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Authors per region:

NL - Netherlands: Nico van der Velden

IT - Italy: Nadia Lombardi, Massimo Giorgini, Michelina Ruocco.

FR - France: Cécile Bresch, Marion Giraud, Pia Parolin, Hicham Fatnassi, Ricardo Suay, Christine Poncet

ES- Spain: Miquel Alonso, Ricardo Suay, Francisco Beitia, Meritxell Pérez-Hedo, Alberto Urbaneja

Institutions, PURE partners

DLO: Wageningen University & Research Centre, LEI, Alexanderveld 5, P.O. Box 29703, 2502 LS The Hague, The Netherlands.

INRA: Institut National de la Recherche Agronomique (INRA), Institut Sophia Agrobiotech, 400 route des Chappes, 06903 Sophia-Antipolis, France.

InVivo AgroSolutions:

CNR: Consiglio Nazionale delle Ricerche, Istituto per la Protezione Sostenibile delle Piante, UOS Portici (NA), Italy.

IVIA: Unidad Asociada de Entomología UJI-IVIA. Centro de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA). Carretera de Moncada -Náquera Km. 4.5, 46113-Moncada. Valencia (Spain).

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GREENHOUS TOMATO CULTIVATOIN
« Results and lessons learnt from PURE »

FEBRUARY 2015

OBJECTIVES

The general objectives of WP7 are:

- To define the starting situation, in terms of greenhouse technologies, pests and pathogens problems of tomato greenhouse cultivation in Europe (mainly Italy, France and Spain).
- To find alternative control methodologies based on IPM principles.
- To compare IPM *vs.* conventional pest management (CPM) both in terms of pest-pathogens control efficacy and economic sustainability.

APPROACH (EXPERIMENTS, ASSESSMENT TOOLS, …)

To reach these goals, the PURE WP7 members followed these steps:

1. We performed an assessment of the situation of the European tomato greenhouse cultivation.
2. We designed IPM solutions for greenhouse tomato cultivation and performed on-station and on-farm tests to validate these solutions. In the on-station trials we mainly focused on the applications of microbial biological control agents (MBCAs) and their metabolites to control plant diseases and pests. On-farm experiments were done in specific crop cycles in different countries (Italy, South of France and Spain) and were primary focused on pests.
3. At the end of 4 years of experimentation, we compared the different IPM systems adopted in Italy (Campania region), France and Spain in terms of used antagonists, pesticides, BCAs and tactics.

PESTS

Introduction

The major pests and diseases in different countries are summarised in the following table (Table 1). In the table the pests (air), diseases (airborne), soil pathogens (soil-borne diseases and pests) and viruses are distinguished as groups.

Table 1 Main pests and disease per country

	Netherlands	France	Spain	Italy
Viruses				
- TYLCV		+	+	+
- TSWV				+
- PepMV	(+)			
Pests				
- greenhouse whitefly (*Trialeurodes vaporariorum*)	+	(+)		+
- whitefly (*Bemisia tabaci*)		+	+	+
- tomato borer (*Tuta absoluta*)		+	+	+
- trips		+	+	+
- spider mite	+	+	+	+
- rust mite	+	+	+	+
- caterpillars	+	(+)	+	+
- aphids	+	(+)		
Diseases; airborne				
- *Botrytis*	+	+	+	+
- downy mildew	+	(+)		
- powdery mildew (*Oidium*)		+	+	+
- *Cladosporium*		+		+
Soli pathogens (diseases; soil born and nematodes)				
- *Phytophthora*		+	+	+
- *Fusarium*				+
- *Pythium*				+
- nematodes		(+)	(+)	+

Regarding pest and diseases results, our ex-ante assessment indicated that:

- Pests and airborne diseases show strong similarities between the countries: sometimes different species are concerned;
- The South America pinworm, *Tuta absoluta* is a problem in the southern countries, although its incidence is starting to decrease after some years of being the main pest;
- Thrips are a growing problem in the southern regions;
- The tomato rust mite, *Aculops lycopersici*, occurs in all countries as a secondary pest, although its incidence is considerably increasing related to the shift from broad spectrum to selective pesticides;
- Viruses are a big problem in the south, especially the tomato yellow leaf curl virus (TYLCV) and tomato yellow leaf curl Sardinia virus (TYLCSV) vectored by the whitefly *Bemisia tabaci* and the tomato spotted wilt virus (TSWV) transmitted by different species of thrips;
- Airborne diseases are more problematic in the south of Europe than in the north and this is partly due to the poorer ventilation and heating control in the south;
- Soil pathogens (nematodes and soil-borne diseases) are mainly a problem in Italy, this is probably due to cultivation in soil and the type of soils.

Main pests and their damages

Tuta absoluta (adult and larvae)

Tuta absoluta damages on plant and fruit

Whiteflies

Bemisia tabaci *Trialeurodes vaporariorum*

Thrips (*Frankliniella occidentalis*) and damages on fruit

TECHNICAL RESULTS

Main control methods in different countries

The main control methods used to control pests and diseases in different countries are summarised in Table 2. In the table viruses, pests, airborne diseases, soil pathogens (soil-borne diseases and nematodes) are distinguished as groups. Subsequently, the most important control methods are given:

- In the southern regions the viruses can be controlled by preventing the entrance of vector insects with nets on the ventilation openings of the greenhouses in combination with the control of whiteflies and thrips and choosing tomato varieties tolerant/resistant to virus disease;
- Biological pest control agents are important in all countries, except in Italy;
- In addition to biological pest control agents also mass traps to catch the pests are used, and in the southern countries the use of nets in the ventilation openings of the greenhouses is widely applied;
- Due to the biological control of pests and the use of bumblebees for pollination the use of broad spectrum chemicals has shifted to selective chemicals;
- In Spain in particular, the predator *Nesidiocoris tenuis* is widely used for pest control. *N. tenuis* occurs freely in nature. Large populations of *N. tenuis* may also be a pest for the tomato crop, because of its phytophagy when the prey are scant on the plants. The tomato growers in France and the Netherlands do not want to use this predator;
- An effective predator for the secondary pest rust mite is not available;
- Biological control of foliar diseases is not used due to the unavailability of antagonists;

- In the control of airborne fungal diseases ventilation of the greenhouses to reduce the level of moisture plays an important role. The use of technologies such as automatic climate control and heating in the north reduce the problem;
- In the southern regions, the tomatoes are not heated during winter and this is not likely to change as a result of high costs to heating and lower tomato prices in the periods with extra production by heating. In the south important improvements are achieved in relation to the ventilation of the greenhouses but the nets used to prevent the entrance of insects reduce the ventilation;
- In the southern regions the control of soil pathogens is done mainly with chemical agents and also with the use of tolerant rootstocks (Spain) and on a smaller scale with soil less cultivation on substrates or soil solarisation. In the north soil less cultivation is largely prevalent;
- In the fight against pests and diseases more attention is paid to soil quality and plant health in relation to the resilience of the crop by stakeholders in all countries. In the north, this includes the influence of the greenhouse temperature on the crop. Higher temperatures are associated with thinner plants.

Table 2 Main control methods of pests and diseases per country

Table 8.3	Main control methods of pests and diseases per country			
	Netherlands	France	Spain	Italy
Viruses				
- chemical (pests)	-	+	+	+
- nets (pests)	-	(+)	+	+
- tolerance	-	+	+	+
Pests				
- chemical	(+)	+	+	+
- nets	-	(+)	+	+
- trapping	+	+	+	+
- biological	+	+	+	(+)
- tolerance	-	-	-	-
Diseases airborne				
- chemical	+	+	+	+
- ventilation	+	+	+	+
- heating	+	(+)	-	-
- biological	-	-	-	-
- tolerance	-	-	-	-
Soil pathogens				
- chemical	-	+	+	+
- rootstock (tolerance)	-	(+)	+	(+)
- substrate	+	+	(+)	(+)
- steam (substrate)	(+)	-	-	-
- solarisation	-	-	(+)	(+)
- tolerance	-	-	-	-

Crop cycles and greenhouse technology

In the north there are mainly long crop cycles and in the south short and long crop cycles. But in the south the long crop cycle is shorter than in the north.

In the north the crop cycle starts in general in November/December and ends in November (The Netherlands and Belgium) or starts in January/December and ends in

November (northwest of France).
In the south the long crop cycle starts in general in August and ends in March/April. This long cycle occurs mainly in the south of Spain. Sometimes there are also short crop cycles in Spain.
In Italy there are mainly short crop cycles. This is related to the lower temperature in Italy compared with Almeria, the biggest tomato area in Spain. In Sicily in the south of Italy there are also long crop cycle but they are not as common as in the south of Spain. The short crop cycles start in August and end in December (summer-autumn crop) or start in January/February and end in May/June (winter-spring crop). The long crop cycle starts in October/December and ends in June.
In the northern regions high tech glasshouses with heating, screens and climate control by computers are used because of the cold temperature outside and the higher prices of additional production in wintertime.
In the south plastic greenhouses without much technology are used. Low tech greenhouses are used because of decrease of tomato prices in spring. The use of heating and other technology increase the yield in wintertime and extend the crop cycle with substantially additional fruit production in spring. However, growers prefer not to invest money for heating and technology to get extra production in periods with low prices.
Because of the longer crop cycle, the high tech greenhouses and the soil less cultivation, the tomato production per square meter per year is much higher in the north than in the south.

The crop cycles of the on-farm experiments in Italy included spring-summer cycles (May-August and June-September in 2012, April-August and June-October in 2013, April-August in 2014). This crop cycle has a low infection pressure at the start but increases rapidly towards the summer because of high temperatures during this period.

On-station and on-farm tests to validate IPM solutions for greenhouse tomato cultivation

The control of pests with IPM is technically largely possible both in the north and in the south. Predators for most pests are commercially available as well as microbiological insecticides. The major challenges with respect to pesticide reduction in greenhouse tomatoes are the diseases (airborne and soil-borne) and nematodes. This is mainly a challenge in the south because of the simple climate control (low tech greenhouses without heating) and growing in the soil. Nematodes are especially a problem in Italy. Probably this has a relation with the soil (clay).

Efficacy of microbial biological control agents (MBCAs) assessed through on-station trials

In the frame of PURE WP7, we demonstrated that the use of selected microorganisms and their metabolites can be successfully applied to the list of soil-borne pathogens *(Fusarium oxysporum, Pythium ultimum, Sclerotinia sclerotiorum, Rhizoctonia solani*). Our approach (Figure 1) includes the use of biological control agents and their products as alternatives to synthetic agro-chemicals. *Trichoderma* spp. are widely studied fungi and are among the most commonly used microbial biological control agents (MBCAs) in agriculture. They are presently marketed as bio-pesticides, biofertilizers, growth enhancers and stimulants of natural resistance. The use of such biocontrol agents and of mycorrhizae is also desirable because in tomato plants they can significantly increase resistance toward insects (aphids), activating both direct and indirect defense mechanisms. Indeed, we also demonstrated (Battaglia et al 2013) that the application of the MK1 strain of *T. longibrachiatum* to tomato affects the performance of *Macrosiphum* *euphorbiae* and its natural antagonists. In fact, when compared with the uncolonized controls, plants whose roots were colonized by *T. longibrachiatum* MK1 showed quantitative differences in the release of specific volatile organic compounds, and although the aphid population growth indices were slightly higher, the attractiveness toward aphid parasitoids and predators and the development rate of an aphid predator greatly increased. These findings support the development of novel IPM strategies based on multi-trophic interactions, which can be effective in defending plants from pests and pathogens at the same time.

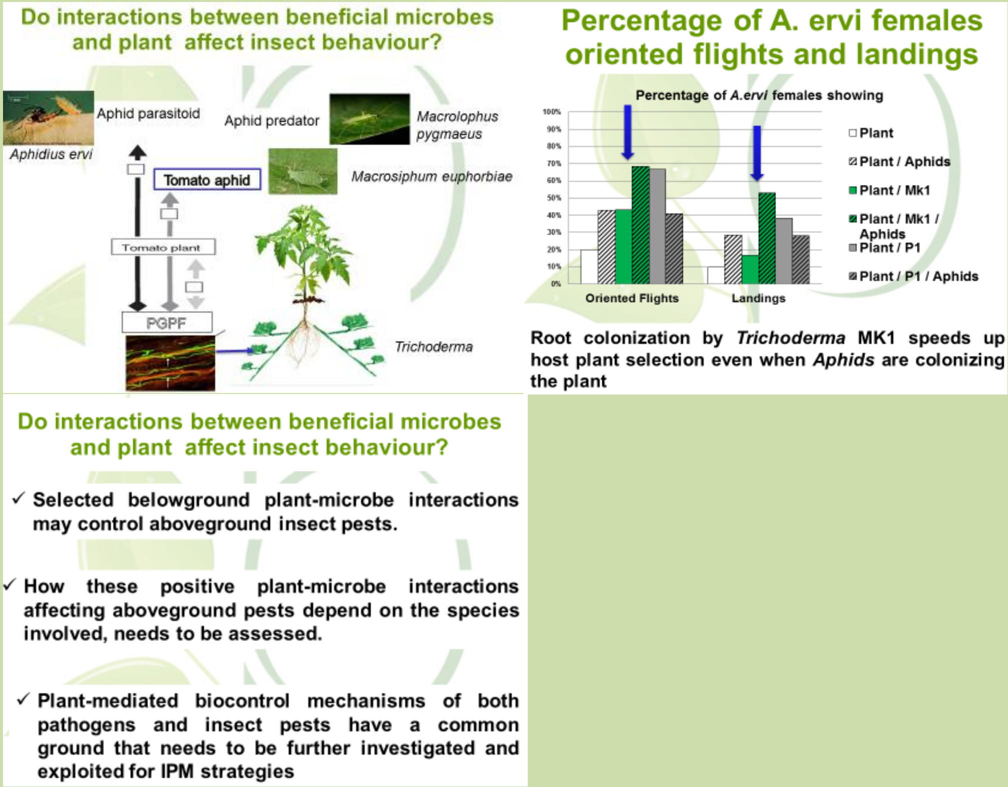


Figure 1 Context and hypothesis of MBCAs tested.

Several experiments were conducted in order to control soil-borne pathogens like *Fusarium oxysporum, Pythium ultimum, Sclerotinia sclerotiorum, Rhizoctonia solani*, by applying to the soil some selected strains of the biocontrol agent *Trichoderma harzianum* instead of the chemical pesticide Previcur. The results obtained (Figure 2) demonstrated that all the applied *Trichoderma* strains can be used as an alternative to Previcur to control natural occurring soil-borne pathogens.

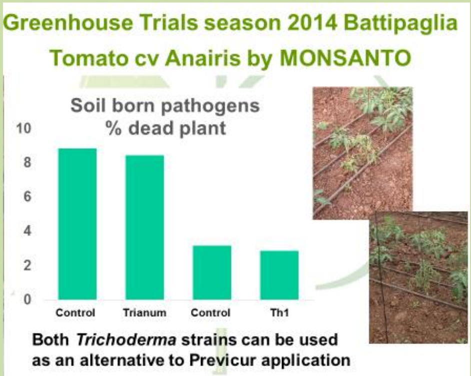


Figure 2 Results of the application of Trichoderma strains vs chemical strategy (Previcur) to control soil-borne pathogens. Y-axis represents % of dead plants.

The results of experiments with microorganisms indicate that

- The use of microorganisms and their metabolites is an added value for IPM of tomato cultivation.
- The use of such biocontrol methods (pest antagonists and beneficial microbial agents) is also desirable because of the indirect effects on the plant fitness, in terms of induction of systemic resistance and plant growth promotion that can make tomato crop more robust against new invasive pests and pathogens with regard to global climate change.

Efficacy of generalist predators as BCAs assessed through on-farm trials

Two mirid species were used in field trials to assess the effectiveness of new IPM solutions in tomato greenhouses in South Europe: *Nesidiocoris tenuis*, in Spain and Italy, and *Macrolophus pygmaeus*, in France. Both predators were used with the main purpose of controlling *Tuta absoluta* and whiteflies. In France, in order to increase the efficacy of *T. absoluta* control, *M. pygmaeus* was used in combination with an egg parasitoid, *Trichogramma acheae*, which parasitizes egg of Lepidoptera, including *T. absoluta*.

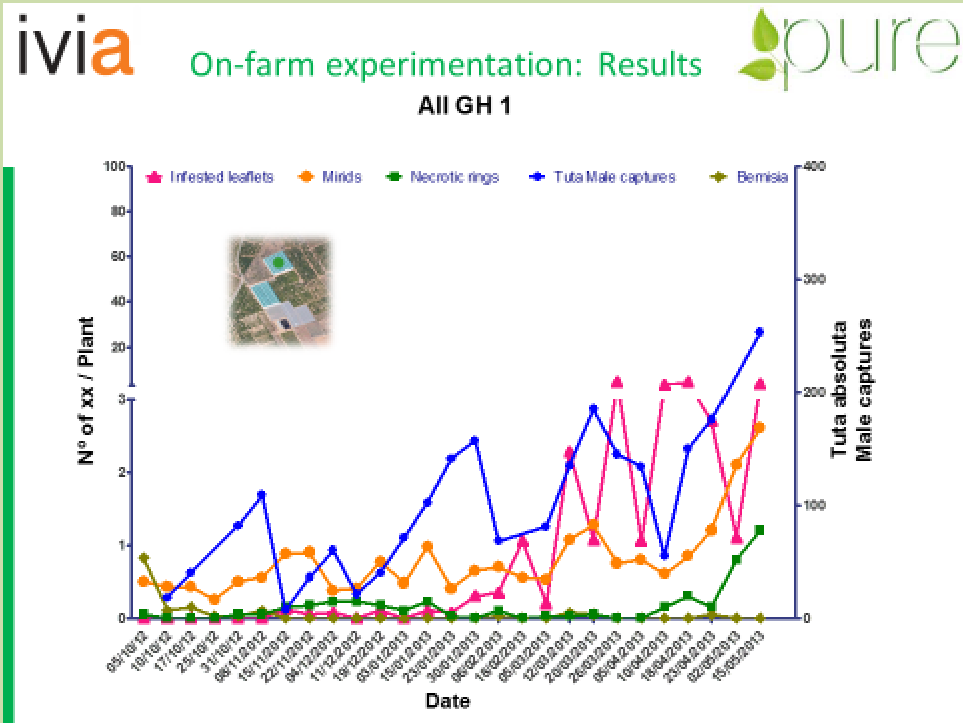
Strategies based on the use of generalist predator (Nesidiocoris tenuis)

Trials took place from 2012 to 2014, in a total of 14 greenhouses pertaining to two different growers (2012 and 2013-2014) and to one tomato cooperative (Coop. V. Unión Protectora del Perelló) specialized in high value tomatoes (Valencian tomatoes; 2013). They concerned different crop cycles and cultivars ("Raf tomato", "El Perelló", "Optima"). The IPM strategy consisted in a release of *Nesidiocoris tenuis* in the nursery (0.5 ind./plant) with the supply of *Ephestia kuehniella* eggs as alternative food (Figure 3) or of *Artemia* cysts (2013-2014) and when needed treatments with *Bacillus thuringiensis*. It was compared to a control strategy consisting of using chemical insecticides allowed in IPM (2012-2013); in 2013-2014 the control strategy was different, with no predators released and conventional supply of *Ephestia* eggs.

Figure 3 General strategy used for *N. tenuis* installation

The IPM strategy allowed saving between 9 and 15 chemical sprayings / greenhouse. *Bt* treatments were reduced and even eliminated as long as the grower was more confident with the IPM strategy. It was extremely successful in terms of control of *Tuta absoluta* (0 to 0.1% of fruits bored vs. 5-10% and 50% in the control strategies of 2012-2013 and 2013-2014, respectively). The incidence of thrips or whiteflies on fruits was negligible.

In 2012, the grower was extremely happy with the PURE results: the crop was finished later in the season due to its healthy status, no chemical treatments were applied due to the extremely efficiency of the predator released. These achievements resulted in an increase of his benefits. Indeed, since this season this grower has adopted this biocontrol strategy in all his greenhouses (Figure 4) and nowadays is an example in the area for biological control in tomato. Before our experiments of 2013, the cooperative which was our partner relied on the use of insecticides for pest management. However, after our successful PURE results, the cooperative exclusively used IPM based on biological control with the use of *Nesidicoris tenuis*. This has resulted in a minimal use of pesticides and therefore a cleaner and healthier yield (Figure 5) which represents an added value to their tomato production. In 2013-2014, we have found that the strategy using *Artemia* cyst instead *Ephestia kueehniella* eggs is much cheaper for the grower. This strategy is currently under development.



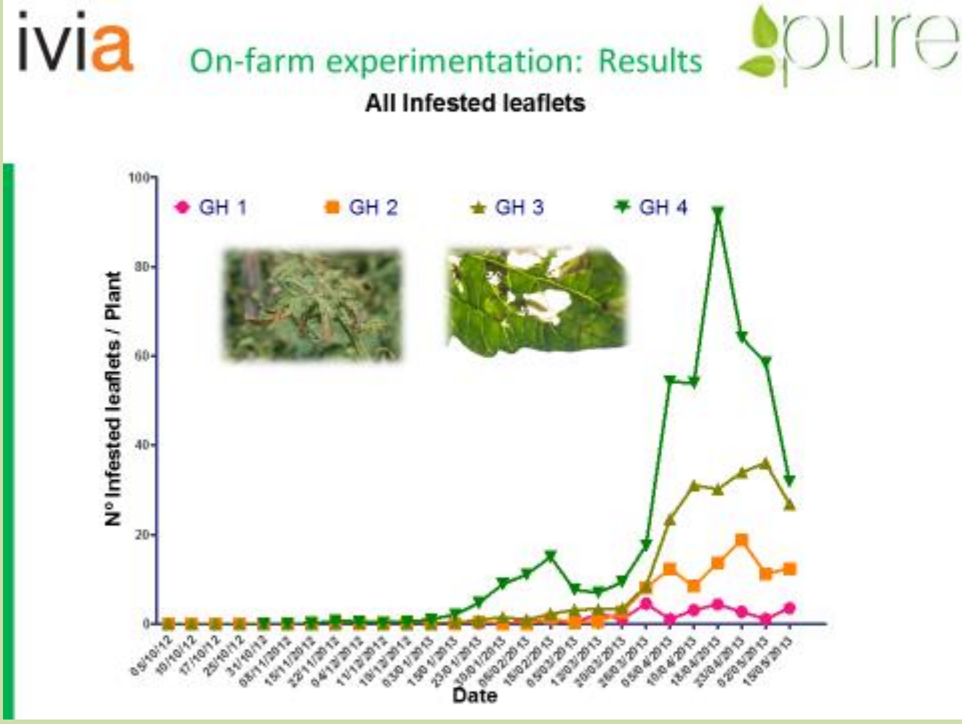


Figure 4 Results of IPM strategy based on the use of generalist predator *Nesidiocoris tenuis* on the crop in 2012-2013 (fall-spring cycle). Four different greenhouses were used for comparison.

- Top: pests (*Tuta* and whitefly) and predator (*N. tenuis*: adults and nymphs) populations, and pest (*Tuta*) and predator (necrotic rings) damages in greenhouse 1.
- Bottom: Pest damages in the four greenhouses used in the trial.

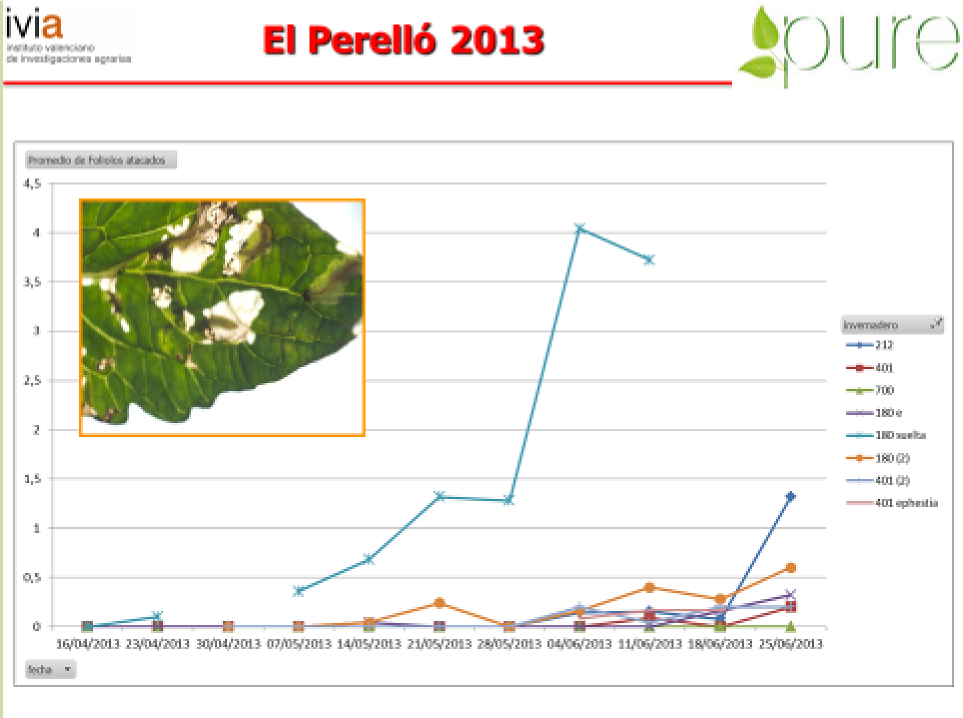


Figure 5 Results of IPM strategy based on the use of generalist predator *Nesidiocoris tenuis* on the crop 2013 (spring-summer cycle).

- Top: Predator (*N. tenuis*) populations in the greenhouses monitored
- Bottom: Pest damages (infested leaflets)

In combination with pheromone traps (Mating disruption)

In South Italy, an IPM strategy based on the use of the sexual pheromone of *Tuta absoluta* was also used. The mating disruption technique (Figure 6) was applied by distributing inside the greenhouse dispensers emitting the sexual pheromone at high density (2000 dispensers/ha). The dispensers attract the male moths and prevent them from mating with females. This is expected to lead to a reduction of the population of the pest.

Mating disruption technique was tested in combination with *N. tenuis* or microbial biological insecticides.

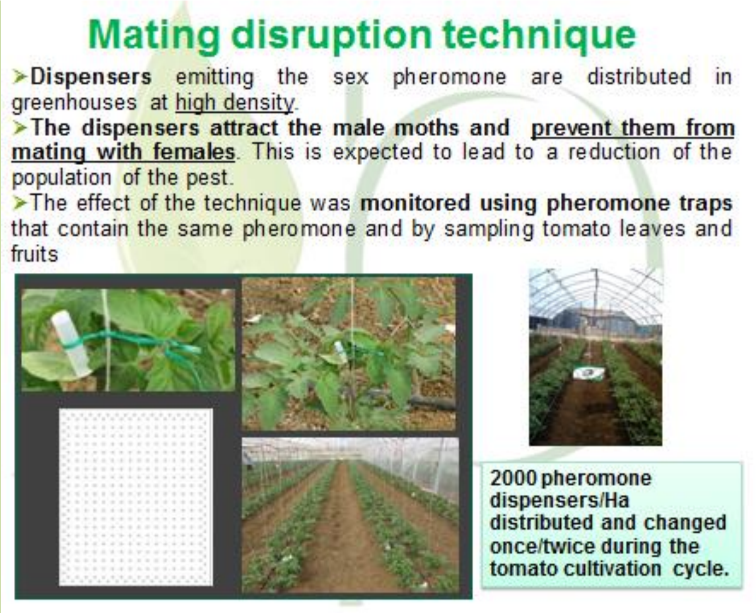


Figure 6 Context for the combined use of generalist predators and pheromone traps

The mating disruption trials took place from 2012 to 2014 in commercial tomato crops of Battipaglia (Salerno, Italy) (Figure 7).

Figure 7 Investigated area in South Italy and typical greenhouses

Trials concerned the spring-summer cycle and two cultivars (Sir Elyan and Ikram). Different strategies were tested and consisted in the use of *Nesidioris tenuis* as BCA, pheromone traps and microbial biological insecticides (*Bacillus thuringiensis* and *Beauveria bassiana*) and their combinations. The IPM strategies were compared to the chemical pest control practices in the area.

In this case, the BCA *Nesidiocoris tenuis* was released in the greenhouse after transplanting (1 ind./m^2). Pheromone dispensers were distributed at the beginning of the tomato culture, and then every two months during the crop cycle. In the first trials microbial biological treatments were realised following a preventive plant of action.

The IPM strategies allowed for important chemical spraying savings: from 9 chemical sprayings in the conventional strategy to none in the IPM strategies in the last year of trials (Figures 8-10). Moreover, the control of *Tuta absoluta* was always better with the IPM strategies (0 to 5% of fruits bored, in the worst case). The incidence of thrips in terms of damaged fruit was important and similar in 2012 and 2013, but it did not have any commercial relevance because fruits were harvested and sold green. In 2014 tomato truss was grown, the incidence of thrips was also important and similar. However, fruit were severely damaged in the conventional chemical strategy and so impacted the commercial yield. In the case of whiteflies, the first two years there was no incidence and so no differences between pest control strategies. However, in 2014 whiteflies were present and produced 15.4% of fruit damages in the chemical strategy and only 0.6% in the IPM strategies.

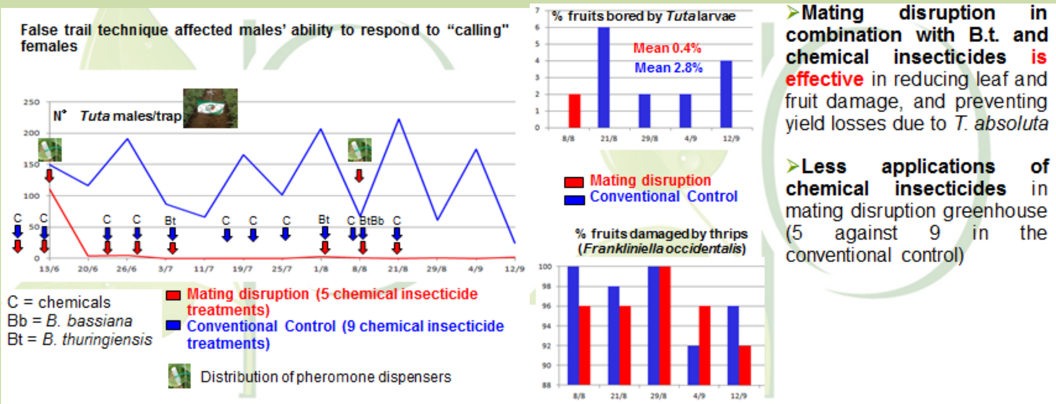


Figure 8 Results of 2012. Combined strategy of generalist predator and pheromone traps: *Tuta absoluta* populations in treatment and control, and *T. absoluta* and thrips damages. Chemical and *Bacillus spp.* treatments are also displayed in left graph.

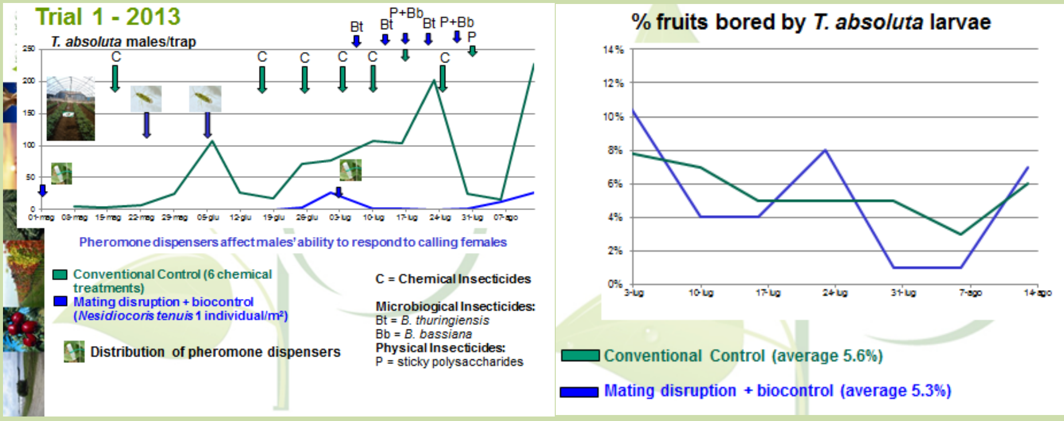


Figure 9 Results of 2013. Combined strategy of generalist predator and pheromone traps: *Tuta absoluta* populations in treatment and control, and *T. absoluta* damages. Chemical and microbial biological (*Bacillus spp.*) treatments are also displayed in left graph

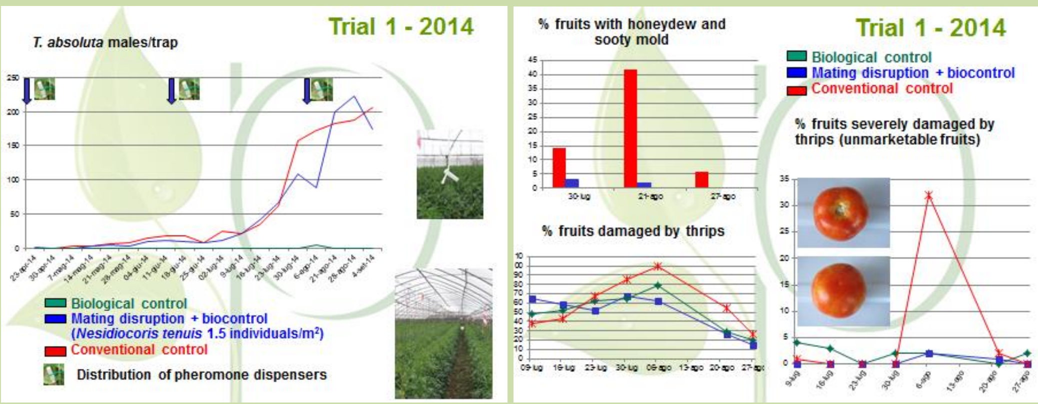


Figure 10 Results of 2014. Combined strategy of generalist predator and pheromone traps (blue lines) vs. generalist predator and microbial biological spraying (green lines):

Tuta absoluta populations in both treatments and control (red line), and *T. absoluta* and thrips damages. Only pheromone dispenser applications are displayed in left graph.

*In combination with parasitoids (*T. achaeae spp.*)*

The release strategy of *T. achaeae* dispensers was set prior to test the combined IPM strategy of the generalist predator, the mirid bug *Macrolophus pymaeus,* and parasitoids (*Trichogramma achaeae spp.)*. The preliminary trials to optimize the release strategy of the parasitoids took place in 2011 in four different greenhouses with four different tomato cultivars in a spring-summer crop cycle. Following this trials, the recommended release method of *T. achaeae* dispensers was fixed at 100 points/ha (Figure 11) and can be resumed as follows:

- Walk along the crop rows distributing the dispensers on the left and on the right alternatively,
- Distribute the dispensers staggered for a more even distribution;
- Intensify the releases in risky areas with supplementary dispensers.

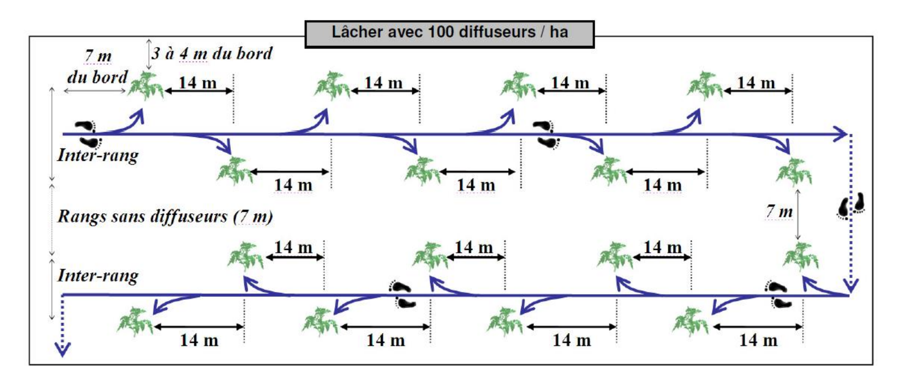


Figure 11 Distribution of *T. achaeae* dispensers in the crop.

The combined IPM strategy trials took place in 2013 in three different greenhouses of three growers in southeast France. They concerned one crop cycle (spring-summer) and three cultivars (“Bonarda”, “Coeur de boeuf”, “Maestria”). The IPM strategy consisted in weekly releases of parasitoids from the first *Tuta* adult was trapped; and a first release of *M. pygmaeus* when *Tuta* traps reached 5-10 adults. A second release was performed if installation was not satisfactory.

The IPM strategy successfully worked (Figure 12) since no chemical treatments were done. No one of the biological control agents alone (*M. pygmaeus* or the parasitoids) could control *Tuta* when pest pressure was high. In this case, the combined strategy performed much better (30 to 12% and 15 to 0% reduction of fruit bored). When pest pressure was medium or low, *Macrolophus* alone provide adequate protection, as far as the mirid installation is good.

Population dynamics

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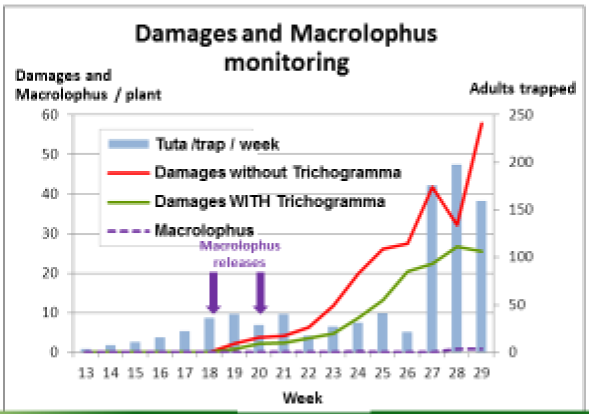
Site 1 (83) :

- Farm in IPM
- Tuta : high pressure

- Farmer practices / Tuta = Macrolophus + Bt
  - → 9 Bt treatments from week 18 to week 28
  - → No Macrolophus installation despite 2 releases



- → Damages reduction by 2 with Trichogramma releases
- → Positive effect, but insufficient in situation of high pressure and absence of Macrolophus

Population dynamics

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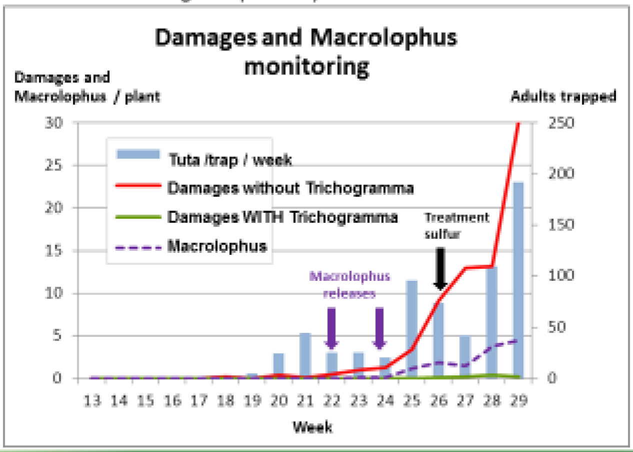
Site 2 (06) :

- Farm in IPM
- Tuta : high pressure
- 1 sulfur treatment against powdery mildew

- Farmer practices / Tuta = Macrolophus
  - → Good Macrolophus installation



- → Macrolophus alone are not sufficient to control *Tuta*
- → Very good *Tuta* control with combinaison Trichogramma + Macrolophus

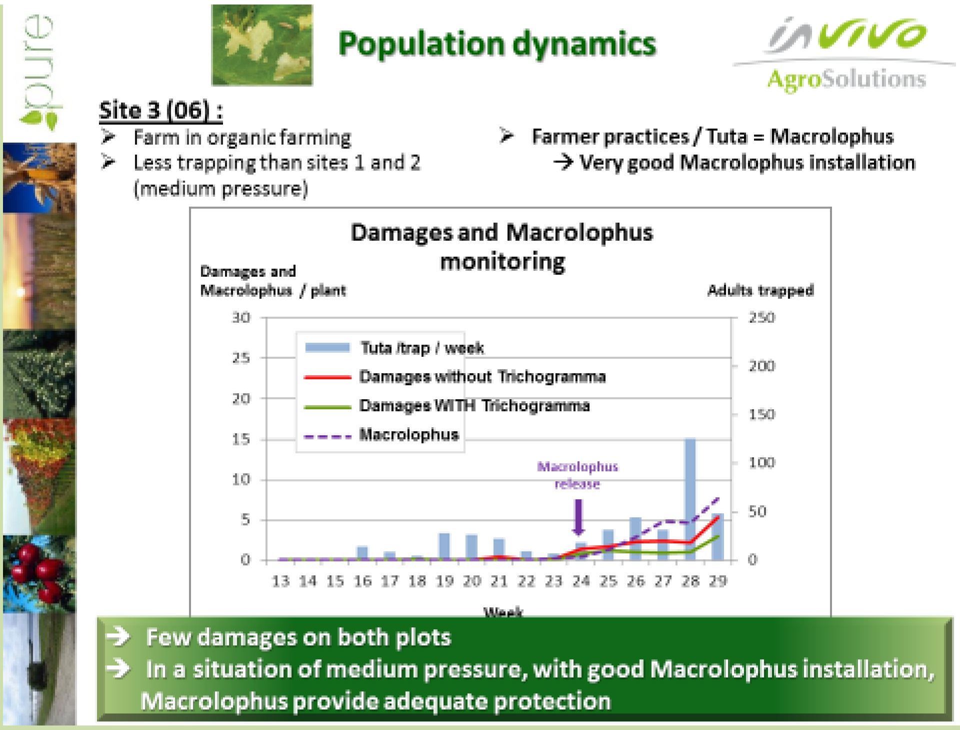


Figure 12 Results of the IPM strategy of generalist predator (*Macrolophus pygmaeus*) alone (red line) or in combination with parasitoids (green line). Graphs present pest (*T. absoluta*) and predator (*M. pygmaeus*) populations as well as pest damages. Each graph represents a different grower and farm site.

In combination with biocontrol plants

Biocontrol plants are plants that are “intentionally added to a crop system to increase the biodiversity of the first trophic level with the intent to enhance crop productivity by mutual benefit, pest attraction and/or pest regulation and thus contributes to an increase of the efficiency of biological control systems, which finally leads to increased crop productivity”. There are different types of plants that play a role on biocontrol that:

- Directly affect pests like repellent, barrier or trap plants
- Indirectly affect pests through the bias of enhancing the presence or the development of populations of natural enemies like insectary or banker plants.

The IPM strategy consisted in using biocontrol plants (banker plants, in this case) to increase the populations of the predatory mirid bug (*Macrolophus pygmaeus*) to control whiteflies (*Trialeurodes vaporariorum*).Trials were carried out on station and took place in 2012 and 2013. They concerned three well-known banker plants (*Ditrichia viscosa*, tobacco and basil) and one tomato cultivar (Saint-Pierre). The crop cycle was spring-summer.

The results ranged from efficient banker plant (tobacco) to not recommended (*D. viscosa*) (Figure 13) through indifferent or neutral (basil) (Figure 14). In the case of tobacco, it also appeared to be efficient for whiteflies. Consequently, none of the banker plants tested are recommended to increase *M. pygmaeus* populations in the presence of whiteflies (*T. vaporariorum*).

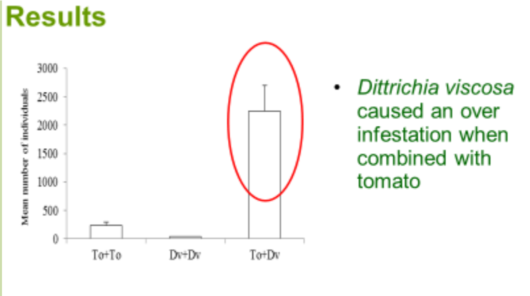


Figure 13 Results of the combined strategy *Ditrichia viscosa* as banker plant for *M. pygmaeus.* Graph displays whitefly populations of the tomato crop system.

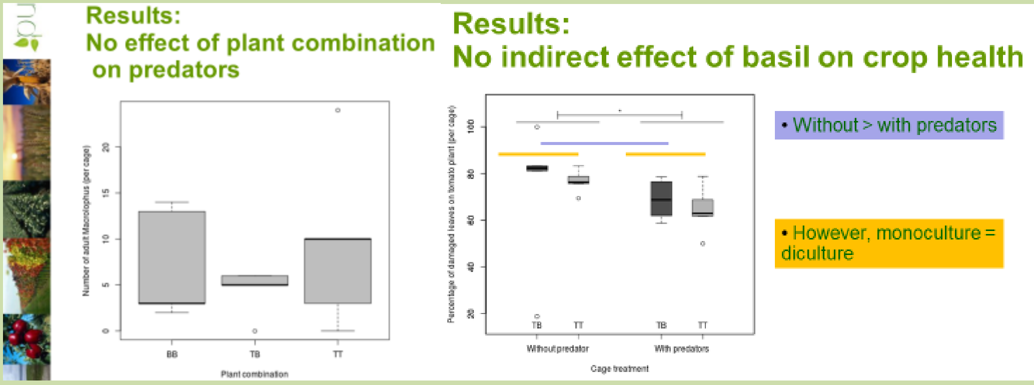


Figure 14 Results of the combined strategy basilic as banker plant for the mirid *M. Pygmaeus*. Results are shown in graphs: box plot of predator populations (bottom left) and box plot of leaf and fruit damages (bottom right).

SUSTAINABILITY OF IPM SOLUTIONS

The IPM solutions displayed have been country and context-dependent. Currently, IPM in protected tomato production seems mature enough to be recommended and applied in European Southern countries with an important reduction of chemical treatments. Main airborne pests (even new invasive ones like *Tuta absoluta*) can be controlled with releasing biological control agents. General predators are now used against main airborne pests like *T. absoluta* and whiteflies. Whenever needed other specialist predators and entomopathogenic bacteria like *Bacillus thuringiensis* can help and reinforced the generalist predators' control of pests. Also other minor pests can be controlled biologically, as it is the case of spider (*Tetranychus urticae*) and predatory mites.

The common trait in all cases has been more production, more quality and marketable production and finally the access to export markets that pay extra prices for this IPM quality production as we have demonstrated in the cost benefit analysis (see D7.2). So IPM in protected tomato production in Southern Europe can be resumed in a much better overall sustainability performance from all points of view: economic, social and environmental.

LIMITS AND CONDITIONS OF SUCCESS, ADAPTATIONS

IPM is mandatory for tomato cultivation and more generally for agricultural production. In the frame of PURE project WP7 we had the opportunity to study, apply and define IPM for tomato in greenhouse cultivation. The biggest opportunity was to have the possibility to compare 3 different greenhouse production systems in 3 of the most important European countries producing tomatoes. The results of these 4 years of experimentations provided evidence of the great differences among the systems cultivation in France, Italy and Spain. These differences can be related to the diverse greenhouse systems, growing

seasons, pest and pathogen pressure, crop cultivars and the reference market.
Regarding IPM guidelines for protected vegetables in Europe we can conclude that is unrealistic to design a protocol applicable for all the defined conditions but, rather, IPM must be designed, case by case, and adapted to the specific cultivation system.

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