Host Response to Biotic Stresses

6

Giandomenico Corrado, Antonio Garonna, Carmen Gómez-Lama Cabanás, Maria Gregoriou, Giovanni P. Martelli, Kostas D. Mathiopoulos, Jesús Mercado-Blanco, Maria Saponari, Konstantina T. Tsoumani and Rosa Rao

Abstract

In olive, several biological processes, including those related to drupe maturation and oil production, are adversely affected by biotic stress. Pesticides are an important, valuable input in modern oliviculture, still central to secure yield and safeguard olive oil quality. However, concerns over the effects of plant protection products on the environment, non-target organisms, and human health prompt the development and implementation of more integrated control strategies. Functional genomics for biotic stress tolerance is a promising area that needs to be explored to increase olive tolerance and reduce reliance on pesticides. A number of studies have recently described, on a genome-wide scale, the participation of genes in olive response to different biotic stresses. Moreover, genes involved in stress tolerance and related signaling networks have been also identified. This chapter presents recent advances in olive molecular response to its major biotic stresses (insects, fungi, bacteria, and viruses). This topic is presented in a larger context that includes the main biological features of the major olive biotic stressors.

G. Corrado · A. Garonna · R. Rao (✉)
Dipartimento di Agraria, Università di Napoli Federico II, Via Università 100, 80055 Portici, Naples, Italy
e-mail: rao@unina.it

C.G.-L. Cabanás · J. Mercado-Blanco
Department of Crop Protection, Institute for Sustainable Agriculture, Agencia Estatal Consejo Superior de Investigaciones Científicas (CSIC), Campus Alameda del Obispo, Avda. Menéndez Pidal, s/n, 14004 Córdoba, Spain

M. Gregoriou · K.D. Mathiopoulos · K.T. Tsoumani
Department of Biochemistry and Biotechnology, University of Thessaly, Larissa 41221, Greece

G.P. Martelli · M. Saponari
Istituto per la Protezione Sostenibile delle Piante, UOS Bari, Consiglio Nazionale delle Ricerche, Bari, Italy

E. Rugini et al. (eds.), *The Olive Tree Genome*, Compendium of Plant Genomes,
DOI 10.1007/978-3-319-48887-5_6

1 Introduction

The olive tree is inextricably linked to every aspect of human life, especially for Mediterranean people. In Greek mythology, the olive was a gift from Athena to the people of Attica, who named their capital city after her. For all religions in the area, Judaism, Christianity, and Islam included, olive oil was revered as the light that illuminated the darkness of temples and houses. Olive trees have always been a source of heat, food, and medicinal compounds.

Today, olive oil is recognized as one the most typical elements of the Mediterranean diet. Biotic stress is an important determining factor of olive oil quality. Many stresses exert a direct and indirect effect on a number of olive parameters, which is assumed to be mainly detrimental. However, it is likely that the interaction between the olive and its biotic stressors can shape the compositional parameters of the drupes in a much more complex way, contributing with both positive and negative reinforcement of features that are under a complex genetic control (Atkinson et al. 2011). As biotic stress can be considered unavoidable in olive, understanding the complex molecular response to stress is important to develop suitable strategies that minimize impact on yield and maximize the amount of compounds that improve olive oil quality. To this aim, "omics" studies based on large-scale and high-throughput methods provide previously inaccessible information on several aspects of plant biology, including the interaction between plants and their enemies. The ever-increasing speed, throughput, and affordability of next-generation sequencing (NGS) approaches have revolutionized the way we can study biological interactions, allowing a level of resolution and depth that was unreachable by earlier tools. However, the full potential of current technologies can be unleashed in the presence of good reference genomes. Many issues have made very difficult the sequencing of the olive genome (Muleo et al. 2012) and a first draft of the genome of the cv. Farga was recently released (Cruz et al. 2016). The genome of the wild olive (*Olea europaea* var. *sylvestris*) was also sequenced and assembled, by the IOGC International Consortium (Unver et al. 2016).

The application of genomic technologies to study olive stress response has been limited by the lack of adequate genomic information. For this reason, as in many plant species, early studies in olive focused on the gene expression analysis of selected genes (Botella et al. 2005; Giannoulia et al. 2007). More recently, efforts were produced to identify, at a larger scale, genes involved in the response to stresses and to describe the network of the multiple signaling pathways involved in olive resistance (Corrado et al. 2012; Gómez-Lama Cabanás et al. 2014, 2015; Leyva-Pérez et al. 2015; Alagna et al. 2016). As many proteins involved in plant resistance against biotic stress are direct gene products, the majority of studies in olive focused on the transcriptome, with particular emphasis on the polyadenylated coding RNAs. Although not all genes induced or repressed in response to a biotic stress necessarily have a direct effect on pest or pathogen performance, studies of responsive genes can yield suitable candidates for further functional investigation. In addition, transcriptomics studies have highlighted the main signaling pathways and metabolic routes activated in response to stress.

The identification of stress-related proteins and secondary metabolites using proteomics and metabolomics are probably the most employed complementary approaches to transcriptome-based gene discovery, although not many studies were performed in this area. Only recently, analysis of some components of the metabolome following biotic stress has been carried out in drupes (Alagna et al. 2016).

This chapter includes an overview of the major biotic stressors of the olive and provides examples of the use of genomics to understand the molecular basis of olive response. This chapter also includes examples of the discovery of genes associated with or involved in olive resistance. This information paves the way for

the development of new integrated strategies to increase stress resistance and for the molecular improvement of the olive tolerance to specific antagonists.

2 The Major Entomological Enemy: The Olive Fly

Bactrocera oleae (Rossi) (Diptera: Tephritidae), the olive fly (OLF), is a strictly monophagous insect pest infesting the fruits of cultivated and wild *O. europaea*. The female fly lays eggs inside ripe and unripe fruits. Hatching larvae feed on the olive pulp, boring galleries inside the fruit mesocarp. OLF infestation makes table olives unmarketable and deteriorates the quality of olive oil (Gucci et al. 2012). Given this tight relationship, the expansion of the OLF is exclusively restricted to the cultivation zone of the olive tree. Population analysis of OLF from different parts of the world showed three separate genetic groups, Pakistan, Africa, and the Mediterranean (Nardi et al. 2005), with Africa considered the likely center of origin. The OLF Mediterranean group was further divided into three genetic groups (Western, Central, and Eastern Mediterranean groups) (Augustinos et al. 2005; Zygouridis et al. 2009). The gradual decrease of fly variability from the Middle East to the Iberian Peninsula indicated a westward expansion of the species, most likely associated with the expansion of the olive cultivation in the Mediterranean (van Asch et al. 2012). A similar East-to-West pattern of expansion was observed in samples from Turkey (Dogaç et al. 2013). Nardi et al. (2010) suggested that most of the evolutionary history of OLF preceded the domestication of cultivated olives and took place on wild olives. In recent years, OLF has also invaded California (Rice 2000; Rice et al. 2003). Genetic analyses of the invasion pointed at an Eastern Mediterranean origin of the flies (Zygouridis et al. 2009).

B. oleae overwinters as adult, as larva in the fruit or as pupa in the soil. The fly is best adapted to develop in the autumn period: A lack of ovarian maturation during late spring and early to mid-summer can be observed (Fletcher et al. 1978; Tzanakakis 2003). The reproductive dormancy ends when suitable fruits become available, usually starting from mid or late summer (Tzanakakis and Koveos 1986). Drupes and temperature determine the number of generations that can be completed before the natural reproduction stop during winter. The number of OLF generations per year varies according to different factors: geographical region, agronomic and climatic conditions, olive canopy microclimate, availability, and quality of the fruits (Gutierrez et al. 2009; Malheiro et al. 2015a). Fruits become susceptible to OLF when the endocarp begins to harden, usually during summer. A single female of *B. oleae* can lay about 10–20 eggs a day and between 200 and 500 eggs in a lifetime (Burrack and Zalom 2008; Burrack et al. 2011). Typically, one egg is laid in an olive, allowing the larvae a direct access to food just after emergence. Once ready for pupae formation, larvae open an exit hole in the olive epicarp and either escape from the fruit to pupate in the soil or pupates inside the fruit and open an exit hole for the adult (Fig. 1). The physiological time scale on which *B. oleae* evolves (from egg to adult), expressed and approximated as cumulated degree-day (CDD), is 379.015 (Crovetti et al. 1982).

2.1 Cultivar Susceptibility to Olive Fly

Different levels of susceptibility are present in olive cultivars (Iannotta et al. 1999, 2006a, b, 2007; Iannotta and Scaliercio 2012; de Alfonso et al. 2014). Malheiro et al. (2015a) summarized the infestation levels from different olive cultivars around the Mediterranean Basin and California and grouped the main stimuli involved in the choice mechanism of olive fly females in three groups: physical, chemical, and molecular. Numerous studies confirmed the importance of fruit size and volume, fruit epicarp parameters, such as elasticity and firmness, and fruit color. OLF prefers large fruits, greener comparatively to ripened fruits, and lower skin elasticity and higher skin firmness (Neuenschwander et al.

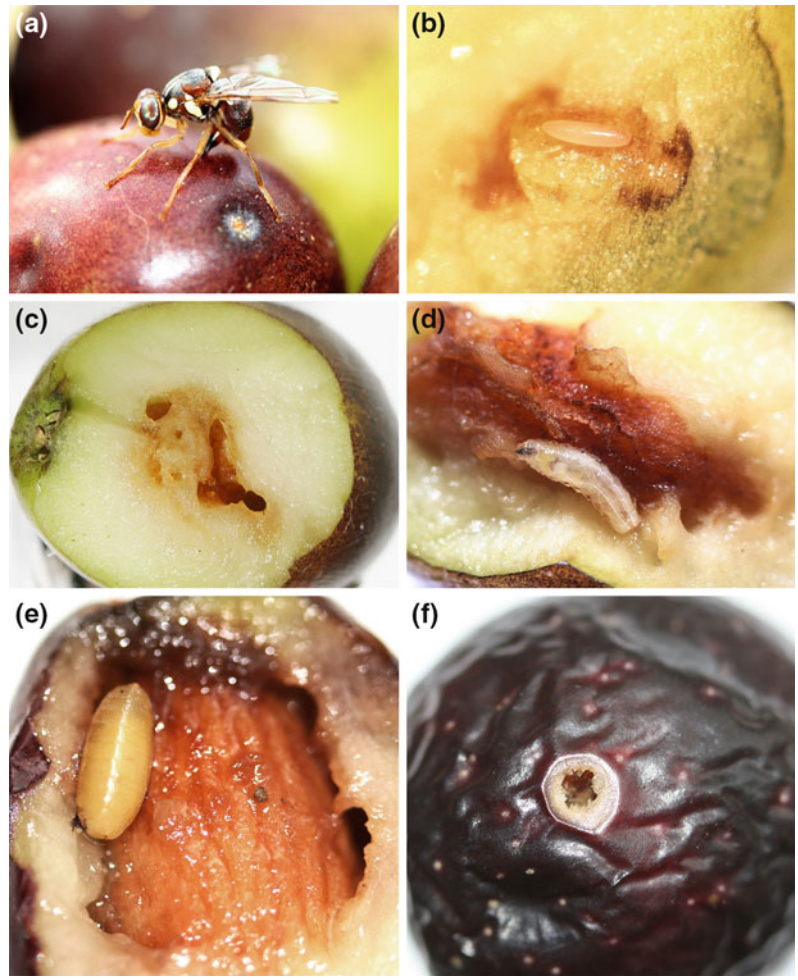

Fig. 1 *Bactrocera oleae* (Rossi). Egg-laying female (**a**); newly deposited egg (**b**); feeding galleries (**c**); intermediate larval stage (**d**); puparium in damaged drupe (**e**); emergence hole (**f**)

1985; Vlahov 1992; Katsoyannos and Kouloussis 2001; Gonçalves et al. 2012; Rizzo et al. 2012; Malheiro et al. 2015b). In relation to chemical parameters, the epicarp compounds may attract or repel OLF females. Cuticular waxes and ammonia may exert opposite effects, respectively, repellent or attractant, such as several volatiles emitted by fruits and leafs. For example, (E)-2-hexenal exerts a repellent action while the olive leaf volatiles toluene and ethylbenzene, stimulated oviposition in the OLF (Scarpati et al. 1993; Lo Scalzo et al. 1994; Scarpati et al. 1996). Another olive volatile α-copaene, a sesquiterpene, is present in higher amounts in more susceptible olive cultivars, promoting *B. oleae* oviposition (de Alfonso et al. 2014). Oleuropein, the main phenolic compound in drupes and leaves, is involved in the defense mechanism against OLF. A higher level of enzymatic hydrolysis of oleuropein is reported for less susceptible olive cultivars inhibiting the early development of OLF (Spadafora et al. 2008; Iannotta and Scaliercio 2012). Laboratory assays with olive leaves essential oils from cultivars with different susceptibilities showed different physiological response of adults OLF dependent from oils chemical composition (Malheiro et al. 2015a). The same authors analyzed leaf volatiles of three cultivars and reported a significantly lower infestation degree and

higher volatile amounts of cv. Cobrançosa than other two cultivars, with a probable deterrent effect for oviposition (Malheiro et al. 2015c, 2016). Among the investigated volatiles, toluene showed a general increase during fruit maturation and positive correlation with olive fly infestation levels (Malheiro et al. 2016). It has also been shown that olive flies are attracted by chemical cues emitted by epiphytic bacteria, which probably contribute to host location (Scarpati et al. 1996; Sacchetti et al. 2007, 2008).

2.2 Olive Fly Control: From Insecticides to SIT and Beyond

The olive fly can reduce more than 30 % of the olive oil production, for an estimated loss of more than 800 million dollars (Mazomenos 1989; Bueno and Jones 2002). During the last fifty years, the control of the fly has been based on chemical insecticides, mainly organophosphates (OPs), pyrethroids, and, more recently, Spinosad. Spinosad is an insecticide based on compounds derived from *Saccharopolyspora spinosa*. Besides the negative impact on the environment, the inconsiderate use of insecticides increases the occurrence of pesticide resistance. The resistance mechanism has been extensively studied for organophosphates, revealing the occurrence of three mutations in the acetylcholinesterase (AChE), the target gene of the insecticide. Two are point mutations in the catalytic gorge of the enzyme (Vontas et al. 2002). The third is a small deletion located in the carboxyl terminal of the enzyme (Kakani and Mathiopoulos 2008; Kakani et al. 2011). Pyrethroid resistance implicates an elevated level of the P450 mixed function oxidases (MFOs), enzymes involved in insecticide detoxification (Margaritopoulos et al. 2008). Spinosad resistance indicates the involvement of several immune system loci (Sagri et al. 2014a). The reduction of reliance on pesticides for crop protection necessitates the development of novel environmentally friendly methods of insect control. Alternative control methods include mass trapping, natural enemies and, the sterile insect technique (SIT). These strategies are not always adequate to control *B. oleae* populations and infestation. Attractive compounds may be used in mass trapping programs, to lure olive fruit flies into traps (Haniotakis et al. 1991; Noce et al. 2009) or to artificial surfaces treated with chemo-sterilantor persistent insecticides, such as that obtained with the *attract and kill* strategy (Broumas et al. 2002; Bueno and Jones 2002; Petacchi et al. 2003). Recently, the bioinsecticide Spinosad has been incorporated into a bait formulation to spray with large droplets (4–5 mm) on minimal parts of the upper tree canopies with limited environmental impact (Yokoyama 2015).

A wide range of natural enemies, mainly parasitoids (e.g., *Bracon celer*, *Eupelmus urozonus*, *Eurytoma martelli*, *Pnigalio agraules*, *Psyttalia concolor*, *Psyttalia lounsburyi,* and *Utetes africanus*) live at the expenses of OLF larvae in different geographical ranges (Boccaccio and Petacchi 2009; Daane and Johnson 2010; Daane et al. 2015). The presence of a high number of OLF parasitoids led to the hypothesis that olive may have evolved indirect defense responses by modulating emitted volatiles to attract natural enemies of OLF (Alagna et al. 2016). However, classical biological control programs for this insect pest have been implemented in several countries without significant success (Daane and Johnson 2010; Hoelmer et al. 2011; Wang et al. 2011). Other potential biological control factors may include the disruption of the relationship between OLF and its endosymbiont, *Candidatus Erwinia dacicola* (Capuzzo et al. 2005; Estes et al. 2012).

In Tephritidae, SIT has been proven reasonably successful. The SIT is an alternative species-specific control approach, whose principle is based on mass rearing, sterilization by irradiation, and subsequent release of the sterilized insects (Knipling 1955). The reproduction of the target population is therefore blocked, since mating between the released sterile males and the wild females leads to offspring reduction. Initial efforts to use the SIT for OLF control in the 1970s were unsuccessful (Economopoulos et al. 1978; Economopoulos and Zervas 1982;

Economopoulos 2002). Mixed sex releases[1] as well as factors of the fly biology are the main problems that lead to poor field performance (reviewed in Estes et al. 2012). They represent unsolved issue that need to be tackled to improve SIT succesful rate.

Genetic engineering provides alternatives to classical SIT. In the medfly *Ceratitis capitata*, the most studied member of the Tephritidae family, substantial progress resulted in transgenic fly lines capable of male-only releases, early embryonic lethality of the progeny between released laboratory males and wild females, and fluorescent marking of the responsible transgene (Gong et al. 2005; Schetelig et al. 2009; Ogaugwu et al. 2013). Such efforts were possible due to extensive classical genetic analysis of the medfly that led to accelerated development of the appropriate modern molecular and genomics tools. On the other hand, the lack of classical genetic tools for OLF (e.g., morphologically stable mutants) makes the early steps of this effort very challenging. Nonetheless, molecular and genomics approaches have now overcome the need for classical genetic analysis and have renewed the interest for OLF SIT.

A breakthrough in *B. oleae* molecular biology was achieved in 2006, when the insect was successfully transformed (Koukidou et al. 2006), generating hopes for the development of a biotechnology-based strategy for its suppression. A significantly improved SIT variant, "Release of Insects carrying a Dominant Lethal" (RIDL; Thomas et al. 2000) had gained ground, mainly because it circumventes the need for sterilization using irradiation. Instead, the transgenic reproductive sterility was achieved by males carrying a dominant lethal gene and killing the offspring in the field. A new transgenic strain was developed based on female-specific RIDL (FsRIDL) (Ant et al. 2012). This method uses sex-alternate splicing sequences from sex determination genes which results in sex-specific-engineered lethality of females at late larval and pupal stages, allowing male-only production and mortality of female progeny in the field. Further studies in medfly engineering resulted in the development of a transgene-based female-specific lethality system for early embryonic sexing (Ogaugwu et al. 2013). This strategy provides a more cost-effective sexing in SIT programs, since the elimination of the fly larval and pupal stages increases the efficiency in the procedure of mass rearing. Such an endogenous effective lethal system for OLF is under way, since the transcriptome analysis of the insect led to the identification of the appropriate genes (the early embryonic *serendipity-α* locus and the pre-apoptotic *head involution defective* gene (Sagri et al. 2014b).

The lowering cost of NGS technologies made it possible the sequencing of several *B. oleae* transcriptomes that focus either on detoxification gene families (Pavlidi et al. 2013), Spinosad resistance (Sagri et al. 2014a), or genes involved in development, reproduction, or olfaction (Sagri et al. 2014b). Since OLF is not a model organism, the overall premise of such analyses is to obtain tools that would lead to novel approaches for its control. The analysis of complex life-history traits, such as mate- or oviposition-choice, fertility or fecundity, now become feasible and may offer the desired alternative approaches. For example, the reproductive and the olfactory systems are of great research interest. The first system is involved in successful mating and egg development while the second controls the basic insect behavior, including the interactions with potential mates, food sources, and appropriate oviposition sites. A possible manipulation of a mechanism regulating these systems would severely affect the insect's fertility thus reducing its destructive ability.

The OLF biology has entered into the molecular era following the recent submission of its genome sequence to GeneBank (GCF_001188975.1). This effort was a combination of sequencing techniques

[1]In original SIT, both male and female insects are released, particularly because the distinction between male and female pupae is practically unfeasible. Released females, however, although sterile, sting fruits with their ovipositors, which generates a source of secondary bacterial or fungal infections at the sting site. Furthermore, co-released sterile females may also cause the sterile males to court these co-released females instead of seeking out wild females.

(Illumina short reads, Illumina mate pairs, and PacBio long reads), as well as a de novo transcriptome assembly with Illumina RNA sequencing. Further exploration of genomic data will enhance our knowledge of genome structure and function, offering access to many dynamic aspects of the biology of this pest. This will form the basis for future research that would i) provide important insights into fundamental biological questions (such as the interaction with the host plant or the evolution within the Tephritidae), ii) elucidate important mechanisms (such as reproduction, olfaction, or insecticide resistance) and iii) offer novel targets for OLF control.

3 Major Viral and Bacterial Enemies

3.1 Olive-Infecting Viruses and Viral Diseases

Olive-infecting viruses. The number of virus-infecting olive trees has increased with time. Currently, 15 different viruses belonging to nine genera in eight families have been identified (Table 1). Four (e.g., the *Olive latent ringspot virus* (OLRV), the *Olive leaf yellowing-associated virus* (OLYaV), the *Olive latent virus 3* (OLV-3), and the *Olive mild mosaic virus* (OMMV), a recombinant between OLV-1 and TNV-D, seem to be specific to olive since they have not been found so far in other host(s) (Cardoso et al. 2005). Whether *Olive vein yellowing-associated virus* (OVYaV), *Olive yellow mottle and decline-associated virus* (OYMDaV), and *Olive semilatent virus* (OSLV) are also host-specific remains to be established. Virus infections have been recorded in 22 different countries (Table 2). Since worldwide systematic surveys have not been carried out, it is reasonable to expect that the virus list will increase following more extensive investigations in countries where the olive industry is expanding (e.g., Argentina, India, China, Australia, New Zealand). The average infection rate, calculated on over 2000 samples of various geographical origins, analyzed in Italy and other countries, approximates 60 %. Such a high infection level apparently does not reflect on olive yield in an equally severe manner. Based on current knowledge, it seems possible to conclude that a viral etiology can be attributed with reasonable confidence only to the affections denoted "Bumpy fruits" and "Leaf yellowing complex," the latter consisting in a foliar discolorations ranging from chlorosis to bright yellowing. Although both diseases appear to have a detrimental impact on the yield, growth rate [OLYaV (Cutuli et al. 2011)], and rooting ability ("Bumpy fruits"), actual losses have not been quantified. A recent analysis of the "Frantoio" and

Table 1 Diseases and associated recognized viruses

Disease and associated virus	Mechanical transmission	Graft transmission	Country and year of record
Bumpy fruits (SLRSV)	+	+	Italy (1986), Portugal (1992)
Olive vein yellowing (OVYV)	+	−	Italy (1994)
Olive leaf yellowing (OLYaV)	−	+	Italy (1996)
Olive yellow mottling and decline (OYMDaV)	+	+	Italy (1996)
Leaf chlorosis, fasciation and deformation of the shoots (OLV-1)	+	−	Portugal (2000)
Leaf and fruit deformation, leaf yellowing (CLRV)	Putative viral agent identified by RT-PCR		Croatia (2011)
Vein banding (TMV)	+	+	Italy (1996)
Vein clearing (OSLV)	+	−	Italy (1996)

+ = Positive transmission, − = Transmission negative or not done

Table 2 Olive-infecting viruses and their geographical distribution

Virus	Taxonomic position (family, genus)	Country and year of first record
Strawberry latent ringspot virus (SLRSV)	*Secoviridae* (genus to be determined)	Italy (1979), Portugal (1990), Spain (1998), USA (2001), Egypt (2001), Turkey (2004), Lebanon (2005), Syria (2005), Croatia (2007), Tunisia (2009), Albania (2009)
Arabis mosaic virus (ArMV)	*Secoviridae*, *Nepovirus*	Italy (1979), Portugal (2000), Egypt (2001), USA (2001) Lebanon (2005), Syria (2005)
Cherry leafroll virus (CLRV)	*Secoviridae*, *Nepovirus*	Italy (1981), Portugal (1990), Spain (1998), Croatia (2011), USA (2001), Egypt (2001), Lebanon (2005), Syria (2005), Tunisia (2009)
Olive latent ringspot virus (OLRSV) Cucumber mosaic virus (CMV)	*Secoviridae*, *Nepovirus* *Bromoviridee*, *Cucumovirus*	Italy (1983), Portugal (1990), Syria (2005), Tunisia (2009) Italy (1983), Portugal (1993), Spain (1998), USA (2001), Syria (2005), Tunisia (2009), Algeria (2011), Australia (2011), France (2011), Cyprus (2011), Chile (2011), Israel (2011), Morocco (2011)
Olive latent virus 1 (OLV-1)	*Tombusviridae*, *Necrovirus*	Italy (1984), Jordan (1994), Turkey (1996), Portugal (2000), USA (2001), Egypt (2001), Lebanon (2005), Syria (2005), Tunisia (2009)
Olive latent virus 2 (OLV-2) Olive latent virus 3 (OLV-3)	*Bromoviridae*, *Oleavirus* *Tymoviridae*, *Marafivirus*	Italy (1984), Lebanon (2005), Syria (2005), Tunisia (2009) Italy (2009), Portugal (2009), Greece (2009), Malta (2009), Tunisia (2009), Lebanon (2009), Syria (2009), Turkey (2009)
Tobacco necrosis virus D (TNV-D)	*Tombusviridae*, *Necrovirus*	Portugal (2002, 2004)
Olive mild mosaic virus (OMMV)	*Tombusviridae*, *Necrovirus*	Portugal (2005)
Olive leaf yellowing-associated virus (OLYaV)	*Closteroviridae*, (genus to be determined)	Italy (1996), Albania (2006), Spain (2006), Croatia (2007), Israel (1999), Egypt (2001), Lebanon (2005), USA (2001), Syria (2005), Tunisia (2009), Cyprus (2011), Chile (2011), Australia (2011), Greece (2011), France (2011), Algeria (2011), Palestine (2011), Morocco (2011)
Olive vein yellowing-associated virus (OVYaV)	*Alphaflexiviride*, *Potexvirus*	Italy (1995)
Tobacco mosaic virus (TMV)	*Virgaviridae*, *Tobamovirus*	Italy (1996)
Olive semilatent virus (OSLV)	Unclassified	Italy (1996)
Olive yellow mottling and decline associated virus (OYMDaV)	Unclassified	Italy (1996)

"Ascolana tenera," two CLRV-infected Italian cultivars grown in Croatian Istria, disclosed that the presence of this virus affects the oil of "Frantoio" by decreasing the yield from 10.9 to 7.6 % and the quality, by lowering the amount of *o*-diphenols and the oleic/linoleic acid ratio (Godena et al. 2011). However, a better evaluation of the detrimental effects of virus infections

on the quality and quantity of the produced fruits and oil will be possible when sanitized clonal selections will be tested in comparative field trials with their infected mother stocks.

Little is known on the epidemiology of olive-infecting viruses. The assessment of virus spread in orchards, if any, is made virtually impossible by the widespread lack of visible symptoms in infected trees. Furthermore, some vectors (e.g., the dorylamoid nematode *Xiphinema diversicaudatum* that transmits SLRSV and ArMV) do not prosper under the climatic conditions found in most of the areas where olives are grown, whereas other vectors (e.g., aphids that are potential CMV vectors) rarely, if ever, colonize olives. In addition, several other viruses (OLV-2, OLV-3, and all those of the "Leaf yellowing complex") do not have recognized vectors. Thus, the only evidence currently available on the actual or potential virus spread in nature is limited to the three olive-infecting members of the genus *Necrovirus* (OLV-1, TNV-D, OMMV). These were experimentally shown to be picked up by the host in the absence of fungal vectors [OLV-1 (Martelli et al. 1996)] or to be transmitted by *Olpidium brassicae* [OMMV (Varanda et al. 2011) and, likely, TNV-D (Felix and Clara 2001]. Thus, except for the established cases of fungus-mediated transmission through the soil, the intervention of other vectors does not seem to be supported by two relevant notions: (i) the generalized and internationally high incidence of the infections, which could only be explained by the presence and activity of the same vectors in widely separated geographical areas, i.e., an unlikely condition to occur; and (ii) the erratic distribution of infected plants in the field, which does not conform to common vector-generated patterns. It seems more plausible that nurseries are the main centers for virus accumulation and subsequent dissemination through trading of their productions. Field surveys revealed a geographical distribution of the virus, consistent with the concept that the main source of infection is represented by propagative material.

Seeds represent another recently discovered source of infection. The presence of OLV-1 was ascertained in the seeds of cv. "Verdeal Alentejana" in Portugal (Lobão et al. 2002) and in cv. "Oliva rossa" in Italy, with an incidence of 82 % in the latter (Saponari et al. 2002a). Seeds of the same variety were infected by CLRV up to 90 % (Saponari et al. 2002b). The infection rate was lower in the seedlings, but still significant, i.e., 36 % (OLV-1) and 41 % (CLRV). Thus, an additional but still little explored mechanism exists, whereby viruses can spread with seeds in natural environments and in agricultural crops with seedlings used as rootstocks.

Most olive-infecting viruses (13 out of 15) are mechanically transmissible to a range of herbaceous hosts using tissue extracts from various organs (flowers, young leaves or drupes, succulent roots). Nevertheless, because of its low sensitivity, the use of manual transmission can hardly be recommended for assessing the sanitary status of olive selections. Thus, the current protocols for virus detection are not based on biotests (mechanical transmission to herbaceous hosts is unreliable and there are no differential woody indicators available) nor on immuno-enzymatic assays, which are also unreliable, but on nucleic acid-based techniques (Albanese et al. 2012).

Serology does not seem always an effective technique for the identification of olive-infecting viruses. For instance, ELISA was successfully applied for SLRV and CMV detection from field samples in Portugal and Spain but not in Italy, except following sample manipulations for virus concentration increase. The unsatisfactory outcome of ELISA applications has prompted the use of nucleic acid-based diagnostic techniques such as: (i) molecular hybridization of crude sap extracts, or denatured dsRNAs, or total nucleic acid (TNA) extracts with virus-specific riboprobes; (ii) one or more of the many RT-PCR protocols (one-step, nested, multiplex) applicable to crude sap or TNA extracts. A well-performing single-step RT-PCR procedure for the detection of eight olive-infecting viruses (ArMV, CLRV,

SLRSV, CMV, OLV-1, OLV-2, OLYaV, and TNV) included in the Italian phytosanitary certification protocol, has recently been developed and validated through an interlaboratory ring test (Loconsole et al. 2010). Real-time PCR protocols are also being developed with encouraging results (Albanese et al. 2012).

Bumpy fruits. This disease was first observed in Italy in cultivar "Ascolana tenera" (Marte et al. 1986), then in Portugal in cv. "Negrinha de Freixo" (Henriques et al. 1992). Infected trees bear pear-shaped, puckered fruits with deformed kernels, show narrow and twisted leaves and bushy growth. The disease has been reproduced in healthy seedlings by grafting. The yield is affected and cuttings have a reduced rooting ability. The latter trait, however, was not confirmed for the Italian cultivar "Raggiola" whose cuttings rooted as well as those of apparently healthy "Frantoio" plants (Roschetti et al. 2009). The interest of this finding lies in the fact that "Raggiola" and "Frantoio" are apparently genetically identical but are retained as different cultivars due to some morphological of "Raggiola" (narrow leaves, small inflorescences), which are attributed to SLRSV infection (Ferretti et al. 2002). The putative agent of bumpy fruits, the aforementioned SLRSV, is a soil-borne (nematode-transmitted) unassigned member of the family *Secoviridae* (Sanfaçon et al. 2011) identified in 15 different Portuguese cultivars and in a number of others in eight different countries (Table 2), very few of which, however, show symptoms. Modifications of olive drupes resembling very much bumpy fruits were observed in Greece, but the presence of SLRV in symptomatic plants was not ascertained.

Leaf yellowing complex. The bright yellow discolorations of the foliage observed in several Italian regions and described under the name of "vein yellowing," "leaf yellowing," and "yellow mottling and decline" constitute the "Leaf yellowing complex." Three different filamentous viruses are associated with this complex: (i) a putative potexvirus, Olive vein yellowing-associated virus (OVYaV) (Faggioli and Barba 1995); (ii) Olive yellow mottling and decline-associated virus (OYMDaV), a virus belonging to an undetermined genus (Savino et al. 1996); (iii) Olive leaf yellowing-associated virus (OLYaV) a member of the family *Closteroviridae* (Sabanadzovic et al. 1990). The leaf yellowing condition which OYMDaV and OLYaV are associated with was reproduced in healthy seedlings by grafting, suggesting the systemic infection ability of the virus. OLYaV has been found in symptomatic or, more often, symptomless trees from 18 different countries (Table 2).

Other diseases. They include the following: (i) low vigor, leaf chlorosis, fasciation, and deformation of the shoots shown by several Portuguese cultivars infected by *Olive latent virus 1* (OLV-1) were suggested as being putatively induced by this virus (Felix et al. 2007); (ii) deformations of leaves and drupes go together with yellowing of the canopy were observed in Croatia in plants infected by *Cherry leafroll virus* (CLRV) which was retained as the putative agent of the disease (Luigi et al. 2011); (iii) "vein banding" and "vein clearing" are two additional disorders reported from Italy (Table 1). Beside the described symptoms (Triolo et al. 1996), there is no information on their origin and the role, if any, played by the viruses associated with them [*Tobacco mosaic virus* (TMV)] and *Olive semilatent virus* (OSLV), respectively.

3.2 *Xylella fastidiosa*

Xylella fastidiosa (*Xf*) is a xylem-restricted pathogenic bacterium native to the Americas, where it has been confined for long time. *Xf* is the agent of destructive diseases of many agriculturally relevant crops (e.g., blueberry, citrus, coffee, grapevine, several stone fruits) and of different shade trees (Hopkins and Purcell 2002). Unfortunately, *Xf* has no longer a geographical distribution limited to the Americas. Its presence in Taiwan is a potential threat to continental Asia, while the recent landing in Italy and France and the ascertained occurrence in Iran contribute

to a permanent modification to its geographic range (Almeida and Nanni 2015). The bacterium continues to spread into new areas, where it may settle in traditional hosts or move into new ones, eliciting destructive diseases.

The most common pathway leading to *Xf* epidemics is the introduction of exotic genotypes into environments that are ecologically adapted to the maintenance of the bacterium in the plant community. One of the most dramatic recent examples of a new *Xf*–host association is that with olive in Southern Italy. The unexpected arrival of the pathogen in the Salento peninsula has created relevant economic issues beyond the agricultural sector, considering the importance of the olive oil production chain in that region.

Xf infections to olive were first reported in 2014 (Krugner et al. 2014) in trees exhibiting leaf scorch and dieback symptoms in California (USA). The putative agent of this condition, whose pathogenicity is still under scrutiny, is a strain of *X. fastidiosa* subsp. *multiplex*., i.e., a bacterium taxonomically different from *X. fastidiosa* subsp. *pauca*. This bacterium is the major if not the unique agent of the olive quick decline syndrome (OQDS), the disease which is devastating the Salentinian olives (Saponari et al. 2013). OQDS is characterized by leaf scorching and scattered desiccation of twigs and small branches which, in the early stages of the infection, prevail on the upper part of the canopy. Leaf tips and margins turn dark yellow to brown, and desiccate. Symptoms become increasingly severe over time and extend to the rest of the crown, which acquires a blighted appearance. Desiccated leaves and mummified drupes remain attached to the shoots. Affected trees, especially those of the major local cultivars, "Cellina di Nardò" and "Ogliarola salentina," decline slowly and die, regardless of their age. Declining trees, especially the aged, century-old ones, very often exhibit a discolored sapwood, which is colonized by fungi of different genera (e.g., *Phaeoacremonium*, *Phaeomoniella*, *Pleumostomophora,* and *Neofusicoccum*) which are thought to act as disease aggravators (Nigro et al. 2013).

Interestingly, olive trees showing symptoms strikingly resembling those of the Apulian OQDS have been reported in Argentina (Haelterman et al. 2015) and Brazil (Coletta-Filho et al. 2016). In both cases, symptomatic plants are infected by *X. fastidiosa* strains genetically closely related to the subspecies *pauca*. Although belonging to the same subspecies occurring in Apulia, the Argentinean, and Brazilian *Xf* strains differ from the Salentinin isolate, known as CoDiRO, whose genome, a DNA molecule of *ca*. 2,500,000 bp in size, has been sequenced (Giampetruzzi et al. 2015) and found to be molecularly identical to a bacterial isolate from Costa Rica.

Although *X. fastidiosa* has not yet been proven to be the only agent causing OQDS as pointed out by Coletta-Filho et al. (2016), a convincingly strong correlation between symptoms in olive trees and the presence of this pathogen appears evident in three distant geographic regions of the world (Southern Italy, Argentina, and Brazil).

Accurate detection of the bacterium in olive trees has been achieved by serological and molecular assays (Loconsole et al. 2014; Yaseen et al. 2015). The bacterium was isolated in pure culture from symptomatic oleander (Cariddi et al. 2014), olives (Saponari et al. 2014), and a number of other naturally infected hosts (Saponari, unpublished). These cultures have been used for artificial inoculation assays of different olive cultivars and hosts. *X. fastidiosa* is exclusively transmitted by xylem-sap feeding insects belonging to the order Hemiptera, sub-order Cicadomorpha. While in the Americas there are numerous sharpshooters species (family Cicadellidae, subfamily Cicadellinae) and almost sixty have been identified as *X. fastidiosa* vectors, very few sharpshooter species are present in Europe. While there is no information about the vector that transmits the bacterium in Argentinean and Brazilian olive groves, search for the putative vector of *Xf* in southern Italy has identified *Philaenus spumarius* as the predominant vector species. Indeed, this spittlebug represents the most common and widespread species, and

the one that more than any other thrives on olive. Populations of hundreds of adults of *P. spumarius* colonize olive trees in spring-late summer, and a high number of individuals are *Xylella*-positive, up to nearly 100 % in summer (Cornara et al. 2014). Thus, *P. spumarius* has a tremendous inoculum potential that is discharged on olive trees, the species with which it entertains a preferential relationship.

As to the risks for Europe and the Mediterranean basin represented by the introduction of *X. fastidiosa* into Italy, and more recently in France, it has been predicted (Purcell 1997; Bosso et al. 2015) how widely the bacterium will spread in these regions, should nothing be done to confine it within its current boundaries. Likewise, a disease management strategy aimed at restraining bacterial dispersal by reducing the inoculum sources and by controlling vector's juveniles (mechanical weeding in late winter) and adults (a pesticide treatment in late spring when they move to olives) had been envisaged.

4 Some of the Major Olive Diseases Caused by Pathogenic Fungi

Verticillium dahliae. Verticillium wilt of olive (VWO), caused by the soil-borne fungus *Verticillium dahliae* Kleb, is one of the most important diseases affecting this woody crop. Loss from Verticillium wilt includes the death of trees and the reduction in fruit yield. The trees may be infected by two pathotypes of *V. dahliae,* classified as defoliating (D) and non-defoliating (ND) based on their aptitude to induce or not defoliation of green leaves, respectively. Severity of attacks depends upon virulence: The ND pathotype is relatively severe, and in infected plants symptoms may resolve completely. On the contrary, infections by the D pathotype can be lethal (Schnathorst and Sibbett 1971). Moreover, the pathogen can survive in the soil for long periods of time and chemical compounds are not effective (Wilhelm 1955). The use of resistant cultivars or rootstocks for grafting of VWO-susceptible varieties may represent a valuable tool to counteract the disease.

During the last 20 years, VWO attacks have considerably increased in many Mediterranean regions. Multiple factors such as (i) the use of infected propagation material or pathogen-infested soils, (ii) the abuse on fertilization and irrigation, (iii) the pathogen's dispersal efficacy and the endurance of its infective structures (microsclerotia), (iv) climatic factors and edaphic variables, (v) the genetic/pathogenic diversity of pathogen's populations (i.e., defoliating [D] and non-defoliating [ND] pathotypes), or (vi) changes in cultivation systems have contributed to boost the disease. This scenario makes necessary to implement an integrated disease management strategy for the effective control of VWO (López-Escudero and Mercado-Blanco 2011).

Spilocaea oleagina. Olive leaf spot (OLS), also called peacock spot disease or *Cycloconium* leaf spot, is caused by the fungus *S. oleagina*, Fries (syn. *Cycloconium oleaginum* Cast). This disease usually arises on the upper surface of the olive leaf and is associated with the fall of leaves and fruit as well as low quality of olive oil, causing considerable losses in many olive-growing areas worldwide (Viruega et al. 2013). Resistance of olive cultivars to *S. oleagina* attacks has been reported to be variable although the underlying mechanisms are not known (Mekuria et al. 2001). Some cultivars have been described as relatively tolerant (based on symptom severity), but not "immune" to the pathogen. The disease may be chemically controlled by the application of fungicides (Sistani et al. 2009), but treatments appear to be not always effective (Obanor et al. 2008). In addition, chemical fungicides may lead to the onset of resistant pathogen races (Vanneste et al. 2003) as well as disorder of the plant metabolism (Obanor et al. 2008).

5 The Interaction of Olive with Its Enemies

5.1 The Molecular Responses of the Olive Fruit to *Bactrocera oleae* Infestation

Despite the dominant importance of the OLF in the vast majority of olive cultivated areas (Malheiro et al. 2015a), research efforts to examine olive response to the fruit fly are scarce. Among others, two main factors may account for the limited information available. Firstly, the study of the interaction is hampered by the virtual impossibility to use the so-called controlled conditions. The cultivation of olive plants in confined environments, such as growth chambers or greenhouses, and the fruit fly rearing pose large structural and economic burdens. Besides being a perennial, slow-growing, shallow-rooted, biennial bearing tree, olives require a specialized workforce and large dedicated space. For instance, trees require some cold for proper fruit setting but they are sensitive to hard freezing. Moreover, although considerable progress has been made, mass rearing of the insect without negative effects on pest performance cannot be considered a routine approach (Rempoulakis et al. 2014; Sagri et al. 2014a). While the absence of controlled conditions likely reduces the reproducibility of the experiments, it is fair to add that not all the results in a confined environment may be necessarily significant in field conditions. On the other hand, the selection of appropriate uninfested drupes from undamaged trees in field conditions is essential to avoid false negatives, and it is a technical challenge that requires entomological expertise. Another factor to consider is the ample genetic variability of the cultivated olive. A very large number of cultivated varieties characterize this species (Belaj et al. 2002). Considering that different cultivars have different levels of tolerance to the fruit fly (Daane and Johnson 2010), it is expected that also molecular response may differ. It is also likely that the same cultivar may display different behavior in different environments, especially in response to different climates or soil types.

The interaction between *B. oleae* and olive drupes is a relatively complex process. Some traits of the plant affect the interaction even before the pest has been in physical contact with its host. The fruit fly can locate potential host plants at a distance and employs non-random behaviors (e.g., based on fruit dimension and phenological state) to increase the probability of landing on a suitable host. Moreover, before oviposition, the OLF evaluates the acceptability by fruit probing. Olive cultivars may have a very different constitutive tolerance to OLF, affected by a high number of factors. These include plant-based traits, pest population density, climate, and their interaction (Lo Scalzo et al. 1994; Scarpati et al. 1996; Massei and Hartley 2000; Burrack and Zalom 2008; Gonçalves et al. 2012). Unfortunately, cultivars that are considered very tolerant to OLF may suffer considerable attacks under intense infestation (Iannotta et al. 2007). The differential susceptibility to the OLF oviposition may involve a number of morphological, physiological, and phenological parameters (Neuenschwander et al. 1985; Kombargi et al. 1998; Rizzo et al. 2012), which make the study of constitutive defense challenging. Some attempts have been made to investigate the molecular reaction of the drupe to the fruit fly sting. Although limited to a small number of genes, it seems clear that inducible genes have a different response to the *B. oleae* puncture, to mechanical wounding, and to the feeding larvae (Corrado et al. 2012). This is expected considering that the larva actively takes away nutrients from the drupe, which is consistent with the higher magnitude of the olive response observed for this interaction.

The first effort to achieve a more comprehensive understanding of the molecular basis and related signaling pathways involved in olive interaction with *B. oleae* larvae was done by PCR-based suppression subtractive hybridization (SSH) (Corrado et al. 2012). The SSH method allows selective PCR amplification of cDNA fragments that differ between a control and

experimental transcriptome without any prior genomic knowledge. For this reason, this technique has been employed to investigate the plant response to biotic and abiotic stress (Ouyang et al. 2007; Estrada-Hernandez et al. 2009). The functional characterization of the subtracted library indicated a higher representation of ESTs involved in the plant response to stress. On the other hand, a noteworthy proportion of sequenced transcripts were similar to uncharacterized olive transcripts, suggesting that the olive response to *B. oleae* also involves a number of olive specific genes yet to be discovered. Considering that in total, less than half of the identified olive transcripts could be annotated, a critical barrier for working with the olive is the dependence gene ontology repertoires and genomic information that are primarily based on model species (Kültz et al. 2007). According to a similarity-based GO-analysis, various classes of genes are affected by the feeding larva. Differentially represented transcripts found a significant similarity with genes involved in the response to biotic stress, such as wounding and pathogen attack, as well as abiotic stress, such as high or low temperature, drought, and NaCl. The identified transcripts were also putatively involved in the production, signal transduction, or response to hormones and molecules involved in pest response (e.g., jasmonic acid and ROS). Transcripts putatively encoding for resistance-related traits such as proteinase inhibitors (i.e., trypsin inhibitors, a type of serine protease inhibitors) were highly overexpressed in two cultivars following larval feeding. Serine inhibitors in plants belong to a large multigene family and, in absence of a reference genome, it was difficult to ascertain the possible contribution or more than one member. Larval infestation maintained high levels of trypsin protease inhibitors in ripe fruits (Alagna et al. 2016). Approximately, a third of the functionally annotated transcripts were homologous to genes that were first described in plant–pathogen interaction. The concurrent presence of genes involved in different signaling pathways suggests that a clear dichotomy between responses to arthropods and pathogens is not present in olive, as also noted for the interaction between olive and *S. oleaginea* (Benitez et al. 2005). It is known that feeding tunnels, as well as stings, may represent an opportunity for "secondary" pathogens. In addition, *B. oleae* larvae require their natural complement of bacteria to growth in unripe olives (Ben-Yosef et al. 2015). The olive defense mechanisms should include also indirect defense that is the ability of the plant to attract natural enemies of the herbivores. OLF induces an ethylene burst and a quantitative and qualitative variation in the volatile organic compounds (VOCs) from fruits (Alagna et al. 2016). Moreover, it has been also proposed that volatiles emitted by olive leaves may interfere in olive fly females' host selection (Malheiro et al. 2016).

As previously mentioned, transcriptome sequencing in olive is not only able to describe differences in gene expression but, with limited genomics information, it is also useful to identify transcripts and biological processes involved in specific plant reactions. Among the various olive transcripts that have been linked to OLF infestation (Corrado et al. 2012), different research lines indicated that genes coding for beta-glucosidases are important element of the olive response (Koudounas et al. 2015). Beta-glucosidase activity promotes the formation of toxic glutaraldehyde-like structure from oleuropein. Oleuropein is a major secoiridoid compound in olive, whose presence has been linked to olive resistance to the fly (Lo Scalzo et al. 1994; Noce et al. 2014), as well as pest resistance in other members of the Oleaceae family (Konno et al. 1999).

Proteomics studies in olive have been mainly focused on fruit development and quality. A study of the effect of the *B. oleae* larvae on drupes indicated that the differentially expressed proteins are primarily involved in carbohydrate metabolism, redox processes, and defense responses, such as a beta-glucosidase (Corrado et al. 2012).

Current evidence indicates that olive tolerance to the OLF is the outcome of a complex response, in which both genetic and environmental variabilities play a role that is worth investigating. Plants make use of pre-existing

physical and chemical barriers to reduce their suitability. The phenological state at the time of the pest outbreak is also a factor that influences host selection, but the relative importance of constitutive and inducible traits to the olive tolerance to the OLF is a question that remains open. Inducible defense mechanisms are activated upon attack and they include both direct and indirect defense mechanisms. The physical interaction between the drupe and the OLF activates components of different signaling pathways and leads to the production of chemically different compounds that directly and indirectly should reduce pest performance.

The complexity of the olive-*B. oleae*-environment interactions requires interdisciplinary approaches in order to elucidate the importance of the genetic factors and of the molecular basis of induced resistance. Future research cannot understand olive defense without a more integrative experimental designs that will require the expertise of different scientific fields. Available information indicates that herbivore-induced response should affect a number of relevant biochemical and morphological features of the drupe. Therefore, the molecular investigation of the effects upon olive yield and quality represent an interesting challenge for the applied research in the field.

5.2 The Olive Responsive Transcriptome to a Vascular Soil-Borne Pathogen

5.2.1 *Verticillium dahliae*

Understanding the genetic and molecular mechanisms triggered in olive upon the infection by *V. dahliae* would be instrumental to design novel control tools to confront the disease. While our knowledge on plant–pathogen interactions has steadily increased over the years, particularly with the development of powerful "-omic" approaches, the information about the genetic and molecular bases underlying plant defense responses against vascular and/or root pathogens is until now scarce (Larroque et al. 2013; Yadeta and Thomma 2013). In the case of *V. dahliae*, plant tissue reactions so far reported can be structural, i.e., the formation of tyloses in the xylem, and/or biochemical, i.e., accumulation of phenolic compounds (Baídez et al. 2007; Markakis et al. 2010). They can be either constitutive (Mueller and Morgham 1993) or induced in response to pathogen infection (Daayf et al. 1997; Markakis et al. 2010). In olive, tolerance of cultivar "Frantoio" to *V. dahliae* has been suggested to be mostly mediated by biochemical mechanisms induced in the root tissues, rather than structural responses such as vascular plugging (Bubici and Cirulli 2012). Overall, these defense reactions seem to take place to a lower extent in susceptible varieties than in tolerant ones (Baídez et al. 2007; Markakis et al. 2010; Bubici and Cirulli 2012).

Our knowledge on the genetic basis underlying *V. dahliae*-olive interaction has been recently enhanced by using the SSH methodology (Diatchenko et al. 1996). The interaction under study was the VWO-tolerant cultivar "Frantoio" and the D, highly virulent pathotype of *V. dahliae* (Gómez-Lama Cabanás et al. 2015). cDNA libraries enriched in up-(FU) or down-regulated (FD) olive genes from above-ground tissues upon root infection by the pathogen were generated. Broad transcriptomics changes taking place in aerial organs were observed as consequence of root infection by *V. dahliae*. It is interesting to emphasize that many of the genes systemically induced or repressed related to defense against diverse stress agents. For instance, genes putatively coding for catalase, calmodulin-binding family protein, ET-responsive transcription factor rap2-12-like, acetone cyanohydrin lyase or thaumatin like-protein were identified as up-regulated genes, whereas defensin, chloroplastic 6-phosphogluconolactonase, or phosphatase 2c 25 were found as down-regulated. Some 2688 expressed sequence tags (EST) were sequenced and analyzed from FU and FD libraries, eventually generating 976 unigenes. A total of 585 transcripts corresponded to up-regulated genes while 381 were down-regulated. Sequence comparison revealed that 37 % of the ESTs matched

to coding sequences previously identified in genomes of woody plants but only 3.5 and 5.9 % of the unigenes found in FU and FD libraries, respectively, showed significant identity with olive genes (Gómez-Lama Cabanás et al. 2015). As also previously stated, this result underlines the scarcity of genetic/genomic information on olive. Bioinformatics analysis showed that colonization of "Frantoio" roots by the *V. dahliae* D pathotype induced (19.8 % of unigenes identified in FU) and repressed (25.7 % of unigenes identified in FD) a broad range of plant defense responses to stresses (i.e., phenylpropanoid biosynthesis or terpenoids, hormones biosynthesis, and salicylic acid [SA]-related proteins). Besides, genes coding for transcription factors (TFs) involved in a/biotic stress such as *GRAS1* and *WRKY*'s (i.e., *WRKY 33* and *20*) were systemically up-regulated, suggesting that these TFs may play important roles in defense against this vascular pathogen. Elongation factors (i.e., *EF-1α*) and genes coding for β-amylases were also found in both cDNA libraries. Recently, β-amylases have been reported as negative regulators in Arabidopsis partial resistance against *V. dahliae* (Gkizi et al. 2015). Nevertheless, the roles of EFs and β-amylases coding genes in the olive-*V. dahliae* interaction remain to be elucidated. Finally, it is interesting to emphasize that 4 % (FU library) and 19 % (FD library) of the identified unigenes are related to the photosynthesis processes. Bilgin et al. (2010) have suggested that down-regulation of photosynthesis-related genes is part of a defense response, particularly against biotic stress.

The study of Gómez-Lama Cabanás et al. (2015) has also analyzed, in "Frantoio" plants, the relative expression pattern along time of seven genes involved in defense responses to different stresses. The genes were: *ACO* (ACC oxidase), *CO-MT* (caffeoyl-O-methyltransferase), *ACL* (acetone cyanohydrin lyase), the TF *GRAS1*, *DRR2* (disease resistance response protein), *PR10* (pathogenesis-related protein), and *DEF* (plant defensin protein). *GRAS1* and *CO-MT* genes were validated indicating that the phenylpropanoid pathway and this TF are systemically induced when *V. dahliae* colonizes olive roots. Four of these genes (*GRAS1*, *DRR2*, *ACL,* and *ACO*) were further assessed on their early- and middle-term expression patterns in olive cultivars showing differential susceptibility to VWO ("Picual" susceptible; "Frantoio" and "Changlot Real" tolerant). Interestingly, similarities in the expression pattern were found depending on the VWO susceptibility/tolerance level of the cv. tested. Thus, *GRAS1* slightly increased its relative expression along time in tolerant cultivars, while it showed a trend to be repressed in "Picual" plants. The putative olive *DRR2* gene, identified in the FD library, showed a trend to be up-regulated in the susceptible cv. unlike the overall down-regulated over time observed in tolerant varieties (Gómez-Lama Cabanás et al. 2015). This transcriptomics approach has provided for the first time crucial information about an as yet poorly understood interaction between the most devastating olive vascular pathogen and a VWO-tolerant cultivar. Data will be useful in terms of both acquisition of fundamental knowledge and the potential development of novel control tools such as genetic markers to evaluate VWO susceptibility/tolerance degree of olive genotypes. While SSH has revealed to be a robust technique, the implementation of more powerful approaches such as RNAseq (Wang et al. 2009) will yield more comprehensive information to understand, among others, this biotic interaction, as already used to study transcriptomics changes taking place during cold acclimation of olive plants (Leyva-Pérez et al. 2015).

5.3 The Olive Responsive Transcriptome to a Foliar Pathogen, *Spilocaea oleagina*

A transcriptomics approach based on differential display was used to elucidate molecular responses during the interaction with *S. oleagina* (Benitez et al. 2005). The interaction of "Lechín de Sevilla," a variety considered to be resistant to "peacock spot" (Trapero and Blanco 2001), with *S. oleagina*, up-regulated 162 olive transcripts.

The relative expression pattern of 21 selected genes revealed differences in gene induction along time. Early induction of several genes involved in signaling, transcriptional control, oxidative stress, a/biotic stresses, as well as a number of genes with unknown function, was observed after infection. However, induction of genes involved in metabolism and cellular maintenance was delayed. In contrast, up-regulation of these 21 selected genes in the susceptible cv. "Picual" was delayed and/or reduced in response to *S. oleagina* inoculation. Basal expression of some genes in control plants of the resistant cv. was higher than in the susceptible one, indicating a constitutive activation of defense responses (Benitez et al. 2005). These results shed light on the underlying mechanisms of this plant–pathogen interaction, indicating that resistance to *S. oleagina* in olive relies on an active genotype-dependent defense response. This conclusion is based on the observation that constitutive expression of defense genes was lower in the susceptible variety than in the resistant one, the latter showing a faster and stronger induction of gene expression after pathogen inoculation.

6 The Interaction of Olive with a Biological Control Agent, *Pseudomonas fluorescens*

Bacterial endophytes are probably present in all plant species, providing benefits to the host plant and positively influencing its growth, fitness, and development (Hardoim et al. 2015). Endophytic bacteria constitute a yet-to-be-explored tool for agricultural biotechnology (Mercado-Blanco and Lugtenberg 2014). For instance, endophytic bacteria exerting biological control may activate control mechanisms once established within plant tissues, potentially setting off a long-term plant protection status (Rosenblueth and Martínez-Romero 2006; Reinhold-Hurek and Hurek 2011). However, many questions on how a plant–endophyte interaction is successfully established remain to be elucidated. To develop such a lifestyle means that endophytes must be adapted to the plant interior and that are able to overcome, elude, or modulate the plant immune response to be recognized by the host plant as beneficial organisms (Reinhold-Hurek and Hurek 2011; Mercado-Blanco 2015).

Pseudomonas fluorescens PICF7 is an indigenous inhabitant of olive roots (Martínez-García et al. 2015). It shows in vitro antagonism against *V. dahliae* (Mercado-Blanco et al. 2004) and is able to endophytically colonize olive roots under different experimental conditions (Prieto et al. 2011; Maldonado-González et al. 2015). It has been previously shown that PICF7 is an effective biological control agent (BCA) against VWO (Mercado-Blanco et al. 2004), and that effective suppression of the disease requires the establishment of the BCA at both the surface and the interior of olive roots, prior to colonization by *V. dahliae* (Prieto et al. 2009).

In the case of olive, our understanding of the transcriptomics changes occurring during the interaction with a beneficial, endophytic bacterium has been recently enhanced. In order to elucidate the genetic processes taking place in roots (Schilirò et al. 2012) and aerial tissues (Gómez-Lama Cabanás et al. 2014) during the colonization of olive roots by strain PICF7, SSH cDNA libraries of the VWO-susceptible cv. "Arbequina" inoculated with strain PICF7 were generated, enabling the identification of a broad set of up-regulated olive genes at both local and systemic levels (Fig. 2). Schilirò et al. (2012) demonstrated that colonization by PICF7 induced a broad set of defense responses in olive root tissues, including genes related to ISR (induced systemic resistance) and SAR (systemic acquired resistance). Computational analysis of 445 unigenes induced in olive roots upon PICF7 inoculation showed that more than 40 % of them were associated with plant defense and response to stresses. A high percentage of unigenes (43.8 %) represented sequences present in genomes of woody plants, although only 2.5 % corresponded to olive, similarly to what observed in the *V. dahliae*–olive interaction.

Relative expression of selected genes involved in plant hormones biosynthesis and responsive transcription (*ACL*; lipoxygenase,

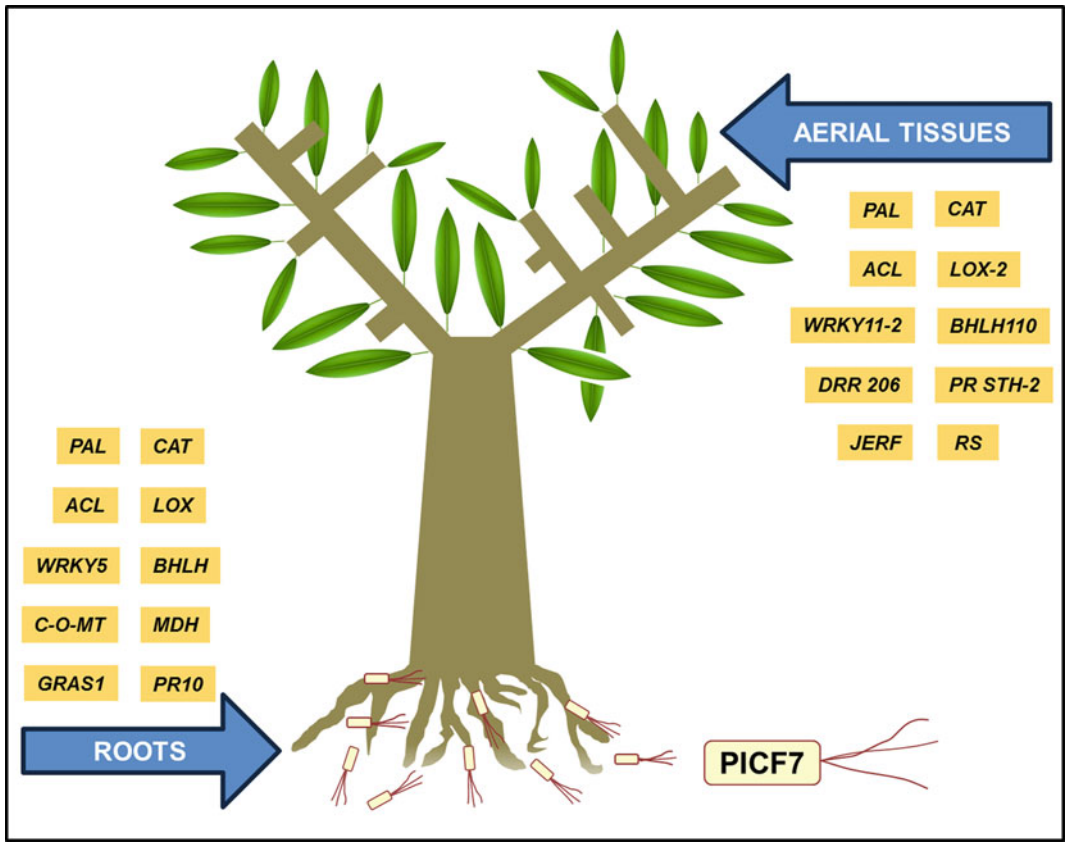

Fig. 2 A schematic representation of some genes involved in defense responses which are induced in olive roots and/or aerial tissues during the interaction of *Pseudomonas fluorescens* PICF7 (a beneficial root endophyte effective against *Verticillium dahliae*) with roots. *ACL* Acetone cyanohydrin lyase; *CAT* Catalase; *C-O-MT* Caffeoyl-O-methyltransferase; *BHLH110* and *BHLH* Basic helix-loop-helix transcription factors; *JERF* Transcription factor JERF; *LOX/LOX-2* Lipoxygenases; *MDH* Malate dehydrogenase; *PAL* Phenylalanine ammonia-lyase; *WRKY5* and *WRKY11-2* WRKY transcription factors; *GRAS1* transcription factor GRAS; *PR10* and *PR STH2* Pathogenesis-related proteins; *DRR 206* Disease resistance response protein; and *RS* Raffinose synthase. Based on Schilirò et al. (2012) and Gómez-Lama Cabanás et al. (2014)

LOX; malate dehydrogenase, and *WRKY5*, *bHLH*; *ARF2* [TFs]), phenylpropanoid biosynthesis (phenylalanine ammonia-lyase, *PAL*) and signal transduction defense response (*GRAS1*, a TF) validated the results from the generated SSH cDNA library. For instance, induction of *PAL* gene transcript suggested that this response pathway may be activated upon PICF7 colonization and may play some role in the biocontrol activity displayed by this bacterium. Up-regulation of *GRAS1* could indicate a modulation of olive defense network signaling after PICF7 treatment. Additionally, the ARF family of TFs regulates a broad range of plant responses to auxin (Tiwari et al. 2003). Up-regulation of *ARF2* and *WRKY5* may contribute to elicit a SAR response in olive as a consequence of PICF7 colonization. Moreover, a vast number of new candidate genes participating in this beneficial interaction, such as intrinsic membrane proteins, catalases (CATs), or purine permeases related to cellular communication, carbohydrates, and ZIP family iron transporters, were identified. Gómez-Lama Cabanás et al. (2014) aimed to elucidate whether similar systemic defense responses were also triggered in "Arbequina" aerial tissues upon

root inoculation by PICF7. In this case, sequencing of 1344 ESTs provided a set of 376 induced unigenes. Computational analysis revealed that many of them were potentially involved, as observed for roots tissues, in plant defense, and response to different stresses. Percentage of sequences homologous to woody plants was somewhat lower than that found in root tissues (34.6 %), while percentage of sequences showing significant identity with olive genes was higher (4.3 %). Interestingly, some genes involved in defense response were up-regulated in both tissues. This suggested that strain PICF7 could play an important role as a BCA against olive pathogens other than *V. dahliae* through a systemic defense mechanism (i.e., ISR). Among others, genes involved in plant hormones and phenylpropanoids biosynthesis (i.e., *PAL*, *ACL*, *ACO*, *LOX-2*), oxidative stress (*CAT*), and Ca^{2+} metabolism implicated in systemic defensive responses were induced in above-ground organs. In addition, expression of several TFs related to plant defense were also up-regulated, i.e., JERF, bHLH, and WRKY (Gómez-Lama Cabanás et al. 2014). In their study, five genes *ACO*, *ACL*, *LOX-2*, *CAT,* and *PAL*, all related to plant defense responses, were then selected for validation of the SSH library at different time points. *ACO* was moderately up-regulated in aerial tissues upon root colonization by PICF7. The putative olive *CAT*, also found as up-regulated gene in root tissues (Schilirò et al. 2012), was highly induced at middle-term after inoculation. Calmodulin (CaM) and other Ca^{2+}-related proteins were induced in aerial tissues as well, suggesting that the complex Ca^{2+}/CaM can decrease H_2O_2 levels in plants by activating CATs, supporting their possible role in plant defense responses as previously reported (Yang and Poovaiah 2002). Olive *LOX-2* was found to be up-regulated in the long term, and another *LOX* gene implicated in biosynthesis of JA was induced in olive roots (Schilirò et al. 2012). The same *ACL* found as up-regulated in roots (Schilirò et al. 2012) was also induced in aerial tissues. The induction of the *PAL* gene in both olive roots (Schilirò et al. 2012) and aerial tissues (Gómez-Lama Cabanás et al. 2014) upon PICF7 treatment indicates that this defense response pathway is activated after PICF7 colonization, and that this BCA seems to be recognized by the host plant, at least transiently (maximum relative expression at middle term), as a stress-inducing agent. Overall, middle-term up-regulation of defense-related genes in olive aerial tissues could be a response of the plant to defend against strain PICF7 colonization, whereas the subsequent decrease in gene expression could indicate that presence of this endophytic bacterium in roots is somehow recognized as "non-hostile." How the plant response is attenuated or overcome by PICF7 remains to be elucidated.

References

Alagna F, Kallenbach M, Pompa A et al (2016) Olive fruits infested with olive fly larvae respond with an ethylene burst and the emission of specific volatiles. J Integr Plant Biol 58(4):413–425

Albanese G, Saponari M, Faggioli F (2012) Phytosanitary certification. In: Muzzalupo I (ed) Olive germplasm—the olive cultivation, table and olive oil industry in Italy. InTech Publisher, Rijeka, Croatia, pp 107–132

Almeida RPP, Nanni L (2015) How do plant diseases caused by *Xylella fastidiosa* emerge? Plant Dis 99 (11):1457–1467

Ant T, Koukidou M, Rempoulakis P et al (2012) Control of the olive fruit fly using genetics-enhanced sterile insect technique. BMC Biol 10:51

Atkinson NJ, Dew TP, Orfila C, Urwin PE (2011) Influence of combined biotic and abiotic stress on nutritional quality parameters in tomato (*Solanum lycopersicum*). J Agric Food Chem 59(17):9673–9682

Augustinos AA, Mamuris Z, Stratikopoulos EE et al (2005) Microsatellite analysis of olive fly populations in the Mediterranean indicates a westward expansion of the species. Genetica 125:231–241

Baídez AG, Gomez P, Del Rio JA et al (2007) Dysfunctionality of the xylem in *Olea europaea* L. plants associated with the infection process by *Verticillium dahliae* Kleb. Role of phenolic compounds in plant defense mechanism. J Agric Food Chem 55:3373–3377. doi:10.1021/jf063166d

Belaj A, Satovic Z, Rallo L et al (2002) Genetic diversity and relationships in olive (*Olea europaea* L.) germplasm collections as determined by randomly amplified polymorphic DNA. Theor Appl Genet 105 (4):638–644

Benitez Y, Botella MA, Trapero A et al (2005) Molecular analysis of the interaction between *Olea europaea* and the biotrophic fungus *Spilocaea oleagina*. Mol Plant Pathol 6(4):425–438

Ben-Yosef M, Pasternak Z, Jurkevitch et al (2015) Symbiotic bacteria enable olive fly larvae to overcome host defences. Roy Soc Open Sci. doi:10.1098/rsos.150170

Bilgin DD, Zavala JA, Zhu J et al (2010) Biotic stress globally downregulates photosynthesis genes. Plant, Cell Environ 33:1597–1613

Boccaccio L, Petacchi R (2009) Landscape effects on the complex of *Bactrocera oleae* parasitoids and implications for conservation biological control. Biocontrol 54:607–616

Bosso L, Di Febbraro M, Cristinzio G et al (2015) Potential distribution of 260 *Xylella fastidiosa* in Italy: a maximum entropy model. Mediterr, Phytopathol. doi:10.14601/Phytopathol_Mediterr-16429

Botella MA, Trapero A, Alsalimya M et al (2005) Molecular analysis of the interaction between *Olea europaea* and the biotrophic fungus *Spilocaea oleagina*. Mol Plant Pathol 6:425–438

Broumas T, Haniotakis G, Liaropoulos C et al (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. J Appl Entomol 126:217–223

Bubici G, Cirulli M (2012) Control of verticillium wilt of olive by resistant rootstocks. Plant Soil 352:363–376

Bueno AM, Jones O (2002) Alternative methods for controlling the olive fly, *Bactrocera oleae*, involving semiochemicals. IOBC wprs Bulletin 25:1–11

Burrack HJ, Zalom FG (2008) Olive fruit fly (Diptera: Tephritidae) ovipositional preference and larval performance in several commercial important olive varieties in California. Ecol Behav 101:750–758

Burrack HJ, Bingham R, Price R et al (2011) Understanding the seasonal and reproductive biology of olive fruit fly is critical to its management. Calif Agric 65:14–20

Capuzzo C, Firrao G, Mazzon L et al (2005) 'Candidatus Erwinia dacicola', a coevolved symbiotic bacterium of the olive fly *Bactrocera oleae* (Gmelin). Int J Syst Evol Microbiol 55:1641–1647

Cardoso JSM, Felix MR, Clara MIE et al (2005) The complete genome sequence of a new necrovirus isolated from *Olea europaea* L. Arch Virol 150:815–823

Cariddi C, Saponari M, Boscia D et al (2014) Isolation of a Xylella fastidiosa strain infecting olive and oleander in Apulia, Italy. J Plant Pathol 96:425–429

Coletta-Filho HD, Francisco CS, Lopes JRS et al (2016). First report of olive leaf scorch in Brazil, associated with *Xylella fastidiosa* subsp. *pauca*. Phytopathol Mediterr. doi:10.14601/Phytopathol_Mediterr-17259

Cornara D, Loconsole G, Boscia D et al (2014) Survey of auchenorrhyncha in the Salento Peninsula in search of putative vectors of *Xylella fastidiosa* subsp. pauca CoDiRO strain. International symposium on the European outbreak of *Xylella fastidiosa* in olive. J Plant Pathol 96(S4):104

Corrado G, Alagna F, Rocco M et al (2012) Molecular interactions between the olive and the fruit fly *Bactrocera oleae*. BMC Plant Biol 12:1–17

Crovetti A, Quaglia F, Loi G et al (1982) Influence of temperature and humidity on the development of the immature stages of *Dacus oleae* (Gmelin). Frustula Entomol 5:133–166

Cruz F, Julca I, Gómez-Garrido J et al (2016) Genome sequence of the olive tree, *Olea europaea*. Giga Sci 5:29

Cutuli M, Campisi G, Marra FP et al (2011) Vegetative growth and ecophysiological aspects in young olive plants inoculated with Olive leaf yellowing-associated virus (OLYaV). Acta Italus Hortus 1:356–361

Daane KM, Johnson MW (2010) Olive fruit fly: managing an ancient pest in modern times. Annu Rev Entomol 55:151–169

Daane KM, Wang XG, Nieto DJ et al (2015) Classical biological control of olive fruit fly in California, USA: release and recovery of introduced parasitoids. Biocontrol 60:317–330

Daayf F, Nicole M, Boher B et al (1997) Early vascular defense reactions of cotton roots infected with a defoliating mutant strain of *Verticillium dahliae*. Eur J Plant Pathol 103:125–136

de Alfonso I, Vacas S, Primo J (2014) Role of a-copaene in the susceptibility of olive fruits to *Bactrocera oleae* (Rossi). J Agric Food Chem 62:11976–11979

Diatchenko L, Lau YF, Campbell AP et al (1996) Suppression subtractive hybridization: a method for generating differentially regulated or tissue-specific cDNA probes and libraries. Proc Natl Acad Sci USA 9:6025–6030

Dogaç E, Kandemir İ, Taskin V (2013) The genetic polymorphisms and colonization process of olive fly populations in Turkey. PLoS ONE 8:e56067

Economopoulos A (2002) The olive fruit fly, *Bactrocera* (*Dacus*) *oleae* (Gmelin) (Diptera: Tephritidae): its importance and control; previous SIT research and pilot testing. Report to International Atomic Energy Agency (IAEA), Vienna, p 44

Economopoulos A, Zervas G (1982) The quality problem in olive flies produced for SIT experiments. In: International symposium on the sterile insect technique and the use of radiation in genetic insect control, Neuherberg, IAEA Proceedings series, 29 June–3 July 1981, p 495

Economopoulos AP, Haniotakis GE, Mathioudis J et al (1978) Long-distance flight of wild and artificially-reared *Dacus oleae* (Gmelin) (Diptera, Tephritidae). Z Angew Entomol 87:101–108

Estes AM, Hearn DJ, Burrack HJ et al (2012) Prevalence of Candidatus *Erwinia dacicola* in wild and laboratory olive fruit fly populations and across developmental stages. Environ Entomol 41:265–274

Estrada-Hernandez MG, Valenzuela-Soto JH, Ibarra-Laclette E et al (2009) Differential gene expression in whitefly Bemisia tabaci-infested tomato

(*Solanum lycopersicum*) plants at progressing developmental stages of the insect's life cycle. Physiol Plant 137(1):44–60

Faggioli F, Barba M (1995) An elongated virus isolated from olive (*Olea europaea* L.). Acta Hortic 386:593–599

Felix MR, Clara MIE (2001) Detection of tobacco necrosis virus and *Olpidium brassicae* in *Olea europaea* L. in soil and in field trees. In: Proceedings of the 11th congress of the mediterranean phytopathological union, Lisbon, Portugal, pp 40–42

Felix MR, Cardoso JMS, Oliveira S et al (2007) Biological and molecular charac-terization of olive latent virus 1. Plant viruses. Global Science Books, Isleworth, UK, pp 170–177

Ferretti L, Faggioli F, Pasquaini G et al (2002) Strawberry latent ringspot virus (SLRSV) cause of differentiation among Raggiola and Frantoio olive cultivars. J Plant Pathol 84(182):187

Fletcher BS, Pappas S, Kapatos ET (1978) Changes in the ovaries of olive flies (Dacus oleae (Gmelin)) during summer and their relationship to temperature, humidity and fruit availability. Ecol Entomol 3:99–107

Giampetruzzi A, Chiumenti M, Saponari M et al (2015) Draft genome sequence of the *Xylella fastidiosa* CoDiRO strain. Genome Announc 3(1) doi:10.1128/genomeA.01538-14

Giannoulia K, Banilas G, Hatzopoulos P (2007) Oleosin gene expression in olive. J Plant Phys 164(1):104–107

Gkizi D, Santos-Rufo A, Rodríguez-Jurado D et al (2015) The β-amylase genes: negative regulators of disease resistance for *Verticillium dahliae*. Plant Pathol 64 (6):1484–1490

Godena LM, S, Dermic E, Barba M et al (2011) Detection of viruses in olive trees in Croatian Istria. Phytopathol Mediteranea 50:150–153

Gómez-Lama Cabanás C, Schilirò E, Valverde-Corredor A et al (2014) The biocontrol endophytic bacterium *Pseudomonas fluorescens* PICF7 induces systemic defense responses in aerial tissues upon colonization of olive roots. Front Microbiol 5:427

Gómez-Lama Cabanás C, Schilirò E, Valverde-Corredor A et al (2015) Systemic responses in a tolerant olive (*Olea europaea* L.) cultivar upon root colonization by the vascular pathogen *Verticillium dahliae*. Front Microbiol 6:928

Gonçalves MF, Malheiro R, Casal S et al (2012) Influence of fruit traits on oviposition preference of the olive fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), on three Portuguese olive varieties (Cobrançosa, Madural and Verdeal Transmontana). Sci Hortic 145:127–135

Gong P, Epton MJ, Fu G et al (2005) A dominant lethal genetic system for autocidal control of the Mediterranean fruit fly. Nat Biotechnol 23:453–456

Gucci R, Caruso G, Canale A et al (2012) Qualitative changes of olive oils obtained from fruits damaged by *Bactrocera oleae* (Rossi). HortScience 47:301–306

Gutierrez AP, Ponti L, Cossu QA (2009) Effects of climate warming on olive and olive fly (*Bactrocera oleae* (Gmelin)) in California and Italy. Clim Change 95:195–217

Haelterman RM, Tolocka PA, Roca ME et al (2015) First presumptive diagnosis of *Xylella fastidiosa* causing olive scorch in Argentina. J Plant Pathol 97:393

Haniotakis G, Kozyrakis M, Fitsakis T (1991) An effective mass trapping method for the control of *Dacus oleae* (Diptera: Tephritidae). J Econ Entomol 84:564–569

Hardoim PR, van Overbeek LS, Berg G et al (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev. doi:10.1128/MMBR.00050-14

Henriques MIC, Rei FT, Leitão FA et al (1992) Virus diseases in *Olea europaea* L. cultivars. I. Immunodiagnosis of Strawberry latent ringspot virus. Phytopathol Med 31:127–132

Hoelmer KA, Kirk AA, Pickett CH et al (2011) Prospects for improving biological control of olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae), with introduced parasitoids (Hymenoptera). Biocontrol Sci Technol 21:1005–1025

Hopkins DL, Purcell AH (2002) *Xylella fastidiosa*: cause of Pierce's disease of grapevine and other emergent diseases. Plant Dis 86:1056–1066

Iannotta N, Scalercio S (2012) Susceptibility of cultivars to Biotic Stresses. In: Muzzalupo I (ed) Olive germplasm and olive cultivation, table olive and olive oil industry in Italy. InTech, Rijeka, pp 81–106

Iannotta N, Perri L, Tocci C et al (1999) The behaviour of different olive cultivars following attacks by *Bactrocera oleae* (Gmel.). Acta Hortic 474:545–548

Iannotta N, Condello L, Perri L et al (2006a) Valutazione di suscettibilità di genotipi di olivo nei confronti di *Bactrocera oleae* (Gmel.). Italus Hortus 13 (2):242–245

Iannotta N, Macchione B, Noce M et al (2006b) Olive genotypes susceptibility to the *Bactrocera oleae* (Gmel.) infestation. In: Proceedings of the second international seminar on "biotechnology and quality of olive tree products around the mediterranean basin", Olivebioteq, Marsala-Mazara del Vallo, vol II, 5–10 Nov 2006, pp 261–266

Iannotta N, Belfiore T, Monardo D et al (2007) Indagine nel germoplasma dell'olivo sul comportamento di numerosi genotipi in relazione alla loro suscettibilità agli attacchi parassitari. Studi Trent Sci Nat Acta Biol 83:215–220

Kakani EG, Mathiopoulos KD (2008) Organophosphate resistance-related mutations in the acetylcholinesterase gene of tephritidae. J Appl Entomol 132:762–771

Kakani EG, Bon S, Massoulié J et al (2011) Altered GPI modification of insect AChE improves tolerance to organophosphate insecticides. Insect Biochem Mol Biol 41:150–158

Katsoyannos BI, Kouloussis NA (2001) Captures of the olive fruit fly *Bactrocera oleae* on spheres of different colours. Entomol Exp Appl 100:165–172

Knipling E (1955) Possibilities of insect control or eradication through the use of sexually sterile males. J Econ Entomol 48:459–462

Kombargi WS, Michelakis SE, Petrakis CA (1998) Effect of olive surface waxes on oviposition by Bactrocera oleae (Diptera: Tephritidae). J Econ Entomol 91 (4):993–998

Konno K, Hirayama C, Yasui H et al (1999) Enzymatic activation of oleuropein: A protein crosslinker used as a chemical defense in the privet tree. Proc Natl Acad Sci USA 96:9159–9164

Koudounas K, Banilas G, Michaelidis C et al (2015) A defence-related *Olea europaea* β-glucosidase hydrolyses and activates oleuropein into a potent protein cross-linking agent. J Exp Bot 66(7):2093–2106

Koukidou M, Klinakis A, Reboulakis C et al (2006) Germ line transformation of the olive fly *Bactrocera oleae* using a versatile transgenesis marker. Insect Mol Biol 15:95–103

Krugner R, Sisterson MS, Chen JC et al (2014) Evaluation of olive as a host of *Xylella fastidiosa* and associated sharpshooter vectors. Plant Dis 98:1186–1193

Kültz D, Fiol D, Valkova N et al (2007) Functional genomics and proteomics of the cellular osmotic stress response in 'non-model' organisms. J Exp Biol 210 (9):1593–1601

Larroque M, Belmas E, Martinez T et al (2013) Pathogen-associated molecular pattern-triggered immunity and resistance to the root pathogen *Phytophthora parasitica* in *Arabidopsis*. J Exp Bot 64 (12):3615–3625

Leyva-Pérez MO, Valverde-Corredor A, Valderrama R et al (2015) Early and delayed long-term transcriptional changes and short-term transient responses during cold acclimation in olive leaves. DNA Res 22:1–11

Lo Scalzo R, Scarpati ML, Verzegnassi B et al (1994) *Olea europaea* chemicals repellent to *Bactrocera oleae* females. J Chem Ecol 20:1813–1823

Lobão DL, Felix MR, Clara MIE et al (2002) Detection of Olive latent virus 1 in Olea europaea L. tis-sues by reverse transcription-polymerase chain reaction. XIII Congreso Nacional de Bioquimica, Lisbon, Portugal, p 102

Loconsole G, Saponari M, Faggioli F et al (2010) Inter-laboratory validation of a PCR-based protocol for detection of olive viruses. Bull OEPP/EPPO Bull 40:423–428

Loconsole G, Potere O, Boscia D et al (2014) Detection of *Xylella fastidiosa* in olive trees by molecular and serological methods. J Plant Pathol 96:1–8

López-Escudero FJ, Mercado-Blanco J (2011) Verticillium wilt of olive: a case study to implement an integrated strategy to control a soil-borne pathogen. Plant Soil 344:1–50

Luigi M, Godena S, Dermic E et al (2011) Detection of viruses in olive trees in Croatian Istria. Phytopathol Mediterr 50:150–153

Maldonado-González MM, Bakker PAHM, Prieto P et al (2015) *Arabidopsis thaliana* as a tool to identify traits involved in *Verticillium dahlia* biocontrol by the olive root endophyte *Pseudomonas fluorescens* PICF7. Front Microbiol 6:266

Malheiro R, Casal S, Baptista P et al (2015a) A review of *Bactrocera oleae* (Rossi) impact in olive products: from the tree to the table. Trend Food Sci Technol 44:226–242

Malheiro R, Casal S, Baptista P et al (2015b) Physico-chemical characteristics of olive leaves and fruits and their relation with *Bactrocera oleae* (Rossi) cultivar oviposition preference. Sci Hortic 194:208–214

Malheiro R, Casal S, Cunha SC et al (2015c) Olive volatiles from Portuguese cultivars Cobrançosa, Madural and Verdeal Transmontana: role in oviposition preference of *Bactrocera oleae* (Rossi) (Diptera: Tephritidae). PLoS ONE 10:1–15

Malheiro R, Casal S, Cunha SC et al (2016) Identification of leaf volatiles from olive (*Olea europaea*) and their possible role in the ovipositional preferences of olive fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae). Phytochemistry 121:11–19

Margaritopoulos JT, Skavdis G, Kalogiannis N et al (2008) Efficacy of the pyrethroid alpha-cypermethrin against *Bactrocera oleae* populations from Greece, and improved diagnostic for an iAChE mutation. Pest Manag Sci 64:900–908

Markakis EA, Tjamos SE, Antoniou PP et al (2010) Phenolic responses of resistant and susceptible olive cultivars induced by defoliating and non-defoliating *Verticillium dahliae* pathotypes. Plant Dis 94:1156–1162

Marte M, Gadani F, Savino V et al (1986) Strawberry latent ringspot virus associated with a new disease of olive in central Italy. Plant Dis 70:171–172

Martelli GP, Yilmaz MA, Savino V et al (1996) Properties of a citrus isolate of Olive latent virus 1, a new necrovirus. Eur J Plant Pathol 102:527–536

Martínez-García PM, Ruano-Rosa D, Schilirò E et al (2015) Complete genome sequence of *Pseudomonas fluorescens* strain PICF7 an indigenous root endophyte from olive (*Olea europaea* L.) and effective biocontrol agent against *Verticillium dahliae*. Stand Genomic Sci 10:10.doi:10.1186/1944-3277-10-10

Massei G, Hartley SE (2000) Disarmed by domestication? Induced responses to browsing in wild and cultivated olive. Oecologia 122(2):225–231

Mazomenos BE (1989) Estimates of the crop losses caused by *Dacus oleae* (Gmel.) (Diptera, Tephritidae) in Crete. In: Robinson AS, Hooper G (eds) Fruit flies of economic importance. Elsevier Science Publishers, Amsterdam, The Netherlands, pp 69–177

Mekuria GT, Collins G, Sedgley M et al (2001) Identification of genetic-markers in olive linked to olive leaf-spot resistance and susceptibility. J Am Soc Hortic Sci 126:305–308

Mercado-Blanco J (2015) Life of microbes inside the plant. In: Lugtenberg B (ed) Principles of plant-microbe interactions. Springer, Switzerland, pp 25–32. doi:10.1007/978-3-319-08575-3_5

Mercado-Blanco J, Lugtenberg BJJ (2014) Biotechnological applications of bacterial endophytes. Curr Biotechnol 3:60–75

Mercado-Blanco J, Rodríguez-Jurado D, Hervás A et al (2004) Suppression of verticillium wilt in olive

planting stocks by root-associated fluorescent *Pseudomonas* spp. Biol Control 30:474–486
Mueller WC, Morgham AT (1993) Ultrastructure of the vascular responses of cotton to *Verticillium dahliae*. Can J Bot 71:32–36
Muleo R, Morgante M, Velasco R et al (2012) Olive tree genomic. In: Mazzalupo I (ed) Olive germoplasm—the olive cultivation, table and olive oil industry in Italy. InTech Publisher, Rijeka, Croatia, pp 133–148
Nardi F, Carapelli A, Dallai R et al (2005) Population structure and colonization history of the olive fly, *Bactrocera oleae* (Diptera, Tephritidae). Mol Ecol 14:2729–2738
Nardi F, Carapelli A, Boore JL et al (2010) Domestication of olive fly through a multiregional host shift to cultivated olives: comparative dating using complete mitochondrial genomes. Mol Phylogenet Evol 57:678–686
Neuenschwander P, Michelakis S, Holloway P et al (1985) Factors affecting the susceptibility of fruits of different olive varieties to attack of *Dacus oleae* (Gmel.) (Dipt., Tephritidae). J Appl Entomol 100:174–188
Nigro F, Boscia D, Antelmi I et al (2013) Fungal species associated with a severe decline of olive in southern Italy. J Plant Pathol 95(3):668
Noce ME, Belfiore T, Scalercio S et al (2009) Efficacy of new mass-trapping devices against *Bactrocera oleae* (Diptera Tephritidae) for minimizing pesticide input in agroecosystems. J Environ Sci Health B 44(5):442–448
Noce ME, Perri E, Scalercio S et al (2014) Phenolic compounds and susceptibility of olive cultivars to Bactroceraoleae (Diptera: Tephritidae) infestations and complementary aspects: a review. Acta Hortic 1057:177–184
Obanor FO, Jaspers MV, Jones EE et al (2008) Greenhouse and field evaluation of fungicides for control of olive leaf spot in New Zealand. Crop Protect 27:1335–1342
Ogaugwu CE, Schetelig MF, Wimmer EA (2013) Transgenic sexing system for *Ceratitis capitata* (Diptera: Tephritidae) based on female-specific embryonic lethality. Insect Biochem Mol Biol 43(1):1–8
Ouyang B, Yang T, Li HX et al (2007) Identification of early salt stress response genes in tomato root by suppression subtractive hybridization and microarray analysis. J Exp Bot 58(3):507–520
Pavlidi N, Dermauw W, Rombauts S et al (2013) Analysis of the olive fruit fly *Bactrocera oleae* transcriptome and phylogenetic classification of the major detoxification gene families. PLoS ONE 8:e66533
Petacchi R, Rizzi I, Guidotti D (2003) The "lure and kill" technique in *Bactrocera oleae* (Gmel.) control: effectiveness indices and suitability of the technique in area-wide experimental trials. Int J Pest Manag 49:305–311
Prieto P, Navarro-Raya C, Valverde-Corredor A et al (2009) Colonization process of olive tissues by *Verticillium dahliae* and its in planta interaction with the biocontrol root endophyte *Pseudomonas fluorescens* PICF7. Microb Biotechnol 2:499–511
Prieto P, Schiliro E, Maldonado-González MM et al (2011) Root hairs play a key role in the endophytic colonization of olive roots by *Pseudomonas* spp. With biocontrol activity. Microb Ecol 62:435–445
Purcell AH (1997) Xylella fastidiosa, a regional problem or global threat? J Plant Pathol 79:99–105
Reinhold-Hurek B, Hurek T (2011) Living in side plants: bacterial endophytes. Curr Opin Plant Biol 14:1–9
Rempoulakis P, Dimou I, Chrysargyris A et al (2014) Improving olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae) adult and larval artificial diets, microflora associated with the fly and evaluation of a transgenic olive fruit fly strain. Int J Trop Insect Sci 34 (Suppl S1):114–122
Rice RE (2000) Bionomics of the olive fruit fly *Bactrocera (Dacus) oleae*. UC Plant Protec Quart 10:1–5
Rice RE, Phillips PA, Stewart-Leslie J et al (2003) Olive fruit fly populations measured in central and southern California. Calif Agric 57:122–127
Rizzo R, Caleca V, Lombardo A (2012) Relation of fruit color, elongation, hardness, and volume to the infestation of olive cultivars by the olive fruit fly, *Bactrocera oleae*. Entomol Exp Appl 145:15–22
Roschetti A, Ferretti L, Muzzalupo I et al (2009) Evaluation of the possibile effect of virus infections on olive propagation. Petria 19:18–28
Rosenblueth M, Martínez-Romero E (2006) Bacterial endophytes and their interactions with hosts. Mol Plant-Microbe Interact 19:827–837
Sabanadzovic S, Abou-Ghanem N, La Notte P et al (1990) Partial molecular characterization and RT-PCR detection of a putative closterovirus associated with olive leaf yellowing. J Plant Pathol 81:37–45
Sacchetti P, Landini S, Granchietti A et al (2007) Attractiveness to the olive fly of *Pseudomonas putida* isolated from the foregut of *Bactrocera oleae*. IOBC/WPRS Bulletin 30:37–42
Sacchetti P, Granchietti A, Landini S et al (2008) Relationships between the olive fly and bacteria. J Appl Entomol 132:682–689
Sagri E, Reczko M, Gregoriou M-E et al (2014a) Olive fly transciptomics analysis implicates energy metabolism genes in spinosad resistance. BMC Genom 15:714
Sagri E, Reczko M, Tsoumani KT et al (2014b) The molecular biology of the olive fly comes of age. BMC Genet 15(Suppl 2):58
Sanfaçon H, Iwanami T, Karasev AV et al (2011) Family Secoviridae. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ (eds) Virus taxonomy. Ninth report of the international committee on taxonomy of viruses. Elsevier-Academi Press, Amsterdam, The Netherlands, pp 881–899
Saponari M, Boscia D, Nigro F et al (2013) Identification of DNA sequences related to Xylella fastidiosa in oleander, almond and olive trees exhibiting leaf scorch symptoms in Apulia (southern Italy). J Plant Pathol 95 (3):668
Saponari M, Loconsole G, Almeida R et al (2014) Isolation, genotyping and preliminary data on the pathogenicity of Xylella fastidiosa CoDiRO strain.

International Symposium on the European Outbreak of Xylella fastidiosa in Olive. J Plant Pathol 96:S4.103
Saponari M, Savino V, Martelli GP (2002a) Preliminary results of sanitation trials of viruses infecting olive. J Plant Pathol 84:176
Saponari M, Savino V, Martelli GP (2002b) Trasmissione per seme di virus dell'olivo. Frutticoltura 64(4):103–105
Savino V, Sabanadzovic S, Scarito G et al (1996) Due giallumi dell'olivo di possibile origine virale. Informatore Fitopatologico 46(5):55–59
Scarpati ML, Lo Scalzo R, Vita G (1993) *Olea europaea* volatiles attractive and repellent to the olive fruit fly (*Dacus oleae*, Gmelin). J Chem Ecol 19:881–891
Scarpati ML, Lo Scalzo R, Vita G et al (1996) Chemiotropic behavior of female olive fly (*Bactrocera oleae* Gmel.) on *Olea europaea* L. J Chem Ecol 22:1027–1036
Schetelig MF, Caceres C, Zacharopoulou A et al (2009) Conditional embryonic lethality to improve the sterile insect technique in *Ceratitis capitata* (Diptera: Tephritidae). BMC Biol 7(1):4
Schilirò E, Ferrara M, Nigro F et al (2012) Genetic responses induced in olive roots upon colonization by the biocontrol endophytic bacterium *Pseudomonas fluorescens* PICF7. PLoS ONE 7:e48646
Schnathorst WC, Sibbett GS (1971) The relations of strains of *Verticillium albo-atrum* to severity of Verticillium wilt in *Gossypium hirsutum* and *Olea europaea* in California. Plant Dis Rep 55:780–782
Sistani F, Ramezanpour SS, Nasrollanejad S (2009) Field evaluation of different fungicides application to control olive leaf spot. Aus J Basic Appl Sci 3(4):3341–3345
Spadafora A, Mazzuca S, Chiappetta FF et al (2008) Oleuropein-specific-b- glucosidase activity marks the early response of olive fruits (*Olea europaea*) to mimed insect attack. Agric Sci China 7:703–712
Thomas DD, Donnelly CA, Wood RJ et al (2000) Insect population control using a dominant, repressible, lethal genetic system. Science 287:2474–2476
Tiwari SB, Hagen G, Guilfoyle TJ (2003) The roles of auxin response factor domains in auxin-responsive transcription. Plant Cell 15:533–543
Trapero A, Blanco MA (2001) Enfermedades. In: Barranco D, Fernández-Escobar R, Rallo L (eds) El Cultivo Del Olivo. Mundi-Prensa-Junta de Andalucía, Madrid, Spain, pp 497–549
Triolo E, Materazzi A, Toni S (1996) An isolate of tobacco mosaic tobamovirus form *Olea europaea*. Adv Hortic Sci 10:39–45
Tzanakakis ME (2003) Seasonal development and dormancy of insects and mites feeding on olive: a review. Netherland J Zool 52(2–4):87–224
Tzanakakis ME, Koveos DS (1986) Inhibition of ovarian maturation in the olive fruit fly *Dacus oleae* (Diptera: Tephritidae), under long photophase and an increase of temperature. Ann Entomol Soc Am 79:15–18
Unver T, Turktas M, Dorado G et al (2016) De novo whole genome sequencing of olive tree (*Olea europaea* L.). In: Abstract of the XXIV international plant & animal genome, San Diego, CA, USA, 8–13 Jan 2016, p 1164
van Asch B, Pereira-Castro I, Rei F et al (2012) Mitochondrial haplotypes reveal olive fly (*Bactrocera oleae*) population substructure in the Mediterranean. Genetica 140:181–187
Vanneste JL, Voyle MD, Zydenbos SM (2003) Genetic basis of copper resistance in New Zealand strains of *Pseudomonas syringae*. In: New Zealand plant protection. Proceedings of a conference, Chateau on the-Park, Christchurch, New Zealand, 12–14 Aug 2003, pp 109–112
Varanda CMR, Silva MS, Felix MR et al (2011) Evidence of olive mild mosaic virus transmission by *Olpidium brassicae*. Eur J Plant Pathol 130:165–172
Viruega JR, Moral J, Roca LF et al (2013) *Spilocaea oleagina* in olive groves of southern Spain: survival, inoculum production, and dispersal. Plant Dis 97:1549–1556
Vlahov G (1992) Flavonoids in three olive (*Olea europaea*) fruit varieties during maturation. J Sci Food Agri 58:157–159
Vontas JG, Hejazi MJ, Hawkes NJ et al (2002) Resistance-associated point mutations of organophosphate insensitive acetylcholinesterase, in the olive fruit fly *Bactrocera oleae*. Insect Mol Biol 11:329–336
Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet 10:57–63
Wang XG, Johnson MW, Yokoyama VY et al (2011) Comparative evaluation of two olive fruit fly parasitoids under varying abiotic conditions. Bio Control 56:283–293
Wilhelm S (1955) Longevity of the Verticillium wilt fungus in the laboratory and field. Phytopathology 45:180–181
Yadeta KA, Thomma BPHJ (2013) The xylem as battleground for plant hosts and vascular wilt pathogens. Front Plant Sci 4:97
Yang T, Poovaiah BW (2002) Hydrogen peroxide homeostasis: activation of plant catalase by calcium/calmodulin. Proc Natl Acad Sci USA 99:4097–4102
Yaseen T, Drago S, Valentini F et al (2015) On-site detection of *Xylella fastidiosa* in host plants and in "spy insects" using the real-time loop-mediated isothermal amplification method. Phytopathol Mediterr 54:488–496
Yokoyama V (2015) Olive fruit fly (Diptera: Tephritidae) in California table olives, USA: invasion, distribution, and management implications. J Integr Pest Manag 6 (1):14. doi:10.1093/jipm/pmv014
Zygouridis NE, Augustinos AA, Zalom FG et al (2009) Analysis of olive fly invasion in California based on microsatellite markers. Heredity 102:402–412