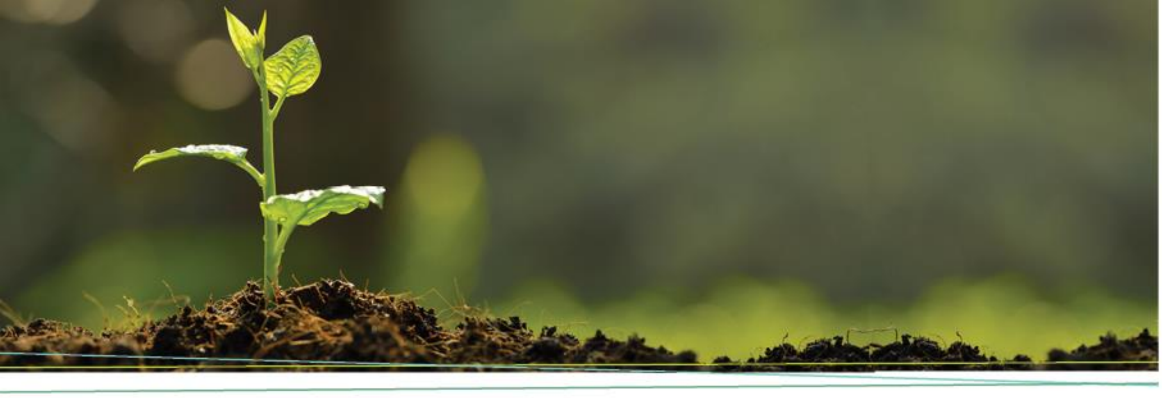

Focus Group SOIL-BORNE DISEASES

Mini-paper – ***The use of microbial biocontrol agents against soil-borne diseases***

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INTRODUCTION

The value of the global biopesticide market is expected to reach $4,556.37 Million by 2019, at a compound annual growth rate of 15.30% from 2014 to 2019 (source: Marketsandmarkets.com, 2014; last access 31/03/2015). The reasons for such growth can be found in: the increasing concerns on the impact of residues and overuse of synthetic chemical pesticides and the increasing relevance of pests and pathogens due to growth in food demand, the withdrawal of several chemical pesticides including soil fumigants, the appearance of new invasive species and pesticide resistant strains of pests, the effect of climate change and the specialised monoculture. The decrease of the cost of several biopesticides, the introduction of technologically advanced products, the increase knowledge of farmers and the strict thresholds in pesticides residues set by many food retailers are also promoting the switch form chemical to biopesticides. In addition in EU the Directive on the Sustainable Use of Pesticides (2009/128/EC) is also expected to stimulate the biopesticide market positively.
Some active ingredients have been included in Annex 1 according Regulation EC 1107/2009 and several new others are most probably under development by multinational agrochemical companies following recent acquisitions. According the present regulation if a microorganism has fungicide activity should be registered in order to be applied for such use. The high cost of registration prevents a large number of potential biocontrol agents to reach the market. Since each registered use should be supported by specific efficacy trials, the companies tend to register the products against those pathogens with the largest potential market. This results in products potentially active against a large number of soilborne pathogens, but authorised only on the specific one on which the registration trails have been carried out.

Several microorganisms (estimated in few hundred of strains) have shown efficacy against soil-borne pathogens and nematodes in literature in the last 50 years. Some of them are the active substances of some existing biopesticides. The full list of approved active substances based on a microbial strain is presented in table 1. Some of these strains are successfully used from several years against soil-borne diseases and pathogens as *Coniothyrium minitans* used to reduce the inoculum of *Sclerotinia sclerotiorum* and *S. minor* on high value vegetable crops, *Pseudomonas chlororaphis* used against soil-borne disease of cereals in northern EU countries or several *Trichoderma* strains applied against a wide spectrum of soil-borne pathogens.

Although several of these strains have been developed a few years ago, they never achieved a large market, mainly because the competiveness with synthetic chemical fumigants, which are commonly cheaper, easy to apply, with a wide spectrum of activity and high efficacy. The banning of methyl bromide and several other compounds has now revived the interest in

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microbial biocontrol agents against soil-borne diseases in particular in integration with other agronomic practices or resistant/tolerant varieties.
The mechanisms of action of microbial biocontrol agents against plant pathogens include direct antibiosis, hyperparasitism, induction of resistance and competition for space and nutrients. Some microbial agents also act as biofertilizer and/or plant growth promoters by fixing N, solubilizing P, chelating Fe, producing hormone-like substances, degrading 1-aminocyclopropane-1-carboxylate (ACC) deaminase, degrading organic matter and releasing nutrients in soil.

Table 1. List of the active substances active against plant pathogens based on a microbial strain and their status; in bold the microorganisms with claimed or possible activity against soil-borne pathogens and nematodes (**http://ec.europa.eu/sanco_pesticides/public/index.cfm?event=homepage&language=EN**, last accessed on 15/03/2015)

Substance	Category	List (*)	Status under Reg. (EC) No 1107/2009	Date of approval	Expiration of approval
Ampelomyces quisqualis strain AQ10	FU	C	Approved	01/04/2005	31/07/2017
Aureobasidium pullulans (strains DSM 14940 and DSM 14941)	FU, BA	C	Approved	01/02/2014	31/01/2024
Bacillus amyloliquefaciens MBI 600	FU	C	Pending		
Bacillus amyloliquefaciens strain FZB24	FU	C	Pending		
Bacillus amyloliquefaciens subsp. plantarum D747	FU	C	Approved	01/04/2015	31/03/2025
Bacillus firmus I-1582	NE	C	Approved	01/10/2013	30/09/2023
Bacillus pumilus QST 2808	FU	C	Approved	01/09/2014	31/08/2024
Bacillus subtilis str. QST 713	BA, FU	C	Approved	01/02/2007	30/04/2018
Candida oleophila strain O	FU	C	Approved	01/10/2013	30/09/2023
Coniothyrium minitans	FU	C	Approved	01/01/2004	31/10/2016
Gliocladium catenulatum strain J1446	FU	C	Approved	01/04/2005	31/07/2017
Purpureocilium lilacinum strain 251	NE	C	Approved	01/08/2008	31/07/2018
Phlebiopsis gigantea (several strains)	FU	A 4	Approved	01/05/2009	30/04/2019
Pseudomonas chlororaphis strain MA342	FU	C	Approved	01/10/2004	30/04/2017
Pseudomonas sp. Strain DSMZ 13134		C	Approved	01/02/2014	31/01/2024
Pseudozyma flocculosa	FU	C	Pending		
Pythium oligandrum M1	FU	A 4	Approved	01/05/2009	30/04/2019
Saccharomyces cerevisiae strain LAS02	FU	C	Pending		
Streptomyces K61 (formerly S. griseoviridis)	FU	A 4	Approved	01/05/2009	30/04/2019
Streptomyces lydicus WYEC 108	FU, BA	C	Approved	01/01/2015	31/12/2024
Trichoderma asperellum (formerly T. harzianum) strains ICC012, T25 and TV1	FU	A 4	Approved	01/05/2009	30/04/2019

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Trichoderma asperellum (strain T34)	FU	C	Approved	01/06/2013	31/05/2023
Trichoderma atroviride (formerly T. harzianum) strains IMI 206040 and T11	FU	A 4	Approved	01/05/2009	30/04/2019
Trichoderma atroviride strain I-1237	FU	C	Approved	01/06/2013	31/05/2023
Trichoderma atroviride strain SC1	FU	C	Pending		
Trichoderma gamsii (formerly T. viride) strain ICC080	FU	A 4	Approved	01/05/2009	30/04/2019
Trichoderma harzianum strains T-22 and ITEM 908	FU	A4	Approved	01/05/2009	30/04/2019
Trichoderma polysporum strain IMI 206039	FU	A 4	Approved	01/05/2009	30/04/2019
Verticillium albo-atrum (formerly Verticillium dahliae) strain WCS850	FU	A 4	Approved	01/05/2009	30/04/2019
Zucchini yellow mosaic virus (ZYMV mild strain)	FU	C	Not Approved		
Zucchini Yellow Mosaik Virus, weak strain	FU	C	Approved	01/06/2013	31/05/2023

FU= fungi; BA= bacteria; NE=nematodes, * A: Existing active substances divided into four lists for phased evaluations, B: Substances not considered in scope of the directive, either because already banned or never notified under directive 91/414/EEC or because not considered as plant protection products, C: New active substances

Main groups of biocontrol agents: characteristics, mechanism of action, use, advantages and limitations

Bacillus spp.

Characteristics

Members of the *Bacillus* genus are among the beneficial bacteria mostly exploited as biopesticides to control plant diseases but also nematodes. The most known species hosting biocontrol agents are *B. subtilis* and *B. amyloliquefasciens*, but also *B. firmus* and *B. pumilus*. They are Gram positive rod-shaped bacteria that can form a protective endospore that can tolerate extreme environmental conditions.

Mechanism of action

Bacillus *spp.* can control fungal pathogens by competition, direct antibiosis and induced resistance. In the rhizosphere, competition takes place for space at the root surface and for nutrients, noticeably those released as seed or root exudates. Competitive colonisation of the rhizosphere and successful establishment in the root zone is a prerequisite for effective biocontrol, regardless of the mechanism(s) involved. Direct antagonism involves the production of several microbial metabolites among which lipopeptides play the major role. Surfactin in particular is involved in the mechanism of resistance induction. The bio-nematicide *Bacillus firmus* also colonizes the rhizosphere of the plant where it parasitizes the eggs and larvae of nematodes especially of the rootknotnematodes

Use

Bacillus strains are effective against a broad spectrum of plant pathogens and they can be used either as foliar application or root application before trasplanting. In case of soil or root

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application the ability of the specific strain to colonise and permanently establish on the roots of the specific crop is crucial. Sometime the colonization is not simply crop-specific, but cultivar-specific. Root application of *Bacillus* spp. should be preferably targeted to improve resistance of the plant or to protect the early stage of seed germination, rather to directly control soil-borne inoculum of pathogens.

Advantages, limitations and ways to improve efficacy

The advantage of *Bacillus* spp.-based products is their long shelf-life at room temperature, given by the fact that they produce endospores. There is also a lot of knowledge gained in the fermentation process and the ways of production are commonly optimised. It is a relatively easy microorganism to ferment with relatively low cost of production compared for example to fungal biocontrol agents.

Main limitation is due to the fact that the *Bacillus* strains should colonise the roots in order to be active. Since no major direct effect on the pathogen's inoculum is expected, its efficacy may be low in case of high pathogen's inoculum in soil or against very aggressive soil-borne pathogens. The highest efficacy is seen on germinating seeds or young plantlets, because of the combination of direct antibiosis, induced resistance and grow promotion of several *Bacillus* strains.

Possible ways to improve efficacy are: verification of compatibility between strains and cultivars, application in strategies where another biocontrol agents are targeted to reducing soil-borne inoculum, application in hydroponic culture or fertigation, selection of *Bacillus* strains with high production of antimicrobial metabolites.

Coniothyrium minitans

Characteristics

Coniothyrium minitans is a coelomycete with a worldwide distribution. In nature it occurs mainly in sclerotia of *Sclerotinia sclerotiorum* and *S. trifoliorum*, but can colonise other Sclerotinia species. Spore germination, mycelial extension and sclerotial infection take place at temperatures between 5 and 25 °C. Field control of *S. sclerotiorum* and *S. minor* can be achieved following soil incorporation of solid-substrate formulations before planting. *C. minitans* can survive and spread in soil for at least 2 years and continue to give some control of disease although limited.

Mechanism of action

Coniothyrium minitans enters through the small pores or a lacerated surface of the target organism, and/or lysing by chitinase and β-1,3 glucanase for entry. Upon entry, *Coniothyrium minitans* penetrates the inter- and intra-cellular sub-cortex and medulla, producing fruiting bodies, through asexual reproduction. The infection process causes the cell to shrink, due to high osmotic potential, and the membrane is detached and/or degraded. *Coniothyrium minitans* is highly specialized antifungal agent that targets sclerotia of Ascomycotina and Deuteromycotina (e.g., *Sclerotinia sclerotiorum* and Sclerotinia minor). *Coniothyrium minitans* requires a host to be in a vegetative stage.

Use

The use is mainly in high value crops as lettuce, beans, peas, but also in sunflower, oilseed rape. Application is commonly by spraying onto soil and incorporating into upper soil layer either in pre-plant, by applying the product to the soil 3 to 4 months prior to the onset of disease or in postharvest, by applying product to harvest residues and incorporate into the soil.

Advantages, limitations and ways to improve efficacy

The main advantage is that *C. minitans* controls specifically *Sclerotinia* species without any interference with the soil microflora, the treatment is easy to apply and with no risk for the operators. Main limiting factors are the cost per hectare (the concentration should be sufficiently high per unit of soil and sufficiently deeply incorporated) and the time needed to reduce the inoculum (few months) which is incompatible with frequent growing cycles as for lettuce in some areas. If the time between the application of the biocontrol agent and the subsequent growing cycle is too short, they biocontrol activity and the reduction of inoculum can be insufficient. Another limiting factor is the habit of yearly renting of land by growers, which is common in some countries. In such context farmers do not invest in a soil treatment the year before, which can benefit the next farmer renting that land.

Gliocladium catenulatum

Characteristics
Gliocladium catenulatum is a naturally-occurring saprophytic fungus which is widespread in the environment.

Mechanism of action
The mode of action is reported to be by an enzymatic mechanism. There are no reports indicating that *Gliocladium catenulatum* produces any toxins or antibiotics of concern.

Use
It can be used to control damping-off, seed- root- and stem-rots, and wilt diseases caused by *Rhizoctonia, Pythium, Phytophthora, Fusarium, Didymella, Botrytis, Verticillium, Alternaria, Cladosporium, Helminthosporium, Penicillium,* and *Plicaria*. It can be applied by soil incorporation, soil drench, foliar spray, and dipping of cuttings, bulbs, and tubers. Timing of application: at sowing, potting or transplanting and 2-6 weeks later for potted plants and 2-8 weeks later for other plants. Cuttings, bulbs, or tubers may be sprayed after planting or treated before planting or storage.
Advantages, limitations and ways to improve efficacy
Advantages, limitations and ways to improve efficacy are the same as reported for the *Trichoderma* spp. (see below).

Purpureocillium lilacinum (previously Paecilomyces lilacinus)

Characteristics
Purpureocillium lilacinum is a naturally occurring fungus commonly found in soils. Unlike many other *P. lilacinum* strains, the registered strain does not produce mycotoxins or paecilotoxins. It grows optimally at 21-27 °C, and does not grow or survive above 36 °C. The registered strain was isolated from infected nematode eggs.

Mechanism of action
P. lilacinum parasitizes and subsequently kills eggs, juveniles, and adult females of various plant parasitic nematodes.

Use
As a pesticide active ingredient, *P. lilacinum* can be used to control plant root nematodes on many food and non-food crops. It acts by infecting eggs, juveniles, and adult females of various plant pathogenic nematodes including *Meloidogyne* spp. (root knot nematodes); *Radopholus similis* (burrowing nematode); *Heterodera* spp. and *Globodera* spp. (cyst nematodes); *Pratylenchus* spp. (root lesion nematodes). It can be applied to agricultural soil through drip irrigation, or water in the suspension around base of each plant. If neither of these methods is possible, spray the soil surface around the base of each plant, and drench in afterwards using the irrigation system. The timing varies with crop. In general, 14 days pre-plant; just before planting, 6 weeks after planting, repeat every 6 weeks to 4 months.

Advantages, limitations and ways to improve efficacy
The main advantages are related to safety and the possibility to treat the soil immediately before planting or after. Main limitations are related to the short shelf-life, temperature and the cost per hectare.

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Phlebiopsis gigantea

Characteristics
Phlebiopsis gigantea is a common and widely distributed saprophytic wood-decay fungus in the coniferous forests of the Northern Hemisphere.

Mechanism of action
Phlebiopsis gigantea is able to prevent colonisation of stumps by *Heterobasidion annosum* through competition for resources.

Use
Phlebiopsis gigantea can be used as a biological control of annosum root rot, caused by *Heterobasidion* spp., in Western Europe. Annosum root rot is primarily a problem in conifer plantations that have been partially cut (e.g. thinned)

Advantages, limitations and ways to improve efficacy
The main advantage is that it can support the phase out the use of chemical agents in forestry. The main limitations are the cost of the products and its application.

Pseudomonas spp.

Characteristics
Pseudomonas spp. are aerobic, gram-negative bacteria, ubiquitous in agricultural soils, and are well adapted to growing in the rhizosphere. Pseudomonads possess many traits that make them well suited as biocontrol and growth-promoting agents

Mechanism of action
The traits related to biocontrol include the ability to rapidly utilize seed and root exudates, colonize and multiply in the rhizosphere and spermosphere environments and in the interior of the plant, produce a wide spectrum of bioactive metabolites (i.e., antibiotics, siderophores, volatiles, and growth-promoting substances), compete aggressively with other microorganisms; and adapt to environmental stresses.

Use
Pseudomonas strains are commonly uses to treat seeds or roots of plants before planting. They can also be used to treat tubers and bulbs.
Advantages, limitations and ways to improve efficacy
One of the major advantages is that they grow rapidly in vitro and can be mass produced at relatively low cost. Other advantages are the good compatibility with several fungicides. The major weakness of pseudomonads as biocontrol agents is their inability to produce resting spores (as do many *Bacillus* spp.), which complicates formulation of the bacteria for commercial use. The identification of new formulations, which can prolong their shelf-life, can dramatically increase their use in practice. Other point to be implemented is the understanding of the best combination between the specific strain and plant species, soil type and environmental conditions.

Pythium oligandrum

Characteristics
Pythium oligandrum is a mycoparasite belonging to the *Pythium* family. This mycoparasite is widely distributed throughout the world. *Pythium oligandrum* is common in soil and in or on plants.
Mechanism of action
Pythium oligandrum acts as a hyperparasite by colonizing other plant pathogenic fungi in and around seeds and the rhizosphere of treated plants, thereby suppressing the growth of several

soil-borne pathogenic fungi, including *Alternaria, Botrytis, Fusarium, Gaeumannonyces, Ophiostoma, Phoma, Pseudocercosporella, Pythium, Sclerotinia* and *Sclerotium*. *P. oligandrum* produces the protein oligandrin and other compounds that stimulate plants' cell walls to fend off pathogen invasion, and also stimulates natural plant defense mechanisms called pathogenesis-related (PR) proteins which help plants resist disease, without harming the plant.

Use

It can be applied as seed dressing, pre-plant soak, overhead spray, soil drench, or through irrigation system application.

Advantages, limitations and ways to improve efficacy

The main advantage is the wide spectrum of activity and the possibility to be applied with irrigation systems. However to function effectively, the *P. oligandrum* requires a moist environment and a temperature range of 20-35°C during the infection period, which can last for three to four hours. Under dry conditions, the onset of infection may be delayed until increased moisture is available. In addition the quantity per hectare should be sufficiently high, which can make the treatment very expensive. The implementation of pre-planting treatments with the irrigation systems and the reduction of the cost per hectare can make this biocontrol agent more appealing.

Streptomyces spp.

Characteristics

The active ingredients, *Streptomyces* spp. and *S. lydicus*, are ubiquitous and naturally-occurring bacteria that are commonly found in soil environments.

Mechanism of action

The mode of action is as follows: *S. lydicus* colonizes growing root tips of plants and acts as a mycoparasite of fungal root pathogens which helps protect the plants. Other possible mechanisms include the production and excretion of antifungal metabolites (e.g., antibiotics and/or low molecular weight antifungal compounds or lytic enzymes like chitinase) after colonization.

Use

These strains can be used as an antifungal agent for greenhouse, nursery, turf grass, and agricultural use sites and control Fusarium, *Rhizoctonia, Pythium, Phytophthora, Phytomatotricum, Aphanomyces, Monosprascus, Armillaria, Sclerotinia, Postia, Verticillium, Geotrichum*, and other root decay fungi. They can be applied as a soil mix/drench to potted plants or turf grass or to plant foliage in greenhouses.

Advantages, limitations and ways to improve efficacy

The main advantage is related that once it has successfully colonised the tip of the roots, it can continuously protect the plant. On the other hand the efficacy is not extremely high and the rhizosphere competence is not validated in many crops. A possible way to improve the efficacy is to combine with other biocontrol agents more active in reducing the inoculum.

Trichoderma spp.

Characteristics

Trichoderma is a genus of asexually reproducing fungi that are often the most frequently isolated soil fungi; nearly all temperate and tropical soils contain 10^1–10^3 cultivable propagules per gram. These fungi also colonize woody and herbaceous plant materials, in which the teleomorph (genus *Hypocrea*) has most often been found. However, many strains, including most biocontrol strains, have no known sexual stage. They show a high level of genetic diversity, and can be used to produce a wide range of products of commercial and ecological interest. Several strains belonging to the genus Trichoderma have been identified so far as biocontrol agents of plant diseases and nematodes and a few of them have been developed and registered as biofungicides. Several species have been found to be very effective as biocontrol agents, namely *T. atroviride, T. asperellum, T. harzianum, T. viride, T. gamsii, T. polysporum*, etc.

Mechanism of action

Trichoderma strains may have one or all mechanisms of action according to species and strain. In some strains prevails the direct, hyper-parasitic activity against pathogens, in others the induction of resistance mechanism prevails or they compete with the pathogens for space and nutrients. Trichoderma strains produce a great variety of lytic enzymes most of which play a great role in biocontrol (cell wall degrading enzymes, CWDEs). CWDEs from different Trichoderma strains showed antifungal activity towards a broad spectrum of fungal pathogens (i.e. species of *Rhizoctonia, Fusarium, Alternaria, Ustilago, Venturia* and *Colletotrichum*, as well as funguslike organisms such as the Oomycetes *Pythium* and *Phytophthora* which lack chitin in their cell walls). Trichoderma produces also many secondary metabolites, some of them specifically involved in the direct antibiosis, like i) volatile antibiotics, i.e. 6-pentyl-a-pyrone (6PP) and most of the isocyanide derivates; ii) water-soluble compounds, i.e. heptelidic acid or koningic acid; iii) peptaibols, which are linear oligopeptides of 12–22 amino acids rich in a-aminoisobutyric acid, N-acetylated at the N-terminus and containing an amino alcohol (Pheol or Trpol) at the C-terminus. The production of lytic enzymes and antibiotic metabolites may greatly vary among strains. Each strain shows specific characteristics and adaptation to the environment. Some strains are very good colonizers of soil other prefer wood material, others are found on dead plant tissue in the phyllosphere or colonizing the rhizosphere of plants and the root tissues. Several strains can also promote growth.

Use

Being soil inhabitants and good colonizers of organic matter the main use of Trichoderma strains is against soil-borne pathogens, wood disease and grey mould (*Botrytis cinerea*). In particular some strains represent the only possible solution against wood diseases of grape (no chemical substance have been shown to be effective so far), in fact they can colonize the pruning wounds on the plant for several months thus preventing the entrance of the pathogens. In infested soils they can significantly reduce several soil-borne pathogens and thus the related diseases. On grape, strawberry, tomatoes and other crops they can colonise the dead plant material as leaf residues, dead leaves, etc. preventing the establishment of *B. cinerea* on those materials and thus reducing the disease. Some strains have been used to induce resistance; however this mechanism seems to be very limited in term of efficacy.

Advantages, limitations and way to improve efficacy

Trichoderma can offer several advantages over synthetic chemical fumigants: they are not toxic for mammals and environment, they do not leave toxic residues, they have no adverse effect on soil microflora and microfauna and they do not negatively affect the natural biocontrol population.

To be effective the *Trichoderma* strains should be carefully chosen according to their mechanism of action and adaptation to the specific environment. The intrinsic capability of the strain of producing lytic enzymes and secondary antimicrobial metabolites can make the difference. The use of poorly competitive strains or not adapted to soil characteristics may also impede the efficacy. Most of the failures in term of efficacy can be related to the use of product with poor viability of conidia (this often happens when Trichoderma-based fertilizers are applied), insufficient concentration in the soil, short time for activity (Trichodermas need a certain amount of time to kill the pathogen), too high disease pressure. In few weeks after its application the concentration of exogenous Trichoderma tends to decrease to the basal level of that specific soil.

There are several possible way to improve its efficacy as: the increase of the applied quantity per unit of soil, which is commonly limited by the cost of the commercial product, the use of carriers or substrates which can guarantee a longer persistency (i.e. cellulose, or chitin rich substrates), to give sufficient time to Trichoderma to reduce inoculum before planting, to apply when the

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temperature of the soil is sufficiently high, to combine with other practices, for example as bioinoculum after solarisation or anaerobic soil disinfestation or biofumigation.

How to improve the efficacy of microbial biocontrol agents?

Besides the specific indication given above for each group of biocontrol agents there are few ways to increase the efficacy of these tools. In the first instance wrong or bad uses/application of such products should be avoided. Most of the time, failure is due to the scarce knowledge on the way or conditions of application which results in a wrong use. Secondly we should distinguish failures caused by the application of low quality bio-inoculum. Often, inefficacy is due to the fact that fertilizers or compost containing biocontrol agents are used instead of registered plant protection products. This bad practice generated a lot of confusion around biocontrol agents.
The specific way to increase efficacy of these products are the following:

- Use in combination with agronomic practices in an IPM strategy: in this case the biocontrol agents are applied not just as substitution of a chemical soil fumigant but in combination of agronomic practices, for example after solarisation, anaerobic soil disinfestation or biofumigation;
- Verify the efficacy of the strain against the specific disease and the right conditions of application;
- Verify the compatibility between the strain and the crop or cultivar, in particular for rhizosphere colonizers;
- Give sufficient time to the biocontrol agent to reduce the inoculum of the disease;
- Apply under the right conditions;
- Calibrate the quantity per unit of soil to a level that can guarantee efficacy (often, the quantity per hectare is limited by the price of the product);
- Develop carriers or specific substrates to increase their efficacy;
- Improve production systems to reduce the cost of biocontrol agents;
- Develop multi-year strategies to control pathogens with biocontrol agents, especially for those supposed to reduce soil-borne inoculum of pathogens;
- Apply only when the inoculum is not too high;
- Identify more effective strains (most of the commercial strains have been isolated more than 20 years ago, most of the time without a targeted selection and wide comparison among strains).

The 'grey zone'

There are several commercial products that are sold as fertilizers or biostimulants which contains biocontrol agents. These products usually do not claim a direct fungicide efficacy however they play with the concept that they may improve plant health and contain the most common species used in biocontrol. This 'grey zone' of product creates confusion not only for farmers, but also for some experts. These products do not have to declare the CFUs neither guarantee the viability of the microorganisms. Most of the time they fail to give any efficacy and thus contribute to give a bad image of the microbial biocontrol agents.
Although for many strains the distinction between plant protection products and biofertilisers is rather difficult (they possess both activities) a more strict regulation is needed to guarantee the quality of microorganisms contained in the biofertilisers and to avoid misuse of biofertilisers (as plant protection products).
Today there are only few genera, species and strains of BCAs [*Coniothyrium minitans* (1 strain), *Gliocladium catenulatum* (1 strain), Pseudomonas chlororaphis and sp. (2 strains), *Streptomyces griseovirides* and *Streptomyces lydicus* and *Trichoderma asperellum, T. atroviride* and T. *harzianum* – several strains) that are registered against some soil borne diseases on certain crops. Regarding nematodes, *Bacillus firmus*, and Purpureocillium lilacinum are the only BCAs approved for use against nematodes, and also, not in any crop. Even if we take the whole list

(Table 1), some of these products will be never registered in some countries, so, finally the availability in different European countries of microbial biocontrol products to be used against soil-borne diseases and nematodes is practically very limited for the growers. In addition in order to improve microbiological control it would be useful to associate several strains of the same genus-specie, or several genus-specie and it would be useful also to associate several modes of action. But from the point of view of regulation, that would mean to register any single strain with unsustainable costs for companies (8-10 or more successful efficacy trials per crop/disease in each zone). Due to this contrain, biological control of soil-borne plant diseases in Europe is still very limited; as stated above there are only a few products registered in Europe and with possibilities to be available to the growers.

The priorities for Research (needs from practice)

The microbial biocontrol agents can target directly the problem and solve it when other agronomic practices are insufficient or cannot be applied. For example crops rotation, which is an excellent agronomic solution, is useless in case of polyphagous pathogens that can grow on several crops or when farms are highly specialised as in horticulture or viticulture (vineyards can be planted on specific areas, i.e. ADC, DOC, terroirs, etc.). Another example is when technologies as solarisation, biofumigation or anaerobic soil disinfestation cannot be applied because of one or more compulsory factors are missing (enough temperature, water, time, …).
The identified needs from practice can be summarised in:

- Isolate a new generation of biocontrol agents (several among the registered ones have been isolated more than 20 years ago, when this field of research was at its infancy) with higher efficacy, good technological characteristics (high productivity in fermenters), good shelf-life and possibility to store at room temperature, high compatibility with other means of control (i.e. survival during solarisation, biofumigation conditions, with low dosages of pesticides, etc.).
- Identification of the best protocols to apply biocontrol agent against soil-borne disease (timing, quantities, integration with other means of control, etc.);
- Implementation of IPM strategies which include the use of microbial biocontrol agents in integration with other means of control;
- Development of decision support systems to guide the right application of the available biocontrol agents;
- Enlarge the knowledge on the efficacy of the microbial biocontrol agents against other soilborne pathogens than the targeted in the label and possible use with carriers which can increase survival in soil, in particular to prove environmental safety;
- Optimize and reduce the cost of production by improving the technologies fermentation or use of low cost substrates;
- Support and push industries to register the microbial products that fit with 'low risk substances' against pathogens especially for the use in minor crops (either speed-up the process of registration of low risk substances or subsidies to farmers choosing low risk substances).
- Improving microbiological control by integrating several strains of the same genus-specie, or several genus-specie

Ideas and priorities that could be interesting for operational groups

The following ideas can be interesting for operational group:

- Development of an integrated strategy (protocols integrating microbial biocontrol agents, choice of crops/varieties and agronomic practices) to reduce the problems related to replanting perennial crops (soil-borne pathogens including replanting disease syndrome), when crop rotation is not a feasible option.
- Development of protocols to reduce the inoculum of soil-borne disease in horticulture, which include targeted use of biocontrol agents.

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- Use of specific biocontrol agents in combination/sequence with anaerobic soil disinfestation, biofumigation or solarisation in order to improve the efficacy.
- Combination of compost and other organic substrates or carriers to increase the efficacy of biocontrol agents.
- Selection of compatible varieties to specific rhizosphere competent biocontrol agents in order to improve the resistance induction mechanism in hydroponic or soilless substrates.
- Use of biocontrol agents to recycle/reuse substrates in greenhouse production (i.e. to 'clean' peat after the growing cycle and reuse it).

Improve the cost/benefits

One of the major limiting factors in the wide use of microbial biocontrol agents is their cost compared to the chemical fumigants. The high cost is due to the cost of production (fermentation), the logistics (they comply should be kept at low temperatures and their shelf life is very short 0.5-2 years) and the cost of registration.
Possible solutions can be found in:

- Improving the production systems and the logistics
- Make them more attractive to companies for example by speeding-up the entrance to the market for those which fit with the 'low risk substance' by speeding up the registration process.
- Subsidies for growers to make them more familiar with these products.

The effects on the whole soil-health

Commonly these biocontrol agents have no effect or only minor and transient effect on the soil microflora and microfauna, because many of them are highly specific against pathogens, their persistence is limited, they are biodegradable and do not bioaccumulate. Therefore if they are implemented in a good agronomic strategy they can be a sustainable tool to reduce the soil inoculum potential and improve soil and plant health.

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