Università degli Studi di Napoli "Federico II"

European PhD "Insect Science and Biotechnology"

Course XXVI

Tri-Trophic Interactions: Impact of Russet Mite on the Induced Defences of Tomato against Spider Mites

Supervisors

Dr. Sauro SIMONI
Prof. Francesco PENNACCHIO

Candidate

Mabrouk BOUNEB

Academic year 2013-2014

﴿قُلْ إِنَّ صَلَاتِي وَنُسُكِي وَمَحْيَايَ وَمَمَاتِي لِلَّهِ رَبِّ الْعَالَمِينَ﴾

[سورة الأنعام الآية: 162]

Index

Summary

Plant and herbivores coexist for millions of years and have developed an arsenal of complex interactions. They can be mutually beneficial or antagonistic. In antagonistic interaction, plants have evolved a wide array of constitutive morphological, biochemical and molecular defences to defend themselves from herbivore attacks (Karban and Baldwin, 1997; Walling, 2000). In addition, plants can activate induced direct defences that often act systemically throughout the plant and are typically effective against a broad spectrum of invaders (Green and Ryan, 1972). Moreover, plants under herbivore attack synthesize and release complex blend of volatiles that attract the third trophic level, predators or parasitoids, resulting in increased attacks on herbivores (Turlings and Wäckers, 2004). This later response is known as indirect defence and include a third trophic carnivore to increase plant fitness and resistance against herbivory. Induced direct and indirect plan defences are mainly orchestrated in jasmonic acid, ethylene and salicylic acid signaling pathways. When plants are attacked by single herbivore species, JA/ethylene pathway commonly regulates plant response to wounding by herbivorous invader, whereas salicylic acid controls systemic acquired resistance (SAR) in pathogen-like induced response. However, in their natural environment plants are exposed simultaneously to multiple herbivory and the interactions are often more complex. Yet, infestations by multiple herbivores having similar or distinct feeding guilds elicited plant defences in different manner compared to single herbivore.

Despite available reports from over 100 plant-herbivore systems concerning plant induced responses to herbivory (Agrawal, 1998), very few studies involved acarine herbivores. Therefore, more studies are required to understand plant-herbivore interaction in a more natural and complex system. The aim of this project was to enhance our understanding of the plant-herbivorous mites interaction in a context of multiple herbivory and to evaluate the effects of such interaction on the third-trophic level. To this goal, I analyzed the transcriptional changes of the main defence genes in tomato (*Solanum lycopersicum*) in response to simultaneous attack by spider mite (*Tetranychus urticae*) and eriophyoid russet mite (*Aculops lycopersici*) and single attack by the corresponding herbivores. The defence genes studied were: tomato lipoxygenase D (TomLoxD) and allene oxide synthase (AOS), two genes in the octadecanoid pathway upstream to jasmonic acid biosynthesis; wound induced proteinase inhibitor II (WIPI-II), a JA-responisive gene; pathogenesis related protein 1 (PR-1), a salicylic-dependent gene;

geranylgeranyl pyrophosphate synthase 1 (GGPS1), a gene involved in terpene synthase. I also evaluated the consequence of the resulted interaction on olfactory choice of specialist and generalist predaceous phytoseiid, *Phytoseiulus persimilis* and *Neoseiulus californicus*, respectively. Here I report in dual infestation, that the eriophyoid russet mite suppresses the induction of upstream and downstream signals of JA triggred by spider mite in local and distant systemic tomato leaves. Russet mite and spider mite both induced PR-1. Due to this interaction, spider mites under dual infestation showed increased performance. Moreover, both specialist and generalist predatory mites were more attracted to tomato plants attacked by spider mites compared to dual attack. The olfactory choice of predatory mites was dependent on population density of russet mites and on the extent of damage. In correspondence with the observed olfactory choice, analysis of volatiles emitted from tomato plants in response to different mite herbivory revealed a clear decrease in total volatiles in plants under dual attack compared to spider mite-attacked plants. Predatory mites seem to respond to tomato volatiles emitted in response to different herbivory as a whole blend and not as specific compounds.

Riassunto

Piante e fitofagi coesistono da milioni di anni e hanno sviluppato un insieme di interazioni e dinamiche complesse e di varia tipologia. Tali meccanismi possono essere, reciprocamente, a carattere vantaggioso o antagonista. Nelle interazioni che determinano antagonismo, le piante hanno sviluppato un'ampia gamma di difese che si basano sulle caratteristiche morfologiche, biochimiche e molecolari per difendersi dagli attacchi dei fitofagi (Karban e Baldwin, 1997; Walling, 2000). In tutta la pianta può essere attivata la difesa sistemica e l'insieme di tali reazioni può consentire una generale efficacia contro un ampio spettro di parassiti/fitofagi (Green e Ryan, 1972). Inoltre, le piante attaccate da fitofagi sintetizzano e rilasciano un mix complesso di sostanze volatili che 'richiamano' predatori o parassitoidi -terzo livello trofico-, con conseguente intensificazione degli attacchi contro i fitofagi (Turlings e Wackers, 2004). Questa ulteriore risposta è conosciuta come difesa indiretta e può includere, quale target, un terzo agente che si nutre sulla pianta al fine di accrescere la fitness della pianta e la resistenza contro i fitofagi. Le difese dirette e indirette della pianta sono regolate principalmente dalle catene metaboliche, 'pathway', di acido jasmonico (JA), etilene (ET) e acido salicilico (SA). Quando le piante sono attaccate da singole specie di fitofagi, il pathway JA/ET comunemente regola la risposta della pianta alle ferite determinate dai fitofagi; il pathway SA recita un ruolo significativo controllando la resistenza sistemica acquisita (SAR) quando i danni sono indotti da patogeni. Frequentemente, in impianti in ambiente naturale e non, le piante sono esposte contemporaneamente all'azione di specie di fitofagi diverse e le interazioni risultanti tra fitofagi e pianta sono spesso più complesse. Infestazioni da parte di fitofagi diversi con differenti abitudini/modalità nutritive può incidere nell'attivazione delle difese della pianta in modo diverso rispetto all'azione di un singolo erbivoro.

Nonostante i dati disponibili dagli studi su oltre 100 sistemi pianta-fitofago sulle risposte indotte della pianta a fitofagi diverasi (Agrawal, 1998), un numero limitato d studi ha riguardato l'azione degli acari fitofagi. Pertanto, ulteriori approfondimenti sono necessari per comprendere l'interazione pianta - fitofago in un sistema più naturale e complesso. L'obiettivo di questo progetto è quello di acquisire dati al fine di: a) integrare la comprensione dell'interazione acari fitofagi-pianta in un contesto, per altro riscontrabile, di presenza di fitofagi diversi; b) valutare gli effetti di tale interazione sul terzo livello trofico. A tal fine, si sono analizzate le modifiche trascrizionali dei

principali geni di difesa in piante di pomodoro (*Solanum lycopersicum*) in risposta ad un attacco contemporaneo da parte di due acari fitofagi facilmente riscontrabili singolarmente in contemporanea su questa solanacea: il ragnetto rosso, tetranichide *Tetranychus urticae*, e l'acaro della rugginosità del pomodoro, l'eriofide *Aculops lycopersici*. I geni di difesa studiati sono stati: la pomodoro lipossigenasi D (*TomLoxD*) e l'allene ossido sintasi (AOS), due geni del pathway octadecanoide a monte della biosintesi di JA; l'inibitore della proteasi II (*WIPI-II*) indotta da ferita, un gene JA-responisive; proteina 1 (*PR-1*) correlata a patogenesi, un gene AS-dipendente; il geranilgeranil pirofosfato sintasi 1 (*GGPS1*), un gene coinvolto nella terpene sintasi . E' stata anche valutata la conseguenza dell'interazione che ha determinato la scelta olfattiva di due acari fitoseidi predatori: lo specialista *Phytoseiulus persimilis* ed il generalista *Neoseiulus californicus*. Nei casi di duplice infestazione l'eriofide *A. lycopersici* sopprime l'induzione di segnali a monte e a valle del pathway JA innescato da ragnetto rosso sia in prossimità dell'azione del fitofago che a distanza. Eriofidi ed acari entrambi inducono PR-1. A causa di questa interazione, acari presenti in concomitanza sulla pianta hanno mostrato una maggiore fitness. Gli acari predatori, sia specialisti che generalisti erano più attratti da piante di pomodoro attaccate da tetranichidi singolarmente rispetto al duplice attacco. La scelta olfattiva degli acari predatori era dipendente dalla densità di popolazione degli eriofidi e dall'entità del danno. In corrispondenza della scelta olfattiva osservato, l'analisi delle sostanze volatili emesse dalle piante trattate in modi diversi ha rivelato una netta diminuzione nella quantità totale di componenti volatili emesse nelle piante sottoposte all'azione combinata tetranichidi+eriofidi rispetto alle piante sottoposte all'attacco del ragnetto da solo.

I. Introduction

1.1. Plant defences against herbivores

In nature, plants are often exposed to numerous environmental threats, including biotic and abiotic factors that compromise their fitness and productivity. To survive, plants have evolved a wide array of morphological, biochemical and molecular defences to counter these threats (Karban and Baldwin, 1997; Walling, 2000). As basic defence, plants have evolved a primary immune response to recognize common features of invaders and to establish a defence response that is specifically directed against the invader encountered (Jones and Dangl, 2006). In addition, plants can activate an induced resistance defence that often acts systemically throughout the plant and is typically effective against a broad spectrum of invaders (Walters *et al.*, 2007). Inducible plant defences was first reported by Green and Ryan (1972), who observed that feeding by the Colorado potato beetle induces the expression of proteinase inhibitors (PIs) in potato and tomato plants, which inhibit the activity of digestive proteinases in the insect gut. When challenged with herbivorous insect or microbial pathogen, plants undergo two types of defence mechanism: resistance or tolerance. Plant's resistance refers to traits that prevent infection by pest or pathogen or limit its extent, while tolerance refers to traits that instead reduce or offset the fitness consequences of infection for the host plant (Strauss and Agrawal, 1999; Roy and Kirchner, 2000). Plant resistance traits against herbivores can be direct and include physical barriers to feeding (such as trichomes, spines, and hardening leaf tissue) and chemical defences that decrease the palatability of plant tissues to herbivores (secondary metabolites, proteinase inhibitors, antinutritive enzymes) or indirect by attracting the natural enemy of the attacker through the emission of volatile organic compounds (VOCs).

1.1.1. Direct defence

1.1.1.1. Anatomical defence

Plants have structural traits that form the first physical barrier involved in the direct defences against herbivore attack. These include various types of spines and thorns (spinescence), hairs (trichomes), toughened or hardened leaves (sclerophylly), and the incorporation of granular minerals into plant tissues (Hanley *et al.*, 2007). Trichomes production plays an important role in plants resistance against herbivorous insects (Southwood, 1986; Karban and Baldwin, 1997). While plants produce trichomes

constrictively, many plant species increase trichome density in new leaves in response to herbivore attacks (Dalin *et al.*, 2008). Trichome density may negatively affect the ovipositional behavior by influencing the security with which the eggs are attached to leaves, feeding and larval nutrition of insect pests (Handley *et al.*, 2005). Bjorkman and ahrne (2005) noted that an induced increase in leaf hairiness in willows in response to leaf beetle grazing, while this trait had no negative effects on the main natural enemies. Agrawal (1999) showed that insects had reduced growth and limited feeding on leaves of induced compared to non-induced plants. *Leptinotarsa decemlineata* feeding behavior and growth were negatively influenced by high density of non-glandular trichomes on tomato leaves, while only high glandular trichome density impaired *Helicoverpa zea* growth (Tian *et al.*, 2012). He *et al.*, (2011) reported that the most resistant chrysanthemum cultivars to aphid infestation produced the longest, highest and densest trichomes, the largest and fullest gland cells, and the most wax on the lower leaf epidermis. Fortifying cell walls is another anatomical defence trait, in fact, plants can limit food supplies to herbivores by enforcing mechanical barriers to herbivore feeding and probing through thick cell walls, particularly for piercing-sucking herbivores (Goussain *et al.*, 2005).

1.1.1.2. Production of secondary metabolites

1.1.1.2.1. Terpenoids

Terpenoids represent the largest family of natural plant products with more than 25,000 members with a variety of biological functions (Sacchettini and Poulter, 1997). They are classified by the homologous series of number of five carbon isoprene units in their structure: hemiterpenes C_5 (1 isoprene unit), monoterpenes C_{10} (2 isoprene units), sesquiterpenes C_{15} (3 isoprene units), diterpenes C_{20} (4 isoprene units), terpenes C_{30} (6 isoprene units), tetraterpenes C_{40} (8 isoprene units), and polyterpenes $(C_5)n$ where 'n' can be 9-30.000 (McGarvey and Croteau, 1995). All terpenoids are biosynthesized from two C_5 precursors: isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Figure 1 describes terpenoids biosynthesis from universal terpene precursors via the cytosolic acetate-mevalonate (MVA) pathway and the methylerythritol 4-phosphate (MEP) pathway (Lichtenthaler, 1999; Rohmer, 1999; Arimura *et al.*, 2009). The volatile fraction of terpenoids predominantly consists of the hemiterpene isoprene (C5), monoterpenes (C10) and sesquiterpenes (C15).

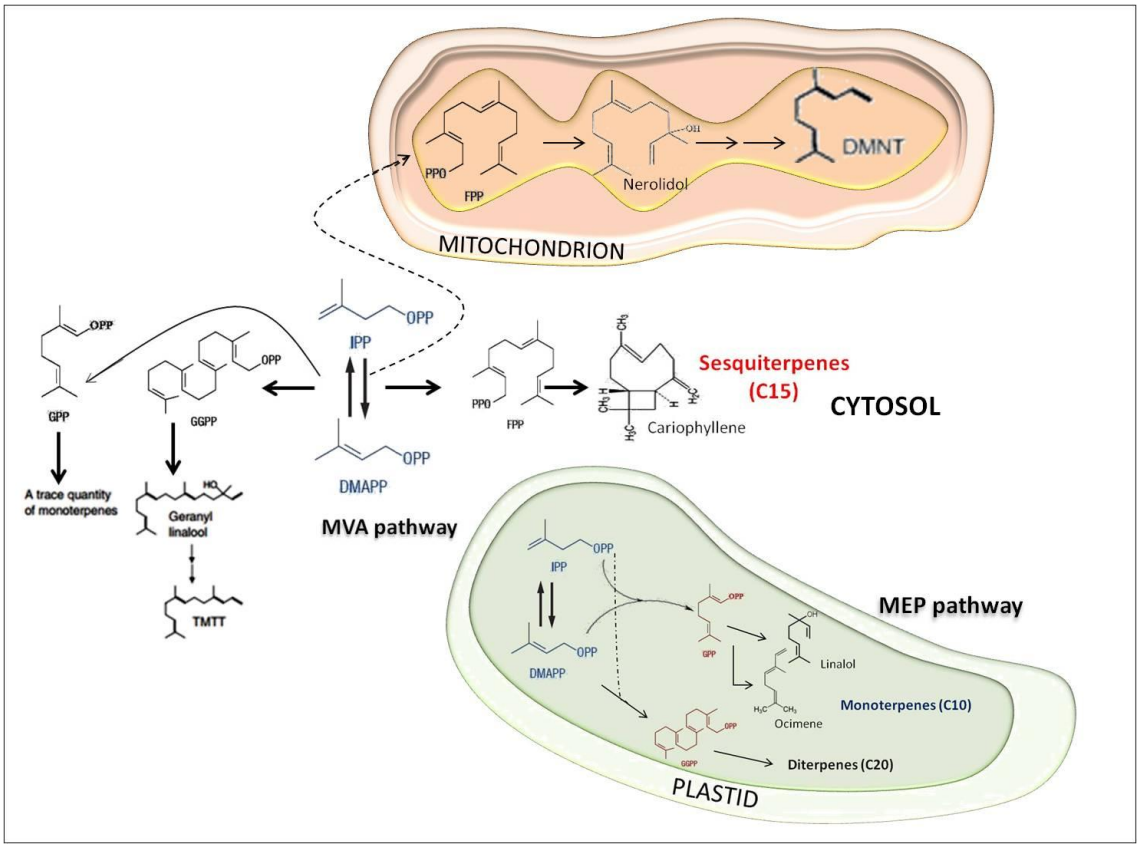

Fig. 1. Schematic representation of the terpenoid biosynthetic pathway in plants. Note that biosynthetic routes of the homoterpees (DMNT and TMTT) from nerolidol and geranyllinalool, respectively are still unclear. DMNT, *(E)*-4,8-dimethyl-1,3,7-nontriene, FPP, farnesyl diphosphate; GGPP, geranylgeraly diphosphate; GPP, geranyl diphosphate; IPP, isopentonyl diphosphate; MEP, 2-C-methyl-o-erythritol-4-phosphate; MVA, mevalonate; TMTT, *(E,E)*-4,8,12-trimethyltrideca-1,3,7,11-tetraene. (Modified from Arimura *et al.*, 2009)

Terpenoids are constitutive chemical reserves accumulated in high levels in specialized glands and trichomes (Paré and Tumlinson, 1997). They have shown many protective functions against abiotic and biotic factors (Holopainen, 2004), due to their physicochemical properties, such as volatility, reactivity, toxicity and aroma. Terpenoids are involved in plant pollinator interactions and have important functions in plant defence against herbivores (Dicke, 1994; Paré and Tumlinson, 1999; Cheng *et al.*, 2007). In undamaged plant a baseline level is constantly released from leaf surface. However, in response to herbivore attack, and the subsequent breakdown of reservoir glands, terpenoids emission is triggered. It was also reported that not only terpenoid quantity is increased under herbivore attack, but instead also new volatile compounds are de novo synthesized (Rose *et al.*, 1996, Paré and Tumlinson, 1998). A series of biochemical reactions, including gene expression, protein synthesis, and/or enzyme induction, may be

required for the synthesis and release of terpenoids after herbivory (Figure 2). This hypothesis is supported by the fact that a consistent (several hours) delay between the occurrence of herbivore attack and terpenoids release (Paré and Tumlinson, 1999). Induced plant volatiles can act directly against the attacking herbivores or other harmful insects by repelling them. For instance, wheat seedlings infested by aphids (*Rhopalosiphum padi*) release VOCs that repel aphids in an olfactory assay (Quiroz *et al.*, 1997). Also, de Moraes *et al.* (2001) showed that tobacco plants *Nicotiana tabacum* release several herbivore-induced volatile compounds exclusively at night that are highly repellent to female moths *Heliothis virescens*. Furthermore, terpenoids may induce defence response to neighboring undamaged plants of the same species or from another species. Zakir *et al.* (2013) showed that emission of volatile terpenoids from damaged cotton increased the resistance of undamaged cotton and alfalfa plants to oviposition by *S. littoralis*. Alteration of terpenoids emission in many plant species has been also observed as response to egg deposition by herbivorous arthropods such as insects and mites. This induction of volatiles by insect egg deposition is known to occur locally at the site of egg laying and systemically at plant tissue adjacent to the oviposition site (Hilker *et al.*, 2002; Mumm *et al.*, 2003; Colazza *et al.*, 2004).

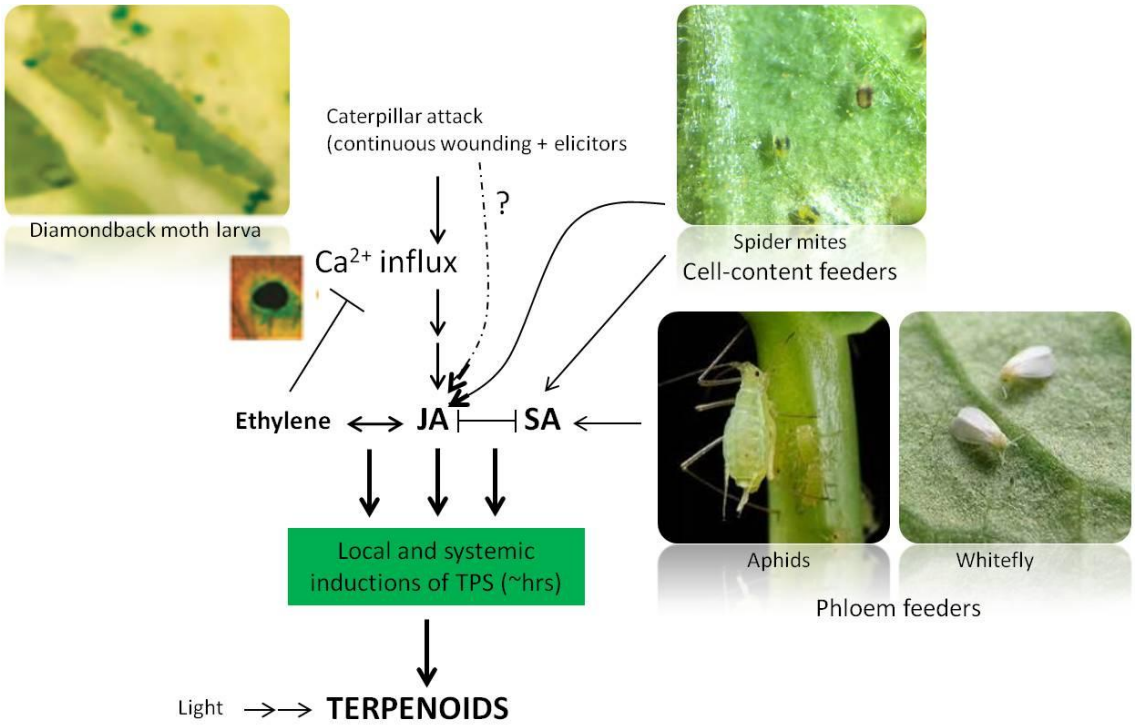

Fig. 2. Model of the signaling network required for terpenoids biosynthesis in chewing and piercing-sucking arthropod-damaged leaves. Arrows and bars indicate positive and negative interactions, respectively. JA: jasmonic acid; SA, salicylic acid. (Modified from Arimura *et al.*, 2009)

1.1.1.2.2. Phenolics

Phenolics are one of the most ubiquitous groups of secondary metabolites found in the plant kingdom (Harborne, 1980; Boudet, 2007). They include a very large group of aromatic compounds characterized by a benzene ring (C6) with one or more hydroxyl groups (Harborne and Simmonds, 1964). Phenolics are constitutively present in plants and accumulate during normal growth and development. They play important roles in lignin and pigment biosynthesis and as physical barriers in constitutive plant defence against herbivores such as cell wall-bound phenolics and stored compounds that have a deterring or directly toxic effect on herbivores (Walling, 2000). They are also produced and accumulated in the subepidermal layers of plant tissues exposed to stress , including trauma, wounding, drought and pathogen attack (Kefeli *et al.*, 2003; Schmitz-Hoerner and Weissenbock, 2003; Clé *et al.*, 2008). Herbivore damages induce the synthesis of new phenolic compounds and accumulation of the constitutively produced compounds. Several types of phenolics have been documented to play a role in plant-herbivore interactions. For example, hydroxycinnamic acids may act as cell wall cross-links that fortify and protect plant cell walls against chewing damage (Santiago *et al.*, 2005). C-

glycosyl flavones function as antibiotic agents after their subsequent conversion to the more toxic quinines which in turn reduce the availability of free amino acids and proteins by binding to -SH and-NH_2 groups (Felton *et al.*, 1989; Wiseman and Carpenter, 1995). Snook *et al.* (1994 and 1995) noted that c-glycosyl flavones in maize silks confer resistance to corn earworm (*H. zea*) larvae. Also, o-dihydroxy phenolics (e.g. caffeic acid and chlorogenic acid) were reported to have toxic effects on *H. zea* mediating the production of superoxide free radical anions, hydroxyl free radicals, and phenolic free radicals which then catalyze lipid peroxidation and destruction of body protein (Summers and Felton, 1994).

1.1.1.2.3. Alkaloids

Alkaloids are low molecular weight nitrogen-based secondary metabolites found in about 20% of plant species and mainly involved in plant defence against herbivores and pathogens. Alkaloids have toxic, deterrent and/or repellent effects on a wide range of generalist herbivores (van Dam *et al.*, 1995; Hartmann and Ober, 2000; Adler *et al.*, 2001). Different classes of alkaloids have been identified in several plant families. Pyrrolizidine alkaloids (PAs) are known to be produced by several species within the angiosperms (Hartmann and Witte, 1995). Mutagenic effects of PAs have been demonstrated in Drosophila (Frei *et al.*, 1992), and acute toxicity in larval development of *Philosamia ricini* (Narberhaus *et al.*, 2005). Steroidal glycoalkaloids, however, are a class of alkaloids found only in many species of the genus Solanum including potato and tomato (Milner *et al.*, 2011). The role of steroidal glycoalkaloids as chemical defence in plant resistance against herbivores has been widely demonstrated (Sinden *et al.*, 1980; Tingey, 1984; Barbour and Kennedy, 1991). They act by inhibition of acetylcholinesterase activity (Roddick, 1989) and disruption of the eukaryotic cell membrane structure through binding with the sterol component of the membranes (Bouarab *et al.*, 2002).

1.1.1.3. Digestibility reducers

Indigestion is considered as best plant's defence against herbivores (Felton, 2005). Proteinase inhibitors (PIs) are considered to play role as antidigestive compounds against many arthropods. PIs are proteins produced by plants and have the capacity to inhibit proteolytic enzymes of insect and microbial origins. Digestibility-reducers that interact with proteins inside the gut of herbivore can exert sublethal effect on herbivores by impairing growth (prolonged development time), lowering resistance to disease and reducing fecundity (Price *et al.,* 1980).Green and Ryan (1972) first claimed a possible role of PIs as plant defence mechanisms against herbivores. They noted an accumulation of proteinase inhibitors in potato and tomato plants upon wounding by adults Colorado potato beetle, *Leptinotarsa decemlineata*, which inhibit the activity of digestive proteinases in the insect gut. Since then, many studies were conducted to understand the mechanism of PIs induction and action inside insect gut (Broadway and Duffey 1986; Ryan, 1990; McManus *et al.,* 1994; Broadway, 1995; Hartl *et al.,* 2010;). Proteomic analysis were carried out by Chen *et al.* (2005) to identify plant proteins that are undigested in the midgut of many caterpillars, including the *Manduca sexta*. JA-inducible plant proteins were found among the most abundant that accumulate in the insect's midgut and include several inhibitors of digestive proteinases and enzymes. This finding enforces the evidence of PIs as induced plant defences against wide range of herbivores.

1.1.1.4. Antinutritive enzymes

In addition to the synthesis of toxic compounds and antidigestive proteins as induced defences, plants, when attacked by herbivory, also produce antinutritive enzymes which interact with other secondary metabolites to starve herbivores of essential nutrients. For instance, plant lipoxygenases are JA-regulated enzymes acting as antinutritive enzymes confer resistance in a number of crops against noctuids (Hildebrand *et al.*, 1986; Felton *et al.*, 1994). Lypoxygenases are O_2-dependent enzymes which rapidly metabolize fatty acids such as linoleic and linolenic acids to highly reactive hydroperoxides, epoxides, and free radicals (Duffey and stout, 1996). These end-products depreciate the nutritive quality of plant tissue and therefore reduce feeding and growth of the attacking herbivore. Also, additional JA-regulated enzymes such as polyphenol oxidases (PPOs) may further intensify the effect of nutrient deprivation. PPOs catalyze the oxidation of

chlorogenic acid to form strongly electrophilic quinones that cause significant losses in alkylatable amino acids (i.e. cysteine, histidine, methionine and lysine) (Felton *et al.*, 1992). For instance, Felton *et al.* (1989) observed a reduction in the growth of the tomato corn earworm *H. zea* and the beet armyworm *S. exigua* after feeding on mature tomato leaves. According to them, this results from the alkylation of amino acids/protein by *o*-quinones, and the subsequent reduction in the nutritive quality of foliage. The loss of essential nutrients caused by the above mentioned defensive plant proteins is predicted to be one of the most ecologically and evolutionarily stable forms of plant defence (Felton, 2005).

1.1.2. Indirect defence

Plants indirect defences are traits that disable or remove herbivores by manipulating tritrophic interactions to the advantage of the plant. Several evidences have demonstrated the interaction between plants and natural enemies of herbivores (Takabayashi and Dicke, 1996; Dicke *et al.*, 1999; Rasmann *et al.*, 2005; Sabelis *et al.*, 2007). Plants release a wide array of volatile compounds from leaves, flowers, and fruits to the atmosphere and from roots into the soil to defend themselves against herbivores and pathogens or to provide reproductive advantages by attracting pollinators (Dudareva *et al.*, 2006). Herbivore attack was shown to increase the emission of volatiles, which attract predators to herbivore-damaged plants in agricultural systems (Kessler and Baldwin, 2001). The specific blends of volatiles emitted by plants in response to herbivore attack are called herbivore-induced plant volatiles (HIPVs). Blends-issued as a result of damage by herbivore allow natural enemies of the herbivore to distinguish the infested plants from those uninfected. For example, lima bean plants and apple trees infested with *Tetranychus urticae* Koch emit volatiles that attract predatory mite *Phytoseiulus persimilis* predators (Takabayashi and Dicke, 1996; Dicke *et al.*, 1999; Sabelis *et al.*, 2007). Maize plants under attack by caterpillar *S. littoralis* emit a specific blend of volatiles that is highly attractive to parasitic wasp *Microplitis rufiventris* (Gouinguené *et al*., 2003). Similarly, a major larval endoparasitoid *Campoletis chlorideae* was attracted to volatiles emitted by *Helicoverpa armigera*- and *Pseudaletia separate*-infested maize plant (Yan and Wang, 2006).

Belowground interactions involving HIPVs were also observed. Van Tol *et al.* (2001) showed that roots of the coniferous plant *Thuja occidentalis* when attacked by the weevil

larvae *Otiorhynchus sulcatus* release volatile chemicals that attract the parasitic nematode *Heterorhabditis megidis*. Rasmann *et al.* (2005) reported that maize roots release a sesquiterpene volatile in response to feeding by larvae of the beetle *Diabrotica virgifera virgifera,* which strongly attracts an entomopathogenic nematode. Furthermore, aboveground-belowground interactions may occur and influence natural enemies. Rasmann and Turlings (2007) reported that simultaneous feeding by aboveground herbivore (African cotton leafworm) and belowground herbivore (*D. virgifera virgifera*) affected the production of HIPVs that in turn affected the attraction of the respective natural enemies. The figure 3 represents possible aboveground and belowground interactions between plant and herbivore mediating volatile organic compounds, and their effects on natural enemies.

Identification of the compounds responsible for plant-natural enemies' interactions has been for long time very complicated. This is believed to be due to the high chemical diversity of volatile mixtures. The failure in identifying specific compound responsible for natural enemy attraction, suggest that mixtures rather one single compounds constitute the active signal (Dicke *et al.*, 1990; Turlings *et al.*, 1991). Despite this early observations, it was shown that the application of individual plant volatiles, such as methyl salicylate and the C16-homoterpene 4,8,12-trimethyl-1,3(E),7(E),11 tridecatetraene [(E,E)-TMTT], can attract predatory mites (De Boer and Dicke, 2004; De Boer *et al.*, 2004). Recently, isolation of genes encoding enzymes responsible for the formation of plant volatile compounds has been achieved (Bohlmann *et al.*, 2000; Ament *et al.*, 2006). This progress allowed investigating the role of individual signaling compounds in mediating tritrophic interactions. For example, the predatory mite *P. persimilis* was attracted to transgenic Arabidopsis over-expressing strawberry nerolidol synthase, a terpene synthase (TPS) which synthesize the sesquiterpene alcohol (3S (E)-nerolidol) (Kappers *et al.*, 2005). Similarly Arabidopsis line expressing other herbivore-induced sesquiterpene hydrocarbons released from maize upon herbivory by lepidopteran larvae, were more attractive to the parasitic wasps Cotesia marginiventris (Schnee *et al.*, 2006).

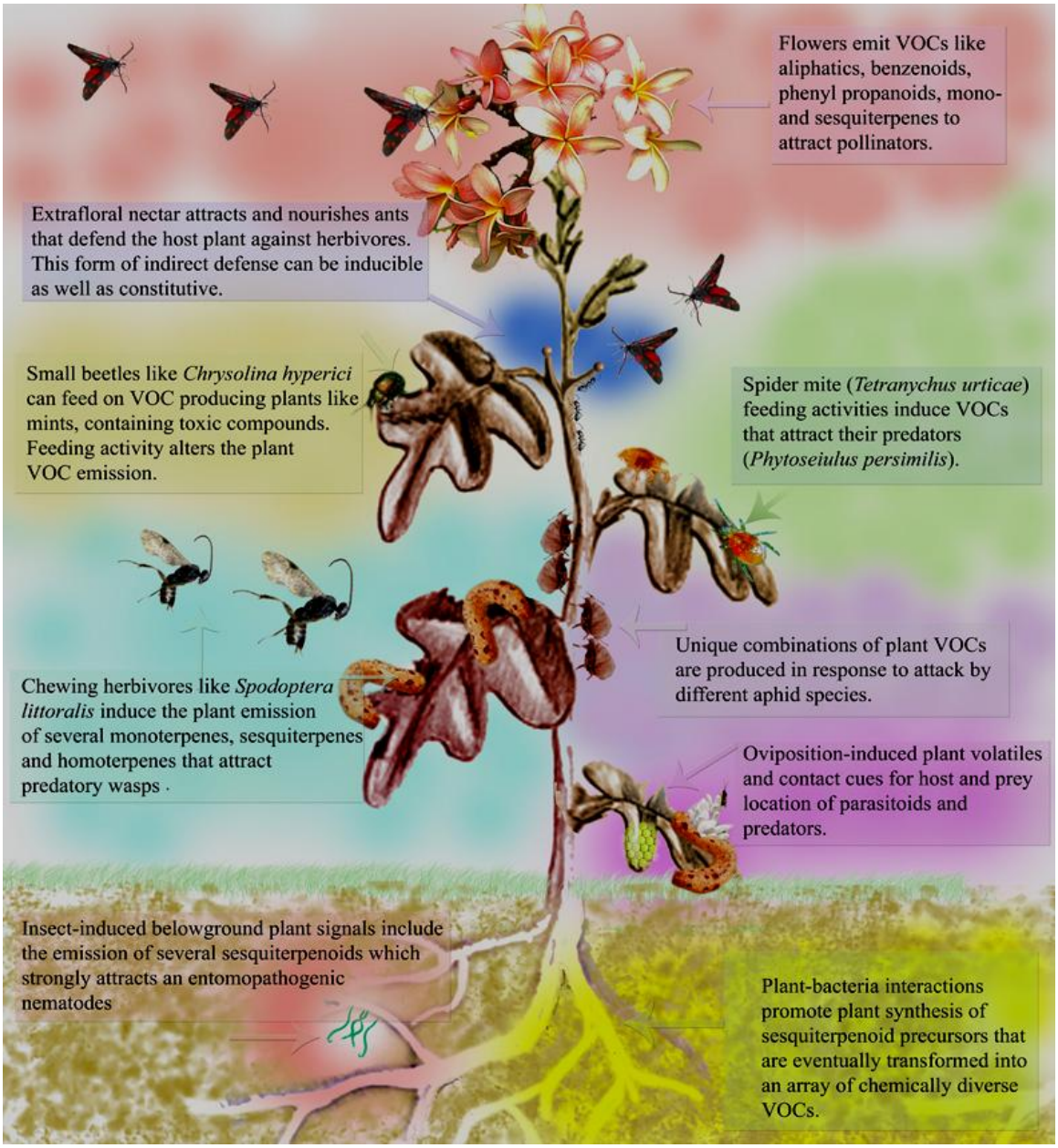

Fig. 3. The plant volatilome: plants emit a wide array of volatile compounds for pollinator attraction and in response to biotic and abiotic stress (Maffei *et al.*, 2007)

1.1.3. Plant responses mediating signal transduction pathways

1.1.3.1. The octadecanoid pathway

Wounding and herbivore damages to leaves of numerous plant species induce the synthesis of defensive proteinase inhibitor proteins in wounded leaves as well as in distal unwounded leaves (Green and Ryan, 1972; Brown and Ryan, 1984; Paré and Tumlinson, 1998). Jasmonic acid and methyl jasmonate have been reported to regulate the

expression of wound-inducible proteinase inhibitor genes (Farmer and Ryan, 1990; Farmer *et al.*, 1992). Also several reports have demonstrated the role of octadecanoid pathway in plant defence response against herbivore attack (Farmer and Ryan, 1992; Doares *et al.*, 1995a; Howe *et al.*, 1996; Howe, 2004; Christensen *et al.*, 2013).

The biosynthesis of jasmonic acid originates from linolenic acid via the octadecanoid pathway (Vick and Zimmerman, 1984; Mueller *et al.*, 1993). The octadecanoid pathway includes dehydration, a reduction and a series of β-oxidationns (Figure 4). Mechanical wounding and herbivores damage activate polygalacturonase (PG) which hydrolyzes pectin in the cell wall to release oligogalacturonides (OGAs). OGAs are potent signals that activate octadecanoid pathway (Doares *et al.*, 1995b; Bergey *et al.*, 1999; Walling, 2000). In some Solanaceae the signal peptide systemin produced and transported through the phloem mediate both local and systemic activation of the octadecanoid pathway (Ryan, 2000). Systemin, OGAs and chitosan, activate plant defensive genes through the octadecanoid pathway (Doares *et al.*, 1995b; Schaller, 1999). After induction, the precursor of this pathway, linolenic acid (18:3) is released from membranes (Narváez-Vásquez *et al.*, 1999) under the action of phospholipases. Linolenic acid is then oxygenated by specific LOXs at C13 to result in (13S)-hydroperoxy-octadecaditrienoic acid (13-HPOT). Allene oxide synthase (AOS) catalyzes the dehydration of (13-HPOT) to form an unstable allene oxide, 12,13(S)-epoxy-octadecatrienoic acid (12,13-EOT), while, the green leaf volatile (n-hexenal) is synthesized from 13-HPOT by a hydroperoxide lyase. Allen oxide undergoes cyclization by allene oxide cyclase to form 12-oxo-phytodienoic acid (OPDA). Subsequently, OPDA is reduced by 12-oxophytodienoate reductase (OPR) to yield (Z)-pentenyl-cyclopentane-1-octanoic acid (OPC-8:0) (Shaller *et al.*, 2000). Finally the shortening of the octanoic side chains in OPC (8:0) to yield JA involves three rounds of β-oxidation (Figure 4).

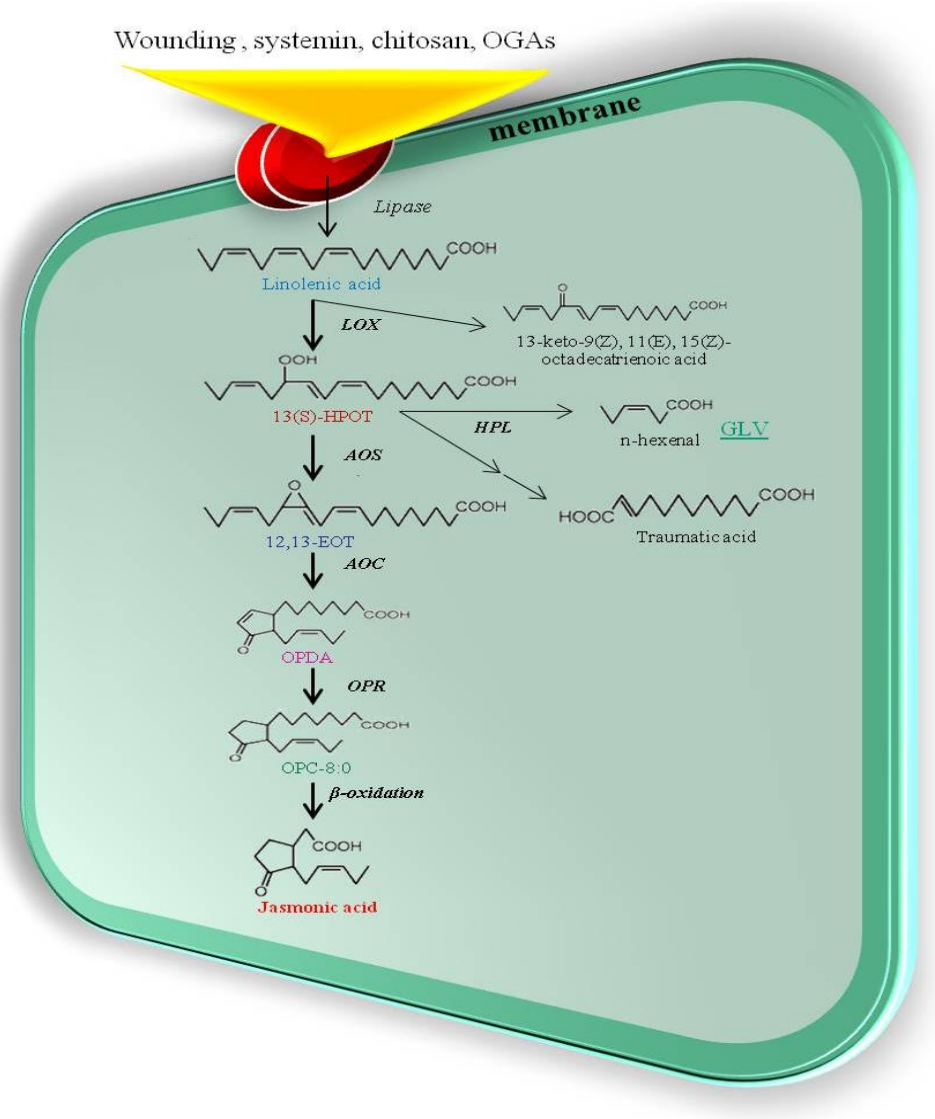

Fig. 4. Biosynthesis of jasmonic acid via the octadecanoid pathway. LOX: lipoxygenase; HPL: hydroperoxide lyase; AOS: allene oxide synthase; AOC: allene oxide cyclase; OPR: 12-oxo-phytodienoic acid reductase; 13(S)-HPOT:(9Z,11E,15Z,13S)-13-hydroperoxy-9,11,15-octadecatrienoic acid; 12,13-EOT: (9Z,11E,15Z,13S,12R-12,13-epoxy-9,11,15-octadecatrienoic acid; OPDA:12-oxo-10,15 (Z)-octadecatrienoic acid ; OPC-8:0: 3-oxo-2(2′(Z)-pentenyl)-cyclopentane-1-octanoic acid; GLV: green leaf volatile (Modified from Shaller, 2001).

1.1.3.2. Cross-talk between signal transduction pathways

Plants encounter numerous herbivorous insects and microbial pathogens with diverse modes of attack. To survive, plants have evolved primary immune response to recognize common features of the invaders and to establish a defence response that is specifically directed against the invader encountered (Jones and Dangl, 2006). In addition, plants can activate an induced resistance defence that often acts systemically throughout the plant and is typically effective against a broad spectrum of invaders (Walters *et al.*, 2007).

Phytohormones are well recognized player in the regulation of the induced defences. Salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) are recognized as key phytohormones in the regulation of the signaling pathways involved (Reymond and Farmer, 1998; Howe, 2004; Lorenzo and Solano, 2005; Grant and Lamb, 2006; Von Dahl and Baldwin, 2007).

Plant responses to herbivores are complex. Distinct signal transduction pathways are activated as response to attacks by herbivores with different feeding mode and variable degree of tissue damage at the feeding site (Walling, 2000; Zhang *et al.*, 2009). Phloem-feeding insects such as whiteflies and aphids produce little injury to plant tissue are perceived as pathogens and dominantly activate the salicylic acid (SA)-dependent (Moran and Thomson, 2001; Kempema *et al.*, 2007; Zarate *et al.*, 2007). Chewing herbivores such as caterpillars and beetles and cell-content feeders such as mites and thrips cause more extensive tissue damage and frequently activate the jasmonic acid (JA) signaling pathway (Kessler and Baldwin, 2002; Zhang *et al.*, 2009). Despite these common thoughts, often, the activation of either SA or JA pathway is not correlated to the feeding behavior of the herbivory. For example, Sarmento *et al.* (2011) observed that two related mite species feeding on cell content on tomato differently activated SA and JA pathways. Similarly, Zhang *et al.* (2009) reported that under simultaneous feeding with the spider mite *T. urticae,* the whitefly *Bemesia tabaci* suppressed the JA and SA pathways on lima been plants. Interactions between defence signal transduction pathways were proposed to play role. In nature, plants often deal with simultaneous or subsequent invasion by multiple herbivores, which can influence the primary induced defence response of the host plant (Van der Putten *et al.*, 2001; Bezemer and Van Dam, 2005; Stout *et al.*, 2006). Cross talk between induced defence-signaling pathways is considered a regulatory mechanism employed by plant to adapt to changes in their hostile environment (Koornneef and Pieterse, 2008). Several lines of evidence suggest the existence of negative cross-talk between the jasmonate and salicylate response pathway (Penna-Cortes *et al.*, 1993; Bostock *et al.*, 2001; Thaler *et al.*, 2002; Jander and Howe, 2008). Cross talk, however, helps the plant to minimize energy costs and create a flexible signaling network that allows the plant to finely tune its defence response to the invaders encountered (Reymond and Farmer, 1998; Bostock, 2005).

1.1.4. Plant responses to multiple herbivory

To date, most studies on the activation of plants defence upon herbivore attack and the effect of resulted interaction on natural enemies of the herbivore have been carried out with single species of herbivore. Yet, in the field, damage by a single herbivore is rare, and plants are likely challenged by different herbivore at the same time (Vos *et al.*, 2001; Rodriguez-Saona *et al.*, 2005). Recently, several studies have attempted to fill this gap by analyzing plant interaction to multiple herbivory and its influence on natural enemies. For example, Zhang *et al.* (2009) reported that the whitefly *bemesia tabaci* interfere with defence signal produced by Lima bean plant attacked by *T. urticae*, and suppressed volatiles that attract the natural enemy predator of spider mite. For instance, *B. tabaci* infestation reduced considerably the emission of terpenoids triggered by the leaf-chewing beet armyworm (*Spodoptera exigua*) on cotton plants (Rodriguez-Saona *et al.*, 2003). In contrast, the aphid *Myzus persicae* caused an increased emission of volatiles triggered by spider mites in pepper plants, and consequently increased the attraction of predators to plants infested with aphids and spider mites (Moayeri *et al.*, 2007). Two volatile compounds of cucumber plants induced by single-species herbivory by *T. urticae or S. exigua* were suppressed upon multi-species herbivory (De Boer *et al.*, 2008). Plants damaged by both herbivores (*S. exigua* and *Macrosiphum euphorbiae*) had similar PI activity, larval growth and survival of *S. exigua* and C*otesia marginiventris* parasitoid, as plants singly damaged by caterpillars (Rodriguez-Saona *et al.*, 2005).

Most studies on multiple herbivory involved species with different feeding behaviors. Recently, Sarmento *et al.* (2011), analyzing plant response to two spider mites belonging to the same feeding guild, found that the invasive spider mite *T. evansi* suppressed the induction of the salicylic acid and jasmonic acid signaling involved in induced plant defences in tomato against the spider mite *T. urticae*.

1.2. Herbivore offences

In confront of the plant anti-herbivore endowments, herbivorous arthropods have evolved a series of behavioral, physiological and biochemical offensive traits. Herbivore offences represent traits that allow herbivores to increase their fitness and reproduction on depend of host plants (Karban and Agrawal, 2002). These traits include, feeding and oviposition choices, morphological adaptations, suppression of plant defence pathway, and enzymatic metabolism of plant compounds.

1.2.1. Feeding and oviposition choices

Close association with few host species is likely to lead to the evolution of more-effective offensive adaptations than association with many hosts. Polyphagous (or highly generalized) herbivores are species that feed on hosts in more than one plant family, whereas, monophagous (or highly specialized) herbivores feed on one or a few closely related plant taxa, often a single genus (Ali and Agrawal, 2012). When damaged, some plants emit exudates from elongate canals. Multiple lineages of caterpillars, beetles and katydids exhibit vein cutting or trenching (Dussourd, 1993). Before feeding, these insects cut through leaf veins, thereby severing secretory canals that occur within the veins. The insects then feed distal to the cuts on the portion of the leaf supplied by the veins. Dussourd (2009) reported that species that exhibit both vein cutting and trenching typically do not have expanded host ranges; most feed exclusively on a single plant family. Moreover, Cardoso (2008) showed that, specialist *H. charithonia* larvae were capable of freeing themselves from entrapment on trichome tips on *Passiflora lobata* by physical force: laying silk mats on the trichomes and removing their tips by biting (trichome tips were found in the faeces). However, trichomes exhibited deterring effects on a non specialist herbivore *H. pachinus*. Caterpillars of several species exhibit window-feeding behavior avoiding edge-feeding on spiny-edged grasses (Keathley and Potter, 2011) or avoiding sclerenchymous bundle sheaths on maple (Hagen and Chabot, 1986). Grasshoppers can avoid nutritionally inadequate foods and foods associated with adverse physical responses (Bernays and Lee 1988; Behmer *et al.*, 1999). Grasshoppers can also learn to associate plant odors with limiting nutrients, and they actively seek these odors (Simpson and White, 1990).

Preference of appropriate host for oviposition is considered an herbivore offense trait (Karban and Agrawal, 2002). The choice of herbivore females where to lay their eggs strongly affects progeny survival and fitness For example, the sawfly *Athalia rosae* beetle laid eggs on plant species that provide larva with better food. However, larvae were more exposed to the predatory wasp *Polistes dominulus* (Muller and Arand, 2007). Plant compounds often serve as cues for phytophagous herbivores to identify suitable hosts for feeding and oviposition.

1.2.2. Suppression of defence signaling

Herbivore feeding elicits defence responses in infested plants, including activation of JA/ethylene signaling cascades and the possible crass-talk with SA signaling pathway. Many herbivores are able to suppress the induced plant defence signaling mediating the oral effectors. Recently, Chung *et al.* (2013) reported the exploitation by Colorado potato beetle (Leptinotarsa decem lineata) of bacteria in their oral secretions to suppress antiherbivore defences in tomato. The associated bacteria were found to suppress the production of JA and JA-responsive antiherbivore defences mediating the induction of SA signaling.

Phloem wound responses, such as coagulating proteins in the phloem sieve elements of the plant and in the capillary food canal in the insect's mouth parts, is a well know plant defence response (Knoblauch and Van Bel, 1998; Eckardt, 2001).During feeding from sieve elements, aphids secret saliva to prevent phloem proteins from clogging inside the capillary food canal (Tjallingii, 2005). Similarly a salivary proteien named C002 was found to be crucial in the feeding of the pea aphid on fava bean (Mutti *et al.*, 2008).

1.2.3. Detoxification

Herbivorous insects have developed also enzymatic metabolisms to detoxify plant allelochemicals. Detoxification systems may be induced when insects are feeding on plants with increasing levels of allelochemicals (Bernays and Chapman, 1994; Schoonhoven *et al.*, 2005). In insects, several detoxification systems have been associated with allelochemical metabolism. Detoxification by cytochrome P450 enzymes, glutathione S-transferases and esterases is generally considered to be the most important (Scott *et al.*, 1998; Salinas and Wong, 1999; Després *et al.*, 2007). P450 monooxygenases metabolize a large diversity of toxic plant compounds to less toxic metabolites (Berenbaum *et al.*, 1992). Glutathione S-transferases act on the toxic by-products of P450 metabolism by increasing hydrophilicity of the metabolites to facilitate their excretion by ATP-binding cassette and other transmembrane transporters from the organism (Misra *et al.*, 2011). Esterases hydrolyze esters and amides, converting them into more polar compounds (Brattsten, 1988). Several studies have reported the occurrence of these detoxification systems in insect against plant toxins. For example, aphids deploy enzymatic detoxification by esterases and glutathione transferases to overcome the toxic effect of hydroxamic acids present in cereals such as wheat, maize,

and rye (Leszczynski *et al.*, 1992; Niemeyer and Perez, 1995; Mukanganyama *et al.*, 2003). Snyder and Glendinnig (1996) observed that larval tobacco hornworms (*M. sexta*) experience a dramatic increase in cytochrome P450 activity against nicotine after ingesting a toxic concentration of nicotine. *H. zea* salivary enzyme glucose oxidase (GOX) was found to inhibit the wound-inducible nicotine production in tobacco (Musser *et al.*, 2005). The polyphagous mite *T. urticae* changes the profile of detoxification enzymes (esterases and GSTs) as response to host shift (Yang *et al.*, 2001). Also the polyphagous arctiid *Estigmene acrea* is well adapted to sequester and specifically handle pyrrolizidine alkaloids of almost all known structural types (Hartmann *et al.*, 2005).

II. Study system

2.1. The host plant

2.1.1. Overview

The domesticated tomato *Solanum lycopersicum* L. (formerly *Lycopersicum esculentum*) is one of the world's most important vegetables, with an estimated total production of about 159.45 million tons in 2011 and China as the far largest producer with almost 1:3 of the world production (Food and Agriculture Organisation [FAO] 2011; Figure 5). In organic tomato production, Italy is occupies the first position with .Tomato fruit is a rich source in micronutrients for human diet and represents the second most widely consumed vegetable after potato (Lugasi *et al.*, 2003).

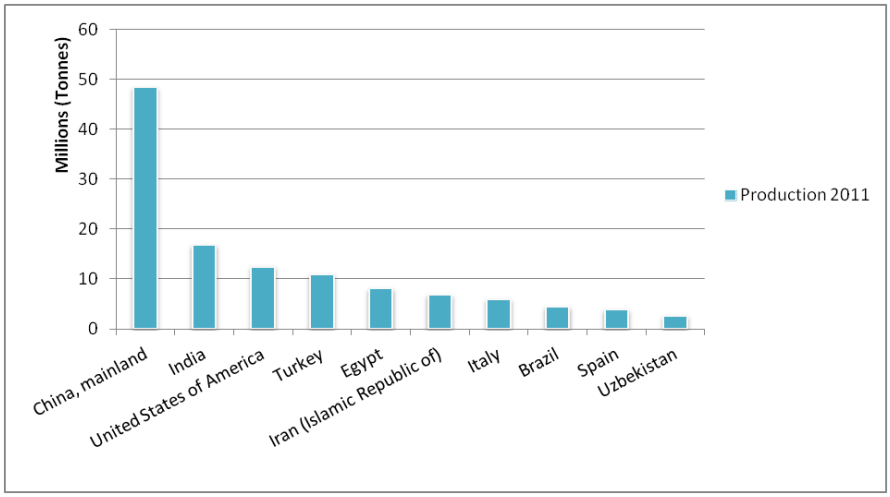

Fig. 5. The top ten tomato producing countries and their production during 2011 (FAOSTAT)

As research material the tomato plant has many interesting features such as fleshy fruit, a sympodial shoot, and compound leaves, which other model plants (e.g., rice and *Arabidopsis*) do not have (Kimura and Sinha, 2008). In addition, tomato belongs to the large commercially important family Solanaceae that include potato, eggplant, peppers, and tobacco. Also there are 13 recognized wild tomato species that display a great variety of phenotypes and can be crossed with the cultivated tomato (Peralta *et al.*, 2005). This Solanum clade containing the domesticated tomato and its wild relatives is very important tool for breeding, and for ecological and evolutionary studies (Moyle, 2008).

Current progress on the tomato genome sequencing project has generated useful information to help in the study of fruit development not just for tomato but also other for fleshy-fruited crops. Recently the tomato genome consortium sequenced and assembled the tomato genome of the inbred cultivar 'Heinz 1706' and its closest wild relative *Solanum pimpinellifolium* (Sato *et al.*, 2012). The predicted tomato genome size is ~900 Mb aligned into 12 chromosomes and shows only 0.6% nucleotide divergence with the wild tomato genome, compared to >8% of nucleotide divergence with potato. Availability of tomato genome will allow breeders to new useful traits for higher yields and better plant resistance to biotic and abiotic aggressions.

Tomato is a natural host to over 100 arthropod herbivores that feed on roots, leaves, or fruit (Lange and Bronson, 1981). Included among the major pests of tomato are adult and larval stages of Coleoptera (beetles), Lepidoptera (moths), Diptera (flies), Thysanoptera (thrips), Heteroptera (true bugs), Homoptera (aphids and whiteflies), and Acari (spider mites and eriophyoid mites). Natural resistance of tomato to many herbivores is attributed to both constitutive and inducible defensive phytochemicals (Farrar and Kennedy, 1992).

2.1.2. Tomato defences against herbivores

Tomato responses to mechanical wounding and herbivory have been widely studied as a model system in which to understand the mechanism of induced resistance (Gatehouse, 2002; Chen *et al.*, 2005; Felton, 2005). Defences include both constitutive such as trichomes on leaf and stem surfaces and induced responses which are initiated upon herbivore feeding. The inducible tomato defence can be direct through initiation of signal transduction pathways followed by increased production of secondary metabolites either toxic or anti-nutritive to herbivores, and indirect defence represented in the emission of HIPVs that attract natural enemies of the attacking herbivores.

Trichomes are outstanding features of the foliage and stems of tomato and among the most important resistance traits to herbivores. According to Luckwill (1943), cultivated and wild species of tomato produce four morphologically distinct glandular trichomes: type I trichomes characterized by a multicellular base, a long (~2mm) multicellular stalk, and a small glandular tip; shorter (~0.3 mm) type IV trichomes, which have a unicellular base, a multicellular stalk shorter than type I, and a small glandular tip; type VI trichomes containing a four-celled glandular head on a short (~0.1 mm) multicellular

stalk; and type VII trichomes consisting of a short (<0.05 mm) unicellular stalk and an irregularly shaped 4- to 8-celled gland. Tomato species also produce several non-glandular trichome types. Gladular trichomes play an important role in tomato resistance to herbivores by impairing their movement and by direct toxicity through chemicals they produce. Particularly, type VI trichomes have been found to produce an array of volatile compounds, among them a methyl kenone, 2-tridecanone known to be lethal to many phytophagous insects and mites, including the aphid *Aphis gossypii* (Williams *et al.*, 1980), the tobacco hornworm *Manduca sexta* (Williams *et al.*, 1980; Barbour *et al.*, 1993; Kang *et al.*, 2010), the tomato fruitworm *Helicoverpa zea* (Williams *et al.*, 1980; Kennedy, 1984), the Colorado potato beetle, *Leptinotarsa decemlineata* (Kennedy and Farrar, 1987) and the two spotted spider mite *T. urticae* (Chatzivasileiadis and Sabelis, 1997, 1998). Also another toxic volatile product emitted from type VI trichome is the 11-carbon methyl ketone, 2-undecanone (Farrar and Kennedy, 1987) which has been found to be toxic to larvae of *H. zea*. Moreover, type IV trichomes of *S. pennellii* were found to produce acyl sugars which confer resistance to numerous insect pests of tomato, such as the aphid *Myzus persicae* (Rodriguez *et al.*, 1993), tomato fruitworm *H. zea* and beet armyworm *S. exigua* (Juvik *et al.*, 1994).

Moreover, trichomes density is a well characterized defence trait in tomato as well as other plants induced by herbivore attack. High densities of glandular trichomes strongly influenced *H. zea* growth, while only high densities of non-glandular trichomes negatively influenced feeding behavior of *L. decemlineata* (Tian *et al.*, 2012).

Beside the defensive features of tomato trichomes against herbivory, negative effects of trichomes (density and chemicals) on both predators and/or parasitoids of herbivores were reported. For instance, van Houten *et al.* (2013) reported that the phytoseiid predatory mite *Amblydromalus limonicus* hampered by tomato trichomes was unable to establish on and control the tomato russet mite before herbivore-associated degradation of trichomes. On the other hand, Farrar *et al.* (1994) observed a reduction of parasitism of *M. sexta* eggs and larvae by *Trichoderma spp* on tomato lines characterized by high trichome densities and increased levels of 2-tridecanone.

Defence responses of tomato to herbivores belonging to different feeding guilds have been commonly known to be orchestrated in JA and SA signaling pathways. Injury to tomato leaves by herbivores or mechanical wounding elicit both local (injured leaf) and systemic (undamaged leaf on damaged plant) responses that results in synthesis of a

complex array of defensive compounds, including antinutritional proteins, signaling pathway compounds, and proteinases (Ryan, 2000). During herbivory or wounding of tomato leaf, systemin, an 18-amino acid wound signaling peptide is proteolytically cleaved from a 200 amino acid precursor protein called prosystemin (McGurl and Ryan, 1992). Systemin subsequently binds a membrane-bound receptor to initiate an intracellular signaling cascade, including the activities of a MAP kinase, a phospholipase, a calcium dependent protein kinase, an extracellular alkalinization, and the release of linlenic acid from membranes. Linolenic acid is converted to the plant hormone jasmonic acid via the octadecanoid pathway (Vick and Zimmerman, 1984; Schaller, 2001). Catalytic activity of polygalaturonase, an early gene, leads to generation of hydrogen peroxide acting as a second messenger for activation of several defence genes (e.g. proteinase inhibitors (PIs), lipoxygenase (TomLox), polyphenol oxidase (PPO)) (Ryan, 1990; Pearce *et al.*, 1991; Ryan and Pearce, 2003; Figure 6). Proteinase inhibitors are the most studied JA-inducible proteins in tomato, which are expressed rapidly and systemically in response to wounding (Gatehouse, 2002). Upon consumption of induced tissues by the herbivore, these proteins bind to and inhibit digestive proteases in the insect gut (Green and Ryan, 1972; Broadway and Duffey, 1986).

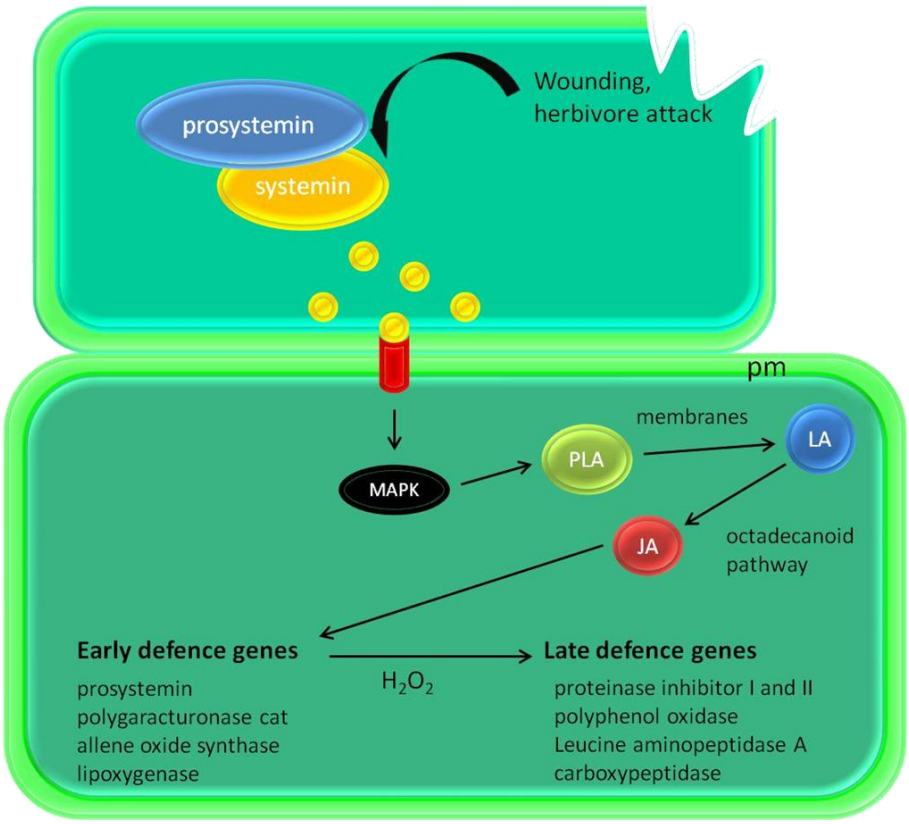

Fig. 6. Model for the activation of defence genes in tomato in response to wounding and insect attack. After wounding, systemin is released from its precursor prosystemin by proteolytic processing. MAPK, MAP kinase; PLA, phospholipase; LA, linolenic acid; JA, jasmonic acid; pm, plasma membrane (modified from Scheer, 2010)

Several lines of evidence support a role of the octadecanoid signaling pathway in the activation of defence responses and resistance of tomato against broad range of herbivores. Field application of exogenous jasmonate promotes resistance of tomato plants to insects (Thaler *et al.*, 1996; Thaler, 1999). Transgenic tomato line that overexpress prosystemin constitutively accumulate high levels of PIs (McGurl *et al.*, 1994) resulting in increased resistance to insect herbivory (Chen *et al.*, 2005). Mechanical wounding, oral secretion of some insects, or systemin results in a rapid and transient accumulation of linolenic acid and JA (Doares *et al.*, 1995a; Conconi *et al.*, 1996; Voelckel *et al.*, 2004). Transgenic tomato plants that express an antisense *prosystemin* are defective in wound-induced systemic expression of *PI* genes and are more susceptible to insects (McGurl *et al.*, 1992). Inhibitors of the octadecanoid pathway block the induction of defence genes by systemin and linolenic acid (Farmer *et al.*, 1994; Doares *et al.*, 1995a). A tomato mutant (*defenceless-1* [*def-1*]) deficient in herbivore-, wound- and systemin-induced JA accumulation and expression of

downstream target genes was shown to be more susceptible to the cell-content feeder *T. urticae* (Li *et al.*, 2002) and chewing feeder *M. sexta* larvae (Howe *et al.*, 1996).

The set of inducible defences of *S. lycopersicum is* differentially induced by different herbivory or combinations of more species (Stout *et al.*, 1999). This variability of tomato responses to herbivory is a result of different feeding behavior and sensitivity of the attacking herbivores. For instance, caterpillar (*S. exigua*) induced three-fold higher PI activity in tomato plants compared to undamaged control, while potato aphid (*Macrosiphum euphorbiae*) had no effects on PIs either alone or when paired with caterpillars (Rodriguez-Saona *et al.*, 2010).

2.2. Herbivores

2.2.1. Spider mite

The two-spotted spider mite, *Tetranychus urticae* Koch is one of the most economically important pests of wide range of plants including fruit, vegetable, grain and ornamental crops in both field and greenhouse worldwide (Lange and Bronson, 1981). It is the most polyphagous species within the family Tetranychidae which contains many harmful species of plant-feeding mites. The mite have been reported infesting over 900 species of plants including vegetables, fruits, ornamentals, herbaceous and woody landscape plants (Agrawal, 2000; Migeon and Dorkeld, 2010). It often forms genetically differentiated populations with somewhat more narrow host ranges (Gotoh *et al.*, 1993, Navajas, 1998).

The spider mite completes development from egg to adult within 7-8 days at 27.5-32.5ºC and all the life stages (Figure 7) are present throughout the year, depending on the environmental conditions (Helle and Sabelis, 1985). Temperature is the most important factor that influences the rate at which mites develop; at low temperature life cycle can be expanded up to four weeks. Host plants, plant nutrition, leaf age, and moisture stress also influence development of *T. urticae*.

As many other spider mites, sex determination in two-spotted spider mites is arrhenotokous. That means, females develop from fertilized eggs and have the normal two sets of chromosomes (diploid); whereas, males develop from unfertilized eggs and have only one set of chromosomes (haploid). Unmated females give rise only to males; mated females can produce either female or male progeny (Helle and Sabelis, 1985).

T. urticae is a cell-content feeder of the mesophyll layer on lower leaf surface, and causes the destruction or disappearance of chloroplasts which then leads to basic physiological changes in the plant. Stomatal closure can be a primary host-plant response, and in such cases, uptake of CO_2 decreases resulting in a marked reduction in transpiration and photosynthesis (Sances *et al.*, 1979). In addition, spider mites produce extensive webbing to protect their eggs, which leads to aesthetic injury, particularly in the case of ornamental plants.

T. urticae has been proposed as candidate model organism for chelicerate (Grbic *et al.*, 2007) and its full genome has recently been sequenced and annotated (Grbic *et al.*, 2011). *T. urticae* genome is the second chelicerate genome available with only 90 megabases is the smallest genome among the sequenced arthropod genomes. The newly available genome shows unique changes in the hormonal environment, and reveals evolutionary innovation of silk production. Transcriptome analysis of mites feeding on different plants shows how this pest responds to a wide changing host environment (Grbic *et al.*, 2011).

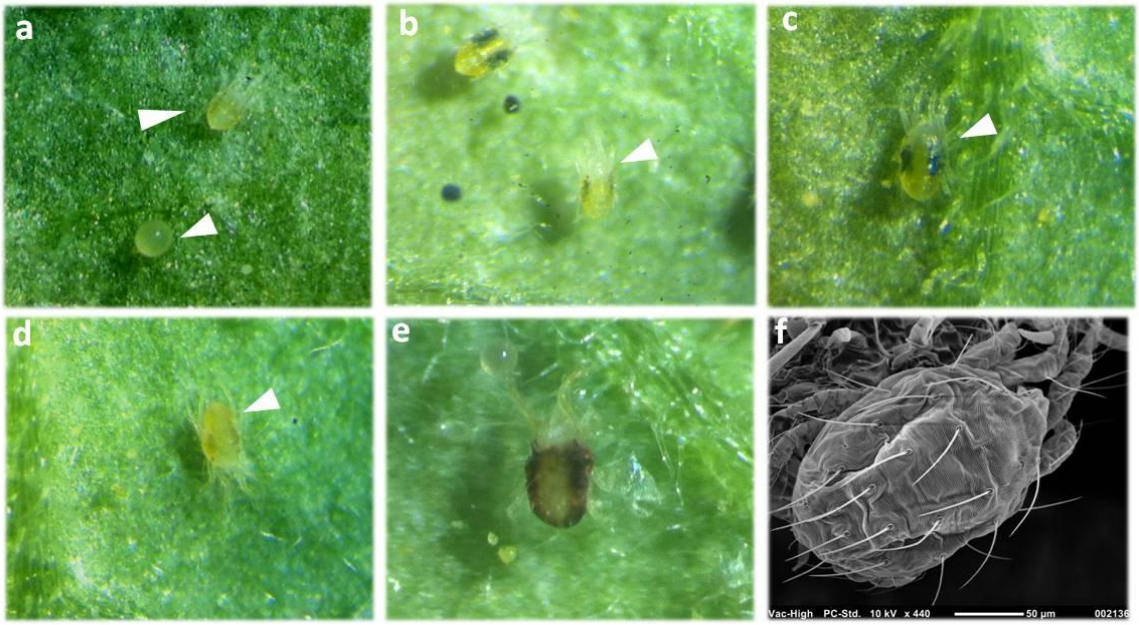

Fig. 7. Life stages of *T. urticae*. Arrow indicates egg and hexapode larva (a), protonymph (b), deutonymph (c), male (d) and adult female (e); (f) represents SEM image of adult female.

Control of *T. urticae* has been and still is largely based on the use of insecticides and acaricides. However, because of the high reproductive rate and fast generation time and the intense selection pressure brought on by chemical control of this pest in the greenhouse, resistance may develop in a comparatively short time (Knowles, 1997; Van

Leeuwen *et al.*, 2008). Indeed, the spider mite has developed resistance to many miticides (Stumpf and Nauen, 2001; Sato *et al.*, 2005; Khajehali *et al.*, 2011), and is considered the "most resistant species" in terms of the total number of pesticides to which populations have become resistant (Van Leeuwen *et al.*, 2010).

2.2.2. Tomato russet mite

The tomato russet mite, *Aculops lycopersici* Massee (Acari, Eriophydae), is an oligophagous vagrant mite, which was found reproducing on host species in many genera of the Solanaceae *(Lycopersicon, Physalis, Solanum, Capsicum, Nicotiana, Datura, Petunia),* but also on field bindweed, *Convolvulus arvensis* (Rice and Strong, 1962; Kay, 1986; Perring and Farrar, 1986). Being oligophagous, *A. lycopersici* is one of very few exceptions of eriophyoid mites which are known to be highly associated to one host species or family. It is cosmopolitan in distribution and widespread in almost all areas where solanaceous crops are grown (Jeppson *et al.*, 1975). On tomato crop, this eriophyoid is considered a worldwide major pest in both open field and greenhouse. It damages plants by piercing epidermal cells which rapidly collapse and die (Royalty and Perring, 1988). Rust mites move away from the injured site and attack other cells, which causes a massive destruction of the epidermis leaf surface visible as russeting, and widespread defoliation followed by considerable reductions in tomato yield (Perring 1996, Petanovic and Kielkiewicz, 2010). Kamau *et al*. (1992), in the process of evaluating commercial tomato varieties, determined that most yield loss was due to mites feeding on the flower stalks and pedicels which withered, causing flower bud death. The selection for high yield and resistance traits in cultivated tomato made the latter very susceptible to russet mite. In fact, high population of russet mite may cause wilting and death of entire tomato plants (Keifer *et al.*, 1982). This is mainly due to leaf mesophyll collapse following destruction of epidermal cells (Royalty and Perring, 1988). In contrast, damages of *A. lycopersici* on field bindweed and some of its natural solanaceous host plants field do not lead to plant death (Rice and Strong, 1962).

The life cycle of russet mite includes egg, larva, nymph and adult stages (Figure 8). The generation time is about one week at 21-25°C. Males develop slightly faster than females. Females live for several weeks and lay ten to 50 eggs. Fertilized eggs produce both males and females, whereas unfertilized eggs give rise to males only (Baradaran-Anaraki and Daneshvar, 1992).

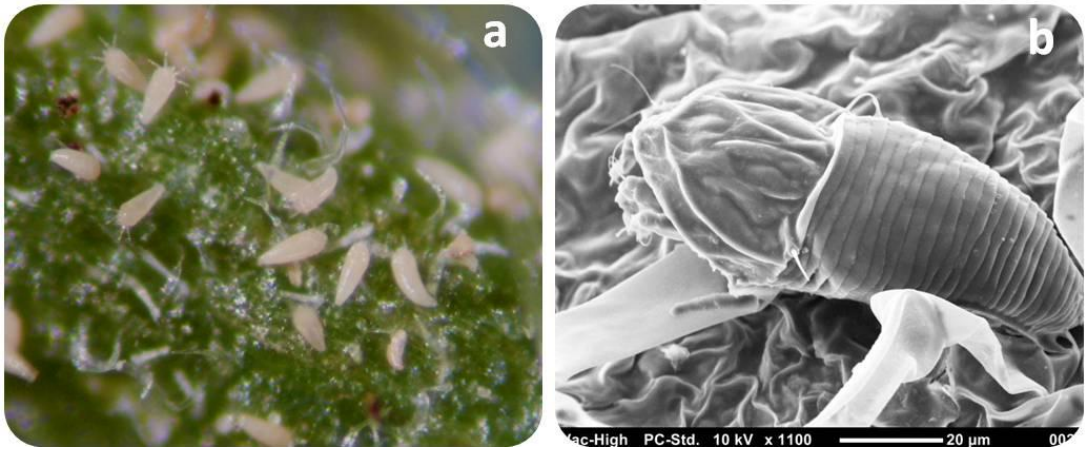

Fig. 8. Tomato russet mite, *A. lycorpersici*. (a): adults and immatures feeding on tomato leaf petiole; (b) SEM image of an adult tomato russet mite.

Russet mite has very small size (female 150-180 µm in length) and preferentially feed in depressions surrounding veins on the leaf of tomato, and can hide among trichomes (Royalty and Perring, 1988). These characteristics made biological control of this pest very problematic. Indeed, to date, all assays mediating release of predatory mites such as *Euseius concordis* (de Moraes and Lima, 1983), *N. Californicus* (Castagnoli *et al.*, 2003), *N. Cucumeris* (Trottin-Caudal *et al.*, 2003), *Amplyseius andersoni* (Fischer *et al.*, 2005) *Amblyseius swirkii* (Park *et al.*, 2010) or *Amblydromalus limonicus* (van Houten *et al.*, 2013), have demonstrated unsufficient control against TRM (Gerson and Weintraub, 2007). Alternative trials with other groups of acarine predators have shown no satisfying results with the stigmaeid *Agistemus exsertus* (Gonzalez) or the tydeid *Homeopronematus anconai* (Baker) (Osman and Zaki, 1986; Brodeur *et al.*, 1997). For chemical control, many products that provide a successful control of TRM have been tested; abamectin and sulphur are very effective and widely used

Molecular data on *A. lycopersici* are unavailable, except a partial cytochrome oxydase subunit I gene sequence I recently deposited at GenBank. Similarly, data on defence responses in tomato-russet mite interactions are very limited and date back to 1996 when Stout *et al.* (1996) reported the induction both locally and systematically of several enzymatic proteins (proteinase inhibitors (PIs), peroxidases (POX), polyphenol oxidase (PPO), lipoxygenase (LOX)) in the leaves of tomato plants in response to *A. lycopersici* short-term feeding.

2.3. Predatory mites

2.3.1. Phytoseiulus persimilis Athias-Henriot

The predatory mite *P. persimilis* belongs to the family Phytoseiidae of the order Mesostigmata. It is the most used and commercialized biological control agent against spider mites (Cote *et al.*, 2002). It has been introduced to many countries and successfully used for over half a century in the biological control of tetranychids phytophagous mites on many vegetable crops and cut flowers (Zhang and Sanderson, 1995; Messelink *et al.*, 2006; Gerson and Weintraub, 2007). It is a specific predator of Tetranychus spider mites and shows reduced reproduction and survival on other spider mites and phytophagous mites. Adulthood of *P. persimilis* is achieved through four developmental stages: egg, larva, protonymph and deutonymph. Development from the egg to adult takes 3.6 days for males and 4.1 days for females at 26°C. The sex ratios of offspring are often highly female-biased (>80% daughters). The larval stage does not feed, but the protonymph and deutonymph will feed on the egg, larva, and protonymph stages of spider mites (Takafuji and Chant, 1976). Depending on the abundance of prey, an adult female (Figure 9) can consume ten to 20 Tetrancyhus spider mite eggs per day and lay as many as five eggs per day and up to 80 eggs during her life (Castagnoli *et al.*, 1998).

Fig. 9. Adult of *P. persimilis* feeding on *T. urticae* on strawberry leaf.

Despite *P. persimilis* mites are specific predators of Tetranychus species (McMurtry and Croft, 1997), its efficiency in the control of *T. urticae* on tomato is often lower than desirable (Gillespie and Quiring, 1994; Drukker *et al.*, 1997). Efforts have been dedicated to obtain other strains of predatory mites with better performance on tomato (Drukker *et al.*, 1997; Gerson *et al.*, 2003).

Although numerous studies on *P. persimilis* biology and behavior have been carried out, molecular data are still rather scarce. In fact, only partial sequences related to the ribosomal RNA units and some mitochondrial DNA genes are available at GenBank. Nevertheless, recently the transcriptome analysis of *P. persimilis* was conducted to study mite reproduction (Cabrera *et al.*, 2011). Also the transcriptome sequencing and annotation of another predatory mite *Metaseiulus occidentalis* was performed to characterize gene expression in all life stages reared under different conditions (Hoy *et al.*, 2013). These initiatives will provide in the near future new insights into predatory mite genomics.

2.3.2. Neoseiulus californicus (McGregor)

N. californicus (Acari: Phytoseidae), is a predatory mite distributed worldwide and used as biological control agent of spider mites in field and greenhouse crops (Castagnoli and Simoni, 1991; Raworth *et al.*, 1994; McMurtry and Croft, 1997). It is a generalist predator that preys and reproduces on wide range of tetranychid mites, dust mites, tomato russet mite and pollen (McMurtry and Croft, 1997; Castagnoli and Simoni, 1999; Castagnoli *et al.*, 2003). The fact that is a generalist predator, its rearing was made easy, in fact for commercial and research implication; it is usually maintained on pollen or dust mites (Figure 10).

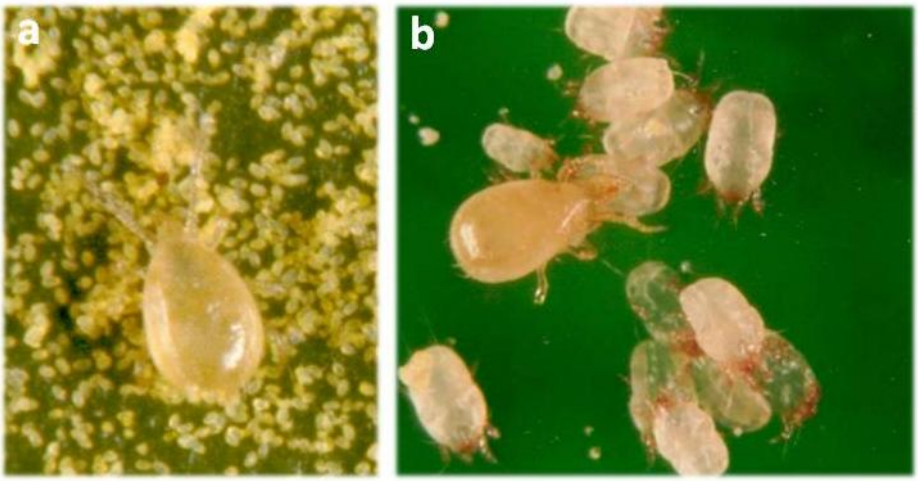

Fig. 10. *N. californicus* adult feeding on Quercus pollen (a) and on dust mite *Dermatophagoides farinae* (b).

Life cycle of *N. californicus* is divided into five stages: egg, larva, protonymph, deutonymph and adult. Development from egg to adult of *N. californicus* fed *T. urticae*

at 25±1°C and 75±10% RH takes 4.3-8.1 days depending on mite strain (Mesa *et al.*, 1990; Castagnoli and Simoni, 1991; Gotoh *et al.*, 2004). The reproductive system is pseudoarrenotoky, males are aploid while females are diploid with 4 and 8 chromosomes, respectively (Simoni, 1992). Mating is essential for oviposition and several matings are necessary to achieve maximum oviposition. Maximum daily oviposition is 3-5 eggs and usually occurs on the second-third day of oviposition (Catagnoli *et al.*, 2003). Like other phytoseiids, sex ratio is female-biased with 64-69.7% offspring are females (Castagnoli and Simoni, 1991; Gotoh *et al.*, 2004).Consumption rate depends on prey nature and density; on *T. urticae, N californicus* can consume up to 17 eggs, 10 immatures or 14 males at the maximum density tested (Laing and Osborn, 1974; Castagnoli and Simoni, 1999).

In natural environments, *N. californicus* use HIPVs from infested plant to locate its prey. For example, *N. californicus* was able to use volatile compounds from the infested host plant (apple), as well as those from the prey (*P. ulmi* and *T. urticae*) as cues in prey location at both short and long distance (Collier *et al.*, 2001; Llusia and Penuelas, 2001).

III. Research objectives

In natural environment, plants are challenged by a multitude of herbivorous arthropods feeding on different plant structures and on different tissues of the same structure. Cell-content feeders are piercing-sucking herbivores that feed on mesophyll layers and on epidermal cells. Inducible defences are activated upon herbivore attack and several phytohormone-mediated signal transduction pathways were found to control induced defence responses. They include the jasmonic acid (JA), ethylene, and salicylic acid (SA) pathways. Herbivores belonging to different feeding guilds induce distinct signal-transduction pathways. For example, chewing herbivores through causing wounds predominantly activate the jasmonic acid (JA) signaling pathway, whereas phloem-feeding insects, such as whiteflies and aphids, frequently activate the salicylic acid (SA) signaling pathway (Moran and Thompson, 2001; Zarate *et al.*, 2007). Moreover, cross-talk between different defence signaling pathways can affect plant response to herbivore attacks. Until recently, plant-herbivore interaction were studied with single species of herbivore, and few studies were carried out with multiple herbivores considered species having the same feeding guild, despite it is widespread phenomenon in nature.

Based on recent reports that infestation by specialist mite may induce SA-dependent genes while suppressing JA-dependent genes and possible cross-talk between JA and SA signaling pathways (Sarmento *et al.*, 2011), I hypothesized that infestation by a specialist eriophyoid mite (*A. lycopersici*) would affect the tomato defence response to a generalist spider mite (*Tetranychus urticae*). Both mites are cell-content feeders, but they exploit different leaf layers: spider mite feed on parenchymal cells, while russet mite feed on epidermal cells. The objectives of this study were to examine the induced direct defence response of tomato plants to attack by russet mite alone, spider mite alone and by two mites. In addition, I investigated the consequence of these interactions on the third trophic level, which is the attraction of predatory mites by volatile organic compounds emitted from tomato plants under different scenarios.

IV. Material and methods

4.1. Material

4.1.1. Plant material

The plant model used in the course of this study is tomato (*Solanum lycopersicum* L. cv. Roma). Seeds were sown in trays in commercial potting soil (Vigorplant®, Italy), and kept inside a growth chamber (25± 2°C, 70-80% R.H., and 16:8 h L/D). Seedlings (3 week-old) were transferred to plastic pots (0.5 L) that contained commercial compost mixed with perlite in a ratio of 3:1 respectively. Tomato plants were further grown inside mite proof screen cage in a greenhouse, and were irrigated with 0.5% solution of fertilizer (NPK 20-20-20) until they were 6 week-old and had at least four completely developed leaves.

4.1.2. Herbivores

Two-spotted spider mites, *Tetranychus urticae* (Acari: Tetranychidae) were obtained from a maintained rearing on strawberry (*Fragaria ananassa* cv. Silva) plants in a greenhouse. In order to obtain the required high number of *T. urticae* females from the same eggs cohort, mass rearing on detached strawberry leaves was carried out in the laboratory under 25± 2°C, 70-80% R.H., and 16:8 h L/D photoperiod.

Tomato russet mites, *Aculops lycopersici* (Acari: Eriophyoidae) were obtained from maintained culture on wild tomato (*Solanum* section ly*copersicum*) plants grown in pots in a greenhouse. All rearing facilities were located at the CRA-ABP Agricultural Research Council-Center for Agrobiology and Pedology (Florence, Italy).

4.1.3. Predatory mites

Two predatory mites were selected for the behavioral study toward differentially infested tomato plants, one specialist and one generalist species. The specialist, *Phytoseiulus persimilis* that feed only on spider mites was supplied by Koppert Italia. Adult mites were maintained for an adaptation period during which they were offered a surplus of spider mites on detached strawberry cv. Silva leaves in the laboratory at 22 ± 2°C, 60 ± 10% RH.

The generalist species *Neoseiulus californicus*, that feed on pollen and may mite species was continuously reared in laboratory at 22 ± 2°C, 60 ± 10% RH, and under natural photoperiod, supplied with detached strawberry leaves infested with *T. urticae*.

4.2. Methods

4.2.1. Evaluation of microscopic damages caused by spider mite and russet mite

Tomato leaves attacked for 3 days by spider mites or russet mites, along with a clean undamaged leaf were observed under Scanning Electron Microscopy (SEM). Leaves were excised into small pieces (~0.5 cm^2) and prepared following the protocol described by Talbot and White (2013) with a small modification. Briefly, leaf tissue was fixed in 100% methanol for 10 min, followed by incubation in 100% dry ethanol for 30 min, and kept to dry at room temperature for 30 min. Dried tissue was then mounted on SEM stub and coated with gold using an auto fine coater (Jeol JFC-1300). Finally leaf tissue was observed and photographed by SEM (NeoScope JCM-5000).

4.2.2. Effects of russet mites and SA treatment on the feeding choice of spider mites

In order to determine the effects of *A. lycopersici* infestation or exogenous salicylic acid application on the feeding behavior of spider mite, we assessed the choice preference of female *T. urticae*. In the tomato russet mite treatment, 6 week-old tomato plants were infested with *A. lycopersici* (at a density of ~200 specimens/ leaflet) for 3 days. Control plants were placed in a mite proof screen cage and left uninfested. In the SA treatment, plants were sprayed with 10 ml/plant of a 1 mM SA solution in water (containing 0.1% Tween 20) and incubated for 3 days, and plants kept under the same conditions were sprayed with 10 ml/plant of water (containing 0.1% Tween 20) and were regarded as controls. Subsequently, spider mites were offered two leaf discs (diameter 2.0 cm): one from *A. lycopersici*-infested plant or from SA-treated plant and one from a control plant. Leaf discs were placed in a Petri dish with moist cotton wool, and were connected by a T-shaped bridge (3.0 cm wide) made from transparent plastic film. The position of the discs was alternated between replicates, 20 replicates for each treatment and control were included. Five newly emerged adult female *T. urticae* were singularly released at the

base of the T-bridge, and after 2h, the number of the spider mite adults on each disc of the two-choice setup was counted.

4.2.3. Effects of russet mites and SA treatment on the performance of spider mites

To assess the performance of female spider mite on russet mite-infested tomato leaf discs or salicylic acid (1 mM) treated discs from same plants used in the choice experiment, I determined the oviposition rate of female adults *T. urticae* after 24 h feeding. Five newly emerged spider mite adult female hatching from the same cohort of eggs were placed on treated and control leaf discs and allowed to feed for 24 h. twenty replicates for each treatment were prepared.

4.2.4. Olfactory choice of predatory mites

4.2.4.1. Y-tube olfactometer set-up

Responses of predatory mites to plant volatiles were tested in an olfactometer set-up as described by Bruin *et al.*, (1992). The set-up consisted of a Y-shaped glass tube (1.2 cm in diameter) with an entry arm (20 cm in length) and two side arms (15 cm in length). Both arms of the Y-tube were connected to a glass cylinder (12 Ø x 30 cm height) containing tomato plant as odour source. Airflow was established from each cylinder through the olfactometer arms via a KNF's LABORPORT® air pump (KNF Neuberger, USA) with the airflow adjusted with a flow meter (key instrument, USA) to 1 l/min. The air passed through activated charcoal before reaching the cylinders and the airflow was measured in the entry arm. The glass Y-tube was positioned horizontally under a light source of about 2000 lux (Figure 11).

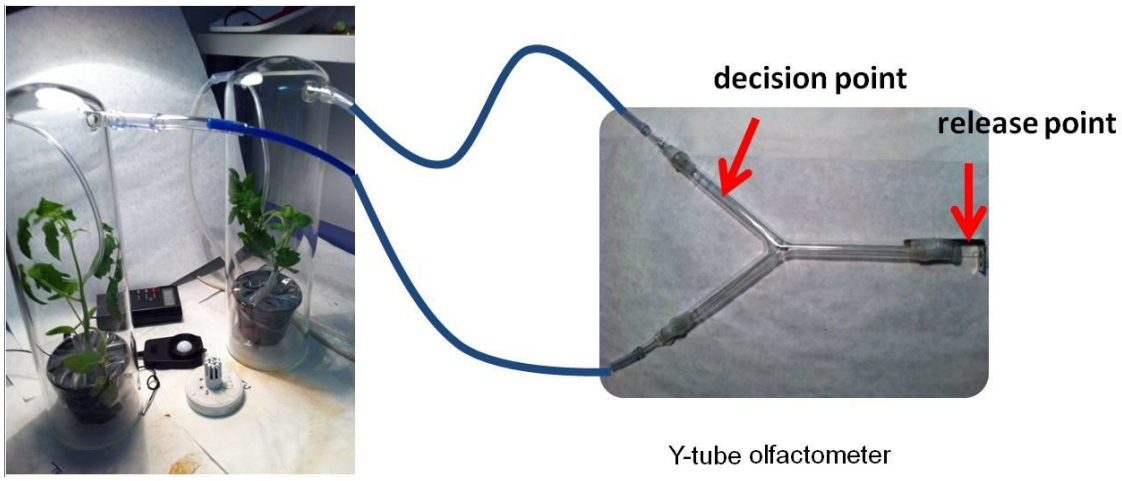

Fig.11. Olfactometer set-up

4.2.4.2. Mites infestations *and olfactometer experiments*

Two separate experiments were conducted to investigate the response of the two predatory mite species to tomato plants infested with *T. urticae*, *A. lycopersici*, and both herbivores. In the first experiment, low populations' density of herbivores: 10 adult females of *T. urticae* and ~ 100 adults of *A. lycopersici* per tomato leaflet was tested. Under single infestation, russet mites were transferred to each of the terminal three leaflets of the third leaves on excised leaf pieces from heavily infested plants. Spider mites were transferred by a fine paintbrush to the upper surface of leaf. Mites were confined to the infested areas by applying homemade insect glue around the petiole of the leaf. For dual infestation, spider mites were applied upon migration of russet mites from the excised leaf pieces to tomato leaflets. In all situations, herbivore mites were allowed to feed for three days, after which olfactory assays and volatiles samplings were performed.

The set of experiments conducted with olfactometer is described in figure 12. In the second experiment, higher infestation density (20 adult females of *T. urticae,* and ~300 adults of *A. lycopersici* per tomato leaflet) were used, and only the comparison between *T. urticae* and dual-infestation was carried out.

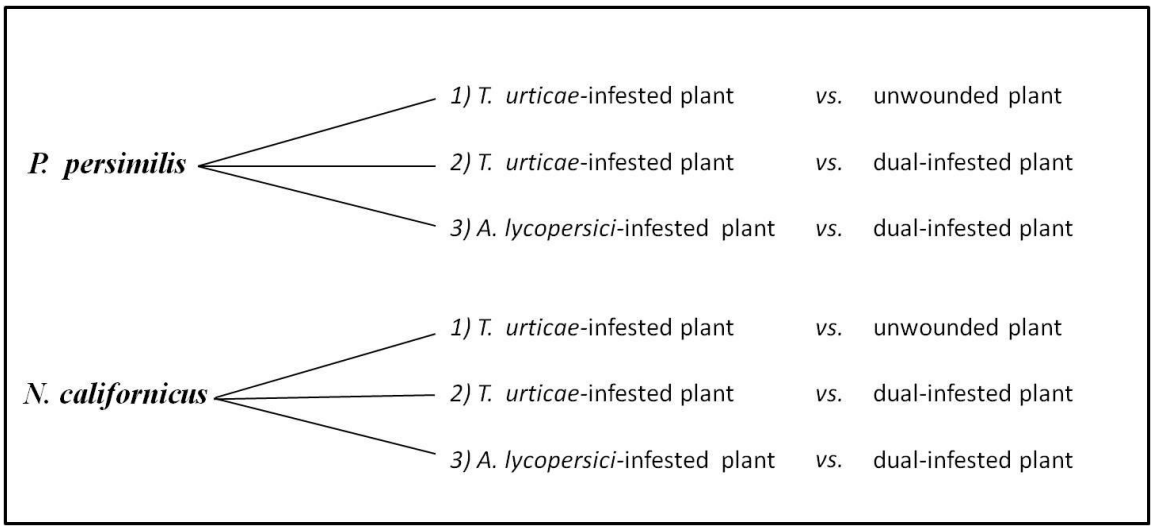

Fig. 12. Scheme of olfactometer experiments with *P. persimilis* and *N. californicus* predatory mites conducted at low population densities of *T. urticae* and *A. lycopersici*

Tomato plants were infested for three days at the time of the olfactometer experiment. Each comparison of infestations was replicated on three different experimental days with new sets of tomato plants.

Before the experiment begin, predatory mites were starved for 24h by enclosing each 10 specimens separately in a 5cm Ø petri dish confined with a piece of moist cotton to supply moisture and avoid escaping. Thirty starved *P. persimilis* and *N. californicus* were tested in each replicate; each individual was tested once. To test the odour choice, individual predatory mite were placed at the entry of the long arm of Y-tube olfactometer with a soft-bristle paintbrush and observed until it had reached at least 2/3 length of one of the arms. Predators that did not choose a side arm within 5 min were excluded from the analysis. Then, the Y-tube was cleaned with a high air flow after testing every single predatory mite. After every 10 runs, the Y-tube was washed with 70% ethanol and left to dry for 5 min, and differentially infested tomato plants were switched between the left-hand and right-hand side arms to minimize any spatial effect on choices. The experiments were conducted on a workbench in the laboratory at 23 ± 0.5°C and 50± 5 % RH.

4.2.5. Gene expression analysis

4.2.5.1. Infestation of plants

In order to obtain molecular evidence of variable plant responses to different mite's infestations, six week-old tomato plants produced as described in section 1.2 were treated as follow: one leaf (composed of 3 fully expanded leaflets) from each three randomly selected plants were infested with 75 adult female *T. urticae* or with ~900 tomato russet mites or with both species, while other leaves were kept clean. Insect glue was applied to the leaf petiole on which mites were released to prevent mites moving to other plant parts. Three clean plants were kept separately under the same conditions and considered as uninfested control.

4.2.5.2. RNA extraction and DNase treatment

Total RNA was isolated from tomato leaf tissue infested with *A. lycopersici*, *T. urticae*, *A. lycopesici+T. urticae* and uninfested at 3 and 6 days after treatment using the RNA Purelink® kit according to the manufacturer's instructions (Ambion, California, USA). Briefly, at room temperature, 200 mg of leaf tissue were ground to a fine powder in liquid nitrogen N_2 using a sterile mortar and pestle. After N_2 evaporation, 1 ml of lysis buffer prepared with 2-mercaptoethanol was added to the extract. The sample was homogenized by vortexing for 2 min, and centrifuged at 12000 x g for 1 min to remove leaf debris. To the recovered supernatant, 0.5 volume of 96-100% ethanol was added,

and the homogenate was passed through a spin cartridge by centrifugation at 12000 x g for 15 s. Thereafter, the spin cartridge was washed once with 700 µl of washing buffer I solution and twice by 500 µl of washing buffer II solution, each time centrifuging at 12000 x g for 15 s. Finally, the total RNA was eluted adding twice 15 µl of RNase-free water and centrifuged at 13000 x g for 1 min. Subsequently, total RNA was treated with 1U DNase (Ambion, California, USA) for 20 min at 37°C, followed by inactivation step using 1X of DNase inactivation reagent. Purified RNA was conserved at -20 for later use.

4.2.5.3. RNA quantification

DNase-treated RNA from each sample was quantified using the RNA high specific kit and a Qubit™ fluorometer (Invitrogen, California, USA). Furthermore, in order to check the quality of the extracted RNA prior to cDNA synthesis, 2 µl from each sample was visualized on 1% agarose gel stained with ethidium bromide under UV illumination.

4.2.5.4. Complementary DNA (cDNA) synthesis

First-strand cDNA synthesis was carried out in 1µg DNase-treated total RNA, 1µl oligo-dT primer (0.25µg/µl) and 1µl (200 units) SuperScript™ II reverse transcriptase (Invitrogen, California, USA) according to the manufacturer's instructions. The resulting cDNA was then stored at -20 until use for qPCR.

4.2.5.5. Target genes and primers design

The response of plants to herbivore attacks is orchestrated mainly in jasmonic acid (JA) and salicylic acid (SA) signal-transduction pathways. Two key enzymes in the octadecnoid pathway upstream to JA synthesis were selected: tomato lipoxygenase D (*TomLoxD*) and allen oxide synthase (*AOS)*. *TomLoxD* is a 13-lipoxygenase that catalyzes the hydroperoxidation of linolenic acid, a key step in JA biosynthesis (Vick and Zimmerman, 1984). *TomLoxD* as oxidative enzyme is associated with the formation of active oxygen species and free radicals, which also exhibit antibiotic properties against herbivores (Elstner, 1980). Overexpression of *TomLoxD* leads to elevated wound-induced JA biosynthesis, increased expression of wound-responsive genes and, therefore, enhanced resistance to insects and necrotrophic pathogens (Yan *et al.*, 2013). *AOS* is an important enzyme in the octadecanoid pathway that catalyzes the first step of JA biosynthesis from lipoxygenase-derived hydroperoxides of free fatty acids (Mueller,

1997). Downstream of JA pathway, the wound-induced proteinase inhibitor-II (*Wipi-II*) was tested; WIPI-II accumulates in tomato leaf cells in response to severe wounds, such as those resulting from insect attacks (Rodriguez-Saona *et al.*, 2010). In addition, gene expression of a pathogen related protein, pathogenesis-related protein 1 (PR-1) was studied as indicator of the SA pathway. Moreover, independent from JA and SA pathways, the trancriptional level of geranylgeranyl diphosphate synthase 1 (*GGPS1*), an enzyme that synthesize the precursor of the volatile TMTT, which is attractive to predators of spider mites (Dicke *et al.*, 1990) was investigated.

In quantitative real-time PCR, for some genes, specific primer pairs were selected from previous studies, while for other genes, Primer3 Plus software (http://www.bioinformatics.nl/ cgi-bin/primer3plus/primer3plus.cgi/) was used to design short amplicons suitable for qPCR (Table 1).

Table 1. Target genes and primers used for quantitative real-time PCR

Gene	Accession number	Primer forward (5'-3')	Primer reverse (5'-3')	Product size (bp)	References
- **Tomato leaf wound-induced proteinase inhibitor II (WIPI-II)**	K03291	gacaaggtactagtaatcaattatcc	gggcatatcccgaaccaaga	152	(Sarmento *et al.*, 2011)
- ***L. esculentum* lipoxygenase D (TomLox D)**	U37840	ctcatttccatcctcaccac	agctaggaacaccgcatac	162	This study
- ***L. esculentum* pathogenesis-related protein 1 (PR-1)**	DQ159948	tccgagaggccaagctataa	ttgcaagaaatgaaccacca	149	This study
- ***S. lycopersicum* geranylgeranyl pyrophosphate synthase 1(GGPS1)**	DQ267902	ggcagattgtggacttggcga	ctcattcgctccacatcaacc	155	(Van Schie *et al.*, 2007)
- ***L. esculentum* allene oxide synthase (AOS)**	AJ271093	gctacaattcccctcgcata	acaggtggtgatgacgatga	153	This study
- ***S. lycopersicum* actin (Actin)**	U60478	gaaatagcataagatggcagacg	atacccaccatcacaccagtat	159	(Løvdal and Lillo, 2009)

4.2.5.6. Quantitative real-time PCR

Real-time PCR reactions were carried out with 1 µl of cDNA (diluted 1:10), 10 µl of SYBR® select master mix (Invitrogen, California, USA), 0.5 µl of each specific primers (0.5 µM), and the final volume was made up to 20 µl with RNase-free water. Three biological replicates were included for each treatment, and 3 technical replicates in each plate. The reactions were prepared in a 96 wells plate, and were performed on a LightCycler 480 platform (Roche Applied Science, USA) following the thermal cycle conditions described in the table 2.

In order to set the primer concentration and efficiency of PCR reaction, a standard curve made with serial dilution of a known sample concentration was included in each plate for the primer pair of target and reference genes. Reaction efficiency above 96% was considered valid for further analysis. The specificity of amplicons was verified by melting curve analysis and agarose gel electrophoresis.

Table 2. Thermal cycling conditions for quantitative real-time PCR

Step	**Temperature**	**Duration**	**Cycles**
UDG Activation	50°C	2 min	Hold
AmpliTaq® DNA Polymerase, UP Activation	95°C	2 min	Hold
Denature	95°C	15 sec	
Anneal	56°C	30 sec	40
Extend	72°C	1 min	
Melting	Default dissociation steps		
Cooling	40°C	30 sec	Hold

To quantify the relative expression of each gene in the treated samples compared to the untreated one, calculations were made according to $2^{-\Delta\Delta Ct}$ method as described by Livak and Schmittgen, (2001). The method describes the fold change of gene expression of a target gene normalized to the reference gene in a given sample compared to a calibrator (non-infested tomato leaves in this case).

4.2.6. Volatile organic compounds (VOCs) emission

4.2.6.1. Volatile sampling

Right after olfactory essay, VOCs samplings were performed with the same set up and at the same conditions. Air was sampled from the headspace of tomato plants within

individual volatile collection chambers. The chambers consisted of 12 cm diam by 30 cm tall glass cylinders that were capped with Teflon® lids. Plants were inserted into the chambers so that the pot and soil completely wrapped in plastic bag were contained within the glass cylinder (Figure 13).

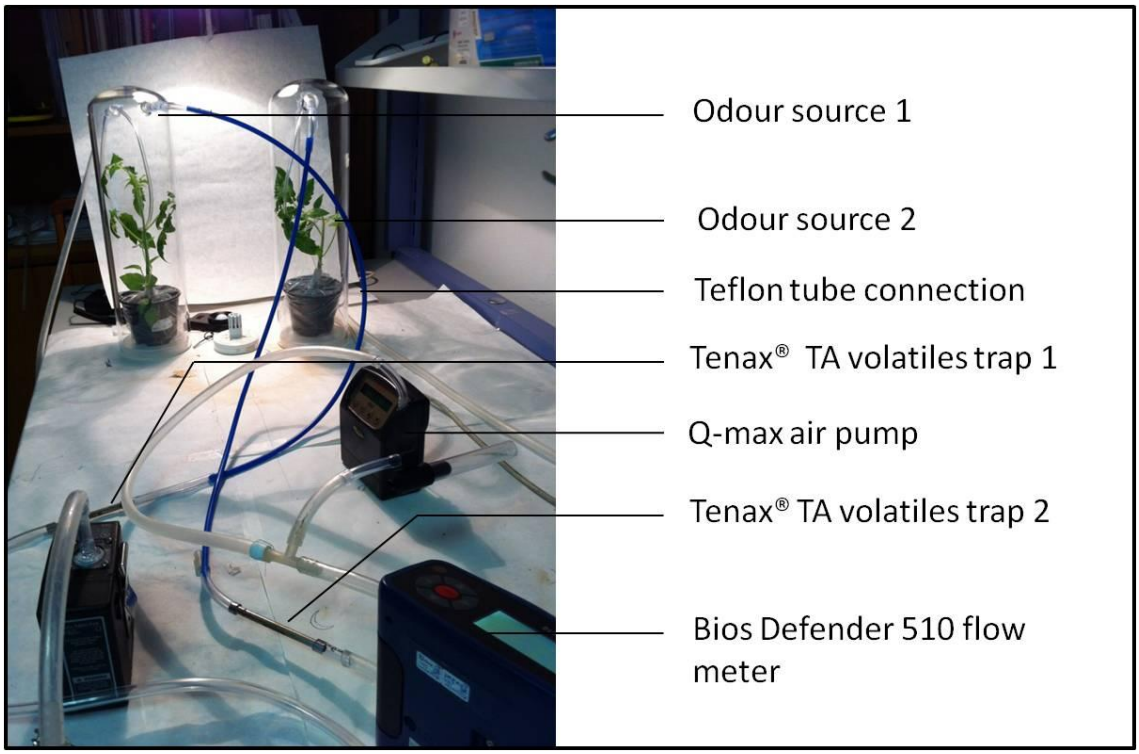

Fig. 13. Set-up for sampling of volatile organic compounds (VOCs)

Q-Max pumps (Supelco, Bellefonte, Pennsylvania), through Tedlar® tubes (Saint Gobain, Akron, USA), sucked the entrapped air of the glass jars containing the plants. This air went through stainless steel tubes (Markes International Inc. Wilmington, USA) filled with 115 mg of Tenax® and 230 mg of Unicarb®, where the VOCs were retained. The sampling time was 20 minutes, with a stable flow of 300 ml min^{-1} controlled by a Bios Defender 510 flow meter (Bios International Corporation, Butler, USA). The VOCs-filled tubes were stored in a -20ºC freezer as soon as the sampling finished. Thereafter, the whole aerial parts of plants used were dried in an oven until constant weight was reached, in order to calculate the dry weight and refer it to the VOCs emissions.

Light and temperature of the glass jars were also controlled in order to have the same conditions in all samplings. Several blanks, consisting in pots with soil wrapped in

plastic bag but without plant, were conducted during the samplings. The glass jars were cleaned with ethanol and distilled water and after every VOCs sampling. All stainless steel tubes used had been previously conditioned during 25 min at 300ºC with a purified stream of helium with a flow of 100 ml min^{-1}.

4.2.6.2. VOCs chemical analysis

The VOCs contained in the tenax TA traps were released with an automatic sample processor (TD Autosampler, Series 2 Ultra, Markes International Inc. Wilmington, USA) and then desorbed using an injector (Unity, Series 2, Markes International Inc. Wilmington, USA) in a GC (7890A, Agilent Technologies, Santa Clara, USA) with an MS detector (5975C inert MSD with Triple-Axis Detector, Agilent Technologies). The chromatographic analyses were performed with a full scan method. The desorbed sample was initially retained in a cryo-trap at -25 ºC. No split was used. The sample was desorbed again at 330 ºC for 25 min and injected into the column with a transfer line at 250 ºC. After sample injection at 35 ºC, the column temperature increased stepwise at 15 ºC min^{-1} to 150 ºC and maintained for 5 min, at 50 ºC min^{-1} to 250 ºC and maintained for 5 min and finally at 30 ºC min^{-1} to 280 ºC and maintained for further 5 min. Total run time was 25.66 min, and the helium flow was 1 ml min^{-1}.

Terpene identification was performed by comparing known standards and the mass spectra with published spectra (Wiley 7n library), while peak quantification was conducted using the fractionation product with mass 93. The MS detection system was operating in SIM mode (Llusia *et al.*, 2012). Calibration curves for quantification were prepared with commercial standards of some of the most abundant compounds in the samples: α-pinene, limonene and γ-terpinene (monoterpenes), α-caryophyllene (sesquiterpene) and o-cymene (sequiterpene), all from Sigma Aldrich, Germany. Terpene calibration curves were always highly significant ($r^2 \geq 0.99$) in the relationship between signal and terpene concentration. The most abundant terpenes had very similar sensitivities, with differences lower than 5%. The rates of terpene emission were expressed in mg of volatiles per m^3 of sampled air (mg/m^3).

4.3. Statistical analysis

When analyzing the differences in oviposition rates of *T. urticae* females under different treatments, Student's t-test was used. Chi-square test was used to analyse predatory mite

olfactory choice. Predators that did not make a choice were excluded from the analysis. For transcriptional changes in the studied gene, the effect of infestation was evaluated on the fold change of gene expression by means of multivariate analysis (MANOVA, main effects). Susbsequently one way ANOVA was performed in order to test the significance of each treatment on each gene separately by Least Significant Difference (LSD) Post Hoc test. ANOVA analysis was also used for the comaprison of volatile emission from differently treated tomato plants. All statistical analyses were carried out on SPSS software® version 13.0 (IBM, USA).

V. Results

5.1. Spider mites and russet mites effects on tomato leaf tissue

Spider mites *T. urticae* having a relatively long stylet which is about 130µm in length (Jeppson *et al.*, 1975), can completely penetrate the epidermal cells and feed on photosynthetically-active mesophyll on both upper and lower leaf surfaces. The feeding causes the destruction or disappearance of chloroplasts which then is visible as yellow to clear spots on the leaf surface (Figure 14b). Under SEM, the feeding site of spider mite is visualized as a circular hole in the epidermal cells layer (Figure 14g).

Tomato russet mite, however, having relatively short stylet which is about 7-20µm in length, can reach only the epidermal cells below the cuticle. The probing site of russet mite is characterized by an irregular hole surrounded by cell content exudates (Figure 14f). Russet mite does not ingest all the epidermal cell content and therefor the cellular liquid is expelled outside the cell. Contineous feeding by rust mites on epidermal cells causes a strong deformation followed by desiccation of the leaf surface as result of excessive transpiration. After a short feeding time, the glandular trichomes, which are the fundamental defence organs in tomato leaf against herbivores, rapidly develop a brownish discoloration after which they dry out and fall over onto the plant surface (Figure 14c and 14g).The damage caused by russet mite appears to be more dispersed and always starting next to the leaf veins.

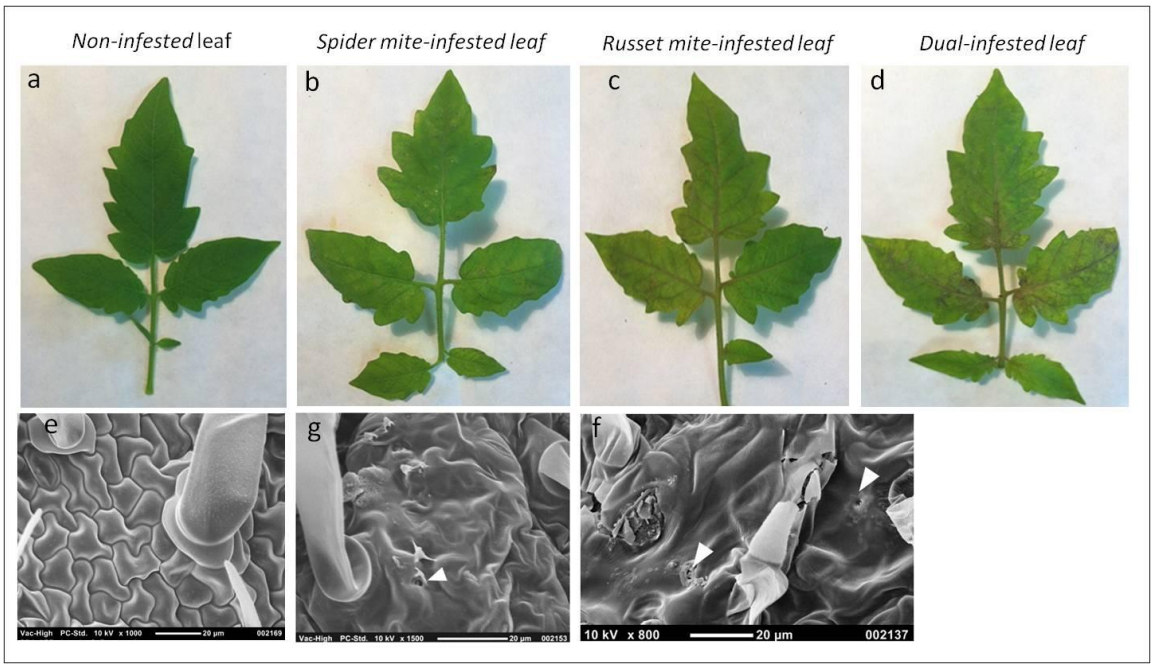

Fig. 14. Comparative description of tomato leaf's damage caused by feeding of different herbivory. Digital photo of a composed tomato leaf (a, b, c and d); SEM photos of the caused damage by the corresponding mite attack (e, g and f).White arrows indicate feeding sites of mites.

5.2. Spider mites feeding preference and performance

Spider mite feeding is perceived by plant as wounds and then activates mainly the JA signaling pathway and its defence-related proteins (Kant *et al.*, 2004; Sarmento *et al.*, 2011). Induction of SA pathway causes the suppression of JA-dependent response triggered by spider mite (Zhang *et al.*, 2009). Supposing that russet mite may induce SA dependent defence response, I used SA-treated tomato leaves to test spider mite feeding choice and performance in comparison to leaves infested by russet mite or untreated. When offered to choose between feeding on tomato leaf discs treated with SA or untreated leaves, spider mite preferred those treated with 1mM SA ($X^2_{test}=6.69;\ P<0.01$) (Figure 15). Surprisingly, spider mite had also a significant preference toward russet mite-infested leaves ($X^2_{test}=8.01;\ P<0.01$) over clean leaves.

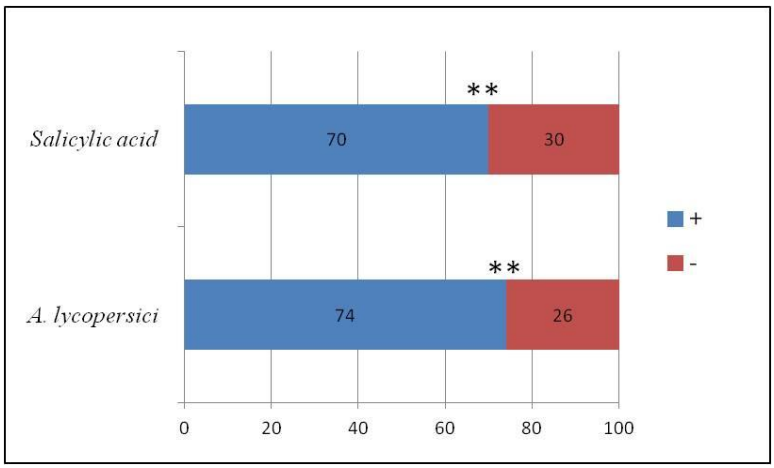

Fig. 15. Feeding choice of *T. urticae* adults between tomato leaf discs treated with *A. lycopersici* or salicylic acid and clean leaf discs. The choice test is performed with twenty replicates per treatment by releasing five spider mites on a plastic T-shape in contact with both leaf discs (at 22ºC and ±70% RH). (+): with treatment; (-): without treatment. *(**: $P<0.01$*).

The oviposition rate is strongly correlated to population growth rate which is a factor reflecting a higher fitness of herbivore on the host plant. In this experiment, the oviposition rate of *T. urticae* was scored at 24h post feeding on *A. lycopersici*-infested or SA-treated and untreated tomato leaf discs. Results showed that *T. urticae* females had significantly higher oviposition rate on tomato leaves infested by *A. lycopersici* (*t*-

test=3.13; P=0.003) or treated by SA (*t-test=2.217*; *P=0.037*) over uninfested/untreated leaves (Figure 16).

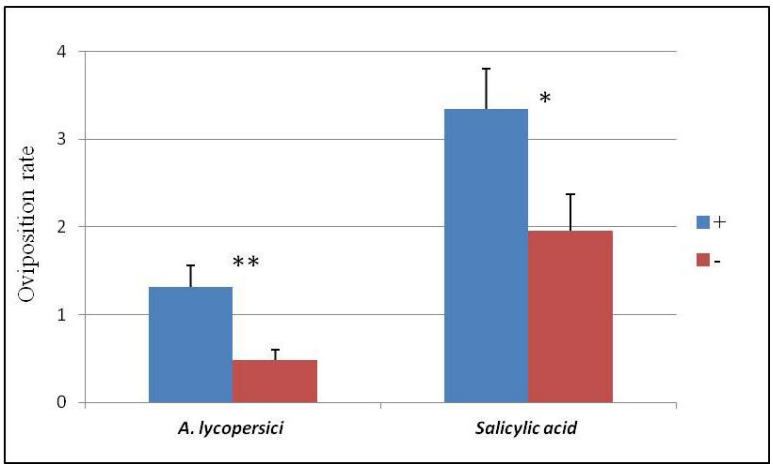

Fig. 16. Performance of spider mite females in the presence of russet mite or SA compared to clean tomato leaves. (+): with treatment; (-): without treatment. Error bars represent the standard error mean of all observations within the same treatment. T_{test} *(*: P<0.05; **: P<0.01*).

These observations underpin that spider mites show better fitness under the dual infestation or after SA treatment. The similar effects recorded with *A. lycopersici* infestation and SA treatment indicates that russet mite might induce the SA signal transduction pathway.

5.3. Transcriptional analysis of defence genes

The transcriptional levels of some defence-related genes were controlled by real time RT-PCR using primers displayed in the table 1. One composed leaf from a six week-old tomato plant was infested by spider mites alone (*T. urticae*), russet mites alone (*A. lycopersici*), or by the two herbivores (*T. urticae* + *A. lycopersici*). Three leaves from 3 different tomato plants were used for each treatment as independent biological replicates. Gene expression levels were monitored on 3 and 6 days following treatment. Actin gene was used as the endogenous reference to normalize the quantification of expression of the genes being analysed, as actin was considered among the most stable genes in tomato (Lovdal and Lillo, 2009). Undamaged tomato plants were used as calibrator. Ct values were used to calculate the relative quantity of every target gene by using the formula $2^{-\Delta\Delta Ct}$. PCR products were also checked on a 2% agarose gel (Figure 17).

The first set of genes included two genes of the octadecanoid pathway upstream to JA biosynthesis (*TomLoxD and AOS*) and a gene downstream to JA, highly responsive to insect wounding (*WIPI-II*). The second set of defence genes consists of the SA-dependent pathogensis related protein 1 (*PR-1*) and a gene involved in terpenoid synthesis (*LeGGPS1*).

The normality of variance was evaluated by levene test. Data were then Ln(x+1) transformed and analysed by one way ANOVA and post hoc comparison of means LSD test ($P<0.05$). The model adopted to evaluate the effect of infestation on the expression of the target genes was highly significant (MANOVA: Pillai's Trace=2.707; F=7.698). Subsequently, one way ANOVA was undertaken to test the effect of infestation on each gene separately.

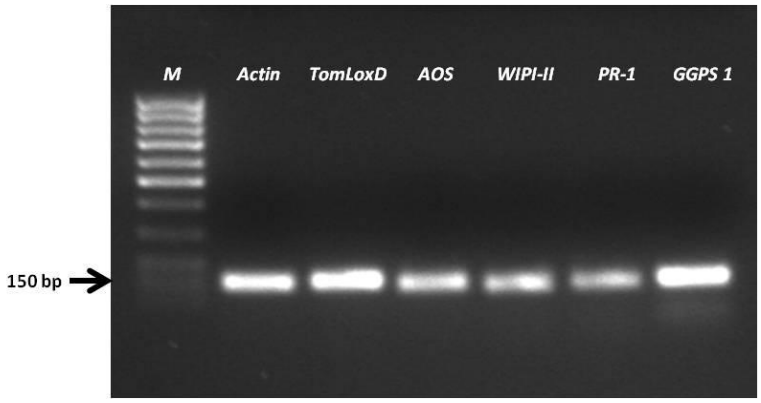

Fig. 17. Agarose gel electrophoresis of RT-PCR of the target defence genes from tomato plant infested by spider mite. **M:** DNA Ladder 100bp (Applichem, USA).

5.3.1. Upstream of JA synthesis

At 3 days of continuous feeding, spider mite induced 25 fold change in the transcrition of *TomLoxD* (*LSD, $df_{3,8}$, F= 9.44, P= 0.005*).Whereas, russet mite, another cell-content feeder did not induced low not significant increase in *TomLoxD* expression (Figure 18). Surprisingly, in simultaneous attack by the two herbivores, the expression of *TomLoxD* triggered by spider mite attack was completely down-regulated by russet mite feeding. Same expression patterns were observed on the 6th day of continuous feeding, with a more intense suppression of *TomLoxD* expression by dual herbivore attack (Figure 18).

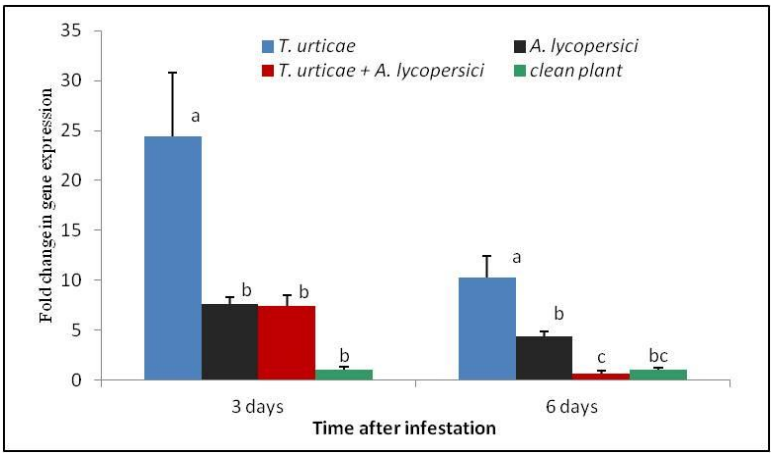

Fig. 18. Fold change in TomLoxD gene expression in tomato leaves infested with spider mites, russet mites, or both herbivores and clean leaves. Leaf samples were collected at 3 and 6 days following infestation. Different letters between treatments at the same sampling time indicate a significant difference in fold change (ANOVA post-hoc test, LSD; P< 0.05).

Alike *TomLoxD*, spider mite induced a significant up-regulation of *AOS* after 3 days ($F_{3,8}$*= 4.13; P= 0.048*) and 6 days ($F_{3,8}$*= 8.36, P= 0.008*) following infestation, while russet mite wounding caused a little increase not significant increase in *AOS* expression (Figure 19). Moreover, russet mite completely suppressed *AOS* gene mRNA expression induced by spider mite attack under dual infestation, which is similar to the effect of russet mite wounding alone.

Taken together, russet mites induce very mild not significant increase in the expression of two key genes in the octadecanoid pathway (*TomLoxD* and *AOS*) upstream to JA in comparison to spider mites. In addition, when feeding simultaneously on the tomato leaf, russet mites were shown to suppress the induction of gene expression triggered by spider mites.

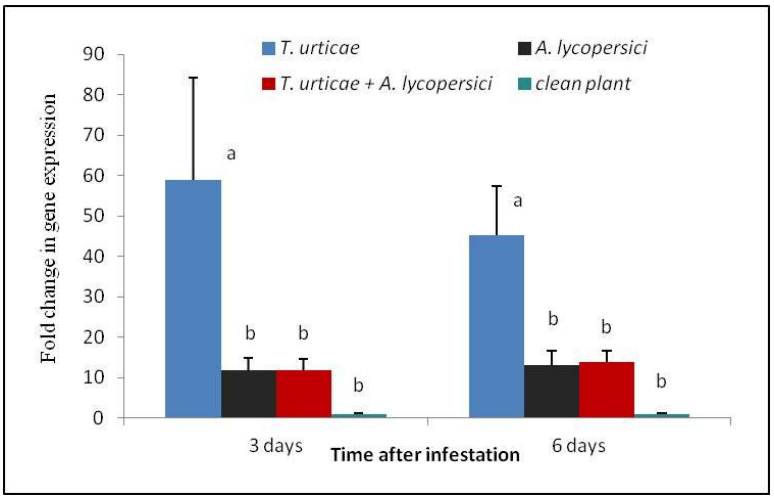

Fig. 19. Fold change in *AOS* gene expression in tomato leaves infested by spider mites, russet mites, or both herbivores and clean leaves. Leaf samples were collected at 3 and 6 days following infestation. Different letters between treatments at the same sampling time indicate a significant difference in fold change (ANOVA post-hoc test, LSD; $P<0.05$)

5.3.2. JA-responsive proteinase inhibitor: WIPI-II

The increase JA production through the octadecanoid pathway after attack by cell-content feeding spider mites was demonstrated to induce transcriptional up-regulation of proteinase inhibitor genes followed by an accumulation of proteinase inhibitor proteins (Kant *et al.*, 2004, Zhang *et al.*, 2009; Sarmento *et al.*, 2011). As expected from *TomLoxD* and *AOS* expression patterns, the common up-regulation of *WIPI-II* gene was observed only in tomato leaves attacked by spider mites alone at 3 days ($F_{3,8}$= 11.62; P= 0.003) and 6 days ($F_{3,8}$= 14.83; P= 0.001), whereas russet mites-attacked tomato leaves did not show any change in *WIPI-II* gene expression compared to undamaged leaves (Fig). Moreover, in accordance with octadecanoid pathway suppression, russet mite entirely suppressed the accumulation of *WIPI-II* mRNA triggered by spider mite (Figure 20).

Over all, russet mites were able to suppress both up- and downstream signals of JA pathway induced by spider mites wounding.

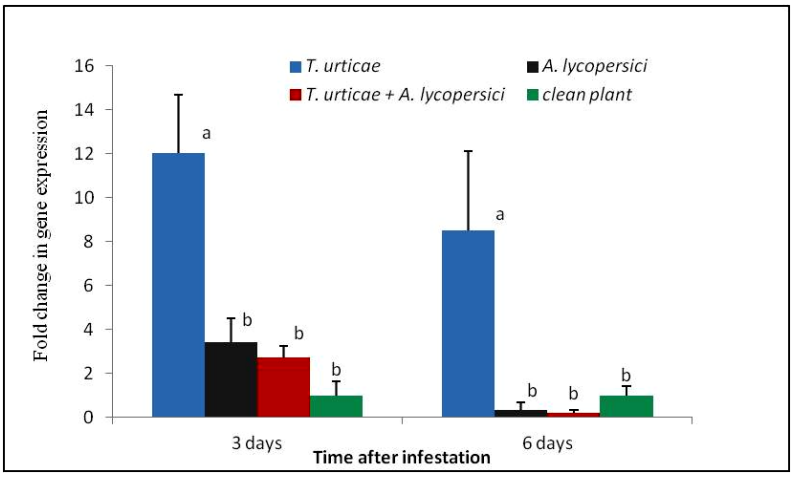

Fig. 20. Fold change in *WIPI-II* gene expression in tomato leaves undamaged or damaged by spider mites, russet mites, or both herbivores. Leaf samples were collected at 3 and 6 days following infestation. Different letters between treatments at the same sampling time indicate a significant difference in fold change (ANOVA post-hoc test, LSD; P< 0.05)

5.3.3. SA-dependent gene: PR-1

Pathogenesis-related (PR) proteins genes are SA-responsive protective factors induced following fungal, bacterial, and viral pathogens challenge (Enyedi *et al.*, 1992; Ryals *et al.*, 1996). Different PR families have been characterized from tomato plants (Fischer *et al.*, 1989; Joosten and Dewit, 1989). PR-1 has been shown to be induced upon herbivore (Puthoff *et al.*, 2010; Rodriguez-Saona *et al.*, 2010) and fungal pathogen attack (Lawrence *et al.*, 1996) to tomato plants. Analysis of PR-1 transcripts of differently infested tomato leaves (Figure 21) revealed that herbivore attack, either by single herbivore or by simultaneous attack by two herbivores induced very high up-regulation (300 to 900 fold change) on 3 days following infestation compared to undamaged leaves ($F_{3,8}$=447.03; P=0.000). On the 6th day following infestation, however, spider mite, russet mite and both herbivores triggered lower but very significant increase in PR-1 gene expression ($F_{3,8}$=270.55; P=0.000) compared to undamaged leaves (Figure 21).

These results are in accordance with previous reports that showed induction of pathogenesis related protein after attack by spider mites. In addition, the activation of a salicylic dependent gene by russet mites and the above observed deactivation of JA pathway indicate that russet mite feeding is perceived by tomato plants in a manner similar to pathogens or phloem-feeding insects.

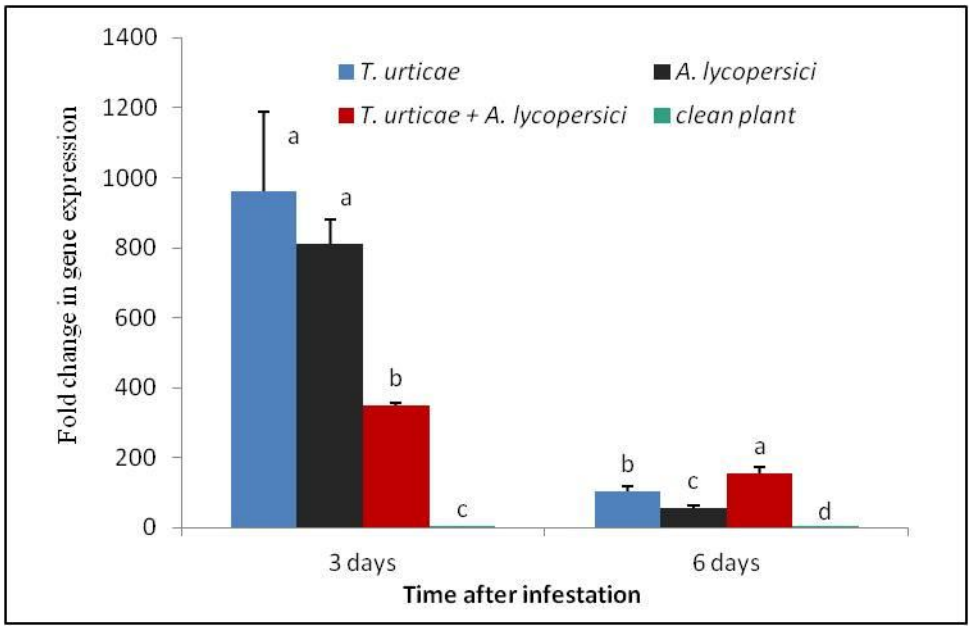

Fig. 21. Fold change in *PR-1* gene expression in tomato leaves undamaged or damaged by spider mites, russet mites, or both herbivores and clean leaves. Leaf samples were collected at 3 and 6 days following infestation. Different letters between treatments at the same sampling time indicate a significant difference in fold change (ANOVA post-hoc test, LSD; P<0.05).

5.3.4. Tomato geranylgeranyl pyrophosphate synthase 1

Mechanical wounding and spider mite damages induced expression of Tomato Geranylgeranyl pyrophosphate synthase 1 (*LeGGPS1*) in tomato leaves (Ament *et al.*, 2006; Sarmento *et al.*, 2011). *LeGGPS1* is involved in the synthesis of the homoterpene TMTT wich has been confirmed to be indced by spider mite feeding and to be an attractant of predatory mites. In this study, the results presented in figure 22, show that spider mite induced 500-fold in *LeGGPS1* gene expression compared to undamaged plants ($F_{3,8}$=85,00; P=0.000) at 3 days following infestation. Russet mite, however, induced 60-fold increase in *LeGGPS1*gene expression. Surprisingly, dual attacked leaves exhibited similar level of *LeGGPS1* expression and very low compared to spider-mite infested leaves.. The up-regulation of *LeGGPS1* with spider mite treatment was maintained on day 6 following infestation ($F_{3,8}$=40,59; P=0.000), whereas, spider mite-mite infested leaves showed the highes increase in gene expression (Figure 22). It results from these observations that russet mites in addition to the deactivation of JA pathway and activation of SA pathway, they also suppress the production of some secondary metabolites triggered by spider mite attack.

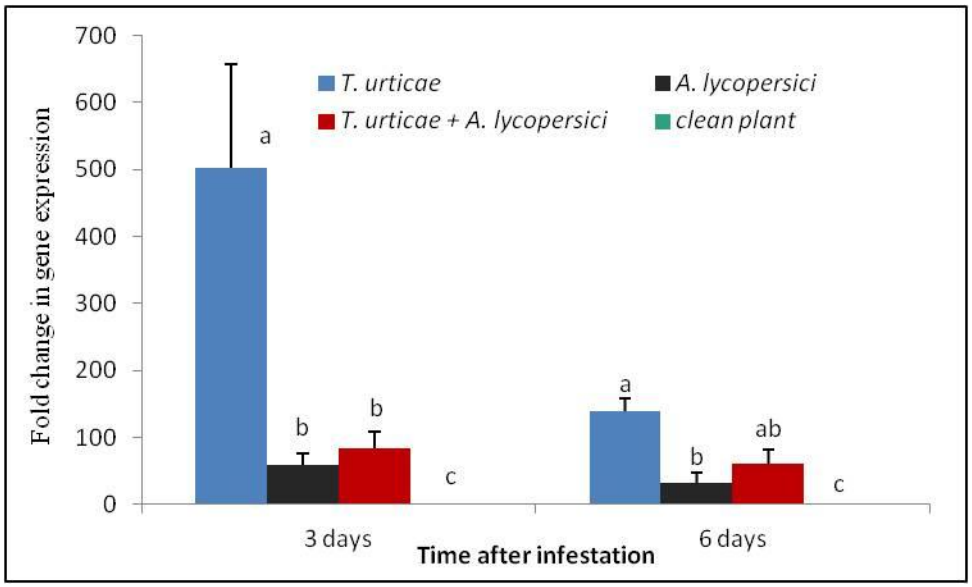

Fig. 22. Fold change in *LeGGPS1* gene expression in tomato leaves undamaged or damaged by spider mites, russet mites, or both herbivores. Leaf samples were collected at 3 and 6 days following infestation. Different letters between treatments at the same sampling time indicate a significant difference in fold change (ANOVA post-hoc test, LSD; $P< 0.05$)

5.3.5. Systemic responses

Since many wound-response genes are expressed systematically and local and systemic responses can be distinct, the levels of the studied genes in distant apical, non-infested leaves were determined. The two octadecanoid genes *TomLoxD* and *AOS* were not induced in distant unwounded leaves in all treatments after 3 days following infestation (Figure 23). Similarly the terpene synthase *GGPS1* gene was not expressed systemically. However, plants attacked by spider mites had systematic induction of *WIPI-II* ($F_{3,8}$=22.85; P=0.0001) and *PR-1* ($F_{3,8}$=19.78; P=0.0001) genes, whereas russet mites or both herbivores did not induce any systemic response (Figure 23).

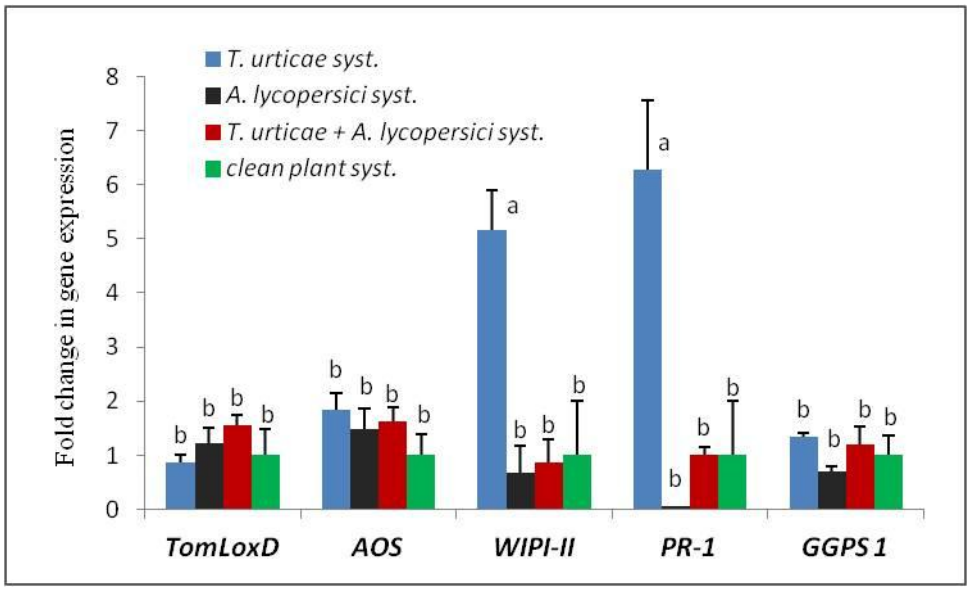

Fig. 23. Fold change in the expression of the selected genes in tomato leaves distant from the wounded leaves by spider mites, russet mites, both herbivores and unwounded clean plants. Leaf samples were collected at 3 days following infestation. Different letters between treatments indicate a significant difference in fold change (ANOVA post-hoc test, LSD; P< 0.05)

5.4. Predatory mites olfactory preference behavior

In this experiment I evaluated whether attack by *A. lycopersici* might affect the indirect plant defence and thus change the attraction of *T. urticae*-infested plants to predatory mites. Two predatory mite species were studied; one specialist (*P. persimilis*) feeds only on tetranychid mites and one generalist (*N. californicus*) feeding on wide prey range including russet mites. Two population densities per tomato leaflet were evaluated; low density and high density. First I tested the olfactory choice of each predatory mite to tomato plants infested by spider mites alone, russet mite alone, or by both herbivores at low population density (10 adult's spider mites, 100 russet mite/ leaflet). A standard test consisted of evaluating the olfactory choice of predatory mites to spider mites-infested plant compared to clean undamaged control plant was considered too. Results showed that when compared to clean tomato plants, significantly more *P. persimilis* ($X^2_{test}=16.40;\ P<0.01$) and *N. californicus* ($X^2_{test}=7.34;\ P<0.01$) moved towards tomato plants infested by *T. urticae* (Figure xx). When given to chose between dual infested (*T. urticae* + *A. lycopersici*) plants and single-infested plants, the specialist *P. persimilis* was significantly more attracted by dual infested tomato plants than by *T. urticae*-infested ($X^2_{test}=5.26;\ P<0.05$) *or A. lycopersici*-infested ($X^2_{test}=10.33;\ P<0.01$) plants (Figure 24). Thus, volatiles from plants under dual infestation are more attractive to the specialist

predatory mite than those under single infestation. In contrast, the generalist *N. californicus* did not show any odour preference, when given to choose between plantes infested by both species, *T. urticae* ($X^2_{test}=0.137$; $P>0.1$) or *A. lycopersici* ($X^2_{test}=1.85$; $P>0.1$) (Figure 25).

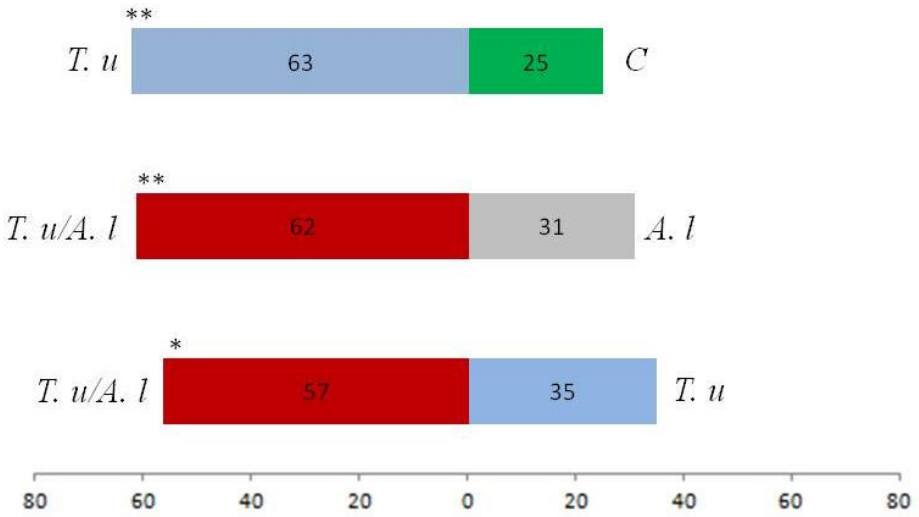

Fig. 24. Olfactory response of *P. persimilis* to tomato infested with *T. urticae* (*T. u*) vs. uninfested (*C*) and infested with both *A. lycopersici* and *T. urticae* (*T. u/A. l*) and *A. lycopersici*-infested vs. dual infested plants. Y-tube olfactory preference is depicted as the absolute number of predatory mites that choose a given choice. Results reported are of three independent replicates with ~30 individuals each. *: $P<0.05$; **: $P<0.01$ (chi-square test).

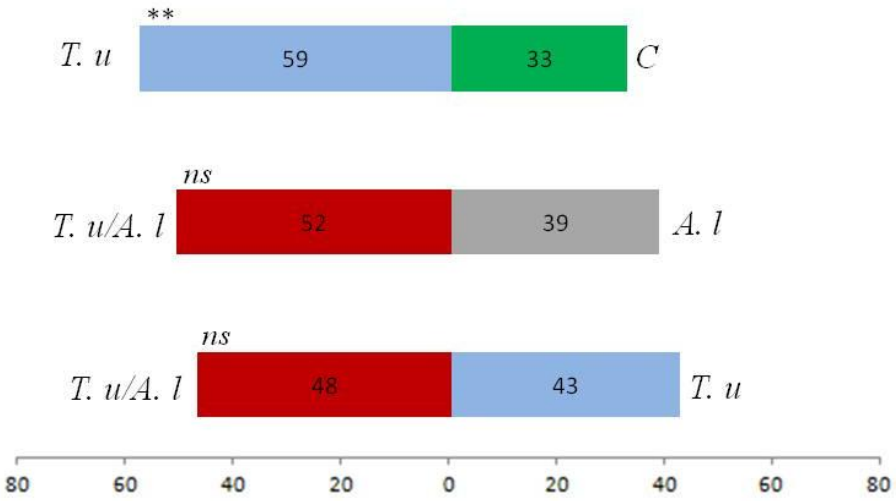

Fig. 25. Olfactory response of *N. californicus* to tomato infested with *T. urticae* (*T. u*) vs. uninfested (*C*) or infested with both *A. lycopersici* and *T. urticae* (*T. u/A. l*) and *A. lycopersici*-infested vs. dual infested plants. Results reported are of three independent replicates with ~30 individuals each. ns: not signifiative; **: P<0.01 (chi-square test).

Further analysis was pursued using higher population density (20 adult's spider mites, 300 russet mites/ leaflet) which is within the infestation ranges in natural conditions. Olfactory choice comparisons were carried out between spider mite-infested and dual infested plants. Results showed that, in contrast to previous observations at low population density of herbivores, the attraction of both specialist *P. persimilis* ($X^2_{test}=4.45;\ P<0.05$) and generalist *N. californicus* ($X^2_{test}=7.33;\ P<0.01$) was shifted towards plants attacked by *T. urticae* alone (Figure 26).

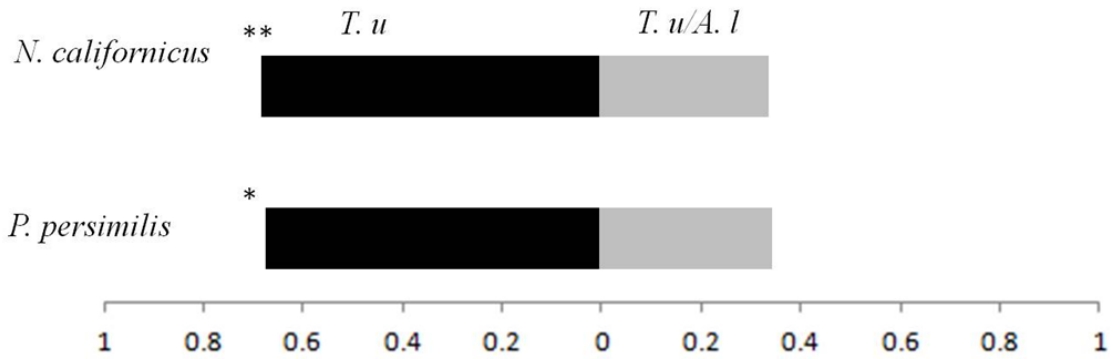

Fig. 26. Olfactory response of *P. persimilis* and *N. californicus* to tomato infested with *T. urticae* (*T. u*) vs. infested with both *A. lycopersici* and *T. urticae* (*T. u/A. l*). Y-tube olfactory preference is depicted as the pourcentage of predatory mites that choose a given choice. Results reported are of three independent replicates with ~30 individuals each. *: $P<0.05$; **: $P<0.01$ (chi-square test).

5.6. Headspace volatiles emitted from tomato plants

To understand the mechanism underlying the different responses of predatory mites towards tomato plants attacked by different herbivores, headspace volatiles were collected from the same plants just after the evaluation of predatory mites' olfactory choices. GC-MC analyses were carried out on volatiles collected from four independent plants for each treatment. Only terpenoids' emission was considered in this analysis because they are known to be induced by herbivores, and also due to the limited availability of the corresponding standards. Figure xxx shows that spider mites induced higher emission of terpenoids ($F_{3,12}=9.85$; $P=0.001$) compared to other treatments. Surprisingly, russet mites alone or in combination with spider mites did not cause any increase of VOCs emission compared to undamaged plants (Figure 27).

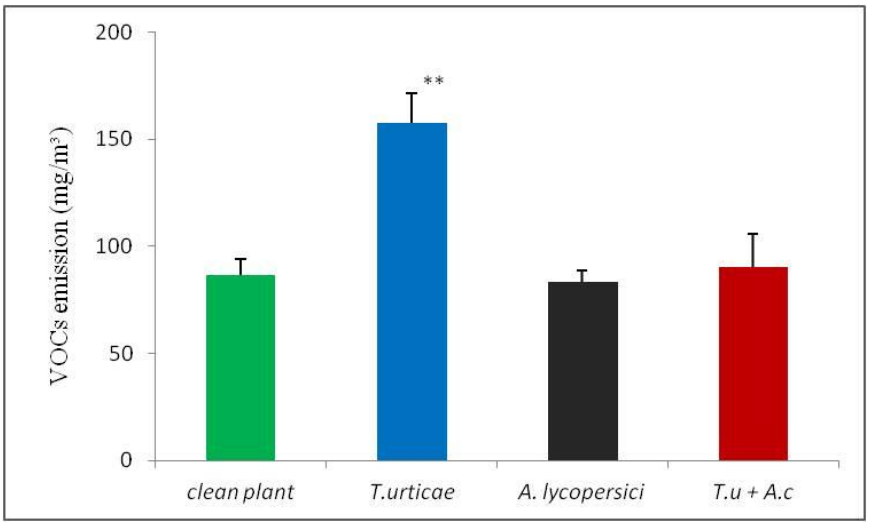

Fig. 27. Total terpenoids emitted (mg/m³) from the differently treated tomato plants
Asterisks above the compound indicate significant differences in VOC emissions between treatments (ANOVA post-hoc test, LSD; **: P< 0.01).

Qualitative analysis of terpenoids composition showed that mainly monoterpenes and sesquiterpenes were detected from tomato plants under various treatments. The table 4 reports the identification of terpenoids and their concentrations in each treatment. Nine monoterpenes and 3 sesquiterpenes were detected in at least two of four examined treatments. The monoterpene β-phellandrene was not detected in control undamaged plants, while it constituted 20-30% of the total terpenoid emissions from tomato plants under herbivore attacks. Similarly, sesquiterpenes (δ-elemene, β-caryophyllene and α-caryophyllene) were not emitted by undamaged plants. This result might indicate the importance of these compounds as herbivore induced plant volatiles (HIPVs).

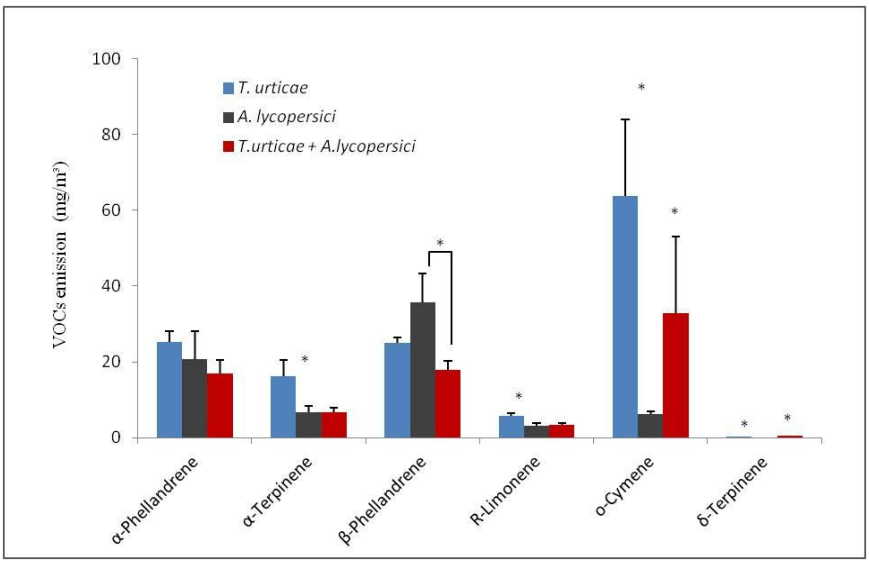

Fig. 28. Monoterpenes emission of tomato plants under different herbivory

Table 4. Volatile organic compound (VOC) emissions from 3 day-old tomato plants damaged by *T. urticae*, *A. lycopersici*, both mites, or clean plants (control).

Compound name	**Retention time (min)**	**Mean ± standard error (mg/m^3)**			
		Clean plant	*T. urticae*	*A. lycopersici*	*T. u + A. l*
(R)-α-Pinene	25.40	0.471 ± 0.129 a	0.541 ± 0.189 a	0.756 ± 0.113 a	0.648 ± 0.433 a
Isolimonene	27.27	12.276 ± 3.989 a	8.956 ± 3.360 ab	2.377 ± 1.281 b	4.922 ± 0.542 ab
2-carene	27.89	1.420 ± 0.276 a	3.774 ± 0.992 ab	5.712 ± 1.347 b	3.786 ± 1.733 ab
α-Phellandrene	28.11	38.137 ± 3.797 a	25.168 ± 2.827 ab	20.695 ± 7.284 b	17.023 ± 3.400 b
α-Terpinene	28.54	22.013 ± 1.401 a	16.123 ± 4.445 a	6.632 ± 1.729 b	6.603 ± 1.195 b
β-Phellandrene	29.13	0.000 a	25.069 ± 1.461 bc	35.694 ± 7.508 c	17.920 ± 2.404 b
(R)-Limonene	29.90	7.967 ± 0.625 a	5.788 ± 0.735 b	3.079 ± 0.822 c	3.465 ± 0.434 c
o-Cymene	28.98	3.194 ± 0.704 a	63.845 ± 20.233 b	6.202 ± 0.745 a	32.876 ± 20.12 ab
δ-Terpinene	30.61	1.324 ± 0.326 a	0.330 ± 0.015 b	0.000 b	0.429 ± 0.094 b
δ-Elemene	37.29	0.000 a	2.176 ± 0.818 b	0.000 a	1.499 ± 0.096 b
β-Caryophyllene	40.85	0.000 a	3.810 ± 1.340 b	2.265 ± 0.949 ab	0.000 a
α-Caryophyllene	42.85	0.000 a	2.139 ± 0.772 b	0.000 a	1.224 ± 0.073 b

Different letters indicate significant difference (ANOVA post-hoc test, LSD; $P<0.05$).

Among monoterpenes, α-terpinene and R-limonene were emitted in higher quantities from spider mite-attacked leaves in comparison to russet mite- and dual-attacked leaves (Figure 28). However higher concentrations were also released from undamaged plants (Table 4). O-cymene was the dominant volatile compound emitted from tomato plants attacked by spider mite or by dual herbivores, whereas in comparison, russet mites induced very low emission of this monoterpene.

VI. Discussion and conclusions

6.1. Differential gene expression in response to mite herbivory

Data on induced defence responses in tomato-eriophyoid mite interaction are very limited. Stout *et al.* (1996) presented evidence for several enzymatic proteins to be induced locally and systemically in the leaves of tomato plants. In response to *A. lycopersici* short-term feeding, total peroxidases (POX, hydrogen peroxide (H2O2) oxidoreductase) activity was induced in local and systemic leaves.

In this study, transcriptional analysis of the main defence genes of tomato leaves showed that eriophyoid russet mite affects the direct tomato defence in a manner different to that of spider mite. Russet mite did not induce the common up-regulation of two keys enzymes of the octadecanoid pathway (*TomLoxD* and *AOS*), which coincided with the lack of up-regulation of *WIPI-II*, a gene that is dependent on the JA defensive pathway. Surprisingly, *A. lycopersici* suppressed the up-regulation of the octadecanoid pathway, upstream and downstream genes of JA synthesis triggered by spider mite under simultaneous infestation.

In addition, transcriptional analysis of PR-1, a SA-dependent gene, suggests that russet mite and spider mite induce the SA pathway, and similar effects where observed under dual infestation. In fact, PRs are vacuolar proteins and herbivore with cell content feeding is reported to induce PRs.

Gene expression of two octadecanoid enzymes LOX and AOS was not induced in unwounded leaf distant from the spider mite-infested leaf of the same plant. Also the lack of up-regulation of these two genes in wounded tomato leaves by russet mite alone or dual herbivores was maintained in systematic leaf. These findings are in complete agreement with the observation that expression of octadecanoid pathway genes is induced by wounding at the site of tissue damage but not systemically (Strassner *et al.*, 2002). Stout *et al.* (1996) reported a non systemic inducton of LOX activity in tomato plants in response to russt mite.Whereas two-fold increase in LOX and PPO activity was observed in local leaf. Furthermore, as reported by (Li *et al.*, 2002; 2003), the induction of defence genes in systemic tissues depends on JA perception and signaling, but not on the capacity to synthesize JA. In contrast, the downstream gene of JA-responsive gene, WIPI-II, was induced in unwounded leaf in spider mite-wounded plant and not in russet

mite and dual attacked plants. These results suggest that systemin acts at the site of herbivore feeding, to strengthen the systemic wound response by boosting the octadecanoid pathway, for the generation of the long-distance signal, maybe JA itself or one of its derivatives (Ryan and Moura 2002; Stratmann, 2003; Schilmiller and Howe, 2005).

Since jasmonate pathway is not involved in the interaction of tomato plant with russet mites, the induction of PR-1 by russet mite attack suggests that plants perceive russet mites like pathogens. Negative cross-talk between JA and SA pathways may explain the suppression of JA-dependent responses induced by spider mite by simultaneous attack of russet mites.

Taken together, these results show that, alike phloem feeder herbivores and pathogens (Bede *et al.*, 2006; Kant *et al.*, 2008; Lawrence *et al.*, 2008), *A. lycopersici* elicits JA-independent and presumably SA-dependent defence response in tomato leaves.

6.2. Terpene synthase: GGPS1

In tomato, *LeGGPS1* has been proposed to be responsible for the biosynthesis of (E, E)-4,8,12-Trimethyltrideca-1,3,7,11-tetraene (TMTT) from geranylgeranyl pyrophosphate GGPP (C20) via geranyllinalol (GL). TMTT was considered as the most abundant spider mite-induced tomato volatile (Ament *et al.*, 2006). In accordance with previous observation, *LeGGPS1* was up-regulated in plants attacked by spider mite *T. urticae* after 3 and 6 days of infestation. In contrast, simultaneous attack of tomato plants by spider mite and russet mite *A. lycopersici* did not induce a high up-regulation of *LeGGPS1* like that observed with spider mite attack, and expression levels were similar to plants attacked by russet mite alone. The response is somewhat similar to the JA-responsive gene WIPI-II, and implies a JA-dependent regulation of *LeGGPS1*. However, the upregualtion of *LeGGPS1* in plants attacked by russet mite and by the two herbivores compared to unattacked plants may indicate that *LeGGPS1* is not a typical JA-responsive gene. In fact, Ament *et al.* (2006) showed that *LeGGPS1* was not induced upon spider mite feeding on SA-deficient line *(NahG)*, while expression of WIPI-II gene was much higher than in wild type. In undamaged plant, a constitutive amount of TMTT is always emitted. In this study, I did not measure the emission TMTT, due to the lack of the corresponding standard; however, it is supposed that spider-mite infested tomato plants would have a higher TMTT emission compared to the other treatments and control.

6.3. Tri-trophic interaction

In agricultural environments, either under field or greenhouse cropping systems, spider mites and russet mites are commonly found feeding simultaneously on tomato plants. Previous studies have showed mutualistic and antagonistic interactions between eriophoid mites and other herbivores feeding on the same host plant (Royalty and Perring, 1996; Stout *et al.*, 1996; Westphal and Manson, 1996; Westphal *et al.*, 1996). Mutualsitic effects were observed between the two herbivores; spider mite webbing hampers the activities of predatory mites against russet mites (Duso *et al.*, 2010). Spider mites are highly polyphagous, while russet mites are generally associated to solanaceous plants. Both herbivores feed on the cell-content of mesophyll and epidermis. In this study, it was observed that the spider mite *T. urticae* had a more preference to and a higher oviposition rate on plants that were previously attacked by russet mites than on non-damaged plants. Also, as previously reported for other plants (Zhang *et al.*, 2009), *T. urticae* had a better performance on SA-treated tomato leaves than on untreated ones. This corresponds to that SA signaling pathway negatively interacts with wounding signaling and alters the induced plant defence. From these results, it seems that russet mite had similar effect like SA treatment and this may indicate the possible involvement of SA signaling pathway in tomato-russet mite interaction.

Cultivated tomatoes are known to be very sucsceptible to russet mite attack. Also, wild tomato relatives were found to be susceptible to this pest. However, resistant genotypes against russet mite were characterized in bittersweet nightshade (*Solanum dulcamara*). In the incompatible interaction, russet mite feeding on epidermal cells causes cell collapse, then plasmolysis and shrinkage of the nucleus in the adjacent cells, leading to cell death and the development of a local hypersensitive reaction (HR) that prevents further development of mites (Westphal *et al.*, 1981). Westphal *et al.*, (1991) reported that attack of *A. cladophthirus* triggers the true HR in resistant *S. dulcamara* plants, which causes the death of *A. cladophthirus* and increases the mortality of the next attacker, *T. solani*. The HR was not effective against subsequent attack by *T. urticae* (Westphal *et al.*, 1990). Indeed, there was a stimulation of *T. urticae* female fecundity and acceleration of *T. urticae* development on leaves previously infested by *A. cladophthirus*. Several other abiotic and biotic stresses (e.g. pathogen infection, mechanical wounding) trigger HR. Hence, the response of a resistant plant genotype to

A. lycopersici feeding cannot be considered as a specific defence reaction (Westphal *et al.*, 1996).

Plants under herbivore attack emit volatile organic compounds to attract natural enemies of the invader. Olfactory choice of two predaceous phytoeiid mites: a specialist (*P. persimilis*) and a generalist (*N. californicus*) was evaluated. Results revealed that the olfactory choice was highly dependent on the population density of the two herbivores. At lower density (10 spider mites and 100 russet mites/leflet), the specialist *P. persimilis* was more attracted to dual-attacked plant than spider mite-attacked ones; whereas, the generalist did not show any preference. In contrast, at higher density (20 spider mites and 300 russet mites/ leafet) both predatory mites were attracted to plant attacked by two herbivores than those attacked by spider mites. Consistent with this observation, Zhang *et al.*, (2009) presented evidence that in simultaneous attack of lima bean leaf with whitefly (*B. tabaci*) and spider mite, increasing whitefly density upto 50 adults/leaf inverted the attaraction of the predatory mite *P. persimilis* towards spider mite-infested leaves.

In correspondence, headspace volatile analysis showed that the volatile blends emitted from tomato plants under different herbivory are quantitatively and qualitatively distinct. Moreover, spider mite-attacked plants emited higher quantities of VOCs than dual-attacked plants. So far, it was not possible to identify a specific volatile compound responsible for the observed response. Zhang *et al.* (2009) found that β-ocymene was responsible for the attraction of *P. persimilis* to lima bean plants attacked by spider mite, whereas, van Wijk *et al.* (2008) found that predatory mite attraction to spider mite-induced plant was not a consequence of attraction to individual herbivore-induced plant volatiles.

Monoterpenes were abundant in all treatments as constitutive plant odors. They were not particularly induced by herbivore attack. However, all wounded plants emitted a blend of sesquiterpenes (table 4), which where not detected in unwounded control plants. Also, the sesquiterpenes were more abundant in tomato leaves attacked by spider mite and both herbivores than in russet mite attacked leaves. Previous reports showed that a sesquiterpene volatile (β-caryophyllene) is released from maize roots in response to feeding by larvae of the beetle *D. virgifera virgifera*, which strongly attracted an entomopathogenic nematode (Rasmann *et al.*, 2005). Overall, predatory mites seem to respond to tomato volatiles emitted in response to different herbivory as a whole blend

and not as specific compounds. This is consistent with what previous studied have reported (van Wijk *et al.*, 2008; 2011).

6.4. How russet mites overcome the tomato defence?

It has been proposed that the increased number of cells damaged by russet mite during a given period of time resulted in gas exchange decrease (Royalty and Perring, 1989), causing an increase in the temperature and the deficit of vapor-pressure of mite-infested leaves. Theses changes stimulate mite feeding and performance. Russet mite probing is commonly considered to be limited to epidermal cells and not reaching mesophyll layers. Therefore, wounding response is not induced in tomato leaves attacked by russet mite. However, russet mite was shown to suppress the wounding response induced by spider mite under dual attack. Hence, the defence reactions of russet mite-infested tomato plant are more likely to be overcome.

The feeding process of eriophyoid mite is not fully understood (Petanovic´ and Kielkiewicz, 2010). Some authors support the idea that eriophyoid mites inject saliva from cheliceral stylets inserted into the cell during mite feeding (Jeppson *et al.*, 1975; Nuzzaci, 1979; Nuzzaci and Alberti, 1996). In contrast, according to Thomsen (1988), first the eriophyoid mite alike aphids, ejects saliva onto the surface of the leaf in order to digest the cuticle and cellulose of the cell wall enzymatically. Then, the marked site on the cell wall is located and punctured by the mite's chelicerae which then sucks out the cell content, taking 10–20 min. This hypothesis was later rejected by Westphal and Manson (1996) who considered that eriophoids need only a few seconds for probing and cell penetration.

Some species of vagrant eriophyoid mites were reported to produce salivary secretions when emmersed in objective lens oil (de Lillo and Monfreda, 2004). The bioassays suggested the presence of lipophilic chemicals with plant growth regulatory effects. Polygalacturonase activity was assessed in salivary secretions of *Aceria caulobia* (Nalepa), which suggests a putative role of this enzyme in eriophyoid-feeding signal release (Monfreda and Spagnuolo, 2004). Yet, there is no available record of salivary secretion by *A. lycopersici*, and wether this eriophyoid uses elicitors in salivary secretions to overcome plant defences is still to be elucidated.

In conclusion, russet mite *A. lycopersici* was found to interfere with the induced direct defence of tomato plant against spider mite *T. urticae* through the suppression of

JA-induced responses. In addition, russet mite altered the indirect tomato defence by suppression of terpenoid emission triggered by spider mite attack which in turn influenced the attraction of predatory mites. A model of the signaling network in tomato-herbivore interaction is updated (Figure 29).

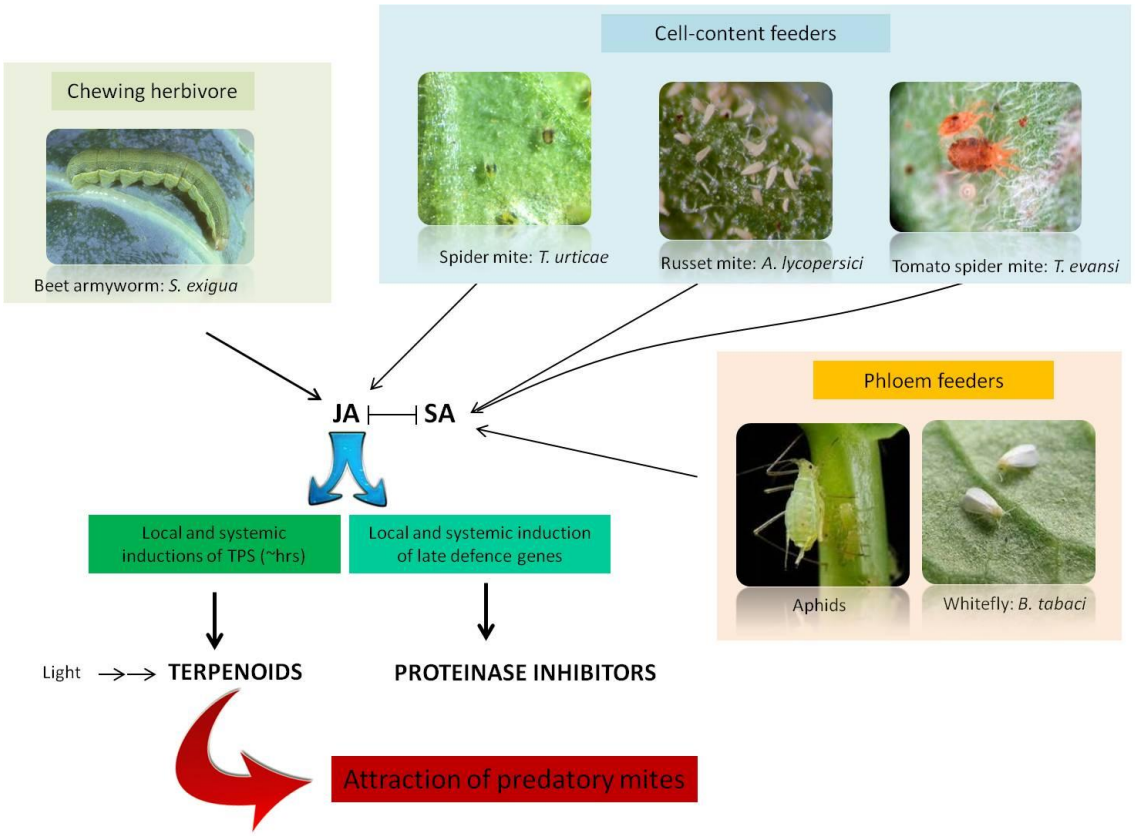

Fig. 29. Model of the signaling network involved in tomato-herbivore interaction in chewing and piercing-sucking arthropod-damaged leaves. Arrows and bars indicate positive and negative interactions, respectively.

Literature cited

1. Adler L.S., Karban R., Strauss S.Y., 2001. Direct and indirect effects of alkaloids on plant fitness via herbivory and pollination. *Ecology* 82(7):2032–2044
2. Agrawal A. A., 1998. Induced responses to herbivory and increased plant performance. *Science 279*:1201-1202
3. Agrawal A.A., 1999. Induced responses to herbivory in wild radish: Effects on several herbivores and plant fitness. *Ecology 80(5):*1713-1723
4. Agrawal A.A., 2000. Host-range evolution: adaptation and trade-offs in fitness of mites on alternative hosts. *Ecology 81(2)*:500–508
5. Ali J.G., Agrawal A.A., 2012. Specialist versus generalist insect herbivores and plant defense. *Trends in Plant Science 17(5)*:293-302
6. Ament K., Van Schie C.C., Bouwmeester H.J., Haring M.A., Schuurink R.C., 2006. Induction of a leaf specific geranylgeranyl pyrophosphate synthase and emission of (E,E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene in tomato are dependent on both jasmonic acid and salicylic acid signaling pathways. *Planta 224:*1197–1208.
7. Arimura G., Matsui K., Takabayashi J., 2009. Chemical and Molecular Ecology of Herbivore-Induced Plant Volatiles: Proximate Factors and Their Ultimate Functions. *Plant Cell Physiology 50(5)*: 911–923
8. Athias-Henriot C., 1957. Phytoseiidae et Aceosejidae (Acarina: Gamasina) d' Algerie, I Genres Blattisocius Keegan, Iphiseius Berlese, Amblyseius Berlese, Phytoseius Ribaga, Phytoseiulus Evans. *Bull. Soc. Hist. Nat. Afrique du Nord* 48: 319-352
9. Baradaran-Anaraki P., Daneshvar H., 1992. Studies on the biology and chemical control of tomato russet mite, Aculops lycopersici (Acari: Eriophyidae), in Varamin. *Applied Entomology and Phytopathology* 59(1-2):25-27
10. Barbour J.D., Farrar R.R., Kennedy G.G., 1993. Interaction of Manduca sexta resistance in tomato with insect predators of Helicoverpa zea. Entomologia Experimentalis et Applicata 68: 143–155
11. Barbour J.D., Kennedy G.G., 1991. Role of steroidal glycoalkaloid α-tomatine in host-plant resistance of tomato to Colorado potato beetle. *Journal of Chemical Ecology* 17(5):989-1005
12. Bede J.C., Musser R.O., Felton G.W., Korth K.L., 2006. Caterpillar herbivory and salivary enzymes decrease transcript levels of *Medicago truncatula* genes encoding early enzymes in terpenoid biosynthesis. *Plant Molecular Biology 60*:519–531
13. Behmer S.T., Elias D.O., Bernays E.A., 1999. Post-ingestive feedbacks and associative learning regulate the intake of unsuitable sterols in a generalist grasshopper. *The Journal of Experimental Biology 202*:739–748
14. Berenbaum M.R., Cohen M.B., Schuler M.A., 1992. Cytochrome-*P*450 monooxygenase genes in oligophagous Lepidoptera. *ACS Symp Ser* 505: 114–124
15. Bergey D.R., Orozco-Cárdenas M.L., De Moura D., Ryan C.A., 1999. A novel wound- and systemin-inducible polygalacturonase in tomato leaves. *Proceedings of National Academy of Sciences USA 96*:1756–176
16. Bernays E. A., Chapman R. F., 1994. Host-plant Selection by Phytophagous Insects. New York: Chapman and Hall.
17. Bernays E.A., Lee J.C., 1988. Food aversion learning in the polyphagous grasshopper *Schistocerca americana* (Drury). *Physiological Entomology 13*: 131–137
18. Bezemer T.M., Van Dam N.M., 2005. Linking aboveground and belowground interactions via induced plant defences. *Trends in Ecology and Evolution 20*:617–624
19. Bjorkman C., Ahrne K., 2005. Influence of leaf trichome density on the efficiency of two polyphagous insect predators. *Entomologia Experimentalis et Applicata115*:179–186

20. Bohlmann J., Martin D., Oldham N.J., Gershenzon J., 2000. Terpenoid secondary metabolism in Arabidopsis thaliana: cDNA cloning, characterization, and functional expression of a myrcene/ (E)-beta-ocimene synthase. *Archives of Biochemistry and Bophysics 375*: 261–269
21. Bostock R.M., 2005. Signal crosstalk and induced resistance: straddling the line between cost and benefit. *Annual Review of Phytopathology 43*: 545–580
22. Bostock R.M., Karban R., Thaler J.S., Weyman P.D., Gilchrist D., 2001. Signal interactions in induced resistance to pathogens and insect herbivores. *European Joirnal of Plant Pathology 107*: 103–111
23. Bouarab K., Melton R., Peart J., Baulcombe D., Osbourn A., 2002. A saponin detoxifying enzyme mediates suppression of plant defences. *Nature* 418:889-892
24. Boudet A., 2007. Evolution and current status of research in phenolic compounds. *Phytochemistry 68*:2722–2735
25. Brattesten L.B., 1988. Potential role of plant allelochemicals in the development of insecticide resistance .In: Barbosa P., Le Tourneau D.K., (eds.) Novel aspects of insect-plant interactions. Wiley, New York. 331-348 pp
26. Broadway R.M., 1995. Are insects resistant to plant proteinase inhibitors? *Journal of Insect Physiology.* 41, 107–116
27. Broadway R.M., Duffey S.S., 1986. Plant proteinase inhibitors: mechanism of action and effect on the growth and digestive physiology of larval Heliothis zea and Spodoptera exigua. *Journal of Insect Physiol*ogy 32:827-833
28. Brodeur J., Bouchard A., Turcotte G., 1997. Potential of four species of predatory mites as biological control agents of the tomato russet mite, *Aculops lycopersici* (Massee) (Eriophyidae). *Canadian Entomology* 129: 1–6
29. Brown W.E., Ryan C.A., 1984. Isolation and characterization of a wound-induced trypsin inhibitor from alfalfa leaves. *Biochemistry* 23:3418-3422.
30. Bruin J., Dicke M., Sabelks M.W., 1992. Plants are better protected against spider mites after exposure to volatiles from infested conspecifics. *Experientia 48*: 525-529
31. Cabrera A.R., Donohue K.V., Khalil S.M.S., Scholl E., Opperman C., Sonenshine E.D., Roe R.M., 2011. New approach for the study of mite reproduction: The first transcriptome analysis of a mite, Phytoseiulus persimilis (Acari: Phytoseiidae). *Journal of Insect Physiology* 57:52–61
32. Cardoso M.Z., 2008. Herbivore handling of a plants trichome: the case of Heliconius charithonia (L.) (Lepidoptera: Nymphalidae) and Passiflora lobata (Killip) Hutch. (Passifloraceae). *Neotropical Entomology* 37(3):247-252
33. Castagnoli M., Liguori M., Simoni S., and Guidi S., 1998. Tomato as host plant: effect on some biological parameters of Phytoseiulus persimilis Athias-Henriot and Tetranychus urticae Koch strains. *Redia* 81:93-99
34. Castagnoli M., Simoni S., 1991. Influenza della temperatura sull'incremento delle popolazioni di Amblyseius californicus (McGregor) (Acari: Phytoseiidae). *Redia 74*: 621-640
35. Castagnoli M., Simoni S., 1999. Effect of long-term feeding history on functional and numerical response of Neoseiulus californicus (Acari: Phytoseiidae). *Experimental and Applied Acarology 23(3)*:217-234
36. Castagnoli M., Simoni S., 2003. Neoseiulus californicus (McGregor) (Acari: Phytoseiidae): survey of biological and behavioral traits of a versatile predator. *Redia* 86:153–164
37. Castagnoli M., Simoni S., Liguori M., 2003. Evaluation of Neoseiulus californicus (McGregor) (Acari: Phytoseiidae) as a candidate for the control of Aculops lycopersici (Tyron) (Acari: Eriphyoidea): a preliminary study. *Redia 86*:97–100
38. Chatzivasileiadis E. A., Sabelis M.W., 1998. Variability in susceptibility among cucumber and tomato strains of *Tetranychus urticae* Koch to 2-tridecanone from tomato trichomes: effects of host plant shift. *Experiemntal and Applied Acarology* 22:455-466
39. Chatzivasileiadis E.A., Sabelis M.W., 1997. Toxicity of methyl ketones from omato trichomes to Tetranychus urticae Kock. *Experiemntal and Applied Acarology 21*:473-484

40. Chen H., Wilkerson C.G., Kuchar J.A., Phinney B.S., Howe G.A., 2005. Jasmonate-inducible plant enzymes degrade essential amino acids in the herbivore midgut. *Proceedings of the National Academy of Sciences of USA 102*:19237–19242
41. Cheng A.X., Lou Y.G., Mao Y.B., Lu S., Wang L.J., Chen X.Y., 2007. Plant terpenoids: biosynthesis and ecological functions. *Journal of Integrative Plant Biology 49*:179–186
42. Christensen S.A., Nemchenko A., Borrego E., Murray I., Sobhy I.S., Bosak L., De Blasio S., et al., 2013. The maize lipoxygenase, ZmLOX10, mediates green leaf volatile, jasmonate and herbivore-induced plant volatile production for defense against insect attack. *The Plant Journal* 74:59–73
43. Chung S.H., Rosa C., Scully E.D., Peiffer M., Tooker J.F., Hoover K., Luthec D.S., Felton G.W., 2013. Herbivore exploits orally secreted bacteria to suppress plant defenses. *Proceedings of the National Academy of Sciences of USA 110(39):*15728-33
44. Clé C., Hill L.M., Niggeweg R., Martin C.R., Guisez Y., Prinsen E., Jansen M.A.K., 2008. Modulation of chlorogenic acid biosynthesis in Solanum lycopersicum; consequences for phenolic accumulation and UV-tolerance. *Phytochemistry 69*:2149–2156
45. Colazza S., Fucarino A., Peri E., Salerno G., Conti E., Bin F., 2004. Insect oviposition induces volatile emission in herbaceous plants that attracts egg parasitoids. Journal Experimental Biology 207**:** 47–53.
46. Collier K.F.S., Albuquerque G.S., Eiras A.E., Blackmer J.L., Araujo M.C., Monteiro L.B., 2001. Estimulos olfactivos envolvidos na localizacao de presas pelo Acaro predator Neoseiulus californicus (McGregor) (Acari: Phytoseiidae) em macieiras e plantas hospedeiras alternativas. *Neotropical Entomology 30*: 631-639
47. Conconi A., Miquel M., Browse J.A., Ryan C.A., 1996. Intracellular levels of free linolenic and linoleic acids increase in tomato leaves in response to wounding. *Plant Physiology 111*:797–803
48. Cote K.W., Lewis E.E., Schultz P.B., 2002. Compatibility of acaricide residues with Phytoseiulus persimilis and their effects on Tetranychus urticae. *HortScience* 37 (6):906–909
49. Dalin P., Agren J., Bjorkman C., Karkkainen P.H.K., 2008. Leaf Trichome Formation and Plant Resistance to Herbivory. In: Induced plant resistance to herbivory.Shaller A., (ed). Springer, 89-105
50. Dam N.M., Vuister L.W.M., Bergshoeff C., Vos H., Meijden E.D., 1995. The 'raison d'etre' of pyrrolizidine alkaloids in Cynoglossum officinale: deterrent effects against generalist herbivores. *Journal of Chemical Ecology* 21:507–523
51. De Boer J.G., Dicke M., 2004. The role of methyl salicylate in prey searching behaviour of the predatory mite Phytoseiulus persimilis. *Journal of Chemical Ecology 30*:255–271
52. De Boer J.G., Hordijk C.A., Posthumus M.A., Dicke M., 2008. Prey and non-prey arthropods sharing a host plant: Effects on induced volatile emission and predator attraction. *Journal of Chemical Ecology* 34:281–290
53. De Boer J.G., Posthumus M.A., Dicke M., 2004. Identification of volatiles that are used in discrimination between plants infested with prey or nonprey herbivores by a predatory mite. *Journal of Chemical Ecology 30*:2215–2230
54. de Lillo E.,vMonfreda R., 2004. «Salivary secretions» of eriophyoids (Acari: Eriophyoidea): first results of an experimental model. *Experimental and Applied vAcarology 34(3–4*): 291–306
55. De Moraes C.M., Mescher M.C., Tumlinson J.H., 2001. Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature* 410:577-580
56. De Moraes G.J., Lima H.C., 1983. Biology of Euseius concordius (Chant) (Acarina: Phytoseiidae) a predator of the tomato russet mite. *Acarology 24*: 251–255
57. Després L., David J. P., Gallet C., 2007. The evolutionary ecology of insect resistance to plant chemicals. *Trends in Ecology and Evolution* 22:298-307
58. Dicke M., 1994. Local and Systemic Production of Volatile Herbivore-induced Terpenoids: Their Role in Plant-carnivore Mutualism. *Journal of Plant Physiology 143(4–5)*:465–472

59. Dicke M., Gols R., Ludeking D., Posthumus M.A., 1999. Jasmonic acid and herbivory differentially induce carnivore-attracting plant volatiles in lima bean plants. *Journal Chemical Ecology* 25:1907–1922.
60. Dicke M., Sabelis M.W., Takabayashi J., Bruin J., Posthumus M.A., 1990. Plant strategies of manipulating predator-prey interactions through allelochemicals: prospects for application in pest control. *Journal of Chemical Ecology 16*: 3091-3118
61. Dicke M., Takabayashi J., Posthumus M.A., Schutte C., Krips O.E., 1998. Plant-phytoseiid interaction mediated by herbivore-induced plant volatiles: variation in production of cues and in responses of predatory mites. *Experimental and Applied Acarology 22*: 311-333
62. Doares S.H., Narváez-Vásquez J., Conconi A., Ryan C.A., 1995a. Salicylic acid inhibits synthesis of proteinase inhibitors in tomato leaves induced by systemin and jasmonic acid. *Plant Physiology* 108: 1741–1746
63. Doares S.H., Syrovets T., Weiler E.W., Ryan C.A., 1995b. Oligogalacturonides and chitosan activate plant defensive genes through the octadecanoid pathway. *Proceeding of National. Academy of Science USA* 92:4095–4098
64. Drukker B., Janssen A., Ravensberg W., Sabelis M.W., 1997. Improved control capacity of the mite predator Phytoseiulus persimilis (Acari: Phytoseiidae) on tomato. *Experiemntal and Applied Acarology* 21: 507–518
65. Dudareva N., Negre F., Nagegowda D.A., Orlova I., 2006. Plant volatiles: recent advances and future perspectives. Critical Reviews in Plant Science 25:417–440
66. Duffey S.S., Stout M.J., 1996. Anutritive and toxic compounds of plant defense against insects. *Archives in Insect Biochemistry and Physiology 32*: 3-37
67. Duso C., Kreiter S., Tixier M.S., Pozzebon A., Malagnini V., 2010. Biological control of mites in European vineyards and the impact of natural vegetation. Trends in Acarology. In: Proceedings of the 12th international congress, Amsterdam, 399–407 pp
68. Dussourd D.E., 1993. Foraging with finesse: caterpillar adaptations for circumventing plant defenses. In: Stamp N.E., Casey T. (eds) Caterpillars: ecological and evolutionary constraints on foraging. Chapman and Hall, New York, 92–131 pp.
69. Dussourd D.E., 2009. Do canal-cutting behaviours facilitate host-range expansion by insect herbivores? *Biological Journal of the Linnean Society 96*:715–731
70. Eckardt N.A., 2001. A calcium-regulated gatekeeper in phloem sieve tubes. *The Plant Cell 13*:989-992
71. Elstner E.F., 1980. Metabolism of activated oxygen species. In: The Biochemistry of Plants. A Comprehensive Treatise. (ed.). D.D., Davies Biochemistry and Metabolism. Academic Press, New York. Volume 11. 388 pp.
72. Enyedi A.J., Yalpani N., Silverman P., Raskin I., 1992. Signal molecules in systemic plant resistance to pathogens and pests. *Cell* 70:879-886
73. Farmer E.E., Caldelari D., Pearce G., Walker-Simmons M.K., Ryan C.A., 1994 Diethylthiocarbamic acid inhibits the octadecanoid signaling pathway for the wound induction of proteinase inhibitors in tomato leaves. *Plant Physiology 106:* 337-342
74. Farmer E.E., Ryan C.A., 1990. Interplant Communication: Airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proceeding of National. Academy of Science* 87:7713–7716
75. Farmer E.E., Ryan C.A., 1992. Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. *Plant Cell* 4:129–134
76. Farrar R.R., Barbour J.D., Kennedy G.G., 1994. Field evaluation of insect resistance in a wild tomato and its effect on insect parasitoids. *Entomologia Experimentalis et Applicata* 71:211–26
77. Farrar R.R., Kennedy G.G., 1987. 2-Undecanone, a constituent of the glandular trichomes of *Lycopersicon hirsutum f. glabratum*: effects on Heliothis zea and Manduca sexta growth and survival. *Entomologia Experimentalis et Applicata 43*: 17–23

78. Farrar R.R., Kennedy G.G., 1992. Insect and mite resistance in tomato. In G Kalloo, ed, Genetic Improvement of Tomato. Monographs on Theoretical and Applied Genetics 14. Springer-Verlag, New York, 122–142 pp
79. Felton G.W., 2005. Indigestion is a plant's best defense. *Proceedings of the National Academy of Sciences of USA* 102:18771–18772
80. Felton G.W., Bi J.L., Summers C.B., Mueller A.J., Duffey S.S., 1994. Potential role of lipoxygenases in defense against insect herbivory. *Journal of Chemical Ecology 20:* 651–666
81. Felton G.W., Donato k., Del Vecchio R.J., Duffey, S.S., 1989. Activation of plant foliar oxidases by insect feeding reduces nutritive quality of foliage for noctuid herbivores. *Journal Chemical Ecology* 15: 2667–2694
82. Felton G.W., Donato K.K., Broadway R.M., Duffey S.S., 1992. Impact of oxidized plant phenolics on the nutritional quality of dietary protein to a noctuid herbivore, *Spodoptera exigua*. *Journal of Insect Physiology 38*: 277–285
83. Fischer S., Klotzli F., Falquet L., Celle O., 2005. An investigation on biological control of the tomato russet mite Aculops lycopersici (Massee) with Amblyseius andersoni (Chant). *IOBC/WPRS Bulletin 28*:99–102
84. Fischer W., Christ U., Baumgartner M., Erismann K.H., Mösinger E., 1989. Pathogenesis-related proteins of tomato II: Biochemical and immunological characterization. *Physiological and Molecular Plant Pathology* 35(1): 67–983
85. Frei H., Luthy J., Brauchli J., Zweifel U., Wurgler F.E., Schlatter C., 1992. Structure/activity relationships of the genotoxic potencies of sixteen pyrrolizidine alkaloids assayed for the induction of somatic mutation and recombination in wing cells of Drosophila melanogaster. *Chem Biol Interact* 83:1–22
86. Gatehouse J.A., 2002, Plant resistance towards insect herbivores: a dynamic interaction. *New Phytologist 156*:145–169
87. Gerson U, Smiley R.L., Ochoa R., 2003. Mites (Acari) for pest control. Oxford, Blackwell Science, 539 p.
88. Gerson U., Weintraub P.G., 2007. Review Mites for the control of pests in protected cultivation. *Pest Management Science* 63:*658-676*
89. Gillespie D.R., Quiring D.J.M., 1994. Reproduction and longevity of the predatory mite, Phytoseiulus persimilis (Acari: Phytoseiidae) and its prey, Tetranychus urticae (Acari: Tetranychidae) on different host plants. *Journal of the Entomological Society of British Columbia* 91:3-8.
90. Gotoh T., Bruin J., Sabelis M.W., Menken S.B.J., 1993. Host race formation in *Tetranychus urticae*: genetic differentiation, host plant preference and mate choice in a tomato and a cucumber strain. *Entomologia Experimentalis et Applicata* 68:171–178
91. Gotoh T., Yamaguchi K., Mori K., 2004. Effect of temperature on life history of predatory mite Amblyseiulus (Neoseiulus) californicus (Acari: Phytoseiidae). *Experimental and Applied Acarology 32*: 15-30
92. Gouinguene S., Alborn H., Turlings T.C.J., 2003. Induction of volatile emissions in maize by different larval instars of Spodoptera littoralis. *Journal of Chemical Ecology 29*:145–162
93. Goussain M.M, Prado E., Moraes J.C., 2005. Effect of silicon applied to wheat plants on the biology and probing behaviour of the greenbug *Schizaphis graminum* (Rond.) (Hemiptera: Aphididae). *Neotropical Entomology* 34(5):807-813
94. Grant M., Lamb C., 2006. Systemic immunity. *Current Opinion in Plant Biology* 9: 414–420
95. Grbic M., et al. 2011. The genome of Tetranychus urticae reveals herbivorous pest adaptations. *Nature 479*:487-492
96. Grbic M., Khila A., Lee K.Z., Bjelica A., Grbic V., Whistelcraft J., Verdon L., Navajas M., Nagy L., 2007. Mity model: *Tetranychus urticae*, a candidate for chelicerate model organism. *Bioessays 29:*489–496
97. Green T.R., Ryan C.A., 1972. Wound-induced proteinase inhibitor in plant leaves: a possible defense mechanism against insects. *Science* 175:776 –777

98. Hagen R.H., Chabot J.F., 1986. Leaf anatomy of maples (Acer) and host use by Lepidoptera larvae. *Oikos 47*:335–345
99. Handley R., Ekbom B., Agren J., 2005. Variation in trichome density and resistance against a specialist insect herbivore in natural populations of *Arabidopsis thaliana. Ecological Entomology* 30:284-292
100. Hanley M.E., Lamont B.B., Fairbanks M.M., Rafferty. C.M., 2007. Plant structural traits and their role in anti-herbivore defence. *Perspectives in Plant Ecology, Evolution and Systematics* 157–178
101. Harborne J.B., 1980. Plant phenolics. In: *Encyclopedia of Plant Physiology*. Bell E.A., Charlwood B.V., (eds) Berlin Heidelberg, New York: Springer-Verlag. 329–395 pp.
102. Harborne J.B., Simmonds N.W., 1964. Natural distribution of the phenolic aglycones. In: *Biochemistry of Phenolic Compounds* (Harborne, J.B., ed.),. London: Academic Press. 77–128 pp.
103. Hartl M., Giri A.P., Kaur H., Baldwin I.T., 2010. Serine protease inhibitors specifically defend Solanum nigrum against generalist herbivores but do not influence plant growth and development. Plant Cell 22(12):4158-4175
104. Hartmann T., Ober D., 2000. Biosynthesis and metabolism of pyrrolizidine alkaloids in plants and specialized insect herbivores. In: Leeper FJ, Vederas JC, editors. Topics in current chemistry, Berlin: Springer 207 p.
105. Hartmann T., Theuring C., Beuerle T., Klewer N., Schulz S., Singer M.S., Bernays E.A., 2005. Specific recognition, detoxification and metabolism of pyrrolizidine alkaloids by the polyphagous arctiid Estigmene acrea. *Insect Biochemistry and Molecular Biology* 35:391–411
106. Hartmann T., Witte L., 1995. Chemistry, biology and chemoecology of the pyrrolizidine alkaloids. In: In: Alkaloids: Chemical and Biological Perspectives. (Ed.) Pelletier S.W., Vol. 9. Pergamon Press, Elmsford, New York. 156–233 pp.
107. He J., Chen F., Chen S, Lv G., Deng Y., Fang W., et al., 2011. Chrysanthemum leaf epidermal surface morphology and antioxidant and defense enzyme activity in response to aphid infestation. *Journal of Plant Physiology* 168:687–93
108. Helle W., Sabelis M.W., 1985. Spider mites: their biology, natural enemies and control. Amsterdam, Elsevier, v. 1B, 458p
109. Hildebrand D.F., Rodriguez J.G., Brown G.C., Luu K.T., Volden C.S., 1986. Peroxidative responses of leaves in two soybean genotypes injured by twospotted spider mites (Acari: Tetranychidae). *Journal of Economical Entomology 79*: 1459-1465
110. Hilker M., Kobs C., Varama M., Schrank K., 2002. Insect egg deposition induces *Pinus sylvestris* to attract egg parasitoids. *Journal of Experimental Biology* 205**:** 455–461.
111. Holopainen J.K., 2004. Multiple functions of inducible plant volatiles.*Trends in Plant Science 9*:529–533
112. Howe G.A., 2004. Jasmonates as signals in the wound response. *Journal of Plant Growth Regulation* 23:223–237
113. Howe G.A., Lightner J., Browse J., Ryan C.A., 1996. An octadecanoid pathway mutant (JL5) of tomato is compromised in signaling for defense against insect attack. *Plant Cell* 11:2067–2077
114. Hoy M.A., Fahong Yu, Meyer J. M., Tarazona O.A, Jeyaprakash A., Wu K., 2013. Transcriptome sequencing and annotation of the predatory mite *Metaseiulus occidentalis* (Acari: Phytoseiidae): a cautionary tale about possible contamination by prey sequences. *Experimental and Applied Acarology 59(3)*:283-296
115. Jander G., Howe G., 2008. Plant Interactions with Arthropod Herbivores: State of the Field. *Plant Physiology* 146:80-803
116. Jeppson L.R., Keifer H.H., Baker. E.W., 1975. Mites Injurious to Economic Plants. University of California Press, Berkeley, CA. 614 pp.
117. Jones J.D.G., Dangl J.L., 2006. The plant immune system. *Nature 444*: 323–329

118. Joosten M.H.A.J., De Wit P.J. G. M., 1989. Identification of Several Pathogenesis-Related Proteins in Tomato Leaves Inoculated with Cladosporium fulvum (syn. Fulvia fulva) as 1,3-β-Glucanases and Chitinases 1. *Plant Physiology* 89(3): 945–951.
119. Juvik J.A., Shapiro J.A., Young T.E., Mutschler M.A., 1994. Acylglucoses from wild tomatoes alter behavior and reduce growth and survival of Helicoverpa zea and Spodoptera exigua (Lepidoptera, Noctuidae). *Journal of Economic Entomology* 87:482–492.
120. Kamau A.W., Mueke J.M., Khaemba B.M., 1992. Resistance of tomato varieties to the tomato russet mite, Aculops lycopersici (Massee) (Acarina: Eriophyidae). *Insect Science and its Application 13(3)*:351-356
121. Kang J.H., Shi F., Jones A.D, Marks M.D, Howe G.A., 2010. Distortion of trichome morphology by the hairless mutation of tomato affects leaf surface chemistry. *Journal of Experimental Botany 61:*1053–1064
122. Kant M.R., Sabelis M.W., Haring M.A., Schuurink R.C., 2008. Intraspecific variation in a generalist herbivore accounts for differential induction and impact of host plant defences. *Proceedings of the Royal Society B, Biological Sciences 275*:443–452
123. Kant M.R., Ament K., Sabelis M.W., Haring M.A., Schuurink R.C., 2004. Differential timing of spider mite-induced direct and indirect defenses in tomato plants. *Plant Physiology* 135:483–495
124. Kappers I.F., Aharoni A., van Herpen T.W.J.M., Luckerhoff L.L.P., Dicke M., Bouwmeester H.J., 2005. Genetic engineering of terpenoid metabolism attracts bodyguards to Arabidopsis. *Science* 309: 2070–2072
125. Karban R., Agrawal A.A., 2002. Herbivore offense. *Annual Review of Ecology and Systematics* 33: 641-664
126. Karban R., Baldwin I.T., 1997. Induced Responses to Herbivory. University of Chicago Press, Chicago.
127. Kay I.R., 1986. Tomato Russet Mite: A Serious Pest of Tomatoes. *Queensland Agricultural Journal 112 (5):* 231-232
128. Keathley C.P., Potter D.A., 2011. Behavioral plasticity of a grass-feeding caterpillar in response to spiny- or smooth-edged leaf blades. *Arthropod-Plant Interactions 5*:339–349
129. Kefeli V.I., Kalevitch M.V., Borsari B., 2003. Phenolic cycle in plants and environment. *Journal of Cell Molecular Biology 2*:13–18
130. Keifer H., Baker E., Kono T., Delfinado M., Styer W., 1982. An illustrated guide to plant abnormalities caused by Eriophyid mites in North America. USDA Agriculture Handbook N 573
131. Kempema L.A, Cui X.P., Holzer F.M., Walling L.L., 2007. *Arabidopsis* transcriptome changes in response to phloem-feeding silverleaf whitefly nymphs. Similarities and distinctions in responses to aphids. *Plant Physiol* 143:849–865.
132. Kennedy G.G., Farrar R.R.., 1987. Response of insecticide-resistant and susceptible Colorado potato beetles, *Leptinotarsa decemlineata* to 2-tridecanone and resistant tomato foliage: The absence of cross resistance. *Entomologia Experimentalis et Applicata 45*: 187-192
133. Kennedy G.G., Soremson C.E., 1985. The role of glandular trichomes in the resistance of *lycopersicon hirsutum* f. *Glabratum. Journal of Economical Entomology* 78:547–551
134. Kessler A., Baldwin I.T., 2001. Defensive function of herbivore-induced plant volatile emissions in nature. *Science 291*(5511):2141-2144
135. Kessler A., Baldwin I.T., 2002. Plant responses to insect herbivory: the emerging molecular analysis. *Annual Review of Plant Biology* 53: 299–328
136. Khajehali J., Van Nieuwenhuyse P., Demaeght P., Tirry L., Van Leeuwen T., 2011. Acaricide resistance and resistance mechanisms in Tetranychus urticae populations from rose greenhouses in the Netherlands. *Pest Management Science 67*:1424–1433
137. Kimura S., Sinha N., 2008. Tomato (*Solanum lycopersicum*): A Model Fruit-Bearing Crop. In: (Eds) Johnson A.D., Krumlauf R.E., Levine M., Patel N., Sinha N. **E**merging Model Organisms: Volume 1: A Laboratory Manual**.** Cold Spring Harbor Laboratory Press, USA .552 p
138. Knoblauch M., Van Bel A.J.E., 1998. Sieve tubes in action. *The Plant Cell 10*:35–50

139. Knowles C.O., 1997. Molecular Mechanisms of Resistance to Agrochemicals, ed Sjut V. Springer, Berlin, 57–77 pp.
140. Koornneef A., Pieterse C.M.J., 2008. Cross Talk in Defense Signaling. *Plant Physiolog 146 (3):* 839-844
141. Laing J.E., Osborn J.A.L., 1974. The effect of prey density on the functional and numerical responses of three species of predatory mites. *Entomophaga 19*: 267-277
142. Lange W.H., Bronson L., 1981. Insect pests of tomatoes. *Annual Review of Entomology 26*:345-371.
143. Lawrence C.B., Joosten M.H.A.J, Tuzun S., 1996. Differential induction of pathogenesis-related proteins in tomato by Alternaria solani and the association of a basic chitinase isozyme with resistance. *Physiological and Molecular Plant Pathology* 48(6):361-377
144. Lawrence S.D., Novak N.G., Ju C.J-T., Cooke J.E.K.., 2008. Potato, Solanum tuberosum, defense against Colorado potato beetle, Leptinotarsa decemlineata (Say): microarray gene expression profiling of potato by Colorado potato beetle regurgitant treatment of wounded leaves. *Journal of Chemical Ecology.34:*1013–1025
145. Leszczynski B., Matok H., Dixon A.F.G., 1992. Resistance of cereals to aphids: the interaction between hydroxamic acids and UDP-glucose transferases in the aphid Sitobion avenae (Homoptera: Aphididae). *Journal of Chemical Ecology* 18, 1189–1200
146. Li C.Y., Williams M.M., Loh Y.T., Lee G.I., Howe G.A., 2002.Resistance of cultivated tomato to cell content-feeding herbivores is regulated by the octadecanoid-signaling pathway. *Plant Physiology 130:* 494–503
147. Lichtenthaler H.K., 1999. The 1-deoxy-D-xylulose 5-phosphate pathway of isoprenoid biosynthesis in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* ***50***:47–65
148. Livak K.J., Schmittgen T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCt method. *Methods 25*: 402–408
149. Llusia J., Peñuelas J., Seco R., Filella I., 2012. Seasonal changes in the daily emission rates of terpenes by Quercus ilex and the atmospheric concentrations of terpenes in the natural park of Montseny, NE Spain. *Journal of Atmospheric Chemistry 69*:215-230
150. Llusia J., Penueles J., 2001. Emission of volatiles organic compounds by apple trees under spider mite attack and attraction of predatory mites. *Experimental and Applied Acarology 25*: 65-77
151. Lorenzo O., Solano R., 2005. Molecular players regulating the jasmonate signalling network. *Current Opinion of Plant Biology* 8: 532–540
152. Løvdal T., Lillo C., 2009. Reference gene selection for quantitative real-time PCR normalization in tomato subjected to nitrogen, cold, and light stress. *Analytical Biochemistry* 387:238–242
153. Luckwill L.C., 1943. The Genus Lycopersicon*:* A Historical, Biological and Taxonomic Survey of the Wild and Cultivated Tomato. Aberdeen, Scotland: Aberdeen Univ. Studies, 120. Aberdeen Univ. Press. 44 pp
154. Lugasi A., Bıro L., Hovarie J., Sagi K.V., Brandt S., Berna E., 2003. Lycopene content of foods and lycopene intake in two groups of the Hungarian population. *Nutrition Research 23*:1035-1044
155. Maffei M., 2010. The plant volatilome. In: Taiz L., Zeiger E. A (eds), Secondary metabolites and plant defense. Plant Physiology online, Fifth Edition.
156. Massee A.M., 1939. A species of gall mite (Eriophyidae) injurious to tomato. *Annals and Magazines of Natural History Series 2 and 3:*617-619
157. McGarvey D.J., Croteau R., 1995. Terpenoid metabolism.*Plant Cell* 7:1015–102
158. McGurl B., Pearce G., Orozco-Cárdenas M., Ryan C.A., 1992. Structure, expression, and antisense inhibition of the systemin precursor gene. *Science 255*: 1570-1573
159. McGurl B., Ryan C.A., 1992. The organization of the prosystemin gene. *Plant Molecular Biology* 20: 405–409
160. McGurl B., Ryan C.A., 1992. The organization of the prosystemin gene. *Plant Molecular Biology 20:* 405-409
161. McManus M.T., White D.W.R., McGregor P.G., 1994. Accumulation of a chymotrypsin inhibitor in transgenic tobacco can affect the growth of insect pests. *Transgene Research* 3:50-58

162. McMurtry, J.A., and Croft B.A., 1997. Life-styles of phytoseiid mites and their roles in biological control. *Annual Review of Entomology 42*:291–321
163. Mesa N.C., Braun A.R., Belotti A.C., 1990. Comparison of Mononychellus progresivus and Tetranychus urticae as prey for five species of phytoseiid mites. *Experimental and Applied Acarology 9(3-4):*159-168.
164. Messelink G.J., Van Steenpaal S.E.F., Ramakers P.M.J., 2006. Evaluation of phytoseiid predators for control of western flower thrips on greenhouse cucumber. *Biocontrol 51*:753-768
165. Migeon A., Dorkeld F., 2010. Spider Mites Web:http://www.montpellier.inra.fr/CBGP/spmweb
166. Milner S.E., Brunton N.P., Jones P.W., O'Brien N.M., Collins S.G., Maguire A.R., 2011. Bioactivities of glycoalkaloids and their aglycones from Solanum species. *Journal of Agriculture and Food Chemistry 59*:3454–3484
167. Misra J.R., Horner M.A, Lam G., Thummel C.S., 2011. Transcriptional regulation of xenobiotic detoxification in Drosophila. *Genes and Development* 25: 1796-1806
168. Moayeri H.R.S., Ashouri A., Poll L., Enkegaard A., 2007. Olfactory response of a predatory mirid to herbivore induced plant volatiles: Multiple herbivory vs. single herbivory. *Journal of Applied Entomoly* 131:326–332
169. Monfreda R., Spagnuolo M., 2004. Enzyme activity of an eriophyoid 'salivary' secretion: preliminary report of polygalacturonase. *Phytophaga 14*:611–614
170. Moran P.J, Thompson G.A., 2001. Molecular responses to aphid feeding in *Arabidopsis* in relation to plant defense pathways. *Plant Physiol 125*:1074–1085.
171. Moyle L.C., 2008.Ecological and evolutionary genomics in the wild tomatoes (Solanum sect. Lycopersicon). *Evolution 62(12):*2995-3013
172. Mueller M.J., 1997. Enzymes involved in jasmonic acid biosynthesis. *Physiologia Plantatrium 100:* 653–663
173. Mueller M.J., Brodschelm W., Spannagl E., Zenk M.H., 1993. Signaling in the elicitation process is mediated through the octadecanoid pathway leading to jasmonic acid. *Proceedings of National Academy of Sciences of USA* 90:7490-7494
174. Mukanganyama S., Figueroa C.C., Hasler J.A., Niemeyer H.M., 2003. Effects of DIMBOA on detoxification enzymes of the aphid Rhopalosiphum padi (Homoptera: aphididae). *Journal of Insect Physiology* 49:223–229
175. Müller C., Arand K., 2007. Trade-offs in oviposition choice? Food-dependent performance and defence against predators of an herbivorous sawfly. *Entomologia Experimentalis et Applicata* 124(2):153–159
176. Mumm R., Schrank K., Wegener R., Schulz S., Hilker M., 2003. Chemical analysis of volatiles emitted by *Pinus sylvestris* after induction by insect oviposition. *Journal of Chemical Ecology* 29**:** 1235–1252
177. Musser R.O., Cipollini D.F., Hum-Musser S.M., Williams S.A., Brown J.K., Felton G.W., 2005 Evidence that the caterpillar salivary enzyme glucose oxidase provides herbivore offense in solanaceous plants. *Archives in Insect Biochemistry Physiology* 58(2):128-137
178. Mutti N.S., Louis J., Pappan L.K., Pappan K., Begum K., Park M-S.C.Y., Dittmer N., Marshall J., Reese J.C., Reeck G.R., 2008. A protein from the salivary glands of the pea aphid, Acyrthosiphon pisum, is essential in feeding on a host plant. *PNAS 105(29)*:9965–9969
179. Narberhaus I., Zintgraf V., Dobler S., 2005. Pyrrolizidine alkaloids on three trophic levels – evidence for toxic and deterrent effects on phytophages and predators. *Chemoecology* 15:121–125
180. Narváez-Vásquez J., Florin-Christensen J., Ryan C.A., 1999. Positional specificity of a phospholipase A activity induced by wounding, systemin, and oligosaccharide elicitors in tomato leaves. *Plant Cell 11*: 2249-2260
181. Navajas M., 1998. Host plant associations in the spider mite *Tetranychus urticae* (Acari: Tetranychidae): insights from molecular phylogeography. *Experimental and Applied Acarology* 22:201–214
182. Niemeyer H.M., Perez F.J., 1995. Potential of hydroxamic acids in the control of cereal pests, diseases, and weeds. In: Inderjit, Dakshini, K.M.M., Einhellig, F.A. (Eds.), Allelopathy:

Organisms, Processes, and Application. ACS Symposium Series 582. American Chemical Society, Washington, DC, 260–270pp

183. Nuzzaci G., 1979. Contributo alla conoszenza dello gnathosoma degli Eriofidi. Entomologica 15:73–101
184. Nuzzaci G., Alberti G., 1996. Internal anatomy and physiology. In: Lindquist E.E., Sabelis M.W., Bruin J. (eds) Eriophyoid Mites their biology natural enemies and control. Elsevier, Amsterdam, 101–150 pp.
185. Osman A.A., Zaki A.M., 1986. Studies on the predation efficiency of *Agistemus exsertus* Gonzalez (Acarina, Stigmaeidae) on the eriophyid mite *Aculops lycopersici* (Massee). *Anz. Schädlingskd. Pfl. Umwelt. 59*: 135–136
186. Paré P.W., Tumlinson J.H., 1998. Cotton volatiles synthesized and released distal to the site of insect damage. *Phytochemistry* 47: 521–526
187. Paré P.W., Tumlinson J.H., 1997. De novo biosynthesis of volatiles induced by insect herbivory in cotton plants. *Plant Physiology* 114: 1161–1167
188. Paré P.W., Tumlinson J.H., 1999. Plant volatiles as a defense against insect herbivores. *Plant Physiology* 121(2):325-331
189. Park H.H., Shipp L., Buitenhuis R., 2010. Predation, development, and oviposition by the predatory mite Amblyseius swirskii (Acari: Phytoseiidae) on tomato russet mite (Acari: Eriophyidae). *Journal of Economic Entomology 103*:563–569
190. Pearce G., Strydom D., Johnson S., Ryan C.A., 1991. A polypeptide from tomato leaves induces wound-inducible inhibitor proteins. *Science 253*: 895–898
191. Penna-Cortes H., Albrecht T., Prat S., Weiler E.W., Willmitzer L., 1993. Aspirin prevents wound-induced gene expression in tomato leaves by blocking jasmonic acid biosynthesis. *Planta* 191: 123–128
192. Peralta I.E., Knapp S., Spooner D.M., 2005. New species of wild tomatoes (*Solannum* Section *Lycopersicon*: Solanaceae) from Northern Peru. Systematic Botany 30:424-434
193. Perring T.M., 1996. Vegetables. In: Lindquist EE, Sabelis MW, Bruin J. (eds) Eriophyoid mites-their biology, natural enemies and control, World Crop Pest Series, vol 6. Elsevier, Amsterdam, 593–610 pp
194. Perring T.M., Farrar C.A., 1986. Historical Perspective and Current World Status of the Tomato Russet Mite (Acari: Eriophyidae). Miscellaneous Publications of the Entomological Society of America. 63: 1-9
195. Petanovic R., Kielkiewicz M., 2010a. Plant-eriophyoid mite interactions: cellular biochemistry and metabolic responses induced in mite-injured plants. *Experimental and Applied Acarology 51*:61–80
196. Petanovic´ R., Kielkiewicz M., 2010b. Plant–eriophyoid mite interactions: specific and unspecific morphological alterations. Part II.
197. Price P.W., Bouton C.E., Gross P., McPheron B.A., Thompson J.N., Weis A.E., 1980. Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. Annual Review Ecol Syst 11: 41–65
198. Puthoff D.P., Holzer F.M., Perring T.M., Walling L.L., 2010. Tomato pathogenesis-related protein genes are expressed in response to trialeurodes vaporariorum and bemisia tabaci biotype b feeding. *Journal of Chemical Ecology* 36: 1271-1285
199. Quiroz A., Pettersson J., Pickett J.A., Wadhams L.J. Niemeyer H.M., 1997. Semiochemicals mediating spacing behavior of bird cherry-oat aphid, *Rhopalosiphum padi*feeding on cereals. *Journal of Chemical Ecology 23(11)*:2599-2607
200. Rasmann S., Köllner T.G., Degenhardt J., Hiltpold I., Toepfer S., Kuhlmann U., et al. 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature 434*: 732–737
201. Rasmann S., Turlings T.C.J., 2007. Simultaneous feeding by aboveground and belowground herbivores attenuates plant mediated attraction of their respective natural enemies. *Ecology Letters* 10: 926 – 936

202. Raworth D.A., Fauvel G., Auger P., 1994. Location, reproduction and movement of Neoseiulus californicus (Acari: Phytoseiidae) during the autumn, winter and spring in orchards in the south of France. *Experimental and Applied Acarology 18:*593–602
203. Reymond P., Farmer E.E., 1998. Jasmonate and salicylate as global signals for defense gene expression. *Current Opinion in Plant Biology 1*: 404–411
204. Rice R.E., Strong F.E., 1962. Bionomics of the Tomato Russet Mite. *Vasates lycopersici* (Massee). *Annal of Entomological Society of America 55:* 431-435
205. Roddick J.G., 1989. The acetylcholinesterase-inhibitory activity of steroidal glycoalkaloids and their aglycones. *Phytochemistry* 28, 2631–2634
206. Rodriguez A.E., Tingey W.M., Mutschler M.A., 1993. Acylsugars of Lycopersicon pennellii deter settling and feeding of the green peach aphid (Homoptera, Aphididae). *Journal of Economic Entomology 86*:34–39
207. Rodriguez-Saona C., Chalmers J.A., Raj S., Thaler J.S., 2005. Induced plant responses to multiple damagers: Differential effects on an herbivore and its parasitoid. *Oecologia* 143:566–577
208. Rodriguez-Saona C., Crafts-Brandner S.J., Canas L.A., 2003. Volatile emissions triggered by multiple herbivore damage: Beet armyworm and whitefly feeding on cotton plants. *Journal of Chemical Ecology* 29:2539–2550
209. Rodriguez-Saona C.R., Musser R.O., Vogel H., Hum-Musser S. M.,Thaler J.S., 2010. Molecular, biochemical, and organismal analyses of tomato plants simultaneously attacked by herbivores from two feeding guilds. *Journal of Chemical Ecology 36*:1043-1057
210. Rohmer M., 1999. The discovery of a mevalonate independent pathway for isoprenoid biosynthesis in bacteria, algae and higher plants. *Natural Products Reports* 16:565–574
211. Rose U.S., Manukian A., Heath R.R., Tumlinson J.H. 1996. Volatile semiochemicals released from undamaged cotton leaves: a systemic response of living plants to caterpillar damage. *Plant Physiolgy* 111: 487–495
212. Roy B.A., Kirchner J.W., 2000. Evolutionary dynamics of pathogen resistance and tolerance. *Evolution* 54(1):51–63
213. Royalty R.N., Perring T.M. 1996. Nature of damage and its assessment. In: Lindquist EE, Sabelis MW, Bruin J (eds) Eriophyoid Mites—their biology, natural enemies and control. Elsevier, Amsterdam 493–512 pp
214. Royalty R.N., Perring T.M., 1988. Morphological analysis of damage to tomato leaflets by tomato russet mite (Acari: Eriophyidae). J Econ Entomol 81(3):816–820
215. Royalty R.N., Perring T.M., 1989. Reduction of photosynthesis of tomato leaflets by tomato russet mite (Acari: Eriophyidae). *Environmental Entomology* 18:256–260
216. Ryals J.A., Neuenschwander U.H., Willits M.G., Molina A., Steiner H.Y., Hunt M.P., 1996. Systemic acquired resistance. *Plant Cell* 8:1809-1819
217. Ryan C.A, Moura D.S., 2002 Systemic wound signaling in plants: a new perception. *Proceedings of National Academy of Sciences of USA* 99:6519–6520
218. Ryan C.A., 1990. Protease inhibitors in plants: genes for improving defenses against insects and pathogens. *Annual Review of Phytopathology 28*: 425-449
219. Ryan C.A., 2000. The systemin signaling pathway: differential activation of plant defensive genes. *Biochemica and Biophysica Acta 1477*:112–21
220. Ryan C.A., Pearce G., 2003. Systemins, a functionally defined family of peptide signal that regulate defensive genes in Solanaceae species. *Proceedings of the National Academy of Sciences USA 100:*14577–14580
221. Sabelis M.W., Takabayashi J., Janssen A., Kant M.R., van Wijk M., Sznajder B., et al., 2007. Ecology meets plant physiology: herbivore- induced plant responses and their indirect effects on arthropod communities. In Ecological Communities: Plant Mediation in Indirect Interaction Webs. (Eds) by Ohgushi, T., Craig, T.P. and Price, P.W. Cambridge University Press, Cambridge. 188–217 pp.
222. Sacchettini J.C., Poulter C.D., 1997. Creating isoprenoid diversity. *Science* 277:1788–1789

223. Salinas A.E., Wong M.G., 1999. Glutathione S-transferases: a review. *Current Medicinal Chemistry* 6:279-309
224. Sances F.V., Wyman J.A., Ting I.P., 1979. Morphological responses of strawberry leaves to infestations of twospotted spider mite. *Journal of Economic Entomology 72*:710–713
225. Santiago R., Malvorlvor R.A., Baamonde M.D., Revilla P., Soulo T.C., 2005. Free phenols in maize pith and their relationship with resistance to Sesamia nonagrioides (Lepidoptera: Noctvidae) attack. *Journal of Economical Entomology 98*: 1349-1356
226. Sarmento R.A, Lemos F., Bleeker P.M., Shuurink R.C., Pallini A., Lima E.R., Kant M., Sabelis M.W., Jansen A., 2011. A herbivore that manipulates plant defence. *Ecology Letters* 14:229-236.
227. Sato M.E., Marcos Z., Da Silva A., Dalton R.., De Souza M.F., 2005. Abamectin resistance in Tetranychus urticae Koch (Acari: Tetranychidae): Selection, cross-resistance and stability of resistance. *Neotropical Entomology 34*: 991-998
228. Sato M.M., de Moraes G.J., Haddad M.L., Wekesa V.W., 2011. Effect of trichomes on the predation of Tetranychus urticae (Acari: Tetranychidae) by Phytoseiulus macropilis (Acari: Phytoseiidae) on tomato, and the interference of webbing. Experimental and Applied Acarology 54:21–32
229. Sato S., Tabata S., Hirakawa H., Asamizu E., Shirasawa K., Isobe S., Kaneko T., Nakamura Y., Shibata D., Aoki K., et al., 2012. The tomato genome sequence provides insights into fleshy fruit evolution. *Nature 485(7400)*:635-641
230. Schaller A., 1999. Oligopeptide signalling and the action of systemin. *Plant Molecular Biology 40*: 763-769
231. Schaller A., 2001. Bioactive peptides as signal molecules in plant defense, growth and development. In: Bioactive natural products. Rahman A.U. (ed). Amsterdan, The Netherlands, Elsevier Vol. 25, 3ó7-411 pp.
232. Schaller F., Biesgen C., Mussig C., Altmann T., Weiler E.W., 2000. 12-Oxophytodienoate reductase 3-(OPR3) is the isoenzyme involved in jasmonate biosynthesis. *Planta* 210:979–984
233. Scheer J.M., 2010. The systemin receptor. In: Taiz L., Zeiger E. A (eds), Secondary metabolites and plant defense. Plant Physiology online, Fifth Edition.
234. Schilmiller A.L., Howe G.A., 2005. Systemic signaling in the wound response. *Current Opinion in Plant Biology 8*:369–377
235. Schmitz-Hoerner R., Weissenbock G., 2003. Contribution of phenolic compounds to the *UV*-B screening capacity of developing barley primary leaves in relation to DNA damage and repair under elevated *UV*-B levels. *Phytochemistry 64*:243–255
236. Schnee C., Kollner T.G., Held M., Turlings T.C.J., Gershenzon J., Degenhardt J., 2006. The products of a single maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores. *Proceedings of the National Academy of Sciences of USA 103*: 1129–1134.
237. Schoonhoven L.M., van Loon J.J.A., Dicke M., 2005. Insect-Plant Biology, Ed 2. Oxford University Press, Oxford
238. Schuman M., Barthel S.K., Baldwin I.T., 2012. Herbivory-induced volatiles function as defenses increasing fitness of the native plant Nicotiana attenuata in nature. *elife 1(e00007)*
239. Scott J.G., Liu N., Wen Z.M., 1998. Insect cytochromes P450: diversity, insecticide resistance and tolerance to plant toxins. *Comparative Biochemistry and Physiology 121C*:147-155
240. Shaller F., 2001. Enzymes of the biosynthesis of octadecanoid-derived signalling molecules. *Journal of Experimental Botany* 52 (354): 11-23
241. Simoni S., 1992. Determinazione della sex ratio primaria in Amblyseius californicus (McGregor), Amblyseius cucumeris (Oud.) e Typhlodromus exhilaratus Ragusa (Acari Phytoseiidae). *Redia 75*: 467-474
242. Simpson, S.J., White P., 1990. Associative learning and locust feeding: evidence for a "learned hunger" for protein. *Animal Behavior 40*:506–513
243. Sinden S.L., Sanford L.L., Osamn S.F., 1980. Glycoalkaloids and resistance to the Colorado potato beetle in *Solanum chacoense* Bitter.*American. Potato Journal* 57:331–343

244. Snook M.E., Widstrom N.W., Wiseman B.R., Byrne P.F., Harwood J.S., Costello C.E., 1995. New C-4-hydroxy derivatives of maysin and 3-methoxymaysin isolation from corn silks (Zea mays). *Journal of Agriculture and Food Chemistry 43*: 2740-2745
245. Snook M.E., Widstrom N.W., Wiseman B.R., Gueldner R.C., Wilson R.L., et al., 1994. New flavone C-glycosides from corn (Zea mays L.) for the control of the corn earworm (Helicoverpa zea) In: Bioregulators for crop protection and pest control. (Eds) Hedin P.A., Symposium Series 557 of the American Chemical Society, Washington, DC. 122–135 pp.
246. Snyder M.J., Glendinning J.I., 1996. Causal connection between detoxification enzyme activity and consumption of a toxic plant compound. *Journal of Comparative Physiology A 179*:255 261
247. Southwood T.R.E., 1986. Plant surfaces and insects - an overview (*In):* Juniper B., Sowthwood T.R.E., (eds.). Insects and the plant surface. Edward Arnold Publishers, London, 360 p
248. Stout M.J., Fidantsef A.L., Duffey S.S., Bostock R.M., 1999. Signal interaction in pathogen and insect attack: systemic plant-mediated interactions between pathogens and herbivores of the tomato: *Lycopersicon esculentum. Physiological and Molecular Plant Pathology 54*:115–30
249. Stout M.J., Thaler J.S., Thomma B.P.H.J., 2006. Plant-mediated interactions between pathogenic microorganisms and herbivorous arthropods. *Annual Review of Entomology* 51: 663–689
250. Stout M.J., Workman K.V., Bostock R.M., Duffey S.S., 1998. Specificity of induced resistance in the tomato *Lycopersicon esculentum*. *Oecologia* 113:74–81
251. Stout M.J., Workman K.V., Duffey, S.S., 1996. Identity, spatial distribution, and variability of induced chemical responses in tomato plants. *Entomologia Experimentalis et Applicata*. *79*:255-271
252. Strassner J., Schaller F., Frick U.B., Howe G.A., Weiler E.W., Amrhein N., Macheroux P., Schaller A., 2002. Characterization and cDNA-microarray expression analysis of 12-oxophytodienoate reductases reveals differential roles for octadecanoid biosynthesis in the local versus the systemic wound response. *The Plant Journal 32(4):*585-601
253. Stratmann J.W., 2003 Long distance run in the wound response – jasmonic acid is pulling ahead. *Trends Plant Science 8:*247–250
254. Strauss S.Y., Agrawal A.A., 1999. The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology and Evolution* 14:179–185
255. Stumpf N., Nauen R., 2001. Cross-resistance, inheritance and biochemistry of mitochondrial electron transport inhibitor-acaricide resistance in Tetranychus urticae (Acarie: Tetranychidae). *Journal of Economic Entomology 94*: 1577-1583
256. Summers C.B., Felton G.W., 1994. Prooxidant effects of phenolic acids on the generalist herbivore Helicoverpa zea (Lepidoptera: Noctuidae): Potential mode of action for phenolic compounds in plant anti-herbivore chemistry. Insect Biochemistry and Molecular Biology 24: 943-953
257. Takabayashi J., Dicke M., 1996. Plant–carnivore mutualism through herbivore-induced carnivore attractants. *Trends in Plant Science 1*:109–113
258. Takafuji A., Chant D.A., 1976. Comparative studies of two species of predacious phytoseiid mites (Acarina: Phytoseiidae), with special reference to their responses to the density of their prey. Research in Population Ecology 17: 255-310
259. Thaler J.S., 1999. Jasmonate-inducible plant defences cause increased parasitism of herbivores. *Nature 399*:686–688
260. Thaler J.S., Karban R., Ullman D.E., Boege K., Bostock R.M., 2002. Cross-talk between jasmonate and salicylate plant defense pathways: effects on several plant parasites. *Oecologia* 131: 227–235
261. Thaler J.S., Stout M.J., Karban R., Duffey S.S., 1996. Exogenous jasmonates simulate insect wounding in tomato plants (Lycopersicon esculentum) in the laboratory and field. *Journal of Chemical Ecology 22:* 1767-1781
262. Thomsen J., 1988. Feeding behavior of Eriophyes tiliae tiliae Pgst. and suction track in the nutritive cells of the galls caused by mites. *Entomologia Mediteranea 56(2)*:73–78

263. Tian D., Tooker J., Peiffer M., Chung S.H., Felton G.W., 2012. Role of trichomes in defense against herbivores: comparison of herbivore response to woolly and hairless trichome mutants in tomato (Solanum lycopersicum). *Planta* 236(4):1053-66
264. Tingey W.M., 1984. Glycoalkaloids as pest resistance factors. *American Potato Journal* 61:157–167.
265. Tjallingii W.F., 2005. Salivary secretions by aphids interacting with proteins of phloem wound responses. *Journal of Experimental Botany 57(4):*739–745
266. Trottin-Caudal Y., Fournier C., Leyre J.M., 2003. Biological control of Aculops lycopersici (Massee) using the predatory mites Neoseiulus californicus McGregor and Neoseiulus cucumeris (Oudemans) on tomato greenhouse crops. In: Proceedings, international symposium on Greenhouse Tomato: integrated crop protection and organic production, 17–19 September 2003. Avignon, France.153–157 pp
267. Turlings T.C.J., Tumlinson J.H., Heath R.R., Proveaux A.T., Doolittle R.E., 1991. Isolation and identification of allelochemicals that attract the larval parasitoid, Cotesia marginiventris (Cresson), to the microhabitat of one of its hosts. *Journal of Chemical Ecology* 17:2235–2251
268. Turlings T.C.J., Wäckers F.L., 2004. Recruitment of predators and parasitoids by herbivore-injured plants. In Advances in insect chemical ecology (eds. Cardé RT, Millar JG). Cambridge University Press, Cambridge. 21–75 pp.
269. Van Dam N.M., Lucienne W.M., Bergshoeff V.C., Van der Meijden E., 1995. The "raison d'etre" of pyrrolizidine alkaloids in Cynoglossum officinale: deterrent effects against generalist hervibores. *Journal of Chemical Ecology 21*:507-523
270. Van der Putten W.H., Vet L.E.M., Harvey J.A., Wäckers F.L., 2001. Linking above- and below-ground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. *Trends in Ecology and Evolution 16*:547–554
271. van Houten Y.M., Glas J.J., Hoogerbrugge H., Rothe J., Bolckmans K.J.F., Simoni S., van Arkel J., Alba J.M., Kant M.R., Sabelis M.W., 2013. Herbivore-associated degradation of tomato trichomes and its impact on biological control of *Aculops lycopersici*. Experiemntal and Applied Acarology 60 (2):127-138.
272. Van Leeuwen T., Vanholme B., Van Pottelberge S., Van Nieuwenhuyse P., Nauen R., Tirry L., Denholm I., 2008. Mitochondrial heteroplasmy and the evolution of insecticide resistance: non-Mendelian inheritance in action. *Proceedings of National Academy of Sciences of USA* 105: 5980-5985
273. Van Leeuwen T., Vontas J., Tsagkarakou A., Dermauw W., Tirry L., 2010. Acaricide resistance mechanisms in the two-spotted spider mite Tetranychus urticae and other important Acari: a review. Insect Biochemistry and Molecular Biology 40:563–572
274. Van Tol R.W.H.M., Van der Sommen A.T.C., Boff M.I., Van Bezooijen J., Sabelis M.W., Smits P.H., 2001. Plants protect their roots by alerting the enemies of grubs. *Ecology Letters 4*: 292-294
275. van Wijk M., De Bruijn P.J.A., Sabelis M.W., 2008. Predatory mite attraction to herbivore-induced plant odors is not a consequence of attraction to individual herbivore-induced plant volatiles. *Journal of Chemical Ecology* 34:791–803
276. Vick B.A., Zimmerman D.C., 1984. Biosynthesis of jasmonic acid by several plant species. *Plant physiology 75*: 458–461
277. Voelckel C., Weisser W.W., Baldwin I.T., 2004. An analysis of plant-aphid interactions by different microarray hybridization strategies. *Molecular Ecology 13*: 3187–3195
278. Von Dahl C.C., Baldwin I.T., 2007. Deciphering the role of ethylene in plant-herbivore interactions. *Journal of Plant Growth Regulation 26*: 201–209
279. Vos M., Moreno Berrocal S., Karamaouna F., Hemerik L., Vet L.E.M., 2001. Plant-mediated indirect effects and the persistence of parasitoid-herbivore communities. Ecology Letters 4:38–45
280. Walling L.L., 2000.The myriad plant responses to herbivores. *Journal Plant Growth Regulation 19*:195–216
281. Walters D., Newton A., Lyon G., 2007. Induced Resistance for Plant Defence: A Sustainable Approach to Crop Protection. Blackwell, Oxford. 272 p

282. Westphal E., Bronner R., Dreger F., 1996 .Host plant resistance. In: Lindquist E.E., Sabelis M.W., Bruin J., (eds) Eriophyoid Mites: their biology, natural enemies and control. Elsevier, Amsterdam, 681–688 pp.
283. Westphal E., Bronner R., Le Ret M., 1981. Changes in leaves of susceptible and resistant Solnum dulcamara infested by the gall mite Eriophyes cladophthirus (Acarina: Eriophyoidea). *Canadian Journal of Botany 59*:875–882
284. Westphal E., Dreger F., Bronner R., 1991. Induced resistance in Solanum dulcamara triggered by the gall mite Aceria cladophthirus (Acari: Eriophyoidea). *Experiemntal and Applied Acarology 12*:111–118
285. Westphal E., Manson D.C.M., 1996. Feeding effects on host plants: gall formation and other distorsions. In: Lindquist E.E., Sabelis M.W., Bruin J., (eds) Eriophyoid Mites–their biology, natural enemies and control. Elsevier, Amsterdam 231–250 pp.
286. Westphal E., Minnot M.J.P., Kreiter S., Gutierrez J., 1990. Hypersensitive reaction of Solanum dulcamara to the gall mite Aceria cladophthirus causes an increased susceptibility to Tetranychus urticae. *Experiemntal and Applied Acarology* 15:15–26
287. Williams W.G, Kennedy G.G, Thacker J.D., Bordner J., 1980. 2-tridecanone: a naturally occurring insecticide from the wild tomato *Lycopersicon hirsutum* f. *glabratum*. *Science 62*: 1427-1431
288. Wiseman B.R., Carpenter J.E., 1995. Growth inhibition of corn earworm (Lepidoptera: Noctuidae) larvae reared on resistant corn silk diets. *Journal of Economic Entomology 88*: 1037–1043
289. Yan L., Zhai Q., Wei J., Li S., Wang B., Huang T., Du M., Sun J., Kang L., Li C.B., Li C., 2013. Role of Tomato Lipoxygenase D in Wound-Induced Jasmonate Biosynthesis and Plant Immunity to Insect Herbivores. *PLoS Genetics 9(12*): e1003964
290. Yan Z.G., Wang C.Z., 2006. Similar attractiveness of maize volatiles induced by *Helicoverpa armigera*and *Pseudaletia separata* to the generalist parasitoid *Campoletis chlorideae. Entomologia Experimentalis et Applicata 118(2):*87-96
291. Yang X., Margolies D.C., Zhu K.Y., Buschman L.L., 2001. Host Plant-Induced Changes in Detoxification Enzymes and Susceptibility to Pesticides in the Twospotted Spider Mite (Acari: Tetranychidae). *Journal of Economic Entomology 94(2):*381-387
292. Zakir A., Sadek M.M., Bengtsson M., Hansson B.S., Witzgall P., Anderson P., 2013. Herbivore-induced plant volatiles provide associational resistance against an ovipositing herbivore. *Journal of Ecology 101(2):*410-417
293. Zarate S.I., Kempema L.A., Walling L.L. 2007. Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. *Plant Physiol* 143:866–875.
294. Zhang P.J., Zheng S.J., Van Loon J.J.A., Boland W., David A., Dick M., 2009. Whiteflies interfere with indirect plant defense against spider mites in Lima bean. *PNAS 106(50)*: 21202-21207
295. Zhang Z.Q., Sanderson J., 1995. Two-spotted spider mite (Acari: Tetranychidae) and Phytoseiulus persimilis (Acari: Phytoseiidae) on greenhouse roses: spatial distribution and predator efficacy. *Journal of Economic Entomology 88*: 352-57