Final executive summary

European and worldwide policies are increasingly working to limit the use of chemical substances for fertilisation and plant protection so as to reduce their negative environmental impacts and to increase soil fertility. In the plant nursery sector there is widespread use of chemical pesticides to repress plant pathogens and of synthetic fertilisers to stimulate plant growth. Both practices lead to a reduction in the complexity of the soil microflora and to a decline in overall plant quality. As a result, innovative nursery plant production processes are urgently needed to produce plants that are more tolerant of soil-borne pathogens and also more efficient in using the natural soil resources (minerals, water, etc.) in all phases of cultivation (in the tissue-culture laboratory for rootstock production, the nursery and the orchard).

The aim of the SITINPLANT project was: ♦ to use mycorrhizae, antagonist bio-control microorganisms, during the acclimation phase of micropropagated rootstocks and in the nursery, ♦ to optimise an innovative micrografting technique, ♦ to obtain mycorrhisated and micrografted fruit trees in four months instead of the usual two years needed to grow the plants to the stage required by consumers and ♦ to produce trees more tolerant of a range of biotic and abiotic stresses in the nursery and orchard. The rootstocks considered during the whole period of the project were: GF677 (*Prunus persica x Prunus amygdalus*), the most commonly used rootstock for peach, nectarine, plum and almond cultivars; Myrobolan 29C, a rootstock for plum and apricot cultivars; GiSeLa®6 a rootstock for sweet and sour cherry cultivars, and OHF 19-89 (Old Hom x Farmingdale) a rootstock for pear. Microorganisms used during the acclimatisation stage: *Trichoderma harzianum* T-22 (a commercial and a fresh culture produced by Fitotechniki); *Trichoderma asperellum* B1 (a fresh culture produced by Fitotechniki) isolated from tomato roots by the University of Thessaloniki during the period of the SITINPLANT project; *Glomus intraradices; Agrobacterium Radiobacter* K84 (a fresh culture produced by Fitotechniki). In the nurseries of the partners: *Streptomyces lydicus* WYEC 108; *Bacillus subtilis* QST 713.

The following partners were involved: 1) SMEs: AGTE (AGRIBIOTECNICA, ITALY); BALKA (BULGARIAN-PLANT, BULGARIA); FITO (FITOTECHNIKI, GREECE); PROFIT (PROFITO-FIDANCILIK, TURKEY); 2) RESEARCH CENTERS: UBAS (UNIVERSITY OF BASILICATA, ITALY); AINS (AGROBIOINSITUTE, BULGARIA); AUTH (ARISTOTELE UNIVERSITY OF THESSALONIKI, GREECE); SELKO (UNIVERSITY OF SELCUCK, TURKEY). The SITINPLANT project, funded by the European Commission under the 7th Framework Programme, started officially on 1 September 2008. A variety of relevant research expertises in the fields of plant physiology and commercial management were involved in the project to ensure optimum support during experimentation and also during the transfer of the new technologies it developed to the industry.

During the two years of activity, the work achieved the following main objectives: ♦ Optimisation of the inoculation method on micropropagated rootstocks; ♦ Development of the correct interaction between mycorrhizae and antagonist biocontrol micro-organisms on controlling disease incidence and improving plant nutrient uptake; ♦ Optimisation of micrografting techniques; ♦ Optimisation of nursery growing techniques for micrografted and mycorrizated plants; ♦ Implementation and testing of the innovated plant materials under a range of pedo-climatic conditions. Colonisation of root systems by mycorrhizae represents a key topic for setting up successful production procedures. To improve the fraction of colonised roots, two inoculation methods (incorporating in the turf and dipping in water solution) were compared during transplantation at the Fitotecniki laboratory. The source of bioagents (commercial or pure culture) and the eventual combination of *Trichoderma harzianum* T22 with others fungi were also tested. Better results were achieved using plant dipping in pure culture solutions. Both commercial and pure cultures of *Trichoderma harzianum* significantly reduced *Phytium ultimum, Fusarium oxysporum and Rhizoctonia solani* incidence during the acclimatisation stage. Both antagonists *T. harzianum* and *asperellum* inhibited the growth of *R. solani* and *P. ultimum* through the production of volatile and non-volatile substances. The *T. harzianum* volatile metabolites were more effective against *P. ultimum* and *R. solani* than those of *T. asperellum*. At the end of the first year of experiments, the SME Fitotechniki transferred the positive results of biological control on all micropropagated rootstocks produced (about 10 million plants), avoiding the use of fungicides, during the acclimatisation stage, delivering enormous advantages from economic, social and environmental points of view and protecting the health of workers. The quality of the plants was also improved and the duration of acclimatisation was shortened by four or five days. One of the problems that can limit the implementation of biocontrol agents and antagonist microorganisms in nurseries is the difficulty in establishing and maintaining symbiosis and colonisation. Growing techniques were optimised, qualitative and quantitative measurements were carried out to check for the presence and persistence of biocontrol agents in pots (UBAS-AGTE) and also under nursery conditions (SELKO-PROFIT and AINS-BALKA). The results of two years experimentation on the persistence of microorganisms showed a general colonisation trend for *Glomus intraradices* and *Trichoderma harzianum* with a peak in June-July followed by a decline in December. However, both these microrganisms survive during the winter period and reactivate in spring. *Bacillus subtilis* and *Agrobacterium radiobacter* do not persist in the field for more than one year after application. *Streptomyces lydicus* was detected everywhere and continued to persist to December 2010 (the final measurement).

The Project also developed a new technique for agamic propagation based on micrografting. This will resolve a number of important economic, organisational and managerial problems of the fruit tree nursery sector. This innovative technique allows extension of the calendar period during which it is possible to produce bimember plants, therefore allowing greater flexibility for the sale of transplants and a greater certainty of high health in the final product. The success of these micrografting techniques was strongly influenced by the adaptability of the species being manipulated and by the environmental conditions. The UBAS-AGTE trials showed “chip budding” to be the best technique for obtaining maximum grafting ‘take’ percentage. FITO SME produced biotisated and micropropagated rootstocks to be used for micrografting (AGTE) and grafting (PROFIT and BALKA) tests. Autumn (October) was shown to be the most suitable period for micrografting using mycorrhizated rootstocks actively growing in pots under controlled conditions and fresh buds selected at the time of the operation. Mycorrhization of rootstocks showed effects on cultivar quality and general vigour after shoot development. There were no significant differences among the mineral element concentrations in the dry matter of the various treatments compared to the controls but, based on the mean total amounts per plant, quantities of macro-elements were greater in treatments with *Glomus* or *Trichoderma* than in the controls. This was due to the greater growth rate of the inoculated plants. Moreover, micrografted plants on inoculated rootstocks showed an increase in water conductance. Field experiments on grafted plants confirmed the previous results. The best nursery management technique was defined which ensured microorganism symbiosis and obtained highest quality micrografted plants in pots and also grafted plants in the nursery. The main outcomes are working protocols and specific technical procedures containing information related to the phases of the optimised cycles. The project’s positive impact will benefit the whole agriculture sector in as much as the efficiency of perennial agriculture production depends very heavily on the quality of the plants obtained from the nursery.