Spanish Journal of Agricultural Research 22 (4)
October-December, 2024, 20852
eISSN: 2171-9292, ISSN-L: 1695-971X
https://doi.org/10.5424/sjar/2024224-20852

RESEARCH ARTICLE

Phenology, biological and cultural control of the new almond pest *Eurytoma amygdali* (Hymenoptera, Eurytomidae) in Spain

Eugenia Rodrigo[1], Vicent Benedito[2], Pilar Xamaní[3], Manuel Baena[4] and Francisco Ferragut[1]

[1]*Instituto Agroforestal del Mediterráneo, Universitat Politècnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain.* [2]*Instituto de Ingeniería del Agua y Medio Ambiente Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain.* [3]*Departamento Ecosistemas Agroforestales, Escuela Técnica Superior de Ingeniería Agronómica y del Medio Natural, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain.* [4]*Departamento de Biología y Geología, I.E.S. Alhaken II, Manuel Fuentes "Bocanegra"; 14005 Córdoba, Spain.*

***Correspondence** should be addressed to Eugenia Rodrigo: erodrigo@eaf.upv.es

Abstract

Aim of study: *Eurytoma amygdali* Enderlein (Hymenoptera, Eurytomidae), the almond wasp, is a new pest detected in Spain. Although the insect is present in Europe, in Spain no damage was detected until 2016, increasing substantially in the following years. The phenology of almond wasp and the natural enemies complex were investigated and the results of the parasitism rate and cultural control measures were analyzed.

Area of study: Five Spanish provinces, important regions for almond production where *E. amygdali* causes serious harvest losses

Material and methods: Samples of different almond varieties were taken from ecological almond plots in the Spanish provinces of Valencia, Alicante, Albacete, Murcia and Cuenca.

Main results: The first almond wasp eggs were observed at the beginning of May and completely developed larvae, early in July. Wasp flight occurred from mid-March to late May. Two natural enemies were associated with the pest, *Pyemotes amygdali* Çobanoğlu & Doğanlar (Acari, Pyemotidae), and *Opilo domesticus* (Sturm) (Coleoptera, Cleridae). *Pyemotes amygdali* parasitized wasp larvae, adult females and males, and *Opilio domesticus* fed on wasp larvae. *O. domesticus* was very scarce, unlike *P. amygdali*. In the plots with parasitized wasps, the parasitism rate ranged from 0.6% to 44% for *P. amygdali*.

Our results suggest that sanitation measure was an important cultural pest control practice, because in the plantations where mummified almonds were removed, 96% of edible almonds were harvested, compared to 18% if mummified almonds were not removed from trees.

Research highlights: Cultural control could be an important measure to control the almond wasp, because it allows biological conservation control, by preserving natural enemies present in the environment and avoid the negative effect of insecticides.

keywords: almond wasp, biological cycle, natural enemies, sanitation, harvest

Fenología, control biológico y cultural de la nueva plaga del almendro, *Eurytoma amygdali* (Hymenoptera, Eurytomidae) en España

Resumen

Objetivo del estudio: *Eurytoma amygdali* Enderlein (Hymenoptera, Eurytomidae), la avispilla del almendro, es una nueva plaga detectada en España. Aunque el insecto está presente en Europa, en España no se detectaron daños hasta 2016, aumentando de forma sustancial en los años siguientes. En este estudio se ha investigado la fenología de la avispilla del almendro y el complejo de enemigos naturales, así como la tasa de parasitismo y el efecto de medidas culturales de control.

Area de estudio: Cinco provincias españolas que son importantes zonas productoras de almendras y donde *E. amygdali* ha provocado graves pérdidas de cosecha.

Materiales y métodos: Se tomaron muestras en parcelas de cultivo de almendro ecológico en las provincias españolas de Valencia, Alicante, Albacete, Murcia y Cuenca.

Principales resultados: Se observaron los primeros huevos de la avispilla a principios de mayo y larvas completamente desarolladas a principios de julio. El vuelo de la avispilla tuvo lugar desde mediados de marzo a finales de mayo. Se han encontrado dos enemigos naturales asociados a la plaga, *Pyemotes amygdali* Çobanoğlu & Doğanlar (Acari, Pyemotidae), y *Opilio domesticus* (Sturm) (Coleoptera, Cleridae). *Pyemotes amygdali* parasitó larvas, hembras y machos adultos de avispilla, y *O. domesticus* se alimentó de larvas de avispilla. *Opilio domesticus* fue muy escaso a diferencia de *P. amygdali*. En las parcelas con avispilla parasitada, la tasa de parasitismo por *P. amygdali* osciló entre 0.6% y 44%.

Nuestros resultados sugieren que el saneamiento fue un importante método de control cultural de la avispilla, ya que en las parcelas donde las almendras momificadas se eliminaron, el 96% de las almendras cosechadas fueron almendras comestibles, frente al 18% de almendras comestibles que se recogieron en las parcelas donde las almendras momificadas no se eliminaron.

Aspectos destacados de la investigación: El control cultural puede ser una importante medida de control de la avispilla del almendro ya que permite el control biológico por conservación, respetando a los enemigos naturales presentes en el medio ambiente y evitando el efecto negativo de los insecticidas..

Palabras clave: avispilla del almendro, ciclo biológico, enemigos naturales, saneamiento, cosecha.

Citation: Rodrigo, E; Benedito, V; Xamaní, P; Baena, M; Ferragut, F (2024). Phenology, biological and cultural control of the new almond pest *Eurytoma amygdali* (Hymenoptera, Eurytomidae) in Spain. Spanish Journal of Agricultural Research, Volume 22, Issue 4, 20852. https://doi.org/10.5424/sjar/2024224-20852

Received: 15/10/2023. **Accepted**: 25/07/2024. **Published**: 28/11/2024

Introduction

The genus *Eurytoma* Illiger (Hymenoptera, Eurytomidae) displays a wide range of hosts and biology, with eight Palearctic species associated with stone fruit. Of these species, the almond wasp *Eurytoma amygdali* Enderlein (Hymenoptera, Eurytomidae) attacks only almond trees and is considered one of the most important pests of this crop. The insect is widely distributed throughout almond-growing areas in SE Europe, and has been recently detected in Romania, some countries of the former Soviet Union and in Middle Eastern countries like Syria, Turkey, Iran, Lebanon and Cyprus (Zerova & Furson, 1991; Noyes, 2019; Cioacă et al., 2022).

Previous studies conducted on *E. amygdali* biology indicate that it has one generation per year (Mentjelos & Atjemis, 1970; Plaut 1971b; 1972; Talhouk, 1977). In the Mediterranean basin where the pest was studied, adult flight and reproduction begin in March-April and end late in May (Plaut, 1971b; Talhouk, 1977; Katsoyannos et al., 1992). Females lay eggs in the endosperm of seeds when the endocarp is still soft. They can lay up to 7 eggs per seed, but only one larva survives (Mentjelos & Atjemis, 1970; Plaut, 1972; Talhouk, 1977). The larva enters diapause in June-July when it has consumed almost all the seed. The majority of larvae break their diapause after roughly 7 to 8 months, although a proportion of larvae can remain in diapause for 1, 2 years or more. Larvae pupate in January-February and wasps emerge in March-April (Mentjelos & Atjemis, 1970; Talhouk, 1977; Cakar, 1980). From June, infested fruits shrivel and remain firmly attached to the trees for a long time (Plaut, 1971b; 1972; Zerova & Fursov, 1991).

The natural enemy complex of *E. amygdali* is composed of the parasitoid hymenoptera *Adontomerus amygdali* Boucek, (Hymenoptera, Torymidae), *Aprostocetus bucculentus* (Kostjukov) (Hymenoptera, Eulophidae), *Gugolzia bademia* Doğanlar (Hymenoptera, Pteromalidae) *Exeristes roborator* (Fabricius) (Hymenoptera, Ichneumonidae), parasitoid mites *Pyemotes amygdali* Çobanoğlu and Doğanlar (Acari, Pyemotidae) and predatory beetles, *Thanasimus* spp Latreille (Coleoptera, Cleridae) (Çobanoğlu and Doğanlar, 2006; Doğanlar et al., 2006; Schäckermann et al., 2015; Abdul-Rassoul & Mohammed, 2017; Tolga & Yoldas, 2018).

Eurytoma amygdali is considered one of the most important almond pests, especially in the SE Mediterranean (OEPP/EPPO, 2004), because it can cause up to 80% harvest loss (Mentjelos & Atjemis, 1970; Talhouk, 1977; Cakar, 1980). Sanitation is used to control almond wasp and consists of collecting and removing infested fruit containing diapausing larvae from trees. Chemical control with insecticides is also applied. Systemic insecticides are applied after females oviposit inside fruit to kill any neonate larvae (Mentjelos & Atjemis, 1970; Plaut, 1971a; Katsoyannos et al., 1992). Tzanakakis et al. (1997), proposed using insecticides to control adult populations before oviposition occurs. The use of resistant cultivars has been suggested to control this pest (Katsoyannos et al., 1992) because some cultivars showed different infestation rates (Tzanakakis et al., 1997; Ibrahim et al., 2008; Saeidi, 2021).

Although the presence of *E. amygdali* is known in several countries in the Mediterranean Basin such as France, Greece and Turkey (Zerova & Fursov, 1991; Noyes, 2019), its presence and any damage it may cause have not been confirmed in Spain. Since 2016, 30% harvest losses have been observed, which increased to 70% the following year. Given this situation, between 2018 and 2019, *E. amygdali* was declared a pest in some Spanish Autonomous Communities like the Valencian Community (DOGV 2018. Resolution of 23 February 2018), the Murcia Region (BORM 2018 Order of 26 December 2018) and Castilla-La Mancha (DOCM 2019 Order 33/2019 of 25 February). These documents indicate that farmers are obliged to take control measures against this insect. As no almond wasp studies are available in Spain, the objectives of the present work were to study the phenology of *E. amygdali*, investigate the natural enemies complex and analyze parasitism rate and the effect of sanitation on the level of almond wasp infestation.

Material and methods

Study sites

In 2018, two organic almond tree plots, managed in the same way, were chosen for having presented severe almond wasp damage the year before (2017). Both of these plots are located in the Valencian Community (eastern Spain): one in the town of Biar (Alicante) (38º 37' 14.74" N – 0º 47' 55.80" W) and the other in Los Isidros district (Requena, Valencia) (39º 25' 52.63" N – 1o 16' 17.96" W). The trees in the two plots were the same age and had a similar crown size. In the Biar plot, almonds were of the "Guara" variety, while the variety in the Los Isidros plot was "Largueta".

In 2019, it was decided to intensify the study, because no natural enemies were found from the 2018 harvest. Collaboration from farmers in the areas affected by almond wasp was requested so they would send us mummified almonds from the 2019 harvest. Samples were taken from ecological almond plots in the Spanish provinces of Valencia, Alicante, Albacete, Murcia and Cuenca. Table 1 shows the plots and the different almond varieties present in fields if they were known.

Phenology and natural enemies of *E. amygdali*

The 2018 trial. Phenology of *E. amygdali*

To determine the flight, egg and larval periods, the "Los Isidros and Biar" plots were periodically sampled from March to November 2018 (fortnightly from May to July, and monthly until November). On each sampling date, in the field, the growth and ripening of the fruits were monitored and the presence of adults in flight was reported. To detect the presence of flying almond wasps, on each sampling date, in the afternoon (from 12:00 to 14:00) the entire plot was walked through by passing along rows of almond trees, looking at the tree canopy, at eye level or slightly higher. Wasp presence was noted, but not the number of wasps observed. During each sampling, 100 fruits were picked at random and taken to the Crop Protection Laboratory at the School of Agricultural Engineering and Environment (ETSEAMN, Universitat Politècnica de València (UPV)). In the laboratory, almond fruits were opened up and the presence of eggs or larvae, the number of larvae per seed, the length of the larvae, and the location on the almond tissue (nucellus, endosperm, and embryo) were recorded.

To determine the emergence period and sex ratio, almonds were harvested. Harvest took place on 25 July 2018 in Biar and on 30 August 2018 in Los Isidros and were placed inside cardboard boxes measuring 15 x 15 x 31 cm. Two holes were made in rearing boxes, one covered with gauze to allow aeration and the other with a transparent glass tube to allow light to enter. Specifically, 90 fruits per tree from Los Isidros (a total of 56 trees) were placed inside the boxes. In Biar, all the collected fruits (about 5,000 from 80 trees) were kept into the boxes. A maximum of 60 almonds was placed in each box, and boxes were stored in a chamber under natural conditions with natural light, temperature between 17 ºC and 28ºC and relative humidity between 70% and 80%. The boxes with almonds remained inside the chamber from the end of July/August 2018 to June 2019. From March 2019, glass tubes were examined every 2 days and the numbers of emerged males and females were recorded. When no wasp was observed inside glass tubes, all the boxes were opened to report natural enemies.

The 2019 trial. Natural enemies of *E. amygdali*

The mummified fruits collected in 2019 from five Spanish provinces were taken to the ETSEAMN (Table 1) and placed inside cardboard boxes (31 x 15 x 15 cm) in a chamber under natural conditions (natural light, temperature between 17ºC and 28ºC and relative humidity between 70% and 80%). As previously indicated, cardboard box interiors were dark with only an opening for airing and another hole covered with a transparent glass tube. These fruits were left inside the chamber from August 2019 to June 2020. From June, fruits were opened and the number of fruits with a wasp exit hole, the fruits with dead wasps inside, the fruits with natural enemies and those that failed for unknown reasons were reported. Identification of natural enemies was carried out by specialist taxonomists based on morphological characters using identification keys (Gerstmeier, 1998; Çobanoğlu & Doğanlar, 2006; Bahillo de la Puebla et al., 2021).

Parasitism rate

The parasitism rate per plot was calculated as the percentage of almonds with a parasitized wasp in relation

Table 1. Spanish provinces where mummified fruits were collected from the 2019 harvest, geographical indications , total number of almonds opened and almond tree variety if known.

Province	Town/District	Lot[a]	Plot[a]	almonds[b]	Variety/Varieties
Valencia[c]	Venta Moro	14	41	47	*Largueta, Guara, Vayro*
		14	165	119	*Largueta*
		16	101	46	*varied*
		61	46	139	*Ferraduel, Largueta roja, Largueta, Constantí, Asperilla*
	Requena	130	389	118	*Largueta, Largueta oja*
		120	435	149	*Largueta, Largueta roja, Ferraduel*
		120	431	103	*Asperilla, Largueta*
		128	46	30	*Constantí, Vayro, Largueta roja, Largueta*
	Los Isidros			287	*Unknown*
Alicante[c]	Villena	49	109	171	*Guara*
	Biar	8	100	230	*Guara*
	Castalla	22	97	103	*Unknown*
Albacete[d]	Fuentealbilla	38	129	93	*Unknown*
	Casas Ibáñez	29	36	100	*Unknown*
	Hellín	31	196	169	*Filippo Ceo*
		33	10	223	*Guara y Filippo Ceo*
		36	3	31	*Filippo Ceo y Guara*
		48	230	752	*Guara*
		28	112	231	*Filippo Ceo*
		31	169	134	*Guara*
	Mahora	40	33	32	*Unknown*
	Almansa	523	5005	112	*Guara*
		523	5001	147	*Guara*
	Tobarra	13	75	191	*Guara and Ferragnes*
	Albacete	138	5086	84	*Guara*
		137	27	82	*Guara*
	Madrigueras			311	*Unknown*
				55	*Lauranne*
	Casas de Ves			152	*Unknown*
	Cenizate	1	10027	101	*Unknown*
	Golosalvo	13	208	185	*Unknown*
Cuenca[d]	Granja Iniesta	15	65	100	*Lauranne*
Murcia[e]	Yecla			99	*Unknown*
	Jumilla			106	*Unknown*

[a]Data from SIGPAC (lot and plot): the Geographical Information System of Spanish Agricultural Plots (source: SIGPAC, 2023) (https://sigpac.mapama.gob.es/fega/visor/). [b]Total number of almonds opened. The province belongs to the Autonomous Communities of: Valencia[c], Castilla-La Mancha[d] and Murcia[e]

to almonds with an exit hole, almonds with a dead wasp inside and almonds with a parasitized wasp.

$$\left[\frac{\text{almonds with a parasitized wasp}}{(\text{almonds with an exit hole} + \text{almonds with a dead wasp inside} + \text{almonds with a parasitized wasp})}\right] * 100$$

Infestation level

The 2018 trial

At harvest time (25 July 2018 in Biar and 30 August 2018 in Los Isidros), 10 fruits were collected per tree in Los Isidros. In Biar the number of collected fruits was half of the fruit of each tree. All the fruits were taken to the laboratory, where they were opened and it was noted whether they were healthy or unhealthy seeds. Of the unhealthy seeds, it was also noted whether they had almond wasps inside or failed for other reasons. It is noteworthy that after the 2017 harvest, all the fruits left on the almond trees in Los Isidros plot were removed, as well as all those from the surrounding plots.

The 2019 trial

The level of infestation in Los Isidros plot was very low in 2018, so it was decided to repeat the experiment in 2019 only in Biar on the same plot from the previous year and where all the almond fruits were manually collected. In 2019 in Biar, almonds were mechanically collected using a trunk shaker with an umbrella hitched to an orchard tractor. After harvesting, on 26 August 2019, all fruits that did not fall after the application of the trunk shaker (58,09 ± 6,01 almonds) were collected manually, in the same way as the previous year. They were placed inside separate bags and taken to the laboratory, where they were opened and examined, following the same criteria applied the year before.

Results

Phenology and natural enemies of *E. amygdali*

The 2018 trial. Phenology of *E. amygdali*

During field sampling, in Biar, the first adults were detected on 17 April 2018 and the last flying wasps were seen on 15 May 2018. In Los Isidros, flight started from 24 April 2018 and ended on 11 May 2018

Inside the kernels, the first eggs were observed at the beginning of May (6 May in Biar). When seed tissue was still soft and transparent, up to four larvae were found per

Table 2. Mean percentage of parasitism due to *Pyemotes amygdali* in the different plots of the same sampled town/district calculated from mummified almonds for the 2019 harvest.

Province	Town/District	Percentage of parasitism
Valencia	Venta del Moro	0.82
	Requena	8.3
	Los Isidros	0.76
Alicante	Villena	0
	Biar	0
	Castalla	3.53
Albacete	Fuentealbilla	4.29
	Casas Ibáñez	44.44
	Hellín	0.26
	Mahora	0
	Almansa	0
	Tobarra	0
	Albacete	0
	Madrigueras	13.235
	Casas de Ves	0
	Cenizate	0
	Golosalvo	0
Murcia	Yecla	1.33
	Jumilla	3.08
Cuenca	Graja de Iniesta	16.42

seed (15 May 2018 in Biar and Los Isidros). When the seed reaches its final size, it begins to dry and harden, the number of larvae decreased to one larva/kernel, sometimes with two larvae/kernel, and very rarely with three larvae/kernel. If one fruit housed two kernels, there was one larva in each kernel.

Larva length varied over time (Figure 1). By mid-May, larvae were 0.46 ± 0.015 mm long and were distributed all over the seed tissue (nucellus, endosperm and embryo), but were not easily detected, except for well-chitinised mandibles. At the end of June and the beginning of July, larval size increased up to approximately 8 mm (the longest size) in both studied areas. In July, the completely developed larva had consumed all the seed content and remained inside the seed coat.

Figure 2 shows the emergence of adult almond wasps from the harvested almond fruits which kept in a chamber under natural conditions. Almond wasps emerged abundantly between 14 and 18 March 2019, with males being more abundant. Flight ended 2.5 months later (on 29 May 2019). In total, 272 females and 192 males emerged from almonds, and no predators or parasitoids were found.

The 2019 trial. Natural enemies of *E. amygdali*

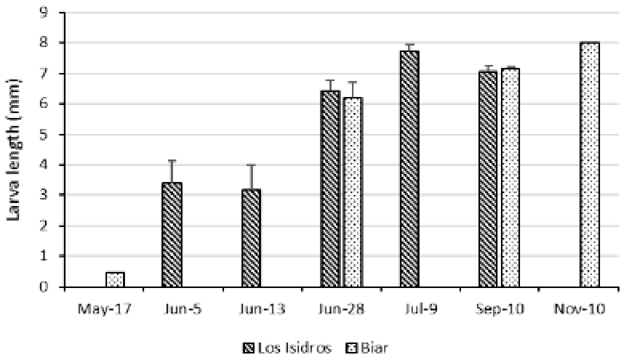

Figure 1. Mean size (± Standard Error) of *Eurytoma. amygdali* larvae on the different sampling dates in 2018 for the two study plots (Los Isidros and Biar) in the Valencian Commmunity (eastern Spain).

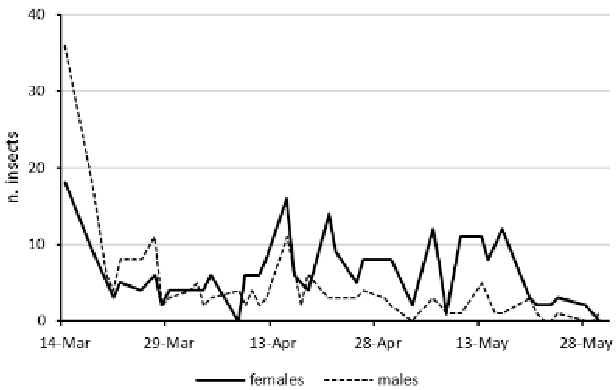

Figure 2. Emergence of adult males/females *Eurytoma. amygdali* wasps from the mummified fruits placed inside a climate chamber from the end of July 2018 to June 2019.

From the 2019 harvest almonds left inside the chamber, 5,032 almonds were removed from boxes and opened. Of all these almonds, 46.2% had an exit opening and the kernel had been consumed, 18.9% had a dead wasp inside, 21.2% failed for unknown reasons, 11.4% contained a live larva in diapause, 1.9% had a mite-parasitized wasp inside, 0.16% contained one larva, adult or exuvial clerid Coleoptera, 0.2% had larval and adult Cerambycidae, and 0.04% had adult Buprestidae.

Four arthropod species, which differed from almond wasp, were detected and identified. *Opilo domesticus* (Sturm) (Coleoptera, Cleridae) (Figures S1 and S2 [suppl.]) and *Pyemotes amygdali* Çobanoğlu and Doğanlar (Acari, Pyemotidae) (Figure S3 [suppl.]) were identified as predator and parasitoid respectively. *Trichoferus fasciculatus* (Faldermann) (Coleoptera, Cerambycidae) and *Anthaxia (Haplanthaxia) millefolii* (Fabricius), are not natural enemies and were eating the almond shell.

Pyemotes amygdali was observed in all the studied provinces, 41% of the plots contained parasitized wasps, and in these plots, the percentage of parasitism ranged from 0.26% to 44.44% (Table 2).

Only four *O. domesticus* specimens were found. Clerids were inside almonds in August 2019 when almonds were harvested from Requena, Almansa and Venta del Moro plots. We found an adult insect when opening a mummified almond in July 2020. The other specimens were larvae, which were reared individually in Petri dishes with alive *E. amygdali* larvae. The petri dishes with clerid larvae remained in the chamber under natural conditions until they became adults in July 2021, approximately 2 years later.

Infestation level

In 2018, 560 almonds were opened in Los Isidros, of which 6.8% had a live wasp inside and 74% were edible. In Biar, 4,791 almonds were opened, of which 71.7% contained a live wasp, and only 17.5% were edible.

In 2019, 8,842 almonds were collected and opened in Biar, of which 19.7% contained a live wasp, and 71% were edible almonds.

In Biar, the plot was the same in both study years. These differences in harvest for both years are shown in Table 3, which also indicates that the percentage of edible almonds from the 2019 harvest was 95.8%, compared to only 17.5% the previous year.

Discussion

In our studied area, *E. amygdali* showed an annual generation in accordance with previous studies (Mentjelos and Atjemis, 1970; Plaut, 1971b; 1972). The first eggs inside seeds were detected at the beginning of May. Newly hatched larvae measured around 0.46 mm long and were observed in the three seed parts (nucellus, endosperm and

Table 3. Percentage of fruits infested by almond wasp and edible nuts for the 2018 and 2019 harvests at Biar

Year	Number of harvested almond trees	Total almond fruits harvested	Percentage of fruits containing a live wasp	Percentage of edible nuts
2018	75	4,791	71.1	17.5
2019	152	61,162	2.9	95.8

embryo), which agrees with Mentjelos & Atjemis (1970) and with Plaut (1972).

According to our results, larva grew and fed on seed content from May to the end of June and beginning of July, when maximum size was reached., Although initially up to three larvae per kernel were detected, finally only one per kernel developed, exactly as Plaut (1972) and Talhouk (1977) reported.

The larva enters diapause when it has consumed all the seed content (Plaut, 1972). The effect of temperature on diapause termination has been recently studied (Tzanakakis & Veerman, 1994; Margaritopoulos & Tzanakakis, 2006). This process begins at the end of May in Israel, which is approximately one month earlier than in our studied area. Similar to Talhouk (1977), at the beginning of July the larva was fully developed, had consumed the seed contents and remained inside the intact seed coat.

While making direct field observations in 2018, we observed adult wasps flying from mid-April to mid-May. In 2019, from the infested almonds placed inside boxes, emergence of adults peaked at mid-March, and ended at the end of May, a period that lasted approximately two months. According to our results, males were emerged earlier than females as a result of the protandry described for this species in the previous studies (Plaut, 1971b; Talhouk, 1977; Katsoyannos et al., 1992; Yiğit et al., 2020).

According to our results, 11% of fruits contained a live larva in diapause. The percentages of larvae in diapause reported by previous studies vary, with 42% for Mentjelos and Atjemis (1970), 23% for Talhouk (1977) or 1% for Plaut (1972). For the three years that our study lasted (from 2018 to 2020), we observed adult almond wasps emerging two years after oviposition. Our results are consistent with other studies showing that the greater majority of the larvae break their diapause after seven to eight months while only a small percentage can remain in diapause for up to three winters (Mentjelos & Atjemis, 1970; Talhouk, 1977; Cakar, 1980).

During the first study year (2018), we did not find that any natural enemies emerged from infested almonds. We intensified the study in 2019 by extending the sampling area to five Spanish provinces affected by the almond wasp. Of the approximately 5,000 opened almonds in June-July 2020, most had been consumed by almond wasp (65.1%) or contained a larva in diapause (11.4%), 21.4% were failed almonds or attacked by other insects, and natural enemies were detected in 2.1% of almonds.

Two natural enemies of almond wasps were found: s *P. amygdali* and the predatory clerid *O. domesticus*. *Pyemotes amygdali* is a gregarious ectoparasitoid that was described as a new species in 2006 (Çobanoğlu & Doğanlar, 2006). This ectoparasitoid mite sucks the hemolymph of its host by attacking the intersegmental membrane and paralysing the insect. The female opisthosoma substantially increases in size when eggs develop inside her (phisogastric females), and eggs hatch within the female. This mite, that parasitized the larvae, pupae and adults of *E. amygdali* have been reported by Çobanoğlu & Doğanlar (2006) and Doğanlar et al. (2006). The study by Doğanlar et al. (2006), was conducted in three provinces in Turkey (Adana, Nizip and Hatay), and in eight localities. *Pyemotes amygdali* was detected only in three localities of the Hatay province, while we found it in the five Spanish provinces that we sampled. This finding indicates that this mite appears where the almond wasp is present. The presence of large phisogastric females makes these mites clearly visible on the parasitized insect. We found groups of mites parasitizing larvae, male adults and female adults of almond wasp. In the plots with parasitized wasps, the parasitism rate in our study ranged among plots from 0.56% to 44.4%, which coincides with the study by Doğanlar et al. (2006), who obtained parasitism rates between 7.56% and 44.53%. According to Çobanoğlu & Doğanlar (2006), this Pyemotidae species could easily reproduces on the host, and may be a good candidate for biological control of almond wasp.

We found *O. domesticus*, preying on *E. amygdali* larvae. According to Bahillo de la Puebla et al. (2021), 37 clerid species are cited in the Iberian Peninsula and Balearic Islands. They present relatively non-specific feeding habits, and both adults and larvae actively prey on xilophagus insects. Adult *O. domesticus* has been detected in the wood of conifers and frondose species, where it consumes mainly anobiidae and some cerambycidae species (Correa de Barros, 1929; Español, 1959). This insect has been reported to appear on the branches of some *Quercus*, beech (*Fagus sylvatica* L.), fig wood (*Ficus carica* L.), carob (*Ceratonia siliqua* L.) and cypress (*Cupressus* L.) species (Bahillo de la Puebla & López-Colón, 2000; Bahillo de la Puebla et al., 2021). Doğanlar et al. (2006) cited *Thanasimus* spp (Coleoptera, Cleridae) as a predator of *E. amygdali*. Therefore, it is the first time that *O. domesticus* is cited to prey on a hymenoptera species and inside an almond fruit. Information about the life cycle of *O. domesticus* and other species of the same family is scarce. One of the most widely studied genera is *Thanasimus*, predator of bark beetles (Coleoptera, Curculionidae), which are serious forest pests. Adult *T. formicarius* lives for 4-10 months and its life cycle completed in 1 year (Gauss, 1954), or 2 years in Scandinavia (Schroeder 1999). *Korynetes caeruleus* (de Geer 1775), predator of *Anobium punctatum* (de Geer

1774) (Coleoptera: Ptinidae), takes 2 years to develop from eggs to adults at 21oC and 75% RH, with a 4-month cold period at 40oC (Haustein et al., 2019). Approximately 2 years had elapsed from the time we observed *O. domesticus* larve until adults emerged, which roughly coincides with previous results.

The methods applied to control almond wasp include cultural measures, which consist in collecting and removing any mummified fruits attached to almond tree branches, and chemical control. Systemic insecticides have been used against recently hatched larvae found inside fruit (Mentjelos & Atjemis, 1970; Plaut 1971a; Katsoyannos et al., 1992), and insecticides applied to the tree canopy against adults (Tzanakakis et al., 1997). In our study area, in almond crops, we can generally state that, to date, not many insecticide applications are carried out. Insecticides were applied in spring to control aphids and copper products to control diseases (leaf curl disease, shot hole disease and red leaf blotch disease). Moreover, in organic almond cultivation, the applied products are non residual contact insecticides; e.g., narrow-range oil. This situation changed because of the damage that the insect was causing and severe crop loss. Between 2018 and 2019, *E. amygdali* was declared a pest in the Valencian Community and other Spanish Autonomous Communities, and farmers were obliged to take control measures against this insect. With the new situation, farmers carried out weekly insecticide applications in March and April, and have also used more persistent and more toxic active ingredients to date. Presently, the allowed active materials to be applied to almond crops are paraffin oil, lambda-cyhalotrin, pyrethrins (authorised for ecological crops) and spirotetramat (https://www.mapa.gob.es/es/agricultura/temas/sanidad-vegetal/productos-fitosanitarios/fitos.asp).

We do not have any data about the effect of authorised insecticides in Spain against the natural enemies that we found: *P. amygdali* and *O. domesticus*. Nevertheless, the secondary effects of these insecticides on a wide range of enemies are known. According to IOBC-WPRS Pesticide Side Effect Database (https://www.iobc-wprs.org/restricted_member/toolbox.cfm), the toxicity level of products lies between 1 (harmless) and 4 (harmful). The most widely used insecticide is lambda-cyhalotrin. This insecticide has a high toxicity level against a wide range of natural enemies. It has toxicity level 4 to *Adalia bibunctata* (L) (Coleoptera, Coccinellidae), level 3 to *Episyrphus balteatus* (De Geer) (Diptera, Syrphidae) both natural enemies of aphids, and level 4 to *Opius concolor* Szepligeti (Hymenoptera, Braconidae), parasitoid of olive fruit fly..

We conclude that sanitation could be an important measure for almond wasp control, as 90% of the mummified almonds contain larvae inside, that will become the population infesting almonds the following year By collecting mummified almonds, the side effects of insecticides (such as lambda-cyhalothrin) are avoided, allowing conservation biological control by *P. amygdali* and *O. domesticus* and other natural enemies present in the fields and in the natural environment.

Supplementary material (Figures S1, S2 and S3)**:** accompanies the paper on SJAR's website

Acknowledgements: We wish to thank Victoria Beltrán (Estación Experimental Agraria de Elche, Generalitat Valenciana) and Juan Salvador Samper (Mañán Sociedad Cooperativa) for their technical assistance and advice. Juan A. Berenguer and Narciso Martínez Gómez for providing the trial field at Biar and Los Isidros and to the technicians of the Servicio de Producción Ecológica Innovación y Tecnología (Generalitat Valenciana) for insecticide applications. Special thanks to the technicians of agricultural cooperatives and farmers for send us mummified almonds from the 2019 harvest. We want to dedicate this paper to Dr. Rafael Laborda Cenjor for his important contribution and who unfortunately passed away in 2021.

Competing interests: The authors declare that no competing interests exist.

Authors' contributions: Eugenia Rodrigo: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. Vicent Benedito: Investigation, Writing – review & editing. Pilar Xamaní: Data curation, Methodology. Manuel Baena: Investigation. Francisco Ferragut: Formal analysis, Investigation, Writing – original draft, Writing – review & editing.

Funding

Funding agencies/institutions:	Project / Grant
Conselleria de Agricultura, Medio Ambiente, Cambio Climático y Desarrollo Rural (Generalitat Valenciana)	S8456000

References

Abdul-Rassoul MS, Mohammed SM, 2017. First record of *Adontomerus amygdali* (Boucek, 1958) (Hymenoptera, Torymidae): a parasitoid of the almond fruit wasp, *Eurytoma amygdali* Enderlein, 1907 (Hymenoptera, Eurytomidae) in Erbil Provence, IRAQ. Bull Iraq nat His Mus 14: 301-306. https://doi.org/10.26842/binhm.7.2017.14.4.0301

Bahillo de la Puebla P, López Colón JI, 2000. El género Opilo Latreille, 1802 en la Península Ibérica (Coleóptera, Cleridae) Boln Asoc esp Ent 24: 213-227.

Bahillo de la Puebla P, López Colón JI, Prieto Piloña F, 2021. La familia Cleridae Latreille, 1802 (Coleoptera) en la Península Ibérica e Islas Baleares. A E 23: 3-98

Cakar L, 1980. *Eurytoma amygdali* End. (Hym., Chalcidoidea, Eurytomidae) a pest of almond in Macedonia. Zastita Bilja, 31, 263-272. (in Bosnian with French abstract)

Cioacă L, Ciceoi R, Stănică F, 2022. Almond seed wasp, *Eurytoma amygdali*, a new fruit pest in the romanian fauna. Scientific Papers. Series B, Horticulture 66: 34-39.

Çobanoğlu S, Doğanlar M, 2006. A new Pyemotes (Acari: Pyemotidae) reared from larvae of the Almond Seed Wasp, *Eurytoma amygdali* (Hymenoptera: Eurytomidae) from Hatay, Turkey. Zool Middle East 39: 101-106. https://doi.org/10.1080/09397140.2006.10638189

Correa De Barros JM, 1929. Notas para o estudo das espécies da Fam. Cleridae existentes em Portugal. Arquivos da secçáo de Biología e Parasitologia do Museo da universidade da Coimbra 1, 1-25.

Doğanlar O, Emin Yıldırım A, Doğanlar M, 2006. Natural Enemy Complex of *Eurytoma Amygdali* Enderlein, 1907 (Hymenoptera, Eurytomidae) in Eastern Mediterranean Region of Turkey; Notes on Their Interaction and Effectiveness. Res J Agric & Biol Sci 2: 282-286.

Español F, 1959. Los Cléridos (Cleridae)de Cataluña e Islas Baleares (Col., Cleroidea). Publicaciones del Instituto de Biología Aplicada Barcelona 30, 105-146

Gauss R, 1954. Der Ameisenbuntkafer *Thanasimus* (Clerus) formicarius Latr. als Borkenkaferfeind. In. Die Grosse Borkenkaferkalamitat in Südwest-Deutschland J944-5J. G. Wellenstein [Ed.]. Ulm.

Gerstmeier R, 1998. Checkered Beetles: Illustrated Key to the Cleridae and Thanerocleridae of the Western Palaearctic Buntkäfer: Illlustrierter Schlüssel zu den Cleridae und Thanerocleridae der West-Paläarktis. Magraf publishers, Weikersheim, Baden-Württemberg, 241 pp, 8 col plates, 304 text figs, maps.

Haustein T, Busweiler S, Haustein V, von Laar C, Plarre R, 2019. Laboratory breeding of *Korynetes caeruleus* (Coleoptera: Cleridae) for the biological control of *Anobium punctatum* (Coleoptera: Ptinidae). Eur J Entomol 116: 362–371. https://doi.org/10.14411/eje.2019.038

Ibrahim MY, Al-Fouzoo T, Al-Naser Z, 2008. Biological and ecological studies of almond fruit wasp, *Eurytoma amygdali* End. (Hymenoptera: Eurytomidae) at Homs Governorate (Syria). JJAS 4(1): 139-151.

Katsoyannos BI, Kouloussis NA, Bassiliou A, 1992. Monitoring populations of the almond seed wasp, *Eurytoma amygdali*, with sex pheromone traps and other means, and optimal timing of chemical control. Entomol Exp Appl 62: 9-16. https://doi.org/10.1111/j.1570-7458.1992.tb00635.x

Margaritopoulos JT, Tzanakakis ME, 2006. Diapause completion in the almond seed wasp, *Eurytoma amygdali* (Hymenoptera: Eurytomidae) following early low temperature treatment. Eur J Entomol 103: 733–742. https://doi.org/10.14411/eje.2006.097

Mentjelos J, Atjemis A, 1970. Studies on the Biology and Control of *Eurytoma amygdali* in Greece. J Econ Entomol 63: 1934-1936. https://doi.org/10.1093/jee/63.6.1934

Noyes JS, 2019. Universal Chalcidoidea Database. World Wide Web electronic publication. http://www.nhm.ac.uk/chalcidoids (accessed June 2023).

OEPP/EPPO 2004, Bulletin OEPP/EPPO Bulletin 34, 427–438. Stone fruits. https://doi.org/10.1111/j.1365-2338.2004.00750.x

Plaut HN, 1971a. Behaviour of phosphamidon within the young almond fruit, and action of translocated phosphamidon and monocrotophos on the egg and the young larva of almond wasp *Eurytoma amygdali*. Pestic Sci 2: 113-114. https://doi.org/10.1002/ps.2780020305

Plaut HN, 1971b. On the biology of adult of almond wasp, Eurytoma-amygdali End (Hym, Eurytomidae), in Israel. B Entomol Res 61: 275-281. https://doi.org/10.1017/S0007485300057795

Plaut HN, 1972. On the biology of the immatures stages of the almond wasp, Eurytoma amygadali End (Hym. Eurytomidae) in Israel. B Entomol Res 61: 681-687. https://doi.org/10.1017/S0007485300047519

Saeidi Z, 2021. Resistance of different almond cultivars/genotypes to almond fruit wasp, *Eurytoma amygdali* (Hymenoptera: Eurytomidae). J Crop Prot 10(3): 535-545.

Schäckermann J, Pufal G, Mandelik Y, Klein AM, 2015. Agro-ecosystem services and dis-services in almond orchards are differentially influenced by the surrounding landscape. Ecol Entomol 40: 12-21. https://doi.org/10.1111/een.12244

Schroeder LM, 1999. Prolonged development time of the bark beetle predator *Thanasimus* formicarius (Col.: Cleridae) in relation to its prey species Tomicus piniperda (L.) and lps typographus (L.) (Col.: Scolytidae). Agr Forest Entomol 1: 127-35. https://doi.org/10.1046/j.1461-9563.1999.00018.x

Talhouk AS, 1977. Contributions to the knowledge of almond pests in East Mediterranean countries V. The fruit-feeding, *Eurytoma amygdali* End., and Anarsia lineatella Z. Z Angew Entomol 83: 145-154. https://doi.org/10.1111/j.1439-0418.1977.tb02384.x

Tolga M, Yoldaş A, Zeynep Y, 2018. Investigation on the damage rate of *Eurytoma amygdali* Enderlin (Hymenoptera: Eurytomidae) with first record of its parasitoid, *Exeristes roborator* (Fabricus) in almond orchards of Mugla and Manisa. Türkiye VII. Bitki Koruma Kongresi (Uluslararas1 Kat1hmh), 14-17 Kas1m 2018, Mugla, Türkiye.

Tzanakakis ME, Papadopoulos NT, Katsoyannos BI, Drakos GN, Manolakis E, 1997. Premature fruit drop caused by *Eurytoma amygdali* (Hymenoptera: Eurytomidae) on three almond varieties. J Econ Entomol 90: 1635-1640. https://doi.org/10.1093/jee/90.6.1635

Tzanakakis ME, Veerman A, 1994. Effect of temperature on the termination of diapause in the univoltine almond seed wasp *Eurytoma amygdali*. Entomol Exp Appl 70: 27-39. https://doi.org/10.1111/j.1570-7458.1994.tb01756.x

Yiğit T, Özgen İ, Canbay A, Koç İ, 2020. Infestation rate of almond seed wasp (*Eurytoma amygdali* Enderlein, Hymenoptera: Eurytomidae) on important commercial almond varieties in Malatya province (Turkey). I J I E A 4: 9-12. https://doi.org/10.46460/ijiea.737755

Zerova MD, Fursov VN, 1991. The Palaearctic species of Eurytoma (Hymenoptera: Eurytomidae) developing in stone fruits (Rosaceae: Prunoideae). B Entomol Res 81: 209-219. https://doi.org/10.1017/S0007485300051294