UNIVERSITÀ DEGLI STUDI DI PALERMO

Department of Agricultural and Forest Sciences

Eco-compatibility Phytosanitary Management in Agroforest and Urban Environments

Tomato leaf miner *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae)

The old and new challenge

Scientific Area Code - AGR/11

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DOTTORATO

"Leave The World Better Than You Found It, Take No More Than You Need, Try Not To Harm Life Or The Environment, Make Amends If You Do"

Paul Hawken

"Our Nature Is Organic … Investing Its' Assets In A Properly Way Is … The Only Way To Achieve A Real Life Sustainability"

Sadek Abbas

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SUMMARY

The term Phytosanitary exists within many of the modern and old scientific terms such as: organic farming, plant protection, integrated pest management, national and international movement of plant germplasm, biosecurity, good agricultural practices and others. There are many aspects within those related terms. All those terms have common interests of which the pests' risk be avoided and the environmental health is achieved. Consequently preventive measures, precaution measures and effective testing procedures are required.

The study problem is the pest *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), the Tomato leaf miner, this pest is one of the most devastating pests affecting Tomato crops worldwide. Management of *T. absoluta* was assessed in open-field tests (2011 and 2012) using three biorational insecticides, Azadirachtin, *Bacillus thuringiensis* var. kurstaki, and *Beauveria bassiana*, and a combination of three synthetic insecticides, Emamectin, Indoxacarb and Metaflumizone, as a control treatment.

Important fact have been proved by this study that only the combination of Azadirachtin – *B. thuringiensis* was able to reduce the impact of Tomato leaf miner regarding to the fruit's marketable production (as a bioassay criteria), similarly to the control treatment (no significant differences). This finding suggests that biorational insecticides are a good alternative than synthetic ones. Possible use of biorational insecticides in the management of Tomato leaf miner in organic farming system is being discussed.

What had been obtained in this study (Chapter 2) demonstrated that treatment based on *Azadirachtin* and *B. thuringiensis* combination can effectively reduce *T. absoluta* damage on Tomato open field cultivation. However, the side effects of that combination of *Azadirachtin* and *B. thuringiensis* on Tomato leaf miner natural enemies should not be ignored.

Therefore, this study has included experiments (Chapter 3,4 and 5) related to the new integrated/biological control agent predator *Dicyphus maroccanus* (Wagner, 1951) (Hemiptera: Miridae). Those experiments have been carried out in IVIA/ Valencia –Spain 2013.

Those experiments included *D. maroccanus* lifecycle parameters (Chapter 3), its host and prey preferences (Chapter 4), and yet its potentials on Tomato leaf miner control *T. absoluta* with and without the implementation of the biorational insecticide combination of *Azadirachtin* and *B. thuringiensis* under semifield (Chapter 5).

In 2008, the omnivorous predator *D. maroccanus* was firstly detected inhabiting Tomato crops in Valencia region (East Coast of Spain). Since then, *D. maroccanus* has been commonly found preying on eggs of *T. absoluta* in this area.

In this study *D. maroccanus* was successfully developed by preying on eggs of *E. kuehniella*; however, none of *D. maroccanus* larvae tested was able to complete development on the plant without supplemental food. Immature development time was approximately 19.5 days for both males and females, being their immature

survival of 85 %. *D. maroccanus* larvae consumed 267 and 312 eggs of *E. kuehniella*, to reach male and female adulthood, respectively. Females of *D. maroccanus* produced 51 larvae per female throughout their 16 days of lifecycle.

The progeny sex ratio was 75.6 females/total offspring. Net reproductive rate (R0) was estimated at 34.52 ± SE 0.52 female eggs/ female, generation time (T) was 28.28 ± SE 0.13 day and the estimate of intrinsic rate of increase (rm) was 0.1254 ± SE 0.0001 females/female/day. Some of these values are pretty higher than those reported for other Mirid predators on Tomato crops like *Nesidiocoris tenuis* and *Macrolophus pygmaeus*, indicating the potential as biocontrol agent of *D. maroccanus* on Tomato crops in the Mediterranean Basin.

Life history variables varied of *D. maroccanus* positively comparing with the two predator species *N. tenuis* and *M. pygmaeus*, when they prey on *E. kuehniella* eggs. Moreover and according to the preference's bioassays that has been conducting in IVIA showed that; *D. maroccanus* has high preference to healthy Tomatoes, to Tomatoes infested with *T. absoluta* eggs and larvae.

Most of *D. maroccanus* lifecycle parameters are showing the possibilities to invest that predator by adopting and then by adapting it within the IPM promising strategies for time to come. Moreover the field observations showed that there were no side effects or harm features for that predator on the Tomato crop (Mollá et al. 2009).

The final chapter (Chapter 5) of this study included the *D. maroccanus* IPM potentials assessments to control *T. absoluta* under semifield condition. This experiment has included *D. maroccanus* (alone) in different releasing rate (high and low) and that predator establishing in high releasing rate along with the biorational insecticides combination of Azadiractine plus *B. thuringiensis,* other treatment included only the biorational insecticide combination of Azadiractine plus *B. thuringiensis* and finally the control treatment.

The results approved the positive effects of: a) the treatments included *D. maroccanus* in *T. absoluta* management, b) the treatments included *D. maroccanus* along with the biorational insecticides combination of Azadiractine plus *B. thuringiensis,* c) the treatments included *D. maroccanus* in different releasing rates (high and low) d) the treatment t included *D. maroccanus* high releasing rate along with the biorational insecticides combination of Azadiractine plus *B. thuringiensis* and that was the best treatment among the other treatments, since this treatment had the lesser number of *T. absoluta* Tomato infested leaflets, and had the highest level of the efficacy. It is clearly appeared that o there will be good and promising opportunities for recruiting that predator within IPM strategies for time to come.

CHAPTER1

Management of *Tuta absoluta* (Myrick, 19170) (Lepidoptera: Gelachiidae) populations in organic farming

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1. INTRODUCTION

The term Phytosanitary exists within many of the modern and old scientific terms such as: organic farming, plant protection, integrated pest management, national and international movement of plant germplasm, biosecurity, good agricultural practices and others.

It clearly appears that there are many aspects within those related terms. All those terms are referred to different global trends and approaches. These trends and approaches have common interests of which the pests' risk be avoided and the environmental health is achieved. And for that reason: preventive measures, precaution measures and effective testing procedures are required and that what is called *"the phytosanitary managements"*.

The term Phytosanitary management includes set of measures those should necessarily implement and yet to be used to achieve the main goal of that management. With regard that the term pest means any species, strains or biotypes of plant, animal or pathogenic agent injurious to plants or plant products (FAO 1990; revised FAO 1995; IPPC 1997)

The main global organizations and agencies that work nationally and internationally on/and within the sanitary and phytosanitary measures/standards are:

Food and agriculture organization (FAO), international plant protection convention (IPPC), international standards of phytosanitary measures (ISPM), international committee for phytosanitary measures (ICPM), European and Mediterranean plant protection organization (EMPPO), National plant protection organization (NPPO), world trade organization (WTO), and others.

By those organizations and agencies the international phytosanitary standards had built up since the recent decades. Moreover, their agreements on application of sanitary and phytosanitary measures are established.

As it mentioned above, the phytosanitary managements represent set of measures those include international phytosanitary standards. The International phytosanitary standards provide specific guidance on;

1- The common key terms' definitions such as: area, commodity, consignment, country of origin (of the consignment of the plants or theirs by products), pest risk analysis (PRA), endangered area, entry of a pest, establishment, introduction, pathway, pest, pest categorization, pest free area, pest free production site, prohibition, regional plant protection organization, quarantine pest and others. And within each single term there are a certain related terms of which the key terms definitions are relatively be readable and yet to be understandable,
2- The international sources of information may include environmental impact assessments and they should be recognized that such assessments usually do not have the same purpose as pest risk analysis and cannot substitute for PRA,
3- Key details about: pest categorization, assessment of the probability of the pest introduction and spread, assessment of potential economic consequences, and degree of uncertainty,
4- Pest risk management, phytosanitary certificates and other compliance measures, building up the final conclusion by which the specific Phytosanitary measure will use,
5- The details for conducting pest risk analysis to determine if pests are quarantine pests. The international phytosanitary standards describe the integrated processes to be used for risk assessment as well as the selection of risk management options. Moreover the main stages of PRA, those include: initiating the process, risk assessment and risk management,
6- The details regarding the analysis of risks of plant pests to the environment and biological diversity, including those risks affecting uncultivated/unmanaged plants, wild flora, habitats and ecosystems contained in the PRA area,
7- The details those related to living modified organisms (LMOs). Finally, the guidance on evaluating potential phytosanitary risks to plants and plant by products posed by living modified organisms (LMOs),

8- The documentation of pest risk analysis (documentation requirements),

9- The structure and content of diagnostic Protocols. Guidance on how these protocols will be initiated reviewed and published. These protocols describe Procedures and Methods for the detection and identification of pests that are regulated by contracting parties and relevant for international trade. They are addressed to diagnosticians/diagnostic laboratories performing official tests as part of phytosanitary measures. They provide at least the minimum (acceptable) requirements for reliable diagnosis of the relevant pests. (FAO, 1990; revised FAO, 1995; IPPC, 1997).

The main principals of the specific guidance items are: how, when and by what to deal with different types of pests, within different areas and different circumstances. In certain circumstances the target plant itself may be actually or potentially a pest thus restricting its movement across borders in a form that may possess some risks.

Moreover, the specific guidance items declared that there are some of the factors those which negatively effect on the plant health such as the pollution, misuse of the agrochemicals (pesticides, sanitation chemical-based substances, fertilizers and soil conditioners), misuse of the genetic propagation techniques, genetic modified organisms (GMOs) and the using of unknown origin propagation different materials.

Nevertheless, the specific guidance items has also declared the factors those related to the general policies and strategies of different states of which deteriorated and fragile factors being implemented and the whole ecosystem being in a real danger (World Health Organization 2005). Finally, the effects of the global environmental crises such as: the global warming and co_2 emission, flooding and dusty storms those being unwisely made by human.

As a result, there were many of the human inventions, interventions and innovations to ensure the acceptable level of Eco-compatibility Phytosanitary Managements through decades ago. Nevertheless, many of the world bilateral and multilateral different protocols flow in the same stream had been conducted.

Our world have different Geographical and Topographical Characteristics (the flora and fauna are included) from area to area, and from country to country within the same area, and from region to region within the same country. In addition, the different characteristics those related to the human nature management for soil, water resources, animal husbandry and the Ecosystem types around and finally the implemented agricultural system/systems being used.

Consequently many countries have their own sanitary and phytosanitary laws (standards, legislations and regulations) those regulate the exchange of plants and theirs materials through their territories. Some of those laws control even internal movement of those plants and plant based materials. Yet those laws are playing great deal roles of which efforts of mitigation against spread of plant diseases and pests are being achieved.

Therefore, phytosanitary laws intending to move plant germplasm both nationally and internationally need to understand and adhere to the prevailing legal requirements (FAO 2010). In cases of a plant material crossing International borders, it is a requirement that it is accompanied with a phytosanitary certificate declaring that the conditions outlined by the importing country have been met.

Most of the sanitary and phytosanitary standards, legislations and regulations based on such common procedures those called *International Standards for Phytosanitary Measures*. Unfortunately, the main goal of those implementing standards is likely to focus on the global trade or commercial dynamic interests (WTO 2011), it is unlikely to be focus on the Environment, Ecosystem and Agro-ecosystem health such as: using different races of Agrochemical substances as sanitation, pesticides, water purifications, and finally and for instance the use of MB (Methyl Bromide) in the treatments of imported wood consignments' fumigation.

Moreover MB using in the green houses (soil treatments) and in the date palm stores in some countries. By this conclusion, the unique question that green scientists are looking forward to achieve it, is: what are the most *"Eco-compatibility of the phytosanitary managements in agro forest and urban environments*" should be?!
Since *Organic Farming* main bases and principles are *"health, environment, fairness and care"* (according to *IFOAM*, international federation for organic agriculture movements). The typical proposed option to provide such an answer for that arisen

question mentioned above is more or less the combination between the *International Standards* for Phytosanitary Measures and the *Organic Agriculture Standards* for Phytosanitary Measures.

That means: the future concerns should flow toward; shortening the gap between those standards and to create the Organic International Standards for Phytosanitary Measures (hybrid unit of standards). By make general comparative study that should start with establishing such a table of which different patterns of phytosanitary measures are included, the first pattern is representing International current phytosanitary measures and the second is representing International Organic phytosanitary standards.

The second step should starting to analyse those both measures and to explore the common concepts and to matching between those different measures. What will be complies with the International Organic phytosanitary standards that will be as a valid part of the new hybrid unit of standards, what will be incompliant with the International Organic phytosanitary standards that will be invalid part of the new hybrid. For the invalid concepts that will be as inputs for future following studies to figure out what will be the possible alternative/ alternatives.

The pest *Tuta absoluta* (Meyrick 1917) (Lepidoptera: Gelechiidae) represents one of the Tomato main pests since it had been explored (Desneux et al. 2010). Its spreading was so fast and its destructive was so harsh, for that reason and others many of the phytosanitary measurements had been taken in different areas all over the world (Desneux et al. 2011).

Unfortunately, most of those measurements were driven under the conventional management's canopy, other measurements those supposed to be oriented toward the organic farming phytosanitary standards were in limit and narrow trends in particular in the academic studies and studies.

This study it comes to be as a one of those trends and efforts by introducing and yet to promote: using such strategy that included the establishing of one of the promising and new zoophytophagous generalist predator *Dicyphus maroccanus* (Hemiptera;

Miridae), along with the use of the biorational insecticides combination of Azadirachtin plus *Bacillus thuringiensis* (Biorational Insecticides)* in controlling and managing *T. absoluta* on Tomato crops.

With regard that strategy is strongly authorized by the organic farming phytosanitary standards, and yet this study comes to be flow in those efforts main stream, those move toward clean and healthy environment. Furthermore, this study had investigated the lifecycle of *D. maroccanus* Wagner (Miridae) and its host and prey preferences and finally its IPM potentials role in controlling and managing *T. absoluta* in semifield trials.

***(Biorational Insecticides)**

The U.S. EPA (Environment Protection Agency) identifies biorational pesticides as inherently different from conventional pesticides, having fundamentally different modes of action, and consequently, lower risks of adverse effects from their use. Biorational has come to mean any substance of natural origin (or man-made substances resembling those of natural origin), that has a detrimental or lethal effect on specific target pest(s), e.g., insects, weeds, plant diseases (including nematodes), and vertebrate pests, possess a unique mode of action, are non-toxic to man and his domestic plants and animals, and have little or no adverse effects on wildlife and the environment.

http://ipmworld.umn.edu/chapters/ware.htm

The current study have applied group of biorational insecticides and for the control trials have applied group of synthesis insecticides as will detailed in the following;

➢ ***Pesticides in a brief***

- *Affirm (Emamectin benzoate)*

Emamectin benzoate (Affirm) is a novel insecticide with potent efficacy against many species of *Lepidoptera* which are damaging fruits and leaves of agricultural crops. The active ingredient belongs to the naturally derived chemical group of avermectine, causing paralysis of Lepidoptera larvae due to the activation of chloride channel at nerves level.

Affirm is acting mainly through ingestion, due to its mode of action and fast activity, it is effective at very low rates and on all instars stages.

- *STEWARD (pa Indoxacarb)*

Indoxacarb is an oxadiazine pesticide developed by DuPont that acts against lepidopteran larvae. It is marketed under the names Indoxacarb Technical Insecticide, Steward Insecticide and A vaunt Insecticide. Indoxacarb is the active ingredient in a number of household insecticides, including cockroach baits, and can remain active after digestion. Its main mode of action is via blocking of nerve sodium channels. This pesticide had been used in this study was at 125 g/100L/ha.

- *ALVERDE (pa Metaflumizone)*

Metaflumizone is a semicarbazone insecticide indicated for the veterinary treatment of fleas and ticks, marketed under the brand name ProMeris. Metaflumizone formulation is waterproof and typically remains effective for 30–45 days in a cutaneous application at the base of the neck. Metaflumizone works by blocking sodium channels in target insects, resulting in paralysation associated with blocking nerve activity. This pesticide had been used in this study was at 100 g/100L/ha.

- *Azadirachtin*

Azadirachtin, a chemical compound belonging to the limuloid group, is a secondary metabolite present in Neem seeds. Azadirachtin has a complex molecular structure, It was initially found to be active as a feeding inhibitor towards the desert locust (*Schistocerca gregaria*), it is now known to affect over 200 species of insect, by acting mainly as an antifeedant and growth disruptor, and as such it possesses considerable toxicity toward insects (LD50 in *Spodoptera littoralis*): 15 μg/g). It fulfils many of the criteria needed for a natural insecticide if it is to replace synthetic compounds.

Azadirachtin is biodegradable (it degrades within 100 hours when exposed to light and water) and shows very low toxicity to mammals (the LD50 in rats is > 3,540 mg/kg making it practically non-toxic).This compound is found in the seeds (0.2 to 0.8 percent by weight) of the Neem tree, *Azadirachta indica* (hence the prefix aza does not imply an “aza” compound, but refers to the scientific species name).

Effects of Azadirachtin different preparations on beneficial arthropods are generally considered to be minimal. Some laboratory and field studies have found Neem extracts to be compatible with biological control. Because pure Neem oil contains other insecticidal and fungicidal compounds in additional to Azadirachtin, it is generally mixed at a rate of (7.8 ml/l) of water when used as a pesticide. This pesticide had been used in this study was at 300 cc /100L/ ha.

- *Bacillus thuringiensis* (Berliner 1915)

Bacillus thuringiensis is a Gram-positive, soil-dwelling bacterium, commonly used as a biological pesticide; alternatively, the Cry toxin may be extracted and used as a pesticide. *B. thuringiensis* also occurs naturally in the gut of caterpillars of various types of moths and butterflies, as well as on the dark surface of plants.

During sporulation, many *B. thuringiensis* strains produce crystal proteins (proteinaceous inclusions), called δ-endotoxins, that have insecticidal action. This has led to their use as insecticides, and more recently to genetically modified crops using *B. thuringiensis* genes. There are, however, many crystal-producing *B. thuringiensis* strains that do not have insecticidal properties.

B. thuringiensis toxins are considered to be environmentally friendly by many farmers and may be a potential alternative to broad-spectrum insecticides. The toxicity of each *B. thuringiensis* type is limited to one or two insect orders; it is nontoxic to vertebrates and many beneficial arthropods, because Bt works by binding to the appropriate receptor on the surface of mid-gut epithelial cells. Any organism that lacks the appropriate receptors in its gut cannot be affected by *B. thuringiensis*. This pesticide had been used in this study was at 150cc/100L/ha.

- *Beauveria bassiana*

Beauveria bassiana is a fungus that grows naturally in soils throughout the world and acts as a parasite on various arthropod species, causing white muscardine disease; it thus belongs to the entomo-pathogenic fungi. It is being used as a biological insecticide to control a number of pests such as termites, thrips, whiteflies, aphids and different beetles. Its use in the control of malaria-transmitting mosquitoes is under investigation. This pesticide had been used in this study was at 150cc/100L/ha.

1.1 The Pest Infection Triangle

As a common knowledge there is a disease triangle, this study was establishing and adopting *the pest infection triangle* (Figure 1).

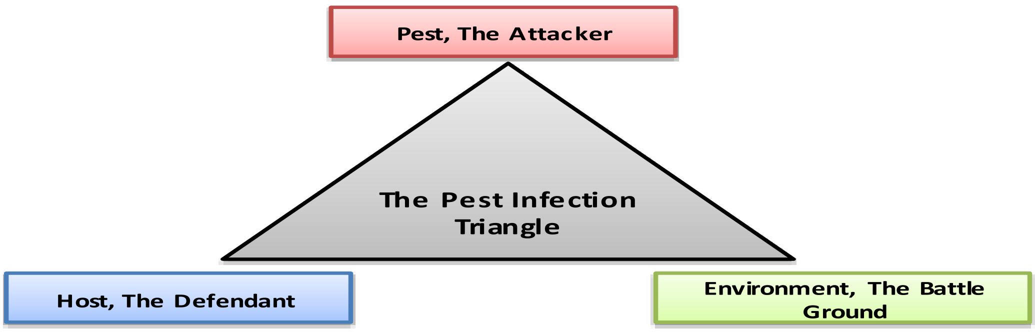

Figure 1, the Pest Infection Triangle

1.2 The pest, the attacker, *Tuta absoluta* (Meyrick, 1917) analysis

Figure 2, *Tuta absoluta* adults in matting behavior

- ***Tuta absoluta* full classification is:**

Kingdom: Animal

Phylum: Arthropod

Class: Insects

Order: Lepidoptera

Family: Gelechiidae

Genus: *Tuta*

Species: *absoluta*

- ***Tuta absoluta* Synonyms are:**

Phthorimaea absoluta (Meyrick, 1917)

Gnorimoschema absoluta (Clarke, 1962)

Scrobipalpula absoluta (Povolny, 1964; Becker, 1984)

Scrobipalpuloides absoluta (Povolny, 1987)

Tuta absoluta represents an old and new challenge of which most of South America, Europe, Middle East and some of Asia countries had been stormed gradually with this pest (Desnaux et al. 2011). The ecological point of view suggests that *T. absoluta* was within the environmental equilibrium limits.

In 1917, *T. absoluta* (was originally described by Meyrick as *Phthorimaea absoluta*, based on individuals collected from Peru, Clarke 1962 declared that *T. absoluta* was reported as *Gnorimoschema absoluta*, *Scrobipalpula absoluta* (Povolny), or *Scrobipalpuloides absoluta* (Povolny), but was finally described under the genus *Tuta* as *T. absoluta* by Povolny in 1994 (Barrientos et al. 1998).

Tuta absoluta is one of the most devastating pests of Tomato in South America (Barrientos et al. 1998; Miranda et al. 1998). This pest was initially reported in eastern Spain in late 2006 (Urbaneja et al. 2007), and has subsequently spread throughout the Mediterranean Basin and Europe (Potting 2009).

T. absoluta is considered a typical invasive species because of its capacity to develop very quickly on tomato cultivations and to spread rapidly in new areas causing economically relevant damage (Desneux et al. 2010; Caparros Megido et al. 2012). Since the time of its initial detection, the pest has caused serious damages to tomato in invaded areas (Germain et al. 2009), and it is currently considered a key agricultural threat to European, Middle East, North African Tomato production and part of Asia.

Tuta absoluta has been a long-time pest of open field and greenhouse Tomato in South America (Vargas 1970; Fernandez and Montagne 1990; Colomo et al. 2002). This moth is a Neotropical species and is considered a key pest of the tomato in South America (Guedes and Picanco, 2012; Luna et al. 2012), where it remained confined until its first record in Western Palaearctic, in Spain in 2006 (Desneux et al. 2010; Tropea Garzia et al. 2012). Afterwards, it rapidly spread throughout the Mediterranean Basin, in Europe, North Africa and the Middle East (Desneux et al. 2011).

Tuta absoluta is considered a typical invasive species because of its capacity to develop very quickly on tomato cultivations and to spread rapidly in new areas causing economically relevant damage (Desneux et al. 2010; Caparros Megido et al. 2012).

Yet this key pest started moving and invading from country to country within one region and from region to region within the same areas and freely invasive species represent a major threat to both natural and agronomic ecosystems (Desneux et al.2010).

Figure 3, *T. absoluta* caused 80-100% crop losses in tomato greenhouse

As a common knowledge, agricultural pests can reduce yield, increase costs (related to their management), and lead to the use of pesticides which ultimately lead to the disruption of existing Integrated Pest Management (IPM) systems (Thomas 1999).

Figure 4, *T. absoluta* caused 80-100% crop losses in tomato open fields

Consequently, *T. absoluta* existence and rapid spreading have created new trends and approaches of which biological and ecological studies and studies being undertaken.

- *Tuta absoluta* biology

T. absoluta lifecycle has four developmental stages: egg, larva, pupa and adult. Females are usually depositing eggs on the underneath of Tomato leaves or stems, and on immature fruits. After hatching, young larvae penetrate leaves, fruits or stems, on which they feed and develop. *T. absoluta* eggs are small cylindrical, creamy white to yellow 0.35 mm long. Egg hatching takes place 4-6 days after egg lying.

Figure 5, *T. absoluta* eggs

T. absoluta has four larval. Fourth instar (Fully-fed larvae) is usually dropped to the ground by its made silk thread of which pupae stage being begun in the soil or dead leaves or at the inner edges of Tomato seedlings' plastic pots.

Figures 6, *T. absoluta* larvae (leaf penetration)

Figures 7, *T. absoluta* larvae

Pupae (length: 5–6 mm) are cylindrical in shape and greenish when just formed becoming darker in colour as they are near adult emergence. *T. absoluta* adults (length: 6–7 mm). Their antennas are filiform type. *T. absoluta* adults colour is silver to grey scales (Coelho and Franc 1987).

Buff mosaic is the dominated colour of the external wings; females are distinguished with shining colour since males are darker and attend to the grey colour. Black spots are present on anterior wings, and the females are abdominally wider and bigger than the males.

Figures 8, *T. absoluta* pupa

In the optimum conditions *T. absoluta* has 10 to 12 generations per year, since the hatching of its eggs takes 4–5 days, the four larval instars takes 13–15 days, pupae phase takes 9–11 days, and yet the one generation total duration is about 26-31days (Desneux et al.2010).

T. absoluta is nightly presence. Usually adults remain hidden during the day, showing greater morning-crepuscular activity with adults dispersing among crops by flying. Among a range of species within the Solanaceae, Tomato (*Lycopersicon esculentum* Miller) appear to be the primary host of *T. absoluta* (Desneux et al. 2010).

T. absoluta has a high reproductive potential. *T. absoluta* developmental cycle duration to a great extent depends on environmental conditions (temperature, relative humidity and light), with average development time of 76.3 days at 14 ^{o}C, 39.8 days at 19.7 ^{o}C and 23.8 days at 27.1 ^{o}C (Barrientos et al. 1998).

Temperature thresholds for egg, larva and pupa were estimated at 6.9 ± 0.5, 7.6 ± 0.1 and 9.2 ± 1.0 ^{o}C, respectively; overall, the threshold for egg-larva-adult is 8.1 ± 0.2_C. Accordingly, thermal constants were 103.8 ± 1.4, 238.5 ± 0.5 and 117.3 ± 5.3 DD for egg, larva and pupa, respectively, whereas the total thermal constant from egg to adult was estimated at 453.6 ± 3.9 DD (As long as food is available, *T. absoluta* attend to reproduce 10–12 generations per year) (Desneux et al. 2010).

Figure 9, *T. absoluta* lifecycle

Vercher et al. (2010) were able to maintain *T. absoluta* larvae alive during several weeks at 4ºC. When *T. absoluta* does not pupate in the soil, a cocoon is usually built (South America Condition).

Under Mediterranean conditions, adults of *T. absoluta* can be detected all around the year (Vercher et al. 2010). Adult lifecycle ranges between (10 and 15) days for females and (6–7) days for males (Estay 2000) .Females mate only once a day and are able to mate up to six times during their lifecycle, with a single mating bout lasting (4–5 h) (Desneux et al.2010).

The most productive ovipositing period is 7 days after first mating, and females lay (76%) of their eggs at that time, with a maximum lifetime fecundity of 260 eggs per female (UchoA Fernandes et al. 1995).

The latest symptoms are easily diagnosed by emerging of the galleries, yet could cause necrosis as a second infestation. Fruits can be attacked as soon as they are formed, and the galleries bored inside them can be invaded by secondary pathogens leading to fruit rot.

An important additional problem is that *T. absoluta* directly feeds on the growing tip, thereby halting plant development. The pest affects Tomatoes destined to fresh market as well as to processing, with larvae causing losses in its area of origin of up to 80–100% (Apablaza1992,; Lopez 1991). Furthermore, feeding activity on fruits directly affects the visual aspect of harvested products.

- ***T. absoluta* look-alikes**

Unfortunately *T. absoluta* has some look-alikes which can confuse the monitoring:

1. *Liriomyza bryoniae* (Diptera; Agromyizidae) mines look almost the same as *T. absoluta* mines. However *T. absoluta* mines become wide and blotch-shaped, while *Liriomyza* mines remain tunnel shaped. The excrement (frass) from *T. absoluta* are scattered through the mine, while *Liriomyza* excrement form a narrow string inside the mine. *T. absoluta* larvae (inside the mine) look like real caterpillars with a distinct head and legs, while *Liriomyza* larvae look more like a maggot, without head and legs.

2. Potato tuber moth (*Phthorimaea operculella*) is also present on *Solanaceae*. Its larvae have a bigger black band and black legs.

3. Guatemaltecan potato moth (*Scrobipalpopsis solanivora*), is present in Sardegna, and possibly in the South of Europe.

4. Tomato pinworm (*Keiferia lycopersicella*), appears among others in USA and Italy.

1.3 The Host, the Defendant, Tomato Varieties

Figure 10, tomato plants *Lycopersicum esculentum* (*Tuta absoluta* key host)

This Tomato is believed to have its origin in the Peru-Ecuador areas from where it spread as a weed throughout many parts of tropical America and then domesticated in Mexico. It was introduced into Europe early in the sixteen-century and the United States of America two centuries later.

Tomato belongs to the family Solanaceae and its scientific name is *Lycopersicum esculentum*. Tomatoes can be considered one of the most important vegetables. The fruits can be cooked or eaten raw. Moreover, this vegetable is used broadly in the canning industry in the production juices, sauces, ketchup and paste

Tomatoes varieties are described as “Creole” and “English”. Within the Creole there are the smell rounded cherry types and the large flat types. “English” are those varieties that are imported and include: Heat Master, Heat wave, Capitan, Alafua Winner, TA, TB, and TC etc.

Although *T. absoluta* prefers Tomato, it can also feed, develop and reproduce on other cultivated *Solanaceae* such as eggplant (*Solanum melongena* L.), potato (*S. tuberosum*), sweet pepper (*S. muricatum* L.) and tobacco, *Nicotiana tabacum* L. (Vargas 1970; Campos 1976), as well as on non-cultivated *Solanaceae* (*S. nigrum* L, *S. eleagnifolium* L., *S. bonariense* L., *S. sisymbriifolium* Lam., *S. saponaceum*, *Lycopersicum puberulum* Ph. etc.) and other naturally available host-plants such as *Datura ferox* L, *D. stramonium* L. and *N. glauca Graham* (Garcia and Espul 1982, Larraın 1986, Desneux et al.2010).

On potato, *T. absoluta* only attacks aerial parts, thereby not directly impeding tuber development. Nevertheless, leaf feeding may indirectly lower potato yield and, under appropriate climatic conditions, *T. absoluta* could become a pest for the potato crop (Pereyra and Sanchez 2006).

Since the time of its arrival in Europe, additional plant species have been reported as alternative hosts. It has been reported in a Sicilian greenhouse of Cape gooseberry (*Physalis peruviana*) (Tropea Garzia 2009) and has been found in Italy on bean, *Phaseolus vulgaris* (EPPO 2009), and on *Lycium sp*. and *Malva sp*. (Caponero 2009).

1.4 Environment, the battle ground

(Natural migration)

Little is known about natural spreading but there are indications that those moths can spread over kilometers by flying or drifting with the wind and can easily survive in/under harsh conditions. In Spain *T. absoluta* has been found tens of kilometers from any Tomato producing farm and even in woodland. This suggests the insect can spread via natural vegetation areas.

1.5 Economic importance and current management

Tomato represents such an economic importance in most of the world countries. South America Countries (Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Paraguay, Peru, Uruguay and Venezuela) are representing the extremely suffering countries of *T. absoluta.*

T. absoluta is considered a devastating pest of Tomato (Barrientos et al.1998; Estay 2000; EPPO 2006). Plants can be attacked at any developmental stage, with females ovipositing preferentially on leaves (73%), and to lesser extent on leaf veins and stem margins (21%), sepals (5%) or green fruits (1%), (Estay 2000).

In Tomato leaves, damages are caused through mine-formation within the mesophyll by feeding larvae, thus affecting the plant’s photosynthetic capacity and consequently lowering Tomato yield (Desneux et al.2010).

1.6 Phytosanitary and quarantine facts

Despite increasing interest in such control approach options (notably because smaller populations of *T. absoluta* are usually observed in organic and. conventional systems, (Medeiros 2007; Medeiroet al. 2009b) they are only scarcely used.

The process of biological invasion can be divided into a series of phases: arrival, establishment and spread (Mack et al. 2002). Such an iterative process is employed to quantify the likelihood and impact of invasion by an exotic pest species in a new geographic region, and could constitute a suitable framework for assessing the invasion of it in Europe and Mediterranean Basin countries.

T. absoluta is thought to have benefited greatly from agricultural trade within the continent for its further spread (Ca´ceres 1992). For example, agricultural trade between Chile and Argentina introduced *T. absoluta* to the Mendoza province (Argentina) in 1964 (Bahamondes and Mallea 1969).

Figure 11, *T. absoluta* larvae mines

Figure 12, *T. absoluta* larvae (Tomato fruits infestation)

Interceptions and measures against expansion despite its initial denomination as a key quarantine pest (i.e. A1 listing in 2004; EPPO 2005), *T. absoluta* was and still is not listed in Plant Health Directive 2000/29/EC. Consequently, Tomato fruits originating in third countries were not subject to a plant health inspection before their entry and movement within the European Community.

This omission could have caused the introduction of *T. absoluta* into Europe, which was reported from the province of Castellón de la Plana (Eastern Spain) in late 2006

(Urbaneja et al. 2007). Based on experiences in South America, spread of *T. absoluta* can be greatly facilitated through agricultural trade.

Despite the presence of well-organized plant protection agencies throughout Europe, contingency measures are proving ineffective to halt the spread of *T. absoluta* throughout the continent. For example, despite recurrent interceptions of *T. absoluta* by the British Food and Environment Study Agency (FERA) from 2006 to 2009, subsequent intensive monitoring of Tomato packing stations and statutory control measures at infested sites, the pest was ultimately reported from a Tomato farm in the British countryside in July 2009.

Similarly, the Netherlands Plant Protection Service recorded *T. absoluta* in a Tomato packaging and sorting facility in 2008 (Potting 2009) and the Russian Phytosanitary Service reported the presence of *T. absoluta* in the Kaliningrad region in Tomato shipments from Spain.

The spread of the pest in Spain is mainly happening by natural means (Spanish Expert Group in Plant Protection of Horticultural Crops, personal communication). Wind currents seem to be especially favourable for its dispersal (though the flight ability of *T. absoluta* remains as an uncertainty, which should be studied further).

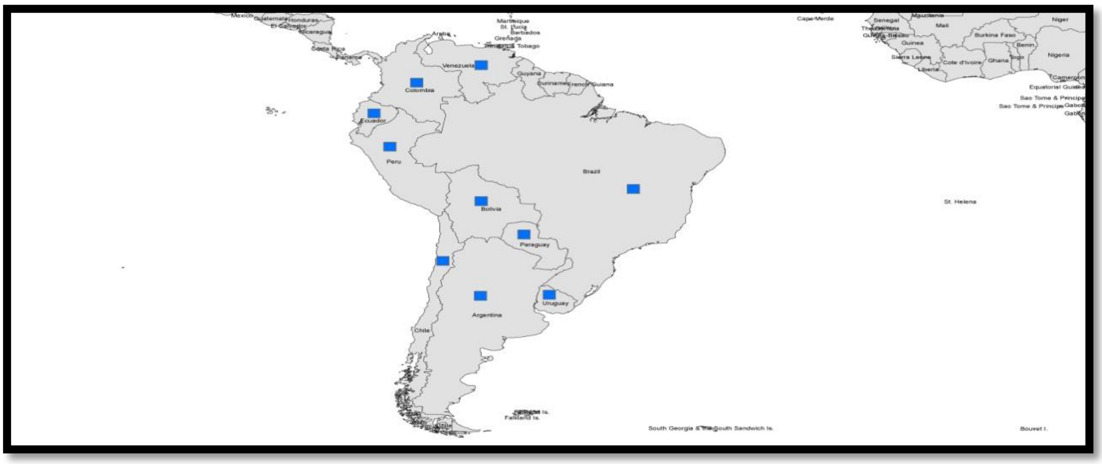

Figure 13, *T. absoluta* in South American countries since 1970

The US Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) is constantly updating the list of countries whose imported products are regulated for *T. absoluta*. Federal Orders were issued in February and May 2009 (Bech 2009) that place severe restrictions on Tomato fruits from infested countries (Albania, Algeria, France, Greece, Italy, Morocco, Netherlands, Portugal, Spain, Switzerland and Tunisia).

USDA-APHIS also prohibits the entry of *Solanum sp*., *Datura spp*. and *Nicotiana spp*., as potential host plants of *T. absoluta* from all of the above mentioned countries, pending the completion of a Pest Risk Analysis and implementation of appropriate mitigation measures.

Spain is currently authorized to export green, pink and red Tomatoes to the USA provided that a system approach to mitigate the risk associated with *T. absoluta* is applied. This requirement is in accordance with the temporary emergency measures for the control of *T. absoluta* established by the Spanish Plant Protection Committee and which have been adopted by all the Spanish Autonomous Regions.

T. absoluta was reported in Tomato crops from Italy, southern France, Greece, Portugal, Morocco, Algeria and Tunisia (Potting 2009). In 2008, *T. absoluta* was reported from five principal Tomato-growing regions (i.e., Liguria, Sicilia, Sardegna, Calabria, and Campania), while in 2009 *T. absoluta* further invaded central and northeast Italy.

T. absoluta distribution in Sicily; National Plant Protect Organization (NPPO) of Italy has recently informed the European and Mediterranean Plant Protection Organization (EPPO) secretariat that *T. absoluta* (Lepidoptera: Gelechiidae) has been found on *Phaseolus vulgaris*, common bean plants in Sicilia, Italy. This has raised the concern of possible movement of the pest to Artichoke plants. In another development, in Italy, the presence of *T. absoluta has* been officially reported in new locations Abruzzo, Liguria and Umbria.

In France, *T. absoluta* was originally found during late 2008 on Corsica island and in various areas of the French Riviera, and further expanded its geographic distribution in 2009 to north (Rhoˆne-Alpes) and southwest (Languedoc-Roussillon) (Germain et al. 2009) and recently to two regions of the Atlantic coast (Decoin 2010).

In Greece, in 2009, the species was present in the mainland (Prevesa, Axaia, Trifilia) and Crete (Roditakis et al. 2010). The pest was initially reported from several locations of Portugal in 2009. In addition, *T. absoluta* has been reported in some European countries with colder climate (Switzerland, UK and the Netherlands); such phenomenon is considered to be confined to the protected Tomato cultivation (Potting 2009).

Finally, the presence of this pest has been reported in southern Germany and Cyprus (EPPO 2010), in Tomato greenhouses in Romania, in both greenhouse and open field Tomato crops in Bulgaria (EC Report 2009), in Turkey (Kılıc 2010), in Lithuania and in Middle East (Bahrain, Kuwait).

Figure 14, *T. absoluta* Geographical distribution in Europe, North Africa and Asia

1.7 Preventive chemical measures against the different stages of *T. Absoluta*

1.7.1 greenhouses

As mentioned above, the incidence of *T. absoluta* in greenhouses is much lower than under open field conditions. However, some measures could be also adopted to reduce its impact.

- **Before planting**
  - Periodically maintenance (no holes),

- Crop perfect isolation (Key point),
- Periodically weed management earlier time (inside and outside). If possible maintain clean of plants 4-6 weeks before planting (During this period all emerging adults should die),
- Placing delta traps (0.5 m high) with *T. absoluta* male pheromone within some weeks before planting to start monitoring the population (Continue after planting),
- Install water traps for male mass trapping with *T. absoluta* male pheromone (20-40 water traps /ha) within some weeks before planting (Continue after planting), especially at the beginning of the season. Be sure to maintain the trap filled of water. A detergent or mineral oil should be mixed with the water to break the superficial tension,
- Powdering powder sulphur on the greenhouse structure and the soil inside within one week before (also good to avoid mite infestations),
- Be sure to use healthy and pest free plants from nursery (Key point).

- After planting

Monitoring

- Producing Delta traps for monitoring, any increasing in adults/trap/week preventive measures should be taken,
- Any infested plants' leaflets or fruits curative measures should be taken,

Chemical treatments

Preventive measures; *o*ne of the following preventive measures should be weekly implemented;

- If possible sprinkle powder sulphur on the plants is useful to avoid mite infestations,
- *B. thuringiensis* (var. kurstaki) weekly. It's also useful to avoid *Helicoverpa* or *Noctuidae*: *Plusidae* infestations.
- Azadiractine (Neem). It's also useful to stop the thrips probably out-break,

Curative treatments; by using selective insecticides only, after these treatments if *T. absoluta* population is being decreased, preventive measures might be an option.

Cultural practices

- Detach infested leaves and fruits. The infested organs must be removed and introduced in a black plastic bag. The bag must be well-closed and be exposed to the direct sun. This practice is very important at the beginning of the crop season,
- Continue during all the season removing weeds (especially Solanaceae),
- Continue isolating the greenhouse. When possible uses mesh (6x9 thread /cm2) in the ventilation openings and in the door (if possible use also double door). This mesh will avoid the entrance of *T. absoluta* and should allow the ventilation of the greenhouse to avoid diseases,
- When possible cover the ground with plastic or mesh as a (mulch), to avoid *T. absoluta* pupation. Also it is good to avoid weeds.

Biological control

- Promote the use of beneficials and especially the appearance of Mirid bugs (*Nesidiocoris sp.* and *Macrolophus sp*. (Hemiptera: Miridae). To increase the probability of appearance of *Nesidiocoris sp*. use only selective pesticides (not only for *T. absoluta*, also against other pests) (Check side-effect list for beneficials). Spinosad and Indoxacarb are harmless to Mirids when they are installed in the crop. If possible release predatory Mirids 2-3 weeks after transplanting when prey is available.

Biotechnical control

- Continue with the use water traps for male mass trapping with *T. absoluta* male pheromone (20-40 water traps /ha). Be sure to maintain the trap filled of water. A detergent or mineral oil should be mixed with the water to break the superficial tension. This technique is especially useful at the beginning of the season when *T. absoluta* is starting to increase its densities.

- **After the yield harvest**

Cultural practices

- If the greenhouse is infested do not abandon the crop. This would be a hot spot to contaminate Tomato crops in open-field. Continue with the greenhouse isolated. Close the greenhouse to increase temperatures.
- Do not take out infested organs from the greenhouse.

Chemical treatments

- If the greenhouse is hardly infested spray with an allowed adulticide and larvicide. Some adulticides could be also applied by drench. If the field is really infested repeat the treatment twice.

1.7.2 open field

- **Before planting**

- Remove all weeds.
- One week before planting hold delta traps (0.5-1 m high) with *T. absoluta* male pheromone to monitor the population (Continue after planting).
- One week before planting install water traps for male mass trapping with *T. absoluta* male pheromone (20-40 water traps /ha) (Continue after planting). Be sure to maintain filled of water the trap. A detergent or mineral oil should be mixed with the water to break the superficial tension.

- **After planting**

Monitoring

- Delta traps monitoring. Count the numbers of captures per trap and week to follow the incidence of the pest. When male captures increase (adults/trap/week), start with the preventive measures (see below).
- Plant sampling. Count the percentages of infested plants and fruits in the field. Only when plants are infested or one infested fruit is detected, start with the curative treatments,

Chemical treatments

As preventive measures it is preferred to weekly alternate one of the following options:

- If possible sprinkle powder sulphur on the plants. Also useful to avoid mite infestations,
- *B. thuringiensis* (var. kurstaki) weekly. Also useful to avoid *Helicoverpa* or *Noctuidae: Plusidae* infestations.
- Azadiractine (Neem). Also useful to stop explosions of thrips,

Curative measures; use only selective insecticides, such as spinosad or indoxacarb, no more than 3 and 6 times per season each one, respectively. After these treatments if population of Tuta decreases, it is possible to come back to preventive treatments. Check if the treatment is effective (dead larvae and clean new leaf flushes).

Cultural practices

- Detach infested leaves and fruits. The infested organs must be removed and introduced in a black plastic bag. The bag must be well-closed and be exposed to the direct sun. This practice is very important at the beginning of the crop season and could assist the compost preparation in the farm.
- Continue during all the season removing weeds (especially Solanaceae).

Biological control

- Promote the use of beneficial and especially the appearance of Mirid bugs *Nesidiocoris sp*. and *Macrolophus sp.* (Hemiptera: Miridae). To increase the probability of appearance of *Nesidiocoris sp.* use only selective pesticides (not only for *T. absoluta*, also against other pests) (Check side-effect list for beneficials). Spinosad and Indoxacarb are harmless to Mirids when they are installed in the crop. If possible release predatory Mirids 2-3 weeks after transplanting when prey is available.

Biotechnical control

- Continue with the use of water pheromone traps for male mass trapping of *T. absoluta* male (20-40 water traps /ha). Be sure to maintain the trap filled of water. A detergent or mineral oil should be mixed with the water to break the superficial tension. This technique is especially useful at the beginning of the season when *T. absoluta* is starting to increase its density.

- **After the end of the season**

- If the field is hardly infested spray with an allowed adulticide and larvicide. If the field is really infested repeat the treatment twice.
-

1.8 Non-chemical measures against the different stages of *T. absoluta*

1. The predatory bugs *Nesidiocoris tenuis* (Nesibug) and *Macrolophus caliginosus* (Mirical) are effective predators of *T. absoluta* eggs and young larvae. A quick establishment of these predatory bugs in the crop gives the best protection against the pest. Introduce the predatory bugs several times during the first weeks of cultivation in a total dose of 1-2 bugs per m^2 or until the bugs are sufficiently established in the crop.

a. Avoid broad spectrum pesticides in open field Tomatoes in order not to disturb the development of indigenous population of predatory bugs.

b. Delayed de-leafing helps to boost the development of both *Nesidiocoris tenuis* and *Macrolophus pygmaeus* in the crop. The impact of *Trichogramma spp*. is being investigated. Only use products that are permitted in your country or state!

2. Pherodis pheromones used in Tutasan water traps catch many male adults, up to 300 per trap per day. This measure will slow down the reproduction of the population. Use 20-50 traps per hectare inside the greenhouse, depending on the situation. Renew the Pherodis pheromone capsules every
6 weeks. Also place some traps in the direct surrounding of the greenhouse.

3 If the pest occurs in hot spots, remove infested leaves (and fruit) with larvae and destroy them.

4. Caterpillars move out of the mines several times in their development. Preventive and regular sprays with *B. thuringiensis* can kill the caterpillars in this phase, thus contributing to the control. However, note that intensive *B. thuringiensis* sprays can leave residue on the fruit, so apply this only in the early phase of the crop.

1.9 Chemical corrections

Many insecticides have traditionally been employed to control *T. absoluta* populations. When the first pest outbreaks appeared in South America, organophosphate products (OP) and cartap were fully used, and were later substituted by the pyrethroids in the 1970s (Desneux et al., 2010).

In the 1980s, alternate applications of cartap and pyrethroids/thiocyclam were made (Lietti *et al.*, 2005). New insecticides were introduced in the 1990s, such as acylurea, spinosad, abamectin, tebufenozide and chlorfenapyr. In addition, new pyrethroid molecules have been shown to be very efficient in Brazil (Silvério *et al.*, 2009).

The efficiency of OP products fell after 1980 with the development of resistance to such products by *T. absoluta* in Brazil and Chile, as well as resistance to cartap, abamectin and permethrin in Brazil (Siqueira *et al* 2000; Siqueira *et al.* 2001), and to pyrethroids in Chile (Salazar and Araya, 1997) and Argentina (Lietti *et al.*, 2005).
This resistance raised doubts on insecticide use, but heavy chemical applications to control *T. absoluta* in these countries are still common. Recent study on plant extracts has demonstrated the efficiency of extracts of *Trichilia pallens* (Cunha *et al.,* 2005; Cunha *et al.,* 2006; Cunha *et al.,* 2008) or Neem (Gonçalves-Gervásio and Vendramim 2007) on *T. absoluta*, but such products are rarely used in South America.

Since *T. absoluta* was detected in the Mediterranean Basin, the most common control practice has been based on the use of chemical insecticides (Bielza, 2010). Nevertheless, these treatments may disrupt the existing integrated pest management programs in Tomato crops based on biological control (van der Blom *et al.* 2009) and may lead to resistance (Bielza 2010), as it occurred in the area of origin of this pest.

Therefore, there was an immediate need to choose pesticides which fulfilled two main objectives:
1) Effectiveness against *T. absoluta* and,
2) Selective, in order to preserve natural enemies in Tomato crops.

In addition to spinosad and indoxacarb, which were the two first available insecticides in the Mediterranean Basin, new effective and selective insecticides are currently available to control *T. absoluta*, such as flubendiamid, emamectin, rynoxapir, abamectin or etofenprox (Araujo-Gonçalves 2010; Torné *et al.* 2010; Espinosa 2010; Robles 2010; López *et al.* 2010; Astor 2010; Gutiérrez-Giulianotti 2010).
Moreover, Azadirachtine (Neem) and sulphur treatments may also help in reducing *T. absoluta* incidence, although efficacies are much lower (Monserrat, 2009). However, repeated applications should be conducted each season in order to control *T. absoluta*

exclusively by chemical means. Rotation of these active ingredients is compulsory to prevent resistance development (Ortega *et al.*, 2008; Bielza 2010), as well as, the use of insecticides compatibles with biological control and integration of other control tactics.

The above mentioned measures may be insufficient for total control of the pest. In that case chemical interventions are needed to keep the pest below the economic threshold correlation. High volume sprays with pesticides based on spinosad or indoxacarb have shown the best effect, but still may have some impact on biological pest control or natural pollination. Always use these products at recommended dose, respecting the local rules. Restrict the number of applications per season to avoid resistance. Consult your consultant to evaluate the effect of the chemical interventions and their effects on the population of the natural enemies.

1.10 The Current management methods

The primary *T. absoluta* management tactic in most South American countries is chemical control (Lietti et al. 2005). Organophosphates (not authorized in organic farming phytosanitary measurements they are as follow;

- **Organophosphate pesticides:**

Organophosphate pesticides irreversibly inactivate acetyl cholinesterase, which is essential to nerve function in insects, humans, and many other animals. Organophosphate pesticides affect this enzyme in varied ways, and thus in their potential for poisoning. For instance, parathion, one of the first OPs commercialized, is many times more potent than malathion, an insecticide used in combating the Mediterranean fruit fly (Med-fly) and West Nile Virus-transmitting mosquitoes.

In health, agriculture, and government, the word "organophosphates" refers to a group of insecticides or nerve agents acting on the enzyme acetyl cholinesterase (the pesticide group carbamates also act on this enzyme, but through a different mechanism). The term is used often to describe virtually any organic phosphorus (V)-containing compound, especially when dealing with neurotoxic compounds.

Commonly used organophosphates have included parathion, malathion, methyl parathion, chlorpyrifos, diazinon, dichlorvos, phosmet, fenitrothion tetrachlorvinphos, and azinphos methyl. Malathion is widely used in agriculture, residential landscaping, public recreation areas, and in public health pest control programs such as mosquito eradication.

In the US, it is the most commonly used organophosphate insecticide. Forty organophosphate pesticides are registered in the U.S., with at least 73 million pounds used in agricultural and residential settings.

Mode of action:

Organophosphates work by irreversibly blocking an enzyme that's critical to nerve function in bugs even at relatively low levels.

Organophosphate pesticides degrade rapidly by hydrolysis on exposure to sunlight, air, and soil, although small amounts can be detected in food and drinking water. Their ability to degrade made them an attractive alternative to the persistent organochlorides pesticides, such as DDT, aldrin and dieldrin. Although organophosphates degrade faster than the organochlorides, they have *greater acute toxicity*; posing risks to people who may be exposed to large amounts.

US Environmental Protection Agency (EPA) banned most residential uses of organophosphates in 2001, but they are still sprayed agriculturally on fruits and vegetables. They're also used to control pests like mosquitos in public spaces such as parks. *They can be absorbed through the lungs or skin or by eating them on food*. Organophosphates were initially used for *T. absoluta* control, which were gradually replaced by pyrethroids during the 1970s".

- **Pyrethroids:**

Pyrethroids are *synthetic* chemical compounds similar to the natural chemical pyrethrins produced by the flowers of pyrethrums (*Chrysanthemum cineraria folium* and *C. coccineum*). Pyrethroids now constitute a major proportion of the synthetic insecticide market and are common in commercial products such as household insecticides.

In the concentrations used in such products, they may also have insect repellent properties and are generally harmless to human beings in low doses but can harm *sensitive individuals*. They are usually broken apart by sunlight and the atmosphere in one or two days, and do not significantly affect groundwater quality.

Pyrethroids are *toxic to fish and other aquatic organisms*, at extremely small levels, such as 2 parts per trillion, Pyrethroids are lethal to mayflies, gadflies, and invertebrates that constitute the base of many aquatic and terrestrial food webs.

Pyrethroids have been found at acutely toxic levels in sediments and waterways in California; the chemical is able to pass through secondary treatment systems at municipal wastewater treatment facilities causing the chemical to be commonly found in the final effluent, usually at levels lethal to invertebrates.

Mode of action:

Pyrethroids are axenic poisons and cause paralysis of an organism. The chemical causes paralysis by keeping the sodium channels open in the neuronal membranes of an organism. The sodium channel consists of a membrane protein with a hydrophilic interior; this interior is effectively a tiny hole which is shaped exactly right to strip away the partially charged water molecules from a sodium ion and create a thermodynamically favourable way for sodium ions to pass through the membrane, enter the axon, and propagate an action potential. When the toxin keeps the channels in their open state, the nerves cannot de-excite, so the organism is paralyzed.

Pyrethroids are usually combined with piperonyl butoxide, a known inhibitor of key microsomal oxidase enzymes. This prevents these enzymes from clearing the pyrethroids from the body of the insect, and assures the pyrethroids will be lethal and not merely a paralyzing agent. Combined, pyrethroids are *toxic* to most beneficial insects such as bees and dragonflies.

During the early 1980s, cartap, which alternated with pyrethroids and thiocyclam, proved highly efficient in controlling *T. absoluta* outbreaks (Lietti et al. 2005). During the 1990s, novel insecticides were introduced, such as abamectin, acylurea IGR, spinosad, tebufonozide and chlorfenapyr (Desneux et al. 2010)

Recently in Brazil, 10 new molecules of pyrethroids proved to be effective in controlling *T. absoluta*, with different toxical effects, and in some cases, up to 100% larval mortality was recorded (Silverio et al. 2009).

Also, some vegetal products were assessed for potential use in the leafminer control, including extracts of *Trichilia pallens* (da Cunha et al. 2006), species belonging to the same family of *Neem* Tree, whose extracts are largely used for insect pest control. However, the use of insecticide, drawing upon a limited set of products, has proven not to be a sustainable management option for this pest in South America.

Since the 1980s, efficacy of organophosphates for *T. absoluta* control has gradually decreased in countries like Bolivia, Brazil and Chile (Salazar and Araya 1997 Siqueira et al. 2000, 2001). In addition, resistance development has been reported against organophosphates and pyrethroids in Chile (Salazar and Araya 1997) and against abamectin, cartap, methamidophos and permethrin in Brazil (Siqueira et al. 2000, 2001).

Resistance to deltamethrin and abamectin has recently been demonstrated for open field and greenhouse populations of *T. absoluta* in Argentina (Lietti et al. 2005).

- **The basic idea behind using insecticides is:**

The decision scheme of using insecticides for management of T. absoluta is largely based on adult captures in sexual pheromone traps (Benvenga et al. 2007), as adult catches are correlated with larval damages and yield losses (Faccioli 1993; Benvenga et al. 2007).

An action level of 45 ± 19.50 *T. absoluta* caught daily using pheromone traps in Brazil (Benvenga et al. 2007), while in Chile extension specialists report an economic threshold of 100 males per pheromone trap per day.

An action threshold could also be based on occurrence of the pest in the Tomato crop with 2 females/plant or 26 larvae per plant (Bajonero et al. 2008) or 8% defoliation (Bayer Crop Science, Colombia) recommended in Colombia.

1.10.1 Mass trapping

The Southern European and North African invasion of *T. absoluta* increased the demand for pheromone monitoring lures, which were then used in mass trapping campaigns for greenhouse pest management. Mass trapping can be an effective management tool in isolated and controlled spaces such as greenhouses. For *T. absoluta* in a Tomato greenhouse setting, placing at least one trap per 500 square meters has been used to significantly reduce moth populations as part of an integrated pest management program (Stoltman *et al.*, 2010). Mass trapping programs must be deployed early in the plant growth cycle, when *T. absoluta* populations are present at low densities; otherwise the program is likely to fail.

Traditional paper and plastic delta traps can also be used in a mass trapping program. These traps may be preferred in larger operations due to their relatively low cost, ease of deployment and disposal. Paper delta traps come with sticky interior walls and are designed for one-time use. They should be disposed of once the trap becomes saturated. Conversely, plastic delta traps come with removable sticky liners. Liners can be replaced once they are filled. One problem with the sticky traps is that the glue liner of the trap becomes rapidly saturated under high *T. absoluta* population densities and requires frequent replacement

In order to reduce the cost of mass trapping, and also avoid trap saturation, growers have started using water traps in their mass trapping programs. Trap designs vary and can be as simple as deep plastic trays filled with soapy water and with the pheromone lure suspended over the center of the tray, just above the water line, so that attracted moths become trapped when they touch the soapy water

1.10.2 Cultural practices

- Prophylaxis is one of the most effective and cheapest ways of reducing pest infestation (Berlinger et al. 1999). Consequently, this is the aim of most of the cultural practices recommended for *T. absoluta* control. The adoption of prophylactic methods could be the key to success in controlling this pest, particularly in greenhouses, and as a result, a great deal of publications with recommendations on cultural control measures have been published and distributed to farmers (Arnó & Gabarra 2010).

- One of the most accepted cultural methods to reduce *T. absoluta* populations includes crop isolation. This can be achieved in greenhouses by screening vents and installing double-doors. Monserrat (2009a) advised the use of mesh of (at least 6 x 9 threads/$cm^{2)}$ to prevent entry of *T. absoluta* adults,
- At the beginning of the growing season, it is important to remove leaves, stems and fruits affected by the presence of *T. absoluta* larvae or pupae, by placing the materials in sealed plastic bags exposed to direct sunlight.
- Before planting and throughout the growing season, removal of weeds that may also host *T. absoluta* is also advised. Furthermore,
- It is recommended that infested crop residues be removed either during the growing season or immediately following harvest by burying the residue or placing the material in closed containers covered with a transparent plastic film to allow fermentation.

In Almería (southeast of Spain) covering crop residues with plastic for no less than three weeks reportedly reduced the number of adult *T. absoluta* by 94% during the fall (Tapia *et al.*, 2010). Crop residues can also be eliminated by burning or grinding combined with insecticide sprays (Robredo and Cardeñoso 2008), although these methods may have some disadvantages such as the need to obtain a permit for burning or the high cost of grinding.

Crop rotation with non-host crops is also imperative. In highly specialized farms where Tomatoes are intensively produced, it is recommended that greenhouses be emptied between crop cycles and sealed during a period of 4 to 8 weeks depending on the temperature (Monserrat, 2009b; Monserrat, 2010). Under this situation, all adults emerging from the soil will die or be captured by pheromone or light traps. In some situations, chemical treatments may be applied in order to reduce *T. absoluta* levels in soil.

- Soil solarization has been advised in warm climates to kill pupae that remain in the soil after harvest.

The use of genetic resistance may be also an alternative to control this pest since some sources of resistance to *T. absoluta* have been reported in some species of wild Tomato. The two mechanisms of resistance detected so far have been the antixenosis and antibiosis (Oliveira *et al.*, 2009) This approach is not been accepted within the organic farming standards.

- Sprinkler irrigation (as in Brazil) has been shown to have a significant impact on populations of T. absoluta eggs and larvae.

1.10.3 Predators and parasitoids of *Tuta absoluta* in brief

Various predators and parasitoids spontaneously attack *T. absoluta* in tomato crops in Europe and in North Africa. Some of these, mainly native Miridae, have been already employed in integrated pest management (IPM) strategies (Castan˜e´ et al. 2011; Molla´ et al. 2011; Cabello et al. 2012; Zappala` et al. 2012; Chailleux et al. 2013). However, several screenings for effective natural enemy species in the invaded area are still ongoing (Chailleux et al. 2012; Gabarra et al. 2013).

More than 70 species of generalist natural enemies have been reported for *T. absoluta* in the Western Palaearctic region* so far. These have been sampled both on open-field and protected susceptible crops as well as on wild flora and/or using infested sentinel plants (Zappala et al. 2013).

***The Western Palaearctic or Western Palearctic is part of the Palaearctic ecozone, one of the eight ecozones dividing the Earth's surface. Because of its size, the Palaearctic is often divided for convenience into two, with Europe, North Africa, northern and central parts of the Arabian Peninsula, and part of temperate Asia, roughly to the Ural Mountains forming the western zone, and the rest of temperate Asia becoming the Eastern Palaearctic.**

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CHAPTER 2

Use of biorational insecticides for the control of *Tuta absoluta* infestations on open field tomato

Chapter 2

Based on Lo Bue, P. et al. the SPECIAL ISSUE NEW MEDIT N. 4/2012

Use of biorational insecticides for the control of *T. absoluta* (Meyrick, 1917) infestations on open field Tomato

P. Lo Bue, S. Abbas, E. Peri, S. Colazza

Abstract

T. absoluta (Meyrick) (Lepidoptera: Gelechiidae), the Tomato leaf miner, is one of the most devastating pests affecting Tomato crops in Italy. Management of T. absoluta was assessed in tomato open-field tests 2011/2012 by using three biorational insecticides, Azadirachtin, Bacillus thuringiensis var. kurstaki, and Beauveria bassiana, and a combination of three synthetic insecticides, Emamectin, Indoxacarb and Metaflumizone, as a control treatment. Our results showed that only the combination of Azadirachtin + B. thuringiensis was able to reduce the impact of Tomato leaf miner on the fruit's marketable production similarly to the control treatment. This finding suggests that biorational insecticides are a good alternative to synthetic ones. Possible use of biorational insecticides in the management of Tomato leaf miner in organic farming system is discussed.

Keywords; Tomato leaf miner, Azadirachtin, Bacillus thuringiensis, Beauveria bassiana

Introduction

The Tomato leaf miner, *T. absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), is a key pest of Tomato, *Lycopersicon esculentum* Mill., originating from South America (Barrientos et al., 1998).

In Europe, *T. absoluta* has been initially detected in eastern Spain in 2006 (Urbaneja et al. 2007), then, a few years later, it was found in most of the countries facing the Mediterranean Sea and in several countries of Europe, where it is causing serious damages open field and greenhouse Tomato (Desneux et al. 2010).

Several cultivated and wild species have been reported as host plants, e.g *Solanum melongena* L., S. *tuberosum Nicotiana glauca* Graham, *Datura stramonium* L., *Capsicum annuum* L. *Phaseolus vulgaris* L. (EPPO, 2009).

In Italy the pest was also reported on *Lycium* sp. and *Malva* sp. (Caponero, 2009), and on greenhouse plants of Cape gooseberry, *Physalis peruviana* L., cultivated in Sicily (Tropea et al., 2009).

To reduce *T. absoluta* infestations, some strategies, currently underway, seem to give encouraging results, as mating disruption technique (Filho et al., 2000), and biological control programs based on indigenous parasitoids and predators (for a review see Desneux et al. 2010). However, to date, applying insecticide treatments is the dominant strategy in *T. absoluta* management. In the present paper we evaluate the efficacy of three biorational insecticides to establish sustainable strategy in controlling the Tomato leaf miner in organic Tomato plantations.

Materials and Methods

The experiments of 2011/2012 were carried out in a Tomato field located near Santa Ninfa (Trapani), West Sicily, at 410 m above mean sea level (AMSL), from March to August 2011 and from March to August 2012. Tomato plants, cv "Patataro", were cultivated in around 7000 m^2 area, including 12 rows of 36 plants each. Separation between rows and plants was 1.5 m and 1 m, respectively.

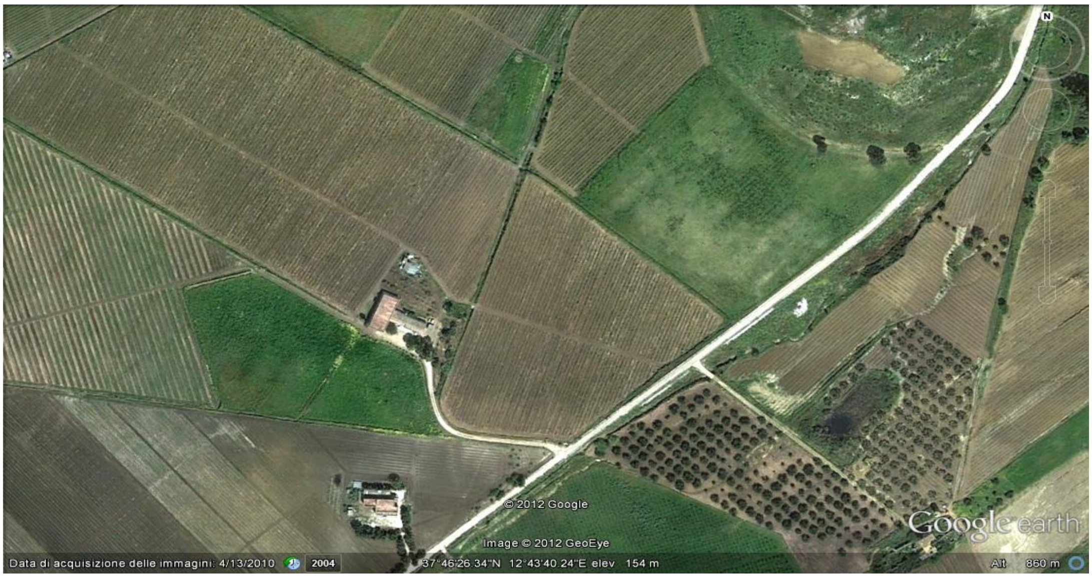

Figure 1, the experimental sites of the years 2011/2012, Sicily, Italy

Plants were cultivated under an organic system, and following typical open field Tomato cultivation techniques applied in Sicily. The plant main stem was trained with plastic rings to a cane structure, secondary shoots and senescent leaves were weekly pruned, and the application of a standard nutrient solution (Guanito, ItalPollina) for Tomato was added directly to the soil. The average temperature for the tow experimental years of 2011/2012 ranged from 13 °C, on March to 33°C, on August. The average relative humidity ranged from 21%, on March, to 13%, on August.

Figure 2, the experimental preparations 2011/2012

Two delta traps supplied with *T. absoluta* pheromone (Intrachem, Italy) were hung at opposite ends of the field at a height of 1 m. The traps were weekly observed. The sticky plate was changed once a week and the pheromone capsule once a month.

The experiments of 2011/2012 three biorational insecticides – *Azadirachtin* (Neemazal - Intrachem), *Bacillus thuringiensis* var. *kurstaki* (EG 2348) (Bt Rapax-Intrachem), and *Beauveria bassiana* (Naturalis- Intrachem) – and three synthetic insecticides - Emamectin (Affirm-Syngenta), Indoxacarb (Steward-DuPont), and Metaflumizone (Alverde-Basf) – were selected.

The experiments used a randomized block design with four treatments replicated three times (12 plots). Each plot was 12 m long and 3 m wide and contained 3 rows with 12 plants, for a total of 36 plants.

The treatments, applied at the dose recommended by the producing companies, were: *Azadirachtin* (*Az*) with the dose of 300 cc/100L/hl, *Azadirachtin* + *B. thuringiensis*

(Az + Bt) with the dose of 300 cc/100L/ha and 150 cc/100L/ha, respectively; *Azadirachtin* + *B. bassiana* (Az + Bb) with the dose of 300 cc/100L/ha and 150 respectively; Emamectin with the dose of 150 g/100L/ha, Indoxacarb with the dose of 12.5 g/100L/ha, and Metaflumizone with the dose of 100 ml/100L/ha (Control).

The synthetic insecticides were applied in rotation. All the treatments started when more than 3 adults were captured into the traps and were weekly repeated. The number of 3 captured adults has been selected to prove the field pest presence without economic threshold correlations.

For both experiments of 2011/2012 plants were weekly visually checked from March 28th to August 25th and fruits were weekly harvested and weighted from July 21st to August 25th. Insecticide efficacy was evaluated in terms of marketable production, i.e. weight (g) of no damaged fruits. Data were compared by **One-way ANOVA**, followed by **Fisher's LSD test**, using Statistica for Windows 6.0 (Stat Soft Italia, 1997).

Based on the results of the first year experiment of 2011, the biorational insecticides; Az plus *Beauveria bassiana* had been replaced with *Bacillus thuringiensis* alone in the consequent year experiment of 2012 and that was the only modification of the second year experiment.

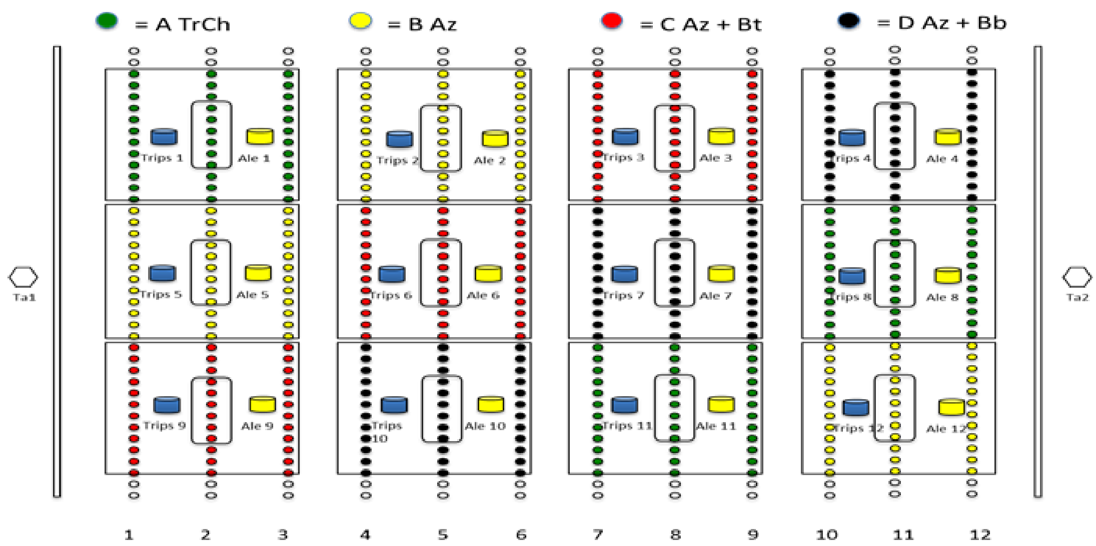

Figure 3, the experimental design (CRBD)

a- **(Black=AZ+Bb, Green= Chemical treatments (control), Red= AZ+Bt, and Yellow=AZ) of the year experiment of 2011.**

b- (Black= Bt alone, Green= Chemical treatments (control), Red= AZ+Bt, and Yellow=AZ alone) of the year experiment 0f 2012.

Results

The capturing of *T. absoluta* males in the pheromone traps started from June 2nd reaching the fixed threshold (3 adults / trap) to start the treatments on June 16th (Figure 1 and 2).

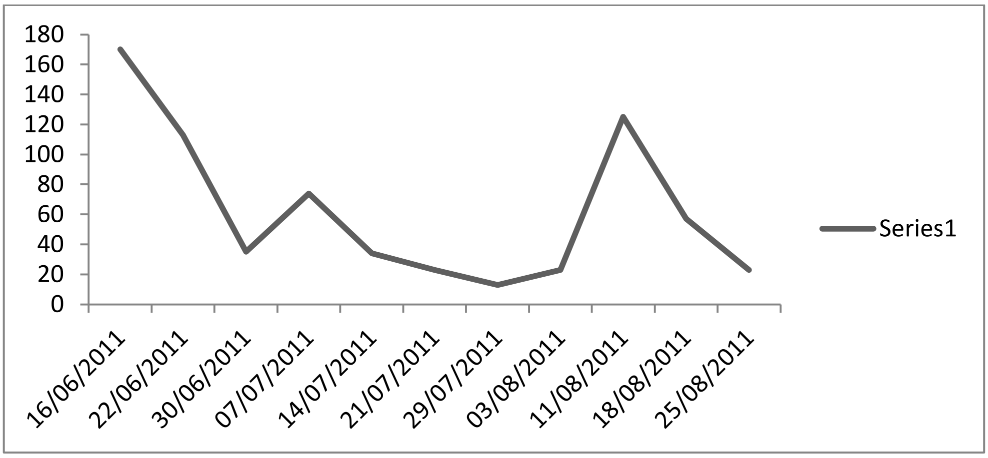

Figure 1, *T. absoluta* pheromone trap no1

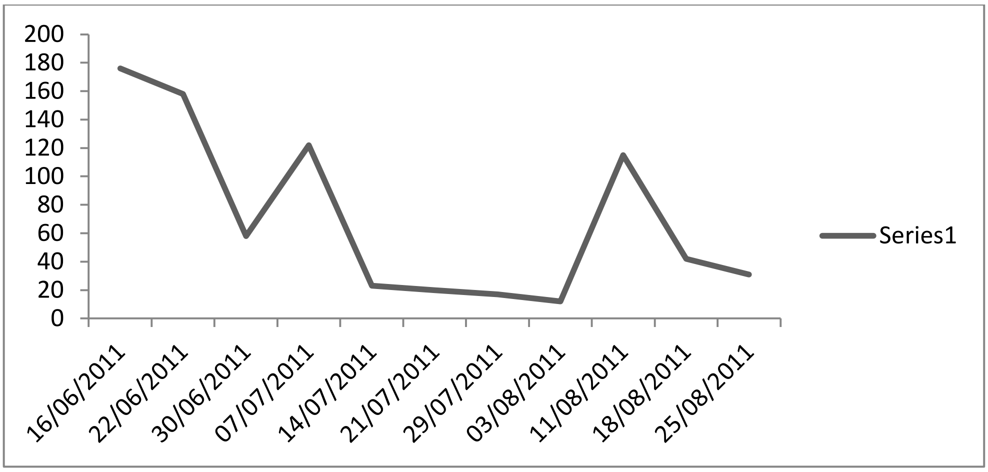

Figure 2, *T. absoluta* pheromone trap no 2

The experiment of the year 2011 in all the plots fruits were damaged by Tomato leaf miner (Figure 3). In terms of weight, in the plots treated with *Az* + *Bt*, damaged fruits never exceed 30% of the total weight of harvested fruits. On the contrary, the weight of the damaged fruits was always upper to 30% in both plots treated with *Az* + *Bb* and with *Az*.

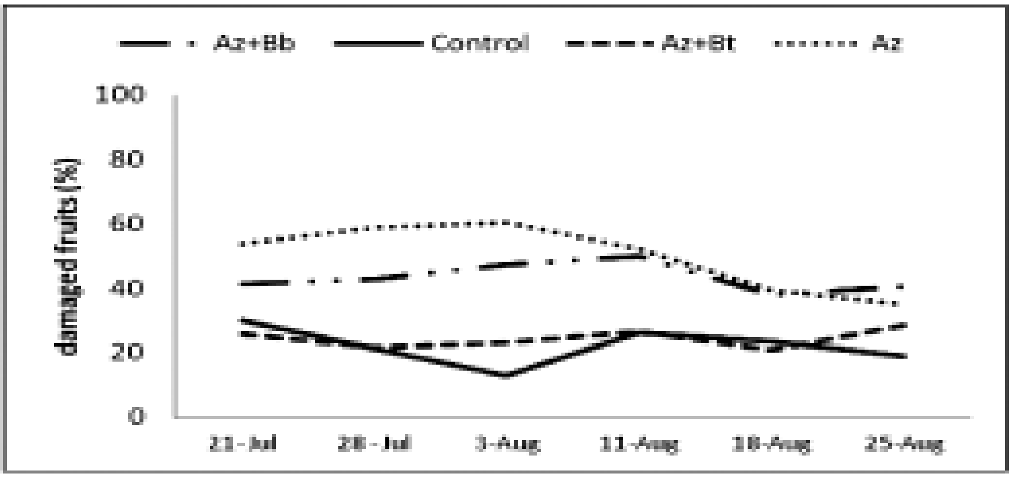

Figure 3, percentage of damaged Tomato fruits (weight of damaged fruits/total weight of harvested fruits*100) from plants differently treated from July 21st to August 25th 2011. Az = Azadirachtin; Az+Bb = Azadirachtin + *B. bassiana*; Az+Bt = Azadirachtin + *B. thuringiensis*; Control = synthetic insecticides.

Considering the mean marketable production, the fruits that were not damaged, for each treatment, (Figure 4), the maximum and minimum production were obtained from plants treated with *Az* + *Bt* and with Az (6471 ± 382 g and 2740 ± 280 g, respectively P = 0.015).

The production from plants treated with *Az* + *Bb* (3703 ± 398 g) was not significant different from the plants treated with *Az* + *Bt* or *Az* (P = 0.064 and P = 0.504, respectively). Compared to the control (8195 ± 420 g), statistic differences were evidenced for *Az* + *Bb* and *Az* (P=0.004 and P=0.001, respectively). On the contrary no significant differences between control and *Az* + *Bt* were found and for both experiments of 2011/2012.

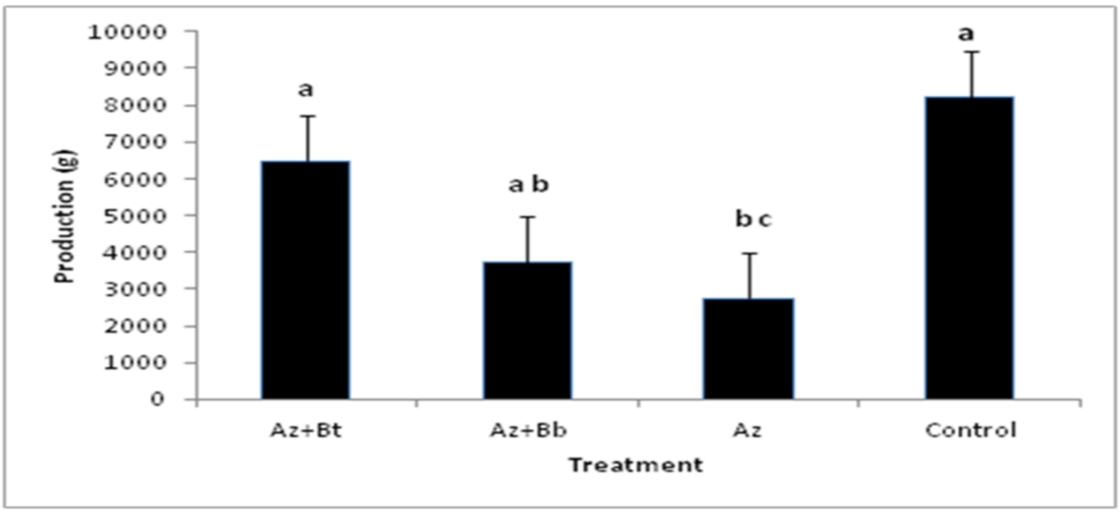

Figure 4, marketable Tomato fruits harvested from plants differently treated from July, 21st to August, 25th 2011. Az = Azadirachtin; Az+Bb = Azadirachtin + *B. bassiana*; Az+Bt = Azadirachtin + *B. thuringiensis*; Control = synthetic insecticides.

In the experiment of 2011 the non-infested tomato fruits/g those treated with *Az* + *Bb* was not significant different from those treated with *Az* alone (P = 0.064 and P = 0.504, respectively). Compared to the control, statistic differences were evidenced for *Az* + *Bb* and *Az* (P=0.004 and P=0.001, respectively) (Figure 5).

Meanwhile, in the experiment of 2012 the non-infested tomato fruits/g those treated with *Bt* alone was not significant different from those treated with *Az* alone. (P = 0.064 and P = 0.504, respectively). Compared to the control, statistic differences were evidenced for *Bt* and *Az* (P=0.004 and P=0.001, respectively) (Figure 6)..

On the contrary and in both experiments of 2011/2012 no significant differences between control and *Az* + *Bt* were found.

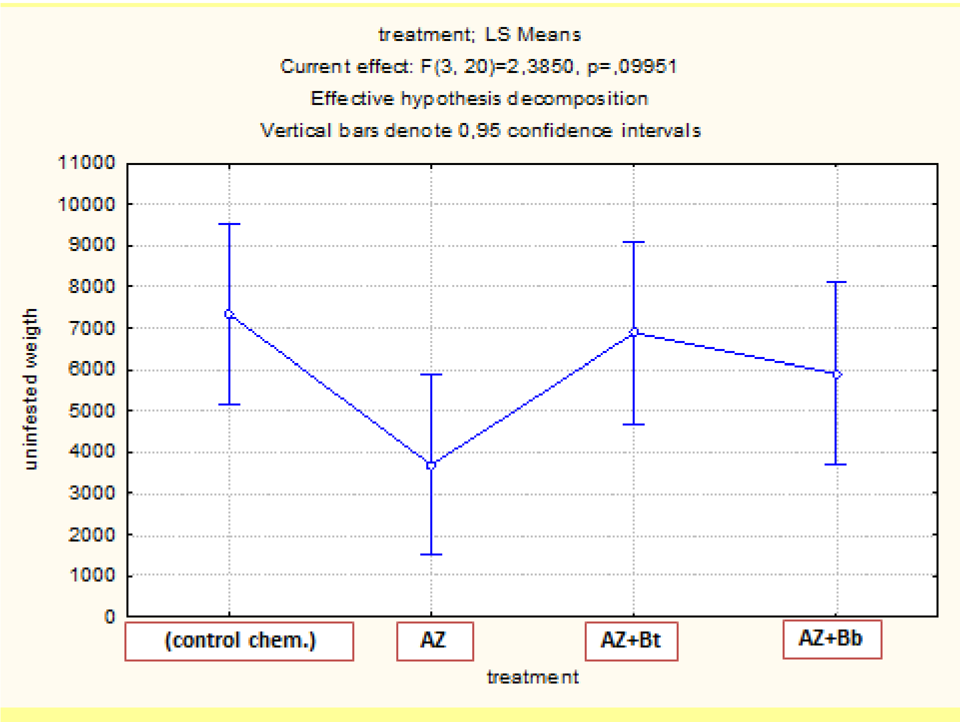

Figure 5, non-infested Tomato fruits/g the experiment of 2011

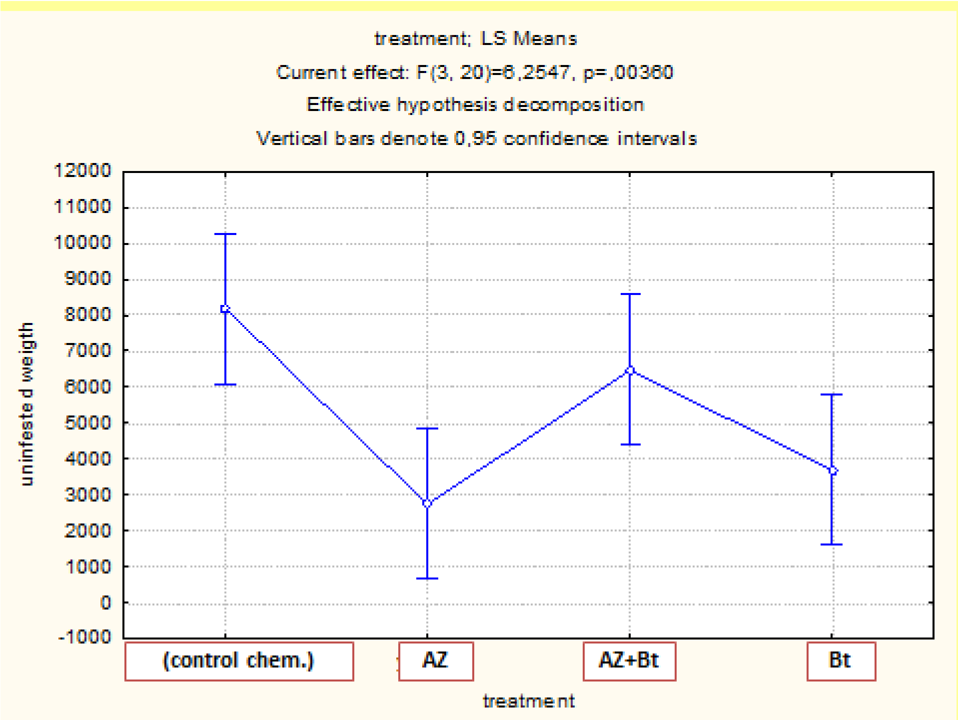

Figure 6, non-infested Tomato fruits/g the experiment of 2012

Discussion

The results obtained in Tomato open-field cultivation experiments of 2011/2012 reveal that it is possible to reduce the Tomato leaf miner impact applying birational insecticides. In particular, the *Azadirachtin* + *B. thuringiensis* combination offers promising results in controlling the pest.

The potential of Bt formulates in controlling *T. absoluta* was clearly demonstrated in laboratory tests (Giustolin et al., 2001; Lolas and Meza-Basso, 2006; Giulianotti, 2010; Gonzáles- Cabrera et al., 2011; Ladurner et al.; 2011). Moreover, Gonzáles-Cabrera et al. (2011) evidenced that Bt strains are able to reduce the pest's impact to very low levels when tested in greenhouse and open-field.

Furthermore, management of *T. absoluta* based on treatments with *Bt* doesn't induce resistance in phytophagous populations, that is a likely cause of field control failures (Silva et al., 2011), and could be associated with the use of parasitoids or predators (DE Medeiros et al, 2009;Mollá et al., 2011).

Azadirachtin has a knock-down power towards larvae of *T. absoluta*, as in laboratory test aqueous Neem seeds extracts induced high larval mortality by both systemic and trans laminar actions (Gonçalves-Gervásio and Vendramim, 2007). However, our results suggest that in open field cultivation treatment with *Azadirachtin* alone is not enough to reduce successfully Tomato leaf miner damages. Moreover, the adding of *B. bassiana* to *Azadirachtin* did not induce a better control of the pest.

Previous studies showed that, in laboratory tests, isolates of *B. bassiana* induce high mortality to Tomato leaf miner eggs and larvae (Giustolin et al., 2001; Rodríguez et al,, 2006), and the eggs are more susceptible than the first instars larvae (Pires et al., 2010). So that, this entomopathogenic fungus was considered a promising agents for control of *T. absoluta* in open Tomato fields (Torres Gregorio et al. 2009), however, our data showed that, in open field, the combination *Azadirachtin* + *B. bassiana* has a lower efficacy than the combination *Azadirachtin* + *B. thuringiensis* in controlling the Tomato leaf miner.

Conclusion

T. absoluta has become a key pest of Tomato in several world regions and its geographic distribution is rapidly expanding. The extensive insecticide use can cause on the one hand several undesired side-effect on human and environment safety, on the other hand resistance development in *T. absoluta*. In this view, is a necessary consequence applying environmentally- friendly strategies.

The results obtained in this study demonstrated that treatment based on *Azadirachtin* and *B. thuringiensis* combination can effectively reduce *T. absoluta* damage on Tomato open field cultivation. However, the sublethal effects of *Azadirachtin* on Tomato leaf miner natural enemies should not be ignored, as it was demonstrated that *Azadirachtin* significantly reduced the offspring of the predator Mirid bug *Nesidiocoris tenuis* females (Arnó and Gabarra, 2011). Therefore, further studies should be carried out to integrate this strategy with other integrated or biological control methods in order to reduce the use of chemicals and, consequently, improve food safety and environment quality.

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CHAPTER 3

Life history traits of the predatory mirid *Dicyphus maroccanus*, a new potential biological control agent in tomato crops

Chapter 3

Based on Abbas, S. et al. (in preparation)

Life history traits of the predatory Mirid *Dicyphus maroccanus* (Wagner 1951) (Hemiptera: Miridae), a new potential biological control agent in Tomato crops

Sadek Abbas, Meritxell Pérez-Hedo, Stefano Colazza,, Alejandro Tena and Alberto Urbaneja

Abstract

In 2008, the omnivorous predator Dicyphus maroccanus (Wagner 1951) (Hemiptera: Miridae) was firstly detected inhabiting Tomato crops in Valencia region (East Coast of Spain). Since then, D. maroccanus has been commonly found preying on eggs of T. absoluta (Meyrick, 1917) (Lepidoptera: Gelechiidae) in this area. To evaluate its potential as biological control agent, the life history traits of this predator were studied under laboratory conditions with [(eggs of Ephestia kuehniella Zeller (Lepidoptera: Pyralidae)] and without prey on Tomato plants. Immature stages of this predator successfully developed preying on eggs of E. kuehniella; however, none of the larvae tested was able to complete development on the plant without supplemental food. The immature development time was approximately 19.5 days for both males and females, being their immature survival of 85 %. Larvae of D. maroccanus consumed 267 and 312 eggs of E. kuehniella, to reach male and female adulthood, respectively. Females of D. maroccanus produced 51 larvae per female throughout their 16 days of lifecycle. The progeny sex ratio (produced females/total offspring) was 75.6. Net reproductive rate (R0) was estimated at 34.52 ± 0.52 female eggs/ female, generation time (T) was 28.28 ± 0.13 d and the estimate of intrinsic rate of increase (rm) was 0.1254 ± 0.0001 females/female/day. Some of these values are well above those reported for other Mirid predators on Tomato crops, indicating the potential as biocontrol agent of D. maroccanus on Tomato crops in the Mediterranean Basin.

Key words: Miridae, T. absoluta, Ephestia kuehniella, development time, intrinsic rate of increase, biological control.

Introduction

During the recent ten years many IPM strategies in horticultural crops have been moved from using specific natural enemies toward the use of generalist predators. Thanks to study and technical efforts devoted during the last decade to survey, select and produce new natural enemies, Spain has currently become one of the pioneers among the global states in using generalist predators.

Generalist predators represent the basis of Spanish IPM strategies in vegetable crops such as Tomato [(*Nesidiocoris tenuis* (Reuter) or *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae)], sweet pepper [*Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae) and *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae)] and cucurbits [*A. swirskii*] (Zappala, L. et al, 2013).

Predatory Mirid bugs (Hemiptera: Miridae) are generalist zoophytophagous predators that naturally appear and attack invertebrate pests in horticultural crops in the Mediterranean basin. Mass rearing systems for some Mirid species have been developed and two species *Nesidiocoris tenuis* (Reuter) and *Macrolophus pygmaeus* (Rambur) have been developed into commercial biocontrol-products (van Lenteren 2012).

Both Mirid predators are primarily released to control whiteflies and the Tomato leaf miner *T. absoluta* (Meyrick) a new invasive pest, which had a devastating effect on Tomato production when it was first introduced into Europe (Desneux et al. 2010). Furthermore, both species also provide partial control a range of other greenhouse pests (including aphids, thrips, mites, leafminers and other lepidopteran) (Messelink et al., 2011; Urbaneja et al. 2012).

Good Mirid establishment has also shown to increase the resilience of greenhouse production systems to new invasive pests (e.g. *T. absoluta*) in greenhouse crops, thus reducing (a) the risk of catastrophic crop losses and/or (b) the need for pesticide based intervention and associated negative effects on biological control agent/natural enemy populations in greenhouse (Urbaneja et al. 2012).

However, a factor limiting the more widespread use of Mirid is that some Mirid predators may also feed on plant tissue and can cause significant damage like the necrotic rings on different parts of Tomatoes plants that the fact of *N. tenuis* on Tomato especially in the absence of invertebrate pests and other prey (Sanchez 2008, Calvo et al. 2009a).

Figure 1, the necrotic rings on Tomatoes because of *Nesidiocoris tenuis* feeding

Looking for alternatives biocontrol agents was the main concerns of scientists worldwide. Through the field observations it's been clear that *Dicyphus maroccanus* has the ability to prey on *T. absoluta* (Mollá et al. 2010).

Although Mirid bugs are one of the most diverse groups of zoophytophagous insects found in natural ecosystems, there is little information on their basic biology traits. This is the case of *D. maroccanus*, a Mirid bug almost unknown until recently when was detected preying on *T. absoluta.*

Therefore, as a first step to evaluate the potential of *D. maroccanus* as biological control agent, the life history traits of this predator were studied under laboratory conditions with and using the eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae as nutrients and without prey on Tomato plants.

Alternatives is became as one of the scientific and ecological and yet environmental priorities here in Spain to be adopted and yet to adapt with. In recent times Instituto Valenciano de Investigaciones agrarias (IVIA of Spain) had found *D. maroccanus* (Wagner 1951) as eggs and young larvae predator of the Tomato leaf miner *T. absoluta* in the year 2009.

Consequently, this study is getting the first step to explore this predator by studying its biological parameters and then to compare its predation potentials with other predatory Mirids.

This study comes to clear those parameters such as “developmental time and juvenile survivorship, reproductive parameters, and demographic growth indexes of which the differences between the predator´s life history traits, such as development time from eggs to adult per each larva stage, the number of consumed eggs from eggs to adult per each larva stage, female longevity, and the progeny sex ratio were analyzed and presented and discussed, yet to consider whether *D. maroccanus* predation potentials as one of the biological control alternative agents or not.

Materials and Methods

- ***Developmental time and juvenile survivorship***

Eight adult couples of *D. maroccanus* were released separately in methacrylate cages with four Tomato plants. *Ephestia kuehniella* eggs were offered as a nutrition source every two days. One week later, plants bearing predator eggs were removed, cut into small sections and placed in petri dishes (60 mm diameter) with filter paper to avoid excess humidity. In order to observe the larvae hatching, the dishes were checked daily. Then, newly emerged larvae ($\leq$ 24 h old) were individually transferred to 60 mm Petri dishes.

Figure 2, *D. maroccanus* lifecycle parameters trials preparations

Predator´s larvae were supplied with *E. kuehniella* eggs, Tomato leaf disks (40 mm diameter) and water-soaked cotton wool as a source of water. The amount of eggs provided was constant according to predator developmental stage and was always higher than the daily requirement. Larval development (the presence of exuviae was used as an evidence of molting), larval survivorship and amount of preyed eggs were checked daily under a stereomicroscope until death or adulthood.

Figure 3, *D. maroccanus* new emerged larvae

Predator´s larvae were transferred daily to new Petri dishes with fresh Tomato leaf discs, moth eggs and water-soaked cotton. The Petri dishes were conserved in a climatic chamber at 25ºC, 60% RH, and 16:8 h L:D.

Figure 4, *D. maroccanus* larva exuviae as a development indicator

- ***Reproductive parameters***

Newly emerged *D. maroccanus* larvae originating from the larva development bioassay, were transferred in pairs to plastic glasses (370 cm3) with a tender apical Tomato flush (approximately 14 cm) in which 100 eggs of *E. kuehniella* were spread to the immature instars.

Following the methodology of Zappalà et al. (2012), adapted from Sánchez et al. (2009), the plastic glasses were placed inside smaller ones (230 cm3) that contained water. The apical Tomato flushes were pushed through a hole in the inner glasses to reach the water inside the bottom glass. Then, the bigger plastic glasses were covered by a fine net and fixed with a rubber band.

Adult couples were transferred to a new plastic glass every two days with 100 new eggs of their respective prey until the female died. To assume that mating occurred during the female lifecycle, each female was provided with a young male (obtained from the corresponding immature bioassay) that was replaced when it died. Daily checks of adult survival were performed, whereas larvae hatching inside the glasses in which the females have oviposited; were assessed every 2 days.

To measure the sex ratio of the progeny, the progeny of 14 females randomly chosen was left to develop until adulthood in the same arenas where they hatched and were provided with *E. kuehniella* eggs. Experimental arenas were maintained in a climatic chamber at 25ºC, 60% R.H., 16:8 h L:D.

- ***Demographic growth indexes***

Life tables were constructed using daily survival values and the number of progeny produced by the females. Therefore, the following demographic growth parameters were generated:

Net reproductive rate or basic reproductive rate:

$$R_o = \sum \mathrm{l_x\ m_x}$$

where x is the pivotal age of individuals in days, lx is the age-specific survival as proportion of individuals still alive at age x and mx is the age-specific fertility (Birch 1948). This parameter corresponds to the number of times a population will multiply per generation.

- ***Generation time:***

$$T = \sum \frac{\mathrm{x\ l_x m_x}}{\mathrm{R_o}}$$

where T represents the average time interval separating female births of one generation from the next (Birch 1948).

Intrinsic rate of increase:

$$r_m = \sum \mathrm{e}^{\mathrm{r_m\,x}}\ \mathrm{l_m\ m_x}$$

where r_m is the innate capacity of a given species to increase in numbers. Biologically, this parameter is the number of times the population will multiply per unit of time, and erm is the antilog of r_m (Birch 1948).

- ***Doubling time:***

$$DT = \frac{log_e 2}{r_m}$$

where DT is the time required for a given population to grow exponentially, without limit, to double in size when increasing at a given r_m (Mackauer 1983).

To estimate the standard error associated with rm, R0, T and DT the Jackknife technique was performed on the raw data to calculate the per capita rm, R0, T and DT, omitting one replicate per trial, and by repeating this process until pseudo-values were calculated for all the possible omission cases. Then, we computed the standard error by applying the Jackknife formula (Meyer et al. 1986).

- ***Statistical analysis***

The differences between the predator´s life history traits, such as development time from eggs to adult per each larva stage, the number of consumed eggs from eggs to adult for each larval stage, female longevity, and the progeny sex ratio were analyzed using student's t-tests.

Differences were considered significant at $P < 0.05$. The data regarding larva survival were analyzed using Kaplan-Meier survivorship curves. Prior to the analyses, the raw data were tested for normality and homogeneity of variance using either Kolmogorov-Smirnov's D test or Cochran's test and were transformed if needed.

Results

- ***Developmental time and juvenile survivorship***

The immature developmental time of *D. maroccanus* when fed *E. kuehniella* eggs and Tomato plant was approximately 19.5 days for both males and females (Table 1) being their immature survival of 85% from first larval instar until adult stage (Figure 5).

Meanwhile, *D. maroccanus* was not able to complete its immature development when only fed on Tomato plant and the major number of larvae died between second and third larval instar (Table 1 and Figure 5).

Instars	With *E. kuehniella*		Statistics		Without *E. kuehniella*
	D. m Male (n=11)	*D. m* Female (n=32)	t	P-value	
L1	4.6 ± 0.2	4.8 ± 0.2	0.64	0.52	2.7 ± 0.2 (n=21)
L2	3.7 ± 0.3	3.5 ± 0.2	0.66	0.50	6.6 ± 0.2 (n=6)
L3	3.6 ± 0.4	3.2 ± 0.2	0.95	0.34	11 (n=1)
L4	4.7 ± 0.4	5.0 ± 0.3	0.51	0.60	
L5	2.8 ± 0.4	2.9 ± 0.3	0.09	0.92	
Total	19.5 ± 0.4	19.4 ± 0.2	0.23	0.81	

Table 1, Developmental time of male and female larval instars of *D. maroccanus*, (days; mean ± SE), with and without addition of *E. kuehniella* eggs in Tomato at 25 ± 1º C and 16:8 h L:D. n= initial number replicates used. Means followed by the same letter within the same row and for the same predator species are not statistically different (P < 0.05)

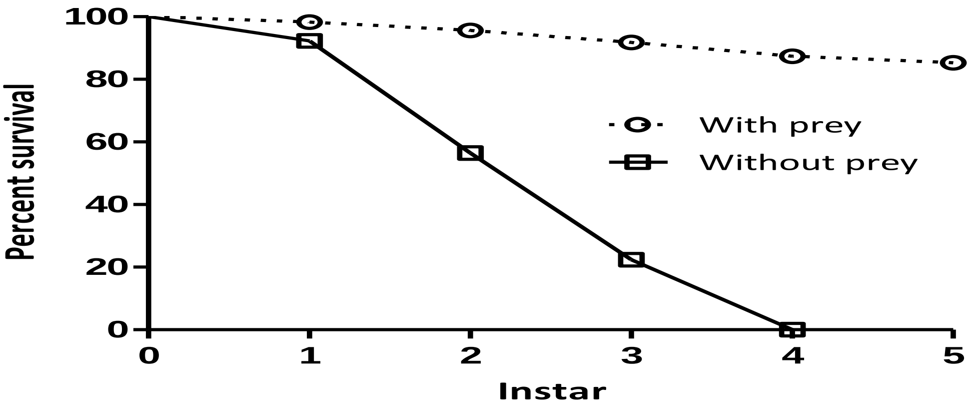

Figure 5, age-specific survival (%) of the immature stages of *D. maroccanus* with and without addition of *E. kuehniella* eggs in Tomato at 25 ± 1º C and 16:8 h L:D.

Larvae of *D. maroccanus* need to consume 267 and 312 to reach male and female stage, with no significant differences between both sexes (Table 2).

Instars	*D. maroccanus*		Statistics	
	Male (n = 63)	Female (n = 64)	t	P-value
L1	42.3 ± 3.5 a	45.0 ± 3.3 a	0.2774	0.7828
L2	56.0 ± 7.0 a	40.3 ± 3.27 b	2.262	0.0291
L3	56.3 ± 6.5 a	56.3 ± 5.5 a	0.007	0.9944
L4	77.1 ± 9.2 a	98.0 ± 9.0 a	1.259	0.215
L5	36.0 ± 9.0 a	72.0 ± 8.5 b	2.333	0.0247
Total	267.2 ± 16.6 a	312.9 ± 14.2 a	1.651	0.1063

Table 2, Mean numbers (± SE) of *E. kuehniella* eggs preyed upon by the different larval instars of *D. maroccanus* on Tomato plants at 25 ± 1º C and 16:8 h L:D.Means followed by the same letter within the same row and for the same predator species are not statistically different (*P* < 0.05)

- ***Reproductive parameters***

Females of *D. maroccanus* produced 50.8 ± 7.7 (n=11) larvae per female throughout their 15.8 ± 2.0 days of lifecycle. These values gave a daily fertility of 3.6 ± 0.7 larvae per female and day. The age-specific fertility curve (the number of larvae produced per day during the lifecycle of *D. maroccanus*) is presented in Figure 5. The higher daily fertility was between day 8 and 12 when tested females produced around ten larvae per day. The progeny sex ratio was 75.6 ± 1.6 (produced females/total offspring).

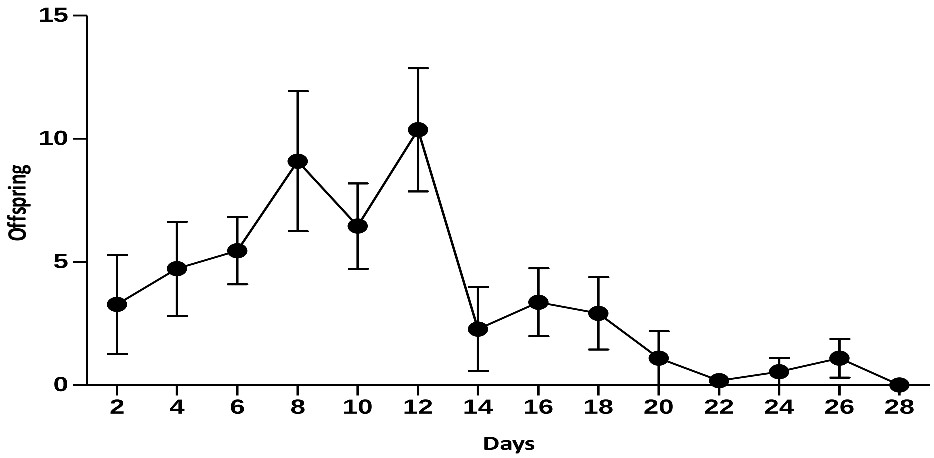

Figure 6, age-specific fertility (number of L1 produced/female/day ± SE) of *D. maroccanus* females when preying on *E. kuehniella* eggs on Tomato plants at 25 ± 1º C and 16:8 h L:D.

- ***Demographic indexes***

Effects of prey on the intrinsic rate of increase rm (females/female/day), net reproductive rate R0 (female eggs/ female), generation time T (days) and generation doubling time *DT* (days) of *D. maroccanus* is presented in Table 3.

Parameter	**Means values (± SE)**
r_m (females/female/day)	0.1254 ± 0.00006
R0 (net reproductive rate (female eggs/ female)	34.52 ± 0.52
T (generation time T/days)	28.28 ± 0.13
DT (generation doubling time/days)	5.54 ± 0.03

Table3. Means (± SE) of the intrinsic rate of increase r_m (females/female/day), net doubling time *DT* (days) of *D. maroccanus* when preying on *E. kuehniella* eggs on Tomato plants at 25 ± 1º C and 16:8 h L:D. Means followed by the same letter within the same row and for the same predator species are not statistically different (*P* < 0.05)

Discussion

Mirid bugs are one of the most diverse groups of zoophytophagous insects found in natural ecosystems, but for most Mirid species there is little information on the (a) efficacy of predation and prey/pests range, (b) ability to establish on different greenhouse crops (c) level of crop damage they may cause and (d) compatibility with other important biological control agents.

IVIA (Instituto Valenciano de Investigaciones agrarias) IPM different strategies were to invest the environment assets to protect and sustain the agroecosystem from the indigenous and exotic pests. The main environment different assets are the generalist predator species. *N. tenuis* and *M. pygmaeus* were as the pioneers to reach this destination.

This study comes to provide a detailed analysis of biological different parameters of *D. maroccanus* when preying upon the eggs of *E. kuehniella* (as a factitious prey) on Tomato plants comparing with *N. tenuis* and *M pygmaeus* since the late predators had been recently studied.

The results of this study show that the life history variables varied of *D. maroccanus* comparing with the two predator species *N. tenuis* and *M. pygmaeus*, when they prey on *E. kuehniella* eggs. The *D. maroccanus* juvenile development time was approximately six days longer than *N. tenuis* and four days longer than *M. pygmaeus*, that fact coms as a first positive indicator of that predator just because it assumes that *D. maroccanus* staying in the field longer than the other two predators, consequently consuming more eggs and finally to effect greatly on the pest population.

The overall number of *E. kuehniella* eggs consumed by the *D. maroccanus* larval instars was 267.2/for males and 319.9/for females (Table 3), and that comes as the three folds than in the other predator species *N. tenuis* and *M. pygmaeus* since they were (104.9 and 114.8) respectively. That fact comes as a second positive indicator because it speculate that high number of the pest eggs being consumed by this predator and then the number of the pest itself will be decreased.

The differences in *E. kuehniella* eggs consuming among those three predator species could be attributed to the habitat preference nature of the predator itself in one hand

and in other that represents such a good indicator among the predator required features.

Analyses of the reproduction data indicate that the reproductive capacity of *D. maroccanus* was slightly higher than *M. pygmaeus* and pretty lesser than *N. tenuis* in terms of daily values and in cumulative lifetime fertility levels.

D. maroccanus produced higher progeny numbers 76.6 than the situation in *N. tenuis* and *M. pygmaeus* 48.6 and 49.2 respectively, when preying upon *E. kuehniella* eggs. And that fact represents the third positive indicator of *D. maroccanus* than the other two predator species since that means producing more progeny effecting greater effects on the pest eggs and consequently a big damage on the its population.

That fact also suggesting that this prey is a suitable food source for the population development of *D. maroccanus*. Since adequate nutrition is essential for organisms to attain optimal fitness and to realize their maximal reproductive potential, these effects need to be investigated in order to determine their overall impact on the population dynamics (Thompson 1999).

Another suitable hypothesis for the differences found in the reproductive capacity of those three Mirids on *E. kuehniella* eggs may be related to the differing degree of omnivore present among those three Mirid species. It is possible that *D. maroccanus* was able to better compensate for the suboptimal nutrition offered by *E. kuehniella* eggs and also integrated by feeding on the Tomato plant different tissues. Further studies are needed to clarify what is driving this difference in fertility levels among those predators.

The estimated population growth indexes of *D. maroccanus* on *E. kuehniella* Tomato crops were in between with the other two predators since the rm values estimated in this study for *D. maroccanus* was 0.125 and for *M. pygmaeus* and *N. pygmaeus* were 0.103 and 0.160 females/female/day , respectively;

whereas the R0 value for *D. maroccanus* was the highest value comparing with the other two predators since it was 34.52 and for *M. pygmaeus* and *N. pygmaeus* were 20.04 and 32.97 female eggs/ female, respectively, when all feeding on *E. kuehniella*. These results are particularly important in regard to the role of predating and rearing features and yet in predator nutrition and finally for assessing effects in terms of population demographics.

Finally *D. maroccanus* female longevity is higher than *N. tenuis* and *M. pygmaeus* since they were 31.6, 18.2 and 22.0, respectively. This fact represents the fourth positive indicator because as longer as the female is staying as good as for the whole predator population presence and then for extending its lasting.

Here is worthy to mention that the necessity of conducting other bioassays in order to clear the biocontrol activity and efficiency of *D. maroccanus* as a new promising alternative generalist predator, and yet to assess the expecting damage on the Tomato leaf miner *T. absoluta* or other Tomato key pests just as it had been presented by Urbaneja et al. (2008) when *N. tenuis* and *M. pygmaeus* been tested under greenhouse conditions. Moreover, to clear the diversified favorability of *D. maroccanus* since the generalist predators are commonly known theirs high prey diversity in the crop (Symondson et al. 2002).

As a conclusion *D. maroccanus* lifecycle parameters advantages of which implementing efforts of that predator under the Mediterranean basin environment and condition being achieved, nevertheless, the spontaneously presence of that predator along with the most of the Solanaceae, or nightshades, family different varieties. *D. maroccanus* lifecycle parameters are showing that there are such possibilities to invest that predator by adopting and then by adapting it and yet to be as one of the promising IPM strategies for time to come. Moreover the field observations showed that there were no side effects or harm features for that predator on the Tomato crop (Mollá et al. 2009).

Conclusion

Most of *D. maroccanus* lifecycle parameters being studied on Tomato crops and *E. kuehniella* eggs such as survivorship, developmental time, female longevity, eggs consumed, and progeny sex ratio of *D. maroccanus* are relatively higher than they are in *N. tenuis* and *M. pygmaeus*, consequently there will be good and promising possibilities to recruit *D. maroccanus.*

This study is highly recommending for future studies to be conducted to explore more about that predator potentials and its roles in IPM strategies in managing Solanaceae main pests such as the Tomato leaf miner *T. absoluta* (Meyrick, Lep. Gelechiidae), the greenhouse whitefly (*Trialeurodes vaporariorum*), tobacco whitefly (*Bemisia tabaci*), two-spotted spider mite (*Tetranychus urticae*), thrips and finally moth eggs.

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CHAPTER 4

Plant preference in the zoophytophagous generalist predator *Dicyphus maroccanus*

Chapter 4

Based on Abbas, S. et al. (in preparation)

Plant preference in the zoophytophagous generalist predator *Dicyphus maroccanus* (Wagner 1951) (Hemiptera: Miridae)

Sadek Abbas, Meritxell Pérez-Hedo, Stefano Colazza, Alejandro Tena and Alberto Urbaneja

Abstract

Dicyphus maroccanus (Hemiptera: Miridae) is an omnivourus predator has been detected in Spain 2010. D. maroccanus spontaneously presented in and around the Tomato open fields along with the presence of the Tomato leaf miner Tuta absoluta. Its lifecycle parameters have been studied in Spain 2013. With the aim to explore the relationship between this predator and its key host plant different status being infested with eggs and larvae of T. absoluta compared with the healthy Tomato have been investigated and studied. Plant preferences were measured by multi-choice host plant selection and olfactometric bioassays based on the least attractive under laboratory conditions. The results showed that D. maroccanus has been strongly attracted by the Tomato plants comparing with no plants jar. D. maroccanus has been attracted strongly to the plants infested with T. absoluta eggs infested tomato comparing with non-infested plants. Its preference ability to the infested plants with T. absoluta larvae higher than the plants being infested with T. absoluta eggs. The fourth trial showed that D. maroccanus been strongly attracted to the Tomato was plants being infested with the T. absoluta larvae than those been infested with T. absoluta eggs. This study suggested that D. maroccanus establishment in the field should take in consideration the continuing presence of that predator to ensure proper and adequate control to T. absoluta different phases in particular the eggs phase since that predator showed high preferences to Tomatoes being infested with T. absoluta eggs.

Key words: Dicyphus maroccanus, Tomatoes, Tuta absoluta, olfactometric bioassays

Introduction

Dicyphus maroccanus (Hemiptera: Miridae) is an omnivourus predator. It has been observed and detected in Spain 2010. *D. maroccanus* is spontaneously presence in and around the Tomato open fields along with the present of the Tomato leaf miner *Tuta absoluta* (Mollá 2010). Its lifecycle parameters have been studied in details in IVIA of Spain 2013.

D. maroccanus (Hemiptera: Miridae) as a species is belonging to the subfamily Bryocorinae, tribe Dicyphini, that represented generalist predators well-known for their role in the control of several pests (e.g. aphids, mites, moths, thrips, whiteflies) in horticultural crops in Europe (Avilla et al., 2004).

In fact, starting from the end of 1980s, their presence was reported in IPM vegetable crops of different regions of southern Europe, as a consequence of the reduced insecticide pressure (Tavella, L., Goula, M., 2001). In particular, unlike other predatory bugs, such as anthocorids of the genus *Orius* that are hampered by glandular trichomes (Coll and Ridgway, 1995), Tomato plants represent a very suitable host for Dicyphini (Riudavets and Castañé, 1998; Tavella and Goula, 2001).

Dicyphini are characterized by zoophytophagous behavior, thus they are strictly related to the plant besides the prey. Zoophytophagy is positive because predators can survive in the crop even when prey is scarce or totally absent (Eubanks and Denno, 1999). The plant can provide not only water essential for predation (Sinia et al., 2004), but also nutrients. In fact, some species can develop and reproduce in the absence of prey by feeding on plants, but only on some plant species or even on some parts of them (Lucas and Alomar, 2001).

Plants release volatile compounds that vary quantitatively and qualitatively that are depending on plant species and attacks of specific pests, and able to attract predators (Paré and Tumlinson, 1999; Dudareva et al., 2006).

Concerning host range, Dicyphini show a preference for glandular and sticky plants; in fact, most of the northwestern Italian species have been collected on hairy plant species those belonging to Solanaceae, Lamiaceae and Geraniaceae (Ingegno et al., 2008; Tavella, L., Goula, M., 2001).

Among the Dicyphini species colonizing Tomato crops in the Mediterranean region, the species initially identified as *Macrolophus caliginosus* Wagner seemed to be the most promising:

Their density within crops in fact can be related to composition and abundance of the surrounding vegetation and to topographic characteristics, suggesting the importance of host plant proximity to enhance early movement of these predators into the fields (Alomar et al., 1994; Gabarra et al., 2004).

Recently, studies on responses of some Dicyphini species to volatile compounds produced by plants and prey were carried out above all in laboratory conditions (McGregor and Gillespie, 2004; Moayeri et al., 2006a,b, 2007a,b). To enhance the presence and activity of Dicyphini in the crops, the factors stimulating predatory bugs to leave the natural host plants and colonize the crop should be investigated thoroughly for an ecologically and economically sustainable farming.

D. maroccanus laboratory lifecycle studies showed that this predator survival, reproduction and intrinsic rate of increase were highly favored comparing with other predators *N. tenuis* and *M. pygmaeus* when fed on *E. kuehniella* frozen eggs and under the same conditions. Moreover the results of those studies showed its predatory habits and its ability to still alive until the larval instar 4 without praying. (Urbaneja et al., 2008))

Thus this study has been aimed at assessing preference and bio-ethological responses of the zoophytophagous *D. maroccanus* on healthy and on *T. absoluta* eggs and larvae infested Tomato seedlings under the laboratory conditions.

Yet to evaluate host plant preference of the predatory species *D. maroccanus* since those bioassays are so important when the decision is being set to establish that predator within the Tomatoes crops in the open and covered fields, finally that parameter will be considerate as a positive indicator among *D. maroccanus* other positive lifecycle parameters been recently gathered by its lifecycle biological parameters IVIA of Spain 2013.

Materials and Methods

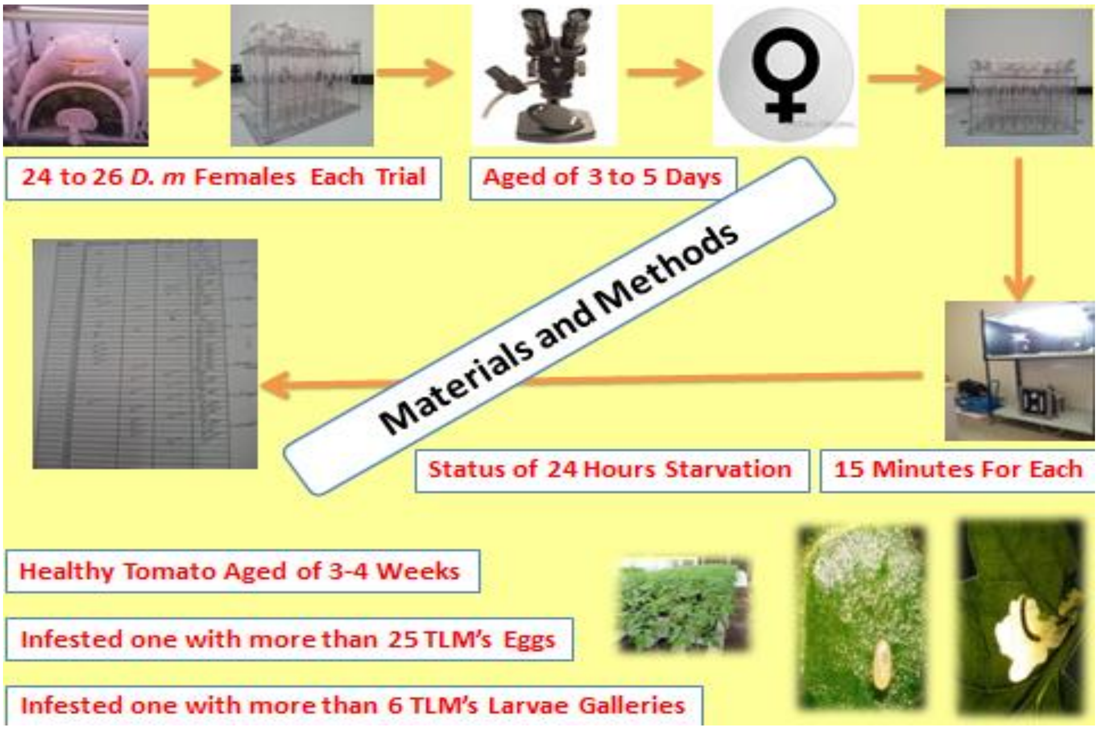

Figure 1; Y-tube bioassay preparations

- **Plants and insects**

Tomato seedlings (var. Bodar) used in those bioassays had been obtained by IVIA greenhouses from a local seed source. *T. absoluta* and *D. maroccanus* individuals were initially collected in Tomato fields located in Valencia province (Spain) and then reared on pesticide-free Tomato seedlings.

T. absoluta rearing took place in screened cages (120 x 70 x 125 cm) in which groups of six Tomato plants (approximately 30 cm high) were introduced weekly (25 ± 1° C, 60 ± 5% R.H.). Into the *T. absoluta* rearing, adults were collected using a mechanical aspirator (Hausherr's Machine Works, Toms River, New Jersey) when necessary. Using a fine paint brush and a stereoscopic binocular microscope, newly laid eggs (≤ 24 h old) were carefully collected from 30 days old Tomato plants that had previously been exposed for 24 hours inside *T. absoluta* rearing.

The same methodology mentioned above was followed to obtain Tomato plants (approximately 30 cm high) infested with *T. absoluta* larvae. Egg infested plants were left undisturbed until the desired larval instar was reached.

D. maroccanus have been obtained from the rearing stock of *D. maroccanus* those have been maintained with *E. kuehniella* frozen eggs, obtained from mass rearing (Koppert Biological System, Águilas, Spain). *D. maroccanus* females have been prepaird to y tube bioassay leted 24 hours of starvation.

- **Y-tube olfactometer**

Y-tube olfactometer (Analytical Research Systems, Gainesville, FL) was used to test the attraction of the omnivorous predator *D. maroccanus* (Hemiptera: Miridae) females prey-related odor. The system consists of a central tube (13.5 cm long, 24mm diam.) and two lateral arms (5.75 cm long, 24mm diam.) which are separately connected to an extending glass tube (14.5 cm long, 19 mm diam.).

There is a sieve inlayed in the extending glass tube 5.25 cm away from the connection to prevent escaping of insects and to serve as an end point of each lateral arm. Humidified and purified air was passed into the extending glass tube through a Teflon connection at 150 mL/min. To minimize visual distraction for the predator females, the Y-tube olfactometer was placed inside an steel bench like the structure of a box, which was allow from the top (for properly illumination) and on the front side (for observation). Illumination was provided by vertically hanging an office lamp (20W, 250 Lux) above (50 cm high) the olfactometer tube.

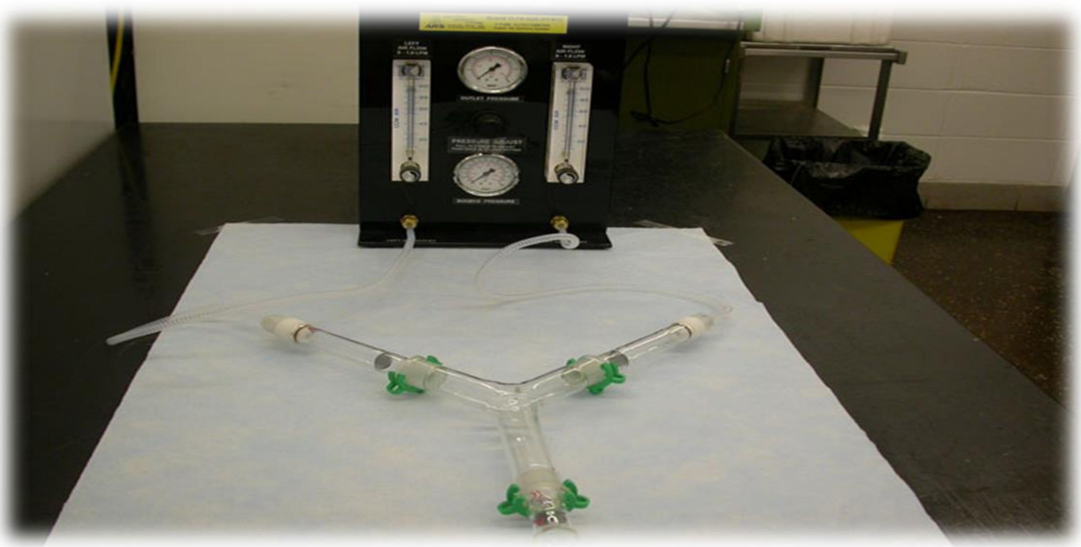

Figure 2; Y-tube bioassay preparations

The first experiment was conducted to test the attractiveness of 24 hour starved females of *D. maroccanus* (aged from 3 to 5 days) to the Tomato seedling odor

comparing with empty Jar in a Y-tube olfactometer. In these experimental 26 females of *D. maroccanus* had been bioassayed.
Tomato plants being used in this experiment were at 5 to 6 week age Tomato seedling with 25 to 35 leaflets. That plant was in a healthy status and growing from plastic pot that including natural soil mixed with local peat moss. *D. maroccanus* females were introduced individually into the central arm of the Y-tube.

The initial choice of a female that responded by walking into one of the arms (choice chambers) and remaining there at least 15 seconds was recorded. If a female had not made a choice within 15 min after being released, it was removed and discarded. Females that did not walk toward any of the arms were not counted.

After five individual females have been tested, the olfactometer arms were flipped around (180°) to minimize positional effect. After 10 females had been bioassayed, females were discarded and replaced with others, and the olfactometer set-up was rinsed with soap water, and acetone, and then air-dried.

In this experiment and the following experiments, 24 to 26 adult females of *D. maroccanus* were bioassayed, and females were used only once. The same protocol being used in this experiment has been repeated in the three following experiments.

A second experiment was conducted to test the attractiveness of *D. maroccanus* females to healthy Tomato seedling and to Tomato plant that infested with up to 40 eggs of *T. absoluta* 24 hours earlier to the experiment conducting time, using similar procedures described in the first experiment.

A third experiment was conducted to test the attractiveness of *D. maroccanus* females to healthy Tomato seedling and to Tomato plant that infested with up to 9 galleries of *T. absoluta* larvae that being prepared 24 hours earlier to the experiment conducting time, using similar procedures described in the first experiment.

A fourth experiment was conducted to test the attractiveness of *D. maroccanus* females to Tomato plant infested with up to 40 eggs of *T. absoluta* that being prepared 24 hours earlier to the experiment conducting time and to Tomato plant infested with up to 9 galleries of *T. absoluta* larvae that being prepared 24 hours earlier to the experiment conducting time, using similar procedures described in the first experiment.

The experimental atmosphere been adjusted by keeping its temperature constantly within 25 ± 1°C range in order to keep *D. maroccanus* females those being kept at that range for the previous 24 hour.

- **Data analysis**

  X^2 goodness of fit tests was also used to test whether the relative numbers of responding and non-responding individuals recorded were independent of odor source treatments.

Results

Odor-Source Pairs	Number of responses to stimulus test	Number of Responses to control Stimulus	Number of Non Responders	X^2	P
Pant and no Plant	**20**	**4**	**0**	**21.33**	**<0.0001**
Plant infested with *T. a* eggs and healthy Plant	**16**	**7**	**3**	**7.043**	**0.008**
Plant infested with *T. a* Larvae and healthy Plant	**22**	**0**	**4**	**44**	**<0.0001**
Plant infested with *T. a* eggs and Plant infested with *T. a* Larvae	**5**	**21**	**2**	**19.69**	**<0.0001**

Table 1, Responses of *D. maroccanus* females to the odors of *Tuta absoluta* egg and larvae infested Tomato plants in a Y-tube olfactometer and number of non-responding individuals recorded for each odor pair. χ^2 statistics test the hypothesis that the distribution of side-arm choices of responding individuals deviated from a null model where odor sources were chosen with equal frequency

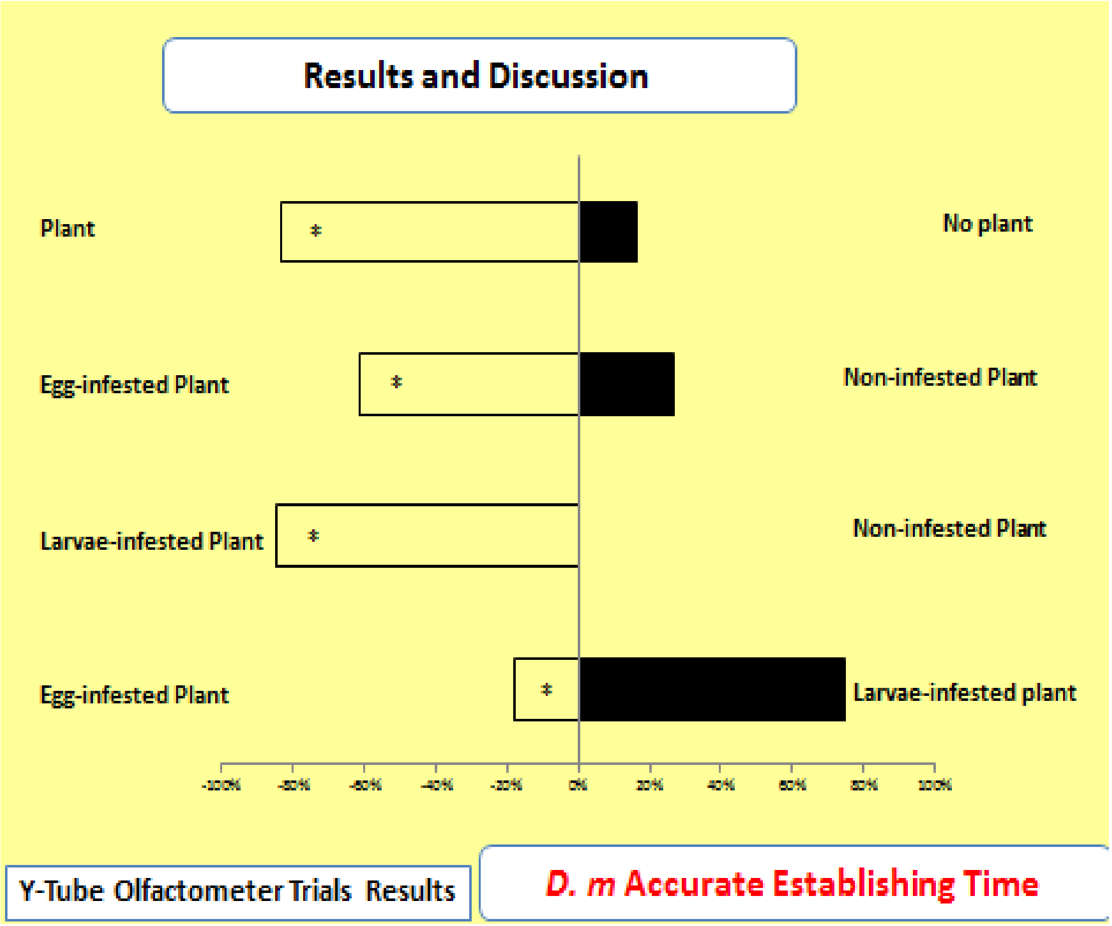

Figure 3; shows responses of *D. maroccanus* females to the odors of *T. absoluta* eggs and larvae infested Tomato seedlings in a Y-tube olfactometer and the non-responding of *D. maroccanus* females.

The results of the first experiment showed the *D. maroccanus* strong attractiveness toward the jar that contained a healthy Tomato seedling. In fact, 20 *D. maroccanus* females of 24 females (almost 80%) were attracted, while 4 females showed either no respond or responding toward the control jar.

The results of the *D. maroccanus* females' movements toward *T. absoluta* eggs/larvae infested Tomato seedlings (experiment 2/3 respectively) comparing with the health Tomato seedling were similarly positive. The responding *D. maroccanus* females in those both experiments were 16 of 26 and 22 of 24, respectively (Table 1).

The results of the fourth experiment showed the *D. maroccanus* females' strong attractiveness toward *T. absoluta* larvae infested Tomato seedling comparing with the *T. absoluta* eggs infested Tomato seedling since they were 21 of 28 and 5 of 28, respectively (Table 1).

Discussion

The results show that there are good and promising opportunities to recruit *D. maroccanus* as a new biocontrol agent for time to come, along with the other Mirids being adopted worldwide like *N. tenuis* and *M. pygmaeus*. 80 percent of 24 hours starved females showed strong attractiveness to the Tomato seedling that comes with the fact that the Tomato seedling blended odors has the attractant effects on the predator as an available nutrition source (Figure 1).

Figure 3 shows that 20% of *D. maroccanus'* females' were attracted toward *T. absoluta* eggs infested Tomato seedlings (experiment 2), meanwhile 80% of *D. maroccanus'* females' were attracted toward *T. absoluta* larvae Tomato seedlings (experiment 3) comparing with the uninfected Tomato seedlings. Similar results have been obtained by McGregor and Gillespie (2004), in which females of *Dicyphus hesperus* were attracted to the odor of Tomato leaflets infested with larvae of *Trialeurodes vaporariorum* compared to uninfected leaflets.

Meanwhile, Table 1 shows the results of the fourth experiment that demonstrated *D. maroccanus* females' attractiveness strongly toward *T. absoluta* larvae infested Tomato seedling comparing with the *T. absoluta* eggs infested Tomato seedling. They were 21 of 28 and 5 of 28, respectively that likely explained by the fact that; the collected odor from the *T. absoluta* larvae infested Tomato have the strong effect on *D. maroccanus* females by attracted them.

The hypothesis behind the case of *D. maroccanus* being attracted to the *T. absoluta* eggs and larvae infested Tomato is likely to have two explanations; the first explanation is related to the Tomatoes being attacked by *T. absoluta* and their emissions, since those emissions might include such a volatile odorants blend released by the plant itself as an emergency call (attracting factor) to the predator *D. maroccanus* in order to attack *T. absoluta* different than those volatile odorants being released by the healthy Tomato seedling (tritrophic relationship)*.

***The tritrophic interactions regulating the plant-prey-predator relationships are very complicated in the case of zoophytophagous Mirid bugs. In spring, predatory bugs, especially fertile females, migrate from winter refuges onto Tomato where, if not disturbed by chemicals, they establish and contribute efficiently to control pest outbreaks (Tavella et al., 1997).**

The second explanation is related to the predator appetite behavior itself, since the starvation for 24 hours enforced the predator's female to be attracted toward to the infested Tomato seedlings whether they were infested with the eggs or with the larvae of *T. absoluta*.

However good and proper establishing of *D. maroccanus* in the field ensures good and proper *T. absoluta* management. An earlier colonization of Tomato seems to be affected by the presence and abundance of natural host plants, rather than by their abundance in the agroecosystem (Alomar et al., 2002; Ingegno et al., 2009). Plant preference of a zoophytophagous as *D. maroccanus* may have a great impact on population dynamics of prey in crops. The high preference might be exploited to enhance the use of the predator in tomate and to develop cultural practices which enhance pest control.

D. maroccanus showed high preference to *T. absoluta* eggs and larvae infested Tomatoes leaflets. *D. maroccanus* didn't show any negative side effects because of its feeding on Tomato along with 27 tested Tomatoes samples and those facts come to be add to *D. maroccanus* other positive indicators and features.

Conclusion

This study showed that zoophytophagous generalist predator *D. maroccanus* has very good preference to Tomato plants and to the presence of *T. absoluta* as a one of herbivores, since Tomato volatiles induced by *T. absoluta* feeding are strongly attracting natural enemies of the herbivores that feature been found in *D. maroccanus*.

That suggests that there will be good opportunities and new options for putting this predator within the sort of valuable assets of any of the IPM strategies against *T. absoluta* or any other key pests all over the world and for generations to come in order to reduce the use of chemicals and, consequently, improve food safety and environment quality.

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CHAPTER 5

Dicyphus maroccanus in IPM of *Tuta absoluta* under semifield conditions

Chapter 5

Based on Abbas, S. et al. (in preparation)

Dicyphus maroccanus (Wagner 1951) in IPM of *Tuta absoluta* (Meyrick, 1917) (Lep.: Gelechiidae) under semifield conditions

Sadek Abbas, Meritxell Pérez-Hedo, Stefano Colazza,, Alejandro Tena and Alberto Urbaneja

Abstract

Spain comes as the ninth among the top ten Tomato producers worldwide. This important economically fact requires specific prerequisites and needs in order to conserve and protect that national production and to keep the agroecosystem in healthy and conserved status. During the recent ten years most of the world IPM strategies are being flowed toward generalist predators. Spain is one of the pioneers among the global states in this field since generalist predators are representing one of the important Spanish biological and integrated pest management strategies and vital assets; along with several studies those have previously stressed the important role of Mirid bugs in controlling leafminers. The biological and integrated pest management have considerably developed these last decades. In this context a large scale of biological control agents is being worldwide commercialized to control plant different pests'. To insure a successful introduction of a native or exotic biological control agent in an agro-ecosystem, appropriate agent should be carefully chosen and yet to be well acquainted with it. A biological control program deeply depends on biological agent intrinsic characteristics' and also on its interactions with all the biotic and abiotic components of the introduction area. The main objectives of this study are; to introduce the predator Dicyphus maroccanus as a native biological control agent in Tuta absoluta (Meyrick, Lep: Gelechiidae) management program and to explore its IPM potentials roles under semifield conditions. Since there was some study that getting the first step to explore the lifecycle and biological parameters of this predator in IVIA of Spain-October 2013, moreover to explore that predator host and prey preference. The current *study had consisted of 5 treatments 4 replications each, seven Tomato plants each replication. The treatments included D. maroccanus alone in 2 different releasing rates (high and low), D. maroccanus with the biorational insecticides combination of Azadiractine plus Bacillus thuringiensis, the combination of Azadiractine plus B. thuringiensis alone, and the control treatment. Azadirachtin was tested as Neem at 3cc/1L/ plant and B. thuringiensis var. kurstaki was used at 0.333g/1L/plant The high releasing rate of D. maroccanus was at one couple each plant and the low releasing rate was one couple each two plants. The results clearly showed that there were no side effects of the combination of Azadiractine plus B. thuringiensis on D. maroccanus; moreover, the treatment of D. maroccanus (high releasing rate) with the biorational insecticides combination of Azadiractine plus B. thuringiensis demonstrated the lowest number of Tomato infested leaflets comparing with other treatments. In addition that combining use showed the highest efficacy in T. absoluta infestation reduction percentage comparing with the other treatments. Finally, this study highly suggests that there are good and promising IPM potentials roles of Dicyphus maroccanus in particular with the biorational insecticides combination of Azadiractine plus B. thuringiensis to control the Tomato leaf miner T. absoluta. Further studies should be carried out to integrate this strategy with other integrated or biological control methods and under different conditions, in order to reduce the use of chemicals and, consequently, to improve our food safety and environment quality.*

Keywords: integrated pest management, biological control, Dicyphus maroccanus, Tuta absoluta, Tomato crops,

Introduction

The total Tomato production of the top ten producers worldwide is 120.733.873 tones (FAOSTAT 2011). Spain comes as the ninth among them with production reached 3.821.490 tones after (China, India, USA, Turkey, Egypt, Iran, Italy, and Brazil). However, this important economically fact requires specific prerequisites and needs in order to conserve and protect that national production on one hand and to keep the agroecosystem in healthy and conserved status on another.

Some common Tomato pests are stink bugs, lepidopteran moths such as Tomato leaf miner (*T. absoluta*), cutworms, Tomato hornworms and tobacco hornworms, aphids, cabbage loopers, whiteflies, Tomato fruit worms, flea beetles, red spider mite, slugs, and Colorado potato beetles.

Unfortunately, human interventions, innovations and creative managements had been made some insects to be moved from just insects to pests and for some extent to key pests (invasive existence pests) such as *T. absoluta* by using carelessly, wrongly and in neglected way different sorts of harmful agrochemicals.

During the recent ten years most of the world IPM strategies are being flowed toward generalist predators. Spain is one of the pioneers among the global states in this field since generalist predators are representing the main and important Spanish IPM strategies and vital assets, by exploring, studying and yet to adopt them in such strategies.

Several studies have previously stressed the important role of Mirid bugs in controlling leafminers (Nedstam and Johansson-Korn 1999; Arno et al. 2003) and lepidopteran pests (Izquierdo et al. 1994; Agusti et al. 1999; Devi et al. 2002) in Tomato crops.

Generalist predators (Hemiptera, Miridae) like *Nesidiocoris tenuis* Reuter (Arno et al. 2009), (Mollá et al. 2009), (Urbaneja et al. 2009), *Macrolophus pygmaeus* Rambur (Arno et al. 2009), (Mollá et al. 2009), (Urbaneja et al.2009) and *Dicyphus maroccanus* Wagner (Mollá et al. 2010) are as eggs and young larvae predators of the Tomato leaf miner *T. absoluta* (Meyrick, Lep.: Gelechiidae) (Desneux et al 2010).

Those generalist predators (zoophytophagous predators) *N. tenuous*, *M. pygmaeus*, *D. tamaninii* and *D. hesperus*…etc. are endemic natural enemies that spontaneously appear in various crops in the Mediterranean basin (Alomar et al. 2002; Sanchez et al. 2009; Stansly et al. 2004; Urbaneja et al. 2005) of which the greenhouse whitefly (*Trialeurodes vaporariorum*), tobacco whitefly (*Bemisia tabaci*), two-spotted spider mite (*Tetranychus urticae*), thrips and moth eggs which are representing group of pests being successfully controlled by those generalist predators.

Recently, predation potential and life history aspects of *N. tenuis* and *M. pygmaeus* on *T. absoluta* have been tested under laboratory conditions (Arno et al. 2009; Mollá et al.2009; Urbaneja et al. 2009). Both predators actively attack *T. absoluta* eggs and larval stages, although they prefer first-instar larvae. Adults of both species consumed 100 eggs per individual per day, and *M. pygmaeus* larvae consumed less *T. absolute* eggs than *N. tenuis* larvae (Arno et al. 2009).

Nevertheless, (Mollá et al. 2009) showed that when *M. pygmaeus* and *N. tenuis* were well established in the crop, they were able to reduce leaflets infestation up to 75 and 97% or fruits infestation up to 56 and 100%, respectively (Desneux et al 2010).

Currently, there were some studies showed that *M. pygmaeus* is not performing well in South Europe. To adopt and yet to adapt alternatives is became as one of the scientific and ecological and yet environmental priorities here in Spain. In recent times Instituto Valenciano de Investigaciones agrarias (IVIA of Spain) had found *Dicyphus maroccanus* (Wagner) as eggs and young larvae predator of the Tomato leaf miner *T. absoluta* in the year 2009 (Mollá 2009).

Through the field observations it's been cleared that *D. maroccanus* has the ability to prey on *T. absoluta* (Mollá et al. 2010). Consequently, some study was gotten the first step to explore its biological parameters and then to compare its predation potentials with other predatory Mirids in IVIA of Spain 2013.

Those parameters included "developmental time and juvenile survivorship, reproductive parameters, and demographic growth indexes of which the differences between the predator´s life history traits, such as development time from eggs to adult per each larva stage, the number of consumed eggs from eggs to adult per each larva stage, female longevity, and the progeny sex ratio were presented, and they have been analyzed and discussed (chapters 3 and 4).

Chapters 3 and 4 of this study revealed that most of *D. maroccanus* lifecycle parameters are very good. Consequently, the same study had suggested further studies to be conducted for time to come to explore whether IPM potentials roles of *D. maroccanus* to control the Tomato leaf miner *T. absoluta* are positive or not. And whether this predator be as one of the biological control alternative agents or not

This study comes as a continuing effort of that *D. maroccanus* lifecycle parameters were studied under laboratory conditions in IVIA of Spain facilities 2013 (chapter 3) since the main objectives of this study are to introduce the predator *Dicyphus maroccanus* as a native biological control agent in *T.* management program and to explore its IPM potentials roles under semifield conditions.

Materials and Methods

This experiment was conducted in one of the Instituto Valenciano de Investigaciones Agrarias IVIA greenhouses in Moncada-Valencia of Spain, 2013. The climatic conditions were 23-27 °C, 65 ± 10 % RH and natural photoperiod.

- **Chemicals**

The biorational insecticides those consisted of Azadirachtin which was tested as a Neem at 3cc/1L/plant and *B. thuringiensis* var. kurstaki which was used at 0.333g/1L/plant. Spraying the combination of Azadirachtin plus *B. thuringiensis* in treatments 3 and 4 was once a week on the day after the samples collecting day.

- **Plants and insects**

Pesticide-free Tomato plants (30 cm high) variety ‘‘Optima” (Seminis Vegetable Seeds, Inc., Almería, Spain) were used in all the experiments’ different treatments. Tomato seedlings were transplanted into 8 x 8 x 8 cm pots.

The experiment was conducted in 20 plastic cagesof 60 x 60 x 60 cm (BugDorm-2; Mega View Science Co., Ltd.; Taichung, Taiwan). Seven Tomato plants (30 cm high) were in each bug dorm.

During the following seven weeks after *D. maroccanus* establishing in the plastic cages and after the *T. absoluta* releasing, one Tomato plant per cage was weekly removed and number of infested leaflets by *T. absoluta* and number of larvae and adults of *D. maroccanus* were counted in the greenhouse.

IPM potentials roles of the predatory Mirid *D. maroccanus* were studied under semifield conditions to Tomato leaf miner *T. absoluta* management. The study experiment was based on 5 treatments with 4 replications for each (7 plants for each replication). The experimental period was 8 weeks from mid of July to mid of September in 2013.

T. absoluta (6 couples / plant) collected from the rearing stock source were been released into those 20 plastic cages. Releasing rate of *T. absoluta* was as had previously mentioned weekly repeated (as reinfesting attempts) just one day after the combination of Azadiractine plus *B. thuringiensis* has been sprayed

Figure 1, the plastic cageswith 7 of Tomato seedlings each

➢ Experimental Design and Treatments

The experimental design was based on CRBD (Complete Randomized Block Design) the 20 plastic cages were being organized (Figure 2).

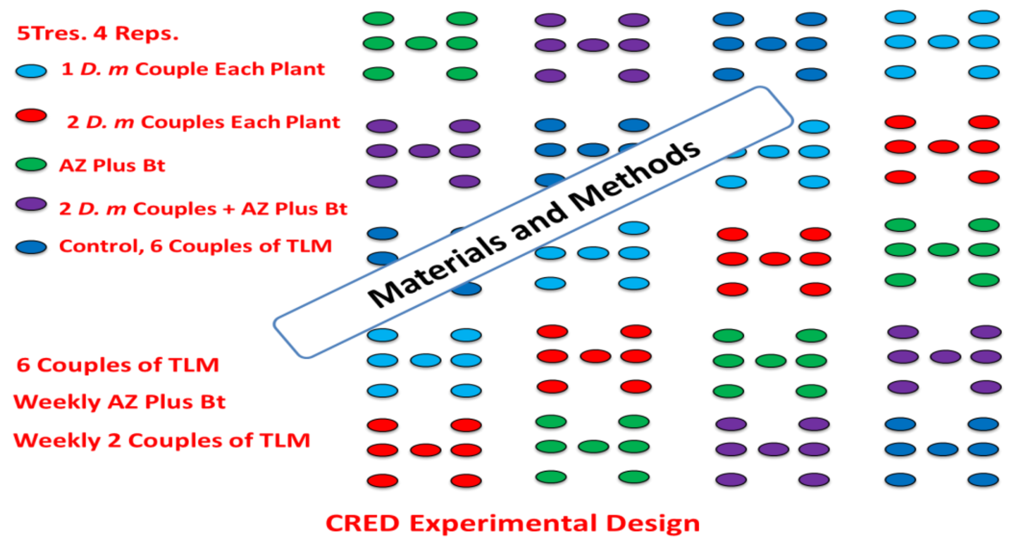

Figure 2, the experimental design (CRBD)

The five treatments of the study experiment are as a follow;

- **<u>Treatment no 1</u>**

D. maroccanus alone, (low releasing rate) releasing rate was at 1 couple every 2 plants and released in pre-transplant conditions, 7 plants multiple 4 replications equal to 28 Tomato seedlings, 14 couples of *D. maroccanus* have been used.

<u>Treatment no 2</u>

D. maroccanus alone, (high releasing rate) releasing rate was at 1 couple per plant and released in pre-transplant conditions, 7 plants multiple 4 replications equal to 28 plants, 28 couples of *D. maroccanus* have been used.

<u>Treatment no 3</u>

Azadirachtin plus *Bacillus thuringiensis*, *Azadirachtin* being used was as a (Neemazal-Intrachem), and *Bacillus thuringiensis* have been used was Costar 24,000 IU.Tni/m. *Azadirachtin* + *B. thuringiensis* (Az + Bt), this combination was at the doses of 3cc/1L/plant and 0.333g/1L/plant, respectively,

- **<u>Treatment no 4</u>**

D. maroccanus (1 couple per plant) combined with *Azadirachtin* plus *Bacillus thuringiensis*, 7 plants multiple 4 replications equal to plants, 28 couples of *D. maroccanus* adults have been used.

- **<u>Treatment no 5</u>**

Control treatment that includes only 7 Tomato seedlings infested by 6 couples of *T. absoluta*. *T. absoluta* was repeatedly released for reinfesting purposes.

➢ **Some notices related to *D. maroccanus* Establishing**

D. maroccanus establishing had taken the following periods:

- One week to let the couples matching to be occurred,
- Two weeks to obtain *D. maroccanus* new larvae to be emerged,
- Together *D. maroccanus* adults and its new emerged larvae are ready to be monitored and yet to explored its role as one of the alternative and new predator Mirid against *T. absoluta* and its harm effects,

- Consequently; the required and proper period for establishing that Mirid is from 21 to 25 days,
- *D. maroccanus* total adults being used are 140,
- *E. kuehniella* frozen eggs were used as an integrated diet been presented to the released *D. maroccanus* couples during the predator establishing period (21 days earlier to *T. absoluta* releasing time),

Figure 3, *D. maroccanus* adult and Azadirachtin plus *Bt*

- **Some notices related to the treatments**

The time factor was the main limited factor for conducting this sort of experiments in order to get good results those related to *T. absoluta* management within the IPM properly strategies. Other important factors were related to the Phytosanitary measures to protect and conserve the entire experiment different assets until the end.

The factors those related to Tomato servicing activities such as irrigation, pruning, fertilizing, and adjustment of the surviving factors of the temperature, humidity and illumination periods…etc. should be taken in consideration.

- **Data analysis**

The number of adults and larvae of *D. maroccanus* in Tomato plants in the different experiments were analyzed using a **Generalized Linear Mixed Model** with repeated measurements. Treatment was considered as a fixed factor and time as a random one. When significant differences were found, pairwise comparisons of the fixed factor levels were performed with the least significant difference (LSD) post hoc test (P<0.05).

Results

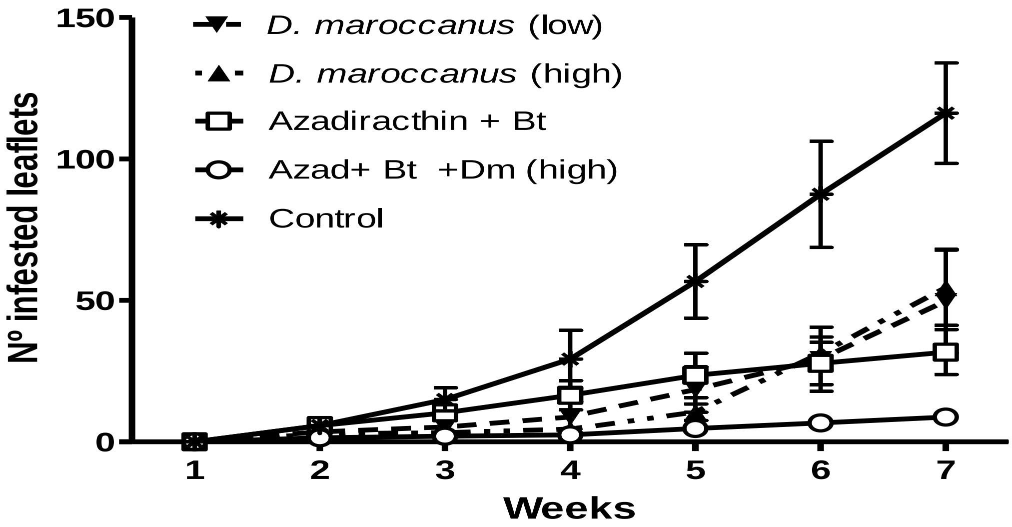

Figure 4, number of infested leaflets per tomato plant (mean ± SE) in a semifield trial comparing the effectiveness of four different strategies *T. absoluta* at different time intervals

The results showed that the treatments had a significant effect on *T. absoluta* infestation levels when they were expressed as a number of infested leaflets per plant ($F_{4,\ 135} = 16.985$, $P < 0.0001$) (Fig. 4-based on table 1). The combining of *D. maroccanus* (high releasing rate) and Azadiractine plus *B. thuringiensis* showed a lower number of infested leaflets per plant.

Meanwhile, the number of infested leaflets per plant was increasing chronologically except the combination of *D. maroccanus* (high releasing rate) and Azadiractine plus *B. thuringiensis* treatment.

Moreover, table 1 showed that there were significant differences between the following compared pair treatments; *D. maroccanus* (low releasing rate) and Az+Bt+ *D. maroccanus* (high releasing rate), *D. maroccanus* (low releasing rate) *and* control, *D. maroccanus* (high releasing rate) and Az+Bt+ *D. maroccanus* (high releasing rate), *D. maroccanus* (high releasing rate) *and* control, Az+Bt and Az+Bt+ *D. maroccanus* (high releasing rate), Az+Bt and control and finally between Az+Bt+ *D. maroccanus* (high releasing rate) and control

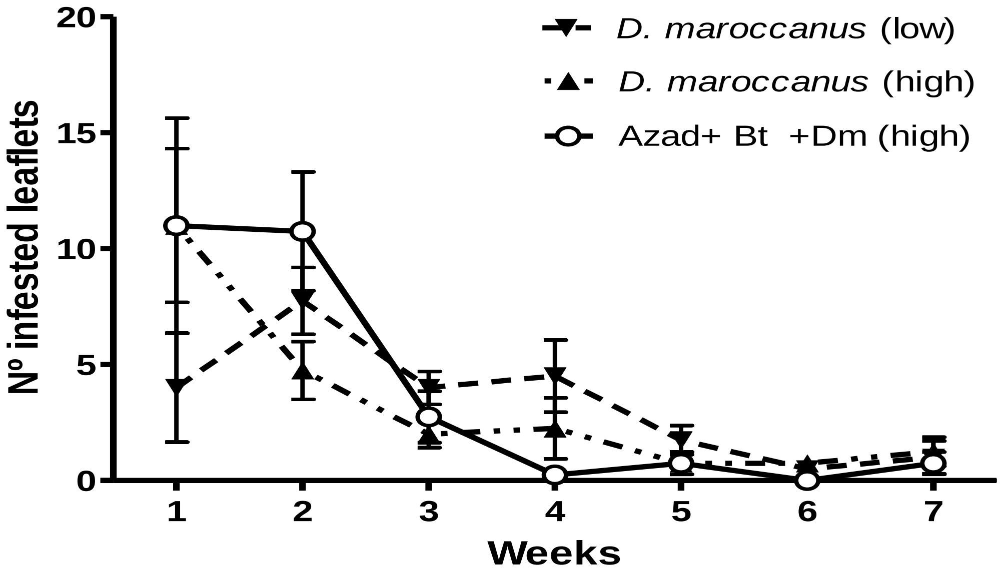

Figure 5, number of Mirids per tomato plant (mean ± SE) in a semifield trial comparing the effectiveness of four different strategies *T. absoluta* at different time intervals

In all the three treatments (1, 2 and 4) those been included *D .maroccanus* alone in (high and low releasing rates), and *D .maroccanus* (high releasing rate) and Azadiractine plus *B. thuringiensis,* the number of Mirids per plant was not significant different among those treatments ($F_{2, 81}$ = 0.157, P = 0.855) (Fig. 5-based on table 1). Since that number has been come gradually descended along with the 8 weeks of the experimental periodically space.

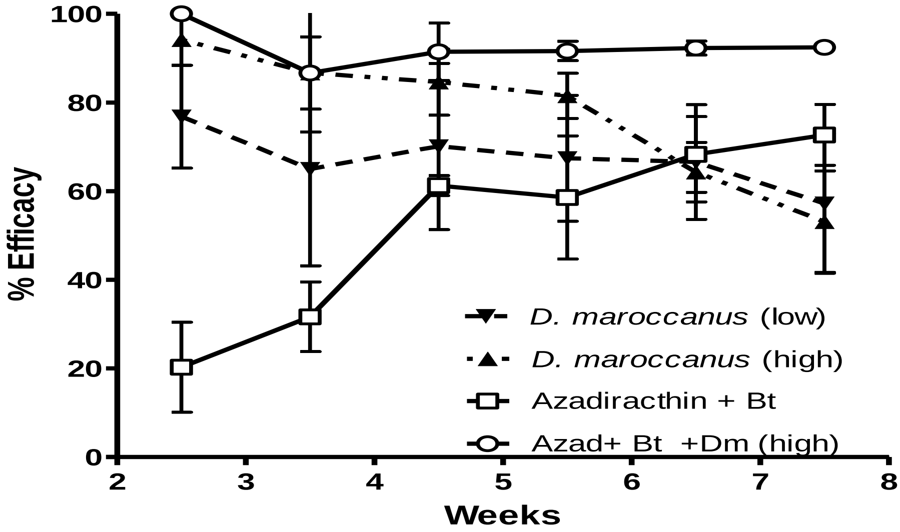

Figure 6. percentage of reduction (Henderson-Tilton) of infested leaflets per tomato plant (mean ± SE) in a semifield trial comparing the effectiveness of four different strategies *T. absoluta* at different time intervals

Figure 6 that based on (table 1) showed clearly that there were significant differences among the four treatments ($F_{3,\ 87}$ = 12.140, P < 0.0001) with regards to *T. absoluta* infestation reduction percentages.

Generally it is been accurately appeared that the treatment of *D .maroccanus* (high releasing rate) and Azadiractine plus *B. thuringiensis* showed high efficacy since the *T. absoluta* infestation reduction percentage was almost 100%, meanwhile there were no significant differences with the other two treatments those included *D .maroccanus* alone (high and low releasing rates).

Moreover, table 1 showed that there were significant differences among the following compared pair treatments *D. maroccanus* (low releasing rate) and *D. maroccanus* (high releasing rate), *D. maroccanus (*low releasing rate) and Az+Bt, *D. maroccanus* (low releasing rate) and Az+Bt+ *D. maroccanus* (high releasing rate), *D. maroccanus* (high releasing rate) and Az+Bt, and finally between Az+Bt and Az+Bt+ *D. maroccanus* (high releasing rate) with regard to the *T. absoluta* infested leaflets per tomato plant reduction percentage.

Treatments	infested leaflets/plant		% efficacy	
	t	P	t	P
D. m Low and *D. m* High	0.229	0.819	**1.503**	**0.0137**
D. m Low and Az+Bt	0.014	0.989	**2.039**	**0.044**
D. m Low and Az+Bt+ *D. m* High	**2.46**	**0.015**	**3.841**	**<0.001**
D. m Low and C	**5.42**	**<0.001**	-	-
D. m High and Az+Bt	0.243	0.809	**3.525**	**0.001**
D. m High and Az+Bt+ *D. m* High	**2.323**	**0.027**	**2.339**	**0.022**
D. m High and C	**5.648**	**<0.001**	-	-
Az+Bt and Az+Bt+ *D. m* High	**2.474**	**0.015**	**5.838**	**<0.001**
Az+Bt and C	**5.406**	**<0.001**	-	-
Az+Bt+ *D. m* High and C	**7.88**	**<0.001**	-	-

Table 1. P-values for the pairwise comparison of the number of infested leaflets by *T. absoluta* by plant (Figure 4 for details) and percentage of efficacy regarding to the control treatment (Figure 6 for details) that received different treatments to control *T. absoluta*: *D. maroccanus* low releasing rate= 1 couple of *D. maroccanus* per 2 plants, *D. maroccanus* high releasing rate= 1 couple of *D. maroccanus* per plant, Az+Bt= Azadirachtin + *B. thuringiensis*, Az+Bt+ D. *maroccanus* high releasing rate= Azadirachtin + *B. thuringiensis* + 1 couple of *D. maroccanus* per plant and C= control treatment. Values in bold correspond to significant differences between treatments.

Discussion

The potential of combining Azadirachtin and *B. thuringiensis* formulations to reduce the impact of economically important pests and diseases is well known in particular in *T. absoluta* management (Lo Bue et al 2012) However, to our knowledge; this is the first report demonstrating the efficacy of both products in combination with the predatory Mirid *D. maroccanus* in *T. absoluta* management.

Clearly the obtained results of this study in tomato semifield cultivation reveal that it is possible to reduce the tomato leaf miner impact by applying biorational insecticides along with *D. maroccanus* high releasing rate. Since that strategy showed a lower number of infested leaflets per plant during the 8 weeks of the experiment (Figure 4).

Moreover it is showed higher efficacy of *T. absoluta* infestation reduction percentage among other four different strategies being implemented in this study. This strategy is offering good and promising results, and yet offering new IPM opportunities and options in *T. absoluta* management.

Recently there were some studies those have emphasized the role of the Azadiractine plus *B. thuringiensis* combination and the relation between those two items. Since these studies suggested that the combined action is not synergistic but complimentary, with azadirachtin particularly facilitating the action of *B. thuringiensis*.

Also the *B. thuringiensis* spray-azadirachtin combination is more economical than combinations that involve isolating the toxic protein, as the *B. thuringiensis* spray formulations can be combined in a spray mixture with Neem (Singh G et al 2007).

These combinations may be useful for controlling some other pests like bollworm populations that have acquired resistance to B. thuringiensis as they may not survive the effect of mixture. Azadirachtin may be useful as a means of reducing the endotoxin concentrations in a mixture (Singh G et al 2007).

Although, Azadirachtin has a knock-down power towards larvae of *T. absoluta*, as in laboratory test aqueous Neem seeds' extracts induced high larval mortality by both systemic and translaminar actions (Gonçalves-Gervasio and Vendramim, 2007), nevertheless there was such study that demonstrated that Azadirachtin significantly reduced the offspring of the predator Mirid bug *N. tenuis* females (Arnó and Gabarra, 2011), this study showed that; there were no side effects of Azadiractine on *D. maroccanus* presence along with experimental period of 8 weeks.

The potential of *B. thuringiensis* formulates in controlling *T. absoluta* was clearly demonstrated in laboratory tests (Giustolin et al., 2001; Lolas and Meza-Basso, 2006; Giulianotti, 2010; Gonzales- Cabrera et al; 2011, Ladurner et al.; 2011). Moreover, Gonzales-Cabrera et al. (2011) evidenced that *B. thuringiensis* strains are able to reduce the *T. absoluta's* impact to very low levels when tested in greenhouse and open-field.

Furthermore, *T. absoluta* management based on treatments with *B. thuringiensis* doesn't induce resistance in phytophagous populations, that is a likely cause of field control failures (Silva et al., 2011), and could be associated with the use of parasitoids or predators (deMedeiros et al, 2009; Mollá et al., 2011).

With regards to *D. maroccanus* most of its lifecycle parameters and its preference to Tomato crops along with the presence of *T. absoluta* have been studied recently in a study conducted in IVIA of Spain 2013 (chapter 3). This conducted study have approved that there are more than 7 positive indicators belong to *D. maroccanus* lifecycle different parameters more likely to present that predator as a new and active biocontrol agent. In addition *D. maroccanus* has not showing any side negative impact on the Tomato.

Moreover, there were no side effects been observed or approved of that combination on the presence of *D. maroccanus* individuals along with the experimental periods of 8 weeks. (For more details is possible to review chapter 3 of this thesis)

However, the 8 weeks experimental period of this part of this study might perfectly explained *D. maroccanus* individual's number descending within that strategy and the other strategies, since *D. maroccanus* needs more time to reproduce new progenies with regards that the examined tomato seedlings samples were not being turned to the cages and that means the probability of hatching new eggs and yet emerging new larvae is ignored.

Although the obtained results pointed out that there were significant differences in *T. absoluta* infested leaflets per tomato plant reduction percentages among all the compared pair treatments (all the implemented strategies), those strategies have effectively decreased *T. absoluta* population since those percentages were at the range between 70 to more than 90 percent.

The high and low *D. maroccanus* releasing rates have no significant differences with regard to the number of the *T. absoluta* infested leaflets number; meanwhile, there were such a significant difference between those different releasing rates levels comparing with the control treatment with regard to *T. absoluta* infestation reduction percentage. That would be a positive feature to be adapted at *D. maroccanus* establishing efforts.

Conclusion

Prior to the writing of this study, *T. absoluta* has still as a key pest of tomato worldwide. Most of the global states different strategies flow into the extensively using of the synthetic insecticides stream of which the health of the environment and all its vital assets are being in very risky status and there will be no considerations or efforts to relieve and mitigate that status, and for generations to come.

As a common knowledge there are many undesired side-effect on human and environment safety by implementing those synthetic pesticides. Those side effects are likely to be more dangerous than using them itself since that might development such a resistance *T. absoluta*.

For that reason and others, most of the global scientific institution working so hard to explore more and more of the environmental and ecological pest managements techniques and strategies by implementing the proper IPM programs.

In this study is demonstrated that treatment based on *D. maroccanus* high releasing rate along with the Azadirachtin plus *B. thuringiensis* combination can effectively reduce *T. absoluta* damage on tomato in semifield cultivation. Thus, adopting this strategy on tomato leaf miner in open field tomato should not be ignored.

Other important concern that related to the lack of Azadiractine effects on *D. maroccanus*. There was some study declared that *Azadirachtin is* significantly reduced the offspring of the predator Mirid bug *Nesidiocoris tenuis* females (Arnó and Gabarra, 2011).

Finally, this study highly suggests that there are good and promising IPM potentials roles of *D. maroccanus* to control the Tomato leaf miner *T. absoluta*. Further studies should be carried out to integrate this strategy with other integrated or biological control methods in order to reduce the use of chemicals and, consequently, improve food safety and environment quality.

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Conclusions

According to the general title of UNIPA doctorate program and for several cycles; *"Eco-compatibility Phytosanitary Management in Agro forest and Urban Environments*" and along with the fact that the term *Phytosanitary* been included within many of the modern and old scientific terms such as: organic farming, plant protection, integrated pest management, national and international movement of plant germplasm, biosecurity, good agricultural practices and others, the pests' risks to be avoided and the environment health to be achieved; that could surly achieved and yet actually obtained only by the properly implementing of such preventive measures, precaution measures, public awareness and others of the IPM and organic farming effective standards and strategies.

Solving the problem of this study; is representing such an effort that been flowed within the most Eco-compatibility Phytosanitary Managements general stream. The study problem is how and by what to manage/control the Tomato leaf miner *T. absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), this pest was considering as one of the most devastating pests affecting Tomato crops in worldwide, and even for time to come.

The first part (Chapter 2) of this study was including one of *T. absoluta* management strategies that have been assessed in open-field tests in Sicily-Italy (2011 and 2012) by using three of biorational insecticides Azadirachtin, *Bacillus thuringiensis* var. kurstaki, and *Beauveria bassiana*, and to the control treatment by using three of synthetic insecticides, Emamectin, Indoxacarb and Metaflumizone, in different combinations (according to the recommended use instruction of the producing companies).

Important fact been approved by this assessment was;

- ***Conclusion 1*** only the biorational insecticides combination of Azadirachtin – *B. thuringiensis* was able to reduce effectively the impact of Tomato leaf miner on the Tomato since that assessment relied on the fruit's marketable production as a bioassay criteria. The efficacy of that combination was similar with the synthetic insecticides in another word there were no significant differences between those implementing treatments.

This finding suggests that biorational insecticides combination is a good alternative than synthetic ones and that fact comes suit to the IPM and organic farming standards and strategies.

Since there were no accurate ideas about the side effect of that biorational insecticides combination on the natural enemies of *T. absoluta*, and yet what would be the situation in case of associate that combination with other of IPM different strategies??!! Coincidently and fortunately there was such a place within IVIA of Spain facilities to provide the answer of that question by studying the predator *Dicyphus maroccanus* (Wagner 1951) (Hemiptera; Miridae) as one of *Tuta absoluta* eggs predator.

The recent three years; *Dicyphus maroccanus* had been observed and detected by IVIA in and around Tomato crops and along with the presence of *T. absoluta*. It is a common known that this predator is belonging to the Miridae family of which most of its species are representing Omniphagus but part of the generalists green bugs such as *Nesidiocoris tenuis* and *Macrolophus pygmaeus.* Studying this predator lifecycle biological parameters was the first step to explore its IPM potential roles against *T. absoluta* (Chapter 3).

The second part (Chapter 3) of this study was conducted in IVIA and the main conclusion been obtained was;

- ***Conclusion 2*** most of *D. maroccanus* lifecycle parameters being studied closely on Tomato crops and *E. kuehniella* frozen eggs under the laboratory conditions such as survivorship, developmental time, female longevity, eggs consumed, progeny sex ratio of *D. maroccanus* are relatively higher than they are in *N. tenuis* and *M. pygmaeus*, consequently there will be good and promising possibilities to recruit *D. maroccanus*.

The third part (Chapter 4) of this study was conducted to explore *D. maroccanus* host plant preference (Tomato) and yet to *T. absoluta* eggs/larvae infested Tomato.

What had been achieved of this part was representing another positive indicator to be added to the others of those predator positive features.

Conclusion 3 *D. maroccanus* showed a very good preference to the healthy Tomato plants (80%), and to *T. absoluta* eggs/larvae infested Tomato (almost 60, 80%) respectively.

The zoophytophagous generalist predator *D. maroccanus* has very good preference to Tomato plants but the presence of *T. absoluta* as one of herbivores that means; Tomato volatiles induced by *T. absoluta* feeding and that strongly attracting *D. maroccanus*.

So there will be such opportunities and new options for considering the name of this predator within the valuable assets IPM strategies' package against *T. absoluta* or any other key pests those negatively affect on the Solanaceae different species all over the world and for generations to come in order to reduce the use of chemicals or/and other of plant protection negative strategies.

The fourth and final part (Chapter 5) of this study was conducted as an attempt to introduce *D. maroccanus* and yet to explore its IPM potentials in *T. absoluta* under semifield conditions after its lifecycle different parameters were being studied closely.

What had been achieved in this part of the study was demonstrated the fact that

- ***conclusion 4*** treatment based on *D. maroccanus* high releasing rate along with the biorational insecticides combination of Azadirachtin plus *B. thuringiensis* can effectively reduce *T. absoluta* damage on Tomato in semifield cultivation. However, adopting this strategy on Tomato leaf miner in open field Tomato should not be ignored.

Other important related facts that

- ***Conclusion 5*** no negative side effects of that combination of Azadirachtin plus *B. thuringiensis* had been monitored on *D. maroccanus*, since its individuals' presence was continuing until the end of the experimental period of 8 weeks.
- ***Conclusion 6*** no negative side effect had been observed of *D. maroccanus* feeding nature on the host plant Tomato different parts.

Finally, this study highly suggests that there are good and promising IPM potentials roles of *D. maroccanus* to manage the Tomato leaf miner *T. absoluta*. Further studies should be carried out to integrate this strategy with other integrated or biological control methods in order to reduce the use of chemicals and consequently, to improve our food safety and our environment quality.

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