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Original article (Orijinal araştırma)

Notes on the genera *Paederidus* Mulsant & Rey, 1878, *Paederus* Fabricius, 1775 and *Uncopaederus* Korge, 1969 from the Palearctic Region (Coleoptera: Staphylinidae: Paederinae)[1]

Palearktik Bölgedeki *Paederidus* Mulsant & Rey, 1878, *Paederus* Fabricius, 1775 ve *Uncopaederus* Korge, 1969 cinslerine bağlı türler üzerine notlar (Coleoptera: Staphylinidae: Paederinae)

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Abstract

The genera *Paederidus* Mulsant & Rey, 1878, *Paederus* Fabricius, 1775 and *Uncopaederus* Korge, 1969 comprise 117 species in the Palearctic Region. In the present study, new and additional distribution data for 16 species of the genera *Paederidus*, *Paederus* and *Uncopaederus* are reported from different countries of the Palearctic Region. The material examined was collected between 1884 and 2017; and contained types and additional specimens in European museums and Alaşehir Zoological Museum. Among them, six species are the first country records: Albania (1), Bosnia Herzegovina (2), Israel (1), Kazakhstan (1), Macedonia (1), Montenegro (2), Morocco (1). In addition, *Uncopaederus signiventris* (Smetana, 1962) is illustrated. Two synonymies are proposed: *Paederus littoralis* Gravenhorst, 1802 = *Paederus pelikani* Reitter, 1884 syn. n., = *Paederus littoralis ilsae* Bernhauer, 1932 syn. n.

Keywords: Paederinae, *Paederidus*, *Paederus*, synonymies, *Uncopaederus*

Öz

Paederidus Mulsant & Rey, 1878, *Paederus* Fabricius, 1775 ve *Uncopaederus* Korge, 1969 cinsleri Palearktik Bölge'de 117 tür ile temsil edilirler. Bu çalışmada, Palearktik Bölge'deki farklı ülkelerde bulunan *Paederidus*, *Paederus* ve *Uncopaederus* cinslerine bağlı 16 türe ait yeni ve ek yayılışsal kayıtlar verilmiştir. İncelenen materyal, 1884-2017 yılları arasında toplanmış olup, Avrupa müzeleri ve Alaşehir Zooloji Müzesi'nde bulunan tip ve diğer örnekleri içermektedir. Bu 16 türden altı tür ilk ülke kaydı niteliğindedir: Arnavutluk (1), Bosna Hersek (2), Israil (1), Kazakistan (1), Makedonya (1), Karadağ (2), Fas (1). Ayrıca, *Uncopaederus signiventris* (Smetana, 1962) türü şekillendirilmiştir. Yeni sinonimler önerilmiştir: *Paederus littoralis* Gravenhorst, 1802 = *Paederus pelikani* Reitter, 1884 syn. n., = *Paederus littoralis ilsae* Bernhauer, 1932 syn. n.

Anahtar sözcükler: Paederinae*, Paederidus*, *Paederus*, sinonimler, *Uncopaederus*

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Introduction

The genera *Paederidus* Mulsant & Rey, 1878, *Paederus* Fabricius, 1775 and *Uncopaederus* Korge, 1969 belong to the tribe Paederini Fleming (Schomann & Solodovnikov, 2017). They are remarkable for the coloring of their body. These species occur in many places, mainly on the banks of rivers, creeks, lakes, dams, wetlands and wet agricultural areas (Anlaş et al., 2017).

According to the recent catalog by Schülke & Smetana (2015), the genera *Paederidus* (13 species) *Paederus* (103 species) and *Uncopaederus* (1 species, known only from Northern Anatolia) comprise 117 species in the Palearctic Region. The genera *Paederidus* and *Uncopaederus* do not include any subgenera, but the genus *Paederus* has been divided into ten subgenera: the nominate subgenus (13 species); *Eopaederus* Scheerpeltz (13 species); *Gnathopaederus* Chapin (6 species); *Harpopaederus* Scheerpeltz (16 species); *Heteropaederus* Scheerpeltz (2 species); *Megalopaederus* Scheerpeltz (8 species); *Nepalopaederus* Scheerpeltz (1 species); *Oedopaederus* Scheerpeltz (1 species); *Oreinopaederus* Scheerpeltz (1 species); and *Poederomorphus* Gautier des Cottes (2 species), with 13 additional species listed as incertae sedis (Assing, 2015; Schülke & Smetana, 2015). The genus *Paederus* is widespread around the world; and is also one of the most studied staphylinid taxa. Despite this, the taxonomy of this genus is very complicated and still far from being resolved, especially from the point of subgeneric concepts. For this reason, to resolve the taxonomical problems, phylogenetic analyses using multiple DNA markers should be made.

In the present study, 16 species of the genera *Paederidus*, *Paederus* and *Uncopaederus* are reported from different countries of the Palearctic Region, including some records of zoogeographic interest. Two synonymies are proposed: *Paederus littoralis* Gravenhorst, 1802 = *Paederus pelikani* Reitter, 1884 syn. n., = *Paederus littoralis ilsae* Bernhauer, 1932 syn. n. Additionally, *Uncopaederus signiventris* (Smetana, 1962) is illustrated.

The main aim of this study was to contribute to the knowledge of the *Paederidus*, *Paederus* and *Uncopaederus* fauna of the Palearctic Region.

Material and Methods

Classification and nomenclature of the genera *Paederidus*, *Paederus* and *Uncopaederus* suggested by Schülke & Smetana (2015) were followed in this study. Morphological studies were conducted using a Stemi 2000-C microscope (Zeiss, Germany). For photographs a digital camera (Zeiss Axiocam ERC5s) was used.

The studied material was collected between 1884-2017 and included types and additional male specimens in museums. The material referred to in this study is stored in the following collections:

AZMM – Alaşehir Zoological Museum, Manisa, Turkey (S. Anlaş);

HNHM – Hungarian Natural History Museum, Budapest, Hungary (G. Makranczy, O. Merkl);

IRSNB – Inst. Royal des Sciences Naturelles de Belgique, Bruxelles, Belgium (W. Dekoninck);

MHNG – Muséum d'Histoire Naturelle, Genève, Switzerland (G. Cuccodoro);

NMNHS – National Museum of Natural History, Sofia, Bulgaria (R. Bekchiev); and

NMPC – National Museum, Praha, Czech Republic (M. Fikáček).

Results

Genus *Paederidus* Mulsant & Rey, 1878

Paederidus albipilis (Solsky, 1871)

Material examined: Turkmenistan: 2 exs, Turcmenia, coll. Reitter (NMPC).

Distribution: This species has been recorded in Afghanistan, Kyrgyzstan, Tajikistan, Turkmenistan and Uzbekistan (Schülke & Smetana, 2015).

Paederidus algiricus antoinei (Koch, 1937)

Material examined: Morocco: 1 ex., 15.IV.1974, Morocco, Sud Valée, du Dra Agdz, leg. Besuchet (MHNG).

Distribution: This species has been recorded in Spain and Morocco (Schülke & Smetana, 2015).

Paederidus rubrothoracicus (Goeze, 1777)

Material examined: Greece: 3 exs, 23.V.1976, Graecia, Peloponnes, Megalopolis, leg. Maroni (MHNG). Italy: 1 ex., 11-12.V.1980, Appennino, Lombardo, Stáffora-Tal, leg. Zwick (MHNG). Montenegro: 2 exs, 10.VII.1934, Crna Gora, Savnik, leg. Fodor (HNHM). Romania: 8 exs, 26.V.1994, Romania, Gorj, Menadiai-hegyseg, Cerna-Sat, 700 m, Cserna-Part, leg. Székely (HNHM). Turkey: 3 exs, 16.IX.2011, Afyonkarahisar, Şuhut, Paşacık, Selevir Dam, 38°30'46" N, 30°42'24" E, 1102 m, leg. Yağmur & Örgel (AZMM). 7 exs, 11 exs, 22.V.2015, Denizli, Acıpayam, Sandalcık, 37°04'58" N, 29°07'24" E, 633 m, leg. Yağmur & Örgel (AZMM). 4 exs, 14.XI.2014, İzmir, Karaburun 5 km SW 38°37'39" N, 26°29'26" E, leg. Anlaş, Yağmur & Örgel (AZMM). 3 exs, 18.IX.1988, Manisa, Salihli, leg. Jaccoud & Zumstein (MHNG). 5 exs, 21.V.2015, Muğla, Dalaman, 36°53'37" N, 28°53'37" E, 127 m, leg. Yağmur & Örgel (AZMM). 3 exs, 19.IX.2013, Uşak, Gediz, Sandıklı 2 km S, 38°55'03" N, 29°525'44" E, 854 m, leg. Özgen & Örgel (AZMM). 2 exs, 24.V.2016, Konya, Hadim, Beyreli Hill, 36°50'37" N, 32°22'26" E, 1537 m, leg. Örgel & Yaman (AZMM). 3 exs, 25.X.2017, Konya, Beyşehir, Dumanlı, 37°29'51" N, 31°20'45" E, 1615 m, leg. Örgel & Yaman (AZMM).

Distribution: This species is widespread from Europe, Turkey and Cyprus (Schülke & Smetana, 2015). Its occurrence in Montenegro is reported for the first time.

Paederidus ruficollis (Fabricius, 1777)

Material examined: Albania: 1 ex., 4.VI.2003, Thirré from conjuction of the Shkodér-Kukés Road 42°01'05" N, 20°12'59" E, 970 m, leg. Eráss & Feher (HNHM). Bosnia Herzegovina: 2 exs, 14.VI.1930, Bosnia, Sarajevo, leg. Fodor (HNHM). 3 exs, 15.VII.1929, Bosnia, Jablanica, Narenta Valley, leg. Fodor (HNHM). Italy: 7 exs, 14.VI.1994, Calabria, Castlglione Cos. (CS) torr. Padula, leg. Angelini (AZMM). FRANCE: 1 ex., V.1930, Gorge du Longi, leg. Fodor (HNHM). Morocco: 5 exs, 02.V.1997, Ijoukak, High Atlas, leg. Batelka & Podrouzkova (NMPC). Turkey: 1 ex., 15.IV.2015, Afyonkarahisar, Hocalar, Kirseli Mts., 38°33'44" N 30°02'17" E, 1460 m, leg. Anlaş & Örgel (AZMM). 3 exs, 19.IV.2015, Denizli, Acıpayam, Elmadağ, 37°37'01" N, 29°26'55" E, leg. Anlaş, Yağmur & Örgel (AZMM). 3 exs, 28.VI.2014, Kütahya, Domaniç, Ortaca 1 km W, 39°49'55" N, 29°29'40" E, 700 m, leg. Yağmur & Örgel (AZMM). 2 exs, 30.XI.2014, Manisa, Spil Mts., 38°33'44" N, 27°23'10" E, 1100 m, leg. Yağmur & Örgel (AZMM). 1 ex., 04.IV.2013, Muğla, Datça, Emecik 2 km SW, 36°46'01" N, 27°48'39" E, 107 m, leg. Yağmur & Örgel (AZMM). 1 ex., 01.XI.2017, Niğde, Çiftlik Road, 38°06'47" N, 34°35'47" E, 2114 m, Örgel & Yaman (AZMM).

Distribution: According to Schülke & Smetana (2015), this species is widespread from Europe, North Africa (Algeria, Morocco and Tunisia) Iran, Turkey and Cyprus. However, it has not been recorded from Albania.

Genus *Paederus* Fabricius, 1775

Subgenus *Eopaederus* Scheerpeltz, 1957

Paederus mesopotamicus Eppelsheim, 1889

Material examined: Iran: 12 exs, 8-9.IV.1973, S-Iran, 13 km SSE Nikshahr, coll. B. Šticha (NMPC). 3 exs, 6-7.X.2002, Fars prov. Zagros, 5 km above Thangebolhayat (to Shiraz), 1750 m, leg. Gyulai & Garai (HNHM). Iraq: 12 exs, 4-5.XII.1977, Iraq, Arbil, Eskikalak, near Great Zab River, leg. Topál & Zilahy (HNHM). 1 ex., Mesopotamia, coll. Reitter (HNHM). Syria: 1 ex., Holotype of *Paederus syriacus* Reitter, 1889, Syria, coll. Reitter (HNHM). Turkey: 1 ex., 31.V.1989, Siirt, Kotum, leg. Podlussány (HNHM). 8 exs, 21.V.2010, 17.XI.2010, Siirt, Baykan 4 km E, ca. 770 m, 38˚11'42" N, 41˚49'03" E, leg. Anlaş & Yağmur (AZMM). 1 ex., 20.V.2011, Elazığ, Keban 2 km S, leg. Anlaş (AZMM). 1 ex., 17.V.2011, Erzincan, Kemah, Özdamar 2 km W, leg. Anlaş (AZMM). 2 exs, 16.V.2011, Gümüşhane, Kelkit, Çimenli, 1689 m, 39°58'06" N, 39°22'48" E, leg. Anlaş & Özgen (AZMM). 8 exs, 19.V.2011, Tunceli, Pülümür 3 km SE, leg. Anlaş,

Özgen & Khachikov (AZMM). 2 exs, 19.V.2011, Tunceli, Pülümür, Kangallı, 1310 m, 39˚25'37" N, 39˚50'16" E, leg. Anlaş, Özgen & Khachikov (AZMM). 4 exs, 02.XI.2017, Sivas, Hafik, Beydili, 40°04' N, 37°16' E, 1700 m, leg. Örgel & Yaman (AZMM).

Distribution: According to Willers (2011), the species is known from Iran, Iraq, Syria and Turkey.

Paederus debilior Eppelsheim, 1892

Material examined: Kazakhstan: 1 ex., Turkestan, coll. Reitter (HNHM). 2 exs, 05.IV.2010, Yuzhno-Kazakhistan Region, Boralday Range, Satur Mts, hole of the Kulan nv., high Krasnye Vorota pass, 1000 m, 42˚35'13" N, 70˚26'53" E, leg. Matalin (AZMM).

Distribution: This species is known from Turkmenistan and Uzbekistan (Schülke & Smetana, 2015). The above specimens from Kazakhstan represent the first record for this country.

Subgenus *Harpopaederus* Scheerpeltz, 1957

Paederus baudii Fairmaire, 1860

Material examined: Italy: 4 exs, 22.VI.1992, Fornovolasco (LU), 700 m, leg. Angelini (AZMM). 2 exs, 22.IX.1996, Basilicata, str. Irsina-Grassano su F. Bradano Mts., 150 m, leg. Angelini (AZMM). 1 ex., 20.V.1998, Toscana, Minucciano (LU), L. Gramolazzo, 680 m, leg. Angelini (AZMM).

Distribution: This species occurs in France, Italy and Switzerland (Schülke & Smetana, 2015).

Paederus schoenherri Czwalina, 1889

Material examined: Bulgaria: 1 ex., 09.VIII.2010, Belasitsa Mts., Kongura hut, 41˚35’10" N, 23˚19’07"1 E, leg. Bekchiev (NMNHS). 1 ex., 30.V.2010, Black sea coast, Primorsko env., 42˚28’59" N, 27˚58’37" E, leg. Bekchiev (NMNHS). 1 ex., 07.VIII.1983, Bulgaria, Cserno More, Burgasz, leg. Kismarjai (HNHM). 1 ex., 29.VI.1969, Pirin, hegyséa, Popina, leg. Kismarjai (HNHM).

Distribution: *Paederus schoenherri* is known from Southeastern Europe and Iran (Schülke & Smetana, 2015).

Subgenus *Heteropaederus* Scheerpeltz, 1957

Paederus alfierii Koch, 1934

Material examined: Egypt: 3 exs, 15.X.1957, Sids, Exp. Egypt, Mus. Nat. Hung., leg. Gozmány (HNHM).

Distribution: According to Schülke & Smetana (2015), this species is widespread in Algeria, Egypt, Iraq and Saudi Arabia.

Paederus fuscipes Curtis, 1826

Material examined: Algeria: 4 exs, 20.V.1988, Algeria, Gde Kabylie, Oued Sébaou, W. Dellys, leg. Besuchet, Löbl & Buchardt (MHNG). Armenia: 1 ex., 20.VIII.1976, Armenia, Tsakhkadzor, 1850 m, leg. Vósárhelyi (HNHM). Azarbaijan: 1 ex., 09.VI.2004, Talysh, Derinsky distr., Gostilyani 10 km N, leg. Kasatkin (AZMM). 1 ex., 02.VI.2008, Lenkoran distr., near Alexeevka (Burgali), leg. Kasatkin (AZMM). Bosnia Herzegovina: 2 exs, 20.VIII.1930, Bosnia, Vrelo, Bosne, leg. Fodor (HNHM). Czech Republic: 1 ex., 28.VIII.1958, Ryb. Rožmberk, Bohemia (NMPC). Egypt: 10 exs, 29.V.1996, Cairo, Dahshur ca. 23 km S Cairo, 29˚48'00" N, 31˚14'30" E, light traps, leg. Ullrich (MHNG). Iran: 2 exs, 03.VI.1975, Azerbaidjan occ., prés de Mahâbâd, 36˚50' N, 45˚47'E, leg. Senglet (MHNG). 3 exs, 14.VI.2000, Mazandran, Sah Mts. Ghorogh, 54˚40'52" E, 36˚52'55" N, 140 m, leg. Fabian & Székely (HNHM). 2 exs, 28-30.V.1973, S-Iran, Korsiah, Exped. Nat. Mus. Praha (NMPC). Israel: 1 ex., 27.IV.1982, Golan, Mahjor, -200 m, leg. Besuchet & Löbl (MHNG). Italy: 1 ex., 27.VI.1979, Sicilia, Coccamo, Tannita, leg. Zombori (HNHM). Kazakhistan: 2 exs, 05.IV.2010, Yuzhno-Kazakhistan Region, Boralday Range, Satur Mts, hole of the Kulan nv., high Krasnye Vorota pass, 1000m, 42°35'13" N, 70°26'53" E, Matalin (AZMM). 1 ex., 05.IV.2010, Yuzhno-Kazakhistan Region, Taskara Mts, Novonikolaevka vill 2,5 km SW 1200m, 42°24'49" N, 70°27'23" E, Makaraov & Matalin (AZMM). 2 exs, 31.III.2010, Yuzhno-Kazakhistan Region, South bank of Kyzylkol Lake, right bank

Ushbas River, near mouth 1200m, 43°43'56" N, 69°30'48" E, leg. Matalin (AZMM). Morocco: 1 ex., 05.V.1960, Morocco, M. Atlas, Ouioname, leg. Besuchet (MHNG). Syria: 2 exs, 18.VI.1998, Deir-er-zu prov., Dura Europas, at light, 34°45' N, 40°44' E, leg. Chvojka (NMPC). Turkey: 2 exs, 31.V.2011, Muş, Varto, leg. Khachikov & Kasatkin (AZMM). 1 ex., 25.V.2010, Kırklareli, İğneada, car net, leg. Bekchiev (NMNHS). 1 ex., 02.V.1967, Turquie, Adana, leg. Wittmer (MHNG). 1 ex., 05.V.1967, Turquie, Adana, 24 km N of Kozan, 1600 m, leg. Wittmer (MHNG). 4 exs, 12.V.1967, Turquie, Erzurum, Azort, leg. Besuchet (MHNG). 2 exs, 16.IV.1969, İzmir, Bahçeliköy, leg. Besuchet (MHNG). 7 exs, 06.V.1978, Kayseri, Sultan Sazlığı, 1000 m, leg. Besuchet & Löbl (MHNG). 1 ex., 27.IV.1989, Diyarbakır, Kavurma vill., Ergani 10 km NE, 1400 m, 39˚41' E, 38˚19' N, leg. Fábián, Ronkay & Ronkay (HNHM). 1 ex., 12.IV.2013, Afyonkarahisar, Düzağaç 2 km N, 38°48'01" N, 30°09'03" E, 1172 m, leg. Anlaş, Yağmur & Örgel (AZMM). 3 exs, 02.V.2015, Afyonkarahisar, Ahır Dağları, Büyükhacet Tepesi, 38°39'52" N, 30°07'17" E, 1556 m, leg Yağmur & Örgel (AZMM). 15 exs, 22.III.2015, Aydın, Dilek Yarımadası Milli Parkı, 37°39'49" N, 27°12'57" E, 969 m, leg. Yağmur & Örgel (AZMM). 1 ex., 30.IV.1975, Aydın, 10 km S of Çine, leg. Besuchet & Löbl (MHNG). 6 exs, 14.X.2013, Denizli, Çivril, Beydilli, Işıklı Lake, 38°15'56" N, 29°55'33" E, 841 m, leg. Özgen & Örgel (AZMM). 4 exs, 01.V.2014, Çivril, Işıklı Lake, 38°15'49" N, 29°55'36" E, 826 m, leg. Yağmur & Örgel (AZMM). 2 exs, 08.V.1979, İzmir, Efes, leg. Besuchet & Löbl (MHNG). 1 ex., 14.VII.1980, Smyrna, (HNHM). 6 exs, 13.IV.2015, Kütahya, Simav, Akdağ, 39°14'58" N, 28°49'41" E, 1670 m, leg. Anlaş, Yağmur & Örgel (AZMM). 7 exs, 26.III.2015, Uşak, Altıntaş vill., 38°43'04" N, 29°30'27" E, 918 m, leg. Yağmur & Örgel (AZMM). 1 ex., 21.VI.2016, Eskişehir, Sarıcakaya, Sakarya River, 40°05'24" N, 30°50'41" E, 286 m, leg. Örgel & Yaman (AZMM). 1 ex., 31.V.2016, Niğde, Çiftlik, Kitreli, Melendiz Mts., 38°07'13" N, 34°22'01" E, 2150 m, leg. Anlaş, Örgel & Yaman (AZMM). 4 exs, 09.VI.2016, Kayseri, Pınarbaşı, Eskiyassıpınar, Gövdeli Mts., 38°44'03" N, 36°38'21" E, 1921 m, leg. Yağmur, Örgel & Yaman (AZMM). 1 ex., 26.IX.2017, Ankara, Kızılcahamam-Çerkeş Road, 40°36'19" N, 32°39'50" E, 1150 m, leg. Örgel & Yaman (AZMM). 2 exs, 30.X.2017, Karaman, Ayrancı, Yüğlük Tepesi, 37°00'49" N, 33°46'55" E, 1967 m, leg. Örgel & Yaman (AZMM). Turkmenistan: 2 exs, 09.V.1991, USSR, Turkmenia, Karakum desert, 200 m, 20 km SW Repetek, 63°09' E, 38°25' N, leg. Gsoroba, Fabian, Herczig, Hrebiay & Ronkay (HNHM).

Distribution: According to Schülke & Smetana (2015), this species is widespread and common in the Palearctic Region. However, it has not been recorded from Bosnia Herzegovina and Morocco.

Subgenus *Paederus* Fabricius, 1775

Paederus balcanicus Koch, 1938

Material examined: Italy: 2 exs, 22.VII.1993, Basilicata, F. Sinni a Episcopia (PZ), leg. Angelini (AZMM). 4 exs, 30.V.1991, Basilicata, Oasi WWF Lago Pantano di Pignola, 770 m, leg. Angelini (AZMM). Romania: 1 ex., Romania, Rum-Mamaia, L. Siut-Ghiol, Smetana, 1961 (NHMH).

Distribution: This species is known from Europe, Iran and Turkey (Schülke & Smetana, 2015).

Paederus melanurus Aragona, 1830

Material examined: Italy: 3 exs, 01.V.1995, Lombardia, Bereguardo (PV), leg. Diotti (AZMM).

Distribution: This species occur in Albania, Greece, Italy and Switzerland (Schülke & Smetana, 2015).

Paederus riparius (Linnaeus, 1758)

Material examined: Russia: 1 ex., 22.VII.1999, Rostov Region, Veshenskaya vill., leg. Khachikov (AZMM). Turkey: 1 ex., 23.IV.2014, Denizli, Babadağ, Akdağ, 37°48'17" N, 28°49'26" E, 1781 m, leg. Anlaş & Örgel (AZMM). 28 exs, 11.IV.2014, İzmir, Ödemiş, Bozdağ, Gölcük, leg. Yağmur & Örgel (AZMM). 3 exs, 30.XI.2014, Manisa, Spil Dağı, 38°33'44" N, 27°23'10" E, 1100 m, leg. Yağmur & Örgel (AZMM). 2 exs, 25.X.2017, Konya, Beyşehir Lake, 37°45' N, 31°40' E, Örgel & Yaman (AZMM).

Distribution: This species is widespread from Europe, Algeria, Egypt, Siberia, Middle Asia, Iran and Turkey (Schülke & Smetana, 2015).

Paederus sabaeus Erichson, 1840

Material examined: Israel: 1 ex., 21.IV.1982, Meron, 900 m, leg. Besuchet & Lobl (MHNG). Oman: 3 exs, 28.III.2012, Oman, Dnolar prov., wadi Darbat, 325 m, northern part near Shihayt, leg. Reiter (NMPC). Yemen: 1 ex., 04.XI.2010, Al Hudaydah gov., Jabal Bura valley, Forest NP, 240-350 m, 14°52'45" N, 43°24'25" E, leg. Bezdék (NMPC).

Distribution: This species is known from Egypt, Oman, Saudi Arabia, Syria and Yemen. The above specimen from Israel represents the first record for this country (Schülke & Smetana, 2015).

Subgenus *Poederomorphus* Gautier des Cottes, 1862

Paederus littoralis Gravenhorst, 1802 (Figures 1A-U; 2A-G)

Paederus pelikani Reitter, 1884: 44 syn. n.

Paederus littoralis ilsae Bernhauer, 1932: 233 syn. n.

Material examined: Armenia: 2 exs, 16.IX.1982, USSR, Armenia, Karashamb, Agveran, netted, 1500 m, leg. Merkl & Ronkay (HNHM); 1 ex., 01.X.1982, USSR, Armenia, Tsakhkadzor, 1800-2300 m, leg. Merkl & Ronkay (HNHM). 1 ex., 10.X.1984, Armenia, Djrvezh, 1100 m, leg. Korsós & Vásárhelyi (HNHM). 3 exs, 16.X.1984, Armenia, Nor Geghi, 1200 m, leg. Korsós & Vásárhelyi (HNHM). 2 exs, 16.X.1984, Armenia, Aghveran, 200 m, leg. Korsós & Vásárhelyi (HNHM). 1 ex., 14-19.VI.1999, Razdansky distr., Tsakhkunyats Range, high Arzakan vill., leg. Nabozhenko (AZMM). Austria: 1 ex., Styria, Reitter (IRSNB). Azerbaijan: 2 exs, 16.VI.2007, Lenkoran, environs Dashytuk and Apo vills., leg. Kasatkin (AZMM). Bosnia Herzegovina: 2 exs, 17.III.1930, Bosnia, Sarajevo, Kosevo, leg. Fodor (HNHM). 2 exs, 03.IV.1932, Bosnia, Sarajevo, Rijeka Miljacka, leg. Fodor (HNHM). 3 exs, 28.IX.1928, Bosnia, Pazariŏ, Krupa valley, leg. Fodor (HNHM). 9 exs, I.1918, Bosnia, Sarajevo, Pale, leg. Fodor (HNHM). Bulgaria: 2 exs, 12.II.2012, Maleshevska Mts., Mikrevo vill., 41°54'29" N, 23°17'58" E, leg. Beckhiev (AZMM). 1 ex., 29.V.2010, Strandzha Mts., Slivarovo vill., leg. Bekchiev (NMNHS). CYPRUS: 2 exs, 24.IV.2015, Lefkoşa, Değirmenlik, 650 m, leg. Yağmur (AZMM). France: 1 ex., 20.VI.1970, Corse (AZMM). Greece: 2 exs, 09.IV.2011, Halkidiki, North of Aston, Stagira vill. env., in a forest, 585 m, 40°31'51" N, 23°43'11" E, leg. Bekchiev (AZMM). IRAN: 1 ex., 28-30.V.1973, S-Iran, Korsiah, Exped. Nat. Mus. Praha (NMPC). 4 exs, VII. 2006, Mazandaran, Savadkooh, leg. Ghahari (AZMM). Iraq: 2 exs, 17-20.V.2008, northern Iraq, ca 10 km NW Suleimaniyah province, leg. Sevinç (AZMM). Israel: 1 ex., 15.IV.1982, Golan, Gilbon, 300 m, leg. Besuchet & Löbl (MHNG). 1 ex., 18.IV.1982, Côte Akko, N. Naaman, leg. Besuchet & Löbl (MHNG). Italy: 1 ex., V.1983, Emilia, plan de Voglio, Apennin, leg. Marggi (MHNG). 1 ex., 26.V.1979, Genova, M. Montanasco, leg. Zombori (HNHM). 3 exs, X.2006, Basilicata, Parco Nazionale del Pollino (AZMM). Macedonia: 3 exs, 07-14.VII.1937, Macedonia, Galicnik, Bistra planina, leg. Fodor (HNHM). Montenegro: 1 ex., 19-20.VII.1938, Crna Gora, Han Garancic (HNHM). Russia: 1 ex., 07.IX.2005, Rostov Region, Sholokhovsky distr., Vehenskaya vill., leg. Khachikov (AZMM). 1 ex., 12.VI.2004, Dagestan, Berikey vill., Ulluchay River, leg. Liyina (AZMM). 1 ex., 03.V.2010, Krasnodar prov., Anapsky disr., B. Utrish vill., leg. Terskov (AZMM). Turkey: 1 ex., 27.V.1989, Adana, Hieropolis, leg. Podlussány (HNHM). 1 ex., 15.IV.2015, Afyonkarahisar, Hocalar, Kirseli Mts., 38°33'44" N 30°02'17" E, 1460 m, leg. Anlaş & Örgel (AZMM). 1 ex., 18.IV.2015, Denizli, Çameli, Değirmentaşı Hill, 37°07'21" N, 29°20'35" E, 1497 m, leg. Anlaş, Yağmur, Örgel & Altın (AZMM). 1 ex., 30.XI.2014, Manisa, Spil Dağı, 38°33'44" N, 27°23'10" E, 1100 m, leg. Yağmur & Örgel (AZMM). 3 exs, Muğla, 10.XI.2013, Fethiye, Seki Plateau, 1483 m, leg. Kesdek (AZMM). 2 exs, 09.VII.2006, Kahramanmaraş, Nurhak 7 km E, leg. Anlaş (AZMM). 2 exs, 06.VI.2006, Trabzon, Maçka, Sümela 3 km N, leg. Yağmur (AZMM). 2 exs, VI.2001, Artvin, leg. Nabozhenko (AZMM). 1 ex., Sinop, Nisi Lake, leg. Koç (AZMM). 5 exs, 13.VIII.2012, Ardahan, Hanak, Sulakçayır, leg. Altın (AZMM). 1 ex., 01.IV.2008, Şanlıurfa, Siverek 15 km S, 37°39'00" N, 39°12'52" E, 700 m, leg. Yağmur (AZMM). 2 exs, 01.V-01.VI.2012, Fethiye, Yanıklar, pastoral valley, by pitfall traps (AZMM). 1 ex., 26.IX.2017, Ankara, Kızılcahamam, Aluç Mts., 40°30'30" N, 32°34'59" E, 1482 m, leg. Örgel & Yaman (AZMM). 2 exs, 24.IX.2017, Ankara, Beypazarı, Köseler, 40°21'46" N, 32°00'35" E, 1360 m, leg. Örgel & Yaman (AZMM). 3 exs, 24.IX.2017, Ankara, Kızılcahamam, Sorgun Lake, 40°19'51" N, 32°13'08" E, 1271 m, leg. Örgel & Yaman (AZMM). 3 exs, 25.IX.2017, Ankara, Beypazarı, Kıbrıscık Road, 40°19'09" N, 31°55'40" E, 1574 m, leg. Örgel & Yaman (AZMM). 2 exs, 25.IX.2017, Ankara, Beypazarı, 40°20'33" N, 31°56'48" E, 1630 m, leg. Örgel & Yaman (AZMM). 11.IV.2017, 3 exs, Ankara,

Beypazarı, Üreğil, 40°17'07" N, 32°04'11" E, 1375 m, leg. Örgel & Yaman (AZMM). 10 exs, 24.VI.2016, 09.IV.2017, Ankara, Kızılcahamam, Sorgun Plateau, leg. Örgel & Yaman (AZMM). Turkmenistan: 1 ex., 21.V.1993, Chilmamedkum sands, Kyzyl-Takir, leg. Arzanov & Ivliev (AZMM).

Paederus littoralis ilsae Bernhauer, 1932: 233 syn. n.

Comments: *P. littoralis littoralis* is widespread in Europe, Algeria, Cyprus, Iran, Turkey and western Siberia (Schülke & Smetana, 2015). *Paederus littoralis ilsae* is known from Caucasus, Ukraine, Middle East, Afghanistan, Iran, Middle Asia and Saudi Arabia (Gusarov, 1997; Schülke & Smetana, 2015). According to Gusarov (1997), this subspecies differs from *P. littoralis littoralis* in having the hooks of the aedeagus broader in lateral view. An examination of material from various parts of the distribution revealed that the species is subject to remarkable intraspecific variation, in both external and aedeagal characters (Figures 1A-U). At the same time, the variation of the breadth of the hooks of the aedeagus in lateral view does not correspond to plausible distribution patterns. In addition, this character is very variable even within the same population.

Figure 1. *Paederus littoralis* Gravenhorst 1802, intraspecific variation of aedeagus in lateral and ventral view: A-B) Turkey, Northern Anatolia; C-D) Russia, Rostov; E-F) Italy, Basilicata; G-H) Turkmenistan, Chilmamedkum; I-J) Turkey, Central Anatolia; K-L) Iran, Mazandran; M-N) Turkey, Southeastern Anatolia; O-P) Armenia, Razdansky; R-S) Azerbaijan, Lenkoran; and T-U) Bulgaria, Maleshevska. Scale bar, A-U) 0.5 mm.

Paederus pelikani Reitter, 1884 syn. n. (Figure 2A-G)

Type material examined: Lectotype 1 ♂: Corfu, Reitter, coll. Reitter; Holotypus 1884 *Paederus pelikani* Reitter; Lectotypus 1 ♂, *Paederus pelikani* Reitter, V. Gusarov des. 1993 (HNHM). Paralectotypes: 2 ♂♂, 2 ♀♀, same data as lectotype; Paratypus 1884 *Paederus pelikani* Reitter, Paralectotypus, *Paederus pelikani* Reitter, V. Gusarov des. 1993 (HNHM). 1 ♂, 1 ♀, same data as lectotype, but 693 14; Paratypus 1884 *Paederus pelikani* Reitter, Paralectotypus, *Paederus pelikani* Reitter, V. Gusarov des. 1993 (HNHM). 1 ♂, 1 ♀, Morea, Taygetus, Brenske, coll. Reitter; Paratypus 1884 *Paederus pelikani* Reitter, (HNHM). Additional material examined: Greece: 1 ♂, Attica, leg. Reitter (MHNG); 1 ♂, Insel Corfu (MHNG). 1 ♂, 1 ♀, Balkan, Corfu, leg. Paganetti (MHNG); 1 ♂, 01.IV.1971, Gréce, Céphalonie, Argostolion, leg. Hauser (MHNG). 1 ♂, 16-23.VI.1995, Greece, Korfu, Rode, leg. Czetö (HNHM).

Comments: According to Schülke & Smetana (2015) *Paederus pelikani* is known Albania, Greece and Turkey. The original description of *P. pelikani* is based on numerous syntypes from "Corfu, Zante, Cephalonia, Morea" (Reitter, 1884) in Greece. All of these localities are now part of Greece. Most of the Reitter collection was deposited in the Hungarian Natural History Museum (HNHM). The syntypes from Corfu in the HNHM were studied and labeled as lectotype and paralectotypes by Vladimir Gusarov who remarked, "…Reitter chose no holotype while describing the new species. The labels seem to have been attached by the curators of the collection and types actually were syntypes." (Gusarov, 1997). The above labeled lectotype designations were published by Gusarov (1997). I studied the type specimens of *P. pelikani* in the collections of the HNHM during a visit in 2015. However, I found only the specimens of this species from Corfu and Morea (Figure 2A-G).

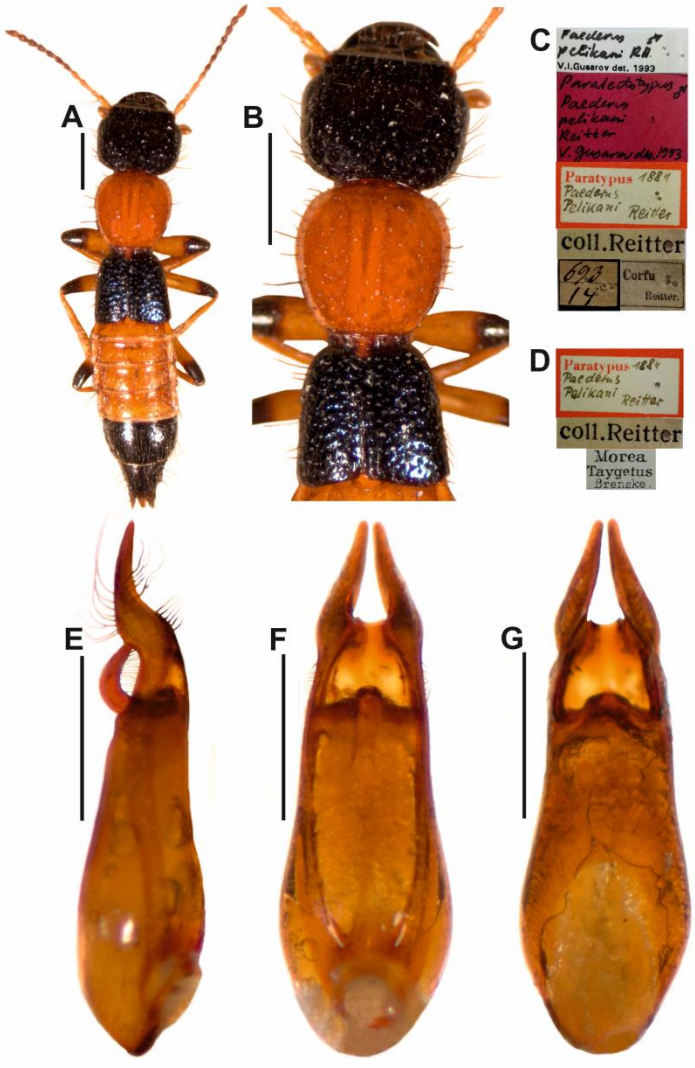

Figure 2. Detail of *Paederus pelikani* Reitter, 1884: A) Habitus; B) forebody; C-D) Paralectotypus labels; E) aedeagus, lateral view; F) aedeagus, ventral view; and G) aedeagus, dorsal view. Scale bars, A-B) 1.0 mm and E-G) 0.5 mm.

Gusarov (1997) states that this species resembles *Paederus littoralis*, except that the head is broader, the temples are more parallel and longer, the elytra are shorter and that the parameres of the aedeagus are longer. An examination of the type series from Corfu and Morea, and additional material from near the type localities revealed that the aedeagus and body is identical to that *P. littoralis*. The aedeagal and external characters, including the length of the aedeagal paramers, the head width, the shape and length of the temples, and the length of the elytra, are subject to some variation, as is usually

the case with widespread *Paederus* species. At the same time, Coiffait (1982) states that this species is mainly distinguished from *P. littoralis* by the reduced and more trapezoidal elytra and different shape of the median lobes of aedeagus, an observation that is not confirmed in present study. Moreover, when I examined of the material of *P. littoralis* from a vast area, this revealed that the species is subject to remarkable intraspecific variation, particularly in the shape and length of the elytra, and in the aedeagus. Consequently, the two new synonymies are proposed above.

The above specimens from Bosnia Herzegovina, Macedonia and Montenegro represent the first records for these countries.

Genus *Uncopaederus* Korge, 1969

Uncopaederus signiventris (Smetana, 1962) (Figure 3A-G)

Material examined: Turkey: 6 exs, 17.V.1976, Kastamonu, Ilgazdağı, prés du col., 1700-1800 m, leg. Besuchet & Löbl (MHNG). 2 exs, 08.VII.2013, Kastamonu, Ilgaz Mountains, 41°06'07" N, 33°44'54" E, leg. Kunt (AZMM). 2 exs, 14.III.2010, Sinop, Ada, 42°02'50" N, 35°11'16" E, leg. Koç (AZMM). 1 ex., 27.VI.2016, Çankırı, Ilgaz Dağı, 41°00'28" N, 33°37'00" E, 1841 m, leg. Örgel & Yaman (AZMM). 1 ex., 27.IX.2017, Ilgaz Dağı, 41°00'09" N, 33°36'32" E, 1835 m, leg. Örgel & Yaman (AZMM).

Distribution: This species is known only from Kastamonu, Rize and Samsun provinces in Northern Anatolia (Anlaş, 2009; Assing, 2010).

Comments: *Uncopaederus signiventris* is the single representative of the genus. Coiffait (1982) illustrated the aedeagus of this species. However, the illustration of the aedeagus in the paper is inaccurate. For that reason, the species illustrated in Figure 3A-G.

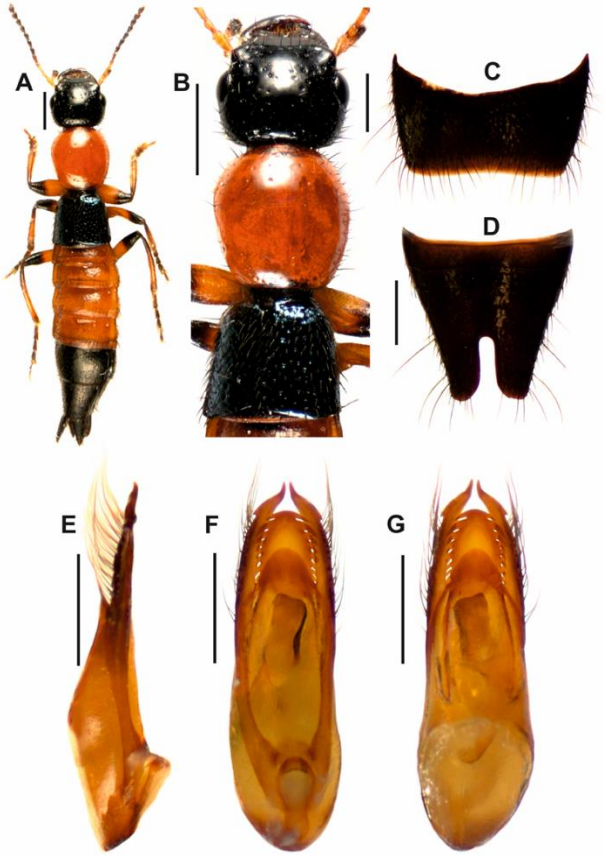

Figure 3. Detail of *Uncopaederus signiventris* (Smetana, 1962): A) Habitus; B) forebody; C) male sternite VII; D) male sternite VIII; E) aedeagus, lateral view; F) aedeagus, ventral view; G) aedeagus, dorsal view. Scale bars, A-B) 1.0 mm and C-G) 0.5 mm.

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Original article (Orijinal araştırma)

Population features of biparental and uniparental forms of the oleander scale, *Aspidiotus nerii* Bouché, 1833 (Hemiptera: Diaspididae) on squash

Zakkum kabuklubiti, *Aspidiotus nerii* Bouché, 1833 (Hemiptera: Diaspididae)'nin tek ve çift eşeyli formlarının kabak üstünde popülasyon özellikleri

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Abstract

Aspidiotus nerii Bouché, 1833 (Hemiptera: Diaspididae) is a cosmopolitan pest, mainly found in tropical and subtropical regions. It has been reported from hosts corresponding to more than 100 plant families. Particularly important is the damage caused on lemon and olive trees and ornamental plants such as oleander. It has both biparental and uniparental forms. To investigate the population dynamics of both forms of pest, life tables were constructed under controlled conditions in 2016. The studies were carried out on squash in climatic cabinet adjusted to 25±1ºC, 65±1% RH and 16:8 h L:D photoperiod. At the end of the study, life table parameters of both forms of pest were calculated. Namely intrinsic rate of increase (r), 0.039 and 0.042 d^{-1}; finite rate of increase (λ), 1.040 and 1.043 d^{-1}; net reproductive rate (R_0), 14.07 and 27.19 d^{-1}; mean generation time (T), 67.51 and 78.49 d, for biparental and uniparental forms, respectively. R_0 and T were statistically significant different between the two populations. Given these differences, it was estimated that the population size of the uniparental form may be 1.9 times higher than the biparental form.

Keywords: *Aspidiotus nerii*, biparental form, fecundity, two-sex life table, uniparental form

Öz

Aspidiotus nerii Bouché, 1833 (Hemiptera: Diaspididae) esas olarak tropik ve subtropik bölgelerde bulunan yaygın bir türdür. Konukçularının bağlı olduğu bitki familyası sayısının 100'den fazla olduğu rapor edilmektedir. Özellikle limon, zeytin ağaçları ve zakkum gibi süs bitkileri üstünde meydana getirdiği zarar önemlidir. Hem çift eşeyli ve hem de tek eşeyli formlara sahiptir. Zararlının her iki formunun popülasyon dinamiklerini araştırmak için 2016 yılında kontrollü koşullarda yaşam çizelgesi oluşturulmuştur. Çalışmalar, 25±1ºC, %65±1 orantılı nem ve 16:8 A:K şartlarına ayarlanmış iklim kabinlerinde kabak üstünde yürütülmüştür. Çalışma sonunda zararlının her iki formunun yaşam çizelgesi parametreleri sırasıyla: kalıtsal üreme yeteneği (r), 0.039 ve 0.042 d^{-1}; artış oranı sınırı (λ), 1.040 ve 1.043 d^{-1}; net üreme gücü (R_o) 14.07 ve 27.19 d^{-1}; ortalama döl süresi (T) 67.51 ve 78.49 gün olarak hesaplanmıştır. Bu parametrelerden R_o ve T istatistiksel olarak önemli bulunmuştur. Bu farklılıklardan dolayı popülasyon tahminlerine göre tek eşeyli formun popülasyon büyüklüğünün çift eşeyli forma göre 1.9 kat daha yüksek olabileceği hesap edilmiştir.

Anahtar sözcükler: *Aspidiotus nerii*, çift eşeyli form, üreme oranı, iki-eşeyli yaşam çizelgesi, tek eşeyli form

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Introduction

Hemiptera species are important insect species that cause economic loses in citrus trees. Some of the most important and economically harmful are the species *Aspidiotus nerii* Bouché, 1833, *Aonidiella aurantii* (Maskell, 1879) (Hemiptera: Diaspididae) and *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae) (Siscaro et al., 2006). Beardsley & Gonzalez (1975) stated that *A. nerii* is among the principal armored scale pests of the world. Oleander scale is a cosmopolitan species with 455 different host plants (Miller & Ben-Dov, 2004). Oleander scale has both biparental and uniparental forms, which were for the first time described by Felt in 1901 (Gerson & Hazan, 1979). Some authors suggest that cryptic multiple species complexes may also be present (Ferris, 1941; Einhorn et al., 1998; Provencher et al., 2005).

Various investigators have also shown that the behavior, host preferences and biological properties of these two forms of pests also vary. Gerson & Hazan (1979) reported that uniparental forms in Israel are specific to one host (*Pittosporum undulatum* Vent., Pittosporaceae) and that biparental forms naturally occur on different host species. Furthermore, DeBach & Fisher (1956) reported that two sympatric populations of these forms were obtained on oleander (*Nerium oleander* L., Apocynaceae) and English ivy (*Hedera helix* L., Araliaceae). Schmutterer (1952) has pointed out that uniparental forms could only survive indoors in Germany, while biparental forms could also survive in the natural environment and tolerate frost.

The oleander scale is a species that must be carefully monitored due to its serious economic damage to many host plants. The development of effective control methods against both forms and the determination of the correct time for applying management methods require that some details on the life cycle are well known. A limited number of studies on the biology of both forms of this scale have been conducted and, in these studies, the rates of reproduction, development and survival of both forms on different conditions and on different hosts were compared (DeBach & Fisher, 1956; Gerson & Hazan, 1979).

Results of previous investigations revealed that the biparental form showed a higher reproductive rate and shorter developmental time. However, these assessments do not provide enough evidence to understand the population dynamics of a species. Life tables provide comprehensive outputs to understand key-aspects of the life cycle of a given species in a more detailed way. For this reason, this study was undertaken and the biological features of both forms of the oleander scale were investigated. Provencher et al. (2005) reported that existence of uniparental forms adapted to specific hosts can provide enormous practical benefits for the quarantine and control methods, and may even allow better understanding of their ecology and evolution. Oleander scale is known as the best host for production of effective biologic control of pests against scale insects, it is appropriate for use in mass production of both predator and parasitoids in biological control of pests. For example, *A. nerii* is a suitable host for the production of *Aphytis melinus* DeBach, 1959 (Hymenoptera: Aphelinidae) for control of a major citrus pest, *A. aurantii* (Karaca & Uygun, 1993; Gonzalez-Zamora et al., 2012). The objective of our investigation was to determine the life table parameters of biparental and uniparental forms of the oleander scale reared on squash fruit.

Material and Methods

Rearing of insects and experimental area

The study was carried out at the Süleyman Demirel University, Agriculture Faculty, Plant Protection Department, and Biological Control Laboratory in 2016. Squash (*Cucurbita moschata* L. cv. Sunset QHI, Cucurbitaceae) fruit were used for rearing and assaying of biparental and uniparental forms of oleander scale. When active nymphs were settled on the fruit, the surface of each squash was divided into 15-20 areas of 4 cm^2 per individual and surrounded by an adhesive (Tangle-Trap, Tanglefoot, Australia). A total of 191 nymphs were used for biparental assay, and 69 for uniparental assay. Insects were checked daily.

When applied insects became adults, reproduction was estimated by counting and removing all newly emerged nymph each day until all individuals had died. The experiments were conducted in a growth chamber set to 25±1ºC, 65±1% RH and 16:8 h L:D photoperiod.

Life table analysis

The raw data obtained in the experiments were analyzed based on the age-stage two-sex life table by using the TWOSEX-MS Chart computer program, described by Chi (1988) and developed by Chi & Liu (Chi & Liu, 1985; Chi, 1988, 2013; Huang & Chi, 2011). The variances and standard errors of the population parameters were estimated using the bootstrap technique (Efron & Tibshirani, 1993; Polat Akköprü et al., 2015; Özgökçe et al., 2018) with 200,000 resampling to obtain stable estimates (Akça et al., 2015).

The age-stage specific survival rate (s_{xj}; x: age, j: period), the age-specific survival rate (l_x), the age-specific fecundity (m_x) and also life table population parameters such as intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R_0) and mean generation time (T) were calculated.

$$l_x = \sum_{j=1}^{k} s_{xj} \tag{1}$$

$$m_x = \frac{\sum_{j=1}^{k} s_{xj} f_{xj}}{\sum_{j=1}^{k} s_{xj}} \tag{2}$$

where, k is the number of stages and s_{xj} is the probability a newly emerged nymph will survive to age x and stage j.

The intrinsic rate of increase (r), Euler-Lotka equation (Goodman, 1982),

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1 \tag{3}$$

Net reproductive rate (R_0),

$$R_0 = \sum_{x=0}^{\infty} l_x m_x \tag{4}$$

Mean generation time (T) is the time required for a population to increase to R_0-fold at stable age-stage distribution,

$$T = \frac{\ln R_0}{r} \tag{5}$$

Finite rate of increase (the rate at which the population increases from one day to the next day) (d^{-1}),

$$\lambda = e^{r} \tag{6}$$

The life expectancy (e_{xj}), which is the time that an individual of age i and stage j is expected to live, was calculated according to Chi & Su (2006),

$$e_{xj} = \sum_{i=x}^{\infty} \sum_{y=j}^{k} s'_{iy} \tag{7}$$

The reproductive value is defined as the contribution of an individual to the future population (Fisher, 1930). The reproductive value, v_{xj}, was calculated according to Huang and Chi (2011) and Tuan et al. (2014a, b) in age-stage two-sex life table.

$$v_{xj} = \frac{e^{r(x+1)}}{s_{xj}} \sum_{i=x}^{\infty} e^{-r(i+1)} \sum_{y=j}^{k} s'_{iy} f_{iy} \tag{8}$$

Population Projection

TIMING-MSChart (Chi, 2014) computer program was used to project the population growth with an initial population of 10 newly emerged nymphs to reveal the growth and age-stage structure of biparental and uniparental forms of oleander scale. The methods developed by Chi & Liu (1985) and Chi (1990).

Results and Discussion

All the biparental and 92% of the uniparental nymphs of oleander scale were able to became adult and generate a new population. The growth, reproduction and survival rates of both forms of the insect and the life table parameters are given in Table 1. According to the comparison tests performed, statistically significant differences were found between the development time, longevity, oviposition period, total pre-oviposition period, fecundity, mean generation time and net reproductive periods of biparental and uniparental forms (Table 1).

Table 1. Life table parameters of biparental and uniparental forms of the oleander scale, *Aspidiotus nerii* at 25±1ºC, 65±1% RH and 16:8 h L:D photoperiod

	Biparental		Uniparental				
	n	Mean±SE	n	Mean±SE	*F*	df	*P*
Development time (d)	191	45.07±0.09	69	59.78±0.42	9743.1	264	0.000*
Adult longevity (d)	191	17.31±1.64	69	28.80±0.42	9982.0	264	0.000*
Mean longevity, female (d)	65	93.29±0.46	69	88.58±0.76	1590.0	132	0.000*
Mean longevity, male (d)	126	46.43±0.14	-	-	-	-	-
Mean longevity, all (d)	191	62.38±1.62	75	84.16±1.91	8796.8	264	0.000*
APOP** (d)	65	12.17±0.08	69	12.06±0.05	84.9	132	0.259
TPOP*** (d)	65	56.74±0.96	69	71.78±0.42	345.9	132	0.000*
Oviposition period (d)	65	18.78±1.09	69	9.45±0.52	4130.4	132	0.000*
Fecundity (nymphs/female)	65	41.34±3.91	69	29.55±1.73	521.4	132	0.006*
Intrinsic rate of increase, (r) (d^{-1})	191	0.039±0.00	75	0.042±0.00	188.3	264	0.173
Finite rate of increase, (λ) (d^{-1})	191	1.040±0.00	75	1.043±0.00	188.6	264	0.173
Mean generation time, (T) (d)	191	67.51±0.43	75	78.49±0.53	9878.3	264	0.000*
Net reproductive rate, (R_0)	191	14.07±1.94	75	27.19±1.83	3142.9	264	0.000*

Standard errors were calculated by using with 200,000 bootstrap replicates. The difference between means in the same row and indicated with * is significant according to P < 0.05 (F test; Sidak); ** Adult pre-oviposition period of female adult; *** Total pre-oviposition period of female counted from birth.

Developmental time (45.07 and 59.78 d), adult longevity (17.31 and 28.80 d), mean longevity of all (62.38 and 84.16 d), the generation times (*TPOP*) (56.74 and 71.78 d), and mean generation times (67.51 and 78.49 d) were significantly shorter in the biparental form than the uniparental form, respectively (Table 1). Mean longevity of female (93.29 and 88.58 d) and oviposition periods (18.78 and 9.45 d) were significantly longer in the biparental form than the uniparental form, respectively (Table 1). Similarly, fecundity (41.34, 29.55 nymphs/female) and net reproductive rate (14.07 and 27.19 nymphs) were significantly higher in the biparental form, respectively (Table 1).

Since life table parameters reflect combined effects of life history parameters, including survival, development and reproduction, they provide an accurate estimate of the growth rate of an insect

population (Uygun & Atlıhan, 2000; Atlıhan & Özgökçe, 2002; Özgökçe & Atlıhan, 2005; Özgökçe et al., 2006; Atlıhan & Chi, 2008; Chang et al., 2016; Tuan et al., 2016, Atlıhan et al., 2017; Bussaman et al., 2017). In this study, no differences were found between the intrinsic rates of increase (0.039, 0.042 d^{-1}) and the finite rate of increase (1.040, 1.043 d^{-1}) for either form (Table 1).

The age-stage-specific survival rate (s_{xj}) of both forms are represented in Figure 1. These curves show the probability that a newly emerged individual will survive to age x and stage j. For example, the probability that a newly emerged nymph survives to the adult stage is 0.24 for males and 0.34 for females for the biparental form and 0.92 for the uniparental form (Figure 1). Given the variation in the developmental rate between biparental and uniparental forms, there are obvious overlapping of stages.

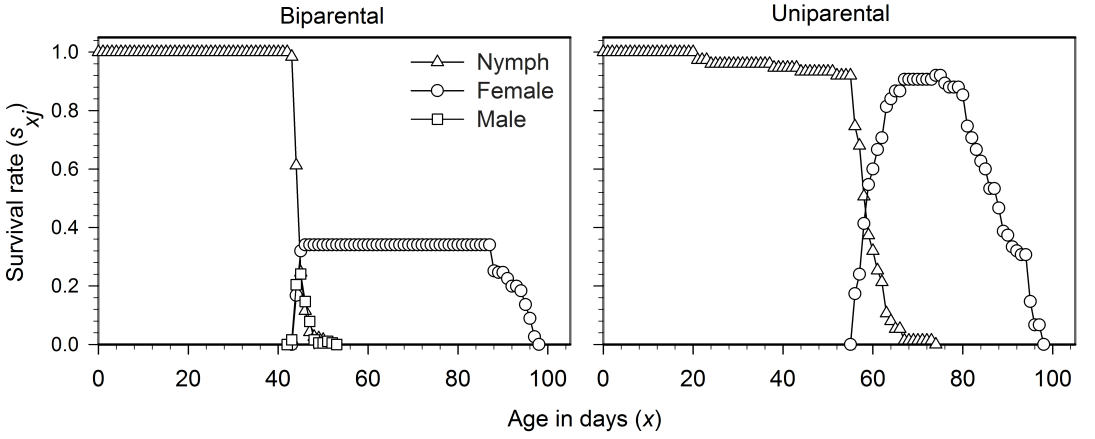

Figure 1. Age-stage specific survival rate (s_{xj}) of biparental and uniparental forms of *Aspidiotus nerii* on squash.

The age-specific survival rate (l_x), the age-specific fecundity (m_x) and the age-specific maternity ($l_x m_x$) curves of both forms of oleander scale are shown in Figure 2. The l_x is the probability that a newly emerged individual survive to x and its curve is a derivate s_{xj} (Marouf et al., 2013). The l_x of the biparental form sharply decreased from 45-52 d due to death of male individuals within 1-2 d and the adult females in the cohort died from 86-98 d. Whereas, the uniparental females died from 75-98 d (Figure 2). While the first reproduction began after 56 d and reached the highest growth rate (3.74 individuals/female) within 6 d in the biparental form, it began after 68 d and reached highest level (3.10 individuals/female) at 87 d in the uniparental form (Figure 2).

The total numbers of nymphs emerging for the whole population were 2687 for biparental and 2039 uniparental forms. Although a shorter developmental period, a longer oviposition period and a higher fecundity was found for the biparental form. The age-specific maternity curve, which was calculated under the effect of sharply decrease in the survival rate at the beginning of the reproduction, showed a gradual departure from the m_x curve in the biparental form (Figure 2).

The expected life time (e_{xj}) of individuals in both forms of the oleander scale is shown in Figure 3 and it estimates the time individuals of age x and stage j are expected to live. For example, the life expectancy of a newly emerged nymph is 62.38 d for the biparental form while it is 84.16 d for the uniparental form. Reproductive value (v_{xj}) for a newly emerged individual is the finite rate of increase (λ) and gives the expectation of future population of individuals of age x and stage j (Fisher, 1930; Pianka, 1994; Kavousi et al., 2009). The peak in reproductive value 27.90 individuals occurred after 57 d in the biparental form, and 19.92 individuals after 68 d in the uniparental form (Figure 4).

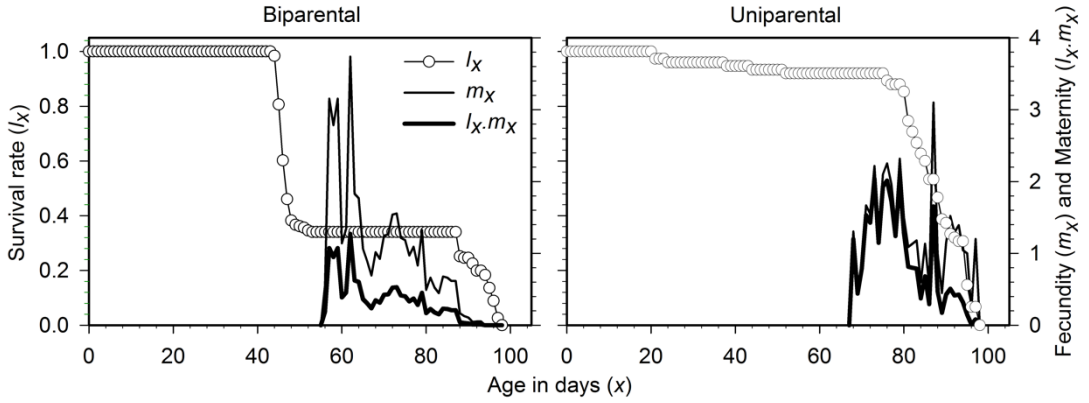

Figure 2. Age-specific survival rate (l_x), age-specific fecundity (m_x), and age-specific maternity ($l_x m_x$) of biparental and uniparental forms of *Aspidiotus nerii* on squash.

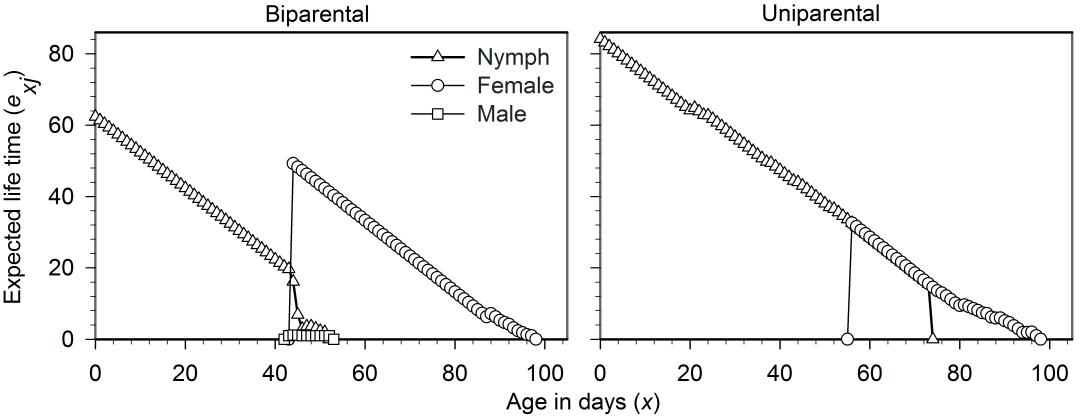

Figure 3. Age-stage-specific life expectancy (e_{xj}) of biparental and uniparental forms of *Aspidiotus nerii* on squash.

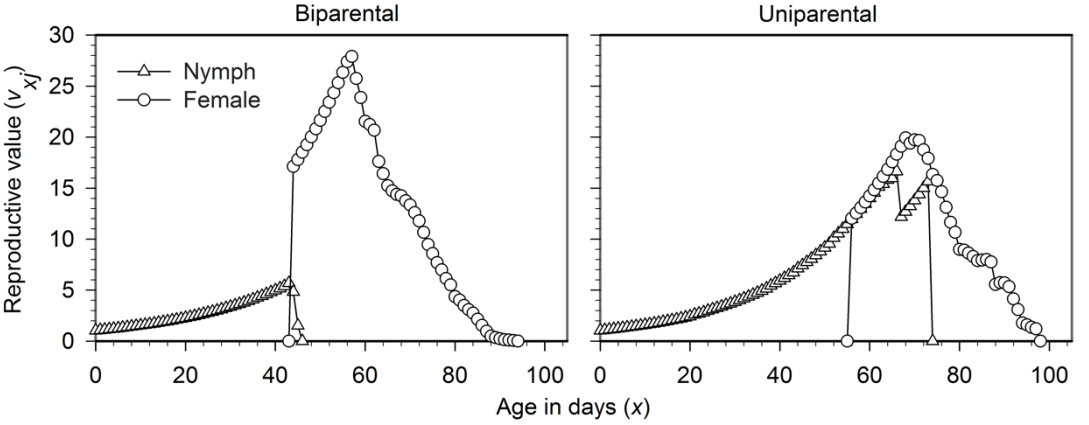

Figure 4. Age-stage-specific reproductive value (v_{xj}) of biparental and uniparental forms of *Aspidiotus nerii* on squash.

The intrinsic rate of increase is most important parameter in life table studies and it gives the most comprehensive description of the growth, development and reproduction of a population, however, it gives no information about the number of individuals. The population projection is an estimate of the future population of a cohort using the basic data (survival rate, development rate and fecundity), and it predicts the growth trends, as well as the stage structure of a population in the short or long term (Farhadi et al., 2011; Huang & Chi, 2011). The population size which a given initial population can reach in a specified time can be estimated by using the TIMING-MSChart program. In this study, 10 newly emerged nymphs were taken as the initial population for the biparental and uniparental forms of oleander scale, and the population size after 100 d according to each stage was calculated and the results are given in Figure 5. Theoretically, it is estimated that the total number of individuals can reach about 140 and 266 individuals, respectively.

Gerson & Hazan (1979) reported that the generation time of the biparental form was shorter than the uniparental form at 19, 24 and 28ºC. They reported that the biparental form completed a generation in about 45 d and the uniparental form in about 64 d at 24ºC, which close to the temperature in this study. Similarly, they emphasized that the number of progeny and oviposition periods were statistically higher for the biparental form at the three temperatures. The same researchers found that the biparental form produced 99.7 nymphs and the uniparental form 41.6 nymphs, and the oviposition periods were 37.2 d and 24.3 d at 24ºC, respectively. Schmutterer (1952) reported that both forms required about 91 d to complete a generation, biparental females each produced an average 127 nymphs and uniparental females 41 nymphs at 25ºC and 80% RH. In a similar study by DeBach & Fisher (1956), the time required for the uniparental form to complete a generation at 23.9ºC was 49 d and the maximum number of nymphs was 94.

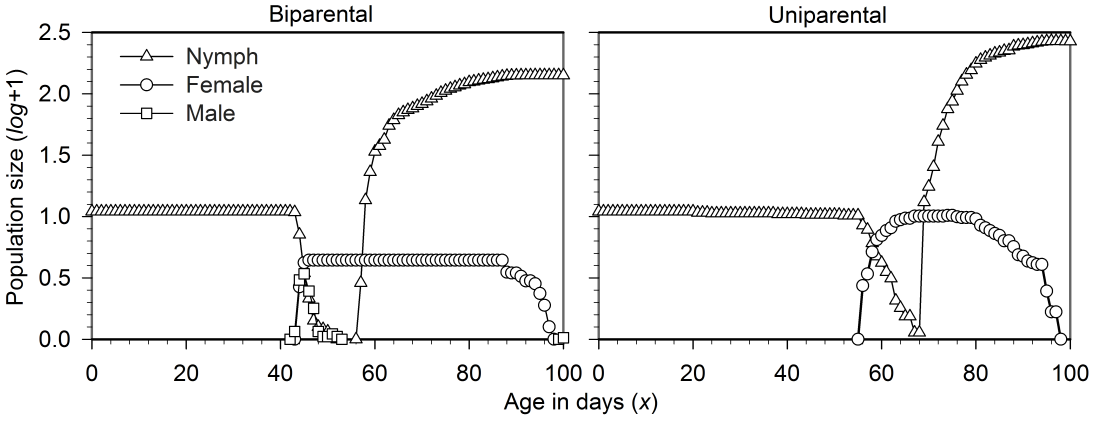

Figure 5. Population projection showing the change of age-stage structure of biparental and uniparental forms of *Aspidiotus nerii* on squash during population growth.

In this study, the biparental form was able to complete a generation in 57 d and the uniparental form in 72 d, and the oviposition periods were about 19 and 9.5 d respectively. During this period, maximum total nymphs were 176 and 67, respectively. Growth conditions and host differences are likely to be the reason for the differences in the results.

However, in previous studies, consistent general results were obtained in terms of development, reproduction and generation times for both forms, that is, the biparental form developed faster, the duration of oviposition was longer, and the number of nymph was greater.

There are some noteworthy differences in the life table results of both forms of the oleander scale. A life table, which is generated based on the development, reproduction, and survival data under certain conditions by an organism, can provide basic information about the entire biology and population dynamics. Among the parameters of a life table, in particular the intrinsic rate of increase is a highly useful parameter for comparing organisms. In this study, although the biparental and uniparental forms of oleander scale have statistically significant differences between the development, reproduction, survival

and some of the life table parameters calculated, there were no significant differences between the intrinsic rate of increase and the finite rate of increase. However, the net reproductive rate and mean generation time parameters were statistically different between the both forms. As found in this study and also in previous studies, the number of nymphs of the biparental form was significantly higher than the uniparental form. The biparental form was found to give 2.6 times more nymphs than the uniparental form in terms of total number of nymphs and net reproductive rate even though the mean generation time was shorter. When the life table parameters are calculated, the sex ratios as well as the rate of reproduction, development and survival ratios are taken into account. The sex ratio in the biparental population was 66% males. These differences may cause significant changes in population dynamics in favor of the uniparental form over the long term. Specifically, according to the population projection, the uniparental form was found to increase the population by 1.9 times compared to the biparental form based on a 100-d estimate. In the comparison of the life table parameters, r and λ are statistically different, while T is shorter in favor of the biparental form, and R_0 is greater in favor of the uniparental form. Therefore, it is difficult to conclude which form has the more advantageous population dynamics. The population projections obtained in this study are important as they provide a more detailed understanding on this pest.

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Original article (Orijinal araştırma)

Neonicotinoid resistance of *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) in cotton fields of Çukurova Region, Turkey[1]

Çukurova Bölgesi (Türkiye) pamuk alanlarında *Aphis gossypii* (Glover) (Hemiptera: Aphididae) neonikotinoid direnci

Selçuk ULUSOY[2*] **Ekrem ATAKAN[3]** **Sadık DİNÇER[4]**

Abstract

Cotton aphid, *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae), is a major pest in cotton fields. Neonicotinoids are important and highly prevalent insecticides currently used against *A. gossypii* and other herbivorous insect pests in the Mediterranean Region of Turkey. However, some insecticide applications against *A. gossypii* in the Çukurova Region have failed despite using high rates. Therefore, bioassays and enzyme analyses were conducted to determine resistance to imidacloprid and thiamethoxam in *A. gossypii* populations collected in 2015-2016 from cotton fields in this region. Resistance factors (RF) were 54.6 to 206.5 fold for imidacloprid and 5.7 to 65.7 fold for thiamethoxam. Populations from Kürkçüler (RF 206.5) had the highest LD_{50} for imidacloprid and from Körkuyu (RF 65.7) for thiamethoxam. Enzyme analysis revealed statistically higher metabolic resistance. Maximum enzyme activities were 17.8, 142.3 and 3.8 nM/min/mg protein for carboxylesterase for in Körkuyu, for glutathione S-transferase in Bahçe and for cytochrome P450 monooxygenase in Körkuyu, respectively. This study revealed the development of resistance in *A.gossypii* to neonicotinoid insecticides in Turkey and the need for new management strategies to break this resistance.

Keywords: *Aphis gossypii*, biyoassay, cotton, neonicotinoid, resistance

Öz

Pamuk yaprakbiti, *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae), pamuk tarım alanlarında ana zararlılardandır. Neonicotinoidler, Akdeniz Bölgesi'nde *A. gossypii* ve diğer herbivor böceklerin mücadelesinde oldukça yaygın kullanılan önemli bir insektisit grubudur. Ancak, Çukurova Bölgesi'nde *A. gossypii*'ye karşı bazı insektisit uygulamaları yüksek oranlarda kullanılmasına rağmen başarısız olmuştur. Bu nedenle, bu bölgedeki pamuk alanlardan 2015-2016 toplanan *A. gossypii* popülasyonlarında imidacloprid ve thiamethoxam dayanıklılık düzeyi belirlemek amacıyla biyoassay ve enzim analizleri yapılmıştır. Analizler sonucunda imidacloprid için 54.6-206.5 (dirençlilik faktörü: RF) arasında, thiamethoxam için 5.7-65.7 arasında LD_{50} dayanıklılık katsayıları bulunmuştur. Kürkçüler (RF 206.55) popülasyonu imidacloprid için, Körkuyu (RF 65.72) popülasyonu da thiamethoxam için en yüksek LD_{50} değerine sahiptir. Enzim analizi istatistiki anlamda yüksek metabolik direnci ortaya çıkarmıştır. Her iki insektisit içinde en yüksek enzim akitviteleri, karboksil esteraz enzimi Körkuyu popülasyonunda 17,8 nM/dk/mg protein, glutatyon S-transferaz (GSTs) enzimi Bahçe popülasyonunda 142,3 nM/dk/mg protein ve Körkuyu popülasyonunda 3,8 nM/dk/mg protein ile en yüksek monooksigenaz P450 enzim aktivitesi bulunmuştur. Bu çalışma, Türkiye'de neonicotinoidlere karşı *A. gosyypii*'de direnç gelişmesini ve bu direncin kırılması için yeni yönetim stratejilerine ihtiyaç olduğunu ortaya koymuştur.

Anahtar sözcükler: *Aphis gossypii,* biyoassay, pamuk, neonicotinoid, dirençlilik

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Introduction

The cotton aphid, *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) is one of the most important pest of cotton plants. It has a polyphagous habit and has a wide host range (Blackman & Eastop, 2000; Tomizawa & Casida, 2005). As the main pest of cotton, *A. gossypii* produces honeydew on the plant that supports the development of sooty molds. It causes direct damage through sucking leaves and indirectly by transmitting some viral pathogens to its host (Kim et al., 1986). One of the most effective control methods being used worldwide for this pest is the application of insecticides. However, the development of resistance to insecticides (organophosphate, carbamate, pyrethroid and neonicotinoids) has been reported in many countries (Ahmad et al., 2003; Wang et al., 2007).

Neonicotinoids are highly effective chemical insecticides that control many important pests; providing good market value (Nauen et al., 2008; Jeschke et al., 2011). They have been used effectively against the coleopteran, dipteran and lepidopteran insects by foliar, soil and seed treatments in more than 120 countries for 25 years (Nauen et al., 2008; Bass et al., 2015). The first neonicotinoid, imidacloprid, was discovered in 1990 and it affects the central nervous system of insects (Elbert et al., 2008; Bass et al., 2015).

Recently, an increasing number of studies associated with neonicotinoid resistance in cotton aphids have been published (Herron et al., 2011; Bass et al., 2015). Thiamethoxam and imidacloprid, which belong to the neonicotinoid group, are the most commonly used insecticides (Herron et al., 2011). These insecticides bind irreversibly to insect nerve cell nicotinic acetylcholine receptors (nAChR), resulting in impaired nerve function in insects (Herron et al., 2011; Jeschke et al., 2011). More than 500 peer-reviewed papers have been published on neonicotinoid resistance issues in different pest insects in which a substantial proportion of these refer specifically on imidacloprid resistance (Bass et al., 2015). Neonicotinoides have been widely used in the management of aphids since 1990. This has led to the development of resistance to imidacloprid and thiamethoxam in the aphids (Herron et al., 2011; Gore et al., 2013). The different mechanisms of insecticide resistance have been a focus of many studies. Particularly, in *A. gossypii* a number of enzymes having been reported to be involved in detoxification of these insecticides.

Metabolic resistance due to overproduction of total esterase causes detoxification of organophosphates, carbamates and pyrethroids in Hemiptera and Diptera (Field et al., 1999; Bass & Field, 2011). In addition, it has been reported that acetylcholinesterase and alpha-naphthyl acetate (α-NA) esterases levels were higher in the imidacloprid resistant *A. gossypii* populations (Wang et al., 2002). High levels of glutathione S-transferases (GST) activity has been widely observed in organophosphate, organochlorine, dichlorodiphenyltrichloroethane (DDT), and pyrethroid insecticide chemical classes in the formation of individual resistance (Ranson & Hemingway, 2005; Li et al., 2007).

Cytochrome P450 occurs widely in nature and is involved in many biological processes, such as hormone synthesis and the metabolism of xenobiotics (Scott & Wen, 2001). In insects, cytochrome P450 monooxygenases (P450) is implicated in resistance to insecticides through the degradation of these foreign compounds to more soluble and less toxic forms (Scott & Wen, 2001; Rauch & Nauen, 2003). In addition, high levels of P450 enzyme activity were found in neonicotinoid insecticide resistant *A. gossypii* populations (Shang et al., 2012; Seyedebrahimi et al., 2015).

Pesticide use in Adana, Mersin and Antalya Provinces accounts for about 40% of Turkey's annual pesticide consumption. While the rate of pesticide usage in Adana is 11%, this rate is 16% in Icel. It has been estimated that about 40% of total pesticide use is on cotton and cereals and this is mostly insecticides (Dağ et al., 2000; Ulusoy et al., 2017a,b). As of 2018, in Turkey there were more than 250 licensed insecticide products for use against *A. gossypii* in cotton fields (Anonymous, 2018). Half of these insecticide are neonicotinoids (40% imidacloprid, 46% acetamiprid, 12% thiamethoxam, 2% clothianidin), 39% organophosphates, <8% pyrethroids and <5% pyridines (Anonymous, 2018). So clearly the highest consumption is of neonictinoid group insecticides. Velioğlu et al. (2008) reported that insecticide resistance resulted in insensitivity to acetylcholinesterase in *A. gossypii* populations in Çukurova cotton fields. Ulusoy et al. (2017a,b) reported varying levels of insecticide resistance in *A. gossypii* populations for clothianidin and acetamiprid in cotton fields of Adana Province. The problem of cotton pest has been rapidly increasing worldwide, especially in irrigated fields. The increase in pest populations leads

extensive chemical applications and occurrence of the resistance problems (Tomlin, 1997). The most intensive insecticide applications are in the Mediterranean Region of Turkey (Velioğlu et al., 2008). Intensive use of chemicals disrupts the existing natural balance in agroecosystems and leads to development of insecticide resistance. Similar to other countries, the cotton agroecosystems in Turkey has been challenged by the insecticide resistance problems (Tomlin, 1997).

The Mediterranean Region of Turkey is one of the most important agricultural areas where polyculture is frequently practiced. For this reason, pest management systems are highly dependent on the use of insecticides. Thus, the objective of this study is to determine the neonicotinoid resistance status in the *A. gossypii* populations collected from the different cotton growing areas of in Adana Province located in Çukurova Region of Turkey.

Material and Methods

Aphid populations

Aphis gossypii populations was sampled in 2015 in the Çukurova Region where intensive insecticide applications have been used (Table 1). A susceptible clone which has been maintained for 20 years under in vitro conditions was obtained from Bayer (Leverkusen, Germany). All populations were routinely reared on *Gossypium hirsutum* grown in net-covered cages, (70×50×40 cm), under greenhouse conditions at 22°C, 65±5% RH and 16:8 h L:D photoperiod. The plants in the cages were replaced every 2 weeks with new ones in order to keep colonies alive. The test population was collected from the cotton fields located in the different areas of Çukurova Region (namely, Körkuyu, Durhasandede, Bebeli, Kürkçüler, Bahçe, Çukurova, Yumurtalık) in Adana Province.

Table 1. *Aphis gossypii* populations from cotton fields and the susceptible population tested in this study

Population	Coordinates	Collection date
Körkuyu	35º57’09.3" N, 35º47’36.9" E	May 2015
Durhasandede	36º56’47.5" N, 35º45’25.6" E	May 2015
Bebeli	36º38'15.2" N, 35º27'08.0" E	June 2015
Bahçe	36º37'26.8" N, 35º25'47.3" E	June 2015
Çukurova	37º01'16.1" N, 35º21'17.6" E	May 2015
Kürkçüler	37º02'16.1" N, 35º33'32.6" E	May 2015
Yumurtalık	36º48'29.0" N, 35º43'10.2" E	June 2015
Susceptible	Germany	1998

Insecticides and bioassays

In the experiments, imidacloprid (350 g/L soluble concentrate) and thiamethoxam (400 g/L soluble concentrate) commercial formulations were used. Aphid samples were taken for bioassay experiments after one to two generations of greenhouse culture conditions. The Insecticide Resistance Action Committee 019 bioassay method was used to determine the resistance status of the aphids to the insecticides (IRAC, 2015). Leaf samples taken from cotton plants were cut into 4 cm diameter discs. The leaves were dipped in the insecticide solutions for 10 s, dried and then placed in Petri dishes containing 1.5% agar. About six different doses, excluding a control, were tested in three replicates. The field-collected populations were tested against 1-100 ppm for imidacloprid and 1-200 ppm for thiamethoxam, and the susceptible population against 0.1-30 ppm for both insecticides. Distilled water containing 0.2% Triton-X (0.2 g/L) was used as the control. About 30 adult aphids were transferred to each Petri dish. After the Petri dishes were covered with Parafilm, they were placed in a controlled environment at 22±1ºC, 70% RH and 16:8 h L:D photoperiod. Mortality was assessed after 72 h.

Biochemical assays

Populations from cotton fields were collected into ice boxes and kept at -80°C until used within two weeks for enzyme analysis.

Determination of carboxylesterase activity

Twenty individual aphids were homogenized in 100 µl sodium phosphate buffer (0.1 M, pH 7.5) (containing 0.1% Triton X-100). This homogenate was used as an enzyme source after centrifugation at 10,000 g, at 4ºC for 5 min. The supernatant used as an enzyme source was diluted 10 times. Supernatant of 25 µl was combined with 25 µl of phosphate buffer (0.2 M, pH 6) in a microplate. The reaction was initiated by the addition of 200 µl substrate solution to the wells. The substrate solution was prepared by dissolving 30 mg fast blue RR salt in 50 ml of 0.2 M sodium phosphate buffer and adding 500 µl of 100 mM α-napthyl acetate to this mixture. Enzyme activity was read at 23ºC with a Multiskan GO Microplate Spectrophotometer for 10 min at 450 nm. Blank cells were read without homogenization. Enzyme readings were made three times (Stumpf & Nauen, 2002). Mean levels of carboxylesterase **(**CE) activity were based on protein content and α- naphthol standard curves. Protein content was determined by the method of Bradford (1976) using bovine serum albumin as the standard.

Determination of glutathione S-transferase activity

The method developed by Stumpf & Nauen (2002) and was used to determine GST activity. About 30 individuals were homogenized in 300 µl Tris HCL buffer (0.05 M, pH 7.5). The supernatant was centrifuged at 10,000 g at 4ºC for 5 min. One hundred 100 µl supernatant, 100 µl 1-chloro-2,4-dinitrobenzene (CDNB) and 100 µl reduced glutathione (GSH) were added to a microplate. CDNB was prepared in 0.1% ethanol and 0.4 mM CDNB was added to the microplate wells at final concentration. GSH was prepared in Tris HCL buffer and 4 mM GSH was added to the wells at final concentration. The change in absorbance was read at 340 nm at 25ºC for 5 min. Enzyme readings were made at three-time replicates. Changes in absorbance per minute were converted into nM CDNB conjugated/min/mg protein using the extinction coefficient of the resulting 2,4-dinitrophenyl-glutatione (Habig et al., 1974).

Determination of cytochrome P450 monooxygenase activity

The method of Hansen and Hodgson (1971) was used to determine the P450 enzyme activity. Accordingly, 90 µl of the enzyme from stock solutions and 100 µl 2 mM p-nitroanisole (substrate) were added to each of the microplate wells. After incubating for 2 min at 27ºC, 10 µl of 9.6 mM nicotinamide adenine dinucleotide phosphate (NADPH) was added to initiate the reaction. Measurement of P450 enzyme activity was made in a microplate reader at 405 nm at 27ºC for 10 min at intervals of 10 s, three-time replicates. Protein quantities were calculated according to Bradford (1976) with OD values determined. The enzyme activity was determined using the extinction coefficient of p-nitrophenol (Kranthi, 2005).

Statistical analysis

Dose-response regressions were computed using Polo-Plus computer program (LeOra Software, Berkeley, CA, USA). In order to estimate the LD_{50} (lethal dose to kill 50% of the test population), resistance factors were calculated by dividing the LD_{50} of the field collected population by the LD_{50} of the susceptible population.

Results

Bioassay

Imidacloprid resistance factors (RF) ranged from 54 to 206 fold. The most sensitive population was from Bebeli, whereas the most resistant was from Kürkçüler. For thiamethoxam the RF ranged from 5 to 65 fold. The most susceptible population was from Yumurtalık and the most resistant from Körkuyu (Table 2).

Table 2. Bioassay of imidacloprid and thiamethoxam in test and susceptible populations of *Aphis gossypii*

	Imidacloprid					Thiamethoxam				
Population	n	Slope±SE	LD_{50} µl/ml confidence	X^2	RF	n	Slope±SE	LD_{50} µl/ml confidence	X^2	RF
Körkuyu	540	1.49±0.457	12.001 (2.909-20.826)	0.986	139,54	540	1.20±1.650	85.111 (45.672-106.868)	0.602	65.72
Durhasandede	540	1.93±0.602	9.768 (3.008-14.669)	0.296	113,58	540	1.26±0.402	33.152 (11.347-76.001)	0.602	25.60
Bebeli	540	1.77±0.391	4.698 (1.758-7.775)	0.958	54,62	540	1.37±0.337	16.284 (7.163-25.954)	0.602	12.57
Bahçe	540	1.38±0.299	5.465 (2.289-8.870)	0.863	63,54	540	2.45±0.661	17.387 (7.674-25.704)	0.602	4.22
Çukurova	540	1.52±0.353	12.360 (3.506-23.590)	0.991	143,72	540	1.48±0.38	68.214 (34.754-109.595)	0.602	52.67
Kürkçüler	540	2.24±0.743	17.764 (9.454-24.930)	0.475	206,55	540	1.75±0.202	18.570 (8.190-28.706)	0.602	14.34
Yumurtalık	540	1.36±0.422	7.741 (1.839-13.006)	0.819	90,01	540	0.90±0.252	7.496 (1.415-15.782)	0.602	5.78
Susceptibe	540	0.50±0.164	0.086 (0.001-0.376)	0.387	-	540	0.745±0.238	1.295 (0.158-3.859)	0.857	-

RF, resistant factor; $X^{2,}$ lower than ($p \leq 0.05$) indicates a significant fit between the observed and expected regression lines.

Enzyme analyses

Maximum enzyme activity and ratios relative to susceptible population were 17.8 nM/min/mg protein (6.4 fold) in Körkuyu for carboxylesterase activity, 142.3 nM/min/mg protein (3.32 fold) in Bahçe for GST activity and 3.8 nM/min/mg protein (75 fold) in Körkuyu for P450 (Table 3). Bebeli, Durhasandede, Körkuyu and Bahçe populations were statistically different from other populations and their CE activity levels were higher than the susceptible populations. Çukurova, Yumurtalık and Kürkçüler populations were in the same statistical group with lower CE activity than the other populations. GST activity ranged from 10 to 140 M/min/mg protein. The Bahçe population had the highest enzyme activity relative to susceptible population. For GST activity, all populations were statistically different from each other. For P450 activity, the Körkuyu population had highest level enzyme activity (3.803 nM/min/mg protein) and was statistically different from the other populations. Metabolic resistance levels were found to be higher in the enzyme analyses.

Table 3. Carboxylesterase (CE), glutathione S-transferase (GTS), cytochrome P450 monooxygenase (P450) enzyme activities of resistant and susceptible populations

Population	n	CE (M/min/mg protein)	CE ratio (rest.pop./sus.pop.)	GSTs (M/min/mg protein)	GSTs ratio (rest.pop./sus.pop.)	P450 (M/min/mg protein)	P450 ratio (rest.pop./sus.pop.)
Körkuyu	3	17.85±0.57 a	6.46	75.17±1.154 c	1.75	3.803±0.57 a	74.57
Durhasandede	3	16.45±1.15 a	5.96	58.93±0.570 e	1.37	1.521±0.57 bcd	29.83
Bebeli	3	16.50±0.57 a	5.97	54.58±0,570 f	1.27	0.338±0.02 ab	6.63
Bahçe	3	17.21±0.57 a	6.23	142.23±1,670 a	3.31	1.876±0.57 cd	36.79
Çukurova	3	9.36±0.57 b	3.39	64.78±0,570 d	1.51	0.761±0.06 ab	14.91
Kürkçüler	3	8.85±0.57 b	3.20	88.02±0,570 b	2.05	2.789±0.57 de	54.68
Yumurtalık	3	8.61±1.15 b	3.11	90.66±0,570 b	2.11	0.507±0.04 ab	9.94
Susceptible (Control)	3	2.76±0.57 c	-	42.87±1,154 g	-	0.051±0.05 e	-

Discussion

Varying resistance levels were observed to imidacloprid and thiamethoxam, neonicotinoid insecticides, in seven populations collected from cotton fields. The Kürkçüler, Körkuyu and Durhasandede populations had the highest LD_{50} values compared to the other populations, while the susceptible populations changed with each insecticide application rate (Table 2). Resistance to imidacloprid was found to be the highest. Imidacloprid is an active ingredient in a large number of licensed insecticide products applied to cotton in Turkey (Anonymous, 2018). Its wide spectrum, very widespread and intensive use will have contributed to the high level of resistance of *A. gossypii* to this agent (Bass et al., 2015). Imidacloprid and thiamethoxam are also being used against major cotton pests, for example, *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae). In addition, these insecticides have also been extensively used on vegetables and other field crops in Çukurova. We consider that the resistance of *A. gossypii* is likely to remain at high levels as long as neonicotinoid insecticides are used for managing aphids, whiteflies and other piercing-sucking insect pests of cotton and vegetables in the study area (Dağ et al., 2000; Ulusoy et al., 2017a). The presence of piercing-sucking pests such as the polyphagous *A. gossypii* and *B. tabacii* in the same region, can lead to exposure to multiple insecticide applications in both cotton and vegetable fields. Intensive applications may create ideal condition for the selection of resistant populations. Furthermore, higher LD_{50} values were obtained in Kürkçüler and Körkuyu populations sprayed with the thiamethoxam and imidacloprid. There is a possibility of cross resistance due to overuse of insecticides of the same group (Marshall et al., 2012). In addition, studies have also reported cross resistance to imidacloprid and thiamethoxam insecticides (mode of action 4A neonicotinoid) (Shi et al., 2011; Wang et al., 2007).

Aphis gossypii exhibits different levels of resistance to insecticides of the neonicotinoid group worldwide. For instance, in China there was 1200 fold resistance to imidacloprid (Chen et al., 2017), but in Australia 85 fold to thiamethoxam was observed (Marshall et al., 2014). Resistance of 66.5 fold to imidacloprid in some cotton fields in the Asian continent (Shi et al., 2011). Furthermore, 6.4, 10 and 22 fold resistance has been recorded to acetamiprid, clothianidin and thiamethoxam, respectively (Herron & Wilson, 2011). Additionally, the resistance of 17 fold to imidacloprid and thiamethoxam were found in Shandong, China (Wang et al., 2007). Similarly, 74 fold-resistances in Körkuyu population was observed in the current study.

In the enzyme analysis, Yumurtalık population had minimal CE activity, whereas, Körkuyu population had the highest CE activity among the seven populations tested, followed by the susceptible population (Table 3). Bahçe population had the highest GST activity. Both these CE and GST activities are consistent with the bioassay results. It was observed that the increase in metabolic enzyme activities responsible for resistance paralleled the measured increase in resistance.

Previous studies have indicated that there was a strong positive correlation among the organophosphate, carbamate, pyrethroid chlorinated hydrocarbon group insecticide resistance levels and general esterase and GST activities in aphids and many insect species (Devonshire & Moores, 1982; Hemmingway & Georghiou, 1984; Rauch & Nauen, 2003). In vivo, high esterase enzyme activity representing up to 3% the amount of total protein was observed in highly resistant aphid populations (Devonshire & Sawicki, 1979; Devonshire, 1989).

Even though organophosphate insecticide resistance was not assayed in the current study, it is predicted that resistance to organophosphate insecticides could be higher because more than 40% of licensed insecticides used in cotton and vegetable fields in this region contain organophosphates (Anonymous, 2018). In a study of Velioğlu et al. (2008), it was reported that insecticide resistance in *A. gossypii* resulted from higher acetylcholinesterase activity in a population from a Çukurova cotton field. It has also been reported that intensive insecticide application causes increased production of detoxifying enzymes, such as acetylcholinesterase, carboxylesterase and P450 group enzymes, in insects (Wang et al., 2002; Field et al., 1999; Bass & Field, 2011). Furthermore, acetylcholinesterase and alpha-naphthyl acetate (α-NA) esterase levels were found to be higher in imidacloprid-resistant *A. gossypii* populations (Wang et al., 2002).

In the current study, P450 analysis of the Körkuyu population of *A. gossypii* population showed a 74-fold increase in resistance (Table 2). P450 is an effective enzyme to give resistance to neonicotinoid insecticides in insects (Wang et al., 2007). P450 activities in Körkuyu and Çukurova populations were also higher than the susceptible populations. The P450 activities also parallel the bioassay results.

Some studies have reported that the resistant aphid populations have higher P450 activity compared to susceptible aphid populations (Shang et al., 2012; Seyedebrahimi et al., 2015). According to some studies, the neonicotinoid resistance mechanism is associated with mutations in the nAChR gene, but is usually directly associated with xenobiotic detoxification enzyme, 7-ethoxycoumarin O-deethylase, which is catalyzed by cytochrome P450 (Karunker et al., 2008; Nauen et al., 2008; Wang et al., 2009).

A. gossypii resistance to imidacloprid and thiamethoxam were determined by the bioassay and biochemical methods in Çukurova Province. Also, increasing resistance to neonicotinoids has been observed in this cotton fields. The suitable climatic conditions along with rich soils enable the establishment of polyculture in Çukurova Region. This region comprises intertwined cotton, vegetable and other agricultural fields. Proximity of vegetable and cotton growing areas to each other in the region facilitates movement of *A. gossypii* between different crops. Furthermore, extensive applications of neonicotinoid insecticides, such as imidacloprid and thiamethoxam, may lead to resistance development. The continuous use of pesticides with the same mode of action in the management of aphids may lead to the selection of resistant *A. gossypii* populations and the elimination of susceptible populations. It may contribute to the development of cross resistance. The results of this study suggest that new management methods and strategies should be developed and implemented for management of insecticide resistant *A. gossypii* in cotton growing areas of the region.

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Original article (Orijinal araştırma)

Investigation into control of cherry fruit fly, *Rhagoletis cerasi* (L., 1758) (Diptera: Tephritidae), in organic cherry production[1]

Organik kiraz yetiştiriciliğinde Kiraz sineği *Rhagoletis cerasi* (L., 1758) (Diptera: Tephritidae)'nin mücadelesi üzerine araştırmalar

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Abstract

The study was conducted at two locations, Pozantı (Adana) and Darboğaz (Ulukışla, Niğde) in 2015-2017. It aimed to determine the effects of the emergence time of cherry fruit fly, *Rhagoletis cerasi* (L., 1758) (Diptera: Tephritidae), the dynamics of adult flight and the control methods that could be used in organic cherry production. It investigated the effectiveness of netting trees, textile mulch, mass capture, plant-based insecticides and insecticide application against cherry fruit fly. Population monitoring revealed that the population of cherry fruit fly was low at Pozantı and slightly higher at Darboğaz. Clear statistical differences were observed between the untreated control and the treatments evaluated. The most effective control was obtained from with netting (100% efficacy). It was concluded that the other methods evaluated could be useful in organic cherry production.

Keywords: Alternative control, cherry, organic farming, *Rhagoletis cerasi,* Turkey

Öz

Çalışma, 2015-2017 yılları arasında Pozantı (Adana) ve Darboğaz (Ulukışla/Niğde) olmak üzere iki alanda yürütülmüştür. Kiraz sineği [*Rhagoletis cerasi* (L., 1758) (Diptera: Tephritidae)]'nin ortaya çıkış zamanı, popülasyon takibi ve Kiraz sineğine karşı organik kiraz yetiştiriciliğinde kullanılabilecek mücadele yöntemlerinin etkilerinin belirlenmesi amaçlanmıştır. Kiraz sineğine karşı mücadelede ağaçları örten net, malç tekstili, kitlesel yakalama tekniği, bitkisel kökenli insektisit ve insektisit uygulamalarının etkinliği araştırılmıştır. Yapılan popülasyon takibi, Kiraz sineği popülasyonunun Pozantı'da düşük, Darboğaz'da biraz daha yüksek olduğunu ortaya koymuştur. Denemeye alınan mücadele yöntemleri ile kontrol karşılaştırıldığında aralarında istatistiksel olarak fark olduğu gözlenmiştir. En etkili mücadele yöntemi net uygulaması (%100 etki) ile elde edilmiştir. Denemeye alınan diğer mücadele yöntemlerinin de organik kiraz yetiştiriciliğinde yararlı olabileceği sonucuna varılmıştır.

Anahtar sözcükler: Alternatif mücadele, kiraz, organik tarım, *Rhagoletis cerasi,* Türkiye

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Introduction

The European cherry fruit fly belongs to the family of Tephritidae, which has a worldwide distribution of about 4,000 described species in about 500 genera (Headrick & Goeden, 1998). The genus *Rhagoletis* Loew, 1862 includes about 65 known species (White & Elson-Harris, 1992). Most species are oligophages, attacking only a few closely related host plants. In addition to *Rhagoletis cerasi* (L., 1758), the American cherry fruit fly species, *Rhagoletis cingulata* (Loew, 1862), *Rhagoletis indifferens* Curran, 1932 and *Rhagoletis fausta* (Osten Sacken, 1877), as well as the apple maggot, *Rhagoletis pomonella* (Walsh, 1867), the blueberry maggot, *Rhagoletis mendax* Curran, 1932, and the walnut infesting species, *Rhagoletis completa* Cresson, 1929 and *Rhagoletis suavis* (Loew, 1862), are pest insects of economic importance (Boller & Prokopy, 1976). Host plants of *R. cerasi* include various *Prunus* spp. (*P. cerasus*, *P. avium*, *P. serotina*, *P. mahaleb;* Rosaceae) (Thiem, 1934; Leski, 1963) as well as *Lonicera* spp. (*Lonicera xylosteum* and *Lonicera tatarica; Caprifoliaceae*) (Mik, 1898; Thiem, 1932, 1939; Wiesmann, 1938; Ranner, 1988; White & Elson-Harris, 1992). The European cherry fruit fly is the most serious pest in cherry orchards in Europe and Turkey, causing fruit damage and yield losses (Ulusoy et al., 1999; Vogt, 2002; Daniel & Wyss, 2003). The adult flies emerge from the soil in May to June and begin to lay eggs under skin of cherry fruit about 10 d after emergence. The larvae develop inside the cherries. The larvae leave the fruit, drop to the soil and within hours start to pupate under the tree canopy. Cherry fruit fly is univoltine and overwinters as pupae (Wiesmann, 1934; Boller, 1966). In addition to cultural and biotechnical methods in the control of cherry fruit fly, the use of alternative substances is at the forefront of recent developments.

Production of cherry in Turkey increases slowly from year to year (about 600 kt in 2016) and the problem with *R. cerasi* has become more important. This situation motivated us to undertake some investigations concerning *R. cerasi* flight activity and possibilities of controlling it with different kinds of management.

The aim of the organic farming system is to produce clean products (pesticide free) of good quality and also to correct the ecological balance which is deteriorated due to traditional agriculture. Therefore, chemical methods should be regarded as a last resource due to their potentially adverse effects on the environment and on consumer health. For this, eco-friendly management techniques and tools are needed. The aim of this study was mainly to develop a reduced-risk management program and predict the first emergence via trapping method of *R. cerasi* flies for optimal timing of insecticide application.

Material and Methods

In this research natural populations of *R. cerasi* in orchards with mid-season and late cherry cultivars were studied. Materials used included yellow sticky traps (13.5x22.5 cm) with ammonia capsules (Trece-Pherocon® AM No-Bait trap with Dual-Pak™ Supercharger™), netting (0.8 x 2 mm mesh size, 8-10% shade), textile mulch, azadirachtin 40 g/l insecticide (a plant-based product) and thiacloprid 240 g/l insecticide.

Studies were conducted in three cherry orchards, located in Çukurova University Pozantı Agricultural Research Center [Pozmer orchard 1 (174 trees) and Pozmer orchard 2 (144 trees)] in Pozantı (Adana) and in Darboğaz (Ulukışla, Niğde) (123 trees) in 2015-2016. In 2017, studies were only conducted in two orchards in Pozantı. The mass capture techniques were used to study mature flight dynamics, and plant-based insecticide, textile mulch, netting, yellow sticky traps and slow-spreading ammonia capsules were evaluated as control measures. Insecticide application was applied for comparative purposes. In the trial orchards, the trees had been fruitful for at least 5 years and the experiments were conducted in large blocks in each orchard. Five treatments were concurrently and randomly applied to blocks with eight replicates per block distributed throughout the orchard, with each replicate consisting of one tree.

Rhagoletis cerasi flight activity

Yellow sticky traps with ammonia capsules were used to monitor the dynamics of adult flight. Three traps were set at Darboğaz and five at Pozantı (orchard 1). The traps were set before the start of adult flight at the beginning of May. They were hung on the southeast side of trees about 1.5-2 m above ground. The traps were checked twice per day until the first mature fly was trapped and then they were checked once per week and cleaned. The traps were removed after three consecutives zero captures. The enabled the first date of *R. cerasi* emergence to be determined for each orchard and annual adult flight graphs to be drawn.

Evaluation of the methods to control *Rhagoletis cerasi*

Mass trapping

The evaluation of mass trapping was done at Pozantı (orchard 2). The cherry cultivars were Sweet heart, 0900 Agriculture, Regina, Metron late and Starks gold as mid-season and late cultivars. During the study, no sprays were applied to control *R. cerasi*. Yellow sticky traps with ammonia capsules were hung at 1.5-2 m above ground in the mid center and outer section of the tree canopies. In order to monitor adult cherry fruit flies, two traps were hung around the orchard at the beginning of May in 2015, 2016 and 2017, and checked as described above. Mass traps were hung after first adult was seen in the traps. Traps were hung at intervals of 15-20 m with 3-4 trap/tree according to the size of each tree. Totally, 38 traps were used. Traps that were very dirty were replaces with new traps. Traps were left in the orchard to check whether the flight period continued after the harvest. The trapping was evaluated for 100 fruits randomly collected from the trees located in the middle part of each plot.

Textile mulch and netting

The evaluation of textile mulch and tree netting was done at Pozantı and Darboğaz on 123 and 144 trees, respectively, most at late ripening. Trees 4-5 m tall were protected by netting from the onset of ripening till the end of harvest. The effectiveness of two different covering methods with the anti-insect net was compared with unprotected trees. In treatment A (15 trees), mulch textile was used as a soil covering. The textile mulch was laid directly on the ground under the trees with its edges buried in the soil (Figure 1a). In treatment B (12 plants), a strip of netting was positioned vertically along both sides of the row, and then stitched to completely cover the trees, and then the netting was stitched together at the trunk level (Figure 1b).

No sprays were applied to control *R. cerasi* in any part of the orchards. The flight of the adults was monitored using one yellow sticky trap each per tree as described above. The percentage of fruits damage was assessed at the harvest time, by individually dissecting 50-100 fruit/tree. The number of fruit collected varied depending on the total yield of the tree. Each sample was collected from around of the entire tree.

Figure 1. a) Soil covering with mulch textile, and b) tree covering with net.

Plant-based insecticide

The assessment of a plant-based insecticide was conducted at both locations. The efficacy of the azadirachtin (plant-based) insecticide (formulated product Nimiks 4,5) was compared with insecticide containing thiacloprid as the active ingredient (formulated product Calypso OD 240). These two insecticides were applied 125 ml and 40 ml/100 l water, respectively, by tractor-mounted equipment and were compared with an untreated control. The flight of the adult flies was monitored with yellow sticky traps as described above. Spraying commenced after one adult fly was trapped. The spraying was repeated depending the numbers of adults trapped. The number of applications per treatment and application dates are detailed in Table1.

Table 1. Insecticide dates during 2015-2017

Year	Pozantı	Darboğaz
2015	28 May	21 June
2016	18 May, 1 June	2 June, 15 June
2017	26 May, 8 June	-

Damage assessment and data analysis

To asses percent fruit damaged at harvest in each plot, 50-100 fruit were randomly collected, damaged and healthy fruits were counted, and the percentage of fruits damaged by *R. cerasi* was determined. The results were evaluated statistically by analysis of variance. Mean differences were compared with Duncan's test ($P < 0.05$). The efficacy of the treatments in reducing fruit damage at harvest was calculated according to Abbott (1925).

Results

Rhagoletis cerasi flight activity

Figures 2 and 3 show the pattern of flight activity at Pozantı and Darboğaz, respectively. In addition, the first adult, highest and last exit dates of *R. cerasi* are detailed in Table 2.

Table 2. First, maximum and last capture, and harvest dates and duration of capture of adult *Rhagoletis cerasi* at Pozantı and Darboğaz

	Pozantı			Darboğaz	
	2015	2016	2017	2015	2016
First capture	25 May	18 May	25 May	27 May	25 May
Maximum capture	25 May	18 May	8 June	1 July	13 July
Last capture	15 June	15 June	22 June	1 July	20 July
Harvest	15 June	14 June	19 June	21 June	29 June
Duration of capture (d)	22	27	28	37	58

At Pozantı, the first adult fly captures occurred on 25 May 2017, 18 May 2016 and 25 May 2015. In 2015 and 2016, the maximum captures on the same dates, where as it was 2 weeks later during the warm and sunny period from 1 May to 8 June, 2017. Figure 2 shows the flight activity for each year. Peak captures were recorded between 18 May to 8 June 2015, 11 May to 8 June 2016, and 1 to 22 June 2017 when the climatic conditions were favorable. The subsequent decline in numbers was because of climatic conditions were no longer suitable. The decline was monitored until the last capture, which was observed at the end of June in three years.

At Darboğaz, the first captures occurred on 27 May 2015 and 25 May 2016 when the fruits were small and still green. The maximum catches were on 1 July 2015 and 13 July 2016, both after harvest. The last adult capture was in July in both two years. In 2015, the population of *R. cerasi* accepted as zero quarantine tolerance was found to be low relative to 2016. In 2016, captures were made had been registered from 25 May to 20 July with two peaks (Figure 3). After the harvest, some adults continued to be captured.

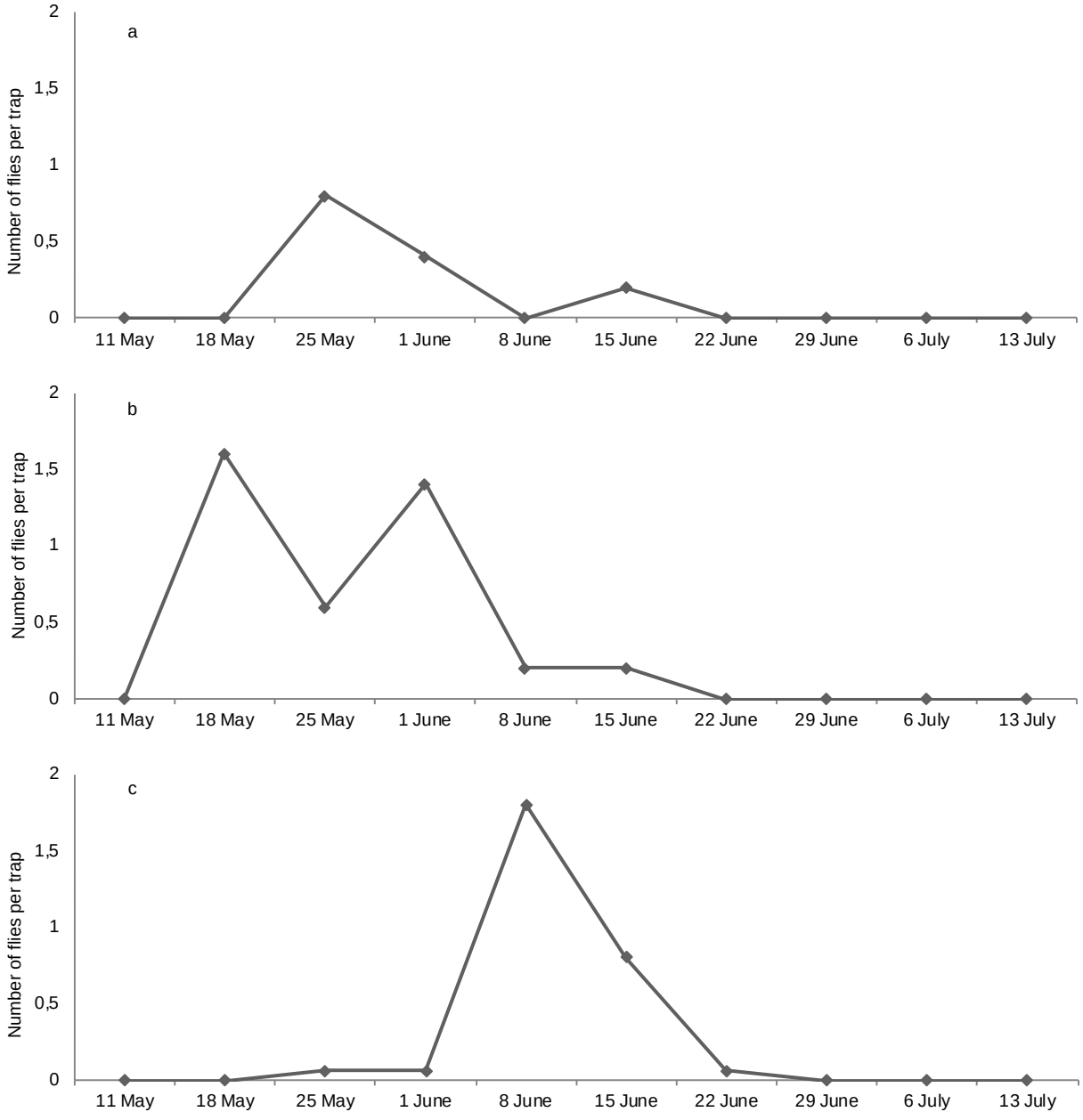

Figure 2. Flight activity of *Rhagoletis cerasi* at Pozantı in a) 2015, b) 2016, and c) 2017.

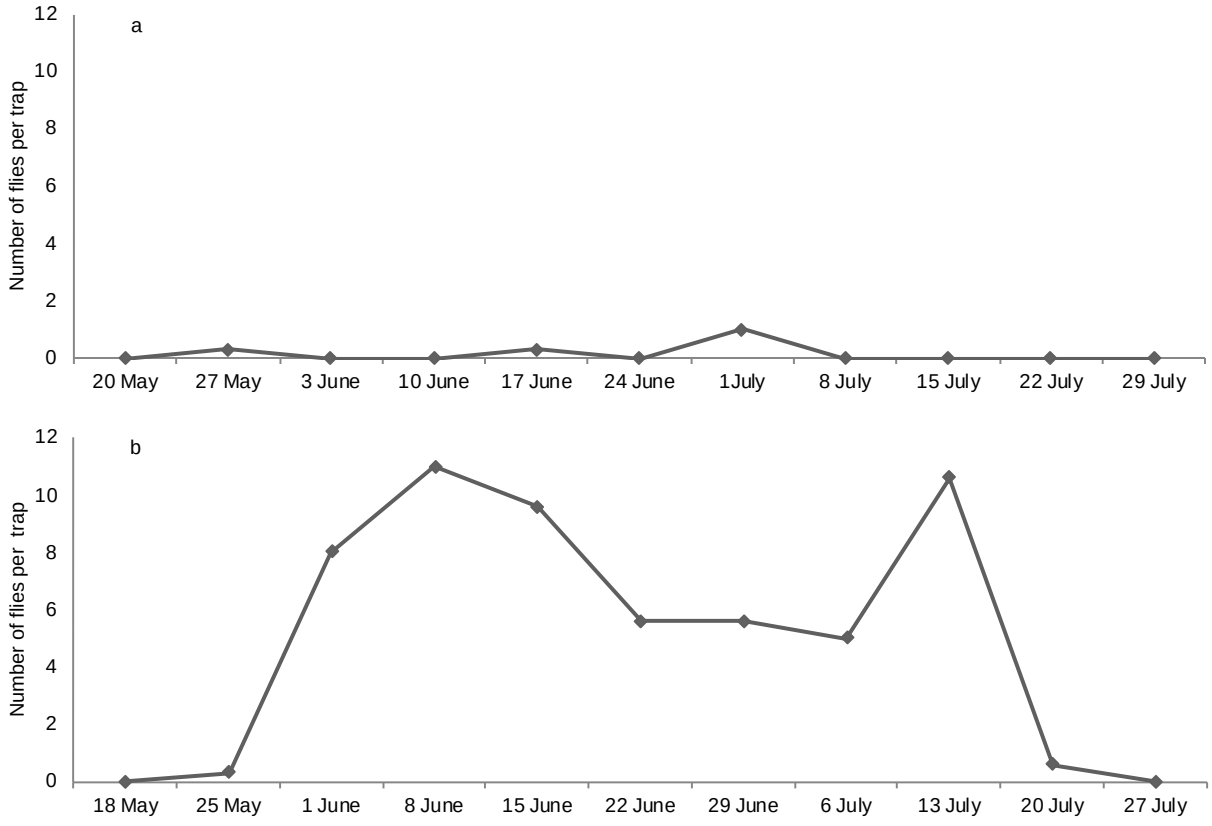

Figure 3. Flight activity of *Rhagoletis cerasi* at Darboğaz in a) 2015, and b) 2016.

Evaluation of methods to control *Rhagoletis cerasi*

At both Pozantı and Darboğaz, significant differences between the treatments in the percentage of fruits damaged by the *R. cerasi* were recorded (Tables 3 and 4). Fruit damage was always significantly higher in the untreated control than the other treatments. At Pozantı, fruit damage in control plot was 9.1, 27.6 and 11.1% in 2015, 2016 and 2017, respectively (Table 3). At Darboğaz, fruit damage in control was 7.5 and 11.6% in 2015 and 2016, respectively (Table 4).

At Pozantı, mass trapping was highly successful with only 4.5, 1.5, 0.8% fruit damage in 2015, 2016 and 2017, respectively, and its efficacy was 50.7, 94.6, 92.7% (Table 3).

At both Pozantı and Darboğaz, netting of trees prevented all damage, so the efficacy of the treatment was 100%.

For textile mulch at Pozantı, the damage was 1.0, 4.4 and 2.2%, with treatment efficacy of 89.0, 84.7 and 80.3% in 2015, 2016 and 2017, respectively (Table 3). At Darboğaz, the damage was 2.9% in both two years, with efficacy of 61.6 and 74.6% in 2015 and 2016, respectively (Table 4).

For azadirachtin at Pozantı, the damage 1.5, 7.8 and 1.6%, with efficacy of 83.6, 71.9 and 85.6% in 2015, 2016 and 2017, respectively. Whereas, with thiacloprid the damage was 3.1, 13.0 and 3.3%, efficacy of 65.7, 52.9 and 70.8% in 2015, 2016 and 2017, respectively (Table 3). At Darboğaz with azadirachtin the damage was 2.0 and 5.5%, with efficacy 73.3 and 53.1% in 2015 and 2016, respectively, compared to damage of 4.5 and 2.5% with thiacloprid, with efficacy of 40.0 and 78.5%, respectively (Table 4).

Table 3. Damage rates (%) determined for various control methods for *Rhagoletis cerasi* at Pozantı (2015-2017)

	2015		2016		2017	
Treatment	Damage (%) mean±SE*	Efficacy (%)	Damage (%) mean±SE *	Efficacy (%)	Damage (%) mean±SE*	Efficacy (%)
Mass trapping	4.5±1.03 b*	50.7	1.5±0.63 e*	94.6	0.8±0.30 d*	92.7
Thiacloprid	3.1±0.97 b	65.7	13.0±2.92 b	52.9	3.3±0.37 b	70.8
Azadirachtin	1.5±0.46 c	83.6	7.8±1.58 c	71.9	1.6±0.50 cd	85.6
Textile mulch	1.0±0.53 c	89.0	4.4±0.75 d	84.2	2.2±0.53 c	80.3
Netting	0.0±0.00 d	100.0	0.0±0.00 f	100.0	0.0±0.00 e	100.0
Control	9.1±2.90 a		27.6±3.99 a		11.1±1.93 a	

* Difference between means followed by the same letter within a column are not statistically significant based on Duncan's test (P<0.05).

Table 4. Damage rates (%) determined for various control methods for *Rhagoletis cerasi* at Darboğaz (2015 and 2016)

	2015		2016	
Treatment*	Damage (%) mean±SE **	Efficacy (%)	Damage (%) mean±SE **	Efficacy (%)
Thiacloprid	4.5±0.96 b**	40.0	2.5±1.04 c**	78.5
Azadirachtin	2.0±0.65 c	73.3	5.5±1.89 b	53.1
Textile mulch	2.9±0.93 c	61.6	2.9±1.07 c	74.6
Netting	0.0±0.00 d	100.0	0.0±0.00 d	100.0
Control	7.5±0.65 a		11.6±2.10 a	

* There was no orchard suitable for mass trapping technique at Darboğaz.
** Difference between means followed by the same letter within a column are not statistically significant based on Duncan's test (P<0.05).

Discussion

This study showed that the adult population density of *R. cerasi* in the orchard corresponded to the phenology of cherry trees. Also, the data collected at Pozantı showed that even at low population density of *R. cerasi* damage occurred, so there needs to be a zero tolerance for this pest. One reason for this is that *R. cerasi* usually pupate directly under the canopy of the cherry trees, especially under the south and southeast parts of the tree where the highest fruit infestation levels are observed (Engel, 1969). For pests that overwinter beneath perennial hosts, there appears to be little impetus for adults to move long distances. Cherry fruit fly does not move far and usually completes its maturation in the fresh shoots of the tree. Adults after mating firstly lay eggs in the fruit on that tree, but when they cannot find fruit, they only move to the nearest tree with fruit to lay their eggs. Cherry fruit fly adults do not tend to leave the environment as long as they can find suitable fruit for maturation, food and egg laying. Researchers reported which their movements are associated with normal activities of feeding, oviposition and mating (Wiesmann, 1934; Katsoyannos et al., 1986). These movements show a daily periodicity and rarely take individuals far from their host plants (Haisch et al., 1976; Katsoyannos et al., 1986). For these reasons, it is thought that if the food-attracting odors from traps are not strong, the flies do not head for such traps. Therefore, the adult density may be low in trees in which traps are hung. Particularly in control studies (mass capture technique), a large number of such traps need to be hung. At Darboğaz, first adult emergence was recorded when the fruits were small and green. After the harvest, some adults continued to be seen in the orchard, so it was concluded that these *R. cerasi* were living on alternative hosts (wild cherry, mahaleb trees and sour cherry trees) around the trial area.

When we compared the two orchards, it was observed that the first adult capture dates were very close to each other, although there was an altitude difference of 400 m between these orchards. This situation might be because some adults which emerged early from the diapause at Darboğaz. Ulusoy & Vatansever (2001) reported that *R. cerasi* adults can be seen between the second and third weeks of May at Pozantı and they can have emerged after another 10-15 d at Pozantı due to altitude and climatic conditions at Darboğaz. It has been reported that adults appear a little later in higher altitude areas than in lower altitudes areas, due to exposure to lower temperatures during post-diapause development (Kovancı & Kovancı, 2006). The cause of the early emergence of adults at Darboğaz might be that the average winter temperature is low and the temperature rises above 7ºC per day in March-April after they have completing the post-diapause development. This conclusion would be consistent with the causes of early emergence of the pest mentioned in the literature.

In both orchards, there were significant differences between treatments in the percentage of fruit damaged. Fruit damage was always significantly higher in the untreated control than the other methods. In both orchards, netting of trees was 100% effective and clearly the best option for fruit fly-free cherry production in ordinary and organic production and should be adopted as routine practice. This result was consistent with the reports of some other researchers. The high protection provides completes control with no side effects due to aphids or fruit rot being reported (Caruso & Cera, 2004; Charlot & Weydert, 2013). The results of this and earlier studies are consistent and it is recommended that been thinked a technique that should be transferred to practice.

It is clearly seen that mass trapping technique with yellow sticky traps is an effective method for cherry fruit fly control. The results were quite good when compared with the untreated control and insecticide application, and demonstrated that mass trapping for control of cherry fruit fly is a real alternative. This is consistent with other research that used yellow sticky traps in the control of cherry fruit fly which successfully prevented infestation of cherry fruit (Tezcan & Gülperçin, 2000; Tezcan et al., 2000; Ulusoy et al., 2001; Grassi et al., 2010).

Fabric mulching of the soil surface under the cherry trees was the next most successful method after netting of trees at both locations. Compared to insecticide application mulching was more successful with efficacy of 80-89% at Pozantı (vs. 53-71% with thiacloprid) and 62-75% at Darboğaz (vs. 40-79% with thiacloprid). One reasons for the success of the mulch is the biology of the pest, which generally only flies short distances. The pest pupates in the ground directly under the cherry tree crown so is preveted from emerging even if suitable conditions occur in the spring. Daniel & Baker (2013) studied the general potential of soil treatments and dispersal and flight behavior of *R. cerasi* within orchards. Their experiments using netting to cover the soil were conducted in two orchards with different pest population densities over two years. The netting reduced flight activity by 77% and fruit infestation by 91%. Therefore, it is reasonable to conclude that mulch application is a viable alternative to insecticide application. Therefore, given that mulch application can also control weeds and reduce water loss from the soil, it should be considered as a cultural control method in organic agriculture. The ability of mulch to prevent of weed emergence in a range or crops and to reduce soil water loss by evaporation had been confirm in a number of studies (Asiegbu, 1991; Monks et al., 1997; Kitiş, 2002; Kitiş et al., 2017).

In conclusion, the best results were obtained from netting (100%) followed by mass capture technique (93-95%) and mulch application (62-89%). The plant-based insecticide, azadirachtin was more effective than the synthetic insecticide, thiacloprid, which gave the lowest level of control. Mass capture and mulch application were shown to be superior than plant-based and synthetic insecticides. In light of these results, we can conclude that the cultural methods are viable alternatives to insecticide application.

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Original article (Orijinal araştırma)

Development of *Lysiphlebus testaceipes* (Cresson, 1880) (Hymenoptera: Braconidae) on different hosts and temperatures[1]

Lysiphlebus testaceipes (Cresson, 1880) (Hymenoptera: Braconidae)' in farklı konukçu ve sıcaklıklarda gelişimi

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Abstract

In this study, the development time, mortality, parasitization rate and sex ratio of *Lysiphlebus testaceipes* (Cresson,1880) (Hymenoptera: Braconidae: Aphidiinae) on *Aphis craccivora* Koch, 1854, *Aphis fabae* Scopoli, 1763 and *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae), were determined. The experiments were carried out at three different, temperatures (17, 22 and 27±1ºC) for each aphid species, 60±10% RH and 16:8 h L:D photoperiod. The development thresholds and thermal constants for the parasitoid were also determined for the three aphid species. The parasitization rate of *L. testaceipes* was 25.0, 53.4 and 20.5% (for 17, 22 and 27ºC, respectively) for *A. craccivora*; 62.7, 71.1 and 37.1% for *A. fabae*; and 54.2, 70.7 and 20.0% for *A. gossypii*. The development time of *L. testaceipes* was 18.5, 10.9 and 7.9 d in *A. craccivora*, 17.6, 10.2 and 7.4 d in *A. fabae*, and 19.8, 12.6 and 9.3 d in *A. gossypii* at 17, 22 and 27±1ºC. The development thresholds and thermal constants for *L. testaceipes* in *A. craccivora*, *A. fabae* and *A. gossypii* were 9.42, 9.69 and 8.12ºC, and 136.99, 128.05 and 175.44 degree days, respectively. Based on the overall results, *A. fabae* is an excellent potential host for the mass rearing of *L. testaceipes* at 20-22ºC.

Keywords: Aphid species, biological control, development threshold, parasitoid, sex ratio

Öz

Çalışmada *Lysiphlebus testaceipes* (Cresson,1880) (Hymenoptera: Braconidae: Aphidiinae)'in *Aphis craccivora* Koch, 1854, *Aphis fabae* Scopoli, 1763 ve *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) üzerinde gelişme süresi, ölüm, parazitleme ve cinsiyet oranı belirlenmiştir. Denemeler her bir yaprakbiti türü için üç farklı sıcaklık (17, 22 ve 27±1ºC), 60±10% RH ve 16:8 L:D koşullarında yürütülmüştür. Gelişme eşiği ve termal konstant üç yaprakbiti türü için hesaplanmıştır. *Lysiphlebus testaceipes*'in parazitleme oranı *A. craccivora* için %25.0, 53.4 ve 20.5, *A. fabae* için %62.7, 71.1 ve 37.1, *A. gossypii* için %54.2, 70.7 ve 20.0 olmuştur. *Lysiphlebus testaceipes*'in gelişme süresi 17, 22 ve 27±1ºC' de sırayla *A. craccivora* üzerinde 18.5, 10.9 ve 7.9 gün, *A. fabae* üzerinde 17.6, 10.2 ve 7.4 gün, *A. gossypii* üzerinde 19.8, 12.6 ve 9.3 gündür. Gelişme eşiği ve termal konstant *A. craccivora*, *A. fabae* ve *A. gossypii* üzerinde sırayla 9.42, 9.69 ve 8.12ºC; 136.99, 128.05 ve 175.44 gün derece olmuştur. Genel sonuçlara dayanarak, 20-22ºC'de *A. Fabae,* L. ***testaceipes***'in kitle üretimi için çok iyi bir potansiyel konukçudur.

Anahtar sözcükler: Afit türleri, biyolojik mücadele, gelişme eşiği, parazitoit, eşeysel oran

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Introduction

Aphid damage is considered one of the major impediments to production in many cultivated crops. They not only feed on plant sap and produce honeydew but they are also responsible for the dispersal of many viruses. Chemical control is the major method used to suppress population levels among the possible control methods, including cultural and biological methods (Parrella et al., 1999). Biological control methods are being implemented for aphid management in open fields and greenhouses (Zamani et al., 2007; Uygun et al., 2010). The Aphidiinae species (Hymenoptera: Braconidae) are all parasitoids of aphids (Mackauer & Starý, 1967) and many species have been successfully used against aphid species in these areas (Ramakers et al., 1989; van Steenis & El-Khawass, 1995; Yoldaş et al., 2011).

Lysiphlebus testaceipes (Cresson, 1880) (Hymenoptera: Braconidae: Aphidiinae) have been accepted one of the effective aphid parasitoids in this group. Originally from Cuba, it was released in France and Corsica to control *Aphis spireacola* Patch, 1914 (Hemiptera: Aphididae). However, the parasitism of *Toxoptera aurantii* (Boyer de Fonscolombe, 1841) rather than *A. spireacola* was recorded (Starý et al., 1988). After release, it spread gradually throughout the Mediterranean and to the western Atlantic with wide host range (Starý et al., 2004). The successful introduction of any parasitoid to a new environment depends on several factors. Firstly, releases in classical biological control should be done in each climatic zone that is occupied by the host, so that the parasitoid has a chance to establish in all areas where the host occurs. Secondly, the releases should be large enough to ensure rapid establishment. Often more than one release in an area is needed for successful establishment. To achieve these goals, the mass rearing of candidate parasitoids is an integral step (Debach, 1974; Uygun et al., 2010). Understanding the factors that regulate interactions between aphid parasitoids and their hosts will improve both conservation and augmentation of the parasitoids. Temperature and host are key abiotic and biotic factors, respectively, that regulate insect population dynamics, development rates and seasonal occurrence (Campbell et al., 1974; Harvey, 2000). Both the host-aphid species and temperature can affect the rate of development and longevity of aphid parasitoids (Deng & Tsai, 1998). There is considerable literature on temperature-dependent biology of *L. testaceipes*. Variation in observed developmental periods of *L. testaceipes* have been attributed to genetic variability among distinct populations and differences among the host-aphid species (Tang & Yokomi, 1995; Elliott et al., 1999; Royer et al., 2001; Rodrigues et al., 2004; Starý et al., 2004).

In the present study, the effects of three host-aphid species and three constant temperatures on the development, parasitism percentage, pupal mortality percentage and sex ratio of *L. testaceipes* were studied to evaluate their potential as hosts for its mass rearing.

Material and Methods

Insect and plant sources

The aphids used in the experiments, namely cowpea aphid, *Aphis craccivora* Koch, 1854 black bean aphid, *Aphis fabae* Scopoli, 1763 and cotton aphid *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae), were collected from horse bean and cotton fields in Balcalı, Adana, Turkey in March 2011 and the experiments were set at the same year. Laboratory colonies of the cotton aphid were established on cotton (*Gossypium hirsutum* L. cv. Çukurova 1518), and black bean and cowpea aphids were established on horse bean, *Vicia fabae* L. Each colony was separately reared in a 70 x 55 x 40 cm cage at 23±2ºC, 65±10% RH and 16 h of artificial light of 6,000 lx in an insect rearing room. The aphids were reared in the laboratory for three generations before being used in the experiments.

Lysiphlebus testaceipes population was originally collected from *A. gossypii* in a citrus orchard in Seferihisar, İzmir, in May 2008, and maintained on *A. fabae* in the laboratory for 10-12 generations before the individuals were used in the current experiments. *Lysiphlebus testaceipes* was reared in a 70 x 55 x 40 cm cage at 20±2ºC, 60±10% RH and 16 h of artificial light of 6,000 lx in an insect rearing room.

Effect of temperature and aphid species on parasitoid development and parasitization rate

The development rates of *L. testaceipes* on *A. craccivora*, *A. fabae* and *A. gossypii* were studied at three different temperatures (17, 22 and 27±1ºC), 60±10% RH and 16 h of artificial light (5,000 lx). The

apterous adults of *A. craccivora, A. fabae* and *A. gossypii* were transferred separately with a fine camel hairbrush to excised leaf disks (Ø 5 cm) of the host plant inverted on wet cotton on the Petri dishes. Offspring born within 24 h were taken from the cotton disk and transferred to potted cotton and horse bean seedlings (3-4 true leaf stage). Each plant had 80 first instar aphids on it and is referred to as a unit. The units, which were transferred to a climate chamber set at 22ºC, were tightly covered with a 5 L plastic cage (30 cm high x 14 cm lid diameter) which had three openings (10 cm Ø) covered with mesh, one on the bottom and two on the sides of the cylinder. These nymphs were used for parasitization when they reached the second or third instar.

Adult parasitoids were obtained from aphid mummies isolated in a 50 ml falcon tube (10 cm by 1.5 cm Ø). Upon adult emergence, the gender was determined under a stereomicroscope. Two male adults and one female were introduced into a 5-L plastic cage for a minimum 4-h mating period. A small piece of fine muslin fabric containing 3% sugar solution was placed in the cage for nutrition. Thereafter, for each temperature, one mated parasitoid female was introduced into each unit covered by the plastic cage for a 24-h oviposition period and then removed. The experimental units were kept at the same temperature and monitored daily for adult parasitoid emergence. Individual development time from oviposition to the beginning of mummy formation and from oviposition to adult emergence was determined for males and females, and combined. The sex of the adult parasitoids was determined under a stereomicroscope. The data were used to calculate the effects of temperature and aphid on the female-male ratio of the parasitoid. The number of unemerged parasitoids from mummies was used to assess the mortality percentage for each cage. The parasitization rate was calculated as the proportion of transferred aphids that became mummies. At each temperature, 10 replicates of each aphid species were used.

Data analysis

The effect of temperature on the developmental periods of *L. testaceipes* on each aphid species from the oviposition to the beginning of mummy formation, and from oviposition to adult emergence, was analyzed by one-way analysis of variance (ANOVA, $\alpha = 0.05$). If a significant difference was detected, multiple comparisons were made by using Tukey's HSD multiple range test. Data from the three aphid species were also pooled to test for the possible effect of aphid species on the development of parasitoids at different temperatures, i.e., an interaction effect, by using two-way ANOVA. The effect of temperature on the parasitization rate and mortality ratio of the mummy stage for each aphid species were analyzed with one-way ANOVA ($\alpha = 0.05$). If a significant difference was detected, the Tukey's multiple range test was applied to separate the means. The data on the mortality rate and the parasitism percentage were arcsine square root transformed before the application of the tests (SPSS Inc. 2008).

Chi-square (χ^2) analysis was applied to determine if there was any effect of temperature and aphid species on the sex ratio of the parasitoid in comparison to a hypothesized sex ratio of 1:1. The analysis was applied separately to the sex ratio of *L. testaceipes* that became adults on each aphid species at the different temperatures. In addition to Chi-square analysis, a phi-Cramer's V test was applied to measure the effect of each aphid species on the sex ratio of *L. testaceipes*. The analyses were carried out with SPSS 17.0 statistical software (SPSS Inc. 2008).

Separately, a linear technique was employed to compute the lower development threshold of the egg-larval stages and total immature stages of the parasitoid by using development rate data as the dependent variable and temperature as the independent variable. The lower development threshold was determined as the intercept point of the linear equation with the x-axis and the degree-day requirements were calculated as the inverse of the linear equation slope (Campbell et al., 1974).

Results and Discussion

Effect of temperature and aphid species on parasitoid development time

The development time of *Lysiphlebus testaceipes* on the three aphid species was shortest at 27ºC, the highest temperature tested, and longest at 17ºC, the lowest temperature tested (Table 1). As the temperature increased, the developmental period shortened, with the three mean development periods significantly different from each other ($\alpha < 0.05$). The shortest development time of the egg and nymphs at both 17 and 27ºC was for *A. gossypii*; but at 22ºC it was for *A. fabae*. However, the development time

from egg to adult for females, males and females-males combined was longest in *A. gossypii* and shortest in *A. fabae* at the three temperatures.

Males of *L. testaceipes* reared on *A. fabae* took a longer time to complete their development than females at 17 and 27ºC but a shorter time at 22ºC. When all the individuals reared on the three aphids at three temperatures were considered collectively, in general, the males developed in a shorter time than females. This difference, however, was often less than half a day (Table 1).

Table1. Development times of *Lysiphlebus testaceipes* on *Aphis craccivora*, *Aphis fabae* and *Aphis gossypii* at three constant temperatures

Host species	Temperature (ºC)	n	Development Time (d, mean±SE)*			
			Egg - Nymph	Female (♀)	Male (♂)	Total (♀&♂)
Aphis craccivora	17	155	11.4±0.06 a	18.6±0.09 a	18.50±0.09 a	18.5±0.07 a
	22	328	6.5±0.03 b	10.9±0.04 b	10.8±0.04 b	10.9±0.04 b
	27	170	4.3±0.04 c	8.0±0.05 c	7.6±0.05 c	7.9±0.04 c
Aphis fabae	17	501	11.2±0.02 a	17.6±0.06 a	17.7±0.08 a	17.6±0.05 a
	22	553	6.1±0.02 b	10.3±0.04 b	10.1±0.37 b	10.2±0.03 b
	27	179	4.1±0.02 c	7.4±0.06 c	7.5±0.07 c	7.4±0.04 c
Aphis gossypii	17	560	10.8±0.04 a	19.8±0.10 a	19.7±0.07 a	19.8±0.08 a
	22	614	6.7±0.03 b	12.7±0.04 b	12.5±0.02 b	12.6±0.06 b
	27	32	4.0±0.00 c	9.6±0.04 c	9.0±0.12 c	9.3±0.24 c

* Within the columns means followed by the same letters are not significantly different ($\alpha > 0.05$, Tukey; $df_{A.craY\text{-}L} = 2, 650$, $F_{A.craY\text{-}L} = 6090$, $Sig_{A.craY\text{-}L} = 0.000$; $df_{A.craTot♀} = 2, 422$, $F_{A.craTot♀} = 6173$, $Sig_{A.craTot♀} = 0.000$; $df_{A.craTot♂} = 2, 225$, $F_{A.craTot♂} = 4296$, $Sig_{A.craTot♂} = 0.000$; $df_{A.craTot♀\text{-}♂} = 2, 649$ $F_{A.craTot♀\text{-}♂} = 10211$, $Sig_{A.craTot♀\text{-}♂} = 0.000$; $df_{A.fabY\text{-}L} = 2, 1230$, $F_{A.fabY\text{-}L} = 4926$, $Sig_{A.fabY\text{-}L} = 0.000$; $df_{A.fabTot♀} = 2, 692$ $F_{A.fabTot♀} = 7478$, $Sig_{A.fabTot♀} = 0.000$; $df_{A.fabTot♂} = 2, 535$, $F_{A.fabTot♂} = 6396$, $Sig_{A.fabTot♂} = 0.000$; $df_{A.fabTot♀\text{-}♂} = 2, 1230$, $F_{A.fabTot♀\text{-}♂} = 13921$, $Sig_{A.fabTot♀\text{-}♂} = 0.000$; $df_{A.gossY\text{-}L} = 2, 1203$, $F_{A.gossY\text{-}L} = 6090$, $Sig_{A.gossY\text{-}L} = 0.000$; $df_{A.gossTot♀} = 2, 10$, $F_{A.gossTot♀} = 6173$, $Sig_{A.gossTot♀} = 0.000$; $df_{A.gossTot♂} = 2, 2$ $F_{A.gossTot♂} = 4296$, $Sig_{A.gossTot♂} = 0.000$; $df_{A.gossTot♀\text{-}♂} = 2, 13$, $F_{A.gossTot♀\text{-}♂} = 10211$, $Sig_{A.gossTot♀\text{-}♂} = 0.000$).

Two way analysis of variance of the total development times of *L. testaceipes* in the three aphids at the three temperatures revealed significant differences attributable to temperature, parasitoid, and temperature by host-aphid species interaction ($\alpha = 0.05$; $df_{species} = 2, 3027$ $F_{species} = 193.759$, $Sig_{species} = 0.000$; $df_{temp} = 2, 3027$ $F_{temp} = 11754$, $Sig_{temp} = 0.000$; $df_{speciesXtemp} = 4, 3027$ $F_{speciesXtemp} = 3.687$, $Sig_{speciesXtemp} = 0.005$). Tukey's multiple range tests ($\alpha = 0.05$) applied after univariate analysis of variance (ANOVA) showed that host species was significant for the development time of *L. testaceipes* ($\alpha < 0.05$) but temperature was not significant ($\alpha \geq 0.05$).

Tang & Yokomi (1995) reported that the development times of *L. testaceipes* in *A. gossypii* on the host plant, *Hibiscus rosa-sinensis* L., 1753, at 15, 18, 21, 24, 27 and 30ºC were 25.0, 23.4, 15.3, 13.7, 10.5 and 9.5 d, respectively. Elliott et al. (1999) reported that the development times of *L. testaceipes* in the wheat aphid *Schizaphis graminum* (Rondani, 1852) on the host plant, *Hordeum vulgare* L., 1753, at 10, 14, 18, 22 and 26ºC were 49.1, 24.1, 15.2, 10.6 and 9.3 d, respectively. A major difference between these two studies is the 8.2 d difference between the developmental times of the parasitoid at 18ºC. However, it may not be correct to attribute this difference in development time only to the aphid host species. Weathersbee et al. (2004) reported that the composition and concentration of secondary plant metabolites can influence insect herbivore fitness, and that these effects are reflected in the parasitoid's development time. The host plant is an important factor in the development of aphids. The mean development times of *A. gossypii* to maturity on cotton at 15, 20, 25 and 30ºC were 12.0, 8.1, 5.7, and 4.5 d (Kersting et al., 1999), and on grapefruit at 20, 25, and 30ºC, they were 7.4, 6.4, and 5.9 d (Satar et al., 1998), respectively. During the development of *L. testaceipes* from egg-laying to egg hatching, the host is not killed by the parasitoid during or soon after egg-laying, i.e., the parasitoid uses the living host as a

source of nutrition for its development. This makes the development period of the parasitoid dependent to some extent on the species of aphid. In this regard, the present study corroborated the assertion of Tang & Yokomi (1995) and Elliott et al. (1999) that the development time of *L. testaceipes* is affected by host-aphid species. Separately, Rodrigues et al. (2004) reported that the development times of *L. testaceipes* in *A. gossypii* fed on chrysanthemum were 26.9, 14.8, 11.3, and 12.2 d at 15, 20, 25, and 30ºC, respectively. The development times obtained from the present study were similar to those determined in these studies, in spite of the host plant difference.

Effect of temperature and aphid species on the parasitization and pupal mortality rate of *Lysiphlebus testaceipes*

The parasitization rate of aphids by *L. testaceipes* at the three temperatures, was highest at 22ºC for the three aphid species, and the rate for *A. fabae* stands out as significantly higher ($\alpha < 0.05$). The lowest rate for the three aphid species was at 27ºC and the highest rate at this temperature was again for *A. fabae*, while the parasitization rates of *A. craccivora* and *A. gossypii* were close to each other. The lowest rates at the three temperatures were for *A. craccivora* (except at 27ºC for *A. gossypii*) (Table 2). When compared to the other two species, *A. craccivora* exhibited the fastest response to antennal or ovipositor contact by the parasitoid, moving its body violently and erratically, especially the abdomen, and throwing themselves to the soil.

When the effects of temperature, aphid species and temperature by aphid species interaction on parasitization rate were examined with two-way analysis of variance, the effect of temperature on the parasitization rate was significant ($\alpha = 0.05$, df = 2, 60, F = 5.925, P = 0.005) but the effect of species was not significant ($\alpha = 0.05$, df = 2, 60, F = 3.178, P = 0.050). The multiple comparison test (Tukey, $\alpha = 0.05$) for parasitization rate and temperature placed the parasitization rate on the three aphid species at 17ºC in one group and the rate for 22 and 27ºC in a different group. Rodrigues et al. (2004) reported that the parasitization rates of *L. testaceipes* for *A. gossypii* fed on chrysanthemum were 76, 68, 65 and 40% at 15, 20, 25 and 30ºC, respectively. In the present study, the lowest parasitization rate was also obtained at the highest temperature. Separately, the parasitization rates for the three aphid species suggest that *L. testaceipes* most successfully parasitized *A. fabae* to the other aphid species at all temperatures.

Table 2. Parasitization rate, pupal mortality rate and female-male ratio of *Lysiphlebus testaceipes* on *Aphis craccivora, Aphis fabae* and *Aphis gossypii* at different temperatures (Mean±SE)*

Host species	Temperature (ºC)	Number of Exposed Unit	Parasitization Rate (%)**		Pupal Mortality Rate (%)***		Female/Male Ratio
	17	6	25.0±4.79	b	0.0±0.00		1:0.79
Aphis craccivora	22	9	53.9±11.00	a	0.0±0.00		1:0.46
	27	6	20.5±6.54	b	0.0±0.00		1:0.40
	17	10	62.7±8.92	ab	0.0±0.00	b	1:0.57
Aphis fabae	22	10	71.1±4.12	a	3.1±2.18	b	1:0.97
	27	7	37.1±8.66	b	20.4±5.63	a	1:0.84
	17	5	54.2±10.02	a	5.9±1.81	b	1:0.80
Aphis gossypii	22	5	70.7±15.49	a	4.7±2.59	b	1:0.63
	27	4	20.0±7.50	a	56.8±6.80	a	1:0.27

* Within the columns means followed by the same letters are not significantly different ($\alpha \geq 0.05$, Tukey).

** Each unit has 80 second or third instars *aphid* nymphs.

*** Parasitization rate and pupal death ratio were arcsine-square root transformed before one-way ANOVA and Tukey; untransformed data are presented ($df_{A.cracPar} = 2,20$, $F_{A.cracPar} = 1.08$ $Sig_{A.cracPar} = 0.36$; $df_{A.fabePar} = 2, 24$, $F_{A.fabePar} = 4.07$ $Sig_{A.fabePar} = 0.030$; $df_{A.gossPar} = 2,10$, $F_{A.gossPar} = 1.37$ $Sig_{A.gossPar} = 0.30$; $df_{A.fabeMortality} = 2,24$, $F_{A.fabeMortality} = 16.02$ $Sig_{A.fabeMortality} = 0.000$; $df_{A.gossMortality} = 2,10$, $F_{A.gossMortality} = 7.45$, $Sig_{A.gossMortality} = 0.01$).

When the aphid species in their immature stage were examined for the mortality of *L. testaceipes* (Table 2), the highest rate was 56.8% in *A. gossypii* at 27ºC (it should be noted that no mortality was observed for *L. testaceipes* in *A. craccivora*.) In addition, *A. craccivora* was the species least parasitized by *L. testaceipes* at any temperature. The reason for this was probably the behavior of *A. craccivora,* which threw themselves from the plant to the soil in the experimental pots when they were disturbed by the parasitoid; this appears to have been a major factor in reducing its parasitization rate below that of the other two species.

The mortality of *L. testaceipes* pupae in *A. fabae* at 27ºC was 20.4%, while there was only low mortality at 17 and 22ºC. Similarly, *L. testaceipes* showed lower mortality in *A. gossypii* at 17 and 22ºC than at 27ºC (Table 2). A study by Takanashi (1990) on the development time of *Lysiphlebia japonica* (Ashmead) between 12 and 30ºC reported that no individuals were able to develop at 30ºC. Also, Deng & Tsai (1998) stated that the mortality rates of *L. japonica* in *Toxoptera citricida* (Kirkaldy) (Hemiptera: Aphididae) were 2.8, 16.2, 27.5 and 73.3% at 15, 20, 25 and 30ºC, respectively. Likewise, Rodrigues et al. (2004) demonstrated that the mortality rates of *L. testaceipes* in *A. gossypii* fed on chrysanthemum were 20, 39, 38 and 86% at 15, 20, 25 and 30ºC, respectively. The highest temperature used in the present study and others studies (Takanashi, 1990; Deng & Tsai, 1998; Rodrigues et al., 2004) caused similar mortality rates.

In the present study, the two-way analysis of variance applied to the mortality rates of *L. testaceipes* individuals separately reared on the three aphid species demonstrated a significant statistical difference between the means and also that this difference was due to temperature, host-aphid species and temperature by host-aphid species interaction. In the multiple comparison tests, both temperature and aphid species formed a separate group (Tukey, $\alpha = 0.05$; $df_{species} = 2, 60$, $F_{species} = 28.34$, $P_{species} = 0.000$; $df_{temp} = 2, 60$, $F_{temp} = 26.22$, $P_{temp} = 0.000$; $df_{speciesXtemp} = 4, 60$, $F_{speciesXtemp} = 7.861$, $P_{speciesXtemp} = 0.000$).

For the three aphids at the three temperatures, the highest female ratio of *L. testaceipes* was obtained on *A. fabae*, followed by *A. gossypii* (Table 2). Temperature affected the sex ratio of the parasitoid in *A. craccivora* and it was statistically significant ($\chi^2_{A.craccivora} = 7.694$, $df_{A.craccivora} = 2$, $Sig_{A.craccivora} = 0.021$). Chi-square tests applied separately to the data on sex ratios for the parasitoids reared on *A. fabae* and *A. gossypii* gave similar results ($\chi^2_{A.fabae} = 18.02$, $df_{A.fabae} = 2$, $Sig_{A.fabae} = 0.000$; $\chi^2_{A.gossypi} = 17.056$, $df_{A.gossypi} = 2$, $Sig_{A.gossypi} = 0.000$). Given that temperature affected the sex ratio of *L.* testaceipes reared on each aphid species, the phi-Cramer's V test was applied to measure the magnitude of this effect. The effect was strong for A. fabae (0.174) and A. gossypii (0.174), but not for A. craccivora (0.109). Furthermore, the Chi-square test revealed that the host-aphid species affected the sex ratio of the parasitoid as strongly as temperature ($\chi^2_{species} = 36.136$, $df_{species} = 2$, $Sig_{species} = 0.000$, phi-Cramer's V value = 0.121).

The sex ratio is one of the most important contributors to the success of released parasitoids. The reproduction of hymenopteran parasitoids begins with the mating of females and males shortly after their emergence from mummies. Mated females store sperm in their spermatheca. The females facultatively alter the gender of their progeny in response to changes in the environmental conditions which can affect the sex ratio by stimulating the release of sperm for fertilization of the eggs, with only females produced (haplodiploid genetic system). In contrast, if the females do not release sperm for the fertilization of eggs, only males hatch from the unfertilized eggs (DeBach, 1974; Godfray, 1994).

The present study overall obtained more male individuals at 17 and 22ºC than at 27ºC, with *L. testaceipes* having a higher mortality rate and male-female ratio for *A. craccivora* than for *A. fabae* and *A. gossypii*. However, the mean for *A. fabae* was similar to the mean for *A. craccivora*, especially at 17 and 22ºC. Rodrigues et al. (2004) reported that the male-female ratios of *L. testaceipes* on *A. gossypii* fed on chrysanthemum were 0.35, 0.43, 0.45, and 0.54 at 15, 20, 25, and 30ºC, respectively. These male-female ratios were quite different from the results obtained in the present study. Environmental conditions and the density of aphid, leaf texture and plant allomones are indicators for the sex ratio of *Diaeretiella rapae* (McIntosh) in *A. gossypii* on chrysanthemum (Shukla & Triphathi, 1993). Our test unit consisted of 80 individuals in 5-L cages. In contrast, Rodrigues et al. (2004) used Petri dishes that contained several aphid nymphs. The test unit differences may have caused a higher number of male *L. testaceipes* in *A. gossypii*. Moreover, *A. gossypii* has clones based on host plant (Satar et al., 2013) that probably have different clones to those on chrysanthemum (Guldemond et al., 1994).

Development thresholds and thermal constants for *Lysiphlebus testaceipes* reared on three aphid species

The thermal constants for *L. testaceipes* reared on *Aphis craccivora*, *A. fabae* and *A. gossypii* in the period from egg to pupa were 68.96 DD (degree days), 64.52 DD and 63.69 DD, and the development thresholds for that period were 11.07ºC, 11.25ºC and 11.61ºC, respectively. Furthermore, the thermal constants required for the period of egg to adult development of the parasitoids in *A. craccivora*, *A. fabae* and *A. gossypii* were 136.99 DD, 128.05 DD and 175.44 DD, the development thresholds were 9.42ºC, 9.69ºC and 8.12ºC, respectively, for the same development period (Table 3 & Figure 1).

Table 3. Development thresholds (ºC) and thermal constants (degree days, DD) of *Lysiphlebus testaceipes* reared on *Aphis craccivora, Aphis fabae* and *Aphis gossypii* in the egg and nymph period, and total adult development period

Host-aphid species	Egg and Nymph Period		Total Development Period	
	Development Threshold (ºC)	Thermal Constant (DD)	Development Threshold (ºC)	Thermal Constant (DD)
Aphis craccivora	11.07	68.96	9.42	136.99
Aphis fabae	11.25	64.52	9.69	128.05
Aphis gossypii	11.61	63.69	8.12	175.44

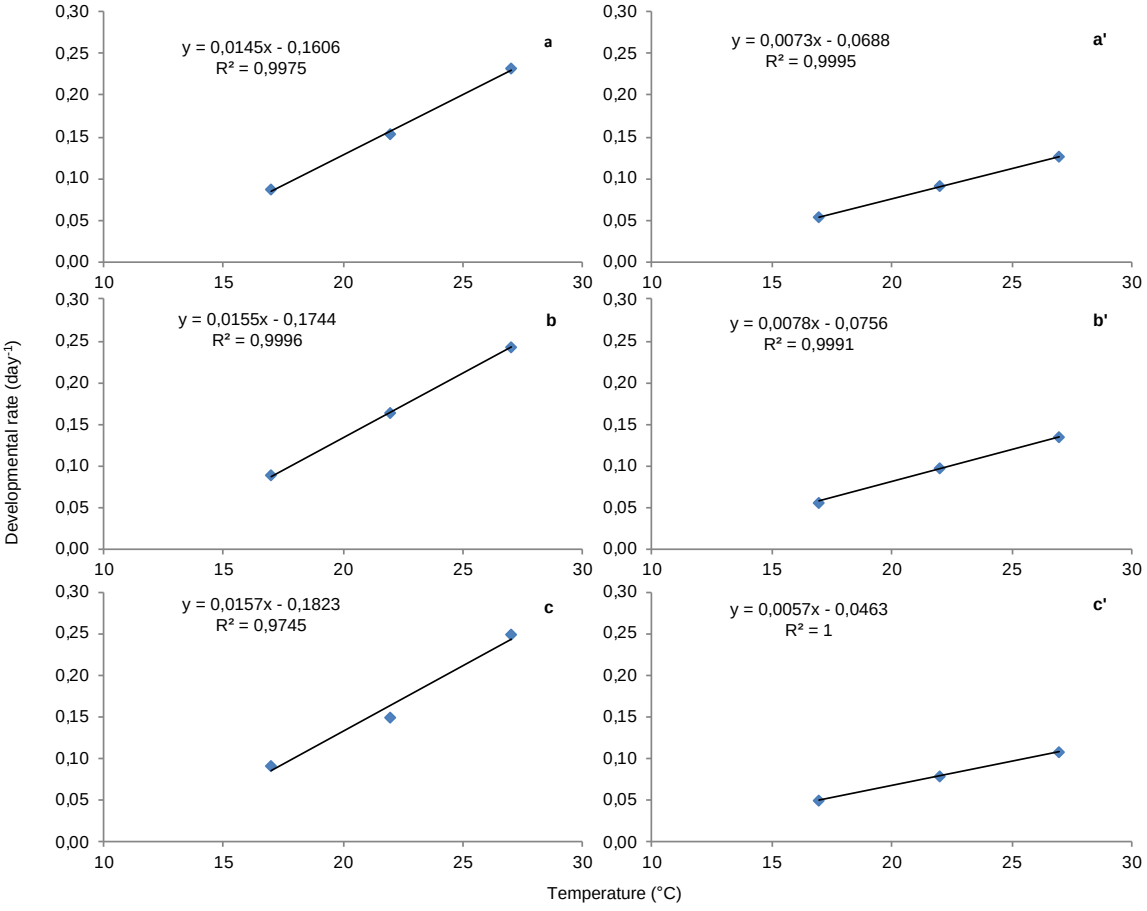

Figure 1. Regression lines and equations for development rates for the egg-larval and adult stages of *Lysiphlebus testaceipes* in (a and a') *Aphis craccivora*, (b and b') *Aphis fabae* and (c and c') *Aphis gossypii*.

The lowest development threshold in the egg-larval period was for the parasitoids reared on *A. craccivora* but the lowest thermal constant was calculated for the parasitoids in *A. gossypii*. As for the development threshold for the total development period, while the lowest threshold for the parasitoid was for *A. gossypii*, the lowest thermal constant was for *A. fabae*. In a study conducted by Tang & Yokomi (1995) on the development time of *L. testaceipes* in *T. aurantii,* the development threshold and the effective temperature to achieve maturity were 7.5ºC and 212.8 DD, respectively. The development threshold calculated by Tang & Yokomi (1995) was lower than for the aphid species obtained in the present study but the thermal constant was higher. *Lysiphlebus testaceipes* has geographical isolates, even for same host plant, and aphids can have different developmental thresholds (Royer et al., 2001).

Rodrigues et al. (2004) stated that the development times of *L. testaceipes* in *A. gossypii* fed on chrysanthemum were 26.9, 14.8, 11.3 and 12.2 d, respectively, the parasitization rates were 76, 68, 65 and 40%, respectively, and the emergence rates were 80, 61, 62 and 14%, at 15, 20, 25 and 30ºC, respectively. On the basis of these results, Rodrigues et al. (2004) recommended 25ºC as the best temperature for both the reproduction and establishment of *L. testaceipes*. Zamani et al. (2007) reported development thresholds and thermal constants for the egg to adult period for *Aphidius colemani* Viereck 1912 (Hymenoptera: Braconidae) in *A. gossypii* and *Myzus persicae* (Sulzer, 1776) of 2.97 and 2.65ºC, respectively, and 256.41 and 270.27 DD, respectively. As in the present study, Zamani et al. (2007) found different development thresholds for *A. colemani* on different host aphids.

In the light of the findings of this study, the three temperatures and the three aphid species could be used for the production of *L. testaceipes*. However, for the reasons stated earlier, *A. fabae* is a better overall host for *L. testaceipes* than both *A. craccivora* and *A. gossypii*. More specifically, *A. fabae* feeding on *V. faba* at 20-22ºC is potentially the most suitable combination of host-aphid host-plant and temperature for the mass production of *L. testaceipes*. However, the high mortality rate of the parasitoid observed in mummies at 27ºC may be a factor limiting its performance in hot areas such as the Çukurova Basin of Turkey. In addition, a study on the interactions between *L. testaceipes* and others parasitoids such as *Lysiphlebus fabarum* (Marshall, 1896) and *Lysiphlebus confusus*, Tremblay & Eady, 1978 in citrus plantations would be beneficial to understanding the prospects of *L. testaceipes* being successful in the same ecological niche as the other parasitoid species.

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Original article (Orijinal araştırma)

Creating a degree-day model of honeydew moth [*Cryptoblabes gnidiella* (Mill., 1867) (Lepidoptera: Pyralidae)] in pomegranate orchards[1]

Nar bahçelerinde Portakal güvesi [*Cryptoblabes gnidiella* (Mill., 1867) (Lepidoptera: Pyralidae)]'nin gün-derece modelinin oluşturulması

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Abstract

This study has been conducted in a pomegranate orchard in Tarsus, Mersin Province between 2008-2010 and 2012-2013. In this study, the sum of effective temperatures based on a degree-day (DD) model was determined to be successful for the scheduling control of honeydew moth [*Cryptoblabes gnidiella* (Mill.,1867) (Lepidoptera: Pyralidae)]. For this purpose, phenological stages, sex pheromone traps, the sum of effective temperatures (ETS), egg hatching time and fruit control were used. ETS values based on the DD model were 80 DD for hanging time of sex pheromone traps, 250 DD for first generation for egg hatching period, 800 DD for the second generation, 1375 DD for the third generation, 1930 DD for the fourth generation, and 2500 DD for fifth generation. However, the first generation of *C. gnidiella*, which had lower population having come from overwintering places, and fifth generation, which emerged at after harvest, should be monitored regularly so that control applications will be more beneficial.

Keywords: *Cryptoblabes gnidiella*, degree-day model, honeydew moth, pomegranate

Öz

Bu çalışma; 2008-2010 ve 2012-2013 yıllarında Mersin iline bağlı Tarsus ilçesindeki nar bahçesinde beş yıl süreyle yürütülmüştür. Çalışmada; Portakal güvesi [*Cryptoblabes gnidiella* (Mill.,1867) (Lepidoptera: Pyralidae)]'nin mücadelesinde daha etkin ve başarılı olabilmek için gün-derece modeline esas etkili sıcaklıklar toplamı değerleri belirlenmiştir. Bu amaçla fenolojik dönem, eşeysel çekici tuzak, etkili sıcaklıklar toplamı (EST), yumurta açılım zamanı ve meyve kontrolü kriterlerinden yararlanılmıştır. Bu çalışma sonuçlarına göre; *C. gnidiella*'nın gün-derece (g.d.) modeline esas EST değerleri; tuzak asım zamanı için 80 g.d., yumurta açılım zamanlarında birinci döl 250 g.d., ikinci döl 800 g.d., üçüncü döl 1375 g.d., dördüncü döl 1930 g.d. ve beşinci döl için ise 2500 g.d. olarak belirlenmiştir. Ancak, mücadele amaçlı yapılacak çalışmalarda *C. gnidiella*'nın kışlaktan gelen döl popülasyonu çok düşük olduğundan birinci döl, hasat sonrasına denk geldiğinden de beşinci dölün düzenli olarak takip edilmesinin ve uygulamanın da buna göre yapılmasının yararlı olacağı düşünülmektedir.

Anahtar sözcükler: *Cryptoblabes gnidiella*, gün-derece modeli, Portakal güvesi, nar

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Introduction

Pomegranate (*Punica granatum* L.) is one of the oldest fruit species mentioned in history and originated in the Middle East and the Caucasus. Considered as a tropical and subtropical climate fruit, pomegranate can be grown in range of soil and climatic conditions and it contains plenty of vitamin C, it protects the heart, reduces sugar and cholesterol, and strengthens the immune system against AIDS and cancer (Lansky et al., 1998). Pomegranate is generally consumed as table and fruit juice. In recent years it has become increasingly recognized as a fruit of interest due to its development in the field, food technology, storage and transportation. Pomegranate can be grown in places where the temperature does not fall below -10ºC and the altitude is up to 1000 m. World pomegranate production is about 2.5 Mt, and Turkey ranks third in the world with nearly 400,000 t produced annually (Anonymous, 2010a, b). The pomegranate production in Turkey is mainly carried out in the Mediterranean, Aegean and Southeastern Anatolia Regions, and new monoculture-pomegranate orchards are also being established in all the regions in recent years (Anonymous, 2010b).

There are many pests that cause crop loss in pomegranates in Turkey and around in the world (Juan et al., 2004; Toledo & Albujer, 2005; Blumenfeld et al., 2007; Öztürk & Ulusoy, 2009). One of these species is the honeydew moth, *Cryptoblabes gnidiella* (Mill., 1867) (Lepidoptera: Pyralidae). This pest feeds on the pomegranate fruit and damages fruit before it ripens, causes rots and reduces market value (Öztop et al., 2002; Öztürk & Ulusoy, 2010). *Cryptoblabes gnidiella*, which is found in many countries i with subtropical growing conditions, is a harmful polyphagous insect (Ronald & Jayma, 1992; Silva & Mexia, 1999). So far, 11 hosts have been identified in Turkey (Öztürk & Ulusoy, 2011). Also *C. gnidiella* has been reported to have caused significant crop losses in recent years by reading high populations in the Mediterranean Region pomegranates (Öztop et al., 2002; Öztürk & Ulusoy, 2011).

Until the recent 10-15 years, chemical control against diseases and pests in Turkey pomegranates had not usually been conducted or recommended. Successful agricultural production, breeding, identification and control of diseases and pests, and development of adequate methods and models of control require adequate study of pest biology. The most important of these models is the degree-day (DD) model, also known as forecast-warning model. Although this model has been applied to many pests globally (Anonymous, 2011), it has been applied to European grapevine moth (*Lobesia botrana* Den-Schiff., 1775), codling moth [*Cydia pomonella* (L., 1758)], oriental fruit moth [*Grapholita molesta* (Busck.,1916)], peach twig borer (*Anarsia lineatella* Zell., 1839), carob moth [*Apomyelois* (=*Ectomyelois*) *ceratoniae* (Zell.,1839)] and olive moth (*Prays oleae* Bern.,1788) in Turkey (Kumral et al., 2005; Anonymous, 2008; Mamay et al., 2014). However, there appears to have been no studies on a DD model for *C. gnidiella*.

In this study, phenological stages, sex pheromone trap, effective temperatures sum (ETS), hatching time and fruit control were used to develop a DD model for providing a more effective control strategy against honeydew moth for the first time in pomegranate orchards. According to the model, ETS values of hanging time of sex pheromone traps, time of first adult emergence and egg hatching time for each generation were determined. Critical ETS values on phenological periods of pomegranate were also determined. According to the data collected, producers can be given a timely warning of the need to control *C. gnidiella,* so the effectiveness and success of insecticide applications will be increased. Thus, the number of insecticide applications will be reduced, benefiting the economy, ecological balance, human health and the environment.

Material and Methods

A pomegranate orchard infested with honeydew moth (*Cryptoblabes gnidiella* Mill.), delta type pheromone traps (Pherocon CAP, Trécé Inc., Adair, OK, USA) and a Hobo (Onset, MA, USA) temperature data logger were used for this study.

The study was conducted for five years (2008-2010 and 2012-2013) in Tarsus, Mersin Province where the pomegranate production is concentrated in the Eastern Mediterranean Region. Daily minimum and maximum temperatures (°C) data were recorded on site.

Degree-day model of honeydew moth

The orchard had 65 ha of 15-year old pomegranate cv. Hicaz and was located in the village of Akarsu, Tarsus, Mersin, Province. DD study of *C. gnidiella* was based on the hanging time of pheromone trap, the first adult emerging time, egg hatching time with ETS for each generation (Rice et al., 1982; Anonymous, 2008). In the calculation of ETS values, the lower threshold (12.0ºC), generation time (564.6 DD) and the egg hatching period from the adulthood (120.0 DD) were used (Ringenberg et al., 2005; Öztürk, 2010).

Sex pheromone traps: Traps were used to monitor the first adult emergence time and adult population changes of *C. gnidiella*. Traps: [(Z)-11-hexadecenal (Z,11-16:Ald), (E)-11-hexadecenal (E,11-16:Ald), (Z)-13-octadecenal (Z,13-18:Ald), (E)-13-octadecenal (E,13-18:Ald)] were hung at the orchard at the end of March every year at a height of 1.5-2 m above the ground in the south facing side of a tree (Anshelevich et al., 1993; Öztürk, 2010). The trap was checked 2-3 times per week until the first adults were caught, after which they were checked weekly and butterflies counted individually. Pheromone-containing capsules were changed every 4-6 weeks according to the manufacture's instructions, and the roof and adhesive tabs were changed as needed. Adult population data for *C. gnidiella* was obtained in each year, and egg hatch and the time of spraying for each generation with hanging time of traps were correlated with the phenological stages of the pomegranates (Rice et al., 1982; Anonymous, 2008; 2011).

Effective temperatures sum: ETS (DD) values used to determination of appropriate spraying times and trapping times for *C. gnidiellia* were calculated according to the formula (Anonymous, 2008).

$$\text{ETS} = \frac{\text{Minimum temperature} + \text{Maximum temperature}}{2} \_ \text{Lower threshold (12.0ºC)}$$

The ETS value, equivalent to about 7-10 d prior to the 5-year average ETS value of the first adults captured in traps from 1 January, was considered as the first trapping time for monitoring purposes. After the first butterfly was caught in the trap, the hatching of the first emerging of eggs was followed and the average ETS values corresponding to the day when the first larvae were determined to be the appropriate spraying time for the first generation of *C. gnidiella*. Control was continued for the other generation of the pest and the average ETS values corresponding to the days in which each egg hatching was detected and calculated separately. In the calculations, development threshold, ETS value corresponding to generation period (Ringenberg et al., 2005; Öztürk, 2010) and the peak point of the *C. gnidiella* flight curve for each offspring was taken into account (Rice et al., 1982; Önder & Zümreoğlu, 1986; Anonymous, 2008, 2011).

Egg hatching time: Theoretically egg laying of the pest occurs within 1-3 d of catching the first butterfly in the trap, which is used to determine the spraying time for control of the first and second generation of *C. gnidiella*. Eggs were monitored on the fruit (stamens), where the great majority of eggs are deposited, on 10 trees for this were selected randomly. Critical phenological development in 5 fruits on 4 sides of the trees (i.e., 25 fruits/tree and a total 250 fruit), were checked 2-3 times a week and egg hatching or larvae emergence were monitored (Öztürk, 2010). The insecticide spraying time for the first generation was when 5% of eggs had hatched of the fruit, and ETS value was determined following the first larvae hatched. When egg had hatched in 5% of the fruit, ETS value were calculated according to required conditions for the first generation insecticide applications. Monitoring of the traps continued to determine the start of the drop in peak flight due to the density of the population, and when the DD value for each generation when 5% of the fruit were infested. The necessary conditions for the timing of spraying against *C. gnidiella* were accepted (Rice et al., 1982; Önder & Zümreoğlu, 1986; Toledo & Albujer, 2005; Anonymous, 2008, 2011).

ETS values obtained annually were calculated from 1 January according to the lower threshold of *C. gnidiella*. One-way ANOVA and Duncan's multiple range test was used for analysis of the data. According to results, difference between years were determined for adult emergence and egg hatching for each generation.

Results and Discussion

Honeydew moth degree-day model

In the first year (2008), the pheromone traps were hung on 19 March (62.1 DD) on the basis of plant phenology and *C. gnidiella* adults were first caught on 4 April (138.7 DD). In 2009, 2010, 2012 and 2013, traps were hung on 25, 26, 31 and 29 March (69.4, 116.8, 23.2 and 80.5 DD) and adults caught on 9 April, 31 March, 18 and 1 April (121.5, 134.7, 121.9 and 117.0 DD), respectively.

The population development curves of *C. gnidiella* according to the numbers of butterflies captured in the sex pheromone traps for the five years are given in Figure 1.

The first adults of *C. gnidiella* were caught in the sex pheromone trap between 31 March and 18 April 18 in the 5 years (Figure 1). However, it was determined that the population of the pest adults generally continued at low density for about 3 months until the second half of July, then started to increase from that date and continued at high density for about 4 months till the middle of November. According to the trap counts, adult flight graphs show that *C. gnidiella* populations had 4-5 peak points during the year and the adult flight ended at the end of November to the beginning of December. Yehuda et al. (1992) reported, for a study carried out in Israel, that *C. gnidiella* were first caught in March-April, the population was low in March-June, did not cause damage in first generation and adult flights ended in late October to early November. In another study carried out in Portugal, *C. gnidiella* adults emerged in the second half of the March, the population gradually increases from the beginning of June until the end of August, generations overlapped with each other and adults were active from the second half of March until the beginning of December (Silva & Mexia, 1999).

Öztürk & Ulusoy (2012) carried out a study in 2008-2009 in pomegranate orchards in the Eastern Mediterranean Region (Adana, Mersin and Osmaniye). They reported that the first adults were caught at the beginning of April, the population showed an increase from the second half of July, reached the highest level in October-November and the moth flight ended at the end of November to the beginning of December. Similarly, a study conducted on pomegranates in Hatay Province (2010-2011) found that *C. gnidiella* population was low in May and November, and increases in June-October, giving four generations per year, May-June, July, August-September and October-November, with mature flight ending in December (Demirel, 2016).

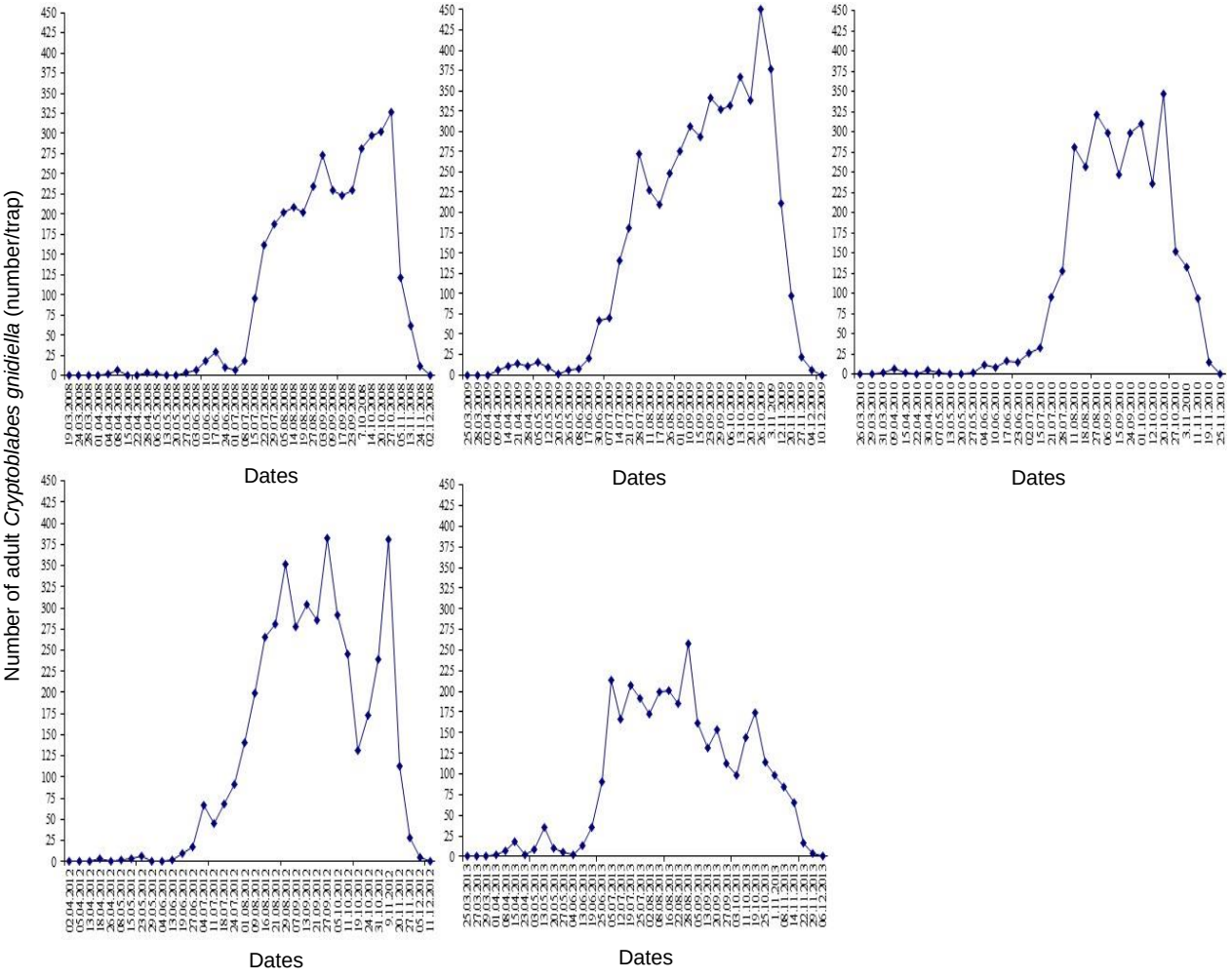

Figure 1. Adult population variation of *Cryptoblabes gnidiella* between 2008-2010 and 2012-2013 at the pomegranate orchard in the village of Akarsu, Tarsus, Mersin Province, Turkey.

For the first generation of *C. gnidiella,* first egg laying and ETS values were on 21 April 2008 and 253.8 DD, 1 May 2009 and 238.9 DD, 24 April 2010 and 253.5 DD, 1 May 2012 and 243.8 DD, and 23 April 2013 and 237.1 DD. During the first egg laying period in the five assessment years, the fruit were still quite small, i.e. walnut sized.

For the second generation, first adult emergence from larvae were on 9, 14, 11, 14 and 6 June (708.2, 685.4, 699.5, 699.7 and 686.8 DD) and the egg hatching on 18, 22, 19, 21 and 17 June (820.2, 796.1, 809.8, 807.4 and 805.2 DD), respectively for 2008-10 and 2012-13. In this period, the great majority of the fruit were about 70 mm in size.

For the third generation in 2018, first adult emergence larvae were on July 17 (1269.3 DD) and the egg hatching on 22 July 22 (1389.7 DD). In the four subsequent years of assessment, these dates were 21 and 28 July (1241.8 and 1361.9 DD) in 2009, 22 and 30 July (1260.9 and 1391.5 DD) in 2010, 19 and 26 July (1248.0 and 1374.9 DD) in 2012, and 18 and 26 July (1247.0 and 1368.1 DD) in 2013, when the majority of the fruit had reached 50% of the mature size.

For the fourth generation in 2018, adult emergence was on 20 August (1834.9 DD) and egg hatching on 27 August in 2008 (1952.2 DD). In the subsequent assessments, these dates were on 25 August (1807.0 DD) and 2 September (1930.6 DD) in 2009, on 24 and 31 September (1825.9 and 1941.8 DD) in 2010, 19 and 27

September (1822.9 and 1929.4 DD) in 2012, and 24 and 31 September 24 (1814.6 and 1919.8 DD) in 2013, when the fruit had start to sweeten and 50% were fully grown.

For the fifth generation in 2018, adult emergence was on 27 September (2398.4 DD) and the egg hatching on 7 October (2514.6 DD). In the subsequent assessments, these dates were 8 and 18 October (2378.4 and 2497.3 DD) in 2009, 30 September (2393.8 DD) and 12 October 12 (2516.3 DD) in 2010, 26 September (2390.2 DD) and 4 October (2509.5 DD) in 2012, and 11 and 27 October (2370.7 and 2495.4 DD) in 2013, when 85-90% of the fruit have matured and where ready for harvest.

In 2008, the pomegranate harvest started on 22 September, and on 25 September, 25 September and 5 October and 27 September in the subsequent assessment years, and was completed in about 15-20 d.

The 5-year pentad temperature (five consecutive days) and DD values based on a DD model of *C. gnidiella* are given in Table 1 and Figure 2.

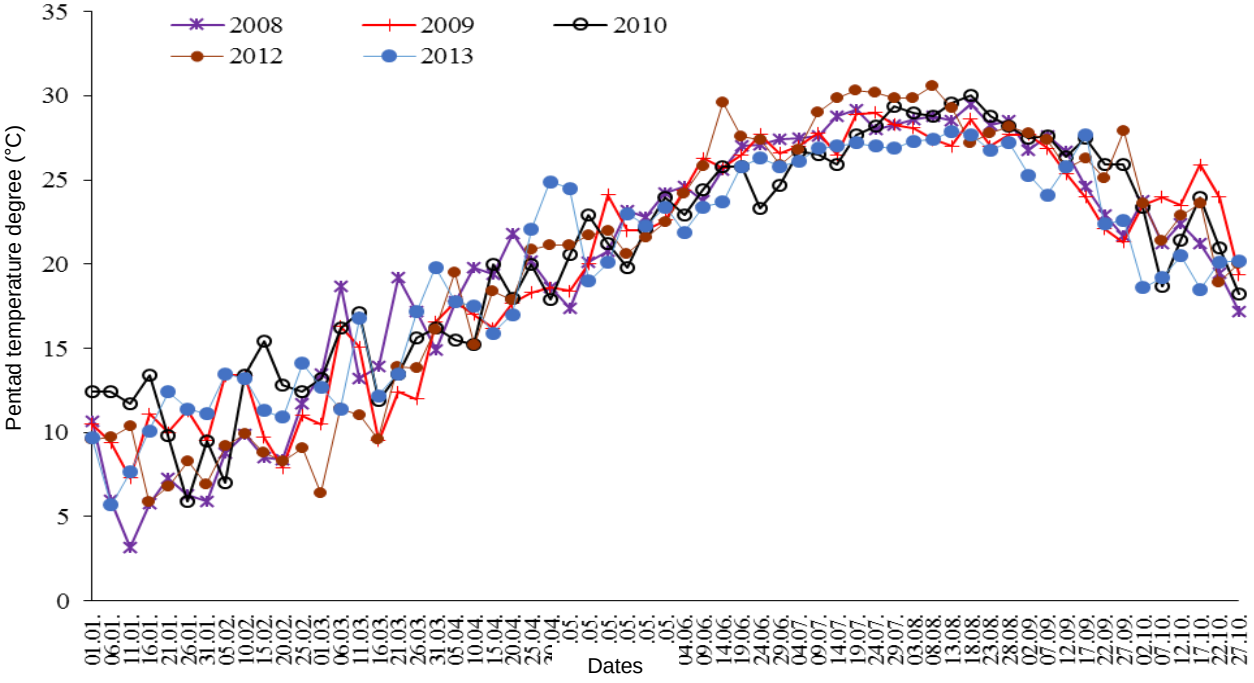

Figure 2. Pentad temperature values in 2008-2010 and 2012-2013 at the pomegranate orchard in Akarsu village in Tarsus/Mersin.

The 5-year pentad temperature values of the pomegranate orchard in Akarsu village ranged between 3.2 and 29.5ºC in 2008 in Figure 2. In the subsequent assessment years, the temperature ranges were 7.3-29.0ºC, 7.0-30.0ºC, 5.9-30.6ºC and 5.7-27.9ºC. The pentad temperatures at the time of the first adult of *C. gnidiella* caught were 14.9, 17.8, 16.2, 18.4 and 19.8ºC for the five years. Therefore, it was concluded that the first adults of *C. gnidiella* could be trapped when the average temperature was about 17.4ºC (15-20ºC).

The number of moths caught in the five years at the highest average temperature values during were 30.7ºC/235 moths (21 August 2008), 30.2ºC/273 moths (23 July 2009), 31.1ºC/321 moths (21 August 2010), 32.1ºC/91 moths and 140 moths (19 and 28 July 2012), and 28.6ºC/186 moths (18 August 2013). The highest number of caught at the mean temperature values were 20.2ºC/326 moths (27 October 2008), 22.5ºC/451 moths (26 October 2009), 22.8ºC/347 moths (20 October 2010), 25.8ºC/383 moths (27 September 2012) and 27.2ºC/258 moths (28 August 2013) (Figures 1 and 2). The adult population of *C. gnidiella* was not negatively affected by temperatures above 30ºC and biological activity (egg, larva, pupa and adult) continued throughout the entire study. According to these results, the most

suitable temperature for development of *C. gnidiella* was 25-30ºC and upper threshold for development was 40ºC. The optimum temperature for development was previously reported as 25-30ºC by (Anonymous, 2016), and Salama (2008) reported that development continued at 35°C. However, the mortality on egg embryo was 100% at 5, 10 and 40ºC.

As shown in Table 1, the average effective temperature calculated from 1 January over the 5 years, and used in *C. gnidiella*'s DD model, were 70.4 DD for hanging traps, 126.76 DD for the first adult emergence, and 245.42 DD for the first egg hatching. In addition, the value for egg hatching in the second to fifth generations were 807.74, 1377.22, 1934.76 and 2506.58 DD, respectively.

Table 1. Degree-day (DD) values of *Cryptoblabes gnidiella* based on a DD model for years 2008-2013

Application periods	ETS values (DD±SE) for each year*										Average ETS value (DD±SE)	Recommended ETS value (DD)
	2008		2009		2010		2012		2013			
Traps hanging	62.1±2,1	b**	69.4±2,4	b	116.8±0,8	c	23.2±2,2	a	80.5±3,5	d	70.40±15.08	80.0
First adult emergence	138.7±2,7	c	121.5±4,5	ab	134.7±4,7	bc	121.9±5,4	ab	117.0±1	a	126.76±4.19	120.0
Egg hatching	253.8±1,8	b	238.9±2,9	a	253.5±3,5	b	243.8±1,2	a	237.1±0,9	a	245.42±3.53	250.0
2nd generation adult emergence	708.2±2,2	b	685.4±0,6	a	699.5±1,7	b	699.7±3,7	b	686.8±3,2	a	695.92±4.31	700.0
Egg hatching	820.2±1,2	c	796.1±3,9	a	809.8±3,8	bc	807.4±2,4	a	805.2±5,2	a	807.74±3.88	800.0
3rd generation adult emergence	1269.3±4,3	c	1241.8±2,8	a	1260.9±1,9	b	1248.0±2	ab	1247.0±7		1253.40±5.06	1250.0
Egg hatching	1389.7±3,7	bc	1361.9±1,9	a	1391.5±1,5	c	1374.9±4,9	a	1368.1±2,1	a	1377.22±5,84	1375.0
4th generation adult emergence	1834.9±4,9	c	1807.0±2,0	a	1825.9±4,1	bc	1822.9±3,1	bc	1814.6±4,6		1821.06±4.78	1820.0
Egg hatching	1952.2±4,2	b	1930.6±2,6	a	1941.8±3,2	b	1929.4±3,6	a	1919.8±2,8	a	1934.76±5.53	1930.0
5th generation adult emergence	2398.4±2,4	c	2378.4±4,6	ab	2393.8±4,2	bc	2390.2±1,2	bc	2370.7±2,7	a	2386.30±5.11	2385.0
Egg hatching	2514.6±3,6	b	2497.3±0,7	a	2516.3±2,3	b	2509.5±3,5	b	2495.4±0,6	a	2506.58±4.34	2500.0

* ETS: Indicates effective temperatures sum (DD) and calculated from 1 January;
****** values within a column followed by the same letters are not significantly different (Duncan, P=0.05).

In a DD-model study in Turkey, to determine the first emergence of the codling moth sexual attractant traps were hung when ETS reaches 40-80 DD from 1 January and when ETS reached 250 and 800 DD, the first and second generations egg hatching occurred, respectively. Traps were hung for the European grapevine moth when the sum of the maximum temperatures reached to 1000°C from 1 January, and when ETS reach to 120, 520 and 1047 DD, the first, second and third generations egg hatching occurred, respectively. Sex pheromone traps were hung when the ETS reached 150 DD from 1 January for the peach twig borer (*A. lineatella*). Following the first adult emergence, egg hatching occurred when the ETS reached 250 DD, consequently spraying should be performed. The calculation of development thresholds for DD values are accepted as 10.0ºC for *C. pomonella*, 12.0ºC for *L. botrana* and 10.0ºC for *A. lineatella* (Anonymous, 2008). Similarly, sex pheromone traps were hung at the end of March and when ETS reached 400 DD, first spraying should be performed, and according to insecticide efficacy second and third spraying should be performed for oriental fruit moth. The average first adult emergence ETS values of carob moth were found to be 403.86 and 294.48 DD in pomegranate orchards in Central and Siverek Regions of Şanlıurfa. Adults were found to have four peaks during the year with ETS values of 1642.19, 2374.25, 2754.76 and 3107.46 DD in the Center Region, and 1218.45, 1595.80,

2109.57 and 2409.71 DD in the Siverek Region of Şanlıurfa. According to these results, development threshold was determined 10.0ºC for *C. pomonella*, *A. lineatella and G. molesta,* 10.85ºC for *P. olea*, and 12.0ºC for *L. botrana* (Kumral et al., 2005; Anonymous, 2008; Mamay et al., 2014).

Regression models and R square values were obtained for mean DD values and mean temperatures of five different generations adult emergence (Table 2).

Table 2. Regression models, parameters between mean degree-day values and mean temperatures for different generations adult emergence in 2008-2013.

Generations	Equations	R^2 values	F values	p values
1st generation	y= 0.0636 x - 19.601	0.490	2.958	0.184
2nd generation	y= 0.0425 x - 4.8642	0.041	0.129	0.743
3rd generation	y=-1.2334 x + 1289.145	0.047	0.149	0.725
4th generation	y= 0.095 x - 144.44	0.890	26.014	0.015
5th generation	y= 0.1147 x - 250.13	0.550	3.667	0.151
Mean	y= 0.0007 x - 25.084	0.130	0.457	0.547

In studies conducted on a DD model for *A. lineatella* in peach orchards in the USA, it is reported that *A. lineatella* has a development threshold of 10.0ºC, a generation completed at 600.0 DD, traps can be hung when ETS reaches 183.3 DD from 1 January, when ETS value from the first adult emergence the traps reach to 222.2-277.7 DD for first generation and 811.0 DD for the second generation the spraying can be applied (Anonymous, 1999; Reding & Alston, 2001). Similarly, studies on the DD model of oriental fruit moth in California (USA) peach orchards revealed that *G. molesta* has a developmental threshold at 7.2ºC, a generation is completed at 535 DD, and when the ETS value reaches at 126.1 DD from 1 January, the first adult are caught at traps, following the catch of the first adult, 175 DD, first spraying, 350 DD second spraying are carried to control the first generation. For the second generation could be controlled by the first spraying when ETS values of 1150-1200 and 1500 DD second spraying, and for the second generations when ETS reach at 2100-2200 DD first spraying and at 2500 DD second spraying can be applied (Croft et al., 1980; Polk et al., 1995).

In conclusion, values for the DD model of *C. gnidiella* from the 5 years of data, taking into account standard errors in the values obtained with literature information, were 80.0 DD for hanging time of trap, and 250.0, 800.0, 1375.0, 1930.0 and 2500.0 DD for first, second, third, fourth and fifth generations egg hatching, respectively. However, when scheduling insecticide applications, first generation of *C. gnidiella*, which had a low population density, having emerged from overwintering places, and the fifth generation, whose time was after harvest, should be controlled. If insecticides are applied according to above information, the applications will be more effective.

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Original article (Orijinal araştırma)

Occurrence of plant parasitic nematode species in important crops in the Southeast Anatolia Region of Turkey[1]

Güneydoğu Anadolu Bölgesindeki önemli kültür bitkilerinde bitki paraziti nematodların belirlenmesi

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Abstract

The Southeast Anatolia Region is one of the major agricultural production areas of Turkey where durum wheat, barley, vegetables and some fruit crops are grown. This study aimed to determine the important plant parasitic nematode species affecting the most commonly cultivated plants in this region. Soil samples were collected in the wheat growing areas of Şanlıurfa, Mardin, Şırnak, Kilis Provinces between May and June in 2011-2012, and pistachio, barley, grapevine, wheat, tomato, watermelon, melon, cotton and tobacco fields of Adıyaman in May, July, October in 2010-2011. The study also reviewed the list of nematode species previously identified in the region. *Aphelenchus avenae* Bastian 1965, *Anguina tritici* (Steinbuch) Filipjev, *Merlinius brevidens* (Allen, 1955) Siddiqi, 1970, *Pratylenchus neglectus* (Rensch, 1924) Filipjev & Schururmans Stekhoven 1941, *P. thornei* Sher & Allen, 1953, *Paratrophurus acristylus* Siddiqi et Siddiqui, 1983 and *Pratylenchoides alkani* Yüksel, 1977 were the most common species found. This study reports *Ditylenchus longicauda* Geraert & Chi, 1988*, Rotylenchus echelimae* Scotto La Massese & Germani, 2000*, Filenchus hamatus* (Thorne & Malek, 1968) Raski & Geraert, 1987*, Helicotylenchus crassatus* Anderson, 1973*, Helicotylenchus goodi* Tikyani et al., 1969, and *Helicotylenchus oleae* Inserra, Vovlas & Golden, 1979 for the first time in Turkey.

Keywords: Plant parasitic nematodes, Southeast Anatolia Region, vegetables, wheat

Öz

Güneydoğu Anadolu Bölgesi Türkiye'nin önemli tarımsal üretim alanlarından birisi olup, makarnalık buğday, arpa, sebze ve bazı meyve üretimleri yapılmaktadır. Çalışmada yoğun tarımsal üretim yapılan bölgelerdeki, önemli bitki paraziti nematod faunasının belirlenmesi amaçlanmıştır. Toprak örnekleri, Şanlıurfa, Mardin, Şırnak, Kilis illeri buğday alanlarından 2011-2012 yılları Mayıs-Haziran aylarında; Adıyaman ili fıstık, arpa, bağ, buğday, domates, karpuz, kavun, pamuk ve tütün alanlarından da 2010-2011 yılları Mayıs-Temmuz-Ekim aylarında toplanmıştır. Çalışmada ayrıca bölgede günümüze kadar saptanmış nematod türlerinin listesi verilmiştir. *Aphelenchus avenae* Bastian 1965, *Anguina tritici* (Steinbuch) Filipjev, *Merlinius brevidens* (Allen, 1955) Siddiqi, 1970, *Pratylenchus neglectus* (Rensch, 1924), *P. thornei* (Sher and Allen 1953), (Sher, 1948), *Paratrophurus acristylus* Siddiqi et Siddiqui, 1983 ve *Pratylenchoides alkani* Yüksel, 1977 en yaygın bulunan türlerdir. Bu çalışmada *Ditylenchus longicauda* Geraert & Chi, 1988, *Rotylenchus echelimae* Scotto La Massese & Germani, 2000*, Filenchus hamatus* (Thorne & Malek, 1968) Raski & Geraert, 1987*, Helicotylenchus crassatus* Anderson, 1973*, Helicotylenchus goodi* Tikyani et al., 1969 ve *Helicotylenchus oleae* Inserra, Vovlas & Golden, 1979 Türkiye'de ilk kez tespit edilmiştir.

Anahtar sözcükler: Bitki paraziti nematodlar, Güneydoğu Anadolu Bölgesi, sebze, buğday

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Introduction

Plant parasitic nematodes are multicellular microorganisms that behave as obligate parasites of plants. Plant parasitic nematodes living outside their host are called ectoparasites and some of them are known as vectors of important plant viruses. Nematodes living inside roots, as migratory or sedentary, are called endoparasites. All plant parasitic nematodes use their stylet to puncture plant cells and to remove the contents. The major symptoms exhibited by plants affected by nematodes include retarded growth, wilting and predisposition to infection by other pathogens (Williamson & Hussey, 1996). Each nematode causes different damage due to secretions of the pharynx. They cause significant yield losses worldwide. The damage caused by plant parasitic nematodes has been estimated at up to $80 billion per year due to the 10-20% production loss of agricultural yield (Sasser & Freckman, 1987; Bongers & Ferris, 1999; Gaugler & Bilgrami, 2004; Nicol et al., 2011).

There are many diseases and pests known as limiting factors in wheat, vegetables, grapevine, barley, melon and other crops, depending on the region. Among these biotic factors, the most common and important are plant parasitic nematodes of which the most damaging to the world agricultural crops are root-knot (*Meloidogyne* spp.) and cyst (*Heterodera* spp. and *Globodera* spp.) nematodes. Root-knot nematodes have wide host range and infect a number of plant species, both wild and cultivated. It has been found that some nematode species of different cultivated plants are new records for the Turkish fauna. The occurrence in Turkey of *Meloidogyne artiella* Franklin, 1961, emphasize the need to take precautions against plant parasitic nematodes (İmren et al., 2014). *Meloidogyne luci* Carneiro et al., 2014, very similar to *Meloidogyne ethiopica* Whitehead, 1968, was detected in Turkey (Gerič Stare et al., 2017). *Meloidogyne chitwoodi* Golden et al., 1980, is known to be quite common in Central Anatolian, East Anatolia, and Aegean regions (Devran et al., 2009; Özarslandan et al., 2009; Yıldız et al., 2009; Ulutaş et al., 2011; Evlice & Bayram, 2012; Özarslandan et al., 2013). Cyst nematodes can cause up to 50% yield losses in wheat plants. Of this group of nematodes determination of pathotype, species, screening for genetic resistance and phylogenetic analyses were made in Turkey (İmren et al., 2012, 2013; Cui et al., 2017). *Helicotylenchus multicinctus* (Cobb, 1893) Golden, 1956 was found in chickpea, whereas *Pratylenchus thornei* Sher & Allen, 1953 and *Heterodera avenae* Wollenweber, 1924 were reported in wheat in the Southeastern Anatolia Region (Kepenekçi, 2014).

A list of the plant parasitic nematodes with their hosts association and distribution in the Southeast Anatolia Region of Turkey is presented. It includes 240 different Tylenchid species collected from different sites of Turkey. Since 2000, agricultural areas in the Southeast Anatolia Region are irrigated with water derived from different dams, and have become more productive. Despite the importance of agriculture in this region, only a few studies had been undertaken to ascertain the occurrence of plant parasitic nematodes (Öztüzün, 1970; Di Vito et al., 1994; İmren, 2007, 2013; Yıldız, 2007; Kılıç, 2011; Öcal, 2012). Therefore, this study was aimed to determine the important plant parasitic nematode fauna associated to wheat, barley, vegetables, grapevine, pistachio, melon, tobacco, cotton, and watermelon in the area.

Material and Methods

Survey

Surveys were conducted in Mardin, Kilis, Şanlıurfa, Adıyaman, Şırnak Provinces between May and October (Figure 1). Soil samples were taken from 275 wheat, 29 pistachio, 69 barley, 16 tomato, 21 watermelon, 23 melon, 23 cotton and 32 tobacco fields, and 45 grapevines in spring, summer and autumn seasons in 2010-2012. Each soil samples were taken from at least 50-60 different points according to a zigzag pattern in each field with a soil corer to 30 cm deep (Southey, 1986).

Figure 1. Map showing the provinces survey in the Southeast Anatolia Region of Turkey.

Laboratory studies

Each sample was thoroughly mixed and a 100-g subsample processed. To extract vermiform nematodes from the soil, a Petri sieving method, a modification of the enhanced Baermann funnel method, was used (Barker, 1985; Southey, 1986).

In order to identify nematodes at species level, permanent slides of them were prepared. Therefore, nematodes extracted from soil were killed at 65ºC and fixed in TAF solution [7 ml formalin (40% formaldehyd), 2 ml triethanolamin and 91 ml pure water] (Hooper, 1986). After the fixation process, nematodes were transferred to solution I (1 part glycerol and 79 parts pure water) at 35-40ºC for 12 h and then in solution II (5 parts glycerin and 95 parts 96% ethanol) at 40ºC for 3 h. Individuals nematode were put in a desiccator for the period of time required for all remaining water to evaporate (Seinhorst, 1959). The nematodes were kept in pure glycerin and were separated according to their genus then permanently mounted on glass slides using the wax-ring method (Hooper, 1986). The method of Hartman and Sasser (1985) developed for root-knot nematodes identification was used. The vulval sections of root-knot nematode females were cut in 45% lactic acid, and processed into glycerin and used for species-level identification. For *Heterodera* spp., the vulval cone were dissected, bleached in H_2O_2 and prepared in glycerin for identification.

Identification of nematodes

Nematodes were identified by morphological and morphometric characters. Ten nematodes per sample were identified. If ten nematodes were not available, all specimens in the sample were identified. Synonyms, systematic position and phylogenetic classification of Tylenchid nematodes are according to Siddiqi (2000). Taxonomic position and synonyms of *Aphelenchoides* and nematode species belonging to Dorylaimida are according to Hunt (1993).

Results and Discussions

Plant parasitic nematode species identified in this study belong to the genera, *Amplimerlinius* (Siddiqi, 1976) (Tylenchida: Telotylenchidae), *Aphelenchoides* Fischer, 1894 (Aphelenchida: Aphelenchoididae), *Aphelenchus* Bastian, 1965 (Aphelenchida: Aphelenchidae), *Bitylenchus* Filipjev, 1934 (Tylenchida: Belonolaimidae), *Ditylenchus* Filipjev, 1936 (Tylenchida: Anguinidae), *Filenchus* Andrassy, 1954 (Tylenchida: Tylenchidae), *Helicotylenchus* Steiner, 1945 (Tylenchida: Hoplolaimidae), *Heterodera* Schmidt, 1871 (Tylenchida: Heteroderidae), *Meloidogyne* Goeldi, 1892 (Tylenchida: Meloidogynidae), *Merlinius* Siddiqi, 1970 (Tylenchida: Telotylenchidae), *Paratrophurus* Arias, 1970 (Tylenchida: Belonolaimidae), *Pratylenchus* Filipjev, 1936 (Tylenchida: Pratylenchidae), *Pratylenchoides*

Winslow, 1958 (Tylenchida: Pratylenchidae), *Quinisulcius* Siddiqi, 1971 (Tylenchida: Belonolaimidae), *Rotylenchus* Filipjev, 1936 (Tylenchida: Hoplolaimidae), *Rotylenchulus* Linford and Oliveira, 1940 (Tylenchida: Rotylenchulidae), *Scutylenchus* Jairajpuri, 1971 (Tylenchida: Telotylenchidae), *Trophurus* Loof, 1956 (Tylenchida: Belonolaimidae), and *Xiphinema* Cobb, 1913 (Dorylaimida: Longidoridae). The nematode species identified in the survey are presented in Table 1.

Among the 39 species found, six species, *Ditylenchus longicauda, Filenchus hamatus, Helicotylenchus crassatus, H. goodi, H. oleae,* and *Rotylenchus echelimae* are new records for the Turkish nematode fauna. A list of plant parasitic nematode fauna in important cultivated plants in the Southeast Anatolia Region was compiled. Also, additional information is given only for the nematodes species that are new records for Turkey.

Table 1. Plant parasitic nematode species found in the soil during this study in the Southeast Anatolia Region of Turkey

Family*	Species**	Host	Locality
Anguinidae	*Ditylenchus longicauda****	barley	Adıyaman
	Ditylenchus myceliophagus	barley, tobacco, wheat	Adıyaman
	Ditylenchus dipsaci	wheat	Mardin
Aphelenchidae	*Aphelenchus avenae*	barley, grapevine, melon, pistachio, wheat	Adıyaman
		wheat	Mardin
Belonolaimidae	*Amplimerlinius vicia*	watermelon, wheat	Adıyaman
	Bitylenchus goffarti	tomato	Adıyaman
		wheat	Kilis
	Paratrophurus acristylus	barley, cotton, grapevine, watermelon, wheat	Adıyaman
		wheat	Kilis
	Paratrophurus loofi	wheat	Adıyaman
	Paratrophurus striatus	barley, cotton, watermelon, wheat	Adıyaman
	Quinisulcius capitatus	tobacco, tomato	Adıyaman
	Scutylenchus cylindricaudatus	wheat	Şanlıurfa
		wheat	Şırnak
		wheat	Mardin
	Scutylenchus quadrifer	wheat	Mardin
	Scutylenchus quadrifer	barley, melon, watermelon, wheat	Adıyaman
	Scutylenchus stegus	tobacco	Adıyaman
	Trophurus imperialis	grapevine, melon	Adıyaman
Heteroderidae	*Heterodera latipons*	wheat	Adıyaman
Hoplolaimidae	*Helicotylenchus crassatus****	barley, wheat	Adıyaman
	Helicotylenchus digonicus	grapevine, pistachio	Adıyaman
	Helicotylenchus exallus	grapevine	Adıyaman
	*Helicotylenchus goodi****	grapevine	Adıyaman
	*Helicotylenchus oleae****	melon	Adıyaman
	Rotylenchus cypriensis	grapevine	Adıyaman
	*Rotylenchus echelimae****	wheat	Mardin

Table 1. (Continued)

Family*	Species**	Host	Locality
Longidoridae	*Xiphinema index*	pistachio	Adıyaman
	Xiphinema pachtaicum	grapevine, pistachio	Adıyaman
Meloidogynidae	*Meloidogyne arenaria*	tobacco, tomato	Adıyaman
	Meloidogyne incognita	tobacco, tomato	Adıyaman
	Meloidogyne javanica	tomato	Adıyaman
Pratylenchidae	*Pratylenchoides alkani*	barley, melon, watermelon, wheat, tobacco	Adıyaman
	Pratylenchus crenatus	melon	Adıyaman
	Pratylenchus neglectus	barley, tobacco	Adıyaman
	Pratylenchus thornei	cotton, grapevine, melon, tobacco, watermelon, wheat	Adıyaman
Rotylenchulidae	*Rotylenchulus macrosoma*	wheat, cotton	Adıyaman
Telotylenchidae	*Merlinius brevidens*	cotton, barley, melon, tobacco, watermelon, wheat,	Adıyaman,
		wheat	Mardin, Kilis
	Merlinius microdorus	barley, grapevine, pistachio, wheat	Adıyaman
		wheat	Mardin, Kilis
Tylenchidae	*Filenchus cylindricauda*	wheat	Adıyaman
	Filenchus cylindricus	wheat	Adıyaman
	*Filenchus hamatus****	tomato	Adıyaman
	Filenchus thornei	wheat	Adıyaman

* Families are listed alphabetically.
** Species are listed alphabetically.
*** The new species for Turkish nematode fauna.

Additional information on the new species records for Turkey

Filenchus hamatus (Thorne & Malek, 1965)

Synonym*: Tylenchus hamatus* (Thorne and Malek, 1968)

Hosts and distribution: Previously reported by Duan et al. (1995) in soybean in South China; Walters et al. (2008) in peach nurseries in Illinois, USA; Baird & Bernard (1984) in wheat and soybean. There is no previous record for Turkey Therefore this is a new record of the species for Turkish nematode fauna and in particular of tomato in Adıyaman Province.

Ditylenchus longicauda Geraert & Choi, 1988

Hosts and distribution: This species was first described by Geraert & Choi (1988) in rice area in Korea. Later it was recorded in Romania and in association with rice in Korea (Choi et al., 1989; Dobrin & Geraert, 1994). During this study, *D. longicauda* was recorded in barley in the Adıyaman Province. This is a new record of the species for the Turkish nematode fauna.

Helicotylenchus crassatus Anderson, 1973

Hosts and distribution: Anderson (1973) found this species in white clover (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.), clover (*Trifolium* sp.), tobacco (*Nicotiana tabacum* L.), apple (*Malus* x *domestica* Borkh) trees, grass and in the bird's-foot trefoil (*Lotus corniculatus* L.) plant in Canada, Quebec and Ontario. It was also reported in carnation and walnut in Iran (Deimi et al., 2008; Bahmani et al., 2013). In Turkey, *H. crassatus* was found in wheat and barley in the Adıyaman Province. This is new record of the species for the Turkish nematode fauna.

Helicotylenchus goodi Tikyani et al., 1969

Synonym*: Helicotylenchus gratus* Patil and Khan, 1983 (syn. by Lal and Khan, 1977)

Hosts and distribution: This species was detected in guava (*Psidium guajava* L.) in India by Tikyani et al. (1969) and Khan et al. (2007). *Helicotylenchus goodi* was recorded in grapevine in Adıyaman Province and is a new record for the Turkish nematode fauna.

Helicotylenchus oleae (Inserra, Vovlas & Golden, 1979)

Hosts and distribution: This species was first recorded by Inserra et al. (1979) in olive in Italy. Additionally, it was found in olive and grapevine in Spain and Greece (Palomares-Rius et al., 2015; 2018). This species was identified in association with melon in Adıyaman Province. This is the first record of *H. oleae* for the Turkish nematode fauna.

Rotylenchus echelimae Scotto La Massese & Germani, 2000

Hosts and distribution: Previously. it has only detected in Menton, France (Scotto La Massese & Germani, 2000). In this study, *R. echelimae* was found in association with wheat in Mardin Province and is the first record for the Turkish nematode fauna.

Discussion

In this study a total of 39 plant parasitic nematode species were found in the Southeast Anatolia Region of Turkey. Among them, 6 species were found for the first time in Turkey and considered as new records for the Turkish nematode fauna.

In previous studies, 37 plant parasitic nematode species were detected in this region (Table 2), totaling 76 the nematode species in the Southeast Anatolia Region. Among these species 16 species have economic importance in other regions of Turkey.

The genus *Ditylenchus* Filipjev, 1936 has more than 80 valid species (Brzeski, 1991), grouped as mycophagous and phytophagous species (Qiao et al., 2016). In this study, three species were identified with *Ditylenchus dipsaci* (Kühn, 1857) Filjpev, 1936, being more important than *Ditylenchus myceliophagus* and *D. longicauda. Ditylenchus dipsaci* is one of the most economically important plant parasitic nematodes; mostly it infests onion and garlic, as well as many other crop plants and weeds worldwide. Population densities of *D. dipsaci* of 10 individuals/500 g of soil may lead to significant crop losses (Palo, 1962). *Ditylenchus dipsaci* has been recorded in onion fields in Turkey (Mennan & Ecevit, 2002; Yavuzaslanoğlu et al., 2015). Investigation on races of *D. dipsaci* and host status in Turkey should be undertaken.

Spiral nematodes, *Helicotylenchus dihystera* and *H. multicinctus* are observed most frequently in banana plantations in the Mediterranean Region of Turkey (Elekcioğlu, 1992; Özarslandan & Dinçer, 2015; Kasapoğlu et al., 2015). However, *H. dihystera* can also be found in vegetables. The economic importance of the *Helicotylenchus* spp., *H. crassatus, H. digonicus, H. exallus, H. goodi, H. oleae,* found in the Southeast Anatolia Region, is not known. Therefore, more research on occurrence, biology, distributions and economic importance of these and other plant parasitic nematodes is needed to understand the role that these nematodes have in the Southeast Anatolia Region.

Pratylenchus thornei, Heterodera avenae, *H. filipjevi* and *H. latipons* are widespread and cause severe yield losses in wheat in Turkey (Gözel, 2001) and other countries (Lasserre et al., 1994; Taheri et al., 1994; Smiley et al., 2004). İmren (2013) and Gözel (2001) detected these species in the Southeast Anatolia and Mediterranean Regions, respectively. Yield losses caused by *H. avenae* were estimated to be up to 26% in the Southeast Anatolia Region of Turkey and up to 57% by *H. avenae*, 40% by *P. thornei* in East Mediterranean Region of Turkey, and 52% by *H. latipons* in İran (Gözel, 2001; Hajihasani et al., 2010; İmren, 2013). It should be taken into consideration that *Heterodera* species may cause economic yield losses in wheat growing areas. Also, it is not feasible to use nematicide for control root lesion and cyst nematodes in wheat. So, studies on screening of resistant wheat genotypes and management strategy have been carried out in Turkey in recent years (Dababat et al., 2015; İmren et al., 2015; Toktay et al., 2015).

Table 2. Plant parasitic species identified previously in the Southeast Anatolia Region of Turkey

Family*	Species**	Host	Locality	Reference
Belonolaimidae	*Amplimerlinius vicia*	wheat	Diyarbakır	İmren, 2008
	Amplimerlinius vicia	lentil, unidentified grass	Şanlıurfa	Yıldız, 2007
Anguinidae	*Anguina tritici*	wheat	Diyarbakır	İmren, 2008
		wheat	Şanlıurfa	Öztüzün,1970
Aphelenchidae	*Aphelenchus avenae*	eggplant, grapevine, pepper, tobacco, tomato, wheat	Diyarbakır	İmren, 2008
		wheat	Mardin	Kılıç, 2011
Aphelenchoididae	*Aphelenchoides bicaudatus*	wheat	Mardin	Kılıç, 2011
Belonolaimidae	*Paratrophurus acristylus*	wheat	Diyarbakır	İmren, 2008
		lentil	Şanlıurfa	Yıldız, 2007
		wheat	Mardin	Kılıç, 2011
	Paratrophurus striatus	wheat	Diyarbakır	İmren, 2008
		barley, lentil, wheat,	Şanlıurfa	Yıldız, 2007
Heteroderidae	*Heterodera avenae*	wheat	Diyarbakır	İmren, 2013
		wheat	Gaziantep, Kilis, Şanlıurfa, Mardin, Şırnak, Kahramanmaraş	İmren et al., 2011
	Heterodera filipjevi	wheat	Şanlıurfa	Yıldız, 2007
	Heterodera latipons	wheat	Şanlıurfa	Yıldız, 2007
Hoplolaimidae	*Helicotylenchus ciceri*	lentil	Diyarbakır	Di vito et al., 1994
		chickpea	Mardin, Şanlıurfa	Di vito et al., 1994
	Helicotylenchus dihystera	grapevine, tomato, pepper, eggplant, tobacco	Diyarbakır	İmren, 2008
		barley, grapevine, lentil, pistachio, unidentified grass	Şanlıurfa	Yıldız, 2007
	Helicotylenchus tunisiensis	eggplant, grapevine, pepper, tomato	Diyarbakır	İmren, 2008
Longidoridae	*Xiphinema diversicaudatum*	grapevine	Diyarbakır	İmren, 2008
	Xiphinema index	grapevine, pistachio, wheat	Şanlıurfa	Yıldız, 2007
	Xiphinema pachtaicum	grapevine	Diyarbakır	İmren,2008
		barley, lentil, wheat	Şanlıurfa	Yıldız, 2007
Meloidogynidae	*Meloidogyne arenaria*	eggplant, pepper, tobacco, tomato	Diyarbakır	İmren, 2008
	Meloidogyne incognita	cucumber, eggplant, grapevine, pepper, tobacco, tomato	Diyarbakır	İmren, 2008
		eggplant, parsley, pepper, tomato	Şanlıurfa	Yıldız, 2007
Paratylenchidae	*Paratylenchus israelensis*	barley, grapevine, lentil, pistachio, wheat	Şanlıurfa	Yıldız, 2007

Table 2. (Continued)

Family*	Species**	Host	Locality	Reference
Pratylenchidae	*Pratylenchoides alkani*	eggplant, grapevine, pepper, tomato	Diyarbakır	İmren, 2008
		cotton, unidentified grass, wheat	Şanlıurfa	Yıldız, 2007
		wheat	Mardin	Kılıç, 2011
	Pratylenchoides erzurumensis	grapevine	Diyarbakır	İmren, 2008
		unidentified grass, wheat	Şanlıurfa	Yıldız, 2007
		chickpea	Mardin	Di vito et al., 1994
	Pratylenchoides leiocauda	chickpea, lentil	Diyarbakır	Di vito et al., 1994
		chickpea	Mardin, Şanlıurfa	Di vito et al., 1994
	Pratylenchoides sheri	wheat	Diyarbakır	İmren, 2008
	Pratylenchus crenatus	wheat	Diyarbakır	İmren, 2008
		cotton	Şanlıurfa	Yıldız, 2007
	Pratylenchus fallax	eggplant, grapevine, pepper, tomato, wheat	Diyarbakır	İmren, 2008
	Pratylenchus flakkensis	cotton	Şanlıurfa	Yıldız, 2007
	Pratylenchus loosi	lentil	Şanlıurfa	Yıldız, 2007
	Pratylenchus mediterraneus	chickpea	Diyarbakır, Gaziantep	Di vito et al., 1994
	Pratylenchus neglectus	wheat	Diyarbakır	İmren, 2008
		unidentified grass, wheat	Şanlıurfa	Yıldız, 2007
	Pratylenchus penetrans	eggplant, grapevine, pepper, tomato, wheat	Diyarbakır	İmren, 2008
		chickpea, lentil	Diyarbakır	Di vito et al., 1994
		chickpea	Gaziantep	Di vito et al., 1994
		chickpea	Şanlıurfa	Di vito et al., 1994
		corn, lentil, unidentified grass	Şanlıurfa	Yıldız, 2007
	Pratylenchus pratensis	wheat	Şanlıurfa	Yıldız, 2007
	Pratylenchus scribneri	unidentified grass	Şanlıurfa	Yıldız, 2007
	Pratylenchus thornei	wheat	Diyarbakır	İmren, 2008
		chickpea, lentil	Diyarbakır, Mardin	Di vito et al., 1994
		barley, cotton, unidentified grass, wheat	Şanlıurfa	Yıldız, 2007
		wheat	Mardin	Kılıç, 2011
Rotylenchulidae	*Rotylenchulus macrosoma*	cotton	Şanlıurfa	Yıldız, 2007
		wheat	Mardin	Kılıç, 2011
Telotylenchidae	*Merlinius brevidens*	grapevine, wheat	Diyarbakır	İmren, 2008
		cotton, lentil, unidentified grass	Şanlıurfa	Yıldız, 2007
		wheat	Mardin	Kılıç, 2011
	Merlinius microdorus	unidentified grass	Şanlıurfa	Yıldız, 2007
		wheat	Mardin	Kılıç, 2011
	Scutylenchus rugosus	grapevine, wheat	Diyarbakır	İmren, 2008
	Tylenchorhynchus usmanensis	cotton	Şanlıurfa	Yıldız, 2007

* Families are listed alphabetically.
** Species are listed alphabetically.

Meloidogyne arenaria (Neal, 1889) Chitwood, 1949*, M. incognita* (Kofoid & White, 1919) Chitwood 1949*,* and *M. javanica* (Treub, 1885) Chitwood, 1949 have been detected in vegetables and known to infest many crops in Turkey. While *Meloidogyne incognita, M. javanica, M. arenaria* have been found in warm areas, *Meloidogyne hapla* Chitwood, 1949 and *M. chitwoodi* occur in cool areas (Kaşkavalcı & Öncüer, 1999). Depending on population density, *Meloidogyne* spp. can cause yield losses of 22% in okra, 15% in peppers, 29% in tomato and 23% in eggplant (Sasser, 1979). Therefore, study on the impact of these nematode on different crop plants in Turkey is suggested.

Xiphinema causes root tip galling and damage a broad range of crop plants by their direct feeding on root tips. They are migratory ectoparasite and polyphagous nematodes. *Xiphinema* includes important species that transmit plant viruses, such as *Xiphinema index* Thorne & Allen, 1950 which is well known as the natural vector of *Grapevine Fanleaf Virüs* to grapevine. *Xiphinema index* and *X. pachtaicum* (Tulaganov 1938) Kirjanova, 1951 have been identified in Mediterranean, Marmara, and Aegean Regions (Elekcioğlu, 1992; Nogay et al., 1995; Mıstanoğlu et al., 2015). As a result of this study, additional harmful plant parasitic nematode fauna was revealed in the Southeast Anatolia Region. Determination of new species from soil samples taken at different times and in different regions is also necessary to ensure early detection of new pests. Given the difficulties of nematode surveys and extraction methods, population densities of existing species and new species need to be determined by systematically sampling from different regions at defined intervals.

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