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Spécialité : Phytiatrie

La mineuse de la tomate *Tuta absoluta* (Lepidoptera : Gelechiidae) : Paramètres biologiques, écologiques et alternatives de lutte

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Résumé

Tuta absoluta (Meyrick) (Lepidoptera : Gelechiidae) est un ravageur important pour les cultures de tomate causant des dégâts significatifs allant jusqu'à 80% dans les nouvelles zones envahies. Les résultats de cette étude montrent une conformité génétique élevée pour ce ravageur et une susceptibilité de se développer sur plusieurs milieux artificiels. *Tuta absoluta* est capable de se développer sous différents niveaux de température (21, 25 et 28°c) et d'humidités relatives (32, 52 et 72%) ainsi que sur différentes plantes hôtes (tomate, pomme de terre et aubergine). Cet insecte est capable de présenter jusqu'à 4-5 pics de vol et 3-4 générations d'œufs et de larves sous serres et en pleins champs. Une corrélation positive est observée et démontrée entre adultes capturés et œufs pondus, adultes capturés et mines avec larves, adultes capturés et mines totales et mines avec larves et mines sans larves. L'utilisation combinée du piégeage de masse et d'insecticides a montré des résultats encourageants vu que les problèmes de résistance n'ont pas été prouvés. La densité de vingt *Trichogramma cocoeciae* (Marchal) (Hymenoptera : Trichogrammatidae) par plant, testée sous serre et en plein champ a été signalée comme étant la plus efficace en réduisant les dégâts causés par ce ravageur. Cette étude démontre, également, la toxicité de quelques insecticides (indoxacarbe, spinosade…), largement utilisés dans les parcelles de tomates en Tunisie, sur tous les stades de développement de *T. cocoeciae* et met en valeur l'absence de nocivité d'autres substances (azadirachtin, *Bacillus thuringiensis* et virus HaNPV). Cette étude a permis, donc, une meilleure connaissance de *T. absoluta* en ce qui concerne sa biologie, sa dynamique des populations et sa caractérisation génétique. Elle a proposé des programmes de lutte efficaces en utilisant des insecticides avec moins d'effets secondaires sur le parasitoïde. Aussi, cette recherche a favorisé un meilleur contrôle biologique utilisant des lâchers innondatifs de trichogrammes. Cependant, des études supplémentaires sont encore nécessaires pour tester de nouvelles stratégies de lutte dans le but d'éviter les problèmes de résistance comme c'est déjà le cas dans plusieurs pays comme le Brésil.

Mots-clés : diversité génétique, effets non intentionnels, plantes hôtes, trait de vie, *Trichogramma cacoeciae*, *Tuta absoluta*

Abstract

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is an important pest of tomato crops causing significant yield losses up to 80% in newly invaded area. Obtained results of this study indicated a high genetic conformity of this pest and its susceptibility to develop in tested artificial diets. Our data showed that this pest was able to develop under different levels of temperature (21, 25 and 28°c) and relative humidity (32, 52 and 72%) as well as various host plants (tomato, potato and eggplants). This pest was able to achieve up to 4-5 flight peaks and 3-4 generations of eggs and larvae under greenhouse and field conditions. A positive correlation between some specific parameters (captured males and eggs laid, captured males and mines with larvae, captured males and total mines and mines with larvae and mines without larvae) was emphasized. The combined use of mass trapping and insecticides gave an encouraging results given that problems of resistances were not found. Dose of twenty *Trichogramma cocoeciae* (Marchal) Hymenoptera: Trichogrammatidae) tested in protected and open filed crops was the most effective in reducing the pest population. This study shows the toxicity of some insecticides (indoxacarb, spinosad…), widely used in Tunisia tomato crops, on all *T. cocoeciae* development stages and highlights the safety of others (azadirachtin, *Bacillus thuringiensis* and virus HaNPV). This study allows a better understanding of *T. absoluta* in terms of biology, population dynamics and genetic characterization. It proposes efficient control strategies when using effective insecticides with less side effects on parasitoid. Also this research promotes a better biological control using *Trichogramma* mass releases. However, additional studies, are still required to test new control strategies and propose new ones in order to avoid problems of resistance as reported for example in many countries such as in Brazil.

Key words: Biological traits, genetic diversity, host plants, side effects, *Trichogramma cacoeciae*, *Tuta absoluta*

ملخّص

تعتبر الحشرة "توتا أبسولوتا" حافرة أوراق الطماطم آفة رئيسية لزراعة الطماطم حيث تتسبّب في أضرار كبيرة تصل إلى 80% في المناطق المحتاجة حديثا. أثبتت النتائج المتحصل عليها في هذه الدراسة وجود توافق وراثي عالي بالإضافة إلى قدرتها على العيش في نظام غذائي بديل. وتشير الدراسة إلى أنّ هذه الآفة قادرة على العيش والتأقلم في درجات حرارة (21، 28°C) ورطوبة مختلفة (32، 52، 72%) بالإضافة إلى عدّة زراعات أخرى (بطاطا، بذنجان).

لهذه الحشرة 4-5 فترات طيران رئيسية و3-4 أجيال للبيض واليرقات على أوراق الطماطم في البيوت المحمية والحقول. أثبتب هذه الدراسة وجود ترابط إيجابي بين (الحشرة والبيض في الأوراق) (الحشرة والبرقات في الأوراق)، (الحشرة والأوراق المصابة). كما أوضحت أن استعمال المصائد الفيرومونية والمبيدات الحشرية قد أدّت إلى نتائج مشجعة حيث لم تثبت أي تأقلم للحشرة بالإضافة إلى الاكتفاء بـ20 *Trichogramma cacoeciae* للنبتة الواحدة في البيوت المحمية والحقول. وتوضح هذه الدراسة خطورة بعض المبيدات التي تستخدم في نطاق واسع في حقول الطماطم في تونس على جميع مراحل عيش *T. cacoeciae* كما تؤيد استعمال مبيدات ذات فعالية عالية والتي ليس لها آثار جانبية على الطفيل. هذه الدراسة أوضحت بيولوجيا توت أبسولوتا، بالإضافة إلى تخصّصها الجيني. اقترحت برامج فعّالة باستخدام مبيدات حشرية ذالت آثار جانبية محدودة على الطفيل *T. cacoeciae* وقد عزّز هذا البحث أفضلية المكانة البيولوجية باستخدام *T. cacoeciae*. ومع ذلك لا تزال هناك حاجة إلى المزيد من الدراسات لاستعمال طرق مكانة فعّالة، متطورة وحديثة من أجل تجنب تأقلم الحشرة كما أشير في عدّة بلدان مثل البرازيل.

الكلمات المفاتيح : تأثيرات جانبية، الخصائص البيولوجية، توتا أسبولوتا، *T. cacoeciae*

Je dédie ce travail doctoral à mes chers parents Belgacem et Radhia,

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Asma cherif

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Liste des publications

1.<u>**Cherif Asma**</u>**,** Marconato Glaucia, Hached Wiem, Barhoumi-Attia Sabrine, Hausmann Axel and Lebdi-Grissa Kaouthar. 2017. Some remarks on the genetic uniformity of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). **Published in Journal of entomology and zoology studies, 5(3): 1380-1382.**

2. <u>**Cherif Asma**</u>**,** Barhoumi-Attia Sabrine and Lebdi-Grissa Kaouthar. 2017. Effect of relative humidity, temperature and host plant on the Biological traits of the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under laboratory conditions. **Submitted.**

3. <u>**Cherif Asma**</u> and Lebdi-Grissa Kaouthar. 2017. Population dynamics of the tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Tunisia natural conditions. **Published in Journal of entomology and zoology studies, 5(4): 427-432.**

4. <u>**Cherif Asma**</u>**,** Barhoumi-Attia Sabrine and Lebdi-Grissa Kaouthar. 2017. Traps and their impact on population dynamics and management strategies of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in protected tomato crops**.**.

5. <u>**Cherif Asma**</u>, Harbaoui Khalil, Zappalà Lucia and Grissa-Lebdi Kaouthar. 2018. Efficacy of mass trapping and insecticides to control *Tuta absoluta* in Tunisia. **Published in Journal of plant diseases and protection, 125: 51-61.**

6. <u>**Cherif Asma**</u>, Barhoumi-Attia Sabrine, Ichaoui Ons, Zappalà Lucia and Lebdi-Grissa Kaouthar. 2017. Can Innundative releases of *Trichogramma cacoeciae* (Marchal) (Hymenoptera: Trichogrammatidae) improve biological control of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Tunisia tomato crops? **Submitted.**

7.<u>**Cherif Asma**</u>**,** Ichaoui Ons, Barhoumi-Attia Sabrine and Lebdi-Grissa Kaouthar. 2018. Life-stage-dependent side effect

of selected insecticides on *Trichogramma cacoeciae* (Marchal) (Hymenoptera: Trichogrammatidae) under laboratory conditions. **Published in Phytoparasitica**
https://doi.org/10.1007/s12600-018-0638-x

Communications orales dans des congrès internationaux

1.**Asma Cherif,** Khalil Harbaoui et Kaouthar Grissa-Lebdi. 2016. Essai d'Elaboration d'un milieu artificiel pour l'élevage en masse de la mineuse de la tomate *Tuta absoluta* (Meyrick, 1917) et étude de sa sensibilité variétale vis-à-vis de deux variétés différentes de tomate plantées sous serres. **27ème forum international des sciences biologiques et Biotechnologie de l'ATSB.** Mars, 2016.

2. **Asma Cherif,** Ons Ichaoui, Sabrine Attia-Barhoumi et Kaouthar Grissa-Lebdi. 2017. Monitoring de la mineuse de la tomate (Meyrick, 1917) en culture de tomate. **28ème forum international des sciences biologiques et e Biotechnologie de l'ATSB.** Mars, 2017.

3. **Asma Cherif,** Sabrine Attia-Barhoumi et Kaouthar Grissa-Lebdi. 2017. Etude de la biologie de *Tuta absoluta* (Meyrick) (Lepidoptera :Gelechiidae) sur hôtes alternatives sous conditions de laboratoire. **Conférence scientifique internationale sur l'environnement et l'agriculture.** Avril, 2017.

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Introduction générale

La tomate (*Solanum lycopersicon* L.) est une culture très populaire à l'échelle mondiale présentant une production d'environ 160 millions de tonnes produites sur une superficie de 5 millions d'hectares en 2011 (Terzidis *et al.*, 2014). Il existe plusieurs variétés de tomates cultivées destinées pour la consommation fraiche ou bien pour la transformation en industries agroalimentaires (Terzidis *et al.*, 2014). En Tunisie, la tomate se cultive sous serres (serres froides, serres chaudes, tunnels plastiques…) et en pleins champs, essentiellement, en trois saisons avec trois périodes de production couvant toute l'année allant jusqu'à 1,2 mille tonnes en 2016 (Lebdi-Grissa *et al.*, 2011 ; Gil, 2016). Cependant, la production de cette culture est sujette aux attaques sévères des maladies et ravageurs principalement, *Tuta absoluta* (Meyrick) (Lepidoptera : Gelechiidae). Dès l'apparition de ce ravageur en Tunisie, une stratégie nationale de lutte intégrée a été mise en place vu la gravité de la situation qui pouvait influencer d'une façon remarquable l'économie nationale. La mineuse de la tomate, *T. absoluta*, est un ravageur oligophage. Les larves consomment les feuilles en produisant des mines caractéristqiues, ce qui peut affecter la capacité photosynthétique de la plante (Fernandez et Montagne, 1990, Uchôa-Fernandes *et al.*, 1995, Pereyra et Sánchez, 2006). Aussi, les fruits peuvent être gravement altérés aboutissant à des pertes de rendements significatives (Colomo et Berta 1995). Par conséquent, diverse approches de lutte ont été mises en place afin de combattre ce ravageur (Consoli et al., 1998 ; Collavino et Gimenez, 2008 ; Desneux *et al.*, 2010).

Dans le cadre de cette thèse, une analyse bibliographique sur le ravageur en question a été abordée dans la chapitre 1.

Dans le but d'étudier la variabilité génétique du ravageur, des prospections ont été réalisées du nord au sud de la Tunisie permettant de collecter des larves de *T. absoluta* et de pratiquer des analyses moléculaires des populations cibles par l'ADN mitochondriale CO1 (5') (Chapitre 2, article 1). Ce travail a été réalisé en collaboration avec le ZSM localisé à Munich, Allemagne.

Afin d'approfondir nos connaissances en termes de biologie et d'écologie, l'élevage de *T. absoluta* qui se fait essentiellement sur des plants de tomate, a été envisagé au laboratoire sur différents milieux artificiels (chapitre 2, article 2).

La mineuse de la tomate est capable de se développer principalement sur la tomate comme elle peut s'adapter à d'autres cultures alternatives ainsi que quelques espèces de mauvaises herbes (Desneux *et al.*, 2010, 2011 ; Tropea Garzia *et al.*, 2012. Portakaldali *et al.*, 2013, Ettaib, 2017). Le potentiel de développement de cet insecte sur tomate (var. Sankara), pomme de terre (var. Spunta) et aubergine (var. A336) a été aussi évalué sous conditions contrôlées (T=25°C et HR=60%). *Tuta absoluta* a pu se disperser sur toute la Tunisie en un temps assez court vu les conditions climatiques favorables. L'effet combiné de deux températures (21 et 28°C) et de trois humidités relatives (32, 52 et 72%) sur les traits de vie de l'insecte a été étudié sous les conditions de laboratoire (Chapitre 2, article 3).

Au niveau de cette étude, la bio-écologie du nuisible et son comportement sous serre et en plein champ ; ainsi que les différentes corrélations entre les adultes capturés par les pièges et l'infestation causée par les larves ont été analysés (Chapitre 3, articles 4, 5).

Différents types de pièges ont été testés tel que les pièges à phéromones sexuelles utilisés principalement pour la surveillance et aussi pour le piégeage de masse ; ainsi que ; les plaques noires engluées conseillées pour le renforcement du piégeage sexuel (Chapitre 3, article 5). L'utilisation des pièges à phéromones sexuelles seuls ou combinés avec d'autres mesures de contrôle telle que la lutte chimique a été abordé au chapitre 4 (Chapitre 3, article 6). De même, l'efficacité de quelques substances chimiques utilisées contre *T. absoluta* a été vérifiée au laboratoire et sous serres (Chapitre 3, article 6).

Un nombre assez large d'ennemis naturels a été signalé pour *T. absoluta* y compris des prédateurs et des parasitoïdes (Zappalà *et al.*, 2015). Le potentiel d'exploitation de ces agents naturels s'accroit rapidement, jour après jour, vu le fort intérêt dispensé par la communié scientifique démontrant l'utilité et l'importance de la lutte biologique comme une alternative prometteuse à la lutte chimique (Terzidis *et al.*, 2014). Parmi ces ennemis naturels, on note les trichogrammes caractérisés par leur forte efficacité de parasitisme de diverses espèces de lépidoptères y compris *T. absoluta*. En Tunisie, la souche autochtone

Trichogramma cacoeciae (Marchal) (Hymenoptera : Trichogrammatidae) a été testé pour son efficacité contre *T. absoluta* sous serres et en plein champ (Cherif and Lebdi-Grissa, 2013 ; Zouba *et al.*, 2013). Des lâchers innundatifs de ce parasitoïde ont été réalisés via différentes doses sous serres (10, 20 et 30 trichogrammes/plante) et en plein champs (20 et 40 trichogrammes/plante) à Takelsa (Cap-Bon, Nord-ouest) (Chapitre 4, article 7).
Finalement, dans le but d'approfondir les connaissances sur les effets non intentionnels des pesticides sur la faune auxiliaire, onze insecticides (Indoxacarbe, spiromesifen, cyromazine, chlorfenapyr, cypermethrine, diafenthiuron, chlorantraniliprole, spinosade, azadirachtine *Bacillus thuringiensis* et virus HaNPV) largement utilisés en Tunisie sur la culture des tomates ont été testés sur les différents stades de *T. cacoeciae* (œufs, larve, pré-pupe et adulte) (Chapitre 4, article 8).
Finalement, cette thèse étudiant la mineuse de la tomate, *T. absoluta*, ait pour objectifs :
*Caractériser sa diversité génétique
*Elaborer un milieu artificiel pour l'élevage de masse
* Etudier l'effet des facteurs biotiques et abiotiques sur ses paramètres biologiques
*Déterminer la dynamique des populations en plein champs et sous serres froides
* établir différents moyens de lutte (piégeage de masse, lâchers de trichogrammes, traitements chimiques)
*Etudier les effets non intentionnels de quelques insecticides sur le parasitoïde *Trichogramma cacoeciae*.

Au total, quatre articles ont été publiés au niveau de cette thèse dans des journaux internationaux (journal of entomology and zoology studies, journal of plant diseases and protection, et journal of phytoparasitica).

Analyse bibliographique

I. Chapitre 1 : Analyse bibliographique

I.1 La culture de la tomate en Tunisie

La tomate est originaire de la région andine du nord-ouest de l'Amérique du Sud, où sa domestication remonte à plus de 5 000 ans. Elle a été introduite au Mexique puis, via l'Espagne, en Europe au XVIème siècle (Perron, 1999). La tomate, en l'absence totale de toute taille, est une plante à port buissonnant. Les feuilles sont impa-ripennées. L'inflorescence – cyme unipare avec un nombre de fleurs à pétales jaunes très variable en fonction du génotype – est disposée en position latérale sur tige ou sur rameau (Perron, 1999). Le fruit est une baie polymorphe et polychrome. La tomate est, à l'origine, une plante allogame mais elle est devenue auto-game préférentielle dans ses aires de domestication (Perron, 1999).

Les cultures maraîchères de plein champ et sous abris occupent en Tunisie une moyenne de 140 mille ha (GIL, 2016). La culture de la tomate s'étend sur une superficie moyenne de 29 mille ha/an, offrant une production moyenne de l'ordre de 1, 2 million de tonnes (GIL, 2016). Cette production est issue des cultures de plein champ (Tomates de saison et tardive) et des cultures sous abri (serres froides et serres chauffées par les eaux géothermales) (GIL, 2016) (Tableau 1). Les exportations tunisiennes des tomates proviennent essentiellement de la tomate fraîche issue principalement des cultures géothermales et de la tomate transformée en concentré de tomate et en tomate séchée (APIA, 2016). Les exportations de la tomate fraiche sont passées de 2 481 tonnes durant la campagne 2004/2005 à 13 981 tonnes durant la campagne 2013/2014 (APIA, 2016).

I.2 Les problèmes associés à la culture de tomate

La culture de la tomate est extrêmement sensible aux attaques de maladies, telles que la maladie des taches noires, la pourriture grise, l'oïdium, la fusariose et certaines viroses, dont le TYLC (Tomato Yellow Leaf Curk virus, transmis par des aleurodes) et de ravageurs tels que les aleurodes, les pucerons, les mineuses, les acariens, les thrips, les noctuelles et *Tuta absoluta* (Trottin-Caudal *et al.,* 1995). Le nombre important de ravageurs et de maladies associés à la tomate, ainsi que le risque constant de voir apparaître de nouveaux ravageurs, conduit à un intérêt certain de la part des producteurs pour le développement des moyens de lutte, qu'ils soient de nature chimique ou biologique (Trottin-Caudal *et al.*, 1995).

Tableau 1 : Données sur la production des tomates en Tunisie (GIL, 2016)

	Saison de culture	Superficie (Ha)	Production (T)	Zones de production	Période de production	Destination
Cultures de plein champ	Tomate de saison	17 900	960 000	Cap Bon, Sahel	juin-août	Tomate fraîche et industrielle
	Tomate tardive			Kasserine, Sidi Bouzid, Bizerte	Septembre-novembre	Tomate fraîche
Cultures sous abri	Serres froides	6300	290 000	Nabeul, Monastir, Sfax, Mahdia, Sidi Bouzid, Kef, Bizerte	décembre-mai	Marché local, exportation
	Serres chauffées			Gabes, Tozeur et Kébili	novembre-mai	Marché local, exportation

I.3 La mineuse de la tomate : *Tuta absoluta*

La position systématique de la mineuse de la tomate d'après Bloem et Spaltenstein (2011) est la suivante :

I.3.1 Position systématique

Phylum : Arthropoda
Classe : Insecta
Ordre : Lepidoptera
Sub-ordre : Glossata
Superfamille : Gelechiidaea
Famille : Gelechiidae
Sub-famille : Gelechiinae
Tribu : Gnorimoschemini
Genre : Tuta
Genre espèce : *Tuta absoluta* (Meyrick, 1917)

Tuta absoluta a été initialement décrit comme *Phthorimaea absoluta* (Meyrick, 1917). Le genre a été successivement remplacé par *Gnorimoschema* (1962) et *Scrobipalpula* (1964). Cette espèce a ensuite été placée dans un nouveau genre, *Scrobipalpuloides* (en 1987). Le nom correct de l'espèce est maintenant *Tuta absoluta* (Povolny, 1994).

I.3.2 Répartition géographique

La mineuse de la tomate, *T. absoluta*, est originaire d'Amérique du sud. Sa présence a été déclarée pour la première fois en Argentine en 1964. Ensuite, ce ravageur a colonisé les autres pays de l'Amérique latine (Bolivie, Brésil, Chili, Colombie, Équateur, Paraguay, Pérou, Uruguay et Venezuela) (EPPO, 2005) à l'exception de la région des Andes à une altitude supérieure à 1000 m (Viggiani *et al.*, 2009). En Europe, la présence de *T. absoluta* a été signalée la première fois en Espagne en 2006, au nord de la province de Castellón (EPPO, 2008 ; Desneux *et al.*, 2010). En Afrique du Nord, *T. absoluta* a été détecté, en 2008, en Algérie (Guenaoui, 2008), au Maroc (OEPP, 2008) et en Tunisie (OEPP, 2009). En Tunisie, *T. absoluta* a été enregistré dans de nombreuses régions, y compris Sousse, Kairouan, Bizerte, Nabeul, Zaghouanet et Quebeli (Harbi *et al.*, 2012, Ettaib, 2017). En 2011, plus de 20 pays en Europe du Sud, Afrique du Nord et Moyen-Orient ont signalé la présence de la mineuse de la tomate (OEPP, FERA, 2009). Actuellement la mineuse de la tomate est signalée en Europe de sud, en Inde et au Népal (Guillemaud *et al.*, 2015 ; Kalleshwaraswamy *et al.*, 2015 ; Bajracharya *et al.*, 2016).

I.3.3 Morphologie

Les adultes ont une longueur de 5-7 mm et une envergure d'environ 10 mm. Il n'y a pas de dimorphisme sexuel évident, bien que l'abdomen des adultes mâles est plus étroit et pointu postérieurement, tandis que celui des femelles est plus large et volumineux (Vargas, 1970 ; Desneux *et al.*, 2010). Les écailles abdominales chez le mâle sont de couleur gris, par contre elles sont de couleur crème chez les femelles (Vargas, 1970 ; USDA APHIS, 2011). Les adultes sont de couleur gris tacheté (Estay, 2000). Les antennes sont longues et filiformes (Vargas, 1970). Les ailes sont frangées et la paire antérieure contient des taches noirâtres (Sannino et Espinosa, 2010). La disposition des franges diffère entre les ailes antérieures et postérieures ; les ailes antérieures présentent des franges sur la moitié distale tandis que tout le contour des ailes postérieures est frangé (Sannino et Espinosa, 2010). Les œufs sont de forme ovale, de 0,38 mm de long et 0,21 de large (Vargas, 1970) (Figure 1). Les œufs nouvellement pondus sont de couleur blanc crème, ensuite jaune et jaune-orange au cours du développement (Estay, 2000). Les œufs matures deviennent sombres et le contour noir de la ligne dorsale peut être vu à travers le chorion (Vargas, 1970 ; Germain *et al.*, 2009). La chenille de premier stade est de couleur crème avec une tête sombre (Figure 1). En passant du 2ème au 4ème stade larvaire, la couleur change du vert au rose clair (Ramel et Oudard, 2008). Le 1er stade larvaire mesure 1,6 mm, le 2ème 2,8 mm, le 3ème 4,7 mm et le 4ème stade larvaire mesure 7,7 mm. Tous les stades larvaires présentent une ligne dorsale rougeâtre au niveau du pronotum (Germain *et al.*, 2009. Lebdi-Grissa *et al.*, 2010) (Figure 1). Nouvellement formées, les chrysalides sont de couleur verdâtre, alors que les plus matures sont de couleur brun foncé (Estay, 2000 ; Sannino et Espinosa, 2010). Les chrysalides mâles sont plus légères (3,04 ± 0,49 mg) et plus petites (longueur 4,27 ± 0,24 mm et largeur 1,23 ± 0,08 mm) que les chrysalides femelles (4,67 ± 0,23 mg de poids ; 4,67 ± 0,23 mm de longueur et 1,37 ± 0,07 mm de largeur) (Fernandez et Montagne, 1990a).

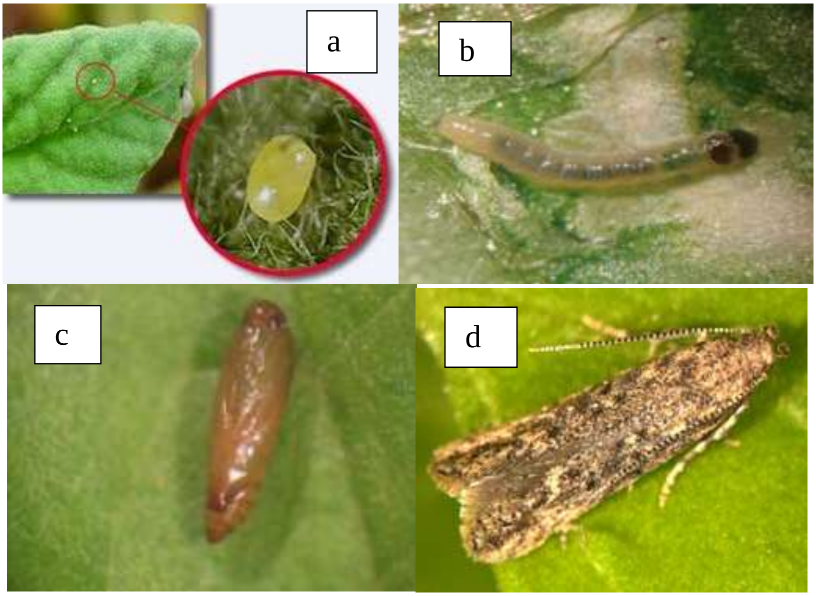

Figure 1 : Stades de développement de *T. absoluta* (a : œufs, b : larve (L2), c : chrysalide, d : adulte) (Anonyme 1, 2017)

I.3.4 Plantes hôtes

La principale plante hôte de *T. absoluta* est la tomate (*Lycopersicon esculentum*), mais elle peut, également, attaquer la pomme de terre (*Solanum tuberosum*), le pepino (*S. muricatum*) et l'aubergine (*S. melongena*) (Galarza, 1984 ; Notz, 1992 ; MPAAF, 2009 ; Viggiani *et al.*, 2009 ; Desneux *et al.*, 2010, 2011 ; Tropea Garzia *et al.*, 2012). Parmi les hôtes alternatifs de la mineuse de la tomate, il y a les mauvaises herbes telles que la morelle noire (*Solanum nigrum*), la stramoine commune (*Datura stramonium*) (Estay, 2000), la stramoine épineuse (*D. ferox*) et le tabac arorescent (*Nicotiana glauca*) (OEPP, 2005). La mineuse de la tomate peut terminer son développement (de l'œuf jusqu'au stade adulte) sur *S. gracilius*, *S. bonariense* et *S. sisymbriifolium*, mais le développement n'est pas achevé (interrompu au premier ou deuxième stades larvaires) sur *N. tabacum* et *S. pseudo-capsicum* (Galarza, 1984). D'autres études montrent que *T. absoluta* peut utiliser *S. elaeagnifolium* comme hôte alternative (Cardozo *et al.*, 1994). *Physalis peruvianna* L., *Phaseolus vulgaris* L., *Malva* sp. et *Lycium* sp. ont été signalés comme hôtes alternatifs pour *T. absoluta* en Italie (OEPP, 2009i; MPAAF, 2009 ; Tropea Garzia, 2009; Desneux *et al.*, 2010). En Turkie, *T. absoluta* a été indiqué sur *Convolvulus arvensis* L. et *Chenopodium album* L. (Portakaldali *et al.*, 2013).

I.3.5 Biologie

T. absoluta est une espèce multivoltine. La durée de son cycle de vie dépend des conditions environnementales, en particulier de la température (Tropea Garzia *et al.*, 2012). Différents travaux ont étudié le cycle de vie de *T. absoluta* à différentes températures. En effet, Barrientos, (1997) a indiqué que le cycle biologique dure de 76,3 jours à 14 °C, de 23,8 jours à 27 °C et il peut y avoir jusqu'à 10 à 12 générations par an. Guenaoui *et al.* (2008) ont démontré que le cycle biologique de ce ravageur peut être accompli en 3 semaines dans les régions méditerranéennes à 27°C. Une étude réalisée en Algérie a montré que le cycle de vie de *T. absoluta* est de 21,1± 0,4 jours à 26 ± 1,6°C et une HR 87 ± 6,4% et 29,4± 2 jours à 234± 2°C et une HR de 75± 3% (Boualem *et al.*, 2012). Alors qu'en Tunisie, Lebdi-Grissa *et al.* (2010) ont signalé qu'à 25°C, le cycle de vie de *T. absoluta* est de 37,5 jours. Potting *et al.* (2013) ont démontré que *T. absoluta* peut survivre à une température inférieure à 0°C. Krechmer et Forester (2015) ont montré que la basse température prolonge le développement de *T. absoluta* qui peut prendre 4 mois à 10°C. Ces auteurs ont indiqué aussi qu'à 10 °C, les mâles et les femelles vivent en moyenne 17,5 jours et 32,3 jours respectivement (Krechmer et Forester, 2015). Toutefois, Cuthberthson *et al.* (2013) ont indiqué qu'à 10°C, l'éclosion des œufs est faible, 3% des larves peuvent atteindre le stade nymphal et que l'émergence des adultes est nulle. Van Damme *et al.* (2014), ont signalé une mortalité des adultes de l'ordre de 90% à une température de 5°C durant un mois. Cuthberthson *et al.* (2013) ont indiqués aussi que le développement de l'insecte est meilleur lorsque la température varie entre 19 et 23°C. Des études ont montré que cet insecte passe l'hiver au stade œuf, chrysalide ou adulte (Barrientos, 1997). En effet, au stade larvaire, *T. absoluta* n'entre pas en diapause tant que la nourriture est disponible. En Tunisie, des études prouvent que l'insecte hiberne entre janvier et mars sous serres (Cherif *et al.*, 2013, 2014). Sitôt éclose, souvent le matin, la larve du premier stade rampe à la surface de la feuille pendant un laps de temps

(5 à 40 minutes) jusqu'à ce qu'elle trouve un point qui lui permette de se faufiler dans le limbe et elle se met à dévorer les tissus situés entre les deux épidermes de la feuille (Ferguson et Shipp, 1994 ; Tropea Garzia *et al.*, 2012). Dans cet abri, la larve continue de ronger les tissus internes des feuilles et les galeries ainsi creusées apparaissent sous forme de taches (Ferguson et Shipp, 1994 ; Tropea Garzia *et al.*, 2012). La larve peut quitter la galerie pour chercher d'autres sites de nutrition (Ferguson et Shipp, 1994 ; Tropea Garzia *et al.*, 2012). Généralement, c'est au niveau de la partie médiane du plant de tomate que se concentrent les larves et les galeries formées (Gomide *et al.*, 2001 ; Cherif *et al.*, 2013). Les larves peuvent, aussi, choisir de s'installer au niveau des fruits (Ferguson et Shipp, 1994). Le nombre de larves qui pénètrent dans un fruit est d'autant plus élevé que la densité de la population est élevée (Estay, 2000). Les larves parvenues à la fin de leur développement se suspendent à un fil et se laissent tomber jusqu'au sol où elles vont se nymphoser, mais cette étape peut, aussi, se produire à l'abri dans un coin de feuille repliée ou à l'intérieur des galeries (Ferguson et Shipp, 1994). Dès leur sortie de la coque de nymphose, les adultes s'accouplent, pondent des œufs et le cycle recommence (Uchoa-Fernandes et *al.*, 1995). Les adultes mâles et les femelles vierges vivent en moyenne 36,2 et 27,8 jours respectivement (Fernandez et Montagne, 1990). Généralement, les mâles émergent avant les femelles (Tropea Garzia *et al.*, 2012). Des études ont montré que l'émergence des adultes se fait à l'aube et que l'accouplement est lent et dure entre 2 et 3 heures (USDA APHIS, 2011). Ettaib (2017) a démontré que la période d'activité est limitée à une heure durant toute la journée. Cette période est située entre une demi-heure avant le lever de soleil et une demi-heure après le lever de soleil. Les adultes ont des habitudes nocturnes et dans les conditions méditerranéennes, ils peuvent être facilement détectés pendant toute l'année (Tropea Garzia *et al.*, 2012). En absence d'accouplement, la longévité des adultes se prolonge (Tropea Garzia *et al.*, 2012). La période séparant l'émergence des adultes et leur accouplement est plus importante chez les femelles (20 à 22 heures) que les mâles (quelques heures) (Tropea Garzia *et al.*, 2012). Le sex ratio est de 1 mâle pour 1 femelle (Rey *et al.*, 2014 ; Attrassi, 2015). Au laboratoire, Lee *et al.* (2014), ont

indiqué que la durée d'accouplement varie de quelques minutes à 6h40 min. De même, ces auteurs ont montré, qu'aux conditions de laboratoire, l'adulte de *T. absoluta* peut s'accoupler jusqu'à 15 fois durant sa vie (Lee *et al.*, 2014). Le pic de ponte est enregistré durant la nuit (Vargas, 1970) avec 76% des œufs pondus le même jour de l'accouplement (Lee *et al.*, 2014). Chaque femelle peut pondre isolement, de 40 jusqu'à 229 œufs de préférence à la face inférieure des feuilles ou au niveau des jeunes tiges tendres et des sépales des fruits immatures (Gomide *et al.*, 2001 ; USDA APHIS, 2011). Des études récentes ont signalé que *T. absoluta* peut se reproduire par voie parthénogénétique ce qui peut limiter l'efficacité de certaines méthodes de lutte surtout celles basées sur l'utilisation des phéromones sexuelles (Caparros Megido *et al.*, 2012 ; Abbes et Chermiti, 2014).

I.3.6 Symptômes et dégâts

Toutes les parties de la plante à différents stades de développement peuvent être infestées par *T. absoluta* (Tropea Garzia *et al.*, 2012 ; Attrassi, 2015). Les symptômes causés par les larves de *T. absoluta* sur plants de tomate peuvent être confondu avec ceux de *Liriomysa* sp. (KOPPERT, 2009). Cependant, les galeries de *T. absoluta* forment des plages tandis que celles de *Liriomysa* sont en forme de tunnel et s'évasent très progressivement (KOPPERT, 2009). Les déjections de *T. absoluta* sont dans la galerie alors que celles de *Liriomysa* forment un étroit filet à l'intérieur de la galerie (KOPPERT, 2009). Les dommages de *T. absoluta* sont directement liés à la réduction de la capacité photosynthétique et des niveaux de production, tant dans les cultures de tomates en serre qu'en plein champ (Tropea Garzia *et al.*, 2012). Au niveau des serres, les différents stades de cet insecte peuvent se manifester durant tout le cycle végétatif des plants de tomate (Mallia, 2009, Cherif *et al.*, 2013). Les chenilles ont une forte préférence pour les feuilles et les tiges, mais elles peuvent être, également, trouvées au niveau des bourgeons, dans ou sous la couronne du fruit et dans le fruit lui-même (Figure 2) (Mallia, 2009 ; USDA APHIS, 2011). Les excréments sont souvent trouvés près du trou d'entrée des larves (Mallia, 2009 ; USDA APHIS, 2011). Après éclosion, les jeunes larves pénètrent dans les feuilles ou les tiges dont elles se nourrissent et se développent, créant ainsi des mines et des galeries visibles (Figure 2 A, B) (Salvo and Valladares, 2007 ; Tropea Garzia *et al.*, 2012 ; Bajracharya *et al.*, 2016). Les fruits peuvent être attaqués dès qu'ils sont formés, et les galeries percées en leur sein peuvent être envahies par des pathogènes secondaires conduisant à la pourriture des fruits (Figure 2C) (OEPP, 2005 ; Desneux *et al.*, 2010, 2011 ; Balzan and Moonen, 2012 ; Caparros Megido *et al.*, 2012). Sur les feuilles, les larves se nourrissent uniquement sur les tissus du mésophile, laissant l'épiderme intact (Figure 2 A). En effet, une seule larve détruit en moyenne 28,7% de la surface foliaire (Attrassi, 2015). La surface foliaire consommée par le premier et deuxième stade larvaire est relativement faible par comparaison à celle consommée par les L3 et L4 ; de ce fait, la chenille de *T. absoluta* devient de plus en plus vorace en passant d'un stade à un autre (Bogorni *et al.*, 2003). De même, Ettaib (2017) a démontré que les descendants d'un œuf peuvent détruire à peu près 30% de la surface verte d'une feuille (contenant 10 folioles) et que 875 descendants de *T. absoluta* sont capables de détruire un plant de tomate entier. Aussi, cet auteur a signalé que 105000 adultes de *T. absoluta* peuvent détruire une serre entière (500 m^2 contenant 1200 plants). Les mines sur les feuilles sont irrégulières et peuvent devenir plus tard nécrotiques (OEPP, 2005 ; Tropea Garzia *et al.*, 2012). Les galeries sur les tiges peuvent modifier le développement général de la plante (OEPP, 2005 ; Desneux *et al.*, 2010) et peuvent entrainer la mort des jeunes plantules (Pereyra et Sanchez, 2006).

Figure 2 : dégâts de *T. absoluta* sur feuilles (A), tige (B) et fruit (C) (Anonyme 2, 2017 ; Bajracharya *et al.*, 2016)

Sur pomme de terre, seules les parties aériennes sont attaquées, *T. absoluta* ne se développe pas sur les tubercules (Notz, 1992 ; Caffarini *et al*., 1999). Les fruits de tomate sévèrement attaqués perdent leur valeur commerciale (Figure 2C) ; 100% de pertes ont été signalées sur la culture de tomate sous forte infestation (Urbaneja *et al.*, 2009).

I.3.7 Moyens de lutte

I.3.7.1 La lutte culturale

a. Mesures prophylactiques

Il faut interdire tout transport de plantes hôtes ou de fruits des zones infestées vers d'autre régions, il faut donc isoler et détruire les cultures infestées. Aussi, il faut s'assurer que les plants soient indemnes avant la plantation (USDA APHIS, 2011).

b. Les différentes pratiques culturales

Parmi les pratiques culturales traditionnellement appliquées par l'agriculteur, on note le désherbage à l'intérieur et aux alentours des serres cultivées. Ceci permet d'éliminer les refuges naturels choyés par les insectes ravageurs connus (Shipp et Ferguson, 1994 ; Germain *et al.,* 2009). Aussi, les agriculteurs sont amenés à installer des doubles-portes ou sas (Arnó et Gabarra, 2010) et à utiliser des insecte-proof au niveau des ouvertures et des portes d'entrée avec une densité minimale de 9*6 mailles/cm² (Germain *et al.*, 2009) et un diamètre inférieur ou égal à 1,6 mm (Oztemiz, 2014). En Tunisie, Harbi *et al.,* (2015) ont testé l'efficacité de l'insect-proof, installé sous serres, ayant une densité de l'ordre de 8*6 mailles/cm² pour lutter contre *T. absoluta*. De même, il est conseillé aux agriculteurs de vérifier l'étanchéité des serres et la réparation des trous (Terrentroy, 2012). Les autres pratiques culturales se font tout d'abord, par une préparation convenable du sol. En effet, le labour profond empêche l'émergence des adultes étant donné que les chrysalides sont enterrées en profondeur, ce qui entraine par conséquent l'interruption du cycle (Larrain, 1992). On peut aussi faire appel à l'élimination des feuilles basales sénescentes au niveau des cultures sous serre (pratique assez courante chez les agriculteurs) puisqu'elles présentent un niveau élevé d'infestation par les larves de *T. absoluta* (Larrain, 1992). Ces feuilles doivent être éloignées de la parcelle cultivée (ONSSA, 2010). La rotation des cultures est possible avec d'autres solanacées non hôte de ce ravageur ou des plants d'autres familles (Larrain, 1992 ; Oztemiz, 2014). Une période de vide sanitaire de l'ordre de 4 à 6 semaines est recommandée entre deux cultures hôtes de *T. absoluta* (Terrentroy, 2012).

c. L'utilisation des variétés résistantes

Différentes études ont examiné l'effet de la sensibilité de différentes variétés de tomate aux attaques de *T. absoluta*. En effet, Proffit *et al.* (2011) ont indiqué que ce ravageur préfère pondre sur la variété Aromata en comparaison aux autres variétés Santa Clara et Carmen. De même, en Tunisie, Cherif *et al.* (2013) ont remarqué que la variété Ferrinz est plus attractive et plus sensible à *T. absoluta* que les variétés Chebli et Chams. La résistance de la tomate aux ravageurs peut être liée à des facteurs chimiques, mécaniques ou génétiques. Le facteur chimique est attribué à la présence d'exsudats libérés des trichomes glandulaires foliaires (Fery et Kennedy, 1987 ; Leite *et al.*, 2001) ; alors que le facteur mécanique est lié à la structure de la cuticule des feuilles et des fruits (Leite *et al.*, 1999). Les variétés de *Lycopersicum* telles que *L. hirsutum, L. pennellii et L. peruvianum* sont connues par leurs résistances à *T. absoluta*. Ce facteur peut être déclenché par la réduction de la variabilité génétique introduite durant la domestication de la tomate et qui a entrainé la perte du gène qui contrôle la production des composés allélochimiques intervenant dans les éléments de défense de la plante (Oliveira *et al.*, 2008). La résistance de *L. hirsutum* à la mineuse de la tomate est attribuée aux allomones tridecan-2-one et undecan-2-one présentes dans les trichomes (petits poils existants à la surface foliaire de quelques plantes) glandulaires foliaires qui sont absents chez *L. esculentum* (Leite *et al.*, 2001). Les trichomes non glandulaires existants chez *L. esculentum* sont, quant à eux moins efficaces dans le mécanisme de défense contre les attaques du ravageur (Leite *et al.*, 2001). De ce fait, *T. absoluta* préfère se développer sur *L. esculentum* que *L. hirsutum*. L'utilisation de *L. peruvianum* pour la lutte contre *T. absoluta* est restreinte vu la difficulté de son croisement avec *L. esculentum* (Lourençào *et al.*, 1984). L'heptadecane, qui est une substance retrouvée dans les feuilles de *L. peruvianum* (CNPH 101), a diminué significativement le taux d'éclosion des œufs de *T. absoluta* ; tandis que le cyclobutanol et l'hexadécane existants dans la même variété ont augmenté le nombre de mines de *T. absoluta* (Suinaga *et al.*, 1999). *L. pimpin ellifolium* Mill est quant à elle résistante à *T. absoluta* grâce à la présence de tomatine et à la dureté de la cuticule des fruits (Juvik et Stevens, 1982). Concernant le facteur génétique, il s'agit d'introduire le gène ARNi dans les plants de tomate (Camargo *et al.*, 2016). En effet, la production de plants transgéniques ayant ce gène (ARNi) vise essentiellement à affecter les gènes des insectes en réduisant l'appétit des larves se nourrissant de ces plants qui finissent par mourir (Camargo *et al.*, 2016). Une étude récente a signalé que la mineuse de la tomate peut être contrôlée moyennant l'introduction du gène ARNi dans les plants de tomate (Camargo *et al.*, 2016). Deux gènes cibles ont été sélectionnés (Vacuolar ATPase-A et Arginine kinase) en se basant sur la réponse d'ARNi rapportée pour ces gènes dans d'autres espèces d'insectes ravageurs (Camargo *et al.,* 2016). Cette étude a indiqué que les larves de *T. absoluta* qui ont consommé des feuilles de tomate ayant ces gènes ont montré une réduction de 60 % dans l'accumulation des gènes cibles transcrits, une augmentation de la mortalité larvaire et moins de lésions foliaires (Camargo *et al.,* 2016).

I.3.7.2 Autres méthodes de lutte

a. Utilisation de bandes engluées

Il existe trois types de bandes engluées conditionnées pour lutter contre la mineuse de la tomate :

***Tuta roll**

Il s'agit d'un film transparent enduit de glu et imprégné de la phéromone de *T. absoluta* à libération lente. Tuta roll s'utilise en piégeage de masse tout en assurant une attraction ciblée du ravageur et permettant une réduction importante des populations. L'utilisation de Tuta roll est recommandée principalement sous serres pour les exploitations utilisant les auxiliaires comme moyen de lutte biologique (RUSSEL IPM, 2011). Ces bandes doivent être placées dans la serre entre les lignes et aux alentours à une hauteur de 1m du sol en laissant un espace de 15m entre les bandes (RUSSEL IPM, 2011).

***Optiroll Tuta+**

Il s'agit d'un film jaune où la phéromone sexuelle de *T. absoluta* est incorporée à la colle gluante (RUSSEL IPM, 2011). La couleur jaune du piège permet de lutter en plus contre la mouche blanche et les pucerons ce qui constitue un meilleur rapport coût-efficacité pour la lutte contre ces trois ravageurs. Optiroll Tuta+ est spécialement adapté aux serres où les auxiliaires ne sont pas utilisés comme moyen de lutte biologique. Ces bandes jaunes doivent être placées autour de la serre et entre les lignes (RUSSEL IPM, 2011).

***Horiver black Tuta**

Il s'agit de plaques noires engluées, ayant une dimension de 25*40cm, utilisées principalement en culture de tomate et aubergine (KOPPERT, 2016). Ces plaques doivent être positionnées à 30 et 50 cm au-dessus du sol (KOPPERT, 2016). L'utilisation de ces plaques est complémentaire au piégeage de masse préconisé contre *T. absoluta* (KOPPERT, 2016).

b. Attract and Kill

Il s'agit d'une pâte imprégnée de phéromone sexuelle et mélangée avec un insecticide à base d'Imidaclopride (Darek-Czokajlo, 2011). En effet, l'insecte qui sera attiré par la phéromone sera ensuite tué au contact de l'insecticide diminuant ainsi fortement la population mâle et par conséquent les œufs fertiles, ainsi que la population de *T. absoluta* (Darek-Czokajlo, 2011). Le produit est appliqué sous forme de gouttelettes sur les feuilles et les tiges ou bien sur les piliers de la serre, les fils et les cordes de palissage ou autre matériaux afin de réduire le contact avec la plante et par conséquent l'effet nocif des résidus de pesticides et le développement de la résistance par le ravageur (Darek-Czokajlo, 2011).

I.3.7.3 La lutte chimique

Le recours à la lutte chimique est indispensable car elle constitue d'une part la ressource sûre à laquelle l'agriculteur a accès tout le temps (Guedes et Picanço, 2012 ; Tropea Garcia *et al.*, 2012) et d'autre part, elle permet de maintenir la population du ravageur au-dessous du seuil de nuisibilité (Guedes and Picanço 2012 ; Tropea Garcia *et al.*, 2012). Dès l'apparition de *T. absoluta* sur tomate, en Argentine, les interventions chimiques ont commencé par l'utilisation des organophosphorés, lesquels ont été remplacés graduellement par les pyréthrinoides en 1970 (Galarza et Larroque, 1984 ; Lietti *et al.*, 2005). Certains essais ont conduit à l'utilisation en alternance les pyréthrénoides et les organophosphorés. Les Thiocyclanes ont, par contre, donné une meilleure efficacité (Galarza et Larroque, 1984). Les recherches ont amené à l'utilisation d'autres substances actives ayant de nouveaux sites d'intervention telles que l'abamectin, le spinosad et le chlorantraniliprole (Lietti *et al.*, 2005 ; Haddi *et al.*, 2012 ; Campos *et al.*, 2015 ; Roditakis *et al.*, 2015). Des études ont montré que l'utilisation de l'imidaclopride sous serre est efficace pour la dilution de 3,5 % appliquée par pulvérisation foliaire ainsi que pour la dilution de 7% appliquée par immersion des racines (Collavino et Gimenez, 2008). D'autres essais ont montré que l'utilisation du triflumuron, de l'abamectine, du chlorfenapyr et du *Bt,* causent une mortalité supérieure à 65 % des larves de *T. absoluta* (Collavino et Gimenenz, 2008). Certains chercheurs ont proposé que les traitements chimiques visent plus la larve néonate de *T. absoluta* avant qu'elle n'entre dans les feuilles et les fruits (Estay, 1998) ; vu que la larve passe une grande partie de sa vie à l'intérieur des galeries et qu'elle échappe par conséquent aux insecticides (Siqueira *et al*., 2000a). De même, les traitements qui visent les œufs n'ont pas donné de bons résultats, car ces derniers sont déposés à la face inférieure des feuilles qui ne sont pas bien trempées par les insecticides (Siqueira *et al*., 2000a). Des études récentes ont testé l'efficacité de certains produits contre les œufs de *T. absoluta* ; les produits testés, zeolites BEA (Beta Polymorph A), FAU (Faujasite), LTA (Linde type A), ainsi que leurs formulations, n'ont aucune activité insecticide contre les œufs de *T. absoluta* (De Smedt *et al.,* 2016). Cependant, l'exposition des œufs aux zéolithes semble affecter leur processus de développement en affaiblissant les larves du premier stade larvaire et en augmentant leur mortalité (De Smedt *et al.,* 2016). De plus, cette étude a signalé, qu'aucune différence significative n'a été observée entre le nombre d'œufs posés sur les feuilles traitées avec les zéolithes et les feuilles témoins (De Smedt *et al*., 2016). L'utilisation excessive des produits chimiques a entrainé non seulement des problèmes toxiques vis-à-vis des êtres humains et la pollution de l'environnement (Devonshire et Field, 1991 ; Weisenburger, 1993 ; Desneux *et al.,* 2007 ; Landgren *et al.,* 2009 ; Biondi *et al.,* 2012) ; mais aussi l'apparition rapide de phénomène de résistance développé par le nuisible vis-à-vis des substances actives (Campos *et al.,* 2010 ; Haddi *et al.,* 2012 ; Reyes *et al.,* 2012 ; Roditakis et *al.,* 2017). Ce problème de résistance a été observé dès les années 80, au Brésil, lorsque *T. absoluta* est devenu résistant à l'abamectine, au cartap et à la permethrine (Siqueira *et al*., 2000a). Des études en Argentine, ont montré que l'application régulière du cartap sur *T. absoluta* (échantillons collectés de différentes régions) a entrainé un problème de résistance de ce nuisible vis-à-vis du produit. Les chercheurs ont trouvé comme solution, l'utilisation d'un synergique du cartap (Siqueira *et al*., 2000b). En effet, l'utilisation du piperonyl butoxide a presque supprimé la résistance de ce ravageur au cartap. Par contre, l'utilisation du diéthyl maleate et du triphenylphosphate ont montré une suppression partielle de la résistance des populations de *T.*

absoluta (Siqueira *et al.*, 2000b). Des études récentes ont souligné le problème de la resistance dévelopée par *T. absoluta* vis-à-vis de certaines molécules chimiques (telles que les pyrethroides synthetiques, l'abamectine, le spinosade, le chlorantraniliprole) en Amérique de sud et en Europe (Lietti *et al.*, 2005 ; Campos *et al.*, 2010 ; Haddi *et al.*, 2012 ; Roditakis *et al.*, 2015).

I.3.7.4 Bio-insecticides à base des micro-organismes

Certains produits biologiques ont montré une meilleure efficacité pour lutter contre *T. absoluta* tels que le spinosad et le bactospeine.

a. Le spinosad

C'est un produit biologique obtenu par la fermentation naturelle d'un micro-organisme appelé *Saccharopolyspora spinosa* appartenant au groupe des actinomycètes (Verpont et Guérineau, 2002). Verpont et Guérineau (2002) ont mentionné que le spinosad est une combinaison de deux métabolites, spinosyne A et spinosyne D, aux propriétés insecticides et qui sont fabriqués naturellement dans le sol par le micro-organisme. Le mode d'action de ce bio-insecticide est de type neurotoxique ; il agit par ingestion et par contact. Ce produit constitue une alternative intéressante pour la lutte contre *T. absoluta* (Benchaâbane *et al.*, 2016). Une étude récente a souligné les effets retardés du spinosad sur la population de *T. absoluta* en l'appliquant sur le 4ème stade larvaire (Benchaâbane *et al.*, 2016). En effet, ces derniers ont indiqué que les analyses biochimiques révèlent un processus de détoxification, un stress oxydatif et une inhibition de l'acétylcholinestérase dans la génération 0 (G0) et la génération 1 (G1). Aussi, ces auteurs ont signalé une diminution des vitellogénines et du contenu de vitellines dans les deux générations, ce qui peut affecter négativement la fertilité et la fécondité des adultes (Benchaâbane *et al.*, 2016). La résistance de *T. absoluta* au spinosad a été signalée dans plusieurs pays à travers le monde tel que le Brésil. En effet, Campos *et al.* (2014a), ont démontré que la résistance au spinosad est monogénique, incomplètement récessive et autosomique avec héritabilité élevée (h2 = 0,71). Ces auteurs ont indiqué, aussi, que cette résistance est instable sans pression de sélection avec un taux de variation négatif du niveau de résistance (= 20,51) indiquant un coût adaptatif associé (Campos *et al.*, 2014a). Ces auteurs suggèrent que l'arrêt de l'utilisation du spinosyn contre *T. absoluta* peut être utile dans la gestion de la résistance au spinosad développé par ce ravageur (Campos *et al.,* 2014a). D'autres études réalisées au Brésil, suggèrent l'utilisation du spinetoram au lieu du spinosad en vue d'éviter le problème de la résistance croisée développée par *T. absoluta* (Campos *et al.,* 2014b). Ces auteurs encouragent à faire une rotation des produits dans le cadre des programmes de lutte préconisés contre cet insecte (Campos *et al.,* 2014b).

b. Le bactospeine

Ce bio insecticide contient une bactérie (*Bacillus thuringiens*) qui se trouve naturellement dans l'environnement sous forme de plusieurs souches, parmi elles, la souche *Bt Kurstaki* qui a été homologuée depuis les années 60 pour la lutte contre diverses chenilles (Linda et Adams, 2002). C'est un insecticide larvicide microbiologique. En effet, ces bactéries forment au moment de la sporulation une toxine cristalline qui agit sur l'épithélium du tube digestif induisant tout d'abord, un arrêt de nutrition avec paralysie de l'insecte, puis la mort de ce dernier. Il s'agit donc d'un produit agissant par ingestion au niveau de l'intestin (Linda et Adams, 2002). Des études ont montré que, deux isolats de *Bt* collectés à partir d'échantillons du sol des régions de Chili, présentent une toxicité spécifique vis-à-vis des larves de *T. absoluta.* Par conséquent, ces souches ayant des gènes appartenant à la famille de cry 1, ont montré une efficacité plus élevée que l'isolat obtenu à partir de la préparation commerciale Dipel *Bt* (*Bt* var Kurstaki) (Neidmann et Meza-Basso, 2006). D'autres études ont montré, que l'applications du *Bt* sur des feuilles de tomate issues de variétés résistantes est toxique pour les larves de *T. absoluta* (Giustalin *et al.,* 2001). En effet, l'application du *Bt*k sur des larves déjà nourrits par ces feuilles peut entrainer une mortalité plus élevée après l'ingestion (Giustalin *et al.,* 2001). L'étude de l'oviposition de *T. absoluta* sur des feuilles non traitées par *Bt*k, montre que le nombre d'œufs déposés dépasse celui observé sur des feuilles traitées par *Bt*k. Ceci montre que le *Bt* peut agir comme répulsif vis-à-vis des femelles de *T. absoluta* lors de l'oviposition (Marques et Alves, 1996). Mollá *et al.,* (2011) ont étudié l'utilisation combinée de la punaise prédatrice *Nesidiocoris tenuis* Reuter (Hemiptera : Miridae) avec des pulvérisations de *Bt*. En effet, le

Bt a été appliquée chaque semaine pendant 2 mois, 3 mois ou tout au long du cycle végétatif de la culture de tomate (Mollá *et al.*, 2011). Dans chaque cas, 1 adulte de *N. tenuis* a été libéré par plant de tomate (Mollá *et al.*, 2011). Mollá *et al.*, (2011) ont démontré que les dégâts foliaires ont été minimisés jusqu'à 97% en les comparant au témoin. Ces auteurs suggèrent l'application du *Bt* en début d'attaque vue la disponibilité des œufs, alors que les lâchers de la punaise doivent être réalisés en fin de culture (Mollá *et al.*, 2011). Des études récentes ont indiqué que l'intégration de l'utilisation du spinosad avec le *Bt* possède un effet additif dans le cadre des programmes de lutte préconisés contre ce ravageur (Hashemitassuji *et al.*, 2014). Ces auteurs suggèrent, aussi, l'utilisation de ces deux produits en presence des ennemis naturels du nuisible (Hashemitassuji *et al.*, 2014).

I.3.7.5 Bio-insecticides à base d'extraits de plantes

Des études au laboratoire ont montré, que l'utilisation d'extraits méthanoïques tirés des grains d'*Annona coriacea* Mart. (Annonaceae) sur des larves récemment écloses et placées sur des feuilles de tomate, produit une mortalité des larves de 86,4 % pour une concentration de 0,5 % et 100% pour une concentration de 1 % (Silva *et al.*, 2007). L'utilisation d'extraits aqueux de feuilles et de branches de *Trichilia pallida* Swartz (Meliaceae) associés aux cultures de tomate *Santa calara* et IPA-5, sur les larves de *T. absoluta*, a causé une mortalité de 86,8 % à une concentration de 50 % après 72 h (Folcia *et al.*, 2003). Des études ont montré que l'utilisation des huiles essentielles comme moyen de lutte alternative contre la mineuse de la tomate *T. absoluta*, aide à développer des stratégies de lutte moins toxiques aux ennemis naturels avec une faible persistance dans l'environnement (Umpiérrez *et al.*, 2012 ; Goudarzvand Chegini et Abbasipour, 2017). Goudarzvand Chegini et Abbasipour, (2017) ont indiqué que l'huile essentielle extraite à partir de cardamom, (*Elettaria cardamomum*) est toxique à *T. absoluta* ayant un fort potentiel dans le contrôle de ce ravageur surtout dans les zones protégées. Ces auteurs ont démontré que les principaux composants de cette huile sont l'acétate d'α-terpinyle (36,61%), le 1,8-cineol (30,42%), l'acétate de linalyle (5,79%) et le sabinène (4,85%). Aussi, ces auteurs ont signalé que les LC50 pour les œufs, 2ème stade larvaire (à l'intérieur et à l'extérieur des feuilles) et les adultes sont de 1,2, 7,9, 1,6 et 1,9 µl d'air L^{-1} respectivement (Goudarzvand Chegini et Abbasipour, 2017). Ibrahim *et al.* (2016) ont démontré que l'utilisation de l'huile essentielle à base de clou de girofle est efficace pour la lutte contre *T. absoluta* sous conditions semi naturelles.

I.3.7.6 La lutte biotechnique par l'utilisation des phéromones sexuelles

La phéromone sexuelle de *T. absoluta* comprend deux composés, l'un majeur : 3, 8, 11-Tétra-decatrienyl acétate (92 %) (Attygalle *et al.*, 1995 ; Attygalle *et al.*, 1996) et l'autre mineur : 3, 8- tétra-decadienyl acétate (8%) (Griepink *et al.*, 1996 ; Svatos *et al.*, 1996). Les études ont montré, que seul le composant majeur a une attraction élevée (avec environ 869 mâles piégés pendant 3 nuits consécutives de la population de *T. absoluta* (Filho *et al.*, 2000). L'addition des deux isomères du composant mineur au composant majeur n'a pas d'effet significatif sur le nombre de mâles capturés par piège (Filho *et al.*, 2000). Les phéromones sexuelles sont utilisées dans le cadre du monitorage et du piégeage de masse. Des capsules à phéromones sexuelles avec différentes concentrations ont été testées en vue de fixer la dose la plus convenable en termes de durée de vie et d'efficacité (Cherif et Lebdi-Grissa, 2014). En outre, la concentration des phéromones sexuelles peut dépendre du niveau d'infestation. En effet, Chermitti et Abbes, (2012), ont démontré que les capsules à phéromones sexuelles faiblement concentrées (0,5mg) sont à utiliser pour le suivi des faibles populations alors que celles fortement concentrées (0,8mg) sont recommandés en cas de fortes infestations. Différents types de pièges sont recommandés tels que les pièges delta et les pièges à eau qui sont les plus utilisés (Hassan *et al.*, 2010a ; RUSSEL IPM, 2012 ; Cherif *et al.*, 2013). Les pièges delta sont construits en plastique ayant une forme triangulaire avec des extrémités ouvertes. Ils contiennent des plaques engluées renouvelables en dessus desquelles sera suspendue la phéromone sexuelle (USDA APHIS, 2011). Les pièges à eau consistent en des bassines en plastique contenant de l'eau et une couche d'huile végétale (USDA APHIS, 2011). Cette huile sert à réduire la tension superficielle et par conséquent réduire la capacité de l'insecte à échapper du piège. Aussi cette huile permet de réduire l'évaporation de l'eau. La phéromone sexuelle est fixée au dessus du contenu par un fil attaché aux deux extrémités du piège (USDA APHIS, 2011). Il est conseillé d'installer les pièges dans la parcelle à une hauteur de 25 cm avant la plantation et à 60 cm au fur et à mesure que les plants de tomate grandissent (Ferrara *et al.*, 2001). En effet, l'utilisation des pièges pour le monitorage aide à suivre le niveau d'infestation et donc à déclencher un programme de lutte approprié une fois le seuil de nuisibilité atteint (Salas, 2004). En fait, ce seuil a été calculé en se basant sur le nombre d'adultes capturés par les piégeages à phéromones sexuelles. Selon Stol *et al.* (2009), le niveau d'infestation est considéré bas pour 1 à 3 adultes capturés par semaine, modéré pour 4 à 30 adultes capturés par semaine et élevé pour plus de 30 adultes capturés par semaine. En Tunisie, le seuil de tolérance économique défini par le Ministère de l'agriculture pour *T. absoluta* est de 50 adultes/piège/semaine (Abbes *et al.*, 2012b). D'autres types de pièges ont été signalés tels que les pièges lumineux (Refki *et al.*, 2016). En effet, ces auteurs ont comparé l'efficacité des pièges à phéromones sexuelles (associés ou pas à une source de lumière) et des pièges lumineux installés sous serres géothermales localisées dans le sud de la Tunisie. Ces auteurs ont démontré que les pièges à phéromones sexuelles attirent plus d'adultes que les autres types de piège avec un nombre moyen d'adultes capturés par piège par semaine de l'ordre de 73,4±142 (Refki *et al.*, 2016). Le piégeage de masse est une technique qui implique de placer un nombre assez élevé de pièges dans la culture afin de capturer la proportion la plus élevée de mâles existants dans la population du ravageur (Jones, 1998). La densité des pièges recommandée est de 20-25 pièges/ha (soit 1 à 2 pièges/500m^2) dans les serres et 40-50pièges/ha en plein champs (Bolckmans, 2009). Des études récentes ont été réalisées en vue de déterminer la densité des pièges optimale à utiliser dans le cadre du piégeage de masse. En effet, Aksoy et Kovanci (2016) ont démontré que l'utilisation de 40 pièges/ha en plein champ est capable de réduire l'infestation dûe à de faibles attaques causées par *T. absolua*. En Tunisie, trois densités de pièges ont été testées (20, 40 et 80 pièges/ha) en plein champs (Braham, 2014). Cet auteur a indiqué qu'aucune différence significative n'a été signalée entre les trois doses testées en ce qui concerne la réduction du taux d'infestation des feuilles et des fruits (Braham, 2014). Des études ont montré que l'utilisation des pièges à phéromones sexuelles seules n'est pas efficace pour la lutte contre *T. absoluta* tant qu'ils sont incapables de réduire les dégâts causés par ce ravageur sur feuilles et fruits (Cocco *et al.*, 2012). Des études réalisées sous serres en Tunisie ont montré que l'utilisation combiné de pièges à phéromones sexuelles avec les filets insect-proof peut limiter les dégâts causés par *T. absoluta* en limitant le nombre de mines foliaires (Harbi *et al.*, 2012 ; Cherif *et al.*, 2013).

La confusion sexuelle est utilisée aussi comme moyen de lutte contre *T. absoluta* et vise à perturber la rencontre des deux sexes. Il s'agit de saturer l'atmosphère avec les phéromones sexuelles synthétiques en vue d'inhiber l'accouplement et par conséquent de réduire l'infestation causée par le ravageur (Cardé, 2007 ; Cocco *et al.*, 2013). Les résultats issus de l'application de cette méthode sont nombreux et différents. En effet, des études ont montré que cette méthode ne garantit pas la réduction des dégâts. En fait, certaines études ont montré que la libération de 30 à 50 g/ha de la phéromone sexuelle de *T. absoluta* en plein champ est efficace pour la perturbation de l'orientation des mâles (60-90%) alors que les dégâts sont semblables aux témoins (Filho *et al.*, 2000). D'autres études ont montré que l'utilisation de la confusion sexuelle avec une dose de 30 g/ha (500 diffuseurs/ha chargés de 60 mg de phéromone sexuelle) dans des serres à haut confinement peut donner des résultats satisfaisants (Vacas *et al.*, 2011). Cocco *et al.* (2013) ont démontré que l'application de 1000 diffuseurs/ha à raison de 60 g/ha au niveau de serres isolées a permis de réduire le taux d'infestation des feuilles et des fruits de 85 et 89% respectivement. La technique de la confusion sexuelle peut être efficace contre *T. absoluta* si elle est appliquée sous serres bien protégées et isolées en vue d'empêcher l'entrée de nouveaux adultes (Cocco *et al.*, 2013). Cette technique dépend de la durée de vie de la phéromone sexuelle ainsi que de son prix (Vacas *et al.*, 2011).

I.3.7.7 La lutte biologique

I.3.7.7.1 l'utilisation des entomophages parasitoïdes

a. L'utilisation des trichogrammes

Les trichogrammes sont des micros hyménoptères de taille inférieure au millimètre, appartenant à la famille des

Trichogrammatidae (Chalcidoidea) (Ksentini, 2004). Ces espèces sont exclusivement oophages et parasitent essentiellement les œufs de plusieurs espèces de Lépidoptères (Parra et Zucchi, 2004). Certaines espèces peuvent cependant parasiter les œufs de certains Coléoptères, Diptères, Hétéroptères, Hyménoptères et Névroptères (Ksentini, 2004).

Sur le plan taxonomique, les trichogrammes se caractérisent par des ailes antérieures de grande taille, aux bords pourvus de longs cils et à la face supérieure garnie de soies (Ksentini, 2004). Plusieurs espèces de Trichogrammes ont été étudiées pour lutter contre la mineuse de la tomate telles que : *Trichogramma pretiosum*, *T. acheaae*, *T. bactrae*, *T. nerudae et T. cacoeciae.* (Virgula *et al.*, 2006 ; Faria et *al.*, 2008 ; Cabello *et al.*, 2012 ; Cherif et Lebdi-Grissa, 2013 ; Zouba et al., 2013). Les chercheurs ont étudié comment *T. pretiosum* exploite la distribution des œufs de *T. absoluta* localisés sur le plant de tomate et l'effet de la morphologie de la plante sur le parasitisme (Faria et *al.*, 2008). Le nombre d'œufs parasités est corrélé avec le nombre d'œufs pondus par le ravageur sur la plante (Faria et *al.*, 2008). En effet, le pourcentage de parasitisme varie de 1,5 à 28 % sur des plants ayant plus de 6 œufs pondus (or la densité des œufs peut varier de 2 à 146 œufs/plant) (Faria *et al.*, 2008). Les résultats montrent que le parasitisme par *T. pretiosum* est lié au nombre d'œufs déposés par *T. absoluta* sur les différentes parties de la plante. Seul le 1/3 supérieur de la plante de la tomate est le site préféré pour l'oviposition de *T. absoluta* et de *T. pretiosum* (Faria *et al.*, 2008). Cette étude montre, également, que ce trichogramme n'est pas forcément influencé par la morphologie de la plante hôte, mais par la densité élevée des trichomes non glandulaires présents sur les feuilles de l'apex de la plante et qui peut affecter son comportement. En plus le parasitisme est corrélé spatialement avec l'oviposition du ravageur (Faria *et al.*, 2008). D'autres chercheurs ont étudié l'effet combiné de l'utilisation de *T. pretiosum* et du bio-insecticide à base de *Bt*, qui a donné une meilleure efficacité lorsqu'on effectue 3 lâchers/semaine du parasitoïde avec une pulvérisation de *Bt*, ceci a permis de réduire de 20 % l'utilisation des insecticides (Haji *et al.*, 1995). França *et al.* (2000) ont obtenu une réduction de 2 % seulement des fruits endommagés lorsqu'ils ont effectué 1 lâcher/ semaine de *T. pretiosum* avec des applications de *Bt.* D'autres auteurs ont montré que l'utilisation de *T. pretiosum* réduit le pourcentage de dégâts sur fruits (seulement 13 %) alors que les cultures traitées par le lefenuron présentent des dégâts allant jusqu'à 58 % (Geraldo *et al.*, 2006). Des études ont montré, également, la toxicité de certaines substances actives vis-à-vis de *T. pretiosum* tels que le lambdacyhalothrin, le chlofenapyr et le Methamidophos (Geraldo *et al.*, 2006). En Espagne, Cabello *et al.* (2009) ont montré que *T. acheae* étudié sous serres et dans les conditions de laboratoire a entrainé un taux de parasitisme allant jusqu'à 83,3 %. Il entraine une réduction des dégâts sous serre allant jusqu'à 91,7% lorsque les plants de tomate sont infestés en moyenne par 75 adultes/m^2 avec des lâchers tous les 3-4 jours, durant les mois d'août et de septembre (Cabello *et al.*, 2009). D'autres études ont évalué l'effet d'une dose sublétale de deltaméthrine sur l'émission des phéromones sexuelles des Trichogrammes. La réponse des mâles traités émetteurs de la phéromone sexuelle ne diffère pas de celle de la femelle mais la cinétique de leur réponse n'est pas la même (Cabello *et al.*, 2009). Par conséquent, l'effet de la dose sublétale de deltaméthrine peut être avantageux ou désavantageux, tout dépend de la difficulté à trouver le sexe mâle et de sa rareté (Cabello *et al.*, 2009). Au Pérou, il y a eu utilisation de *T. bactrae* Nageraja pour lutter contre *T. absoluta* (Virgula *et al.*, 2006), comme parasitoïde oophage. Les chercheurs ont étudié l'effet de 3 insecticides (Triflumuron, abamectine et chlorfenapyr) sur ce parasitoïde. Tous les insecticides testés sont nocifs à cette espèce de Trichogramme. Seuls les insecticides biologiques à base de *Bt* ne sont pas toxiques à *T. bactrae* (Virgula *et al.*, 2006). Au Chili, des essais ont évalué l'efficacité *de T. nerudai* (espèce indigène) et ont pu obtenir un taux de parasitisme allant jusqu'à 79,3 %. Ce taux est indépendant de l'âge des œufs de *T. absoluta* (Gerding et Torres, 2003). En Turquie, des études ont signalé que 7 lâchers de *T. evanescens* sous serres à raison de 75 adultes/m² tous les 3-4 jours ont montré un taux de parasitisme des œufs de *T. absoluta* de l'ordre de 60,3% (Oztemiz *et al.*, 2012). Selon ces auteurs, cette dose a entrainé une réduction du nombre d'œufs, de larves et de fruits infestés de l'ordre de 63,3, 54,6 et 85,3% respectivement (Oztemiz *et al.*, 2012). En Tunisie, différentes doses de *T.*

cacoeciae ont été testées sous serres et en plein champs (Cherif et Lebdi-Grissa, 2013 ; Zouba *et al.*, 2013). Dans le nord tunisien (Cap bon), Cherif et Lebdi-Grissa, (2013) ont indiqué que 3 lâchers de *T. cacoeciae* à raison de 30 adultes/plant/lâcher en plein champ ont montré un taux de parasitisme des œufs de *T. absoluta* de l'ordre de 54,7%. Ces auteurs ont indiqué aussi, que le nombre moyen de larves dans la parcelle traitée a diminué et n'a pas dépassé 3 larves/120 feuilles en le comparant à celui du bloc témoin (8 larves/120 feuilles) (Cherif et Lebdi-Grissa, 2013). Dans le sud tunisien, les lâchers de *T. cacocciae* à la dose de 40 adultes/plant libérés tous les 3 à 4 jours durant les mois de février et mars sous serre ont donné des résultats satisfaisants (Zouba et Mahjoub, 2009). En effet, le taux de réduction des dégâts a dépassé les 75% (Zouba et Mahjoub, 2009). Zouba *et al.* (2013) ont étudié l'efficacité de *T. cacoeciae* et *T. bourarachae* suite à des lâchers innodatifs (25 000 parasitoïdes/ semaine) sous serres de tomate (480 m^2) chauffées par les eaux géothermales dans le Sud-Ouest Tunisien. Ces auteurs ont signalé que ces deux espèces sont capables de localiser et parasiter les œufs de la mineuse de la tomate (Zouba *et al.*, 2013). Les taux de parasitisme des œufs par *T. bourarachae et T. cacoeciae* étaient respectivement de 63,9% et 57,1%. Une réduction du nombre de galeries sur feuilles de l'ordre de 87,6% et de 78,9% a été signalée aussi, respectivement pour *T. bourarachae et T. cacoeciae* (Zouba *et al.*, 2013).

b. L'utilisation d'autres parasitoïdes

Les larves de *T. absoluta* peuvent être parasitées par divers parasitoïdes appartenant principalement à l'ordre des hyménoptères et à la famille des Eulophidae tel que *Stenomesius japonicus* signalé en Espagne (Urbaneja *et al.*, 2012). Cet ectoparasitoïde idiobionte est capable de parasiter les 2éme et 3éme stades larvaires de *T. absoluta* (Urbaneja *et al.*, 2012).

Les espèces *Necremnus artynes* et *N. tutae* sont notées aussi comme étant des parasitoïdes idiobiontes parasitant préférentiellement le troisième stade larvaire de *T. absoluta* (Dehliz et Guenaoui, 2015 ; Gebiola *et al.*, 2015). Des études ont montré que *N. tutae* testé en plein champ à une température comprise entre 17 et 23°C, présente un taux de parasitisme moyen de l'ordre de 20,4% (Dehliz et Guenaoui, 2015). Pour les Braconidae, diverses études ont signalé la présence du parasitoïde *Bracon nigricans* dans divers pays à travers le monde tels qu'en Italie, Espagne et Tunisie (Urbaneja *et al.*, 2012 ; Zappalà *et al.*, 2012 ; Abbes *et al.*, 2013 ; Biondi *et al.*, 2013). Des études effectuées au laboratoire ont montré que les femelles nouvellement fécondées de *Pseudopanteles dignus* Muesebeck, détectent et parasitent l'hôte même à de faibles densités. Le taux de parasitisme va jusqu'à 30 % (Luna *et al.*, 2007). Pour *Retisympiesis phthorimea*, qui est un ectoparasite de 2ème et 3ème stade larvaire, le taux de parasitisme sur *T. absoluta* s'approche de 40 % (Rojas, 1981). Cette étude, qui a été conduite en Bolivie a montré que la femelle est capable de découvrir la larve hôte à l'intérieur de la galerie, y enfonce sa tarière et dépose les œufs dans la larve mineuse. Dès son éclosion, la larve parasite est capable de consommer graduellement son hôte (Rojas, 1981). D'autres parasitoïdes sont spécialisés des larves tel que le genre *Conura* sp. (Hymenoptera ; Chalcididae) possèdant un taux de parasitisme d'environ 2,6 % (Marchiori *et al.*, 2003).

I.3.7.7.2 l'utilisation des entomophages prédateurs

En Espagne, des études menées au laboratoire ont montré que *Nabis pseudoferus* (Hemiptera : Nabidae) est capable de réduire le nombre d'œufs de *T. absoluta* jusqu'à 92-96 %. Ce prédateur est connu pour son efficacité contre les pucerons et les œufs et les larves de lépidoptères (Cabello *et al.*, 2009). On trouve aussi, comme prédateurs, *Nesidiocorus tenuis* et *Macrolophus pygmaeus* qui s'attaquent aux œufs et aux larves *de T. absoluta* (Cabello *et al.*, 2009 ; Urbaneja *et al.*, 2009). Des études ont montré que l'inoculation de *M. pygmaeus* en plein champ a entrainé une réduction des dégâts sur feuilles et fruits de tomate l'ordre de 76 et 56% respectivement (Mollà *et al.*, 2009). En Sardaigne, l'introduction de ce prédateur à raison de 2 adultes/m²/semaine sous serre, où l'application d'insecticides se fait d'une façon régulière, n'a pas pu diminuer les dégâts causés par le ravageur (Nannini *et al.*, 2012). Trottin-Caudal *et al.* (2012) ont indiqué que l'introduction de *M. pygmaeus* est conseillée durant les premiers stades de développement de la plante voire avant le repiquage. Mollà *et al.* (2014) ont démontré que le rôle de *M. pygmaeus* dans le contrôle de *T. absoluta*, peut être plus efficace s'il existe d'autres sources d'alimentation tels que les œufs d'aleurodes ou d'*Ephestia*. D'autres études ont montré que la punaise *N. tenuis* peut être efficace dans le contrôle de *T. absoluta*. En effet, Mollà *et al.* (2009) ont indiqué que cette punaise introduite sous serre a entrainé une réduction du taux d'infestation des feuilles et des fruits de 97 et 100% respectivement. Les piqûres d'alimentation de cette espèce sur les plants de tomate, les effets conséquants et les stratégies de lutte ont été l'objet de diverses études (Biondi *et al.*, 2016 ; Naselli *et al.*, 2017 a, b). Parmi les autres prédateurs de ce ravageur, on trouve *Chrysoperla externa* Hagen (Nevroptera : Chrysopidae). Les larves de cet auxiliaire sont des oophages et des prédatrices des jeunes stades larvaires (Iannacone et Reyes, 2001). Un autre prédateur est noté comme efficace pour la lutte contre *T. absoluta* ; il s'agit de *Podisus nigrispinus* (Dallas) (Heteroptera : Pentatomidae). Ce prédateur est capable de maintenir sa population en se nourrissant seulement de *T. absoluta* (Vivan *et al.*, 2002). Cependant, la mineuse de la tomate produit plusieurs générations par an et possède une croissance naturelle des populations plus rapide que son prédateur *P. nigrispinus* (Vivan *et al.*, 2002). Des études ont, également, visé l'effet du spinosad sur *P. nigrispinus*, qui s'avère 5 fois moins sensible au spinosad que les larves de *T. absoluta* (Torres *et al.*, 1999).

I.3.7.7.3 La lutte par les champignons entomopathogènes

Parmi les microorganismes utilisés dans le contrôle biologique de *T. absoluta*, il y a les champignons, *Metarhizium anisopliae* (Metschn) et *Beauveria bassiana* (Bals). Inanli *et al.* (2012) ont montré que *M. anisopliae* affecte significativement les œufs et le premier stade larvaire de *Tuta*, tandis que *B. bassiana* est efficace principalement sur les œufs. *M. anisopliae* possède une spécificité élevée et une compatibilité avec les insecticides (França *et al.*, 1999). Une étude a porté sur l'identification de l'effet de l'isolat UFRPE-6 de ce champignon sur la fécondité et la mortalité des femelles de *T. absoluta* et le mécanisme d'infection des œufs (Pires *et al.*, 2008). L'infection des femelles par le champignon n'affecte, ni leur oviposition, ni leur fécondité. Elle affecte, par contre, les individus issus des femelles infectées avec un taux de mortalité de 54,2 % ; ce qui ramène le taux de mortalité à 37,1% pour l'ensemble de la population (femelles infectées et la première génération de leur descendance) (Pires *et al.*, 2008). Contreras *et al.* (2014) ont signalé une efficacité totale de *M. anisopliae* appliqué à une dose de 5,58 10^9 conidies/litre sur les chrysalides de *T. absoluta.*

I.3.7.7.4 la lutte par des nématodes entomopathogènes

Certains nématodes (tels que *Steinermena carpocapsae*, *S. feltiae* et *Heterorhabditis bacteriophora*) sont capables de parasiter les larves et les chrysalides de *T. absoluta* (Battala-Carrera *et al.*, 2012 ; Terzidis *et al.*, 2014). Des études précédentes ont indiqué que l'application de *S. feltiae* et *S. carpocapsae* peut causer une mortalité du premier stade larvaire de *T. absoluta* de l'ordre de 100% au bout de 6 jours (Jacobson et Martin, 2011). Des essais au laboratoire ont été réalisés afin de tester la capacité de ces nématodes à atteindre la larve et à la tuer dans les galeries sur feuilles de tomate (Battala-Carrera *et al.*, 2012 ; Terzidis *et al.*, 2014). Battala-Carrera *et al.* (2012) ont signalé la présence de mortalités larvaires élevées de *Tuta* allant de 78,6 jusqu'à 100% après avoir réalisé des applications foliaires de ces 3 espèces de nématode (*S. carpocapsae*, *S. feltiae* et *H. bacteriophora*) sur les plants de tomate infestés par *T. absoltua*. En effet, selon ces auteurs, le traitement par ces nématodes réduit l'infection par cet insecte avec un pourcentage allant de 87 à 95% **(**Battala-Carrera *et al.*, 2012). D'autres études ont indiqué que les traitements du sol, visant les larves qui vont se nymphoser dans le sol, par des préparations à base des nématodes cités ci-dessus ont entrainé de fortes mortalités larvaires allant de 52,3% pour *S. feltiae* jusqu'à 96,7% et 100% pour *H. bacteriophora* et *S. carpocapsae* respectivement (Garcia-Del-Pino *et al.*, 2013).

Etude de quelques paramètres biologiques de *Tuta absoluta*

II. Chapitre 2 : Etude de quelques paramètres biologiques de *Tuta absoluta*

II.1 Quelques remarques sur l'uniformité génétique de *Tuta absoluta* (Meyrick) (Lepidoptera : Gelechiidae)

II.1.1 Introduction

Le but de ce travail est de caractériser la diversité génétique de *T. absoluta* dans divers sites. De ce fait, des prospections ont été réalisées dans des parcelles de tomate de pleins champs (Béja, Bizerte, Nabeul et Ariana) et sous serres géothermales (Tozeur, Kebeli, Gabés) réparties du nord au sud tunisien. Au total, sept populations de *Tuta absoluta* ont été examinées. Ce travail a permis d'étudier la variabilité génétique de ce ravageur tout en évaluant l'ADN mitochondriale CO1 (5') des populations examinées. Les résultats de cette prospection ont fait l'objet d'une publication dans la revue <Journal of Entomology and Zoology studies 2017, 5 (3) : 1380-1382>.

II.1.2 Some remarks on the genetic uniformity of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Article 1)

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Running title: Genetic variability of *Tuta absoluta*

Abstract

Genetic variability of a lepidopteran pest was studied by investigating the mitochondrial DNA CO1 (5') of seven Tunisian populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). We have found a high genetic homogeneity which may confirm the hypothesis that this species was introduced only once in Tunisia. Alternatively, a selective sweep could be responsible for the absence of variability in the mitochondrial DNA.

Keywords: Pest, tomato, Solanaceae, crop, damage, barcode

1.Introduction

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae), is considered in Tunisia, since its first detection in 2008, as one of the most serious pest causing extensive damages mainly to tomato (*Solanum lycopersicum* L.) crop [6]. Other cultivated solanaceous plants including potato, (*S. tuberosum* L.), eggplant (*S. melongena* L.), sweet pepino (*S. muricatum* L.) and tobacco (*Nicotiana tabacum* L.) as well as wild plant species were also reported as secondary hosts for *T. absoluta* [9, 10, 12, 13, 15, 19, 21]. *Tuta absoluta*, which is a multivoltine non-diapausing species, was characterized by a high potential of growth performing several generations per year depending on climatic conditions and quality of existed hosts [8, 15]. This invasive pest can reduce significantly the tomato productions up to 80-100% in newly invaded areas both in protected and open fields if no control procedures are taken [1, 9]. Damages are caused by larvae which can feed on all aerial parts of tomato plants (leaves, buds, stem and fruits) by making large galleries [1, 18]. Secondary pathogens may invade later damaged fruits leading to their rot which can reduce the crop production and therefore inducing heavy cost losses to farmers [2, 9, 10, 11, 16]. Control programs based mainly on pesticides applications were carried out to manage this pest since its first detection in all production countries around the world [6]. Alternative pest management has been also investigated as sustainable tools used in IPM strategies deployed against this pest [5]. The study of genetic variability of *T. absoluta* is essential to establish an efficient IPM programs [3]. To identify possible genetic diversity which may have been developed by this invasive species molecular markers, such as AFLP and microsatellites markers were tested respectively for Brazilian and Tunisian populations [4, 20]. The aim of this work was to investigate if any genetic variation is expressed in Tunisian populations of *T. absoluta* using the standard DNA barcode (CO1) as a marker.

2.Material and methods

Infested tomato leaves with *Tuta* larvae were sampled from different regions located in Northern and Southern Tunisia as indicated in Table 1. Only L2 instars were preserved for DNA analysis. Second segment of larval thorax was placed into lysis plates containing one droplet of ethanol (96%) for DNA barcoding (cytochrome c oxidase subunit I, COI 5'). Three replicates were considered for each region resulting in a total of 21 individuals analyzed. All DNA analysis steps, including DNA extraction, PCR and DNA sequencing were carried out following standard high-throughput protocols [14] at the Canadian Centre for DNA Barcoding, Guelph, Canada (CCDB) (http://ccdb.ca/resources.php). Tunisian data were blasted against >100 other COI sequences of *T. absoluta* on BOLD Data Systems (Ratnasingham & Hebert 2007) using the identification engine of BOLD. All data are accessible on BOLD in the public dataset DS-ABSOLUTA.

Table 1: Sampling data of collected larvae

Collection date	Province	Region	Host plant/site	Locality	Latitude	Longitude
18/12/2015	Tozeur	Mzara	Tomato/greenhouse	South-west	33°55.1808′N	8°8.0112′E
18/12/2015	Guebeli	Lymeguez	Tomato/greenhouse	South-west	33°42.2634′N	8°58.1418′E
17/12/2015	Gabes	Ben Guilouf	Tomato/greenhouse	South-East	33°52.8876′N	10°5.892′E
28/10/2015	Beja	Testour	Potato/open field	North-West	36°43.5384′N	9°10.9014′E
22/10/2015	Bizerte	Teskreya	Tomato/open field	North-west	37°16.4652′N	9°52.4346′E
16/10/2015	Nabeul	Bouargoub	Tomato/open field	North-East	36°27.3636′N	10°44.2578′E
07/10/2015	Ariana	Kalaat el Andalous	Tomato/open field	North-East	36°51.6072′N	10°11.6022′E

3.Results and discussion

A total of 18 barcodes sequences were obtained from 21 specimens of Tunisian *T. absoluta*. All sequences resulted belong to one single haplotype for all *T. absoluta* specimens regardless of their host-plants and localities. The Tunisian COI haplotype exactly corresponds to that of >100 examined COI barcodes including vouchers from countries like France, Netherlands, Portugal, Serbia, Bosnia and Hercegovina, Montenegro, Egypt, Saudi Arabia, Kenya, Tanzania, India. This uniformity in the mtDNA may indicate a genetic bottleneck and/or a selective sweep at a global level. Our results are supported by those obtained by [7] which indicated high genetic homogeneity found in *T. absoluta* populations from the Mediterranean Basin and South America, based on mtCOI and ITS rDNA sequence analysis. Other studies on Tunisian *T. absoluta* using Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) technology on genomic DNA, however, resulted in a comparatively high genetic diversity as well as in a significant differentiation between populations [3].

Further research is needed to investigate the genetic variability for *T. absoluta* basing on different markers and involving larger samples from many locations and host-plants.

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II.3 Conclusion

Le présent travail a pu confirmer que la population de *T. absoluta* présente sur tout le territoire tunisien a une seule origine de provenance expliquée par l’homogénéité génétique très élevée des populations existantes en Tunisie. Ce resultat confirme le caractère invasif de l’espèce puisqu’un seul point d’entrèe du nuisible lui a permis rapidement de s’adapter, de s’intaller et de se propager dans les différentes régions du pays sans difficultés (Cap Bon, Sahel, Oasis…).

II. 2 Elevage de *Tuta absoluta* sur milieux artificiels sous conditions de laboratoire

II.2.1 Introduction

Ce travail avait pour objectif de mettre au point un milieu artificiel adapté pour l'élevage de masse de *Tuta absoluta* afin de bien étudier sa biologie sous conditions de laboratoire. Durant cette expérimentation, six milieux artificiels ont été testés. Les résultats figurent dans l'article ci-après qui a été présenté sous forme d'une communication orale <27ème forum international des sciences biologiques et Biotechnologie de l'ATSB>.

II.2.2 Rearing of *Tuta absoluta* on artificial diets under laboratory conditions

Abstract

Tuta absoluta (Meyrick, 1917) (Lepidoptera: Gelechiidae) is noted as a key tomato pest in Tunisia and other countries around the world. To get better knowledge of its biological traits and to establish efficient control strategies, it is important to develop adequate rearing systems. For this, six artificial diets were tested for *T. absoluta* mass rearing as alternative to tomato leaves under laboratory conditions. Only the diet based on bean as protein source showed the best viability up to 30%. This result is promising and needed to be improved.

Key words: artificial diets, mass rearing, *T. absoluta*

1. Introduction

The oligophagous pest *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) is a devasting pest of tomato crops causing losses up to 80-100% if management strategies are absent (Desneux et al. 2010, 2011; Rostami et al. 2015; Bajracharya et al. 2016). This pest feeds an all aerial parts at all stages of tomato plant growth causing large galleries (Salvo and Valladares, 2007; Bajracharya et al., 2016; Bajonero and Parra, 2017). Secondary pathogens may have invaded the damaged fruits through the wounds made by the insect (Salvo and Valladares, 2007; Dos Santos et al., 2011; Bajracharya et al., 2016). *Tuta absoluta* was reported on alternative host plants including potato (*Solanum tuberosum* L.), eggplant (*Solanum* *melongena* L.), sweet pepino (*S. muricatum* L.) and tobacco (*Nicotiana tabacum* L.). (Siqueira et al., 2000; Pereyra and Sa´nchez, 2006). Weed plants were noted also as hosts for the tomato leaminer (Abbes et al. 2015, Bawin et al. 2015, Salas Gervassio et al. 2016; Bajonero and Parra, 2017). Chemical control has been the main method used to manage this pest, however, problems of resistance were reported in many countries around the world such as Argentina (Siqueira et al. 2000; Roditakis *et al.*, 2015, 2016, 2017). Other control strategies are developed to control this pest such as the use of predators and parasitoids (Hoffmann et al., 2006; Hegazi et al., 2007; Chailleux et al., 2012, 2013; Cherif and Lebdi-Grissa, 2013; Abbes et al., 2015; Refki et al., 2016). Alone and/or in combination with selective compounds (Zappalà *et al.*, 2012). To develop new and successful management strategies, it is necessary to improve rearing techniques.

The aim of this work was to test different artificial diets used for mass rearing of *T. absoluta* and to select the most suitable.

2. Material and methods

2.1 Rearing of *T. absoluta*

Colonies of *T. absoluta* were maintained for several generations in an experimental greenhouse containing tomato plants grown in pots (var: Sankara). Infested leaves with larvae were collected and set in plastic box kept in climatic controlled chamber (T= 25 ± 2°C, RH= 70±10%) in order to allow the larvae to achieve their development until pupae. Then, pupae were recovered and set in other boxes until emergence of adults. Obtained adults were released in cages containing tomato plants for mating. Eggs were collected, placed in petri dishes containing tomato leaflets and kept in climatic controlled room until the larvae hatch. Second instar larvae were used in this study.

2.2 Composition of tested artificial diets

Six artificial diets were tested during this study. For each diet, five larvae were introduced and four repetitions were considered (table 1).

Table 1: Composition of six tested artificial diets for rearing of *T. absoluta*

D1		D 2		D 3	
Ingredient	**Quantity**	**Ingredient**	**Quantity**	**Ingredient**	**Quantity**
Water	150 ml	Water	90ml	Water	30ml
Agar	3g	Saccharose	0.2g	Agar	3g
Starch	9g	Mixed salts	3.33g	Mixed salts	0.7g
Wheat germ	11g	Wheat germ	100 g	Wheat germ	11 g
yeast	9g	Yeast	0.33 g	Yeast	9 g
Ascorbic acid	0.9g	Glycerin	16.66ml	Ascorbic acid	0,9 g
Benzoic acide	0.3g	Ascorbic acid	1.11 g	Lysin	0.1g
Corn oil	0.3ml	Lysin	0.1g	Corn oil	0,3 ml
Nipagin	0.3g	Starch	5g	Nipagin	0,3 g
Casein	0.3g	Nipagin	0,22g	Casein	3 g
		Aureomycin	0.11g		

D4		D 5		D 6	
Ingredient	**Quantity**	**Ingredient**	**Quantity**	**Ingredient**	**Quantity**
water	150 ml	Water	1200 ml	water	1200 ml
Agar	3 g	Agar	23 g	Agar	23 g
Amidon	4 g	Wheat germ	60 g	yeast	37.5 g
Wheat germ	11 g	Yeast	37.5 g	Bean	265 g
yeast	9 g	Bean	105 g	Ascorbic acid	3.6 g
ascorbic acid	0.9 g	Acide ascorbique	3.6 g	Benzoic acid	1.8 g
Benzoic acid	0.3 g	Benzoic acid	1.8 g	Starch	5 g
Corn oil	0.3 ml	Starch	5 g	Nipagin	3 g
Nipagin	0.3 g	Nipagin	3 g	Casein	30 g
		Casein	30 g		
		aldehyd form	3.6 ml		

3. Results

Comparing the performance of the six artificial diets after inoculation of larvae (L2) showed that diet (D6) presented aproximateley 30% of viability. In this diet, six larvae, which changed color (Figure 1), achieved their development and reached pupal and adult stage (figure 2). A 100 % mortality of larvae was observed for the other tested diet.

Figure 1: Comparison between natural larvae (a) and a color changing larvae (b) of *T. absoluta* (Personal picture, 2014) (*1.6)

Figure 2: Comparison between pupae of *T. absoluta* (a: artificial diet; b: natural diet) (Personal picture, 2014) (*1.6)

4. Discussion

In this study, we observed the development of *Tuta* larvae on six artificial diets. Our data indicated that only the diet based on bean as protein source gave encouraging results and confirmed those obtained by Mihsefeldt and Parra (1999). According to these authors, this diet had a phagostimulant effect, low larval mortality and promoted higher total viabilities (Mihsefeldt and Parra, 1999). A recent study evaluated the suitability of different artificial diets for rearing of *T. absoluta* under laboratory conditions (Bajonero and Parra, 2016). The authors indicated that the diet based on casein, wheat germ and cellulose allowed the best development of *T. absoluta*, showing higher viability with no negative effects on larval instars and pupal weight **(**Bajonero and Parra, 2016).

In conclusion, results presented in this study are promising but further studies are needed in this issue to develop adequate rearing protocols.

II.2.3 Conclusion

Cette étude a pu démontrer l'aptitude de *Tuta absoluta* à se développer sur milieu artificiel à base d'haricot présentant un taux de survie des larves plus ou moins important (30%). Les résultats trouvés sont préliminaires et nécessitent d'être améliorés. Des travaux supplémentaires sont nécessaires soit pour améliorer les doses des différents ingrédients déjà retenus ou bien pour tester d'autres milieux ayant différentes sources protéiniques.

II.3 Effets de l'humidité relative, de la température et de la plante hôte sur les traits biologiques de la mineuse de la tomate, *Tuta absoluta* (Meyrick) (Lepidoptera : Gelechiidae) sous conditions de laboratoire

II.3.1 Introduction

Cette étude vise à étudier le cycle de vie de *Tuta absoluta* (longévité des adultes, fécondité des femelles…) sur cultures de tomate, pomme de terre et aubergine sous conditions contrôlées ; comme elle s'intéresse à l'effet de deux températures (21 et 28°C) et trois humidités relatives (32, 52, et 72%) sur le cycle biologique de l'insecte élevé sur plants de tomate. Cet aricle a été soumis au journal <Entomologia generalis> et il est sous revision et correction.

II.3.2 Effect of relative humidity, temperature and host plant on the Biological traits of the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under laboratory conditions (Article 2)

Running title: Biological parameters of *T. absoluta*

Abstract

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is a serious threat of tomato productions worldwide. This pest can occur on various cultivated Solanaceae. Here, we investigated the insect performance (life cycle, fecundity of females and longevity) on tomato, potato and eggplant plants under controlled conditions (25°C and 60% RH). Because of its worldwide distribution and invasive potential, the biological traits of *T. absoluta* on tomato were further investigated at two temperatures (21 and 28°C) and three levels of relative humidity (32, 52, and 72%). These results show the higher capacity of *T. absoluta* to develop on tomato with a shorter life cycle (30.95 ± 3.04 days), high fecundity (30.71 ± 27.31 eggs/female) and higher longevity (14.05 ± 6.30 days) compared to potato and eggplant. The development time of *T. absoluta* was longer at 21°C compared to 28°C and the longevity of adults was higher for all tested relative humidity (32, 52 and 72%). Fecundity of *T. absoluta* females was highest at 28°C and 52% of relative humidity, with an average number of eggs laid per female of about 41.58 ± 11.33. The present study provides valuable information to understand the effect of host plant, temperature

and relative humidity on the life cycle of *T. absoluta* which is useful for developing control strategies for this pest.

Key- Words: life cycle, climatic conditions, host plant, fecundity, longevity

1. Introduction

The horticultural crop is an important sector in Tunisia with a harvested area of 140000 Ha (Gil 2016). Tomato (*Solanum lycopersicon* L.) and potato (*Solanum tuberosum* L.) represent the most important cultivated crops among this sector with areas of about 29000 and 25000 and average yields of about 1.2 and 370 million tons respectively (Gil 2016). The eggplant (*Solanum melongena* L.) crop is considered in Tunisia as a new product destined mainly for export with an average quantity exported in last 5 years of about 187 tons (Gil 2016). Among horticultural pests, the tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), can cause serious damage to these crops. This lepidopteran specie is a key pest of the tomato in South America (Urbaneja et al. 2007; Guedes & Picanço 2012; Luna et al. 2012). After its first record in Spain in 2006 (Desneux et al. 2010; Tropea Garzia et al. 2012), this invasive pest was reported in several countries around the world until India Nepal and Burkina Faso (Desneux et al. 2011; Kalleshwaraswamy et al. 2015; Bajracharya et al. 2016; Son et al. 2017). *Tuta absoluta* can develop on other hosts including cultivated Solanaceae such as potato ((*S. tuberosum* L.), eggplant (*S. melongena* L.), sweet pepino (*S. muricatum* L.) and tobacco (*Nicotiana tabacum* L.), as well as on wild plant species [Jimson weed (*Datura stramonium* L.), black nightshade (*S. nigrum* L.) and deadly nightshade (*Atropa belladonna* L.)] (Desneux et al. 2010, 2011; Tropea Garzia et al. 2012; Cocco et al. 2015). Other plants such as *Physalis peruviana* L., *Phaseolus vulgaris* L., *Malva sp.* and *Lycium sp.* have been reported as hosts for *T. absoluta* (Tropea Garzia 2009; Desneux et al. 2010). In Turkey, *T. absoluta* has been found on *Convolvulus arvensis* L. and *Chenopodium album* L. (Portakaldali et al. 2013). This pest has the capacity not only to grow faster on tomato plants but also to quickly spread in new areas causing major damages to crops and consequently heavy cost losses (Desneux et al. 2010, 2011; Caparros Megido et al. 2012). Larvae feed on leaves' mesophyll, as well as on stems and fruits (green and red), resulting in significant yield losses, cosmetic damages, as well as rot diseases to fresh market tomatoes (Bawin et al. 2015). Chemical control has been the principal management tactic used against *T. absoluta* (Guedes & Picanço 2012; Calvo et al. 2013); but this strategy has become problematic. In fact, repeated pesticide applications increases not only the risk of developing insecticide resistance to some active ingredients (Siqueira et al. 2000; Campos et al. 2014; Roditakis et al. 2015, 2017); but also implies additional production costs and negative side effect on beneficial organisms (Arno´ & Gabarra 2011; Biondi et al. 2012, 2013), worker, consumer safety and environment (Calvo et al. 2013). Moreover, global change (climate and land use changes) is expected to impact the severity and timing of pest outbreaks (Hance et al. 2007; Battisti and Larsson, 2015). Species respond in different ways to climate change. Variation in the distribution, phenology, and abundance of species will lead to inevitable changes in species interactions and communities structuring. These changes impose a high threat to population viability (Bellard et al. 2012). Temperature and relative humidity have been cited among the major factors that affect the insect biological traits (Willmer 1982; Guarneri et al. 2002; Broufas et al. 2009). These abiotic factors may influence *T. absoluta* life cycle and might play a role in its adaptability to local climate. An analysis on how host-plant systems react to changes in temperature is needed so that researchers may manage the consequences of global change at the ecosystem level. Altogether, the need for improved control of agricultural pests and diseases and the necessity to reduce the use of chemicals require a change in the paradigm of crop protection, especially in the context of climate change.

Therefore, the aim of this research was firstly to study the insect performance (development time, fecundity of females and longevity) on three different host plants (tomato, potato and eggplant) under controlled conditions, and secondly to evaluate the effect of two temperatures (21 and 28°C) and three relative humidity levels (32, 52 and 72%) on its development, reproduction and longevity on tomato.

2. Materials and Methods

2.1 Insect rearing

Infested leaves with *T. absoluta* larvae were collected from tomato greenhouses in March 2014 from tomato greenhouses (Gouvernorate of Nabeul, North-Eastern of Tunisia) and transferred to the laboratory of Entomology at the National Agronomic Institute of Tunisia. The strain was reared on tomato leaves keept in ventilated plastic boxes and placed in a climate-controlled room [25°C, 50–60% RH, 16: 8 (L: D)] until obtaining pupae. Fresh leaves were added whenever necessary. Pupae were then placed in new plastic boxes containing small cotton soaked in diluted honey, which is considered as a food for newly emerged *T. absoluta* adults. Then, 300 young adults (24 h old) were released inside 3 cages (100 adults/cage) covered by an insect-proof net, containing 20 pots/cage of untreated host plants (1 plant/pot). These cages, which were placed inside an experimental greenhouse (25°C), contained respectively pots (20*20 cm) of tomato plants (var. “Sankara”), eggplants (var. “A336”) grown from seed and potato (var. “Spunta”) grown from tubers (1 tuber/pot). *Tuta absoluta* population was allowed to increase in each different host plant separately during several generations, in order to avoid any possible lingering effects of egg vitellus and food reserves on emerged larvae (Hassan et al. 2011; Caparros Megido et al. 2012, 2013). Then, *T. absoluta* population from each host plant was transferred in new plastic boxes to allow larvae achieving their development until pupae formation.

2.2 Effect of host plant and abiotic factors on the biological traits of T. absoluta

Two trials were conducted in this study; the first one aimed to study the effect of three different host plants (tomato (var. “Sankara”), potato (var. “Spunta”) and eggplant (var. “A336”)) on *T. absoluta* fitness when both temperature (25°C) and relative humidity (60%) were fixed. The second one was carried out on tomato and aimed to test the effect of two temperatures (21 and 28°C), three relative humidity levels (32, 52 and 72%) and their interactions on the development time, the fecundity and the longevity of *T. absoluta* adults. For each assay, 50 *T. absoluta* eggs were taken separately from each host plant (tomato, potato and eggplant) depending on the trial, placed inside Petri dishes covered by meshes and kept inside the controlled climatic chamber. Each Petri dish contained leaflet of each host as a food for larvae. The petiole of each leaflet was rolled in cotton soaked in water to avoid their wilting. New leaflets were added when needed. Every day, obtained pupae (males and females), were placed by couples for mating in tubes containing leaflets. Male and female pupae were identified based on the charecteristics as indicated by Nayana and Kalleshwaraswamy (2015). Daily checks were carried out to record development and survival of individual insects as well to count the number of eggs laid. Eggs were examined daily for hatching and the change in their colour was recorded. Dates were marked in each tube. Dead adults were counted and eliminated. Leaflets were taken from each tube to count the number of laid eggs. Then, each leaflet containing eggs was placed inside one new Petri dish containing a new and fresh leaflet and covered with meshes to allow larvae achieve their development. For each trial, some biological parameters of the insect were recorded, such as, hatching egg rate, egg period (days from egg laying to hatching neonate larvae), larval period (days from hatching of eggs to pupa stage), pupal period (days from pupae to adult emergence), adult emergence, adult longevity and daily fecundity rate of the female. Anlysis were based on data recorded only on individuals that survived to adult stage.

3 Statistical analysis

The effect of host plant on the biological traits of *T. absoluta* was analysed using one way anova and the interaction between relative humidity and temperature was checked using a Generalised Linear Model (GLM) followed by one way anova. Normality and homoscedasticity were checked using Shapiro-wilk tests and Kruskal Wallis tests were applied when needed. Comparison of viability percentages was realized using Chi-square tests of independence. Significant means were separated using Duncun post hoc test (SPSS statistical software version 21®).

3. Results

4.1 Evaluation of T. absoluta fitness on tomato, potato and eggplant

Significant differences in the embryonic period ($F_{2, 77}$=233.196, *P*<0.0001), larval period ($F_{2, 78}$=363.272, *P*<0.0001) and egg to adult period ($F_{2, 78}$=618.333, *P*<0.0001) were observed between the different host plants. Embryonic development of *T. absoluta* is longer on potato and eggplant compared to it on tomato leaves. Also, eggs viability is highest on tomato (55.22 %) than on potato (15.58 %) and eggplant leaves (15,41 %). Also, the larval development time differered depending on the host plant, being longer on potato and eggplant than on tomato.

Pupal development, longevity and fecundity of females did not differ significantly among the three host plants (Table 1). Egg to adult period was shorter on tomato leaves compared to it on potato and eggplant leaves. Longevity of *T. absoluta* did not differ significantly among the three host plant used in this study (Table 1). Adults lived longest on tomato and shortest in potato and eggplant. *T. absoluta* fecundity was not significantly different between the three host plants. Nonetheless the table 1 demonstrated a difference among tomato which has the greatest number of eggs compared to other host plants.

Table 1. Biological traits of *T. absoluta* on different host plants: tomato (c.v Sankara), potato (c.v Spunta) and Eggplant (c.v A336)

Biological parameters	**Host plants (T°: 25±2°C ; RH: 60±10%)**			**Statistics**	***P* values**
	Tomato (c .v Sankara)	**Potato (c.v Spunta)**	**Eggplant (c.v A336)**		
Egg period (days)	3.36±1.32 a	4.82±2.1 b	4.12±1.65 c	$F_{2,77}$=233.196	0.000
Egg viability (%)	55.22 a	15.58 b	15,41 ab	χ^2=32.000	0.036
Larval period (days)	21.37±3.84 a	28.36±3.29b	28,79±3,77b	$F_{2,78}$=363.272	0.000
Pupal period (days)	6.22±1.93 a	8,42±1.6 a	9,58±2,24 a	$F_{2,78}$=0.246	0.783
Egg to adultperiod (days)	30.95±3.04 a	41.6±2.33 b	42,49±7,67c	$F_{2,78}$=618.333	0.000
Adult longevity (days)	14,05±6,30 a	6,31±2,98 a	9,54±4,14 a	$F_{2,33}$=0.652	0.528
Fecundity rate (eggs/female)	30.71±27.31a	10.45±2.36a	11,68±8,87a	$F_{2,33}$=0.016	0.985

*Means followed by the same letter(s) are not significantly different at 5%.

4.2 Effect of relative humidity, temperature and their interactions on the development time, longevity and fecundity of *T. absoluta*

Table 2 shows that that the investigated relative humidity levels (32, 52 and 72%) does not affect the development pre-imaginale stages (eggs, larvae and pupae), as P>0.05 at both 21 and 28°C. Conversely, relative humidity significantly affects the egg-to-adult development time at both 21 ($F_{2,83}$= 6.366; *P*<0.0001) and 28°C ($F_{2,62}$=70.333; *P*<0.0001). For RH $_=$ 32%, all biological parameters (egg, larval, pupa and egg to adult periods) are influenced by the low relative humidity. We noted mortality of large individuals' number of each stage. For the interactions between temperature and relative humidity, statistical analysis shows significant differences for egg (*P*<0.0001), larval (*P*<0.0001) and egg to adult periods (*P*<0.0001).

Table 2. Development time of *T. absoluta* at T= 28 and 21°C and RH=72, 52 and 32%

Biological parameters	**T° C**	**RH %**			**Statistics**	***P* values**
		72±10%	**52±10%**	**32±10%**		
Egg period (days)	**21±2°C**	3.36±0.97 aB	3.39±1.02 aA	3,36±1,29 aA	$F_{2,83}$= 0.783	*P*=0.460
	28±2°C	2.84±1.06aA	2.88±1.01aA	3,32±1,27aA	$F_{2,62}$=2.843	*P*=0.066
Statistics		$F_{1,53}$=15.211	$F_{1,50}$=0.195	$F_{1,45}$=0.524		
***P* values**		*P* =0.000	*P* =0.661	*P* =0.473		
Larval period (days)	**21±2°C**	30.75±5.89 aB	30.42±6.00aB	18,08±4,00aA	$F_{2,83}$= 0.197	*P*=0.821
	28±2°C	17.46±3.72 aA	15.48±3.62aA	11,24±3,65aA	$F_{2,62}$=0.005	*P*=0.995
Statistics		$F_{1,53}$=4.691	$F_{1,50}$=32.926	$F_{1,45}$=0.133		
***P* values**		*P* =0.035	*P* =0.000	*P* =0.719		
Pupal period (days)	**21±2°C**	9.52±2.84 aB	9.49±2.86 aB	10,58±2,93aA	$F_{2,83}$=0.151	*P*=0.860
	28±2°C	6.12±2.67 aA	6.18±2.59 aA	6,37±1,31 aA	$F_{2,62}$=0.006	*P*=0.994
Statistics		$F_{1,53}$=5.597	$F_{1,50}$=28.470	$F_{1,45}$=0.076		
***P* values**		*P* =0.022	*P* =0.000	*P* =0.784		
Egg to adult period (days)	**21±2°C**	43.63±3.23 aB	43.30±3.29 aB	32,03±8,23bA	$F_{2,83}$= 6.366	*P*=0.003
	28±2°C	26.42±2.48 aA	24.54±2.40bA	20,94±6,24bA	$F_{2,62}$=70.333	*P*=0.000
Statistics		$F_{1,53}$=75.525	$F_{1,50}$=7.796	$F_{1,45}$=0.923		
***P* values**		*P* =0.000	*P* =0.007	*P* =0.342		

*Means followed by the same letter(s) within a row (lower case letter) and within a column (upper case letter) are not significantly different at 5%.

Table 3 shows that relative humidity does not statistically affect the egg viability and adult longevity. In fact, the eggs viability was greater (40.88 %) when T=28°C and RH= 52%. However, with RH=32%, eggs viability was lower and did not exceed 2 and 4% for T=21 and 28°C respectively. Adult longevity was better at 21°C. Fecundity rate was higher at T=28°C and RH= 52% compared at T=21°C with the same RH. Table 3 indicates that all tested biological parameters were influenced by low relative humidity. Statistical analysis showed that there were no significant differences for the interactions between relative humidities and temperatures for eggs viability (P=0.141) and fecundity rate (P=0.092); However, there was a significant interaction in adult longevity (P=0.001).

Table 3. Eggs viability, adult longevity and fecundity rate of *T. absoluta* at T=28 and 21°C and RH=72, 52 and 32%

Biological parameters	T (°c)	RH (±10%)			Statistics	*P values*
		72±10%	52±10%	32±10 %		
Eggs viability(%)	21± 2°C	23,2484076aA	23,11aA	1,53aB	χ^2=13.6	*P*=0.032
	28± 2°C	34.58aA	40.88aA	4,18aA	χ^2=23.139	*P* =0.282
Statistics		χ^2=8.171	χ^2=8.571	χ^2=4.550		
P values		*P* =0.417	*P* =0.199	*P* =0.103		
Adult Longevity (day)	21± 2°C	12,35±4,02 aA	11,33±3,82aA	8,33±3,49 aA	$F_{2,36}$=0.103	*P* =0.903
	28± 2°C	8,78±3,69 aA	10,48±4,55aA	6,85±3,61 aA	$F_{2,31}$=1.193	*P* =0.317
Statistics		$F_{1,26}$=0.611	$F_{1,27}$=0.416	$F_{1,17}$=0.418		
P values		*P* =0.442	*P* =0.524	*P* =0.527		
Fecundity rate(eggs/female)	21± 2°C	5,29±3,59 aB	6,38±4,96 aB	4,86±6,36 aA	$F_{2,28}$=0.401	*P* =0.673
	28± 2°C	13,90±6,96 aA	41,58±11,33bA	8,92±6,10aA	$F_{2,77}$=28.77	*P* =0.000
Statistics		$F_{1,17}$=7.126	$F_{1,15}$=37.284	$F_{1,16}$=2.515		
P values		*P* =0.017	*P* <0.0001	*P* =0.134		

*Means followed by the same letter(s) within a row (lower case letter) and within a column (upper case letter) are not significantly different at 5%.

5. Discussion

The tomato borer *T. absoluta* is considered as among the most devasting pest that can cause major damages to tomato and other crops (Bawin et al. 2015). Its life cycle seems to be complicated and influenced by abiotic factors such as temperature and relative humidity, as well as cultivar choice. In this study, the values of temperature and relative humidity were chosen related to two Tunisian regions (Nabeul and Zaghouan) where *T. absoluta*'s attack levels in tomato crops differ when the temperature is the same. The working hypothesis was, therefore, to modify the relative humidity which are different for the two regions. This polyphagous pest was reported to develop on economically important cultivated solanaceous crops, as well as non-cultivated solanaceous species (Bawin et al. 2015). Host plant choice seems to be a key factor to *T. absoluta* fitness. Previous studies demonstrated that for Lepidoptera species fitness, host plant choice is very important (Thompson 1988a, b; Thompson and Pellmyr 1991). Our study shows that tomato was the most suitable host plant for *T. absoluta* despite no significant differences shown, with a maximum eggs laid compared to potato and eggplant. Also, eggs laid on tomato were more viable but did not significantly differ among tested host plants. The life cycle was shorter and the longevity of adults was highest on tomato. Previous reports indicate that tomato was the most suitable host plant for *T. absoluta* which had a better nutritional quality compared to potato (Periera et al., 2006). Caparros Megido et al. (2013) examined the potential of development of *T. absoluta* on tomato and on four varieties of potato, under laboratory conditions. These authors demonstrated that the survival rate of *T. absoluta* did not differ between the 5 tested host plants; however, its development time (egg to pupation) was significantly affected (Caparros Megido et al. 2013). The same results confirm the ability of *T. absoluta* to develop on potato crops (Pereyra et al. 2006; Caparros Megido et al. 2013). Our study shows that RH= 52% allowed a higher fecundity rate compared to RH=32%. Low relative humidity (RH = 32%) induced a high mortality rate of all *T. absoluta* instars. Relative humidity seems affect the development of this pest, but information about its effect on *T. absoluta* biological development are incomplete. This study showed that development, reproduction and longevity of *T. absoluta* were affected by both temperature and relative humidity. In fact, the high temperature (28°C) was more suitable to *T. absoluta* development compared to 21 °C. The development time was slower and the fecundity rate was lower at 21°C than at 28°C. Thus, low temperatures may lengthen the development time by reducing metabolic rate which is confirmed by (Roy et al. 2002). Moreover, our study showed that the temperature at 28°C and relative humidity at 32% gives the shortest life cycle. Our results showed that the life cycle of *T. absoluta* was longer at T=25°C, RH=60% when compared to other published results which may be due to the different tomato cultivar as highlighted by Nouri-Ganbalani et al., (2016). In a previous study, Silva et al., (2015) demonstrated that at 25°C egg to adult development time of *T. absoluta* was 20.52 ± 0.30 days on tomato plants (var. Tex 317). Barrientos et al. (1998) indicated that *T. absoluta* makes 23.8 days to develop from egg to adult at 27.1°C. In another study, Boualem et al. (2012) demonstrated that the life cycle of *T. absoluta* was 21.1 ± 0.4 days at 26 ± 1.6°C, RH 87 ± 6.4%. However, Lebdi-Grissa et al. (2010) showed that the development time of *T. absoluta* from egg to adult emergence is 37.5 days at 25°C. Martins et al. (2016) found in a previous that the optimum temperature for the development of *T. absoluta* is 30°C with upper and lower developmental thresholds of about 34.6 and 14°C, respectively. Krechemer & Foerster (2015) demonstrated that this pest required 416.7 degree days to achieve its entire cycle from egg to adult. They showed that the lower and the upper temperature thresholds were estimated to be 8.0 and 37.3°C respectively (Krechemer & Foerster 2015). The same authors showed that the highest fecundity was obtained at 20 and 25°C respectively. However, at 10 and 30°C, only one egg clutch was laid by *T. absoluta*, which is in contrast with our result where *T. absoluta* ‘s fecundity rate and eggs viability were higher at 28°C. So, *T. absoluta* populations are particularly affected by climatic changes as exemplified from several studies that showed that a change in the temperature altered the population dynamics that may lead to the extinction of part of the system (Parmesan et al. 2003; Van Baaren et al. 2005; Hance et al. 2007). Hance et al. (2007) estimate in a previous study that the negative impact of climate changes on multi-trophic interactions will increase the severity and timing of pest outbreaks in addition to ecosystem functioning. In the present study, we pointed out that on tomato the viability of *T. absoluta* eggs differs among the tested relative humidity which coincides with other results reported by Osman et al. (2015). These authors showed that the eggs viability on tomato at T=25°C and RH = 60 ± 10% was higher and it reached 93.33% which differ to our results. This finding may be explained by differences in cutivars used (Osman et al. 2015). Relative humidity can affect the physiology and thus the biological traits of many insects including development, longevity and oviposition (Norhisham et al. 2013). Low relative humidity causes loss of lubrication and cuticular softness in insect which may affect embryo development and egg hatching (Guarneri et al. 2002), therefore development may be retarded (Norhisham et al. 2013). Furthermore, low humidity on the leaf surface due to stomatal closure or hypersensitive response by formation of necrotic tissue at the site of egg deposition may affect eggs hatching by leading to their desiccation (Hilker & Meiners 2011; Woods 2010). Adult longevity may differs depending on the host plant, the temperature and relative humidity tested. In our study, we found that the adult longevity was between 6 and 14 days for the tested host plants, temperatures and relative humidity. Boualem et al. (2012) demonstrated in a previous study that adult longevity was on average of 12.5±3.6 days at T=26±1.6°C and RH= 87±6.4%. But in general, adults of *T. absoluta* reared on tomato at 25°C and a relative humidity ranging from 65 to 70% live between 16 and 20 days, with females living a few days longer than the male (Erdogan & Babaroglu 2014; Krechemer & Forester 2015). Our study shows that the interaction between temperature and relative humidity affects significantly the biological parameters of *T. absoluta* including egg, larval and egg- to- adult development as well as adult longevity.

We conclude that *T. absoluta* had a better performance on her favorite host plant (tomato) when T°= 25±2°C and RH= 60±10%. Furthermore, we demonstrated that low relative humidity has a negative effect on the biological parameters of *T. absoluta* which can limit its invasion. Basic climate parameters (temperature and humidity) may influence pests both directly and indirectly (Jaworski and Hilszczański 2013). The impact of climate change on insects may be reflected in their distribution, phenology, activity, number of generations and, indirectly, through impact on their natural enemies (Jaworski and Hilszczański 2013). Moreover, an understating of the exact impact of climat changes on pest population may anticipate crop losses and help to plan well-timed pest control measures (Aasman 2001).

The factors controlling the abundance and distributions of *T. absoluta* are mostly unknown and there is no data until yet about the effect of climate change on this pest. It is then of tremendous importance to explore new ways to decrease pest effects by biological control.

6. Acknowledgements

The authors would like to thank the farmers for their contribution to this work.

7. Disclosure statement

There is no conflict of interest.

II.3.3 Conclusion

Ce travail a souligné l'aptitude de *T. absoluta* à se maintenir sur des hôtes alternatifs (comme la pomme de terre et l'aubergine) avec une préférence de développement sur l'hôte principal (la tomate), sous conditions de laboratoire. Les résultats trouvés indiquent aussi que la température et l'humidité relative peuvent influencer le cycle biologique de l'insecte qui a montré une meilleure performance à la température de 28°C et une humidité relative variant entre 52 et 60%. De telles informations pourraient être utiles pour une meilleure gestion de l'insecte sous les conditions naturelles permettant de comprendre la dynamique de ses populations (vol des adultes et abondance des différents stades sur feuilles) sur terrain et d'intervenir au moment opportain soit par l'application d'insecticides ou bien par des lâchers de prédateurs ou de parasitoïdes.

Dynamique des populations de *Tuta absoluta* (Meyrick) (Lepidoptera : Gelechiidae) et moyens alternatifs de lutte

III. Chapitre 3 : Dynamique des populations de *Tuta absoluta* (Meyrick) (Lepidoptera : Gelechiidae) et moyens alternatifs de lutte

III.1 Introduction

Le travail a été réalisé dans deux régions tunisiennes (Zaghouan et Nabeul) productrices de tomates et ayant des conditions climatiques favorables aux attaques de *Tuta absoluta*.

Ce Chapitre a été formulé et présenté sous forme de 3 articles portant sur la dynamique des populations de la mineuse sous serres froides et en plein champ ; comme il a abordé quelques moyens de lutte soit par application d'insecticides chimiques et biologiques ou alors par piégeage de masse. L'article 3 (publié dans Journal of Entomology and Zoology studies 2017, 5(4), 427-432 est intitulé **« Population dynamics of the tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Tunisia natural conditions ».** L'étude avait pour but d'étudier la dynamique des populations de ce ravageur sur des cultures de tomate de saison en plein champ, deux années de suite (2014-2016) dans les régions de Takelsa et de Zaghouan.

L'article 4 (soumis et en cours de correction dans Journal of African Entomology) est intitulé **« Traps and their impact on population dynamics and management strategies of *Tuta absoluta* (Meyrick) (Lepidoptera : Gelechiidae) in protected tomato crops ».** Le suivi de *T. absoluta* a été enregistré sur trois années consécutives (2013-2016) sous serres froides à Takelsa. Ce travail a permis, également, de tester l'efficacité du piègeage de masse et des pièges noirs englués. **L'article 5** (soumis et en cours de correction dans Journal of plant diseases and protection) est intitulé **« Efficacy of mass trapping and insecticides to control *Tuta absoluta* in Tunisia ».** L'objectif était de juger l'efficacité de certaines substances actives, utilisées souvent par les agriculteurs tunisiens pour la lutte contre *T. absoluta* (au laboratoire et sous serres). Les traitements chimiquescombinés au piégeage de masse étaient testés sous serre froide à Tekilsa.

III.2 Dynamique de la population de la mineuse de la tomate (Meyrick) (Lepidoptera: Gelechiidae) sous conditions naturelles tunisiennes

Population dynamics of the tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Tunisia natural conditions (Article 3).

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Abstract

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is a key pest of tomato crops in Tunisia causing heavy losses to its production. The population dynamics of this pest was surveyed during spring-summer tomato growing season in Zaghouan and Nabeul provinces between 2014 and 2016. *T. absoluta* was monitored using two sex pheromone water traps. Tomato leaves were harvested and inspected weekly in order to determine the number of generation accomplish by each alive instar (eggs, larvae and pupae). Mines with and without larvae are also counted. Results showed that this pest was able to achieve 4-5 flight peaks. Three generations of eggs and larvae were recorded with a significant preference to laid eggs in the underside of leaves. High correlations were found between traps catches, eggs laid, active and total mines. Obtained data may help Tunisian farmers to detect early infestations, therefore to establish and to apply efficient control methods.

Key-words: *Tuta absoluta*, tomato open field crops, population dynamics, linear regression

1.Introduction

Tomato (*Solanum lycopersicon* L.) is considered in Africa and especially in Tunisia as a strategic culture thanks to income, food and nutrition that provides to farmers [6, 16, 19, 29]. Tomato was cultivated throughout the year under protected and open field crops [21]. Nevertheless, this important vegetable crop may be threatened by several diseases and pests [9, 22, 29]. Among pests, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) was cited as the most harmful lepidopteran which can cause serious damages mainly to this crop [8, 9, 22]. Other cultivated and weed species belonging to the families of Solanacae and fabacae have been classified as host plants [2, 15, 20]. Damages were caused by larvae which feed on all areal parts of the plant (leaves, stem and fruits) causing characteristic mines [3, 21]. *Tuta absoluta* may perform several generations per year in field conditions which may reach 7-8 generations as reported for Chile [27]. In Turkey, this pest completed 5 generations during summer-winter growing season [21]. In Tunisia, studies have demonstrated that *T. absoluta* was able to achieve 3 generations in protected crops for the period extending from January to May [7]. Development cycle of *T. absoluta* depends on availability of host plants as well as suitability of climatic conditions especially temperature [11, 13, 20]. Data studying its biological and ecological characteristics are needed to establish effective management of this pest [21]. Thus, various factors must be taken into consideration such as climate interactions with the biology of the pest and multi-species interactions such as competition and predation [24]. Several control strategies were carried out to limit damages caused by this pest including chemical sprays, the use of sex pheromone for mass trapping or mating disruption and releases of natural enemies [4, 5, 10, 12, 26, 28].

The aim of this work was to investigate the population dynamics of *T. absoluta* in open field tomato crops over two years in two Tunisia regions (Bou Slim and Takelsa). The relationship between male captures and specific parameters (eggs laid, mines with larvae and total mines), correlations between mines with and without mines as well as distribution of eggs per side of leaf, was also examined

2. Materials and methods

2.1 Study sites

This study was carried out in two Tunisia counties: Bou Slim (North-East, Governorate of Zaghouan, Latitude. 36° 24' 10.48" N, Longitude. 10° 08' 34.51" E) and Takelsa (North-East, Governorate of Nabeul, Latitude. 36°27.3636′N, Longitude. 10°44.2578′E), characterized by favorable climatic conditions to *Tuta* attacks.

2.2 Insect monitoring

Population dynamics of *Tuta absoluta* was surveyed over two years under open field crops through spring-summer main growing season. *T. absoluta* was monitored using two sex pheromone water traps (Pherodis®) set up on 15/04/2014 and 14/04/2016 respectively for Bou slim and Takelsa regions and placed at a distance of 40 cm above the ground. A distance of about 20 m was respected between these traps. The renewal of sex pheromone capsules was realized every 4 weeks. Dirty contents of traps (water and a thyn vegetable oils) were removed and replaced by others. For each open field crop, tomato plants were drip-irrigated under plastic mulch. Chemical applications were done by farmers by spraying to runoff insecticides at the recommended doses using a compressed air sprayer. Insecticides sprays were started when captures in water traps reached 50 moths/trap as recommended by the Tunisian ministry of Agriculture. Specific characteristics of studied sites are given by Table 1.

Table 1: Some characteristics of selected open field crops in the two study sites

Years	**Harvest**	**Area**	**Tomato plants**	**Distance (m)**		**Insecticides application**
				Row	**Plant**	
2014	*Main crop season *2 open field crops in Bou slim region	1 Ha each	*33000 plants each *Crop 1: var. cxd 255 *Crop 2: var. Podium	1.6	0.20	*23/04/2014: Cyromazin 750, 30g/hl *10/06/2014: Indoxacarb, 50cc/hl *11/07/2014: Spirotetramat, 50cc/hl
2016	*Main crop season *1 open field crops in Takelsa region	1 Ha	*30000 plants *var. Chams	1.5	0.3	*21/04/2016: Flubendiamid, 30g/hl *01/06/2016: Indoxacarb, 50cc/hl *02/07/2016: Diafenthiuron, 100cc/hl

2.3 Sampling

Samplings were carried out in the three chosen open field crops. Forty tomato leaves taken weekly at random, were put individually in plastic bugs and then brought back to the laboratory. Sampled leaves were inspected for their both leaflets surfaces as well stems using a binocular microscope (Leica® Model MS5). Thus, the number of eggs, larvae, pupae and mines found on the whole leaves were counted.

2.4 Climatic data

The climatic data for Zaghouna and Nabeul provinces are given by the Tunisian Meteorological Institute (Table 2).

Table 2 (A, B): Climatic data for Zaghouan (A) and Nabeul (B) provinces (year 2014 and 2016)

Year	Parameter (A)	April	May	June	July	August
2014	Temperature (°C)	16.70	19.50	25.20	27.20	28.60
	Relative humidity (%)	66	63	50	37	35

Year	Parameter (B)	April	May	June	July	August
2016	Temperature (°C)	20.40	19.50	23.20	26.30	26.20
	Relative humidity (%)	79	69	68	68	70

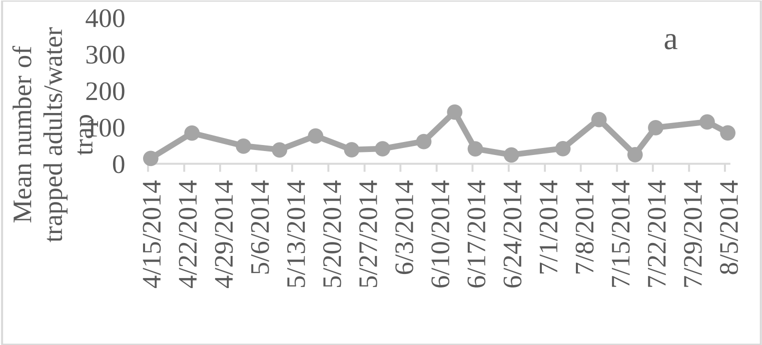
2.5 Statistical analysis

A one-way ANOVA and a post hoc mean comparison test (Duncan test at $p<0.05$) was carried out to test linear regression of parameters cited above and the distribution of eggs per side of leaf. Statistical analysis was performed using SPSS version 21 (2012).

3. Results

3.1 Male flight activity

The study was initiated in middle April and continued to 05/08/2014 and 12/07/2016 respectively for tomato growing areas of Bou Slim and Takelsa counties. Five generations were recorded on April, May, June, July and August in Bou Slim region. However, in Takelsa, only 4 peaks were registered within the he above mentioned time frame (Figure 1). Figure 1 showed that catches were more frequent in summer at high temperature especially in Takelsa. The highest trap counts were recorded on 13/06/2014 in Bou Slim (142 males/trap/week) and on 03/06/2016 in Takelsa (380.5 males/trap/week).



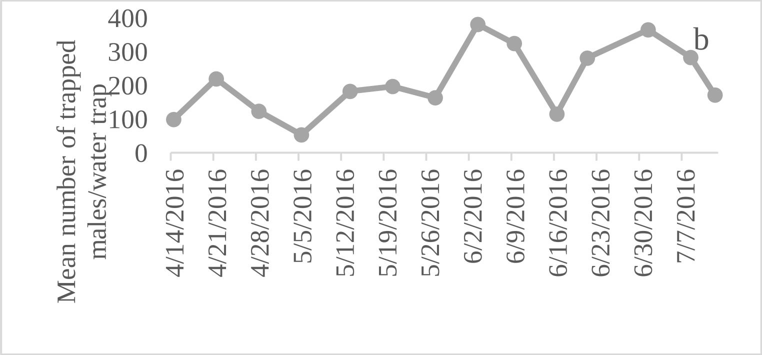


Fig 1: Male flight activity of *T. absoluta* in Bou slim (a) and Takelsa (b) tomato open field crops

3.2 Population dynamics

Figure 2 showed that, in Zaghouan province, *T. absoluta* was able to achieve three generations of eggs and larvae recorded in June, early and late July, 2014. The number of larvae found was the greater compared to it of eggs and may reach 9.5 larvae/40 tomato leaves on 11/07/2014 (Figure 2a). Likewise, in Nabeul province, *T. absoluta* performed three generations for both eggs and larvae for the study period extending from April to July, 2016 (Figure 2b). Oviposition on leaves started low and rose up to 7.5 on 24/06/2014 and 164 on 03/06/2016 respectively for Bou Slim and Takelsa locations (Figure 2). Whereas, during the survey, all development stages were more abundant in Takelsa and started to appear early compared to them in Bou Slim (Figure 2). The number of pupae recorded in the two study sites was very low and did not exceed 5 pupae /40 tomato leaves for example in Takelsa region on 03/06/2016.

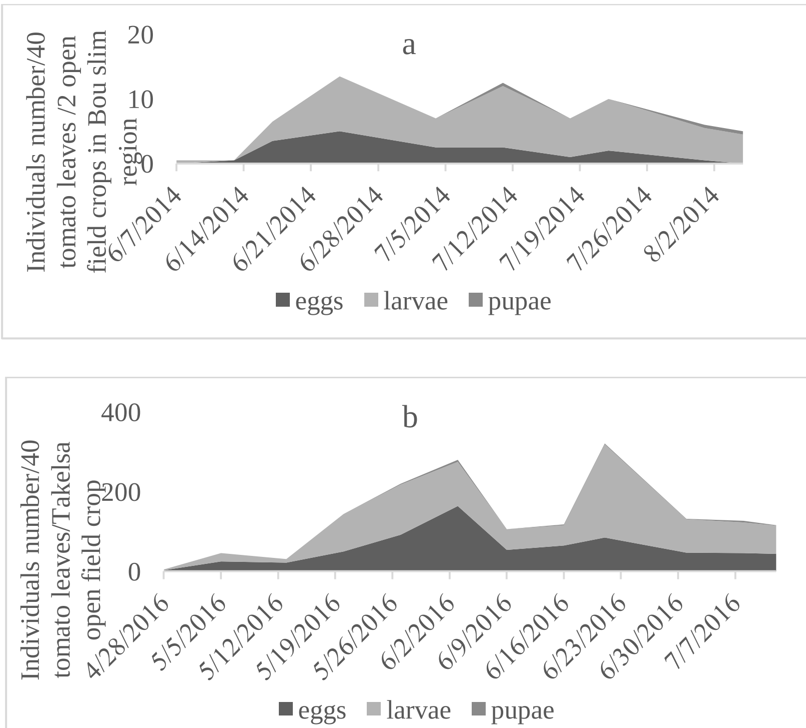


Fig 2: Leaf density of *T. absoluta* eggs, larvae and pupae in Bou Slim (a) and Takelsa (b) open field crops

3.3 Distribution of eggs per side of leaf

For the two study sites, figure 3 showed that the number of eggs laid in the underside of tomato leaves (85%) was significantly more important compared to it found in the upper side ($F_{1, 43}=10.67$; $p=0.002<0.05$).

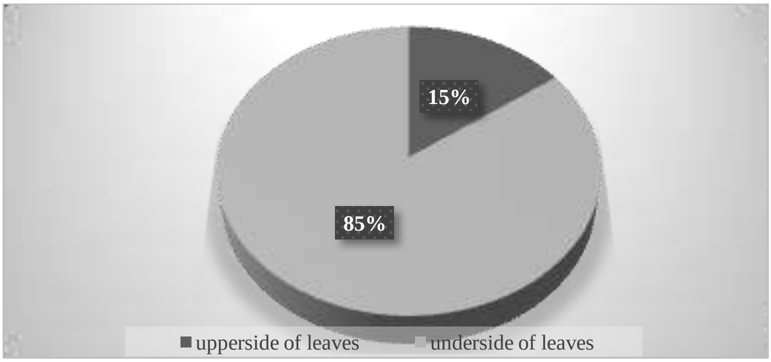


Fig 3: Number of eggs laid per side of leaf recorded in Bou Slim and Takelsa open field crops

3.4 Relationship between trap catches, number of eggs laid and mines

Figure 4a indicated a significant linear regression demonstrated for trap catches/eggs laid in the surveyed open field crops (R^2=0.78; $F_{1,\ 43}$=27.81; p=0.000<0.05). Likewise, for the two experimental sites, captured adults/mines with larvae were highly and significantly correlated (R^2=0.74; $F_{1,\ 87}$= 25.88; p=0.000<0.05) (Figure 4b). A high linear regression between trapped adults/ total mines (with and without larvae) was noted, despite there is no significant statistical analysis found (R^2=0.75; $F_{1,\ 87}$=0.722; p=0.398) (Figure 4c).

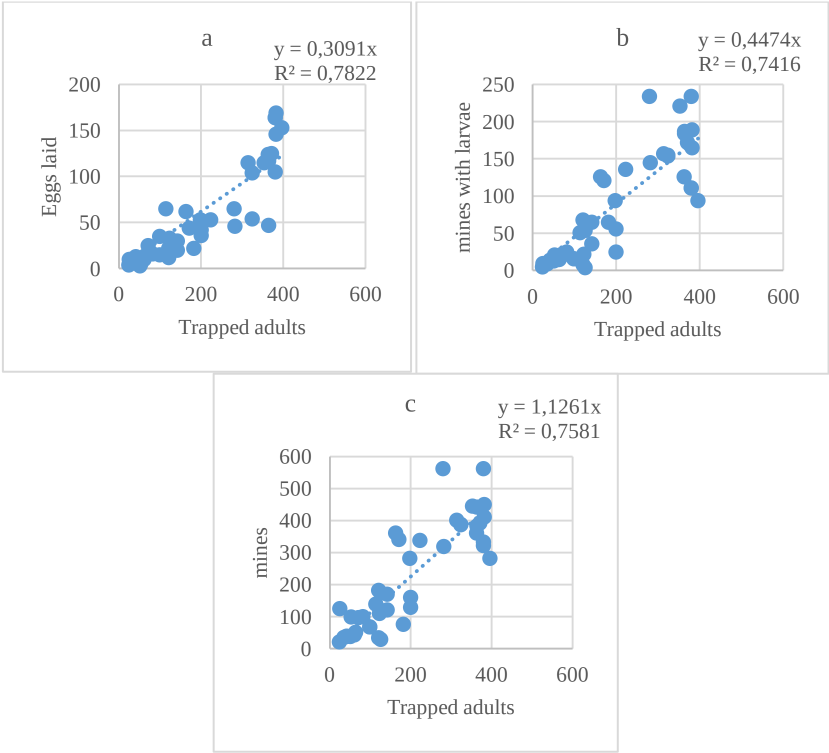


Fig 4: Relationship between trap catches and eggs laid (a); trap catches and mines with larvae (b) and trap catches andtotal mines (with and without larvae) (c) in Bou Slim and Takelsa counties

Figure 5 indicated a high and significant linear regression between mines with larvae/ mines without larvae in tomato open field crops for Bou Slim and Takelsa counties (R^2= 0.83; $F_{1, 87}$= 7.76; p=0.007<0.05).

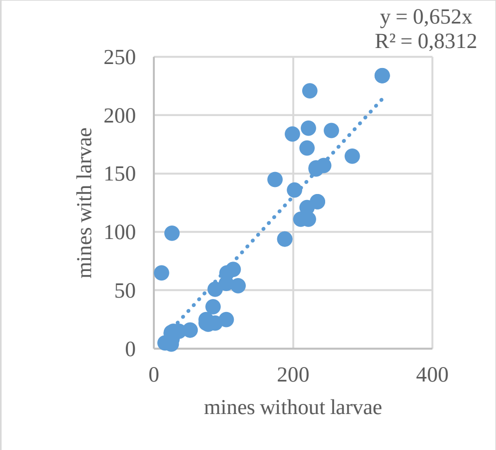


Fig 5: Linear regression between mines with larvae/ mines without larvae in tomato open field crops (Bou Slim and Takelsa regions)

4. Discussion

Tuta absoluta is a major pest of tomato crops in Tunisia causing heavy losses [1, 7, 8]. The knowledge of its population dynamics under field conditions is considered as major step to plan effective management strategies. Our results highlighted the occurrence of 4 and 5 flight peaks registered respectively in Takelsa and Bou Slim locations. Also, three generations of eggs and larvae were detected in the two study regions. Pupae were found with a low number on tomato leaves given that pupation occurs in most of case in the soil [18]. Several research studied the population dynamics of *T. absoluta*. In Tunisia, Cherif and Lebdi-Grissa, [8] indicated that *T. absoluta* developed two flight peaks during autumn-winter late growing season in Zaghouan province. According to Polat et al. [21], *T. absoluta* may accomplish 5 generations in Çanakkale province, Turkey, through summer-winter growing season. The number of adults, eggs and larvae recorded differs for the two studied counties (Zaghouan and Nabeul provinces) which may be explained mainly by differences in environmental conditions especially temperature and relative humidity. In fact, from April to August, the temperature values ranged from 16.70 to 28.60 and from 20.40 to 26.20 respectively in Zaghouan and Takelsa provinces. Relative humidity values are higher in Takelsa compared to those in Zaghouan. For example, the relative humidity recorded during the month of July was 37% in Zaghouan and 68 % in Takelsa. In this study period, the number of larvae recorded was 8/40 leaves on 22/07/2014 in Zaghouan region. However, in Takelsa, on 12/07/2016, the number of recorded larvae was 71/40 leaves.

These data explain that, the severity of *Tuta* attacks was related to relative humidty, therefore, attacks decrease with low relative humidity values. Our findings are in accordance with other results indicating that environmental conditions such as temperature affected generation time and biological proprieties of *T. absoluta* [21]. Likewise, Cherif and Lebdi-Grissa, [8] proved in a previous study realized in Zaghouan province, that the low density of recorded eggs and larvae was linked to cold weather registered in the study period extending from October to December, 2011. In the present study, we assessed the preference of *T. absoluta* to lay eggs on the underside of leaves (85%) which proved results of Torres et al. [25]. Here, we indicated the existence of a high and significant linear regression between trap catches and eggs laid (R^2=0.78) or trap catches and actives mines(R^2=0.74). Likewise, mines with and without larvae were highly correlated (R^2=0.75). In a previous study, Abbes and Chermitti, [1] indicated the presence of a significant relationship between trapped adults and infested leaves in protected tomato crops.

This paper provides population dynamics data in two Tunisian provinces (Zaghouan and Nabeul) where *T. absoluta* attacks level differs. With the resulting information's, we will be able to estimate infestation rate caused by this pest. Obtained data can be considered as a vital step towards early detections and therefore developing efficient Integrated Pest Management programs against this pest.

5. Acknowledgements

We thank the farmers for their contributions to this work.

III.3 les pièges et leurs effets sur la dynamique de la population et les stratégies de lutte contre *absoluta* (Meyrick) (Lepidoptera : Gelechiidae) sous serres

Traps and their impact on population dynamics and management strategies of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in protected tomato crops (Article 4).

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Running title: Population dynamics of *Tuta absoluta* in Tunisia

Abstract

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is a key pest of tomato worldwide. The population dynamics of this pest as well as the efficacy of two trap types were studied in protected tomato crops (Takelsa, Northeastern Tunisia). Our results indicated that *T. absoluta* has up to four flight peaks and passes through four generations of eggs and larvae on tomato leaves. The male trap catches and pest densities decreased in autumn-winter and increased steadily in spring to reach up to 120 males/trap/week and 74.66 larvae/40 leaves in May, 13th, 2016. Furthermore, there was a significant linear relationship between trapped adults and eggs laid (R^2 = 0.81, P <*0.0001*), trapped adults and mines with larvae (R^2 = 0.76, P <0.0001) and trapped adults and mines (R^2 = 0.70, P <0.05) respectively. Likewise, mines with and without larvae were highly correlated (R^2 = 0.72, P <0.0001). There was no significant difference between the number of eggs laid and the number of emerged larvae in greenhouses using only sex pheromone traps or sex pheromone traps combined with black sticky traps. Our study demonstrated that the use of pheromone traps for *T. absoluta* monitoring may help farmers in view to develop efficient management strategies such as chemical control by planning effective insecticide spays.

Key words: Tomato leaf miner, traps, IPM, population dynamics, tomato greenhouse

1. Introduction

Tomato *Solanum lycopersicon* (Mill) is the second most commonly consumed vegetable in the world with a harvested area devoted to its production of about 5 million ha **(**Umpiérrez *et al.* 2012). In Tunisia, tomato cultivation and production resulted in a total of 29 thousand ha/ year and 1.2 million tons respectively (Gil 2016). This strategic crop may be threatened by many diseases and pests such as the tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelichiidae) (Cherif *et al.* 2017; Ebdah *et al.* 2016; Ettaieb *et al.* 2016 a, b; Gil 2016). This pest is noted as a major pest of tomato cultivation originating from South America causing losses over of 80% in newly invaded area (EPPO 2005; Desneux *et al.* 2011; Cherif & Lebdi-Grissa 2013; Lopez *et al.* 2013; Bajracharya *et al*. 2016; Biondi *et al.* 2018). *Tuta absoluta* can occurs on various cultivated solanceaous crops as well wild species (Notz 1992; Desneux *et al*. 2010, 2011; Tropea Garzia *et al.* 2012) causing damages mainly to leaves, stems and fruits creating mines (Salvo & Valladares 2007; Cocco *et al.* 2015; Bajracharya *et al.* 2016). The knowledge of the population dynamics of *T. absoluta* as well its biology in terms of host range, voltininsm, and overwintering and pest plant distribution is important to develop efficient control programs (Radcliffe *et al.* 2009; Cocco *et al.* 2015; Cherif & Lebdi-Grissa 2017). *Tuta absoluta* is not only a multivoltine non-diapausing species but has also a high potential of population growth (Pereyra & Sánchez 2006). In the Mediterranean area, this pest was detected during the entire year and over winter in all life stages (EPPO 2005; Vercher Aznar *et al.* 2010; Delrio *et al.* 2012; Tropea Garzia *et al.* 2012). Life cycle of *T. absoluta* depends on climatic conditions as well quality of available hosts (Cocco *et al.* 2015). Variable number of *Tuta* generations have been detected. In South America, up to 10-12 generations were detected (EPPO 2005) whereas, in Tunisia, population dynamics of *T. absoluta* was studied within single growing season (Abbes & Chermiti 2011; Harbi *et al.* 2012; Cherif *et al.* 2013; Cherif & Lebdi-Grissa 2017). Management strategies were based mainly on pesticides applications, but other control strategies had been deployed such as the use of natural enemies and resistant varieties of tomato plants (Desneux *et al.* 2010; de Oliveira *et al.* 2012). A large number of wasps have been cited and tested against *T. absoluta* including parasitoids belonging mainly to the families of Trichogramatidae and Braconidae (*Trichogramma cacoeciae* (Marchal), *Bracon nigricans* (Szépligeti)…) and predators such as mirids (*Nesidiocoruris tenuis* (Reuter), *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae)…) **(**Desneux *et al.* 2010; Cherif & Lebdi-Grissa 2013; Zappalà *et al.* 2013). Moreover, the use of sexual synthetic pheromones for mass trapping, mating disruption, or the sterile insect technique have been successfully tested against *T. absoluta* (Caparros-Megido *et al.* 2012; Cocco *et al.* 2013). Mass trapping as control method was widely used within Integrated Pest Management (IPM) programs in Tunisia and other countries around the world (Chermiti & Abbes 2012; Cocco *et al.* 2012; Lobos *et al.* 2013; Cherif & Lebdi-Grissa 2014; Braham & Nefzaoui 2015; Roda *et al.* 2015; Askoy & Kovanci 2016; Mohamedova *et al.* 2016). Several studies have judged the effectiveness of pheromone traps (Chermiti & Abbes 2012; Cherif & Lebdi-Grissa 2014; Braham & Nefzaoui 2015; Roda *et al.* 2015; Askoy & Kovanci 2016; Braham & Nefzaoui 2015; Ettaieb *et al.* 2016 b; Mohamedova *et al.* 2016)**.** Likewise, various commercial brands of *T. absoluta* sex pheromone lures have been tested in both protected and open field crops (Abbes & Chermiti 2011; Abbes & Chermiti, 2012; Cherif & Lebdi-Grissa 2014; Ettaib *et al.* 2016 b; Mohamedova *et al.* 2016). However, few researchers have investigated the correlation between trap catches and infestation level caused by *T. absoluta* (Caffarini *et al.* 2000; Abbes & Chermitti 2011; Mohamedova *et al.* 2016). Due to the excessive use of pesticides such as abamectin and the associated problems of resistance and environmental pollution, there is an increasing demand for sustainable, environmentally friendly control methods. In addition, control methods based on the use of synthetic insecticides sometimes fail to keep the number of insects below the economic threshold level (Arno & Gabarra 2011; Biondi *et al.* 2012, 2013; Calvo *et al.* 2013). So to avoid such problems and to get a successful integrated pest management (IPM) programs, integration of chemical control with other control methods such as cultural, biological, and biotechnological methods become a necessity and must be applied by farmers (Gonzalez-Cabrera *et al.* 2011; Zappalà *et al.* 2012; Cocco *et al.* 2013; Biondi *et al.* 2016).

Until now, as we know there are no studies conducted on the population dynamics of *T. absoluta* over more than one year in protected tomato crops in Tunisia. Furthermore, few studies demonstrated relationship between trapped adults, and damages caused by this pest (Abbes & Chermitti 2011). Moreover, the effectiveness of black sticky traps used in combination with sex pheromone traps under greenhouse conditions are not yet confirmed.

The main goal of this work was firstly to study the population dynamics of *T. absoluta* and understand the linear regression between trapped adults, eggs laid and infestation level over three consecutive years. Secondly, to test the efficiency of the combined use of black sticky and sex pheromone traps under greenhouse conditions.

2. Materials and methods

2.1 Population dynamics

2.1.1 Experimental site

Population dynamics of *T. absoluta* was investigated in protected tomato crops (Takelsa region, Gouvernorate of Nabeul, Northeastern Tunisia) over three years of autumn-spring growing season during 2013-2014, 2014-2015 and 2015-2016 respectively. A total of 18 greenhouses were selected for this study. Six greenhouses were chosen each year for the survey of *T. absoluta*. Each greenhouse (500 m^2, 60 m length, 8 m wide and 3 m height) contains 1400 tomato plants. Distance between rows and plants was 1.5 and 0.5 m respectively. Three different cultivars chosen by farmers including Sankara, Galaxy and Mirano, were used separately in 2013-2014, 2014-2015 and 2015-2016 (Table 1). Tomato plants were drip-irrigated under plastic mulch. Lateral and upper openings of protected crops were equipped with insect-proof screens and were opened during times of high solar radiation which not alter *Tuta* population dynamics inside the greenhouse (Cocco *et al.* 2015). Air should circulate from the outside to the inside of the greenhouse in order to remove excess heat and to avoid the risk of diseases. Basal leaves and lateral shoots were pruned by farmers when needed. For each study year, the surveyed greenhouses had the same calendula in terms of fertilization practices and insecticides sprays. In 2013-2014, three insecticides (cyromazin, flubendiamid and azadirachtin) were applied on 06/05/2014 only in three selected greenhouses. In 2014-2015, no insecticides application was carried out. In 2015-2016, two insecticides sprays were done, the first was on 15/03/2016 using azadirachtin, while on 14/04/2016, indoxacarb was used. Characteristics of applied insecticides are cited in table 1. Insecticides were sprayed by farmers until runoff at the recommended doses using a hydraulic knapsack hand sprayer of 10 liters**.** The seasonal phenology of the pest was less influenced by insecticides applications compared to its populations abundance (Cocco *et al.* 2015).

Table 1. Characteristics of insecticides sprayed in Takelsa tomato greenhouse

Years	Varieties used	Characteristics of tested insecticides				
		Active ingredient	**Formulation type**	**Trade name**	**Concentration of active ingredient**	**Dose (g/hl or ml/hl)**
2013-2014	Sankara	cyromazin flubendiamid azadirachtin	WP WG EC	Clave Takumi Fortune aza	75% 20% 32 g/hl	30 g/hl 30 g/hl 150 ml/hl
2014-2015	Galaxy	Without insecticides sprays				
2015-2016	Mirano	azadirachtin indoxacarb	EC SC	Fortune aza Amiral	32 g/hl 150 g/hl	150 ml/hl 50 ml/hl

EC Emulsifiable Concentrate, SC Suspension Concentrate, WP Wettable Powder, WP Wettable Granules

2.1.2 Monitoring of *T. absoluta*

Tuta absoluta were monitored using two sex pheromone water traps (Pherodis®) per greenhouse set up regularly on 10/10/2013, 19/11/2014 and 24/11/2015. Visual inspections of tomato plants were also recommended to study its seasonal fluctuations as well as its population's dynamics (Witzgall *et al.* 2010; Cocco *et al.* 2015). A distance of 25 m was kept between the water traps placed at 40 cm above the ground. Trapped adults were counted and removed from traps weekly. The contents of traps (water and a thin layer of vegetable oil) were replaced when needed. Sex pheromone capsules were renewed every 4 weeks. The number of generations achieved by *T. absoluta* was evaluated through the developmental structure of immature stages (eggs, larvae and pupae). Insect presence was assessed weekly by sampling at random 40 tomato leaves by greenhouse. The number of eggs, mines with or without alive larvae and pupae per leaf were recorded. Sampled leaves were inspected using a binocular microscope (Leica® Model MS5).

2.2 Efficacy of traps used under greenhouse conditions

The efficacy of sex pheromone traps used alone or combined with Horiver sticky black traps was studied in greenhouses described above in 2015-2016. Three greenhouses baited with only two pheromone traps were compared to those containing both two pheromone traps and black Horiver sticky traps (Horiver *Tuta*, Koppert ®) (25*40 cm) tested at three rates as recommended by koppert (1/500 m^2, 1/200 m^2 and 1/100 m^2 respectively for preventive, curative and curative heavy use) (https://www.Kopper.com/products/monitoring/horiver-tuta/).
Three untreated greenhouses containing neither sex pheromone traps nor black sticky traps were left as untreated control. In total, nine greenhouses were considered for this trial. Sex pheromone traps were set up on 24/11/2015. The three rates of Horiver traps were tested separately on 19/11/2015, 15/03/2016 and 28/04/2016 respectively in parallel with increases of *T. absoluta* infestation level. These traps were placed at 15-25 cm above the ground surface changed and replaced by others whenever necessary. Captured adults were counted and removed from pheromone and sticky traps weekly. All studied greenhouses were sprayed with the same insecticides when threshold (50 moth /sex pheromone trap) fixed by the Tunisian ministry of agriculutre, was reached (Table 1) (see for details http://www.neppo.org/wp-content/uploads/2015/12/Tunisia.pdf).

Statistical analysis

Experimental data corresponding to linear regressions, distribution of eggs per side of leaf and efficacy of traps in reducing pest densities were analyzed using one-way ANOVA. Means of treatment were separated using Duncan's multiple range test at 5% level of probability (SPSS 21, 2012).

3. Results

3.1 Population dynamics in protected crops

Results of male flight activity indicated that, after three consecutive study years, *T. absoluta* performed up to four peaks under Takelsa greenhouse conditions. The first peak was recorded from October to December, the second was registered between January and February. However, the third and fourth peaks were seen in April and May (Figure 1). *Tuta absoluta* adults started to appear with low number in late autumn-early winter when tomato plantation began, whereas the highest catches were registered under spring at favorable climatic conditions. Trap catches during the autumn-winter period were low, averaged 6.04; 2.27 and 8.36 males/trap/greenhouse respectively for the three surveyed growing season and then increased steadily in spring in parallel with the increasing of the temperature values (Figure 1).

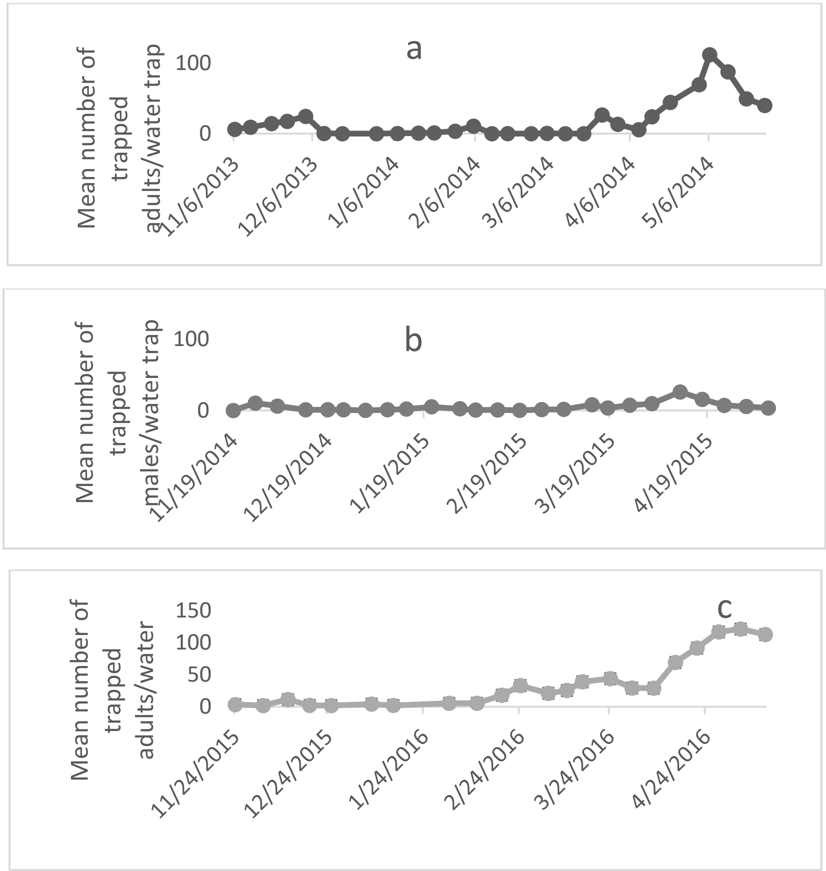


Figure 1. Male flight activity of *T. absoluta* under Takelsa tomato greenhouses ((a): year 2013-2014; (b): year 2014-2015: (c): year 2015-2016)

Population dynamics of *T. absoluta* indicated the occurrence of 4 generations of eggs and larvae from February to May during the three study years (2014, 2015 and 2016) (Figure 2). In autumn-winter period, the proportion of all larval stages was almost constant. The population density was low due to a long oviposition and a slow development period (Figure 2).

Figure 2 indicated that eggs and larvae were frequent in the spring compared to those recorded in autumn-winter. This finding may be due to a faster development time reached at favorable climatic conditions (high temperatures) that concentrated the laying period of females. As example, on 14

April, 2015, the number of eggs and larvae recorded was respectively 32 and 76 per 40 sampled leaves. Moreover, the larval age structure indicated the continuous presence of the fourth development stages during three growing season (2013-2014; 2014-2015 and 2015-2016 respectively) (Figure 2). The number of recorded pupae were very low compared with the other development stages and did not exceed 4.25/40 leaves on 05 May, 2016 (Figure 2).

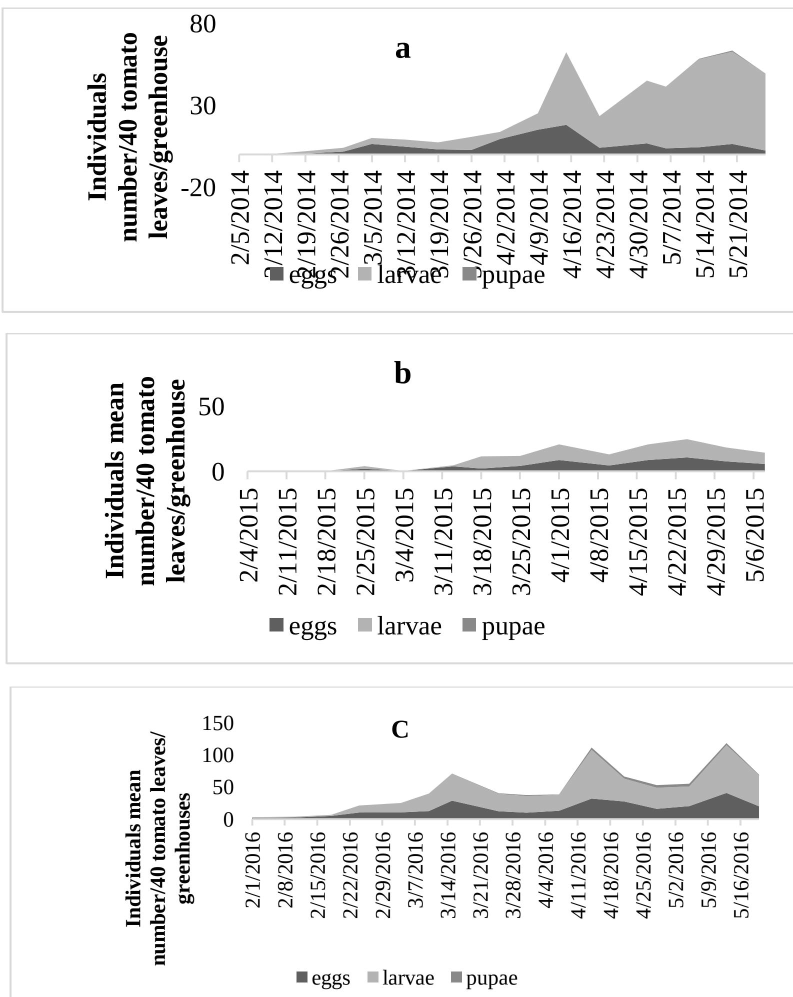


Figure 2. Population dynamics of *T. absoluta* on tomato leaves under greenhouse conditions in Takelsa region (February-May, 2014, 2015, 2016)

3.2 Distribution of eggs per side of leaf

There was a significant difference between the number of eggs laid on the underside of tomato leaves which correspond to 79 % of eggs and those laid on the upper side of tomato leaves ($F_{1, 95}$=11.386; P=0.001<0.05) (figure 3).

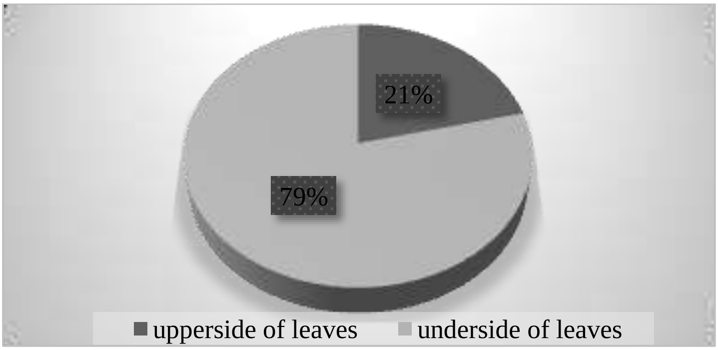


Figure 3. Percentage of eggs in upper side and underside of sampled tomato leaves in Takelsa greenhouses

3.3 Relationship between trap catches, number of eggs laid and infestation levels

Results from One-way ANOVA showed that there was a significant linear relationship between trapped adults and eggs laid, trapped adults and mines with larvae, trapped adults and total mines (with and without larvae) and mines with and without larvae which correspond to (R^2= 0.81; $F_{1, 191}$=35.275; P<0.0001) (Figure 4a) (R^2 = 0.76; $F_{1, 191}$= 20.065; P <0.0001) (Figure 4b) (R^2 = 0.70; $F_{1, 191}$= 25.583; P <0.0001) (Figure 4c) and (R^2 = 0.72; $F_{1, 191}$= 56.442; P <0.0001) (Figure 4d) respectively.

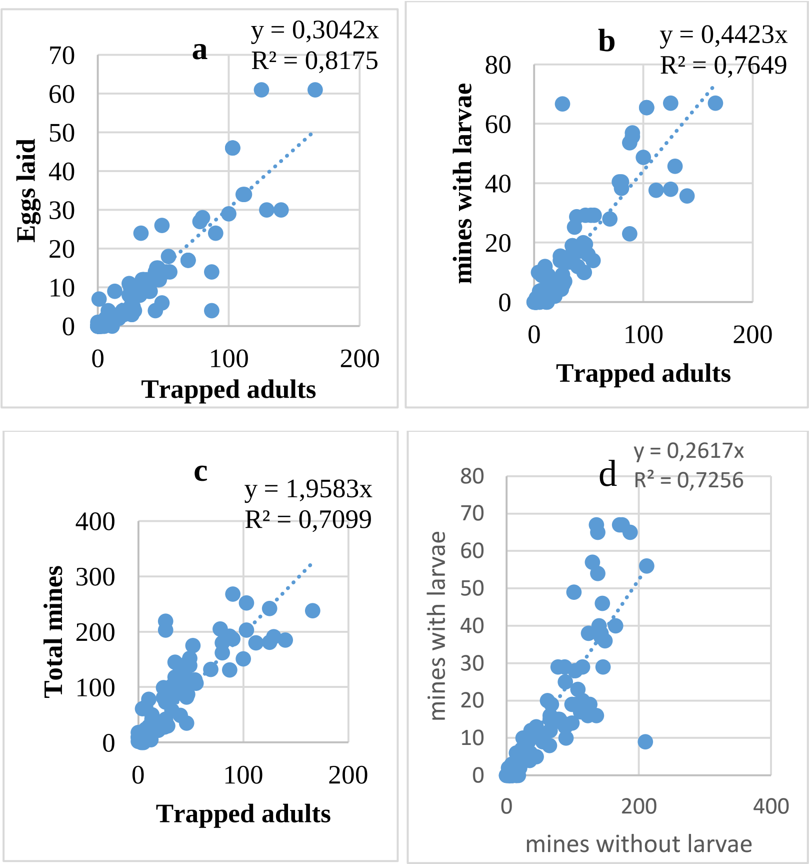


Figure 4. Linear regression between trapped adults/eggs laid (a); trapped adults/mines with larvae (b); trapped adults/total mines (c); mines with and without larvae (d) in Takelsa greenhouses (years 2013-2014; 2014-2015; 2015-2016)

3.4 Efficacy of traps tested under greenhouses conditions

In terms of trap catches, there was no significant difference between greenhouses containing only sex pheromone traps and those equipped with both sex pheromone and black Horiver sticky traps for the period extending from late November to middle May ($F_{1,43}$= 0.838; P=0.365). Figure 5 indicated that since early April, the number of captured adults in sex pheromone and black sticky traps increased progressively in parallel with increases of temperature values.

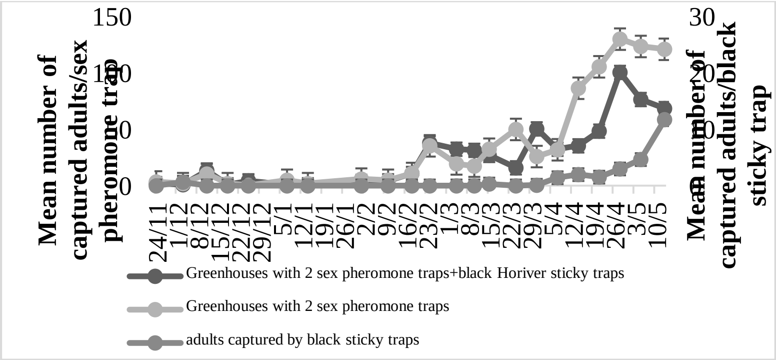
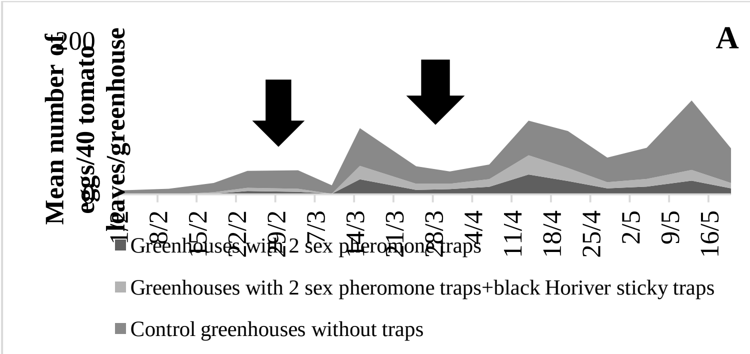


Figure 5. weekly evolution of captured adults per sex pheromone and black sticky traps set up at 3 rates (1/500m^2 on 19/11/2015, 1/200m^2 on 15/03/2016 and 1/100m^2 on 28/04/2016) under Takelsa greenhouses

The mean number of eggs and larvae (34.93 and 47.8/40 tomato leaves respectively) was statistically greater in greenhouses without traps compared to those containing traps ($F_{2, 44}$=5.55; *P* = 0.007 <0.05 and $F_{2, 44}$=8.77; *P* = 0.001 <0.05 respectively for eggs and larvae) (Figure 6, Table 2). The same was not true when comparing greenhouses containing only pheromone traps to those containing two trap types ($F_{12, 31}$=0.892; *P* = 0.622 and $F_{18, 31}$=1.101; *P* = 0.437 respectively for eggs and larvae) (Figure 6, Table 2).



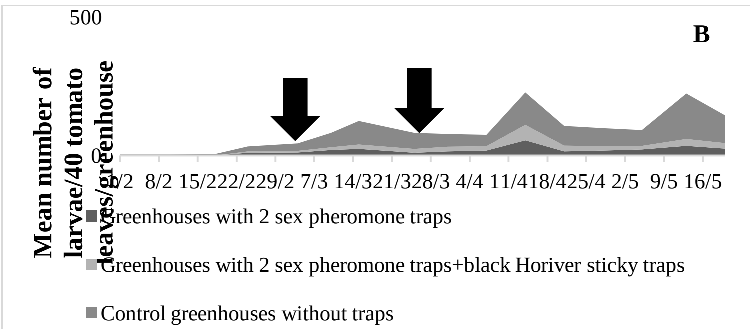


Figure 6. Effect of sex pheromone traps used alone or combined with black sticky traps on *T. absoluta* eggs (A) and larvae (B) under greenhouses in Takelsa region in 2016 (2 insecticides sprays were done by the farmer: 1st application with Azadirachtin on 15/03/2016 and 2nd with Indoxacarb on 14/04/2106 for all studied greenhouses

Table 2. Effect of traps on *T. absoluta* eggs (a) and larvae (b)

Treatment (a)	**Mean number of eggs/40 tomato leaves/2 greenhouses (a)**	**Mean number of larvae/40 tomato leaves/2 greenhouses (b)**
Control without traps	34.93a	47.8a
Greenhouses with pheromone and sticky traps	5.66b	11.33b
Greenhouses with pheromone traps	5.33b	14.20b

* Means followed by the same letter are not significantly different at $P \leq 0.05$ within each column.

4. Discussion

4.1 Population dynamics in protected crops

Tuta absoluta is a key pest of tomato cultivation worldwide (Cherif *et al.* 2013; Cherif & Lebdi-Grissa 2017; Mohamadi *et al.* 2017; Biondi *et al.* 2018). In Tunisia, tomato crop is grown all year round through main and late growing season which have great economic impact but represent optimal trophic conditions for occurrence of this pest. Moreover, Tunisia had a Mediterranean climatic conditions which favor the development of *T. absoluta***.**

Here, population dynamics of *T. absoluta*, investigated through unheated greenhouses, indicated the presence of 4 flight peaks from October to May and 4 generations of eggs and larvae recorded on tomato leaves from February to May. Our findings are in accordance with other results realized in the Mediterranean area including Tunisia showing that *T. absoluta* was able to perform several generations per year (Abbes *et al.* 2011; Lebdi-Grissa *et al.* 2011; Allache *et al.* 2012; Harbi *et al.* 2012; Cherif *et al.* 2013, 2014; Cocco *et al.* 2013, 2015; Mahmoud *et al.* 2015). . Pupae are present in a few numbers on leaves which confirm the idea that pupation occurs usually in the soil (Michereff & Vilela 2001; Cherif & Lebdi-Grissa 2017). In protected tomato crops, initial infestation depends on residual populations from the previous cultivation while the pest density depends on length of the intercrop period (Cocco *et al*. 2015). Our study showed that trap catches increased in the spring, when temperature rose, with remarkable numbers compared to autumn-winter favoring the development of the pest. Our results confirm those realized by other authors in Tunisia (Abbes *et al.* 2011; Harbi *et al.* 2012; Cherif *et al.* 2013; Cherif & Lebdi-Grissa 2014; Cherif & Lebdi-Grissa 2017) and in other regions such as Mediterranean zones (Cocco *et al*. 2015; Mahmoud *et al*. 2015).

Adults, eggs and larvae were simultaneously recorded during the tomato growing season from February to May for all study years. Our result confirms the idea of overlapping of generations which may be due firstly to the long oviposition period of *Tuta* female fixed to up to 24 days and secondly to variable development time of individuals caused by change in microclimatic conditions in the protected crop (Lee *et al.* 2014;

Cocco *et al.* 2015). In a previous study, Cocco *et al.* (2015) indicated that the capture pattern in protected crops was largely unimodal with low catches in winter, increasing population density from the spring, in conjunction with highest temperature. The same authors demonstrated that, the development time of *T. absoluta* is influenced by the presence of tomato plants known as its principal host as well as high temperatures values (Cocco *et al.* 2015).

4.2 Distribution of eggs per side of leaf

Our findings showed that *T. absoluta* prefer laying eggs on the underside than on the upper side of tomato leaves confirming other results (Torres *et al.* 2001; Cherif & Lebdi-Grissa 2017).

4.3 Relationship between trap catches, number of eggs laid and infestation levels

Here, we demonstrate a significant linear regression between trapped males and eggs laid, trapped males and active infestation and trapped males and total infestation which confirm results obtained by Abbes & Chermitti (2011) that found a significant relationship between trapped males and infestation level in protected crops. However, Mohamedova *et al.* (2016) indicated in another study that there was no correlation between the number of trapped moths and damaged leaves and fruits.

Our study indicated a significant relationship between active and total infestation, while Caffarini *et al.* (2000) found a non-significant regression between infested leaves and damaged fruits at low pest densities (R^2=0) in protected crops.

Our study approved the decrease of damaged leaves at the end of growing season. Abbes & Chermitti (2011) explain these findings by the widespread devastation of tomato plant canopies due to the high number of feeding which may limit the presence of eggs laid as well juvenile larvae on leaves. In this case, females lay eggs on stems and sepal of fruits causing heavy yield losses (Abbes & Chermitti 2011).

4.4 Efficacy of traps tested under greenhouses conditions

Our survey demonstrated that the use of sex pheromone traps combined with chemical sprays is sufficient to control *T.*

absoluta under tomato greenhouses. However, the use of black sticky traps is not necessary to enhance the efficacy of sex pheromone traps.

According to previous studies, pheromone traps, allows not only the detection of early infestations but also may reduce the number of chemical sprays and therefore avoid heavy cost losses and unwanted effects on human health and biodiversity **(**Abbes & Chermitti, 2011). Several studies highlighted the negative impact of uncontrolled chemical sprays on natural enemies such as the Merid bug *Nesidiocorus tenuis* and the egg parasitoid *Trichogramma pretiosum* (Riley) (Cônsoli *et al.* 1998; Michereff Filho *et al.* 2000). In Tunisia, Braham & Nefaaoui (2015) compared the efficacy of mass trapping and cultural techniques to control *T. absoluta* in tomato greenhouses. For this, a density of 120 pheromone Jackson traps per ha was used and a manual removal of damaged leaves and fruits was carried out (Braham & Nefaaoui 2015). The authors demonstrated that both techniques showed low effectiveness in the reduction of the infestation level compared to the control (Braham & Nefaaoui 2015). In Italy, Cocco *et al.* (2012) tested the efficacy of light and pheromone water traps against *T. absoluta* under plastic commercial greenhouses equipped with insect-proof nets in both winter-summer and summer-winter tomato growing seasons. These authors indicated that pheromone traps evaluated at the density of 1 trap/350m^2, 1/250m^2 or 1/100m^2 were not effective in reducing leaf and fruit damage in both seasons. However, light traps tested at the density of 1 trap/1000m^2, 1/700m^2, 1/500m^2, or 1/350m^2 reduced significantly the leaf damage at low/moderate *T. absoluta* population density only during the summer-winter season (Cocco *et al.* 2012). In southern Tunisia**,** Ettaib *et al.* (2016 b) tested the effectiveness of pheromone traps (associated or not with a source of light) and luminous traps (associated or not with water, with limed buckets for limed covers) to control *T. absoluta* under heated greenhouses. These authors proved that the highest number of adults was recorded by pheromone traps with an average number of trapped males of about 73.4 (± 142) (Ettaib *et al.* 2016 b). Moreover, this study highlighted the advantage of using luminous traps in terms of production low costs and simultaneously capturing males and females of *T. absoluta* (Ettaib *et al.* 2016 b). In Turkey, Aksoy

& Kovanci (2016) tested the efficacy of three trap types (pheromone delta traps, pheromone water pan traps and water pan traps with both pheromone and a light source) to manage *T. absoluta* using a density of 40 traps/ha in tomato open field crops. This study showed that delta traps caught significantly more moths than the other two trap types and female moths were only captured in pheromone-baited water traps with a light source (Aksoy & Kovanci 2016). The authors demonstrated that mass trapping with delta traps significantly reduced the percentage of infested leaves and fruits compared with insecticide-treated controls (Aksoy & Kovanci 2016). Lobos *et al.* (2013) evaluated several commercial trap designs for capture of *T. absoluta* and they found that the most effective trap is a small plastic container with entry windows cut on the sides filled with motor oil over water (Lobos *et al.* 2013**)**. The authors suggest that these traps are most effective when placed near ground level and ideal trap baits were loaded with 0.5 mg of pheromone (Lobos *et al.* 2013). In another study, two trapping systems (dry bucket traps or delta traps with either hot melt pressure sensitive adhesives (HMPSA) or cool melt adhesives) were evaluated for their effectiveness in trapping *T. absoluta* (Roda *et al.* 2015). The study showed that delta traps with HMPSA and cool melt adhesives both trapped *T. absoluta* with equal efficacy (Roda *et al.* 2015). Mohamedova *et al.* (2016) tested the efficacy of three brands of pheromone lures [(Russel IPM® (*Tuta absoluta*, 0.5 mg; *Tuta absoluta*-Optima, 0.8 mg; TUA-100N, 3 mg); Koppert® (Pherodis *Tuta absoluta*, 0.8 mg), BioBest® (*Tuta absoluta* pheromone, 0.5mg)] while using delta traps at a density of 1 per 0.05-0.1 ha for *T. absoluta* monitoring. This study indicated that the most attractive lure was the formulation of *Tuta absoluta*-Optima, followed by Pherodis *Tuta absoluta*. However, during the cooler summer, the lures of Pherodis *Tuta absoluta* (Koppert®) and *Tuta absoluta* pheromone (BioBest®) were more attractive (Mohamedova *et al.* 2016). Sex pheromones may be used within mating disruption program considered as a potential control method used against *T. absoluta* (Marti *et al.* 2010; Navarro *et al.* 2010). Cocco *et al.* (2013) proved the efficacy of the mating disruption technique to control *T. absoluta* and its potential use in protected tomato crops over an integrated pest management programs.

These authors indicated that in greenhouses treated with 1000 dispensers/ha of pheromone loaded with 60 mg of the natural blend of the major and minor sex pheromone component (rate 90: 10), a reduction of trap catches (93–97%) was observed, compared with untreated (control) greenhouses (Cocco *et al*. 2013). Moreover, in disrupted greenhouses, leaf and fruit damages were significantly reduced throughout the growing season ranging from 57% to 85% and 62 to 89% respectively (Cocco *et al.* 2013). Previous study highlighted the limited effectiveness of this technique when used in small plots (0.01 hectares) of tomato crop (Michereff Filho *et al.* 2000). These authors proved that sex pheromones applied at doses ranging from 0 to 80 g a.i./ha in cages containing tomato plants were unable to reduce the percentage of damages or the frequency of mating compared to the control plots (Michereff Filho *et al.* 2000). These authors attributed the failure of mating disruption technique to the composition of the synthetic pheromone, doses used, high pest population density, and mated female migration to the treated area (Michereff Filho *et al.* 2000).

In conclusion, in protected crops, sex pheromone traps may be used for monitoring or mass trapping program, but cannot be used alone as control tool (Cocco *et al.* 2012). Mass trapping is not sufficient to reduce pest densities; it must be combined by other control tools such as chemical sprays or releases of wasps (Cocco *et al.* 2012). Abbes & Chermitti (2011) recommended the use of sex pheromone trap as indicator of pest densities in tomato crops.

Moreover, the success of mass trapping used within IPM strategies depends on the longevity of sex pheromone capsules renewed usually each 4-5 weeks, rate of pheromone releases, traps design and prices (Fernando *et al.* 2001; Abbes & Chermitti 2011). However, tomato greenhouses must be equipped by insect-proof in order to limit the entering of adults from outside (Abbes & Chermitti 2011).

Furthermore, the knowledge of the population dynamics of *T. absoluta* in terms of number of generations and population structure during the crop growing season is necessary to plan effective control methods. Control management strategies recommended against *T. absoluta* would be using sex pheromone traps for monitoring and planning insecticides applications accordingly to action thresholds taking into account the safety of tested active ingredients towards natural enemies present in the crop.

Further researches must be performed such as the study of the impact of climatic changes on the ecosystem level which may influence the efficacy of crop protection strategies.

4. Acknowledgments

We thank the farmers for their contribution to this work.

III.4 Efficacité du piègeage de masse et des insecticides pour le contrôle de *Tuta absoluta* en Tunisie

Efficacy of mass trapping and insecticides to control *Tuta absoluta* in Tunisia: Accepted in Journal of plant diseases and protection (Article 5).

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Running title: Integrated Management of *Tuta absoluta* in Tunisia

Acknowledgements

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Abstract

Since its first detection in Tunisia in 2008, the tomato pinworm, *Tuta absoluta* has become a serious problem to tomato crops by causing heavy yield losses. The aim of this work was to evaluate the efficacy of some actives ingredients, commonly used by Tunisian farmers, under laboratory and greenhouse conditions in controlling *T. absoluta* larvae.

We also tested the role of mass trapping as control method for *T. absoluta* while performing two greenhouse trials; one to test the most effective number of pheromone traps to be used and the second evaluating the combination of insecticides and pheromone traps.

Our study demonstrated that the product combining abamectin and chlorantraniliprole was the most effective 2h after the treatment in laboratory trials. Moreover, this study indicated that the dose of two sex pheromone traps/greenhouse was the most effective compared to the other tested dose. Also, mass trapping

alone (2 sex pheromone traps/greenhouse) was not effective in reducing larval populations; flubendiamid and cyromazin caused 96 and 77% larval mortality respectively.

Key-words: chemical control, traps, insecticide resistance, Tomato borer, IPM.

1. Introduction

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is a devastating pest of tomato originating from South America (Desneux et al. 2010). This tomato leafminer can cause several damages by feeding on the leaves, stems and fruit (Desneux et al. 2010; Gonzalez-Cabrera et al. 2010), and losses caused by this pest can reach in some cases 60 to 100% (Desneux et al. 2010; Gonzalez-Cabrera et al. 2010)**.** In a few years, *T. absoluta* has undergone a rapid expansion in its range and is now present across Europe, North Africa and parts of Asia (Desneux et al. 2011; Tropea et al. 2012). It was recently reported for India, Nepal and Burkina Faso (Kalleshwaraswamy et al. 2015; Bajracharya et al. 2016; Son et al. 2017). In Tunisia, *T. absoluta* was recorded in many regions such as Sousse (coastal Tunisia), Kairouan (central part of Tunisia), Bizerte (northeastern part), Nabeul (Cap-Bon region, north-east of Tunisia) and Zaghouan (northern half of Tunisia) (Harbi et al. 2012). Although many natural enemies of this exotic pest have been recorded in the newly invaded areas (Urbaneja et al. 2012; Zappalà et al. 2013; Abbes et al. 2014), several management strategies were carried out to control this pest. The success of the use of natural enemies to control *T. absoluta* under the tomato growth conditions is variable and depends on several factors such as in the case of *Trichogramma* spp. The effectiveness of releases may depend on the biological characteristics of selected parasitoids, doses of releases, populations levels of *T. absoluta* as well as on the presence of other natural ennemis in the crop (Tabone et al. 2010; Andrade et al. 2011; Chailleux et al. 2012; Yuan et al. 2012). Among natural enemies, the omnivorous predator *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) is becoming increasingly important for its biocontrol activity against *T. absoluta*, although it can produce feeding damage to the plants and therefore needs to be rationally managed (Biondi et al. 2016; Naselli et al. 2016). Integrated pest management

(IPM) based essentially on the use of adult sex pheromone and mass trapping applications has been widely adopted to control this pest in many countries around the world (Chermiti and Abbes 2012; Cocco et al. 2012; Cherif and Lebdi-Grissa 2014). However, *T. absoluta* control still largely relies on chemical applications both in its native region and in the newly invaded areas (Guedes and Picanço 2012; Tropea et al. 2012). Repeated pesticide applications, however, could have unwanted effects, such as toxicity towards non-target organisms and the environment in general (Devonshire and Field 1991; Weisenburger 1993; Desneux et al. 2007; Landgren et al. 2009; Biondi et al. 2012; 2013; 2015) and could also lead to resistance, which is a limiting factor in the control of this pest (Campos et al. 2010; Haddi et al. 2012; Reyes et al. 2012)**.** Problems of resistance to synthetic pyrethroids, abamectin, spinosad, chlorantraniliprole, have been reported in many countries around the world such as in South America and Europe (Lietti et al. 2005; Haddi et al. 2012; Campos et al. 2010; Roditakis et al. 2015). For this, integration of chemical control with other control methods such as cultural, biological, and biotechnological methods should be performed and may lead to a successful IPM (Gonzalez-Cabrera et al. 2010; Zappalà et al. 2012; Cocco et al. 2013; Biondi et al. 2016).

The aim of this work was first to test under laboratory and field conditions the susceptibility of the tomato leaf miner to some insecticides commonly used by Tunisian farmers; so to check if these insecticides were still effective and if *T. absoluta* deployed resistance to the tested actives ingredients in Takelsa region which is the first tomato growing in Tunisia where farmers use insecticides widely. Second, to test the efficacy of three trap densities (1, 2 and 3 sex pheromone traps/greenhouse) used for mass trapping against *T. absoluta* and to study the effect of mass trapping used alone or combined with chemical treatment in reducing the population of *T. absoluta* under greenhouse conditions.

2. Materials and methods

2.1 Laboratory bioassay

2.1.1 Plant and insect material

In order to rear *T. absoluta* in the laboratory, pesticide-free tomato plants *Lycopersicon esculentum* Mill. (cv. Sankara) were cultivated in pots in a greenhouse under natural environmental conditions. To establish a *T. absoluta* colony, leaves infested with larvae were collected on February, 2014 from commercial tomato greenhouses located in Takelsa (Cap-Bon region, governorate of Nabeul) and transferred to the laboratory. These infested leaves were placed in plastic boxes and kept in a rearing chamber at 26 ± 2°C, 60 ± 10% RH (%) and a 16:8 (L: D) photoperiod. Fresh leaves were added to the infested material in order to help the larvae complete their development until pupation. Finally, pupae were recovered and placed in tubes until their emergence. Adults of *T. absoluta* were then released on the tomato plants in the experimental greenhouse. Infested leaves were collected from tomato plants located in the greenhouse and kept inside plastic ventilated boxes in the rearing chamber until adult emergence, adding periodically fresh leaves to allow larvae complete their development.

The newly emerged adults were re-inoculated in the experimental greenhouse and from this rearing the second instar larvae for the insecticide bioassay were taken.

2.1.2 Insecticide bioassays under laboratory conditions

In order to study the effect of eight insecticides (table 1), widely used in tomato crops under Tunisia conditions, on *T. absoluta* larvae, a leaf-dip bioassay method was performed following IRAC protocol (http://www.irac-online.org/methods/tuta-absoluta-larvae). Tomato leaves were dipped in the insecticide solution prepared with tap water using recommended field rates and allowed to air dry for one hour. Petioles of tomato leaves were rolled in cotton soaked in water to avoid their wilting. Fifteen L2 instars were placed individually on leaves with completely dried insecticide solution inside Petri dishes (one larva/leaf/Petri dish), then placed in a rearing chamber at 26 ± 2 °C, 60 ± 10% RH (%) and a 16:8 (L: D) photoperiod. Three replicates were performed for each insecticide. Each replicate consisted of 15 larvae so a total of 45 larvae were subjected to each treatment. Tap water was used for the control treatment. Mortality was recorded 2, 4, 6, 24, 48, 72 and 96 hours after larvae exposure and larvae were considered dead when they were unable to restore their ventral position when placed on their dorsum.

Table 1: List of active ingredients used for bioassays and their respective trade names, concentrations and doses

Active ingredient	Trade names	Conc. a.i.	Dose
Flubendiamid	TAKUMI 20 WG	20%	30g/hl
cypermethrin	CYPERCAL 250 EC	250g/L	50cc/hl
Cyromazin	CLAVE	75%	30g/hl
Chlorfenapyr	CHALENGER	240g/L	50cc/hl
abamectin+chlorantraniliprole	VOLIAM TARGO 063 SC	1.8% (18g/L) abamectine+4.5% chlorantraniliprole (45g/L)	60cc/hl
Diafenthiuron	PEGASUS 500 EC	500g/L	100ml/hl
Chlorantraniliprole	CORAGEN 20 SC	200g/L	50ml/hl
Azadirachtin (0.03%) + neem oil (90.5%)	NIMBICIDINE	0.3 g/L or 0.03% + 90.5%	250cc/hl

2.2 Field experiment

2.2.1 Study site

Greenhouses located in Takelsa (Cap-Bon region, governorate of Nabeul) were considered for this study. Each greenhouse had an area of 500 m^2 (62.5 m length, 8 m width and 3 m height). The greenhouses contained 1400 tomato plants (var. Sankara) cultivated on 5 doubled rows with a distance between rows and plants of about 1.5 and 0.5 m respectively. All greenhouses had the same crop calendar in terms of fertilization, irrigation and chemical treatments (see www.avfa.agrinet.tn/fr/resultatsupport.php for details).

2.2.2 Efficacy of mass-trapping in reducing *T. absoluta* infestations

Mass trapping trials were performed in the greenhouses described above and two trials were carried out during this study. The first trial (2.2.2.1) was conducted from 07/12/2009 to 02/06/2010 while the second trial (2.2.2.2) from 10/10/2013 to 06/05/2014.

2.2.2.1 Comparison of three doses of sex pheromone water traps

Three doses of sex pheromone water traps (1, 2 and 3 Traps/500m^2) (Pherodis®, Koppert Biological System) were tested separetly in greenhouses and compared to others without sex pheromone traps. All greenhouses were treated with the same insecticides on 19/02/2010 (BACTOSPEIN, *Bacillus thuringiensis*, 450 g/hl) and 25/04/2010 (CLAVE, cyromazin, 30g/hl). Three replicates were considered for each treatment and a total of 12 greenhouses were used. The threshold was fixed by the Tunisian ministry of agriculture as 50 moths/pheromone trap.

2.2.2.2 Efficacy of sex pheromone water traps used with or without insecticides applications

Untreated greenhouses, left as control, were compared to greenhouses where two sex pheromone traps (Pherodis®, Koppert Biological System) were tested alone or combined with insecticide applications. Three treatments with three replicates each were tested, therefore a total of 9 greenhouses were used for this trial. Traps consisted in plastic containers (12 cm deep and 20 × 30 cm size), containing approximately 5 liters of water and a thin layer of vegetable oil. The pheromone dispenser was secured above the water level with a wire. Traps were placed above the soil at a height of 40 cm and a distance of 20 m was kept between traps. The content of the traps was replaced whenever necessary. Trapped males were recorded and removed from traps weekly. Change of sex pheromone lures was performed every four weeks. For the two trials, the effect of mass trapping in reducing *T. absoluta* population density was evaluated counting weekly the number of larvae on 40 randomly sampled leaves per greenhouse.

2.2.3 Chemical control of *T. absoluta* under greenhouse conditions

In order to control this leaf miner under greenhouse conditions, two synthetic pesticides (cyromazin and flubendiamid) and one biopesticide (azadirachtin), were tested in three greenhouses containing sexual pheromone lures (Pherodis®, Koppert Biological Systems) fixed for the second trial in Takelsa region. The three tested insecticides were considered as the most commonly used by Tunisian farmers. Each greenhouse was divided into four blocks. Each block contained 466 tomato plants (1.5 m high) and received one of the following treatments: cyromazin, flubendiamid, azadirachtin and untreated control (Table 1). Three replicates were performed per each treatment. Insecticide treatments were triggered on 09/05/2014 when threshold was reached. From each block, 40 leaves from apical and middle part of the plant were collected at random and transferred to the laboratory for examination.

In order to evaluate the insecticide efficacy on *T. absoluta* larvae, the corrected mortality according to Abbott's formula was calculated:

$$\% \text{ efficacy} = [(A - B / A) \times 100]$$

where:

A: Mean number of *T. absoluta* larvae on untreated tomato plants

B: Mean number of *T. absoluta* larvae on treated tomato plants

2.2.5 Statistical analysis

The data were analyzed using repeated measures analysis of variance, using a GLM procedure, followed by one way anova to determine differences between different mortality rates and numbers of *T. absoluta* larvae after using insecticides in the laboratory and field conditions and also to determine the effect of trap densities as well as mass trapping used alone or combined with insectcides in reducing *T. absoluta* larvae under greenhouse conditions. Means were separated using least significant different test (LSD) and Duncan at P=0.05. All statistical analyses were performed using the software SPSS 17 (SPSS Inc. 2009).

3. Results

3.1 Insecticide bioassay under laboratory conditions

All active ingredients tested were efficient in controlling *T. absoluta* larvae (Table 2). The association of abamectin and chlorantraniliprole demonstrated the most important effect 2h after the treatment compared to the other active ingredients. It ensured a mean (±SE) number of dead larvae of 14.33±0.47. Mortality caused by azadirachtin was lowest among tested active ingredients and ranged from 2.33±0.47 to 10±2.82 respectively 2h and 96h after the treatment. The efficacy of flubendiamid, cypermethrin, diafenthiuron and chlorfenapyr on larvae appeared 4h after the treatment with a mean larval mortality (± SE) of about 8±0.81, 8±1.41, 12.33±1.69 and 12±1.63 respectively. The effect of chlorantraniliprole and cyromazin on *T. absoluta* larvae instead began one day after the insecticide application (larval mortality of about 8±1.63 and 9±0.81 respectively). As for the untreated control, larval mortality was very low and did not exceed 1.66±0.94 96h after the test. Statistical analysis showed that all the tested insecticides had an effect on mortality of *T. absoluta* larvae ($F_{1,7}$= 81.162, *P*= 0.001).

Table 2: Mean number of dead larvae 2, 4, 6, 24, 48, 72 and 96h after the treatment (HAT) by commercial products under laboratory conditions

Active ingredient	Larvae mortality (mean±SE)							Statistical analysis
	2 HAT	4 HAT	6 HAT	24 HAT	48 HAT	72 HAT	96 HAT	
Control	0Aa	0Aa	0Aa	0.66±0.47AB a	1.33±0.47Ba	1.66±0.94Ba	1.66±0.94Ba	F=3.9; *P*=0.17
Flubendiamid	2.66±0.47Ab	8±0.81B c	13.66±1.88Cd	14±1.41C c	14±1.41C c	14.33±0.94Ccde	14.33±0.94C cd	F=28.188; *P* <0.0001
Chlorantraniliprole	4±0.81A b	4.66±1.24Ab	6±1.63A b	8±1.63B b	15±0C c	15±0C e	15±0Cd	F=66.640; *P* <0.0001
Cyromazin	4±0.81A b	4.66±0.47A b	5.66±1.24A b	9±0.81B b	11±0.81BC b	12±1.63C bcd	12±1.63C bc	F=18.894, *P* <0.0001
Cypermethrin	3±0.81A b	8±1.41Bc	9±1.41B Cc	9.33±2.62C b	10.33±0.47C b	11.66±1.69C bc	13.33±1.24C cd	F=19.097; *P* <0.0001
Diafenthiuron	7.66±1.24A c	12.33±1.69 Bd	14±1.63C d	14.66±0.47C c	15±0C c	15±0C e	15±0Cd	F=3.333; *P* =0.03
Abamectin+chlorantraniliprole	14.33±0.47A d	14.33±0.47B d	15±0BC d	15±0C c	15±0C c	15±0C e	15±0C d	F=13.788; *P* <0.0001
Chlorfenapyr	7.33±1.69A c	12±1.63B d	13.33±0.47B d	14.33±0.94BC c	14.66±0.47BC c	14.66±0.47CD de	14.66±0.47CD cd	F=16.900; *P* <0.0001
Azadirachtin	2.33±0.47A b	4.66±1.69AB b	7.66±0.47BC bc	8.33±0.94BC b	9.66±0.47C b	10±2.82C b	10±2.82C b	F=6.007; *P* =0.003
Statistical analysis	F=45.313; *P* <0.0001	F=29.343; *P* <0.0001	F=41.990; *P* <0.0001	F=96.328 ; *P* <0.0001	F=101.672; *P* <0.0001	F=23.770; *P* <0.0001	F=23.906; *P* <0.0001	

*In each column (lower case letter) and row (capital letter), means followed by the same letter are not significantly different at $P \leq 0.05$.
*HAT: Hours After Treatment

3.2 Efficacy of different doses of sex pheromone water traps in reducing *T. absoluta* infestation

The *T. absoluta* male flight activity, in the experimental greenhouses, took place during the whole experimental period in both years. Catches were initially very low and increased progressively to reach the first, second, third and fourth peak on December, February, March and May respectively (Figure 1, Figure 2).

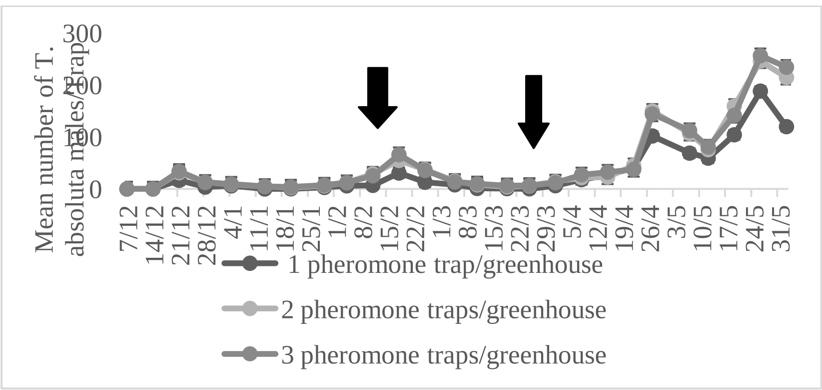


Figure 1: Weekly captures of *T. absoluta* males in sex pheromone water traps under greenhouses in 2009-2010 (insecticide applications: 19/02/2010 and 25/04/2010)

The number of captured adults, for the first trial, was higher when using 2 to 3 pheromones traps**.** For example, on 26/05/2010, this number was 256.33 males/trap for both 2 and 3 pheromones traps used, while, it was only 188.33 males/trap when using 1 pheromone trap (Figure 2). However, statistical analysis showed there was no significant difference for the number of captured males between the three tested trap densities ($F_{2, 77}$=0.737; p=0.482).

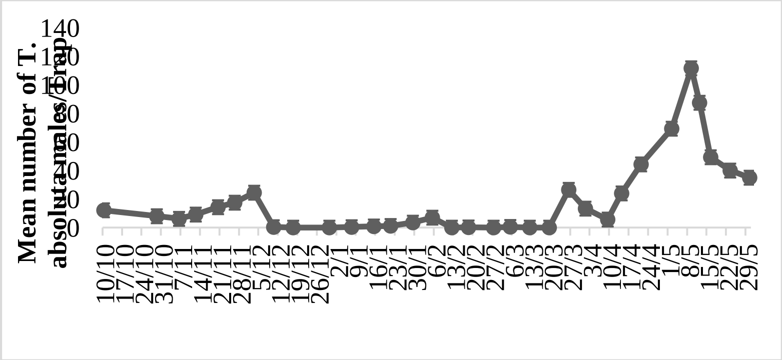


Figure 2: Weekly captures of *T. absoluta* males in sex pheromone water traps under greenhouse in 2013-2014

The number of *T. absoluta* larvae was compared in greenhouses where mass trapping was applied at three different doses. Two insecticides applications were done on 19/02/2010 and 25/04/2010. The number of larvae did not differ significantly between greenhouses without (control) or with 1 pheromone trap (treatment 1) (Figure 3, Table 3). In contrast, the infestation data of greenhouses with 2 or 3 pheromone traps (treatment 2 and 3) showed a significant reduction in pest populations (Figure 3, Table 3). On 22/04/2010, the number of alive larvae did not exceed 17 larvae/40 leaves/greenhouse for both treatment 2 and 3 compared to 45 and 38 larvae/40 leaves/greenhouse respectively for the control and the treatment 1 (1 trap/greenhouse) (Figure 3). Statistical analysis shows that there was a significant difference between the tested greenhouses ($F_{3, 83}$=3.132; p=0.03).

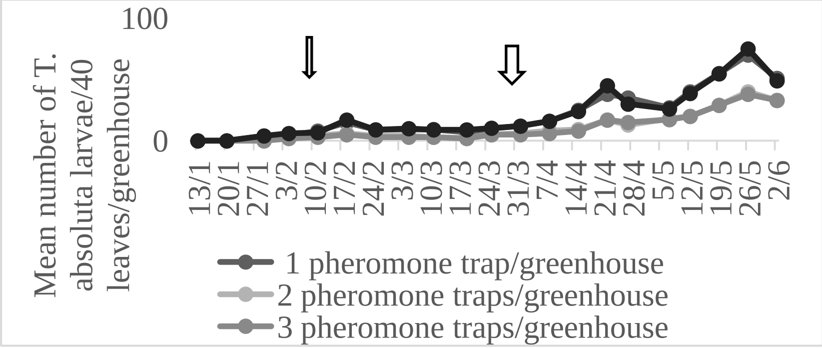


Figure 3: Effect of three doses of pheromone traps in reducing *T. absoluta* populations in greenhouses conditions in 2009-2010 (⇩ Insecticides applications: 19/02/2010 and 25/04/2010)

3.3Efficacy of sex pheromone water traps used with or without insecticide applications

The number of *T. absoluta* larvae was highest in the untreated greenhouses (Figure 4). Also, for the treated greenhouses, the first peak was higher in the greenhouses where insecticide treatments combined with mass trapping were performed (27 larvae/40 leaves) than in greenhouses where mass trapping was tested alone (17 larvae/40 leaves).

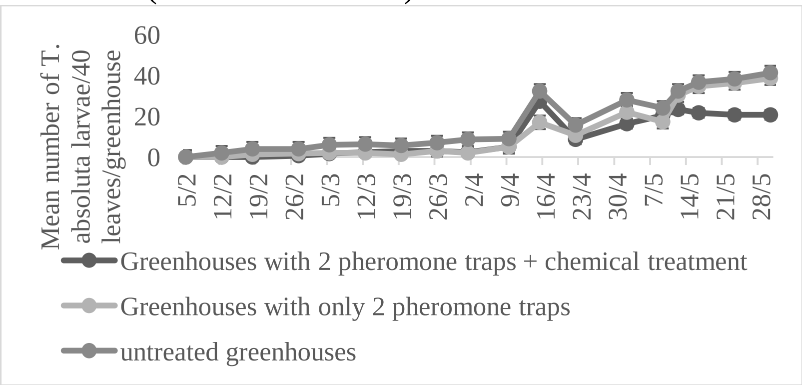


Figure 4: Mean number of *T. absoluta* larvae on the leaves in greenhouses where mass trapping was used alone or combined with chemical treatment in 2013-2014

Statistical analysis showed that sex pheromone traps used alone as a means of control didn't have a significant effect in reducing *T. absoluta* population density ($F_{2,\ 41}$=1.289; *P*=0.287)**.** However, after insecticide application (06/05/2014), pest populations decreased significantly compared to the untreated control and to the greenhouses where mass trapping was used alone ($F_{2, 14}$=6.043, P=0.015) (Table 3).

Table 3: Effect of sex pheromone traps used alone or combined with insecticide applications on *T. absoluta* larvae on tomato plants

Treatments	Mean number of larvae/40 leaves/ 3 greenhouses
Control	34.53a
Only sex pheromone trap	31.46a
Sex pheromone trap with insecticide applications	21.33b

*Means followed by the same letter are not significantly different at $P \leq 0.05$.

This result led us to conclude that mass trapping used alone as a control technique in greenhouses may not be efficient in reducing larval population, however it reduces significantly the infestation when used jointly with insecticide applications (Figure 4, Table 3).

3.4 Chemical control of *T. absoluta* under greenhouse conditions

Insecticide treatments were triggered on May, 9th, 2014 in three greenhouses containing two sex pheromone water traps considered in the second trial. In the assay period, flubendiamid and cyromazin were proved highly effective in reducing *T. absoluta* larvae on tomato plants, with an average mortality of larvae of about 96 and 77% respectively 21 days after insecticide application, compared to azadirachtin whose efficacy did not exceed 40% in the same period (Figure 5, Figure 6, Table 4). Insecticide applications significantly reduced *T. absoluta* larvae density ($F_{4, 74}$ =45.971; $p \leq 0.001$), when compared to untreated control (Table 4).

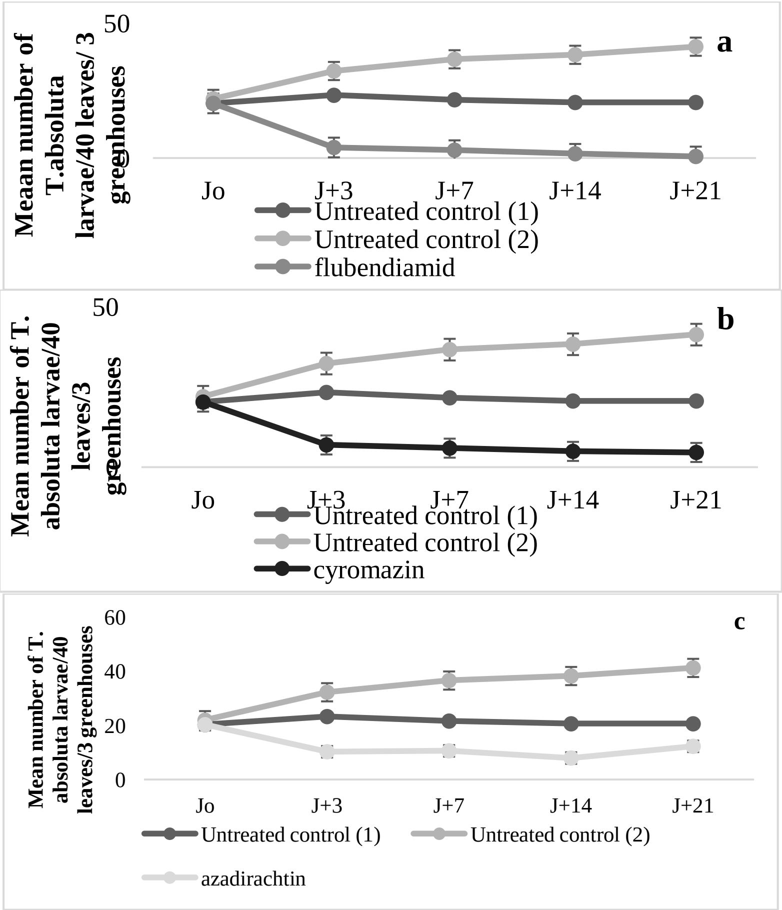
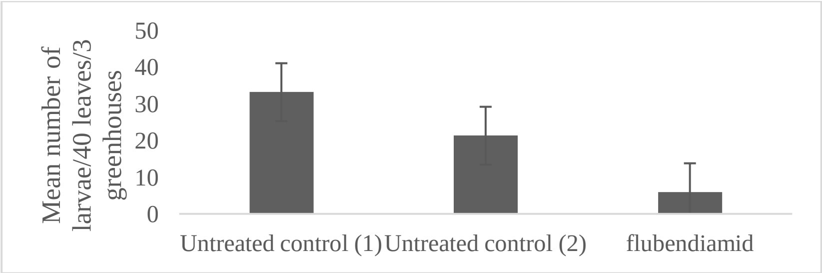
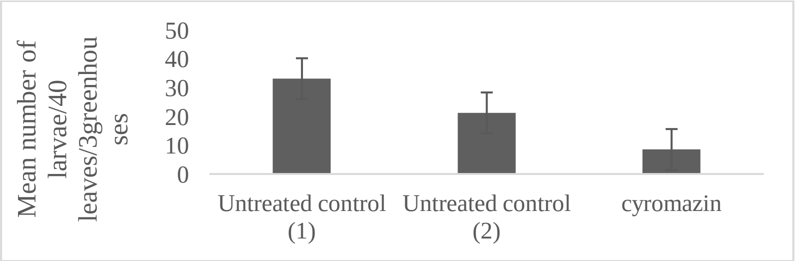


Figure 5 (a, b, c): Effect of the three insecticides on *T. absoluta* larvae after 3, 7, 14 and 21 days of the treatments under Takelsa greenhouses in 2013-2014 (Untreated control (1) from greenhouses containing pheromone water traps and treated with insecticides; Untreated control (2) from greenhouses with no insecticides neither mass trapping treatment)





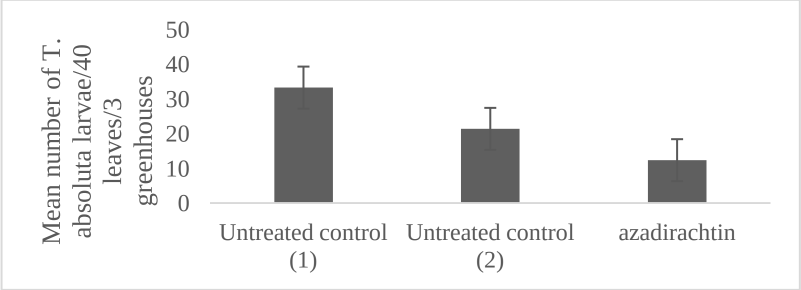


Figure 6 (a, b, c): Effect of three insecticides treatment on *T. absoluta* larvae in Takelsa tomato greenhouses (Untreated control (1) from greenhouses with no insecticides neither mass trapping treatment; Untreated control (2) from greenhouses containing pheromone water traps and treated with insecticides)

Insecticide applications significantly reduced *T. absoluta* larvae density ($F_{4, 74}$ =45.971; p≤0.001), when compared to untreated control (Table 4).

Table 4 (a, b, c): Effect of three insecticide treatments on *T. absoluta* larvae in Takelsa tomato greenhouses

(a) Treatments	Mean number of larvae/40 leaves/ 3 greenhouses
Untreated control (1)	33.20a
Untreated control (2)	21.33 b
Flubendiamid	5.93 d

(b) Treatments	Mean number of larvae/40 leaves/ 3 greenhouses
Untreated control (1)	33.20a
Untreated control (2)	21.33 b
Cyromazin	8.6 cd

(c) Treatments	Mean number of larvae/40 leaves/ 3 greenhouses
Untreated control (1)	33.20a
Untreated control (2)	21.33 b
Azadirachtin	12.33 c

*Untreated control (1) from greenhouses with no insecticides neither mass trapping treatment
*Untreated control (2) from greenhouses containing pheromone water traps and treated with insecticides
*Means followed by the same letter are not significantly different at $P \leq 0.05$

4. Discussion

This study shows, for the two years of study, that *T. absoluta* performed up to 4 peaks under greenhouse conditions in the period extending from December to May, 2010 and from October, 2014 to May, 2015 in Takelsa region in Tunisia. Also, we note that the number of *T. absoluta* males recorded in sex pheromone traps increased progressively from the month of April in parallel with the increases in the temperature values during spring-summer, which confirms what was reported in the literature (Lacordaire and Feuvrier 2010). In a previous study, Cherif et al. (2013) suggested that, under Takelsa greenhouses and using pheromone water traps during the period mid January - mid May, *T. absoluta* males realized three flight peaks with the highest trap counts recorded in spring. Also, Allache et al. (2012) demonstrated that three flight peaks of *T. absoluta* males were recorded, from mid December to late May, in a greenhouse located in Biskra (Center-east Algeria). This study demonstrated, that the dose of 2 traps/ 500 m^2 was the most suitable rate to control *T. absoluta* compared to 1 and 3 traps/500m^2. Although this study showed that the use of water traps with sexual pheromone is important for monitoring *T. absoluta* but cannot be used alone as a single control method. It may be integrated with both chemical and biological control. Previous results explain this finding by the inability of traps to catch all *T. absoluta* males and the possible development of parthenogenesis for females' reproduction (Silva 2008; Capparos Megido et al. 2012). In this context, using sex pheromone traps is helpful for planning insecticides application or releases of wasps accordingly to action thresholds (Abbes et al. 2011). In another study realized in Tunisia, Abbes et al. (2012) stated that monitoring in the IPM cropping field with sex pheromone water traps showed fluctuations in the number of males caught per week between November 18th, 2009 and February 10th, 2010. Also, these authors pointed out that the level of weekly catches of males increased gradually, despite its instability, especially from the second half of February. In order to choose the optimal dose of sex pheromone traps used to control *T. absoluta*, many studies were carried out. Indeed, Cocco et al. (2012), demonstrated that all tested doses of sex pheromone traps (1 trap/350 m^2, 1/250 m^2 or 1/100 m^2) used alone for controlling *T. absoluta* populations under greenhouse conditions were not effective in reducing leaf and fruit damage. In another study, Blockmans, (2009) suggested the use of 20-25 traps/ha inside greenhouses and 40-50 traps/ha in open fields for mass trapping of *T. absoluta*. In a study performed under greenhouses located in the region of Teboulba, in Tunisia, the efficacy of the use of insect-proof screens alone or in combination with one sex pheromone water trap was evaluated compared to a control greenhouse which was not equipped with either (Harbi et al. 2012). In this study, the authors confirmed that the use of one sex pheromone water trap combined with insect-proof screens is sufficient to guarantee an effective control and they stated that the combined control system allowed a low density of the pest (less than 2 individuals per leaf) with a mean number of mines below 1 mine per leaf (Harbi et al. 2012). The sexual pheromone lures may be also used through a mating disruption program by creating a sexual confusion in *T. absoluta* males (Caparros-Megido et al. 2013; Cocco et al. 2013; Michereff-Filho et al. 2000). Cocco et al. (2013) showed in a previous study that a rate of 1000 dispensers/ha loaded with 60 mg of the sex pheromone component used under greenhouse conditions, is able to reduce the leaf and fruit damage at level ranging from 57 to 85% and from 62 to 89% respectively. According to previous results, the success of this technique depends on the high pest population density, the entering of new moths from other areas, the synthetic pheromones's composition and dose as well its end price (Vacas et al. 2011; Caparros-Megido et al. 2013). The leaf-dip bioassay (IRAC protocol), which is an efficient and simple method to test insecticide efficacy, led us to distinguish the susceptibility of *T. absoluta* to insecticides used to control this pest. In this study, all tested insecticides showed a high level of larval mortality except azadirachtin (for which the mean number of dead larvae did not exceed 10 in the end of the experiment). Under the laboratory conditions and despite their different modes of action (MoA), only diafenthiuron, known as inhibitor of mitochondrial ATPsynthase, and chlorantraniliprole, which is a ryanodine receptor modulator used alone or associated with abamectin (an allosteric modulator), gave an average mortality rate of about 100% 96h after the treatment. Concerning the chemical treatment under greenhouse condition, the presence of *T. absoluta* larvae on tomato plants was significantly affected after the application of the three tested insecticides (flubendiamid, cyromazin and azadirachtin) compared to the untreated control. The chemical insecticides, flubendiamid (classified as ryanodine receptor modulator) and cyromazin (a moulting disruptor) showed a better efficacy compared to azadirachtin (neem extract) whose MoA is still uncertain (http://www.irac-online.org/modes-of-action/). The efficacy shown by the azadirachtin based compound tested, may be due to the neem oil contained in it. In the laboratory tests, mortality of *T. absoluta* larvae caused by azadirachtin increased steadily starting 3 days after the treatment, which may be related to its biological characteristics taking more time to act compared to synthetic insecticides. The effectiveness of tested insecticides including azadirachtin may depend on other factors such as variation in temperature and relative humidity values which are more controlled under laboratory conditions. Moreover, in the field trial, tomato leaves were sampled at random and in this case some larvae may have not been reached by the insecticide sprays. Nonetheless, using pesticides with different modes of action can delay the onset of resistance in *T. absoluta*, and this strategy may be considered as a key point in IPM programs (Devkota et al. 2016). Hafsi et al. (2012) tested the efficacy of 13 insecticides on *T. absoluta* eggs and larvae in semi-natural conditions in Tunisia. In this study, it was found that only *Bacillus thuringiensis* Berliner var. kurstaki among all tested bio-insecticides, showed an average mortality of about 72.5% on all instars of *T. absoluta* larvae. Also, these authors suggested that the two insecticides spinosad and spinetoram showed high efficiency in controlling *T. absoluta* larvae with an average percent of mortality of about 66.5% and 85.6% respectively. Also, this study revealed that neem oil based insecticides induced a 43.8 % of egg mortality. In another study, Braham and Hajji (2012) demonstrated under laboratory conditions that the neem extract decreased the mean number of *T. absoluta* larvae from 1.5 to 0.75, 0.75, 0.5 and 0.5 larvae/ tomato plant respectively 3, 5, 8 and 12 days after the treatment. Also, this study showed that abamectin applied alone had a good performance with an average percent of mortality of about 71%. Under field conditions, Braham and Hajji (2012) showed that only indoxacarb compared to other tested insecticides (diafenthiuron and triflumuron) had a good control efficacy after the first application while after the second and third spray all insecticides performed very well compared to the control. The leaf-dip bioassay may be considered as a good method also to monitor resistance (Sauphanor et al. 2000; Charmillot et al. 2001, 2002; IRAC, 2012). In fact, in a previous study, Haddi et al. (2013) demonstrated, through insecticide bioassays, the response of five *T. absoluta* strains collected from fields located in Europe and Brazil to pyrethroids. The authors demonstrated in this study that high levels of resistance to ʎ-cyhalothrin and tau-fluvalinate were observed in all five strains tested. Also, Haddi et al. (2012) suggested the presence, with high frequencies, of three kdr/super-kdr-type mutations (M918T, T929I and L1014F) in the tested strains. These authors concluded that pyrethroids were ineffective for the control of *T. absoluta* and explained in part the idea that the rapid expansion of this pest was linked to its resistance to chemical insecticides. In another study, Reyes et al. (2012) used a diagnostic concentration of the insecticide spinosad to determine the susceptibility of different *T. absoluta* strains (five strains collected from the field and a laboratory reference strain), to spinosad. The authors showed that larval mortality of strains collected from field populations was significantly lower than in the laboratory strain. Also, the authors suggested that the evaluated mechanisms would be involved in spinosad resistance by populations of *T. absoluta*, presenting an increased Mixed-Function Oxidase activity in all populations.

In conclusion, the results of this study revealed that *T. absoluta* performed up to 4 peaks during the period extending from October to May, under greenhouse conditions in Takelsa region. Also in this study, we demonstrated that the dose of 2 pheromone water traps/greenhouse are the most efficient considering both moth catches and handling time for traps. Moreover, pheromone water traps alone may not be sufficient for control, while they are effective together with insecticides. Trials testing insecticides alone, which may be sufficient only if no resistance occurs, would be needed in order to compare the integrated treatment with only chemical treatment. Also this work stated that all the tested insecticides were still effective on *T. absoluta* in 2014. So, an integrated pest management program based on pheromone traps and the use of chemical treatment to control the pest when threshold was reached, is sufficient to maintain *T. absoluta* population under effective control. However, an adequate resistance management strategy must be adopted to minimize the risk of dispersal/introduction of *T. absoluta* resistant strains in new areas of introduction. Moreover, the use of some insecticides such as spinosad, should be carefully monitored to prevent the rapid spread of resistance in new area (Campos et al. 2014; Roditakis et al. 2016). Despite the use of sex pheromone for the control of *T. absoluta* may be compromised due to the possible development of facultative deuterotokous parthenogenesis favoring the dispersal of insecticide resistant populations, farmers were strongly advised to monitor this pest using sex pheromone traps which help them to apply effective insecticides when thresholds were reached (Gontijo et al. 2012; Caparros Megido et al. 2013). In this context, other studies based on sustainable and alternative control methods, such as biological control, must be performed on *T. absoluta* to avoid future problems of resistance development to some active ingredients as already reported in various regions worldwide.

III.5 Conclusion

Les résultats obtenus mettent en valeur l'importance de la surveillance du ravageur moyennant soit des pièges à phéromones sexuelles soit des prélèvements de feuilles. Ceci aide à bien programmer les dates d'application des insecticides ou bien les lâchers de parasitoïdes et de prédateurs. Le nombre d'adultes capturés par les pièges ainsi que les stages immatures présents sur feuilles sont abondants au printemps où les conditions climatiques sont favorables au développement de cet insecte. Des corrélations positives ont été trouvées entre les adultes piégés et les œufs pondus, les adultes capturés et les mines avec larves et les adultes piégés et le nombre total des mines. De même, les mines avec larves et les mines sans larves sont hautement corrélées. On a pu, également, démontrer que le nombre optimal de pièges à phéromones sexuelles à utiliser dans le cadre du piègeage de masse, est de deux par serre. De même, les insecticides testés au laboratoire ou sous serres ont été jugés efficaces pour le contrôle de *T. absoluta*. Nos résultats ont montré, aussi, que l'utilisation des plaques noires engluées dans des serres équipées de pièges à phéromones sexuelles et traitées par des insecticides, ne différent pas statistiquement avec celles où ces plaques n'ont pas été fixées.

Ainsi, une stratégie de lutte efficace contre cet insecte doit être basée sur l'utilisation des pièges à phéromones sexuelles pour la surveillance et le piégeage de masse avec une application raisonnée des insecticides tout en respectant le seuil de nuisibilité.
La lutte biologique peut donner des résultats encourageants et peut être considérée comme une alternative satisfaisante à la lutte chimique.

Lutte biologique contre *Tuta absoluta* (Meyrick) (Lepidoptera : Gelechiidae) par des lâchers de *Trichogramma spp.*

VI. Chapitre 4 : Lutte biologique contre *Tuta absoluta* (Meyrick) (Lepidoptera : Gelechiidae) par des lâchers de *Trichogramma spp.*

VI.1 Introduction

Le contrôle biologique est considéré comme un moyen de lutte efficace pouvant remplacer l'utilisation des insecticides pour la gestion de *Tuta absoluta*. Ainsi, l'utilisation de parasitoïdes, tels que les trichogrammes, peut donner des résultats prometteurs. Dans le but d'étudier l'effet des lâchers innondatifs de *Trichogramma cacoeciae* (Marchal) (Hymenoptera : Trichogrammatidae) sur la population de *T. absoluta*, deux expérimentations ont été réalisées dans la région de Takelsa (Nord-ouest de la Tunisie) moyennant trois lâchers hebdomadaires. Le premier essai servait à évaluer l'efficacité de trois doses de trichogrammes (10, 20 et 30 *Trichogramma*/plante) sous serres froides alors que le deuxième consistait à tester deux doses (20 et 40 *Trichogramma*/plante) sur culture de tomate en plein champ. Ce travail a fait l'objet d'un article soumis au journal<Crop protection>

VI.2 Can Innundative releases of *Trichogramma cacoeciae* (Marchal) (Hymenoptera: Trichogrammatidae) improve biological control of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Tunisia tomato crops? (Article 6)

Abstract

Tuta absoluta (Meyrick, 1917) (Lepidoptera: Gelechiidae) is a major pest of tomato crop causing heavy losses worldwide. As an alternative to pesticides, biological control using *Trichogramma* parasitoids is considered a promising management tool to this pest. Innundative releases of *Trichogramma cacoeciae* (Marchal) (Hymenoptera: Trichogrammatidae) for suppression of *T. absoluta* were made in Takelsa county (Nabeul Province, Northeastern Tunisia). Three weekly releases of three (10, 20 and 30 *Trichogramma*/plant) and two rates (20 and 40 *Trichogramma*/plant) were tested respectively in protected and open field crops. Our study indicated that density of 20 *Trichogramma*/plant is the most suitable and able to reduce the infestation level caused by this pest. A mean emergence rate, up to 67.14% and 79.66% was found under greenhouses and open field conditions. Parasitism rate was more important in apical leaves compared to middle leaves. Furthermore, the number of eggs and larvae was significantly reduced after releases of *Trichogramma*. This suggested that *T. cacoeciae* could be an efficient control agent of *T. absoluta* in tomato crop.

Keyword: *T. absoluta*, *T. cacoeciae*, tomato crop, biological control, innudative releases

Running title: *T. cacoeciae* a biological control agent against *T. absoluta*

1. Introduction

The oligophagous tomato borer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a key pest that can cause significant yield losses in tomato crops worldwide (Sabbour et al., 2014; Cascone et al., 2015). This insect is currently considered a threat to both greenhouse and open-field tomato production. Yield losses can approach for 80 to 100% in newly invaded area (Cagnotti et al., 2016). To control *T. absoluta*, several management programs have been tested including chemical control. This strategy was based on a widely use of synthetic insecticides, despite their failure to reach effectively the larvae protected inside the leaf mesophyll (Desneux et al., 2010; Guedes and Picanço, 2011; Calvo et al., 2012; Cagnotti et al., 2016). Furthermore, this practice can cause not only negative side effect on biocontrol agents (Croft, 1990; Desneux et al., 2007; Arno´ and Gabarra, 2011; Biondi et al., 2012, 2013) but also can lead in some cases to the selection of resistant populations of *T. absoluta* to synthetic pyrethroids, abamectin, spinosad, chlorantraniliprole as reported for south America and Europe. (Siqueira et al., 2000; Lietti et al., 2005 ; Campos et al. 2010 ; Haddi et al., 2012; Roditakis et al. 2015, 2016, 2017). Most organophosphates and pyrethroids registered for *T. absoluta* control are highly toxic to bees and beneficial insects (USDA–APHIS, 2011). Due to the excessive use of pesticides and the associated problems of resistance and environmental pollution, there is an increasing demand for biological control. This sustainable and environmentally friendly control strategy, has been achieved in glasshouses as an alternative method to chemical control, therefore, can be a promising solution against

T. absoluta (De Oliveira et al., 2008; Desneux et al., 2010). Various natural enemies have been recorded on *T. absoluta* (Zappalà et al., 2013; Cascone et al., 2015), such as egg parasitoids considered as the most efficient wasps known by their highly efficacy in controlling and regulating lepidopteran pests (Hoffmann et al., 2006; Hegazi et al., 2007). *Trichogramma cacoeciae* (Marchal) as well other *Trichogramma* species have been employed in agricultural cropping system through innundative releases in many countries of the word against different lepidopteron species such as *Ectomylois ceratoniae* and *Prays oleae.* (Hassan, 1993 ; Smith, 1996 ; Zouba et al., 2009 ; Desneux et al., 2010; Ksentini et al., 2010; Tabone et al., 2010; Chailleux et al., 2013; Cherif et al., 2013. Blibech et al., 2015). Abiotic factors such as temperature and relative humidity fluctuations may influence the effectiveness of *Trichogramma* releases by affecting different developmental stages of parasitoids such as the emergence, the fecundity, the longevity, and the sex ratio of the progeny. Furthermore, releases may depend on the biological characteristics of the parasitoid strains used and their interaction with a specific host (Tabone et al., 2010, Andrade et al., 2011, Yuan et al., 2012) as well as other factors such as introduction rate, frequency and density of *Trichogramma* (Smith, 1996; Wang and Ship, 2004). In order to estimate the potential for re-introducing *Trichogramma* to control *T. absoluta* in Tunisia tomato crops, *T. cacoeciae* was selected as the most promising native strain for biological control based on previous research conducted in Tunisia (Zouba et al., 2009; Cherif et al., 2013).
Until now, as we know, there is no data studying the effect of different *T. cacoeciae* releases rates to reduce damages caused by *T. absoluta* in Northeastern Tunisia.

For all of this, this study was conducted to determine whether innundative releases of *T. cacoeciae* can reduce *T. absoluta* densities both in protected and open field crop in Northeastern Tunisia.

2. Materials and methods

2.1 Experimental site

Experiments conducted between March and June 2016, aimed to evaluate the effectiveness of *T. cacoeciae* in greenhouses and open field crop located in Takelsa county (Nabeul Province, Cap Bon region, North-Eastern of Tunisia). The experimental greenhouse (60 m length, 8 m wide and 3 m height) had an area of about 500 m^2 containing 1400 plants (cv. Galaxy) planted in 5 doubled rows on 15/11/2015. Distance between rows and plants was 1.5 and 0.4 m respectively. In order to avoid problems of excess temperature and relative humidity which constitute a favorable microclimate for diseases establishment during time of high solar radiation, laterl and upper openings of the greenhouse equipped with insect proof screens were opened (Cocco et al., 2015). However, the efficiency of *T. cacoeciae* releases as well as *T. absoluta* population dynamics will not be altered (Cocco et al., 2015). The open field crop covered an area of 1 Ha, planted on 01/04/2016 with 16000 plants (cv. chams) spaced by a distance between rows and plants of 1.5 and 0.5 m respectively. For both greenhouse and open field crop, tomato plants were drip-irrigated under plastic mulch. No chemical sprays were carried out during the experiments.

2.2 *T. cacoeciae* mass releases

The parasitoid *T. cacoeciae* tested in the experiment has been already commercialized in Tunisia to control *T. absoluta* and other Lepidopteran pests. Cardboards (5*1 cm^2) containing eggs of *Ephestia kuehniella* Zell (Lepidoptera: Pyralidae) parasitized by *T. cacoeciae* were provided by a private company in Tunisia. Three consecutive releases of *T. cacoeciae* spaced one week each were realized in both greenhouse and open field crop. For greenhouse trial, three doses (10, 20 and 30 *Trichogramma*/plant) were tested. This greenhouse was divided into 4 plots having the same dimensions (7.5*13.5 m) containing each 300 plants and covered by insect-proof (8*6 mesh/cm^2), corresponding to the untreated control, dose 1, dose 2 and dose 3. Releases were made on 15/03, 21/03 and 31/03/2016. 50 plants were kept between each plot to minimize the chance of *T. cacoeciae* dispersal from release to control plots. For each release; 6, 12 and 18 cardboards were considered respectively for dose 1 (10 *Trichogramma*/plant), dose 2 (20 *Trichogramma*/plant) and dose 3 (30 *Trichogramma*/plant).

Each cardboard contained 500 *E. kuehniella* eggs parasitized by *Trichogramma* and placed each 50, 25 and 17 plants respectively for dose 1, dose 2 and dose 3. These cardboards were fixed on the support wire of the greenhouse at 1.5m height and distributed in fixed plots. Concerning the open field crop trial, two doses of 20 and 40 *Trichogramma*/plant, were tested in two plots containing each 300 plants distributed on three rows. In the same way, three untreated rows of 300 plants were left as control. A total of 12 and 24 cardboards containing each 500 *E. kuehniella* eggs parasitized by *T. cacoeciae* were considered respectively for dose 1 and 2 described above. In this context, one cardboard was introduced each 25 and 12 plants respectively. Releases were done on 20/03/2016, 27/03/2016 and 03/06/2016. Plots were separated from each other by 300 plants to avoid possible *T. cacoeciae* dispersal. *Trichogramma* cardboards were fixed by a fine wire on the apical part of tomato plants.

Trichogramma cardboards used for the two experiments were protected by pieces of insect proof (6*2 cm^2) to avoid direct sun light and predators.

To assess the emergency rate of *Trichogramma* for protected and open field crop, 200 parasitized eggs were taken randomly from each piece of cardboard after each release and the number of hatched and unhatched eggs were recorded. The emergency and parasitism rate were calculated according to the following formulas:

Emergency rate (%) = (number of hatched eggs of *E. kuehniella*/total number of released eggs) *100

Parasitism rate (%) = (number of parasitized eggs of *T. absoluta*/ total number of eggs recorded) *100

2.3 sampling of tomato plants

The efficiency of *Trichogramma* mass releases in reducing *T. absoluta* densities, was evaluated by collecting tomato leaves. For this, 30 apical and 30 middle leaves/plant were sampled randomly from each 100 plants. Three replications were considered for each plot described above for both greenhouse and open field trial. Tomato leaves (approximately 10 leaflets/leave) were inspected under binocular microscope (Leica® model MS5) in the laboratory of entomology at National Agronomic Institute of Tunisia.

2.4 Statistical analysis

The software SPSS 21 (SPSS Inc. 2012) was used to perform all statistical analysis. Percentage data were checked for homoscedasticity and normality using Levene and Shapiro-wilk test respectively and were normalized by an arcsine square root transformation when needed. Obtained data corresponding to the emergence rate of *T. cacoeciae*, parasitism rate of *T. absoluta* as well as the effectiveness of *T. cacoeciae* against *T. absoluta* were subjected to repeated measures analysis of variance (GLM procedure). Additional one-way ANOVA followed by Duncan post hoc tests at p=0.05 inside each treatment was carried out. Untransformed data are presented in all tables.

4. Results

4.1 Emergence rate of *T. cacoeciae*

Data of table 1 (A) show that the emergence rate of *T. cacoeciae* under greenhouse conditions ranged from 61.73 to 73.36%. For the open field crop (Table 1 (B)), the emergence rate was higher and reached for the dose 1 (20 *Trichogramma*/plant) in the 3rd release 81.33%. In fact, for both protected and open field crops, for each release, statistical analysis showed that there was no significant difference between the tested doses (table 1 (A, B)). However, and only for open field, there was a significant difference between the three releases only for dose 1 (Table 1 (A, B). The statistical analysis showed that also the interaction between the two factors (doses and release date) had not an effect on the emergence rate of *T. cacoeciae* both in greenhouse greenhouse (df = 2; *P*=0.244) and open field trials (df = 2; *P* =0.187).

Table 1 Emergence rate of *T. cacoeciae* under greenhouse (A) and open field (B) conditions

(A)	**Dose 1**		**Dose 2**		**Dose 3**		**ANOVA**
Date of releases	**AE[1] (Mean ±SE)**	**ER [2] (%)**	**AE (Mean± SE)**	**ER (%)**	**AE (Mean ±SE)**	**ER (%)**	
1st release (15/03/2016)	137.80 ±15.98 aA	68.90	133.20± 37,16aA	66.60	146.72 ±5.59aA	73.36	$F_{2,8}$=0.226; *P* =0.804
2nd release (25/03/2016)	133.45 ±16.77 aA	66.72	146.32± 16.33aA	73.16	136.97 ±13.94 aA	68.48	$F_{2,8}$=0.565; *P* = 0.596
3rd release (31/03/2016)	142.78 ±12.64 aA	71.39	123.47± 26.35aA	61.73	140.27 ±7.08aA	70.13	$F_{2,8}$=1.070; *P* =0.401
ANOVA	$F_{2,8}$=0.278; *P* =0.766		$F_{2,8}$=0.517; *P* =0.621		$F_{2,8}$=0.808; *P* =0.489		

(B)	**Dose 1**		**Dose 2**		**ANOVA**
Date of releases	**AE[1](Mean ±SE)**	**ER[2] (%)**	**AE (Mean±SE)**	**ER (%)**	
1st release (20/05/2016)	155.66±5.92aA	77.83	155.83±8.47aA	77.91	$F_{1,11}$=0.002; *P* =0.969
2nd release (27/05/2016)	159.66±2.65abA	79.83	159.33±5.68aA	79.66	$F_{1,11}$=0.017; *P* =0.899
3rd release (03/06/2016)	162.66±2.87bA	81.33	162.16±4.44aA	81.08	$F_{1,11}$=0.54; *P* =0.822
ANOVA	$F_{2, 17}$=4,405 ; *P* =0,031		$F_{2, 17}$=1,463 ; *P* =0,263		

[1] AE=Adults Emerged. Mean ± Standard Error (SE) followed by the same letter do not differ by Duncan test (P < 0.05). Capital and lower letters following the means represent comparisons within the line and the column respectively. [2] ER= Emergence Rate

4.2 Parasitism rate of *T. absoluta* eggs

Although the effect of the interaction between the distribution of leaves on the plants and the release dose, was not significant for both greenhouse and open field crop (df=0), a higher parasitism rate of *T. absoluta* eggs was recorded on apical leaves. For example, under greenhouse conditions, the parasitism rate recorded on apical leaves was 58.63% and 49.91% respectively for dose 2 (20 *Trichogramma*/plant) and dose 3 (30 *Trichogramma*/plant) (Fig. 1A). Concerning the open field crop, this level was 55.95% on the apical leaves compared to 45.26% in the middle leaves when releasing 40 *Trichogramma*/plant (dose 2) (Fig. 1B)

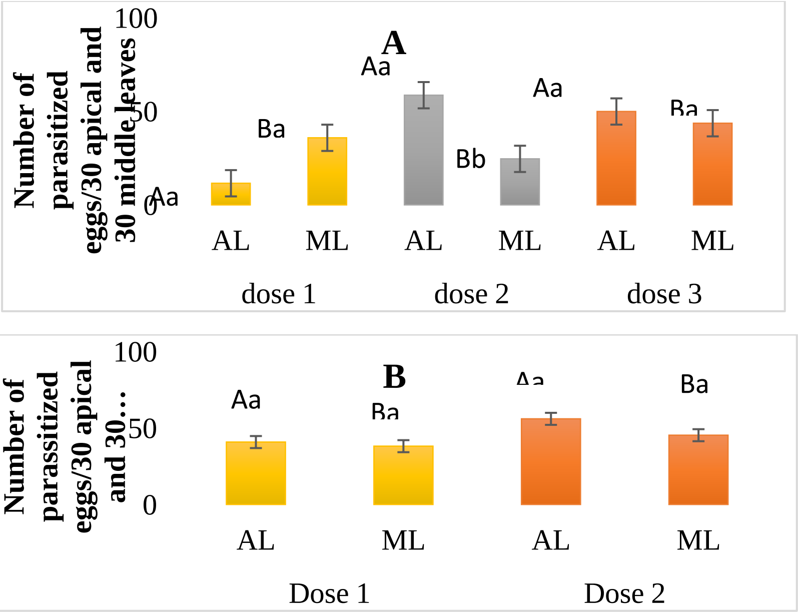


Fig. 1 (A, B). Parasitism rate of *T. absoluta* eggs under greenhouse (A) and open field plot (B) conditions (AL=Apical Leaves; ML=Middle Leaves) (Parasitism rate (%) followed by same letter do not differ by Duncan test ($P < 0.05$). Capital letters represent comparisons within doses for apical and middle leaves separately; Small letters represent comparison within distribution of leaves for each dose)

Statistical analysis that the parasitism rate doesn't differ statistically between the tested doses for both greenhouse and open field trial (Table 2).

Table 2 Statistical analysis results of parasitized eggs in the different parts of the plants sampled (apical and middle leaves) for each dose (ANOVA followed by Duncan test (P < 0.05). (Parasitism rate (%) followed by the same letter do not differ significantly).

	Dose 1	**Dose 2**	**Dose 3**	**One way Anova**
Apical Leaves	$F_{1,15}$=1.946 ; *P* =0.185	$F_{1,15}$=3.109 *P* =0.100	$F_{1,15}$=0.000 ; *P* =0.996	$F_{2,23}$=2.769; *P* =0.086
Middle Leaves				$F_{2,23}$=0.570; *P* =0.574

	Dose 1	**Dose 2**	**One way Anova**
Apical Leaves	$F_{1,17}$=0.230; *P* =0.638	$F_{1,17}$=0.004; *P* =0.950	$F_{1,17}$=0.496; *P* =0.492
Middle Leaves			$F_{1,17}$=0.107; *P* =0.748

4.3The effectiveness of *T. cacoeciae* against *T. absoluta*

4.3.1 Greenhouse trial

4.3.1.1 For eggs

For apical leaves only dose 2 (20 *Trichogramma*/plant) and dose 3 (30 *Trichogramma*/plant) decreased significantly the number of eggs compared to the control untreated ($F_{3,\ 31}$=10.424; *P* <0.0001) (Table 3). However, for middle leaves, the dose 2 and dose 3 gave better results compared to dose 1 (10 *Trichogramma*/plant) in reducing the number of eggs recorded ($F_{3,\ 31}$=5.901; *P* =0.003). For example, on 14/04/2016, on the apical leaves the number of eggs was 13.33±1.24, 8.33±1.24, 4±1.63, 3.66±1.69 respectively for the control untreated dose1, dose 2 and dose 3 (Fig. 2A). On the middle leaves, the number of eggs was on 07/04/2016, 5.66±0.47 for the control untreated while in the treated plots this number was 5.66±0.47, 0 and 1.33±1.88 for dose1, dose 2 and dose 3 respectively (Fig.2B). Also, table 3 indicate that the number of eggs vary significantly between apical and middle leaves for the control untreated as well the three tested doses. However, the interaction between the two factors doses and the distribution of leaves (apical and middle leaves) did not affect significantly the number of eggs after releases of *Trichogramma* (df = 5; *P* =0.213).

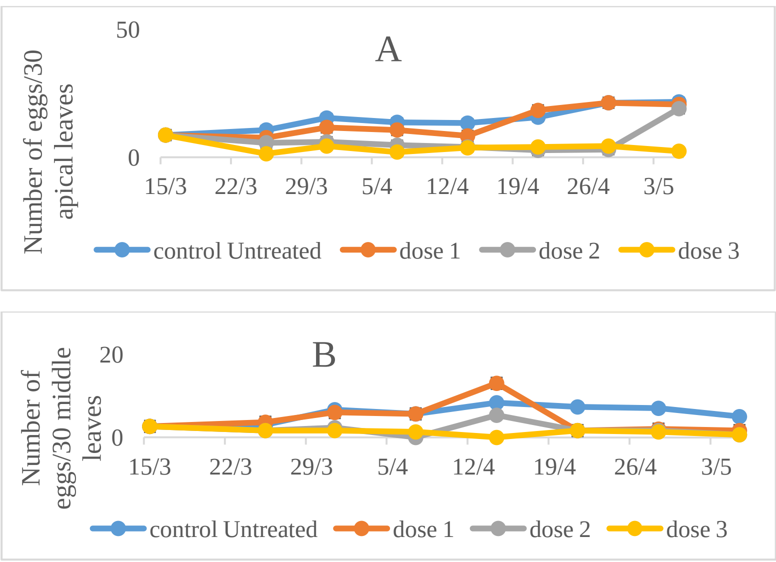


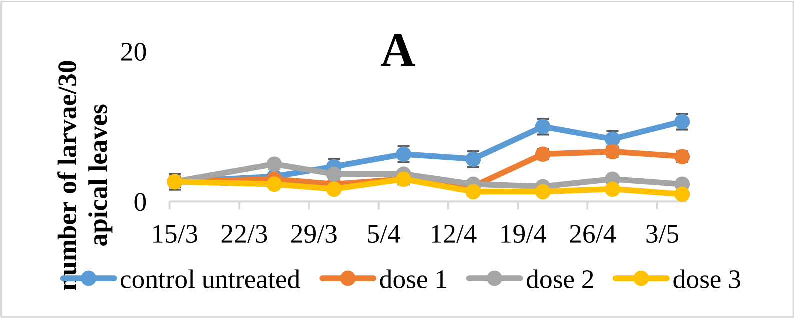
Fig.2 (A, B). Density (Mean ± SE) of *T. absoluta* eggs in the apical (A) and middle leaves (B) per tomato plants after three releases in the control untreated and treated plots under greenhouse conditions (curve with the same letter do not differ by Duncan test (P < 0.05); Capital letter represent comparisons within doses for apical and middle leaves separately; small letter represent comparison within distribution of leaves for each dose).

Table 3 Analysis of variance for *T. absoluta* eggs between the control untreated and treated plots after releases of 3 doses of *Trichogramma* under greenhouse (Cases with the same letter do not differ by Duncan test (P < 0.05)).

	Control	Dose1	Dose2	Dose3
Apical Leaves	$F_{3,31}$=10.424; *P* <0.0001			
Middle Leaves	$F_{3,31}$=5.901; *P* =0.003			
One way ANOVA	$F_{1,15}$=27.444; *P* <0.0001	$F_{1,15}$=14.121; *P* =0.002	$F_{1,15}$=5.751; *P* =0.031	$F_{1,15}$=8.440; *P* =0.012

4.3.1.2 For Larvae

For apical leaves, there was a significant difference between the three doses and the control untreated in decreasing the number of alive larvae ($F_{3, 31}$=8.512; *P* <0.0001) (Table 4). For example, on 21/04/2016, the number of alive larvae was 10±1, 6.33±2.51, 2±1 and 1.33±0.57 for the control and the three doses respectively (Fig. 3A). However, for middle leaves, statistical analysis shows that only dose 2 (20 *Trichogramma*/plant) and 3 (30 *Trichogramma*/plant) had an effect in reducing the pest population (Table 4) ($F_{3, 31}$=14,555; *P* <0.0001). In fact, the number of larvae recorded on 21/04/2016 was 30.33±1.52 for the control untreated while for dose 1, dose 2 and dose 3 this number was 19±1, 5.66±0,57 and 5±1 respectively (Fig. 3B). Additionally, table 4 demonstrates that the number of larvae vary significantly between apical and middle leaves and the interaction between the two factors doses and the distribution of leaves (apical and middle leaves) affect the number of larvae (df=5; *P*<0.0001).



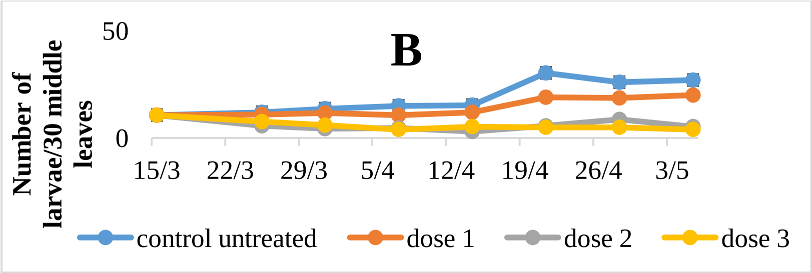


Fig.3 (A, B). density (Mean±SE) of *T. absoluta* larvae in the apical (A) and middle (B) leaves per tomato plants after three releases in the control untreated and treated plots under greenhouse (curve with the same letter do not differ by Duncan test ($P < 0.05$); Capital letter represent comparisons within doses for apical and middle leaves separately; small letter represent comparison within distribution of leaves for each dose).

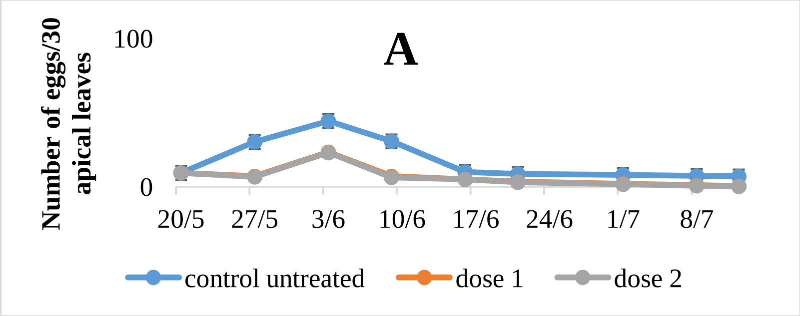
Table 4 Analysis of variance for *T. absoluta* larvae in the control untreated and treated plots after releases of 3 doses of *Trichogramma* under greenhouse (Cases with the same letter do not differ by Duncan test ($P < 0.05$)).

	Control	Dose1	Dose2	Dose3
Apical Leaves	$F_{3,31}$=8.512; *P* <0.0001			
Middle Leaves	$F_{3,31}$=14.555; *P* <0.0001			
One way ANOVA	$F_{1,15}$=17.662; *P* =0.001	$F_{1,15}$=38.851; *P* <0.000	$F_{1,15}$=9.538; *P* =0.008	$F_{1,15}$=24.235; *P* <0.000

4.3.2 Open field trial

4.3.2.1For eggs

Fig.4A indicates that on 12/07/2016 the number of eggs decreased to reach 0.33±0.57 for both dose 1 (20 *Trichogramma*/plant) and dose 2 (40 *Trichogramma*/plant) compared to the control untreated (7±1.73) on the apical leaves. For the middle leaves the number of eggs was 0 on the same date for the two doses compared to the control untreated (7.66 ±0.57) (Fig.4B). Analysis of variance showed that there was a significant difference between the control untreated and the two tested doses for the apical and middle leaves in decreasing the number of eggs (table 5). Statistical analysis showed also that the interaction between the two studied factors (doses and the distribution of leaves (apical and middle)) affect the number of eggs recorded (df= 2; *P* <0.0001) (Table 5).



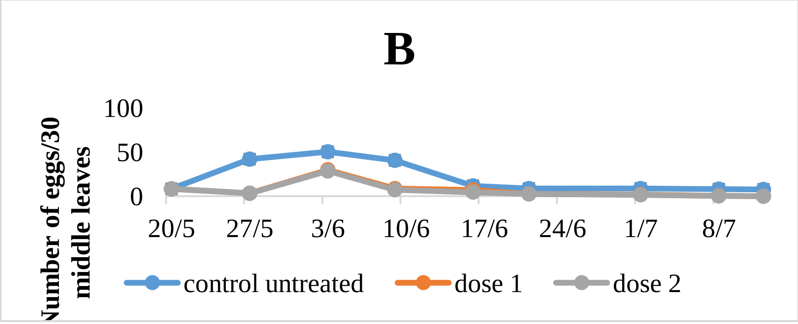


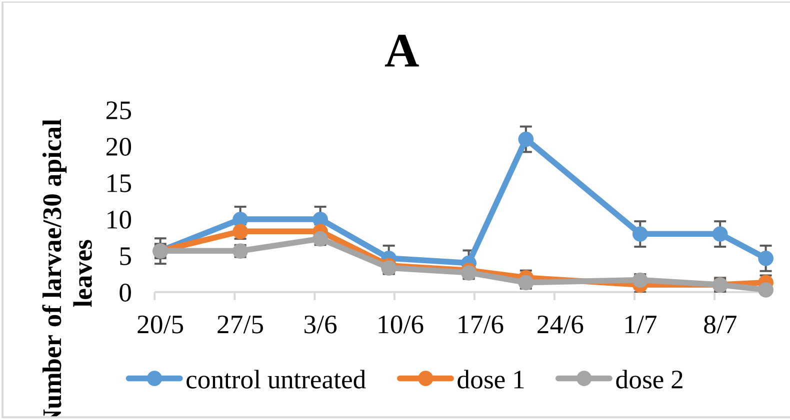
Fig.4 (A, B). Density (Mean±SE) of *T. absoluta* eggs in the apical (A) and middle (B) leaves per tomato plants after three releases in the control and treated plots in the open field plot (curve with the same letter do not differ by Duncan test ($P < 0.05$); Capital letter represent comparisons within doses for apical and middle leaves separately; small letter represent comparison within distribution of leaves for each dose)

Table 5 Analysis of variance for *Tuta* eggs in the control and treated plot after releases of 3 doses of *Trichogramma* in the open field crop (Cases with the same letter do not differ by Duncan test ($P < 0.05$)).

	Control	**Dose1**	**Dose2**
Apical Leaves	$F_{2,26}$=3.680; *P* =0.040		
Middle Leaves	$F_{2,26}$=3.672; *P* =0.041		
One way ANOVA	$F_{1,17}$=0.212; *P* =0.649	$F_{1,17}$=0.016; *P* =0.902	$F_{1,17}$=0.001; *P* =0.977

4.3.2.2 For larvae

Fig. 5 (A, B) showed that the number of larvae decreased significantly compared to the control untreated for dose 1 (20 *Trichogramma*/plant) and dose 2 (40 *Trichogramma*/plant) for the apical leaves as well as the middle leaves. On 21/06/2016, on the apical leaves, the number of larvae decreased to reach 2±1 and 1.33±0.57 respectively for dose 1 and dose 2 compared to the control (21±1.73) (Fig. 5A). For middle leaves and in the same date the number of larvae was 4.33±1.15 for dose 1 and 3.66±0.57 for dose 2 compared to the control untreated (57.66±2.30) (Fig. 5 B). Analysis of variance showed that the number of larvae varied significantly between apical and middle leaves only for the control treatment (Table 6). Also, statistical analysis showed that the number of larvae was influenced by the interaction between doses and distribution of the leaves (apical and middle leaves) (df=2; $P < 0.0001$) (Table 6).



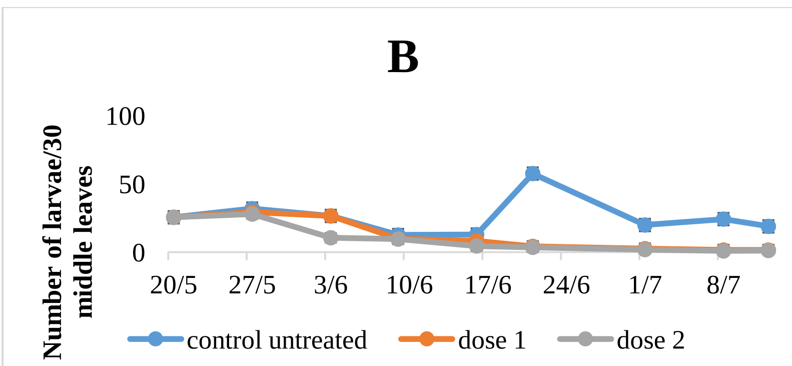


Fig. 5 (A, B). Density (Mean±SE) of *T. absoluta* larvae in the apical (A) and middle (B) leaves per tomato plants after three releases in the control and treated plot in the open field (curve with the same letter do not differ by Duncan test ($P < 0.05$); Capital letter represent comparisons within doses for apical and middle leaves separately; small letter represent comparison within distribution of leaves for each dose).

Table 6 Analysis of variance for *T. absoluta* larvae in the control and treated plot after releases of 3 doses of *Trichogramma* in the open field crop (Cases with some letter do not differ by Duncan test (P < 0.05)).

	Control	**Dose1**	**Dose2**
Apical Leaves	$F_{2,26}$=5.231; *P* =0.013		
Middle Leaves	$F_{2,26}$=4.687; *P* =0.019		
One way ANOVA	$F_{1,17}$=12.623; *P* =0.003	$F_{1,17}$=4.0469; *P* =0.051	$F_{1,17}$=3.263; *P* =0.090

5. Discussion

T. cacoeciae is considered as a promising biocontrol agent of Lepidopteran insect pest especially *T. absoluta*. In protected and open field crops, a significant reduction in the infestation level was recorded. Obtained results showed that the emergence rate of *T. cacoeciae* under greenhouse was up to 73.36% and differ to it in the open field crop which can reach 81.33%. This difference may be related to the differences in climatic data values may be more adequate in the field condition. In a previous work, Cherif and Lebdi-Grissa, (2013), studied the efficacy of *T. cacoeciae* to control *T. absoluta*, through releases of 30 *Trichogramma*/tomato plant performed in open field conditions in Northeastern Tunisia. These authors indicated that the emergence rate of this parasitoid was in average more than 63% (Cherif and Lebdi-Grissa, 2013). The same authors demonstrated that differences in *T. cacoeciae* emergence rates may be linked either to abiotic conditions such as differences in temperature and relative humidity values or to the quality of *E. khueniella* eggs used as host for mass rearing to this parasitoid (Cherif and Lebdi-Grissa, 2013). Data of the present study indicated, for the different releases rates, that the parasitism rate of *T. absoluta* eggs was as important as that find by Cherif and Lebdi-Grissa, (2013). In fact, these authors indicated that *T. cacoeciae* caused an average rate of parasitism of about 54.7%

on *T. absoluta* eggs (Cherif and Lebdi-Grissa, 2013). Moreover, the high parasitism rates found in this study may be linked to the low pest density present before *T. cacoeciae* releases (11.32 and 17.66 eggs/60 leaves found on 15/03 and 20/05/2016 respectively under greenhouse and open field crop) and the growth stage of the tomato crop (before the increase of plant architecture complexity). Various factors may affect the efficiency of the parasitoid in finding hosts including the increasing plant architecture complexity (Rutledge and O'Neil 2005, Tabone et al. 2012) and the small size of the parasitoids emerging from *T. absoluta* eggs which make parasitoid females mature fewer eggs (Kazmer and Luck 1995). Other *Trichogramma* species tested on *T. absoluta* eggs showed higher parasitism rate such as *T. achaeae* (Nagaraja and Nagarkatti) which gave a parasitism rate of about 83.3% under laboratory conditions (Cabello et al., 2009). The number of parasitized *T. absoluta* eggs varied greatly depending on the different strains used. Khanh et al., (2012) showed that the proportion of *Trichogramma* females that parasitized *T. absoluta* eggs was significantly different between strains used and it was between 0 and 100%. In a previous research, Sarhan et al., (2015) showed that *T. bactrae* (Nagaraja) was the most effective parasitoid characterized by higher percentages of parasitism, followed by *T. evanescens* (Westwood), *T. cacoeciae* (Marchal) and *T. pretiosum* (Riley). Cabello et al. (2012) evaluated the biotic potential of two species of *Trichogramma* against tomato leaf miner and they found that *T. achaeae* was better at controlling *T. absoluta* populations than *T. urquijoi*. Here, despite no significant difference was found between parasitim rates, it has been demonstrated that apical and middle leaves showed a high parasitism rate when releasing 20, 30 and 40 *Trichogramma*/plant compared to dose of 10 *Trichogramma*/plant. Previous studies showed that the parasitism rate for all the parasitoid species was influenced by the number of released parasitoids and spacing among release points (Agamy, 1994; Sarhan et al., 2015) given that species of *Trichogramma* were characterized by their reduced capacity of flight and they reach their host eggs by walking and jumping (Chailleux et al., 2013). We investigated through this study that apical leaves host a high parasitism rate of *T. absoluta* eggs in both greenhouse (on average 13.47%; 68.40% and 58. 23% respectively for dose 1, dose 2 and dose 3) and open field crop (on average 46.61 and 63.95% respectively for dose 1 and dose 2). This result may be linked to the higher number of eggs laid by *T. absoluta* on apical leaves compared to the leaves of medium portion. In fact, previous studies demonstrated, *T. absoluta* females exhibited a preference to lay eggs on leaves of the tomato plant apex characterized by their lower calcium content and their tender side **(**Leite et al., 1999; Cherif et al., 2013). In the same way, Muniappan (2013) indicated that *T. absoluta* lays 73% on leaves compared with 21%, 5%, and 1% on veins, stems, sepals and fruits, respectively. Furthermore, previous studies demonstrated that *Trichogramma* performance may be affected negatively by trichomes of tomato plant (Kauffman and Kennedy, 1989; Farrar et al., 1994; Chailleux et al., 2013) as well as the size of *T. absoluta* eggs which were 3-fold narrower than the eggs of *E. kuehniella* used in the mass rearing as a substitute host (Roriz et al., 2006; Chailleux et al., 2012). In this context, Cascone et al. (2015) found in a previous research that one generation of *T. achaeae* (Nagaraja and Nagarkatti) reared on *T. absoluta* eggs laid on tomato leaf was able to improve significantly *T. achaeae* parasitism rate. Moreover, the authors noted, that the combined effect of rearing system (plant + host egg) and temperatures (during development and use) may improve the longevity and fertility of the parasitoid (Cascone et al., 2015). Obtained data indicated that the number of *T. absoluta* eggs vary significantly between apical and middle in protected and open field crops. Our result was in accordance with previous studies indicating that *T. absoluta* prefer laying eggs in the apical leaves of tomato plants as explained above (Torres et al., 2001; Leite et al., 1999; Leite et al., 2004; Cherif et al., 2013). Our results demonstrated also that the number of *T. absoluta* larvae was significantly higher on the middle leaves for both greenhouse and open field crops which may be explained by the fact that larvae migrate through the plant and condenses in the medium portion containing more leaves (Cherif at al., 2013). Cuthbertson et al. (2013) showed that the larvae after hatching wandered around the leaf surface for an average of 12 minutes and about 15 mm from its egg shell before starting to graze on the leaf surface. Also, our result showed that the interaction between the two factors doses and distribution of leaves had an effect on decreasing the number of larvae in protected and open field crops. However, this interaction wasn't demonstrated statistically for eggs in greenhouse area. Concerning the effectiveness of *T. cacoeciae* against *T. absoluta*, under greenhouse, dose 2 (20 *Trichogramma*/plant) and dose 3 (30 *Trichogramma*/plant) decreased significantly the number of pest eggs compared to the control and gave better results than dose 1 (10 *Trichogramma*/plant). Likewise, in the open field crop, the two tested (20 and 40 *Trichogramma*/plant), which are not different statistically, decreased significantly the number of eggs compared to the control untreated. Moreover, the number of alive larvae decreased after releases of 20, 30 and 40 *Trichogramma*/plant for both greenhouse and open field crop. Only dose of 10 *Trichogramma*/plant tested in the greenhouse was not efficient to control *T. absoluta*. Previous studies demonstrated that the two tested doses (30 and 40 *T. cacoeciae*/tomato plant) were proved efficient to control *T. absoluta* in north and south Tunisia (Zouba and Mahjoubi, 2009; Cherif and Lebdi-Grissa, 2013). In other countries, southern Spain as example, Cabello et al., (2009) showed that 30 adults per tomato plant released every 3 or 4 days under greenhouse were able to reduce infestations levels caused by the tomato leaf miner *T. absoluta*.

In summary, this study highlights the effectiveness of *T. cacoeciae* to control *T. absoluta* and proved that density of 20 *T. cacoeciae* adults/tomato plant was chosen as the most suitable (from a coast point of view) compared to the other released doses (10, 30 and 40 *T. cacoeciae* adults/tomato plant) in both greenhouse and open field conditions. However, to improve the biological control of *T. absoluta*, recent studies suggest the combined use of *Trichogramma* with predator such as *Macrolophus pygmaeus* (Rambur) (Chailleux et al., 2013). Chailleux et al., (2013) suggested the use of several inundative releases of *Trichogramma* parasitoids for biological control of *T. absoluta* in tomato crops taking into consideration the presence of generalist predators either artificially released, naturally occurring, or both.

So a successful biological control program against *T. absoluta* when releasing *Trichogramma sp.* in combination with a predator, a high number of the pest should be suppressed and the predator must be able to discriminate against parasitized prey in order to reduce the intraguild predation (Colfer and Rosenheim, 2001) which is the case for example when studying the relationship of *T. achaeae* and *T. urquijoi* with *Nesidiocoris tenuis* (Reuter) (Cabello et al., 2012). Our results proved that *T. cacoeciae* releases are promising tools for controlling *T. absoluta* populations in tomato crops in Tunisia. Nevertheless, in order to pursue and to optimize such control methods, more field trials are needed. Moreover, biological and chemical pest management strategies for the control of *T. absoluta* adopted by farmers must be reviewed.

6. Acknowledgements

Special thanks to farmers for their valuable contribution in this work.

VI.3 Conclusion

Les résultats obtenus prouvent l’efficacité de *T. cacoeciae* comme agent de lutte biologique contre *T. absoluta*. La dose recommandée est de 20 adultes par plant de tomate à utiliser sous serres et en plein champ. Cependant, plusieurs programmes de lutte signalés comme efficaces, ont été basés sur l’utilisation de trichogrammes en combinaison avec des prédateurs ou bien des insecticides. Cependant, l’application de pesticides peut influencer l’efficacité des lâchers de trichogrammes.

Effets non intentionnels des insecticides contre *Trichogramma cacoeciae* (Marchal) (Hymenoptera : Trichogrammatidae) sous conditions de laboratoire

V. Chapitre 5 : Effets non intentionnels des insecticides contre *Trichogramma cacoeciae* (Marchal) (Hymenoptera : Trichogrammatidae) sous conditions de laboratoire

V.1 Introduction

Afin d'étudier les effets secondaires et non intentionnels des pesticides vis-à-vis des ennemies naturels présents dans les parcelles de tomate en Tunisie, onze insecticides (l'indoxacarbe, le spiromesifen, la cyromazine, le chlorfenapyr, la cypermethrine, le diafenthiuron, le chlorantranliprole, le spinosad, l'azadirachtine, le *Bacillus thuringiensis* et le virus HaNPV) ont été testés sur les différents stades de développement de *T. cacoeciae* sous conditions de laboratoire.

Le travail a permis de mettre en évidence l'utilisation de produits chimiques à la fois efficaces pour le contrôle de *Tuta absoluta* et non toxiques vis-à-vis de *T. cacoeciae*.

Les résultats obtenus sont présentés dans l'article 6 qui a été publié dans le journal <Phytoparasitica>.

V.2 Life-stage-dependent side effects of selected insecticides on *Trichogramma cacoeciae* (Marchal) (Hymenoptera: Trichogrammatidae) under laboratory conditions (Article 6).

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Running title: Side effect of some insecticides on *Trichogramma cacoeciae*

Abstract

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is classified as one of the most harmful pest of tomato crops. Many species of predators and parasitoids including *Trichogramma cacoeciae* (Marchal) (Hymenoptera: Trichogrammatidae) are noted as potential candidates used for biological control of this pest.

Therefore, the use of selective insecticides is critical to conserve and protect natural enemies in the field**.** This study assessed the side effects of insecticides on different development stages of *T. cacoeciae* under laboratory conditions. For this, eleven pesticides such as: Indoxacarb, spiromesifen, cyromazin, chlorfenapyr, cypermethrin, diafenthiuron, chlorantraniliprole, spinosad, azadirachtin, *Bacillus thuringiensis (Bt)* and virus HaNPV were tested. This study shows that indoxacarb, spiromesifen, chlorfenapyr, cypermethrin, diafenthiuron and spinosad had a negative effect on immature stages of *Trichogramma*. All insecticides residues on tomato leaves were found to be toxic to *Trichogramma* adults except azadirachtin, *Bt* and virus HaNPV. Therefore, the use of the tested natural products (azadirachtin, *Bt* and HaNPV) at the recommended doses is viable, having no negative impact on *T. cacoeciae* in tomato crops.

Key words: pesticide toxicity, *T. absoluta*, *T. cacoeciae*, tomato

1. Introduction

Tomato (*Solanum lycopersicon* L.) is considered as a strategic culture in Tunisia. It is cultivated in an area of about 29,000 Ha producing an average yield of about 1.2 thousand tons (GIL 2016). Tomato crops may be threatened by a large number of diseases and pests including mites, aphids and lepidopteran species belonging to the families Noctuidae and Gelechiidae. (Santini 2001; Fernandes 2003; Brust and IPM Vegetable Specialist 2008; Cherif and Lebdi-Grissa 2015). The tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is considered as one of the most harmful pest of tomato crops, causing up to 100% yield losses in newly invaded areas if control methods are absent (Desneux et al. 2010). Although various management strategies such as cultural practices, biological control and mating disruption technique were carried out to control *T. absoluta* in Tunisia and other countries around the world (Caparros Megido et al. 2012; Chermitti and Abbes 2012; Cocco et al. 2012, 2013; Cherif and Lebdi-Grissa 2013, 2014), chemical products are still the main pest control method presently used by farmers worldwide (Carvalho 2010). However, repeated application of insecticides may cause pesticides resistance (Gassen 1996; Lietti et al. 2005; Moreno et al. 2012). Resistance of *T. absoluta* to many active ingredients

including abamectin, spinosad, chlorantraniliprole has been reported in South America and recently in Europe (Lietti et al. 2005; Haddi et al. 2012; Campos et al. 2015; Roditakis et al. 2015). Biological control may be a promising alternative to insecticides application (Da silva and Bueno 2015). Various natural enemies have proven to be effective against *T. absoluta* such as mirid predators [*Nesidiocoris tenuis* (Reuter), *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae)] and parasitoids (Chailleux et al 2013; Abbes et al. 2016; Biondi et al. 2016; Naselli et al. 2016). Egg parasitoid belonging to the *Trichogramma* genus (Hymenoptera: Trichogrammatidae) was considered one of the most important beneficial organisms known for being highly effective against a large number of lepidopteran pests such as *T. absoluta* (Hassan and Abdelgader 2001; Braga Maia 2013; Chailleux et al. 2013). In Tunisia, the autochthone strain *Trichogramma cacoeciae* (Marchal) is mass reared and has shown a good efficacy on reducing *T. absoluta* densities when released in both open field and protected crops in Tunisia (Cherif and Lebdi-Grissa 2013; Zouba et al. 2013). Often, to manage *T. absoluta* the integration of chemical and biological control may lead to a successful Integrated Pest Management program (IPM) (Smilanick et al. 1996; El-Wakeil and Vidal 2005; El-Wakeil et al. 2006; Volkmar et al. 2008). Therefore, the use of selective insecticides is critical to conserve and protect natural enemies in the field (Carvalho et al. 2003). A significant advantage of selective insecticides is their effectiveness against the pest, with minimal side effects on non-target arthropods. Thus, the study of side effects of insecticides is crucial to enhance the combined effect of chemical and biological control tools (Youssef et al. 2004).
Thus, the study is to investigate the side effects of some insecticides commonly used mainly against *T. absoluta* in tomato crops in Tunisia on immature stages and adults of *T. cacoeciae* under laboratory conditions.

2. Material and methods

2.1 Insecticides

Eleven insecticides currently authorized on tomato crops by Tunisian ministry of Agriculture (AVFA 2012) were evaluated (Table 1). Pesticides were prepared accordingly to the rates normally recommended to be used in tomato crops and considering the volume of mix per hectar.

Table 1: Characteristics of tested insecticides

Trade name, Supplier name	**Active ingredient, concentration, Formulation type**	**Doses**	**Pests**	**Main group and primary site of action**
AMIRAL, EL MOUSSEM AGRI.	Indoxacarb, 150g/l, SC	50 cc/hl	*T. absoluta*	Voltage-dependent sodium channel blockers: Nerve action
OBERON, ATLAS AGRICOLE	Spiromesifen, 240g/l, SC	60 ml/hl	Mites	Inhibitors of acetyl CoA carboxylase **:** growth regulation
TRACER, INNOVA	Spinosad, 240g/l, SC	60 ml/hl	Noctuidae	Nicotinic acetylcholine receptor (nAChR) allosteric modulators **:** Nerve action
CLAVE, ETS. MEZGHANI	Cyromazin, 750g/kg, WP	30 g/hl	*T. absoluta*	Moulting disruptor: Growth regulation
BACTOSPEIN, SEPCM	*Bacillus thuringiensis*, 16000 UI/mg, WP	450 g/hl	*T. absoluta*	Microbial disruptors of insect midgut membranes
NIMBICIDINE, BIOLCHIM TUNISIE	Azadirachtin (0.03%) + neem oïl (90,5%), EC	250 cc/hl	*T. absoluta*	UN * Compounds of unknown or uncertain MoA
CHALENGER, AGRIPROTEC	Chlorfenapyr, 240g/l, SC	50 cc/hl	*T. absoluta*	Uncouplers of oxidative phosphorylation via disruption of the proton gradient: Energy metabolism
CYPERCAL 250 EC,	Cypermethrin, 250g/l, EC	50cc/hl	*T.*	Sodium channel modulators:

BIOPROTECTION			*absoluta*	Nerve action
PEGASUS 500 EC, BIOPROTECTION	Diafenthiuron, 500g/l, SC	100 ml/hl	*T. absoluta*	Inhibitors of mitochondrial ATP synthase: Energy metabolism
CORAGEN 20 SC, SEPCM	Chlorantraniliprole, 200g/l, SC	50 ml/hl	*T. absoluta*	Ryanodine receptor modulators: Nerve and muscle action
HELICOVEX, STIMA	*Helicoverpa armigera* 7.5 10^{12} cfu/l, SC	0.2 l/ha	*H. armigera*	Naturally occurring insecticidal virus that specifically infects and kills larvae of *H. armigera*

EC emulsifiable concentrate, SC suspension concentrate, WP wettable powder

2.2 Insects and tomato plants

The experiments were carried out in the Department of Entomology and Acarology at National Agronomic Institute of Tunisia. Fifty cm height untreated tomato plants (Var. Galaxy; 40-days old; number of leaves/plant) used for experiments were grown from seed in plastic pots (Ø 48 * H 40 cm) set in an experimental greenhouse under natural environmental conditions. In total seventy-two tomato plants were used for the experiments. Colony of *T. cacoeciae* used in this study was collected in various locations in the oasis of the south-west of Tunisia from infested dates by the carob moth *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae). Parasitoids were reared on *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs, considered the best alternative host to keep this parasitoid colony in laboratory (Gomes and Parra 1998). *Ephestia küehniella* (Zeller) (Lepidoptera: Pyralidae) was reared in plastic boxes containing whole wheat flour and maintained in climatic chamber in controlled conditions (25 ± 2° C, 60 ± 10% RH, and 16:8 L: D). *Ephesia kuehniella* eggs were UV sterilized, glued on cardboard strips (5 x 1 cm^2) with diluted Arabic gum and offered for 24 hours to recently emerged *T. cacoeciae* females in vials of 40 ml (8.5*2.5 cm^2). The number of females used was enough to guarantee close to 100% of parasitism during the first 48h of females' life time (Blibech et al. 2011; Ksentini et al. 2011). After that, the cards were placed in plastic vials (40 ml) (8.5*2.5 cm^2) and kept in controlled conditions (25 ± 2° C, 60 ± 10% RH and 16:8 L: D). Adults of *T. cacoeciae* were fed with honey droplets smeared in the inner wall of the vials.

2.3 Toxicity bioassay

2.3.1 Toxicity on immature stages

For each treatment, six cards (5 * 1 cm^2) containing each approximately 300 *E. kuehniella* eggs were exposed to 15 females' wasp confined in vials for each treatment. After 24 h, *T. cacoeciae* adults remaining on eggs were gently removed using a fine brush. Then, these cards were dipped into insecticides solutions at the recommended rates or in tap water used as control (Table 1) for 10 seconds either 3 day (larvae), 6 days (pre-pupae) and 9 days (pupae) after initial parasitism (Knutson 1998; Ksentini et al. 2010). Treated cards were left to dry in the laboratory for one hour to eliminate the excess of

humidity. After that, the cards were placed inside new clean vials (xx ml, 8.5 * 2.5 cm^2) and incubated to the growth chamber (25 ± 2° C, 60 ± 10% RH, and 16: 8 L: D).
In order to check mortality during immature development, treated cards were inspected daily until parasitoid adult emergence. A final assessment of emergence was made 15 days after initial parasitism.

2.3.2 Residue toxicity on adults

In order to test the residual toxicity, seventy-two tomato plants described above were sprayed individually with the insecticides mentioned on table 1. Tap water was used for the control. Sprays were done separately for each treatment using two-liter plastic bottle sprayer until runoff. Treated tomato plants were kept inside the experimental greenhouse and six leaflets were taken randomly from each plant, 1, 2 or 6 days after treatments. Each leaflet was placed individually in vials (8.5 * 2.5 cm^2) containing 30 individuals of *Trichogramma* adults (<24 hours) fed with honey droplets smeared in the inner wall of the vials. Each treatment was replicated six times.
Vials were kept under the controlled conditions described above. Mortality was assessed at 24 hours and 10 days' post exposure.

2.4 Statistical analysis

Data were tested for homoscedasticity and normality using Levene and Shapiro-wilk test respectively. Arcsine square root transformation was performed for all percentage data before analysis to stabilize error variance (Gomez and Gomez 1984). The untransformed means are presented in table 2 and 3. Data were subjected to repeated measures analysis of variance (GLM procedure) to test the interaction between the effects of insecticides and timing after pesticide exposure, followed by one-way ANOVA (Duncan test for mean separation at $P < 0.05$) (SPSS 21, 2012). To determine the toxicological categories, reduction (R %) in emergence and survival rate of adults is calculated using this formula: Reduction (R %) =100-((insecticide)/control water) *100) (Hassan 1998). The value R (%) calculated for each pesticide treatment was classified accordingly to the International Organization of Biological Control (IOBC) where: class 1 = harmless (R<30 %), class 2 = slightly harmful (30 ≤R<80 %), class 3 = moderately harmful

(80 % ≤ R ≤ 99 %) and class 4 = harmful (R>99 %) (Sterk et al. 1999; Boller et al. 2005).

3. Results

3.1 Toxicity bioassay

3.1.1 Toxicity on immature stages

Results of the side effects of widely used insecticides in Tunisia tomato crops, on the development of immature stage of *T. cacoeciae* are given in the table 2. The tested chemicals differed markedly in their toxicity. The emergence of wasps was significantly reduced after insecticide treatments at the three immature developmental stages of the parasitoid (larva, pre-pupa and pupa) ($F_{11, 71}$=7.467; *P*<0.0001). Table 2 shows that *Bt*, cyromazin, HaNPV, azadirachtin and chlorantraniliprole were found to be harmless (Class 1) to all immature developmental stages of *T. cacoeciae*. However, diafenthiuron, chlorfenapyr, cypermethrin, indoxacarb and spinosad were slightly harmful (Class 2). Only spiromesifen was moderately harmful on *T. cacoeciae* larva (Class 3) and gave the lowest emergency rate for all development of immature stage (20.90 ± 9.06, 31.78 ± 8.41 and 31.78 ± 13.14 respectively for the larva, pre-pupa and pupa). Table 2 shows that there was no significant difference between the three treated stages regarding their responses to each insecticide. Statistical analysis showed that there was a significant difference on the interaction between insecticides and timing of exposure (df=65, *P*=0.002).

Table 2: Effect of insecticides on the immature development stage of *T. cacoeciae* treated at 3, 6 and 9 days (25 ± 1 °C; 60 ± 10% RH).

Treatement	Larva			Prepupa			Pupa			Statistical analysis
	ER [1]	R^2	C [3]	ER	R	C	ER	R	C	
Diafenthiuron	58.76±15 .17Ab	35.2 9	2	64.41 ±15.13Acde	32.32	2	61.31 ± 9.07 A bc	32.68	2	$F_{2, 17}$=0.279 ; *P* =0.760
Cypermethrin	32.18±12 .33Aa	61.4 6	2	29.98 ±10.86Aa	68.87	2	44.85±16.59 Aab	53.90	2	$F_{2, 17}$=0.2005 ; *P* =0.169
Spiromesifen	20.90± 9.06Aa	79.4 4	3	31.78±8.41Aa	73.17	2	31.78±13.14 Aa	68.38	2	$F_{2, 17}$=1.751 ; *P* =0.207
Chlorantraniliprole	58.06±15 .70Ab	29.5 8	1	73.81±5.74Adef	21.70	1	63.77±11.83 Abcd	29.18	1	$F_{2, 17}$=2.618 ; *P* =0.106
Azadirachtin	79.29 ± 13.96Acd	6.50	1	87.42±8.04Aef	7.26	1	83.67±8.04Ac de	5.84	1	$F_{2, 17}$=0.699 ; *P* =0.513
Indoxacarb	25.74± 19.57Aa	70.3 0	2	42.14±41.73Abc	51.12	2	44.23±16.30 Aab	45.25	2	$F_{2, 17}$=0.618 ; *P* =0.552
Spinosad	34.72±28 .80Aa	59.9 4	2	47.89±36.46Abcd	68,46	2	59.59±26.88 Abcd	4348	2	$F_{2, 17}$=1.069 ; *P* =0.368
HaNPV	66.18±17 .45Abc	23.6 6	1	79.40±22,02Aef	17.75	1	84.73±13,61 Ade	3.81	1	$F_{2, 17}$=1.959 ; *P* =0.175
Cyromazin	83.20±7. 44Acd	4.03	1	73.01±20.40Adef	17.86	1	87.84±8.62Ae	0.62	1	$F_{2, 17}$=1.941 ; *P* =0.178
Bt	90.14±7.	-3.96	*	85.38±15.4Aef	9.14	1	82.88±13.38	7.10	1	$F_{2,}$

	57Ad						Acde			$_{17}$=0.661 ; *P* =0.531
Chlorfenapyr	30.08±8.76Aa	65.32	2	28.11±6.93Aa	69.94	2	41.42±37.07 Aab	68.85	2	$F_{2, 17}$=0.401 ; *P* =0.677
Control water	86.70±8.87Ad	-	-	94.14±5,05Af	-	-	88.75±10.91 Ae	-	-	$F_{2, 17}$= 0.947 ; *P* =0.410
Statistical analysis	$F_{11, 71}$=16.585 ; *P* <0.0001			$F_{11, 71}$=7.467 ; *P* <0.0001			$F_{11, 71}$=7.164 ; *P* <0.0001			

[1]ER= Emergence rate (%). Means ± Standard Error (SE) followed by the different alphabets are statistically different by Duncan test (P < 0.05). Capital and small case letters following the means represent respectively comparisons within a line and a column.
[2]R=Reduction (%)
[3]C=Class: Class 1 = harmless (R < 30%), Class 2 = slightly harmful (30 ≤ R ≤ 79%), Class 3 = moderately harmful (79 ≤ R ≤ 99%) and Class 4 = harmful (R > 99%) (Sterk et al. 1999; Boller et al. 2005).
*Emergency rate given by the insecticide was greater compared to the control water.

3.1.2 Residual toxicity on adults

Table 3 shows that *Trichogramma* adults were significantly affected by insecticide treatments ($F_{11,\ 71}$=51.714; *P*<0.0001) and the insecticides' toxicity toward the parasitoid decreased significantly with time as shown for diafenthiuron, cypermethrin, spiromesifen, chlorantraniliprole, indoxacarb, spinosad and chlorfenapyr (table 3). Statistical analysis showed that there was a significant difference for the interaction between treatments and exposure time (df=65, *P*=0.002). Our results showed that only azadirachtin, *Bt* and virus HaNPV can be considered harmless (Class 1) to *Trichogramma* adults 1, 2 and 6 days after treatments. However, spinosad and chlorfenapyr were considered to be the most toxic and classified harmful (Class 4) to moderately harmful (Class 3), respectively, 1 to 2 days after treatments. Diafenthiuron, cypermethrin and spiromesifen were characterized as moderately harmful (Class 3) 1 day after treatment, but their toxicity decreased to become harmful (Class 2) after 2 and 6 days. Indoxacarb, cyromazin and chlorantraniliprole were slightly harmful (class 2) 1 and 2 days after treatments and harmless (Class 1) 6 days after treatments.

Table 3: Percentage of *T. cacoeciae* adult survival after 24 h of exposure to insecticides residues on tomato leaves at 1, 2 and 6 days (25 ± 1 °C; 60 ± 10% RH).

Treatement	1d			2d			6d			Statistical analysis
	PS [1]	R^2	C [3]	PS	R	C	PS	R	C	
Diafenthiuron	13.33± 5.96Ac	85.27	3	47.77±11.65Bcd	50.57	2	59.44±6.78Bb	39.54	2	$F_{2, 17}$=42.599 ; *P* <0.0001
Cypermethrin	7.22±2.5Abc	92.02	3	42.22±10.48Bc	56.32	2	60.55±7.04Cb	38.41	2	$F_{2, 17}$=102.035 ; *P* <0.0001
Spiromesifen	6.11±5.34Ab	93.25	3	43.33±5.09Bc	55.17	2	58.88±6.57Bb	40.11	2	$F_{2, 17}$=57.415 ; *P* <0.0001
Clorantraniliprole	31.11±14.24Ad	65.64	2	55±6.59Bcde	43.10	2	70.55±7.30Bbc	28.24	1	$F_{2, 17}$=15.684 ; *P*<0.0001
Azadirachtin	64.44±14.70Ae	28.83	1	70.55±10.78Aefg	27.01	1	78.88±9.55Abcde	19.77	1	$F_{2, 17}$=2.089 ; df=17 ; *P* =0.158
Indoxacarb	22.22±19.05Acd	75.46	2	57.22±16.60Bcdef	40.80	2	77.22±21.72Bbcd	21.46	1	$F_{2, 17}$=10.093 ; *P* =0.002
Spinosad	0.55±1.36Aa	99.38	4	3.88±6.21 Aa	95.97	3	12.22±6.57Ba	87.57	3	$F_{2, 17}$=9.167; *P* =0.003
HaNPV	68.33±9.12Aef	24.53	1	75.55±10.30Afg	21.83	1	75.55±19.68Abcd	23.16	1	$F_{2, 17}$=0.373 ; *P* =0.695
Cyromazin	62.77±17.30Ae	30.67	2	62.77±15.56Adefg	35.05	2	82.77±17.15Acde	15.81	1	$F_{2, 17}$=2.402 ; *P* =0.124

Bt	82.77±14.81 Aef	8.58	1	81.11±6.57 Agh	16.09	1	95±4.19Ade	3.38	1	$F_{2, 17}$=3.336 ; p=0.063
Chlorfenapyr	0±0Aa	100	4	13.33±8.81 Bb	82.20	3	20.55±15.32Ba	79.09	2	$F_{2, 17}$=12.588 ; *P* =0.001
Control water	90.55±6.46Af	-	-	96.66±3.84 Bh	-	-	98.33±2.54Be	-	-	$F_{2, 17}$=4.487 ; *P* =0.03
Statistical analysis	$F_{11, 71}$=51.714 ; *P* <0.0001			$F_{11, 71}$=35.402 ;; *P* <0.0001			$F_{11, 71}$=26.679 ; *P* <0.0001			

[1]PS=Percent Survival (%). Means ± Standard Erreur (SE) followed by the different alphabets are statistically different by Duncan test (P < 0.05). Capital and lower case letters following the means represent respectively comparisons within a line and a column.
[2]R=Reduction (%)
[3]C=Class: Class 1 = harmless (R < 30%), Class 2 = slightly harmful (30 ≤ R ≤ 79%), Class 3 = moderately harmful (79 ≤ R ≤ 99%) and Class 4 = harmful (R > 99%) (Sterk et al. 1999; Boller et al. 2005).

4. Discussion

Our results showed that insecticides had a negative effect on immature stages (larval; pre-pupal and pupal stages) of *T. cacoeciae* except chlorantraniliprole, cyromazin, azadirachtin, *B*t and virus HaNPV classified as harmless (Class 1). Our results coincide with previous studies which demonstrated that these insecticides cited above are not toxic to many species of *Trichogramma* such as *T. pretiosum* (Riley) (Carvalho et al. 2003; Da Silva et al. 2015), *T. chilonis* (Ishii) (Hussain et al. 2012; Khan et al. 2014) and *T. nubilale* (Ertle & Davis) (Chen et al. 2013). The rest of tested compound acts differently with other species of *Trichogramma*. Hewa-Kapuge et al. (2003), and Chen et al. (2013) showed that indoxacarb (10 g/hl) was safe for all development stages of *T. nubilale*. Ksentini et al. (2010) studied the effect of two doses (70 and 100 g/hl) of *Bt* (Berliner) on immature stages of *T. cacoeciae*, *T. bourarachae* (Pintureau & Babault) and *T. evanescens* (Westwood). These authors showed that the higher dose of *Bt* (100 g/hl) was slightly harmful only to *T. bourarachae* prepupae stage compared to the low dose (70 g/hl) considered as harmless towards the tested species of *Trichogramma* (Ksentini et al. 2010). In the same context, the dose of 450 g/hl of *Bt* tested on *T. cacoeciae* in our study was considered to be harmless to this parasitoid which suggest that the effect of this insecticide depends on the doses and on the species of *Trichogramma* tested. In another study, Jader-Braga et al. (2013) investigated the effect of spinosad (0.16 g/l) and chlorfenapyr (0.6 g/l) on different stages of *T. atopovirilia* (Oatman & Platner) and they found that these two active compounds were slightly harmful (Class 2) to the different stages tested of this parasitoid such as egg larval, prepupal and pupal stages which confirm our results. Other studies showed that spinosad may be more toxic to *Trichogramma*. Indeed, Cônsoli et al. (2001) demonstrated that spinosad (48 g/hl) was harmful when tested against any immature stage of *T. galloi* (Zucchi). In another study, Ksentini et al. (2010) proved that spinosad (60 cc/hl) was harmful to the development of different stages of the three tested species of *Trichogramma*.

Concerning insecticides persistence on tomato leaves, we found that only *Bt*, azadirachtin and virus HaNPV were safe to *T.*

cacoeciae adult with the dose of 450 g/hl, 250 cc/hl and 0,2 l/ha respectively, however, spinosad (60 ml/hl) and chlorfenapyr (50 ml/hl) were the most toxic. Previous studies showed that chlorantraniliprole (72 ml/ha) was harmless to *T. chilonis* adults (Hussain et al. 2012) compared to cypermethrin (0.113 kg/ha) which had relatively lower residual toxicity to *T. brasiliensis* **(**Ashmead) (Navarajan and Agarwal 1989). Ksentini et al. (2010) tested the effect of *Bt* (70 g/hl and 100 g/hl) and spinosad (60 cc/hl) on adults' survival of the three *Trichogramma* species cited above. These authors demonstrated that spinosad was very toxic and harmful compared to *Bt* considered as harmless towards *T. bourarachae*, *T. cacoeciae* and *T. evanescens*. Cônsoli et al. (2001) tested the toxicity of spinosad (48 g/hl) to adults of *T. galloi* and they showed that this insecticide is toxic to this species of *Trichogramma* (Cônsoli et al. 2001). Likewise, Hewa-Kapuge et al. (2003) found that chlorfenapyr (36 g/hl) tested on *T.* nr. *brassicae* (Haliday & Walker) caused 100% of mortality of individuals when applied directly to adults, and 95% of mortality when adults were exposed to residues 24 h after application. Here, we show that insecticides are more toxic to *T. cacoeciae* adults compared to immature stages developing within host eggs which is almost similar for different species of *Trichogramma*. This result could be explained by the presence of egg chorion which could help to protect pre-imaginal stages from different insecticides compared to adult *Trichogramma* (Bull and House 1983, Bull and Coleman 1985, Li et al. 1986, Singh and Varma 1986, Brar et al. 1991, Cônsoli et al. 1998) but cannot be considered as enough for all insecticides as shown in this study (Ksentini et al. 2010). Previous studies suggested that some insecticides, such spinosad, were unable to penetrate into the host egg chorion and *Trichogramma* were affected during the opening of the emergence hole (Plewka et al. 1975). This product induces the paralysis of newly emerged adults after exciting the nervous system by activating the nicotinic receptors of acetylcholine (Cônsoli et al. 2001). The difference of *T. cacoeciae* response to tested insecticides may be explained by many factors. In fact, the rate of penetration of the insecticide to its target site may be affected by the octanol–water partition coefficient (log Kow) and the molecular weight of the compound as well as some characteristics of the host egg (Stock and Holloway 1993; James 2002; Sechser et al. 2002). The toxicity of some products may be due to their high log Kow values, which are for example, 4.91 and 4.83 respectively for spinosad and chlorfenapyr (Guedes et al. 1992; Moura et al. 2005). The high log Kow values allows better and higher lipophilicity in addition to a further penetration of the insecticide through the corium of the egg (Hoffmann et al. 2008; Guedes et al. 1992). Likewise, difference in the toxicity of insecticides may depend on the species of *Trichogramma*, host egg they were reared upon, doses of the insecticides as well as the methodology used in the experiment (Bai et al. 1992; Ksentini et al. 2010). Our results showed that insecticides tested under laboratory conditions had more negative effects on adults than on immature stages of *T. cacoeciae* except *Bt*, HaNPV and azadirachtin classified as harmless for all development stages. However, under field conditions, obtained results can vary largely depending upon various factors. Indeed, Suh et al. (2000) demonstrated in a previous study that under field conditions, parasitized eggs laid on the underside of leaves may escape from insecticides treatment or may receive a lower dose. So these eggs may produce viable adults of *Trichogramma*. Also, these authors showed that the short residual activity of some insecticides or the timing of *Trichogramma* releases, which occurred 4-5 days after insecticide applications, may not affect the viability of *Trichogramma* adults (Suh et al. 2000).

In conclusion, this study brings valuable data to select harmless pesticides with short-lived residues compatible with releases of *T. cacoeciae* in IPM programs. This research helps also to plan the timing of these releases when combined with insecticides applications. But, it would be necessary to study the transgenerational effects of tested insecticides on parasitism rates and emergence of *T. cacoeciae* which survived to pesticide exposure to complete results presented here. Moreover, for a pest management strategy against *T. absoluta* which can cause serious problems to tomato crops in Tunisia, insecticides classified as toxic to non-target organisms, must not be included in IPM programs. The work presented here provides helpful information for selecting insecticides, but can produce extreme results that may not occur under natural conditions (Abreu Costa et al. 2014). For this, further studies needed to be done under greenhouse and field conditions in order to study the safety of insecticides use towards natural enemies, and the slowing of the development of resistance.

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V.3 Conclusion

Ce chapitre a mis en évidence la non toxicité de certains insecticides vis-à-vis de *T. cacoeciae* sous conditions contrôlées ; comme ils peuvent renseigner sur le moment convenable d'application des insecticides. Les produits biologiques testés (azadirachtin, *Bt* et virus HaNPV) sont fortement recommandés dans les programmes de lutte intégrée vu non seulement leurs efficacités mais aussi leurs inoffensivités vis-à-vis du parasitoïde en question. Cependant, les résultats obtenus ne peuvent pas refléter exactement le déroulement de ces processus sous conditions naturelles. Par conséquent, des essais sur terrain doivent être réalisés en vue de confirmer ces résultats.

Conclusion générale

La mineuse de la tomate, *Tuta absoluta,* s'est propagée par tout dans le monde et a pu envahir les continents d'Europe, d'Afrique et d'Asie en temps plus ou moins court grâce à sa forte capacité de dispersion. Cette invasion a transformé ce lépidoptère d'un ravageur régional de l'Amérique du sud en un ravageur majeur et mondial provoquant d'énormes dégâts sur les cultures de tomate. La situation de ce ravageur dans le monde, sa description morphologique, biologique ainsi que la description des dégâts occasionnés et les différents moyens de lutte adoptés ont été exposés dans le premier chapitre.

Dans un deuxième chapitre, l'étude moléculaire des populations larvaires de *T. absoluta* collectées à partir de sept régions tunisiennes (Tozeur, Guebeli, Gabes, Béjà, Bizerte, Nabeul et Ariana) a confirmé, en étudiant l'ADN mitochondriale CO1 (5'), l'absence de variabilité génétique des spécimens étudiés due à un balayage sélectif. Ces résultats confirment l'hypothèse que *T. absoluta* s'est introduite une seule fois en Tunisie.

Dans ce chapitre, des essais de milieux artificiels pour l'élevage de masse du ravageur ont été mis au point. Cependant, seul le milieu artificiel à base d'haricot a montré des résultats prometteurs.

Ce chapitre a permis de démontrer que *T. absoluta* peut se développer sur des hôtes alternatifs comme la pomme de terre et l'aubergine sous conditions contrôlées (T=25°C et HR=60%). Cependant, la tomate reste son hôte principal préféré présentant un cycle de vie le plus court, une meilleure longévité des adultes et une fécondité la plus élevée sous les mêmes conditions citées ci-dessus. La température et l'humidité relative semblent avoir un effet sur le cycle biologique de cet insecte. En effet, notre étude a montré que les deux températures testées (21 et 28°C) en combination avec trois niveaux d'humidités relatives (32, 52 et 72%) influencent les traits de vie de cet insecte. Nos résultats étudiant l'effet combiné de la température et de l'humidité relative sur les traits biologiques de *T. absoluta* sont signalés pour la première fois. Jusqu'à maintenant aucune étude n'a abordé ce volet, bien que cet effet a été démontré pour d'autre insectes, cas de *Lygus pratensis* (L.) (Hemiptera : Miridae).

Pour le troixième chapitre, nous avons démontré que *T. absoluta* développe 4 générations pour les adultes, les œufs et les larves sous serres froides dans la région de Takelsa.
Sous serres et en plein champ, les corrélations obtenues (entre les adultes piégés et les œufs pondus, les adultes capturés et les mines avec larves, les adultes piégés et les mines totales et les mines avec larves et les mines sans larves) sont positives et hautement significatives. On a pu, également, démontrer que *T. absoluta* préfère pondre les œufs sur la face inférieure des feuilles apicales des plants de tomate. L'acquisition de ces résultats nécessitent un suivi continu du ravageur durant au moins deux-trois ans, ce qui est plus ou moins difficile due à plusieurs contraintes telles que le choix et l'accès aux sites d'étude.
La densité optimale de pièges à phéromones sexuelles à installer dans les serres a été fixée. Nos résultats ont indiqué que l'installation de deux pièges à phéromones sexuelles/serre avec l'application d'insecticides est suffisante pour le contrôle de *T. absoluta* en cultures protégées. On a pu, également, démontrer que l'utilisation des plaques noires engluées pour le renforcement du piégeage de masse n'a pas d'effet significatif sur la population de l'insecte ; ces plaques noires sont même inutiles suite aux coûts élevés se traduisant par des charges supplémentaires pour l'agriculteur (Chapitre 3).
Les stratégies de lutte recommandées contre *T. absoluta* accordent à la lutte biologique une place importance jour après jour. Ceci, vu les problèmes signalés suite à l'utilisation excessive de la lutte chimique vouée à l'échec dans certains cas.
Dans un quatrième chapitre, l'effet des lâchers innondatifs de *Trichogramma cacoeciae*, moyennant différentes doses sous serres et en plein champ a été exploité. Nos résultats ont signalé le fort potentiel de ce parasitoïde à diminuer l'infestation causée par ce ravageur avec une dose optimale à recommander aux agriculteurs tunisiens de l'ordre de 20 *Trichogramma*/plant.
Dans un dernier chapitre, les effets non intentionnels de certaines molécules chimiques utilisées d'une façon intense dans les parcelles de tomate, vis-à-vis de *T. cacoeciae* ont été étudiés. Les résultats obtenus ont permis de classifier ces insecticides selon leurs toxicités. Certains produits sont à éviter dans les programmes de lutte intégrée vu leurs toxicités accrues vis-à-vis

de ce parasitoïde. D'autres sont fortement conseillés surtout ceux ayant une base biologique. Des essais sur terrain sont fortement recommandés en vue de confirmer ces résultats.

Finalement, le succès des stratégies de gestion préconisées contre *T. absoluta* dépend d'une meilleure connaissance de sa biologie vu son potentiel de développement sur plusieurs hôtes et sous différentes conditions climatiques. De plus, l'utilisation des pièges à phéromones sexuelles facilite le choix de la méthode de lutte à appliquer. Ainsi, ces pièges renseignent sur le moment convenable de l'application des insecticides ou bien des lâchers de prédateurs et de parasitoïdes en tenant compte du seuil de nuisibilité toléré pour ce ravageur. L'intégration de différentes méthodes de lutte et leur adaptation dans un calendrier de traitements s'avère nécessaire d'autant plus que le problème de la résistance de *T. absoluta* vis-à-vis de certaines molécules chimiques s'accroit et s'intensifie, tel est le cas de l'abamectine et du spinosade.

Comme perspectives, de nouvelles approches étudiant les mécanismes génétiques impliqués dans la dispersion de ce ravageur doivent être fixées en vue de stopper la colonisation de nouvelles localités. Aussi, des nouvelles stratégies de lutte à recommander doivent tenir compte de plusieurs facteurs tels que l'impact du réchauffement climatique sur le ravageur en question ainsi que sur son cortège d'auxiliaires pour une production durable des tomates en Tunisie.

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Résumé : *Tuta absoluta* (Meyrick) (Lepidoptera : Gelechiidae) est un ravageur clé pour les cultures de tomate causant des dégâts significatifs allant jusqu'à 80% dans les nouvelles zones envahies. Les résultats de cette étude montrent une conformité génétique élevée pour ce ravageur et une susceptibilité de se développer sur plusieurs milieux artificiels. *T. absoluta* est capable de se développer sous différents niveaux de température (21, 25 et 28°c) et d'humidités relatives (32, 52 et 72%) ainsi que sur différentes plantes hôtes (tomate, pomme de terre et aubergine). Cet insecte est capable de présenter jusqu'à 4-5 pics de vol et 3-4 générations d'œufs et de larves sous serres et en pleins champs. Une corrélation positive est observée et démontrée entre adultes capturés et œufs pondus, adultes capturés et mines avec larves, adultes capturés et mines totales et mines avec larves et mines sans larves. L'utilisation combinée du piégeage de masse et d'insecticides a montré des résultats encourageants vu que les problèmes de résistance n'ont pas été prouvés. La densité de vingt *Trichogramma cocoeciae* (Marchal) (Hymenoptera : Trichogrammatidae) par plant, testée sous serre et en plein champ a été signalée comme étant la plus efficace en réduisant les dégâts causés par ce ravageur. Cette étude démontre, également, la toxicité de quelques insecticides (indoxacarbe, spinosade…), largement utilisés dans les parcelles de tomates en Tunisie, sur tous les stades de développement de *T. cocoeciae* et met en valeur l'absence de nocivité d'autres substances (azadirachtin, *Bacillus thuringiensis* et virus HaNPV). Cette étude a permis, donc, une meilleure connaissance de *T. absoluta* en ce qui concerne sa biologie, sa dynamique des populations et sa caractérisation génétique. Elle a proposé des programmes de lutte efficaces en utilisant des insecticides avec moins d'effets secondaires sur le parasitoïde. Aussi, cette recherche a favorisé un meilleur contrôle biologique utilisant des lâchers innondatifs de trichogrammes. Cependant, des études supplémentaires sont encore nécessaires pour tester de nouvelles stratégies de lutte dans le but d'éviter les problèmes de résistance comme c'est déjà le cas dans plusieurs pays comme le Brésil.

Mots-clés : diversité génétique, effets non intentionnels, plantes hôtes, trait de vie, *Trichogramma cacoeciae*, *Tuta absoluta*

Abstract: *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a key pest of tomato crops causing significant yield losses up to 80% in newly invaded area. Obtained results of this study indicated a high genetic conformity of this pest and its susceptibility to develop in tested artificial diets. Our data showed that this pest was able to develop under different levels of temperature (21, 25 and 28°c) and relative humidity (32, 52 and 72%) as well as various host plants (tomato, potato and eggplants). This pest was able to achieve up to 4-5 flight peaks and 3-4 generations of eggs and larvae under greenhouse and field conditions. A positive correlation between some specific parameters (captured males and eggs laid, captured males and mines with larvae, captured males and total mines and mines with larvae and mines without larvae) was emphasized. The combined use of mass trapping and insecticides gave an encouraging results given that problems of resistances were not found. Dose of twenty *Trichogramma cocoeciae* (Marchal) Hymenoptera: Trichogrammatidae) tested in protected and open filed crops was the most effective in reducing the pest population. This study shows the toxicity of some insecticides (indoxacarb, spinosad…), widely used in Tunisia tomato crops, on all *T. cocoeciae* development stages and highlights the safety of others (azadirachtin, *Bacillus thuringiensis* and virus HaNPV). This study allows a better understanding of *T. absoluta* in terms of biology, population dynamics and genetic characterization. It proposes efficient control strategies when using effective insecticides with less side effects on parasitoid. Also this research promotes a better biological control using *Trichogramma* mass releases. However, additional studies, are still required to test new control strategies and propose new ones in order to avoid problems of resistance as reported for example in many countries such as in Brazil.

Key words: Biological traits, genetic diversity, host plants, side effects, *Trichogramma cacoeciae*, *Tuta absoluta*