Proceedings

New biocontrol agents for powdery mildew on grapevine

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SELECTION OF NEW *AMPELOMYCES* STRAINS FOR THE DEVELOPMENT OF A NOVEL BIOFUNGICIDE AGAINST GRAPEVINE POWDERY MILDEW

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The fungal culture collection of the Plant Protection Institute of the Hungarian Academy of Science contains several hundreds of strains of *Ampelomyces* mycoparasites isolated in different parts of the world from many different powdery mildew species infecting their respective host plants. To select strains which could be developed as commercialized biocontrol agents of grapevine powdery mildew, caused by *Erysiphe necator*, a comprehensive screening protocol was developed and used to compare cultural characteristics and mycoparasitic activities of more than 700 *Ampelomyces* strains. In particular, strains were screened for (i) growth and sporulation rate in culture, and (ii) sporulation rate and intrahyphal spread in *E. necator* colonies on grapevine leaves. At the end of the screening procedure, 10 strains were selected based on their high sporulation rates in culture and promising mycoparasitic activities in grapevine powdery mildew colonies. These were further tested during field experiments carried out in spring, summer and autumn in different vineyards in Hungary and Italy.
To distinguish the newly selected *Ampelomyces* strains from (i) commercialized strains used as biocontrol agents of *E. necator*, and (ii) strains that occur naturally in *E. necator*, molecular markers were developed based on rDNA ITS and actin gene sequences, microsatellite (SSR) loci and ISSR loci. Several newly developed and/or newly selected markers were successfully applied to achieve strain-specific identifications in spite of the fact that many genetically different *Ampelomyces* strains occur naturally in *E. necator*. Thus, the environmental fate of *Ampelomyces* strains applied as biocontrol agents of grapevine powdery mildew can be monitored using the newly developed set of molecular markers.

APPLICATION OF *AMPELOMYCES* AGAINST *ERYSIPHE NECATOR*

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Grapevine powdery mildew is one of the major plant diseases that affects grapevine production world-wide. Soon all European culture shall be managed following the integrated pest control and alternatives to fungicides are currently widely studied. The EU-funded project "BCA_grape", aimed at developing new biocontrol agents for powdery mildew on grapevines fits in with this prospective. A wide *in vitro* screening of more than thirty strains of *Ampelomyces,* a group of hyperparasitic fungi affecting several powdery mildews of different cultivated and wild plants, belonging to the culture collection of the Plant Protection Institute of the Hungarian Academy of Sciences was carried out within this project. The best screened strains were applied on potted grapevine plants bearing powdery mildew colonies in different stages of the life cycle, i.e. sporulating colonies and colonies with developing ascocarps, in order to evaluate the ability of the different strains of the hyperparasite in reducing the conidiogenesis or the production of ascocarps of powdery mildew. Some of the new selected strains significantly reduced both the production of conidia and of ascocarps after an incubation period of 10 and 21 days, respectively.
Moreover, the selected strains were applied in five vineyards of Italy and Hungary, for two grape-growing seasons. Trials were managed in order to statistically compare efficacy of the new *Ampelomyces* strains with an untreated control and with a commercial *Ampelomyces*-based product. The strains were applied repeatedly in late Summer in 2008 and 2009 during formation and development of the *Erysiphe necator* chasmothecia; onset and development of powdery mildew epidemics in Spring 2009 and 2010 was assessed to evaluate effectiveness of the strains. A delay in disease onset and in the initial progress of epidemics was observed, with significant differences among *Ampelomyces* strains and the untreated control, as well as among some strains and the commercial product. These results show the potential effect of *Ampelomyces*–based biocontrol products for reducing the dose of overwintering ascocarps in a vineyard and delaying the progress of the grapevine powdery mildew epidemics.

MASS PRODUCTION OF *AMPELOMYCES*

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In BCA grape project Agrifutur and Bioved tested a number of various inexpensive nutritive media to grow 10 *Ampelomyces* strains highly selected by RTD partners. Fermentation in submerged and solid state were tested at a pilot level performing mass production in monoaxenic systems montoring and controlling the growth paramenters. It was obtained with the best 2 strains a high viable concentration of *Ampelomyces* conidia. Formulation of the active ingredients with drying and freeze drying techniques was performed. Final formulations were done considering the shelf life, dispersion in water, UV sunscreen and other co-formulants. In formulation were involved also Vellsam and Byotar.
The SMEs partners have been trained continuously during the project by the RTD performers: how to grow *Ampelomyces* strains, to induce sporulatation, and to check the viability and the infectivity.
2 product prototypes were obtained with a good shelf life. An estimation of cost production was done and a first feasability study gave good result.
Field tests for efficacy and for demonstration were performed using the BCA grape *Ampelomyces* strains.

RESULTS IN DEMONSTRATION FIELDS

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In autumn 2009 SMEs partners organized 15 demonstration fields across Europe (France, Greece, Germany, Hungary, Italy, Slovakia, Spain and Turkey) to test the prototype bio-fungicide in the most important grape-growing regions. Each field was divided in two plots, one plot was treated as usual while the other plot was treated as usual plus 2 applications of *Ampelomyces* product at the dosage of 7000 g/ha in two autumnal sprays. In spring 2010, periodic observations were performed to assess the presence and severity of the disease in the demonstration plots in order to demonstrate the efficacy of the late season *Ampelomyces*-based prototype sprays. In first observations performed in the demonstration fields settled in Hungary and Slovakia the *Ampelomyces* product achieved 69,47% of efficacy on the incidence and 76,49% on the severity of powdery mildew infection. In Italy only the field located in Taranto (south of Italy) developed symptoms of powdery mildew on leaves and bunches later in the season. The plot treated with *Ampelomyces* showed in the first and second observation on leaves and in second and third observation on bunches less symptoms of powdery mildew than the farmer plot. The other two fields located in the north of Italy showed light incidence of symptoms only in the last observation on bunches in both plots. In the second and third observations in Spanish demonstration field the powdery mildew showed a lower incidence in the plot treated with *Ampelomyces* based product, the symptoms on bunches appeared only in the third observation and also in this case the plot with *Ampelomyces* achieved better results than the farmer. In Greece the symptoms were detected only during the third observation on bunches with high incidences of powdery mildew in both plots. No symptoms developed in the trials settled in France and Germany. In Turkish demonstration trials no symptoms developed in the first and second observations, whether in the last observations the crops were completely destroyed by downy mildew in two demonstration fields and the grapes were not harvested. In the third field no symptoms developed either.
Statistical analysis performed on the data collected during the first observation on leaves showed a good efficacy, i.e. reduction of the disease incidence by 58,29% and of the disease severity by 70,82%. Also on bunches the product showed an efficacy of 32,89% on powdery mildew incidence and an efficacy of 38,84% on its severity. These results achieved by *Ampelomyces* prototype in demonstration fields confirmed that the late season treatments can reduce the incidence and the severity of the disease in the following season as already showed by experimental plots.