EIP-AGRI Focus Group

‘Pests and diseases of the olive tree’

Biocontrol agents and cropping practices to control olive diseases

BELÉN ÁLVAREZ, WILLY COUANON, JUAN OLIVARES, FRANCO NIGRO

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1. Introduction

Non chemical-based plant protection strategies such as the application of biological control agents (BCAs) are emerging as innovative technologies in modern sustainable agrosystems, supporting farmers and favouring implementation of the Sustainable Use of Pesticides Directive (Anonymous, 2009; IBMA, 2018) by promoting Integrated Pest Management and the use of ecologically safe substitutes to conventional pesticides. In accordance to that, a greater interest has been observed among scientists and agrochemical companies focused on developing new biocontrol methods based on BCAs as viable alternatives for management of plant pathogens.

The BCAs tested should have an unfavorable effect on the pathogen, either directly mainly by destroying it, producing substances that inhibit its growth, or competing with it for space and/or nutrients; or indirectly by stimulating plant defense responses. To achieve that, the BCAs must remain active in the susceptible crop during the presence of the pathogen.

However, in practice, there are some problems that often need to be overcome, based on:

***In vitro* activity observed for many BCAs in the laboratory does not necessarily correlate with *in vivo* or *in planta* activity in real field conditions**	↔	**Ecological processes taking place in crops during biocontrol activity by BCAs are very complex, limiting the success in the field (Xu *et al.*, 2011)**

Despite difficulties, a variety of biological control methods are available for use against some olive tree diseases, either alone or in combination with other disease management practices including conventional chemical methods or cultural practices. Further focus on a greater understanding of the complex interactions among plants, pathogens, BCAs and the environment would be required to optimize efficacy of BCAs in the field, which would be essential to minimize the losses caused by diseases, trying to reach full benefits of their use and ensuring profitability for the farmer.

2. Biocontrol agents against olive tree diseases

Verticillium wilt caused by the fungal pathogen ***Verticillium dahliae*** is the disease affecting olive trees for which more potential BCAs have been searched, since it is one of the most difficult to control by conventional methods due to the pathogen survival structures (called microsclerotia) which are able to persist in soil for several years remaining viable. However, despite the wide host range of the pathogen, BCAs against this disease have mainly been investigated in eggplant, cotton and oilseed rape, and just in few woody species including olive. This is probably due to the fact that BCAs are harder to find in woody plants and more research efforts would be needed (Deketelaere *et al.*, 2017).

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Potential bacterial and fungal BCAs against Verticillium wilt in olive plants are summarized in Tables 1 and 2, respectively.

Application of BCAs isolated from the host plant is usually considered a valid approach since they are better adapted to the pathogen ecological niche. In this sense, the rhizosphere of olive plants proved to be a source of indigenous bacteria with biocontrol potential. In general, endophytic *Bacillus* and *Pseudomonas* were the most frequently reported bacterial BCAs (Table 1), and non-pathogenic xylem-colonizing *Verticillium* and *Fusarium* the most frequent fungal BCAs (Table 2). Main biocontrol activities were inhibition of germination, plant growth promotion, competition and induced plant resistance. All of those BCAs were obtained from soils, organic amendments or healthy plants in infested fields (Deketelaere *et al.*, 2017). One of the best BCA known is *P. fluorescens* PICF7, a colonizer of olive roots that showed antagonism against *V. dahliae* and suppression of the disease in nursery-produced olive plants (Mercado-Blanco *et al.*, 2004; Maldonado-González, 2015). Also *Trichoderma* species, which are fungal BCAs targeting several diseases caused by soil-borne plant pathogens, were effective against *Verticillium*, being *T. asperellum* and *T. gamsii* the two species present in the only commercial bioformulation against this pathogen to be used in olive plants (Table 2).

Mycoviruses affecting *Verticillium* have also been searched as potential BCAs. However, plants inoculated with *V. dahliae* infected by mycoviruses just showed a delay in symptoms without significant differences in disease incidence (Mulero-Aparicio *et al.*, 2018), indicating that more research is needed to find effective viral BCAs against this pathogen.

Fungi comprising the *Colletotrichum acutatum* species complex are the causal agents of **olive anthracnose**, which can be controlled by different BCAs. Application under field conditions of *B. subtilis* (Table 1) and a sulfur-based product was effective in controlling the disease, whereas endophytic isolates of *Aureobasidium pullulans* (Table 2) provided high protection (Nigro *et al.*, 2018). On the other hand, fungal isolates belonging to *Alternaria*, *Diaporthe*, *Nigrospora* and *Xylariaceae* genera (Table 2) naturally present in olive trees induced growth inhibition of *C. acutatum* by production of volatile substances (Landum *et al.*, 2016), while epiphytic and endophytic fungal communities from olive trees were able to inhibit *C. acutatum* growth, sporulation and germination (Preto *et al.*, 2017).

BCAs were also found against **olive leaf spot** caused by *Spilocaea oleagina*. In this case, bacterial isolates of *Pseudomonas* and *Bacillus* (Table 1) were observed to have an inhibitory effect on conidial germination (Salman, 2017), whereas other *Bacillus* along with *Burkholderia* and *Corynebacterium* isolates (Table 1) showed antagonistic activity against the pathogen (Al-Khatib *et al.*, 2010).

The survival and multiplication of *Pseudomonas savastanoi* **pv.** *savastanoi*, the causal agent of **olive knot disease**, can be prevented by the phylloplane bacterium *Bacillus mojavensis* A-BC-7 (Table 1), which was isolated from symptomless olive trees and proved to be able to decrease pathogen populations and knot weights, with a production of less necrotic tumors after its application to olive plants (Ghanney *et al.*, 2016).

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Table 1. Potential bacterial biocontrol agents against olive tree diseases.

Bacterial Biocontrol Agent	Target Pathogen	References
Paenibacillus alvei K-165 *Acetobacter aceti* VIN02	*Verticillium* spp.	Deketelaere *et al.* (2017)*
Bacillus cereus NB-4, NB-5 *B. megaterium* NB-3	*Spilocea oleaginea*	Al-Khatib *et al.* (2010)
B. mojavensis A-BC-7	*Pseudomonas savastanoi* pv. *savastanoi*	Ghanney *et al.* (2016)
Bacillus sp. BAT	*Spilocaea oleagina*	Salman (2017)
Bacillus spp. PIC28	*Verticillium dahliae*	Cabanás *et al.* (2018b)
B. subtilis HNEB-1 *B. subtilis* NB-6	*Spilocea oleaginea*	Al-Khatib *et al.* (2010)
B. subtilis sp.	*Colletotrichum* spp.	Nigro *et al.* (2018)
Burkholderia mallei NB-8 *Corynebacterium xerosis* NB-2	*Spilocea oleaginea*	Al-Khatib *et al.* (2010)
Paenibacillus polymyxa PIC73 *P. terrae* PIC167	*Verticillium dahliae*	Cabanás *et al.* (2018b)
Pseudomonas aeruginosa group *PIC25, PIC105*	*Verticillium dahliae*	Cabanás *et al.* (2018a)
P. fluorescens PICF4, PICF6, PICF8 *P. fluorescens* PICF7	*Verticillium* spp.	Deketelaere *et al.* (2017)
P. indica PIC105 *P. mandelii* subgroup PICF141	*Verticillium dahliae*	Cabanás *et al.* (2018a)
P. putida PICP2 *P. putida* PICP5	*Verticillium* spp.	Deketelaere *et al.* (2017)
Pseudomonas sp. ORS3	*Spilocaea oleagina*	Salman (2017)

* Deketelaere *et al.* (2017) reviewed and referenced the original works.

Table 2. Potential fungal biocontrol agents against olive tree diseases.

Fungal Biocontrol Agent	Target Pathogen	References
Alternaria sp. 1, 2	*Colletotrichum acutatum*	Landum *et al.* (2016)
Aureobasidium pullulans AP06	*Verticillium* spp.	Deketelaere *et al.* (2017)*
Aureobasidium pullulans sp.	*Colletotrichum* spp.	Nigro *et al.* (2018)
Diaporthe sp.	*Colletotrichum acutatum*	Landum *et al.* (2016)
Fusarium moniliforme FM01 *F. moniliforme* FM02 *F. oxysporum* FO03, FO04 *F. oxysporum* FO12 *F. oxysporum* FO47 *Gliocladium roseum* GR01 *G. roseum* GR02	*Verticillium* spp.	Deketelaere *et al.* (2017)
Nigrospora oryzae sp.	*Colletotrichum acutatum*	Landum *et al.* (2016)

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Phoma sp. PH01	*Verticillium* spp.	Deketelaere *et al.* (2017)
Phoma sp. PH02		
Trichoderma asperellum Bt2, Bt3, T25	*Verticillium* spp.	Carrero-Carrón *et al.* (2016) Deketelaere *et al.* (2017)
T. asperellum + *T. gamsii* (BIOTEN®)	*Verticillium* spp.	Deketelaere *et al.* (2017)
T. asperellum + *T. gamsii* (REMEDIER®)		
Claroideoglomus claroideum (*G. claroideum*)		
Funneliformis mosseae (*G. mosseae*)		
Rhizophagus intraradices (*G. intraradices*)		
Xylariaceae	*Colletotrichum acutatum*	Landum *et al.* (2016)

* Deketelaere *et al.* (2017) reviewed and referenced the original works.

Further studies on the application of BCAs to increase their biocontrol efficacies in the field would be required to evaluate the optimal conditions or timing needed to reach successful implementation of their activities in olive groves, paving the way for biotechnological development of new formulations of microorganisms effective against olive diseases.

New ecological solutions to be explored for disease management are based on the potential of leaf and root-associated microbiomes (Syed Ab Rahman *et al.*, 2018), which should be applied in olive production in the next future. It is important to understand the contribution of microorganisms to plant growth promotion and disease biocontrol (Umesha *et al.*, 2018). In this sense, bacteria from soil and rhizosphere from cover crops in an olive grove pointed out to their suitability to be used as biofertilizers and biopesticides against *V. dahliae* and other pathogens (Pérez-Díaz *et al.*, 2018).

How to increase the use of Biological Control Agents against olive tree diseases in the field?

Despite the fact that there are a number of reported bacterial and fungal species and isolates with an activity against any of the main pathogens of the olive tree (Tables 1 and 2), in practice, they are rarely used by the farmer. This is because the antagonistic or deleterious activity against those pathogens initially observed for many of these isolates in the laboratory may not be maintained when applied as a treatment in a greenhouse and/or the field, resulting in scarce marketing and implementation of effective BCAs in the olive groves at field scale.

To solve this, a greater effort in carrying out more complete tests on the activity of the potential BCAs *in planta* either in the field or under controlled conditions resembling those of the field should be made, with a number of key points to be considered, mainly:

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- *Proper selection of candidates to be tested as BCAs,* based on: (i) the infection process (entrance in plant host tissues, latency period, colonization, survival) of the pathogen to be controlled, and (ii) the environmental factors (moisture, temperature) favorable for a successful establishment of the pathogen in the olive tree and development of the disease.

- *Type of soil,* especially for BCAs to be applied in the olive rhizosphere, since factors such as composition, structure and size of the soil particles, texture, pH values, water retention capacity and organic and inorganic material contents may have a direct effect on the activity of the tested BCAs.

- *Indigenous olive microbiota* from either the rhizosphere or phyllosphere, because they constitute well-established communities among which the BCAs should break through and carry out their activity against the pathogen. The BCA should be ecologically competent in the habitat where it will be applied.

- *The olive cultivar,* as different olive genotypes may exhibit different degrees of susceptibility or tolerance against a pathogen that may have a synergistic effect, or not, on the performance of the tested BCAs.

- *Aggressiveness or virulence of the pathogen.* This can have an influence in the variability of the biocontrol efficiency, since the potential BCAs will cope differently with isolates of the same pathogenic species that show different virulence towards the host.

- *Climatic conditions of the area concerned,* since weather changes from one area to another, which may influence BCA performance. It would be necessary to evaluate the effect of varying seasonal temperatures, relative humidity, and rainfall and wind events, among other environmental factors, on the effectiveness of the tested BCAs.

- *Adjustment of application parameters to the local conditions* of the olive grove where the BCA has to be effective. In addition to the above-mentioned factors, it should also be considered: (i) BCA concentration, (ii) type of treatment according to plot size, (iii) time, number and mode of application, and (iv) period of pathogen activity. Biocontrol efficiency requires continuous adaptation to each particular orchard condition.

- *Lack of environmental impact.* The potential BCAs should be tested to prove that they have no deleterious effects on other microorganisms, the olive tree and the environment, apart from the target pathogen.

- *Wide-scale evaluation of BCAs* regarding their performance in the field with the aim of establishing the best framework to achieve an effective implementation at field scale of the treatments with the most successful BCAs.

Once field-effective BCAs have been obtained against any of the olive tree diseases, the specialized biotechnology companies will have to deal with the factors determining the pace of market integration, as bioformulation, cost-effectiveness of the products and registration requirements which are also major limitations of the scheme. However, although the

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biotechnological development and marketing of new control products based on BCAs is complex and challenging, the major constriction of the whole process is to find BCAs that can be used at the field scale by farmers, with demonstrated biocontrol activity against olive tree diseases over a highest range of conditions as possible.

Another aspect that needs to be addressed is the transfer of the results (new knowledge, new products) to farmers, which can be done through agricultural extension services and/or advisers, ensuring that farmers can successfully implement these new available bioproducts based on BCAs in the right way to fit their needs and according to their local conditions.

3. Effect of cropping practices on olive tree diseases

Olive plantations are complex biodiverse ecosystems, hosting many microorganisms whose activities contribute to an optimal development of the cropping system, maintaining production without compromising sustainability. In some cases, **agricultural practices** as frequent **tillage** and the use of **agrochemicals** can have an impact on biodiversity, leading to ecosystem imbalance, increased pathogen resistance, loss of beneficial and/or antagonistic microorganisms and appearance of new pathogens due to elimination of competitors (Wezel *et al.*, 2014). **Fertilization** can also have an effect on the incidence of pathogens in olive groves. While appropriate mineral supply to the olive trees improves their nutritional status and defense mechanisms, nitrogen excess and potassium deficiency increase susceptibility to Verticillium wilt and some fungal foliar diseases such as olive leaf spot (Fernández-Escobar *et al.*, 2013; Fernández-Escobar, 2019). **Water supply** can be another factor having an impact on olive diseases, since irrigation favours root pathogen dissemination, increasing the possibility of infection by *V. dahliae*, *Phytophthora* spp. or other soil-borne pathogens, especially in high-density plantations. Also in dense plantations, moisture can accumulate on the leaves, favouring infections by aerial pathogens (Trapero and Blanco-López, 2010). On the other hand, **pruning** can favour control of aerial pathogens, decreasing the incidence of *S. oleagina*, *Colletotrichum*, *Pseudocercospora cladosporioides* and *P. savastanoi* pv. *savastanoi* due to an inoculum reduction after removal of affected parts as well as to a microclimate alteration of the tree canopy. Moreover, avoiding and/or protecting pruning wounds can decrease the incidence of wood decay fungi and some aerial pathogens (Fernández-Escobar *et al.*, 2013). In that sense, **harvesting methods** causing wounds in the olive tree favour olive knot development by *P. savastanoi* pv. *savastanoi*. Early harvesting prevents olives from alteration by *Colletotrichum* spp. and other fruit rot fungi.

Olive soil management strategies such as the use of **cover crops** can have beneficial effects against the development of some diseases. Main advantages would be:

- Cover crops protect the soil from erosion, increase organic matter and biological activity, as well as soil biological diversity and ecological complexity (Sharma *et al.*, 2018), since their root systems increase porosity, creating habitats for organisms.

- The plant species constituting the cover can affect soil- and/or air-borne pathogens, favouring population decreases of these pathogens and/or their survival forms.

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- The use of cover crops considered non-host for certain pathogens, or for which disease-causing organisms have no affinity, can either be an effective physical barrier to their dispersal or an effective way to decrease their populations or pathogenic activities.

- Cover plant species can be established in such a way that the weeds that could constitute natural sources or reservoirs of pathogens are suppressed by direct competition and/or the release of plant growth–inhibiting substances.

- Roots of cover species can produce sugars and/or aminoacids during their growth that can directly influence the composition and biomass of soil microorganisms, activating some beneficial ones with antagonistic activity against plant pathogens.

However, until now there is not much data available about the application of cover crops for disease management. Even when there seems to be an epidemiological risk involved, the eradication of cover crops in the olive groves is not recommended, since it might generate more inconveniences than advantages. In these cases, a proper management of the cover crops based on deep knowledge of biological cycles of the pathogen present in an orchard and its vectors would be more convenient. Moreover, cover crops can be beneficial to allow the development of possible predators of the disease vectors involved. With the use of cover crops and the incorporation of aerial biomass into the soil, a reduction in the incidence of diseases, an increase in yield and an improvement in the quality of crops is thought to be possible but, more research would be needed to prove that.

In olive groves and vineyards, increase in biodiversity was observed when using legumes as cover crops, as well as a decrease in soil erosion and nutrient washing, a progressive increment of C and N in the soil and, in parallel, there was a tendency to obtain a more sustainable although irregular production (Pastor *et al.*, 2007). Likewise, when comparing tillage in relation to three different cover crops: spontaneous, grass, and legume in an olive grove, the last one displayed a tendency to host higher numbers of nematodes, fungi, and bacteria in soil and in the cover crop's roots (Andrés *et al.*, 2019).

Cover crops in olive orchards mostly include barley, oat, vetch, and several *Brassicaceae* species as *Eruca vesicaria*, *Moricandia moricandioides* or *Sinapis alba* (Jiménez-Díaz *et al.*, 2012). Residues of wild white mustard *S. alba* subsp. *mairei* introduced as a winter cover in olive groves reduced summer weed infestation and delayed weed appearance compared with bare soil (Alcántara *et al.*, 2011). The use of the crucifer species *Brassica carinata*, *E. vesicaria* and *S. alba* as cover crops in olive groves would contribute to reduce *V. dahliae* in soil when incorporated as amendments (Cabeza-Fernández *et al.*, 2008). In any case, choice of plants to be used as cover crops should be made with caution until more research unveils the interactions between them and the potential pathogens of the production areas, since some could act as reservoirs of these plant pathogens (Jiménez-Díaz *et al.*, 2012).

Although it seems that the application of cover crops in olive groves may have a big potential for the management of olive diseases in the future, there are other issues to be addressed. Many plant species can be used as cover crops although only a few have been considered for their potential benefits in relation to disease management. The concept of

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functional cover crops should be explored, and research on the diverse roles that cover crops may have in the field according to their composition may be worth it to be taken into account to maximize their benefits. Also, it is needed to adjust cover crops into well-planned rotations, or to develop new rotations. Intercropping according to the push-pull system (Stenberg *et al.*, 2015) with a cover crop that produces volatile organic compounds with antifungal or repellent properties seems to hold great potential for biological control. Nevertheless, the effects of cover crops on pathogen populations are just beginning to be understood.

All these low-input sustainable agricultural strategies open a range of possibilities that would be worth developing in olive groves as appropriate innovative alternatives. However, those practices should be adapted to each region and locally implemented into an integrated pest management programme to control olive tree diseases.

4. Research needs and key issues to be considered on biocontrol agents and cropping practices for controlling olive tree diseases

Here is a revision of the most important research needs regarding BCAs (a - k) and/or cover crops (l - q) proposed. Some of them were already mentioned in previous sections:

a) Broadening of the screening of BCAs from different olive niches, since most of them were isolated from soil and rhizosphere and less number from either the phyllosphere, in spite of the numerous air-borne diseases affecting the olive tree, or from the endosphere (specially the xylem tissues), since two of the most important olive diseases are caused by xylem-inhabiting microorganisms (*V. dahliae* and *Xylella fastidiosa*).

b) Specific mechanisms of action of BCAs, secondary metabolites produced by them against olive pathogens, such as volatile organic compounds, and their genetic underlying production bases.

c) Resistance of BCAs to physiological stresses in olive trees caused by environmental factors, mainly drought or temperature.

d) Variability in the response of strains of plant pathogens (according to races, subspecies, pathotypes, etc.) causing the same olive disease, to the activity of BCAs.

e) Field assays to test BCAs against main olive plant pathogens and evaluation of the optimal conditions for their successful implementation in olive groves. Most of the research has been carried out *in vitro*, and *in planta* field trials are lacking or scarce. Obtaining satisfactory results for biocontrol efficacy has consistently proved to be difficult. Consider dosage, timing of applications, persistence of BCAs that will be directly related to durability and extension of the period of the activity, the establishment of the environmental impact of BCAs in olive groves and other key points (see section 2).

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f) Search for tailored consortia of BCAs effective against olive diseases. The use of mixtures of BCAs may help to increase and/or maintain their activity when individual agents may not be effective. In these cases, synergistic or antagonistic interactions between different BCA populations should be considered *in vitro* and *in planta* to reach a satisfactory control level.

g) Breeding of microbe-optimized crops (Syed Ab Rahman *et al.*, 2018) through the application of microorganisms to the olive tree that could have an activity not only as BCAs but also as biofertilizers, plant growth promoters, and/or modulators of plant defense mechanisms, which indirectly will help to increase the activity of BCAs.

h) Use of plant-optimized microbiomes (Syed Ab Rahman *et al.*, 2018) which, in the olive tree, could be modified to enhance the presence of those indigenous microorganisms with useful biocontrol activity against any of the pathogens causing olive diseases.

i) Biotechnological development of formulations based on field-effective BCAs (one, tailored mixtures, other combinations) and their application methods to obtain commercial bioproducts for prevention and/or control of olive tree diseases that can be easily stored and applied by farmers.

j) Application of BCAs for controlling vector insects of olive pathogens, in combination or not with the use of arthropod natural enemies.

k) Combination of BCAs with other non-chemical sustainable disease control alternatives, and incorporation into integrated pest management programmes in olive groves.

l) Exploration of the relationships between diversity and biological control, especially with respect to microbial diversity provided by cover crops in olive groves.

m) Epidemiological studies on the potential interactions between candidate plant species to be used as cover crops in olive groves and the pathogens of interest, to know if any of these plants could act as a reservoir.

n) Design of functional cover crops against diseases in olive groves in such a way that these cover crops could perform their role mainly by affecting soil-borne pathogens (inhibition or reduction of pathogen inoculum or infectivity) or by interference in the dynamics of insect vector populations.

o) Development of specific rotation plans for optimized use of functional cover crops in olive groves to better exploit their beneficial effects over time.

p) Contribution of volatile organic compounds from cover crops against plant pathogens, and possibility of using the push-pull system to fight either olive diseases or vector insects involved in pathogen dissemination, since this system has already been proposed mainly against pests.

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q) Combination of cover crops with other non-chemical sustainable olive control alternatives, and incorporation into integrated pest management programmes in olive groves.

The interest in the technical progress of products and methods based on the beneficial effects of biocontrol agents and cropping systems to fight olive tree diseases in the field depends to a large degree on the people concerned. Advisers and farmers should be trained to know and use these strategies instead of the usual agrochemicals, and many of them already seem to agree with the convenience of this change. On the other hand, although the biotechnological production of BCAs copes with limitations, as the size of the targeted market, the costs of production and registration, and the business profitability, which is lower than that of agrochemicals, there is an increasing demand and a positive market trend. However, more research would be needed, mainly focused on finding field-effective BCAs and functional cover crops against olive diseases. Closer interactions among the sectors involved (researchers, biotechnology companies, advisers, farmers and consumers) would be needed to successfully include the use of BCAs and some cropping practices in integrated pest management programmes that can allow for an optimistic future in which olive protection against diseases is achieved through sustainable control strategies.

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