

# Biological control of tree and woody plant diseases: an impossible task?

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**Abstract** The social demand for novel, sustainable and environment friendly approaches, while ensuring the health and productivity of our crops, is increasingly growing. Research on biological control of tree/woody crop diseases is scarce compared to that conducted on herbaceous, annual plants. In addition to their large biomass, complicated anatomy, longevity and perennial nature, peculiarities in the management of tree crops and forestry also contribute to the complexity of the processes of developing effective biological control measures in these agro-ecosystems. Although biological control in woody species poses challenges, difficulties and limitations, its implementation either alone or in combination with other disease management strategies is feasible. As a result, examples of successful application of biocontrol measures

based on the use of bacteria, fungi or hypovirulent mycoviruses against tree/woody plant diseases are available. The aim of this special issue is to provide interested readers with an overview and updates on the active research field of biological control of tree and woody plant diseases. Such effort includes updates ranging from the generation of fundamental knowledge to examples of successful application of biological control strategies.

**Keywords** Chestnut blight · Citrus (*Citrus sinensis* L.) nematode · Dutch elm disease · Endophyte · Guava (*Psidium guajava* L.) · Grapevine (*Vitis vinifera* L.) · Microbiome · Verticillium wilt of olive (*Olea europaea* L.)

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## Introduction: importance of trees and woody plants

Trees and woody crops are crucial for life on Earth. In fact, forests and other wooded lands cover about 40 % of the world's land surface (FAO 2010). Crowther et al. (2015) have recently estimated that the global number of forest trees is approximately 3.04 trillion, exceeding previous estimates by far and stressing the magnitude of this massive biomass. Besides forests, huge acreages of agricultural soils are devoted to the cultivation of a large variety of trees and woody plants worldwide (e.g. *Camellia sinensis* (L.) Kuntze, *Citrus* spp., *Coffea* spp., *Malus domestica* B., *Olea europaea* L., *Phoenix*

*dactylifera* L., *Persea americana* Mill., *Prunus* spp., *Theobroma cacao* L., *Vitis vinifera* L., etc.). Domestication and cultivation of woody species is part of the human history and these commodities became essential to the economy of many countries. Extraction of non-timber products from the forest may be considered as the initial phase of domestication of valuable tree species (Prance 1994). Thus, the first woody plants to be cultivated were those yielding food and other non-timber products, such as olive, edible fig and grapes (around 4000 BC) in the Mediterranean area, or diverse fruit trees (around 2000 BC) in Asia (Turnbull 2002). Over the centuries, these activities have shaped particular landscapes, generating outstanding agroecosystems in many regions around the world. Trees and woody plants do not only provide edible products essential for human and animal diets, but also important goods (e.g., wood, paper, etc.). Moreover, they play key roles in nutrient and water cycling processes, preventing soil erosion, mitigating the effects of climate change acting as carbon dioxide sink, and supporting microbial, animal and plant biodiversity (Ruano-Rosa and Mercado-Blanco 2015). The health of forests and woody cropping systems is therefore of particular relevance. However, a range of biotic constraints due to the attacks of a diversity of viruses, phytoplasmas, bacteria, oomycetes, fungi, nematodes, arthropods and parasitic plants continuously compromise the fitness, development and production of trees and woody plants (Fig. 1). For instance, many soil-borne phytopathogens provoke serious losses in economically-relevant tree crops and forestry. Among them, different fungi (e.g. *Fusarium*, *Armillaria*, *Heterobasidion*, *Rosellinia*, *Verticillium*, etc.) and oomycetes (e.g. *Phytophthora*, *Pythium*, etc.) genera (García-Jiménez et al. 2010), and pathogenic bacteria such as *Agrobacterium tumefaciens* (de Cleene and de Ley 1976) are particularly harmful and may cause great losses. Additionally, many foliar pathogens provoke severe diseases on woody plants, such as fungal-based powdery mildew or cankers (Agrios 2005; Amano 1986; Manion 1991), or the aggressive aerial phytopathogenic bacteria *Erwinia amylovora* (causing fire blight; Vanneste 2000) and *Pseudomonas syringae* (causing bacterial blights; Kennelly et al. 2007). To complete the catalogue of important pathogenic organisms causing relevant diseases on tree and woody plants, we must not forget pathogenic viruses (Cooper 1979) and nematodes (Ruehle 1973).

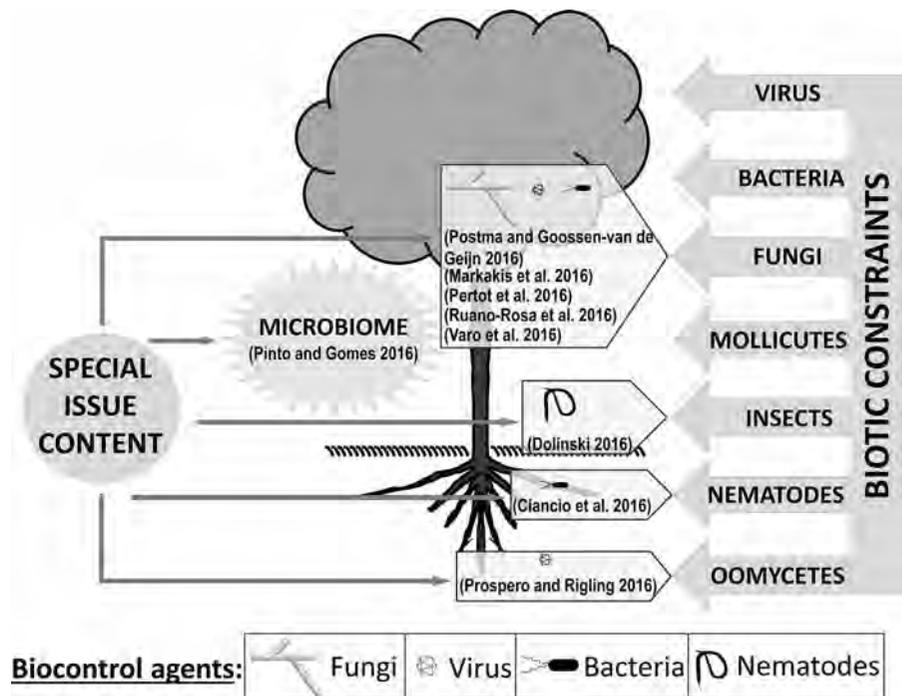
New emergent diseases are gaining relevance worldwide (Santini et al. 2013). These are usually characterized by the presence of severe symptoms, whose control is challenging. Diseases such as the citrus Huanglongbing (previously known as “citrus greening”, and caused by different “*Candidatus Liberibacter* spp.”; Wang and Trivedi 2013), the oak decline (caused by the oomycete *Phytophthora cinnamomi*; Camilo-Alves et al. 2013) or the olive quick decline (caused by the bacterium *Xylella fastidiosa*; Martelli et al. 2016) are catching the interest of nearly all societies and pose new and urgent research challenges. Finally, it is worth mentioning that the development of new cultivation systems (Connor et al. 2014) or either sudden or long-term changes in weather/climate conditions (Ponti et al. 2014) can have an as yet poorly-understood influence on woody crop diseases, as well as unknown effects on the effectiveness of the disease management strategies traditionally deployed to contain them. These scenarios warrant future in-depth studies that, in our opinion, should be conducted based on a holistic perspective involving multidisciplinary research teams together.

### Challenges in biocontrol of tree and woody plant diseases

Plant diseases are one of the main limiting factors in modern agriculture, holding potential for a devastating effect on plant health and yield. In addition to agronomic and cultural practices, growers are usually forced to resort to the use of chemical treatments. However, growing public concern about environmental pollution and harmful effects of chemicals in humans and animals, are paving the way for searching new, more environment friendly disease control methods. Mitigation strategies are effective only in some areas, and solutions to woody plant diseases and pests mainly focus on integrating different management approaches. Within this global strategy, biological control is considered as an interesting complement to other management practices. However, the different facets of this scenario have so far been rather poorly explored.

The utilization of biological control agents (BCA) to suppress pathogens has been investigated in a number of pathosystems involving woody hosts (Milgroom and Cortesi 2004; Pliego and Cazorla 2012, and

**Fig. 1** Approaches to study biological control in different trees and woody plants contained in this special issue. These studies (reference included) use diverse biocontrol agents (represented by *figures* of fungi, bacteria, viruses, nematodes) to fight against some major biotic constraints of trees and woody plants



references therein). Nevertheless, a survey in the scientific literature reveals that, overall, the utilization of BCAs as a disease control strategy has been implemented to a lesser extent in trees and woody plants compared to that in herbaceous species, annual crops and seedlings. A number of reasons could explain the lower implementation and/or success of biological control approaches in trees and woody plants. These may include challenges that have not been encountered so far, or that are not as easily overcome when dealing with annual crops. Factors such as a larger biomass, a more complex anatomy, a greater longevity, the perennial nature, and/or inherent particularities in the management of tree crops and forests (e.g. absence of crop rotation aiming to reduce the pathogen inoculum level, etc.) make it more difficult to develop and implement effective biological control approaches. For instance, in the case of soil-borne pathogens, the large root systems of trees and their architecture may facilitate repeated infection events from any given pathogen persisting in soils as dormant, quiescent or resistant propagules (e.g. chlamydospores, microsclerotia, etc.). Moreover, infection episodes can take place either in the same season or in successive ones. This fact may contribute to reduce the effectiveness of biocontrol strategies

(Pliego and Cazorla 2012). In other cases, such as for vascular pathogens, a tree can only be partially affected by the disease during a growing season. The syndrome is thus not leading to the death of the entire plant but to severe symptoms of individual branches, thereby limiting or stopping tree growth and production. This may pose difficulties when making a decision on which type of disease managing strategy is more appropriate to implement. For instance, López-Escudero and Mercado-Blanco (2011) have highlighted the complexity to control *Verticillium dahliae* in olive (*Olea europaea*) due to, among other factors, the pathogen’s location within the vascular vessels, a tissue difficult to be reached either by chemical or biological treatments. Another example is found in Burr and Otten (1999) who reported that attempts to control crown gall disease, caused by *A. tumefaciens*, have largely failed, except by using the biological control bacterium *Agrobacterium radiobacter* K84 (New and Kerr 1972). However, biocontrol cross-protection is restricted to only certain *Agrobacterium* strains, so these approaches face limitations when they need to be implemented (Anand et al. 2008). In other cases, propagules of pathogens infecting woody hosts (e.g. *Uncinula necator* causing powdery mildew in grapevine [*Vitis vinifera*],

*Rosellina necatrix* causing white root rot on avocado [*Persea Americana*], or *Venturia inequalis* causing apple [*Malus domestica*] scab) overwinter either on plant debris or plant organs/tissues (Pearson and Gadoury 1987; Holb et al. 2004; Pliego et al. 2012; Melnick et al. 2013), making the application of effective control measures difficult. Finally, olive knot caused by the bacterial pathogen *Pseudomonas savastanoi* pv. *savastanoi* (Ramos et al. 2012) can be considered as a model for woody plant diseases in which the causal agent is constantly present in plant tissues. Interestingly, the use of the biocontrol root endophyte *Pseudomonas fluorescens* PICF7 resulted in a reduction of necrotic tumors and confinement of the pathogen at inner regions of the knots, although disease development was not impaired (Maldonado-González et al. 2013).

### Biocontrol approaches for woody plant diseases

In spite of these difficulties, a range of biocontrol-based measures have been developed and are practicable for woody plants. So far, however, they mostly focus on the seedling phase, young plants and/or during the nursery propagation stage (Mercado-Blanco et al. 2004; Abraham et al. 2013; González-Sánchez et al. 2013; Vitullo et al. 2013). The question ahead yet to be asked when implementing biocontrol strategies for woody plants, either alone or in combination with other disease management approaches, is whether they can be consistently and effectively used with adult individuals, under field conditions, and on large scales (orchards, forests, etc.). One more relevant question to be addressed is whether the use of biological control measures is feasible from an economical perspective, considering the idiosyncrasy of tree and woody agro-ecosystems. For instance, deep root systems usually developed by trees are less accessible to chemical- or physical-based disease management approaches, thereby reducing their effectiveness (López-Herrera et al. 2003). It is conceivable to think that deep soil layers will be also less accessible to biologically-based control approaches (e.g. deploying formulations harboring biocontrol agents by irrigation).

Biocontrol approaches to confront tree and woody plants diseases are diverse, encompassing strategies such as the use of hypovirulent viruses and fungi (El

Hassni et al. 2004; Milgroom and Cortesi 2004), beneficial bacteria and/or their secondary metabolites to control, for instance, bayoud disease (*Fusarium oxysporum* f. sp. *albedinis*) in date palm (*Phoenix dactylifera*) (El Hassni et al. 2007) or diverse grapevine pathogens (Compant et al. 2012, and references therein), fungi displaying a multiplicity of biocontrol mechanisms such as *Trichoderma* spp. against avocado (*Persea americana*) white root rot (Ruano-Rosa and López-Herrera 2009) or *Phlebiopsis gigantea* against the root and butt rot disease of conifers caused by *Heterobasidion annosum* (Mgbeahuruike et al. 2011; 2012), and organic amendments with antagonistic microorganisms (Moreira et al. 2007) which in addition can stimulate soil-resident beneficial microbiota (Bonilla et al. 2015). Thus, the use of organic amendments or mulches has been successfully incorporated into integrated management of certain diseases, such as those affecting avocado trees (Pérez-Jiménez 2008; Bonilla et al. 2012). This is mainly due to the fact that avocado roots are easily accessible since they are mostly in the upper centimeters of the soil (60 % of the roots in the first 60 cm of the soil; Salazar-García and Cortés-Flores 1986). An interesting and promising approach, although insufficiently explored, is the use of endophytic microorganisms (Hardoim et al. 2015). Beneficial bacteria and fungi showing effective biocontrol activity and displaying endophytic lifestyle offer the advantage to be adapted and to endure within plant tissues for long periods of time, conferring them great potential in agricultural biotechnology (Mercado-Blanco and Lugtenberg 2014). Adaptation to inner tissues and endurance within them make endophytes good candidates as biocontrol agents of long-living, woody plant diseases. Indeed, tree endophytes have been proposed as an excellent tool aiming to sustain forest health (Pautasso et al. 2015). Examples of effective and/or promising use of bacterial endophytes to control diseases in trees are already available, such as in olive against the soil-borne vascular pathogen *V. dahliae* (Maldonado-González et al. 2015), against poplar (*Populus* spp.) canker caused by *Cytospora chrysosperma*, *Phomopsis macrospora* and *Fusicoccum aesculi* (Ren et al. 2013), diverse cacao (*Theobroma cacao*) pod diseases (Melnick et al. 2011), or against *Xylella fastidiosa* sbsp. *pauca* causing citrus variegated chlorosis (CVC) disease of sweet oranges (*Citrus sinensis* L.) (Lacava et al. 2004, 2007). Noteworthy, this pathosystem poses

an additional interest. Indeed, the vector of CVC, the sharpshooter insect *Bucephalonia xanthophys*, also transports the endophytic biocontrol bacterium *Methylobacterium mesophilicum* (Gai et al. 2009). This offers stimulating perspectives to develop innovative biocontrol approaches for this pathogen. Overall, while some results have been obtained using endophytes in different pathosystems under controlled conditions, yet the actual challenge is to develop appropriate strategies for their formulation, application and efficient use in woody agro-ecosystems and forestry.

### Aim and content of this special issue

In this volume, several examples of on-going research on biocontrol approaches in different pathosystems involving trees and woody plants are presented (Fig. 1). They confirm that even though challenges and difficulties are encountered when confronting woody plant diseases by biocontrol approaches, fundamental knowledge is progressively gathered and promising results are obtained. Moreover, successful application of biocontrol measures is a reality in some cases.

Microbiomes of plants have a strong impact on their health, growth and productivity. Likewise, several factors influence the abundance, diversity and composition of the plant-associated microbial communities. The complexity of the plant microbiome is not yet fully understood, and several attempts to unravel its influence on the host have been performed in different crops of interest, such as grapevine (Pinto et al. 2014; Zarraonaindia et al. 2015). In this special issue, two studies deal with different aspects of biocontrol in grapevine. Pinto and Gomes (2016) review how basic research on grapevine microbiome could lead to new technological developments to improve agriculture productivity and sustainability. This can be achieved by exploiting microorganisms harboring beneficial traits for the plant, identifying new genes and enzymes with potential to be used in novel strategies for crop protection. In this work, it is proposed that the knowledge gathered about the plant-associated microbiome can assist in the elucidation of plant-microbial interactions taking place, thus revealing microorganisms that could be involved in plant protection processes. By this basic knowledge, and facing a

possible disease scenario, different microbial-based tools could be developed thanks to the previous research performed on the plant microbiome. Pertot et al. (2016) present one of the few works focused on the promising use of the well-known biocontrol agent *Trichoderma* spp. during the grapevine nursery process. *Phaeoacremonium aleophilum* and *Phaeoaniella chlamydospora* are pathogens commonly associated with wood discoloration symptoms in tracheomycotic syndromes of esca, and are thus considered the causal agents of this phaeotracheomycotic complex. Infections commonly occur in vineyards or derive from infected mother plants. However, the grafting process in nurseries can also pose an additional risk of infection. The analysis of different *Trichoderma* spp. biocontrol agents applied during this special stage of the plant revealed that although new approaches to reduce the risk of fungal pathogen infection in nurseries can be proposed, the preventive use of strain SC1 of *Trichoderma atroviridae* could be a feasible and relatively cheap control measure.

Verticillium wilt is one of the most threatening biotic constraints for olive cultivation in many areas where this tree is cultivated. So far, the best approach to confront the disease is by implementing an integrated disease management strategy (López-Escudero and Mercado-Blanco 2011), in which biological control measures can play a role, mainly when applied in a preventive way. In this special issue, three studies devoted to the biocontrol of this disease are included. Ruano-Rosa et al. (2016) describe in detail the behavior of *Trichoderma harzianum*, a widely-used BCA, once released in the olive rhizosphere. Using a fluorescently-labeled derivative of *T. harzianum* CECT 2413 and confocal laser scanning microscopy, they observed for the first time events related to mycoparasitism of *V. dahliae* by *T. harzianum* in vitro. Moreover, they found that most of the biomass of CECT 2413 was mainly visualized as chlamydospores, a resistant structure, soon after being released in the olive rhizosphere, regardless of the experimental conditions used and the absence or presence of the pathogen. This observation suggests that this BCA seems not to be able to persist in a metabolically active form when applied as a spore suspension, raising interesting questions about the way this BCA should be formulated and deployed to reach full effectiveness. In another study, Markakis

et al. (2016) evaluated the biocontrol efficiency of the bacterium *Paenibacillus alvei*, strain K165, against the same pathogen under both greenhouse and field experimental conditions. While bioassays conducted in greenhouse revealed that strain K165 significantly decreased disease development in a susceptible olive cultivar, a major step forward of this study was to confirm the suppressive effect of K165 in an established olive orchard naturally infested with *V. dahliae*. Among other interesting observations, this study reports for the first time effective biological control of this disease under field conditions, a scenario not frequently explored in biocontrol research, particularly with woody plants. Finally, Varo et al. (2016) compared diverse pathogen inoculation methods to screen for potential biocontrol agents against *V. dahliae* in olive, addressing one of the major restrictions when developing control measures against this disease: the lack of consistent inoculation methods to evaluate, for instance, the effectiveness of biocontrol agents. The authors eventually propose a promising inoculation method to assess potentially effective BCAs against Verticillium wilt of olive, an important step before evaluation under field conditions.

Dutch elm (*Ulmus* spp.) disease, caused by species of the fungal genus *Ophiostoma*, is a devastating disease affecting elms in Europe and North America. Infections mostly occur by pathogen transmission by several species of bark beetles, mainly from the genus *Scolytus*, although infection by means of root contact between infected and healthy trees can also take place. Biological control of Dutch elm disease under field conditions has already a long history (Scheffer et al. 2008). In this issue, Postma and Goossen-van de Geijn (2016) report on the successful use of a biological control approach to protect a tree over a long period of time (>20 years) and under natural (and urban) conditions. Moreover, this is an interesting case on the use of a pathogenic fungus (*Verticillium albo-atrum* strain WCS850) behaving as a protective agent in a non-host species. While the product under study does not protect already-infected elm trees or trees connected with diseased individuals via root grafts, it does prevent healthy trees from pathogen infection through elm bark beetles.

The ascomycete *Cryphonectria parasitica* is a wound parasite that causes lethal bark lesions on the stem and branches of chestnut (*Castanea* spp.), developing chestnut blight (Prospero and Rigling

2013). The consequences of this disease are less dramatic in Europe on the European chestnut (*Castanea sativa* Mill.) than in North America on the American chestnut (*Castanea dentata* (Marshall) Borkh.) due, among other factors, to the spontaneous appearance and spread of natural hypovirulence caused by an unencapsidated RNA virus (Heiniger and Rigling 1994). This made chestnut blight as one of the few tree diseases in Europe for which biological control is possible. In this special issue, Prospero and Rigling (2016) revealed that therapeutic canker treatment with a virus is mostly effective. However, *Cryphonectria* virus could yield contradictory results, due to competition with naturally occurring viruses. This study also emphasizes the usefulness of molecular markers to track biological control agents and to evaluate the success of their application.

Another hot topic in biological control of trees and woody plants are the pests which severely affect plant performance and yield. Even though this volume is focused on diseases, we also aimed to include an example illustrating biocontrol strategies implemented to manage tree pests. One study model is the common guava (*Psidium guajava* L.), where different insect pests can be found, such as the fruit fly (*Bactrocera* spp.), the fruit borer (*Deudorix Isocrates* Fab.) or different types of Lepidoptera, Hemiptera and Homoptera (Souza et al. 2003). Management of insect pests involves several applications of chemicals, mainly targeting the adult form. To avoid the environmental concerns caused by the effects of pesticides, alternative measures are being developed, including those based on biological control. Dolinski (2016) presents in this special issue a review on how natural pathogens of insects, such as the entomopathogenic nematodes, often play an important role in regulating insect pest populations, and proposes them as components of Integrated Pest Management (IPM) programs.

Another model to study biocontrol on woody plants is the citrus phytopathogenic nematode *Tylenchulus semipenetrans*, which causes heavy attacks on young feeder citrus roots. Moreover, its pathogenicity can be enhanced by stress related to soil and/or water availability (Duncan and Noling 1987). In this issue, Ciancio et al. (2016) confirm the potential of the bacterium *Pasteuria* spp. to effectively control the citrus nematode. Moreover, by modeling obtained data, a clear relationship between *T. semipenetrans*

and *Pasteuria* sp. is shown, making it possible to have a management strategy of this nematode based on the use of this bacterium.

### Concluding remarks

Research conducted on biological control of woody plant diseases, including the examples compiled here, are stimulating and encourage further research in all aspects related to the use of biological control in these agro-ecosystems, particularly as a preventive measure. Moreover, biocontrol approaches, or more particularly the use of BCAs, can be complementary to other disease control measures (e.g. the use of pathogen-free certified planting material, the use of tolerant/resistant cultivars, their combined use with organic amendments, etc.) and/or integrated with appropriate agronomical or forestry practices. Nevertheless, our knowledge on the mechanisms underlying biocontrol, on the influence of environmental factors on the effectiveness of BCAs, on the interaction between a given BCA and the plant-associated microbiome once the former is released in the target host, on the colonization ability of the BCA, or on how the plant respond to the presence of the BCA, are still scant. The implementation of currently available, powerful ‘-omic’ technologies will definitively boost our understanding on woody plant-BCA-pathogen interactions (Massart et al. 2015). So far, these approaches have been used in trees and woody plants to a lesser extent than in herbaceous species, although examples are increasingly available (Sun et al. 2011; Palmieri et al. 2012; Mgbеаhuruikе et al. 2013; Gómez-Lama Cabanás et al. 2014; Calderón et al. 2015; Martínez-García et al. 2015), and many more will be on hand soon. Likewise, proper understanding of the causes behind inconsistencies observed with BCAs is urgent, particularly when they are released under field conditions and a multiplicity of (a)biotic factors is dynamically interacting. Working with trees and woody plants, and considering the peculiarities of these plants which have been briefly described above, can accentuate these inconsistencies. In spite of these difficulties, the use of biocontrol measures, either alone or complementary to other actions within IPM frameworks, is feasible and should have a promising future as sustainable and environmental-friendly approaches.

Finally, we would like to express our sincere gratitude to all researchers who collaborated by submitting their latest results in the field of biological control of tree and woody plant diseases. Their works demonstrate that the biocontrol of tree and woody plant diseases is not an impossible task. We are also in debt to Dr. Eric Wajnberg for his continuous encouragement and enthusiasm to make this special issue a reality.

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