FRONT PAGE

PROJECT PERIODIC REPORT

BIOCOMES
New biological control products
for sustainable farming and forestry

Grant Agreement number:	**612713**
Project acronym:	**BIOCOMES**
Project title:	**Biological control manufacturers in Europe develop novel biological control products to support the implementation of Integrated Pest management in agriculture and forestry**
Funding Scheme:	**Collaborative Project (large-scale integrated project targeted to SMEs)**

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Periodic report: **1st X 2nd ☐ 3rd ☐ 4th ☐**

Period covered: **from 01-12-2013 to 31-05-2015**

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[1] Usually the contact person of the coordinator as specified in Art. 8.1. of the Grant Agreement.

[2] The home page of the website should contain the generic European flag and the FP7 logo which are available in electronic format at the Europa website (logo of the European flag: http://europa.eu/abc/symbols/emblem/index_en.htm logo of the 7th FP: http://ec.europa.eu/research/fp7/index_en.cfm?pg=logos). The area of activity of the project should also be mentioned.

3.1 Publishable summary

3.1.1 Summary description of project context and objectives

The objective of BIOCOMES (www.biocomes.eu) is to develop 11 new biological control agents (BCAs) for key markets in European agriculture and forestry. BCAs were identified through market analysis by six manufacturers of biological control products. BCAs will primarily be for use in open field crops of vegetables (3), of which 2 are also for use in protected crops, arable crops (3), fruit crops (3), and three different types of forests (2). Primary targeted pests are: gypsy moth (*Lymantria dispar*), pine weevil (*Hylobius abietis*), tomato pinworm (*Tuta absoluta*), white flies, aphids of fruit tree crops and *Mamestra brassicae*. Primary targeted pathogens are: damping-off diseases in forest nurseries, soilborne pathogens of oilseed rape and cereals, brown rot (*Monilinia* spp.) of stone fruit, and powdery mildew of cereals (*Blumeria graminis*). The economic sustainability during the entire development process will be assessed by the responsible industrial partners. The environmental sustainability will be quantified for each BCA by means of the Sustainable Process Index method. The entire developmental process for each of the 11 BCA products is guided by a consultancy partner specialized and leading in (bio-) pesticide registration including risk assessments for European (bio-) pesticide industries. In vitro production of entomopathogenic viruses as new innovative technique will be developed aiming at a breakthrough in economic production. Downstream-technology and shelf life for entomopathogenic nematodes will be improved. BIOCOMES will communicate project results with all stakeholders with special attention to European IPM networks throughout the whole project duration. BIOCOMES combines the expertise of 10 industrial SME partners, 3 larger industrial partners and 14 research partners with 38% of the requested EU contribution supporting SMEs. All 11 BCA solutions will be novel IPM tools and new alternatives to replace major pesticide applications in European agriculture and forestry.

3.1.2 Description of the work performed and the main results achieved

Diseases

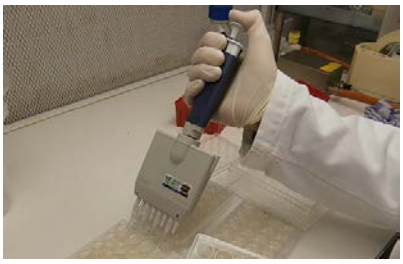
Brown rot. Relevant metabolites produced by *Penicillium frequentans* and *Bacillus subtilis* have been characterized and the mode of action from both biocontrol agents has been determined. In view of the optimization of formulation processes, researchers have been developing new formulations for *Penicillium frequentans* application as well as liquid and solid formulations *for Bacillus subtilis* which did show good survival results. Two formulations of each biological control agent have been tested under field conditions in preliminary assays. Further improvement of formulations will be carried out in 2015 followed by new field trials.

Fungal root diseases in forestry. Several strains of biological control agents were tested against pathogens causing damping-off. The strains were tested on seedlings of Scots pine, common beech and pedunculate oak in Poland in greenhouse experiments. Strains of *Serratia plymuthica, Paenibacillus polymyxa* and *Trichoderma harzianum* have been used. The experiments will be repeated in 2015, because of a very large variation in the results until now. The effect of the potential biological control agent *Trichoderma harzianum* has also been investigated in more detail by checking for the presence of *Trichoderma harzianum* strain INAT 11 and other naturally occurring *Trichoderma* strains in nursery soil samples. Also root colonization by strain INAT 11 was evaluated on root samples of seedlings of common beech, pedunculate oak, and Scots pine. After setting up an adequate study protocol, the inoculum of INAT 11 has been assessed in soil used in nursery trials of the tree species. Treatments with

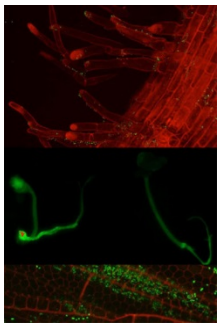
control soil and inoculated soil have been carried out. Soil and root samples were collected and analysed for the colonization of *Trichoderma harzianum*. So far we can conclude that the *Trichoderma harzianum* strain INAT 11 did survive in inoculated soil in which the 3 tree species were grown. It colonized the common beech, pedunculate oak and Scots pine seedlings well and could therefore be a potential biocontrol product to control damping-off.

***Fusarium* spp.** In the first year of the BIOCOMES project, DNA primers for *Trichoderma harzianum* strain INAT 11 have been developed to allow strain-specific detection. Afterwards the influence of environmental factors such as temperature, water activity (a_w) and acidity (pH) on the growth of *Trichoderma harzianum* strain INAT 11 have been investigated. Lab experiments could show that temperature, a_w and pH were limiting factors for fungal growth, however pH had no effect on the survival rate of this antagonist in soil. In vitro trials were carried out to determine the influence of selected fungicides, herbicides and insecticides on mycelial growth of *Trichoderma harzianum* strain INAT 11. All fungicides tested up to date were toxic to the strain at the dose of 1 ppm, while one of the two insecticides and two out of five herbicides tested did not affect mycelial growth. Experiments to define the minimum effective dose of *Trichoderma harzianum* strain INAT 11 and its effect on root colonization by *Fusarium* spp. just started. Seed treatment and semi-field tests will be performed in the course of 2015.
The potential of developing an effective commercial formulation of *Trichoderma harzianum* strain INAT 11 and the optimization of shelf-life have been investigated. *Trichoderma harzianum* can be produced by liquid or solid fermentation. The spores have to be mixed with adequate co-formulants in order to produce a proper formulation. Further formulation experiments will focus on improving shelf-life, long spore viability and easy distribution on seeds.

Powdery mildew. In 2014 leaf samples were collected from cereal crops, grasses and other plant species affected by powdery mildews. 504 leaf samples were collected in the Netherlands, Northeast-, Central- and Western Germany and Southern Sweden. 1.237 fungal isolates could be obtained from the pustules of the powdery mildew fungus *Blumeria graminis*. Almost 900 isolates have been screened with the selection criteria for this including mass production, safety, cold- and drought tolerance and UV-B resistance. Identification of selected isolates was achieved by ITS amplification and sequencing. The next steps are a risk assessments of each identified species, the optimization of the bioassay for antagonist efficacy screening and investigation of the potential of selected isolates for mass production.



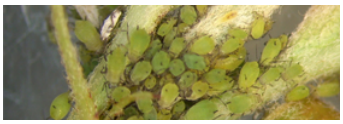
Soil-borne Verticillium wilt. Ten bacterial strains of the genera *Serratia sp.* and *Paenibacillus sp.* were pre-selected for their anti-fungal activity and are being tested for potential application on oilseed rape and cauliflower. Previously the research team aimed to find the optimal plant colonizer and plant growth-promoting in a set of ten bacterial strains. The goal was the selection of one strain per genus and the development of them into a biological control agent for seed treatment.



Based on plant growth promoting effects on oilseed rape and cauliflower in sterile germination pouches, and on the ability to suppress growth of a pathogenic *Verticillium* strain *in vitro,* two potential biocontrol strains have now been selected. The beneficial effect on plant growth by these potential biocontrol strains were tested further on oilseed rape in a sterile and an unsterile soil system. In order to gain a better understanding of the mode of action of these potential biocontrol agents towards pathogens and their hosts, the genomes of the selected strains were sequenced. The genomes revealed several genes that might contribute to its antagonistic and plant growth promoting activity.

The biocontrol efficacy of the different selected antagonistic bacterial strains are being investigated in the greenhouse using pot experiments with cauliflower and oil seed rape. First field trials are also planned for next year.

Pests



Aphids. From an extensive literature survey 201 tritrophic (plant-aphid-parasitoid) associations could be determined in Europe on *Prunus* (peach, plum and cherry), *Malus* (Apple) and *Pyrus* (Pear) species with 32 aphid species present on those fruit tree crops. 21 parasitoid species were identified as potentially parasitizing those 32 aphid species. Among the 21 parasitoids identified in the literature review, 10 were selected as potential new biological control agent for the natural aphid control in commercial fruit tree orchards. Those 10 target species will be collected in the field and screened in the laboratory on parasitism efficiency (for the most problematic aphid species in commercial fruit tree orchards) and on production efficiency (small scale first). The best species will then be tested on plants (in cages first and then in the field). Until now 94 samples with parasitoids have been received. The samples came from Belgium, Serbia and Sweden. The first production tests and parasitism efficiency tests done on these samples look promising for some parasitoid species and the first tests on plants will be carried out next year.

Cabbage moth. During the first year of BIOCOMES information about the parasitoid *Telenomus* sp was collected. Researchers have worked on the characterization of *Telenomus* sp. population structures. Due to the small size of this parasitoid (<1mm) identification of the species is very difficult. Therefore a species specific primer has been developed as a tool for species determination. The primer will also be used (2015) for population characterisation throughout Europe to understand the potential use of *Telenomus* sp. as a biological control agent. In 2015 the laboratory reared lepidoptera eggs will be dispatched into the field to collect naturally occurring egg parasitoids. After that the developed primer will be used to identify *Telenomus* sp. among all the collected egg parasitoids and amplicons belonging to *Telenomus* sp. will be used to determine the intraspecific variability within the different European populations.

Gypsy moth. At two different experimental sites in Poland with 30 year old black alder (*Alnus glutinosa*) stands egg masses of the gypsy moth have been collected in March 2014 to estimate the occurrence of wild virus in the tested population of gypsy moths. In April 2014 20 trees were sprayed with suspensions in two different concentrations of the entomopathogenic virus at these locations. Three to five weeks after these treatments alder leaves were collected. Damage caused by the caterpillars appeared to be reduced 2-3 fold after treatments. Three weeks after the treatments also caterpillars were collected. These caterpillars have been reared for 2 weeks in the laboratory. After two weeks the mortality appeared to be 3-4 fold higher among caterpillars from the treated trees than in those from the untreated trees.

Large pine weevil. In the first year two entomopathogenic nematode species (*Steinernema carpocapsae* and *Heterorhabditis downesi*) were compared in their capability to suppress pine weevil in roots of stumps. The effect of soil type is being investigated, because nematodes are predicted to be more effective in deep peat soils. Also the application method of the biological control agent has to be assessed and optimized. Three small scale field trials have taken place in Ireland and in Poland. In Ireland the *Steinernema* and *Heterorhabditis* nematode species appeared effective in deep peat soils in field tests. The effectiveness in shallow peat is still under investigation. In Poland the use of nematodes caused 15-17% mortality of *Hylobius* larvae developing in treated stumps. A low level of

parasitism was caused by unfavourable conditions for nematode development (drought). In the next year, different methods of nematode application will be tested to minimize the impact of weather conditions on the nematode activity. The best application method of the nematode treatment appeared to be different for the two nematode species: *Heterorhabditis downesi* provided better control when applied on top whereas *Steinernema carpocapsae* provided better control when applied around the stump. The application method on dispersal and efficacy of both species are being further investigated in field trials in Poland and Ireland.

Potato moth and Tomato leaf miner. During the first year naturally occurring virus isolates were gathered in Greece, Italy and on the Canary Islands. Soil samples and infested potatoes were collected. After that the biological activity of isolates in terms of virulence and host range will be determined using bioassays. A bioassay protocol has been developed to test for specific virulence of the PhopGV isolates towards potato moths and tomato leaf miner. Molecular methods will be applied for strain identification.

White fly. The overall objective is to develop a microbial biological control product based on the entomopathogenic fungus *Isaria fumosorosea* for the control of white fly in vegetables under different environmental conditions in the greenhouse and in the open field. In the first year growth temperature and humidity requirements of *Isaria fumosorosea* isolates were compared. In 2015, trials under controlled laboratory conditions trials will be performed with the two most promising isolates to asess the control efficacy of the strains against white fly. The potential of the *Isaria fumosorosea* isolates to produce high spore yields and maximum shelf life was determined. Fungal growth at different environmental conditions, sporulation in solid state fermenter systems and the biological efficacy against several pest insects and beneficial insects were compared for the different *Isaria fumosorosea* isolates. Three strains fulfilled the criteria for further investigation. Some strains showed good efficacy against a range of pest insects but the conidia yield on solid substrates was not sufficient and vice versa. For some strains the standard processing protocol was not feasible. Because the strain preferences for virulence and productivity did not correspond, strains were selected with acceptable virulence and yield. The development of specific markers for two strains is in progress.

Production technologies

In the first year of the project a *Lymantria dispar* cell line for the *in vitro* production of entomopathogenic viruses has been established. Shaking rate, inoculum cell density and filling volume were tested. Results show that high cell densities were obtained which provides a basis for future large scale production of the virus.

Research to trace the responsible genes for desiccation tolerance has been started with entomopathogenic nematodes. About one hundred differentially expressed genes have been identified between virulent and nonvirulent strains, these could serve as genetic markers in order to support the genetic selection.

3.1.3 Expected final results and their potential impact and use

The expected final result of BIOCOMES is the generation of knowledge and innovative solutions for pest and disease management targeted to new biological control products that are effective and can readily and sustainably be introduced into the market. This will support the policy (i.e. Directive 2009/128/EC) laying down that all farmers will have to apply the general principles of integrated pest management by January, 2014.