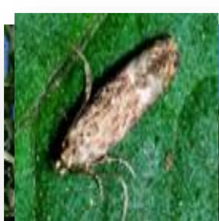
Insecticide Resistance Action Committee

Best Management Practices to Control *Tuta absoluta* and Recommendations to Manage Insect Resistance

IRAC Tuta IRM Task Team – 2017 (v6)





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1. Update Tuta Presence and Pest Status Globally

1. Update Tuta presence and pest status globally.

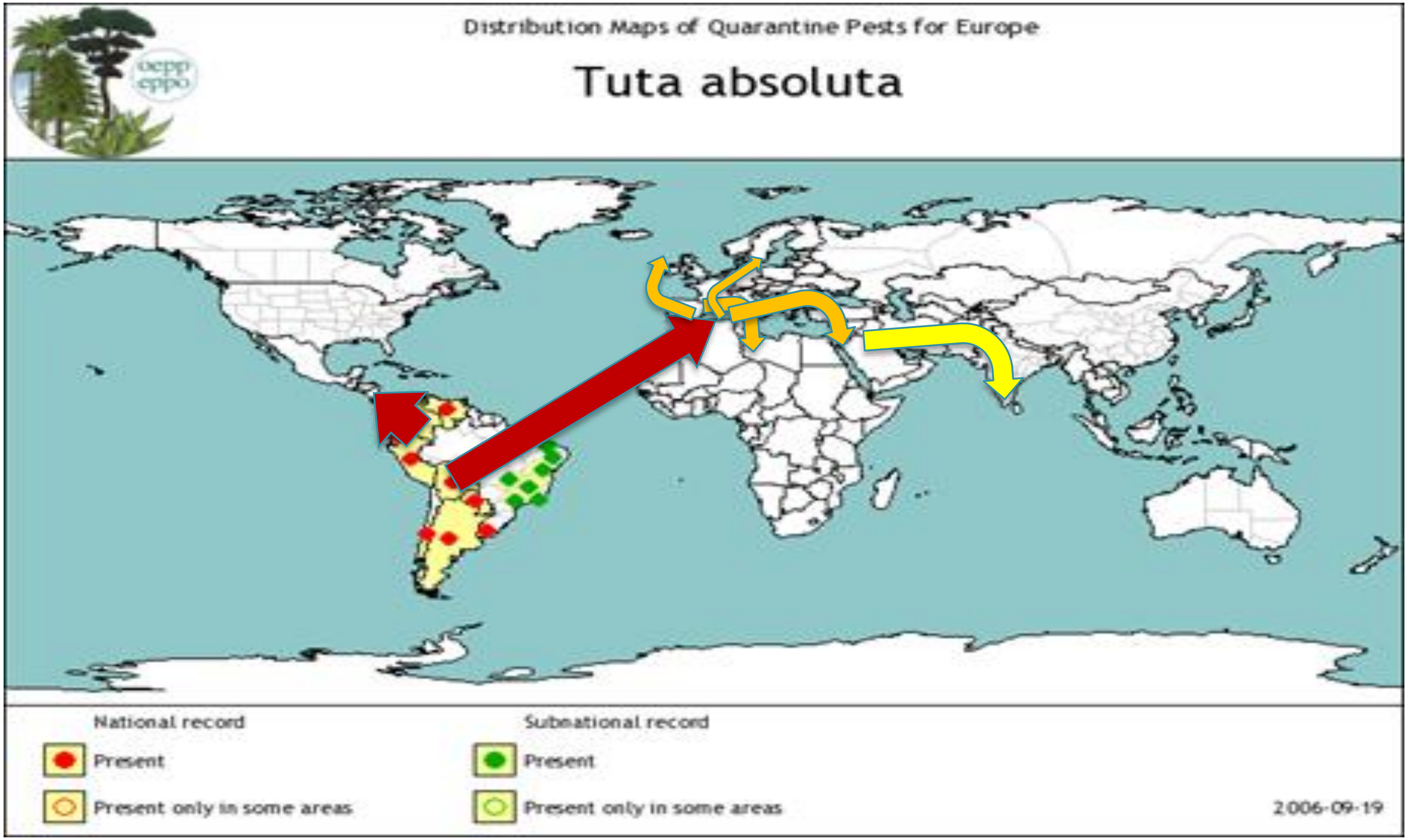
Tuta absoluta, Success spreading around the globe due to:

- **High capacity of dispersion; kilometers by flight and drifting in winds (Willem, S. et col, 2009)**
- **High level of fruit transit from endemic areas to new areas**
  - **South America to Mediterranean areas**
  - **Mediterranean areas to rest of Europe and other areas as distribution bridge**
- **Pest dispersion to favorable climatic conditions**
- **Lack of natural predators of the pest in the new colonized areas**
- **Fast development pest cycle**
- **Species capacity of adaptation and resistance to not optimal conditions during transport (9ºC)**
- **Favorable crop growing systems with optimal hosts (tomato)**

IRAC

1. Update Tuta presence and pest status globally.

Origin of pest



Mainly initial spreading in South America from Chile region **(Lietti et al , 2005)**
From South America to Central America and Europe

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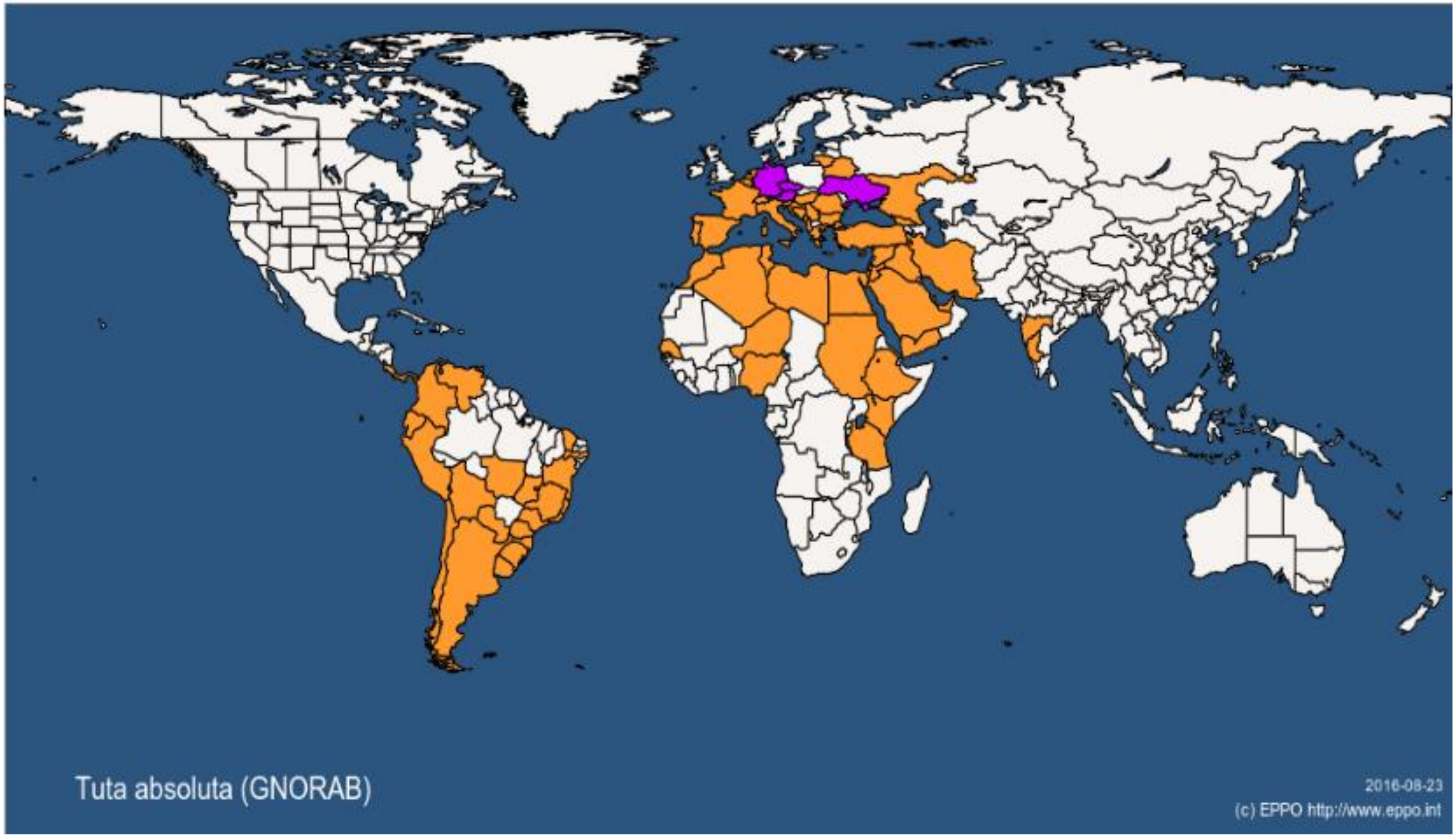
1. Update Tuta presence and pest status globally.

Current distribution



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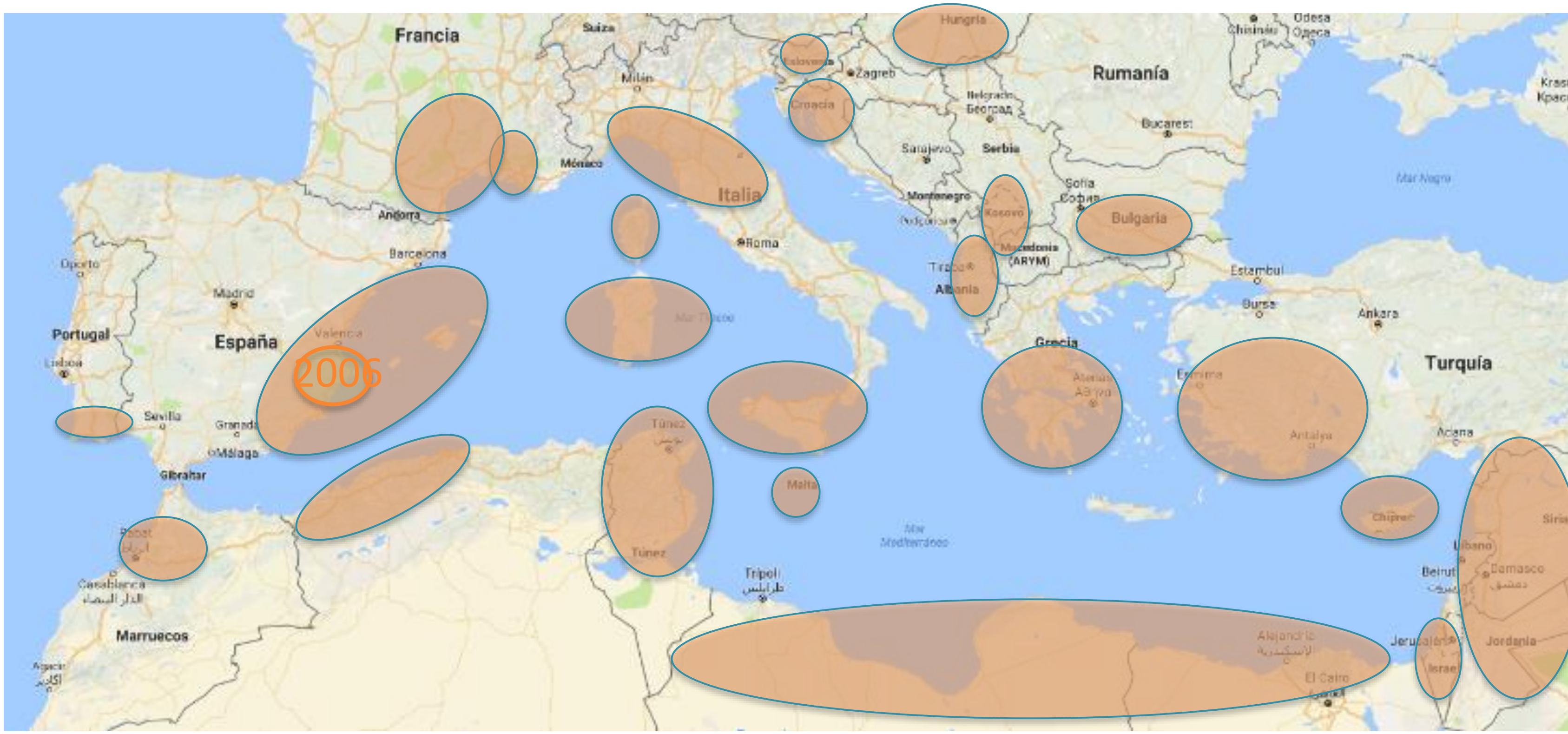
1. Update Tuta presence and pest status globally.

Sequential distribution in the Mediterranean basin (EPPO data base , 2016)

2008 ;
SPAIN/ ALGERIE /MOROCCO/FRANCE (Mediterranean)

2009 ;
ITALY/TUNISSIA/PORTUGAL/ALBANIA/ MALTA/FRANCE/BULGARIA/SLOVENIA/ CROATIA/GREECE/TURKEY/LEBANON/ EGIPT/JORDAN/SYRIA/LYBIA

2010;
ISRAEL/CYPRUS/HUNGARY/KOSOVO



Spread to north European countries mainly through packaging and distribution facilities due to fruit trade

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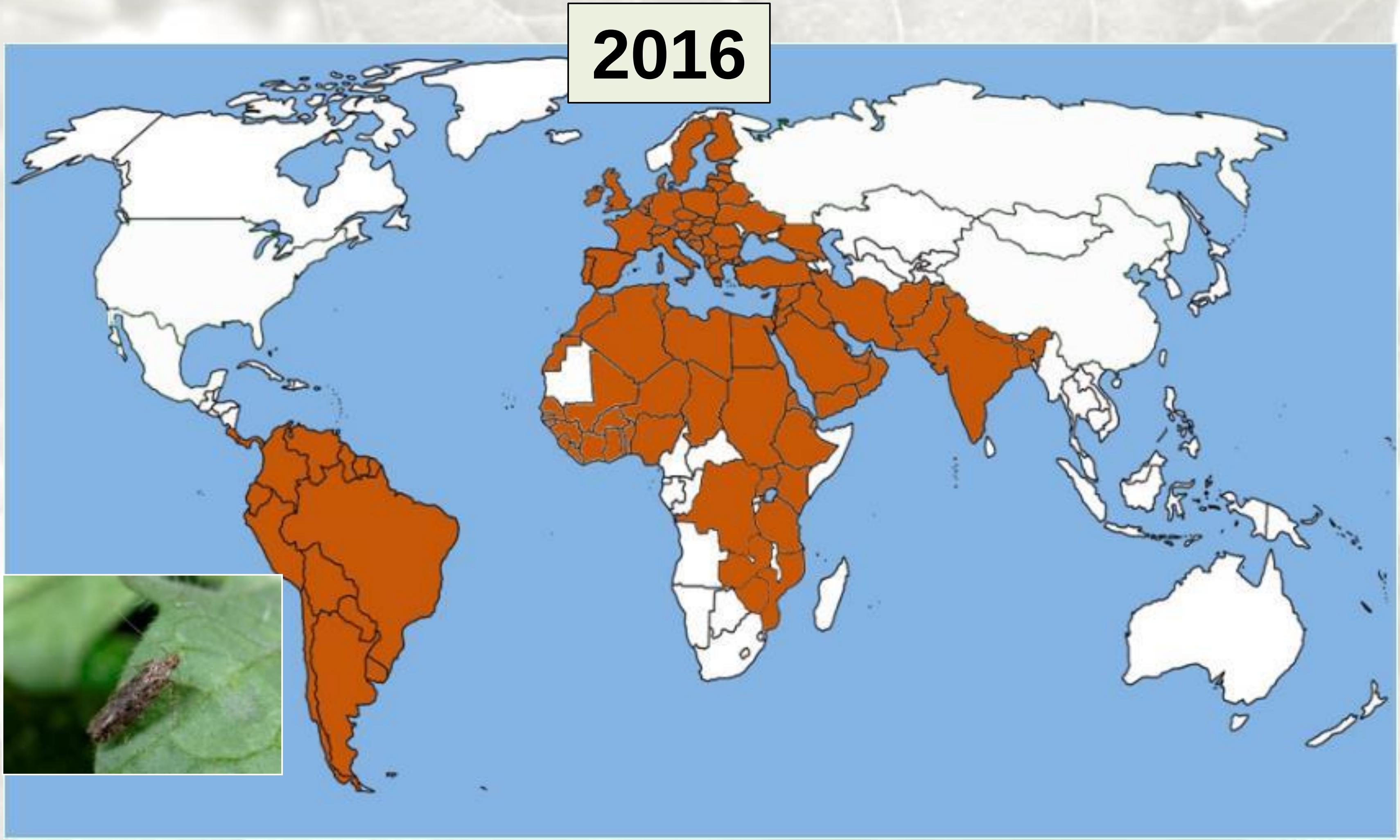
1. Update Tuta presence and pest status globally.



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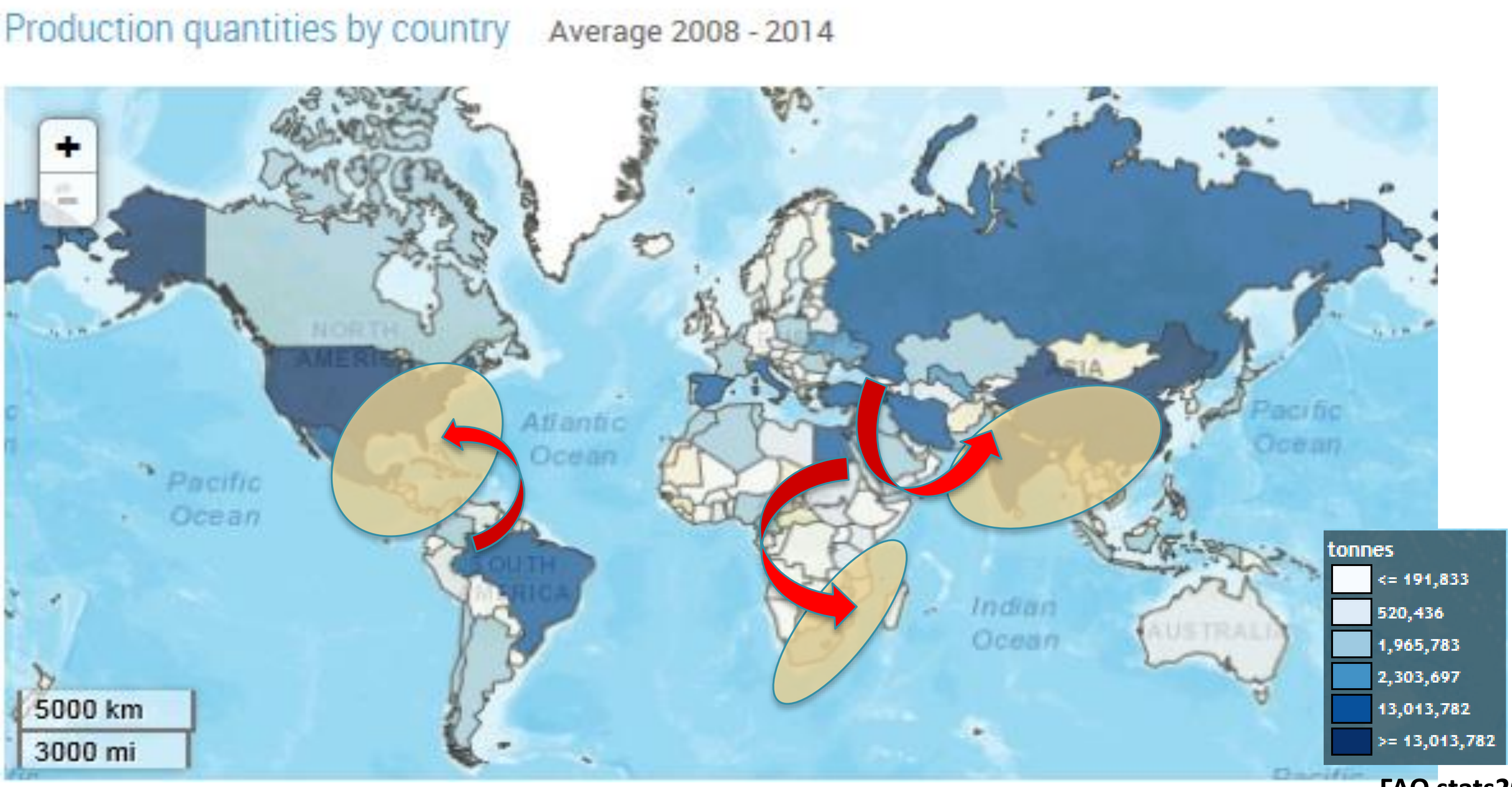
In 10 years from 3% to 60% of tomato crops
2.8 million hectares

1. Update Tuta presence and pest status globally.

Possible areas under risk of pest spread

Main world tomato production areas at risk



FAO stats2016

- **Confirmation of presence in India and Middle east makes Afganistan, Pakistan, India, Nepal and China as risk areas (**Nepal: "Sensitizing workshop on *Tuta absoluta*: An impending threat to tomato production" 2015 Sponsored by IAPPS)
- **Actions at OIRSA (International Regional Organization for Agricultural Health) Including : El Salvador, Costa Rica Honduras, Guatemala, Mexico, Belice, Nicaragua and Panamá**
- **Presence in Kenia and Tanzania makes that Mozambique, Malawi, Zimbabwe, Zambia, Botswana as well as South Africa are at risk**

Lietti, M. M. M., E. Botto, and R. A. Alzogaray, 2005. Insecticide resistance in Argentine populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Neotrop. Entomol. vol.34, no.1. Londrina Jan./Feb. 2005.

***Tuta absoluta*: a new pest for tomato growing in Europe**

Willem Stol, Frans C. Griepink, Peter van Deventer.
Plant Research International PHEROBANK, Wageningen, Holanda.

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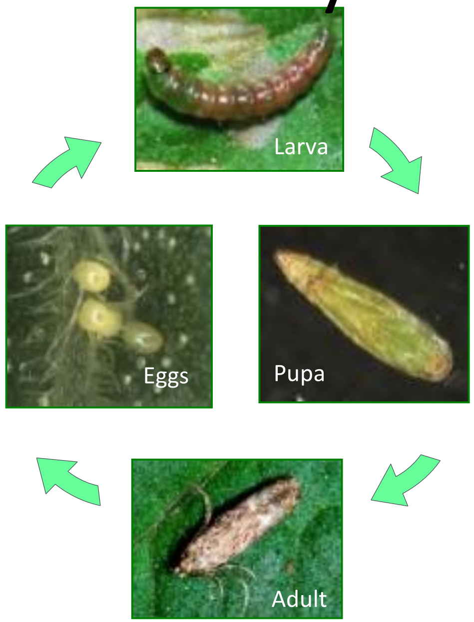
IRAC

2. Recognize Tuta life stages, life cycle, damage, and plant symptoms

Source: IRAC

2. Recognize Tuta life stages, life cycle, damage, and plant symptoms

Life Cycle



Larval developmental time (days) at different temperatures

14° C	76 days
20° C	40 days
27° C	24 days

Modified from Barrientos et al. (1998)

Tuta absoluta is a micro-lepidopteran insect. The adults are silvery brown, 5-7 mm long. The total life cycle is completed in an average of 24-38 days, with the exception of winter months, when the cycle could be extended to more than 60 days. The minimal temperature for biological activity is 4° C. After copulation, females lay up to 300 individual small (0.35 mm long) cylindrical creamy yellow eggs, which are often found alongside the rachis. Freshly hatched larvae are light yellow or green and only 0.5 mm in length. As they mature, larvae develop a darker green color and a characteristic dark band posterior to the head capsule. Four larval instars develop. Larvae do not enter diapause when food is available. Pupation may take place in the soil, on the leaf surface, within mines or in packaging material. A cocoon is built if pupation does not take place in the soil. 10-12 generations can be produced each year. *Tuta absoluta* can overwinter as eggs, pupae or adults depending on environmental conditions. Under open-field conditions *Tuta absoluta* is usually found up till 1000 m above sea level.

2.

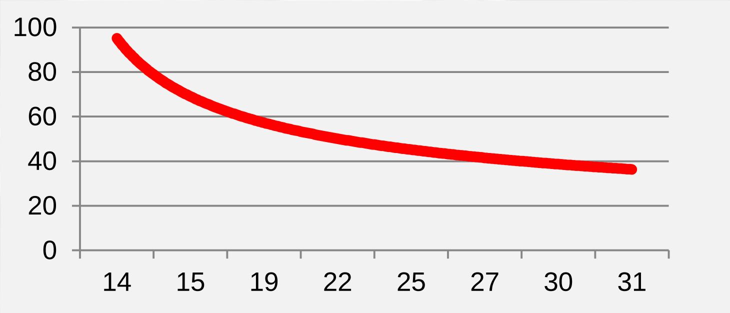
Tuta aboluta Life Cycle

Egg
3 - 8 d

Larva
9 - 30 d

Pupa
6 - 20 d

Adult
6 - 15 d ♂
10 - 25 d ♀



- *Egg to adult*: 25 - 80 days
- *Thermal threshold for juvenile development*
  - – lower: 10 > x < 13 °C
  - – upper: 30 > x < 35 °C

(Various authors;Cuthbertson et al. 2013)

- *Up to 13 generations per year*
- *Good cold resistance*
  - – supercooling points: larvae (-18.2°C), pupae (-16.7 °C), adults (-17.8°C)
  - – the lower lethal time for adults at 0°C (17.9 d), 5°C (27.2 d)

(Van Damme et al. 2015)

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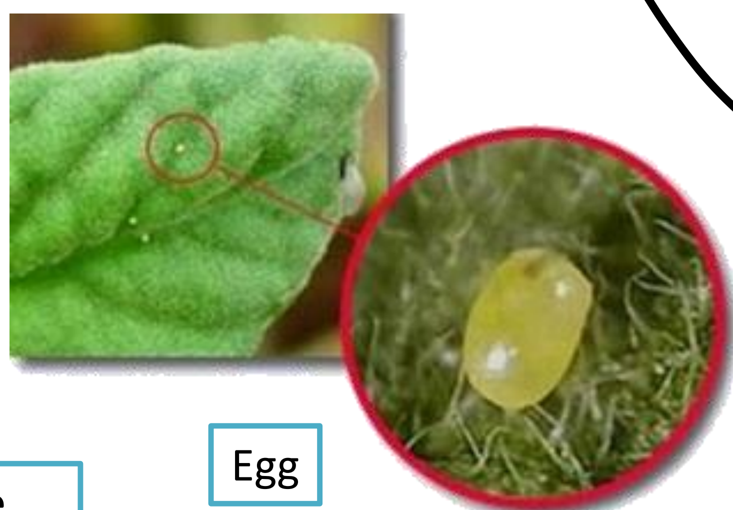
2.

Tuta aboluta Life Cycle

5th Instar Larva

3rd Instar Larva

New Shoot Damage



Egg

Tuta: Biology Cycle

Adult

Eggs
3-5 Days

Larva
4 Life Stages
11 - 19 Days

Pupa
6 - 10 Days

	15°	20°	25°	30°
Uova	10	7	4	3
Larva	36	23	15	11
Pupa	21	12	7	6
Total	67	42	26	20
Life span	23	17	13	9

2. *Tuta absoluta* Adult

- *Adults*: 5-7 mm
- *Hindwings*: narrow, with posterior margin with long hairs
- *Labial palpi*: long and up-curved
- Antennae: filiform, banded with gray and dark brown

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2.

Moth Species Similar in Appearance to Tuta absoluta

Tuta absoluta

Potato tuberworm (*Phthorimaea operculella*)

Tomato pinworm (*Keiferia lycopersicella*)

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Photo credit: Sangmi Lee

2.

Tuta absoluta Reproduction

- *Thermal threshold for reproduction* (Marcano 1995)
  - lower: 10 > x < 15 °C
  - upper: 25 > x < 30 °C
- *No-refractory period for both sexes* (Lee et al. 2014)
- *Female pheromone:* TDDA + TDTA (Svatoš et al. 1996)
- *Re-mating for both sexes* (+fertility +longevity) (Lee et al. 2014)
- *Potential for deuterotokous parthenogenetic*: virgin females can lay fertile eggs (Caparros Megido et al 2013)
- *Eggs/female: 50÷350*

  >90% of eggs laid in the first 4 days (Pereyra & Sánchez 2006)

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2.

Tuta absoluta Eggs

- Yellowish
- 0.2÷0.4mm
- Laid singularly or in small groups, mostly in the upper side of young leaves or in stems

Antonio BIONDI, Lucia Zappalà, Giovanna Tropea Garzia, Gaetano Siscaro University of Catania, ITALY; antonio.biondi@unict.it

Photo credit: Gaetano Siscaro

2.

Tuta absoluta

Larvae

- 0.4 - 8mm
- 4 instars
- Prothoracic plate with dark posterior band
- Mostly endophytic

Pupae

- 3 ÷ 5mm
- Inside a silky and sand small cocoons in the soil

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Photo credit: Gaetano Siscaro

2.

Tuta absoluta larvae

2. Tuta absoluta Damage and Symptoms

Infestation of tomato plants occurs throughout the entire crop cycle. Feeding damage is caused by all larval instars and throughout the whole plant. On leaves, the larvae feed on the mesophyll tissue, forming irregular leaf mines which may later become necrotic. Larvae can form extensive galleries in the stems which affect the development of the plants. Fruit are also attacked by the larvae, and the entry-ways are used by secondary pathogens, leading to fruit rot. The extent of infestation is partly dependent on the variety. Potential yield loss in tomatoes (quantity and quality) is significant and can reach up to 100% if the pest is not managed.

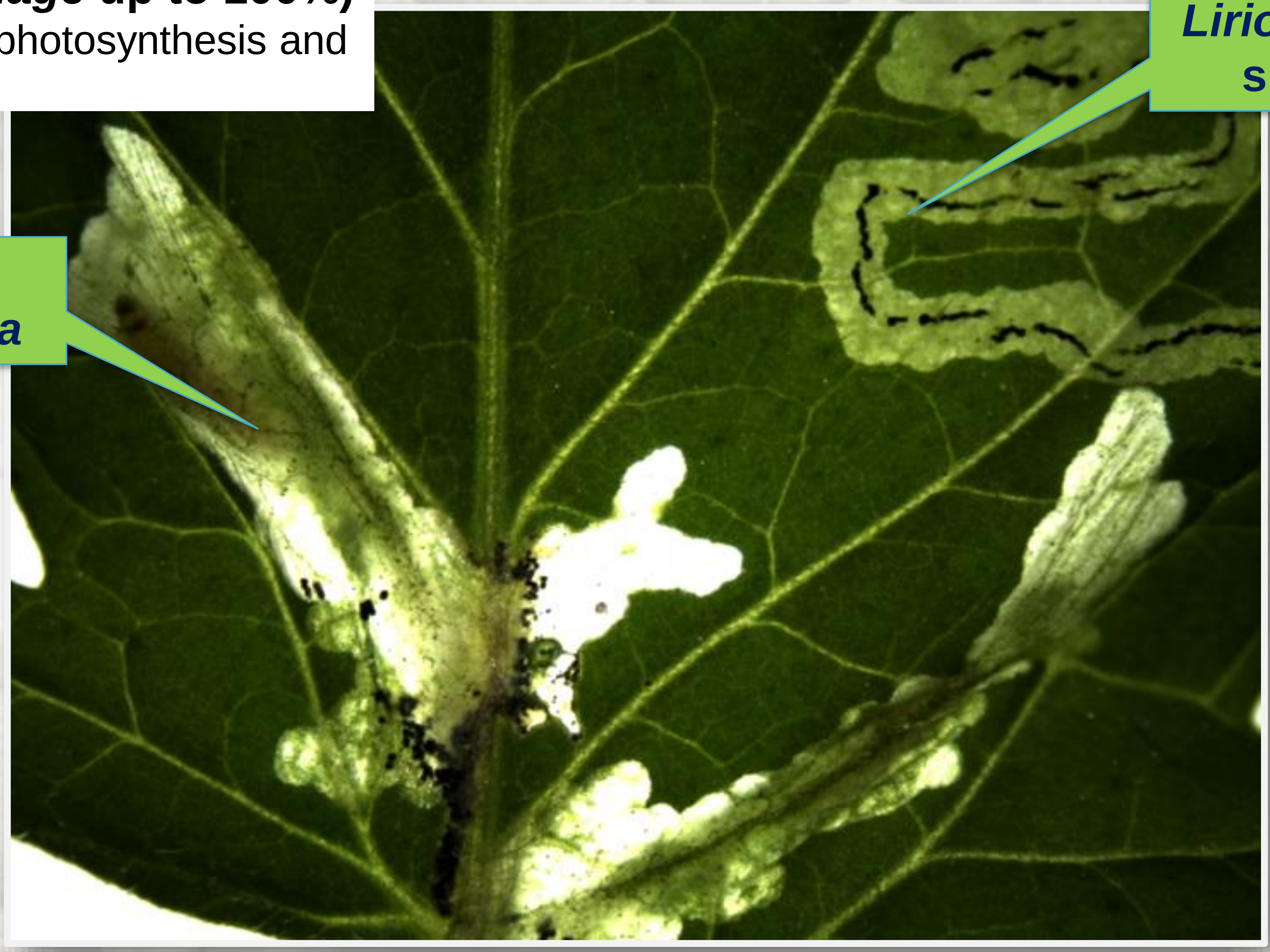




2. *Tuta absoluta* vs *Liriomyza* Damage

Typical damage

Leaf damage **up to 100%)**
Decrease photosynthesis and yields



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2.

Tuta absoluta Mining *and* Leaf Damage

2. *Tuta absoluta* Damage to Growing Point

2.

Tuta damage on stem

Tuta entry points at node

2.

Tuta absoluta Fruit Damage

2. *Tuta absoluta* Leaf and Fruit Damage

2.

Tuta absoluta – Host plants

- *Solanum lycopersicum* (tomato)
- *Solanum tuberosum* (potato)
- *Solanum melongena* (eggplant)
- *Capsium annuum* (pepper)
- *Nicotiana tabacum* (tobacco)
- *Solanum nigrum*
- *Datura stramonium*
- *Solanum eleagnifolium*
- *Physalis peruviana (Cape gooseberry)*
- *Solanum nigrum (European black nightshade) main wild host*
- *Solanum bonariease*
- *Solanum sisymbriifolium*
- *Solanum sapponaceum*
- *Lycopersicum puberulum*
- *Datura ferox*
- *Lycium* sp.

Occasional reports on non-solanacaous plants

- *Beans*
- *Malva spp.*

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2.

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3. Tuta control products, resistance publications, and method to evaluate efficacy.

Source: IRAC

3. Tuta control products, resistance publications, and method to evaluate efficacy.

IRAC Mode of Action Groups for Registered* Tuta Control Products

Chemical Class	IRAC Group	Mode of Action
Organophosphates	1B	Acetylcholinesterase (AChE) inhibitors
Pyrethroids	3A	Sodium channel modulators
Spinosyns	5	Nicotinic acetylcholine receptor allosteric modulators
Avermectins, Milbemycins	6	Chloride channel activators
Pyrroles	13	Uncouplers of oxidative phosphorylation via disruption of the proton gradient
Nereistoxin analogues	14	Nicotinic acetylcholine receptor channel blockers
Benzoylureas	15	Inhibitors of chitin biosynthesis, type .
Diacylhydrazines	18	Ecdysone receptor agonists
Oxadiazine	22A	Voltage-dependent sodium channel blockers
Semi-carbazone	22B	Voltage-dependent sodium channel blockers
Diamides	28	Ryanodine receptor modulators
Tetranortriterpenoid	UN	Compounds of unknown or uncertain MoA

* Insecticide registrations from multiple countries and regions

IRAC

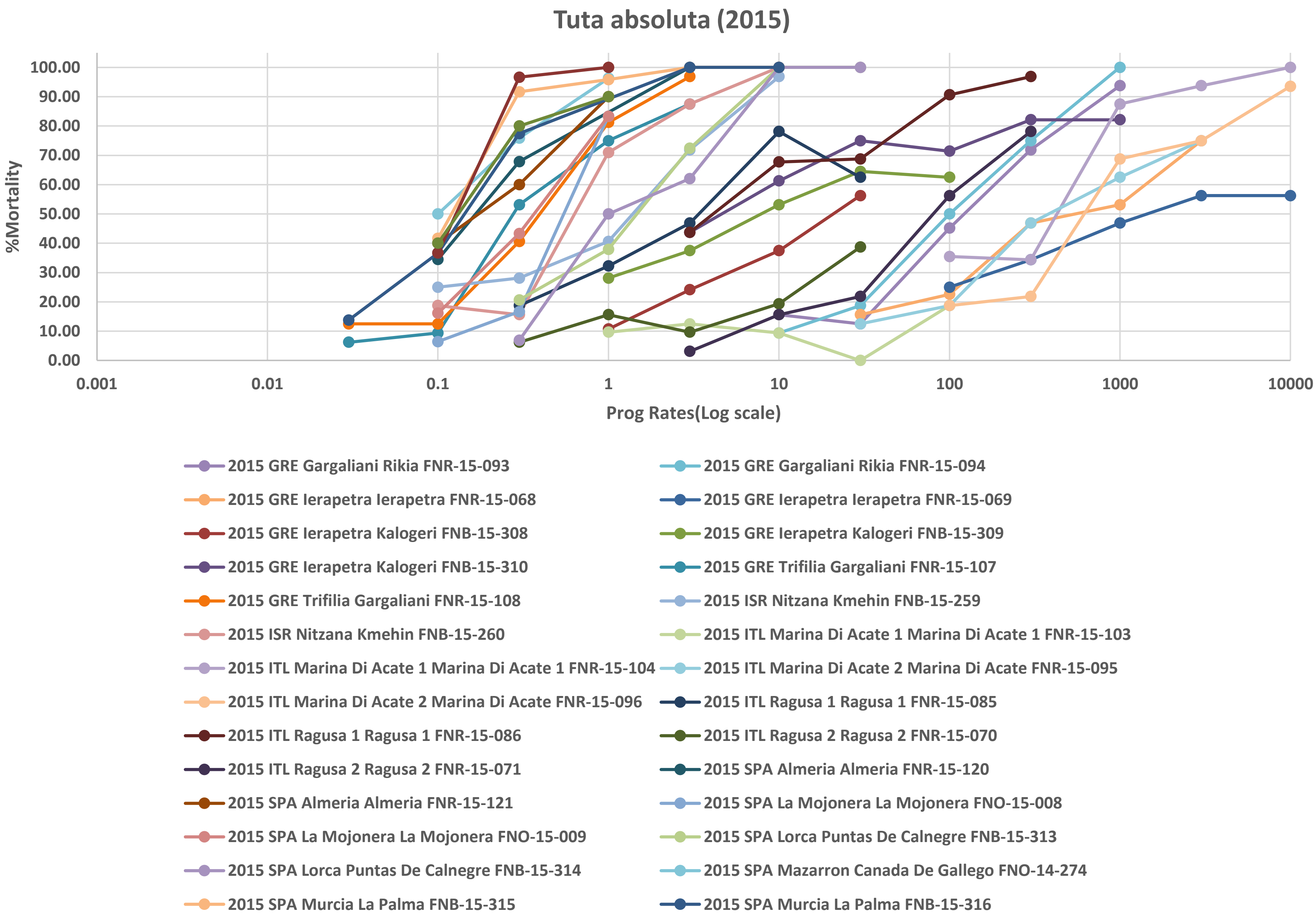
3. Tuta control products, resistance publications, and method to evaluate efficacy.

List of compounds registered for control of *Tuta absoluta* (source IRAC).

Chemical Class	Compounds
Organophosphates	Chlorpyrifos, Methamidophos
Pyrethroids	Bifenthrin, Cyfluthrin, beta-Cyfluthrin, gamma-Cyhalothrin, lambda-Cyhalothrin, Cypermethrin, alpha-Cypermethrin, beta-Cypermethrin, zeta-Cypermethrin, Delthamethrin, Esfenvalerate, Etofenprox, tau-Fluvalinate, Fenpropathrin, Permethrin
Spinosyns	Spinetoram, Spinosad
Avermectins,	Abamectin, Emamectin benzoate
Biopesticide	*Bacillus thuringiensis aizawai, Bacillus thuringiensis kurstaki*
Pyrroles	Chlorfenapyr
Nereistoxin analogues	Cartap
Benzoylureas	Diflubenzuron, Flufenoxuron, Lufenuron, Novaluron, Noviflumuron, Teflubenzuron, Triflumuron
Diacylhydrazines	Chromafenozide, Methoxyfenozide, Tebufenozide
Oxadiazine	Indoxacarb
Semi-carbazone	Metaflumizone
Diamides	Chlorantraniliprole, Flubendiamide
Tetranortriterpenoid	Azadirachtin

3. Tuta control products, resistance publications, and method to evaluate efficacy.

Example Rynaxypyr®: Susceptibility of Tuta -2015 trials in Mediterranean Basin



3. Tuta control products, resistance publications, and method to evaluate efficacy.

Example Rynaxypyr®: 2015 trials in Mediterranean Basin EC50

Location	EC50	EC50 Lower	EC50 Upper
2015 Andalucia, Almeria, Almeria	0.185	0.129	0.249
2015 Andalucia, Almeria, La Mojonera	0.473	0.376	0.592
2015 Extremadura, Merida, Villaverde	0.130	0.005	0.260
2015 Lasithion, Lasithi, Ierapetra	1584.124	871.436	3672.996
2015 Lasithion, Lassithi, Kalogeri	16.030	9.930	25.867
2015 Messinia, Messinia, Gargaliani	0.431	0.332	0.551
2015 Messinia, Messinia, Rikia	122.581	93.813	157.751
2015 Murcia, Region de, Cartagena, La Palma	0.132	0.099	0.170
2015 Murcia, Region de, Murcia, Canada De Gallego	0.114	0.048	0.175
2015 Murcia, Region de, Murcia, Puntas De Calnegre	1.426	1.101	1.801
2015 Not Specified, Negev, Kmehin	0.812	0.463	1.333
2015 Siracusa, Syracuse, Marina Di Acate	694.217	495.238	970.000
2015 Siracusa, Syracuse, Marina Di Acate 1	348.705	195.208	621.830
2015 Siracusa, Syracuse, Ragusa 1	4.690	2.794	7.262
2015 Siracusa, Syracuse, Ragusa 2	85.619	57.547	145.509

Examples of Tuta resistance to insecticides:

Abamectin, cartap, permethrin, methamidophos

Siqueira, H.A.A., Guedes, R.N.C., Fragoso, D.D.B. and Magalhaes, L.C., 2001. Abamectin resistance and synergism in Brazilian populations of *Tuta absoluta* (Meyrick)(Lepidoptera: Gelechiidae). *International Journal of Pest Management*, *47*(4), pp.247-251.

Failures in the control of the tomato leafminer Tuta absoluta (Meyrick) by means of abamectin in Brazil, and a recent report of abamectin resistance in Brazilian populations of this pest species, led to the investigation of the possible involvement of detoxification enzymes using insecticide synergists. Resistance to abamectin was observed in all populations when compared with the standard susceptible population, with resistance ratios ranging from 5.2- to 9.4-fold. Piperonyl butoxide was the most efficient synergist with abamectin synergism ratios ranging from 3.0- to 5.3-fold and providing significant resistance suppression, but complete suppression of abamectin resistance was only obtained in one population of T. absoluta . Triphenylphosphate was an abamectin synergist which was not as efficient as piperonyl butoxide, but it provided complete suppression of abamectin resistance in four of the six resistant populations studied, suggesting a major involvement of esterases as an abamectin resistance mechanism in these populations. The importance of cytochrome P450, inhibited by piperonyl butoxide, seems secondary to esterases. Diethyl maleate also synergized abamectin in nearly all populations, but provided only partial suppression of abamectin resistance in the leafminer populations studied. Therefore, glutathione-S-transferases seem to be of minor importance as an abamectin resistance mechanism in Brazilian populations of T. absoluta .

Spinosad

Campos, M.R., Rodrigues, A.R.S., Silva, W.M., Silva, T.B.M., Silva, V.R.F., Guedes, R.N.C. and Siqueira, H.A.A., 2014. Spinosad and the tomato borer Tuta absoluta: a bioinsecticide, an invasive pest threat, and high insecticide resistance. PloS one, 9(8), p.e103235.

The introduction of an agricultural pest species into a new environment is a potential threat to agroecosystems of the invaded area. The phytosanitary concern is even greater if the introduced pest's phenotype expresses traits that will impair the management of that species. The invasive tomato borer, Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae), is one such species and the characterization of the insecticide resistance prevailing in the area of origin is important to guide management efforts in new areas of introduction. The spinosad is one the main insecticides currently used in Brazil for control of the tomato borer; Brazil is the likely source of the introduction of the tomato borer into Europe. For this reason, spinosad resistance in Brazilian populations of this species was characterized. Spinosad resistance has been reported in Brazilian field populations of this pest species, and one resistant population that was used in this study was subjected to an additional seven generations of selection for spinosad resistance reaching levels over 180,000-fold. Inheritance studies indicated that spinosad resistance is monogenic, incompletely recessive and autosomal with high heritability ($h2 = 0.71$). Spinosad resistance was unstable without selection pressure with a negative rate of change in the resistance level ($= -0.51$) indicating an associated adaptive cost. Esterases and cytochrome P450-dependent monooxygenases titration decreased with spinosad selection, indicating that these detoxification enzymes are not the underlying resistance mechanism. Furthermore, the cross-resistance spectrum was restricted to the insecticide spinetoram, another spinosyn, suggesting that altered target site may be the mechanism involved. Therefore, the suspension of spinosyn use against the tomato borer would be a useful component in spinosad resistance management for this species. Spinosad use against this species in introduced areas should be carefully monitored to prevent rapid selection of high levels of resistance and the potential for its spread to new areas.

3. Tuta control products, resistance publications, and method to evaluate efficacy.

Tuta populations resistant to diamides have been found in: Crete, Greece, Sicily, Italy, SW Spain, and Israel – Jan 2017

Diamides

Roditakis, E., Vasakis, E., Grispou, M., Stavrakaki, M., Nauen, R., Gravouil, M. and Bassi, A., 2015. First report of Tuta absoluta resistance to diamide insecticides. *Journal of pest science*, *88*(1), pp.9-16.

Insect ryanodine receptors (RyR) are the molecular target-site for the recently introduced diamide insecticides. Diamides are particularly active on Lepidoptera pests, including tomato leafminer, Tuta absoluta (Lepidoptera: Gelechiidae). High levels of diamide resistance were recently described in some European populations of T. absoluta, however, the mechanisms of resistance remained unknown. In this study the molecular basis of diamide resistance was investigated in a diamide resistant strain from Italy (IT-GELA-SD4), and additional resistant field populations collected in Greece, Spain and Brazil. The genetics of resistance was investigated by reciprocally crossing strain IT-GELA-SD4 with a susceptible strain and revealed an autosomal incompletely recessive mode of inheritance. To investigate the possible role of target-site mutations as known from diamondback moth (Plutella xylostella), we sequenced respective domains of the RyR gene of T. absoluta. Genotyping of individuals of IT-GELA-SD4 and field-collected strains showing different levels of diamide resistance revealed the presence of G4903E and I4746M RyR target-site mutations. These amino acid substitutions correspond to those recently described for diamide resistant diamondback moth, i.e. G4946E and I4790M. We also detected two novel mutations, G4903V and I4746T, in some of the resistant T. absoluta strains. Radioligand binding studies with thoracic membrane preparations of the IT-GELA-SD4 strain provided functional evidence that these mutations alter the affinity of the RyR to diamides. In combination with previous work on P. xylostella our study highlights the importance of position G4903 (G4946 in P. xylostella) of the insect RyR in defining sensitivity to diamides. The discovery of diamide resistance mutations in T. absoluta populations of diverse geographic origin has serious implications for the efficacy of diamides under applied conditions. The implementation of appropriate resistance management strategies is strongly advised to delay the further spread of resistance.

3. Tuta control products, resistance publications, and method to evaluate efficacy.

Λ-cyhalothrin, tau-fluvalinate

Haddi, K., Berger, M., Bielza, P., Cifuentes, D., Field, L.M., Gorman, K., Rapisarda, C., Williamson, M.S. and Bass, C., 2012. Identification of mutations associated with pyrethroid resistance in the voltage-gated sodium channel of the tomato leaf miner (Tuta absoluta). *Insect biochemistry and molecular biology*, *42*(7), pp.506-513.

The tomato leaf miner, Tuta absoluta (Lepidoptera) is a significant pest of tomatoes that has undergone a rapid expansion in its range during the past six years and is now present across Europe, North Africa and parts of Asia. One of the main means of controlling this pest is through the use of chemical insecticides. In the current study insecticide bioassays were used to determine the susceptibility of five T. absoluta strains established from field collections from Europe and Brazil to pyrethroids. High levels of resistance to λ cyhalothrin and tau fluvalinate were observed in all five strains tested. To investigate whether pyrethroid resistance was mediated by mutation of the para-type sodium channel in T. absoluta the IIS4–IIS6 region of the para gene, which contains many of the mutation sites previously shown to confer knock down (kdr)-type resistance to pyrethroids across a range of different arthropod species, was cloned and sequenced. This revealed that three kdr/super-kdr-type mutations (M918T, T929I and L1014F), were present at high frequencies within all five resistant strains at known resistance 'hot-spots'. This is the first description of these mutations together in any insect population. High-throughput DNA-based diagnostic assays were developed and used to assess the prevalence of these mutations in 27 field strains from 12 countries. Overall mutant allele frequencies were high (L1014F 0.98, M918T 0.35, T929I 0.60) and remarkably no individual was observed that did not carry kdr in combination with either M918T or T929I. The presence of these mutations at high frequency in T. absoluta populations across much of its range suggests pyrethroids are likely to be ineffective for control and supports the idea that the rapid expansion of this species over the last six years may be in part mediated by the resistance of this pest to chemical insecticides.

IRAC

Chlorantraniliprole, flubendiamide

Roditakis, E., Vasakis, E., Grispou, M., Stavrakaki, M., Nauen, R., Gravouil, M. and Bassi, A., 2015. First report of Tuta absoluta resistance to diamide insecticides. Journal of pest science, 88(1), pp.9-16.

The tomato borer Tuta absoluta (Lepidoptera: Gelechiidae) is an invasive pest of tomato crops that is rapidly expanding around the world. It is considered a devastating pest and its control heavily relies on application of insecticides. Diamides are a novel class of insecticides acting on insect ryanodine receptors and are highly effective against lepidopteran pests. To date, chlorantraniliprole and flubendiamide have been registered in the market and they have been extensively used to manage T. absoluta. In this study, a survey was conducted in Greece and Italy monitoring diamide resistance. The populations originating from Sicily (Italy) exhibited LC50s that ranged between 47.6–435 for chlorantraniliprole and 993–1.376 for flubendiamide, while for Crete (Greece) LC50s ranged between 0.14–2.45 for chlorantraniliprole and 1.7–8.4 for flubendiamide (LC50s in mg L^{-1}). Comparing this result to the susceptible reference strain, high resistance levels for the Italian populations were detected, i.e., up to 2,414- and 1,742-fold for chlorantraniliprole and flubendiamide, respectively. Resistance ratios for Greek populations were found up to 14-fold for chlorantraniliprole and 11-fold for flubendiamide, suggesting that diamide resistance is low but increasing considering monitoring data over time. Hereby, we report for the first time, cases of resistance development to diamide insecticides in T. absoluta. These findings underline the importance of committing to the resistance management strategies for diamide insecticides.

3. Tuta control products, resistance publications, and method to evaluate efficacy.

UK Data

Tuta absoluta investigating resistance to key insecticides and seeking alternative IPM compatible products

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Final Report December 2015
Dr C Bass, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ

: The British Tomato Growers' Association Technical Committee requested the following actions which became the focus of this project:

1. Spinosad and chlorantraniliprole resistance tests be undertaken by the Insecticide Resistance Team at Rothamsted Research (IRT RR) to establish the current status of populations of *T. absoluta* in the UK.
2. A desk study to search for all products used to control *T. absoluta* and other leaf mining caterpillars in the Americas, Africa, southern Europe, Middle East and Far East, and then to categorise them according their potential value within the UK tomato IPM programme.

Summary

Part one: The original objective was to test the sensitivity of four UK strains of *T. absoluta* to spinosad and chlorantraniliprole. However, one of the growers who had reported poor results with spinosad in the early part of 2015 stopped producing tomatoes and no insects were available from that site. That population was replaced with one from Denmark that was associated with spinosad treatment failure in 2015. The Danish population provided added value as one resistance test had already been completed on that strain and it was therefore possible to investigate whether 'tolerance' declined when spinosad selection pressure was removed for 7-8 months. Two IRT RR 'susceptible' laboratory strains were also incorporated in the study to provide a base line. Full-dose response bioassays were performed using the standard leaf-dip bioassay procedure outlined in the IRAC Susceptibility Test Method 22. The LD50s (*i.e.* the amount of insecticide required to kill 50% of the population) were determined for each population and resistance ratios calculated by dividing the LD50 of the test population by the LD50 of the most susceptible laboratory strain.

UK Data Continued

Tuta absoluta investigating resistance to key insecticides and seeking alternative IPM compatible products

In summary, the bioassays confirmed that *T. absoluta* populations at two locations in the UK exhibited high levels of resistance to spinosad. The levels of resistance were high enough to seriously compromise control as both strains would show very significant survivorship at the field rate commonly used for spinosad (87-100 mg L^{-1}). No spinosad resistance was detected in the third UK population and other possible causes of treatment failure are being investigated at that site. The original Danish strain showed some tolerance to spinosad but only 8-fold greater than the most susceptible laboratory strain. This had declined to approximately twice that of the most susceptible laboratory strain at the second test. The interim period of 29 weeks equates to 8-9 generations of *T. absoluta* at the usual temperatures in a commercial tomato crop. It would therefore appear that in the absence of spinosad selection pressure the more susceptible individuals in a population have some developmental advantage and gradually become more dominant. This is good news for growers as it indicates that spinosad should still have some value within the IPM programme if treatments are restricted to no more than one application per growing season.

None of the tested populations showed significant levels of resistance to chlorantraniliprole. However, published information from Italy and Greece has confirmed that resistance to this chemical is present within southern Europe. The fact that there is currently unrestricted importation of tomatoes infested with *T. absoluta* from Italy suggests that British growers could inherit this problem at any time.

Two key test method documents for *Tuta*:

IRAC

Insecticide Resistance Action Committee
www.irac-online.org

IRAC Susceptibility Test Methods Series
Version: 3

Method No: 022

Details:

Method:	No: IRAC No. 022	 *Tuta absoluta* larvae *Photograph Courtesy of: DuPont Crop Protection*
Status:	Approved	
Species:	*Tuta absoluta*	
Species Stage	Larvae L2 (size: 4-5 mm)	
Product Class:	Oxadiazins (IRAC MoA 22), anthranilic diamides (IRAC MoA 28), spinosyns (IRAC MoA 5)	

Comments:
In order to obtain homogeneous *Tuta absoluta* larvae (same age, nutritional and general health condition), it is highly recommended that insects collected from the field (F_0 generation) are brought to a laboratory and reared to the F1 generation for evaluation of insecticide susceptibility.

Objectives:

Susceptibility Baseline: ☒ Resistance Monitoring: ☒

Description:

Materials:
Insect-proof containers, scissors, fine forceps, fine pointed brush, seeking pin, beakers and syringes / micropipettes for test liquids (solutions and EC formulations), accurate balance for solids and SC liquid formulations, syringes/pipettes/micropipettes for making dilutions, binocular microscope or hand lens, wire net or paper towels, 10-15 cm^2 cell plates with sealable lid*, filter papers, protective gloves, maximum/minimum thermometer, untreated tender/ young tomato leaflets.
Optional: a light box (glass surface table with a fluorescent light source underneath).

* Suggested model: Bio-Serv, Rearing tray white ref: RT32W and Bio-assay Tray Lid-4 cells ref: RTCV4

Methods:
This method is a leaf-dip bioassay to be performed preferably with F1 L2 larvae (4-5mm in size).

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Research Article

SCI

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Determination of baseline susceptibility of European populations of *Tuta absoluta* (Meyrick) to indoxacarb and chlorantraniliprole using a novel dip bioassay method

Emmanouil Roditakis,[a*] Christina Skarmoutsou,[a] Marianna Staurakaki,[a] Maria del Rosario Martínez-Aguirre,[b] Lidia García-Vidal,[b] Pablo Bielza,[b] Khalid Haddi,[c] Carmelo Rapisarda,[c] Jean-Luc Rison,[d] Andrea Bassi[e] and Luis A Teixeira[f]

Abstract

BACKGROUND: *Tuta absoluta* (Meyrick) is one of the most serious pests of tomato recently introduced in the Mediterranean region. A novel bioassay method designed for the accurate determination of insecticide toxicity on *T. absoluta* (IRAC method No. 022) was validated by three different laboratories [Greece (NAGREF), Italy (UC) and Spain (UPCT)] on European populations.

RESULTS: The insecticides indoxacarb and chlorantraniliprole were used as reference products. The IRAC leaf dip method is easy to perform, producing repeatable, homogeneous responses. LC_{50} values for indoxacarb ranged between 1.8 and 17.9 mg L^{-1} (NAGREF), 0.93 and 10.8 mg L^{-1} (UC) and 0.20 and 0.70 mg L^{-1} (UPCT), resulting in a tenfold, 12-fold and fourfold difference between the least and most susceptible populations at each laboratory respectively. For chlorantraniliprole, LC_{50} values ranged between 0.10 and 0.56 mg L^{-1} (NAGREF), 0.23 and 1.34 mg L^{-1} (UC) and 0.04 and 0.24 mg L^{-1} (UPCT), resulting in a sixfold difference in all three cases. Overall, UPCT reported lower mean LC_{50} to indoxacarb, while UC reported higher LC_{50} to chlorantraniliprole.

CONCLUSIONS: The new bioassay is reliable, providing a useful tool in the design of IRM strategies. Within each country/lab, the variability observed in the results for both indoxacarb and chlorantraniliprole can be attributed to natural variation. Future research is necessary to determine the extent to which it is possible to compare results among laboratories.

Keywords: *Tuta absoluta*; indoxacarb; chlorantraniliprole; baseline toxicity; leaf dip assay; Greece; Italy; Spain

1 INTRODUCTION

Tomato borer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one of the most important pests of tomato, *Lycopersicon esculentum* Mill.[1] It has been reported to have caused significant damage to tomato crops in South America in the 1980s and 1990s.[2–4] *T. absoluta* was found in Europe in 2006 and since then has rapidly invaded numerous countries, mainly in the Mediterranean basin.[5] In particular, the insect was first recorded in Spain in 2006,[6] and shortly afterwards in Italy (2008)[7,8] and Greece (2009).[9] Based on national survey reports, the pest was soon considered widespread, as it was detected in most of the regions of these three countries.[10–12] Currently, the tomato borer invasion is expanding into the Middle East.[13]

T. absoluta is a pest with high damage potential. It has 10–12 continuous generations per year, and females can lay approximately 260 eggs, mainly on the top leaves of tomato plants. Hatching larvae penetrate the leaf/fruit epidermis and bore galleries in the plant tissues. Infested fruits have no market value, as secondary infestations allow the development of fruit rot, while cases of severe defoliation may destroy the tomato plants.[5,14]

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f DuPont Crop Protection, Stine Haskell Research Center, Newark, DE, USA

Method comparison for *Tuta*:

The 32-well repli-dish assay:

- One leaflet per cell
- One larva per cell

The compound leaf assay:

- One compound leaf per cell
- 10 larvae per leaf

3. Tuta control products, resistance publications, and method to evaluate efficacy.

IRAC Susceptibility Test Methods Series
Version: 3

Method No: 022

Details:

Method:	**No: IRAC No. 022**
Status:	**Approved**
Species:	***Tuta absoluta***
Species Stage	**Larvae L2 (size: 4-5 mm)**
Product Class:	**Oxadiazins (IRAC MoA 22), anthranilic diamides (IRAC MoA 28), spinosyns (IRAC MoA 5)**

***Tuta absoluta* larva**
Photograph Courtesy of: DuPont Crop Protection

Comments:
In order to obtain homogeneous *Tuta absoluta* larvae (same age, nutritional and general health condition), it is highly recommended that insects collected from the field (F_0 generation) are brought to a laboratory and reared to the F1 generation for evaluation of insecticide susceptibility.

IRAC Susceptibility Test Methods Series

Version: 3

Method No: 022

Objectives: Susceptibility Baseline: ☒ Resistance Monitoring: ☒

Description:

Materials:
Insect-proof containers, scissors, fine forceps, fine pointed brush, seeking pin, beakers and syringes / micropipettes for test liquids (solutions and EC formulations), accurate balance for solids and SC liquid formulations, syringes/pipettes/micropipettes for making dilutions, binocular microscope or hand lens, wire net or paper towels, 10-15 cm2 cell plates with sealable lid*, filter papers, protective gloves, maximum/minimum thermometer, untreated tender/ young tomato leaflets.
Optional: a light box (glass surface table with a fluorescent light source underneath).

* Suggested model: Bio-Serv, Rearing tray white ref: RT32W and Bio-assay Tray Lid-4 cells ref: RTCV4

Methods:
This method is a leaf-dip bioassay to be performed preferably with F1 L2 larvae (4-5mm in size):

a) Collect a representative sample of insects from a field. These may be larvae suitable for immediate testing (least preferred as these larvae may be contaminated from unknown previous field treatments or otherwise parasitized, etc.) or individuals (larvae/ pupae/ eggs) to be reared to second instar larvae F1 generation (preferred, homogeneous cohort). The insects should not be subjected to temperature, humidity or starvation stress after collection. In order to obtain a representative sample of insects from any given field, ideally a minimum of 100 larvae or pupae should be collected from each field to be tested, in order to establish a colony of at least 50 adults. The collection of late stage larvae (e.g. 4th instar) is recommended because they will require less plant material to develop, and will have shorter rearing time in the lab, and moth emergence will be synchronous. These moths are then reared to obtain enough L2 larvae for the bioassays.

b) Collect sufficient non-infested, untreated tomato leaves. Although the test will be done using single leaflets, it is preferable to collect entire leaves uniform in size. Tender young whole leaves are preferred. Do not allow leaves to wilt by keeping them in a moist environment (sealed plastic bag).

c) Prepare accurate dilutions of the test compound from the identified commercial product. For initial studies, six widely spaced rates are recommended. The use of a wetter/spreader (non ionic adjuvant) is highly recommended in order to obtain optimal leaf coverage. The adjuvant solution should be used for the “untreated” control solution in place of water alone. As the addition of a wetting agent can significantly affect the performance of an insecticide product in a bioassay, it is essential that details of the wetting agent and concentration used are recorded with any summary data and that only data generated with the same type of wetting agent and concentration are compared for susceptibility measurements.

d) Dip leaflets individually in the test liquid for 3 seconds with gentle agitation, ensuring the entire surface is emerged equally. Then dry the treated leaflets on a wire net with upper leaf surface (abaxial surface) facing skywards, or on paper towels (least preferred). Do not allow the leaflets to wilt. Dip the same number of leaflets per treatment (dose) and treat sufficient leaf material to avoid starvation stress in the “untreated” during the test. Do the same procedure for all the doses, starting with the “untreated” control (wetter solution), then followed by the more diluted dose and advancing progressively to the higher concentrations.

e) Prior to placing the leaflets in the bioassay cell units, place a slightly moistened filter paper covering the bottom of each cell. Around 0.2 ml distilled water should be sufficient to moist the filter paper and keep the leaf material turgid throughout the bioassay period. Excess water or water drops need to be removed.

f) When the surface of the leaflets is completely dry, place the leaflets in the labeled containers (one leaflet per cell unit), which must be suitable for holding enough leaf material in good condition for 3 days.

Note. Tomato leaves are quite fragile and sensitive. Maintaining the tomato leaflets intact – avoiding cutting them into measured pieces - helps keep the leaflets in good conditions for the period of the bioassay.

g. Begin the transfer of L2 larvae to the bioassay cell units, using a fine soft brush and taking extreme care not to damage the very fragile larvae. The following method is recommended in order to minimize larvae mortality due to handling: place leaves infested with L2 larvae on a light box (glass surface table with a fluorescent light source underneath), so that larvae can be clearly viewed through the leaf epidermis. The larvae can be easily located by forcing them to move with softly touching the leaf surface using a fine paintbrush. Once an L2 larva is detected, a small leaf square is cut around it with a sharp scalpel. The leaf square (with the larva) is lifted with a brush or fine forceps and is placed on the tomato leaf in the bioassay tray. In a few minutes the larva will start looking for food on the fresh tomato leaf provided in the bioassay tray. Start the infestation process with the untreated control cell units (1 larva per cell) and then continue by ascending order of concentration of insecticide. Avoid cross contaminations, e.g. brush touching treated leaflets (in case it happens, immediately wash the brush well, before continuing the infestation). Each dose should have at least 32 larvae (32 cell units or one tray if using suggested model from Bio-Serv*). **Note.** As developmental time can vary between populations and slight differences in rearing / environmental conditions, the following length measurement can be used to classify L2 larvae: 4-5mm.

h. When the infestation is finished, close the trays carefully, sealing the cells with their lids (each lid closes 4 cells, if using suggested model from Bio-Serv*).

i. Store the bioassay trays in an area where they are not exposed to direct sunlight or extreme temperatures. Record maximum and minimum temperatures. If possible, maintain a temperature of 25 ± 2°C, 60-70% RH, and 16:8 light:dark photoperiod regime.

j. Perform evaluations 72 hours after placing the larvae in the trays:

<u>Evaluation of the effects on the larvae</u>: Larvae which are unable to make coordinated movement from gentle stimulus with a seeking pin or fine pointed forceps to the posterior body segment are to be considered as dead (combination of dead and seriously affected).

IRAC Susceptibility Test Methods Series

Method No: 022

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Anti-feeding effects (percentage damage to the leaf or larval growth) may also be recorded for additional information.
Evaluation of leaf damage: **Since uniform leaves were chosen at the beginning of the assay registering leaf damage as % of total leaf area mined is the preferred method.**

Express effects on larvae as percentage "affected", correcting for untreated control (wetter solution) mortality using Abbott's formula. It is recommended that the mortality data is utilized to perform a probit or logit dose-response analysis to provide LC_{50} and LC_{90} estimates for each insecticide and insect population tested. If the % of "affected" larvae in the untreated control is above 20%, the bioassay is considered to be of inferior quality and should be repeated. Ideally, control mortality should not exceed 10-15%.

Precautions & Notes:

1. **Disposable plastic equipment is preferred provided that it is not affected by the formulation constituents; glass equipment may be used but must be adequately cleaned with an appropriate organic solvent before re-use.**
2. **Insecticide products contain varied concentrations of active ingredient(s). Ensure insecticide dilutions are based on active ingredient content (g a.i.). Some diamide insecticides are sold as pre-mixtures with other insecticides, these products should not be used to determine the susceptibility of insect populations to the single insecticide, as the mixture partner may have a significant impact on the mortality data generated.**

References & Acknowledgements:

This IRAC method is based on a method developed by DuPont Crop Protection in Brazil. The method has been validated by several researchers in Europe: Dr. T. Cabello (University of Almeria, Spain), Dr. P. Bielza (University of Cartagena, Spain), Dr. E. Roditakis (NAGREF, Greece) and Pr. C. Rapisarda (University of Catania, Italy).

Tuta Testing Method Videos

A video version of the IRAC test method can be found at https://youtu.be/PSE_MwIAV0s

If the address does not open by double clicking then cut and paste into your internet browser

Additional information is available at the IRAC website http://www.irac-online.org/

If the link does not open by double clicking then cut and paste into your internet browser

3. Tuta control products, resistance publications, and method to evaluate efficacy.

Determination of baseline susceptibility of European populations of *Tuta absoluta* (Meyrick) to indoxacarb and chlorantraniliprole using a novel dip bioassay method

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The compound leaf assay. Cut compound leaves were immersed in serial insecticide concentrations and were allowed to dry as previously described. Tween 20 (0.05% v/v) was used as non-ionic wetting agent. A moist cotton plug was attached at the cut end of the leaf to provide water during the assay, and the leaf was then placed in a transparent box (dimensions 12 × 10 × 5 cm). Ten second-instar larvae were placed on the leaf in the box as previously described, and the box lid was immediately fitted to prevent larvae from escaping. Three boxes were used for each treatment, resulting in a total of 30 larvae per concentration.

All treatments were placed in a large insect rearing room with a controlled environment (26 ± 1 °C, 50–60% RH, 16:8 h L:D). Rangefinder assays for each insecticide were conducted 2–3 days prior to the toxicological test. Concentrations tested resulted in 0–100% mortality approximately. Mortality was assessed after 72 h. A larva was considered dead if no movement could be observed. A larva was recorded as moribund if no coordinated movement or deficient response to external stimulus was observed (i.e. after gentle probing with a fine paintbrush). Mortality was estimated from the total number of dead and moribund insects. Observations could be performed while the larvae were still in the gallery by using a light-bed. If insect vitality could not be clearly determined (live or moribund), the larvae were carefully extracted from the leaf to observe the responses when undistracted by the leaf epidermis.

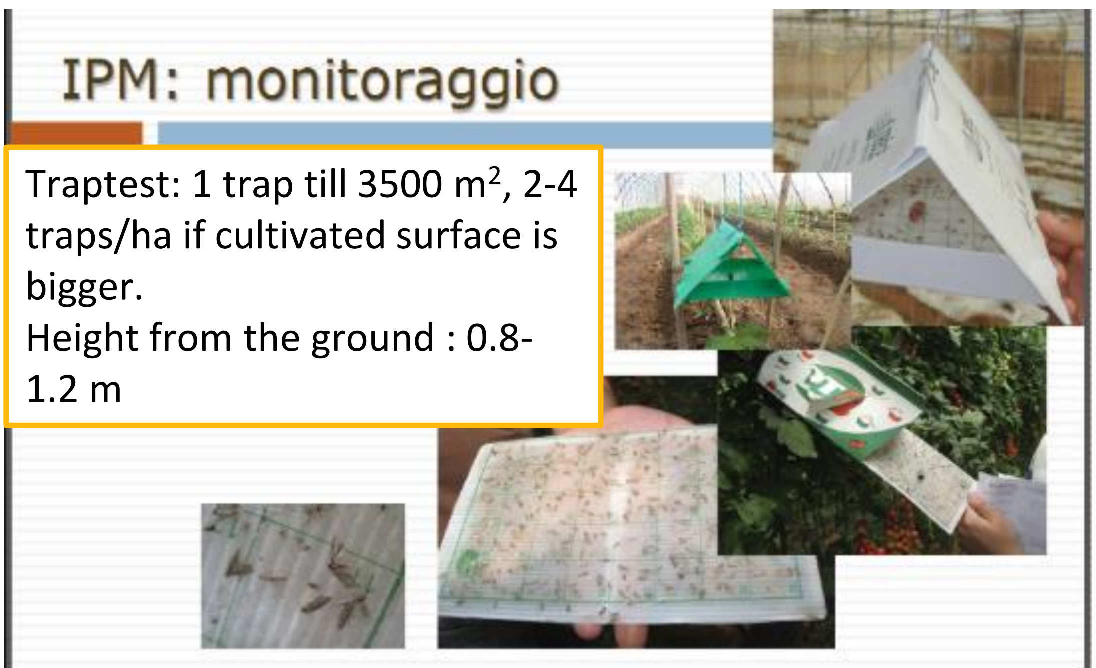
4. Monitor Tuta Populations

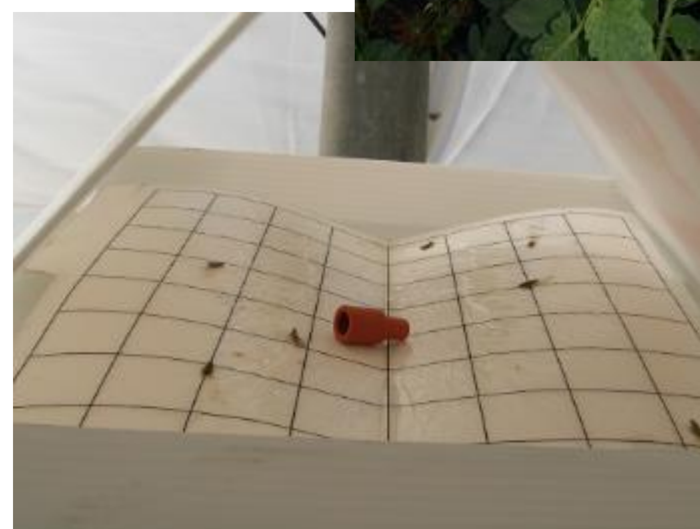
Source: IRAC

4. Monitor Tuta populations

Monitoring - Integrated Pest Management

- Place pheromone-baited traps to monitor all stages of tomato production, i.e. nurseries, farms, packaging, processing and distribution centers. Start monitoring 2 weeks before planting
- As soon as more than 3-4 moths per trap are captured each week, start mass trapping of moths
- Use locally established threshold to trigger insecticide applications





IRAC

4. Monitor Tuta populations

IPM - Monitoring and mass trapping

- For mass trapping of moths, use sticky traps or water+oil traps (20-40 traps/ha) baited with pheromone.
- Position water+oil mass traps between the ground and 0.8 m. height maximum.
- Keep using pheromone traps for at least 3 weeks after removing the crop; this catches remaining male moths

IRAC

5. Integrate key Tuta control strategies

Source: IRAC

5. Integrate key Tuta control strategies

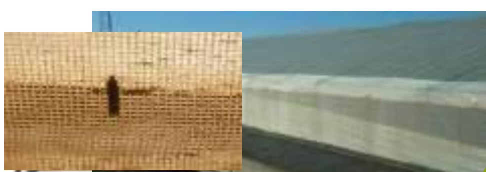
Integrated Pest Management

The basis for effective and sustainable management of Tuta absoluta is the integration of cultural, behavioural, biological and chemical control

- Suitable PPP

IPM

- Natural Enemies & BCO



- Physical & Cultural

IRAC

5. Integrate key Tuta control strategies

Integrated Pest Management – non chemical key tactics:

1. **GH cleaning and sanitation**
   - Prevent carry over of the pest from the previous crop; sanitation of the GH for a better start; use pest-free transplants; remove and destroy attacked part plants
2. **Physical control - Insect exclusion**
   - GH modern structures; insect netting; double doors; climate control
3. **Cultural control - Mass trapping**
   - Water/oil based and sticky traps pheromone-baited
4. **Biocontrol – Natural enemies**
   - Establish populations of effective biological control agents; select crop protection spray programs safe vs beneficials
5. **Mating disruption**
   - Mating disruption contribution when low density population of *T. asboluta*

IRAC

IPM – GH cleaning and sanitation

For a good start:

- Allow a minimum of six weeks from crop destruction to planting the next crop to prevent carry-over of the pest from previous crop
- Between planting cycles, cultivate the soil and cover with plastic mulch or perform solarisation
- Control weeds to prevent multiplication in alternative weed host (especially *Solanum, Datura, Nicotiana*)
- Use pest-free transplants
- Prefer modern greenhouses, that allow insect nettings, double doors, and climate control

5. Integrate key Tuta control strategies

IPM – GH cleaning and sanitation:

Remove and destroy previous tomato crop residues from GH

… and outside, in the neighborhood of cultivated area

Inside the GHs…

IRAC

5. Integrate key Tuta control strategies

IPM – GH cleaning and sanitation:

Tomato crop residues abandoned next to the GH are source of infestation

5. Integrate key Tuta control strategies

IPM – GH cleaning and sanitation:

Remove and destroy attacked part plants is limiting source of infestation

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5. Integrate key Tuta control strategies

IPM – GH cleaning and sanitation:

Remove and destroy attacked part plants

Attacked tomato fruits by *Tuta absoluta* were abandoned on the ground during picking-up / cleaning operations

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IPM – GH cleaning and sanitation:

DESTROY all Solanaceous plants near greenhouses. They harbor Tuta populations

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5. Integrate key Tuta control strategies

IPM – Physical Control

IRAC

5. Integrate key Tuta control strategies

IPM – Insect exclusion

IRAC

5. Integrate key Tuta control strategies

IPM – Insect exclusion:

Double doors

Inside view: in the safety area a sticktrap was positioned in front of the external door to catch insects that were introduced accidentally

IPM – Insect exclusion:

Double doors

Safety area with air assistance.
A fan is starting automatically when the doors open and insects entrance is more difficult

IPM – Insect exclusion:

Double doors

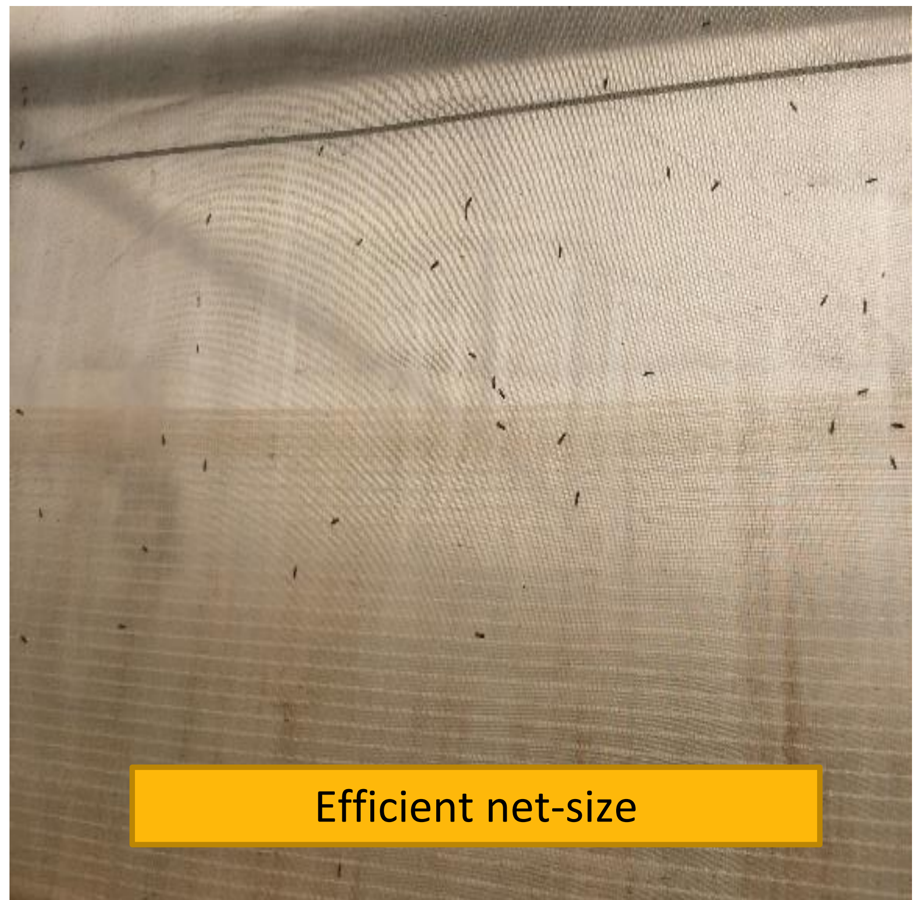
Other examples: even simple, but very effective double doors

IPM – Insect exclusion:

Insect netting

Seal greenhouse with high quality nets suitable for *Tuta absoluta*

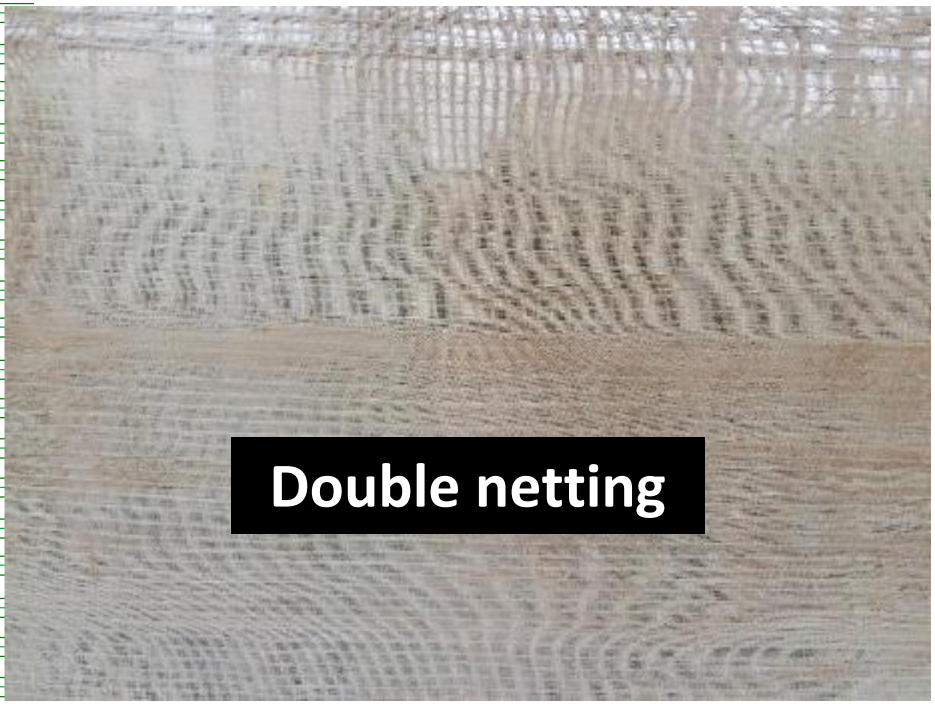
Size: min. 9 x 6 / cm²



IPM – Insect exclusion:

Insect netting

Double net for (1) bumble bees and (2) *Tuta absoluta* well positioned



IPM – Insect exclusion:

Insect netting

Avoid rips and breaks in the insect nets

Insect nets not overlapping

5. Integrate key Tuta control strategies

IPM – Insect exclusion:

Insect netting in modern GH

Protection of ventilation openings with insect nets

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5. Integrate key Tuta control strategies

Integrated Pest Management – mass trapping:

Home made mass traps: pots with water/oil and addition of pheromone bait

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5. Integrate key Tuta control strategies

Integrated Pest Management – mass trapping:

Attractive chrome sticky traps, pheromone-baited or not

Integrated Pest Management – mass trapping:

Home made sticky traps: position sticky traps at the level of *Tuta absoluta* attack on the crop. These traps should follow vegetation growing (around 2/3 of crop height)

5. Integrate key Tuta control strategies

Mating Disruption - Integrated Pest Management

Mating disruption can contribute significantly to control Tuta absoluta if:

- Low population density of *Tuta absoluta*
- Modern greenhouse structure with climate and air movement control

Integrated Pest Management – biological control

1. Establish populations of effective biological control agents (**Nesidiocoris tenuis**, **Macrolophus**, Nabis, Necremnus, Trichogramma, Pseudoapanteles, Podisus, Amblyseius)
   - Use of attractive crops: natural/local pest enemies
   - Biological control: release of beneficials (augmentative)
1. Select crop protection spray programs safe vs beneficials (conservation)

Egg parasitoids

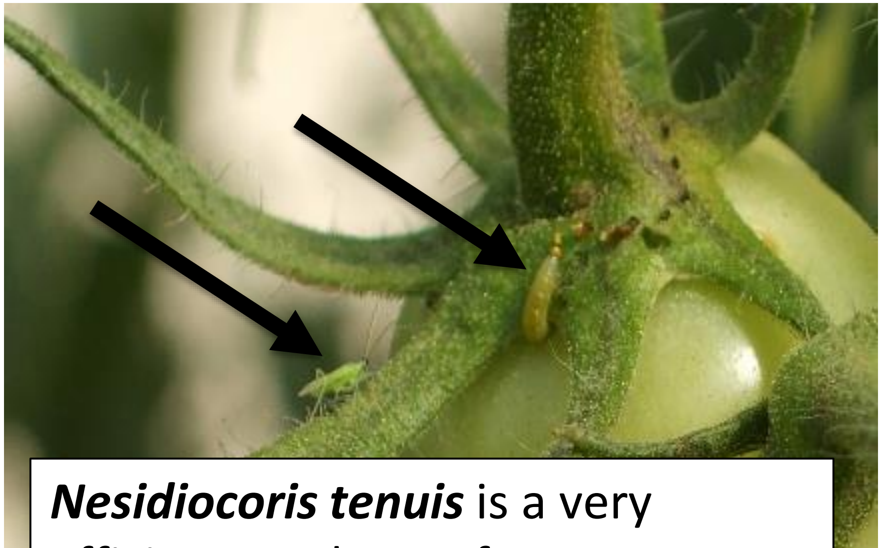
Trichogramma exiguum (South America)
Trichogramma nerudai (South America)
Trichogramma pretiosum (South America)
Trichogramma achaeae (Mediterranean)

Larval parasitoids

Necremnus artynes .
Stenomesius sp. .
Neochrysocharis formosa
Habrobracon hebetor .
Diadegma ledicola
Apanteles gelechiidivoris
Dineulophus phthorimaeae
Pseudoaphanteles dignus

IRAC

Integrated Pest Management – biological control



Nesidiocoris tenuis is a very efficient predator of *Tuta absoluta*'s eggs and occasionally young larvae

Attractive crops for *Nesidiocoris tenuis* (i.e. **Pumpkin**) are planted at the beginning of the crop cycle to attract the beneficials. This establishes populations near the crop

5. Integrate key Tuta IPM control strategies

Key Management Strategy - Integration of Control Measures

The basis for effective and sustainable management of *Tuta absoluta* is the integration of cultural, behavioural, biological and chemical control.

Key Management Tactics

- Allow a minimum of 6 weeks from crop destruction to planting the next crop to prevent carry-over of the pest from previous crop
- Between planting cycles, cultivate the soil and cover with plastic mulch or perform solarisation
- Control weeds to prevent multiplication in alternative weed host (especially *Solanum, Datura, Nicotiana*)
- Prior to transplanting, install sticky traps
- Use pest-free transplants
- Seal greenhouse with high quality nets suitable for *T. absoluta*
- Place pheromone-baited traps to monitor all stages of tomato production, i.e. nurseries, farms, packaging, processing and distribution centers. Start monitoring 2 weeks before planting
- Inspect the crop to detect the first signs of damage

10

IRAC

5. Integrate key Tuta IPM control strategies

Key Management Strategy - Integration of Control Measures

Key Management Tactics

- As soon as more than 3-4 moths per trap are captured each week, start mass trapping of moths.
- For mass trapping of moths, use sticky traps or water + oil traps (20-40 traps/ha) baited with pheromone
- Keep using pheromone traps for at least 3 weeks after removing the crop; this catches remaining male moths
- Remove and destroy attacked plant parts
- Establish populations of effective biological control agents (*e.g. Nesidiocoris tenuis, Necremnus, Trichogramma, Macrolophus, Pseudoapanteles, Podisus, Nabis / Metarhizium*)
- Use locally established thresholds to trigger insecticide applications
- Select insecticides based on known local effectiveness and selectivity
- Rotate insecticides by MoA group, using a window approach (see page 13 & 14)
- Use only insecticides registered for control of *T. absoluta* or lepidopteran leaf miners and always follow the directions for use on the label of each product
- Maintain population levels below economic threshold
- Distribute pheromone dispensers to disrupt mating

6. Understand Action Thresholds for chemical and microbiological control

Source: IRAC

6. Understand Action Thresholds for chemical and microbiological control

Why monitor my farm?

- Detect first occurrences as *Tuta absoluta*
- Monitor local presence/absence
- Monitor populations in individual fields to make decisions on Tuta management.
- The use of Action Treatment Thresholds depend on accurate assessment of pest populations. This allows farmers to time sprays before economic crop damage occurs.

Use the action spray thresholds developed and recommended by local experts within your respective countries

6. Understand Action Thresholds for chemical and microbiological control

Tuta absoluta: how can we monitor the presence and the pressure

➢First action: visual monitoring and level of damages

The experience from Argentina. 2 possibilities:

- Weekly observation on 20 plants/1000 sqm or 10 for a surface < 500 sqm: record the number of infested leaflets (with lived larva) → with more than 2 infested leaflets are detected → TREATMENT!

- Weekly observation on 100 plants: record the number of juvanile stages (larva+pupa) → with more than 8-12 specimens→ TREATMENT!

6. Understand Action Thresholds for chemical and microbiological control

Tuta absoluta: how can we monitor the presence and the pressure

➢Second action: pheromone traps positioning

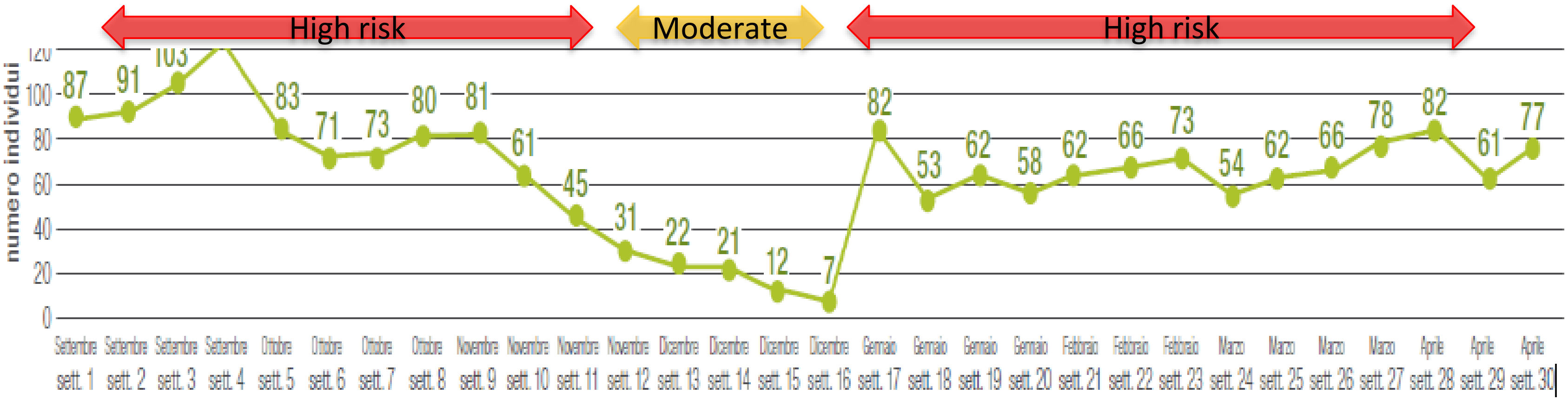
The experience from Spain.

- <u>Weekly observation of the male captures: </u>1 trap/3.500 mq in GH or 2-4 traps/hectar with a GH surface > 3.500 mq.

Nb captures	Level of Risk	Action
0	N	• None
< 10 / month <3 / week	Very low	• Mass trapping (15-20 traps/ha) with water traps
3-30 / week	Moderate	• Mass trapping (15-20 traps/ha) with water traps • Treatments (every 10-15 days) with azadiracthin, mineral oil, *Bacillus thuringensis*
> 30 / week	High	• Mass trapping (15-20 traps/ha) with water traps • Treatments (every 10-15 days) with azadiracthin, mineral oil, *Bacillus thuringensis* • Treatments with chemical compounds like Indoxacarb, Spinosad, Emamectine, Clorantraniliprole, etc.

6. Understand Action Thresholds for chemical and microbiological control

Example of flight curve (September to April) in Italian situation

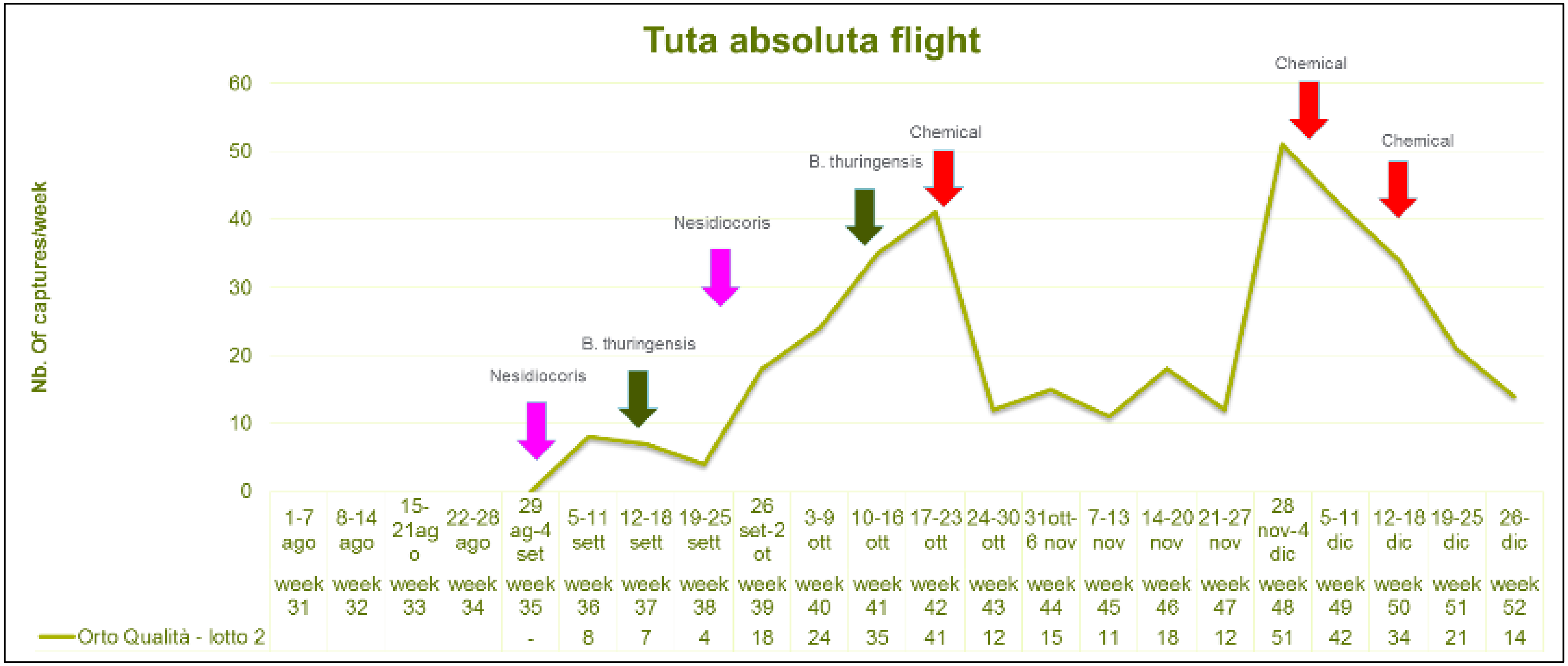


Fonte dati: Giacomo Purromuto

Long presence of high pressure → high risk: important to manage the pest according the the MoA rotation

6. Understand Action Thresholds for chemical and microbiological control

Example of spray application using different solutions according to the flight

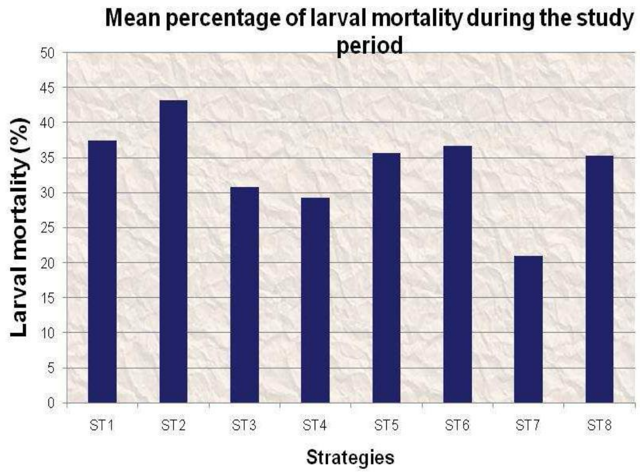


Source: G. Purromuto

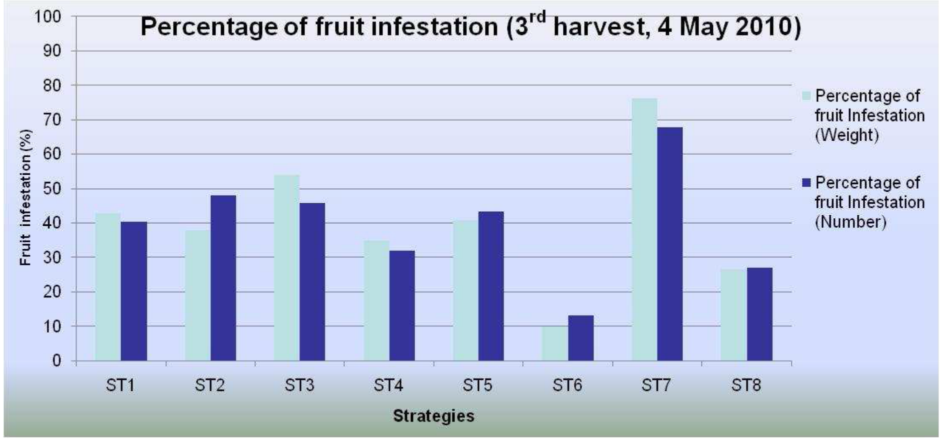
6. Understand Action Thresholds for chemical and microbiological control

The Tunisian study for different strategies according to monitoring

Strategies	Thresholds
ST1	0<ST1=<4 mines or larvae per plant
ST2	4<ST2 =<8 mines or larvae per plant
ST3	8<ST3=<16 mines or larvae per plant
ST4	>16 mines or larvae/plant
ST5	Chemical control
ST6	Organic sprays (spinosad)
ST7	Control (no sprays)
ST8	Both (chemical and organic sprays)



To evaluate the proposed thresholds (strategies), weekly scooting of 5 randomly plants per treatment per block was conducted to determine if thresholds had been reached (from April 18 to June 12, 2010). Concerning the strategies ST1, ST2, ST3 and ST4, the first spray was undertaken within 48 hours when the thresholds had been reached followed by regular spraying (every 10 to 12 days). For strategies ST5, ST6, and ST8 The first spay was undertaken when infestation appeared followed by regular spraying every 10 to 12 days



Source: Control Action threshold for Tuta absoluta (Lep., Gelechiidae) in tomato raised under greenhouse, M. BRAHAM & A. BENSALEM

7. Maximize pest control using adjuvants and app tech equipment

7. Maximize pest control using adjuvants and app tech equipment

Tuta Control is Difficult:

- Controlling the immature leafmining stage of Tuta is difficult since it is protected from foliar applied insecticides while within the leaf cuticle.
- Crop growth, plant structure, and production practices (particularly if trellised) make it more difficult to obtain good spray coverage.

To Maximize Management of Tuta Populations:

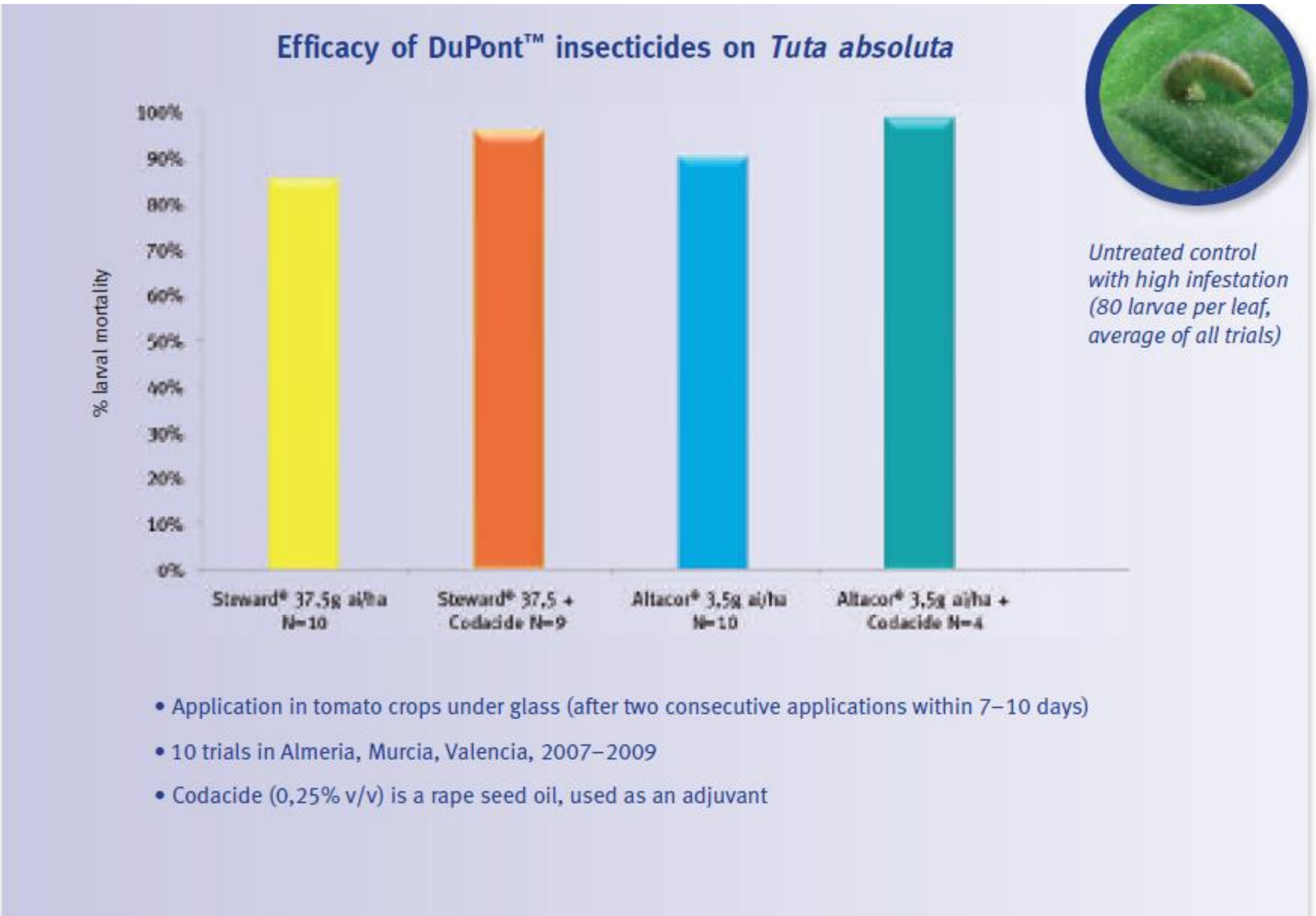
- The addition of oils or adjuvants help products penetrate the plant cuticle to reach the mining larvae in the leaf.
- It is critical to use and maintain the best possible spray equipment to ensure excellent coverage and acquire the highest possible insecticidal efficacy.

IRAC

7. Maximize pest control using adjuvants and app tech equipment

Steward® shows an efficacy of about 85% against *T. absoluta* larvae. The addition of a suitable registered adjuvant (e.g. a rape seed or paraffinic oil) increases efficacy to about 95% by helping more product penetrate the leaves.

Altacor® shows even higher efficacy on *T. absoluta*, about 90% without, up to 98% with the addition of a suitable adjuvant.

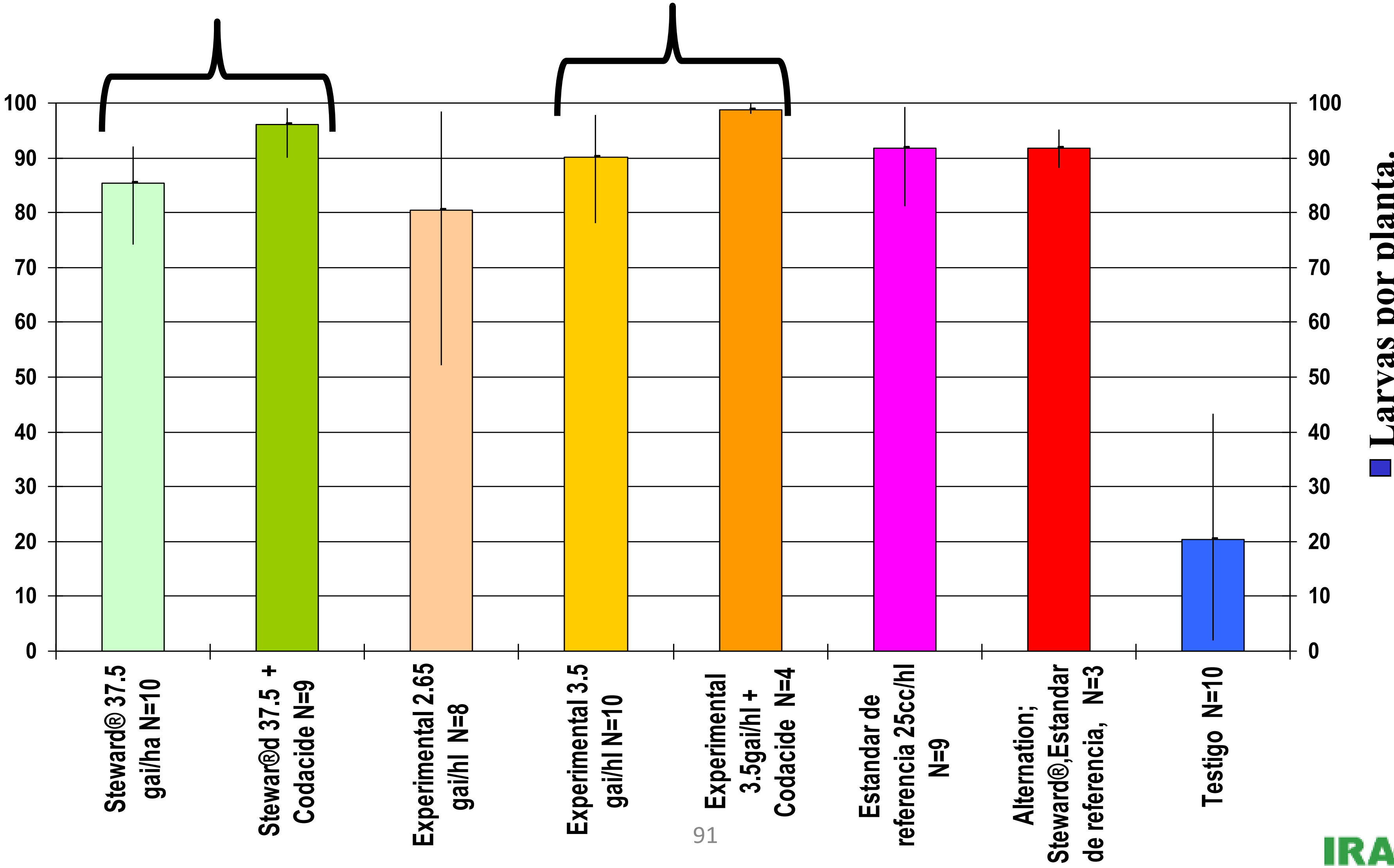


IRAC

7. Maximize pest control using adjuvants and app tech equipment

Increase in Tuta control using Codacide adjuvant.

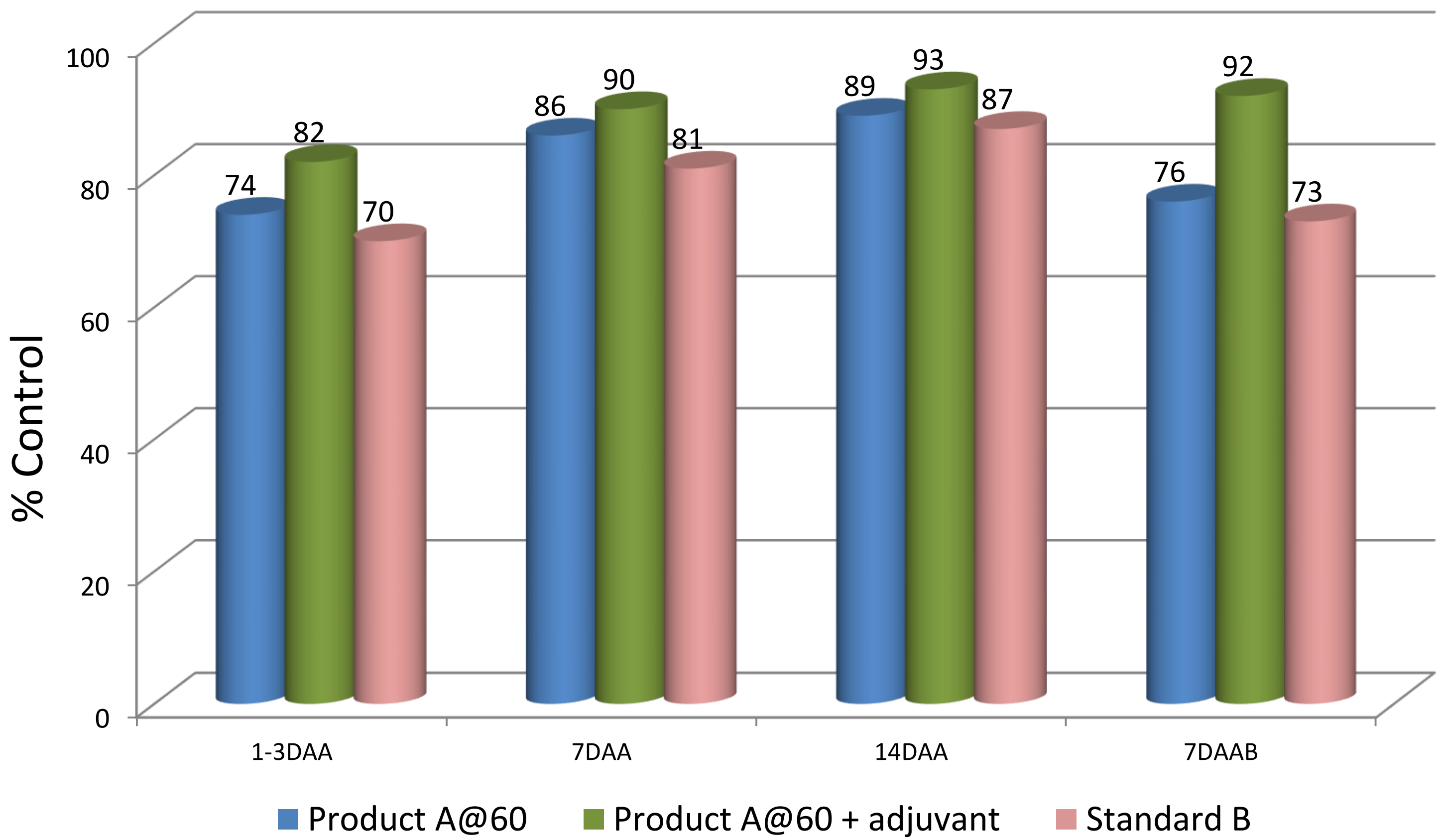
Percent control after two applications: 2007-2009



IRAC

7. Maximize pest control using adjuvants and app tech equipment

- **This is a summary of average efficacy across 12 trials*, carried out in Italy and Spain during 2012 and 2013 in *Tuta absoluta*, comparing the efficacy of a new product with and without adjuvants:**



* Internal data from Dow AgroSciences

IRAC

7. Maximize pest control using adjuvants and app tech equipment

- Different equipment used may affect final efficacies: Data from A. Monserrat

This equipment my give only 50-70% of the potential efficacy of the products (so it may loose 30-50% efficiency)

This equipment my give only 70-90% of the potential efficacy of the products (so it may loose 10-30% efficiency)

This equipment my give only 80-99% of the potential efficacy of the products (so it may loose 1-10% efficiency)

7. Maximize pest control using adjuvants and app tech equipment

South Africa:

Various foliar application methods and equipment.


D van Heerden


D van Heerden


D van Heerden

IRAC

7. Maximize pest control using adjuvants and app tech equipment

South Africa:

Various foliar application methods and equipment.

7. Maximize pest control using adjuvants and app tech equipment

Formulation Mixing Sequence

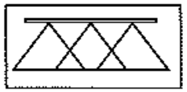
All crop protection products are provided as formulations, most of which are designed to disperse in water. The chemistry of formulation science requires that mixing in water take place in a defined order to assure that the applicator ends up with a sprayable mixture. In the universe of pesticide active ingredients, most are water insoluble, and their inherent nature is to separate from the spray water unless their protective shields of surface-active agents have been fully activated.

The Formulation Science mixing sequence is:

1. Water soluble bags (WSB)
2. Water soluble granules (SG)
3. Water dispersible granules (WG)
4. Wettable powders (WP)
5. Water based suspension concentrates (aqueous flowables) (SC)
6. Water soluble concentrates (SL)
7. Oil based suspension concentrates (OD)
8. Emulsifiable concentrates (EC)
9. Surfactants, oils, adjuvants
10. Soluble fertilizers
11. Drift retardants

7. Maximize pest control using adjuvants and app tech equipment

Sprayer Calibration



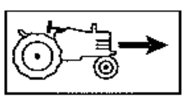
Broadcast Application

Sprayer calibration **(1) readies your sprayer for operation** and **(2) diagnoses tip wear.** This will give you optimum performance of your TeeJet® tips.

Equipment Needed:

- TeeJet Calibration Container
- Calculator
- TeeJet Cleaning Brush
- One new TeeJet Spray Tip matched to the nozzles on your sprayer
- Stopwatch or wristwatch with second hand

STEP NUMBER 1



Check Your Tractor/Sprayer Speed!

Knowing your real sprayer speed is an essential part of accurate spraying. Speedometer readings and some electronic measurement devices can be inaccurate because of wheel slippage. Check the time required to move over a 100- or 200-foot strip on your field. Fence posts can serve as permanent markers. The starting post should be far enough away to permit your tractor/sprayer to reach desired spraying speed. Hold that speed as you travel between the "start" and "end" markers. Most accurate measurement will be obtained with the spray tank half full. Refer to the table on page 140 to calculate your real speed. When the correct throttle and gear settings are identified, mark your tachometer or speedometer to help you control this **vital** part of accurate chemical application.

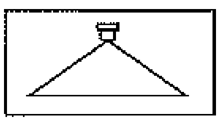
7. Maximize pest control using adjuvants and app tech equipment

STEP NUMBER 2

$A = \frac{B+C}{D}$ **The Inputs**

Before spraying, record the following:	**EXAMPLE**
Nozzle type on your sprayer (All nozzles must be identical)	TT11004 Flat Spray Tip
Recommended application volume (From manufacturer's label)	20 GPA
Measured sprayer speed	6 MPH
Nozzle spacing	20 Inches

STEP NUMBER 3



Calculating Required Nozzle Output

Determine GPM nozzle output from formula.

FORMULA: $GPM = \frac{GPA \times MPH \times W}{5{,}940 \text{ (constant)}}$

EXAMPLE: $GPM = \frac{20 \times 6 \times 20}{5{,}940} = \frac{2{,}400}{5{,}940}$

ANSWER: 0.404 GPM

7. Maximize pest control using adjuvants and app tech equipment

STEP NUMBER 4

Setting the Correct Pressure

Turn on your sprayer and check for leaks or blockage. Inspect and clean, if necessary, all tips and strainers with TeeJet brush. Replace one tip and strainer **with an identical new tip and strainer** on sprayer boom.

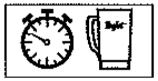
Check appropriate tip selection table and determine the pressure required to deliver the nozzle output calculated from the formula in Step 3 for your new tip. Since all of the tabulations are based on spraying water, conversion factors must be used when spraying solutions that are heavier or lighter than water (see page 141).

Example: (Using above inputs) refer to TeeJet table on page 7 for TT11004 flat spray tip. The table shows that this nozzle delivers 0.40 GPM at 40 PSI.

Turn on your sprayer and adjust pressure. **Collect and measure the volume of the spray from the new tip for one minute in the collection jar.** Fine tune the pressure until you collect .40 GPM.

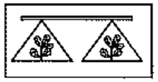
You have now adjusted your sprayer to the proper pressure. It will properly deliver the application rate specified by the chemical manufacturer at your measured sprayer speed.

STEP NUMBER 5



Checking Your System

Problem Diagnosis: Now, check the flow rate of a few tips on each boom section. If the flow rate of any tip is 10 percent greater or less than that of the newly installed spray tip, recheck the output of that tip. If only one tip is faulty, replace with new tip and strainer and your system is ready for spraying. However, if a second tip is defective, **replace all tips on the entire boom**. This may sound unrealistic, but two worn tips on a boom are ample indication of tip wear problems. Replacing only a couple of worn tips invites potentially serious application problems.



Banding and Directed Applications

The only difference between the above procedure and calibrating for banding or directed applications is the input value used for "W" in the formula in Step 3.

For single nozzle banding or boomless applications:

W = Sprayed band width or swath width (in inches).

For multiple nozzle directed applications:

W = Row spacing (in inches) divided by the number of nozzles per row.

8. Understand Insecticide Resistance Management PRINCIPLES

Source: IRAC

8. Understand Insecticide Resistance Management Principles

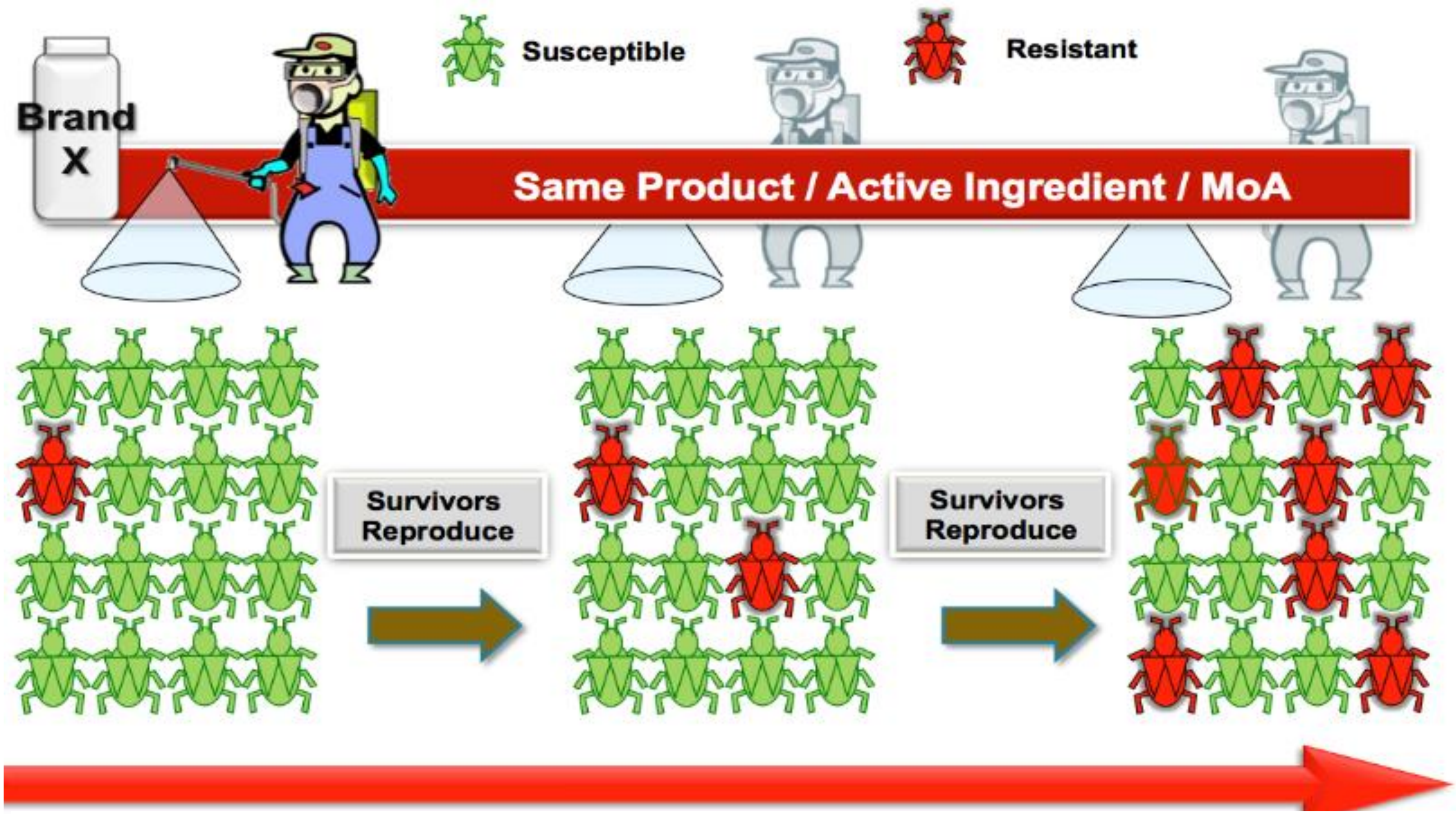
Continued Use of the Same MoA Products Throughout the Season Will Increase # of Resistant Individuals and Spray Expenses

- **Number & timing of applications influence speed of resistance**
- **When insecticides with the same mode of action (MoA) are used repeatedly, <u>exposing multiple consecutive pest generations</u>, less sensitive individuals survive and resistance can evolve.**
- **Continued use accelerates resistance and multiplies the resistant genes in the population**
- **Farmers will increase rates to improve control, accelerating resistance.**
- **Excessive tank mixing with adjuvants and other insecticides increases**
- **Pest control becomes expensive**

8. Understand Insecticide Resistance Management Principles

Continuous use of the same Mode of Action removes the susceptible individuals leaving a tolerant population that survives the insecticide application

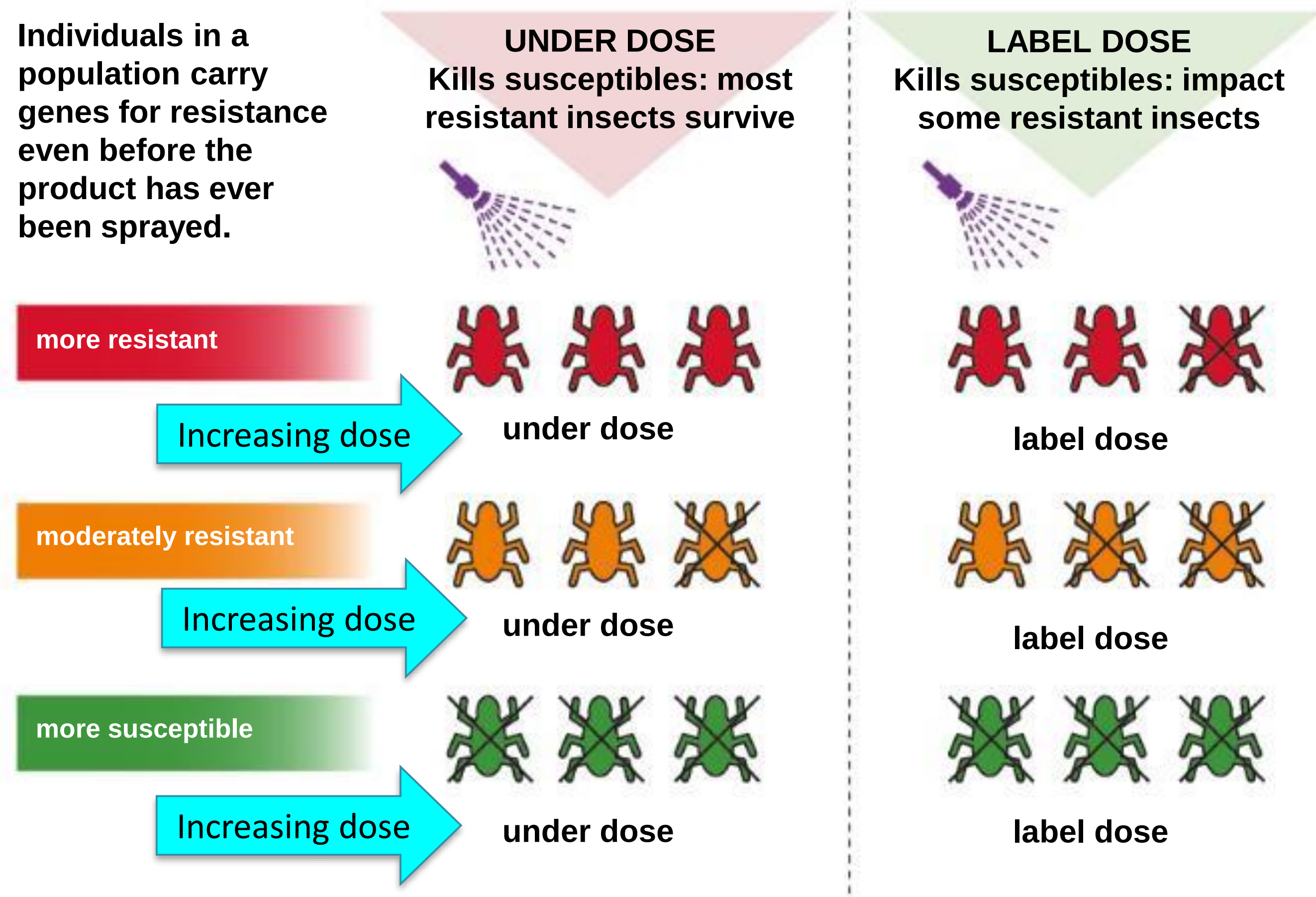
Possible Scenario for Resistance Development in an Insect Population



8. Understand Insecticide Resistance Management Principles

Under-dosing Speeds the Rate of Resistance:
Maximize Insect Kill With Every Spray

An under-dosed insecticide application may not remove moderately resistant insects from a pest population. This can accelerate the evolution of resistance



Always apply the recommended label rate that will remove susceptible, some moderately resistant, and even a portion of resistant insects.

8. Understand Insecticide Resistance Management Principles

Acquiring the highest level of pest control within a generation removes Resistant genes.

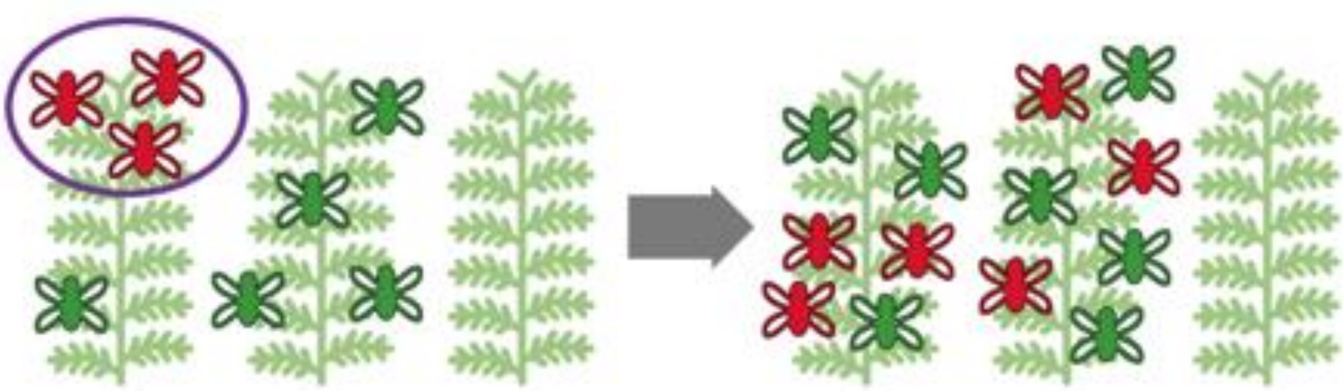
- Need to remove individuals with at least one resistant gene (RS)
- Need high level of control for an entire insect generation – prevent gene transfer
- Need back to back sprays of products with different or same mode of action if adult flights and egg laying continues

IRAC

8. Understand Insecticide Resistance Management Principles

Insect Migration (exchange of Resistant insects) Influences the Speed of Resistance.

- Resistance levels in pest populations can be INCREASED through immigration of resistant insects. Therefore, the evolution of resistance in the pest population may accelerate.



Immigration of resistant insects into a population of sensitive pest insects

Result: The percentage of resistant insects in the population is increased. The inter-breeding between sensitive and resistant insects will likely increase the level of resistance in the next generation.

8. Understand Insecticide Resistance Management Principles

Reproductive Capacity Influences the Speed of Resistance

- Species with a higher reproductive capacity have a higher risk of developing resistance.
- *Tuta absoluta* can have up to with up to 10 - 14 generations per year.
- Temperature drives reproductive capacity. High temperatures increase the number of generations per year and can accelerate rate of resistance.

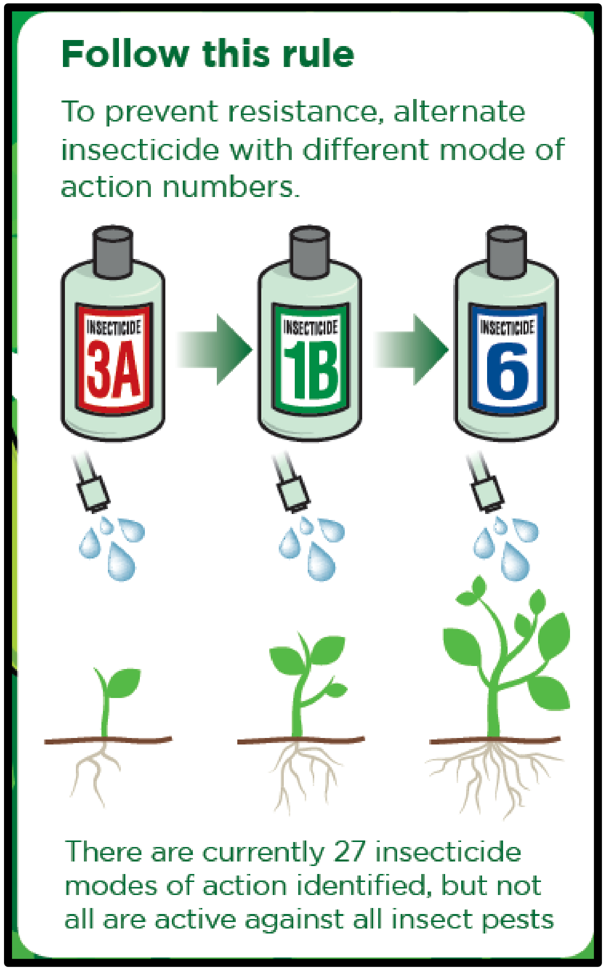
8. Understand Insecticide Resistance Management Principles

Implementing IPM Removes Resistant Individuals from the Population and Improves Level of Pest Control

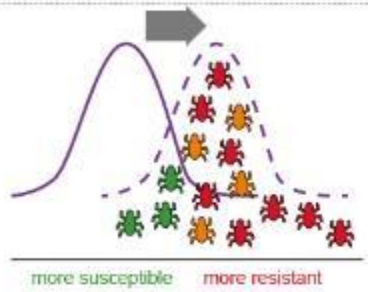
- Diversify insect control methods: Integrate cultural (sanitation), physical (mass trapping, netting to exlude), biological (beneficials, pheromones), and chemical control methods
- Monitor pest populations to determine the correct timing of application at the action spray threshold
- Apply the right product at the recommended life stage
- Follow labeled application rates and intervals
- Calibrate sprayer and maintain nozzles and equipment
- Use optimal spray volumes and best management technique
- Select insect control products that are compatible with natural enemies. Allow the simultaneous use of both strategies to more completely reduce a pest population.
- Avoid using products that will reduce non-target organisms
- Adjust water pH and use adjuvants if necessary

Rotating insecticides with Different Modes of Action Reduces Selection Pressure for Resistance

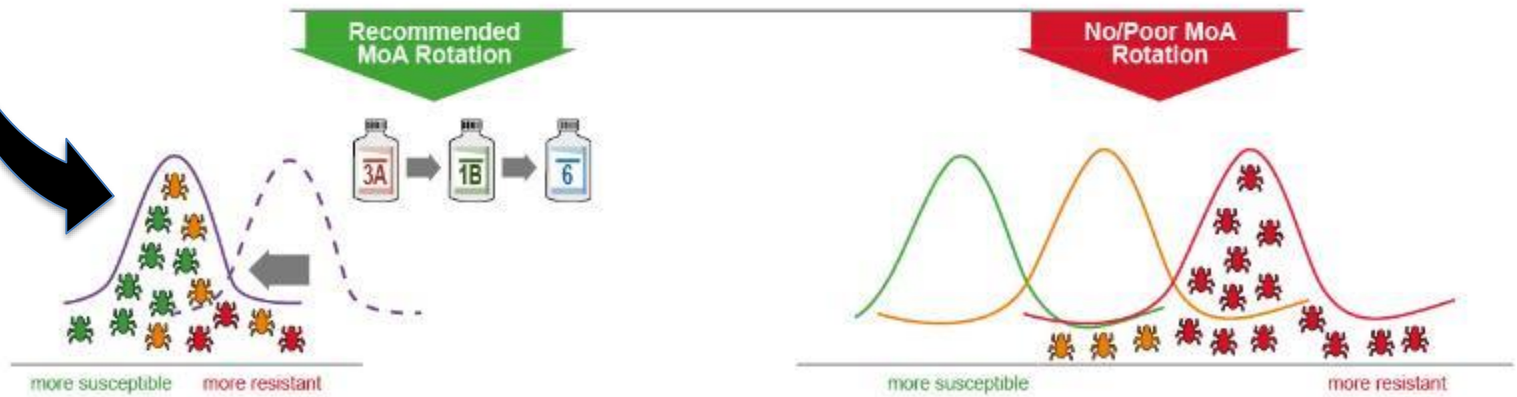
- Repeated exposure of pest populations to insecticides with the same Mode of Action will select for resistant insects.
- Two successive insect generations shouldn't be treated with insecticides that have the same Mode of Action number (examples 3, 1, 6). Products in Mode of Action subgroups (example 3A) should not be rotated among products within the same MoA group (example 3).



Rotating insecticides with Different Modes of Action Reduces Selection Pressure for Resistance



After an insecticide is sprayed, the surviving insects will reproduce and the offspring will be less sensitive.



Rotation of insecticides with different modes of action prevent the build up of resistant individuals in the field. This IRM strategy ensures that most resistant survivors from the MOA 1 spray(s) will be killed by the subsequent rotation of products containing different modes of actions.

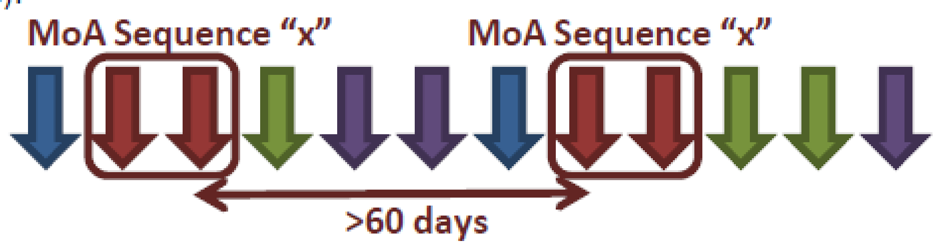
Under permanent selection pressure, the overuse of the same insecticide mode of action can select for less and less susceptibility and a resistant population will evolve.

Exposing fewer pest generations in a season to insecticides with the same MoA reduces selection pressure for resistance

Rotate MoA Products Within Windows of Time

Mode of Action Gap Approach:

- The basic rule for adequate rotation of insecticides by mode of action (MoA) is to avoid treating consecutive generations of the target pest with insecticides of the same MoA group, by using a scheme of "MoA gap".
- A MoA gap is here defined as a period of 60 consecutive days, based on the maximum duration of a single generation of *T. absoluta*.
- A MoA sequence is here defined as one or more consecutive applications of insecticides belonging to a particular MoA group.
- After the last treatment of a MoA sequence, wait at least 60 days for new applications with insecticides of that MoA (follow label for maximum number of consecutive applications and per crop cycle).



Different coloured arrows represent different MoA groups

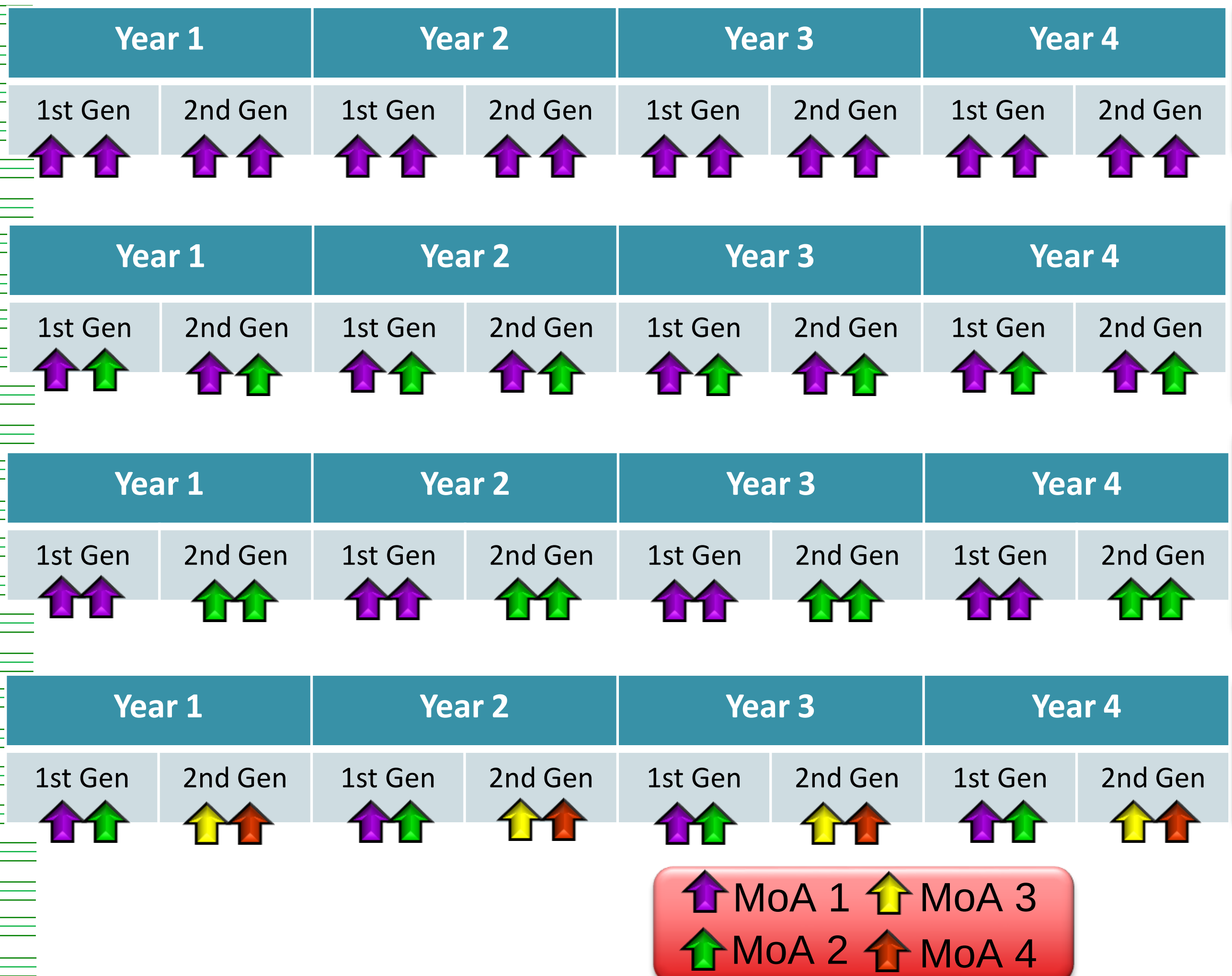
- The proposed scheme seeks to minimize the selection of resistance to any given MoA group by allowing a gap between MoA sequences, ensuring that consecutive generations of *T. absoluta* are not exposed to the same insecticide MoA group.

8. Understand Insecticide Resistance Management Principles

Rotate MoA Products Within Windows of Time

IRM guidelines below show least to best product rotaton recommendations

Maintaining insect susceptibility greatly depends on rotation of insecticides with effective products with a different MOA that eliminate resistant individuals. Rotation with products that provide poor control of the target pest increases the risk of developing Diamide resistance.



No alternation/rotation
High selection pressure
No recover of sensitive population

Rotation within generation
Consecutive generation exposed to same MoA. Selection pressure doesn't change between generation. Risk of resistance development for both ai's

Rotation among generations
Following generations are not exposed to same MoA. Selection pressure doesn't increase within the generation. Recovery of susceptible population.

Rotation within and between
Ideal situation (very low risk) Not alway applicable with good efficacy

IRAC

8. Understand Insecticide Resistance Management Principles

Practicing Resistance Management is a Benefit to the Grower

- **Save money**
  - No need to increase number of insecticide applications
  - Reduces need for more expensive products or control methods
  - Helps achieve better pest control and improved yield

- **Save time**
  - Spend less time in the field making repeat applications
  - Less effort and worry trying to achieve effective pest control

- **Enhance safety of produce**
  - Better assurance of consistent crop protection
  - Minimizes residue risk on produce

- **Protect your health and your land**
  - Less active ingredient applied to ecosystem
  - Better worker safety due to fewer applications and less exposure

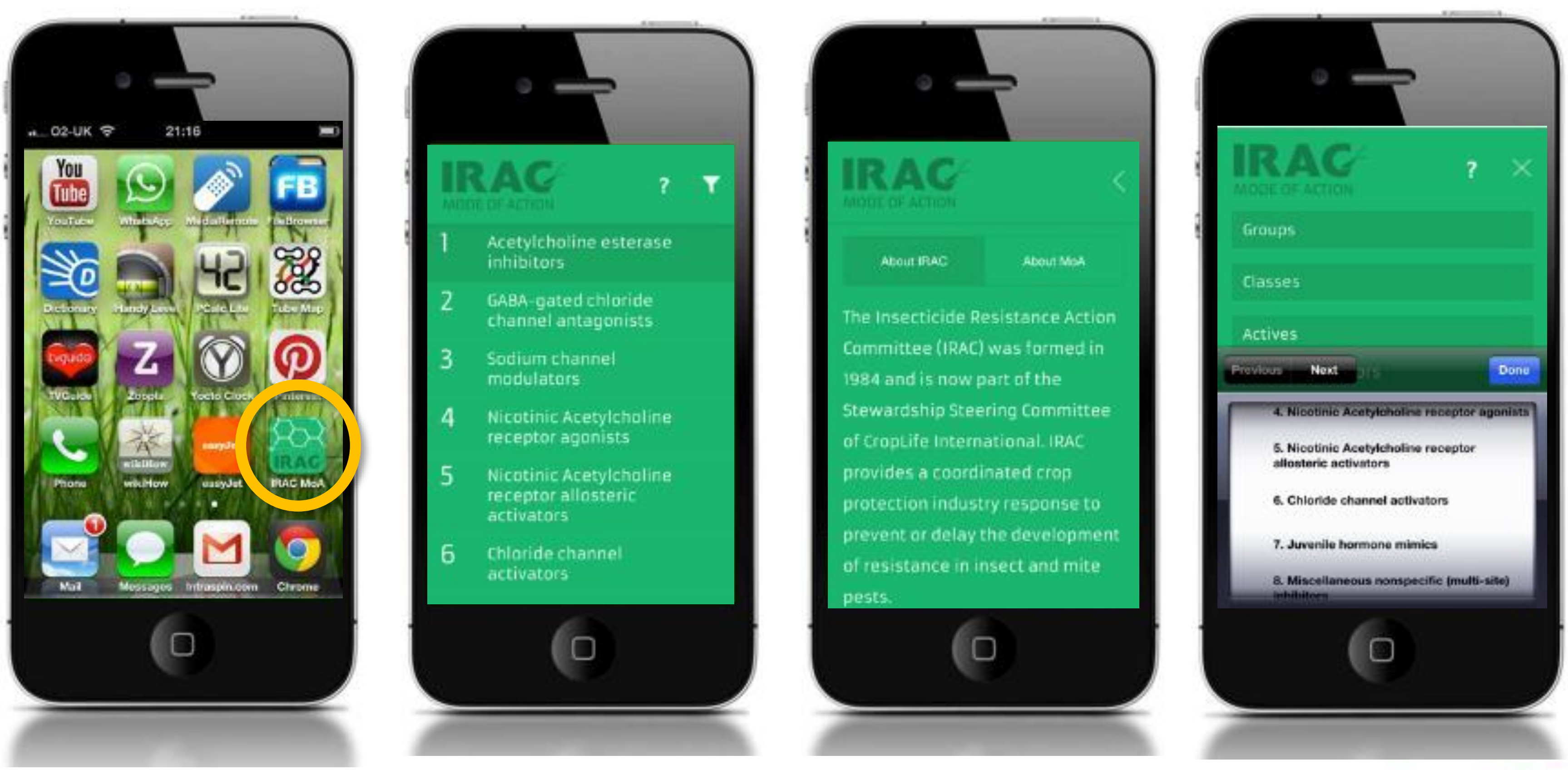
Source: IRAC

IRAC

A Tool to Help You Identify Different Product Chemistries

Mode of Action Classification: Phone/Tablet App (Its Free!!)

Search for: IRAC moa



CropLife

9. Understand Insecticide Resistance Management STRATEGIES

Source: IRAC

Manage Insecticide Resistance: Follow These Recommendations

- The following IRM recommendations have been developed by the International IRAC organization, Country IRAC Groups, Country Ressitance Action Goups, with leading local experts.
- This information is intended to provide the basis for developing an effective pest management program that minimizes the risk of insecticide resistance.
- These are general guidelines and will not fit all crop production systems. Adapt these recommendations and strategies to your local needs.

Source: IRAC

IRM Recommendations for Tuta absoluta on Tomato - 1

- **Practice Integrated Pest Management**
  - Remove and destroy infested cull tomatoes and plant material
  - Remove all wild Solanaceous and other host plants near greenhouse
  - Rennovate greenhouse to exclude Tuta adults
  - Use phermones and sticky traps to monitor and mass trap adults
  - Augment and conserve natural enemy populations
  - Apply entomopathic nematodes (*Steinernema feltiae*) in a foliar spray
  - Use optimal spray volume, maintain and calibrate spray equipment
  - Treat large areas to same MoA
  - CALIBRATE/ MAINTAIN sprayers. Clean/replace nozzles.

- **Apply insecticides at economic pest thresholds**
  - Follow locally established economic pest thresholds for the application of foliar insecticides in order to optimize insecticide use.
  - Always use labeled rates and water volumes.

- **Use windows of insecticide application**
  - Use windows of application to minimize exposure of sequential generations of a insect pest species to the same insecticide modes of action.
  - Each window should be approximately 30 days.

- **Rotate insecticides with different modes of action.**
  - If more than one insecticide application is required during an application window then it is recommended to use an insecticide with a different mode of action.
  - Multiple applications of insecticides with the same mode of action within a single window are acceptable as long as combined effects (residual activity) of the applications do not exceed approximately the 30-day window.

- **Maximum Number of MoA Applications**
  - It is preferred to use the same MOA products in only 2 windows per seasn
  - Aoid using the same Mode of Action products in more than 3 windows.

- **Insecticide mixtures**
  Tank mixing products:
- Do not tank-mix insecticide products with the SAME MoA.
- When tank-mixing insecticide products with DIFFERENT MoA's, follow label rates for each insecticide.
- Respect maximum number of applications, PHI and REI stated in the label of each product.
- Product(s) applied on subsequent window/pest generation should have an MoA that is different from both tank-mix partners.

- **Avoid insecticides with Tuta resistance**
  Consult with local experts to determine which insecticides are affected by resistance in your locality. A preference to insecticides which are not affected by resistance should be given.

- **Preserve non-target & beneficial organisms**
  The use of selective insecticides with reduced impact on non-target and beneficial organisms is recommended whenever possible.

- **Manage the removal of in-season infested stems and fruit**
  In addition to practicing clean sanitation pre and post season it is critical to remove and destroy plant stems pruned during the season and all cull/waste tomato after each harvest.

- **Rotate crops and Incorporate a Host Free Period**
  - Subsequent crop plantings should be of a different crop type, which is not a host to the insects which are pests of Tuta.
  - Institute an area-wide fallow period where only non-host crops to Tuta can be planted disrupting the life cycle of Tuta.

IRM Recommendations for Tuta absoluta on Tomato - 2

MULTIPLE MoA PRODUCTS AVAILABLE

Different MoA products can be used in the **same** window but they must be rotated to different MoA products in the next window.

Gen 1	Gen 2	Gen 3
MoA **A & B**	MoA **C & D**	MoA **A & D**

FEW MoA PRODUCTS AVAILABLE

Apply products with the same MoA in the **same** window.

Gen 1	Gen 2	Gen 3
MoA **A & A**	MoA **B & B**	MoA **C & C**

- **Rotating products with different Modes of Action delays resistance.** Don't apply the same Mode of Action continuously:
  - Rotate insecticides with different modes of action using the window approach to minimize exposure of sequential generations of a pest species to the same insecticide MoA.
  - Each "treatment window" should be approximately 30 days.
  - Multiple applications can be made in a window:
    - If more than one insecticide application is required then attempt to use an insecticide with a different mode of action.
    - Multiple applications of insecticides with the same mode of action within a single window are acceptable if their combined residual activity does not exceed approximately the 30-day window.
  - After a "treatment window" of approximately 30 days rotate to a window with different MoA products for approx 30 days.. Allow at 30-60 days before applying the same mode of action again.
- For crops longer than approx. 100 days, use the same MoA products in only 2 windows per season
- For crops less than approx 100 days then use same MoA products in only one window within the crop cycle.
  A short cycle crop (< 50 days) is a "treatment window". Rotate products with different MoA in the next planting.
- Don't treat the crop for more than approximately 50% of the cropping season or 50% of the total number of applications with same MoA

IRM Recommendations for Tuta absoluta on Tomato - 3

- Select insecticides based on known local effectiveness and selectivity to beneficials.
  - Know the attributes of your pest control products (adulticide, ovicide, larvicide, safety to beneficials, residual, spectrum)
  - Use larvicides to treat young larvae
  - Do not underdose. Follow label rates and intervals
  - Use surfactants (wetting agents) to assure better coverage or methylated seed oil to acquire leaf cuticle penetration. Surfactants may be important to improve the activity of some insecticides.
  - In high populations combine larvicide with adulticide or ovicidal product

- Use sufficient spray volume.
  - Maximize coverage to maximize pest kill
  .
- Whenever possible, use products and mixes that are selective and conserve natural enemies and pollinators
  - Conserve natural enemies early season so they can assist in pest control season-long.
  - Use B.t's and non-chemical products against low Tuta populations.

- Stop using products that are not providing good efficacy. Try that product again next season.
- Ideal to treat large areas with the same mode of action product and follow the same window rotation strategy
- Tank mix insecticides to control different life stages and manage pest populations.
- Rotate solanaceous crops with crops that are not a host to Tuta.

IRAC
Insecticide Resistance Action Committee

9. Implement Insecticide Resistance Management Strategies

Example: Application Windows for Tuta absoluta on Tomato

Do not to use the same insecticide MoA used in a previous window

Window 1	Window 2	Window 3	Window 4	Window 5	Window 6
Insecticides A....A or A....B or A.....B....C	Insecticide D....D or D....E or D....E.....F	Insecticide A....A or A....B or A....B....C	Insecticide D....D or D.....E or D....E....F	Insecticide A....A or A....B or A....B....C	Insecticides D....D or D....E or D....E....F

Rotation of different MoA Groups

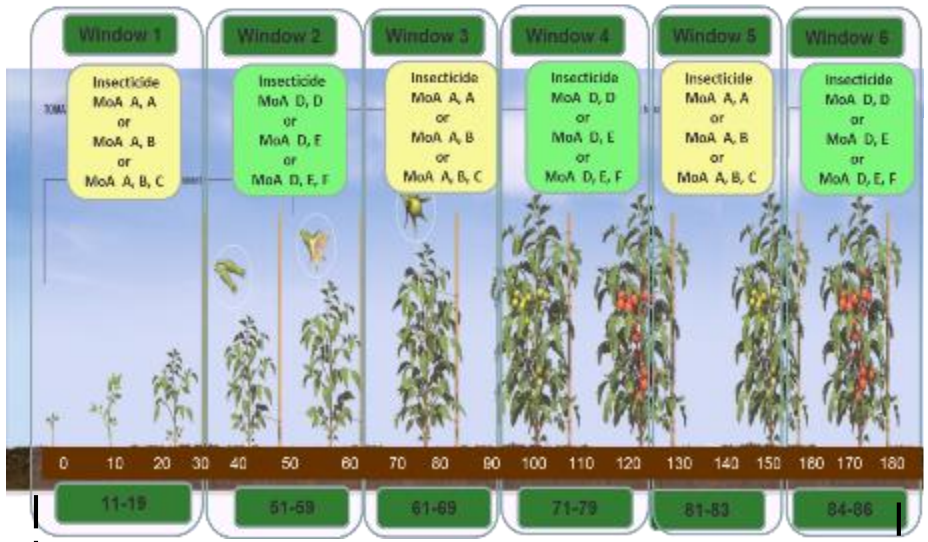
DAP 0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180

Crop Stage	11-19	51-59	61-69	71-79	81-83	84-86

CropLife INTERNATIONAL

9. Implement Insecticide Resistance Management Strategies

Pest Population Control and IPM Activities



Pre-Season

- Remove cull piles
- Kill weed hosts
- Renovate GH
- Moth-proof GH (fix screens)
- Monitor adults-Ph Traps
- Choose tolerant varieties
- Use pest free transplants

During-Season

- Manage the removal of in-season infested pruned stems and fruit
- Use phermones and sticky traps to monitor and mass trap adults.
- Use phermone dispensers for Mating Disruption
- Sprat entomopathic nematoeds and nonchemical products that will not select for insecticide resistance.
- Augment and conserve natural enemy populations
- Use optimal spray volume, maintain and calibrate spray equipment

Post-Season

- Remove cull piles
- Kill weed hosts
- Renovate GH
- Moth-proof GH
- Solarize soil
- Rotate to non-host crop & Incorporate a host free period:
  - subsequent crop plantings should be of a different crop type, which is not a host to the insects which are pests of Tuta.
  - Institute an area-wide fallow period where only non-host crops to Tuta can be planted disrupting the life cycle of Tuta

9. IRAC Poster: Implement Insecticide Resistance Management Strategies

Insecticide Resistance Management

Resistance status in L. America vs. Europe, N. Africa, and Middle East: In L. America, high level and widespread resistance is known to exist in field populations of *T. absoluta* mainly to organophosphates (MoA group 1B), synthetic pyrethroids (MoA group 3), and benzoylureas (MoA group 15). However, resistance has also developed to newer classes of insecticides. Because it is likely that resistant populations from L. America may have spread to Europe, N. Africa and the Middle East, it is urgent that regional technical experts understand the susceptibility profile of *T. absoluta* field populations to the available insecticides so that local recommendations can be made.

Evaluation of Insecticide Susceptibility: IRAC has a standard "leaf-dip" larval bioassay method to assess susceptibility of field populations to insecticides. See IRAC Method No. 022 on the IRAC Website.

Insecticide Resistance Management (IRM):
The recommendations for sustaining the effectiveness of available insecticides is centred on integration of as many pest management tools as possible, use of insecticides only when needed and based on established thresholds, and rotation of effective insecticides with different modes of action.

Mode of Action Window Approach:

- The basic rule for adequate rotation of insecticides by mode of action (MoA) is to avoid treating consecutive generations of the target pest with insecticides in the same MoA group, by using a scheme of " MoA treatment windows".
- A treatment window is here defined as a period of 30 consecutive days, based on the minimum duration of single generation of *T. absoluta*.
- Multiple applications of the same MoA or different MoA's may be possible within a particular window (follow label for maximum number of applications within a window and per crop cycle).
- After a first MoA window of 30 days is completed and if additional insecticide applications are needed based on established thresholds, different and effective MoA's should be selected for use in the next 30 days (second MoA window). Similarly, a third MoA window should use different MoA's for the subsequent 30 days etc.
- The proposed scheme seeks to minimize the selection of resistance to any given MoA group by ensuring that the same insecticide MoA group will not be re-applied for at least 60 days after a window closes, a wise measure given the potential of a longer life cycle based on temperature fluctuations throughout the growing season.
- This scheme requires a minimum of three effective insecticide MoA groups but ideally more MoA groups should be included, if locally registered/effective against *T. absoluta*.

Example: Insecticide Mode of Action (MoA) "Window" Approach – 150 day cropping cycle

Notes:

- Within a "window" (MoA x, y or z in the diagram above) more than one application of the same MoA or different MoA's can be applied if necessary and depending on label advice, as long as these MoA's are not re-applied for 60 days as indicated above.
- Following the "window rotation scheme", example above, use as many effective MoA groups as locally registered/available and always follow product labels for specific directions of use.
- For a comprehensive list of existing insecticides classified by MoA group visit the IRAC website (www.irac-online.org/teams/mode-of-action).

Key Management Strategy Integration of Control Measures

The basis for effective and sustainable management of *Tuta absoluta* is the integration of cultural, behavioural, biological and chemical control.

Key Management Tactics

- Use pest-free transplants
- Prior to transplanting, install yellow sticky traps
- Monitor pest using delta pheromone indicator traps
- Between planting cycles, cultivate the soil and cover with plastic mulch or perform solarisation
- Allow a minimum of 6 weeks from crop destruction to next crop planting
- Seal greenhouse structure with high quality nets suitable for *T. absoluta*
- Inspect the crop regularly to detect the first signs of damage
- For massive trapping, use water + oil traps (20-40 traps/ ha)
- Constantly, remove and destroy attacked plant parts
- Control weeds to prevent multiplication in alternative host
- Establish populations of effective biological control agents (*e.g. Nesidiocoris tenuis*)
- Use locally established thresholds to trigger insecticide applications
- Select insecticides based on known local effectiveness and selectivity
- Rotate insecticides by MoA group using a gap/sequence approach
- Use only insecticides registered for control of *T. absoluta*
- Always follow the directions for use on the label of each product

IRAC general IRM strategy recommendations available as handout and poster

10. Factors That Influence Grower Adoption of Tuta IRM

Source: IRAC

10. Factors That Influence Grower Adoption of Tuta IRM (1)

"*Why farmers don't practice resistance management*"

- **Don't understand Insect Resistance or its impact.**
- **Lack of education on product positioning, IRM strategies, proper product rotation, and timing of execution.**
- **Don't understand pest biology and ideal time to control pests**
- **Don't know Mode of Action of products.**
- **Poor application procedures, unmaintained equipment, and proper use of adjuvants.**
- **Label is difficult to understand or read.**
- **Depend on a few products that have delivered the best efficacy.**

10. Factors That Influence Grower Adoption of Tuta IRM (2)

"*Why farmers don't practice resistance management*"

- **Farmers do not follow the product label to save money or increase product efficacy/residual:**
  - **o drip in greenhouse; apply low rates**
- **No other effective alternative product available for rotation.**
- **Difficult to find value in Resistance Management when neighbors are ignoring it.**
- **Product sustainability/stewardship is a low priority.**
- **Focus is on the current season/crop and will do what is necessary to maximize yield.**
- **Distrust chemical companies.**

IRAC

10. Factors That Influence Grower Adoption of Tuta IRM (3)

"*Why farmers don't practice resistance management*"

- **Farmers do not take responsibility:** Believe it is problem of company; new products will be available.
- **Food chain**: external push to minimize the number of CPC residues forces growers to use few products over and over.
- **Economic**: the smaller farmers cannot afford changing the greenhouse structure and buying multiple insecticides for the same target.
- **Relationships**: small farmers defer to dealers. Dealers margin is often higher for non-CPC (*e.g.* biostimulants, natural substances, biologicals) claiming positive side-effects on pests & diseases. The insecticide transaction becomes secondary.

11. Examples of country IRM programs with Mode of Action rotation: Spain, Italy, Greece, Portugal

Source: IRAC

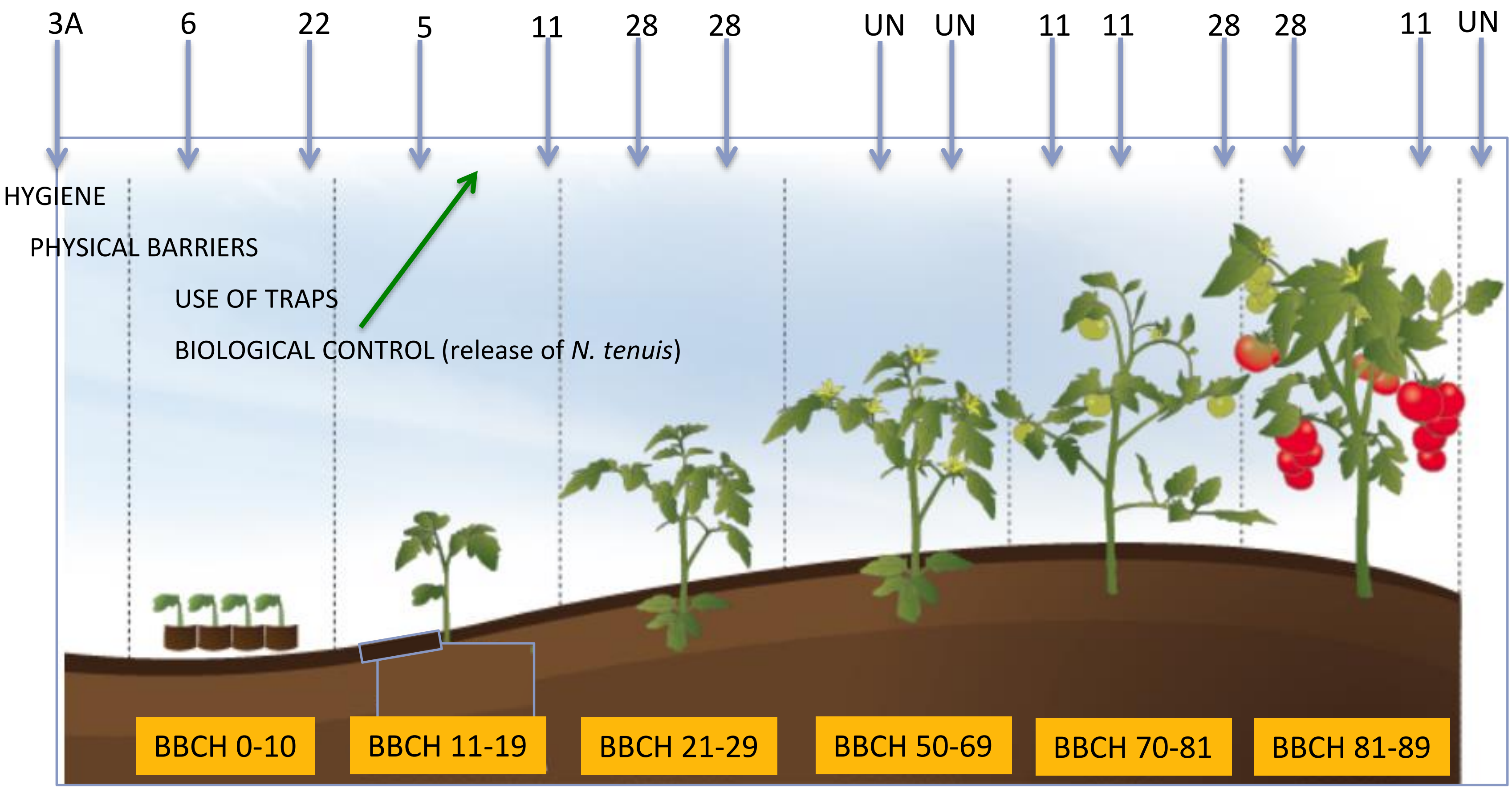
11. Examples of country MoA alternation programs:
Spain

Pest control practices (general example):

Example: planting Sep and crop removal July.

12-16 applications (as average in a long crop cycle)

Product rotation in this case (by MoA):



IRAC

11. Examples of country MoA alternation programs:

Spain

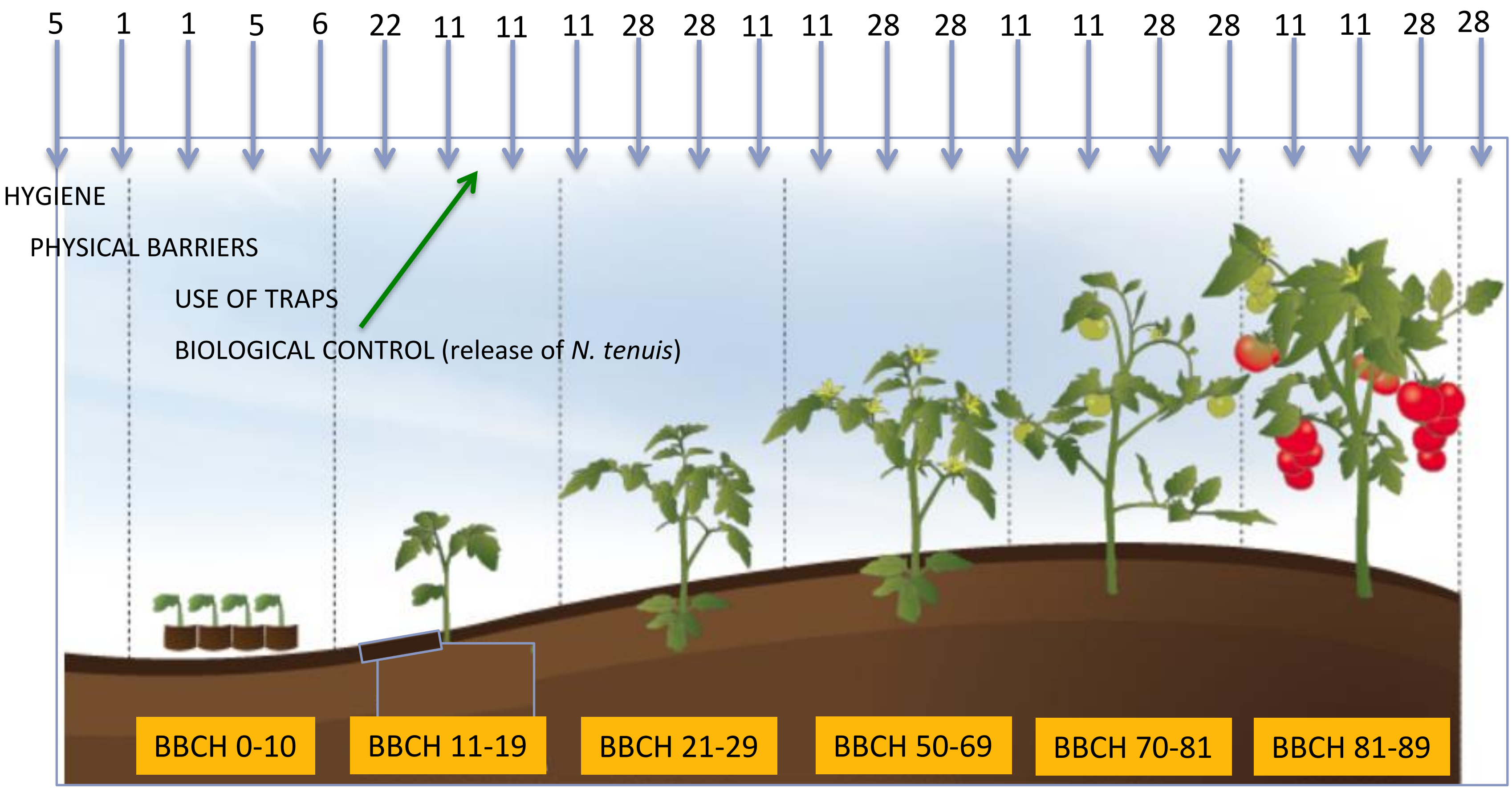
Pest control practices (worse case scenario example):

Example from Murcia: planting 3rd Sep 14 and crop removal 10th July 15

23 applications: 9 BT; 8 Diamides, 2 Spinosad, 1 Emamectine, 1 Indoxacarb and 2 Methomyl.

Up to 11 generations/crop cycle => shorter intervals with warm Tª and longer day light.

Product rotation in this case (by MoA):



11. Examples of country MoA alternation programs:

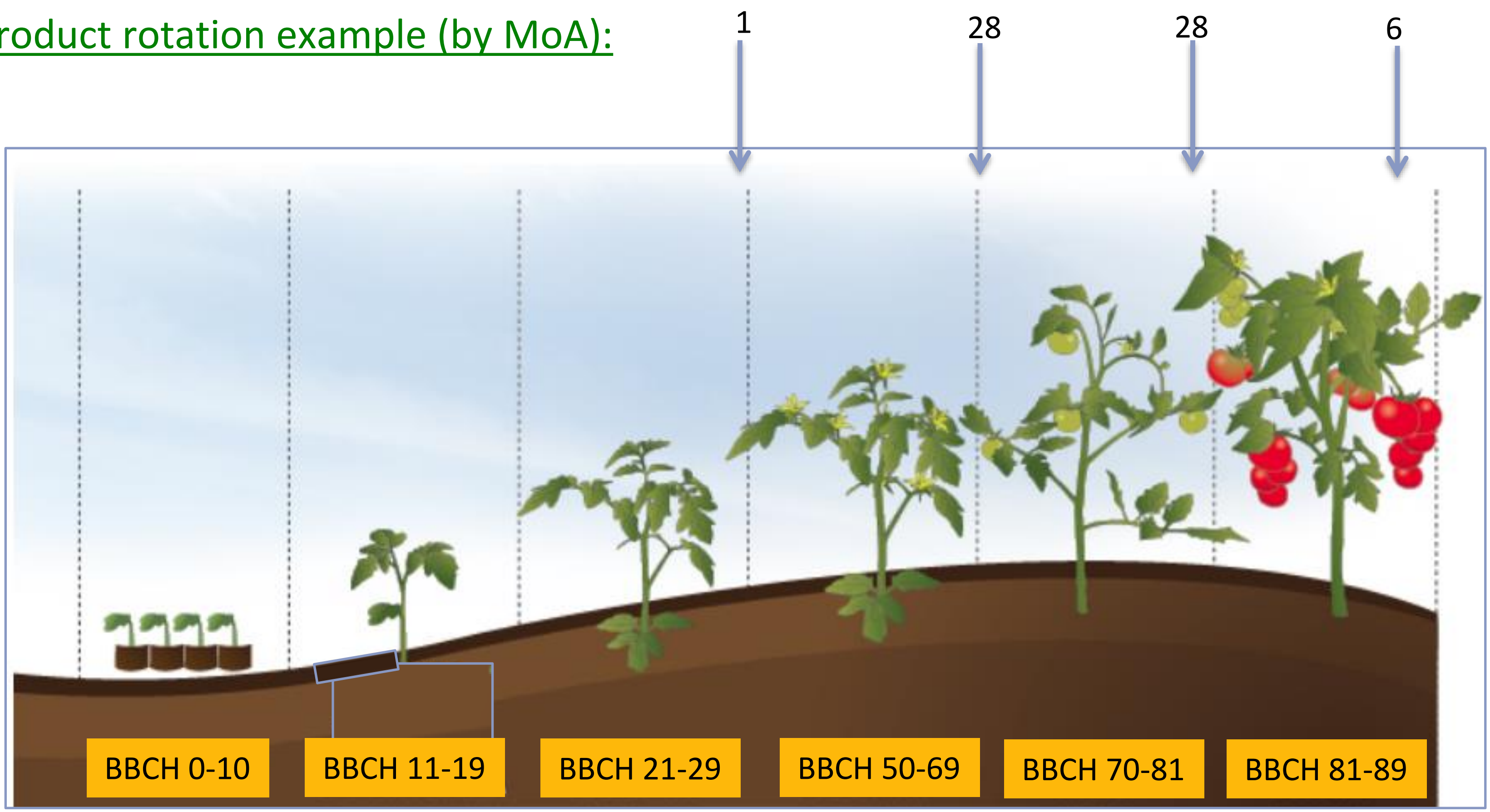
Portugal – Open Field

Pest control practices (general example):

Example from Portugal industry –open field-: planting Mar-Jun and crop removal Aug-Oct

3-5 applications: Diamides, Emamectine, Pirethrins (farmers try to rotate)

Product rotation example (by MoA):



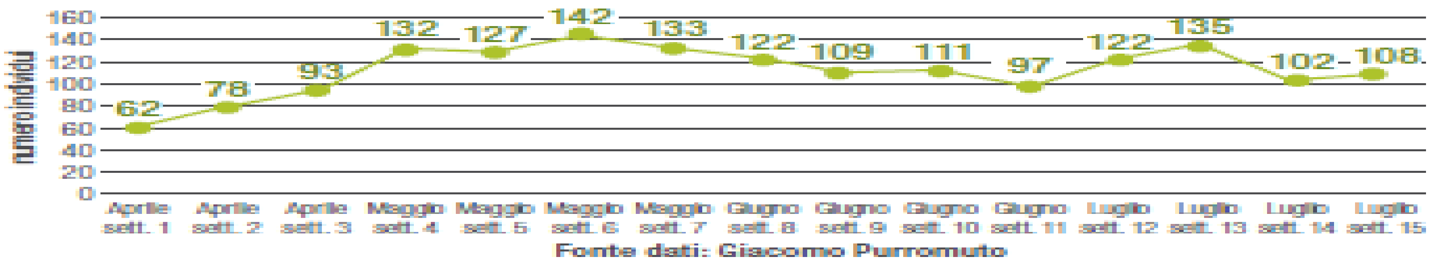
IRAC

11. Examples of country MoA alternation programs:

Italy (Syngenta)

SPRING-SUMMER CYCLE: example of sustainable program

Flight example



Spray proposal

0-30 giorni	30-60 giorni	60-90 giorni	90-120 giorni
2 applications with emamectine	2 applications with clorantraniliprole	Apply products with different MoA (eg. Indoxacarb, metaflumizone)	1 application with emamectine
In case of persistant infestation, make also 1 application with *Spinosad*			In case of persistant infestation, make also 1 application with *Spinosad*

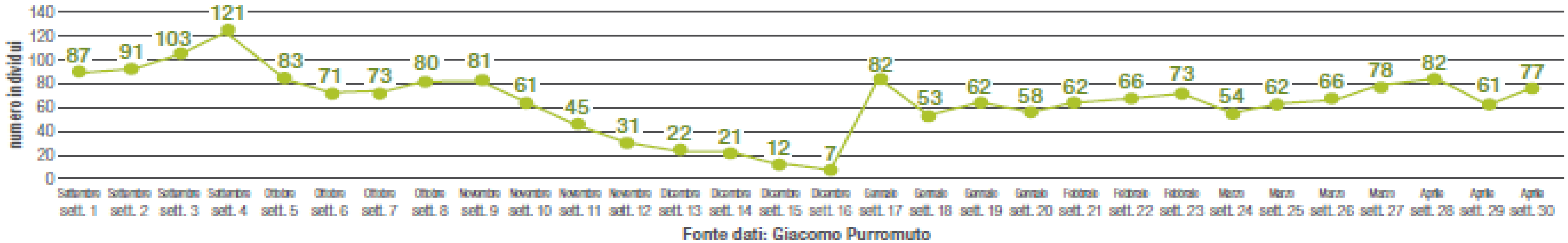
- ➢ In case of high Tuta pressure, and with applications made with short spray interval (7-10 days), integrate the spray calendar with other MoA (eg. Metaflumizone, Spinosad, *B. thuringensis*), FOLLOWING the IRAC recommendations
- ➢ In case of control of other Lepidopteran species, consider insecticides with different Moz (e.g. Lufenuron – IGR)

DO NOT APPLY INSECTICIDES WITH SAME MoA WITHIN 60 DAY FROM THE LAST APPLICATION

11. Examples of country MoA alternation programs:
Italy (Syngenta)

Flight example

SPRING-SUMMER CYCLE: example of sustainable program



2 applications of clorantraniliprole	2 applications of Indoxacarb	2 applications of emamectine	2 applications of *B. thuringensis*	2 applications of Indoxacarb	1 application of emamectine
			In case of more consistent infestation, make 1-2applications of *metaflumizone*		In case of persistant infestation, make also 1 application with *Spinosad*

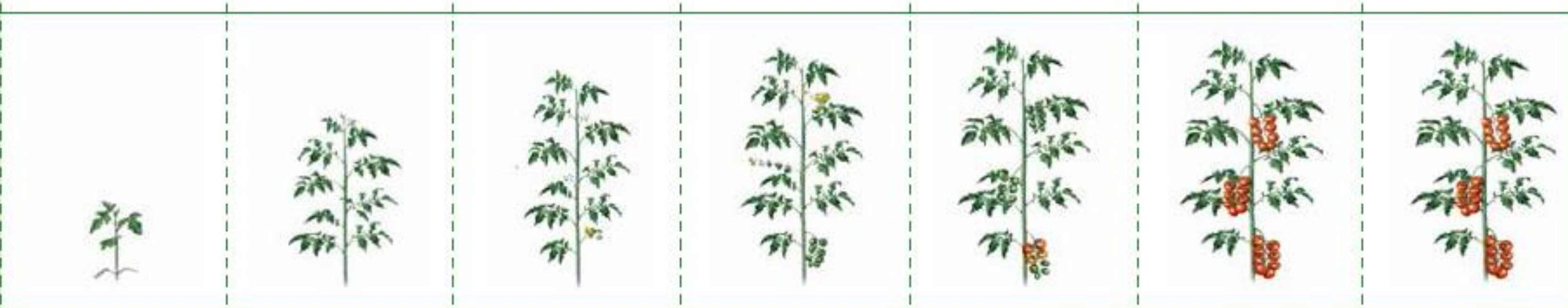
- ➢ In case of high Tuta pressure, and with applications made with short spray interval (7-10 days), integrate the spray calendar with *B. thuringensis*)
- ➢ In case of control of other Lepidopteran species, consider insecticides with different Moz (e.g. Lufenuron – IGR)

DO NOT APPLY INSECTICIDES WITH SAME MoA WITHIN 60 DAY FROM THE LAST APPLICATION

IRAC

11. Examples of country MoA alternation programs:

Italy DuPont™ Greenhouse fall cycle

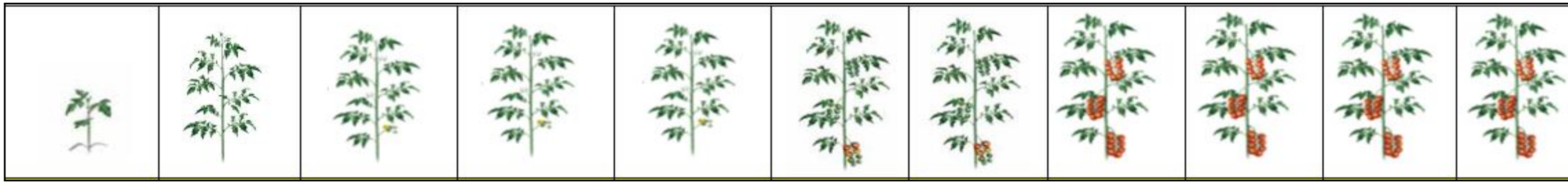
Descrizione stadio	Post-trapianto (prime foglie sviluppate)	Fase di preparazione palchi fiorali	Fase di fioritura	Continua fioritura e comparsa prime bacche	Colorazione bacche e inizio primi stacchi	Termine fioritura e proseguimento raccolta	Raccolta
Periodo indicativo per trapianti campagna autunnale	1 - 20 sett.	20 sett. - 10 ott.	10 - 31 ott.	1 - 30 nov.	1 dic. - 28 febb.	1 - 31 marzo	1 - 30 aprile
Caratteristiche periodo	Pianta in attiva crescita, elevata pressione *Tuta*	Pianta in attiva crescita, elevata pressione *Tuta*	Immissione bombi nelle serre	Presenza bombi nelle serre e calo pressione *Tuta*	Calo temperatura e quiescenza *Tuta*	Ripresa pressione *Tuta*	Ripresa pressione Tuta
							
Stadio BBCH	11 - 19	51 - 59	61 - 69	71 - 79	81 - 83	84 - 86	87 - 89
Prodotti e dosi per ettolitro	2 trattamenti con Steward® 12.5 g + bagnante (intervallo 10-12 gg fra primo e secondo tratt.)	2 trattamenti con Spinosad 25 ml (intervallo 10 gg fra primo e secondo tratt.)	2 trattamenti con Altacor® 12 g + Codacide® (intervallo 7-10 gg fra primo e secondo tratt.)	2 trattamenti con ememectinabenzoato® 150 g (intervallo 7-10 gg fra primo e secondo tratt.)	Trattamenti con *Bacillus thuringensis* (intervallo 8-10 gg fra i tratt.)	2 trattamenti con Steward® 12.5 g + bagnante (intervallo 10-12 gg fra primo e secondo tratt.)	Trattamenti con *Bacillus thuringensis* (intervallo 8-10 gg fra i tratt.) o con Spinosad in caso di infestazioni perduranti

x2	x2	x2	x2	x2/3	x2	x2/3
Group 22A Oxadiazine	Group 5 Spynosins	Group 28 Diamides	Group 6 Avermectines	Group 11 Bacillus	Group 22A Oxadiazine	Group 11 Bacillus

IRAC

11. Examples of country MoA alternation programs:

Italy DuPont Greenhouse spring/summer cycle



Product	Steward + Codacide	Steward + Codacide	Spinosad + bagnante	Spinosad + bagnante	Altacor + Codacide	Altacor + Codacide	Emamectina Benzoato 0,95% + Bagnante	Emamectina Benzoato 0,95% + Bagnante	Steward + Codacide	Steward + Codacide
Rate 1000 m^2	12,5g + 150 ml	12,5g + 150 ml	30 ml + 0,02 V/V	30 ml + 0,02 V/V	12 g + 150 ml	12 g + 150 ml	150 ml + 0,02 V/V	150 ml + 0,02 V/V	12,5g + 150 ml	12,5g + 150 ml
IRAC	Group 22	Group 22	Group 5	Group 5	Group 28	Group 28	Group 6	Group 6	Group 22	Group 22

Rules for the proper use of insecticides and the best of resistance management strategy:

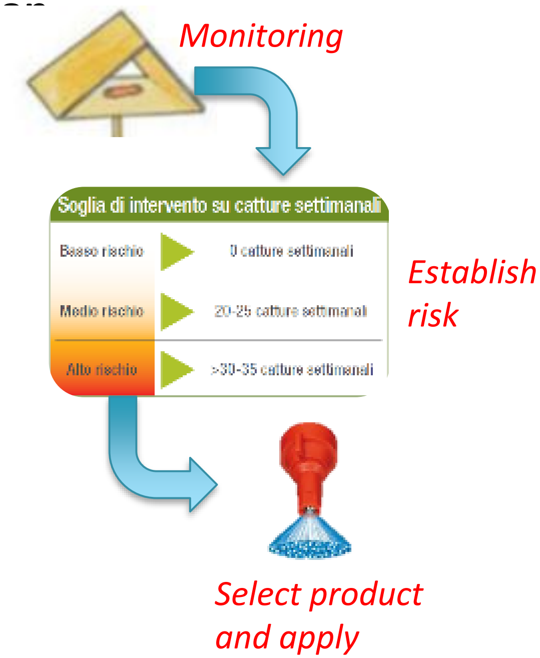
1. Respect label rates and do not use the products in drip irrigation if not provided on the label.
2. Respect intervals between treatments provided and max. number of applications per year.
3. Use only products and active ingredients registered on the crop.
4. Make the required rotations of active ingredients suggested by the label and IRAC.
5. During the crop cycle use the greatest number of active ingredients effective against *Tuta*
6. Do not use insecticides in mixtures.
7. Do not use active ingredients with low activity in the control of Tuta absoluta.

11. Examples of country MoA alternation programs:

ITALY

Tuta absoluta: pest control practice

1. Buy **plants** from nursery **free of infestations**
2. Clean the field from **crop residues**, use **mulching, solarizati**on and **nets** for insect exclusion
3. **Monitoring**: use pheromone traps for monitoring the flight curve and then decide the control strategy to adopt
4. **Remove the infested parts** from the GH and destroy them
5. **Select the product** to be applied according to the **label reccomendation** (dose, spray interval, number of applications)
6. **Rotate the insecticides** available, following the IRAC reccomendations
7. Relese favorite **beneficials**

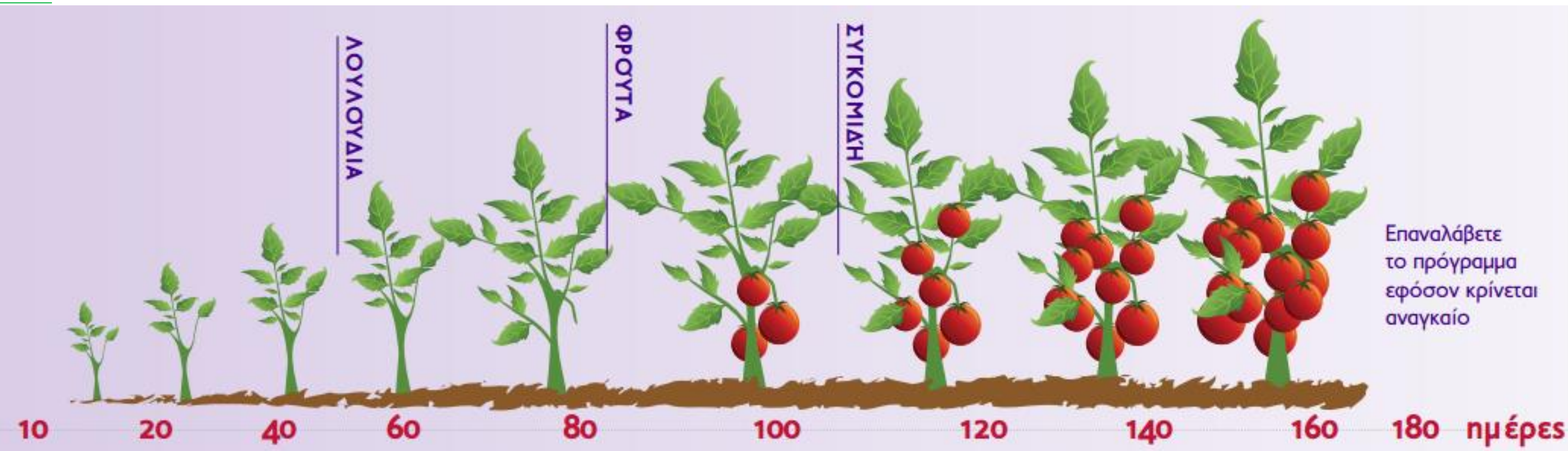


IRAC

11. Examples of country MoA alternation programs:

DuPontGreece Greenhouse

Tuta absoluta: Διαθέσιμα εργαλεία και διαχείριση ανθεκτικότητας



Steward X2	Coragen X2	Affirm X2	Laser X2	Alverde X2	Belt X2	Affirm X2	Laser X2
	B.t.			B.t.			B.t.
Group 22	Group 28	Group 6	Group 5	Group 22	Group 28	Group 6	Group 5

IRAC

11. Examples of country MoA alternation programs:

Greece Greenhouse – Roditakis et al

Πίνακας 1.

Σκευάσματα εγκεκριμένα από το Υ.Π.Α.Π.ΕΝ. για την αντιμετώπιση του εντόμου Tuta absoluta στην τομάτα

Ομάδα δράσης (1)	Εγκεκριμένα σκευάσματα	Δραστική ουσία	Τομάτα Υ: υπαίθρου Θ: θερμ/πίου	Ημέρες προ της συγκομιδής	Μέγιστος αριθμός εφαρμογών /καλ. περίοδο	Τοξικότητα στα αρπακτικά Miridae(2)	Διάρκεια επίδρασης στα αρπακτικά
1A	LANNATE 20 SL, LANNATE 25WP	methomyl	**Υ**	7	2	Τοξικό	8–12 εβδομάδες
5	LASER 480 SC	spinosad	**Υ/Θ**	3	2	Ελαφρώς έως μετρίως τοξικό	2 εβδομάδες
6	AFFIRM 095 SG	emamectin benzoate	**Υ/Θ**	3	3	Ασφαλές	–
	CAL EX	abamectin	**Υ/Θ**	3	3	Τοξικό	≥3 εβδομάδες
11A	BELTHIRUL 32000 WP	*Bacillus thuringiensis ssp. kurstaki*	**Υ/Θ**	0	3	Ασφαλές	–
	BACTOIL SC		**Υ/Θ**	0	3	Ασφαλές	–
	BACTOSPEINE 6,4 WG		**Υ/Θ**	0	8	Ασφαλές	–
22A	BOLERO 30 WG	indoxacarb	**Υ/Θ**	1	3	Ελαφρώς έως μετρίως τοξικό	2–3 εβδομάδες
	STEWARD 30 WG		**Υ/Θ**	1	3	Ελαφρώς έως μετρίως τοξικό	2–3 εβδομάδες
22B	ALVERDE 24 SC	metaflumizone	**Θ**	1	2	Τοξικό	>3 εβδομάδες
28	ALTACOR 35 WG	chlorantraniliprole	**Υ/Θ**	1	2	Ασφαλές	–
	BELT 24 WG	flubendiamide	**Θ**	3	2	Ασφαλές	–
28/6	VOLIAM TARGO 063 SC	chlorantraniliprole + abamectin	**Θ**	7	2	Τοξικό	≥3 εβδομάδες
28/3A	AMPLIGO 150 ZC	chlorantraniliprole + λ-cyhalothrin	**Υ**	3	2	Τοξικό	8–12 εβδομάδες

(1): Κωδικοποίηση τρόπου δράσης κατά I.R.A.C. (International Resistance Action Committee, www.irac-online.org).
Η αναγραφόμενη αποσκοπεί στην οπτική ομαδοποίηση των εντομοκτόνων κατά τρόπο δράσης.
(2): Τα στοιχεία αφορούν στα είδη *Macrolophus pygmaeus* και *Nesidiocoris tenuis*.

Πηγές: α) http://side-effects.koppert.nl
β) http://ipmguidelinesforgrains.com.au/ipm-information/chemical-control/pesticide-impact
γ) http://www.ipm.ucdavis.edu/PMG/r783900111.html
δ) http://www.biobestgroup.com/en/side-effect-manual
ε) Arno, J. and R. Gabarra. 2011. J. Pest Sci. 84: 513–520
στ) Lopez, J. A. et al. 2011. Sp. J. Agric. Res. 9(2): 617–622
ζ) Martinou, F.A. et al. 2014. Chemosphere 96: 167–173

ΠΡΟΣΟΧΗ
Η αλόγιστη εφαρμογή χημικών σκευασμάτων

- **έχει αρνητική επίδραση στους βομβίνους (κοινώς: αβούραι, μέλισσες) που χρησιμοποιούνται για την επικονίαση των φυτών στη θερμοκηπιακή καλλιέργεια τομάτας**
- **επιταχύνει την ανάπτυξη ανθεκτικότητας από τον εχθρό μειώνοντας την δραστικότητα των εντομοκτόνων**
- **αυξάνει το κόστος παραγωγής και τέλος**
- **αυξάνει την πιθανότητα εμφάνισης μη επιτρεπτών υπολειμμάτων γεωργικών φαρμάκων στα γεωργικά προϊόντα.**

Στον Πίνακα 2 παρουσιάζονται συνοπτικά σε γράφημα οι οδηγίες διαχείρισης της ανθεκτικότητας, όπως αυτές περιγράφονται από τις ετικέτες των σκευασμάτων και τις συστάσεις από τον IRAC.

Οδηγίες διαχείρισης ανθεκτικότητας		Καλλιεργητική περίοδος – Χρόνος από την έναρξη των ψεκασμών (σε ημέρες)					
		0–30 ημέρες	30–60 ημέρες	60–90 ημέρες	90–120 ημέρες	120–150 ημέρες	150–180 ημέρες
Εναλλαγή ομάδων εντομοκτόνων	Έναρξη ψεκασμών	Ομάδα Χ (*) μέγιστος αρ. εφαρμογών/καλ. περ.	Ομάδα Ψ (*) μέγιστος αρ. εφαρμογών/καλ. περ.	Ομάδα Ζ (*) μέγιστος αρ. εφαρμογών/καλ. περ.	Ομάδα Χ (*) μέγιστος αρ. εφαρμογών/καλ. περ.	Ομάδα Ψ (*) μέγιστος αρ. εφαρμογών/καλ. περ.	Ομάδα Ζ (*) μέγιστος αρ. εφαρμογών/καλ. περ.
Οδηγίες IRAC			Μην εφαρμόζετε εντομοκτόνα από την Ομάδα Χ			Μην εφαρμόζετε εντομοκτόνα από την Ομάδα Χ	
				Μην εφαρμόζετε εντομοκτόνα από την Ομάδα Ψ			Μην εφαρμόζετε εντομοκτόνα από την Ομάδα Ζ
					Μην εφαρμόζετε εντομοκτόνα από την Ομάδα Ζ		

(*) Προσοχή στον μέγιστο αριθμό εφαρμογών ανά καλλιεργητική περίοδο (αναφέρεται στην ετικέτα των σκευασμάτων)

Πίνακας 2.
Σύνοψη των οδηγιών διαχείριση της ανθεκτικότητας όπως αυτές περιγράφονται από τις ετικέτες των σκευασμάτων και τις συστάσεις του IRAC (www.irac-online.org). Οι Ομάδες Χ, Ψ ή Ζ αντιστοιχούν σε εγκεκριμένα σκευάσματα που περιέχουν δραστικές ουσίες από την ίδια ομάδα δράσης (Mode of action). Ο χρόνος στον πίνακα αρχίζει με την έναρξη των ψεκασμών για τα *Tuta absoluta*.

Ενέργειες που πρέπει να γίνουν σε προσβεβλημένες καλλιέργειες

α) Απομακρύνουμε και καταστρέφουμε με **θάψιμο** τα υπολείμματα της προσβεβλημένης καλλιέργειας, ώστε να περιορίσουμε την εξάπλωση του εχθρού σε γειτονικές καλλιέργειες. Αν αυτό δεν είναι εφικτό, τότε μπορούμε να στοιβάξουμε τα υπολείμματα σε σωρούς και να τα **καλύψουμε ερμητικά** με πλαστικό θερμοκηπίου για 2 μήνες. Οι ελεύθερες άκρες του πλαστικού να παραχωθούν επιμελώς.

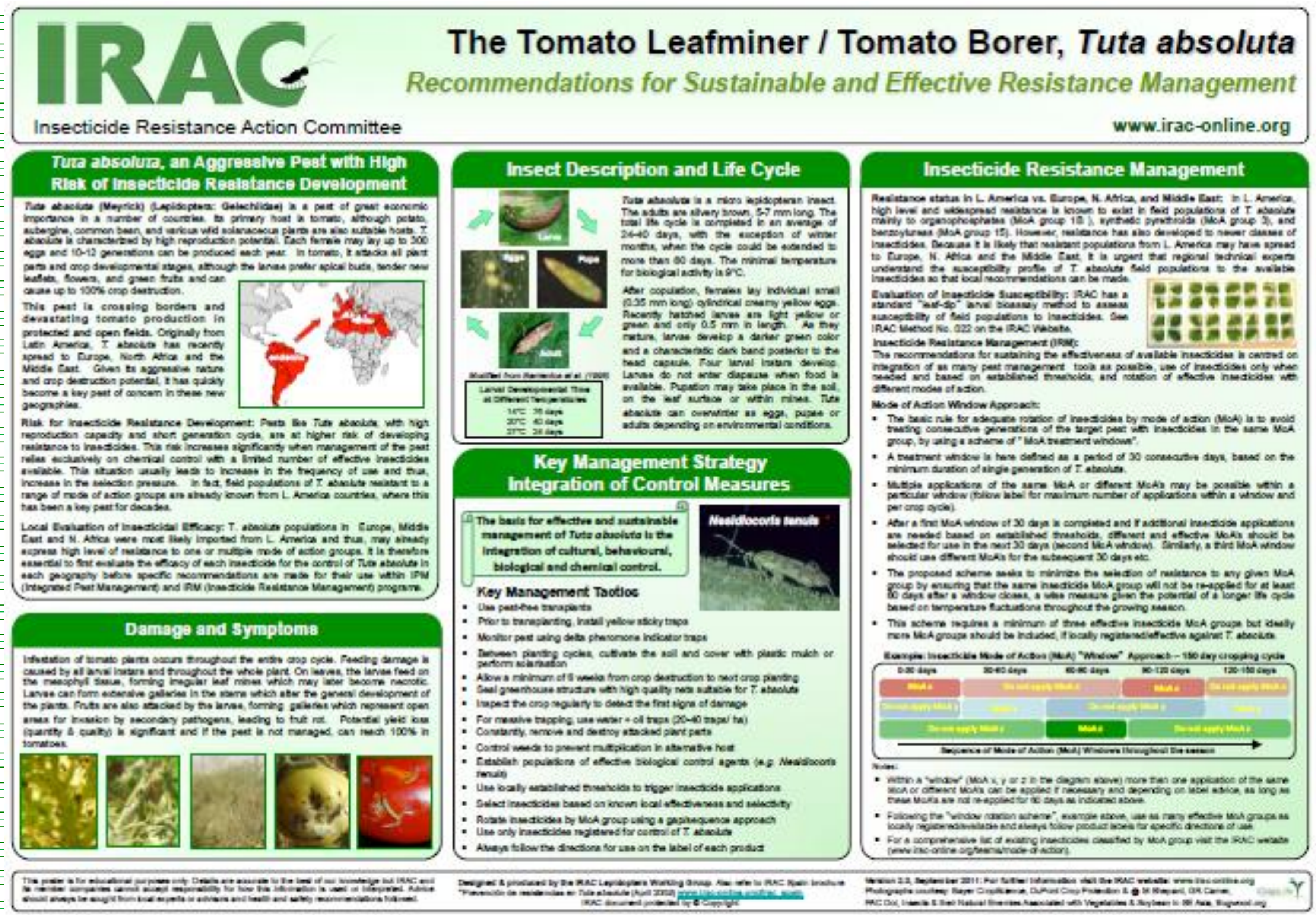
β) Απολυμαίνουμε το χώρο του θερμοκηπίου πριν την νέα φύτευση.
Η εφαρμογή **ηλιοαπολύμανσης** του εδάφους για 4 έως 8 εβδομάδες (ανάλογα με την εποχή εφαρμογής της) μπορεί να συντελέσει στη μείωση των πληθυσμών του εντόμου στο έδαφος του θερμοκηπίου, πριν την έναρξη της νέας καλλιέργειας τομάτας.

Γενικές παρατηρήσεις

Η αντιμετώπιση του ***Tuta absoluta*** είναι ιδιαιτέρα δύσκολη και οι πρώτες ενδείξεις για ανάπτυξη ανθεκτικότητας έχουν δημοσιευτεί. Μόνο ο συνδυασμός διαφορετικών μεθόδων, στα πλαίσια της ολοκληρωμένης διαχείρισης των εχθρών της καλλιέργειας, μπορεί να δώσει ένα ικανοποιητικό αποτέλεσμα με χαμηλό κόστος.

11. Examples of country MoA alternation programs:

IRAC Training Tuta Poster



12. Guidance for Locally Adapting and Implementing IRM Strategies

Source: IRAC

12. Guidance for Locally Adapting and Implementing IRM Strategies

Checklist for country teams to initiate and maintain IRM efforts

Step I   Organize-Meet-Align: A team of industry, university, local experts, and consultants

Step II   Understand the common objectives and expectations of the team – Pick a Leader

Step III   Review IRAC's Code of Conduct & Antitrust Rules

Step IV   Select target locations/growers/areas of common farming practices to focus effort

Step V   Adapt regional Tuta IRM BMP guidelines to local area

Step VI   Develop a plan to implement the IRM BMP strategies to focus areas

Step VII   Develop plan to best communicate MOA to growers and Tuta industry

Step VIII   Develop plan to educate growers & the ag community

Step IX   Communicate advantages of IRM & grower's responsibility to practice IRM

Step X   Implement MoA communication, IRM strategies, grower/influencers education plans

Step XI   Plan to take a leadership role once resistance occurs

Insecticide Resistance Action Committee

12. Review IRAC's Code of Conduct & Antitrust Rules

The Code is designed as a point of reference to establish standards of Conduct when IRAC Committees or individual IRAC Members are representing IRAC. This, along with the IRAC Antitrust Guidelines, forms the basis by which all IRAC Committees should operate.

The Code is also intended to reassure individuals and groups that interact with IRAC that the sole objective of the Committee is to counter the development of insecticide or acaricide resistance through joint technical strategies.

DO:

- Have an agenda and adhere to prepared agendas for all meetings.
- Take minutes and object if they do not accurately reflect the discussion.
- Consult legal counsel on all antitrust questions relating to meetings.
- Protest against any discussions or meeting activities which appear to violate the antitrust laws and leave any meeting in which they continue.

DON'T

….in fact or appearance, in meetings or other forum, formally, informally or even in jest, discuss or exchange information regarding:

- Pricing policies/changes, credit terms, production, capacity, inventories
- Changes in industry production, capacity or inventories.
- Bids on contracts
- Distribution or marketing plans of particular products
- Matters relating to actual or potential individual customers or suppliers