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Review

Biological control of ornamental plant diseases caused by *Fusarium oxysporum*: A review

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HIGHLIGHTS

- Pathogenic Fusarium oxysporum are highly dangerous pathogens for ornamental production.
- Many microorganisms proved efficient in reducing diseases on ornamentals but too few are marketed.
- Known and new antimicrobial properties of botanicals may control pathogenic fungi of ornamentals.
- Biological control using microbes and botanicals is part of integrated disease management strategies.
- European legislation must change to encourage the marketing of products used in biological control.

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ABSTRACT

Ornamentals include all decorative plants suitable for indoor or outdoor uses. A large variety of plants is produced and sold on a worldwide market. One of the most destructive pathogenic microorganisms for ornamental production is the soil-borne fungus *Fusarium oxysporum*. Many *F. oxysporum* pathogenic strains can infect numerous ornamental plants during production and/or post harvest storage. Once the disease has broken out, plants are rarely suitable for commercialization. No curative control method is currently available. The best management of the diseases can only be achieved through an integrative approach in which biological control can play a major role in complement of varietal selection for resistance, provided that lines are available, which is rarely the case. Biological control methods on ornamentals are limited to the use of microbial biological control agents and botanicals, *i.e.* essential oils or plant extracts. An overview of the studies about botanical and microorganism use against *F. oxysporum* on ornamentals highlighted that the use of these methods is less than 2 decades and that they arouse increasing interest. Microorganism and botanical sources are countless; consequently, the choice of a screening method to select good candidates is critical. Both microorganisms and botanicals display various modes of action that are not all fully understood, especially for botanicals. As soon as a promising microorganism or botanical candidate is identified, different parameters linked to the development of the product (mode of application, dose, formulation, production) need to be defined and standardized to optimize the quality of the final product. These steps also determine the success or failure of a product on the market. Once the product has been elaborated, the registration process can start. Depending on the country, requirements are different and the whole process is more or less tedious. However, 26 biological control products are currently available for *F. oxysporum* control on ornamentals, and the biological control market is growing. Moreover, biological control methods can be combined with one another or with other control methods but much additional research is required to develop methodologies for incorporating biologicals into other control strategies for ornamental disease management.

Abbreviations: EO, essential oil; EPA, Environmental Protection Agency; IBMA, International Biocontrol Manufacturers Association; ISO, International Organization for Standardization; MBCA, microbial biological control agent; PE, plant extract; PGPR, plant growth-promoting rhizobacteria; ROS, reactive oxygen species.

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Contents

1. Introduction

Ornamentals include all plants grown for decorative purposes for indoor or outdoor uses. They are produced in potting mixture, in soilless culture medium, or directly in the field. Crops are divided into several categories: cut flower or cut foliage crops, bulb and corm crops, potted plants, and woody ornamentals. The world production of flowers and potted plants is dominated by the European Union, with 34% of the production. The second producer is China (16%), and the third one the USA (14%). In Europe, The Netherlands produce 32% of ornamentals, followed by Italy (12%), and Germany (13%) (European Commission, 2015). Global annual sales of flowers range between US$ 40 and 60 billion. Consumers constantly seek for new products, and the demand is rising. Globally, the floriculture sector is growing, especially in developing countries and to a lower extent in Western Europe, North America and Japan. Nevertheless, Europe, America and Asia productions provide for more than 90% of current needs (Intracen, 2016).

Fusarium oxysporum Schltdl. is a well-described soil-borne fungus (Gordon and Martyn, 1997). The species includes a wide diversity of strains responsible for wilts or rots on many plant species (Kistler, 1997; Ortoneda et al., 2004; Dean et al., 2012). Among its broad range of host plants, many are ornamental crops. *F. oxysporum*-induced diseases cause serious damage during both production and storage (Gullino et al., 2015). Pathogen control and disease management can only be achieved through an integrative approach in which biological control can play a major role. The aim of this review is to present an overview of biological control methods against *F. oxysporum* in ornamentals, their strengths and weaknesses, and their integration into a global disease management approach.

2. *F. oxysporum* diseases on ornamentals

Several fungi can cause diseases on ornamentals, but the most worrying one for worldwide production is the soil-borne fungus *F. oxysporum*. This morphological species is now recognized as a species complex because of its high level of phylogenetic diversity (O'Donnell et al., 2009). The species complex includes a wide diversity of saprophytic and phytopathogenic strains. The pathogenic strains are characterized by narrow host specificity; they are named *formae speciales* according to their species specificity. When resistance genes are identified in the plant, *formae speciales* are further subdivided into races according to their cultivar specificity (Gordon and Martyn, 1997). More than 150 *formae speciales* and races are currently described. *F. oxysporum* host plants include some of the most valuable ornamental plants, such as *Chrysanthemum* spp. *Dianthus* spp., *Gerbera* spp., *Gladiolus* spp. and *Lilium* spp. (Massey, 1926; Imle, 1942; van Arx, 1952; Nelson, 1964; Engelhard and Woltz, 1971). On the whole, more than 30 ornamental plant genera are known targets of *F. oxysporum* (Table 1). Surprisingly, only 21 *formae speciales* are described on ornamental crops. A strain from a given *forma specialis* may infect different ornamentals, and a same ornamental species can be infected by strains of *F. oxysporum* belonging to several *formae speciales*. For example, the *forma specialis chrysanthemi* can cause disease on Barberton daisy, chrysanthemum, daisybush, and Paris daisy, which are all plants from the *Asteraceae* family, while the *forma specialis tracheiphilum* is pathogenic on Barberton daisy and chrysanthemum (Engelhard and Woltz, 1971; Minuto et al., 2007; Troisi et al., 2010). Likewise, *F. oxysporum* f. sp. *gladioli* affects several plants from the *Iridaceae* family (corn lily, crocus, freesia, gladiolus, iris) (McClellan, 1945). Consequently, in ornamentals, the "narrow specificity of *formae specialis*" concept might not apply, or it applies at the botanical family level rather than at the species level (Gullino et al., 2012).

On ornamentals, diseases caused by *F. oxysporum* occur during cultivation and/or during storage of bulbs and corms. Two kinds of symptoms are associated with the fungus: vascular wilt, and crown and root rot (Engelhard and Woltz, 1971; Linderman, 1981; Brayford, 1996). Wilting is usually described on whole plants and on some storage organs, as on gladiolus corm or tulip bulb (Fig. 1) (Bald et al., 1971). *F. oxysporum* penetrates the host roots until it reaches the xylem vessels, which it colonizes upwards (Olivain and Alabouvette, 1999). The first visible symptoms of Fusarium wilt are aerial. They begin with a progressive yellowing of the foliage, often on one side only. Then, leaves gradually wilt, until the whole plant collapses (Fig. 1). Symptoms associated with crown and root rot are described only on some storage organs such as those of lily, crocus, narcissus, or tulip (Bald et al., 1971; Gullino et al., 2012). The fungus degrades the cortex layers, and causes the formation of severe brown to black necrotic spots that lead to basal plate rot (Fig. 1) (Bald et al., 1971; Baayen and Rijkenberg, 1999). Storage organs, such as the corm or bulb, are characterized by a shortened stem. In this context, we may wonder if the symptoms considered as rotting may in fact be advanced wilting symptoms on these peculiar organs.

Table 1
List of ornamental plants affected by *Fusarium oxysporum*.

Host plants	*Formae speciales*	References
Actinotus helianthi	Undefined f. sp.	Bullock et al. (1998)
Anoectochilus sp.	f. sp. *anoectochili*	Huang et al. (2014)
Argyranthemum frutescens	f. sp. *chrysanthemi*	Garibaldi et al. (1998)
Callistephus chinensis	f. sp. *callistephi*	Baker (1953)
Cattleya sp.	f. sp. *cattleyae*	Sepúlveda Chavera and Cortés (1999)
Cereus marginatus var. *cristata*	Undefined f. sp.	Garibaldi et al. (2014)
Cereus peruvianus	Undefined f. sp.	Bertetti et al. (2012)
Chrysanthemum sp.	f. sp. *chrysanthemi*, f. sp. *tracheiphilum*	Engelhard and Woltz (1971)
Coreopsis verticillata	Undefined f. sp.	Elmer et al. (2007)
Crassula ovata	f. sp. *crassulae*	Garibaldi et al. (2011a) and Ortu et al. (2013)
Crocus sativus	f. sp. *gladioli*, f. sp. *croci* and undefined f. sp.	Boerema and Hamers (1989), Gupta and Vakhlu (2015), Yamamoto et al. (1954)
Cyclamen persicum	f. sp. *cyclaminis*	Gerlach (1954)
Delphinium sp.	f. sp. *delphinii*	Laskaris (1949)
Dianthus sp.	Undefined f. sp., f. sp. *dianthi*	Garibaldi (1977) and Garibaldi et al. (2011b)
Echeveria agavoides	f. sp. *echeveriae*	Bertetti et al. (2013)
Echeveria tolimanensis	f. sp. *echeveriae*	Bertetti et al. (2015)
Euphorbia mammillaris var. variegata	Undefined f. sp.	Garibaldi et al. (2016a)
Eustomae sp.	f. sp. *eustomae*	Hahm (1998)
Exacum sp.	Undefined f. sp.	Elmer and O'Dowd (2001)
Freesia sp.	Undefined f. sp., f. sp. *gladioli*	Farr et al. (1989) and McClellan (1945)
Gerbera sp.	f. sp. *chrysanthemi*, f. sp. *tracheiphilum*	van Arx (1952)
Gladiolus sp.	f. sp. *gladioli*	Massey (1926)
Gypsophila sp.	Undefined f. sp.	Werner and Irzykowska (2007)
Hibiscus sp.	f. sp. *rosellae*	Ooi and Salleh (1999)
Hosta sp.	Undefined f. sp.	Wang and Jeffers (2000)
Hyacinthus sp.	f. sp. *hyacinthi*	Boerema and Hamers (1988)
Iris sp.	f. sp. *gladioli*	McClellan (1945)
Ixia sp.	Undefined f. sp., f. sp. *gladioli*	Fantino et al. (1985) and McClellan (1945)
Lewisia rediviva	Undefined f. sp.	Gullino et al. (2015)
Lewisia cotyledon	Undefined f. sp.	Garibaldi et al. (2005)
Lilium sp.	f. sp. *lilii*	Imle (1942)
Mammillaria zeilmanniana	f. sp. *opuntiarum*	Garibaldi et al. (2016b)
Mandevilla sp.	Undefined f. sp.	Sella et al. (2010)
Mimosa sp.	f. sp. *perniciosum*	Hepting (1939)
Narcissus sp.	f. sp. *narcissi*	Gregory (1932)
Osteospermum sp.	f. sp. *chrysanthemi*	Minuto et al. (2007)
Papaver nudicaule	f. sp. *papaveris*	Garibaldi et al. (2012) and Ortu et al. (2015b)
Philodendron oxycardium	Undefined f. sp.	Wang et al. (2015a)
Protea sp.	Undefined f. sp.	Swart et al. (1999)
Ranunculus asiaticus	f. sp. *ranunculi*	Martini et al. (2006)
Rhus sp.	f. sp. *callistephi*	Armstrong and Armstrong (1971)
Sanguinaria canadensis	Undefined f. sp.	Elmer and Marra (2015)
Tulipa sp.	f. sp. *tulipae*	Bergman (1965)

Losses can have a high economic impact, e.g. on carnation and chrysanthemum production. On gladiolus, *F. oxysporum* f. sp. *gladioli* colonizes corms during storage. This can result in 60–80% losses (Barrera-Necha et al., 2008). Since its first outbreaks in 1930 in Germany, Fusarium wilt of cyclamen had spread to all production areas worldwide and led some growers to stop cyclamen production and/or diversify their production (Gerlach, 1954; Rouxel and Grouet, 1974). Furthermore, in the last decade *F. oxysporum* diseases have been newly described on ornamental crops such as *Anoectochilus* spp., *Crassula* sp., *Lewisia* spp., or *Ranunculus* sp. (Table 1) (Ortu et al., 2015a).

3. Control methods

3.1. Available control methods

Pathogenic *F. oxysporum* strains represent a serious threat to production, and no curative control method is currently available. The best control is achieved by integrating several management tools throughout the production cycle. Prophylactic measures and cultural practices are the first methods to be used to prevent the introduction and further dispersal of pathogens. In practice, they consist in: (i) keeping the greenhouse and equipment (machinery) in good sanitary conditions, (ii) using *F. oxysporum*-free plant materials, (iii) disinfecting soils and substrates, (iv) managing water in an appropriate manner, (v) paying special attention to crop monitoring, and (vi) rotating crops (Katan, 2000; Elmer, 2001; Riaz et al., 2009; Mehta et al., 2014; Raudales et al., 2014). In an attempt to control the disease, several chemicals have been used over the years. For instance, Benomyl has long been used to reduce disease severity (Minuto et al., 1995; Someya et al., 2000). However, its carcinogenic effect on mammals led to its removal in several countries at the beginning of the 21st century (McCarroll, 2002). Methyl bromide has also been extensively used as a soil fumigant, but it is now banned because of its ozone-depleting effect (Gullino et al., 2005). Today, the use of chemicals is restricted since more and more molecules are phased out for environmental and health purposes. Crop breeding to select resistant cultivars is a useful and efficient method to manage the disease; however, it takes time to obtain resistant cultivars with suitable ornamental characteristics. Dutch studies reveal that partial *F. oxysporum* resistance exists in most bulbous plants, such as tulip and lily (Straathof et al., 1996). On carnation, the progression of *F. oxysporum* f. sp. *dianthi* in the xylem of a resistant cultivar is limited by vessel occlusion as compared to a susceptible cultivar (Ouellette et al., 1999). Nevertheless, virtually no resistant cultivar is available yet among ornamentals. The first objective of breeding in ornamentals is to improve yield and valuable quality traits, generally at the expense of resistance to pathogens. Moreover, the main drawback of plant breeding is undoubtedly its unpredictable durability due to possible overcome by pathogens.

Biological control can provide effective solutions for managing *F. oxysporum* diseases. It is currently defined by the International Biocontrol Manufacturers Association (IBMA) as the use of agents or products that naturally affect crop pests and pathogens by limiting their propagation. This definition fits with the European directive 2009/128/CE, which promotes the reduction of pesticide use by developing non-chemical methods for disease and pest management in agriculture, including physical, mechanical and biological practices, and biological control (EU, 2009a). The agents or products that naturally affect crop pests and pathogens include macroorganisms, microorganisms, chemical mediators, and

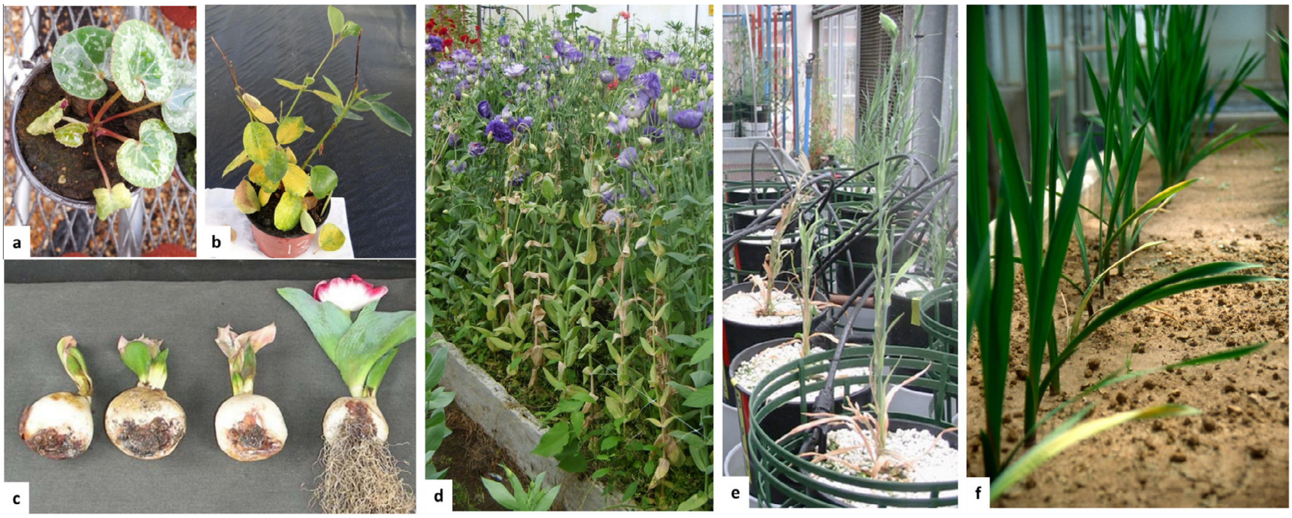

Fig. 1. Symptoms caused by *Fusarium oxysporum* on ornamental plants: *Cyclamen* sp. (a), *Mandevilla* sp. (b), *Tulipa* sp. (c), *Eustoma* sp. (d), *Dianthus* sp. (e), *Gladiolus* sp. (f).

natural substances. A large variety of substances can be considered as "natural", like those originating from animals, microorganisms or plants. In this review, we consider the IBMA definition and focus on microorganisms and natural substances originating from plants (*i.e.* botanicals), the only products supposed to be able to control *F. oxysporum* on ornamentals.

3.2. Microorganisms to control *F. oxysporum*

The sources of potential microorganisms able to control soil-borne pathogens are diverse. However, historically, in the 1960s, studies on suppressive soils, *i.e.* soils with a low disease incidence despite the presence of the pathogen, emphasized the existence of antagonistic microorganisms in the soil (Stover, 1962; Stotzky and Martin, 1963). The first symposium dedicated to the ecology of soil-borne plant pathogens was held in 1965. Different biological control methods using resident microorganisms were considered. Biological control *via* the introduction of organisms was later proposed by Baker and Cook (1974) in their book "Biological control of plant pathogens". Several strategies have been developed to identify potential Microbial Biological Control Agents (MBCA) through *in vitro* or *in vivo* tests. These MBCA are mostly fungi and bacteria. They are able to control soil-borne diseases by directly antagonizing the pathogens or indirectly competing for common trophic and/or spatial resources, or stimulating plant defense reactions, or both (Alabouvette et al., 2009). The different methods used to screen MBCA, their modes of action, and their production and registration processes are addressed in part 5.

3.3. Botanicals to control *F. oxysporum*

Botanicals include plant extracts (PE) and essential oils (EO). They are mixtures composed of 20–60 volatile or/and aromatic components, including acetones, acids, alcohols, aldehydes, alkaloids, esters, terpenes, and phenols (Bakkali et al., 2008; Negi, 2012; Azmir et al., 2013). They accumulate in glandular trichomes or in secretory cavities of plant cell walls and represent between 1% and 2% of plant biomass. Since these peculiar cells can be found in most part of the plant, botanicals can be extracted from any part of the plant (Koul et al., 2008; Bakkali et al., 2008). Two to three major components of a mixture are present in higher concentrations, whereas the others are minor components present at trace levels. According to the International Organization for Standardization (ISO), an EO is a "product obtained from a natural raw material of plant origin either, by steam distillation, by mechanical processes or by dry distillation, after separation of the aqueous phase, if any, by physical processes", while a PE is "a product obtained by treating raw material with one or several solvents" (ISO, 2013). The methods for screening botanicals, their modes of action, and the production and registration processes are addressed in part 5.

4. Biological control of *F. oxysporum* on ornamentals

We compiled peer-reviewed articles about MBCA and botanicals as control agents of *F. oxysporum* diseases on ornamentals published between 1977 and 2015. Health and environmental concerns led to the phasing out of several chemicals, reinforcing the interest for alternative control methods. This growing interest is illustrated by the increasing number of works on the biological control of *F. oxysporum* on ornamentals published in the last 10 years (40 since 2004) as compared to the small amount (30) published between 1977 and 2004 (Fig. 2). The first publication dealing with the use of MBCA dates back to 1977, while the first one focusing on botanicals was published in 2000 (Langerak, 1977; Bowers and Locke, 2000). MBCA have been known for a long time, and numerous studies deal with their use as fungicides. However, the bibliometric data show that among the 45 publications on the subject, only 19 were published during the last 15 years. Similarly, botanicals have been known and used for decades in various sectors (agri-food, medicine, perfumery,...) and for various uses (antimicrobial activity, flavor, fragrance, preservative,...), but their use to control *F. oxysporum* diseases of ornamentals is recent (Philogène et al., 2005; Baser and Buchbauer, 2009). Since 2000, 25 publications about botanicals have been reported. This growing interest for biological control is probably supported by socio-ecological concerns and stimulated by the different safety protocols implemented since 1985 (Isman, 2000; Koul et al., 2008; Mohan et al., 2011; Gurjar et al., 2012).

Among microorganisms, fungi have been assessed more often than bacteria to control *F. oxysporum* (68% *versus* 32%). More attention has been paid to the genus *Trichoderma* (53% of the fungi), which gathers species well-known for their biological control activity (Vinale et al., 2008; Gajera et al., 2013). Other microorganisms well represented in the literature are non-pathogenic *Fusarium* (23%) and *Penicillium* (10%). Bacteria tested as MBCA mainly belong to the genus *Pseudomonas* (44%), followed by *Bacillus* (13%) and *Streptomyces* (9%). All these bacteria belong to the plant

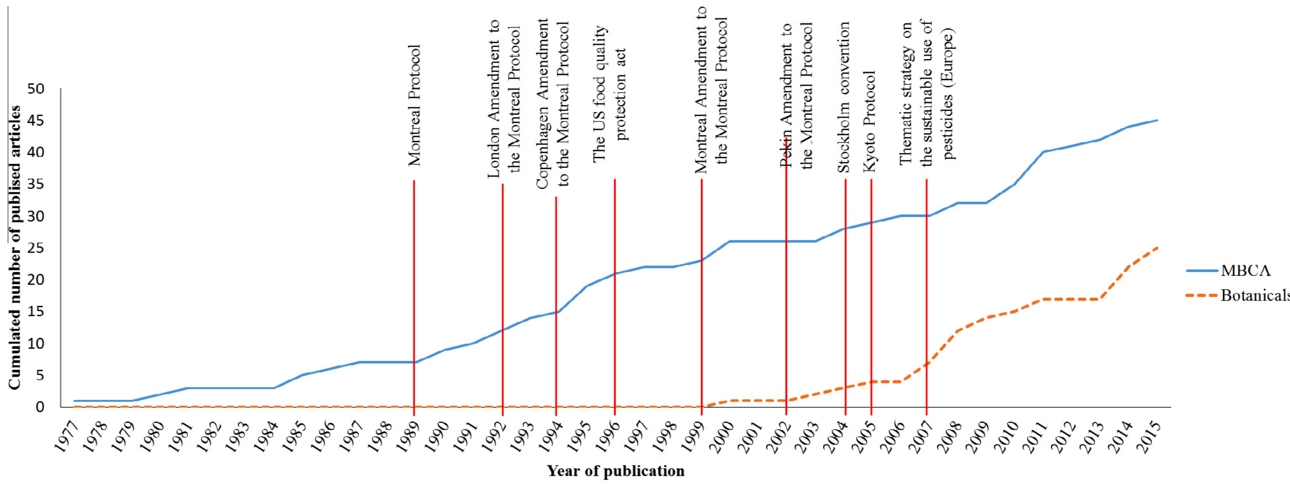

Fig. 2. Dynamics of publishing on biological control of *Fusarium oxysporum* on ornamentals using microorganisms (full line) or botanicals (dotted line) over the years (references available in supplemental data).

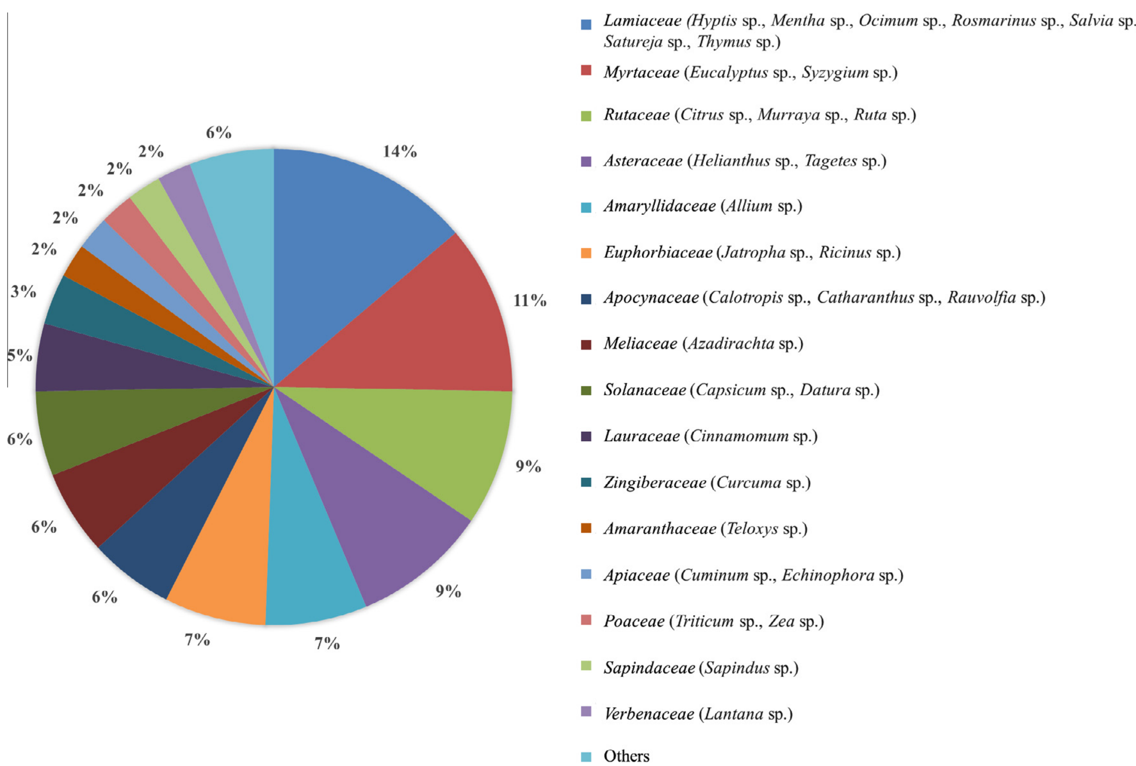

Fig. 3. Most studied plant families to control *Fusarium oxysporum* on ornamentals (references available in supplemental data).

growth-promoting rhizobacteria (PGPR) known to promote plant growth and induce protection against abiotic stresses (Antoun and Prévost, 2006). Bibliometric data also reveal that 57 plant species distributed across 20 plant families have been investigated with the aim to control *F. oxysporum* on ornamentals using botanicals (Fig. 3). The most studied families are *Lamiaceae* (14%), followed by *Myrtaceae* (11%), *Rutaceae* (9%), and *Asteraceae* (9%). They include plants such as citrus, clove, eucalyptus, marigold, and mint, which are well-known for the antimicrobial activity of their oils or extracts (Salie et al., 1996; Bozin and Mimica-Dukic, 2006; Chaieb et al., 2007; Viuda-Martos et al., 2008; Ben Marzoug et al., 2010).

Regarding MBCA as well as botanicals, the most targeted *formae speciales* are *dianthi* and *gladioli*, probably because of the economic importance of the crops they attack. The other *formae speciales* (*chrysanthemi*, *cyclaminis*, *lilii*, *narcissi*, and *tulipae*) have been less studied. Microorganisms and botanicals can both display a wide range of efficiency levels. Among potential microbial candidates for biological control of *F. oxysporum* diseases on ornamentals, 16% provided 80–100% efficiency, while 19% provided between 60% and 80% and 19% provided 40–60% efficiency (Fig. 4). The most efficient microorganisms belong to the *Bacillus*, *Fusarium*, *Glomus*, or *Trichoderma* genera (Postma and Luttikholt, 1996; Singh and Vijay, 2011; Maya and Matsubara, 2013; Hassan et al., 2014). Yet, almost one third (28%) of the microorganisms tested so far reduced the disease by only 10–40%. As for botanicals, most of the studies performed so far were only *in vitro* tests, so a similar kind of categorization is not possible yet. These categories raise the question of

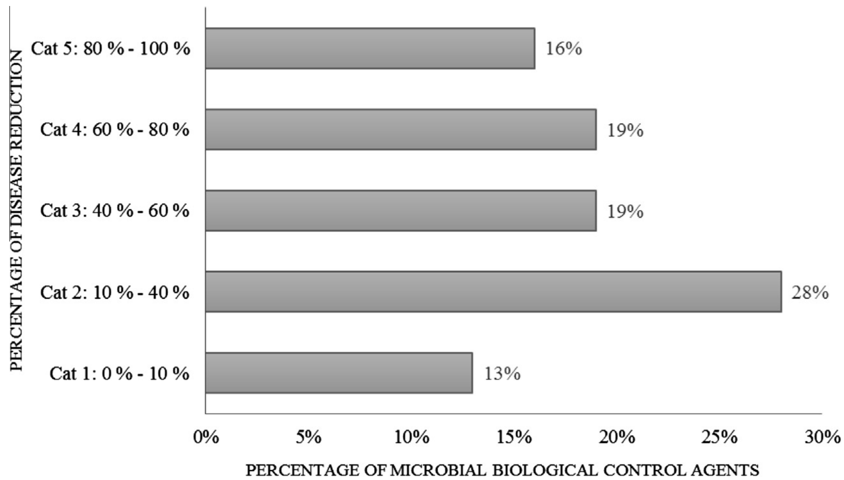

Fig. 4. Ranges of efficiency of biological control microorganisms evaluated *in vivo*. We organized the efficiency rates of disease reduction (incidence or severity) by biological control microorganisms in *in vivo* tests into 5 categories. For 5% of the MBCA, the data did not allow us to calculate an efficiency rate (references available in supplemental data).

the level of efficiency required to consider the commercialization of a biological control product. Actually, 40% reduction of the disease is already of real interest for producers.

5. Screening for candidates

Sources of biological control candidates, whether microorganisms or botanicals, are highly diverse. The most part of the current microorganisms of interest comes from different types of soils (e.g. bulk soil, rhizosphere soil) or from suppressive soil (Beale and Pitt, 1995; Gupta and Vakhlu, 2015). The antagonistic abilities of microorganisms isolated from the rhizoplane, the phylloplane, or from the endophytic compartment (mainly roots) of healthy plants have also been tested (Carver et al., 1996; Someya et al., 2000; Ajit et al., 2006). MBCA can also be found in microorganism collections or from currently commercialized products registered towards other pathogens (Elmer and McGovern, 2004; Shanmugam et al., 2011). More unusually, potential MBCA have been isolated from mangrove, raised bog, or vinegar waste compost (Samuel and Muthukkaruppan, 2011; Szentes et al., 2013; Lin et al., 2014). Moreover, knowing the ecological competence and adaptability of the microorganism is essential to understand suitable conditions of growth of the microorganism in the environment and ensure the best control. Such information is anyway required for the registration process. Similarly, there is a wide diversity of sources among botanicals. They can be extracted from a whole plant or from a plant part (aerial part, branch, bulb, flower, fruit, leaf, peel, root, seed), and from fresh or dry plant material (Okwu et al., 2007; Chohan et al., 2011; Cordova-Albores et al., 2014; Wang et al., 2015b). The composition of botanicals fluctuates in quantity and quality depending on the plant (species, variety, subspecies, and chemotype), the organ, the age or the life stage of the plant, the soil composition, the season, and the climate (Pitarokili et al., 2003; Baser and Buchbauer, 2009; Raeisi et al., 2015; Licata et al., 2015; Moghaddam and Mehdizadeh, 2015). Plant candidates for the search for potential sources of botanical compounds are selected among plants already well known for their interest in medicinal or food uses. They represent a wide diversity of plant species, among which garlic, lemon, cinnamon, mint, Madagascar periwinkle, or eucalyptus (Chandel et al., 2004; Okwu et al., 2007; Barrera-Necha et al., 2008). Another research strategy focuses on plants involved in biocidal functions for other organisms through the production of specific metabolites, e.g. allelopathic plants (Riaz et al., 2010). A practical and timely perspective also consists in using and exploiting by-products from plant production to get botanical candidates, as in the case of *Citrus* juice production (Crupi and Rispoli, 2002).

The first step of the screening for microorganisms or botanicals of interest is performed *in vitro* in 53% and 96% of the studies, respectively. The antifungal activity of a microorganism or a botanical is mainly evaluated by measuring its impact on pathogen growth in liquid or agar culture media. Conidial germination percentages and agar well diffusion are commonly used to assess MBCA and botanical efficiency (Duijff et al., 1993; Shobha and Kumudini, 2012). Other methods are used to test microorganisms or botanicals specifically. The classical dual culture method on Petri dishes was set up in 1960 by Johnson et al. to test the antagonistic abilities of potential MBCA. Microorganisms can also be selected according to their ability to produce enzymes such as chitinase (Someya et al., 2000). The commonly used poisoned food technique defines the minimum inhibitory concentration of botanicals needed to inhibit fungal growth, while the less frequent vapor phase test is used to evaluate the effect of botanical volatiles on fungi (Zentmeyer, 1955; Nakahara et al., 2003). Based on the poisoned food technique, citronella oil failed to inhibit nine different fungal species, while it inhibited the growth of all of them in the vapor phase test (Nakahara et al., 2003). Beale and Pitt (1990) isolated 135 microorganisms from plant materials and soils. They screened all isolates for their antagonistic activity against *F. oxysporum* f. sp. *narcissi* and for their tolerance to a fungicide. Among this collection, 30 microorganisms were found to be antagonists. In another example, the strain *Trichoderma* sp. 075 gave the best result to inhibit *F. oxysporum* f. sp. *narcissi* growth on Petri dishes, while the same strain evaluated in the greenhouse resulted in a higher disease severity compared to the pathogen alone (Beale and Pitt, 1990). These examples emphasize the well-known irrelevance of *in vitro* experiments to assess the potential of a microorganism or botanical to control *F. oxysporum*, since *in vitro* results can be diametrically opposite to *in vivo* results.

The second screening step is performed *in vivo* in 78% of the studies on MBCA, but only in 20% of the studies on botanicals. The tests are performed on storage organs in containers or on whole plants in the greenhouse or in the field. Biological control efficiency is mostly evaluated from the incidence and/or severity of the disease according to a defined disease index. Even so, it can also be assessed by recording data on plant growth parameters such as plant height, the dry or fresh weight of certain plant parts, or the flowering date (Beale and Pitt, 1990; Nosir et al., 2010; Shanmugam et al., 2011). For example, among the 30 antagonistic microorganisms selected *in vitro* against *F. oxysporum* f. sp. *narcissi* by Beale and Pitt (1990), 17 were further tested in greenhouse trials, and 5 were tested in field experiments. The fungus *Minimedusa polyspora* gave the best disease reduction (only 33% of diseased bulbs), and significantly increased bulb weight and flower yield.

The screening can be based on the physiological status of the plant through measurements of the water status (e.g. transpiration, stomatal conductance), of changes in antioxidant activity (e.g. enzymatic activity levels), or the production of plant defense molecules (e.g. phytoalexins) (van Peer et al., 1991; Sant et al., 2010; Maya and Matsubara, 2013). More rarely, the production of molecules such as fusaric acid by the pathogen is monitored (Nosir et al., 2011). However, the results of *in vivo* assays really depend on external parameters such as the plant cultivar, the plant growth stage, cultivation conditions, the growth substrate, the way microorganisms are applied, and the pathogen/MBCA ratio (Sneh et al., 1985; Garibaldi et al., 1987; Minuto et al., 1995; Postma and Luttikholt, 1996).

6. Modes of action

A large amount of studies has been performed on the modes of action of microbial antagonists and reviewed elsewhere (Compant et al., 2005; Harman, 2006; Alabouvette et al., 2009; Lioussanne, 2010). Antagonistic microorganisms act directly or indirectly against *F. oxysporum* and thus reduce its development. These actions include mycoparasitism, antibiosis, competition for nutrients or for an ecological niche, and induction of plant defense reactions (Baker, 1968).

Mycoparasitism is defined as the parasitism of a fungus by another fungus. It is mediated by the development of peculiar organs (haustoria) and by the synthesis of enzymes or secondary metabolites to finally allow nutrient uptake from the pathogenic fungus (Manocha, 1991; Daguerre et al., 2014). Some strains of *Trichoderma* are known for their mycoparasitic ability on *F. oxysporum* hyphae (John et al., 2010). Secondary metabolites, antibiotics or enzymes are also involved in antibiosis. For example, a strain of *Bacillus thermoglucosidasius* produces antibiotics, resulting in growth inhibition and control of *F. oxysporum* f. sp. *lilii* (Chung et al., 2011). Competition for resources is another kind of interaction between microorganisms. Some microorganisms have developed beneficial strategies to be ahead of others when they compete for a same ecological niche or for a same source of nutrients. The secretion of peptides that have high affinity for iron, called siderophores, is a good example of competition for nutrients. This strategy is used by some strains of *Pseudomonas* to control *F. oxysporum* f. sp. *dianthi* (Elad and Baker, 1985; Lemanceau et al., 1992, 1993; Duijff et al., 1993). Other molecules produced by microorganisms trigger plant defense reactions. St-Arnaud et al. (1997) hypothesized that *Glomus intraradices* induces *Dianthus caryophyllus* disease resistance mechanisms against *F. oxysporum* f. sp. *dianthi* to explain reduced disease severity. Similarly, the non-pathogenic strain *F. oxysporum* Fo47 primes the defense responses of tomato plants to control *F. oxysporum* f. sp. *lycopersici* (Aimé et al., 2013).

In comparison to MBCA, the mechanisms whereby botanicals act against microorganisms are even less known. These mechanisms rely on the composition of botanicals, which is multifactor dependent. A few studies reveal that the major components are mainly responsible for the biological activity of botanicals, but others conclude that several components act in synergy (Pitarokili et al., 2003; Bakkali et al., 2008). Furthermore, as botanicals contain a mixture of diverse components, their antifungal activity is probably not attributable to a single mechanism. The main mechanisms reported so far are membrane disruption, metal chelation, interaction with DNA, and induction of plant defense reactions (Cowan, 1999; Arzoo et al., 2012). Several studies report that EO or some of their components are able to disrupt cell wall and membrane integrity and to easily penetrate into the cells (Li et al., 2015; Pontin et al., 2015). This disruption causes mitochondrial membrane damage, which induces changes in the electron transport chain. Consequently, free radicals are produced, and they oxidize and damage lipids, proteins, and DNA. In contact with reactive oxygen species (ROS), EO phenolic compounds are oxidized and release reactive phenoxyl radicals (Bakkali et al., 2008; Alam et al., 2014). The induction of plant defenses by EO has also been investigated. Thyme oil application on tomato roots efficiently triggered peroxidase accumulation in roots, which are well-known to be part of the plant defense mechanisms (Kawano, 2003; Ben-Jabeur et al., 2015). Similarly, Arzoo et al. (2012) found evidence of the induction of plant defense responses against *F. oxysporum* f. sp. *lycopersici* using different plant extracts. Although the antimicrobial mechanisms of action of botanicals have been carefully studied for their pharmaceutical or food preservative uses, less information is available concerning their use to control plant pathogenic microorganisms (Burt, 2004; Buchbauer, 2009; Negi, 2012).

7. Elaboration of the products

The parameters of the processes for mass production and formulation of MBCA and botanicals have to be carefully defined to optimize the quality and yield of the final product. In fact, these steps affect the efficiency, shelf-life, ease of handling and of application of the final product (Montesinos, 2003; Fravel, 2005). Microorganisms are produced in high-volume fermenters to perform liquid or solid fermentation. Mass production of a given microorganism has to be optimized by selecting optimal growth conditions (aeration, pH, substrate, temperature). The product concentration should also be optimized. For MBCA, it is commonly accepted that efficient control can only be achieved when the MBCA is present in higher concentrations than the pathogen (Fravel et al., 2003). Then, the choice of a formulant (e.g. alginate, coal, talc) is of prime importance (Fravel et al., 1998). In a commercialized MBCA product, the amount of formulant makes up the most part of the product. Companion® (Table 2) for example, contains only 0.03% of a strain of *Bacillus subtilis*, and 99.97% of formulant. Garibaldi et al. (1987) described that using poplar bark to formulate an inoculum of non-pathogenic strains of *Fusarium* spp. decreased its biological control efficiency as compared to wheat kernels or wheat bran. The mixture of microorganisms sold by Premier Tech Horticulture as Pro-mix BX biofungicide + mycorrhizae® is composed of a combination of lime, peat, perlite, vermiculite, and a wetting agent. The choice of the formulant is so determining that it is kept secret for most of the marketed products.

Conversely, little information is available about the formulation and mass production processes of botanicals for plant disease management. However, more studies have examined these processes for pharmaceutical or food uses. The main processes used to obtain EO are hydrodistillation, steam or dry distillation, and mechanical extraction (e.g. expression) in large-volume distillation containers (Schmidt, 2015). The extraction parameters, such as temperature, pressure, and total time also influence on the quantity and quality of the final product (Baser and Buchbauer, 2009). As an example, a lower yield but a higher quality of EO from *Curcuma longa* leaves was obtained with water distillation (Babu et al., 2007). Thus, the extraction technique needs to be carefully chosen. Similarly, the

Table 2
Biological control microorganisms commercialized to control *Fusarium oxysporum* on ornamentals in the world.

Antagonistic microorganism	Strain	Product*	Targeted crop	Use	Company
Bacillus pumilus	GB34	Yield Shield	Ornamentals and others	Seed treatment	Bayer Crop Science
Bacillus pumilus, *Bacillus subtilis* and *Glomus intraradices*	NM	Pro-mix BX Biofungicide + Mycorrhizae	Ornamentals (indoor gardening, perennials, potted flowering plants, annuals, foliage plants) and others including greenhouse vegetables, vegetable transplants and young plant propagation	Potted crop	Premier Tech Horticulture
Bacillus subtilis	GB03	Companion	Ornamentals (greenhouse plug production) and others including vegetables, herbs, hydroponics, small fruits, berries and nut trees	Foliar spraying, seed treatment, soil drenching	Growth products
	GB03	Kodiak	Ornamentals and others including barley, bean, corn, cotton, pea, soybean and wheat	Seed treatment	Bayer Crop Science
	QST 713	Rhapsody	Ornamentals (annual and perennial bedding plants, potted flowers) and others including conifer, deciduous trees, landscape plants, shrubs and trees	Foliar spraying, tank mixing, soil drenching	Bayer Crop Science
	MBI 600	Subtilex	Greenhouse and nursery ornamentals and others including fruits, herbs and vegetables	Soil drenching or mixing	BASF
	FZB24	Taegro	Ornamentals and others including cucurbits, fruit and leafy vegetables	Cutting or root dipping, seed treatment, soil drenching	Novozymes and Syngenta
Gliocladium catenulatum	J1446	Prestop*	Ornamentals and others including fruits and vegetables	Dipping, foliar spraying, soil drenching or mixing	Lallemand
Pseudomonas cepacia	NM	Intercept	Ornamentals and others including bulb, field crops, fruit, greenhouse crops, herbs, spices, vegetable and vine crops	Root dipping, soil drenching	Soil technologies
Pseudomonas chlororaphis	63-28	AtEze	Greenhouse ornamentals and others including nursery crops and vegetable transplants	Drenching	EcoSoil Systems
Pythium oligandrum	M1	Polyversum*	Ornamentals and others including cereals, forest nursery, grape, hop, strawberry, turf and vegetables	Root dipping, seed treatment, spray application, watering suspension	Biopreparaty
Streptomyces griseoviridis	K61	Mycostop*	Ornamentals and others including herbs, seedling production and vegetables	Bulbs or cuttings dipping, seed coating, soil drenching or mixing, watering	Verdera oy
Streptomyces lydicus	WYEC 108	Actinovate SP	Ornamentals and others including all greenhouse and nursery crops and landscape plants	Bulb or crop dusting, cutting or root dipping, seed treatment, soil drenching, spray application, watering	Novozymes
Trichoderma asperellum	T34	T34 biocontrol	Carnation	Root dipping, spraying, watering	Biocontrol technologies
Trichoderma harzianum	DSM 14944	Agroguard	Ornamentals and others including fruits and vegetables	Soil drenching, watering	Live systems technology
	T-22	Trianum*	Greenhouse ornamentals and others including, carrot, cucumber, lettuce, tomato and turf	Soil drenching, mixing, or spraying, watering	Koppert
	OBTh55	Tricho-D WP	Ornamentals and others including fruit, greenhouse crops and vegetables, nurseries, perennial crops, seeds and tubers, short-cycle crops and vegetables	Soil drenching, spraying	Orius biotecnologia
	T-22	Plantshield	Ornamentals and others including hydroponic crops, shadehouse and outdoor nursery crops, cucurbit vegetables, fruiting vegetables, herbs, leafy vegetables, mints, pome fruits, spices, stone fruit and tree nuts	Cutting or root dipping, soil drenching, spraying	BioWorks
	T-22	RootShield granule	Ornamentals and others including asparagus, berries, bulb vegetables, cereal grains, citrus fruits, cucurbit vegetables, fruiting vegetables, herbs, leafy vegetables, legume vegetables, mints, oilseed crops, small fruits and spices	In-furrow, soil mixing	BioWorks
	KRL-AG2	Rootshield WP	Ornamentals and others including berries, bulb crops, citrus fruits, cucurbit vegetables, fruiting vegetables, herbs, hydroponic crops, leafy vegetables, mints, pome fruit, shadehouse and outdoor nursery crops, small fruits, spices, stone fruit, tree nuts and tuber crops	Cutting or root dipping, dusting, in-furrow, soil drenching or spraying	BioWorks
Trichoderma harzianum and *Trichoderma polysporum*	NM	Binab TF WP*	Ornamentals (cut flowers, flower bulbs, ornamental trees) and others including, berries, fruit, grass fields, nurseries, pot plants and vegetables	Soil mixing or praying, watering	Bio-innovation AB
Trichoderma harzianum and *Trichoderma virens*	T-22 and G41	RootShield plus WP	Ornamentals and others including asparagus, berries, bulb vegetables, cereal grains, citrus fruits, conifer tree and seedlings cucurbit vegetables, fruiting vegetables, herbs, hydroponic crops, leafy vegetables, legume vegetables, mints, oilseed crops, peanuts, pome fruits, shadehouse and outdoor nursery crops, small fruits, spices, stone fruits, root and tuber vegetables, and tree nuts	Bulb, cutting or root dipping, in-furrow, soil drenching, dusting, spray	BioWorks

(*continued on next page*)

Table 2 (*continued*)

Antagonistic microorganism	Strain	Product*	Targeted crop	Use	Company
Trichoderma virens	GL-21	SoilGard	Ornamentals and others including field crops and transplants	Drip irrigation, soil drenching or spraying, sprinkler	Certis USA

NM: Not mentioned.
* An asterisk denotes that the product is commercialized in Europe.

nature of the solvents determines the chemical composition of PE. For example, anthocyanins can be obtained with methanol or water extraction, while alkaloids can be recovered using ether or ethanol as solvents (Azmir et al., 2013). As for MBCA, the amount of product to be used has to be defined to achieve best efficiency and to avoid undesirable effects (Bowers and Locke, 2000). Special attention has to be devoted to the standardization of these parameters to obtain a consistent and uniform final product. Asparagus extract eluted with ethanol proved to be phytotoxic for asparagus seeds, while it was not phytotoxic when other solvents were used to extract the PE (Rosado-Álvarez et al., 2014). Anyway, application of botanicals presents limitations as a high amount of product is required to reach a good efficiency. Kadoglidou et al. (2011) calculated that 1 ha of oregano is needed to obtain the 5 t of carvacrol required to treat 1 ha of field at an efficient concentration. Thus, the feasibility of a large-scale use of botanicals is questionable.

The mode of application of the product can at least partially solve the scarcity issue. Applications have to fit with the cultural practices of the diseased crop. The tuning of the mode of application with cultural practices influences the success or failure of a biological control product. The method of application also impacts the efficiency of the formulated product. MBCA and botanical products can be prepared under liquid or solid forms (Jones and Burges, 1998; Soliman, 2013). Essential oils or plant extracts can also be prepared in a semi-liquid form (e.g. gel, liposomes) (Soliman, 2013). Therefore MBCA or botanicals can be incorporated into horticultural potting mixes or in-furrow when they are under a solid form (e.g. granular, powder), while a wettable powder formulation is more adapted for spraying or dipping applications (Cook et al., 1996; Spadaro and Gullino, 2005). These methods of application are probably more suitable for use in the greenhouse. For open cultures, application by seed coating or by micro-encapsulation is probably a better option (Minuto et al., 2007; Mohan et al., 2011; Soliman, 2013; El-Mougy et al., 2015).

All these steps of product development are essential to obtain a cost-effective products for biological control. One of the main factors is the economic feasibility of the product, which highly influences the grower's choice. Thus it will contribute to its good competitiveness on the market of plant disease management.

8. Registration processes

To commercialize a biological product, companies have to go through registration processes. Registration requirements depend on countries. In Europe, both biological (MBCA and botanical) and chemical plant protection products are submitted to regulation No. 1107/2009 (EU, 2009b). The active substance is first evaluated by a reporter member state which assesses if its application is acceptable and prepares a report. This report is then evaluated by the European Food Safety Authority (EFSA). Finally, the Commission's Directorate General for Health and Food Safety decides for its final approval or not. Once the active substance is authorized at the European level, the preparation, *i.e.* the formulated product, is evaluated by national authorities (Hauschild et al., 2011). Botanicals are considered as low-risk active substances or basic substances according to regulation No. 1107/2009, hence an easier registration process (EU, 2009b; Tamm et al., 2011).

In Europe, biological products are evaluated in the same way as chemicals although they do not behave as such (Alabouvette et al., 2012). The registration process can take up to 5 years and cost more than 2 million euros (Ehlers, 2011). The prohibitive impact of such a procedure on companies is then easily understandable. Estimations show that only 0.1% of the tested microorganisms reach the market, which explains why the registration process is often considered as a real bottleneck for commercialization (Pertot, 2014). In the USA, regulatory approval conditions for MBCA and botanicals are less complicated as they are not evaluated in the same way as chemicals. They are divided into microbial pesticides and biochemical pesticides. Products are submitted to the registration eligibility decision of the US Environmental Protection Agency (EPA), which only examines hazard and risk assessments, not efficiency. Some of the biological control products can also be considered as minimal risk pesticides; in this case, they can be used without any registration (Hauschild et al., 2011). Consequently, the commercialization of these products is especially well developed in the USA (Regnault-Roger et al., 2012).

Anyhow, these registration processes aim at listing the risks that need to be assessed prior to registration in order to safely commercialize a product. In 1996, Stine Microbial Product Company registered a bio-fungicide based on a strain of *Burkholderia cepacia*, but growing concerns regarding the threat it represents for immunocompromised patients led to its removal several years later (Holmes et al., 1998; Wozniak, 2007). In Europe, according to regulation No. 1107/2009, requirements include a precise identification and characterization of the microorganism at the strain level; a description of its biological properties and ecology; all available details on the product composition and production; information on the methods used to characterize the microorganism and the possible contaminants of the product; a full study of the potential effects of the microorganism on human health; a check on the residues likely to be present in the product or on the treated plant; specifications about the fate and behavior of the microorganism in the environment and its potential effects on non-target organisms (EU, 2009b; Alabouvette and Cordier, 2011). For botanicals, if the product is registered as an active substance, the same requirements as those presented above are needed (Hauschild et al., 2011). However, if the product is considered as a low-risk active substance or as a basic substance, the conditions to be fulfilled are described in annex II.5 and in article 23 of regulation No. 1107/2009, respectively (EU, 2009b). These requirements are only related to the active substance of the product. Details about the formulated products, such as the mode of application, the composition, the targeted pathogen or the conditions of use, can be requested at the national level (Ehlers, 2011).

9. Promising development and success stories

Despite the difficulties encountered to reach commercialization, 23 MBCA products and 3 botanical products are currently available round the world to manage *F. oxysporum* diseases on ornamentals (Table 2). Among these products, only 5 MBCA are authorized in Europe, while no botanical is yet available. In 2010, Sant et al. published interesting results on the control of Fusarium wilt of carnation using strain T34 of *Trichoderma asperellum*. The strain proved able to colonize the substrate and to reduce disease incidence and severity by 33% and 48%, respectively. In 2011, the product was registered by the EPA, in charge of recording all pesticides allowed for sale in the United States (EPA, 2011). In 2012, the European Food Safety Authority gave its first conclusion about the potential risk of T34. The product is about to be commercialized in Europe (EFSA, 2012). In 2013, Canada's Pest Management Regulatory Agency decided to register the product for sale to control *F. oxysporum* on greenhouse ornamentals (PMRA, 2013). The majority of the MBCA products commercialized to control *F. oxysporum* on ornamentals are composed of *Trichoderma* strains.

More globally, the main microbial species efficient against *F. oxysporum* on ornamentals are already represented on the market (Table 2). However, only a few formulations based on *Fusarium* spp., *Streptomyces* spp. or *Pseudomonas* spp. are sold, while many strains of these genera have been largely assessed in the literature. The low number of studies published in this domain and assessed in the present review does not permit to draw hypotheses about this observation. Nevertheless, when considering the entire market, it is obvious that mainly microorganisms studied for a long time are commercialized, which highlights the lack of diversity on the market (Pertot, 2014).

Regarding botanicals, even fewer products are marketed. However, they should provide a sustainable control method: they display various modes of action, so the risk of pathogens bypassing them is reduced (Chiasson et al., 2008). They are biodegradable, so they are naturally catabolized and non-persistent in soil and water. This makes them an eco-friendly solution (Isman, 2000; Regnault-Roger et al., 2012). Even if a high amount of biomass is needed, it is possible to use only part of the plant or plant by-products from production (Batish et al., 2008). Furthermore, botanicals can be applied in microcapsules or seed coating to facilitate their use and avoid any excessive input (El-Mougy et al., 2015; Soliman, 2013). In addition, in some cases they promote plant yield and quality (Granja et al., 2014). Three products are currently registered round the world to control *F. oxysporum* on ornamentals. The first one, Armorex®, is commercialized by SoilTech (Iowa, USA). It is composed of a combination of EO from garlic, pepper, sesame, rosemary, and clove. The second one, Fungastop®, is also marketed by SoilTech. It is composed of citric acid, mint oil, citrus pulp, fish oil, glycerol, and vitamin C. These two products are sold in the USA. The last one, named Regalis®, is produced by Marrone bio innovations. It consists of an extract from giant knotweed, *Reynoutria sachalinensis*. This product is approved for use nearly in the whole American continent.

As a whole, the market of biological control is making progress, although an improvement of the European regulation process is certainly required to facilitate marketing. In 2004, the global biological control market was $588 million, among which 40% for microorganisms. The main sales were localized in North America (43%), followed by Europe and Asia with 21% and 12% of the market, respectively. The European market doubled between 1985 and 2004, and is expected to keep rising in the years to come (Bolckmans, 2008). These facts are confirmed by a study focusing on biological control perspectives in Europe, which highlights an increasing interest for these products. In 2012, the European market for biological control microorganisms was estimated around 52 million euros (Nicot et al., 2012). Similar figures are not available for botanicals, but the increasing number of scientific publications in this area highlights the promising future of these products. Globally, the potential of MBCA and botanical resources is not fully exploited yet, and there is ample space for improvement.

10. Integrated disease management

The two control methods presented here have proved efficient alone, but they would be more effective once combined and integrated with other control methods. Combination can be done simultaneously or successively. As an example, Minuto et al. (2008) tested the effect of biological control and physical methods by modifying the pH and the disinfection protocol of the nutrient solution (using UV radiation, slow sand filtration, or slow rockwool filtration), and application of MBCA (Fusaria mix, *Streptomyces griseoviridis*, or *Trichoderma* mix) to reduce the incidence of *F. oxysporum* on chrysanthemum. The best result was achieved by combining a neutral pH of the nutrient solution, slow sand or rockwool filtration of the nutrient solution, and *S. griseoviridis* or *Trichoderma* mix application. The use of each method alone revealed less efficient. On gladiolus corms, the combined use of treatments inhibiting both the growth and the proliferation of the fungus (using EO, UV-C, and hot water) was more effective in reducing the fungus population during storage than each treatment used alone (Sharma and Tripathi, 2008). Biological control products can be combined with cultural practices (soil or nutrient solution disinfection, management of the pH of the substrate) or chemicals at different times during a production cycle (Duijff et al., 1995; Elmer and McGovern, 2004; Mishra et al., 2000). The use of these different practices often allows for better and longer disease management.

11. Conclusion

In the last years, new or updated control methods have emerged to offer plant growers alternatives to chemicals to manage plant diseases. Among them, MBCA and botanicals have sparked interest. The main ideas of this work are listed below.

1) Although pathogenic *F. oxysporum* are highly dangerous pathogens for ornamental production, only few publications dealing with biological control of these fungi have been recorded in comparison to the biological control of Fusarium wilts of vegetables. However the restriction of chemical use for environmental and human health concerns has stimulated the study of these alternatives.
2) Among the microorganisms evaluated so far, a majority has proved efficient in reducing the disease. However, only a few of them are commercialized. Thus, these microorganisms represent good candidates that should be exploited.
3) Botanicals for the control of pathogenic fungi have been poorly studied, although their antimicrobial properties for other uses have been well known for decades. Nevertheless, studies show a good potential of these mixtures. In addition, they display interesting features such as their low risk for the environment and for human health, and their biodegradability. Botanicals will soon be part of integrated disease management strategies.
4) Numerous techniques and parameters are available to evaluate the biological control competences of microorganisms and botanicals. However, *in vivo* tests under controlled conditions are undoubtedly the best technique. Although *in vitro* tests are more convenient, *in vivo* tests should first be performed to obtain reliable screening results.
5) In this context, research and development should play a key role in the development of these products to define and optimize the parameters of production and use. They should be the link between research and the market.
6) This development will only succeed if the training of agricultural stakeholders about the use of biological control products, their advantages and drawbacks is encouraged in parallel.

7) Producers are expecting solutions to face the threat of diseases on crops, and they should be a driving force for the development and the understanding of alternative control methods such as biological control. The increasing number of studies on MBCA and botanicals highlights that research is already on-going. However, the low number of products available in Europe shows that legislation clearly slows down the commercialization of these products. This is even more obvious when comparing the number of products available in the USA, where procedures are different.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biocontrol.2016.06.004.

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