PURE

Pesticide Use-and-risk Reduction in European farming systems with Integrated Pest Management

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1. Summary

Protected tomato crops in southern countries are evolving from a purely chemical management to a **strategy based on biological control** of pests where pesticides against pests are nowadays rarely used. This process began with the use of bumblebees for pollination, which conditioned farmers to choose those pesticides selective with these pollinators. Consequently, the use of broad-spectrum pesticides was significantly decreasing as the use of bumblebees was being adopted in most tomato crops in these areas. The use of **selective pesticides** opened the door to the first releases of natural enemies in this crop, although chemical control still was the main control measure to fight against pests. At this period, greenhouse crops have been confronted with new invasive pests and diseases coming from countries with warmer climates (*e.g.* Tomato yellow leaf curl virus). Work to implement IPM based on biological control of pests has been destroyed by these new entries that induced a come back to broad spectrum pesticides to control vectors. The appearance of an invasive pest *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in 2006 in Europe, prompted to develop strategies to control this threatening tomato pest based on the use of mirid predators. The use of predatory mirid bugs resulted in very effective control of tomato key pests: the whiteflies *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), and *T. absoluta.* Due to the high polyphagy of mirid predators, most tomato pests are under control, with the exception of *Aculops lycopersici* (Masse) (Acari: Eriophydae) and in some context, thrips (*Frankliniella occidentalis*) which escape to the control of this predator. The success of the use of mirids **has minimized the use of pesticides in tomato** and the specific treatments conducted are currently mainly targeted to control *A. lycopersici* with selective acaricides or thrips. Complementary work has been performed on secondary plants intentionally added to the crop system with the aim to of enhancing crop biocontrol services and, by the way, increasing the robustness of tomato agro-ecosystem. Although it is still possible to improve the IPM in tomato especially on diseases, the current IPM strategies tested within PURE project undoubtedly increase **tomato crop resilience**, and may make this crop **more robust against invasive pests** with regard to global climate change.

2. IPM in protected tomato crops increases crop resilience

IPM based on mirids

The cornerstone of the IPM strategies currently in use and tested within the context of PURE project, is **biological control** with either biological or selective insecticides. To this end, and depending on the type of tomato crop and the area of cultivation, two polyphagous predator species *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) are released and/or preserved. These two predators are currently key factors for the success of IPM in protected tomato crops.

M. pygmaeus and *N. tenuis* are endemic natural enemies that commonly appear in tomato crops in Europe (*M. pygmaeus* in the north and *N. tenuis* in the south). Both predators are mass-reared and released by biocontrol commercial companies. Both possess a high degree of **polyphagy** and due to this characteristic are able to contribute to the control of thrips, leafminers, aphids, spidermites, and Lepidoptera species. Phytophagy has multiple benefits, including facilitating the establishment of these predators in the crop and preserving them when prey is scarce. Two strategies based on the use of these two mirids are nowadays employed which therefore affect the subsequent tomato crop pest management:

1. **Inoculative releases.** Inoculative releases of *N.tenuis* or *M. pygmaeus* (1-2 individuals/m^2) are usually conducted several weeks after transplanting. This strategy has been successfully used to control pest populations once a certain number of mirids are present in the crop. Because mirids must be established in the crop before pest arrival, this strategy is employed on northern areas where pest pressure is not too high or in the south in those crops planted at the end of winter where pest pressure is still low. The releases of mirids must be obviously integrated with use of selective pesticides which do not interfere with the establishment of the mirids.
2. **Predator in first**. To shorten the establishment period and improve the distribution of *N. tenuis* in the crop, especially when weather conditions are less favorable to them, releases of predators can be made in the seedling nurseries (predator in first). This strategy entails transplanting tomato plants on which *N. tenuis* individuals have already laid eggs in the nursery. 0.5-1 mirids per plant are released in the nursery with *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs as an alternative prey.

Although both strategies with both predators are effective to manage tomato pests, the case of the zoophytophagous predator *N. tenuis* released in the nursery has been extremely effective in controlling tomato key pests as the tobacco whitefly *B. tabaci* and the invasive *T. absoluta*.

Due to the high degree of polyphagy of both predators, most tomato pests are under control, with the exception of *Aculops lycopersici* (Masse) (Acari: Eriophydae) which escapes to the control of these predators. The success of the use of mirids has minimized the use of pesticides in tomato and the specific treatments conducted are currently mainly targeted to control *A. lycopersici* with selective acaricides. The incidence of the tomato yellow leafcurl viruses (TYLCV and TYLCSV), which were the limiting factors to cultivate tomatoes in the past, has also decreased significantly since the use of this predator against *B. tabaci* has become widespread and probably also thanks to the use of tolerant tomato cultivars.

In areas where thrips are key pests for tomato (e.g. in South Italy), *N. tenuis*, although effective in controlling *T. absoluta* and whiteflies, could not be enough to control thrips population below the level determining yield losses due to direct fruit damage. In this situation mirids should be combined with insecticide treatments to prevent yield losses. Active ingredients (e.g. spinosad) effective to control thrips can impact negatively mirid establishment and biocontrol efficacy if frequently sprayed, with consequent increased attack of *T. absoluta* and whiteflies, eventually leading to abandonment of IPM and exclusive use of insecticides. On-farm trials carried out in WP7 suggest a IPM strategy where prevention of negative feedback of eventual chemical insecticides against thrips can be achieved by using *N. tenuis* in combination with pheromone-based control (mating disruption applied since the start of the crop cycle) and microbiological insecticides (e.g. *Bacillus thuringiensis*) against *T. absoluta*. With this strategy, if thrips infestation should be high, the application of only few insecticide treatments may result in good thrips control and negligible impact on *N. tenuis*, whose temporary reduction of efficacy against *T. absoluta* is compensated by the application of mating disruption and *B. thuringiesis*. Overall, this IPM strategy may be as effective as standard insecticide-based control strategies in preventing fruit damage by *T. absoluta*, noctuids, thrips and whiteflies in greenhouse tomato crops.

Added benefits of using mirids

Mirids are zoophytophagous predators, which are defined as predators feeding on prey and plants during the same developmental stage. By feeding on plants, mirid predators can activate the same **defense mechanisms** as strict herbivores. As example, the feeding activity of *N. tenuis* activates abscisic acid (ABA) and jasmonate acid (JA) pathways in tomato plants, which makes them less attractive to *B. tabaci* and more attractive to the whitefly parasitoid *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae), respectively. In addition, herbivore induced plant volatiles (HIPVs) from *N. tenuis*-damaged plants can induce plant defenses in neighboring, undamaged plants via JA, resulting in attraction of parasitoids. These results might be one reasonable explanation for the great success achieved by mirids as a key biocontrol agent in tomato cultivations. Indeed, these results are a clear evidence that zoophytophagous mirid predators can activate plant responses and these responses can be an added benefit to their effectiveness as direct predators, making tomato crops more robust against possible accidental introductions of exotic pests.

Biocontrol plants added in the cropping system to maintain stable populations of mirids.

The litterature is quite rich on the secondary plants used in crops or surrounding habitats and even on the most important functional characteristics that enhance pest management (Parolin et al. (2012 a, b). The addition of such plants may act as efficient biocontrol tools we proposed the introduction of a new term to deal with those secondary plants that are specifically suited to enhancing biological control in Integrated Pest Management that we named "biocontrol plants", for simplification and categorization (Parolin, 2013). Within this project, the objective was to maintain stable populations of mirids in the crop system in order to better anticipate new pest entries in the greenhouse and to avoid damage due to phytophagous activity of mirids when prey level was too low.

1 Basil as a banker plant

Within PURE programme, we tested basil as banker plant in IPM tomato crops. As an aromatic herb, basil (*Ocimum basilicum L., Lamiaceae*) has repellent effects. Several studies state its qualities as repellent or companion plant to decrease aphid, thrips and hornworm attacks when intercropped in fields, particularly of tomato crops. We analyzed basil as banker plant in a greenhouse IPM tomato crop system testing different plant combinations in monocultures vs. dicultures.

The main question was whether basil is a suitable biocontrol plant enhancing crop productivity, and more specifically if it acts as banker plant for predatory insects in a tomato crop greenhouse system in Mediterranean climate. The hypothesis is that basil directly favors the presence of a stable reproducing population of predators, indirectly contributes to a significant reduction of whitefly pests in the tomato crop greenhouse system and enhances crop fruit production and plant health. If basil is an efficient biocontrol / banker plant in this species combination which is commonly used in IPM in the Mediterranean region, we hypothesize that the presence of basil plants in the greenhouse crop system has 1) direct positive effects on the population of predators which increases due to favourable conditions provided by basil plants and causes a higher presence of predators when basil is present; 2) indirect negative effects on the population of pests which decreases, via the increase of the population of predators on basil, causing a) lower pest numbers when basil is present, which is b) due to higher predator presence;

Our experiment showed that basil has an effect on the control of whitefly pests in tomato crops employing the predator *M. pygmaeus*. However, we could not show that the predators produced more stable populations when basil was present. Furthermore, the presence of basil did not result in increased plant health and crop yield. Therefore, despite the negative development of pest populations, with the present experiment we cannot define basil as an efficient banker plant.

In our experiment we found that highly significantly fewer pests were found in the diculture tomato + basil or when basil alone was present, but we cannot state the reasons why pest numbers were reduced. The low number of predators on basil may still have been sufficient to control the pests. This needs to be tested in more detail. Crop yield and leaf health were not statistically different in the treatments of differing plant combinations, so that the ultimate goal of a biocontrol plant was not achieved (Parolin *et al*, 2015, *Accepted in IJPM*).

2 Tobacco as a banker plant

Within this programme, we tested also the efficiency of Tobacco as a banker plant for *Marcrolophus pygameus* to control *Trialeurodes vaporariorum* in tomato crops.

Different combinations were used: crop plant and pest were always present, banker plant (abbreviated as BP hereafter) and predators were present or absent. Numbers of individuals of *M. pygmaeus*, *T. vaporariorum*, and plant health were assessed. *M. pygmaeus* reproduced efficiently on tobacco, with the highest reproduction when only BP was present. The number of pests was significantly reduced on the plants where predators had the highest densities. However, without the presence of predators, tobacco acted as an attractive plant for *T. vaporariorum*. Plant growth in terms of height and leaf number was not significantly different between the treatments with different species combinations. Leaf damage was higher when the BP was in a cage with tomato plants Tobacco acted as incubator for the pests when it was in a cage with tomatoes without predators present. This points to a complementarity of these two plant species to provide good reproductive conditions for the pest *T. vaporariorum*, an undesired synergy of plants to increase the presence of pests. Therefore, tobacco was an efficient banker plant to support the population of the predatory mirid bug *M. pygmaeus*, but under absence of predators it enhanced the proliferation of the pests. Its employment as BP in this combination of species is only efficient as long as predators are present *(*Parolin et al, 2014*, UJAR).*

IPM based on use of microorganisms and their products

Although IPM of pests in tomato cultivations is a reality, the same has not been achieved for the control of tomato pathogens. In the frame of PURE WP7, by on station and on farm trials, we demonstrated that the use of selected microorganisms and their metabolite is a strategy successfully applicable at the list for the control of soil-borne pathogens (*Fusarium oxysporum, Pythium ultimum, Sclerotinia sclerotiorum, Rhizoctonia solani*). Our approach 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, biofertilisers, growth enhancers and stimulants of natural resistance. The efficacy of this fungus can be attributed to their ability to protect plants, enhance vegetative growth and contain pathogen populations under numerous agricultural conditions, as well as to act as soil amendments/inoculants for improvement of nutrient ability, decomposition and biodegradation. The living fungal spores (active substance) are incorporated in various formulations, both traditional and innovative, for applications as foliar sprays, pre-planting applications to seed or propagation material, post-pruning treatments, incorporation in the soil during seeding or transplant, watering by irrigation or applied as a root drench or dip. *Trichoderma*-based preparations are marketed worldwide and used for crop protection of various plant pathogens or increase the plant growth and productivity in diverse cultivated environments such as fields, greenhouses, nurseries; in the production of a variety of horticultural, fruits, trees and ornamental crops.

The use of such biocontrol agents and of mycorrhizae is also desirable because of in tomato plants significantly they can increase resistance toward insects (aphids), activating both direct and indirect defense mechanisms. Indeed, we also demonstrate (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 VOC, and although the aphid population growth indices are slightly higher, the attractiveness toward aphid parasitoids and predators and the development rate of an aphid predator greatly increase. These findings support the development of novel IPM strategies based on multitrophic interactions effective in defending plants from pests and pathogens at the same time.

3. Conclusion

In the frame of WP7 a lot of work has been done in order to develop a global IPM strategy of pests and pathogens of tomato greenhouse cultivation, obtaining results that clearly demonstrate:

- The current IPM strategies based on the use of the polyphagous predators, *M. pygmaeus* and *N. tenuis* minimize the use of pesticides and result in an increase of the tomato crop resilience.
- Use of microorganisms and their metabolites is an added value for IPM of tomato cultivations.
- The use of such biocontrol methods (mirids and beneficial microbial agents) is also desirable because of the indirect effects on the plant fitness, in terms of induction of systemic resistance, plant growth promotion that can make tomato crop more robust against new invasive pests and pathogens with regard to global climate change.
- The quantification of the pest control potentially provided by biocontrol plants has to be investigated deeply for several reasons. The abundance of auxiliary arthropods and pests is not a suitable indicator in the majority of cases. In the least case, an accurate assessment of the dynamics of pathogens and auxiliary arthropods is needed, as well as the species complexes and/or intraspecific differentiation processes requiring rigorous approaches.
- Even if the greenhouse agrosystem is considered as a simple production ecosystem from an ecological and physical point of view, complex interactions can occur between the different components, which are sufficiently important to deeply impact system functioning and, in the end, crop yields.
- To conclude, the design of robust solutions to manage sustainable greenhouse production systems requires not only interdisciplinary investigations mixing technological and ecological engineering but also basic research on complex systems.
- However, this advanced IPM strategy that is looking for long-term establishment of generalist beneficials, allows the agro-ecosystem to respond quickly to disturbances induced by a large panel of pests and diseases. This includes potentially emerging or re-emerging pest invasions as well as periodic massive entries or endemic explosions of many major pests present within European regions. More generally, we assume that this strategy constitutes a very promising approach with the goal to reduce the magnitude and duration of further biological disturbances caused by climate change.