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REVIEW

Bacterial symbiosis in *Bactrocera oleae*, an Achilles' heel for its pest control

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Abstract Investigations on microbial symbioses in Tephritidae have increased over the past 30 years owing to the potential use of these relationships in developing new control strategies for economically important fruit flies. *Bactrocera oleae* (Rossi)—the olive fruit fly—is a monophagous species strictly associated with the olive tree, and among all the tephritids, its symbionts are the most investigated. The bacterium *Candidatus* Erwinia dacicola is the major persistent resident endosymbiont in wild *B. oleae* populations. Its relationship with *B. oleae* has been investigated since being identified in 2005. This endosymbiont is vertically transmitted through generations from the female to the egg. It exists at every developmental stage, although it is more abundant in larvae and ovipositing females, and is necessary for both larvae and adults. Studying *B. oleae*–*Ca*. E. dacicola, or other *B. oleae*–microbe interactions, will allow us to develop modern biological control systems for area-wide olive protection and set an example for similar programs in other important food crops. This review summarizes the information available on tephritid–microbe interactions and investigates relationships among fruit flies, bacteria and host plants; however, its focus is on *B. oleae* and its strict association with *Ca*. E. dacicola to promote environmentally friendly control strategies for area-wide pest management.

Key words bacterial symbiosis; esophageal bulb; IPM; olive fly; Tephritid

Introduction

Relationships between true fruit flies (Diptera: Tephritidae) and microorganisms, in particular bacteria, have been studied for well over 100 years. Much is known about the biology and behavior of these flies; however, like many studies that address symbioses, challenges remain in fully defining microbial influences on fly hosts and vice versa. Interest in symbiotic interactions among bacteria and hosts, as well as the benefits that these associations bring to both, has increased from the beginning of the last century, as reviewed by Moran (2006) and Dale and Moran (2006). In particular, investigations on tephritid microbial symbioses have increased over the past 30 years owing to the potential use of these symbionts to develop new control strategies for economically important fruit flies, such as those in the genera *Bactrocera*, *Rhagoletis*, *Anastrepha* and *Ceratitis* (Lauzon, 2003; Behar *et al*., 2009; Noman *et al*., 2020; Raza *et al*., 2020). This review focuses on the important olive pest, *Bactrocera oleae* (Rossi), the olive fruit fly, a monophagous species that is strictly associated with the olive tree and the most investigated of all the tephritids in the area of symbioses, but it also includes some general information on tephritid–microbe interactions.

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[Correction added on 31 Dec 2021, after first online publication: The copyright line was changed.]

Pioneering research on symbiosis in B. oleae

Petri was the first scientist reported to study tephritid–bacterial interactions using *B. oleae* in the early 1900s,

and he has been credited with initiating the study of tephritid microbial symbioses. He described many "long-shaped bodies" (later determined to be bacterial masses) inside midguts of both larvae and adult flies, noting that as larvae molted, the bacteria were not lost and, in fact, seemed to increase. He also found these masses in a specialized foregut eversion that he described as a cephalic vesicle or pharyngeal gland. Later, researchers called this organ the esophageal bulb (Girolami, 1973; Drew & Lloyd, 1987; Stamopoulos & Tzanetakis, 1988; Capuzzo *et al*., 2005; Sacchetti *et al*., 2008). Petri isolated two bacterial species from larval guts, *Bacterium savastanoi* (*Pseudomonas savastanoi*) and *Ascobacterium luteum* (*Pantoea agglomerans*). Petri hypothesized that these bacteria were important in larval digestion, adult female egg production and male spermatogenesis (Petri, 1909); however, he never proved these roles. Interestingly, both of the bacterial species isolated by Petri have been implicated in olive knot disease, and *P. agglomerans* is a symbiont of tephritids in *Bactrocera*, *Rhagoletis, Anastrepha* and *Ceratitis* (Jang & Nishijima, 1990; Lauzon *et al*., 2000; Marchini *et al*., 2002; Robacker & Lauzon, 2002; Robacker *et al*., 2009). Approximately 70 years later, Yamvrias (1970) and Girolami (1973) cultured a variety of Gram-negative bacteria from olive flies. Yamvrias focused on the esophageal bulb and eggs of adults, and he cultured primarily *Pseudomonas* spp. and unidentified species within the families Enterobacteriaceae and Achromobacteriaceae. None of the pseudomonads were identified as *P. savastanoi*. Girolami carried out morphological and histological analyses of alimentary organs from several fruit fly species in the subfamilies Dacinae, Trypetinae and Tephritinae. His comparisons revealed structural differences among the esophageal bulbs and cephalic organs, as well as the bacterial contents in each. In Tephritinae species, the esophageal bulbs lacked microbial symbionts, while in the other pest fruit flies, they contained bacterial masses (Girolami, 1973).

As more researchers described the abundant presence of bacteria in the esophageal bulbs of olive fruit flies (e.g., Poinar *et al*., 1975), as well as bacterial masses in alimentary organs and, in some cases, on egg surfaces, attention turned to their potential roles in fruit fly biology. Luthy *et al*. (1983) incorrectly hypothesized that, owing to their similarity in cellular morphology, the bacterial inhabitants belonged to the same species. Furthermore, they speculated that in female flies, the bacteria provided essential factors that reached the egg and that bacteria from the esophageal bulb entered into the hemolymph and migrated to the ovaries, where they entered the eggs (Poinar *et al*., 1975). Mazzini and Vita investigated this hypothesis further and concluded that the vertical transmission most likely followed a gut-to-ovary route. As shown in previous studies, they found bacterial masses inside the esophageal bulb, as well as in the remaining part of the gut and in the last tract of the hindgut, which is common to both the alimentary canal and the reproductive system. They highlighted the presence of many bacterial masses inside the gut, and they hypothesized that these microorganisms were not damaged by gastric juices. Symbionts were more abundant in the midgut compared with in the last part of the digestive tract. However, they observed many bacterial masses (between 60 and 150) in every finger-like process (approximately 25) present in the last tract of the gut. Additionally, Mazzini and Vita noticed that these organs were blind and arranged in paired groups, with each group being joined to the last part of the anal tract inside the ovipositor. Finally, sagittal and transverse sections of laid eggs showed many bacterial colonies that were internalized inside the micropylar area, as well as on the external surface. Thus, they showed that bacteria passed from the female to the progeny through vertical transmission (Mazzini & Vita, 1981).

Other investigations focused on identifying the bacterial members in the Family Enterobacteriaceae that dominated the alimentary canal organs of *B. oleae*, such as *Erwinia herbicola*, *Klebsiella pneumoniae* and *Serratia marcescens*. Other Gram-negative bacteria, but nonfermenters, such as *Pseudomonas fluorescens* and *Xanthomonas campestris*, were also isolated frequently. Gram positive *Bacillus* spp., *Lactobacillus plantarum* and *Micrococcus luteus* were isolated as well (Tsiropoulos, 1983). Many of these bacterial species have also been isolated from other pest tephritids, such as *Bactrocera tryoni* (Drew & Lloyd, 1989), *Anastrepha ludens* (Robacker *et al*., 1998; Robacker & Lauzon, 2002), *Rhagoletis pomonella* (MacCollom *et al*., 2009) and *Ceratitis capitata* (Behar *et al*., 2009; Lauzon *et al*., 2009). Additionally, many of these bacterial species were found to reside on the olive phylloplane (Ercolani, 1978), and Tsiropoulos later described this commonality in terms of a host plant–microbe–fly interaction (Tsiropoulos, 1983).

Relationships among fruit flies, bacteria, and the host plants

Insects encounter numerous and diverse microorganisms in their environments. The impacts of microbial residence on tephritid host plants have been notably described by Drew and Lloyd for subtropical and tropical members of the Dacinae (Drew & Lloyd, 1987). Briefly,

they found that enteric bacteria that typically inhabit the alimentary canals of adult Dacines are also found on host plant structures, but their presence on the latter may be influenced by the occurrence of foraging fruit flies (Drew & Lloyd, 1987). Volatiles released by the plant and by its resident microorganisms likely influence the chemical ecology of the host plant, and these odors attract adult flies to food and a reproductive site. Tephritids are attracted to microbial-produced odors (Drew & Lloyd, 1987; MacCollom *et al*., 1994; Robacker *et al*., 1998; Robacker *et al*., 2009; Liscia *et al*., 2013), and in one report, bacteria that were attractive to *Anastrepha suspensa* produced 3-methyl-1-butanol, a known fruit odor (Epsky *et al*., 1998).

Stamopoulos and Tzanetakis cultured 28 strains of bacteria from *B. oleae* and found that microbial population was dominated by Gram-positive bacteria (22/28 isolates) and not Gram-negative bacteria (Stamopoulos & Tzanetakis, 1988) as reported previously and presently. They concluded that *B. oleae* collected these bacteria, which are common to soil and dust, incidentally. They also stated that these microorganisms may serve as a food source or suppliers of substances, or both, useful for insect survival. Ercolani sought to define more completely the role of phylloplane microorganisms on *B. oleae* biology (Ercolani, 1991). The aim of the work was to evaluate the chronological distribution of several types of bacteria on olive leaf surfaces. He examined leaves of different ages at different times of the year. Many of the bacterial species that were isolated from leaves were similar to those retrieved from *B. oleae* esophageal bulbs: *Bacillus*, *Erwinia*, *Acetobacter*, *Escherichia*, *Klebsiella*, *Pseudomonas*, *Serratia* and *Xanthomonas*, to name a few (Ercolani, 1991). Others expanded on this work by examining twigs and olives, and they reported that *B. oleae* density was positively correlated with bacterial load on olives. The authors speculated that *B. oleae* influences the microbial ecology of host plant structures, being also responsible for the bacterial spread on the olive phylloplane (Granchietti *et al*., 2007; Sacchetti *et al*., 2008).

Candidatus *Erwinia dacicola: an important symbiont of* B. oleae

In 2005, a novel bacterial species was identified as a symbiont of *B. oleae*. On the basis of the 16s RNA gene and phylogenetics, the symbiont was putatively named *Candidatus* Erwinia dacicola (Capuzzo *et al*., 2005). Nucleotide sequencing of the entire 16s RNA gene consistently yielded a single sequence that showed marked similarity with enterobacterial lineages, including 97% matches with *Erwinia persicina* and *Erwinia rhapontici* (Savio *et al*., 2012). Analyses were carried out on dissected esophageal bulbs and midguts, and this bacterium dominated these samples. Attempts to culture this bacterium on standard nutrient media failed, and thus, the bacterium was defined as an unculturable bacterial species (Capuzzo *et al*., 2005). Molecular techniques have thus expanded what was currently known about microbial symbionts in *B. oleae*. The presence of this bacterium was later confirmed, and another bacterial species was also identified as *Asaia* sp. (Sacchetti *et al*., 2008).

In addition to *Ca*. E. dacicola, other culturable bacteria have been cultured from the esophageal bulbs of *B. oleae* (Tsiropoulos, 1983; Belcari *et al*., 2003). It is unclear whether any or all of these bacteria contribute to *B. oleae* physiology; some could be transient in nature. While different bacteria have been found in alimentary canal organs (and are discussed later in this review), most have not been described to remain in *B. oleae* through all its life stages. It is possible that they enter a viable but nonculturable state in a particular life stage, which would complicate the symbiotic story. Molecular means for determining the microbial presence assist greatly in bacterial detection, but the presence of DNA does not necessarily mean the presence of a viable bacterium. Another complication along a similar line of thought is that in *B. oleae*, *Ca*. E dacicola seems to switch from an intracellular existence to an extracellular one during larval to adult development. Estes *et al*. (2012a) suggested that this transition allows for bacterial survival and their continued presence within all life stages of the insect. This phenomenon is interesting because in the related tephritid, *Ceratitis capitata*, vertically transmitted symbionts were shown be to extracellular (Robacker *et al*., 2009) and culturable, and this intracellular life of *Ca*. E. dacicola may reflect some early strategy of symbionts that existed in other tephritids long ago. The mechanism of *Ca*. E. dacicola's survival during *B. oleae* metamorphosis remains unclear; however, Estes *et al*. (2009) speculated that bacterial cells present in regenerative cells may recolonize the adult gut.

If Estes *et al*. are correct then this would help to explain why newly eclosed wild *B. oleae* harbor a few bacterial cells and why *B. oleae* factory flies lack *Ca*. E. dacicola. It would also substantiate, in part, that the bacteria switch to an intracellular state to escape threats to their survival. When antibiotics are added to wild captured *B. oleae* and they are then reared in the laboratory, *Ca*. E. dacicola is eliminated. In fact, *Ca*. E. dacicola has never been detected in *B. oleae* reared on an artificial diet, while *B. oleae* laboratory colonies are usually associated with several bacteria, which are commonly

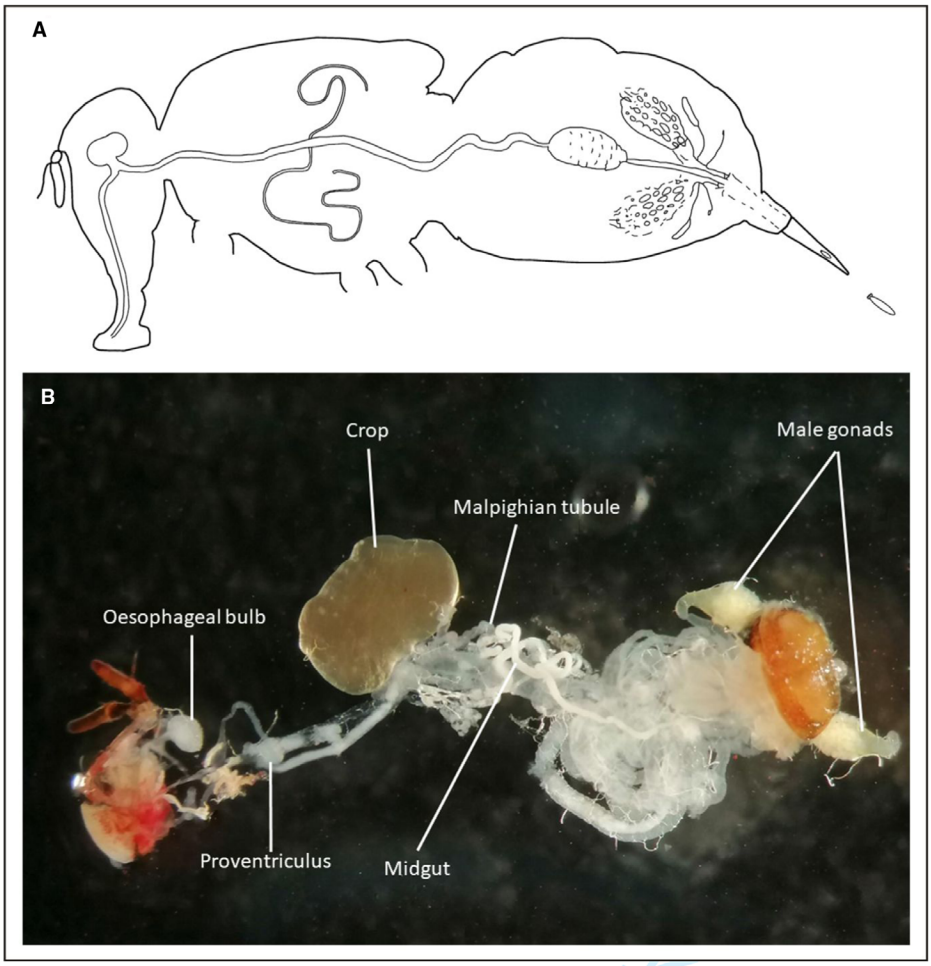

Fig. 1 (A) Schematic illustration of *Ca*. E. dacicola path in the gut apparatus of an olive fly female. Esophageal bulb, Malpighian tubules and ovaries are drawn. (B) Light microscopy micrograph showing the internal gut apparatus of a *B. oleae* male. The picture highlights the milky esophageal bulb, filled with bacterial masses. Other organs are arrowed.

found in laboratory-reared insects (Rempoulakis *et al*., 2014; Augustinos *et al*., 2019). Thus, *Ca*. E. dacicola may be a persistent, resident endosymbiont that is vertically transmitted through generations from the female to the egg. It has been found in every fly developmental stage but is more abundant in larvae and in ovipositing females. A drawing depicting the vertical transmission of the endosymbiont, as well as the adult organs known to be involved in the symbiosis, is displayed in Figure 1.

Savio *et al*. (2012) surveyed over 300 esophageal bulbs from *B. oleae* captured in 26 different olive-producing areas in Italy over a 3-year period. They provided evidence for the existence of two "lineages" or "haplotypes," called htA and htB. The frequencies of htA and htB differed and were related to the season and geographical location, except for two island populations. Sardinian fly populations harbored htA and Sicilian fly populations harbored htB. They subsequently attempted to determine if fly haplotype was correlated with bacterial haplotype. They found that 16 different fly haplotypes existed with no apparent correlation between the symbiont and host fly lineages.

The genetic variation of *Ca*. E. dacicola necessitates determining the best means of detecting its presence in *B. oleae*. Varying results using different primer sets (Estes *et al*., 2009) and approaches, such as standard PCR, DGGE or ARDRA, or both, have been achieved. Further analyses of the genome of *Ca*. E. dacicola will likely result in designing improved primer sets. More recently, draft whole-genome sequences have been reported for *Ca*. E. dacicola (Blow *et al*., 2017; Estes *et al*., 2018b) that revealed the closeness of this bacterium to an *Enterobacter* sp., which is commonly isolated from a variety of pest tephritids (Estes *et al*.,

2018a). Comparative genomic analyses have resulted in a suggested name change for some *Ca*. E. dacicola isolates to *E. dacicola* Oroville (Estes *et al*., 2018b); however, this change has not been adopted at present. The change reflects the possibility that other bacteria are important in the life history of *B. oleae* as suggested by Koundatidis *et al*. (2009) after finding *Acetobacter tropicalis*, as well as *Ca*. E. dacicola, in larval, pupal, and adult stages of both wild and laboratory-reared populations. A variety of bacterial species have since been found in association with *B. oleae* and are members of the following genera: *Klebsiella*, *Pluralibacter*, *Providencia*, *Pseudocitrobacter*, *Stenotrophomonas*, *Deinococcus*, *Enterococcus*, and *Streptococcus* (Koskinioti *et al*., 2019). Bigiotti *et al*. (2019b) found the specific bacterial species, *Ewingella americana*, *Rosenbergiella collisarenosi*, *Erwininia aphidicola*, *Enterobacter muelleri*, *S. marcescens*, *Rahnella woolbedingensis*, *Morganella morganii*, *Cedecea lapagei*, and *Acinetobacter septicus*. They also found a *Lactococcus* sp. and an *Acidobacter* sp. The roles, if any, for these bacteria in *B. oleae* remain undetermined but may involve any number of important biological processes. An up-dated list of bacteria detected using culture-independent methods is provided in Table 1.

The role of Ca. E. dacicola

The elucidation of the role of *Ca*. E. dacicola began in 1966 when Hagen added the antibiotic streptomycin to the adult *B. oleae* diet and found that their 1st instar larvae did not survive when reared on olives (Hagen, 1966). Additional work by Hagen showed that the antibiotic hampered protein hydrolysis in the larvae, and thus, he speculated that the symbiont's role was to assist in protein hydrolysis. The symbiont was referred to as *P. savastanoi* owing to Petri's work in the early 1900s, but no bacterium was identified. Some years later, Tsiropoulos (1985) conducted experiments on the relevance of dietary nitrogen and vitamins for *B. oleae* using fecundity, fertility and survival as metrics. Female *B. oleae* that fed on a diet enriched with nitrogen and vitamins produced more eggs that those that fed on diets lacking these components, and pyrodoxin (vitamin B6) was found to be particularly important for amino acid synthesis in adult *B. oleae*. It was then assumed that flies obtain vitamins during larval development through their associated microbiota (Tsiropoulos, 1983; 1985). The assumption was further tested, and the data showed more strongly, yet not definitively, that *Ca*. E. dacicola was involved in amino acid synthesis and sustaining egg production (Ben-Yosef *et al*., 2010). In that work, they found that female *B. oleae* that contained *Ca*. E. dacicola produced eggs even when provided a diet lacking essential amino acids, while females lacking the symbiont that fed on the same diet did not produce eggs (Ben-Yosef *et al*., 2010). The role of *Ca*. E. dacicola in nitrogen assimilation was later confirmed (Ben-Yosef *et al*., 2014).

While more solid data regarding the dietary contribution of *Ca*. E. dacicola are emerging, recent studies suggest that the symbiont may contribute to larval survival in unripe olives (Ben-Yosef *et al*., 2015). Oleuropein is a secoiridoid, a phenolic glycoside and a known allelocompound of olives (Omar, 2010). The presence and concentration of oleuropein decrease during ripening, with higher levels in green olives and lower levels during maturation to black olives (Omar, 2010). Ben-Yosef *et al*. (2015) showed that *B. oleae* larvae without *Ca*. E. dacicola did not develop to completion on unripe olives, but could do on ripe olives. The symbiont expresses genes to support its own detoxification of oleuropein (Pavlidi *et al*., 2017; Estes *et al*., 2018a), but it is not known whether oleuropein is toxic to *B. oleae*. Regardless, the symbiont affords some benefit to the developing larvae.

Applied management of bacterial symbiosis and conclusions

Insect symbiosis' potential manipulation has been reviewed by several authors (Zindel *et al*., 2011; Ras *et al*., 2017; Noman *et al*., 2020; Raza *et al*., 2020). Nobre (2019) presented a comprehensive review that addressed insect pest–symbiont relationships and their potential use in pest management strategies. The *B. oleae–Ca*. E. dacicola relationship was highlighted owing to the present pressing need to protect olives, an undeniably important economic crop. Clearly, we are closer to understanding the roles of *Ca*. E. dacicola in the life history of *B. oleae*, but more research is needed to refine symbiont-based strategies for efficient pest control. This includes the development of new attractants and pesticides that aim to disrupt symbioses, as well as the use of probiotics in rearing systems that aim to promote symbioses. Epiphytic bacteria emit volatiles that act as attractants, enabling *B. oleae* to locate the host plant, representing a food source (Scarpati *et al*., 1993). Thus, *B. oleae* follows bacterial volatile compounds that act as natural attractants. This was assumed and then confirmed in more recent years by laboratory observations of *B. oleae*'s behavioral responses to volatiles emitted by *Pseudomonas putida*, a commonly associated epiphytic bacterium. A *P. putida* bacterial filtrate acted as a good attractant of adult *B. oleae* (Landini *et al*., 2007; Sacchetti *et al*., 2007). Later,

Table 1 Bacterial genera and species retrieved in different *B. oleae* stages and organs. Bacteria founded both in association with the olive fly and the olive phylloplane are also cited.

Bacterial species or genus	*B. oleae* life stage	*B. oleae* organ	Olive tree	References[†]
Acetobacter tropicalis	Adults, larvae, pupae			14
Acidibacter spp.		Esophageal bulb		16
Acinetobacter spp.		Gut		15
Acinetobacter septicus		Esophageal bulb		16
Alcaligenes spp.		Esophageal bulb		7
Agrobacterium luteum		Gut		2
Asaia spp.		Esophageal bulb, gut		12
Ascobacterium luteum		Esophageal bulb		1
Bacillus sp.	Pupae			11
Bacillus subtilis	Pupae	Esophageal bulb	Phylloplane	6, 7, 8, 11
Bacillus licheniformis		Esophageal bulb		7
Bacillus megaterium	Pupae	Esophageal bulb	Phylloplane	6, 8, 11
Bacillus pumilus		Esophageal bulb		7
Bacillus cereus	Pupae			11
Bacillus thuringiensis	Pupae			11
Brevundimonas vescicularis		Esophageal bulb		9
Brucella spp.		Esophageal bulb		9
Ca. E. dacicola	Adults, larvae, pupae	Esophageal bulb, gut, crop, rectal sacs, ovipositor, larval midgut		10, 13, 14, 16
Cedecea lapagei		Esophageal bulb		16
Citrobacter freundii		Esophageal bulb		6
Deinococcus spp.		Gut		15
Enterobacter spp.		Esophageal bulb		3, 6, 13
Enterobacter cloacae		Esophageal bulb		7
Enterobacter muelleri		Esophageal bulb		16
Enterococcus spp.		Gut		15
Enterococcus faecalis	Adults, larvae, pupae			14
Erwinia aphidicola		Esophageal bulb		16
Erwinia herbicola		Esophageal bulb		6, 8
Ewingella americana		Esophageal bulb		16
Geobacillus spp.		Gut		15
Hafnia alvei		Esophageal bulb		6
Klebsiella pneumoniae		Esophageal bulb		6
Kokuria rosae		Esophageal bulb		9
Kurthia spp.		Esophageal bulb		7
Lactobacillus plantarum		Esophageal bulb	Phylloplane	6, 8
Lactococcus spp.		Esophageal bulb		16
Micrococcus roseus		Esophageal bulb		7
Micrococcus luteus		Esophageal bulb	Phylloplane	6, 8
Meiothermus spp.		Gut		15
Moraxella nonliquefaciens		Esophageal bulb		7
Morganella morganii		Esophageal bulb		9, 16
Paenibacillus glucanolyticus	Adults, larvae, pupae			14
Pasteurella sp.		Esophageal bulb		9
Pluralibacter spp.		Gut		15

(to be continued)

Table 1 Continue.

Bacterial species or genus	*B. oleae* life stage	*B. oleae* organ	Olive tree	References[†]
Proteus mirabilis		Esophageal bulb		6
Providencia sp.	Pupae	Gut		11, 15
Providencia alcafaciens	Pupae			11
Providencia stuartii	Pupae	Esophageal bulb		6, 11
Providencia rettgeri	Pupae			11
Pseudocitrobacter		Gut		15
Pseudomonas sp.	Pupae	Esophageal bulb, gut		3, 6, 7, 11, 15
Pseudomonas aeruginosa		Esophageal bulb	Phylloplane	6, 8
Pseudomonas mendocina		Esophageal bulb		7
Pseudomonas fluorescens	Pupae	Esophageal bulb	Phylloplane	6, 9, 11, 8
Pseudomonas putida		Esophageal bulb	Phylloplane	6, 9
Pseudomonas savastanoi		Esophageal bulb	Phylloplane	1, 2, 9
Rahnella woolbedingensis		Esophageal bulb		16
Rosenbergiella collisarenosi		Esophageal bulb		16
Serratia marcescens		Esophageal bulb	Phylloplane	6, 8, 9, 16
Shigella sp.		Esophageal bulb		9
Sphyngobacterium multivorum		Esophageal bulb		9
Staphylococcus sp.		Esophageal bulb		7
Stenotrophomonas spp.		Gut		15
Streptococcus spp.		Gut		15
Vulcaniibacterium spp.		Gut		15
Xanthomonas campestris		Esophageal bulb	Phylloplane	6, 8

[†]Reference number cited in the table corresponds to the following authors: 1. Petri (1909); 2. Hellmuth (1956); 3. Yamvrias *et al*. (1970); 4. Girolami (1973); 5. Ercolani (1978); 6. Tsiropoulos (1983); 7. Stamopoulos and Tzanekakis (1988); 8. Ercolani (1991); 9. Belcari *et al*. (2003); 10. Capuzzo *et al*. (2005); 11. Rempoulakis *et al*. (2014); 12. Sacchetti *et al*. (2008); 13. Estes *et al*. (2009); 14. Kounatidis *et al*. (2009); 15. Koskinioti *et al*. (2019); 16. Bigiotti *et al*. (2019b).

morphological, electrophysiological and behavioral investigations demonstrated that *B. oleae* antennal and palpal sensilla were responsive to bacterial filtrate odors, proving that adults are influenced by bacterial volatiles (Liscia *et al*., 2013). The abilities of compounds that act as symbionticides, including copper products, to interrupt bacterial symbiosis have been investigated. Tzanakakis was one of the first scientists to investigate the possibility of indirectly controlling *B. oleae* using symbionticides. He tested the effects of antibiotics on larval growth both in the laboratory and in field trials as well as the possibility of spraying copper fungicides in the field (Tzanakakis & Stavrinides, 1973; Tzanakakis & Lambrou, 1975; Tzanakakis, 1985). The efficacies of copper-based products (such as Bordeaux mixture, copper hydroxide and oxychloride) to control *B. oleae* populations in several field trials in different Mediterranean countries were evaluated, providing more evidence that copper could play an important role as a symbionticide (Belcari & Bobbio, 1999; Belcari *et al*., 2005; Caleca & Rizzo, 2007; Caleca *et al*., 2010; Caleca *et al*., 2012; Rosi *et al*., 2007; Gonçalves & Torres, 2012) not just as a repellent (Prophetou-Athanasiadou *et al*., 1991). This hypothesis was ultimately proven through laboratory investigations, in which copper hydroxide significantly reduced the symbiont load in adult *B. oleae* (Bigiotti *et al*., 2019a). In the same study, the symbionticide effect of propolis, to a less extent, was also proven, opening new avenues for sustainable *B. oleae* control. Very recently, it was shown that both copper oxychloride and a fungal metabolite produced by *Trychoderma* sp. are active against the symbionts in adult *B. oleae* (Sinno *et al*., 2020). As our knowledge of the microbial ecology of *B. oleae* increases, the establishment of efficient biological and biotechnological control strategies against *B. oleae* becomes more likely. Additionally, Sterile Insect Techniques need improvement before they can be applied to control this species' population, and laboratory rearing techniques need to be optimized (Ben-Ami, 2010; Gavriel *et al*., 2011; Estes *et al*., 2012b). Understanding the symbiotic relationships of *B. oleae* will aid in the mass rearing of

this species. Enterobacteriaceae bacteria have been used in probiotic diets of different fruit flies to improve mass-reared insect quality (Augustinos *et al*., 2015; Kyritsis *et al*., 2017; Yao *et al*., 2017), restore fitness to irradiated fruit flies (Niyazi *et al*., 2004; Hamden *et al*., 2013; Cai *et al*., 2018) and improve the rearing process, as highlighted by *P. putida* in *B. oleae* (Sacchetti *et al*., 2014). Increasing both juvenile instar and adult fitness levels are a primary goals in adult mass rearing for sterilization.

The potential to improve lures through the addition of novel bacteria-produced chemicals remains great. Thus, additional research should focus on chemical compounds that are characterized in bacterial filtrate profiles and that show attractive effects during laboratory and field trials. The discovery of new powerful attractants will enhance modern biological control strategies in olive systems. Further studies on the changes that occur in the gut microbiota's composition after sterilization are needed, because irradiation may affect the presence of the bacteria that positively influence insect fitness, as recently demonstrated in the oriental fruit fly *Bactrocera dorsalis* (Stathopoulou *et al*., 2019). Additionally, the use of products having antimicrobial activities should be avoided in the *B. oleae* rearing process; indeed, common disinfectants and antimicrobials used in egg collection strongly affect the endosymbiont transmission from the mother to the progeny (Sacchetti *et al*., 2019). Of course, additional studies to fully understand the roles of *Ca*. E. dacicola or other bacterial species, or both, harbored in the guts of *B. oleae* adults, as well in the gastric caeca cells in young larvae, are still needed to develop new tools against this pest fly owing to the effective roles played by microbiota in both insect physiology and behavior (Dillon & Dillon, 2004; Yuval, 2017; Jose *et al*., 2019; Hosokawa & Fukatsu, 2020).

By studying *B. oleae–Ca*. E. dacicola or other *B. oleae*–microbe interactions, we acquire new knowledge that will aid in developing modern biological control systems for area-wide olive production and set an example for such programs in other important food crops.

Disclosure

The authors have declared that no competing interest exists.

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