WP7: IPM solutions for protected vegetables

"*List of scenarios allowing the highest efficacy of pest control*"

IOR Results
Generalist predator efficacy

The aim of the laboratory tests was to determine the efficacy of the predators: *Macrolophus melanotoma, Amblyseius californicus* and *Phytoseiulus persimilis* in reducing the number of the two-spotted spider mite (*Tetranychus urticae*) in greenhouse grown tomato crops. It was shown that a high efficacy (86%) to reduce the pest population was obtained after joint use of both predatory mite species: *M. melanotoma* and *A. californicus*. The results of the studies showed that these two predatory species might be applied jointly as biocontrol agents, without any advertise interaction effect (a phenomenon of neutralism).The joint use of the predatory mites *P. persimilis* and *A. californicus* for the control of the two-spotted spider mite caused a high mortality (72%) of the pest. The efficacy of this treatment was statistically different as compared to the treatment with a separate use of the predatory species tested. Both predators *P. persimilis* and *A. californicus* could be applied jointly, however their joint use seemed to be unjustified from the economical point of view.

The results of the greenhouse experiments with tomato plants infested by the two-spotted spider mite and red spider mite showed a phenomenon of interspecies competitiveness, and *T. cinnabarinus* was a dominant species.

Under the greenhouse condition, the efficacy of two predatory species *M. melanotoma* and *A. californicus* for the control of the two-spotted spider mite, as well as the effect of grey mould on the pest level and efficacy of its natural enemies were studied. The number of spider mites increased by fivefold in the treatment with tomato plants infected by grey mould (*Botrytis cinerea*), and all those treatments showed a lowered efficacy of the applied predators *M. melanotoma* and *A. californicus* in reducing the pest level.

IVIA results
Optimized establishment of *Nesidiocoris tenuis* against *Tuta absoluta*

The predatory mirid bug *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) is a zoophytophagous predator which commonly appears in horticultural crops and natural vegetation in the Mediterranean basin. *N. tenuis* is a predator which is augmentative released and conserved against whiteflies (Hemiptera: Aleyrodidae) and *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in tomato crops. Once established in the crop, *N. tenuis* is also able to regulate populations of other pests such as thrips, mites and some lepidopterans.

Two strategies are nowadays underway to control *T. absoluta* and whiteflies in tomato greenhouses using *N. tenuis*. The first technique is based on the release of 1-2 individuals of *N. tenuis*/m^2 between two and four weeks after transplanting tomato plants in the greenhouse (spring-summer crop cycle). To shorten its establishment period and improve its distribution in the crop, especially when pest pressure is high after

transplanting (summer-spring crop cycle*), N. tenuis* is now released in seedling nurseries (preplant release). This strategy entails transplanting tomato plants on which *N. tenuis* individuals have already laid eggs in the nursery. For this, 0.25 to 0.5 *N. tenuis* couples per plant are released in the nursery with an alternative prey for feeding: *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs or others such as decapsulated *Artemia* cysts. Once the plants are in the field, the factitious prey is weekly added during 2-3 weeks to improve *N. tenuis* establishment..
At IVIA, these two releasing strategies have been successfully used to control *T. absoluta* and whiteflies in both, small research (500 m^2) and commercial greenhouses (1 ha), during lasts tomato cropping cycles of 2012 and 2013.

DLO results

Innovative tools for tomato russet mite control.

The inventory among stakeholders in task 7.2 showed that the tomato russet mite, *Aculops lycopersici* is increasingly appearing as a major pest species in the Mediterranean area since pesticide use is reduced. Other serious pests are greenhouse whiteflies and tobacco whiteflies. DLO selected biological control agents (BCA's) that are potentially able to control both russet mites and whiteflies. The candidate BCA's are the generalist predatory mites *Amblyseius swirskii, Amblydromalus limonicus* and *Amblyseius montdorensis.* The best performing predatory mite was *A. limonicus*, showing the highest predator densities and strongest reduction of russet mites. However, none of the predators was able to control russet mites, because all mites were hampered by the glandular trichomes (Type VI). An IPM strategy in practice could be to overdose plants infested by russet mites with frequent releases of predatory mites, whether or not released from rearing sachets. Biological control of russet mites and whiteflies with predatory mites may be enhanced by selecting cultivars with lower densities of Type VI glandular trichomes on the stems. DLO selected 3 commercially cultivars that varied in trichome densities and tested the performance of russet mites and assessed the control of russet mites with the predator *A. limonicus*. These experiments are still running. Further experiments will test methods that enhance the performance of predatory mites on the stems by adding walking structures ("predatory mites high ways").

INRA Sophia Results

Efficiency of *Ocimum basilicum* and *Dittrichia viscosa* as secondary plants for the predatory mirid bug *Macrolophus pygmaeus* used in biological control of whiteflies

In a greenhouse of South-Eastern France, the predatory mirid bug *Macrolophus pygmaeus* developed well on basil (*Ocimum basilicum*). As the insect is useful in biological pest control of whiteflies, we analyzed the potential efficiency of basil as secondary plant to enhance the presence of the predatory insect in a crop system to control whiteflies. The purpose of the present study was to identify the role of basil as secondary plant for biocontrol processes in an IPM tomato crop system. In a 6 week experiment, we analysed the presence of the predatory mirid bug *M. pygmaeus* and its prey, the pest *Trialeurodes vaporariorum* (whitefly) on tomato and basil in cages. We counted the number of individuals of *M. pygmaeus* and *T. vaporariorum* , and evaluated growth and vitality of the plants at weekly intervals. At the end of the experiment, a destructive sampling was performed to assess the number of present predators (larvae, adults) and pests (eggs, larvae, pupae, adults) under the binocular. Data-processing and analysis are ongoing. First result indicate that both predator and pest prefer tomato to basil as host plant but develop well also on basil.

Dittrichia viscosa is a highly entomophilous ruderal plant of the Mediterranean region. It is known to be efficient in enhancing the presence of predatory arthropods in crop fields which feed on crop pests, e.g. for mirid bugs of the genus *Macrolophus*. We tested *D. viscosa* as banker plant which enhances the presence of the predator *Macrolophus pygmaeus* in tomato crops inoculated with the whitefly pest *Trialeurodes vaporariorum*. In an experiment in a greenhouse in Southern France, we tested its function to act as multiplier for the beneficial arthropod. We measured the population development of *M. pygmaeus* and *T. vaporariorum* on *D. viscosa* and on the tomato crops.
The main questions we addressed were whether *D. viscosa* fulfils the functions of a banker plant, i.e. if it is able to maintain and multiply the population of the predator, and if it causes a reduction of the presence of pest insects in the system, and which mechanisms lead to this role in the tritrophic interactions. Unexpectedly, the results showed that not only the predators did not install on *D. viscosa*, but the combination of *D. viscosa* + tomato caused an explosion of the pest *T. vaporariorum*. On *D. viscosa* grown in combination with tomato, 2246 individuals of *T. vaporariorum* (adult and larvae) were identified after 8 weeks, in comparison to 241 on the treatment with only tomato plants present and 34 with only *D. viscosa*. Although *D. viscosa* is certainly efficient for other species combinations, it was not suited for the protection of tomatoes against *T. vaporariorum* in the greenhouse, and did not act as banker plant for *M. pygmaeus*.

Climate control as an efficient tool for IPM under greenhouses

Gaining knowledge's on the biophysics mechanisms underlying protected climate setting and more particularly the conditions prevailing at leaf level is crucial because air humidity considerably increases close to leaf surface and can promote the damages caused by pests (fungi and insects). It is interesting to control the distributed climate in the ecological niche sheltering the pests, diseases and BCAs threatening the crops and thus to implement more efficiently the various alternative methods (climate, physical, etc.) to the use of chemical products.
The main research activity for this task concerns the Computer Fluid Dynamics based modeling of the distributed climate in the greenhouse.
We have focused on a crucial issue concerning the novel numerical coupling of radiative and convective transfers within the CFD software, together with the coupling between crop activity and the distributed microclimate. Both improvements have more precisely

allowed (i) studying pest occurrence distribution with respect to climate patterns and (ii) modeling the microclimate in the leaf boundary layer in the ecological niche of pests. (iii) Studying the interferences between convection, climate control and use of insect screening as a substitute to pesticide use.
In addition, an ongoing study concerns the effects of different airflow rates on the behavior of the pest (*Trialeurodes vaporariorum*) in tomato crop.

Studying Thrips occurrence distribution with respect to climate patterns

Thrips *Frankliniella occidentals,* originally from western USA, is present on most of ornamental and vegetable crops and has become a major pest for the protected crops .
Alternative control methods require additional knowledge about thrips development in greenhouse conditions and particularly about the effects of micro-climate (air temperature and humidity) in the thrips habitat together with the precise knowledge of their biotical potential characteristics: longevity, fertility, development duration.
Climate and thrips distributions within a greenhouse were therefore compared and information's deduced concerning their dependences. A specific statistical model was derived to define the most favourable climate conditions for thrips adults and larvae, highlighting that diurnal temperature and humidity exert a direct effect on the distribution of thrips (*F. occidentalis*) within the greenhouse space.

Modeling microclimate in the leaf boundary layer, ecological niche of pests.

Knowing the biophysical mechanisms underlying the development of the greenhouse climate, particularly climate prevailing at the ecological niche of beneficial insects and pests, is of particular interest if one needs to implement alternative methods to chemical control.
Leaves boundary layer, the ecological niche of major biotic agents (pests and diseases), is affected by numerous factors such as the transpiration, temperature, humidity and air speed around the leaf . In addition, the uniformity and distribution of these parameters inside the greenhouse influences strongly the uniformity of the microclimate in the boundary layer and the kinetics of germination, development and sporulation of fungi and more generally all pests activities.
We have therefore combined fine measurements of microclimate and modeling by means of analytical and numerical approaches. The temperature and humidity patterns inside greenhouse were simulated using the commercial software solver Fluent which was customized for simulating the sensible and latent heat exchanges .
These results evidenced that the microclimate (air temperature and humidity) close to the lower leaf surface is quite different from the climate prevailing in the greenhouse air, particularly during daytime when crop transpiration is maximum.
The observed discontinuities between the climate in the boundary layer and in the ambient air prove that for improving the crop diseases control, the boundary layer micro climate conditions must be directly targeted rather than controlling the ambient air climate. More specifically, air humidity, the crucial parameter controlling pest activity, must not be considered in the air at the center of the greenhouse near the growing point but in the leaf boundary layer. More generally one must also deploy techniques and strategies, such as localized heating or ventilation aiming at directly controlling the

microclimate at this level.

Studying the interferences between convection, climate control and use of insect screening as a substitute to pesticide use

Modelling greenhouse climate requires taking into consideration all the exchange mechanisms involved in the greenhouse mass and energy balances.
However extensive researches in this field, few studies have considered large-scale greenhouses which constitute the vast majority of commercial greenhouses. Validations performed in greenhouses equipped with insect-proof nets over the vent openings are even rarer. Insect screening which is now a widespread practice in Mediterranean regions reduces ventilation, which in turn causes air temperature and relative humidity to rise significantly thus reducing crop activity as well as fostering the development of fungal diseases. In addition, it favours condensation seen on the plastic cover, altering the optical and thermal properties of the plastic cover.
Spurred on by these important issues, we have developed a model able to evaluate both the sensible and latent heat transfers and the resulting inside climate in a large insect-screened greenhouse taken as a multi-layer system composed of four media: i.e. soil - vegetation – inside air - cover, separated from the outside area by the plastic cover. The results were validated using air temperature and humidity measurements obtained in a large commercial greenhouse with tomato crops.
This model was then exploited to estimate the climatic consequences of the use of various insect screens, showing that both anti-Thrips and anti-Aphid nets induce sharp increases in inside air temperature and humidity. Such climate conditions are not compatible with the normal growth of tomato crops and might lead to an increase of fungal diseases. Combining fine-mesh screens providing good protection against insects and a good climatic balance would require using additional climate control techniques such as reducing incoming solar energy by means of shadow screens or white-washing the plastic cover with reflective paint. However, this would be insufficient and other air-conditioning methods are needed, such as increasing the vent opening area or maximizing crop transpiration. The developed model can be adapted to new greenhouse design and its equipment.

Studying the effects of mechanical plant perturbation on the behavior of the pest (Trialeurodes vaporariorum) in tomato crop.

This study aimed to investigate how mechanical plant perturbation influences the activities of *Trialeurodes vaporariorum* through a permanent air stream maintained at a constant velocity by means of series of fans located immediately in front of each crop row. The hypothesis to be tested is if the mechanical disturbance induced by the airflow can disrupt the insect activities and more especially the egg-laying.
The experiment was performed using a soilless tomato crops in a four compartments glass house with alternating rows with and without fan. The position of the fan at the beginning of the crop row creates an air velocity gradient within the plants that will be used to find the threshold of air speed to disturb the plant pest's development.
The data-processing and analysis phase are currently going and the results will be completed for the next deliverable.

INRA Alénya Results
Design of a set of decision rules

A IPM strategy has been designed, experimented and improved to be able to manage tomato crop in a soil-less and heated glasshouse. The objective of this experiment is to design a tomato crop management that deals with a pool of at least ten pests (e.g. *Tuta absoluta*, *Trialeurodes vaporariorum*, *Aculops lycopersici*) and diseases (e.g. *Botrytis cinerea*, *Oidium neolycopersici, Leveilula taurica*) using an association of different but connected tools in a final purpose of reducing pesticides use. In 2011, a set of decision rules has been designed and has been set up in two production crop compartments (320m² each) in the experimental station, during the whole tomato cycle (from November 2011 to July 2012). Through a socio-technical evaluation, we have been able to make an assessment of this prototype. Considering the damage and occurrence of the main pests and pathogens and the efficiency of the available tools in 2012, some rules have been changed. The new set of rules is currently tested in two crops compartments in the same conditions. So far, the main pests and diseases problems have been controlled without pesticides except for *Aculops lycopersici* in one compartment that required chemical interventions.

InVivo AgroSolutions results
***Trichogramma achaeae* against *Tuta absoluta* in tomato greenhouse**

Trichogramma achaeae is an oophagous parasitoid sold by the company Biotop (member of INVIVO group), for the control of the tomato leafminer, *Tuta absoluta*. *Trichogramma* female looks for tuta's eggs and lays her eggs inside. The development of *Trichogramma* larva inside destroyed the egg.
Great interest of *Trichogramma* is its ovicidal effect, before any occurrence of damage on the crop. Since 2011, INVIVO AgroSolutions carries out trials on strategies for Trichogramma use in tomato culture. The lessons of these three years' trials are:
- *Trichogramma* are effective on *Tuta absoluta*'s eggs. During trials, we observe a reduction in the number of mines per plant compared to the control without Trichogramma or maintaining of the number of mines to low level during the season despite strong adults pressure.
- The dose of *Trichogramma* is between 250 000 and 1 million individuals per hectare, to adjust according to male trapping and installation of mirids.
- 100 releases points per hectare
- Releases are made every week
- *Trichogramma* can be used in addition to other biological control: *Macrolophus* releases, Bt spraying
- But *Trichogramma* are very sensitive to insecticides and to some fungicides, which must be used sparingly in case of *Trichogramma*'s release

CNR results
Innovative control methods against *tuta absoluta*

The insect *Tuta absoluta* is a serious pest in tomato. The aim of WP7 task 7.3b field trials in South Italy is to develop a control strategy with biodegradable pheromone dispensers to confuse the insects (False Trail Following) resulting in control of the pest, reduced damage in tomato and lower chemical inputs. Biodegradable dispensers are developed by DLO (Dr. Willem Jan de Kogel) in WP 11.

In 2012 slow release prototype no-biodegradable polyethylene dispensers with *Tuta absoluta* pheromone have been produced in Wageningen by DLO. By using these dispensers CNR performed two false trail following experiments in two tomato greenhouses in South Italy in 2012. Together with partner DLO a field trial plan and schedule were designed. 2000 pheromone dispenser/ha, containing 10 mg of *Tuta absoluta* pheromone (2 components) each, were hung in the crop and replaced twice per season. Samples of dispensers from the field were sent regularly to DLO to estimate the release rate (this estimation was done by DLO in the frame of WP11).

The effect of the confusion of male *Tuta absoluta* by the pheromone dispensers was checked by placing pheromone traps in the treated and untreated greenhouses. The results showed that in the pheromone treated greenhouses almost no males were captured whereas in the control greenhouses high numbers were caught. This indicates that the confusion was effective and the pheromone release sufficient.

Plant damage, insect counting and combination of sustainable control techniques against *Tuta absoluta* were also performed by CNR.

In tomato greenhouse experiment 1 (May-August crop cycle) the efficacy of the false trail following techniques was compared with a biological control strategy and the farm chemical control strategy. The biological control treatment was the inoculation into the greenhouse of a new biocontrol agent, the parasitoid *Necremnus artynes*.

The results obtained were:

- false trail following technique affected males' ability to respond calling females;
- both false trail following technique and *N. artynes*, when applied as the only mean to control *T. absoluta*, were not effective in reducing leaf and fruit damage, and preventing yield losses;
- at the end of the harvest, % fruits bored by *Tuta* larvae was ~23% both in false trail and *Necremnus* greenhouses, while in farm chemical control greenhouses ~5%;
- parasitization level by *N. artynes* was always low (<20%) when the density of *Tuta* leafminer larvae was high.

Efficacy of false trail technique could improve in greenhouses wider than 300 m^2 tunnels used as replicates in experiment 1. Because greenhouses were not isolated (only bumble bee-proof screens were used that limit *Tuta* entrance partially), mated females can arrive from the outside. This risk should decrease as the treated greenhouse area increase.

In tomato greenhouse experiment 2 the false trail following techniques was combined with *Bacillus thuringiensis* and chemical control of thrips. This strategy was compared with the farm control strategy which included the same treatments with *B. thuringiensis* and chemical insecticides against thrips plus specific chemical insecticides against *T.*

absoluta. The experimental greenhouse were 1000 m^2.
The results obtained were:

- false trail following technique affected males' ability to respond calling females;
- false trail technique in combination with B.t. and chemical control of thrips is effective in reducing leaf and fruit damage, and preventing yield losses due to *T. absoluta*;
- at the end of the harvest, % fruits bored by *Tuta* larvae was ~0.4% in false trail greenhouse, while in farm chemical control greenhouse ~2.8%;
- applications of chemical insecticides were reduced in false trail greenhouse (5 treatments against 9 in the farm control);
- 1000 m^2 greenhouse could be a good minimum area for effective application of the false trail technique in combination with other IPM means.

In 2013 the experiments will be repeated on a larger scale. Prototypes of biodegradable dispensers produced by DLO in WP11 will then be tested in the field to check if their release of pheromone is comparable to the present effective dispenser. False trail following techniques will be used in combination with biological control agents against *Tuta absoluta* and other tomato pests or in combination with reduced chemical control (insecticides against pests other then Tuta). These two strategies will be compared with the farm chemical control. Finally in 2014 the best biodegradable dispenser will be tested for effectiveness in the field.