Chapter 4
Case Study 1: Olive Fruit Fly (*Bactrocera oleae*)

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Population Biology

The olive fruit fly *Bactrocera oleae* (Rossi) (Diptera, Tephritidae) (former name: *Dacus oleae*) is a phytophagous insect associated to olive trees (*Olea europaea*, Oleaceae). With its larvae feeding monophagously on olive fruits, the fly is considered the most severe pest of olive cultivation causing tremendous economic losses. The current distribution of *B. oleae* encompasses the Mediterranean basin, Africa, the Canary Islands, the Middle East, California and Central America (Daane and Johnson 2010; Nardi et al. 2005).

Phenology

Female olive fruit flies oviposit their eggs underneath the skin of ripening olive fruits (Nardi et al. 2005). Usually only one egg per fruit is laid, a process taking six to 13 min (Christenson and Foote 1960; Genç and Nation 2008a; Gutierrez et al. 2009). Studies found that females lay an average of four to 19 eggs per day and a total of 200–350 eggs in their lifetime (Genç and Nation 2008b; Kokkari et al. 2017; Sharaf 1980; Tsiropoulos 1980). To this end, undamaged and unripe olive fruits are preferred for oviposition (Genç and Nation 2008b; Yokoyama and Miller 2004). After hatching inside the fruit, larvae feed monophagously on the pulp and pass through three instar stages to complete larval development (Daane and Johnson 2010; Sharaf 1980). Prior

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to pupation, the last instar leaves the fruit, drops to the soil and pupates at a depth of one to nine centimetres (Dimou et al. 2003; Sharaf 1980). When conditions are favourable, e.g. during summer, pupation may also take place inside the olive fruit (Dimou et al. 2003; Sharaf 1980). Finally, mature adults emerge from the exuviae of the pupa.

Over the course of a year, the olive fruit fly exhibits three to five overlapping generations with seasonal fluctuations of fly densities (Boccaccio and Petacchi 2009; Comins and Fletcher 1988; Kokkari et al. 2017; Pontikakos et al. 2010; Voulgaris et al. 2013). The population dynamics of a Mediterranean population and phenological characteristics of investigated *B. oleae* populations are summarized in Table 4.1. In the Mediterranean, *B. oleae* eggs first appear in August, with a peak of oviposition occurring in mid-October (Bento et al. 1999; Kapatos and Fletcher 1984; Petacchi et al. 2015). Larvae are observed from the end of August and pupation was recorded from the middle of September onwards (Bento et al. 1999). However, life stage-specific developmental times and thus *B. oleae* population dynamics may considerably vary between locations and are strongly driven by climatic conditions (Marchi et al. 2016; Ordano et al. 2015).

While *B. oleae* may be able to even lay eggs in fall and early winter in mild environments (Castrignanò et al. 2012; Petacchi et al. 2015), harsh winter conditions induce overwintering for populations in most Mediterranean regions. The fly overwinters in its adult- or, more commonly, in its pupa-stage in the soil (Kapatos and Fletcher 1984; Neuenschwander et al. 1981; Sharaf 1980). Low winter temperatures regularly cause extremely high mortalities (Table 4.2), with reductions of the adult population of up to 99.7% (Arambourg and Pralavorio 1970; Bigler and Delucchi 1981; Gonçalves et al. 2012). A maximum of two flies per tree was determined to be the overwintering population in a Greek olive orchard (Krimbas and Tsakas 1971). Pupae disappear in high quantities of up to 98.5% over the winter (Arambourg and Pralavorio 1970; Gonçalves et al. 2012). Consequently, *B. oleae* is assumed to frequently go through a bottleneck during winter (Ochando and Reyes 2000). Since all populations in the same area suffer the bottleneck more or less simultaneously, a recolonization by surrounding populations is unlikely (Ochando and Reyes 2000). The spring population consists of the surviving adults, insects arising from overwintering pupae or individuals produced from a spring infestation of unharvested fruits or wild occurring olive trees (Economopoulos et al. 1982; Marchini et al. 2017).

Population Characteristics

In order to assess the inheritance of genetic features, certain qualities of the regarded population need to be considered. Therefore, this section reviews two important aspects determining gene propagation in *B. oleae* populations: sex ratio and mating behaviour.

Table 4.1 Summary of the population dynamics of Mediterranean *Bactrocera oleae* adult populations as reported in the scientific literature

Generations per year	Time of fly presence	Maximum fly density	Adult emerging from overwintering	Study area	References
3	July–late autumn			Tuscany, Italy	Boccaccio and Petacchi (2009)
4	June–November			Corfu, Greece	Kapatos and Fletcher (1984)
4			May	Tripolitania	Sharaf (1980)
			February/March	Arnasco and Levanto, Italy	Petacchi et al. (2015)
		April–May and August–October			Economopoulos et al. (1982)
		September–October		Spain	Ortega and Pascual (2014)
	April–November	October	April	Trasos-Montes, Portugal	Bento et al. (1999)

Life stage	Overwintering mortality (%)	References
Immature stages	98.5	Gonçalves et al. (2012)
	62.2–91.3	Bigler and Delucchi (1981)
Adults	98.5–99.7	Arambourg and Pralavorio (1970)

Table 4.2 Overwintering mortalities of different life stages of *Bactrocera oleae* reported in the literature

Sex Ratio

Trap catches constitute the major data source for population analyses of the olive fruit fly. The attractiveness of traps to olive fruit flies varies over the course of a year (Neuenschwander and Michelakis 1979a) with different effects of various trap systems (e.g. pheromone based traps, food odours) on the two sexes (Economopoulos and Stravropoulou-Delivoria 1984; Neuenschwander and Michelakis 1979a). These aspects need to be considered when looking at trap-determined sex ratios of wild populations. However, most studies report a sex ratio close to 1:1 (Ant et al. 2012; Moore 1962; Speranza et al. 2004). Katsoyannos and Kouloussis (2001) observed a slightly higher number of males (52.2% males) captured on coloured spheres over the course of two years and a total of 7518 flies. In contrast to these observations, in laboratory trials, adults emerged with a ratio of 1:2 (male:female) from reared larvae (Genç and Nation 2008b). Compared to trap catches, the knock-down method or so-called "sondage" (use of insecticides) is considered to be a relatively unbiased method for population analyses. With this technique, Neuenschwander and Michelakis (1979a) demonstrated that the sex ratio of wild populations stays closer to 1:1 than is suggested by trap catches.

Mating Behaviour

After emergence from the pupa, adults complete gonad maturation and reach sexual maturity within three to eight days (Canale et al. 2012; Mazomenos 1984). After mating, females remain unreceptive to further mating for a few days or weeks and store the received semen until ovulation (Solinas and Nuzzaci 1984; Tzanakakis et al. 1968; Zervas 1982). Studies demonstrated that female *B. oleae* are oligogamous, mating only one to three times (Zervas 1982; Zouros and Krimbas 1970). Zouros and Krimbas (1970) demonstrated monogamy for a vast proportion of female flies and estimated a frequency of 17% for female polygamy (Zouros and Krimbas 1970). In contrast to these findings, males are polygamous, mating daily when receptive females are available (Zervas 1982).

Strong intraspecific competition and sexual selection was observed for olive fruit flies (Benelli 2014; Benelli et al. 2012, 2013, 2015). Competition among male flies occurred for single leaf territories suitable for courtship display (wing vibration),

whereas females competed for oviposition sites (Benelli 2014; Benelli et al. 2012). Aggressive behaviour was expressed by wing waving, fast running towards the opponent, pouncing and boxing on the head and thorax of the foe (Benelli 2014).

Environmental Tolerances

The life cycle of *Bactrocera oleae* is closely linked to environmental conditions, in particular to local climatic conditions (Fletcher et al. 1978). This section gives an overview on the two main climatic factors impacting the development of the olive fruit fly in Mediterranean regions: temperature and relative humidity.

Temperature

Local temperature is a major driver of olive fruit fly development and a determinant of the population dynamics (Marchi et al. 2016; Ordano et al. 2015). Consequently, many models on *B. oleae* phenology and population evolution are driven by local temperature data. For this, comprehensive knowledge on the effect of temperature on survival and development of different life stages of *B. oleae* is indispensable. A number of scientific studies have assessed temperature-dependent mortalities and developmental times of the olive fruit fly, e.g. in laboratory trials, the summarized data is displayed in Tables 4.3 and 4.4. In general, high mortalities of the fly are observed at high summer temperatures and at low temperatures over the winter months (Gonçalves et al. 2012). Thus, temperature conditions of the previous seasons were found to determine the rate of *B. oleae* infestation (Marchi et al. 2016). Mild winters are characteristically followed by high olive fruit fly infestations (Marchi et al. 2016).

Strong temperature effects have been observed on the reproduction of *Bactrocera oleae* (Tzanakakis and Koveos 1986). No ovarian development was observed at temperatures lower than 12 °C (Fletcher and Kapatos 1983). The upper maturation threshold was found to be 29.3 °C, with very few females maturing at 29 °C (Fletcher et al. 1978; Fletcher and Kapatos 1983). In some Mediterranean regions, these temperature requirements annually cause two periods when no reproduction occurs: during the winter months and at the beginning of summer, a period characterized by high temperatures and low humidity (Economopoulos et al. 1982; Fletcher et al. 1978). In mild climates, however, *B. oleae* might even be actively reproducing in winter (Economopoulos et al. 1982).

Relative Humidity

The development of *Bactrocera oleae*, i.e. longevity, maturation and survival, is strongly correlated with relative air humidity (Broufas et al. 2009; Broumas et al.

Table 4.3 Temperature-dependent mortalities of *Bactrocera oleae* life stages reported in the scientific literature

Life stage	Temperature (°C)	Mortality (proportion of population)	References
Egg	8	No hatching	Tsiropoulos (1972)
	10–30	<20%	Tsiropoulos (1972)
	16	45–51%	Genç and Nation (2008a)
	22	27–33%	Genç and Nation (2008a)
	25	13–31%	Sánchez-Ramos et al. (2013)
	27	13–26%	Genç and Nation (2008a)
	35	98%	Genç and Nation (2008a)
	35	No hatching	Tsiropoulos (1972)
Larvae	10	100%	Tsitsipis (1980)
	16	38–63%	Genç and Nation (2008a)
	22	18–47%	Genç and Nation (2008a)
	27	10–31%	Genç and Nation (2008a)
	32.5	100%	Tsitsipis (1980)
	35	92–94%	Genç and Nation (2008a)
Pupae	12.5	15.8%	Tsitsipis (1980)
	16	44–70%	Genç and Nation (2008a)
	22	26–56%	Genç and Nation (2008a)
	27	18–37%	Genç and Nation (2008a)
	30	48%	Tsitsipis (1980)
	35	No development	Genç and Nation (2008a)
Adult	16	53–76%	Genç and Nation (2008a)
	22	31–64%	Genç and Nation (2008a)
	27	25–50%	Genç and Nation (2008a)
	35	No development	Genç and Nation (2008a)

2002; Fletcher et al. 1978). Especially long life spans of adult flies have been observed in high air humidity (Broufas et al. 2009). A summary of the effect of humidity on different life parameters of *B. oleae* is displayed in Table 4.5. Humidity may also impact the effectiveness of specific trapping systems for fly monitoring (Bueno and Jones 2002; Mazomenos et al. 2002).

Dispersal Dynamics

Scientists have utilized various techniques to determine the dispersal potential of adult olive fruit flies, ranging from traditional capture–recapture experiments and

Table 4.4 Temperature-dependent development of *Bactrocera oleae* as reported in scientific literature

Stage	Temperature (°C)	Developmental time/Life span	References
Egg	10	3 days	Tsitsipis (1977)
	15	1 day	Tsitsipis (1977)
	16	11 days	Genç and Nation (2008a)
	20	1 day	Tsitsipis (1977)
	22	4 days	Genç and Nation (2008a)
	24	2 days	Genç and Nation (2008b)
	25	1 day	Tsitsipis (1977)
	27	3 days	Genç and Nation (2008a)
	30	1 day	Tsitsipis (1977)
	35	2 days	Genç and Nation (2008a)
Larvae	12.5	37 days	Tsitsipis (1980)
	15	25 days	Tsitsipis (1980)
	20	15 days	Tsitsipis (1980)
	16	33 days	Genç and Nation (2008a)
	22	14 days	Genç and Nation (2008a)
	25	10 days	Tsitsipis (1980)
	25	7 days	Neuenschwander and Michelakis (1979b)
	27	11 days	Genç and Nation (2008a)
	30	10 days	Tsitsipis (1980)
	35	5 days	Genç and Nation (2008a)
Pupa	12.5	49 days	Tsitsipis (1980)
	14	34.5 days	Tsiropoulos (1972)
	15	35 days	Tsitsipis (1980)
	16	28 days	Genç and Nation (2008a)
	20	15 days	Tsitsipis (1980)
	22	12 days	Genç and Nation (2008a)
	25	10 days	Tsitsipis (1980)
	25	13 days	Tsiropoulos (1972)
	27	8 days	Genç and Nation (2008a)
	30	9 days	Tsitsipis (1980)
	35	No development	Genç and Nation (2008a)
Adult (male)	25	47 days	Sánchez-Ramos et al. (2013)
Adult (female)	25	34 days	Sánchez-Ramos et al. (2013)
	25	19–31 days	Broufas et al. (2009)

Table 4.5 Life parameters of *Bactrocera oleae* depending on relative humidity as reported in scientific literature

Relative humidity	Temperature	Longevity	Proportion of mature females (%)	Mean number of eggs per female	Egg hatching (%)	References
12	25 °C	19 days	4	109	12	Broufas et al. (2009)
33		34 days	41	215	77	
55		48 days	61	444	82	
75		50 days	67	581	86	
94		31 days	92	234	87	
<90	20 °C				0	Tsitsipis and Abatzis (1980)
95					72	
100					97	

performance analyses on flight mills to the use of molecular techniques. This section provides a literature review in order to provide data appropriate for dispersal models. Experimentally determined maximum and typical dispersal distances as well as observed impacts of laboratory treatments and genetic parameters are summarized for Mediterranean *Bactrocera oleae* populations.

Dispersal Distances

In suitable environmental conditions, non-dispersive movements are assumed for adult olive fruit flies (Remund et al. 1976), with typical distances of 180–190 m overcome within two weeks (Fletcher and Economopoulos 1976; Fletcher and Kapatos 1981). Radioactive labelling revealed that the major proportion of a wild *B. oleae* population did not disperse further than 1000 m within one month and no migration was observed (Pelekassis et al. 1963). However, extreme conditions, such as high population densities (overcrowding) or lack of oviposition sites, might induce long distance dispersal of olive fruit flies (Economopoulos et al. 1978; Fletcher and Kapatos 1981; Remund et al. 1976). A release experiment by Fletcher and Kapatos (1981) illustrates this situation for *B. oleae* populations on Corfu, Greece. After release to a grove with no new season crop, flies travelled more than 400 m during the first seven days, but only dispersed over 180 m when 30% of olives in the release grove bore fruit (Fletcher and Kapatos 1981). Maximum dispersal distances for *B. oleae* reported in the literature range from 4000 to 5000 m (Economopoulos et al. 1978; Pelekassis et al. 1963; Remund et al. 1976). A summary of dispersal distances recorded in scientific studies is displayed in Table 4.6.

Table 4.6 Dispersal distances of *Bactrocera oleae* in the Mediterranean region

Distance (m)	Conditions	Duration	Survival (%)	References
50	Irradiated laboratory-reared olive fruit flies			Rempoulakis and Nestel (2012)
60	Laboratory-reared flies (olive diet)			Economopoulos et al. unpubl., reported in Remund et al. (1976)
180	30% fruit crop in release grove	1 week		Fletcher and Kapatos (1981)
180–190	Suitable environmental conditions, laboratory-reared flies	2 weeks	13	Fletcher and Economopoulos (1976)
400	No fruit crop in release grove	1 week		Fletcher and Kapatos (1981)
<1000	Wild flies, semi-mountainous olive grove, Greece, 86% of population dispersed up to 1000 m	35 days		Pelekassis et al. (1963)
1000–2500	Wild flies, semi-mountainous olive grove, Greece, 10% of population dispersed 1000–2500 m	35 days		Pelekassis et al. (1963)
2000	Wild flies, closest olive grove 2000 m distance		<1	Economopoulos et al. (1978)
2000	Artificially-reared flies, closest olive grove 2000 m distance		<1	Economopoulos et al. (1978)
2000–3000	Overcrowding, laboratory-reared flies (olive diet)			Economopoulos et al. unpubl., reported in Remund et al. (1976)
4000	Wild and artificially-reared flies, closest olive grove 2000 m distance		<1	Economopoulos et al. (1978)
2500–4300	Wild flies, semi-mountainous olive grove, Greece, 4% of population dispersed 2500–4300 m, maximum dispersal distance	35 days		Pelekassis et al. (1963)
3000–5000	Laboratory-reared flies (olive diet)			Economopoulos et al. unpubl., reported in Remund et al. (1976)

The flight potential of *B. oleae* is likely to vary with fly characteristics, e.g. fly age and sex. Remund et al. (1976) determined flight performance by means of flight propensity, number of flights and total distance flown for laboratory-reared *B. oleae* on flight-mills. The authors observed a threefold increase of the distance overcome by 14-day-old adult flies compared to their 2-day-old conspecifics. At the same time, females displayed a higher flight capacity with regard to all parameters assessed than their male counterparts. This effect of fly sex, however, was not detected in a release experiment by Fletcher and Economopoulos (1976).

Some strategies to reduce population densities of *B. oleae* in the Mediterranean area depend on the release of laboratory-reared individuals that have been sterilised by irradiation treatments. As a consequence, experimenters investigated the impact of laboratory-rearing as well as different rearing conditions on the fly's dispersal potential, assessing both non-sterilised and sterilised specimens. Based on results of field experiments, Fletcher and Economopoulos (1976) suggested greater dispersal distances for wild-compared to laboratory-reared olive fruit flies. In their experiment, no additional negative impact of the irradiation procedure of laboratory-reared *B. oleae* was observed. Remund et al. (1976) found a significant impact of diet on flight performance of olive fruit flies. Compared to individuals reared on an artificial diet, flies feeding on olives flew 2–3 times greater distances (Remund et al. 1976).

Genetic Variability and Gene Flow

Genetic parameters of *Bactrocera oleae* indicate a high degree of genetic variability for Mediterranean populations, as displayed by polymorphism and the number of alleles per locus (Augustinos et al. 2005; Ochando and Reyes 2000; Segura et al. 2008). Compared to polyphagous fruit flies, a lower genetic variability was expected for *B. oleae* due to its high host specialization. However, actual genetic variability was comparable or even higher (Augustinos et al. 2005; Ochando and Reyes 2000). At the same time, analyses detected more variability within than among Mediterranean *B. oleae* populations, indicating the occurrence of gene flow among these populations (Segura et al. 2008). In the regarded studies, gene flow (Nm, with N as the effective population size and m as the net migration rate per generation) was estimated after Wright (1931). Despite the relatively short regular dispersal distances of *B. oleae* individuals observed in field experiments, molecular analyses indicate a high level of gene flow among Mediterranean populations (Augustinos et al. 2005; Ochando and Reyes 2000; Segura et al. 2008). Gene flow was as high as N$m = 8.9$ between different populations from Spain that were separated by hundreds of kilometres of dry climate (Ochando and Reyes 2000). According to Wright (1931), an Nm equal to 1 would be sufficient to prevent differentiation among populations. In addition to ecological dispersal, social-ecological aspects, such as trade with olive cultivars, have to be considered as potential source of gene flow (Augustinos et al. 2005; Segura et al. 2008).

With regard to the fly's dependence on its olive host and its winter disappearance, a high potential for the occurrence of bottlenecks was acknowledged in different studies (Augustinos et al. 2005; Ochando and Reyes 2000). As bottlenecks might result in inbreeding, heterozygote deficiency detected in Spanish fly populations substantiated this assumption (Ochando and Reyes 2000). An interesting result was an inhomogeneity of genetic variability among loci, which indicates selective processes (Augustinos et al. 2005). Natural selection due to agricultural practices may be the factor responsible for the pattern of genetic variability (Ochando and Reyes 2000).

Eggs

Hosted underneath the olive's epidermis, *B. oleae* eggs are protected from most natural enemies (Bateman 1972; Daane and Johnson 2010). However, predation may occur by birds feeding on whole olives, such as thrushes (Bigler et al. 1986; Neuenschwander et al. 1983), or by the midge *Prolasioptera berlesiana* (Kalaitzaki et al. 2014). Larvae of the latter occur in olive punctures infested with the fungus *Macrophoma dalmatica* and were observed to destroy olive fly eggs (Solinas 1967). Recognized as a so-called biocomplex, relationships and potential dependencies between *B. oleae*, *M. dalmatica* and *P. berlesiana* have extensively been studied and tested for the use in biological pest management. Even though olive fly oviposition holes appear to be the main entrance for the fungus as well as for the midge (González et al. 2006; Neuenschwander et al. 1983), neither of the insects plays an important role as disseminating agent of *M. dalmatica* spores (Harpaz and Gerson 1966). According to Neuenschwander et al. (1983) *P. berlesiana* oviposition immediately followed *B. oleae* attacks. The midges may, to a minor extent, prey on olive fly eggs and young larvae, but rely on fungal diet for survival (Harpaz and Gerson 1966; Solinas 1967). Maximum *B. oleae* egg mortality induced by *P. berlesiana* was estimated at 30–50% for Cretan populations (Neuenschwander et al. 1983). Overall predation of eggs, however, is low, with 0–6% predation-induced egg mortality determined for traditional groves in Portugal (Gonçalves et al. 2012).

Larvae

After hatching, *B. oleae* larvae feed monophagous on the pulp of their olive hosts (Daane and Johnson 2010). Within the tissue of an unripe fruit, olive fruit flies depend on the presence of symbiotic microorganisms for growth and survival (Bateman 1972; Ben-Yosef et al. 2010, 2014). The identity of the fly's symbiotic microflora is in the focus of scientific controversy. For many decades a mutualistic relationship with the extracellular bacterium *Pseudomonas savastanoi* has been suspected (Hagen 1966). However, most recently discovered associations involve the bacterium *Acetobacter tropicalis* (Kounatidis et al. 2009) and the before undescribed gut bacterium *Candidatus Erwinia dacicola,* which was numerically dominant inhabitant of the fly's oesophagus bulb (Ben-Yosef et al. 2014; Capuzzo et al. 2005; Estes et al. 2009;

Sacchetti et al. 2008). Based on the absence o*f* Ca. *E. dacicola* in other investigated tephritids and the peculiar morphological structure of the bacteria-hosting oesophagus bulb of *B. oleae*, Capuzzo et al. (2005) proposed a coevolution of *B. oleae* with Ca.*E. dacicola.* A total of 16 different bacteria were found to be associated with wild olive flies in Greece, displaying varying bacteria compositions between generations of the same year (Tsiropoulos 1983). It is important to note, that a lower number of different bacteria, as well as a reduced compositional variability was detected in laboratory-reared olive flies (Tsiropoulos 1983).

Life Stages at the Soil

Life stages associated to the soil, such as late instar larvae, pupae and emerging adults, are most exposed to predation (Bateman 1972; Daane and Johnson 2010). Common predators at the soil surface are arthropods like beetles, myriapods and ants (Daane and Johnson 2010; Neuenschwander et al. 1983). Ants are considered to play a major role in the destruction of *B. oleae* developmental stages. Individual ants or groups are capable of transporting *B. oleae* to the nest, eventually damaging their prey (Neuenschwander et al. 1983). Ants were also reported to be the main predators of *B. oleae* pupae in recently invaded habitats in California (Orsini 2006; Orsini et al. 2007). Additionally, food searching birds, e.g. the black bird (*Turdus merula*), the robin (*Erithacus rubecula*) or the starling (*Sturnus vulgaris*), may destroy life stages of *B. oleae* on the soil surface (Neuenschwander et al. 1983). Pupae removal by birds can account for up to 70%, depending on orchard type (Bigler et al. 1986).

Adults

In contrast to their monophagous larvae, adults of *B. oleae* feed on a variety of organic sources, including insect honeydews, plant nectar and pollen, fruit exudates, bird dung, bacteria and yeast (Christenson and Foote 1960; Daane and Johnson 2010; Tsiropoulos 1977). As a result, adult olive flies may be found in vegetation other than olive trees, e.g. walnut, apple or plane trees (Economopoulos et al. 1982). Despite this range of food sources, the diet of *B. oleae* may be poor or unbalanced in its amino acid composition (Ben-Yosef et al. 2010). Experimental evidence suggests that the gut symbiontic bacterium Ca.*E. dacicola*, that was present in all life stages of *B. oleae* (Estes et al. 2009) enables adult olive fruit flies to utilize non-essential amino acids and urea as a source of nitrogen (Ben-Yosef et al. 2010, 2014). The authors proposed that *B. oleae* depends on the symbiont for protein synthesis and egg production.

Birds as well as cursorial and web spiders are considered to be important predators in olive orchards, capable of suppressing *B. oleae* population densities (Bigler et al. 1986; Picchi et al. 2016). These predators were found to be considerably more numerous around organic olive orchards compared to conventionally managed groves
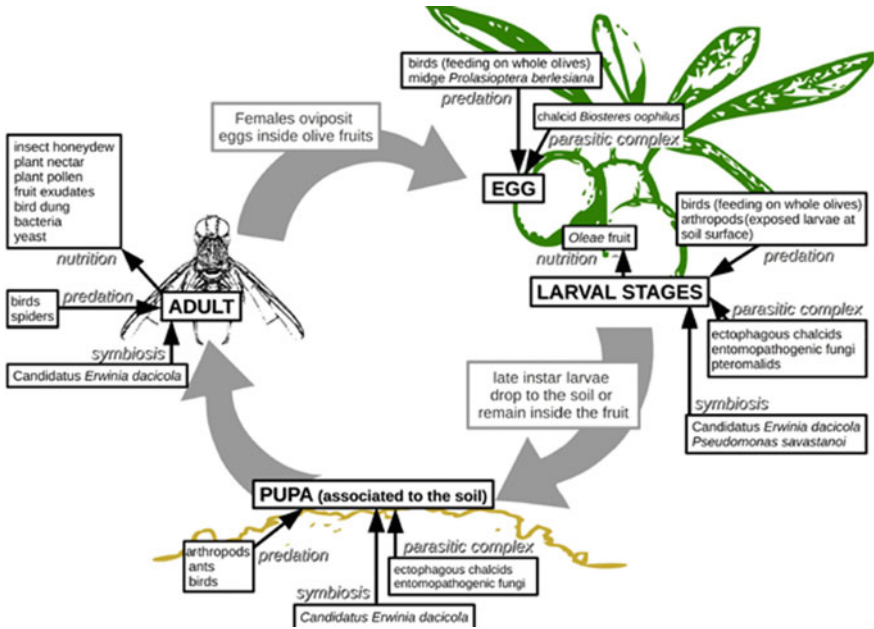
Fig. 4.1 Schematic representation of the ecological network of the olive fruit fly *Bactrocera oleae*

(Bigler et al. 1986; Picchi et al. 2016, 2017). However, the general lack of scientific literature on further potential predators of adult olive flies and predation rates highlights avenues for future research.

The life stages of *B. oleae* and interspecies relations are depicted in Fig. 4.1.

Competition for Olive Fruits

A number of olive tree pests have been identified besides the olive fruit fly. A comprehensive list of pests and diseases occurring on the Maltese Islands is provided by Haber and Mifsud (2007). The authors report different species of insects and eriophyid mites, as well as fungal and bacterial diseases. Most of these species occur primarily on leaves, stems and fresh shoots of the trees (Haber and Mifsud 2007; Martin et al. 2000), which indicates a rather low potential for direct competition for olive fruits with *B. oleae*. However, some hemiptera species also directly impact olive fruits, e.g. *Pollinia pollini*, *Aspidiotus nerii*, *Hemiberlesia rapax*, *Leucaspis riccae* (Alford 2014; Daane et al. 2005; Haber and Mifsud 2007). Even though these species do not penetrate the olive fruit skin, potential surface modifications might impede oviposition by *B. oleae*. In general, no literature references could be detected concerning the fly's competition with other olive pests.

At the same time, different studies highlight aggressive intraspecific interactions for adult olive fruit flies (Benelli 2014; Benelli et al. 2013, 2015). The intense intra-specific competition indicates that genetically modified flies, which might be released for eradication purposes, need to be capable of competing with wild types in order to mate and successfully spread the modified gene.

Potential Hazards with Regard to Gene Drive Release

Based on the ecological network analysis, a number of potential hazards, associated to the release of gene drive-equipped olive fruit flies to wild populations can be identified and are briefly discussed in this section.

Unintentional Long-Distance Transport of the Genetically Modified Organism

A serious increase of exposure associated with the release of genetically modified organisms is the potential for unintentional transport to non-target locations. Speci-mens might arrive in new locations via natural dispersal or by human agency. The lat-ter bears potential for long-distance transport of the altered organism. In the receiving habitat, the newcomer may become invasive or co-exist with related species, enabling a potential transfer of modified genes across species boundaries. In this section poten-tial pathways for long-distance transport of the olive fruit fly *Bactrocera oleae* are evaluated.

There are three main vectors for unintentional long-distance transport of *B. oleae*:

- Transport of adult individuals
  Living adult insect specimens might be transported as hitchhikers on aircrafts, e.g. in the passenger area or within the airline baggage. A prominent example of insect transport along this pathway is the commonly known airport malaria (Isaäcson 1989). In a similar manner, also agricultural pests may travel by this vector (Liebhold et al. 2006). The increase of public air traffic is expected to enhance the chances of insect transport by this vector in coming decades.
- Transport of immature life stages within the olive host
  The olive fruit fly undergoes egg and larval development within its olive fruit host. During this time, transport of the fruit might, in favourable conditions result in transport of the living insect to distant locations. Inspection records of 1962 indeed revealed that, within its host, the olive fruit fly arrived in the US via ships and aircrafts (Rainwater 1963). Both transport of single olives or entire trees may serve as a vector for unintentional introductions. Especially the trade of olive cultivars may result in unintentional introductions of the olive fruit fly and was suggested for the arrival of *B. oleae* in the New World (Nardi et al. 2005).

- Transport of immature stages (larvae, pupae) within the soil
Life stages associated to the soil, such as larvae and pupae, may be transported within their host material. Soil adhering to shoes of outgoing tourists or soil associated to traded olive trees might serve as vector for immature stages of the olive fruit fly.

Hybridization and Horizontal Gene Transfer Across Species Boundaries

Ecological risk assessment for the release of gene drive-carrying organisms to natural populations is urged to consider the risk of unintentional transfer of the altered gene across species boundaries. Vertical inter-species transfer of altered genes will depend on the organism's ability to produce hybrids with related species.

Three fruit flies from the *B.* (subgenus *Bactrocera*) *dorsalis* complex (*B. dorsalis*, *B. papayae*, and *B. philippinensis*) were found capable of cross-mating (Schutze et al. 2013) and sperm transfer was observed between *B. dorsalis* and *B.* (*Bactrocera*) *carambolae* in outdoor field cages (McInnis et al. 1999). For the olive fruit fly, no studies testing the hybridization potential with related species were detected. *B. oleae* belongs to the subgenus *Daculus*, a sister group to the subgenus *Bactrocera* (Smith et al. 2003; Zhang et al. 2010). Hybridization trials should focus on the nearest African congeners (*Daculus*), *B. biguttula* and *B. munroi*, which are also associated with *Oleaceae* (Copeland et al. 2004; Drew and Hancock 2000) and exhibited a mean genetic divergence of 3.4% to *B. oleae* (Bon et al. 2016). Morphological features, i.e. length of terminalia, pheromone composition and variation in courtship signals might play a role for mating compatibility (Iwaizumi et al. 1997; Schutze et al. 2013).

The classification of Tephritidae species and their geographic distribution is shown in Fig. 4.2.

Ecological Niche Filling by Other Species

The declared aim of planned releases of gene drive-equipped insects is the local suppression or eradication of pest species. However, after extinction, the vacant ecological niche might be occupied by a related or competing species. In order to assess this possibility for the case study of the olive fruit fly, potential competitors of the olive fly were investigated. Despite the existence of other olive pests, no studies could be detected on competition with other olive pests. As a consequence of this potential lack of direct competitors, a genetically induced reduction in olive fruit fly densities may be unlikely to cause another pest population to increase.
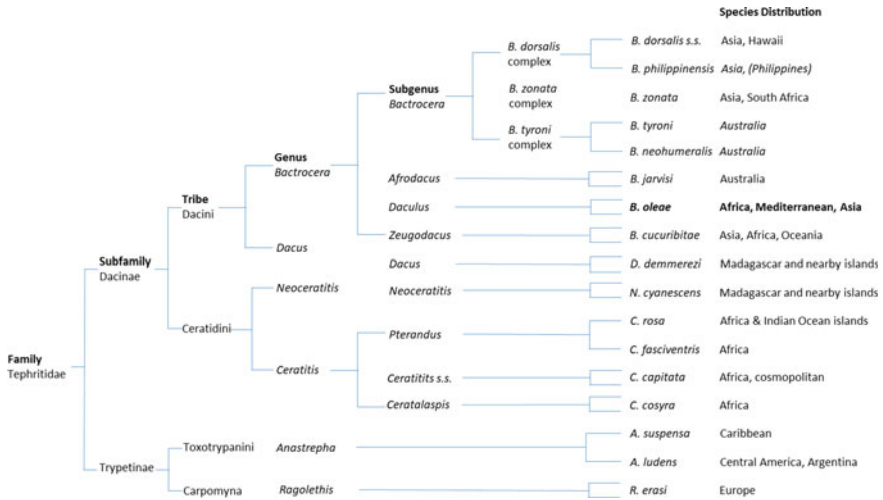
Fig. 4.2 Classification and distribution of 17 Tephritidae species. Adapted from Bonizzoni et al. (2007)

The close co-evolution of *B. oleae* with symbiotic microorganisms, which is essential for its development inside an unripe fruit, already indicates that the ecological niche of the olive fruit fly might not easily be occupied by another organism.

Concluding Remark

With regard to its physiological and ecological characteristics, the olive fly can be considered a prototypic case to discuss the implications of SPAGE application in an economically significant, European context. Furthermore, *B. oleae* is a suitable candidate for a potential target organism in suppression drives, as it already has been the object of pest control efforts for several decades. Compared to other cases, the narrow specialisation and the involved network of ecological relations make this case relatively easy to survey. It appears well suitable to expand risk assessment experience with a wild species with a high dispersal potential, high rates of gene flow and even some risk of hybridisation with related species. It is easy to study under laboratory conditions and reproduces quickly. This case study revealed that uncertainties exist with regard to the dispersal capacity of gene drive-bearing olive flies, as well as concerning the high gene flow between different populations and most importantly with regard to the population bottlenecks that regularly occur in winter. These would significantly increase or decrease genetic variability between subpopulations and thereby severely jeopardize the intended outcome of any SPAGE-application. Nevertheless, there remain significant knowledge gaps in particular on the role of relations to symbiotic and other microflora and wider aspects of the

ecological relations. However, experience gained in the study of this species can be useful to compare assessments of organisms with a more complex or more difficult to survey life cycles for which gene drive applications are discussed.

The Control of pest populations is one of the main objectives for possible SPAGE applications. The olive fly, as the main pest in olive cultivation poses a good example of a potential target organism in a European context. This case study revealed that uncertainties exist with regard to the dispersal capacity of gene drive-bearing olive flies, as well as concerning the high gene flow between different populations and most importantly with regard to the population bottlenecks that regularly occur in winter. These would significantly increase or decrease genetic variability between subpopulations and thereby severely jeopardize the intended outcome of any SPAGE-application. In summary, it can be said that the naturally occurring variability, in contrast to comparatively homogeneous laboratory conditions, leads to considerable and easily underestimated uncertainties about possible post-release effects. This is further exemplified in the stochastic model concerning winter bottlenecks and the individual based model, explored in Chap. 6. These models clearly show that even with a highly efficient gene drive, fluctuations in a single ecological factor may lead to vastly different outcomes.

References

Alford, D. V. (2014). *Pests of fruit crops: A colour handbook* (2nd Ed.). Boca Raton: CRC Press.

Ant, T., Koukidou, M., Rempoulakis, P., Gong, H.-F., Economopoulos, A., Vontas, J., & Alphey, L. (2012). Control of the olive fruit fly using genetics-enhanced sterile insect technique. *BMC Biology, 10*, 51. https://doi.org/10.1186/1741-7007-10-51.

Arambourg, Y., & Pralavorio, R. (1970). Survival of *Dacus oleae* Gmel. during the winter. *Annales de Zoologie Ecologie Animale, 2*, 659–622.

Augustinos, A. A., Mamuris, Z., Stratikopoulos, E., D'Amelio, S., Zacharopoulou, A., & Mathiopoulos, K. D. (2005). Microsatellite analysis of olive fly populations in the Mediterranean indicates a westward expansion of the species. *Genetica, 125*, 231–241. https://doi.org/10.1007/s10709-005-8692-y.

Bateman, M. A. (1972). The ecology of fruit flies. *Annual Review of Entomology, 17*, 493–518. https://doi.org/10.1146/annurev.en.17.010172.002425.

Benelli, G. (2014). Aggressive behavior and territoriality in the olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae): Role of residence and time of day. *Journal of Insect Behavior, 27*, 145–161.

Benelli, G., Bonsignori, G., Stefanini, C., Raspi, A., & Canale, A. (2013). The production of female sex pheromone in *Bactrocera oleae* (Rossi) young males does not influence their mating chances. *Entomological Science, 16*, 47–53. https://doi.org/10.1111/j.1479-8298.2012.00538.x.

Benelli, G., Canale, A., Bonsignori, G., Ragni, G., Stefanini, C., & Raspi, A. (2012). Male wing vibration in the mating behavior of the olive fruit fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae). *Journal of Insect Behavior, 25*, 590–603.

Benelli, G., Desneux, N., Romano, D., Conte, G., Messing, R. H., & Canale, A. (2015). Contest experience enhances aggressive behaviour in a fly: When losers learn to win. *Scientific Reports, 5*, 9347. https://doi.org/10.1038/srep09347.

Bento, A., Torres, L., Lopes, J., & Sismeiro, R. (1999). A contribution to the knowledge of *Bactrocera oleae* (GMEL) in Tras-os-Montes region (Northeastern Portugal): Phenology, losses and control. *Acta Horticulture, 474*, 541–544.

Ben-Yosef, M., Aharon, Y., Jurkevitch, E., & Yuval, B. (2010). Give us the tools and we will do the job: Symbiotic bacteria affect olive fly fitness in a diet-dependent fashion. *Proceedings of the Royal Society B: Biological Sciences, 277*, 1545–1552. https://doi.org/10.1098/rspb.2009.2102.

Ben-Yosef, M., Pasternak, Z., Jurkevitch, E., & Yuval, B. (2014). Symbiotic bacteria enable olive flies (*Bactrocera oleae*) to exploit intractable sources of nitrogen. *Journal of Evolutionary Biology, 27*, 2695–2705. https://doi.org/10.1111/jeb.12527.

Bigler, F., & Delucchi, V. (1981). Evaluation of the prepupal mortality of the olive fly, *Dacus oleae* Gmel. (Dipt., Tephritidae), on oleasters and olive trees in western Crete Greece. *Journal of Applied Entomology, 92*, 189–201.

Bigler, F., Neuenschwander, P., Delucchi, V., & Michelakis, S. (1986). Natural enemies of preimaginal stages of *Dacus oleae* Gmel. (Dipt., Tephritidae) in Western Crete. II. Impact on olive fly populations. *Bollettino del Laboratorio di Entomologia Agraria Filippo Silvestri, Portici, 43*, 79–96.

Boccaccio, L., & Petacchi, R. (2009). Landscape effects on the complex of *Bactrocera oleae* parasitoids and implications for conservation biological control. *BioControl, 54*, 607–616. https://doi.org/10.1007/s10526-009-9214-0.

Bon, M. C., Hoelmer, K. A., Pickett, C. H., Kirk, A. A., He, Y., Mahmood, R., & Daane, K. M. (2016). Populations of *Bactrocera oleae* (Diptera: Tephritidae) and its parasitoids in Himalayan Asia. *Annals of the Entomological Society of America, 109*, 81–91. https://doi.org/10.1093/aesa/sav114.

Bonizzoni, M., Gomulski, L. M., Malacrida, A. R., Capy, P., & Gasperi, G. (2007). Highly similar piggyBac transposase-like sequences from various Bactrocera (Diptera, Tephritidae) species. *Insect Molecular Biology, 16*, 645–650. https://doi.org/10.1111/j.1365-2583.2007.00756.x.

Broufas, G. D., Pappas, M. L., & Koveos, D. S. (2009). Effect of relative humidity on longevity, ovarian maturation, and egg production in the olive fruit fly (Diptera: Tephritidae). *Annals of the Entomological Society of America, 102*, 70–75. https://doi.org/10.1603/008.102.0107.

Broumas, T., Haniotakis, G., Liaropoulos, C., Tomazou, T., & Ragoussis, N. (2002). The efficacy of an improved form of the mass-trapping method, for the control of the olive fruit fly, *Bactrocera oleae* (Gmelin) (Dipt., Tephritidae): Pilot-scale feasibility studies. *Journal of Applied Entomology, 126*, 217–223. https://doi.org/10.1046/j.1439-0418.2002.00637.x.

Bueno, A. M., & Jones, O. T. (2002). Alternative methods for controlling the olive fly *Bactrocera olea* involving semiochemicals. *IOBC/WPRS Bulletin, 25*, 1–11.

Canale, A., Carpita, A., Conti, B., Canovai, R., & Raspi, A. (2012). Effect of age on 1,7-dioxaspiro-[5.5]-undecane production in both sexes of olive fruit fly, *Bactrocera oleae* (Diptera Tephritidae). *IOBC/WPRS Bulletin*, 219–225.

Capuzzo, C., Firrao, G., Mazzon, L., Squartini, A., & Girolami, V. (2005). "*Candidatus Erwinia dacicola*", a coevolved symbiotic bacterium of the olive fly *Bactrocera oleae* (Gmelin). *International Journal of Systematic and Evolutionary Microbiology, 55*, 1641–1647. https://doi.org/10.1099/ijs.0.63653-0.

Castrignanò, A., Boccaccio, L., Cohen, Y., Nestel, D., Kounatidis, I., Papadopoulos, N.T., et al. (2012). Spatio-temporal population dynamics and area-wide delineation of *Bactrocera oleae* monitoring zones using multi-variate geostatistics. *Precision Agriculture, 13*, 421–441. https://doi.org/10.1007/s11119-012-9259-4.

Christenson, L. D., & Foote, R. H. (1960). Biology of fruit flies. *Annual Review of Entomology, 5*, 171–192.

Comins, H. N., & Fletcher, B. S. (1988). Simulation of fruit fly population dynamics, with particular reference to the olive fruit fly. *Ecological Modelling, 40*, 213–231.

Copeland, R. S., White, I. M., Okumu, M., Machera, P., & Wharton, R. A. (2004). Insects associated with fruits of the Oleaceae (Asteridae, Lamiales) in Kenya, with special reference to the Tephritidae (Diptera). *Bishop Museum Bulletin in Entomology, 12*, 135–164.

Daane, K. M., & Johnson, M. W. (2010). Olive fruit fly: Managing an ancient pest in modern times. *Annual Review of Entomology, 55*, 151–169. https://doi.org/10.1146/annurev.ento.54.110807.090553.

Daane, K. M., Rice, R. E., Zalom, F. G., Barnett, W. W., & Johnson, M. W. (2005). Arthropod pests of olive. In G. S. Sibbett & L. Ferguson (Eds.), *Olive production manual* (pp. 105–114). University of California, Agriculture and Natural Resources.

Dimou, I., Koutsikopoulos, C., Economopoulos, A. P., & Lykakis, J. (2003). Depth of pupation of the wild olive fruit fly, *Bactrocera* (*Dacus*) *oleae* (Gmel.) (Dipt., Tephritidae), as affected by soil abiotic factors. *Journal of Applied Entomology, 127*, 12–17. https://doi.org/10.1046/j.1439-0418.2003.00686.x.

Drew, R. A., & Hancock, D. L. (2000). Phylogeny of the tribe Dacini (Dacinae) based on morphological, distributional, and biological data. In M. Aluja & A. L. Norrbom (Eds.), *Fruit flies (Tephritidae): Phylogeny and evolution of behaviour* (pp. 491–504). Boca Raton: CRC Press.

Economopoulos, A., & Stravropoulou-Delivoria, A. (1984). Yellow sticky rectangle with ammonium acetate slow-release dispenser: An efficient long-lasting trap for *Dacus oleae*. *Entomologia Hellenica, 2*, 17–23.

Economopoulos, A. P., Haniotakis, G. E., Mathioudis, J., & Missis, N. (1978). Long-distance flight of wild and artificially-reared *Dacus oleae* (Gmelin) (Diptera, Tephritidae). *Journal of Applied Entomology, 87*, 101–108.

Economopoulos, A. P., Haniotakis, G. E., Michelakis, S., Tsiropoulos, G. J., Zervas, G. A., Tsitsipis, J. A., et al. (1982). Population studies on the olive fruit fly, *Dacus oleae* (Gmel.) (Dipt., Tephritidae) in Western Crete. *Journal of Applied Entomology, 93*, 463–476. https://doi.org/10.1111/j.1439-0418.1982.tb03621.x.

Estes, A. M., Hearn, D. J., Bronstein, J. L., & Pierson, E. A. (2009). The olive fly endosymbiont, "*Candidatus Erwinia dacicola*", switches from an intracellular existence to an extracellular existence during host insect development. *Applied and Environment Microbiology, 75*, 7097–7106. https://doi.org/10.1128/AEM.00778-09.

Fletcher, B. S., & Economopoulos, A. P. (1976). Dispersal of normal and irradiated laboratory strains and wild strains of the olive fly *Dacus oleae* in an olive grove. *Entomologia Experimentalis et Applicata, 20*, 183–194.

Fletcher, B. S., & Kapatos, E. (1981). Dispersal of the olive fly, *Dacus oleae*, during the summer period on Corfu. *Entomologia Experimentalis et Applicata, 29*, 1–8.

Fletcher, B. S., & Kapatos, E. T. (1983). The influence of temperature, diet and olive fruits on the maturation rates of female olive flies at different times of the year. *Entomologia Experimentalis et Applicata, 33*, 244–252. https://doi.org/10.1111/j.1570-7458.1983.tb03264.x.

Fletcher, B. S., Pappas, S., & Kapatos, E. (1978). Changes in the ovaries of olive flies (*Dacus oleae* (Gmelin)) during the summer, and their relationship to temperature, humidity and fruit availability. *Ecological Entomology, 3*, 99–107. https://doi.org/10.1111/j.1365-2311.1978.tb00908.x.

Genç, H., & Nation, J. L. (2008a). Survival and development of Bactrocera oleae Gmelin (Diptera: Tephritidae) immature stages at four temperatures in the laboratory. *African Journal of Biotechnology, 7*, 2495–2500.

Genç, H., & Nation, J. L. (2008b). Maintaining *Bactrocera oleae* (Gmelin.) (Diptera: Tephritidae) colony on its natural host in the laboratory. *Journal of Pest Science, 81*, 167–174. https://doi.org/10.1007/s10340-008-0203-3.

Gonçalves, F. M., Rodrigues, M. C., Pereira, J. A., Thistlewood, H., & Torres, L. M. (2012). Natural mortality of immature stages of *Bactrocera oleae* (Diptera: Tephritidae) in traditional olive groves

from north-eastern Portugal. *Biocontrol Science and Technology, 22*, 837–854. https://doi.org/10.1080/09583157.2012.691959.

González, N., Trapero, A., & Vargas Osuna, E. (2006). Dalmatian disease of olive fruits, 1: Biology and damages in olive orchards of the Seville province [Spain]. *Boletin de Sanidad Vegetal Plagas*.

Gutierrez, A. P., Ponti, L., & Cossu, Q. A. (2009). Effects of climate warming on Olive and olive fly (*Bactrocera oleae* (Gmelin)) in California and Italy. *Climatic Change, 95*, 195–217. https://doi.org/10.1007/s10584-008-9528-4.

Haber, G., & Mifsud, D. (2007). Pests and diseases associated with olive trees in the Maltese Islands (Central Mediterranean). *The Central Mediterranean Naturalist, 4*, 143–161.

Hagen, K. S. (1966). Dependence of the olive fly, *Dacus oleae*, larvae on symbiosis with *Pseudomonas savastanoi* for the utilization of olive. *Nature, 209*, 143–146.

Harpaz, I., & Gerson, U. (1966). The "biocomplex" of the olive fruit fly (*Dacus oleae* Gmel.), the olive fruit midge (*Prolasioptera berlesiana* Paoli), and the fungus *Macrophoma dalmática* Berl. & Vogl. in olive fruits in the Mediterranean basin (pp. 81–126, pp. ref.31/2 pp).

Isaäcson, M. (1989). Airport malaria: A review. *Bulletin of the World Health Organization, 67*, 737–743.

Iwaizumi, R., Kaneda, M., & Iwahashi, O. (1997). Correlation of length of terminalia of males and females among nine species of *Bactrocera* (Diptera: Tephritidae) and differences among sympatric species of *B. dorsalis* complex. *Annals of the Entomological Society of America, 90*, 664–666.

Kalaitzaki, A., Perdikis, D., Marketaki, M., Gyftopoulos, N., & Paraskevopoulos, A. (2014). Natural enemy complex of *Bactrocera oleae* in organic and conventional olive groves. *IOBC/WPRS Bulletin, 108*, 61–68.

Kapatos, E. T., & Fletcher, B. S. (1984). The Phenology of the olive fly, *Dacus oleae* (Gmel.) (Diptera, Tephritidae), in Corfu. *Journal of Applied Entomology, 97*, 360–370. https://doi.org/10.1111/j.1439-0418.1984.tb03760.x.

Katsoyannos, B. I., & Kouloussis, N. A. (2001). Captures of the olive fruit fly *Bactrocera oleae* on spheres of different colours. *Entomologia Experimentalis et Applicata, 100*, 165–172. https://doi.org/10.1023/A:1019232623830.

Kokkari, A. I., Pliakou, O. D., Floros, G. D., Kouloussis, N. A., & Koveos, D. S. (2017). Effect of fruit volatiles and light intensity on the reproduction of *Bactrocera* (*Dacus*) *oleae*. *Journal of Applied Entomology*, 1–9. https://doi.org/10.1111/jen.12389.

Kounatidis, I., Crotti, E., Sapountzis, P., Sacchi, L., Rizzi, A., Chouaia, B., et al. (2009). *Acetobacter tropicalis* is a major symbiont of the olive fruit fly (*Bactrocera oleae*). *Applied and Environmental Microbiology, 75*, 3281–3288. https://doi.org/10.1128/AEM.02933-08.

Krimbas, C. B., & Tsakas, S. (1971). The genetics of *Dacus oleae*. V. Changes of esterase polymorphism in a natural population following insecticide control—selection or drift? *Evolution (N. Y), 25*, 454–460.

Liebhold, A. M., Work, T. T., McCullough, D. G., & Cavey, J. F. (2006). Airline baggage as a pathway for alien insect species invading the United States. *American Entomologist, 53*, 48–54. https://doi.org/10.1093/ae/52.1.48.

Marchi, S., Guidotti, D., Ricciolini, M., & Petacchi, R. (2016). Towards understanding temporal and spatial dynamics of *Bactrocera oleae* (Rossi) infestations using decade-long agrometeorological time series. *International Journal of Biometeorology, 60*, 1681–1694.

Marchini, D., Petacchi, R., & Marchi, S. (2017). *Bactrocera oleae* reproductive biology: New evidence on wintering wild populations in olive groves of Tuscany (Italy). *Bulletin of Insectology, 70*, 121–128.

Martin, J. H., Mifsud, D., & Rapisarda, C. (2000). The whiteflies (Hemiptera: Aleyrodidae) of Europe and the Mediterranean basin. *Bulletin of Entomological Research, 90*, 407–448.

Mazomenos, B. E. (1984). Effect of age and mating on pheromone production in the female olive fruit-fly, *Dacus oleae* (Gmel). *Journal of Insect Physiology, 30*, 765–769.

Mazomenos, B. E., Pantazi-Mazomenou, A., & Stefanou, D. (2002). Attract and kill of the olive fruit fly *Bactrocera oleae* in Greece as a part of an integrated control system. *IOBC/WPRS Bulletin, 25*.

McInnis, D. O., Rendon, P., Jang, E. B., van Sauers-Muller, A., Sugayama, R., & Malavasi, A. (1999). Interspecific mating of introduced, sterile *Bactrocera dorsalis* with wild *B. carambolae* (Diptera: Tephritidae) in Suriname: A potential case for cross-species sterile insect technique. *Annals of the Entomological Society of America, 92*, 758–765.

Moore, I. (1962). Further investigations on the artificial breeding of the olive fly—*Dacus oleae* GMEL.—under aseptic conditions (1). *Entomophaga, 7*, 53–57.

Nardi, F., Carapelli, A., Dallai, R., Roderick, G. K., & Frati, F. (2005). Population structure and colonization history of the olive fly, *Bactrocera oleae* (Diptera, Tephritidae). *Molecular Ecology, 14*, 2729–2738. https://doi.org/10.1111/j.1365-294X.2005.02610.x.

Neuenschwander, P., Bigler, F., Delucchi, V., & Michelakis, S. (1983). Natural enemies of preimmaginal stages of *Dacus oleae* Gmel. (Dipt., Tephritidae) in Western Crete. I. Bionomics and phenologies. *Bollettino del Laboratorio di Entomologia Agraria Filippo Silvestri, Portici, 40*, 3–32.

Neuenschwander, P., & Michelakis, S. (1979a). McPhail trap captures of *Dacus oleae* (Gmel.) (Diptera, Tephritidae) in comparison to the fly density and population composition as assessed by sondage technique in Crete, Greece. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft, 52*, 343–357.

Neuenschwander, P., & Michelakis, S. (1979b). Determination of the lower thermal thresholds and day-degree requirements for eggs and larvae of *Dacus oleae* (Gmel.) (Diptera: Tephritidae) under field conditions in Crete, Greece. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft*.

Neuenschwander, P., Michelakis, S., & Bigler, F. (1981). Abiotic factors affecting mortality of *Dacus oleae* larvae and pupae in the soil. *Entomologia Experimentalis et Applicata, 30*, 1–9. https://doi.org/10.1111/j.1570-7458.1981.tb03577.x.

Ochando, M. D., & Reyes, A. (2000). Genetic population structure in olive fly *Bactrocera oleae* (Gmelin): Gene flow and patterns of geographic differentiation. *Journal of Applied Entomology, 124*, 177–183. https://doi.org/10.1046/j.1439-0418.2000.00460.x.

Ordano, M., Engelhard, I., Rempoulakis, P., Nemny-Lavy, E., Blum, M., Yasin, S., et al. (2015). Olive fruit fly (*Bactrocera oleae*) population dynamics in the Eastern Mediterranean: Influence of exogenous uncertainty on a monophagous frugivorous Insect. *PLoS One, 10*, 1–18. https://doi.org/10.1371/journal.pone.0127798.

Orsini, M. (2006). Mortality and predation of olive fly (*Bactrocera oleae*) pupae on the soil in a Davis, California Olive Orchard (pp. 1–18).

Orsini, M., Daane, K. M., Sime, K. R., & Nelson, E. H. (2007). Mortality of olive fruit fly pupae in California. *Biocontrol Science and Technology, 17*, 797–807.

Ortega, M., & Pascual, S. (2014). Spatio-temporal analysis of the relationship between landscape structure and the olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae). *Agricultural and Forest Entomology, 16*, 14–23. https://doi.org/10.1111/afe.12030.

Pelekassis, C. E. D., Mourikos, P. A., & Bantzios, D. N. (1963). Preliminary studies of the field movement of the olive fruit fly (*Dacus oleae* Gmel.) by labelling a natural population with radioactive phosphorus (P32). In *Radiation and radioisotopes applied to insects of agricultural importance* (pp. 105–114).

Petacchi, R., Marchi, S., Federici, S., & Ragaglini, G. (2015). Large-scale simulation of temperature-dependent phenology in wintering populations of *Bactrocera oleae* (Rossi). *Journal of Applied Entomology, 139*, 496–509. https://doi.org/10.1111/jen.12189.

Picchi, M. S., Bocci, G., Petacchi, R., & Entling, M. H. (2016). Effects of local and landscape factors on spiders and olive fruit flies. *Agriculture, Ecosystems & Environment, 222*, 138–147. https://doi.org/10.1016/j.agee.2016.01.045.

Picchi, M. S., Marchi, S., Albertini, A., & Petacchi, R. (2017). Organic management of olive orchards increases the predation rate of overwintering pupae of *Bactrocera oleae* (Diptera: Tephritidae). *Biological Control, 108*, 9–15.

Pontikakos, C. M., Tsiligiridis, T. A., & Drougka, M. E. (2010). Location-aware system for olive fruit fly spray control. *Computers and Electronics in Agriculture, 70*, 355–368. https://doi.org/10.1016/j.compag.2009.07.013.

Rainwater, H. I. (1963). Agricultural insect pest hitchhikers on aircraft. *Proceedings of the Hawaiian Entomological Society, 18*, 303–310.

Rempoulakis, P., & Nestel, D. (2012). Dispersal ability of marked, irradiated olive fruit flies [*Bactrocera oleae* (Rossi) (Diptera: Tephritidae)] in arid regions. *Journal of Applied Entomology, 136*, 171–180. https://doi.org/10.1111/j.1439-0418.2011.01623.x.

Remund, U., Boller, E. F., Economopoulos, A. P., & Tsitsipis, J. A. (1976). Flight performance of *Dacus oleae* reared on olives and artificial diet. *Journal of Applied Entomology, 82*, 330–339. https://doi.org/10.1111/j.1439-0418.1976.tb03420.x.

Sacchetti, P., Granchietti, A., Landini, S., Viti, C., Giovannetti, L., & Belcari, A. (2008). Relationships between the olive fly and bacteria. *Journal of Applied Entomology, 132*, 682–689. https://doi.org/10.1111/j.1439-0418.2008.01334.x.

Sánchez-Ramos, I., Fernández, C. E., González-Núñez, M., & Pascual, S. (2013). Laboratory tests of insect growth regulators as bait sprays for the control of the olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae). *Pest Management Science, 69*, 520–526. https://doi.org/10.1002/ps.3403.

Schutze, M. K., Jessup, A., Ul-Haq, I., Vreysen, M., Wornoayporn, V., Vera, M., et al. (2013). Mating compatibility among four pest members of the *Bactrocera dorsalis* fruit fly species complex (Diptera: Tephritidae). *Journal of Economic Entomology, 106*, 695–707. https://doi.org/10.1603/EC12409.

Segura, M. D., Callejas, C., & Ochando, M. D. (2008). *Bactrocera oleae*: A single large population in Northern Mediterranean basin. *Journal of Applied Entomology, 132*, 706–713. https://doi.org/10.1111/j.1439-0418.2008.01366.x.

Sharaf, N. S. (1980). Life history of the olive fruit fly, *Dacus oleae* Gmel. (Diptera: Tephritidae), and its damage to olive fruits in Tripolitania. *Journal of Applied Entomology, 89*, 390–400.

Smith, P. T., Kambhampati, S., & Armstrong, K. A. (2003). Phylogenetic relationships among *Bactrocera* species (Diptera: Tephritidae) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution, 26*, 8–17.

Solinas, M. (1967). Osservazioni biologiche condotte in puglia sulla Prolasioptera berlesiana Paoli, con particolare rife-rimento al rapporti simbiotici col *Dacus oleae* Gmel. e con la Sphaeropsis dalmatica (Thüm.) gigante. *Entomologica, 3*, 129–176.

Solinas, M., & Nuzzaci, G. (1984). Functional anatomy of *Dacus oleae* Gmel. female genitalia in relation to insemination and fertilization process. *Entomologica, 19*, 135–165. https://doi.org/10.15162/0425-1016/584.

Speranza, S., Bellocchi, G., & Pucci, C. (2004). IPM trials on attract-and-kill mixtures against the olive fly *Bactrocera oleae* (Diptera Tephritidae). *Bulletin of Insectology, 57*, 111–115.

Tsiropoulos, G. J. (1972). Storage temperatures for eggs and pupae of the olive fruit fly. *Journal of Economic Entomology, 65*, 100–102. https://doi.org/10.1093/jee/65.1.100.

Tsiropoulos, G. J. (1977). Reproduction and survival of the adult *Dacus oleae* feeding on pollens and honeydews. *Environmental Entomology, 6*, 390–392.

Tsiropoulos, G. J. (1980). Major nutritional requirements of adult *Dacus oleae*. *Annals of the Entomological Society of America, 73*, 251–253. https://doi.org/10.1093/aesa/73.3.251.

Tsiropoulos, G. J. (1983). Microflora associated with wild and laboratory reared adult olive fruit flies, *Dacus oleae* (Gmel.). *Journal of Applied Entomology, 96*, 337–340. https://doi.org/10.1111/j.1439-0418.1983.tb03680.x.

Tsitsipis, J. A. (1977). Effect of constant temperatures on eggs of olive fruit fly, *Dacus oleae* (Diptera: Tephritidae). *Annales de Zoologie Ecologie Animale, 9*, 133–139.

Tsitsipis, J. A. (1980). Effect of constant temperatures on larval and pupal development of olive fruit flies reared on artificial diet. *Environmental Entomology, 9*, 764–768. https://doi.org/10.1093/ee/9.6.764.

Tsitsipis, J. A., & Abatzis, C. (1980). Relative humidity effects, at 20 °C, on eggs of the olive fruit fly, *Dacus oleae* (Diptera: Tephritidae), reared on artificial diet. *Entomologia Experimentalis et Applicata, 28*, 92–99.

Tzanakakis, M. E., & Koveos, D. S. (1986). Inhibition of ovarian maturation in the olive fruit fly, *Dacus oleae* (Diptera: Tephritidae), under long photophase and an increase of temperature. *Annals of the Entomological Society of America, 79*, 15–18.

Tzanakakis, M. E., Tsitsipis, J. A., & Economopoulos, A. P. (1968). Frequency of mating in females of the olive fruit fly under laboratory conditions. *Journal of Economic Entomology, 61*, 1309–1312. https://doi.org/10.1093/jee/61.5.1309.

Voulgaris, S., Stefanidakis, M., Floros, A., & Avlonitis, M. (2013). Stochastic modeling and simulation of olive fruit fly outbreaks. *Procedia Technology, 8*, 580–586. https://doi.org/10.1016/j.protcy.2013.11.083.

Wright, S. (1931). Evolution in Mendelian populations. *Genetics*, 97–159. https://doi.org/10.1007/BF02459575.

Yokoyama, V. Y., & Miller, G. T. (2004). Quarantine strategies for olive fruit fly (Diptera: Tephritidae): Low-temperature storage, brine, and host relations. *Journal of Economic Entomology, 97*, 1249–1253. https://doi.org/10.1603/0022-0493-97.4.1249.

Zervas, G. (1982). Reproductive physiology of *Dacus oleae* (GMELIN) Diptera Tephritidae. Comparison of wild and laboratory reared flies. In *Geoponika*.

Zhang, B., Liu, Y. Y. H., Wu, W. W. X., & Wang, Z. Z. L. (2010). Molecular phylogeny of Bactrocera species (Diptera: Tephritidae: Dacini) inferred from mitochondrial sequences of 16S rDNA and COI sequences. *Florida Entomology, 93*, 369–377. https://doi.org/10.1653/024.093.0308.

Zouros, E., & Krimbas, C. B. (1970). Frequency of female digamy in a natural population of the olive fruit fly *Dacus oleae* as found using enzyme polymorphism. *Entomologia Experimentalis et Applicata, 13*, 1–9. https://doi.org/10.1111/j.1570-7458.1970.tb00080.x.