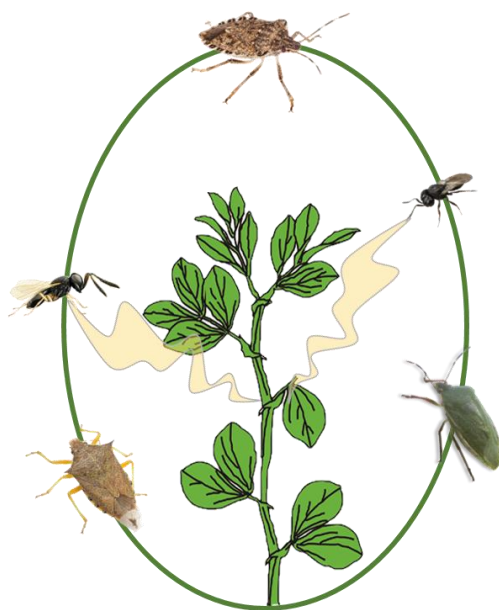UNIVERSITÀ DEGLI STUDI DI PALERMO

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Dipartimento di Scienze Agrarie, Alimentari e Forestali
AGR/11 Entomologia generale e Applicata

Impact of an invasive pest in local semiochemical networks

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Table of contents

Chapter 3

Chapter 4

Abstract

During the host location process, egg parasitoids rely on a series of chemical stimuli from the host/plant complex. In particular, in tritrophic systems consisting of plants, herbivorous stink bugs and their egg parasitoids, previous studies demonstrated that wasps are attracted by oviposition-induced plant volatiles (OIPVs). These compounds act as indirect plant defence mechanism allowing plants to recruit egg parasitoids, which are in general the most important natural enemies of stink bugs. Moreover, when wasps land on a plant, they can exploit chemical footprints left by male and female stink bugs walking on the leaves as indirect host-related cues, since chemicals lead them in an area where newly laid host eggs are likely to be found. Finally, since host female chemical footprints are the most promising signals of host eggs, wasps evolved the ability to distinguish between footprints left by females and males of their associated hosts. These foraging behaviours are adopted by egg parasitoids searching for native and associated hosts, but invasive insect herbivores can interfere with local multitrophic interactions by affecting the chemical cues emitted by plants and disrupting the attraction of egg parasitoids.

In this thesis the potential impact of the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae) on local infochemical networks at long and short range was evaluated. *Halyomorpha halys* is a species native to Asia, which was recently introduced in the Europe and North America, and to evaluate the effect of its establishment, laboratory experiments were conducted using two local tritriphoc systems, in Europe, the one consisting of *Vicia faba* L. - *Nezara viridula* (L.) (Heteroptera: Pentatomidae) - *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae), and in North America, the one consisting of *V. faba* - *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae) - *Telenomus podisi* (Ashmead) (Hymenoptera: Scelionidae). The results showed that both egg parasitoid species, *T. basalis* and *T. podisi*, were attracted by the OIPVs of their associated hosts, *N. viridula and P. maculiventris*, respectively, but not by the OIPVs of non-associated host *H. halys*. However, in both systems, the parasitoid attraction toward OIPVs emitted following the attack of the associated host was disrupted when host and non-associated host were concurrently present on the same plant. Concerning the substrate-born cues, *T. basalis* females (naïve or rewarded by an oviposition experience) ability to discriminate between female and male of *H. halys* were tested. Naïve *T. basalis* wasps did not discriminate between areas contaminated by chemical footprints left by the non-associated host female or male on the contrary experienced female of *T. basalis*, re-encountering after 1 hour, discriminated between male

and female chemical footprints but they were not able to discriminate *H. halys* gender after 24 hours. This indicates that invasion by an alien herbivore can interfere with local established semiochemical webs. Overall the outcomes are discussed by evaluating the possible consequences of alien insect spread on local parasitoid foraging behaviour.

Riassunto

Durante il processo di localizzazione dell'ospite i parassitoidi oofagi si affidano a una serie di segnali chimici provenienti dal complesso pianta/ospite. In particolare studi precedenti hanno dimostrato che nei sistemi tri-trofici costituiti da piante coltivate, insetti fitofagi e i loro parassitoidi oofagi associati, questi ultimi sono attratti dagli "oviposition induced plant volatile" (OIPVs). Questi composti agiscono come meccanismo indiretto di difesa delle piante per attrarre i parassitoidi oofagi, che sono in generale tra i più importanti antagonisti degli insetti fitofagi tra cui i Pentatomidi. Inoltre quando i parassitoidi riescono ad arrivare sulla pianta sfruttano le tracce chimiche lasciate sulle foglie dagli ospiti di entrambi i sessi per essere guidati nelle zone dove è più probabile che trovino le uova dell'ospite. Infine poiché le tracce appartenenti alle femmine dell'ospite sono i segnali più promettenti per localizzare l'ovatura i parassitoidi oofagi hanno evoluto la capacità di distinguere tra le tracce lasciate dai maschi e delle femmine dei loro ospiti. Questo comportamento di foraggiamento è adottato dai parassitoidi per la ricerca degli ospiti associati, ma cosa accadrebbe se un insetto alieno erbivoro entrasse a fare parte di questo network infochimico? Per valutare il possibile impatto su di un sistema tri-trofico locale sia a breve che a lunga distanza, della cimice asiatica *Haliomorpha halys* (Stål) (Heteroptera: Pentatomidae), una specie nativa dell'Asia che recentemente è stata introdotta nel vecchio e nel nuovo mondo sono stati condotti esperimenti in condizione di laboratorio su due sistemi, uno Europeo costituito da *Vicia faba* L. - *Nezara viridula* (L.) (Heteroptera: Pentatomidae) - *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae), e l'altro tipico del Nord America costituito da *V. faba* - *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae) - *Telenomus podisi* (Ashmead) (Hymenoptera: Scelionidae). I risultati hanno evidenziato che entrambi i parassitoidi *T. basalis* e *T. podisi* sono stati attratti dagli OIPVs indotti dall'azione di ovideposizione dei loro ospiti associati, rispettivamente *N. viridula* e *P. maculiventris,* ma non dagli OIPVs indotti dall'ospite non associato *H. halys*. In entrambi i sistemi tritrofici l'attrazione verso gli OIPVs indotti dai rispettivi ospiti associati *N. viridula* e *P. maculiventris* è risultata essere interrotta quando l'ospite associato e l'ospite non associato infestavano contemporaneamente la stessa pianta. Questo indica che l'invasione da parte di un insetto fitofago alieno interferisce con la ben stabilita rete semiochimica locale. Per quanto riguarda le tracce chimiche lasciate sul substrato dall'insetto non associato, è stata testata l'abilità delle femmine di *T. basalis* (con o senza precedente esperienza di esposizione a ovature) di distinguere il genere sessuale di *H.*

halys. Le femmine di *T. basalis* senza esperienza non sono state in grado di discriminare tra le aree contaminate dalle tracce dei maschi e quelle delle femmine di *H. halys*, al contrario le femmine con esperienza sono state in grado di discriminare il genere dell'ospite non associato, tuttavia tale abilità è di breve durata, in quanto l'effetto dell'esperienza svanisce dopo ventiquattro ore.

Chapter 1

1.1 Introduction

Female egg-parasitoids live in highly complex environments, thus they are continuously exposed to several information involving visual, auditory, physical, gustative and olfactory cues through which, by using successive behavioural steps, regulate the ability of the wasp females to locate and attack the host (Godfray 1994; Vinson 1998; Ode 2013; Wäschke et al. 2013). During the host location process wasp females explore these great variety of stimuli, at long and short-range, among which play a relevant role semiochemicals, i.e the chemical cues that mediate the interactions among organisms at intraspecific (pheromones) or inter-specific level (allelochemicals) (Dicke and Grostal 2001; Wyatt 2003). Moreover, allelochemicals are classified into different categories depending on whether the benefit is for the emitter or the receiver: allomones are favourable to the organism that emits the substance, kairomones are favourable to the receiver and synomones are favourable to both the organisms (Dicke et al. 1990; Meiners and Peri 2013). To orientate through the semiochemicals the parasitoids can adopt different strategies based on stimuli exploitation explained by the reliability-detectability theory (Vet and Dicke 1992). Wasp female are under selection pressure, the time that can adopt for location and exploitation of these plethora of stimuli is limited, for this reason female parasitoids need to optimize exploitation of available cues and discriminate those most reliable in indicating the presence of a suitable host (Hilker and McNeil 2007). However, the location and recognition of a suitable host is a complex process, especially for egg parasitoids, because exist different constraints including small sizes of both the host and the parasitoid itself. Eggs are usually unapparent, especially when they are small, dispersed in the habitat, and concealed in plant tissue. As such, cues that are directly related to the presence of eggs may have high reliability, but low detectability (Vinson 1994; Vet et al. 1995). Additionally, suitable host eggs are generally available for only a short time due to their rapid development (Vinson 1998). Wasp female can use for a successful parasitism cues originated from the host eggs or not and termed "direct host-related cues" and "indirect host related cues" respectively (Fatouros et al. 2008; Colazza et al. 2010). To get through the reliability-detectability dilemma, wasp females can adopt three different strategies

based on the exploitation of either cues. First, parasitoids may detect volatiles from non target instars of the host, that is, adults or juveniles, to reach the vicinity of the host eggs eventually enabling them to the point where are present the eggs using additional long- and/or short-range cues (Vet and Dicke 1992). Second, parasitoids may exploit plant volatiles induced as a consequence of herbivory, which are emitted in large quantities and are, therefore, easily detectable by foraging parasitoids but not necessarily highly reliable (Fatouros et al. 2008). Third, egg parasitoids have been observed to associate, through learning, highly detectable but less reliable cues with the presence of suitable hosts, thus increasing reliability of such cues in experienced wasp females (Peri et al. 2006; Dauphin et al. 2009). An example of indirect host-related cues are plant synomones induced by oviposition and/or feeding activity of *Nezara viridula* (L.) (Heteroptera: Pentatomidae) exploited by its associated egg parasitoid *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) (Colazza et al. 2004a; Conti and Colazza 2012). In a hierarchical context, whether volatiles from host adults or from the host plants of host adults are exploited from the furthest distance has yet to be elucidated. In the case of *T. basalis*, because host oviposition is necessary for volatile induction in bean plants, such synomones appear more reliable compared to kairomones from non-target instars or to feeding-induced synomones (Colazza et al. 2004b). Therefore, it can be hypothesized that kairomones from adults and feeding and oviposition activity-induced plant synomones act as a long-distance, indirect cue used to localize the host community, whereas oviposition-induced plant synomones are shorter-range cues used to find plants that actually have host eggs (Conti et al. 2010). While land on a plant, wasp female searching for the host eggs on the leaf, using kairomones left behind from the host. Indeed, the stink bugs that move on a plant cuticle leave chemical footprints that are exploited by egg-parasitoids to short distance (Colazza et al. 1999; Salerno et al. 2006, Frati et al. 2017). Once in contact with host chemical footprints, which are perceived through gustatory sensilla (Colazza et al. 2009; Conti et al. 2012), female egg-parasitoids evidence an innate arrestment response characterized by an intense searching behaviour on host-contaminated areas (Colazza et al. 1999; Peri et al. 2006). These cues are not directly related to the host eggs, but become quite reliable following the finely tuned adaptations of the egg parasitoid. The arrestment behaviour is a step of the host location strategy commonly observed in several host-egg parasitoid associations such as *N. viridula* – *T. basalis*, *Murgantia histrionica* (Hahn) (Heteroptera: Pentatomidae) – *Trissolcus brochymenae* (Ashmead) (Hymenoptera: Scelionidae), and *Euschitus heros* (F.) (Heteroptera: Pentatomidae) – *Telenomus podisi* (Ashmead)

(Hymenoptera: Scelionidae). Wasp females respond to footprints left by immature and adult stages, but with a clear preference for those left by adult females (Colazza et al. 1999, Conti et al. 2003). Moreover, it was demonstrated that *T. basalis* and *T. brochymenae* are able to distinguish and prefer footprints left by virgin females or by mated females (Colazza et al. 1999, 2007). Overall, these kairomones allow the parasitoids to optimize their searching behaviour and to distinguish associated from non-associated hosts (Salerno et al. 2006; Peri et al. 2016). Interestingly, in addition to chemical footprints, *T. podisi* also uses vibratory signals mediating sexual behaviour of the host species, *E. heros* (Borges et al. 1999). Finally, last step of the host process is the host recognition, which is mediated by contact chemicals cues. In *Trissolcus* species it is elicited by contact kairomones present on the egg surface, although physical factors such as shape and size could also affect wasp behaviour (Bin et al. 1993; Conti et al. 2003).

Several studies have investigated the role of the indirect host related cues on tri-trophic systems in a laboratory condition, including a host plant, a herbivore species and its associated parasitoid species (Hunter 2002; De Rijk et al. 2013; Gols 2014). It is well known that insect damage induces the emission of volatile organic compound (VOCs,) acting as indirect defence mechanism, since they are exploited by wasp females to find their hosts in the most efficient way (Meiners and Hilker 1997; Meiners and Hilker 2000; Hilker et al. 2002; Colazza et al. 2004ab; Fatouros et al. 2008, 2009). These VOCs emitted after the feeding activities are known as herbivore-induced plant volatiles (HIPVs) while those induced by egg-deposition activities are known as oviposition-induced plant volatiles (OIPVs) (Hilker and Fatouros 2015). The systems used for the bioassay in laboratory condition may differ from those present in the field conditions as plants are often attacked by several herbivore species and thus are involved in multi-trophic interactions (Moujead et al. 2014; Cusumano et al. 2015; Ponzio et al. 2015, 2017). Furthermore, these multi-trophic systems may be much more complex if there are involved a local and an alien herbivore insect (Dousermont et al. 2018). Overall, alien herbivores may affect several aspects of info-chemical and behavioural ecology of local natural enemies, such as parasitoid learning ability, foraging behaviour, patch exploitation strategy and contest behaviour, as consequence of the modifications of HIPVs and/or OIPVs linked to the lack of plant-herbivore co-evolution (Dicke et al. 2009; Desurmont et al. 2014). Data from the literature (e.g. Moujead et al. 2014; Cusumano et al. 2015) suggest that, under multiple herbivore attack, the emission of induced volatile blends can be altered in a specific manner that is dependent on the insect feeding guild (biting–chewing or piercing–sucking),

plant organ attacked (root damage or leaf damage) and herbivore density, among others (de Rijk et al. 2013; Meiners 2015). In the case of multiple herbivore attack for instance, Dusermont et al. (2014) found that the presence of the exotic herbivore *Spodoptera littoralis* (Boisd) (Lepidoptera: Noctuidae) on *Brassica rapa* L. plants infested by the native herbivore *Pieris brassicae* (L.) (Lepidoptera: Pieridae)*,* makes the plants with the concurrent infestation unattractive to the main parasitoid of *P. brassicae*, the braconid wasp *Cotesia glomerata* (L.) (Hymenoptera: Braconidae). The presence of the alien insect likely disrupted the host attraction of egg-parasitoid as a consequence of altered volatile emissions when plants are simultaneously exposed to herbivore species (Zhang et al. 2009; Dusermont et al. 2014, 2018). Since, invasive herbivores affecting the behaviour of local natural enemies have a big impact in terms of biological control efficiency it is interesting to explore the foraging behaviour of local parasitoids in a multi-trophic context which involve simultaneously a local and an alien insect herbivore.

In this view, the aim of this thesis project was understand the impact of an alien herbivore invasion on local info-chemical communication at long and short range among plants, herbivores and natural enemies, considering that when an alien species invades a new area, it interacts with local species and can modify the structure of local food webs and the strength of existing links with effects cascading through trophic levels.

In laboratory conditions, were observed the impact of the alien insect *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae), on the long range cues, with bioassay on plant volatile-mediated signalling in two tri-trophic webs, one in Europe consisting of *V. faba – N. viridula – T. basalis* and one in North America represented by *V. faba - Podisus maculiventris* (Say) (Heteroptera: Pentatomidae) *– T. podisi*. Furthermore for investigate the effect of an alien insect on the short-range cues exploited by a local egg parasitoid, *T. basalis* wasp females (naïve or rewarded by an oviposition experience) ability to discriminate between female and male *H. halys* chemical footprints were investigated. Finally, host acceptance and host preference between *N. viridula* (associated host) and *H. halys* (non-associated host) egg masses were observed. The outcomes were discussed by evaluating the possible ecological consequences of alien insect spread on local parasitoid foraging behaviour.

1.2 Study systems

1.2.1 Plant species

Vicia faba L., commonly known as faba bean or broad bean, is widely cultivated for its nutritious seeds and pods, which are consumed, by millions of people throughout the world and is a host plant for many herbivorous insects (Cubero 1973). *Vicia faba*, recognizes early infestation by phytophagous insect, and it is able to activate physiological and chemical processes that function as defence (Rondoni et al. 2018). The VOCs emitted from *V. faba* plant after an herbivorous attack are involved in the indirect defence mechanism and are deeply investigated in the tri-trophic system composed by *V. faba* plant, the herbivorous pentatomid *N. viridula* and the egg parasitoid *T. basalis. Vicia faba* plants showed quantitative differences in their odor blends when *N. viridula* feeding activity was combined with oviposition on leaf surfaces, in particular, the plants emitted more (E)-β-caryophyllene in response to the deposition of egg masses (Colazza et al. 1999; Colazza et al. 2004a,b). Furthermore, Frati et al. (2017) detected 23 organic compounds from plant of *V. faba* treated with *N. viridula* walking on adaxial leaf surface from plants with *N. viridula* walking on abaxial leaf surface and plants with *N. viridula* feeding and oviposition activity on abaxial or adaxial leaf surface. In particular, the volatile blend emitted by plants with footprints left on abaxial leaf surface is different from that emitted by plants with footprints left on adaxial leaf surface and from plants without any treatments.

1.2.2 Phytophagous insect

Nezara viridula (**L.**) (**Heteroptera Pentatomidae**), probably originated in the Ethiopian region of eastern of Africa, is one of the most important pentatomid insect pests in the world. It is cosmopolitan and highly polyphagous on many important food crops (Todd 1989; Panizzi 2000). Its host range includes over 30 families of dicotyledonous plants, although it has strong preference for leguminoseae and solanaceae such as soybeen, bean, tomato (Todd 1989). *Nezara viridula* has a complex communication system between sexes and uses both chemical and vibratory signals. Behavioural studies revealed that males of *N. viridula* use vibrational song emitted by females to find them on a host plant. A stationary female continuously emits a female calling song (FCS), which triggers male

searching behaviour and also provides directional cues (Prešern et al. 2018). Furthermore, volatile collections from males or cuticular extracts revealed the presence of five compounds produced by males (Brezot et al. 1994; Miklas et al. 2000), in fact sexually mature males produce a pheromone blend that plays a role in long-range location for attracting female bugs (Brezot et al. 1994). Laboratory experiments showed that *N. viridula* releases volatiles that attract female of the egg-parasitoid *T. basalis*, the main compound identified was the (E)-2-decenal, a kairomone associated with the metathoracic glands of the adults (Mattiacci et al. 1993). Furthermore Colazza et al. (2004) demonstrated that the combination of feeding and oviposition activity of *N. viridula* adults are the principal responsabiles for the induction of OIPVs by been plants for attracting its associated egg-parasitoid *T. basalis*.

***Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae)**, native to Asia, is an invasive pest with a broad host-plant range (Rice et al. 2014). It has a worldwide interest for its rapid diffusion and for the substantial economic loses that had caused (Leskey et al. 2012; Morrison et al. 2018). *Halyomorpha halys* has recently spread to several areas in Europe, with established populations in Switzerland, France, Greece and Hungary (Nielsen 2018). The first detection in Italy occurred in the north, in Emilia Romagna region, in 2012 (Cesari et al. 2015, 2018); established breeding populations were detected in 2013 in the same region and also in Lombardy, damages were recorded also in Piedmont (Pansa et al. 2013). The Italian climate, with suitable average temperatures, together with the abundance and high diversity of crops is an excellent condition for the establishment of large populations of *H. halys* throughout the country from the north to the south (Rice at al. 2014; Maistrello et al. 2017). The ecological impact of *H. halys* could have serious consequences on native ecosystems (Kenis et al. 2009; Simberloff et al. 2013). *Halyomorpha halys* was also regarded as a nuisance pest because large numbers frequently invade human dwellings and commercial structures to overwinter (Inkley 2012, Leskey et al. 2012). In its native range, the most important egg parasitoids of *H. halys* are *Trissolcus* species (Avila and Charles 2018; Stahl et al. 2018a). Although some North American and European *Trissolcus* wasps were found in fresh eggs sentinel of *H. halys*, few were able to successfully develop (Haye et al. 2015; Zhang et al. 2017)). In Europe, *Anastatus bifasciatus* (Geoffroy) (Hymenoptera: Eupelmidae) is among the few native egg parasitoid species capable of developing on viable *H. halys* eggs (Haye et al. 2015; Roversi et al.

2016; Abram et al. 2017). In Switzerland and Italy, it is the most abundant native parasitoid reared from sentinel *H. halys* eggs (Haye et al. 2015, Roversi et al. 2016; Costi et al. 2018) and thus, it was selected as potential candidate for biological control in Europe (Sthal et al 2018b).

1.2.3 Zoophytophagous insect

Podisus maculiventris **(Say) (Heteroptera: Pentatomidae)** is a zoophitophagous insect endemic to North America; it know biological control agent able to kill insect pests to many agricultural, horticultural and ornamental plants. *Podisus maculiventris*, the spined soldier bug, is a predator of several agricultural and forest pests in North America (McPherson 1980, 1982), such as the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) (Biever and Chauvin 1992; Hough-Goldstein and McPherson 1996), and the Mexican bean beetle, *Epilachna varivestis* Mulsant (Coleoptera: Chrysomelidae) (Sant'Ana et al. 1997). When preys are scarce, *P. maculiventris* can feed on plants, but this feeding activity normally does not resolve in crop injury (Ruberson et al. 1986). The aggregation pheromone of *P. maculiventris* has been identified, synthesized, and is commercially available (Aldrich 1988). Techniques were developed to utilize the synthetic pheromone to promote augmentation and conservation of this predator (Sant'Ana et al. 1998; Aldrich 1999). The eggs of *P. maculiventris* are attaked by some scelionids wasp among which: *T. podisi*, *Trissolcus euschisti* (Ashmead) (Hymenoptera: Scelionidae) (Yeargan 1979; Okuda and Yeargan 1988), and *Telenomus calvus* Johnson (Hymenoptera: Scelionidae) (Aldrich et al. 1988; Orr et al. 1986). For instance, Bruni et al. (1999) demonstrated that females of *T. calvus* were attracted by the pheromone of *P. maculiventris* and they used it to facilitate their phoretic behaviour. On the contrary, *T. podisi* and *T. euschisti* do not seem to use this predator's attractant pheromone as a kairomone, evidently these generalist parasitoids rely on other cues to locate host eggs (Bruni et al. 1999).

Podisus maculiventris is highly mobile predator even during the early instars when preys are scarce, it may feed on plant juices (Orr 1988). Moreover, study conduct in laboratory condition showed that *P. maculiventris* reared on larvae and water (in the absence of plants) suffered high mortality and reduced developmental rates (Drummon 1984). Furthermore, Rubenson et al. (1986) demonstrated that the addition of potato foliage to the

nymphal diet of *P. maculiventris* significantly increased the size of both males and females and decreased the length of the preoviposition period. Finally, Coudron et al. (2002) demonstrated that a zoophytophagous diet appears to have good potential for the artificial rearing of *P. maculiventris* and should constitute a good starting formulation for rearing a colony.

1.2.4 Parasitoid insects

***Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae)** is a solitary egg parasitoid that attacks eggs of several phytophagous pentatomid bugs and it is now distributed worldwide as a result of extensive deliberate introductions for use as a biological control agent for *N. viridula* (Jonesm1988; Clarke 1990). During the host location process *T. basalis* females explore a set of volatile and contact cues from the host-plant complex. The system *T. basalis - N. viridula* was deeply investigated. Studies have shown that the wasp female was attracted by oviposition of *N. viridula* females on bean plants combined with feeding punctures, specifically it is attracted by (*E*)-*β*-caryophyllene (Colazza et al. 1999; Colazza et al 2004 a,b; Frati et al. 2017; Salerno et al. 2017). Furthermore, wasp females of *T. basalis* are able to discriminate chemical footprints left by *N. viridula* females, to which they respond more strongly than to chemical traces left by walking males or nymphs. Indeed, Colazza et al. (2007) revealed that *n*-nonadecane (n-C_{19}), sex-specific cuticular hydrocarbon from *N. viridula* males, allows *T. basalis* females to distinguish between male or female host chemical footprints.

***Telenomus podisi* (Ashmead) (Hymenoptera: Scelionidae)**, has been demonstrated to be a suitable biological control agent in a herbaceous plant environment (Okuda and Yeargan 1988; Orr 1988); it is a common egg parasitoid of several pentatomid species in North America, in the field it attacks also the eggs of the zoophytophagous *P. maculiventris* (Yeargan 1982; Orr et al. 1986; Koppel et al. 2009; Laumman et al. 2009). In the literature, is reported that *T. podisi* respond to chemical cues, for istance HIPVs and also vibratory signals of the host species, *Eschistus heros* (F.) (Heteroptera: Pentatomidae) (Borges and Aldrich 1994; Borges et al. 2003; Moraes et al. 2005; Laumann et al. 2009; Michereff et al.

2011, 2013, 2016). Like *T. basalis* also wasp females of *T. podisi* were attracted by metathoracic scent gland secretions of its associated host *E. heros,* as they specifically responded to (*E*)-2-hexenal and 4-oxo-(*E*)-2-hexenal (Conti and Colazza 2012). Furthermore, in the case of *T. podisi*, Moraes et al. (2005) demonstrated the wasp's attractiveness to volatiles emitted by two Leguminosae plants, soybean, (*Glycine max*) and pigeon pea (*Cajanus cajan*) damaged by feeding activity of phytophagous bug *E. heros*. However, this attraction does not occur when the plants are exposed to oviposition and feeding activities of *E. heros* due to the particular behaviour of the host. Indeed *E. heros* prefers to oviposit on plants previously damaged by feeding of conspecifics, therefore, the emission of HIPVs optimizes the wasp recruitment, as they indicate to the *T. podisi* females the probable presence of host females ready to oviposit (Moraes et al. 2008).

1.3 Outline of this thesis

Chapter 2 addresses the impact of an alien infestation by the herbivorous insect *H. halys* on a European local tri-trophic system made by *V. faba. - N. viridula -T. basalis*. Responses of *T. basalis* females to volatile emitted from *V. faba* plants infested by *H. halys*, and by concurrent infestation of the alien insect *H. halys* and the local insect *N. viridula* were investigated. This study conducted at the University of Palermo investigated the response of the naïve wasp females to the treated plants in a dual-choice (Y-tube) olfactometer. The goal was to understand if the presence of the alien insect in a concurrent infestation with a local pest affected the foraging behaviour of a local egg-parasitoid.

Chapter 3 focuses on a Canadian tri-trophic system composed by the bean plant *V. faba*, the zoophytophagus *P. maculiventris*, the egg-parasitoid *T. podisi* and the impact of the alien insect *H. halys* on this local net. Laboratory bioassays were conducted in North America at the University of Montréal using a Y-tube olfactometer in order to understand if plants damaged by the zoophytophagus *P. maculiventris* release HIPVs or OIPVs to recruit the egg-parasitoid *T. podisi*. Furthermore, to assess if the presence of an alien insect affect the foraging behaviour of *T. podisi* the attraction of the indigenous egg parasitoid toward plant volatiles induced by the alien phytophagous *H. halys,* alone or in concurrent infestation with *P. maculiventris* were evaluated.

Chapter 4 examines, in laboratory condition, the foraging behaviours adopted by female parasitoids searching for chemical traces left by native and associated hosts when also an alien herbivore invades these info-chemical networks. In particular the ability of *T. basalis* females (naïve or rewarded by an oviposition experience) to discriminate between female and male of *H. halys* chemical footprints was investigated; moreover, host suitability using fresh egg masses and host preference between *N. viridula* (associated host) and *H. halys* (non-associated host) were observed in choice-test.

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Chapter 2

An invasive insect herbivore disrupts plant volatile-mediated tritrophic signalling[*]

2.1 Abstract

Plants respond to insect attack by emission of volatile organic compounds, which recruit natural enemies of the attacking herbivore, constituting an indirect plant defence strategy. In this context, the egg parasitoid *Trissolcus basalis* is attracted by oviposition-induced plant volatiles emitted by *Vicia faba* plants as a consequence of feeding and oviposition by the pentatomid host *Nezara viridula*. However, this local tritrophic web could be affected by the recent invasion by the alien pentatomid bug *Halyomorpha halys,* an herbivore that shares the same environments as native pentatomid pests. Therefore, we investigated in laboratory conditions the possible impact of *H. halys* on the plant volatile-mediated signalling in the local tritrophic web *V. faba – N. viridula –T. basalis*. We found that *T. basalis* wasps were not attracted by volatiles induced in the plants by feeding and oviposition activities of *H. halys,* indicating specificity in the wasps' response. However, the parasitoid attraction towards plant volatiles emitted as a consequence of feeding and oviposition by the associated host was disrupted when host, *N. viridula*, and non-associated host, *H. halys,* were concurrently present on the same plant, indicating that invasion by the alien herbivore interferes with established semiochemical webs. These outcomes are discussed in a context of multiple herbivory by evaluating the possible influences of alien insects on local parasitoid foraging behaviour.

2.2 Introduction

The reproductive success of insect parasitoids and their efficacy in controlling herbivorous insect pest populations in biological control programmes is closely related to their ability to locate hosts at the suitable stage (Pickett and Khan 2016; Kaiser et al. 2016). In field conditions, the inflight host-searching ability of insect parasitoids is largely based on chemical cues, among which the volatile organic compounds (VOCs) emitted by plants in response to herbivorous insect activities play a central role (Dicke 2016; Kaiser et al. 2017). It is now well established that the main insect activities that induce VOCs are feeding (herbivore induced plant volatiles, HIPVs) or egg deposition (oviposition- induced plant volatiles, OIPVs) (Hilker and Fatouros 2015; Pashalidou et al. 2015). From the plant perspective, the emission of VOCs that recruit natural enemies represents an indirect defence strategy, as these volatiles do not have direct impact on the attacking herbivore. Specifically, OIPVs are able to attract egg parasitoids that might improve plant fitness, as the herbivorous insect can be killed before plant damage occurs (Pierik et al. 2014; Fatouros et al. 2016). In a natural environment, plants are subject to stress by various herbivorous insects and, as a result, the plant's phenotype changes significantly reflecting differences in blends of the volatiles they produce (Moayeri et al. 2007; Dicke 2016). Alteration of induced volatile blends in plants under multiple and simultaneous herbivore attack may depend, among several factors, on the insect feeding habits (chewing, piercing or sucking), the plant organ attacked (above- or belowground) or the strength of the herbivore damage (herbivore density, timing and location of the different attackers) (De Rijk et al. 2013; Ponzio et al. 2014; Kroes et al. 2015). In this context, the colonization of a new environment by an alien herbivore could interfere with the plant indirect defences due to the lack of plant–herbivore coevolution (Desurmont et al. 2014). The attack of alien herbivores could influence parasitoid foraging behaviour in different ways, for example, through direct attraction of parasitoids towards infested plants, or modification of normal attraction towards plants with concurrent infestation with local host herbivore.

Studies focused on multiple attacks are rather limited and most consider only endemic herbivores. The females of the egg parasitoid *Trissolcus basalis* (Wollaston) (Hymenoptera: Platygastridae) are attracted by OIPVs emitted by *Vicia faba* L. plants as a consequence of feeding and oviposition by *Nezara viridula* (L.) (Heteroptera: Pentatomidae) (Colazza et al. 2004a, b). Recent observations demonstrate that this infochemical network is affected by biotic and abiotic stress. In fact, the ability of *T.*

basalis females to exploit *V. faba* OIPVs is disrupted when the plants are simultaneously infested by *N. viridula* and the non-host, *Sitona lineatus* (L.) (Coleoptera: Curculionidae) (Moujahed et al. 2014), or is enhanced when *N. viridula* attack plants that are under water stress conditions (Salerno et al. 2017). The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae), native of east Asia (Lee et al. 2013), is a very polyphagous herbivore of over 100 host plants, including agricultural, horticultural and ornamental plants (Leskey et al. 2012; Haye et al. 2014). After its introduction to North America and Europe, *H. halys* rapidly spread becoming a harmful invasive species that can cause severe economic losses in orchards and field crops (Rice et al. 2014; Haye et al. 2015). In Italy, *H. halys* was detected for the first time in September 2012 in the province of Modena, and to date, its presence is limited in the northern regions where it causes damages mainly in nectarine orchards (Cesari et al. 2015; Roversi et al. 2016); nonetheless, it is expected to spread widely and to increase its importance as crop pest. From an ecological point of view, *H. halys* has the potential to share the same local community structures of local pentatomid pests adding a resource to the environment for their indigenous parasitoids. Therefore, the presence of invasive *H. halys* could determine important ecological consequences for plant – pentatomid herbivore – parasitoid interactions, depending on the parasitoid's ability to recognize the herbivore and successfully develop in *H. halys* eggs. In this view, the deeply investigated interactions among *V. faba – N. viridula – T. basalis* could represent a model system for evaluating the ecological effects of the of invasive *H. halys* on local tritrophic web. Moreover, preliminary observations under laboratory conditions showed that *T. basalis* is able to reproduce in *H. halys* eggs albeit with very low rates of parasitism (M.C. Foti, personal observations). Therefore, the wasp's ability to complete host selection sequence could determine the development of a 'new association' between *T. basalis* wasps and the potential new host *H. halys*. In the present work, laboratory experiments were conducted to determine the impact of *H. halys* on the plant volatile-mediated signalling in the local tritrophic web *V. faba–N. viridula–T. basalis*. The impact of *H. halys* was addressed both as a single stress factor, to investigate whether *T. basalis* females were attracted to *V. faba* plants infested by *H. halys*, and as a concurrent infestation by *H. halys* and *N. viridula*, to investigate whether the activity of the alien *H. halys* disrupts the attraction of parasitoid females towards volatiles emitted by *V. faba*-damaged plants.

2.3 Materials and methods

2.3.1 Plants

Seeds of broad bean plants (*V. faba* cv. Superaguadulce) were immersed for 24 h in a slurry of water and soil (1:4) to start germination and then individually planted in plastic pots (9 x 9 x 13 cm) filled with a mixture (1:1) of agriperlite (Superlite, Gyproc Saint-Gobain, PPC Italia, Italy) and vermiculite (Silver, Gyproc Saint-Gobain, PPC Italia, Italy). Plants were grown in a climate-controlled chamber (24 ± 2 °C, 45 ± 10% RH, 12 h:12 h L:D), watered daily and, from 1 week post-germination, fertilized with an aqueous solution (1.4 g/l) of fertilizer (5–15– 45, N–P–K, Plantfol, Valagro, Italy). For the treatments, 3-week-old broad bean plants, with approximately seven fully expanded leaves, were used.

2.3.2 Insect rearing

The colonies of *N. viridula* and *H. halys* were established from field collected materials around Palermo and Perugia, Italy, and reared separately in insect rearing cages (47.5 x 47.5 x 47.5 cm) (Bug-Dorm-44545, MegaView Science Co. Ltd., Taichung, Taiwan) under controlled conditions (24 ± 2 °C; 70 ± 5% RH; 16 h:8 h L:D). Both colonies were fed with tomato and/or broad bean plants, fresh organic vegetables, and soybean and sunflower seeds.

Food was renewed every 2 days, and water was provided with soaked cotton wool in small containers. Egg masses were collected daily and used to maintain colonies. Stink bugs used in the experiments were from the 1st to the 5th laboratory generations.

The colony of *T. basalis* was originally established from wasps emerging from *N. viridula* sentinel egg masses located in fields around Palermo, Italy, and was reared on *N. viridula* egg masses (Fig.11). Wasps were maintained in 85-ml glass tubes, fed with a honey–water solution (80:20 v/v) and kept in a controlled environment room (24 ± 2 °C; 70 ± 5% RH; 16 h:8 h L:D). After emergence, male and female wasps were kept together to allow mating. For all bioassays, wasp females, from the 1st to the 7th laboratory generation, 3–5 days old and näive, were individually isolated in small vials for 12 h and then transferred to the bioassay room to be acclimatized around 1 h before the tests.

2.3.3 Plant treatments

Potted broad bean plants were exposed to one stink bug female, caged for 24 h on the abaxial surface of an expanded leaf using a clip-cage (Fig. 12), which consists in two modified plastic Petri dishes (Ø = 3.5 cm; h = 1 cm) with a mesh-covered hole (Ø = 3 cm) and the rim covered by a small sponge ring. In these conditions, the stink bugs were allowed to feed and oviposit (exposed plants). The egg masses laid by *H. halys* on the exposed plants ranged from 25 to 30 eggs (N = 10, weight average 0.043 ± 0.001 g), while those laid by *N. viridula* ranged from 65 to 75 eggs (N = 10, weight average 0.040 ± 0.001 g). Treated plants with empty clip-cage, kept on a leaf for 24 h, were used as control (unexposed plants). At the end of the treatments, the stink bugs and the clip-cages were removed, and after 24 h, the plants were bioassayed according to different combinations of treatment versus control. All the treatments were performed using 10- to 20-day-old stink bug adult females, fed and mated.

To determine the response of *T. basalis* to constitutive volatiles of *V. faba* plants, unexposed plants were tested versus air.

To determine the response of *T. basalis* to volatiles induced by plants damaged by *H. halys*, the following combinations were performed (Fig.1):

• Plants exposed to *H. halys* feeding versus unexposed plants

• Plants exposed to *H. halys* feeding and oviposition versus unexposed plants

To determine the response of *T. basalis* to volatiles induced by plants subjected to concurrent infestation of *H. halys* and *N. viridula*, the treatments consisted of:

• Plants exposed to *N. viridula* feeding and oviposition versus unexposed plants

• Plants exposed to *H. halys* feeding and *N. viridula* feeding and oviposition versus unexposed plants

• Plants exposed to *H. halys* feeding and oviposition and *N. viridula* feeding versus unexposed plants

• Plants exposed to *H. halys* feeding and *N. viridula* feeding and oviposition versus plants exposed to *N. viridula* feeding and oviposition.
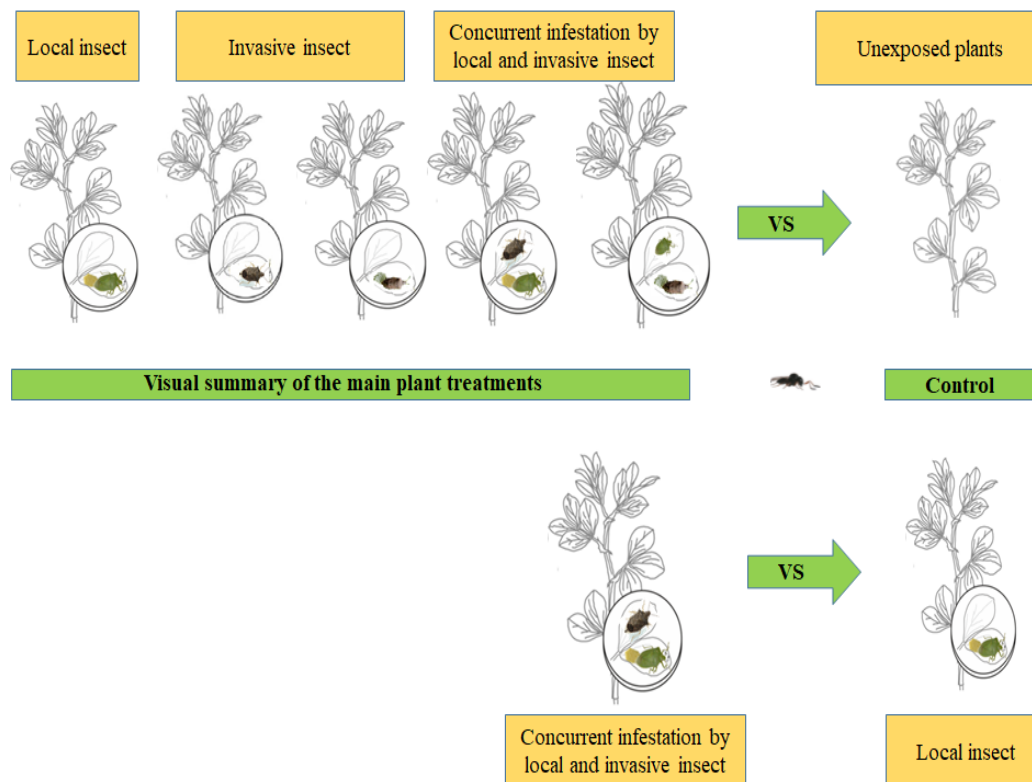
Fig. 1: Treatments conducted to test the responses of *Trissolcus basalis* to volatiles induced by plants damaged by the non-associated host *Halyomorpha halys* and the associated host *Nezara viridula* alone or in concurrent infestation

2.3.4 Y-tube olfactometer bioassays

Wasp responses to the treated plants were investigated with a dual-choice Y-tube olfactometer (Fig. 2) made from a polycarbonate body (stem 9 cm; arms 8 cm at 130° angle; ID 1.5 cm) sandwiched between two glass plates. A stream of medical-grade compressed air (approximately 80:20, N_2:O_2) coming straight from the cylinder, humidified by bubbling through a water jar, was regulated in each arm by a flow-meter at about 0.5 l min^{-1}. The device was illuminated from above by two 22-W cool white fluorescent tubes (full spectrum 5900 K, 11 W; Lival, Italy) and from below by an infrared source (homogeneous emission of wavelengths at 950 nm provided by 108 LEDs). Before entering in the olfactometer arms, each air stream passed through a cylindrical glass chamber (Ø = 12 cm; h = 52 cm) containing a treated plants odour source. The stimuli were randomly assigned at the beginning of the bioassays and were reversed after testing three parasitoid females. At every switch, the whole system was changed with cleaned parts. At the end of the bioassays, the polycarbonate olfactometer and all glass parts were cleaned with fragrance-free soap, rinsed with demineralised water and dried. The glass

parts were then baked overnight at 180 °C. Wasp females were singly introduced into the Y-tube olfactometer, and their behaviour was recorded for 10 min using a monochrome CCD video camera (Sony SSC M370 CE) fitted with a 12.5–75 mm/F 1.8 zoom lens. The camera lens was covered with an infrared pass filter (Kodak Wratten filter 87 Å) to remove visible wavelengths. Analogue video signals from the camera were digitized by a video frame grabber (Studio PCTV–Pinnacle Systems, Mountain View, CA). Digitized data were processed by XBug, a video tracking and motion analysis software as described in Colazza et al. (1999).
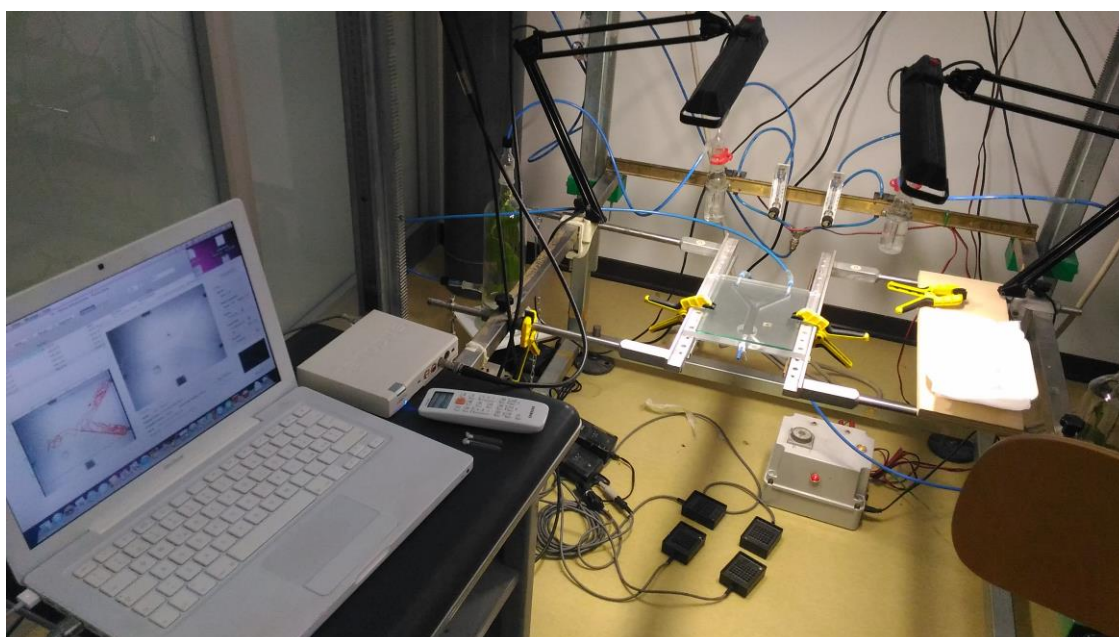
Fig. 2: Tools and equipments of the dual-choice Y-tube olfactometer.

Wasp response was measured in terms of residence time, i.e. the time spent by the wasps in each arm during the bioassay. The Y-tube olfactometer bioassays were carried out as paired choices, in which the test odour sources were always tested versus a control odour as detailed above. Bioassays were conducted from 10:00 to 13:00 h under controlled conditions (26 ± 1 °C; $50 \pm 5\%$ RH).

2.3.5 Statistical analysis

For the bioassays, the time spent by wasp females in each arm was statistically compared by parametric paired t tests for dependent samples. The time spent by the wasps in the common arm was excluded from the analysis. Data were analysed using the STATISTICA 7 software (StatSoft 2001).

2.4 Results

Response of *T. basalis* to volatiles induced by plants damaged by *H. halys* (Fig. 3). *Trissolcus basalis* females were not attracted to unexposed plants when tested versus air (t = -0.05; df = 29; p = 0.96). Wasp females were not attracted to VOCs emitted by *V. faba* plants infested by *H. halys*. Over the observation period, all tested females made a response to volatiles. In particula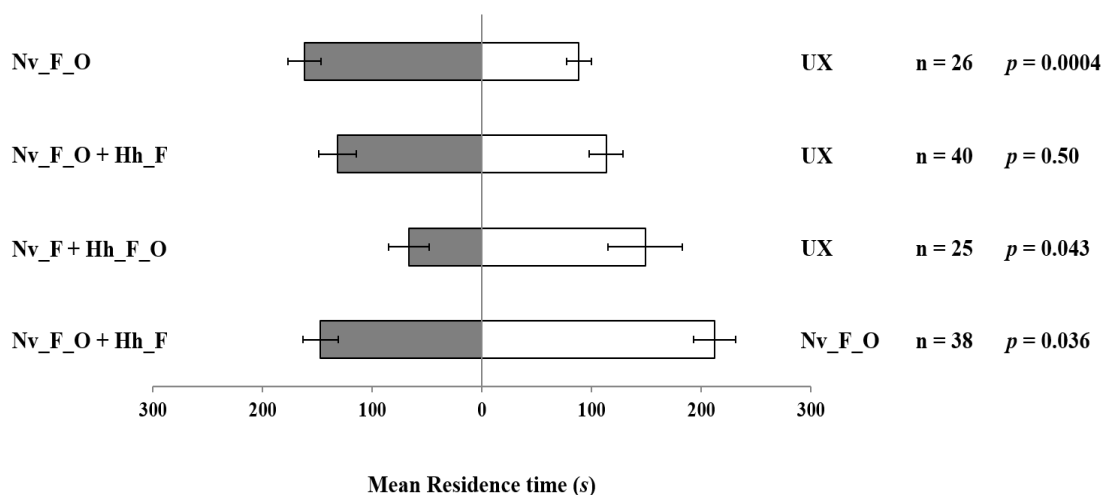r, *H. halys* feeding and oviposition on the plant stimulated a marginally not significant response in the wasps compared to unexposed plants (t = -1.81; df = 29; p = 0.08). Instead, the wasps significantly preferred the volatiles released by unexposed plants, compared to volatiles from plants damaged by *H. halys* feeding activity (t = -2.42; df = 39; p = 0.02).
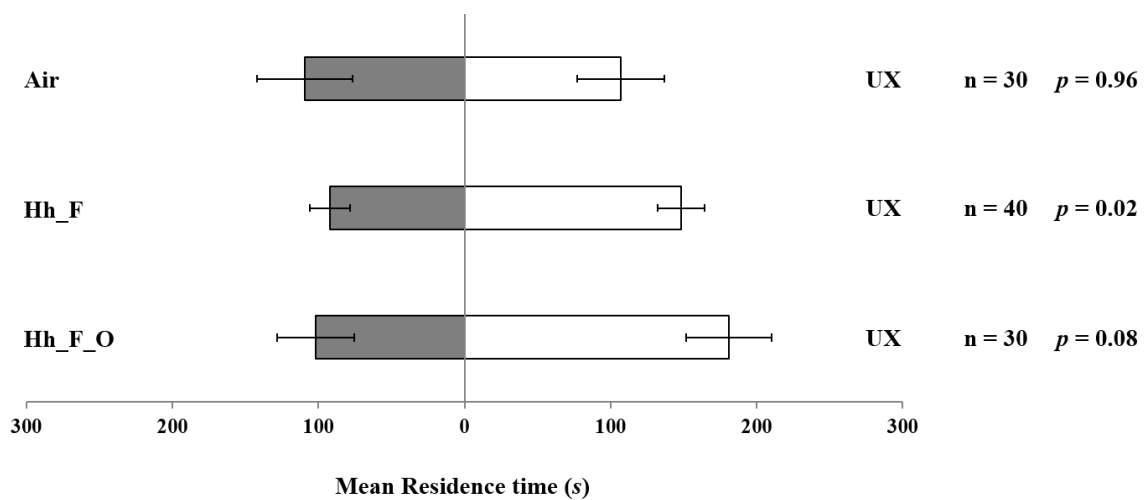
Fig 3: Response of *Trissolcus basalis* females to *Vicia faba* plant volatiles induced by *Halyomorpha halys*. Plant treatments: *H. halys* feeding and oviposition (Hh_F_O); *H. halys* feeding (Hh_F); unexposed (UX). n = number of replicates. Bars represent mean (±SE) of the time spent by wasp females in each arm of the Y-tube olfactometer over an observation period of 300 s (paired t tests)

Response of *T. basalis* to volatiles induced by plants subjected to concurrent infestation of *H. halys* and *N. viridula* (Fig. 4). The response of *T. basalis* females to VOCs emitted by *V. faba* plants infested simultaneously by *H. halys* and *N. viridula* was affected by the plant treatments. Over the observation period, all tested females made a response to volatiles. Wasps were significantly attracted to volatiles emitted by plants attacked by *N. viridula* feeding and oviposition compared to unexposed plants (t = 4.12; df = 25; p = 0.0004), whereas they were not attracted when plants were concurrently exposed to *N. viridula* feeding and oviposition activity and *H. halys* feeding, over unexposed plants (t = 0.68; df =

39; p = 0.50). Female wasps exhibited a significant preference for volatiles released by unexposed plants when tested versus plants concurrently damaged by *N. viridula* feeding and *H. halys* feeding and oviposition (t = -2.10; df = 24; p = 0.043). When volatiles from plants damaged by *N. viridula* feeding and oviposition were tested against plants exposed to concurrent infestation of *N. viridula* and *H. halys*, *T. basalis* females showed significant preference for plants attacked only by *N. viridula* (t = -2.17; df = 37; p = 0.036).

Fig. 4: Response of *Trissolcus basalis* females to *Vicia faba* plant volatiles induced by concurrent infestation of *Halyomorpha halys* and *Nezara viridula*. Plant treatments: *N. viridula* feeding and oviposition (Nv_F_O); *N. viridula* feeding and oviposition and *H. halys* feeding (Nv_F_O + Hh_F); *N. viridula* feeding and *H. halys* feeding and oviposition (Nv_F + Hh_F_O); unexposed (UX). Bars represent mean (±SE) of the time spent by wasp females in each arm of the Y-tube olfactometer over an observation period of 300 s (paired t tests)

2.5 Discussion

In the present study, we demonstrated that the alien herbivore *H. halys* can interfere with the local tritrophic system V. faba – *N. viridula* – *T. basalis*. First, the attack of *H. halys* on *V. faba* plants modifies the response of *T. basalis* to VOCs emitted by uninfested plants. Indeed, the wasp females are not attracted by uninfested plants, but in the presence of plants damaged by *H. halys*, they choose constitutive *V. faba* VOCs, showing a preference that is statistically significant towards plants with *H. halys* feeding activity and marginally not significant towards plants with *H. halys* feeding and oviposition activities. Moreover, we detected that *T. basalis* females, which usually respond to plant volatiles induced by *N. viridula* feeding and oviposition (Colazza et al. 2004a, b), are not attracted by plants that were exposed to *H. halys* feeding and oviposition. The lack of response of *T. basalis* to OIPVs from *H. halys* infested plants could be interpreted as a direct consequence of the

absence of coevolution among the plant, alien herbivore and parasitoid. Plants have evolved adaptive indirect defence responses against coevolved herbivores, by recruiting natural enemies, but they could be more exposed when they have to deal with a novel non-coevolved herbivore (Desurmont et al. 2014). From a parasitoid point of view, this lack of attraction to plant infested by an alien and non-associated host herbivore may have beneficial or negative consequences in terms of its foraging efficiency, depending on its ability to successfully develop on alien host. Indeed, if the parasitoid is able to develop in the new host, its inability to use the new chemical cues provided by the plant–herbivore complex reduces the possibility to use a valuable host. In this case, the lack of attraction could be detrimental to the wasp fitness. On the contrary, when the parasitoids could not complete the development in the new host but they still respond to cues from the plant–herbivore complex, they might invest time and energy in unproductive foraging behaviour. In this respect, the alien herbivore may represent a sink or an 'evolutionary trap' for the natural enemies that undergo a reduction in their populations. Indirectly, this might be an advantage for local herbivore stink bugs, which might exhibit significant outbreaks due to a lower demographic pressure by natural enemies (Abram et al. 2014). Our preliminary laboratory tests suggest that *H. halys* is a potential suitable host for *T. basalis*, even if its reproductive rate on *H. halys* fresh eggs is very low (about 10%, M.C. Foti personal observation), consistent with the results obtained by Haye et al. (2015), which observed a poor development of Trissolcus spp. on *H. halys* fresh eggs. Therefore, this host specificity could allow *T. basalis* to optimize time and energy by exploiting on cues emitted by suitable host species. This ability of *T. basalis* was already observed during orientation induced by substrate-borne kairomones. Indeed, foraging wasp females exploit more deeply chemical traces of the associated host, showing capacity to distinguish chemical traces of associated species from those of non-associated species (Salerno et al. 2006), and host sex discrimination ability only in the presence of chemical footprints from their associated host species (Peri et al. 2006, 2013). Our data also show that herbivory by the alien stink bug *H. halys* disrupts *T. basalis* attraction towards OIPVs emitted by *V. faba* plants following attacks by *N. viridula*. The effect of multiple herbivory attacks on plant volatile emission and therefore on natural enemy recruitment is well reported in the literature, showing that it is a widespread ecological phenomenon, since it can occur not only when the attackers have similar feeding habits (results in this study; Shiojiri et al. 2001; Bukovinszky et al. 2012), but also when plants are under concurrent attacks by above and belowground herbivores (Rasmann and Turlings 2007; Soler et al. 2007), by

piercing–sucking and chewing herbivores (Erb et al. 2010; Cusumano et al. 2015), by herbivorous insects and mites (de Boer et al. 2008; Zhang et al. 2009), and by insect herbivores and plant pathogens (Ponzio et al. 2014). Concerning alien insect herbivores, their impact on tritrophic interactions has been investigated in a few cases, and the results generally indicate that the attraction of parasitoids towards HIPVs and OIPVs is disrupted when both host and non-host herbivores were simultaneously present on the same plant (Desurmont et al. 2014; Chabaane et al. 2015; Clavijo McCormick 2016). For example, Cusumano et al. (2015) demonstrated that the attraction of the egg parasitoids *Trichogramma brassicae* Bezdenko and *T. evanescens* Westwood towards *Brassica nigra* L. is disrupted when the plants are under the attack of a naturally associated host, *Pieris brassicae* L., and by an alien and invasive herbivore, *Spodoptera exigua* (Hübner). The effect exerted by *H. halys* in our tritrophic system not only provides an additional example of disturbance of an infochemical web caused by an invasive insect herbivore, but it also raises the doubt whether the *V. faba* plant – *T. basalis* signalling is a stable communication that can benefit the organisms involved. Indeed, the alteration reported here represents the third case for this plant–parasitoid interaction. Previous studies have shown that the *T. basalis* attraction to *V. faba* OIPVs, emitted after *N. viridula* egg deposition and feeding activity, is altered by biotic and abiotic stresses. Moujahed et al. (2014) demonstrated that the concurrent infestation of non-host beetle *S. lineatus*, either adults feeding on leaves or larvae feeding on roots, reduces the attraction of *T. basalis* towards OIPVs emitted by *V. faba* plants infested by *N. viridula*. On the contrary, the volatile blend emitted by *V. faba* plants that were under simultaneous water stress and *N. viridula* attack enhances the attraction of *T. basalis* (Salerno et al. 2017). However, to better understand the role of OIPVs in mediating broad bean plant–egg parasitoid interaction, the influence played by parasitoid learning should be considered. In fact, it has been suggested that egg parasitoids could rely on learning abilities when foraging for hosts in complex and dynamic environments (Fatouros et al. 2008; Colazza et al. 2010; Cusumano et al. 2012). Indeed, a partially adaptive learning was demonstrated in *T. basalis* females, which are attracted to volatiles induced in *V. faba* plants infested by a non-host herbivorous species (*S. lineatus* adults or larvae) when they are naïve, but not after an oviposition experience (Moujahed et al. 2014). Similarly, Cusumano et al. (2015) suggested that associative learning could be important in foraging behaviour of *Trichogramma* species, such as *T. evanescens* and *T. brassicae* when exploiting OIPVs emitted by *Brassica* plants under multiple herbivore attacks. In summary, our laboratory study evidences the disruptive impact of an alien

insect herbivore on plant volatile- mediated signalling in a local tritrophic web. However, further research on multitrophic interactions under field or semi-field conditions is required to better determine the impact of *H. halys* on *T. basalis* recruitment by infested plants and, consequently, on egg parasitoid efficacy in controlling pentatomid hosts.

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Chapter 3

Attraction of the egg parasitoid, *Telenomus podisi*, to oviposition plant volatiles induced by the zoophytophagous predator, *Podisus maculiventris*, under biotic interactions with the invasive phytophagous pest, *Halyomorpha halys*.
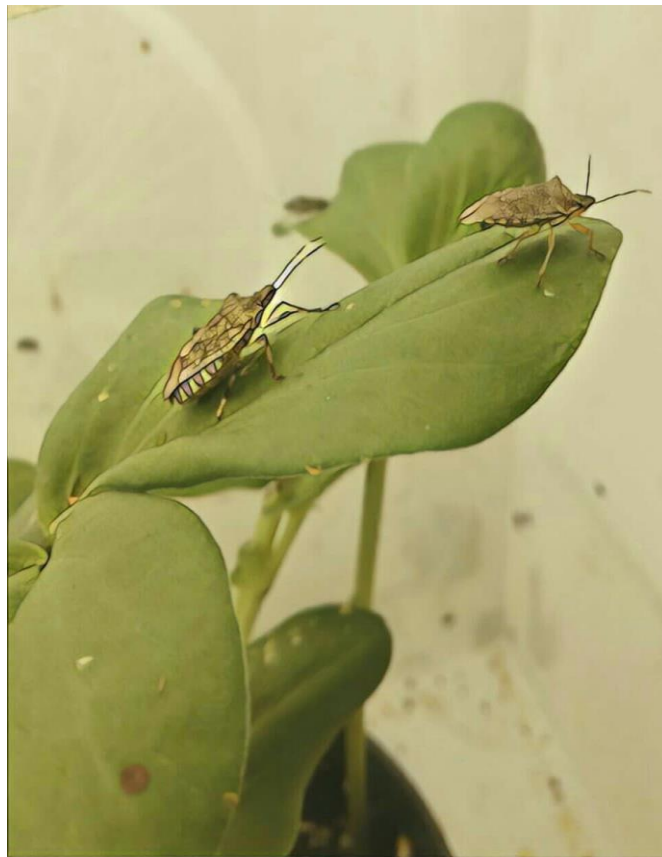
3.1 Abstract:

Plants under attack of phytophagous insects emit volatile organic compounds that act as indirect plant defence mechanism since they recruit egg parasitoids of the attacking pest. However, in nature, plants can be also attacked by predator insects with facultative phytophagy that feed on plant resources to obtain food supplements. Moreover, invasive alien insects can affect existing native infochemical networks. Here we investigated in laboratory conditions the plant volatile-mediated signalling in a local system including *Vicia faba*, the zoophytophagous predator *Podisus maculiventris* and its associated egg parasitoid *Telenomus podisi* and whether the alien phytophagous and non-associated pest *Halyomorpha halys* interfere this infochemical network. We found that: 1) *T. podisi* wasps were attracted by oviposition induced plant volatiles (OIPVs) from *P. maculiventris* attacked plants, indicating the capacity of a zoophytophagous predator to induce plant indirect defence mechanism; 2) *T. podisi* wasps were not attracted by OIPVs from *H. halys* attacked plants, suggesting specificity in the parasitoid response; 3) *T. podisi* wasps were not attracted by plants concurrently infested by *P. maculiventris* and *H. halys*, indicating that the alien herbivore disrupts the established local infochemical network. These outcomes are discussed in a multitrophic context by evaluating the possible ecological and applicative consequences.

3.2 Introduction

Phytophagous insects are known to induce changes in the volatile profile emission in plants they attack (Dicke 2016; Kaiser et al. 2017). In response to phytophagous insect activities, plants emit volatile organic compounds (VOCs), i.e. plant synomones, that act as an indirect plant defence mechanism since they recruit parasitoid species allowing them to improve their ability to localize the colonizing herbivores (Meiners and Peri 2013). These plant synomones are called herbivore induced plant volatiles (HIPVs) and/or oviposition induced plant volatiles (OIPVs), if they are emitted by the plants as a consequence of insect feeding damage and/or insect oviposition activity, respectively (Hilker and Fatouros 2015; Pashalidou et al. 2015). The exploitation of OIPVs is one of the main strategies adopted by egg parasitoids to efficiently optimize their foraging search (Vinson 1998; Fatouros et al. 2008; Colazza et al. 2010). Indeed, egg parasitoids have a short time window to locate and exploit host since host eggs are usually suitable for parasitism for few days, due to their rapid development; moreover eggs are a small component of a composite environment, therefore it is not easy for egg parasitoids detect at long range the quantitatively negligible cues from the hosts (Vinson 1994, 2010); on the contrary OIPVs facilitate host egg location as they are easily detectable as produced in huge quantities by the plants and are reliable indicators of the presence of host eggs (Hilker and Meiners 2011; Conti and Colazza 2012).

Plants can also provide food supplements to omnivorous arthropods that feed on both prey and plant resources and the plant-feeding is a common phenomenon in many arthropod predaceous (Coll and Guershon 2002). Insect predators can show different strategies, e.g. cannibalism, intraguild predation, and omnivory, to integrate their diet, mainly when the prey availability is low (Torres et al. 2010). In particular, omnivorous insects that feed at more than one trophic level, not only can determine ecological consequences, but it might affect biological control programmes (Castané et al. 2011). In fact, zoophytophagous predators, also called plant-feeding predators or facultative predators (Albajes et al. 2006), are efficient natural enemies of several pests (for example, several mirid species, such as *Dicyphus tamaninii* Wagner, *Dicyphus hesperus* (Knight), *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* (Reuter) are predators of whiteflies, leafminers, spider mites), but they might also damage plant tissues and, as a consequence, determine economic losses by feeding on crop plants when their preys become scarce. However, the provided benefits seem higher, as significant plant damages are relatively rare (Albajes and

Alomar 2008). Some papers have discussed anatomical, biochemical and physiological issues of zoophytophagous insects to explain how they can move from a feeding specialty to a totally different food source (Cohen, 1996, 1998; Zhang and Cohen 2000). In the context of HIPVs, it was recently demonstrated that feeding and/or oviposition activities of zoophytophagous predators can activate in the host plants defence mechanisms as well as herbivorous pest, that affect performance of other insect herbivores (De Puysseleyr et al. 2011; Pappas et al. 2015; Zeng et al. 2018) or determine different attractiveness to pests and their parasitoids (Pérez-Hedo et al. 2015a,b; Naselli et al. 2016).

In the last decades, it has become evident that these widely investigated tritrophic interactions need to be studied in a multitrophic context as they can be affected by biotic and abiotic factors (Moujahed et al. 2014; Salerno et al. 2017). Multitrophic interactions involving exotic species may have ecological consequences for native communities, for example, by adding new resources or by introducing novel interactions into native communities (Harvey and Fortuna 2012; Hopper and Mills, 2016; Carrasco et al. 2018). In particular, invasive phytophagous pests may modify the defensive response of plants to local phytophagous insects, indirectly interfering existing native infochemical networks (Desurmont et al. 2014; Chabaane et al. 2015).

In this chaphter was undertook a study to investigate a multitrophic infochemical web, including *Vicia faba* L. plants, the zoophytophagous predator *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae), its associated egg parasitoid *Telenomus podisi* (Ashmead) (Hymenoptera: Scelionidae) and the alien phytophagous pest *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae), in order to evaluate: 1) if the attack of *P. maculiventris* is able to induce in *V. faba* attacked plant the emission of synomones that recruit *T. podisi*; 2) the impact of *H. halys* on the native tritrophic network *V. faba - P. maculiventris - T. podisi*.

Podisus maculiventris, the spined soldier bug, is a predator of several agricultural and forest pests in North America (McPherson, 1980, 1982), such as the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) (Hough-Goldstein and Whalen 1993; Hough-Goldstein and McPherson 1996), and the Mexican bean beetle, *Epilachna varivestis* Mulsant (Coleoptera: Chrysomelidae) (Sant'Ana et al. 1997). When preys are scarce, *P. maculiventris* can feed on plants, but this feeding activity normally does not resolve in crop injury (Ruberson et al. 1986).

Halyomorpha halys (Stål) (Heteroptera: Pentatomidae), the brown marmorated stink bug, is a pest of Asian origin that has recently spread in several North American and European

countries becoming quickly very common and destructive pest in orchards and field crops of the invaded countries (Rice et al. 2014; Haye et al. 2015).

Telenomus podisi parasitizes the eggs of various phytophagous pentatomids such as *Acrosternum hilare* (Say), *Euchistus heros* (F.) and *Nezara viridula* (L.). To locate its associated hosts, it can use cues from host habitat (Bruni et al. 2000) and HIPVs (Moraes et al. 2005, 2008).

As show the fig. 5, all these species directly interact with each other. *Telenomus podisi* is one of the main egg parasitoids of *P. maculiventris* (Bruni et al. 2000), as a consequence, its parasitism activity could affect the ecosystem function provided by this beneficial predator in the field. Recently, it was reported that the egg parasitoid is also able to attack *H. halys* eggs, although it does not complete the development (Abram et al. 2014).

Podisus maculiventris, as reported above, can prey several phytophagous insect species. Among these, it can attack also pentatomid pest, such as *N. viridula* (De Clercq et al, 2002), similarly to congeneric *P. nigrispinus* (Dallas) that has been reported to attack several pentatomid pests in soybean and alfalfa fields (Saini 1994). Recently, test under laboratory conditions showed that the spined soldier bug could prey on *H. halys* eggs (Abram et al. 2015).

Halyomorpha halys has the potential to inhabit same areas of *P. maculiventris* (Boyle 2017). Moreover, the brown marmorated stink bug is a non-associated host of *T. podisi,* as its eggs under laboratory condition were accepted by the parasitoid, although they were not suitable for parasitoid development (Abram et al. 2014). Non-associated hosts could be considered for example species occasionally attacked in the field, factitious host used in laboratory or species eliciting parasitoid responses but not suitable for parasitoid development (Peri et al. 2013). Finally, it was recently demonstrated that this phytophagous alien species might disrupt local semiochemical networks (Martorana et al. 2017).

Specifically, in this chapther were performed a series of bioassays to evaluate: 1) the response of *T. podisi* to volatiles induced by plants damaged by the zoophytophagous predator and associated host *P. maculiventris* in order to evaluate the local infochemical web; 2) the response of *T. podisi* to volatiles induced by plants separately infested by the alien pest and non-associated host *H. halys*, in order to evaluate the ability of a local egg parasitoid to start host selection sequence towards an invasive stink bug by exploiting HIPVs. Based on the results of these experiments we then evaluated the response of *T. podisi* to volatiles emitted by plants subjected to concurrent infestation of *P. maculiventris*

and H. halys, in order to address how the spread of *H. halys* could shape volatile-mediated signalling in the local tritrophic web and impact on the structured insect communities.
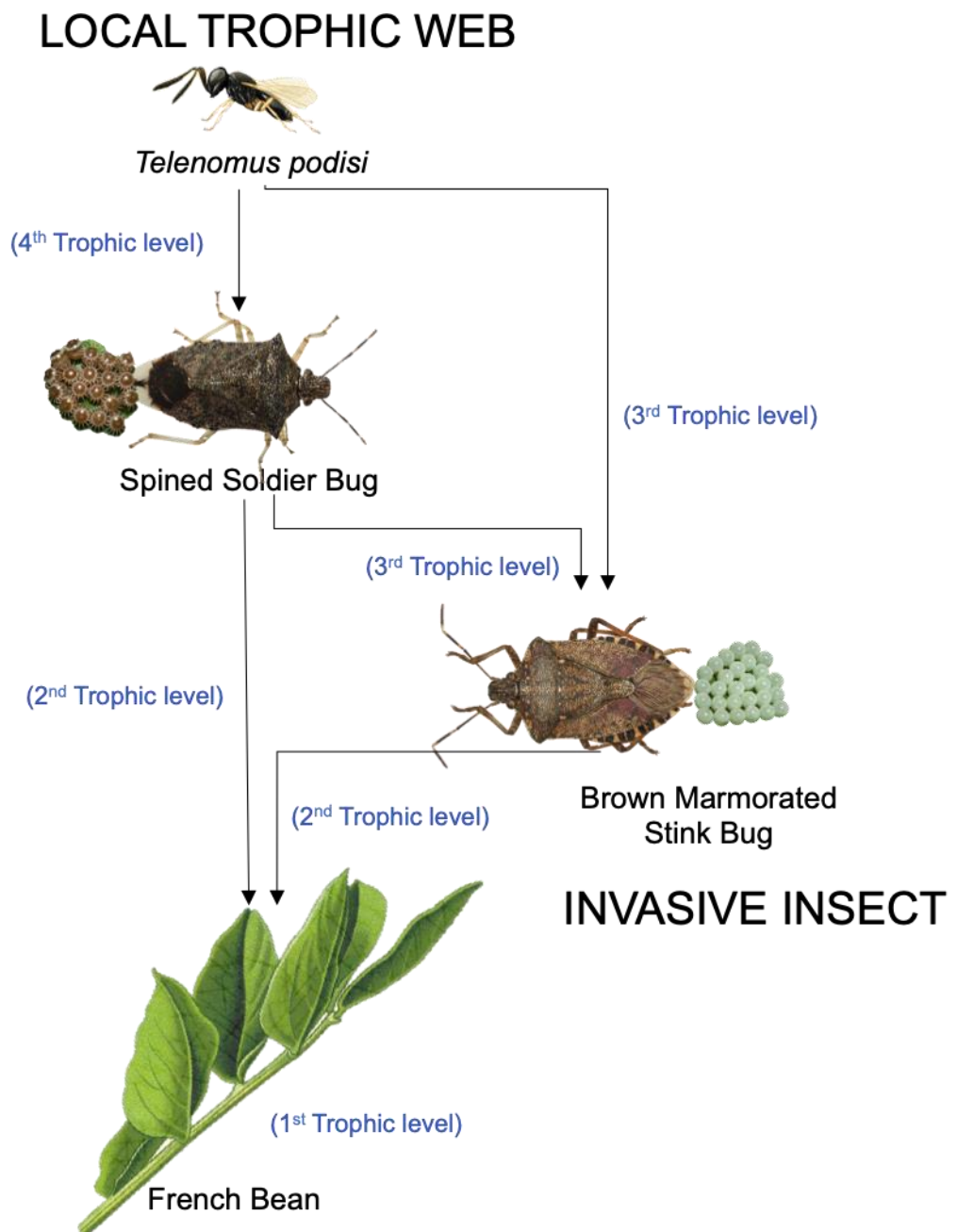
Fig. 5: Multi-trophic system composed by the bean plant *Vicia faba*, the zoophytophagus *Podisus maculiventris*, the egg-parasitoid *Telenomus podisi* and the alien insect *Halyomorpha halys.* The black arrows refer to trophic levels and how each species is related to the others.

3.3 Materials and methods

3.3.1 Plants

Seeds of broad bean plants (*Vicia faba* cv. Superaguadulce) were individually planted in plastic pots (5 x 5 x 10 cm) filled with fertilized commercial soil (BM6, Berger, Québec, Canada).

Plants were grown in a climate-controlled (22 ± 1° C, 50± 5% RH, 16 h:8 h L:D) and equally irrigated every two days. For the experiments, plants of 2-3 weeks old, with approximately 7–8 fully expanded leaves, were used.

3.3.2 Insect rearing

The colonies of *H. halys* and *P. maculiventris* were established from adults collected from fields around Hamilton and Ottawa (Ontario, Canada) and reared separately in insect ventilated cages (30 x 30 x 30 cm) under controlled conditions (24 ± 2 °C; 50 ± 5% RH; 16 h:8 h L:D). The colony of *H. halys* was fed with raw pumpkin seeds, carrots, green beans, potted soy plants. Food was renewed every 2 days, and water was provided with soaked cotton wool in small containers. The colony of *P. maculiventris* was provided a diet consisting of live mealworm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae, fresh green beans and *V. faba* potted plants. Egg masses were collected daily and used to maintain both colonies. Stink bugs used in the experiments were from the 1st to the 5th laboratory generations.

The colony of *T. podisi* was established from wasps emerging from *P. maculiventris* sentinel egg masses located inside the Botanical Garden of the Institute de Recherché en Biologie Végétale of Montreal (Québec, Canada) and was reared on *P. maculiventris* egg masses. Wasps were maintained in a cage (17 x 17 x 17 cm) (Bug-Dorm-44545, MegaView Science Co. Ltd., Taichung, Taiwan), fed with a honey-water solution (80:20 v/v) and kept in a controlled environment room (24 ± 1 C; 50± 5% RH; 16 h:8 h L:D). Daily, about 10-15 egg masses were glued on paper strips and introduced into the cage. After emergence, male and female wasps were kept together to allow mating. For the experiments, wasp females, from the 1st to the 7th laboratory generation, 3–5 days old and naıve with respect to both oviposition experience and contact with cues released by plants and host were individually isolated in 1.2 mL Eppendorf tubes for 24 h.

3.3.3 Y-tube olfactometer bioassays

The responses of *T. podisi* to the treated plants were tested in a dual-choice olfactometer comprised of a Y-shaped glass body (2 cm uniform internal diameter, 7 cm main body (stem) length, and 17 cm arm length at 165° angle). A stream of medical-grade compressed air (approximately 80:20, $N_2:O_2$) flowed through both arms. The flow was cleaned with a charcoal filter to reduce possible environmental cues, humidified by bubbling through a distilled water jar and regulated by flow meters to creating an airstream of about 0.5 l min^{-1} per arm. The device was illuminated from above by two 18-W cool white fluorescent lamps. Before entering in the olfactometer arms, each air stream passed through a 4 l-glass jar (Ø =15 cm;) containing the odour sources. The stimuli were randomly assigned at the beginning of the bioassays and were reversed after testing three parasitoid females to avoid any bias due to eventual side preferences by the parasitoids. At every switch, the system was cleaned with fragrance-free soap, rinsed with demineralised water and dried. Wasp females were singly introduced into the Y-tube olfactometer, and their behaviour was recorded for 10 min using a HDD video camera (Sony HDR-XR500); the video were analysed by CowLog software (Hänninen and Pastell, 2009) and their responses were measured in terms of residence time, i.e. the time spent by each wasp in the test/control arm during the bioassays. The Y-tube olfactometer bioassays were carried out as paired choices, in which the test odour sources were always tested versus a control odour as detailed above. Odour sources and wasp females were used only once. For each treatment, 35 replicates were conducted. The experiments were conducted from 09:00 to 14:00 in a dark room to avoid directional light, under controlled conditions (24 ± 1 °C; 50 ± 5% RH). Wasps were allowed to acclimatize for at least 1 h in the room before the experiment.

3.3.4 Plant treatments

To evaluate the response of *T. podisi* in olfactometer bioassays, we used the protocol of Martorana et al. (2017). Potted broad bean plants were exposed to one stink bug female, caged for 24 h on the abaxial surface of an expanded leaf using a clip-cage, which consists in two modified plastic Petri dishes (Ø = 3.5 cm; h = 1 cm) with a mesh-covered hole (Ø = 3 cm) and the rim covered by a small sponge ring. Inside the clip-cage the stink bugs were allowed to feed and oviposit (exposed plants). The egg masses laid by *P. maculiventris* on the exposed plants ranged from 13 to 25 eggs (weight average 0.056 ± 0.0011 g N = 10),

while those laid by *H. halys* ranged from 25 to 30 eggs (weight average 0.043 ± 0.001 g N = 10,). Treated plants with empty clip-cage, kept on a leaf for 24 h, were used as control (unexposed plants). At the end of the treatments, the stink bugs and the clip-cages were removed, and after 24 h, the plants were bioassayed according to different combinations of treatment versus control. All the treatments were performed using from 10 to 20 days-old stink bug adult females, fed and mated.

To determine the response of *T. podisi* to volatiles induced by plants damaged by the zoophytophagous predator and associated host, *P. maculiventris*, the following combinations were performed:

- Plants exposed to *P. maculiventris* feeding versus unexposed plants
- Plants exposed to *P. maculiventris* feeding and oviposition versus unexposed plants

To determine the response of *T. podisi* to volatiles induced by plants damaged by the alien pest and non-associated host, *H. halys*, the following combinations were performed:

- Plants exposed to *H. halys* feeding versus unexposed plants
- Plants exposed to *H. halys* feeding and oviposition versus unexposed plants

To determine the response of *T. podisi* to volatiles emitted by plants subjected to concurrent infestation of *P. maculiventris and H. halys,* the following combinations were performed:

• Plants exposed to *P. maculiventris* feeding and oviposition and *H. halys* feeding versus unexposed plants

• Plants exposed *P. maculiventris* feeding and *H. halys* feeding and oviposition versus unexposed plants

• Plants exposed *P. maculiventris* feeding and oviposition and *H. halys* feeding versus plants exposed to *P. maculiventris* feeding and oviposition.

3.3.5 Statistical analysis

For the bioassays, the time spent by wasp females in each arm was statistically compared by parametric paired t tests for dependent samples. The time spent by the wasps in the central arm was excluded from the analyses. Data were analysed using the STATISTICA 12 software (StatSoft, 2014).

3.4 Results

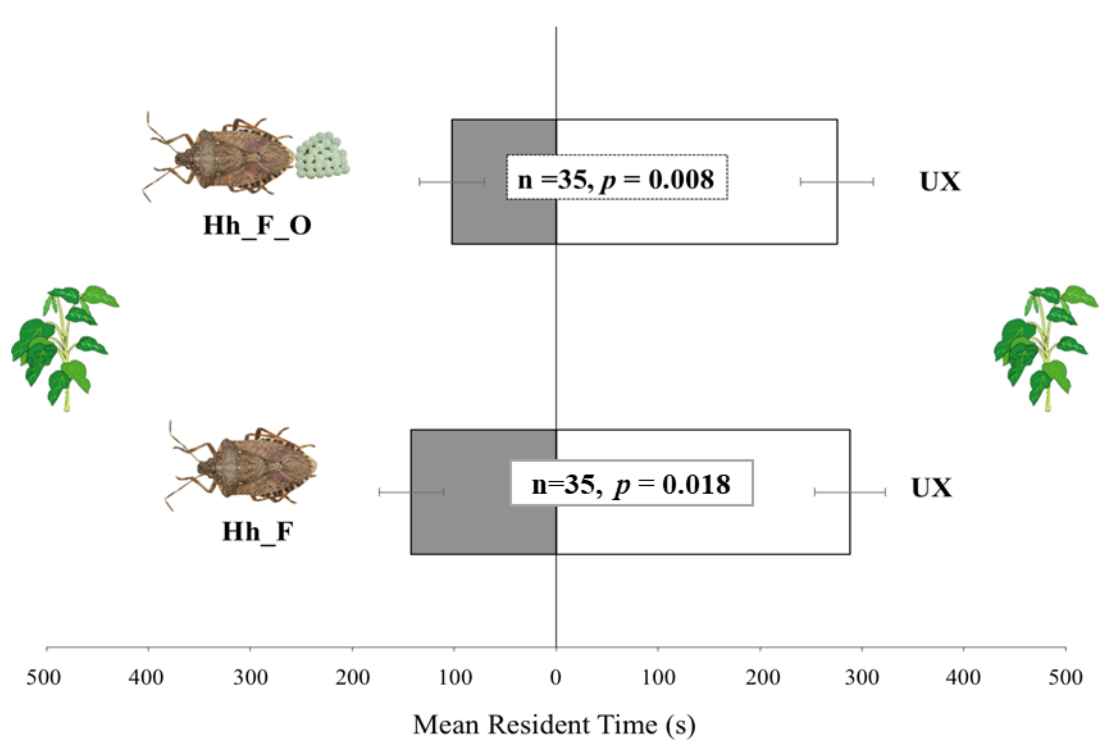The response of *T. podisi* to plant volatile induced by the zoophytophagous predator and associated host, *P. maculiventris,* is reported in Fig. 6. Over the observation period, all tested females of *T. podisi* made a response to volatiles. In particular, wasps were significantly more attracted to VOCs from broad bean plants damaged by feeding and oviposition activity of *P. maculiventris* than to VOCs from unexposed plants (t = 4.12; df = 34; p = 0.0002), while VOCs from broad bean plants damaged by *P. maculiventris* feeding were no more attractive to *T. podisi* females than those from unexposed plants (t = -0.006; df = 34; p = 0.99).
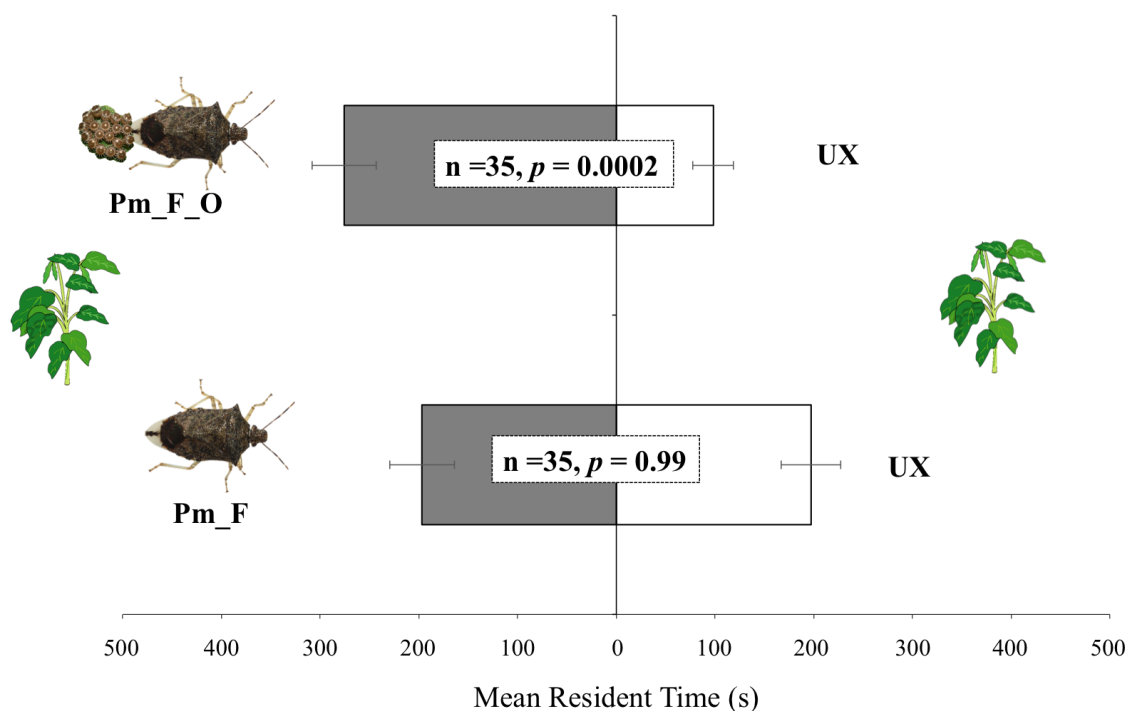
Fig. 6: Response of *Telenomus podisi* females to *Vicia faba* plant volatiles induced by *Podisus maciliventris*. Plant treatments: *P. maculiventris* feeding and oviposition (Pm_F_O); *P. maculiventris* feeding (Pm_F); unexposed (UX). n = number of replicates. Bars represent mean (± SE) of the time spent by wasp females in in each arm of the Y-tube olfactometer over an observation period of 300 sec (paired t-tests).

The response of *T. podisi* to plant volatile induced by the alien pest and non-associated host, *H. halys*, is reported in Fig. 7. Over the observation period, all tested females of *T. podisi* made a response to volatiles. Wasp females were not attracted to VOCs emitted by *V. faba* plants infested by *H. halys*. Indeed, VOCs emitted by unexposed plants determined a significant attraction in the wasps compared to both VOCs induced by *H. halys* feeding and oviposition activities (t = -2.8; df = 34; p = 0.008) and those induced by *H. halys* feeding (t = -2.47; df = 34; p = 0.018).

Fig. 7: Response of *Telenomus podisi* females to *Vicia faba* plant volatiles induced by *Halyomorpha halys*. Plant treatments: *H. halys* feeding and oviposition (Hh_F_O); *H. halys* feeding (Hh_F); unexposed (UX). n = number of replicates. Bars represent mean (± SE) of the time spent by wasp females in in each arm of the Y-tube olfactometer over an observation period of 300 sec (paired t-tests).

The response of *T. podisi* to plant volatile emitted by plants subjected to concurrent infestation of *P. maculiventris* and *H. halys*, is reported in Fig. 8. Over the observation period, all tested females of *T. podisi* made a response to volatiles. The response of wasps to VOCs emitted by *V. faba* plants infested simultaneously by *P. maculiventris* and *H. halys* depended on the plant treatments. Indeed, wasps did not show attraction to VOCS emitted by plants that were concurrently exposed to *P. maculiventris* feeding and oviposition activity and *H. halys* feeding, over unexposed plants (t = -0.83; df = 34; p = 0.4). When volatiles from plants damaged by *P. maculiventris* feeding and *H. halys* feeding and oviposition were tested against unexposed plants, *T. podisi* females were attracted by the latter (t = -2.62; df = 34; p = 0.01). Female wasps exhibited a significant preference for volatiles released by plants damaged by *P. maculiventris* feeding and oviposition when tested versus plants concurrently damaged by *P. maculiventris* and *H. halys* (t = -2.48; df = 34; p = 0.017).
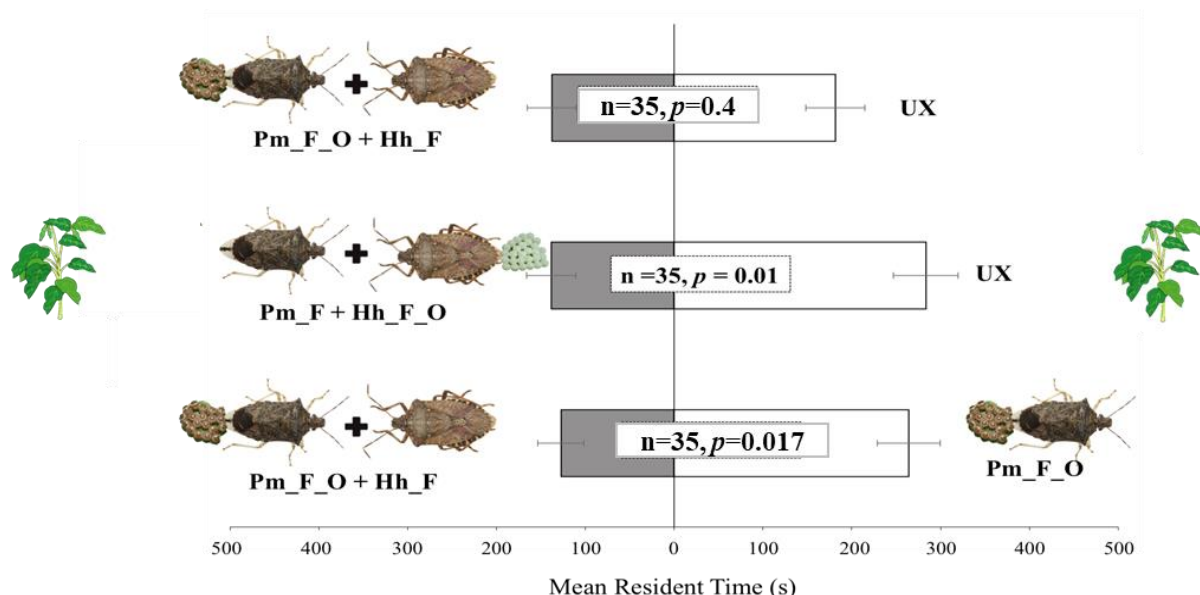
Fig. 8: Response of *Telenomus podisi* females to *Vicia faba* plant volatiles induced by concurrent infestation of *Halyomorpha halys* and *Podisus maculiventris*. Plant treatments: *P. maculiventris* feeding and oviposition (Pm_F_O); *P. maculiventris* feeding and oviposition and *H. halys* feeding (Pm_F_O + Hh_F); *P. maculiventris* feeding and *H. halys* feeding and oviposition (Pm_F + Hh_F_O) unexposed (UX). Bars represent mean (± SE) of the time spent by wasp females in each arm of the Y-tube olfactometer over an observation period of 300 sec (paired t-tests).

3.5 Discussion

In this chapther first, was show that oviposition by the zoophytophagous predator, *P. maculiventris*, on the host plant, *V. faba,* induces emission of plant volatiles that recruit its associated egg parasitoid, *T. podisi*. On the contrary, *T. podisi* is not attracted by VOCs from broad bean on which the alien herbivore and non-associated host, *H. halys*, deposit eggs, even more, when the alien insect lay its eggs concurrently with the zoophytophagous predator, the egg-laden leguminous plants are no longer attractive for the egg parasitoid.

Previous investigations have been elucidated that scelionid egg parasitoids exploit plant synomones released as a consequence of oviposition by pentatomid phytophagous pests to locate the host eggs. Indeed, these egg-induced plant cues can be perceived at long distance, e.g., *Trissolcus basalis* (Wollaston) is attracted to volatiles by *N. viridula* egg-laden Leguminosae plants (Colazza et al. 2004a,b); or at short distance, e.g. *Trissolcus brochymenae* (Ashmead) that remain longer on the cabbage leaves exploiting oviposition-induced contact synomones induce by the oviposition by the harlequin bug, *Murgantia histrionica* (Hahn) (Conti et al. 2010). In the case of *T. podisi*, Moraes and coahutors (2005) demonstrated the wasp's attractiveness to volatiles emitted by two Leguminosae plants, soybean, (*Glycine max*) and pigeon pea (*Cajanus cajan*) damaged by feeding

activity of phytophagous bug *E. heros*, one of the preferred hosts of *T. podisi*. However, this attraction does not occur when the plants are exposed to *E. heros* oviposition and feeding activities. The authors suggested that attraction to HIPVs is linked to the particular behaviour of the host that prefer oviposit on plants previously damaged by feeding of conspecifics, therefore HIPVs exploitation optimize wasp recruitment as more reliable indicator of host females ready to oviposit (Moraes et al 2008). In details, (E,E)-a-farnesene and methyl salicylate, two of the main compounds emitted by damaged plants (Michereff et al. 2011), seem be involved in *T. podisi* attraction although minor volatile compounds from soybean plants play also a role in the parasitoid host-searching behaviour (Michereff et al. 2013).

In the system *P. maculiventris - T. podisi*, our findings are different from the system *E. heros - T. podisi* as the egg parasitoid show attractiveness towards OIPVs emitted by the plants infested by the host, while the HIPVs are nor enough to recruit the wasps. This finding is remarkable as it shows that also the attack of a predator with facultative phytophagy might induce the host plant to emit VOCs that recruit egg parasitoids as well as a phytophagous pest, for example in the system *V. faba - N. viridula – T. basalis* (Colazza et al. 2004a). Facultative phytophagy is important in biological control as it allows predators to survive and reproduce when the preys are scarce (Coll and Guershon 2002). Several papers discussed about plant feeding site of zoophytophagous predators, wich nutrients they exploit during feeding activity on the plants,and if they can develop using a diet consisting of vegetable materials. Armer et al (1998) showed that *Orius insidiosus* (Say) (Heteroptera: Anthocoridae) obtain water feeding on the xylem, and small amounts of nutrients from the mesophyll tissues. In some case, zoophytophagous insects are also able to complete the development adopting a diet based exclusively on vegetable substrate, although this capacity depend on the characteristics of the host plant; for example, the nutrients obtained from Solanaceous plants are insufficient for *N. tenuis* development (Urbaneja et al. 2005), while are suitable those from *Sesamum indicum* (L.) (Pedaliaceae) (Biondi et al. 2016), due to the huge amount of protein, carbohydrates, and minerals (Mbaebie et al. 2010). In general, however, it seems that predators benefit from complementing or supplementing carnivorous diets with plant nutrients as they improve their fitness (Castañé et al, 2011).

Heteropteran predators feed on plants to acquire from vegetable tissues water and nutrients they need to optimize their extra-oral digestion (Gillespie and McGregor 2000). In fact, from their salivary glands they inject into their prey huge quantities of digestive enzymes

that are diluted in water (Cohen, 1995). However, the presence of a-amylase in the salivary glands of *Geocoris punctipes* (Say) (Heteroptera: Lygaeidae), a predator with facultative phytophagia, evidences that the bug can actively acquire plant nutrients in addition to water (Zeng and Cohen, 2000). Therefore, since they have these enzymes (a-amylase) zoophytophagous predators can produce damage to host plants, although damages are lesser than phytophagous pest, as the amount of a-amylase are lower than those of phytophagous pest (Zeng and Cohen, 2000).

Similarly to phytophagous insects, has been evidenced that zoophytophagous insects, can determine biochemical changes in wounded tissue that result in production of defence responses in host plants, as strict herbivores. Feeding and/or oviposition activities of *N. tenuis* activate on tomato plants the signaling pathway of abscisic acid that determine a repellent effect towards the whitefly *Bemisia tabaci* (Gennadius) (Heteroptera: Aleyrodidae) and the jasmonic acid signaling pathway that induce an attractive effect towards the *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae) one of the main whitefly parasitoids (Pérez-Hedo et al. 2015b; Naselli et al. 2016); moreover HIPVs from *N. tenuis* damaged plants induce attraction of parasitoids via JA signaling pathway also in neighboring undamaged plants (Pérez-Hedo et al. 2015b). Similarly, also the feeding activity of other two zoophytophagous mired, *Macrolophus pygmaeus* (Rambur), and *Dicyphus maroccanus* Wagner activate in tomato plants the JA signaling pathway determining the recruitment of *E. formosa* (Pérez-Hedo, et al. 2015a).

In our system, however, the defensive response of the plants infested by *P. maculiventris* affect the predator, as the plants recruit *T. podisi* by OIPVs emission. On the contrary, the same indirect defensive responses do not occur when plants are attacked by the alien herbivore *H. halys*; indeed, in this case, the egg parasitoid is not attracted by plants on which the alien pest fed and/or oviposited. Martorana et al. (2017) have yet demonstrated that a local parasitoid, *T. basalis*, is not able to exploit OIPVs emitted by *V. faba* plants infested by *H. halys*, suggesting that this lack of response of the wasps is a consequence of the absence of coevolution among the plant, alien herbivore and parasitoid. For *T. basalis*, this inability to use OPIVs to locate *H. halys* eggs could be detrimental to the wasp foraging efficiency, since *H. halys* is a potential suitable host for *T. basalis*; however it avoid that *T. basalis* invest time and energy to locate poor quality host eggs, as *T. basalis* reproductive rate on *H. halys* eggs is low (Martorana et al. 2017). In our system, the inability of *T. podisi* to locate *H. halys* eggs has beneficial consequences in terms of wasp fitness, as *T. podisi* is not able to successfully develop in the alien host eggs (Abram et al.

2014). In this way, therefore, the egg parasitoid avoids that the alien host becomes an 'evolutionary trap' for its progeny (Abram et al. 2014) and, in meantime, it allows the wasps to optimize time and energy by exploiting on cues emitted by suitable local herbivore stink bugs.

Finally, our results show that feeding damages caused by the alien stink bug *H. halys* affect the recruitment of *T. podisi* via OIPVs emitted by *V. faba* plants infested by *P. maculiventris*. The impact of alien insect herbivores on plant volatile-mediated tritrophic signalling is reported in a few cases. In general, the effect of concurrent infestation of both alien and local insects is the disruption of attraction of parasitoids towards HIPVs and OIPVs (Desurmont et al. 2014; Chabaane et al. 2015; Cusumano et al. 2015; Clavijo McCormick 2016). In particular, Martorana et al (2017) demonstrated that *H. halys* is able to disrupt the attraction of the egg parasitoid *T. basalis* towards infested *V. faba* plants when the alien pest and the associated host, *N. viridula* are concurrently present. From the parasitoid point of view, this disruption caused by the alien herbivore may have negative effect as prevent the exploitation of OIPVs making difficult the host searching of *T. podisi*. However, from an applicative point of view, this disruption may have beneficial consequences in terms of biological control perspectives. Indeed, taking into account that in general the damages caused by the predators with facultative phytophagia are relatively low (Albajes and Alomar, 2008), it can be presumed that, due to the *H. halys* disruptive effect on egg parasitoid host location, 1) the progeny of the *P. maculiventris* is less affected by *T. podisi* parasitism; therefore the predator could conduct its predation beneficial activity on phytophagous pests, including *H. halys* that, as reported by Abram et al (2014), is a potential prey; 2) the risk that *T. podisi* locates *H. halys* eggs by exploiting OIPVs from *P. maculiventris* attacked plants is reduced; therefore is also reduced the 'evolutionary trap' risk for *T. podisi* progeny, since the egg parasitoid do not successfully develop in *H. halys* eggs .

However, further study under field conditions is required to better determine this multitrophic interactions and, consequently, efficacy of both predator and egg parasitoid in controlling phytophagous pest.

3.6 References

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Chapter 4

Role of contact semiochemicals in mediating response of local egg parasitoids to an invasive non-associated host

4.1 Abstract

When land on the plant. *T.basalis* females are able to discriminate the gender of its associated host *Nezara viridula* and to distinguish associated from non-associated hosts by exploiting chemical footprints as host-related contact kairomones that induce in *T. basalis* an arrestment and a motivate searching behaviour. Considering that *Halyomorpha halys,* shares the same environments of *N. viridula,* in the field is possible that *T. basalis* arrive on plant with a concurrent infestation of both pentatomid, with leaves where the associated and the non-associated host laid them eggs. In laboratory condition it was investigated if *T. basalis* is able to develop in the eggs of *H.halys*, which of the two pentatomid's egg masses prefer and if *T. basalis* female naïve or rewarded by an oviposition experience has the potential to exploit the chemical footprints left by the non-associated host *H. halys.*

The results showed that: 1) Naïve *T. basalis* spy the chemical footprints left by *H. halys* but did not discriminate between areas contaminated by chemical footprints left by the non-associated host female or male; 2) experienced female of *T. basalis,* re-encountering after 1 hour, discriminated between male and female chemical footprints but they were not able to discriminate *H. halys* gender after 24 hours; 3) *T. basalis* did not shows any difference between the eggs masses of the two pentatomid; 4) *T. basalis* develop in the egg masses of *H. halys* but the successful development occurred with a significant lower percentage on the egg masses of the non-associated host *H. halys* compared to the development percentage of the associated host, finally a high number of egg abortion where observed on the egg masses of *H. halys.*

4.2 Introduction

Successful reproduction of insect parasitoids are linked to adult female behavioural decisions that lead them to find suitable hosts often living in highly complex environments (Mainers and Peri 2013). During their foraging behaviour, parasitoids rely on a series of visual, tactile and chemical stimuli, although the chemical cues play the major role (Vinson 1991; Godfray 1994). Wasps use volatile compounds from the plant or host/plant complex to locate a suitable host habitat at long distance, and low volatile compounds for host location at short distance and for host recognition and acceptance. When the wasps land on a plant they are able to exploit stimuli at short range like chemical footprints (contact kairomones) left by hosts walking on the leaves that induce to wasp females an arrestment and motivated searching behaviour in the contaminated area where the parasitoid are more likely to find the host egg masses (Frati et al. 2013). The system composed by the egg parasitoid *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae), and the southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae) was deeply investigated. Studies have shown that the wasp female was attracted by oviposition activity of *N. viridula* females on bean plants combined with feeding activity (Colazza et al. 1999a; Colazza et al 2004 a,b; Frati et al. 2017; Salerno et al. 2017). Furthermore wasp females of *T. basalis* are able to discriminate chemical footprints left by *N. viridula* females, to which they respond more strongly than those left by walking males or nymphs. Indeed, Colazza et al. (2007) revealed that *n*-nonadecane (nC19), which is present in footprints of *N. viridula* males, acts as a "host sex recognition" cue. The host-induced plant volatiles are probably used from the wasp to locate a potential host community, whereas the chemical footprint left by adults and nymphs elicit parasitoid arrestment and stimulate searching. Finally, the process continues with host recognition, which is mediated by contact chemicals present in the ovariole secretion used by the host as an egg adhesive (Bin et al. 1993; Conti et al. 2003). Comparable cues are used in other *Trissolcus* spp. – pentatomid associations such as *Trissolcus simoni* (Mayr) – *Eurydema ventrale* (Kolenati) (Conti et al. 2003) and *Trissolcus brochymenae* (Ashmead) – *Murgantia histrionica* (Hahn), where the parasitoid also responds to chemical footprints from nymphs and short-range volatiles from the host's eggs (Conti et al. 2003). Indirect host related cues, such as adult footprints, represent reliable cues for egg parasitoids to optimize energy and time by restraining their search to areas where newly laid host eggs are likely to be found (Vet and Dicke 1992; Vinson 1998; Fautoros et al. 2008; Colazza et al. 2010). The systems used for the bioassay in laboratory

condition may differ from those present in the field conditions as plants are often attacked by several herbivore species and thus are involved in multi-trophic interactions (Moujahed et al. 2014; Cusumano et al. 2015; Ponzio et al. 2014, 2016). In fact in the field, host plants could be infested by several local or invasive herbivores species and thus contaminated by a plethora of chemical traces. Consequently, wasp females have developed the ability to distinguish footprints left by associated from those of non-associated host. For example *T. simoni* searches longer on chemical footprints left by its associated host, *E. ventrale*, and responds weakly to contact cues of the non-associated species *M. histrionica* and *N. viridula*. Similarly, *T. brochymenae* strongly responds to chemical footprints left by its associated hosts *M. histrionica*, and weakly to footprints from the non-associated species *E. ventrale* and *N. viridula* (Colazza et al. 2010). Moreover, studies by Borges et al. 2003 showed that also *Telenomus podisi* (Ashmead) (Hymenoptera: Scelionidae) females specifically recognize chemical footprints left on the substrate by walking females of the associated host *Euschistus heros* (F.) (Heteroptera: Pentatomidae). Since host female chemical footprints are the most promising signals of host eggs, for example *T. simoni* and *T. brochymenae* responded strongly only to contact cues from their associated host females *E. ventrale* and *M. histrionica* respectively, wasp females developed and enhanced this strategy to distinguish between footprints left by females and males of their associated hosts (Colazza et al. 1999a; Conti et al. 2004a; Colazza et al. 2007; Salerno et al. 2009). In this scenario, is possible to hypothesize that host sex discrimination ability was strictly related to host specificity. In other words the *T. basalis* evolved the ability to distinguish male and female footprints only to find associated hosts, in this way the wasps invest their energy resources only when they can obtain a reward (Colazza et al. 2009; Conti et al. 2004; Salerno et al. 2009) but what if an alien herbivore invades these infochemical networks?

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae), native from Asia (Lee et al. 2013), is a polyphagous herbivore of over 100 host plants, including agricultural, horticultural and ornamental plants (Leskey et al. 2012; Haye et al. 2014). After its introduction in Italy six years ago, this species is rapidly spreading, and has become a harmful invasive species that causes severe economic losses in orchards and field crops (Wermelinger et al. 2008; Fogain and Graff 2011; Leskey et al. 2012; Rice et al. 2014; Joseph et al. 2015; Haye et al. 2015). From an ecological point of view, *H. halys* has the potential to share the same local community structures of local pentatomid pests, such as *N. viridula*, and like observed previous in this thesis work could determine

important ecological consequences on local tri-trophic webs. In Europe *H. halys*, is known to be attacked in the invaded area by some parasitoid species (Haye et al. 2014; Gariepy et al. 2015; Maistrello et al. 2016; Rice et al. 2014). A few parasitoid species have been recorded from frozen sentinel eggs of this species. Haye et al. (2015) recorded *Trissolcus cultratus* (Mayr) (Hymenoptera: Scelionide) and *Anastatus bifasciatus* (Geoffroy) (Hymenoptera: Eupelmidae) from two areas in Switzerland. Roversi et al. (2016) reported *A. bifasciatus* and *Ooencyrtus telenomicida* (Vassiliev) (Hymenoptera: Encyrtidae) in Tuscany, Italy. In a different Italian region, Umbria, *T. basalis* was collected from soybean fields using frozen *H. halys* egg masses (Rondoni et al. 2017). Among the species collected in Europe to date, only *A. bifasciatus* and *O. telenomicida* are able to complete development in fresh *H. halys* eggs, whereas *T. cultratus* only rarely developed (Haye et al. 2015, Sthal et al, 2018). Until now, it was observed that *T. basalis* was able to develop only from freeze-killed eggs (Rondoni et al. 2017). Furthermore considering that in a previous chapter of this thesis work it was observed that *T. basalis* was not attracted by the Oviposition Induced Plant Volatiles (OIPVs) of non-associated host *H. halys*, to try to complete the association process in this chaphter was investigated if *T. basalis* can spy the chemical footprints of the non-associated host *H. halys* when it is in contact with them.In a scenario where the three species, *H. halys*, *N. viridula* and *T. basalis*, interact with each other, the egg parasitoid *T. basalis* could land on a plant where both pentatomids laid eggs, it was interesting investigate1) if *T. basalis* is able to find host eggs, 2) which egg masses prefer between the egg masses of it associated host *N. viridula* and the non-associated host *H. halys* and 3) if it is able to develop in the eggs of the non-associated host.Moreover, in laboratory condition it was investigated the females ability of *T. basalis* to discriminate between female and male of *H. halys* chemical footprints in a open arena moreover host suitability using fresh egg masses and host preference in arena choice-test between the associated and the non-associated host were investigated. Overall, the outcomes are discussed by evaluating the possible consequences of alien insect spread on local parasitoid foraging behaviour.

4.3 Materials and methods

4.3.1 Insect colonies

Nezara viridula and *H. halys* colonies were held in reared in insect cages ($47.5 \times 47.5 \times 47.5$ cm, BugDorm-44545, MegaView Science Co. Ltd, Taichung, Taiwan), in a environmental room ($24 \pm 1°C$, $70 \pm 5\%$ RH, 16 h:8 h L:D), and fed with a diet of sunflower seeds and seasonal fresh organic vegetables. Food was changed every 2–3 days, and separate cages were used for immatures and adults. Newly developed adults were regularly removed from the nymphal rearing cages and evenly distributed among the adult rearing cages. Paper towels were placed inside each adult cage as an ovipositional substrate. Daily collected egg masses were used to maintain both colony and to carry on the experiments afterwards described. *Trissolcus basalis* colonies were established from wasps emerging from sentinel and or naturally laid *N. viridula* egg masses on cultivated and uncultivated crops. Adult parasitoids were separately reared in 16-ml glass tubes, fed with a solution of honey–water (80:20 v/v), and kept in an incubator ($24 \pm 1°$ C, $80 \pm 5\%$ RH, 16 h:8 h L:D). Collected *N. viridula* egg masses were exposed to parasitoids for 48 h in 16-ml glass tubes, then the eggs were removed and stored for incubation. Male and female parasitoids, after emergence, were kept together to allow for mating.

4.3.2 Parasitoid response to host chemical footprints

Bioassays were conducted in an open arena consisting of a square sheet of filter paper (25X25 cm; wasp/arena ratio: 0.003%). In the centre of the arena, a circular area (6·cm diameter; 28.26 cm^2, about 4.5% of the entire arena; wasp/arena ratio: 0.071%), defined by a cardboard mask put on the filter paper, was exposed for 30·min to a single male or female adult of *H. halys* leaving the surrounding area untreated. To ensure bug legs were in constant contact with the filter paper and at the same time, to avoid surface contamination with bug volatiles, adults were constrained under a steel mesh cover (6·cm diameter, 1·cm high, 0.01·cm mesh) and forced to walk with a special device (Conti et al. 2004). Filter papers contaminated by bug's faeces were not used for bioassays. After removing the bug, one female wasp was gently released in the middle of the circular area (Fig. 9) and its arrestment behaviour was followed by a video tracking system XBug, a video tracking and motion analysis software as described in Colazza et al. (1999) (Fig. 10). When the parasitoid flew away or walked off the arena bioassays were stopped.Adults of *H. halys*

used to treat the inner circular area were either males or females taken from the colony and kept isolated about three days before the bioassays. Experiments were carried out in an isolated room at 25 ± 1 °C illuminated by two 18·cm long fluorescent tubes. All experiments were carried out from 09:00·h to 12:00·h and at 25 ± 1°C. Wasps' walking patterns were scanned with a CCD camera (Sony M370) equipped with a zoom lens and mounted above the centre of the arena. The arrestment responses of the female wasps were quantified over the entire arena (pooling both outside and inside the circular contaminated area) by means of the total arena residence time (s). Three-five days old and mated egg parasitoid females were tested; about 24 h before the start of experiments, wasps were isolated in small vials (1.5 x 5 cm) with a drop of honey/water solution. Naïve wasps (without oviposition experience and contact with host chemical traces) were allowed to acclimatize for at least 1 h in the room before the experiment and after were tested on host female or male chemical footprints. Regarding the experiment with the influence of a successful oviposition experience on the wasps' behavioural responses, single naive *T. basalis* females were released onto a circular area contaminated with chemical footprints of *H. halys* host males or females and with a *H. halys* host egg-mass (five to six eggs) in the middle. After that the wasp parasitized at least one egg, were recaptured and kept isolated in a small vial for one and for twenty-four hours. Experienced wasps were then tested in open arenas treated with chemical footprints from host females or males.

Fig. 9: Responding *Trissolcus basalis* female in arrestment position with antennae kept in touch with the substrate
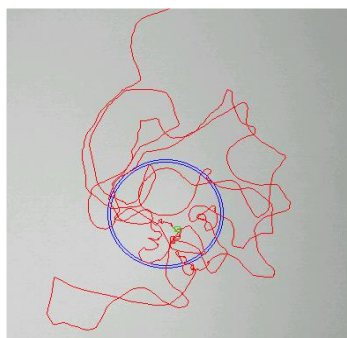
Fig. 10: Screen shot by X-bug software. Red track represents walking path of *Trissolcus basalis* females on *Halyomorpha halys* footprints. Inner circle was the treated area, remaning arena was untreated

4.3.3 Host preference

Two egg masses were created, using respectively tree eggs of the alien host *H. halys* and tree eggs of the associated host *N. viridula.* The two egg masses were glued on filter paper, far 5 mm from each other, inside a small arena (1.5 cm in diameter) made from a Plexiglas body sandwiched between two glass plates (Fig. 11). All eggs used in bioassays were up to 24 h old. Egg parasitoids three-five days old, mated, fed and naive to both egg masses of *N. viridula* and *H. halys* were used for the bioassays. About 24 h before the experiments, wasp females, were isolated in small vials (1.5 x 5 cm) with a drop of honey/water solution. Wasps were allowed to acclimatize for at least one hour in the room before the experiment. A single *T. basalis* female was introduced trough a small hole (place on the side of the small arena) in the middle of the arena between the two artificial egg masses. The behaviour of the parasitoids was observed using an electronic microscope AxioCam ERc 5s (Carl Zeiss Microscopy GmbH). It was considered first choice when the wasp finish to parasitized one egg of the two eggs masses, when the parasitoid marked the egg by sweeping its ovipositor on the chorion surface the bioassay were stopped and the wasp was removed from inside the arena. Finally the two artificial egg masses were stored at the same rearing conditions until parasitoids or nymphs emergence.

Fig. 11: Choice test arena made from a Plexiglas body sandwiched between two glass plates. Inside there are the two egg masses glued on filter paper made each one with three eggs of *Halyomorpha halys* (on the right) and three eggs of *Nezara viridula* (on the left) and a *Trissolcus basalis* female in the moment that choose and start to parasitize the *Halyomorpha halys* egg mass.

4.3.4 Host suitability

The main aim of the exposure of *H. halys* egg masses to local egg parasitoid was to evaluate their potential host suitability ability on alien host eggs. Fresh *H. halys* egg masses were offered to three-day-old mated *T. basalis* in a 16-ml glass tubes for 24 h. Parasitoids were fed with honey water solution and kept in the incubator (24 ± 1° C, 80 ± 5% RH, 16 h:8 h L:D). After 24 h, parasitoids were removed and the containers were stored at the same rearing conditions until parasitoids or nymphs emergence. Emergence of parasitoids, mortality of *H. halys* eggs and nymphal emergence were recorded (Fig. 12).

Fig. 12: *Trissolcus basalis* female that parasitize a *Halyomorpha halys* egg masses.

4.3.5 Statistical analysis

For the response to chemical footprints the residence time of wasp females were statistically analysed using Student's t-tests for paired comparisons. All statistical analyses were done using Statistica 6.0 for Windows (StatSoft Inc., Tulsa, OK, USA). First choose for the host preference and number of *T. basalis*, nymphs emergenced and egg abortion for the host suitability were statistically analysed with χ^2 tests, using Bonferroni correction to find significant differences. These computations were carried out with the R 2.14.1 software (R Development Core Team, 2011).

## 4.4 Results

Parasitoid response to chemical footprints

Naïve *T. basalis* females, spy chemical footprints but did not discriminate between areas contaminated by chemical footprints left by a host female versus host male. Arena residence time of female wasps was almost equal when placed on areas contaminated by host female versus host male chemical footprints (t = 0.888, *P* = 0.38;df = 24) (Fig.13).
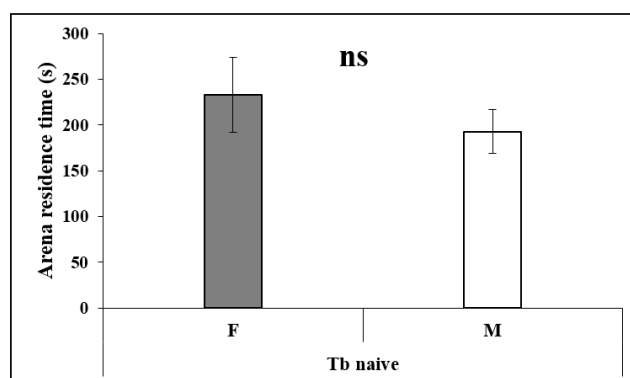
Fig. 13: Searching time of naïve females of *Trissolcus basalis* exploring an artificial substrate contaminated with chemical footprints laid by adult males (M) and females (F) of the non-associated host *Halyomorpha halys*. Bars indicate the duration of the residence time of wasp females (mean ± s.e.m); ns = not significant (Student's *t-test* for independent samples).

Trissolcus basalis females were influenced by previous successful oviposition experience. Rewarded females re-encountering after 1-h on host female footprints showed longer arena residence time and increased arrestment responses compared to rewarded females encountering host male chemical footprints (t = -3.72; *P* = 0.001; df = 21) (Fig 14a). Finally female wasps re-encountering *H. halys* chemical footprints after 24-h showed no

discrimination between areas contaminated by chemical footprints left by a host female or host male (t = -0.005; *P* = 0.99; df= 9) (Fig.14b).

a)

b)
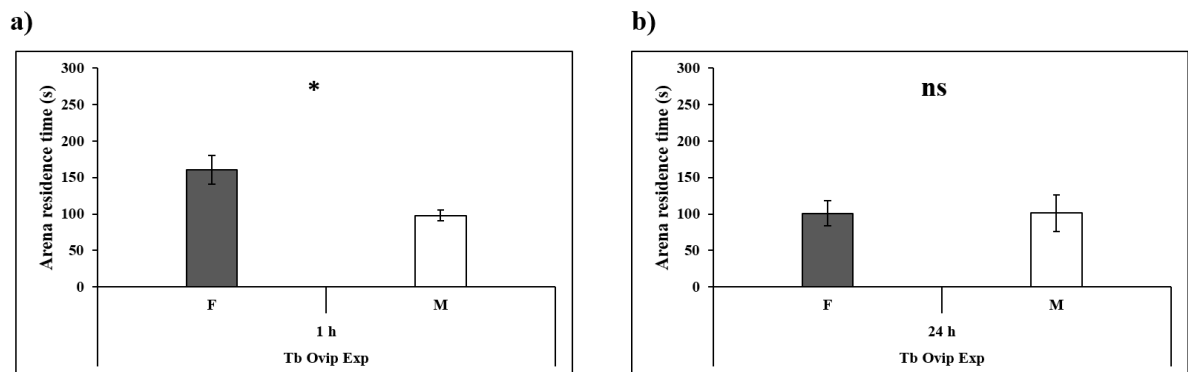
Fig. 14: Searching time of experienced females of *Trissolcus basalis* re-encountering after 1-h (a) and after 24-h (b) exploring an artificial substrate contaminated with chemical footprints laid by adult males and females of the non-associated host *Halyomorpha halys*. Bars indicate the duration of the residence time (mean ± s.e.m) of wasp females. Asterisks (*) indicate p<0.05, ns indicate not significant (Student's t-test for independent samples).

Host preference

No preference were observed when naïve *T. basa*lis females were tested in arena choice test between the two artificial egg masses of the associated host *N. viridula* and the non-associated host *H. halys*. ($\chi^2 = 0.0324$, df = 1, P = 0.8572; n = 27) (Fig.15).
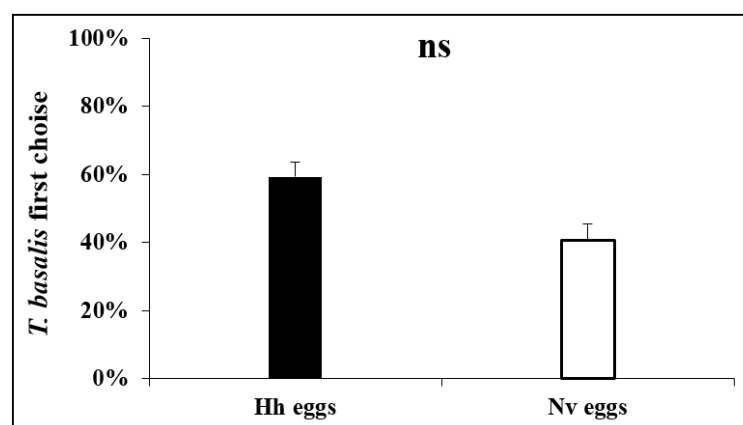
Fig. 15: Host preference of the parasitoid *Trissolcus basalis* in arena choice test between the two a egg masses of the associated host *Nezara viridula* (Nv) and the non-associated host *Halyomorpha halys* (Hh) Bars indicate the percentage of first choice ; ns indicate not significant by χ^2 tests .

Host suitability

Trissolcus basalis develop in the eggs of the non-associated host *H. halys* but with a low percentage (6%) compared with the highest percentage (81%) of development of the wasp on the eggs of the associated host *N. viridula* (χ^2 = 64.707, df = 1, P <0.01; n = 27); besides on the eggs of the non-associated host *H. halys* an highest percentage of egg abortion were observed (75%) compered with the percentage of eggs abortion observed on the egg of the associated host (2%) (χ^2 =68.214, df = 1, P < 0.01; n = 27). Finally no difference were observed on the percentage of nymphs emerged on the egg masses of the associated and the non-associated host (respectively 14% and 19%) (χ^2 = 0.00030788, df = 1, P < 0.986; n = 27) (Fig 16).
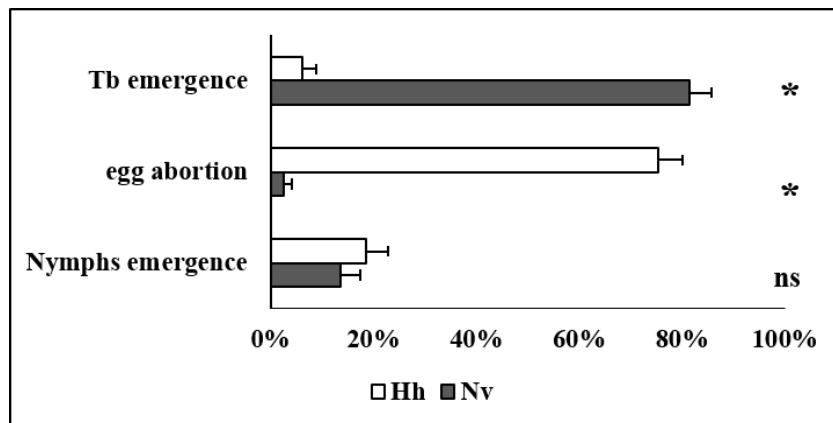
Fig. 16: Host suitability of the parasitoid *Trissolcus basalis* on fresh egg masses of the associated host *Nezara viridula* (Nv) and the non-associated host *Halyomorpha halys* (Hh). Bars indicate the percentage of *Trissolcus basalis* emergence, the percentage of nymphs emergence and the percentage of no emergence. Asterisks (*) indicate p<0.05 ns, indicate not significant by χ^2 tests

4.5 Discussion

In the second chapter of this thesis work was demonstrated that in the laboratory tests the host selection sequence between *T. basalis* and the non-associated host *H. halys* was not completed due to the lack of parasitoid responses to volatile cues. In the present chapter, appeared more evidentet that the development of a new association between *T. basalis* and the non-associated pentatomid *H. halys* could be more unreliable under field conditions due to the partial response to contact cues and to the low host egg suitability. For first was demonstrated that *T. basalis* perceive chemical footprints of the non-associated host *H.*

halys, but it does not have the ability to discriminate female from male differently in presence of chemical footprints of it associated host *N. viridula T. basalis* is able to discriminate between the two sex (Colazza et al. 2007).The inability to discriminate host sex in non-associated hosts could be explained in terms of costs that insects should sustain to acquire the relevant information to tell the host apart from non-host, costs that are not acceptable when the information is poor (Peri et al 2013). Chemical footprints represent, for *Trissolcus* species, indirect host related contact cues that induce wasp females to search longer ('motivated searching') towards the target indeed they reinforce the response by systematically returning to stimuli after losing contact (Vinson 1998; Colazza et al. 2010). If not rewarded by successful oviposition, wasps gradually lose their motivated searching response and regress to general host searching behaviour, as reported for *T. basalis* on *N. viridula* chemical footprints (Peri et al. 2006). The strength of wasp female responses to chemical footprints left by associated hosts could be considered a step of a pairwise coevolution of insect host-parasitoid associations shaped by the natural selection (Goodfray 1994). For example in laboratory analysis by Salerno et al. (2006), *T. basalis* females showed a motivated search behaviour when in contact with chemical footprints left on filter paper by three species of pentatomid bugs, e.g. *E. ventrale*, *M. histrionica* and *Graphosoma semipunctatum* (F.) (Heteroptera: Pentatomidae) but the response was less intense than in the presence of chemical footprints left by the associated host *N. viridula*. Moreover, Tognon et al (2018) confirmed this theory; indeed in experiment on multi-choose test on eggs masses of three pentatomid *Euschistus heros* (F.) (Heteroptera: Pentatomidae), *Piezodorus guildinii* (Westwood) (Heteroptera: Pentatomidae) and *N. viridula,* they observed that *T. basalis* had a specific preference to *N. viridula* egg masses, independently of the host from which the wasp emerged. Moreover, *T. basalis* females are also able to adjust their innate behavioural response towards chemical traces according to prior experience. Here were observed that rewarded females of *T. basalis* re-encountering after 1-h increased the intensity of response to host female chemical footprints towards host male chemical footprints of the non-associated host *H. halys* suggesting that experience is likely to play an important role in the host location process of egg parasitoids. A possible explanation is that foraging insect parasitoids are known to learn which environmental stimuli are associated with rewarding or aversive outcomes to improve their chance of future host location and hence their reproductive success (Lewis and Tumlinson 1988; Lewis and Martin 1990; Vet and Groenewold 1990; Petitt et al. 1992; Turlings et al. 1993; Dutton et al. 2000). *Trissolcus basalis* re-encountering on *H.*

halys chemical footprints after 24-h showed a significantly reduced arrestment response compered with females re-encountering after 1-h and lost the ability to discriminate between areas contaminated by chemical footprints left by a host female or host male. These results presented here shows that oviposition experience enhanced the arrestment responses of the wasps when they were associated with host female chemical footprints, and this appears to be congruent with the form of experience defined as 'α-conditioning' (Vinson, 1998) where host or host's products can provide a reward to foraging that increases the innate response to a stimulus. Generally, experience effects on 'α-conditioned' wasps are not permanent confirming the results found, where after 24 hours *T. basalis* showed a significantly reduced arrestment response and lost the ability to discriminate between areas contaminated by chemical footprints left by a host female or host male (McAuslane et al. 1991; Peri et al. 2006, 2013, 2016).

Furthrmore, considering that *H. halys* share the same host plants of *N. viridula* was interesting try to understand what happens when *T. basalis* could has the possibility to choose between the egg masses of the two pentatomid.. The results in arena-choice test showed that female of *T. basalis* did not have any preference between the two egg masses. It is not the first time that this result has been observed with *T. basalis* in the confined arena, in fact Salerno et al (2006) reported that wasp females responded more strongly to the eggs of *G. semipunctatum* than to those of its associated host, *N. viridula.* The reason for this kind of response may be due to the concentration and/or composition of the chemicals involved, which need to be better investigated. Finally, our results shows that *T. basalis* develop in the egg masses of *H. halys* but the successful development occurred with a significant lower percentage on the egg masses of the non-associated host *H. halys* compared to the development percentage of *T. basalis* on the egg masses of the associated host. An interestingly result was the high number of egg abortion when *T. basalis* parasitized the egg masses of *H. halys*. Parasitoid-induced host egg abortion may be particularly common in novel host-parasitoid associations, as a result of a lack of shared evolutionary history (Schlaepfer et al. 2005). Previous laboratory experiments conducted with North American (*T. podisi*) and European [*Trissolcus semistriatus* (Nees), *Trissolcus scutellaris* (Ashmead), *T. cultratus* and *Telenomus chloropus* (Thomson)] scelionids demonstrated that these parasitoids readily oviposited in *H. halys* eggs (Abram et al. 2014; Haye et al. 2015). However, from fresh egg masses parasitoid offspring rarely or never completed their development. Considering the high number of egg abortion probably in the field the number of parasitoids that attack *H. halys* eggs could be underestimated (Haye et

al. 2015). High levels of host egg abortion may be particularly common, and would generally be considered desirable (compared to not killing the host) for biological control, when assessing the impact of indigenous parasitoids on invasive pests (Abram et al. 2016). The results, presented in this chapter, support the potential of such an approach for improving our understanding of the mechanisms of host specificity in parasitoids, for further biological control program. Future research exploring how associated and non-associated species influence parasitoids host foraging behaviours and host preferences are needed to understanding the possible threats to successfully control of the alien pest *H. halys*

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Concluding remarks

Successful reproduction of egg parasitoids is linked to adult female behavioural decisions that lead them to find suitable hosts often living in highly complex environments. It is know that wasps female use volatile compounds from the plant or host/plant complex to locate a suitable host habitat at long distance, and low volatile compounds for host location at short distance and for host recognition and acceptance.

The dissertation focuses on the important question of what happens when an alien insect arrives in a new environment and has to interact with the local info-chemical network.

In detail, on the first chapter is presented a general introduction on the study system and on the strategy of host searching behaviour of egg parasitoids outcomes are discussed in the chapter by evaluating the possible consequences of alien insect spread on local parasitoid host location process.

In the second chapter it was demonstrated that the alien herbivore *Halyomorpha halys* could interfere with the local tritrophic system *Vicia faba* L. – *Nezara viridula* (L.) (Heteroptera: Pentatomidae) – *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae). First, the plants of *V. faba* infested by *H. halys* do not recruit the egg parasitoids differently with what happen when them are infested with the local host *N. viridula.* Moreover, was detected that *T. basalis* females, which usually respond to plant volatiles induced by *N. viridula* feeding and oviposition are not attracted by plants that were exposed to *H. halys* feeding and oviposition. The lack of response of *T. basalis* to OIPVs from *H. halys* infested plants could be interpreted as a direct consequence of the absence of coevolution among the plant, alien herbivore and parasitoid. The results also show that the alien stink bug *H. halys* disrupts *T. basalis* attraction towards OIPVs emitted by *V. faba* plants.

In the third chapter first, it was showed that the feeding and oviposition of the zoophytophagous predator, *Podisus maculiventris* (Heteroptera: Pentatomidae), on the host plant, *V. faba,* induces emission of plant volatiles that recruit its associated egg parasitoid, *Telenomus podisi* (Ashmead) (Hymenoptera: Scelionidae). On the contrary, *T. podisi* is not attracted by VOCs from broad bean on which the alien herbivore and non-associated host, *H. halys*, deposit eggs, even more, when the alien insect lay its eggs concurrently with the zoophytophagous predator. This finding is remarkable as it shows that also the attack of a predator with facultative phytophagy might induce the host plant to emit VOCs that recruit egg parasitoids as well as a phytophagous pest, for example in the system *V. faba - N.*

viridula – T. basalis. However, in both systems described in the second and in the third chapter, the parasitoid attraction toward OIPVs emitted following the attack of the associated host was disrupted when associated host and non-associated host were concurrently present on the same plant remarking that the invasion by an alien herbivore disrupt the local established semiochemical webs. In the fourth chapter the influences of the non-associated host *H. halys* affecting the host location behaviour was discussed by using the parasitoid explotation of the chemical footprints by the non-associated host *H. halys* as a tool. The results showed that naïve *T. basalis* females spy the chemical footprints left by *H. halys* but did not discriminate between chemical footprints left by the non-associated host female or male. Furthermore, experienced female of *T. basalis,* re-encountering after 1 hour, discriminated between male and female chemical footprints but they were not able to discriminate *H. halys* gender after 24 hours. Furthermore, considering that *H. halys* share the same enviroments of *N. viridula* in the fourth chapter was investigated the preference of *T. basalis* between the egg masses of the associated host *N. virdula* and the non-associated host *H. halys.* The results showed that female of *T. basalis* did not have any preference between the egg masses of both pentatomid may be due to the concentration and/or composition of the chemicals involved, which needs to be better investigated. The last step studied was the host suitability of *T. basalis* towards *H. halys* and *N. viridula* egg masses, the results showed that *T. basalis* develop in the egg masses of *H. halys* but the successful development occurred with a significant lower percentage on the egg masses of the non-associated host *H. halys* compared to the development percentage of *T. basalis* on the egg masses of the associated host. Moreover the emergence of nymphs is lower from the egg masses of both pentatomid. A high number of egg abortion when *T. basalis* parasitized the egg masses of *H. halys* where observed. Parasitoid-induced host egg abortion may be particularly common in novel host-parasitoid associations, as a result of a lack of shared evolutionary history. High levels of host egg abortion may be particularly common, and would generally be considered desirable (compared to not killing the host) for biological control, when assessing the impact of indigenous parasitoids on invasive pests.

Further prospective

Halyomorpha halys has the potential to share the same local community structures of local pentatomid and determine important ecological consequences for the local tritrophic webs depending on the wasp ability to recognize the herbivore and successfully develop in *H. halys* eggs. Determine the host selection strategies in egg parasitoids would be important to define release methods and spreading, select egg parasitoids, evaluate their specificity and assess the risk of their introduction in classical biologiocal control. More generally, these studies would be fundamental for biological control program. However, some limitations to the acquisition of such knowledge depending that bioassays were conducted mainly in the laboratory. For one hand laboratory investigations clearly tend to simplify the systems, they are useful to understand single interactions or more complex interactions but for the other hand for understand the real complexity of multitrophic interactions is important to carry out studies in more realistic conditions, i.e. semi-field and/or field. Furthermore, the measure of the parasitoid induced egg abortion could be a new tool for the biological control.

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