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INDUCTION OF RESISTANCE IN THE CONTROL OF PHYTOPLASMA DISEASES

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Summary

Phytoplasma are wall-less prokaryotes that causing severe diseases in many plants, for which no effective means of control are at present available. However, plants infected by phytoplasma can undergo spontaneous symptom remission, or recovery, a known long-term phenomenon. A strategy to reduce the number of symptomatic plants might thus arise from stimulation of plant defence systems to induce recovery. Several experiments have been carried out on different phytoplasma-infected plant species, which have shown that recovery can be induced artificially by abiotic stress, treatment with resistance inducers or antimicrobial molecules, and application of mycorrhiza and rhizobacteria. Moreover, several recent findings have indicated the importance of endophytic fungi and bacteria in the recovery phenomenon. Examples of different approaches for control of phytoplasma infection are reported here, and some of the possible mechanisms of action involved are discussed.

Key words: Abiotic stress, Endophitic fungi and bacteria, Mycorrhiza, Resistance inducers.

Riassunto

Induzione di resistenza per il controllo delle malattie da fitoplasmi

I fitoplasmi sono Procarioti privi di parete cellulare agenti di gravi malattie delle piante, per le quali al momento non sono note possibilità di cura. Le piante infette da fitoplasmi possono andare incontro alla remissione spontanea dei sintomi di malattia, fenomeno conosciuto da tempo anche con il termine recovery. Una delle poche possibilità di controllo delle fitoplasmosi consiste quindi nel tentare un incremento della quota di recovery mediante l'elicitazione delle difese della pianta. Una serie di indagini condotte su piante infette da fitoplasmi hanno evidenziato la possibilità di incrementare il numero di piante recovered mediante l'applicazione di stress abiotici, induttori di resistenza e molecole ad attività antimicrobica, micorrize e rizobatteri. Inoltre, recenti indagini hanno evidenziato l'importanza di funghi e batteri endofiti nel fenomeno recovery. Nella review vengono riportati esempi di applicazione dei diversi approcci per il contenimento delle fitoplasmosi delle colture e sono discussi alcuni dei meccanismi di azione coinvolti.

Parole chiave: Stress abiotici; Funghi e batteri endofiti; Induttori di Resistenza; Micorrize.

Introduction

Phytoplasma are small, insect-transmitted, cell-wall-less bacteria that cause numerous diseases in economically and environmentally important plant species worldwide (McCoy *et al.*, 1989; Lee *et al.*, 2000; Seemüller *et al.*, 2002; Martelli and Boudon-Padieu, 2006; Weintraub and Beanland, 2006; Bertaccini *et al.*, 2007; Firrao *et al.*, 2007; Hogenhout *et al.*, 2008). The impossibility of cultivating them *in vitro* has blocked the development of methods of control against these pathogens. Indeed, other strategies have been tested, namely: (i) production of transgenic plants expressing antibodies against the major phytoplasma membrane protein (Le Gall *et al.*, 1998; Malembic-Maher *et al.*, 2005); (ii) production of transgenic plants expressing antimicrobial peptides (Zhao *et al.*, 2004; Du *et al.*, 2005); and (iii) protecting the plants using elicitins (cryptogein or oligandrin), small proteins that stimulate P protein plugs and callose release in phloem sieve elements (Lherminier *et al.*, 2003). Other approaches for the control of phytoplasma are not directed against the pathogen, but are based instead on the selection of resistant, tolerant or immune plant varieties. They have so far been investigated by phytoplasma inoculation, symptom observation and variety selection (Jaraush *et al.*, 1999; Sinclair *et al.*, 2000), and also marker-assisted selection programmes (Cardena *et al.*, 2003). This undirected control strategy is not always possible, because not all phytoplasma-infected plant species show resistance or tolerance *versus* to disease. A promising approach has become more prominent recently through significant studies of different groups that have addressed the exploitation of knowledge concerning natural or induced resistance to plant bacteria, and in particular to phytoplasma. Indeed, the existence of different forms of life within the plant is a basic concept that can be developed for the design of possible alternatives and strategies for phytoplasma disease control.

Recovery

Plants infected by phytoplasma can undergo spontaneous symptom remission, which is also known as recovery (Caudwell, 1961). In grapevines, this natural phenomenon has been observed in different varieties and viticultural regions (Osler *et al*., 2003; Garau *et al*., 2004; Maixner, 2006a; Romanazzi *et al*., 2007). Recovery appears to be induced by different factors (Kunze, 1976), and it can be correlated to various biological events. These include the presence and dominance of hypovirulent strains of the pathogens (Kison and Seemüller, 2001; Loi *et al*., 2008; Marcone *et al*., 2008), the presence of antagonists or phytoplasma parasitoids (Marzorati *et al*., 2006), the activity of particular substances or plant secondary metabolites, and the induction of systemic acquired resistance (SAR).

In grapevines, the recovery phenomenon depends on factors such as phytoplasma identity, host-plant variety (e.g. cv. Prosecco shows recovery, whereas cv. Perera does not) (Bellomo *et al*., 2007; Garau *et al*., 2007; Romanazzi *et al*., 2007), rootstock combination (Romanazzi and Murolo, 2008), environmental conditions (Braccini and Nasca, 2008), and agronomic practices such as pruning or transplanting. Recovery can be complete or partial, temporary or permanent, and common or rare, and, consequently, it can be practically significant or not for an infected crop. As recovery can be a temporary or permanent phenomenon, Maixner (2006b) recently proposed that an originally infected plant can be considered recovered only after a minimum of three consecutive years without symptoms. In north-eastern Italy (Province of Treviso), between 1995 and 1998, more than two million Prosecco grapevines completely recovered from Flavescence dorée, and normal production was then re-established (Osler *et al*., 2003). In the heavily Flavescence-dorée-infected north-western part of Italy, in vineyards planted with cvs. Dolcetto, Barbera, Bonarda and Cortese, between 1999 and 2003, the average number of healthy plants decreased and the numbers of both recovered plants and those with symptoms increased. Among these cvs., although the productivity of recovered vines was lower than that of healthy ones, it was always higher than that of vines with symptoms and it was not influenced by the time elapsed from the date of recovery (Morone *et al*., 2007). In trials carried out in Sardinia on cv. Chardonnay, in the first year after recovery, the production was intermediate between infected and healthy plants, and not significantly different as compared to the symptomatic plants; however, two years later, the production reached a level similar to that of healthy plants and greater than that of symptomatic plants (Garau *et al*., 2007). This behavioural trend confirms the evidence found previously in Friuli-Venezia Giulia (Mutton *et al*., 2002) and Emilia Romagna (Credi *et al*., 1989). Moreover, recovery was not seen for phytoplasma-infected Perera grapevines (Pavan *et al*., 1997).

In recovered plants, molecular analysis of leaf veins has failed to reveal the presence of phytoplasmas in several Italian and German areas for Bois-noir-infected and Flavescence-dorée-infected plants (Osler *et al*., 2003; Maixner, 2006b; Morone *et al*., 2007; Romanazzi and Murolo, 2008).

The physiological basis of recovery is not yet completely known. It has been seen that in apple (Musetti *et al.*, 2004), apricot (Musetti *et al.*, 2005a) and grapevines (Musetti *et al.*, 2007a), recovery from phytoplasma-associated diseases was accompanied by an overproduction of hydrogen peroxide (H_2O_2), which was localised to the phloem tissues. Such H_2O_2 accumulation was not detected in symptomatic diseased plants, nor in healthy control plants. Overproduction of H_2O_2 requires the intervention of antioxidant systems, which include metabolites such as ascorbate and reduced glutathione, and scavenging enzymes, such as superoxide dismutase, catalase and hydrogen-donor-aspecific peroxidases, e.g. guaiacol peroxidase. In recovered plants the activities of the two enzymes primarily involved in H_2O_2 scavenging, namely catalase and ascorbate peroxidase, significantly decreased when compared to healthy or diseased plants. Therefore, it has been hypothesised that decreased scavenging, rather than enhanced synthesis, is the probable cause of the increased H_2O_2 levels in plants recovering from phytoplasma infection. In turn, this led us to hypothesise an active role of H_2O_2, and possibly other reactive oxygen species, in counteracting pathogen virulence and contributing to promote recovery.

The expression of chalcone synthase increased in recovered plants of cvs. Chardonnay and Sangiovese, in both September and June (Landi and Romanazzi, 2009). It has also been hypothesised that Ca^{2+}-dependent signalling activities, in particular those connected with plant resistance, increase in recovered plants. Indeed, it has been shown recently that Ca^{2+} concentrations in the cell cytosol are remarkably increased in recovered apple plants, as compared to healthy or infected plants (Musetti *et al.*, 2008).

Together with the demonstration that recovered plants can be re-infected in nature to a lesser extent than plants that have never been previously infected, these observations indicate that a type of SAR is involved in the induction of recovery. Current investigations are aimed at studying the expression of defence-related genes and determining the genetic bases of recovery (Albertazzi *et al.*, 2009; Hren *et al.*, 2009a, 2009b).

Possible strategies for the control of phytoplasma diseases

Application of abiotic stress

Recovery can be promoted by exposing grapevines to abiotic stress, such as uprooting followed by immediate transplanting (Osler *et al.*, 1993) and partial uprooting or pulling (Romanazzi and Murolo, 2008), and by agronomical practices, such as pruning and pollarding (Borgo and Angelini, 2002; Zorloni *et al.*, 2002). Partial uprooting has been effective in the induction of recovery in almost all of the plants of cvs. Chardonnay, Verdicchio and Sangiovese grafted onto Kober 5BB rootstock, but it proved less effective when cv. Chardonnay was grafted onto 420A rootstock. Although both of these rootstocks are hybrid *Vitis berlandieri* x *V. riparia*, a possible interaction between the rootstock and the effectiveness of partial uprooting was seen, although the significance of this relationship remains to be understood

(Romanazzi and Murolo, 2008). Pulling has been partially effective in the induction of recovery in Bois-noir-infected plants. Indeed, this practice is easy and practical to apply by a common machine that is also used in viticulture for ploughing, and thus its application should worth testing in large scale trials. In cv Primitivo, the first year after recovery from Bois noir obtained by partial uprooting the plants had a trend in photosynthesis and respiration similar to the healthy ones, although at times with lower values (Murolo *et al*., 2009).

Treatment with resistance inducers and antimicrobial agents

An innovative possibility for the control of plant yellows diseases involves spraying the plant canopy with resistance inducers. Induced resistance is a non-specific form of disease resistance in plants that can act against a wide range of pathogens, and it can be activated by several non-specific inducers, known also as elicitors (Vallad and Goodman, 2004). A great variety of commercial resistance inducers is today available, some of which are registered in Italy for use as plant protectant products, while a longer list of adjuvants includes the property of plant resistance promoters. To date, there are no treatments that can sanitise phytoplasma-infected plants. The use of antibiotics against phytoplasma infection has been suggested, but in the European Union these *via* has been banned. Moreover, the impossibility of cultivating phytoplasma *in vitro* has increased the difficulties in testing active agents that may eventually inhibit their growth. Under these circumstances, one of the few choices that remain is to promote disease resistance in plants. This strategy has been successfully applied to *in-vitro*-grown periwinkle shoots infected with different ‘*Candidatus* Phytoplasma’ species, with treatments with indole-3-acetic acid and indole-3-butyric acid (Perica, 2008). Both these auxins induced the recovery of phytoplasma-infected periwinkle shoots, with indole-3-butyric acid more effective than indole-3-acetic acid.

Several trials have been carried out in vineyards to test the effectiveness of resistance inducers in the control of Bois noir. In Emilia Romagna (northern Italy), for the control of grapevine yellows on cvs. Lambrusco Salamino and Ancellotta, Mazio *et al*. (2008) tested the effectiveness of treatments with several organic fertilisers based on nitrogen, humic and fulvic acids, and with algal extracts applied to the plant canopy and the soil at four different phenological stages. The percentages of recovered plants were affected by both the climatic conditions of the year and the cultivar, although no significant differences were seen between vines treated with these bioactivators and the control plants. In Sardinia (Italy), Kendal (Valagro, Atessa, Italy) was applied three times in 2005 and 2006 to the canopy of Bois-noir-affected vines of cvs. Chardonnay and Vermentino (Garau *et al*., 2008). However, there were no significant effects of the Kendal treatment on the qualitative and quantitative parameters of healthy, symptomatic and symptomless plants, or on the frequency of recovered vines. In central-eastern Italy, a more intense treatment schedule (7-13 applications a year) has been tested, and it was shown to indeed influence the number of recovered cv. Chardonnay plants. The best results after two years of application have been obtained with the use of Kendal, Bion (Syngenta, Syngenta Crop Protection, Basel, Switzerland) and Olivis (Agrisystem, Lamezia Terme, Italy), which decreased the number of symptomatic plants from 35 to 6, 7 and 8 respectively, over two years of treatment (Romanazzi *et al*., 2009). The application of Aliette (Bayer Crop Science, Monheim, Germany) and Chito Plant (ChiPro GmbH, Bremen, Germany) has also showed a tendency to increase recovery rates, as compared to control plants. Several studies have shown that Bion can successfully induce resistance to various pathogens, by increasing the production of pathogenesis-related proteins in many plant species (Vallad and Goodman, 2004). Bion has also been shown to provide some protection and a delay in symptom appearance in chrysanthemums infected by '*Ca.* P. asteris' (D'Amelio *et al*., 2007), a protectant activity against X-disease phytoplasma, and a reduced leafhopper survival when applied to *Arabidopsis thaliana* (Bressan and Purcell, 2005). Treatments with resistance inducers carried out on phytoplasma-infected *Catharanthus roseus* have shown different responses of the plants sprayed with Phosetyl-Al, contained in Aliette, and chitosan (Prati *et al*., 2004; Chiesa *et al*., 2007), the former being more effective. In field trials carried out with grapevines, which represent a completely different pathosystem, the treatment of the plant canopy with Phosetyl-Al and chitosan did not show significant differences in recovery induction (Romanazzi *et al*., 2009). So, data obtained for recovery promotion for one crop are not directly transferable to any other plant–phytoplasma interaction.

From the practical point of view, however, it is worth noting that Bion is not registered for use on grapevines, Aliette has a 40-day preharvest use interval (and Chardonnay is harvested in central-eastern Italy by mid August, while the other cultivars are usually harvested in September or October), and Kendal, Olivis and Chito Plant are registered as promoters of plant resistance, and not as plant protection products.

Natural and synthetic peptides and essential oils have antimicrobial activities against several plant-pathogenic bacteria, fungi and nematodes, but only a few have been tested to the control phytoplasma diseases. A preliminary evaluation of the antimicrobial activities of some chemicals on apple-proliferation disease was conducted on *in-vitro* cultures of apple shoots infected by '*Ca*. P. mali', and this showed that phytoplasma are not detectable by quantitative real-time PCR in the presence of pyrithione (Aldaghi *et al*., 2008). Physiological modifications in the composition of the phloem sap and in the balance of plant growth regulators that are seen to occur after phytoplasma infection will have important roles in the elucidation of the activities of agents with antimicrobial activities.

Application of mycorrhiza and rhizobacteria

Arbuscular mycorrhizal (AM) fungi form mutualistic associations with most plants, as they colonise the roots of over 80% of plant species and are present in all soil ecosystems. AM fungi might increase plant growth by improving mineral nutrition, and especially for phosphatics, through modifying root architecture and topology, and enhancing plant tolerance towards biotic and abiotic stresses. Moreover, the synthesis of volatile compounds and the secondary metabolic pathways are affected in mycorrhizal plants. Previous studies have shown the positive effects of AM fungi in increasing tolerance to damage caused by soil-borne pathogens (Lindermann, 1994; Lingua *et al*., 2001), enhancing plant nutrition (Azcon-Aguilar and Barea, 1997), and conferring protection against root lesion nematodes (López *et al*., 1997). The role of AM fungi in phytoplasma infection has been investigated in several pathogenic systems. In stolbur infection of tomato, agglutinations and degeneration of phytoplasma cells, coupled to reduced symptom expression, was seen in plants treated with AM fungi, and these correlated with mycorrhizal hormone activity (Lingua *et al*., 2002). In a different pathogen–host system, a preliminary report has suggested that inoculation with *Glomus intraradices* significantly improves plant-health parameters and increases tolerance to pear-decline-infected pear trees (Garcia-Chapa *et al*., 2004). Significant protection from '*Ca*. P. asteris' (a chrysanthemum yellow [CY] strain) infection of chrysanthemum plants, coupled to lower symptom severity, has also been reported (D'Amelio *et al*., 2007). Recently, the evidence that *G. mosseae* BEG 12 inoculation does not decrease periwinkle tolerance to mild and severe "*Ca*. P. asteris" strains (Kaminska *et al*., 2009) has indicated that the effects of AM fungi on phytoplasma infection are complex and probably dependent on a combination of host plant, AM fungus and phytoplasma isolate.

Plant-growth-promoting rhizobacteria represent another group of microorganisms that can activate plant-defence responses. These rhizobacteria have beneficial effects on plant development through a number of mechanisms (Glick, 1995). Direct stimulation of plant growth is usually related to hormone synthesis (i.e. indole acetic acid), mineral nutrition improvement (i.e. phosphate solubilisation, nitrogen fixation), and modifications of root architecture. Indirect stimulation relies mainly on plant health improvements through biocontrol of phytopathogens or enhancement of plant tolerance to environmental stress by production of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase or degradation of the ethylene precursor ACC by bacterial ACC deaminase. Hence, the presence of beneficial rhizospheric microorganisms that can increase plant tolerance to biotic stresses or can behave as biocontrol agents might represent a valid alternative to the control of pathogen diseases. A preliminary study on the rhizosphere microflora of root-(wilt)-resistant/ tolerant palms and diseased coconut palms showed a reduction in the bacterial population in the rhizosphere of resistant/ tolerant trees. However, although the diseased palms had greater bacterial populations, they included only 0.21% of beneficial microbes, while overall the root-(wilt)-tolerant palms had lower numbers of bacteria, these included 3.6% of beneficial microorganisms, suggesting a possible role of the rhizosphere microflora in the mechanisms that allow these palms to evade phytoplasma infection (Gopal *et al*., 2005). Indeed, a recent preliminary report indicated that the application of a pseudomonad slightly reduced the number of CY-infected daisies and extended the life span of infected plants (D'Amelio *et al*., 2007). Besides affecting plant growth and health, bacteria living in the rhizosphere can interact with AM fungi. Stimulation of AM fungi symbiosis development by rhizospheric bacteria has been reported. The inoculation of plants with both mycorrhizal fungi and beneficial rhizospheric bacteria might induce synergistic effects on the health of plants infected with phytoplasma, improving their efficacy as biocontrol treatments. These interactions might be of crucial importance within sustainable, low-input agricultural cropping systems that rely on biological processes, rather than agrochemicals, to maintain soil fertility and plant health.

Fungal endophytes

The endophyte populations within crop plants have sometimes been shown to influence the susceptibility of the plants to disease. However, the role of endophytic microorganisms in relation to systemic, non-curable diseases, such as those associated with phytoplasma, has still to be investigated. For phytoplasma diseases and recovery, it has been hypothesised that endophyte microorganisms might have roles in this resistance phenomenon. Endophytes are microorganisms that live inside host plants without causing disease symptoms or apparent injury. It is recognized that endophytes are of great importance for their hosts, and their role in the protection of plants against insects, nematodes, and pathogenic microorganisms has been well demonstrated (Gimenez *et al*., 2007). They also increase plant fitness, causing physiological modifications in their host, such as making them more resistant to abiotic stress (i.e. water stress). It is known that many endophytes can produce secondary metabolites and other compounds of biotechnological value, such as antibiotics and anti-tumour agents (Schultz *et al*., 2002). The relationships between endophytes and their host plants are not very clear.

Fungal endophytes that belong to interesting taxonomic groups that can produce antibiotics are already used as antagonists against a number of phytopathogenic fungi (Madrigal *et al*., 1991), and they have been isolated from both grapevines and apple plants grown in areas where recovery occurs (Musetti *et al*., 2005b; Martini *et al*., 2009). Musetti *et al*. (2007b) reported the cytological effects caused in phytoplasma-infected *C. roseus* cuttings by treatment with endophytic strains of *Epicoccum nigrum* and *Aureobasidium pullulans*. Ultrastructural observations performed on leaves of endophyte-inoculated cuttings revealed phytoplasma structural modifications similar to those decribed by Lingua *et al*. (2002) in stolbur-infected tomato plants treated with AM fungi. Plant-cell modifications related to an increase in host defence responses have been also seen, such as the formation of phloem protein plugs and callose occlusions, and the presence of vacuolar phenolic deposits in the lumen of sieve elements. Similar ultrastructural changes have been reported in phytoplasma-infected tomato tissues treated with elicitors (Lherminier *et al*., 2003). The modifications described lead to an enhancement of the physical barriers preventing phytoplasma movement and are related to the increasing of the host defence responses.

Moreover, using whole *C. roseus* plants grown in greenhouses and infected with '*Ca.* P. mali', it has been possible to note reductions in symptom severity when the plants were previously inoculated with the endophytic strain of *E. nigrum* (Musetti *et al*., 2009). In particular, in endophyte-treated periwinkles, the flowers appeared normal in shape and size, and were thus not different from non-infected controls. Real-time PCR has demonstrated that '*Ca*. P. mali' concentrations in *E. nigrum*-treated plants was about 2.3-fold lower than in non-treated plants.

The mechanisms by which the fungal endophyte *E. nigrum* affects phytoplasma in periwinkle plants are not known yet. Expression analyses for genes involved in plant defence mechanisms, as well as the characterisation of fungal secondary metabolites, are in progress for the clarification of plant–pathogen–endophyte relationships.

Endophytic bacteria

Over the last few years, interest for the use of endophytic microrganisms to control plant pathogens has considerably increased (Gimenez *et al*., 2007). Bacterial endophytes live inside the host plants without causing disease symptoms (Wilson, 1995; Hallman *et al*., 1997), and they are thought to enter plant tissues in different ways, such as insect sucking, or by passive diffusion or active selection from the adjacent rhizosphere, and they can inhabit different parts of plants, such as roots, tubers, stems and leaves (Hallmann, 2001; Gray and Smith, 2005). Bacterial endophytes are mainly localised in the vascular system, the intercellular space, and/or the cell cytoplasm, and their presence *in planta* is also believed to be related to modifications to plant fitness, through the production of secondary metabolites (Petrini *et al*., 1992), making the plants more resistant to abiotic stress and to attack of parasites such as insects, nematodes, fungi and bacteria. Also, endophytes can produce active compounds that have antibacterial and antifungal properties against pathogens (Schultz *et al*., 2002). Moreover, many endophytes are resistance inducers, as they can promote plant-defence reactions (Gimenez *et al*., 2007), and it has been demonstrated that endophytes have a role in the control of pathogen spread into plant tissues (Lodewyckx *et al*., 2002).

Endosymbiontic bacteria can reduce and prevent damage caused by pathogens according to different strategies such as: (i) competition for an ecologic niche or for a medium (Glick, 1995); (ii) production of allelochemic inhibitors (antibiotics, lytic enzymes); and (iii) induction of systemic resistance (ISR) (Van Loon *et al*., 1998).

Most of the information available about the possible roles of endophytes in phytoplasma disease aetiology has emerged from studies on the phenomenon of recovery. This information has shown that SAR is involved in recovery (Osler *et al*., 1999; Musetti *et al*., 2005a, 2007a). In these studies, phytoplasma are not usually found in the recovered plants, while some studies have reported their presence in the roots of recovered apple trees (Carraro *et al*., 2004).

Recently, Bulgari *et al*. (2009a, 2009b) reported different bacterial community profiles in healthy, recovered and yellows-infected grapevines. The first studies carried out on endophytic fungi and bacteria in non-infected, Flavescence-dorée-infected and recovered grapevines showed different colonisation in relation to the sanitary status of the plants (Bulgari *et al*., 2009a, 2009b), suggesting their involvement in the recovery. Besides, among the prokaryotes, *Pantoea agglomerans* was shown to be the predominant species, using length-heterogeneity-PCR (Bulgari *et al*., 2008). Some *P. agglomerans* strains can produce the antimicrobial compounds Pantocin A and B, which makes them active in the control of *Erwinia amylovora*. Moreover, *P. agglomerans* can elicit ISR by production of an exopolysaccharide that increases production of reactive oxygen species (Ortmann *et al.*, 2006). Some interesting studies have been carried out on the interactions among and between potential biocontrol agents or resistance inducer microrganisms and phytoplasma, both in host plants and insect vectors (Lingua *et al.*, 2002; Lherminier *et al.*, 2003; Marzorati *et al.*, 2006).

These studies, however, need further and appropriate investigation concerning any risks associated with the use of endophytic microrganisms, since it has been shown that some of them have potential risks for human health, like with *P. agglomerans* (Cruz *et al.*, 2007).

Concluding remarks

To date, no appropriate remedial treatments applicable to plants that are already infected with phytoplasma, and no chemical substances or particular approaches are known to be effective against the associated diseases. Moreover, no interesting plant genotypes are available that show reliable resistance to phytoplasma diseases. The use of genetically engineered plants for phytoplasma resistance could be promising, while not underestimating the risk that this might be unstable, and be overcome under conditions of natural pressure of infection. As a consequence, the effective indirect control of these epidemiological diseases has mainly focused on insecticidal treatments against the natural vectors, and on rogueing of the infected plants that act as sources of inoculum. In such a complex and crucial context, the possibility of resorting to natural or induced recovery is certainly an opportunity that should be taken and exploited.

The data reported in this review demonstrate an innovative and promising alternative for the control of phytoplasma diseases. Investigations in this field continue apace, as the induction of recovery appears to be the only possibility for the reduction of the damage caused by phytoplasma. Most of these treatments need to be applied in large scale tests and evaluated according to the economical costs and benefits. As most of the applications are still in the experimental phases, it will be necessary to extend these trials into different environments and eventually into the pathosystems where they work best, such that in a few years time protocols that have been developed for the control of phytoplasma diseases can be suggested to the growers. However, the available experimental results reviewed here are indeed already useful for technicians involved in plant protection, allowing selection of the best agricultural practices for the promotion of recovery in phytoplasma-infected plants.

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