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# Biological Control of Root-Knot Nematodes (*Meloidogyne* spp.): Microbes against the Pests

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## ABSTRACT

Root-knot nematodes (*Meloidogyne* spp.) are important pests of many cultivated plants. Recently, the most efficient chemical control products (e.g. methyl bromide) have now been restricted due to their toxic characteristics. Research on agents that work against root-knot nematodes and do not have a detrimental impact on the environment is becoming increasingly important. Advances in the last decades produced quite a number of biocontrol products that are already marketed. Some of the well-accepted commercial products contain bacteria *Bacillus firmus* and *Pasteuria penetrans*, and fungus *Purpureocillium lilacinus*. In this review we summarize the antagonistic activity of bacteria and fungi, with their advantages and limitations in biocontrol of root-knot nematodes.

**Key words:** biological control, *Meloidogyne* spp., antagonisms, bacteria, fungi, commercial products

## IZVLEČEK

### BIOTIČNO ZATIRANJE OGORČIC KORENINSKIH ŠIŠK (*Meloidogyne* spp.): MIKROORGANIZMI PROTI ŠKODLJIVCEM

Ogorčice koreninskih šišk (*Meloidogyne* spp.) uvrščamo med pomembne škodljivce številnih kmetijskih rastlin. Najbolj učinkovita kemična sredstva za njihovo zatiranje so močno strupena, zato je njihova uporaba močno omejena ali celo prepovedana (npr. metil bromid). Razvoj na področju pripravkov za zatiranje ogorčic koreninskih šišk z okoljsko sprejemljivimi lastnostmi se povečuje. Napredek v zadnjih desetletjih je viden v večjem številu biotičnih pripravkov, mnogi med njimi se danes že tržijo. Aktivne snovi v uveljavljenih biotičnih sredstvih sta bakteriji *Bacillus firmus* in *Pasteuria penetrans* ter gliva *Purpureocillium lilacinus*. V članku je predstavljen pregled zaviralnih mehanizmov delovanja bakterij in gliv, prav tako omenjamo največje prednosti in slabosti njihove uporabe v biotičnem zatiranju ogorčic koreninskih šišk.

**Ključne besede:** biotično varstvo rastlin, *Meloidogyne* spp., antagonizem, bakterije, glive, tržni pripravki

## 1 INTRODUCTION: OLD VS. MODERN PLANT PEST CONTROL STRATEGIES

The success of pesticides in the middle of the 20th century enabled control of many harmful organisms. Unfortunately, the adaptation of plant-damaging organisms was not accounted for. The pesticides introduced new environmental conditions to which plant pathogens had to adapt, frequently by becomming resistant. Recently, the importance of healthly food and identification of environmental hazards inclined the research field toward alternative control disease strategies by focusing on biological control agents.

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Plant parasitic nematodes are important pests of many cultivated plants. The *Meloidogyne* genus belongs to a group of root-knot nematodes (RKN) and is represented by over 90 species that have been described so far (Moens *et al*., 2009). These are ubiquitous soil organisms with a wide host range. From financial standpoint the most damaging species are *M. incognita*, *M. javanica*, and *M. arenaria* (Sasser et al., 1982). The RKN produce galls on roots that eventually lead to reduced water uptake to shoots. The severeness of yield loss can range from minimal to total depending on the infesting RKN species and crop variety, season, soil type and use of crop rotation (Sikora and Fernández, 2005; reviewed in Wesemael *et al*., 2011). The tropic group of *Meloidogyne* spp. thrive in hot climates but can survive in temperate climate conditions also (Strajnar *et al*., 2011). Importing plants and seedlings infested with RKN from tropic to temperate climates promotes their spread, which is especially important in greenhouses where temperatures are suitable for RKN reproduction (reviewed in Wesemael *et al*., 2011).

The concerns at this point are methods of controlling *Meloidogyne* spp. in soil because no effective nematicides are available. The public concern over the chemical nematicides is not only their toxicity but also their loss of efficiency after a prolonged use. In 2005, the EU banned the use of methyl bromide which was the most effective nematicidal agent. The use of other nematicides has been restricted or withdrawn recently (reviewed in Wesemael *et al*., 2011). Still useful but not entirely effective are management strategies focusing on prevention rather than curation. These practices are an improvement of old practices. Among them are agrotechnical measures to restore and maintain healthy soils (removal of plant debris, solarisation of soil, crop rotation with plant species immune to pathogens that harm other rotation crops, soil fallow, and addition of organic amendments), use of pathogen-free seeds and resistant varieties, and biological control, which emerged as an alternative to chemical control (reviewed in Collange *et al*., 2011).

## 2 BIOLOGICAL CONTROL – NATURAL INTERACTIONS IN FOCUSED ACTIONS

Soil is a complex ecosystem, one that harbours many different organisms with a complex network of interactions. In rhizosphere where nutrients are abundant the soil organisms have to compete for food sources. Biological control exploits these interactions to either protect the host plant from infections or to reduce the severity of the disease. In short, biological control uses microbes to control plant pathogens. The pioneer of nematode biocontrol was Duddington in 1951. Since then the research has led to a production of various commercial biological control products containing live microorganisms or their metabolites that target specific nematode hosts, though their low efficacy on the fields remains an issue. We will focus on live microbe action towards the RKN; products based on microbial metabolites are classified as biopesticides and their registration resembles that of chemical pesticides.

### 2.1 The action: specific vs. non-specific

The microorganisms with the ability to control plant parasitic nematodes belong to bacteria, fungi, and actinomycetes. They exert antagonistic action through various mechanisms. Non-pathogenic bacteria antagonize the nematodes by (1) inducing plant resistance (induced or systemic resistance), by (2) degrading signalling compounds to which the nematodes are attracted to, or (3) simply by colonizing the roots thus blocking the penetration of infective juveniles. Some microbes produce toxic compounds that kill the nematodes, others (e.g. fungi) parasitize on them. All these mechanisms can be affected by multiple factors, biotic or abiotic, which limit their use in biological control (Sikora, 1992).

## 3 ACTIVE INDIGREDIENTS IN BIOLOGICAL CONTROL PRODUCTS

Each soil has the capacity to limit the *Meloidogyne* spp. reproduction to a certain degree, the rest depends on the activity of native microbial community in soil (Sikora, 1992). Research on *Meloidogyne*-suppresive soils revealed a high microbial diversity (Bent et al., 2008). Microbial groups with highest suppressive potential are (1) pathogenic fungi infecting nematode eggs; (2) rhizobacteria; (3) fungi with a general antagonising effect; (4) endophytic fungi, and (5) obligate parasitic bacteria (Whipps and Davies, 2000).

Most promise for RKN (*Meloidogyne* spp.) biological control show fungi from *Trichoderma* and *Purpureocillium* genera (Dababat *et al*., 2006; Affokpon *et al*., 2011; Wilson and Jackson, 2013), endospores of *Pasteuria penetrans*, and rhizobacteria (e.g. *Bacillus firmus*) that are already marketed (Wilson and Jackson, 2013).

### 3.1 Bacteria and antagonists

Plant-parasitic nematodes co-exist in rhizosphere with biologically diverse bacterial communities. These bacteria impact the nematode life cycle as endoparasites or antagonists (Table 1). Most of the antagonistic bacteria are saprophytes living in the rhizosphere.

### 3.1.1 Endoparasites: *Pasteuria penetrans*

Well-studied endoparasites of nematodes are bacteria from the *Wolbachia* genus. These are bacteria with a virus-like lifestyle; they are obligate intracellular parasites of invertebrates. Isolation of bacteria from *Meloidogyne* sp. revealed the presence of *Pasteuria* sp., an endoparasite of many economically important plant parasitic nematodes and water fleas (*Daphni*a spp.) (Starr and Sayre, 1988). The genus *Pasteuria* belongs to a *Bacillus*-*Clostridium* group that produces very resilient endospores (Charles et al., 2005). The most common endoparasite of *Meloidogyne* spp. is *P. penetrans* (Stirling, 1985) and *P. hartismeri* in *Meloidogyne ardenensis* (Bishop *et al*., 2007).

*Pasteuria*-infected female nematodes produce low numbers of eggs. The endospores are resistant to drying and have good shelf-life; they also reduce infectivity of the juveniles and fecundity of the females (Mankau and Prasad, 1977; Davies *et al*., 1988; Chen *et al*., 1996). Unfortunatelly, their narrow host range limits their wide use, and mass endospore production is currently hard to achieve. The *Pasteuria Biosciences* LLC (recently aquisited by Syngenta) is the only company able to produce enough endospores in a bioreactor to accomodate small field trials (Hewlett *at al*., 2004; 2006). They overcame the obstacle of obligate living conditions by regulating the activity of the sporulating protein Spo0F (Kojetin *et al*., 2005).

Endospores have different binding affinities to infective juveniles J2. The attachment of endospores to cuticle varies between and within populations of *P. penetrans* (Davies *et al*., 2001). Further, the nematode cuticle which determines the success of the endospore attachment shows equal variability in composition (Wishart *et al*., 2004). The level of soil suppression depends on the density of the *P. penetrans* endospores with the lowest limit of $10^4$ endospores per gram of soil (Stirling , 1991). It is extremely difficult to assess adequate endospore concentration in soil. Endospore detection limit is currently around 100 endospores per gram of soil as achieved with immunological and molecular techniques. Currently, no mathematical equation correctly describes the relationship between the number of soil endospores and the level of soil suppression (reviewed in Hallmann *et al*., 2009).

**Table 1**: Bacterial pathogens and antagonists affect different developmental stages of *Meloidogyne* spp. (adapted from Hallmann *et al*., 2009).

| Developmental stage | Nematode behaviour intercepted | Mode of action | Place of action | Examples of Bacteria | References |
|---|---|---|---|---|---|
| Egg or egg mass | Development, hatching | Toxins, lytic enzymes, parasitism | soil | *Telluria chitinolytica, Bacillus firmus* | Spiegel *et al*., 1991; Wilson and Jackson, 2013 |
| Infective juveniles | Vitality, host attraction, host recognition, penetration | Toxins, lectins, degradation of root exudates, induced resistance, parasitism | Soil, rhizosphere | *Pasteuria penetrans, Pseudomonas fluorescens, Pseudomonas aeroginosa, Rhizobium etli* | Kretchel *et al*., 2002; Siddiqui and Shaukat (2004); Siddiqui *et al*., 2006; Sikora *et al*., 2007; Oliveira *et al*., 2007 |
| Sedentary juvenile | Formation of feeding site, development | Toxins, induced resistance, parasitism | endorhiza | *P. penetrans, R. etli* | Davies *et al*., 1991; Reitz *et al*., 2002 |
| Female | Fecundity | | Rhizosphere, endorhiza | *P. penetrans* | Davies *et al*., 2008 |

### 3.1.2 Endosymbionts of entomopathogenic nematodes

Lewis *et al*. (2001) found that entomopathogenic nematodes exibit biocontrol activity toward *Meloidogyne* spp. These nematodes (*Steinernema* and *Heterorhabditis*) carry endosymbiotic bacteria that produce exo- and endometabolites with a suppressive effect on *Meloidogyne* spp. (Grewal *et al*., 1999; Vyas *et al*., 2006). The symbiotic bacteria from genera *Xenorhabdus* and *Photorhabdus* produce metabolites that reduce egg hatch and juvenile's penetration, exibit repellent effect and can also paralyse juveniles (Hu *et al*., 1999). The metabolites are only effective in soil and do not affect nematode development inside the roots. Both genera of entomopathogenic nematodes were classified among exotic organisms in Slovenia until 2008, and consecutively their usage in biocontrol was prohibited according to the Rules on Biological Protection of Plants (the Official Gazette of the Republic of Slovenia, No. 45/06). Between 2007 and 2009 the presence of *Steinernema affine* (Laznik and Trdan, 2007), *S. carpocapsae* (Laznik *et al*., 2008), *S. feltiae* (Laznik *et al*., 2009a), *S. kraussei* (Laznik *et al*., 2009b), and *Heterorhabditis bacteriophora* (Laznik *et al*., 2009c) was confirmed in Slovenia, and the last four species and now allowed to use in biological control programs.

### 3.1.3 Rhizobacteria

Soil microbiota is attracted to roots. Root exudates are excellent food source for soil organisms that accumulate around the roots. Diversity of microbes in this area called the rhizosphere transcends the diversity in bulk soil. The bacteria that colonize the rhizosphere of the host plant are called rhizobacteria. These are mostly non-pathogenic bacteria that provide the first line of defence much like microbiota in human intestines (Weller, 1988). By colonizing the host roots the bacteria can also benefit the plant. Many rhizobacteria can stimulate the plant growth and are termed as plant-growth promoting rhizobacteria or PGPR (Kloepper *et al*., 1980; reviewed in Ahemad and Kibret, 2013). Most frequently studied antagonistic rhizobacteria to affect the RKN are *Bacillus subtilis*, *B. sphaericus* and *Pseudomonas fluorescens* (Becker *et al*., 1988; Sikora, 1992; Tian *et al*., 2007). Among other representatives are genera of *Agrobacterium*, *Alcaligenes*, *Aureobacterium*, *Chryseobacterium*, *Corynebacterium*, *Enterobacter*, *Klebsiella*, *Paenibacillus*, *Phyllobacillus*, *Rhizobium*, *Telluria*, and *Xanthomonas* (Spiegel *et al*., 1991; Kloepper *et al*., 1992; Hallmann *et al.*, 1995; Krechel *et al.*, 2002; Oliveira *et al.*, 2007; Son *et al.*, 2009).

Plant parasitic nematodes are also attracted to roots. Moreover, they use the exudate concentration and $CO_2$ gradient in the rhizosphere to sense the root's proximity (reviewed in Curtis, 2008). Rhizobacteria consume the exudates thereby truncating the nematode's recognition of root penetration points. They are also able to provoke a plant defence response that controls *Meloidogyne* spp. on tomato (Siddiqui and Shaukat, 2004) and other plant pathogens (Ramamoorthy *et al.*, 2001). Root-nodulating bacterium *Rhizobium etli* G12 can induce systemic resistance by cell surface lipopolysaccharides (LPS) (Reitz *et al.*, 2002). The resistance response decreases the nematode penetration but has no effect on nematode attraction and only slight effect on development inside the roots. Actually, the application of plant defence response elicitors could potentially provide a broad-spectrum and a long-term protection against different plant pathogens (reviewed in Hallmann *et al.*, 2009). In support, it has been established that some pesticides act by primming plant defence to enable a rapid response to pathogen attack (Beckers and Conrath, 2007).

The rhizobacteria are easily grown *in vitro* and in bioreactors. Besides having a beneficial effect on host plant they also reduce plant damage. To maximise the biocontrol efficiency many of the marketed products are sold as seed treatments (Oostendrop and Sikora, 1989). It is vital that bacteria colonize the root surface before the nematodes can compete for entry points. Due to many positive effects, the rhizobacteria are considered ideal for nematode biocontrol, but are limited by a number of factors. The seed treatment provides a short-term control even though it induces systemic resistance and reduces the root invasion of the juveniles. The protection is only effective against nematodes having a single generation in a growing season. Also, the activity of the rhizobacteria is affected by the crop cultivar and nematode species (Kerry, 1990; 1992). The antagonistic activity of rhizobacteria is affected by factors that are difficult to control. The key factors are field conditions, environmental or edaphic factors, nematode species, and developmental stage of the nematode (Table 1), or physiological and genetic characteristics of the host plant (Sayre and Walter, 1991; reviewed in Hallmann *et al.*, 2009).

#### 3.1.3.1 *Bacillus firmus*

*Bacillus firmus* is a Gram-positive, endospore-producing soil bacterium sparsely represented in nature. Not all strains exhibit nematicidal activity. Those that do, destroy the eggs of *Meloidogyne* spp. by colonising egg sacs (Keren-Zur *et al.*, 2000), some have also suggested the involvement of toxins (Mendoza *et al.*, 2008). Recently, Wilson and Jackson (2013) examined the interest of growers for bionematicides, and *B. firmus* preparations received the most attention. Bayer CropScience markets a seed-treatment product (VOTiVO$^{TM}$) and a drench product (Nortica$^{TM}$) (see Table 2) that are currently being sold in the USA.

### 3.1.4 Actinomycetes

Another group of soil bacteria with potent antagonistic activity toward *Meloidogyne* spp. are actinomycetes. These bacteria are known producers of secondary metabolites with antibiotic activity towards many fungi and bacteria. Most studied are *Streptomyces* species that act against various fungal species and *Meloidogyne* spp. (Krechel *et al.*, 2002). *S. avermitilis* produces antibiotic compounds avermectins that are the most effective nematicides. This antibiotic kills infective juveniles, reduces egg hatching, and it has been suggested recently that avermectins inhibit RNA synthesis (Takatsu *et al.*, 2003). A commercial product available on the market is Avicta (Syngenta, Switzerland) used as a seed treatment for vegetables and cotton.

## 3.2 Fungal biocontrol agents

Well-known anatagonists of *Meloidogyne* spp. are ubiquitous soil fungi from genera *Trichoderma* and *Fusarium*. They live in the rhizosphere and colonize the root surface. Their antagonistic activity is focused at fungal pathogens, but they affect the RKN life cycle also (reviewed in Sikora *et al.*, 2008). *Trichoderma* spp. prevents nematode penetration and improves plant growth. The conidia of *Trichoderma* attach to nematode cuticle or to egg shell and parasitize on them (Sharon *et al.*, 2007). The attachment affinities to *Meloidogyne* spp. eggs, cuticle or gelanious matrix of egg masses are species-specific (Sharon *et al.*, 2001). Like rhizobacteria the *Trichoderma* species should be present in soil before the crop planting to completely colonize the root (Dababat *et al*., 2006). Adding organic amendments to the soil (e.g. chicken litter) can maximize the *Trichoderma* control activity (Islam *et al*., 2005).

Production of fungi for wide use is fairly simple, and some even produce resistant resting spores (*Pochnia* sp.). Most soil fungi are rhizosphere competent with a wide host range. Endophytic fungi may improve plant growth and reduce damage caused by the nematodes. Like bacteria, fungi have specific temperature, moisture, and density requirements; therefore it is difficult to predict their control activity in soil. The biocontrol efficiency depends on the nematode species, plant host and their root exudates, and other crops in rotation (reviewed in Hallman *et al*., 2009).

### 3.2.1 Nematode-trapping fungi

Some fungi are predators and feed on nematodes, either by attacking eggs or juveniles and/or by forming special hyphal structures to prey on moving nematodes. Nematophagous fungi are classified into Hyphomycetes species, Zygomycetes (*Stylopage* and *Cystopage*) and Ascomycetes (*Monacrosporium cionopagum*) (Stirling, 1991). Hyphae of nematophygous fungi form trapping structures with an adhesive to catch the nematodes. Most commonly found structures are adhesive nets of *Arthrobotrytis* spp. with a three-dimensional network. The fungal hyphae form rings which constrict upon nematode passage then the hyphae penetrate through the cuticle and feed on nematode (review in Hallmann *et al*., 2009). Adding *A. dactyloides* to soil at an early developmental plant stage provides protection against *M. incognita* penetration for 10 weeks (Kumar and Singh, 2006); long enough to prevent major plant damage.

### 3.2.2 Parasites of eggs and females

Fungi that parasitize on eggs and/or females are facultative parasites. The most important and well studied pathogen of *Meloidogyne* spp. is *Pochonia chlamydosporia* (= *Verticillium chlamydosporium*). The fungus wraps abound the egg, penetrates the shell and destroys the insides of the egg with a cocktail of proteases (reviewed in Hallmann *et al*., 2009; Esteves *et al*., 2009). *Pochonia chlamydosporia* densities in soil can maintain high levels for up to five months in controlled conditions, which makes this fungus suitable for biological control (Atkins *et al*., 2003). There are a few limitations, though. Siddiqui *et al*. (2009) found biotypes of the fungus with a preference to RKN nematodes but with high differences in virulence. The RKN-biotypes with highest virulence had lowest soil densities indicating a fitness cost.

Widely used in marketed control products is *Purpureocillium lilacinus* (former *Paecilomyces lilacinus)* (Table 2) that parasitizes on eggs and other developmental stages of several nematode species. Its antagonistic activity resembles that of *P. chlamydosporia* (Jatala *et al*., 1986). Strain PL251 reduces infestation with *M. incognita* by 66 %, but does not provide a long-term protection. Establishment of *P. lilacinus* in soil varies with soil type and one single application of condia might not suffice, even if the inoculum was high ($10^6$ conidia/g soil), as proposed by Kiewnick and Sikora (2006). Anastasiadis *et al*. (2008) suggested repeated applications to soil and addition of fungicides to prevent secondary infections by soil fungi. Commercial products with *P. lilacinus* are marketed in Europe (in Italy), North Africa and Central America (Wilson and Jackson, 2013).

### 3.2.3 Endoparasitic fungi

Other biocontrol fungi are endoparasitic soil fungi of *Hirsutella* spp. Similar to *Pasteuria penetrans* the fungi produce adhesive conidia that attach to nematode cuticle in a manner much like *P. penetrans,* and also have special requirements to grow *in vitro* (Stirling, 1991). The *H. rhossiliensis* and *H. minnesotensis* have the potential to be used in biological control though they are limited by their low density in soil and short-term protection (Tedford *et al*., 1993; Mennan *et al*., 2007).

### 3.2.4 Mycorrhizal fungi

Symbiotic association between plant roots and fungi is termed mycorrhiza. Mycorhizas form on the root surface (ectomycorrhiza) or grow inside the roots (endomycorrhiza). Endomycorrhizae with hyphae extending inside were found to effectively control the RKN which spend majority of their life-time settled inside the gall. The fungal appresorium penetrates the root cortex, grows inter- and intracellulary forming vesicles and arbuscules. The fungal-plant symbiosis provides the plant with nutrients and protects the plant against the RKN attack. The mechanisms underlying biocontrol activity of mycorrizae are (1) alteration or reduction of plant exudates upon endomycorrhizae symbiosis which affects egg hatch or nematode attraction, (2) competition for nutrients and impediment of nematode reproduction, and (3) parasitism on female nematodes and their eggs (reviewed in Hallmann *et al.*, 2009).

The arbuscular mycorrhizal fungus *Glomus mosseae* gave the most successful results in controlling *Meloidogyne* spp. (Stirling, 1991; Robab *et al.*, 2012). Recently, Vos *et al.* (2012) demonstrated an induction of systemic resistance response in tomato roots colonized by *G. mosseae* against *M. incognita*. The combination of *G. intraradices* with mycorhiza-helper bacteria (e.g. *Rhizobium etli* G12) could further enhance the protection of crops plants and extend the time-frame of the biocontrol activity to whole growing season (Reimann *et al.*, 2008).

### 3.2.5 *Myrothecium verrucaria*

*Myrothecium verurrucaria* is an ascomycete that produces nematicidic compounds. These compounds are the result of *in vitro* fermentation in bioreactors. The biocontrol activity of the fermented broth is not clear at the moment. It is known, however, that the product reduces egg hatching, inhibits development or even kills the nematodes, hinders nematode perception of the host, and enhances microbial antagonism in the rhizosphere (reviewed in Wilson and Jackson, 2013).

**Table 2:** Commercially available biological control products to control RKN (adapted from Hallman *et al.*, 2009).

| Product | Antagonist | Product Form | Application | Crop | Company/ country |
|---|---|---|---|---|---|
| Bioact WG<br>PL Gold | *Purpureocillium lilacinus* | Water-dispersible granulate;<br>Wettable powder | Drench, drip irrigation | Vegetables, banana<br>Tobacco, citrus | Bayer CropScience, USA ;<br>BASF Worldwide |
| BioNem-WP<br>Nortica<br>VOTiVO | *Bacillus firmus* | Wettable powder;<br>Solution | Drench, drip irrigation,<br>Seed treatment | Vegetables;<br>Turfgrass;<br>Corn, soybean, cotton | AgroGreen, Israel;<br>Bayer CropScience, USA |
| KlamiC | *Pochonia chlamydosporia* | Granulate | Soil incorporation | Vegetables | Cuba |
| Econem | *Pasteuria penetrans* | Solution or powder | Irrigation, kapljično namakanje | Vegetables, turf, soybean | Syngenta; Nematech, Japan |
| Deny<br>Blue Circle | *Burkholderia cepacia* | Powder or Solution | Seed treatment, Irrigation | Alfalfa, barley, beans, clover, cotton, peas, grain sorghum, vegetable crops and wheat | CCT Corp, USA;<br>Stine Microbial Products, USA; |
| Biostart | *Bacillus* spp. mixture | Liquid | Soil drench, irrigation | General use | Microbial Solutions, S Africa |
| Nemix | *Bacillus* spp. | Powder | Drench/drip | Vegetables, Fruit trees | AgriLife/Chr Hansen, Brazil |
| DiTera | *Myrothecium verrucaria* | Powder | Ground or chemigation | Almonds | Valent Biosciences Corporation, Canada |

## 4 BIOCONTROL PRODUCTS ON THE MARKET

In the last few decades the number of marketed biological control products has increased substancially. Some are summarized in Table 2. In recent years the multinational companies acquisited small biotechnology companies. In 2012-2013, the BASF acquisited Becker Underwood, Bayer CropScience merged with Agraquest and Prophyta, and Syngenta aquisited Pasteuria Bioscience. According to a study of Wilson and Jackson (2013), the key products at the moment are VOTiVO (*B. firmus*), DiTera (*Myrothecium verrucaria*), and BioAct (*P. lilacinus*). The factors affecting selection of an appropriate biocontrol agent are summarized in Hallmann et al. (2009).

## 5 CONCLUSION: MANY CHALLENGES AHEAD

Many of the biocontrol agents are effective at a specific nematode developmental stage. Attacking the infective juveniles of the RKN may decrease the infection but will not decrease the nematode population, especially of those RKN that have more than one generation in a growing season. On the other hand, the control of females and eggs does not prevent the root invasion and plant damage, but the multiplication of the nematodes is reduced. Another issue is a sedentary stage of RKN that cannot be parasitized by all rhizosphere fungi. The life cycle of the *Meloidogyne* completes when a sedentary female inside the gall produces eggs that extrude from the root surface. The female, however, stays hidden inside the gall. At high temperatures the eggs hatch early and the egg-parasitizing fungi are unable to destroy the eggs in time. Introducing the chitin-degrading bacteria that degrade soil amendments into ammonium can kill most of the nematodes in soil (Kerry, 1992; reviewed in Hallmann *et al.*, 2009).

To maximise the antagonistic control activity many of the commercial products contain one or a few biocontrol organisms. The combinations of biocontrol agents in a product have to be carefully selected as they might not compatibly interact (Roberts *et al.*, 2005). Recently, it has been demonstrated that addition of one microbial species to soil has low impact on indigenious microbial community structure (reviewed in Shade *et al.*, 2012). This finding will hopefully facilitate biocontrol product registration. One thing to keep in mind though, is the possible facultative pathogenesis to human as many rhizobacteria and soil fungi (e.g. *Trichoderma*) can be excellent biocontrol agents and simultaneously opportunistic human pathogens (Berg *et al.*, 2005; Druzhinina *et al.*, 2011).

The EU now faces a challenge. We have reduced or banned many of the toxic chemical nematicides even though the yield losses due to RKN are increasing. Moreover, the climatic changes have presented favourable conditions for RKN that are already spreading or are expected to spread throughout the Medditerranean countries (Strajnar *et al.*, 2011; Castagnone-Sereno, 2012). In Slovenia, four species of RKN have been found since 2003: *M. incognita*, *M. hapla*, *M. arenaria*, and *M. ethiopica* (reviewed in Strajnar, 2012). The infestation is found mainly in greenhouses on tomato and pepper. Controlling RKN in greenhouses is challenging and expensive as frequently the whole greenhouse is contaminated. According to data from Agricultural Institute of Slovenia infestations with RKN are increasing. Like in many EU countries we try to restrain the spread with agrotechnical management techniques and recommend the planting of resistant varieties (Širca S., personal communication).

In conclusion, biological control will never be a substitute for chemical control because of its inherent limitations: inconsistency and lower effectiveness. But, its added value on a long-term scale is much higher: clean environment, safe food and water, and most importantly healthy people. Based on current knowledge we have a long road ahead. Fortunately, the use of biocontrol agents is widely accepted among the growers, which is a strong stimulus for a continued research. On the other hand, the most important impediment that we have to deal with is the bureaucracy of product registration.

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