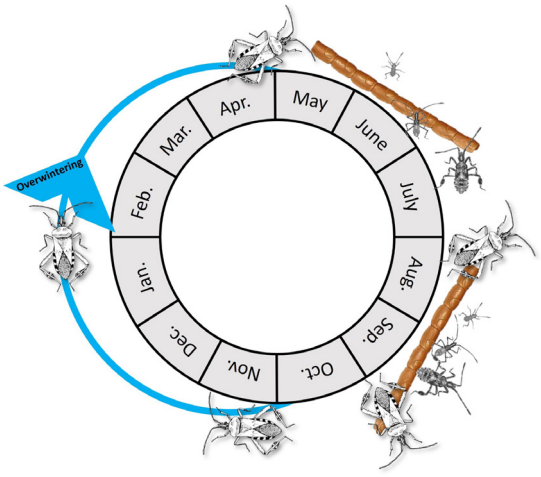

Figure 4. Life cycle of *Leptoglossus occidentalis*
Şekil 4. *Leptoglossus occidentalis*'in yaşam döngüsü

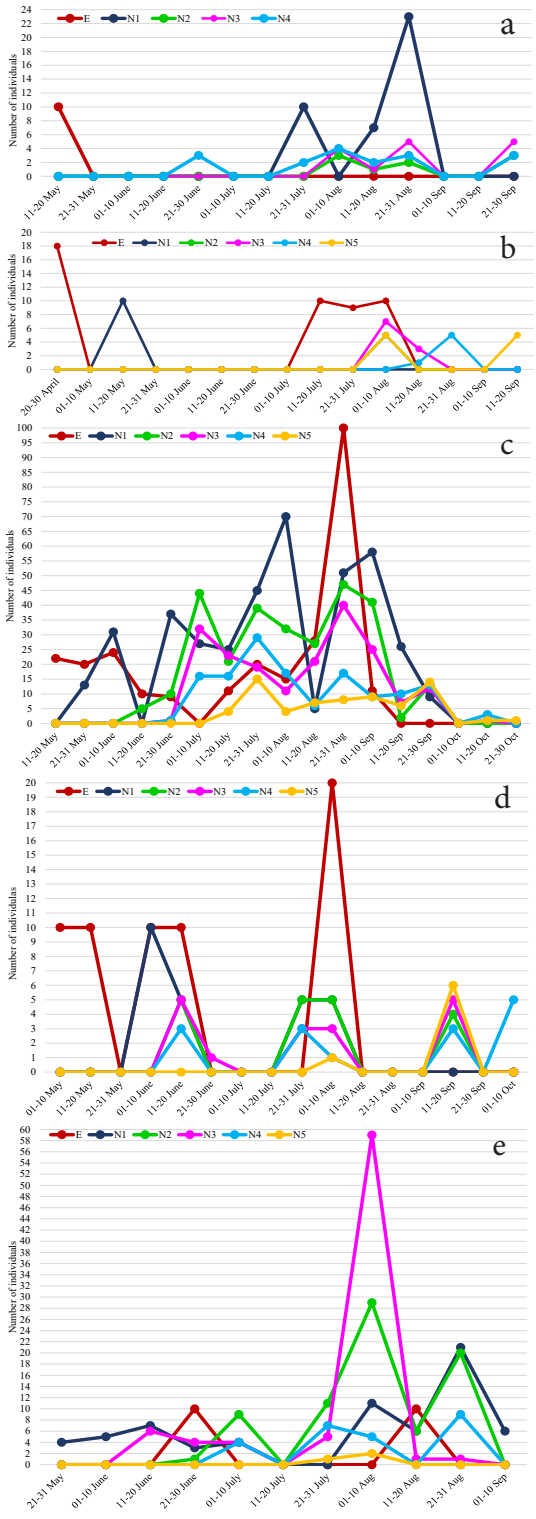

Figure 5. Life cycle of *Leptoglossus occidentalis* in Central Anatolia (a), Eastern Black Sea (b), Inner Western Mediterranean (c), Marmara (d), Aegean and Mediterranean regions (e)

Şekil 5. *Leptoglossus occidentalis*'in İç Anadolu (a), Doğu Karadeniz (b), İç Batı Akdeniz (c), Marmara (d) Ege ve Akdeniz bölgelerindeki yaşam döngüsü (e)

Tamburini et al. (2012) showed that *L. occidentalis* emerged from overwintering sites at 220-390 m in early April, at 1040 m in early May, the first generation at 220-390 m emerged in early July and the second generation in early September and at 1040 m a single progeny emerged in September, whereas overwintering started in early October at 220-390 m and in early September at 1040 m. They also found that another generation started between September and November at 220-390 m, but nymphs could not complete the development. In the present study, we found second generation adults in buildings and tree trunks during the overwintering period which showed that the pest completed the second generation.

3.3. Fungal associations of *L. occidentalis*

3.3.1. Morphological detection of *D. sapinea* from plant samples

We obtained 287 fungal isolates from 19 trees in eight locations in the Aegean (İzmir), Central Anatolia (Çankırı) and Western Mediterranean (Antalya, Isparta) regions (Table 5). Among them, 24 isolates were found to have colony growth characteristics similar to those of *D. sapinea* (Table 5). These isolates were obtained from four tree samples in Antalya-Manavgat (tree no. 13), Antalya-Aksu (15), İzmir-Kozbeyli (18), *İ*zmir-Kozak-Karaveliler (22) (Table 5).

D. sapinea-like fungi isolates were obtained from the seeds of the healthy-looking one-year-old cones from tree 13 in Manavgat, from shoots and buds in Aksu, and from healthy-looking shoots and buds in Kozak (Karaveliler). However, the isolates obtained from Kozbeyli were obtained from a cone damaged by the pine cone butterfly (*Dioryctria mendacella*).

3.3.2. Molecular detection of *D. sapinea* from plant and insect samples

As a result of RT-PCR analyses, no CT value could be obtained for the presence of *D. sapinea* in the DNA of both extracted from plant tissues and insect samples.

3.3.3. Molecular detection of entomopathogenic fungi from insect samples

In total, 25 fungal colonies developed from 50 insect samples (Table 5). We morphologically identified *Alternaria alternata*, *Aspergillus niger*, *Penicillium* sp., and *Phoma* sp. none of which are entomopathogenic species.

Leptoglossus occidentalis can damage its hosts not

Table 5. *Diplodia sapinea*-like isolates obtained from asymptomatic shoots, buds, cones and seeds
Tablo 5. Asemptomatik sürgün, tomurcuk, kozalak ve tohumlardan elde edilen *Diplodia sapinea* benzeri izolatlar

Region	Province	Tree no	Tree species	Number of *Diplodia sapinea*-like isolates from shoots, buds, and cones	Number of *Diplodia sapinea*-like isolates from seeds
Southwestern Mediterranean	Isparta	8	*Pinus nigra*	5/0	-
		9	*Pinus nigra*	4/0	-
		10	*Pinus nigra*	16/0	10/0
		11	*Pinus nigra*	13/0	-
		12	*Pinus brutia*	12/0	-
	Antalya, Manavgat, Sarılar	13	*Pinus brutia*	6/1	8/4
	Antalya, Aksu, Kurşunlu*	14	*Pinus brutia*	8 /0	-
	Antalya, Aksu	15	*Pinus brutia*	14/3	-
	Antalya, Aksu, Yeşilkaraman	16	*Pinus brutia*	7/0	-
Aegean	İzmir, Foça, Kozbeyli	17	*Pinus brutia*	14/0	-
		18	*Pinus brutia*	14 /4**	-
		19	*Pinus brutia*	18/0	-
	İzmir, Bergama, Kozak*	20	*Pinus pinea*	9/0	-
		21	*Pinus pinea*	25/0	-
		22	*Pinus pinea*	29/12	-
		23	*Pinus pinea*	34/0	-
Central Anatolia	Çankırı, Eldivan	24	*Pinus nigra*	17/0	5/0
		25	*Pinus nigra*	12/0	-
		26	*Pinus nigra*	7/0	-

* In these locations, samples of fallen cones were also collected and *D. sapinea* pycnids and spores were detected on them.
**Isolates obtained from a *Dioryctria mendacella* infested cone.

only by feeding on the seeds but also by transmitting some fungal disease agents such as *D. sapinea* (Luchi et al., 2012; Tamburini et al., 2012). The presence of *Diplodia* spp. in Türkiye was first reported in 1993 (Ünligil and Ertaş, 1993; Sümer, 2000). In the present study, although we found *D. sapinea*-like fungi on the host plants by morphological methods, we did not find it on host plant and insect samples by molecular methods. We also did not find any entomopathogenic fungi on the insect samples.

4. Conclusions

Türkiye is an important pine nut seed producer (Awan and Pettenella, 2017; *Özden* et al., 2022). However, a significant decrease has been seen since the early 2000s (Kılcı et al., 2013), which causes great economic concern in pinenut economy-dependent communities. Although the introduction of *L. occidentalis* in Türkiye coincides with this decrease in pine nut production, it is not clear if it is the only causing agent. Nevertheless, as a seed feeder, it must have some effect on seed yields as shown by Farinha et al. (2018). The impact of the pest is not only pinenut production as it feeds on almost all conifer seeds. Thus, it threatens the natural regeneration of forests. In Türkiye, seed stands and gardens are the most important sources of seeds required for basic forestry activities such as regeneration of forests, establishment of new forests, and reforestation of post-fire areas. It should not be ignored that any biotic and abiotic factors that may affect the productivity and continuity of these resources are a threat to the continuity of our country's forests. Therefore, finding efficient control methods against *L. occidentalis* is of paramount importance. It is also important to reveal the introduction pathways of the pest in order to prevent new introductions.

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