UNIVERSITÀ DEGLI STUDI DI PALERMO

Dottorato di ricerca in "Scienze Agrarie e Forestali"
"Gestione fitosanitaria eco-compatibile in ambienti agro-forestali ed urbani"
Dipartimento Scienze Agrarie e Forestali
Settore scientifico disciplinare AGR/11

The role of chemical cues in the host finding behaviour of *Trissolcus basalis* from a Conservation Biological Control perspective

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CICLO XXV
2015

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Acknowledgement

One of the joys of completion is to look over the journey past and remember all the people I had next to me who have helped and supported me along this fulfilling road. I wish to thank all the people met, my friends and my family. Without them, this thesis would not have been achievable. It is true that “When it is all over, it is not who you were, it is whether you made a difference” and this last three years taught me that. It was not easy to write this thesis. This was the first research I did and I was really struggling to combine everything I learned about doing research in an actual dissertation. Sometimes I experienced setbacks and at times I even doubted whether I would be able to write a whole PhD thesis. Fortunately, I had experienced researched that really supported any my step. For this I am very grateful. I found that taking into account the diverse opinions not only I made decisions more balanced, but also I improved them.

First I want to thank Prof. Stefano Colazza to be always available to offer me his contribution during all phases of my research work. I am grateful for giving me the chance to be involved in the BUGSIE project and the opportunity to carry out a wonderful international experience.

I gratefully acknowledge my tutor, Dr. Ezio Peri for the esteem he showed me, for providing thoughtful and detailed comments on this dissertation and for having always lavished to ensure that I could do experiences constructive to my growth as a PhD, sharing with me his professional and personal experience. This meant a lot to me.

I would like to express my heartfelt gratitude to Dr. Michael Rostàs, which made the “kiwi year” at Lincoln University in New Zealand a great time. I thank him for providing me a rich and fertile environment to study and explore new ideas. He taught me to not stop asking new questions every time they run out and how to start

over in front of new hypotheses. I appreciate his dedication to help me in my research and his example of intelligence, joy, enthusiasm and love for the research.

I thank Prof. Steve Wratten for his kindness, for the encouragement to keep on and for his constant interest and enthusiasm for the results of this work.

I thank all the staff of The Bio-Protection Research Centre in New Zealand for the warm welcome from the first day. I felt myself as part of their group, as a part of their family. The group has been a source of friendships and fun. I miss you all very much.

I thank Dr. Kye Chung Park for the months spent in "New Zealand Institute for Plant and Food Research Ltd". I very much appreciated his advices and the important knowledge I have gained during the conduction of electrophysiological experiments, included in the third chapter of this thesis.

New Zealand has been enjoyable and unforgettable time as well as growth opportunity. My time In New Zealand was just great and in large part due to the many friends that became a part of my life. I am grateful for the time spent with my flat mates and friends and the homemade dinners we enjoyed together, for the memorable trips around New Zealand and the "pictures" I will keep in my mind forever.

I wish to thank all my colleagues and the PhD students I met during the PhD period with whom I shared commitments, anxieties, worries and frustrations, as well as ideas and satisfaction. Some of them are Friends now and this was a treasure discovered by accident in this adventure and without whom this PhD would never be so valuable.

Lastly, I thank, immensely, my brothers and my friends, for all their love and encouragement, and my parents who raised me with love and to whom I dedicate this thesis. There are no words to convey how much I love all of you.

Palermo, January 13th, 2015

Thank you!

Maria Cristina

Chapter 1: Conservation biological control

Abstract

Conservation biological control (CBC) aims to improve conditions for natural enemies in agricultural landscapes and has the goal of reducing pest species below threshold level to avoid the need for synthetic chemicals. Conservation biological control in agroecosystems requires a landscape management perspective, using non crop plants within the crop. The plant species pool in the surrounding landscape and the distance of crop from natural habitat are important for the conservation of enemy diversity, indeed, structurally complex landscapes with high habitat connectivity may enhance the probability of pest regulation. Within-crop habitat manipulations have the potential to increase the biological control of pests. Wildflower strips are an important tool in habitat management and have been shown to increase the abundance of natural enemies. However, this enhancement might be achieved by providing the "right" diversity. The ultimate goal of the use of wildflowers is to provide shelter for the natural enemies to enhance their persistence in perennial and annual crops and to represent a good nectar sources, to yield longevity and fecundiy to the natural enemies. Moreover, food sources from plants that are highly attractive to parasitoids are more likely to be visited than food sources that are poorly detectable. Finally, it turn obvious that include biodiversity may dial with some issues such as the possibility that the food sources could benefit the pest itself or the antagonists of the natural enemies.

Keyword: Conservation biological control, Habitat management, wildflowers strip.

Riassunto

Conservation biological control (CBC) si pone l'obiettivo di migliorare le condizioni per i nemici naturali nei paesaggi agricoli e ridurre il numero di specie fitofaghe per evitare la di sostanze chimiche di sintesi. Conservation biological control negli agroecosistemi richiede una prospettiva di "Habitat managment", utilizzando specie spontanee all'interno degli impianti colturali. Infatti, paesaggi strutturalmente più complessi possono migliorare il controllo dei nocivi da parte dei parassitoidi. Di conseguenza, all'interno dei sistemi colturali, l'Habitat managment ha il potenziale di favorire ed aumentare l'efficienza dei prarassitoidi per il controllo biologico. Le strisce fiorali (wildflower strips) sono uno strumento importante nell'Habitat managment e favoriscono la persistenza e l'abbondanza dei nemici naturali. Questo miglioramento può essere ottenuto fornendo la "giusta" biodiversità. L'obiettivo finale dell'utilizzo delle strisce fiorali è (i) fornire un riparo per i nemici naturali per migliorare la loro persistenza in colture sia perenni che annuali e (ii) rappresentare una fonte di cibo (es. il nettare fiorale), incrementando così la longevità e fertilità dei nemici naturali. Inoltre, selezionare piante che sono attrattive per parassitoidi incrementa la possibilità di localizzazione di tali risorse fiorali da parte dei parassitoidi. Infine, risulta anche importante considerare che, aumentando la biodiversità, fonti di cibo utili per i nemici naturali, potrebbero anche beneficiare il fitofago stesso o gli antagonisti dei nemici naturali.

Parole chiave: Conservation biological control, Habitat management, strisce fiorali.

Introduction

The term biological control was coined in 1919 by Smith defining it as the control or regulation of a pest population by natural enemies. The concept of this approach is the use of living organisms such as predators, parasites and other antagonists to suppress the population of specific pest organisms or making them less damaging (Eilenberg et al., 2001). Biological control strategies deal with issues related to the fact that chemical pesticides can cause negative side-effects, *e.g.* on human health and on the conservation of the complexity of the environment. The biological control approach includes a variety of strategies that are used in different contexts. One of these strategies is called "Classical biological control" which involves the intentional introduction of exotic natural enemies. Generally, these are host specific and respond in a density-dependent way, meaning that the natural enemy population increases with the host population. The ultimate purpose is to establish the natural enemy, *i.e.* to have a permanent occurrence of an imported predator or parasitoid in a new environment, which ideally results in the complete control of the pest organism. If successful, no other control methods are required due to the effectiveness of the biological control agent. Classical biological programs have been criticized because their success rate is often low. Indeed, Greathead and Greathead in 1992 and Gurr and Wratten in 2000 concluded in their analyses of the success rates of predator and parasitoid introductions to control insect pests that only 33.5% of predators and parasitoids released have become established and only 11.2% resulted in complete control of the insect pests. Classical biological control programs are more successful in relatively stable habitats, orchards and forests but less so in simpler systems which lack complex food webs (Gurr and Wratten 2000). Bianchi et al., (2006) reviewed various studies and concluded that the density of the natural enemies tends to be higher in more complex agroecosystems. Moreover, Gurr and Wratten in 1999 suggested that low availability of key resources for natural enemies such as

alternative food and overwintering sites in many agroecosystems is one of the reasons that limits biological control effectiveness. It is therefore evident that landscape structure affects biodiversity and natural pest control effectiveness. Several reviews, such as Barbosa in 1998, Pickett and Bugg in 1998, Landis et al. in 2000 and Gurr et al. in 2000, underline the upsurges of "actions that preserve and protect the natural enemies". These actions characterize a biological control strategy called Conservation Biological Control (CBC) (Wratten et al., 2000). While classical biological control is considered a "stand alone" strategy, CBC is part of an integrated pest management (IPM) approach based on modifications of the environment to protect and enhance natural enemies and thus increase their impact on the agroecosystem (DeBach 1964; Barbosa 1998; Coll 2009). Conservation biocontrol practices involve the provision of supplementary resources for parasitoids through habitat management (Landis 2000), which can reduce their mortality and enhance permanent establishment (Rahat et al., 2005). Habitat management may be applied at the within-crop, within-farm, or landscape level. Many of the proximate factors, *e.g.*, lack of adult food, alternative hosts, shelter, overwinter sites, are identified as limiting the effectiveness of the natural enemies in the biological control. In general the diversity in the agroecosystems may favour the reduction of the pest pressure by enhancing the activity of natural enemies. In this context, it begin first necessary identify the important elements of diversity to provide in the agroecosystem. (van Emden 1990; Wratten and van Emden 1995; Gurr et al. 1998; Barbosa 1998). The following paragraph will address this topic.

Some parasitoids are able to obtain resources from hosts, others require access to non-host foods. For example, floral nectar is taken by many species, and can yield in increased rates of parasitism, and thus parasitoid's fecundity, due to the suitable sugar sources effects the longevity of the natural enemies (Schmale et al., 2001; Wäckers 2001 and 2003).

Therefore, CBC thought the environment manipulation may influence the parasitoid's nutritional state and their behaviour (*e.g.*, searching efficiency), increasing their effectiveness and impact on target pests (Landis et al., 2000; Wratten et al., 2003; Jervis et al., 2004).

Mechanism for Habitat Management: ecological value of non-crop habitats

Habitat manipulation approaches seek to identify the potential mechanisms to conserve natural enemies such as provision of shelter, alternative hosts or prey, overwintering sites and food plants from which nectar and pollen may be obtained (Landis et al., 2000; Gurr et al., 2004; Jonsons et al., 2010).

Manipulating crop and non-crop plant species within an agroecosystem can play a key role in pest control (Hickman and Wratten 1996; Baggen and Gurr 1998). Unlike in non-agriculture system, in cropping system interactions between natural enemies and herbivores are more limited and this is even more the case for annual monocultural cropping systems where non-crop vegetation is reduced or removed (Landis 2000; Balmer 2014). A specific tool in habitat management is the addition of non-crop plants to a crop area in form of companion plants or wildflower strips. Companion plants are expected to have repelling and/or intercepting effects on pests and pathogens and to attract natural enemies by providing them with food (Gurr and Wratten 1999; Parolin 2012) such as nectar in the case of adult parasitoids (Jervis et al., 1992; Heimpel and Jervis 2005). Food provision may enhance the abundance and diversity of natural enemy species (Bianchi 2006). Wildflower strips are defined by Nentwig et al., (2000), as ecological compensation areas giving for many species attractive biodiversity and nutritional resources. The intentional provision of flowering plants and plant communities in managed landscapes to enhance natural enemies is a growing aspect of CBC (Gurr et al., 2004). This is accomplished by

selecting and establishing plants within the managed system in order to provide a limited resource such as floral nectar but also additional ecosystem services as *e.g.* regulating the microclimate, affecting hydrological and biochemical cycles and a variety of biological processes. Landscape complexity favourably enhances the establishment of natural enemies and their success in controlling the target pest (Landis 2000). The food webs offer ecosystem services (Farber et al., 2002), increasing the effect of natural enemies on pest populations, potentially mitigating harmful conditions (such as the use of pesticides) or enhancing favourable influences on the natural enemies. This enhancement might be achieved by providing the "right" diversity of alternative food sources, (Landis et al., 2000). Several authors emphasized the need to identify the most important resources for the natural enemy (*e.g.*, shelter, accessibility and quality of the nectar resources) to optimize habitat manipulation (Wratten and van Emden, 1995; Jonsson, 2010).

Shelter is important to enhance the persistence of natural enemies in perennial and annual crops. The presence of a diverse vegetation throughout the year, including winter, warrants a high abundance of the natural enemies. In the late season suitable overwintering sites are rare (particularly in agricultural fields) and non-crop habitats thus become very attractive (Barbosa 1998 and Bianchi et al., 2006). Non-crop habitats in rural landscapes are often associated with beneficial organisms supporting alternative hosts and prey for parasitoids and predators, such as carabid beetles (Varchola and Dunn 2001), spiders (Schmidt and Tscharntke 2005), coccinellids (Honek 1989), syrphids (Cowgill et al., 1993) and parasitoids (Kruess and Tscharntke 1994).

Laboratory experiments have been used to identify which flowering plants may represent good nectar sources for parasitoids. Significant differences exist in the accessibility of nectaries as a result of floral architecture (Vattala et al., 2006). Plant morphology traits such as surface area, foliar pubescence or waxy leaf surface can also influence nectar accessibility (Vinson 1976). For example, a negative effect of

plant pubescence on the level of parasitism has been shown in the whitefly parasitoid *Encarsia formosa* Gahan (Van Lanteren et al., 1977). Patt et al. in 1999 showed that floral architecture influenced the suitability of flowering plants for two parasitoids of the Colorado potato beetle (*Leptinotarsa decemlineata* Say). They determined that *Edovum puttleri* Grissell fed effectively only on flowers with exposed nectaries, while *Pediobius foveolatus* Crawford could also utilize flowers with partially concealed nectaries. Furthermore, the suitability of a flower species for providing nectar to a parasitoid is also dependent on the quality of the nectar. As a consequence, accessible nectars need to be analyzed for sugar composition and for the effect of the sucrose/(glucose + fructose) ratio. Longevity studies indicate that distinct differences exist between insects in their ability to utilize particular sugars (Ferreira et al., 1998). Vattala et al., in 2006, for example, investigated the effects of seven flower species on the longevity of *Microctonus hyperodae* Loan, a parasitoid of the Argentine stem weevil, *Listronotus bonariensis* (Kuschel). They found that *M. hyperodae* was unable to access nectar of red clover, white clover, alyssum and phacelia flowering plants, but was able to gain access to the nectar of buckwheat, coriander and white mustard. However, only buckwheat and coriander increased its longevity. Analysis of the sugar composition showed that buckwheat nectar was characterized by a higher sucrose/(glucose+fructose) ratio than the nectar from coriander and white mustard.

Based on the nectars' sucrose/hexose (mainly glucose and fructose) ratios, Baker and Baker (1983), classified floral nectar in four ratio classes: hexose-dominant (<0.1), hexose-rich (0.1–0.499), sucrose-rich (0.5–0.99) and sucrose-dominant (>0.99). Many examples are reported about parasitoid preference to sucrose-dominant floral nectars (Watt et al., 1974; Baker and Baker, 1983; Patt et al., 1999 and Wäckers et al., 1996).

The olfactory attractiveness and plant-associated visual cues play an essential role for foraging parasitoids (Belz et at 2013; Barbosa 1998). Food sources that are highly attractive are thus more likely to be visited than food sources that are poorly

detectable (Wäckers 2004). Experiments in a Y-tube olfactometer to the parasitoid *Microplitis mediator* (Haliday) (Hymenoptera: Braconidae) to test the attractiveness of bishop's weed, cornflower, buckwheat, candytuft, and oregano flowers showed that cornflower and candytuft were equally attractive and more attractive than buckwheat (Belz et al., 2013).

Similarly, some studies have shown that parasitoids respond to colour and other visual cues (Wäckers and Lewis, 1994). Way and Murdie in 1965, found that Brussels sprout cultivars with light green glossy leaves were more attractive to parasitoids than the darker green, waxy-leaf cultivars. In contrast, other parasitoids do not appear to respond to colour. For example, the number of visits by *Diadegma insulare* (Cresson) (a parasitoid of the diamondback moth) to yellow and white flowers of several crucifer species did not differ (Idris and Grafius, 1997).

Therefore, the attractiveness of flowers is a key aspect for parasitoid population dynamics and that should be taken into account in selecting flowering plants used for habitat management in CBC (Bianchi and Wäckers 2008).

Negative aspects of added Habitat Diversity

Probably the most obvious potential disadvantages of increasing habitat diversity are that some land may be taken out of production and that the provision of resources can provide benefit to the pest itself or to antagonists of natural enemies (Araj et al., 2008, 2009). The former may be a major concern for high-value crops where the economic benefit is predominant. The latter may occur if the resources also benefit the pest. Bagged et al., (1999) showed that care must be taken to select the appropriate pollen and nectar source. For example, flowering buckwheat and dill benefitted both *Copidosoma koehleri* Blanchard and its host the potato pest

Phthorimaea operculella Zeller. In contrast, other plants such as phacelia and nasturtium benefited only the parasitoid.

Understanding the ecological mechanisms underlying CBC by knowing the effects of non-crop plant addition to agroecosystems is one of the first issues that need to be addressed in order to maximize control efficiency while avoiding unwanted side-effects.

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Chapter 2: Host finding behaviour of *Trissolcus basalis*

Abstract

Trissolcus basalis (Wollaston) is a solitary egg parasitoid of several pentatomid bugs. In particular, it is the most important biological control agent of the green vegetable bug *Nezara viridula* (L.), a pest of a wide variety of economically important crops. During the host location process *T. basalis* females explore a great variety of volatile and contact semiochemicals from the host-plant complex. Indeed, wasps are able to exploit volatile oviposition-induced synomones, volatile cues from virgin males and preovipositing females, and, when they land on a plant, chemical footprints left by bugs walking over the leaves. In particular, chemical footprints represent a set of indirect host-related contact kairomones that induce arrestment and motivated searching behaviour, that allow wasps to optimize energy and time by restraining their search to areas where there is a high probability of finding newly laid host eggs. Wasp females have an innate response to host chemical residues, with a strong preference for *N. viridula* female footprints; however, this response can be modified by the experience.

Keyword: Trissolcus basalis, footprints, host-indirect related cues.

Riassunto

Trissolcus basalis è un sparassitoide oofago olitario di diversi pentatomidi e il più importante agente di controllo biologico della cimice verde *Nezara viridula*, fitofago di numerose colture economicamente importanti. Durante il processo di localizzazione dell'ospite, femmine di *T. basalis* esplorano una grande varietà di volatili e semiochimici di contatto originati dal sistema ospite-pianta. Infatti, le femmine di *T.*

basalis sono in grado di esplorare sinomoni indotti dall'ovideposizione, segnali provenienti da maschi vergini e da femmine in stato pre-ovideponente e, una volta raggiunta la pianta, dalle tracce chimiche lasciate dall'ospite sulle foglie. In particolare, le tracce rappresentano un insieme di cairomoni di contatto indiretti indotti all'ospite che inducono arresto e un comportamento di ricerca motivato che permette alle femmine di ottimizzare tempo ed energia restringendo l'area di ricerca dove c'è una più alta probabilità di trovare ovature appena deposte. Femmine di *T. basalis* mostrano una risposta innata ai residui chimici lasciati dall'ospite con una più accentuata preferenza per le tracce lasciate da femmine di *N. viridula*, sebbene questa risposta possa essere modificata dall'esperienza.

Parole chiave: *Trissolcus basalis*, trace chimiche, segnali indiretti indotti dall'ospite.

Introduction

The host finding behaviour of parasitoids consists of steps known as host habitat location, host location, host recognition, and host acceptance (Vinson 1998). At distance, parasitoids of herbivorous insects use visual signals and volatile chemicals emitted by the infested plant (Vet and Dicke 1992; Turlings and Wäckers 2004) and by the surrounding vegetation. Parasitoids of herbivorous insects need to cope with this complexity to locate their suitable hosts in this such complex chemical environments, while searching on one hand for hosts and on the other hand for food. During the host location process, females encounter and explore a great variety of stimuli termed semiochemicals (Vet and Dicke 1992; Godfray 1994; Vinson 1998). Semiochemicals are chemicals mediating the interactions among organisms on different trophic levels, either within the same species (pheromones) or from different species (allelochemicals). Allelochemicals are involved in interspecific interaction and have been subdivided into allomones, when the communication favors the emitter, kairomones, when the receivers keep vantage from the information, synomones, when both emitter and receiver are favorite.

To get through this variety of stimuli, the parasitoid can adopt different strategies based on the stimuli exploitation (Vet and Dicke 1992). One of these strategies is the use of the cues originated from stages different from the one attacked (infochemical detour). In the specific case of egg parasitoids to find their hosts, they can use stimuli originated from the host eggs or not and termed “direct host-related cues” and “indirect host-related cues” respectively.

Contact and volatile kairomones from eggs and synomones induced by egg deposition are “direct host -related cues”. Host scales, host traces and synomones induced by the feeding activity of the larvae or adults are “indirect host-related cues”. The indirect host-related cues do not provide information on egg location, but they lead females into the close vicinity of their potential host egg. Indeed, once the parasitoids

landed on a plant will search the habitat for chemical cues that are indicative of host presence (Wajnberg 2006). Furthermore, host eggs are generally suitable for the egg parasitoids during a short time due to their rapid development (Vinson 1998). Therefore, the efficiency of the natural enemies to locate their host into the crops is important for reducing the interval between pest build up and the control by the natural enemies due to the host signals interact with the complex odorous environment. One kind of indirect host-related cues is the kairomone from the traces left behind by adult hosts while moving on the plant. Indeed, the insects that move over a plant cuticle leave footprints, hydrocarbon compounds from the surface of host insects remaining on the surface of plants (Rostás and Wölfling 2009), that can be detected by other insects, showing to regulate intraspecific interactions. Parasitoids and predators have evolved the capability to exploit and use footprints in the processes of host location. Footprints are produced by the directed attacked host stage, or by previous stages, such as host adult for the location of host eggs, or caterpillar footprints for cocoon location. For example, Rostás et al., in 2008 found that naïve, *i.e*. no previous contact with the chemical cues, *Cotesia marginiventris* Cresson can recognize chemical cues on the wax surface of leaves produced by its host by walking second-instar caterpillars of *Spodoptera frugiperda* Smith. Similarly, parasitoids exploit the footprints as kairomones to track their hosts as stimuli indirectly associated with the presence of the host. The examples of host searching by exploiting host footprints are characterized by two different search strategies: systematic or random search (Godfray 1994). Parasitoids might use a systematic search when the cues are guiding them directionally to the host. One example is the system *Poecilostictus cothurnatus* Gravenhorst, a larval parasitoid, and the pine looper moth, *Bupalus pinarius* L. In this system the parasitoid follows the chemical trails left by the host larvae to locate its target (Klomp 1981). Parasitoids might use a random search when the cues are indirectly associated with the hosts and induce them to increase the host searching behaviour in the area where hosts are more likely

to be found. This strategy is reported for example for platygastrid egg parasitoids (Colazza et al., 2010). Often, such behaviour is expressed at short range of the host target with peculiar searching patterns.

In the following paragraph will be detailed the host finding behaviour of the egg parasitoid *Trissolcus basalis* (Wollaston) once in contact with the traces left by its adult host *Nezara viridula* (L.).

The system *Nezara viridula – Trissolcus basalis*

The green vegetable bug (GVB) (Fig. 1) *Nezara viridula* (L.) (Heteroptera: Pentatomidae), probably originated in the Ethiopian region of eastern Africa, is a widely distributed agricultural pest throughout the world (Todd, 1989).

Fig. 1 *Nezara viridula* adult

It is found throughout the tropical and subtropical regions of the world including the Americas, Asia, Australasia, and Europe (Waterhouse 1998). Both authors suggest that human activity spread the pest from Africa. However, *N. viridula* is a strong flier and may have expanded its range due to weather systems as well as human systems transport (Knight and Gurr 2006). The bug is highly polyphagous feeder, attacking many important food crops (Panizzi 2000). Its host range includes over 30 families of dicotyledonous plants and a number of monocots (Todd 1989) including weeds and non-cultivated plants, although it has strong preference for leguminoseae and solanaceae such as soybean, beans, tomato, etc. Worldwide, there have been many attempts to develop sustainable practices to control *N. viridula* as an alternative to the traditional chemical control with non-selective insecticides such as deltamethrin. Example of biological control of *N. viridula* is the use of sterile-insect technique that might have application to prevent reproduction, however, the high cost of the technique and the lack of the efficacy in the case of *N. viridula*, where adults as well as nymphal stages can cause crop damage, preclude use of this method (Knight and Gurr 2006). The entomopathogenic fungi have greater potential as biopesticides for sucking pests such as *N. viridula*. The time to death is however the crucial point. *Metarhizium anisopliae* takes approximately 14 days to kill adult *N. viridula*, with infection incidence in the pest population increasing for up to 20 days after treatment (Sosa-Gómez and Moscardi 1998), reducing the efficiency of this method where the bugs are present in low tolerance crops. Trap crops are used to prevent the pest from reaching the crop and to concentrate it in a certain part of the field where it can be strategically destroyed (Knight and Gurr 2006). A border planting of white mustard (*Sinapsis albus*) was used as a trap crop with organic sweet corn (*Zea mays*) in New Zealand. *N. viridula* numbers were much higher in the mustard plots (8–12/m2) than in the sweet corn (<1/m2) (Rea et al., 2002). These data show that the trap crops are a potentially useful tactic for an integrated management of *N. viridula* but only if is controlled in the trap crop to prevent its spreading into adjacent or main crops

(McPherson and Newsom, 1984). Indeed, adult of *N. viridula* will quickly move out of trap crops into adjacent crops if they are at a more attractive stage.

Trissolcus basalis (Wollaston) (Hymenoptera: Platygastridae) (Fig.2), a solitary egg parasitoid, is the most important biological control agent of *N. viridula* around the world (Jones 1966) even though it is a parasitoid of other heteropteran species (Lock and Walter 2000).

Fig. 2 *Trissolcus basalis* adult female on *Nezara viridula* egg mass

The parasitoid is widely distributed over North and South America, in the Mediterranean area, in the Middle East and in Australia (Jones 1988). Johnson in 1988 hypothesized the origin of the parasitoid in Africa where other close species have also been found.

To locate *N. viridula* eggs, *T. basalis* exploits cues from its host and from host–associated plants. It is generally accepted that allomones and synomones play the main role in host habitat and community location (Weseloh, 1981).

The behaviour of the egg parasitoid *T. basalis* to plants damaged by feeding activity of *N. viridula* and to volatile and contact chemicals from its host *N. viridula* was deeply investigated (Bin et al., 1993; Mattiacci et al., 1993; Colazza and Bin, 1995; Clemente and Colazza, 1997; Colazza et al., 1999; Colazza et al., 2010; Salerno, 2000; Peri et al., 2013). Colazza et al., showed that bean plants damaged by *N. viridula* feeding activity and onto which an egg mass had been laid, produce volatiles that attract the egg parasitoid (Colazza et al., 2004a) and, specifically, they found that *T. basalis* is attracted by the (E)-ß-caryophyllene, a volatile compound produced by bean plants when *N. viridula* feed and lay upon them (Colazza et al., 2004b). *Trissolcus basalis* orientation within the host community is led by *N. viridula* adult odours and traces. Indeed, the volatile and contact chemicals from *N. viridula* were studied in a Y-tube olfactometer and under open arena conditions respectively (Colazza et al., 2010). The Y-tube olfactometer is a device made in transparent Plexiglas where humidified and compressed air flow through the arms allowing the insects tested to perceive the different odour sources. The “open arena” consists on a filter paper, which served as an area where the insects tested can move in an unconstrained field. It was demonstrated that females of *T. basalis* react to cues emitted by *N. viridula* adults. The responses observed were a more frequent preference for the Y-tube olfactometer arm containing adult host. Particularly the wasps were attracted by volatile cues released by *N. viridula* virgin males and mated females in preovipositional state but were not attracted by those released by *N. viridula* virgin females. In open arena bioassays when a *T. basalis* female encounters a patch contaminated by chemicals deposited by walking of the adults of *N. viridula*, varies its locomotory path showing an arrestment response, characterized by a flight delay and an intensive antennal drumming of the substrate (Colazza et al., 2010).

The arrestment response of wasps to residue of host adults is a host location strategy commonly observed in Platygastridae egg parasitoids of pentatomid bugs. The presence of arresting kairomones has been observed for example in the host–egg parasitoid associations: *Murgantia histrionica* Hahn – *Trissolcus brochymenae* (Ashmead) (Conti et al., 2003), *Eurydema ventrale* Klt. – *Trissolcus simoni* (Mayr) (Conti et al., 2004) and *Euschistus heros* (F.) – *Telenomus podisi* (Ashmead) (Borges et al., 2003). Those chemicals deposited by walking activity of the insects are indirect host-derived cues and can be used to evaluate their hierarchical value on the behavior of *T. basalis* females. For example naïve *T. basalis* females discriminate between areas contaminated by chemical residues left by a host female or host male, with a clear preference for the former (Peri et al., 2006). Moreover, the same authors found that the oviposition experience enhanced the arrestment responses of the wasps when they were associated with host female residues. The effect of chemical residues left by adults of *N. viridula* on *T. basalis* females play an essential role on its host finding behavior and the use of this "footprints" begin a tool for enhancing parasitoids efficiency.

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Chapter 3: Understanding the role of floral nectar in enhancing the fitness of the parasitoid *Trissolcus basalis*: chemical analysis and olfactory responses

Abstract

In the last years an increasing attention has received the idea of habitat manipulation. This approach aims to enhance the impact of biological control in agricultural systems, by creating suitable ecological infrastructures to recruit and to conserve natural enemies populations. The increasing of the vegetational diversity can offer several benefits for arthropod predators and parasitoids as, for example, shelter or alternative food sources such as floral and extrafloral nectar, pollen, alternative prey or hosts. In particular, flowering plants, with volatile odours and nectar resources, can increase the recruitment and fitness of parasitoids. Here, it is investigated the suitability of four flowering plants, alyssum, buckwheat, French marigold and sweet basil, for enhancing and the fitness of *Trissolcus basalis*. In laboratory were conducted: (i) bioassays to test the effect of flowering plants on parasitoid fecundity; (ii) four-chambers olfactometer bioassays to test the attractiveness of the flowers for wasps; (iii) GC-MS analysis to chemically describe organic volatile compounds collected from flowering plant headspaces; (iv) GC–EAD analysis to evaluate the neural activity of antennal olfactory receptors of *T. basalis* in response to volatile extracts from buckwheat flowering plants. Results of experiments on the effect of flowering plants on parasitoid fecundity show that the food type provided to *T. basalis* significantly affected its total fecundity; indeed, only females fed on buckwheat flowers were able to lay eggs during the whole observed period. Four-chambers olfactometer bioassays show that wasp females spend significantly more time in the sectors of the olfactometer with the flowers over the stems only when the odour sources were from buckwheat. Analysis of the odour bouquets emitted from the flowering plants are considerably different in their compositions and quantities of volatile emissions. The preference of *T. basalis* females for the buckwheat flowers is strengthened by the GC-EAD results. Indeed, the results showed consistent GC-EAD

responses in correspondence of two carboxylic acids, i.e. isovaleric acid and butanoic acid, 2 methyl, present on the chemical profile of volatiles collected from only buckwheat flowers. In conclusion, the experiments evidence a positive effect of buckwheat plants on *T. basalis* fitness, in terms of longevity, fertility and flower attractiveness, suggesting a possible role of buckwheat as suitable flowering plants for conservation biological control programmes.

Keywords: *Trissolcus basalis*, Conservation biological control, flowering strip, fecundity, Electrophysiological and olfactometric responses.

Riassunto

Negli ultimi anni l' Habitat managment ha ricevuto crescente attenzione. Questo approccio si pome l'obiettivo di migliorare l'efficienza del controllo biologico nei sistemi agricoli, mediante la creazione di infrastrutture ecologiche idonee a reclutare e conservare le popolazioni dei nemici naturali. L'aumento della diversità vegetazionale è in grado di offrire numerosi vantaggi per i predatori e parassitoidi, come per esempio, riparo o cibo alternativo, quale il nettare floreale e extra-fiorale o il polline, prede oppure ospiti alternativi. In particolare, le piante, con gli odori che emettono e risorse alimentari, possono aumentare il reclutamento e la fitness dei parassitoidi. In questo capitolo sono dettagliate gli esperimenti condotti con l'obiettivo di studiare quale risorsa fiorale tra quattro selezionate, quali l'alisso, il grano saraceno, la calendula francese e il basilico, migliora la fitness di *Trissolcus basalis*. In laboratorio sono stati condotti: (i) biosaggi per verificare l'effetto delle piante a fiore sulla fertilità del parassitoide; (ii) biosaggi usando un olfattometro a quattro camere per verificare l'attrattiva dei fiori per il parassitoide; (iii) analisi GC-MS

per descrivere i profili chimici dei composti volatili raccolti dalle piante a fiore; (iv) l'analisi GC-EAD per valutare l'attività neurale dei recettori olfattivi antennali di *T. basalis* in risposta agli estratti volatili dalle piante di grano saraceno. I risultati degli esperimenti dimostrano che il tipo di cibo fornito al parassitoide influenza significativamente la sua fertilità. Inoltre, i biosaggi usando l'olfattometro a quattro camere mostrano che le femmine di *T. basalis* spendono molto più tempo nei settori del olfattometro in cui sono presenti i fiori rispetto ai settori in cui sono presenti solo foglie, solo quando le sorgenti di odore sono di grano saraceno. L'analisi GC-MS dei volatili emessi dalle piante a fiore evidenzia che il bouquet di odori emessi dalle quattro piante a fiore sono molto diversi tra loro nella qualità e quantità dei volatili emessi. La preferenza di *T. basalis* per i fiori di grano saraceno è rafforzata dai risultati ottenuti negli esperimenti di elettroantennografia. Infatti, i risultati hanno mostrato risposte GC-EAD in corrispondenza di due acidi carbossilici, quali l'acido isovalerico e l'acido butirrico, 2 metile, presente sul profilo chimico di volatili raccolti dai soli fiori di grano saraceno. In conclusione, gli esperimenti evidenziano un effetto positivo di piante di grano saraceno sul parassitoide *T. basalis*, in termini di fertilità e attrattività, suggerendo un possibile interessante ruolo del grano saraceno nei programmi di conservazione del controllo biologico.

Parole chiave: Trissolcus basalis, Conservazione del controllo biologico, strisce fiorali, fertilità, risposte elettrofisiologiche e olfattive.

Introduction

Conservation biological control aims to increase the impact of local populations of native or introduced natural enemies on pest insects within a given habitat (Barbosa 1998; Rahat 2007). This is put into effect by manipulating plant-based resources in the landscape (Bugg and Pickett, 1998 - Fiedler 2008). This is accomplished by selecting plants that provide a limiting resource such as floral nectar, establishing these plants, or plant communities, within the managed system and providing the provision of many additional ecosystem services (Gurr 2004). The intentional provision of flowering plants and plant communities in managed landscapes to enhance natural enemies is called "habitat management" and is a growing aspect of conservation biological control. The floral nectar can be considered an additional food and in many case become the primary reward (Wäckers 2005). Nectar-producing plants can improve biological control of pests by supplying parasitoids with sugar, which is often limited in monocultures (Heimpel and Jervis 2005). Moreover, asking which are the more effective plant species in order to be included in conservation biological control programs, begin essential take into account also the role of the attractiveness of the flowering plants for foraging parasitoids (Belz et al., 2012).

The green vegetable bug (GVB) *Nezara viridula* (L.) (Heteroptera: Pentatomidae) is a widely distributed agricultural pest (Todd 1989). It is found throughout the tropical and subtropical regions of the world including the Americas, Asia, Australasia, and Europe (Waterhouse 1998). The bug is a highly polyphagous sap feeder, attacking many important food crops. Its host range includes over 30 families of dicotyledonous and a number of monocotyledonous plants (Todd 1989). The green vegetable bug was first recorded in New Zealand in 1944 and quickly established to become a common pest in the North Island (Cameron, 1989). The adult overwinters in areas of weeds

and rank growth where populations increase in spring and subsequently invade crops during summer. In New Zealand this pest has caused economic damage to process sweet corn crops in the East Cape region (Rea et al., 2002).

There have been many attempts to develop sustainable practices to control *N. viridula* as an alternative to the traditional chemical control with non-selective insecticides such as deltamethrin. Chemical control has been shown to have negative effects on the bug's most important biological control agent *Trissolcus basalis* (Wollaston) (Hymenoptera: Platygastridae) by altering the parasitoid's behaviour and influencing its foraging ability (Salerno 2002). Examples of biological control of *N. viridula* are the use of sterile-insect technique that might have application to prevent reproduction (Knight and Gurr 2007) and the entomopathogenic fungi, such as *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin (Sosa-Gómez and Moscardi, 1998). While these methods are attractive because they can minimise the adverse effects on natural enemies that result from the use of conventional insecticides, they have rarely succeeded in providing acceptable control of *N. viridula*. Another example of sustainable practices to control *N. viridula* is the use of trap crops. This practice is used to prevent the pest from reaching the crop. For example, a border planting of white mustard (*Sinapsis albus*) was used as a trap crop with organic sweet corn (*Zea mays*) in New Zealand. *Nezara viridula* infestation was much higher in the mustard plots (8–12 bugs/m^2) than in the sweet corn (<1/m^2) (Rea et al., 2002).

On a global scale there are many examples of biological control agents that have been used to control *N. viridula.* The majority of these natural enemies are hymenopterous egg parasitoids, including *T. basalis,* and tachinid parasitoids such as *Trichopoda giacomellii* (Blanchard) (Waterhouse 1998) that attack adults or the late nymphal stages. *Trissolcus basalis*, a solitary and generalist egg parasitoid, was introduced into New Zealand from Australia during the summer 1948-49 (Cumber, 1952) in an attempt to control GVB. After this initial introduction *T. basalis* was released again

during the summer 1996-97. Before these releases the parasitoid was absent or rare; subsequently the last inoculative release the presence of the parasitoid has been reported only after the first week but not one month later. The variability in parasitism suggested that the lack of suitable plant shelter and nectar sources rendered some release sites unsuitable for parasitoid reproduction (Cameron, 1998).

The target of several biological control strategies is to re-establish the level of self-sustained natural control through introducing a natural enemy for permanent establishment and non-crop plants in form of companion plants or flowering strips to enhance the natural enemies activity.

The flowering strip to provide in cropping system must yield the optimal interactions with the natural enemies, increasing their longevity, fecundity and motivation to seek hosts and decreasing the lack of effectiveness of some biological control programs (Gurr et al., 1998; Landis et al., 2000). Broadly, the flower shape (Vattala et al., 2006; Nicolson and Thornburg, 2007), flower colour (Kugimiya et al., 2010) and the amount and quality of nectar (Johanowicz and Mitchell, 2000) can affect the extent to which natural enemies benefit. Moreover, an additional complication is that some flowering plants are unattractive to natural enemies whilst some of those that are attractive have nectaries (a nectar-secreting glandular organ in a flower) inaccessible (Wäckers and van Rijn, 2012).

One example of how conservation biological control was studied for enhancing effectiveness of parasitoid, was carried out by Coombs, 1997 using *T. giacomellii*. Fecundity and longevity of *T. giacomellii* was reduced when only fed water. Indeed, females fed only water had significantly reduced ovarioles and were unable to mature any eggs other than those that were present in the abdomen at the time of emergence (Coombs, 1997). It is obvious then that the availability of food (nectar and other sugar sources) has a significant influence on the effectiveness of *T. giacomellii* as a biological control agent. Equivalent work on the effects of food sources on adult *T. basalis* is not represented in the literature. Recently there is only one investigation

on the longevity of *T. basalis* fed on different floral nectars. Indeed, Rahat et al., in 2005 conducted laboratory studies showing that adult female longevity is increased several-fold by access to flowers of species such as French marigold, basil and buckwheat compared to others flowering plants.

In this scenario this research aims to improve the understanding of the biological control system and, in a more focused target, the management of *N. viridula* in organic and conventional crops. The investigations started asking whether the floral resources increase the fitness of the egg parasitoid *T. basalis* in term of progeny, whether the floral scent attract *T. basalis* and which of the volatiles emitted by the flowering plants perceived by the antennae of female wasps elicit a positive electrophysiological response. To address these questions, it was assessed the suitability of four flowering plants, alyssum, buckwheat, French marigold and sweet basil, for enhancing the fecundity and the fitness of *T. basalis*. To test the attractiveness of the flowers behavioural observations were conducted using a four-chambers olfactometer. The organic volatile compounds collected from all flowering plant headspaces where analysed in GC-MS to describe their chemical profiles. In addition, the volatile extracts from buckwheat flowering plants were used to evaluate the neural activity of antennal olfactory receptors of *T. basalis* using the GC–EAD technique. The ecological implications of the obtained results are discussed with a conservation biological control perspective.

Materials and methods

Insects

A colony of *Nezara viridula* was established with about 30 male and female adults collected during October 2013 from fields located in Maraetai, Auckland, New Zealand. The *N. viridula* colony was kept in insect rearing cages (47.5 x 47.5 x 47.5 cm) (BugDorm-44545, Mega View Science Co., Ltd. Taichung, Taiwan) and fed with organic cabbage, silver beet, beans and sunflower seeds. Food was exchanged every 2 days and water was provided through soaked cotton wool in small containers. Paper towels were placed inside each adult cage as an ovipositional substrate. Egg masses were collected daily and used to maintain cultures of both *N. viridula* and *T. basalis*. A colony of *T. basalis* was established from about 180 females that had emerged from four parasitized sentinel egg masses *of N. viridula.* The sentinel egg masses had been attached to crop plants in organic gardens in Karaka, Auckland and Gisborne, New Zealand during February 2014. The wasps were reared in 16 mL glass tubes, fed with drops of honey-water solution (80:20 v/v). Single *N. viridula* egg masses were removed from the original oviposition substrate, glued (Elmer's glue) onto a strip of filter paper (Whatman quantitative ashless filter paper- Grade No. 40) and exposed to three female wasps in a 16 ml glass tube. Both colonies were reared in an environmental room at 25±1°C, 50±10% R.H. and 16L:8D. In the bioassay experiments female wasps were 1 day old, mated and unfed; for the GC-EAD experiments females were 3 days old, mated and fed with the honey-water solution. Voucher specimens of the insects used in the experiments are deposited in the Entomology Research Collection (LUNZ), Lincoln University, New Zealand.

Flowering plants

Seeds of alyssum (*Lobularia maritima* L.) *cv* carpet of snow, buckwheat (*Fagopyrum esculentum* Moench) *cv* kaitowase, French marigold (*Tagetes patula* L.) *cv* crackerjack and sweet basil (*Ocimum basilicum* L.) were grown in 10-cell plug trays filled with standard potting mix containing slow-release fertilizer. The selection of plant species was based on a previous study showing that the maximum longevity of adult *T. basalis* females was on flowers of French marigold, basil and buckwheat and low on alyssum (Rahat et al., 2007). After germination, one week old seedlings were transplanted into 1 L plastic pots, filled with the same soil used for sowing and watered three times weekly or more often as deemed necessary. New plants were sown weekly. All plants were covered by 30-mesh anti-insect net and grown in a greenhouse. Mean heating set point was 19.8°C. In all experiments plants were used when in full bloom (6-8 weeks) (Figg. 3 and 4).

Fig. 3 Alyssum (*Lobularia maritima* L.); b: Sweet basil (*Ocimum basilicum* L.).

Fig. 4 a: French marigold (*Tagetes patula* L.); b: Buckwheat (*Fagopyrum esculentum* Moench)

Volatile organic compounds collection

Volatile organic compounds were collected from flowering plants using stems, leaves and flowers. In addition, volatile organic compounds were collected separately from buckwheat flowers and leaves. For both volatile collections, the biomass was placed in glass vessels of the volatile collection system (Fig. 5) and the cut end placed in 25 mL of water to prevent drying during the volatile collection.

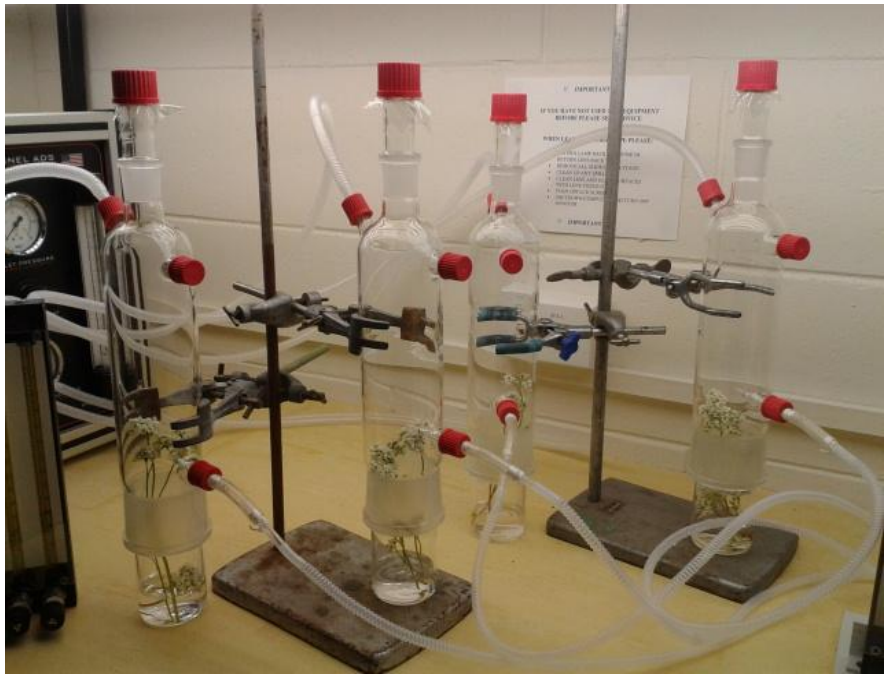


Fig. 5 Volatile collection system. Example of volatile organic compounds collection from buckwheat flowers.

This system consists of a chamber (35 cm long and 6.8 cm outside diameter) that has a sintered glass frit at the upwind end and a joint outlet with a single-port collector base. Filtered air (activated charcoal filter, 400 cc, Alltech, Deerfield, IL, USA)

originating from a compressed air cylinder was pushed into the vessel through a teflon tubes at a rate of 300 ml min^{-1}. With a vacuum pump (ILMVAC GmbH, Germany) 300 ml min^{-1} of air was pulled out through a trapping filter containing 30 mg SuperQ (ARS Inc., Gainesville, FL, USA). Before each experiment, the traps were cleaned by rinsing with 1 ml methylene chloride. Each collection lasted 2 h for French marigold's sample, 3 h for buckwheat, sweet basil and alyssum's samples. Empty odour source vessels and vessels filled with 25 mL of water were also sampled to check for background contaminations. The adsorbed compounds were eluted from the trapping filter with 150 µl of methylene chloride and 200 ng tetralin (Sigma-Aldrich, Australia) was added as an internal standard. All the extract were stored at −80°C until used for the following experiments.

Cage bioassays

In order to evaluate the effect of the flowering plants on the fitness of *T. basalis* in terms of number of progeny and sex ratio cage bioassays were developed. The bioassays were conducted using cages (6 x 6 x 11 cm) made from clear rigid plastic of 2 mm thick. Top side of the cage was made with synthetic mesh screen. Two holes were cut at the bottom and in one of the lateral side of the cage, 5 cm and 3 cm diameter respectively. The bottom hole was used to insert the plant and then was closed by cotton wool wrapped around the stem. The lateral hole was used to introduce a single *T. basalis* females subsequently closed by cotton wool. Twenty-four hours later one fresh (24-48 hours old) *N. viridula* egg mass (92.5 ± 1.1 eggs/mass) was introduced through the lateral hole and removed the day after. (Fig. 6)

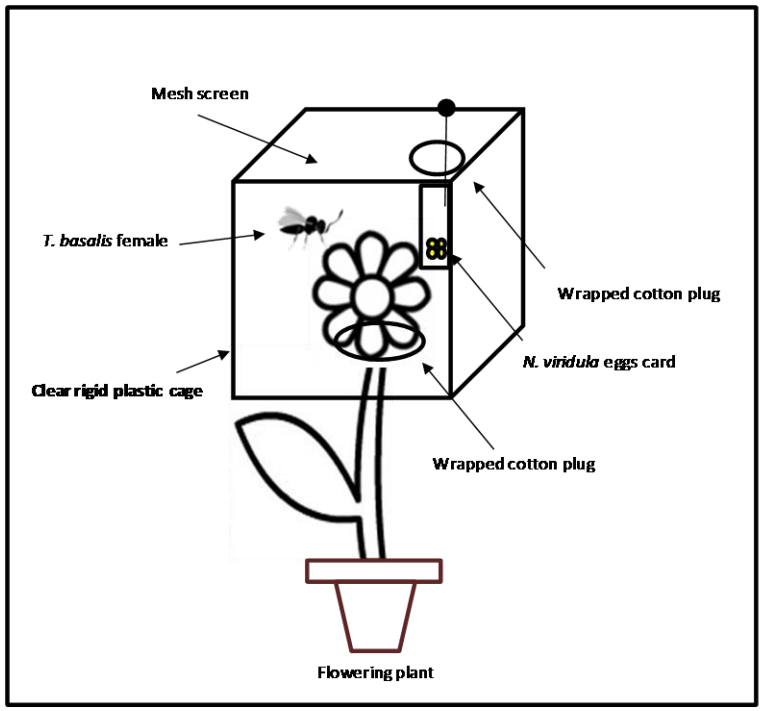


Fig. 6 Cage bioassay

Egg mass replacement was extended over five days. Each exposed egg mass was held individually in glass tubes in the environmental room, until parasitoid emergence. The adults emerging from each egg mass were counted and sexed to determine number of the progeny and the sex ratio respectively. Sex ratio was expressed as the proportion of males on the total of the progeny. The experiments have been set in a repeated block designs. Each block consisted of five cages for the five treatments started on the same day. Throughout the experiments and in any block, cages were checked between 09:30 and 10:30. Fifteen replicates of each block were set up in a controlled environmental room (25±1°C, 50±10% R.H and 16L:8D). Potted plants and cages were replaced per each block.

4-chambers olfactometer bioassays

The attraction to odour resources for *T. basalis* was tested in the 4-chamber olfactometer (Fig. 7).

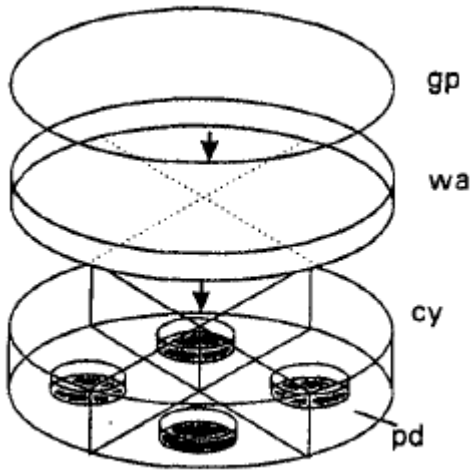


Fig. 7 4-chamber olfactometer . "gp": glass plate; "wa": walking arena with gauze; "cy": cylinder with the four chambers; "pd": samples and control.

The device was made in acrylic glass, consisting in a cylinder (4,5 cm high and 20 cm diameter) divided by vertical plates made in acrylic glass into for chambers. No airflow was generated. On the top of cylinder was placed a removable walking arena (1,5 cm high and 20 cm diameter) made in a plastic gauze. The walking arena was covered with a glass plate to avoid escape of parasitoid. The samples were placed in two opposite chambers. Any odour sample was covered by mesh to avoid colour influences. The position of the samples was changed after every test to avoid influenced results due to the side preference by the parasitoids. The device was clean after every bioassay. The odour sources and wasp females were used only once and

discarded after the behavioural test. A single adult female was isolated in 2 mL glass vials and tested within 12 hours. All the females used for the experiments were food deprived and naïve, *i.e*. no contact with the tested odour sources previous to the experiments. For the bioassays the parasitoids were released in the centre of the walking arena that was covered by glass plate. The time spent by each wasp in each chamber (residence time) was recorded for 5 minutes using the computer software The Observer XT 7.0 (Noldus, Information Technology, Wageningen, Netherlands). Each treatment was replicated twenty times. The experiments were carried out in a dark room to avoid directional light and the olfactometer was situated inside a white cardboard basket and illuminated from above by two cool white fluorescent tubes. The temperature in the bioassay room was continuously maintained at 24 ± 1 °C. Wasps were allowed to acclimatise for at least 1 h in the room before experiments. The following odour sources were tested:

(1) Inflorescence *vs* leaves: for each plant inflorescence and leaves were cut and the stems wrapped in a wet piece of cotton and sealed with parafilm in order to prevent wilting and minimise odours from damaged tissue. The amount of inflorescences used as odour source was visually quantified to match approximately throughout the treatments. The inflorescences and leaves were used within half an hour after cutting and once per wasp tested.

(2) Buckwheat extract *vs* solvent (methylene chloride). Extracts of buckwheat plants (see "*Volatile organic compounds collection"*) and the solvent were tested by pipetting 80 µl of solution onto a circle (13 cm diameter) of filter paper. The solution was allowed to dry for at least two minutes and then the circles were put in the chambers of the olfactometer.

(3) Buckwheat synthetic blends *vs* solvent (methylene chloride). Two buckwheat synthetic blends were prepared as methylene chloride solutions. The first synthetic

blend, “Stock solution”, contained (in mg/ml) butanoic acid (0.4), isovaleric acid (1.3), 2-methyl butanoic acid (1.1), hexanoic acid (0.1), 3-hexen-1-ol,acetate (Z) (0.8), α-farnesene (0.8). This composition was defined on the calculated amounts from buckwheat extract analysed by GS-MS. The second synthetic blend, “1/10 dilution” was prepared as the 1/10 dilution of the “Stock solution” and in details contained (in mg/ml) butanoic acid (0.04), isovaleric acid (0.13), 2-methyl butanoic acid (0.11), hexanoic acid (0.01), 3-hexen-1-ol,acetate (Z) (0.08), α-farnesene (0.08). All chemicals were from Sigma-Aldrich (Australia). The synthetic blends were stored at −80°C until bioassayed. The odour sources were prepared as described for the buckwheat extract using circles of filter paper and by pipetting 40 µl. The solution was allowed to dry for at least two minutes and then the circles were put in the chambers of the olfactometer.

Gas Chromatography-Mass Spectrometry (GC-MS) Analyses

The flowering plant extracts were separated using a Shimadzu GCMS-QP2010 (Shimadzu Corporation, Japan) gas chromatograph - mass spectrometer fitted with a Restek Rxi-1ms fused silica capillary column (30.0 m x 0.25 mm i.d. x 0.25 µm, Bellefonte, PA, USA). Of each sample, 1 µl was injected in pulsed splitless mode at a temperature of 220 °C and with a pulse of 168 kPa for 40 s. Oven temperature was held at 50 °C for 3 min and then raised to 320 °C at 8 °C/min and held at this temperature for 8 min. Helium was used as carrier gas at a constant flux of 1.5 ml/min. Compounds were identified using GCMS solution v. 2.72 (Shimadzu Corporation, Japan) software with NIST 11 and Wiley 10 mass spectrum libraries and by using the software MassFinder4/Terpenoids library (Hochmuth Scientific Software, Hamburg, Germany). Standards were used to confirm identities of compounds that were commercially available (Sigma-Aldrich, Germany; Treatt Ltd., UK). Quantification was obtained by comparing the area of the compounds to the area of the internal standard.

Electrophysiology experiments

Female wasps were anaesthetized by CO_2, and the head was cut. Glass capillaries grounding electrode in contact with a silver wire were filled with conductivity gel. The reference electrode was connected to the neck of the isolated head, while the recording electrode was connected to the antenna tip (with half of the last antennomere severed) (Fig. 8) using a micromanipulator (Leitz, Leica, Germany and P-225, Sutter Instruments, USA).

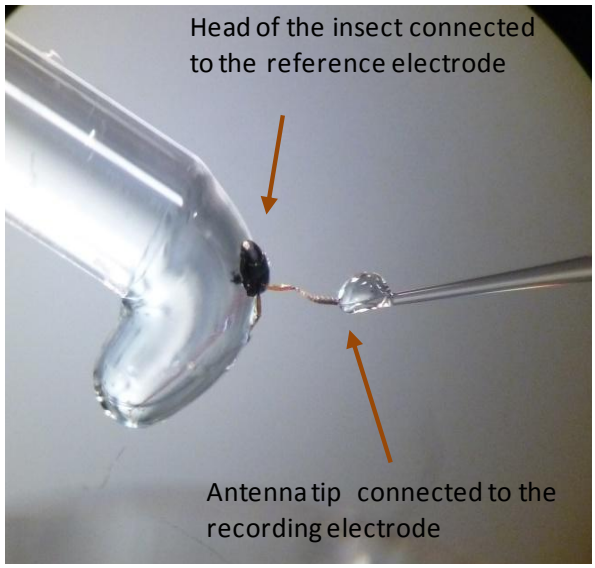


Fig. 8 Antenna preparation for electrophysiology experiments

The capillary glasses to use for the recording electrode were drawn to a fine point using a microelectrode puller to get an inner diameter wide enough to enable contact of the antenna tip. Antennal responses, for all the electrophysiology experiments, were monitored using a high input-impedance AC/DC probe, a data acquisition controller (Type IDAC-4) and software (Autospike 32) (Syntech, Hilversum, The Netherlands).

Trissolcus basalis antenna response to buckwheat plant extracts was performed using coupled GC-EAD (Gas Chromatography-Electroantennogram Detection) analysis to determine what compounds could elicit electrophysiological activity.

One µl of the buckwheat extract was injected into the GC Agilent 7890A (Agilent, USA) with a flame ionization detector (FID) and a split/splitless injector. A 30 m x 0.25 mm i.d. x 0.25 µm DB-5 capillary column (Agilent Technologies, USA) was used for the analyses. The oven temperature was programmed from 60°C (held for 1 min) to 240°C at 10°C min^{-1}. Helium was used as the carrier gas. The column effluent was split 1:1 with one part going to the flame ionization detector (FID) of the GC and the other through a heated transfer line into a humidified and charcoal-filtered airstream directed at the antennal preparation. Based on the GC-EAD results 2 series of EAG experiments were performed. In the first experiment, *T. basalis* antenna response to the main 5 identified compounds of buckwheat extract, *i.e.* isovaleric acid, 2-methyl butanoic acid, 3-hexen-1-ol, acetate (Z), α-farnesene, and butanoic acid, and to 2 general plant volatiles, *i.e.* linalool and geraniol, was evaluated. EAG responses were recorded from 5 wasps using one antenna per individual. In the second one, wasp antenna responses to carboxylic acids, *i.e.* propanoic acid, butanoic acid, pentanoic acid, hexanoic acid, heptanoic acid and octanoic acid, were evaluated. EAG responses were recorded from 7 wasps using one antenna per individual. For odour presentation in EAG experiments, each chemical was dissolved in hexane to yield 100 mg/ml solutions. In both experiments the control was the solvent and caryophyllene was used as standard to normalize the antenna response. In both experiments, *i.e.* response to main compounds of buckwheat extract or to carboxylic acids, for each tested antenna the first stimulus was the solvent and then the sequence of stimuli presentation was randomized. The responses to solvent control were deduced from all responses and mV responses to all stimuli were converted to percentage values of the mean responses to the standard.

In addition, a series of EAG tests was conducted to produce dose – response curves with the 3 major buckwheat compounds, *i.e.*, isovaleric acid, 2-methyl butanoic acid and 3-hexen-1-ol,acetate (Z).

These compounds were serially diluted in hexane (purity 99%) to obtain the following concentrations (µg/ml): 100, 10 and 1.

The same antenna was used to test all of the concentrations of a single compound. Each compound was tested on 4 antennae, using one antenna per wasp. The sequence of the tested compounds was provided starting from the weakest concentration and followed by increasing concentrations.

For all EAG experiments the stimulus applicators were prepared by pipetting 10 µl of hexane solution onto a 6×0.5 cm strip of Whatman no. 1 filter paper, then the solvent was allowed to evaporate for 10s and the filter paper was placed inside a 14.5-cm long glass Pasteur pipette. The tip of the glass pipette was placed about 3 mm into a small hole in the wall of a L-shaped glass tube (130 mm long, 12 mm i.d.) oriented towards the antennal preparation (around 5 mm away from the preparation). The stimuli were provided as 1s puffs of charcoal-filtered air into a continuous humidified main air stream, at 2100 ml min^{-1} continuous flow, that was flowing over the antennal preparation and generated by an air stimulus controller (CS-55, Syntech, Kirchzarten, Germany). The stimulus controller was configured to compensate for pulse flow (550 ml/min) during stimulus delivery. In order to recover and to prevent adaptation for the antenna the stimuli were presented at 1 min intervals. EAG responses were measured by using a measurement marker tool available with the GC-EAD software as maximum amplitude of depolarization (mV). All chemicals used were purchased from Sigma-Aldrich (Australia).

Statistical analysis

Means of the total number of emerged adults and sex ratio (males proportion on the total of the progeny) were analysed using One-way ANOVA and multiple comparisons were performed using Tukey's HSD test. Prior to the ANOVA data set were tested for homogeneity of variances (Levene's test).

Wilcoxon Matched Pairs Test was used to analyse the bioassays performed using the 4-chambers olfactometer.

The composition of the volatile organic compounds from the headspace samples was decrypted by the mean±SE of the amounts identified over the 4 replicates for each flower plant. Principal components method of factor analysis (PCA) was used to examine the covariance relationships among the volatiles present in the headspace of any flower.

In EAG experiments, to obtain the dose–response curves, the data expressed as means of relative responses were analysed by repeated-measures ANOVA, followed by Fisher's LSD test.

EAG responses to main compounds of buckwheat extract or to carboxylic acids were control-adjusted with the hexane only control and expressed as proportional responses relative to the caryophyllene standard. ANOVA single factor was used to analyse the EAG experiments and Fisher's LSD post hoc test for multiple comparisons was performed to compare differences between the means of the EAG amplitudes.

Statistical analysis was conducted by using Statistica 7 software.

Results

Cage bioassys

The results show that the food type provided to *T. basalis* significantly affected its total fecundity. Significantly more adults emerged from egg masses parasitized by females provided with buckwheat (Mean ± SE; 152.93 ± 5.06) and basil (Mean ± SE; 128.33 ± 8) flowers than from egg masses parasitized by females fed on alyssum (Mean ± SE; 105.67 ± 5.59), French marigold (Mean ± SE; 104.33 ± 7.32) or provided with water alone (Mean ± SE; 88.60 ± 5.42) (Fig. 9). Within the treatments there was a highly significant difference in mean of the adults emerged between the feeding treatments "buckwheat" and "alyssum" ($P<0.001$), "French marigold" ($P<0.001$) and "water" ($P<0.001$), while marginal difference was found between "buckwheat" and "basil" ($P=0,060$). No significant difference was found among the three feeding treatments "basil", "alyssum" and "French marigold". The mean fertility trend was similar in each treatment with the peak of oviposition period on the first experimental day (Fig. 10). Only females fed on buckwheat flowers were able to lay eggs during the whole observed period. Longevity of *T. basalis* caged without flowers but with access to water was 2 days.

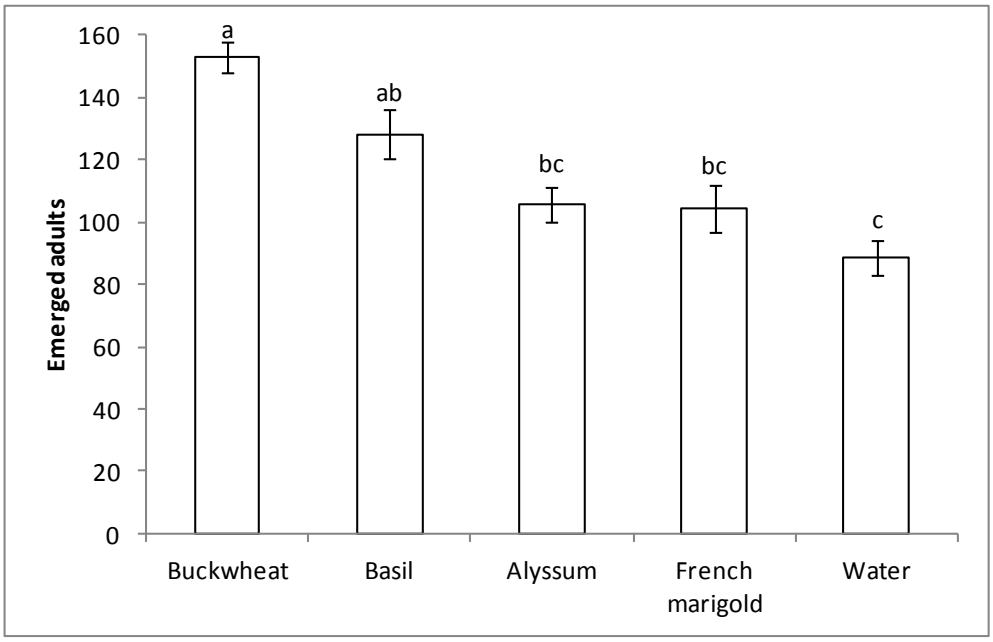


Fig. 9 Cage bioassays: Mean number of emerged adults of *T. basalis* during the observation period. Error bars show ± SE and letters indicate where differences among treatments are significant (Tukey's HSD test with P < 0.05).

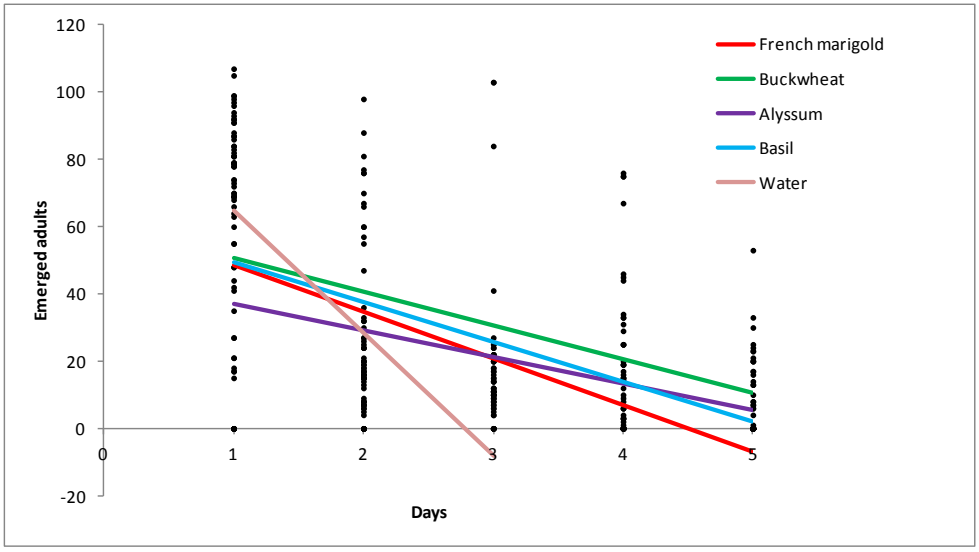


Fig. 10 Cage bioassays: Mean number of emerged adults of *T. basalis* per day.

The food provided to *T. basalis* adults did not affect the sex ratio of their progeny (Fig. 11). Sex ratio (MM/total progeny) of *T. basalis* was 0.15 ± 0.01 (Mean ± SE) for the buckwheat treatment, 0.14 ± 0.01 for the basil treatment, 0.13 ± 0.02 for the alyssum treatment, 0.11 ± 0.01 for the French marigold treatment and 0.12 ± 0.01 for the water treatment.

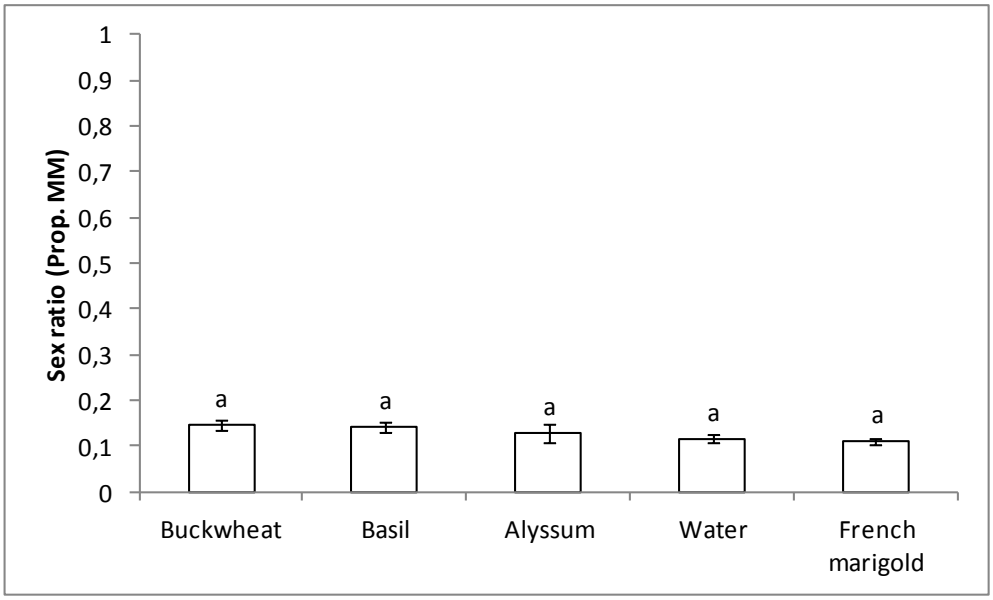


Fig. 11 Cage bioassays: sex ratio average (MM/total progeny). Error bars show ± SE and letters indicate where differences between treatments are significant (Tukey's HSD test with P < 0.05)

4-chambers olfactometer bioassays

(1) Flowers *vs* leaves (Fig. 12)

The parasitoids showed to spend significantly more time in the sectors of the olfactometer with the flowers over the stems when the odour sources were from buckwheat (Mean (sec) ± SE; 185.5 ± 6.27 *vs* 116.35 ± 6.64; n=20; *P*<0.001). On the contrary, *T. basalis* spent more residence time on the leaves over the flowers when the odour sources were from alyssum plants (Mean (s) ± SE; 171.40 ± 6.42 *vs* 128.45 ± 6.38; n=20; *P*=0.004).

Fig. 12. 4-chambers olfactometer bioassays: flowers *vs* leaves. Grey and white bars indicate the duration (means ± SE) of the residence time (sec) of wasp females on the chambers with flowers and leaves respectively. Asterisks (*) indicate $p < 0.05$ by Wilcoxon Matched Pairs Test.

Not significant differences in residence time were found when the odour sources present on the chambers of the olfactometer contained the flowers over the stems

for basil (Mean (s) ± SE; 157.35 ± 5.90 *vs* 142.70 ± 5.99; n=20; *P*>0.05) and French marigold (Mean (s) ± SE; 148.10 ± 7.35 *vs* 151.20 ± 7.31; n=20; *P*>0.05).

(2) Buckwheat extract *vs* solvent (Fig. 13)

Trissolcus basalis showed to spend significantly more time in the chambers of the olfactometer with the buckwheat extracts over the solvent (Mean (sec) ± SE; 160,75 ± 4.07 *vs* 140.75 ± 4.40; n=20; *P*=0.028).

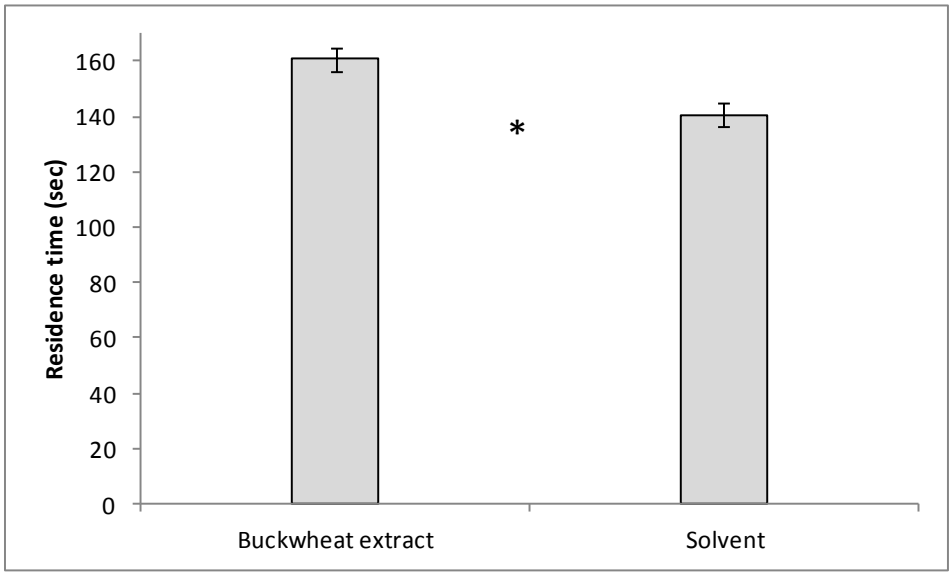


Fig. 13 4-chambers olfactometer bioassays: Buckwheat extract *vs* solvent. Bars indicate the duration (means ± SE) of the residence time (sec) of wasp females on the chambers with "buckwheat extract" and "solvent". Asterisks (*) indicate p < 0.05 by Wilcoxon Matched Pairs Test.

(3) Synthetic solutions of the major indentified buckwheat volatiles *vs* solvent (Fig. 14 and 15)

Trissolcus basalis showed to spend significantly more time in the chambers of the olfactometer with the "1/10 dilution" over the control (Mean (s) ± SE; 174.95 ± 7.28

vs 125.05 ± 7.28; n=20; *P*=0.008). Conversely, the wasps showed to spend significantly more time in the chambers with the control when was tested the "Stock solution" (Mean (sec) ± SE; 178.75 ± 9.32 *vs* 121.25 ± 9.32; n=20; *P*=0.011).

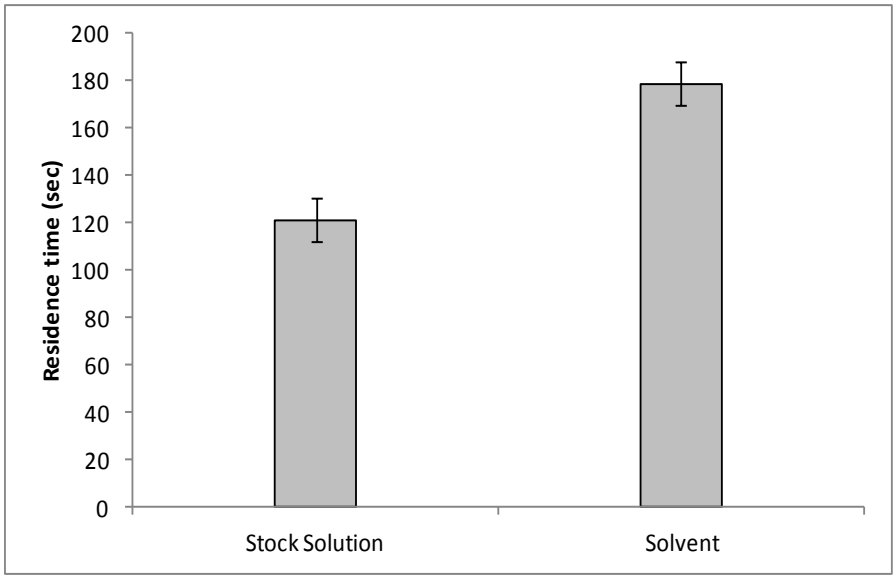


Fig. 14. 4-chambers olfactometer bioassays: Synthetic solutions of the major indentified buckwheat volatiles *vs* solvent. Bars indicate the duration (means ± SE) of the residence time (sec) of wasp females on the chambers with "Stock solution" and "solvent".

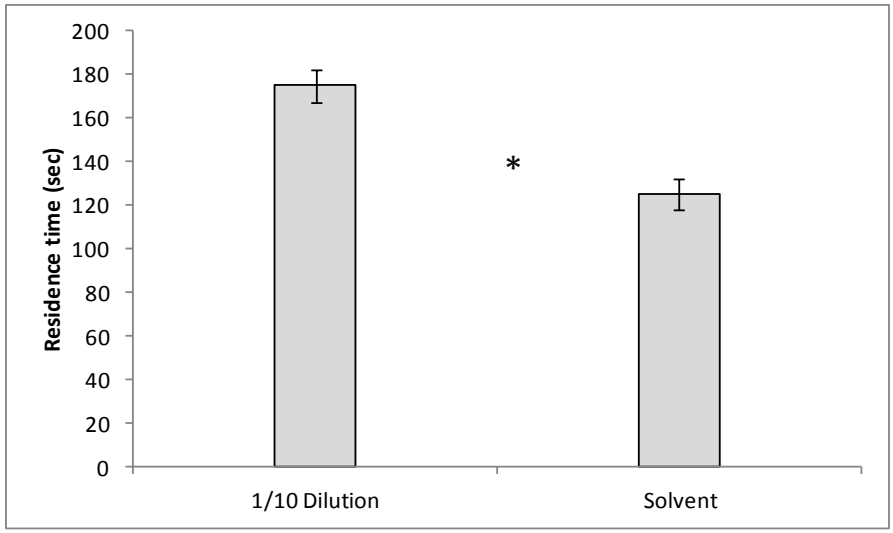


Fig. 15. 4-chambers olfactometer bioassays: Buckwheat extract *vs* solvent. Bars indicate the duration (means ± SE) of the residence time (sec) of wasp females on the chambers with "1/10 dilution" and "solvent". Asterisks (*) indicate $p < 0.05$ by Wilcoxon Matched Pairs Test.

Gas Chromatography-Mass Spectrometry (GC-MS) Analyses

The odour bouquets emitted from the flowering plants are considerably different in their compositions and quantities as showed in Fig. 16 and in Tabs. 1-4. Indeed, there were 4 compounds overlapping between the 52 compounds that were identified from the GC-MS chromatograms (Figg. 17-20). The volatiles collected separately from flowers and leaves of buckwheat plants showed different chemical profiles (Fig. 21 and Tab. 5). The chromatograms show that the carboxylic acids identified from buckwheat plants are present only on the chemical profiles from the flower volatiles.

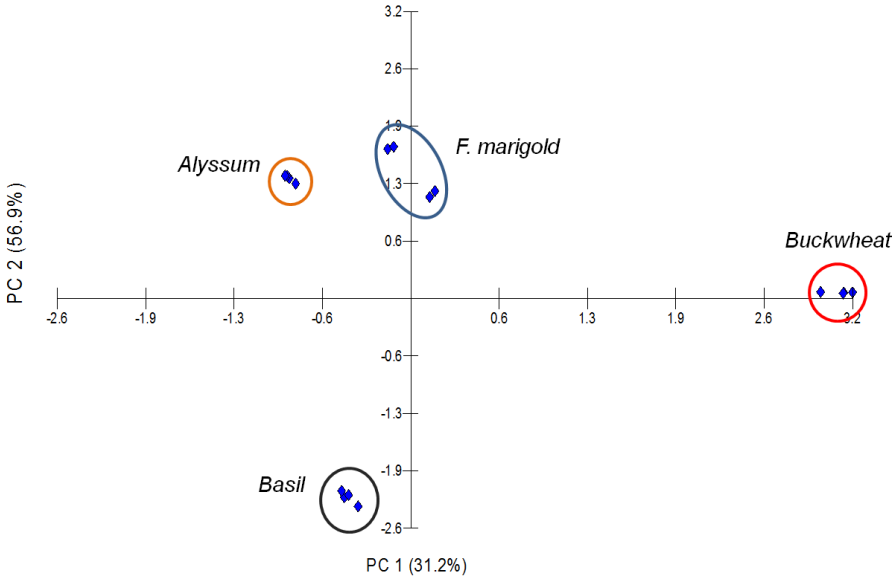


Fig. 16 Principal component analysis (PCA) of volatile organic compound (VOCs) emitted by flowering plants of alyssum (*Lobularia maritima* L.), buckwheat (*Fagopyrum esculentum* Moench), French marigold (*Tagetes patula* L.) and basil (*Ocimum basilicum* L.).

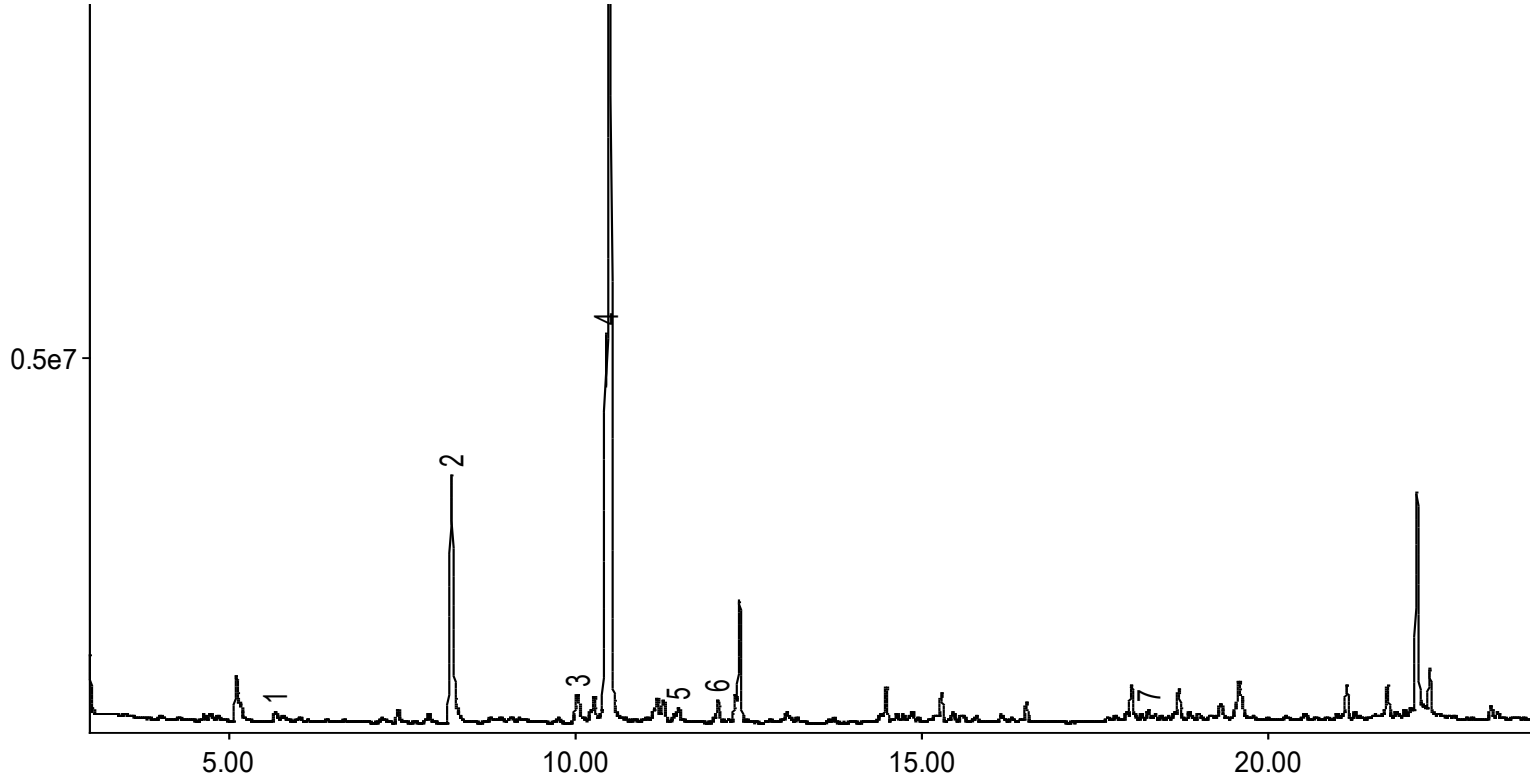


Fig. 17. Representative gas chromatogram of volatile organic compounds collected from alyssum (*Lobularia marittima* L.) plants. See Table 1 for peak identities and "Materials and Methods" for analysis conditions.

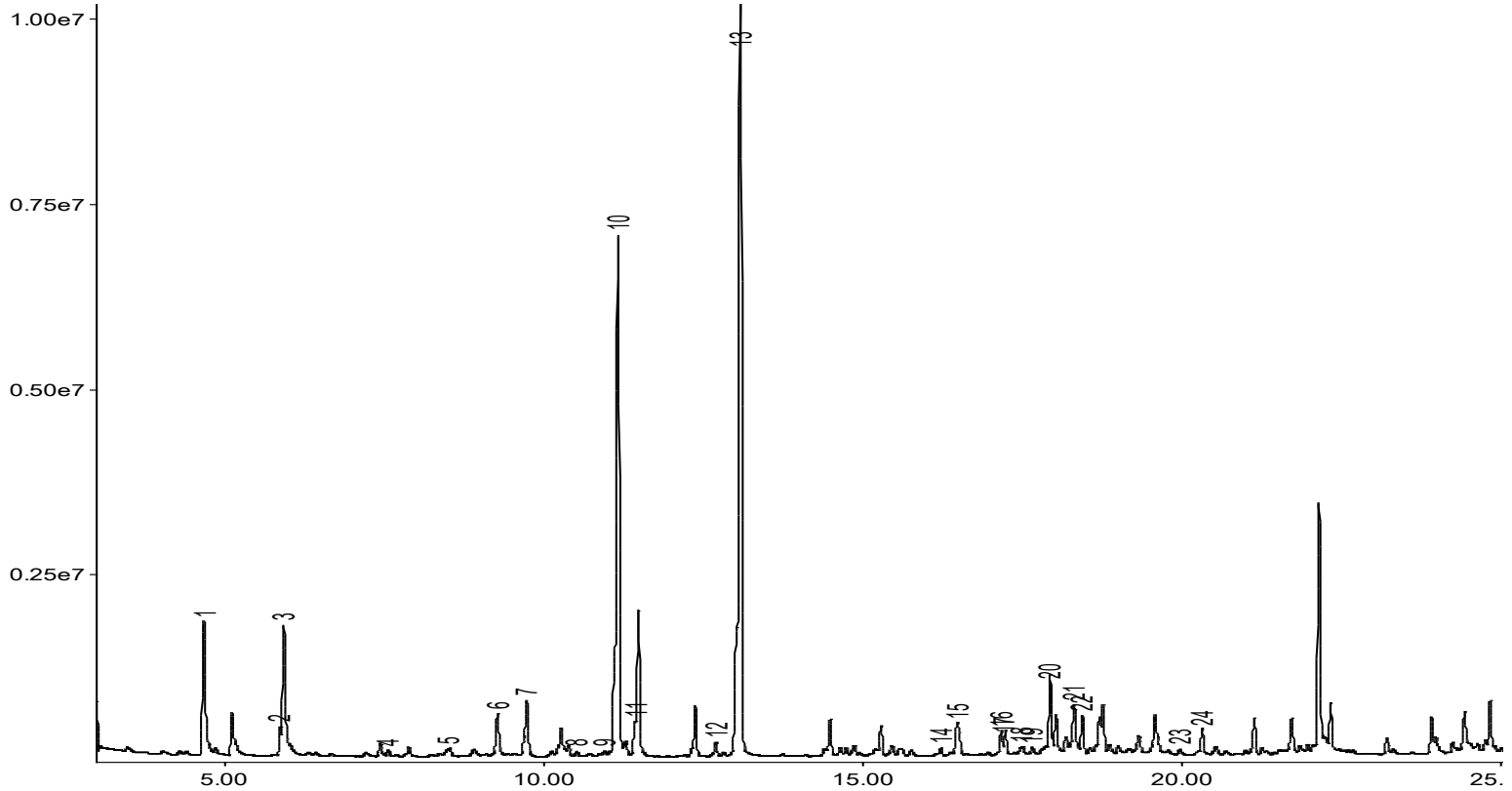


Fig. 18. Representative gas chromatogram of volatile organic compounds collected from sweet basil (*Ocimum basilicum* L.) plants. See Table 2 for peak identities and "Materials and Methods" for analysis conditions.

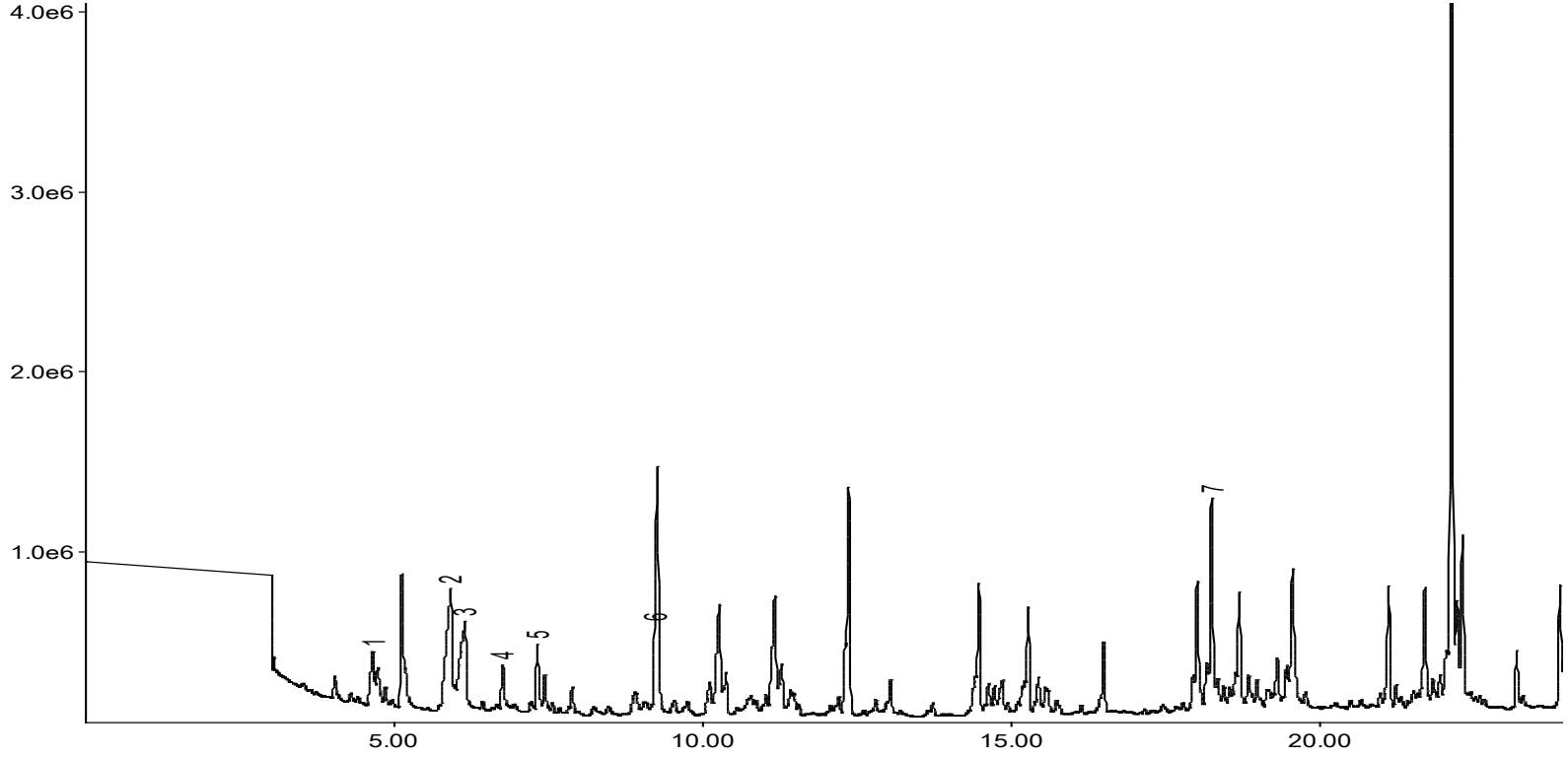


Fig. 19 Representative gas chromatogram of volatile organic compounds collected from buckwheat (*Fagopyrum esculentum* Moench) plants. See Table 3 for peak identities and "Materials and Methods" for analysis conditions.

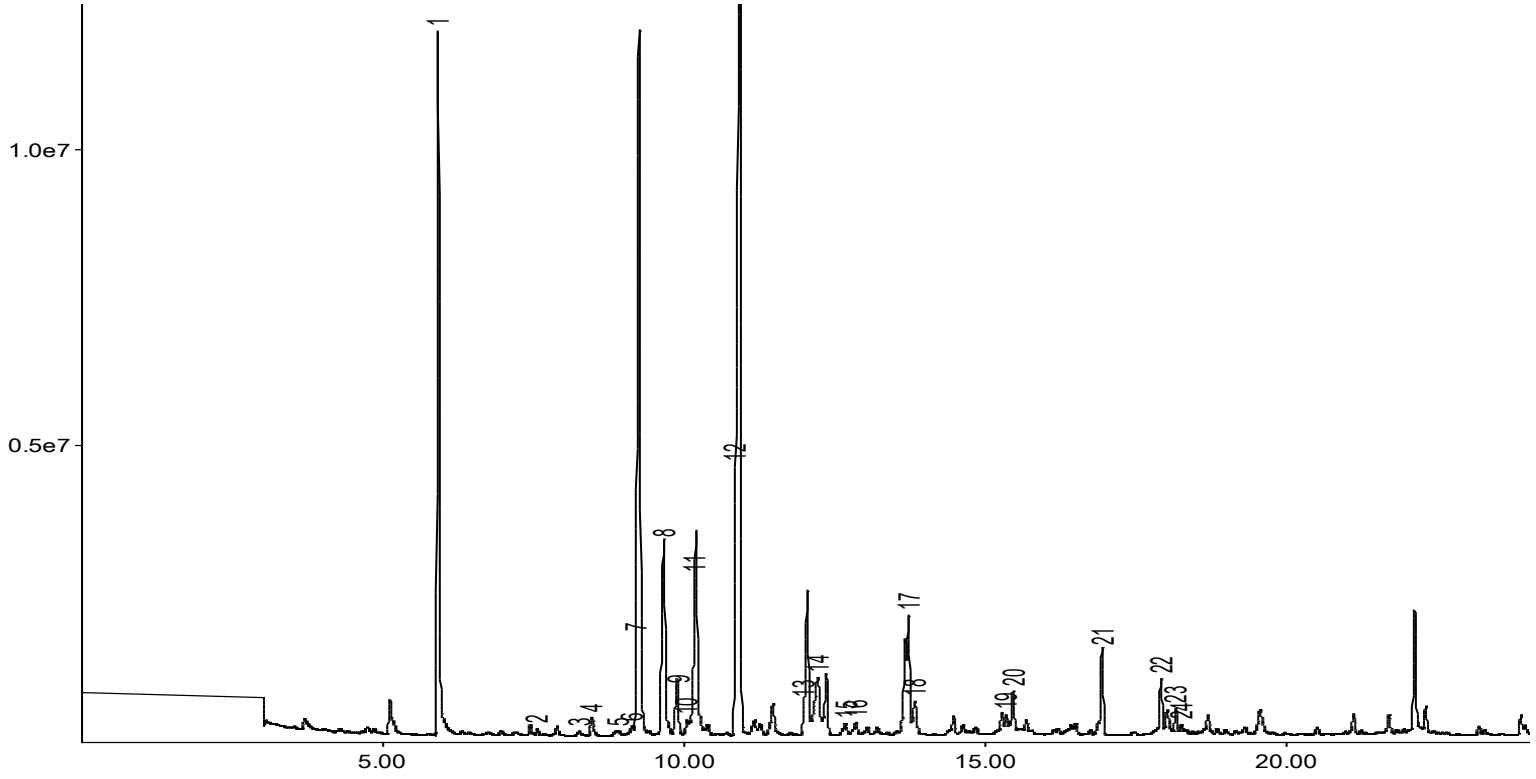


Fig. 20 Representative gas chromatogram of volatile organic compounds collected from French marigold (*Tagetes patula* L.) plants. See Table 4 for peak identities and "Materials and Methods" for analysis conditions.

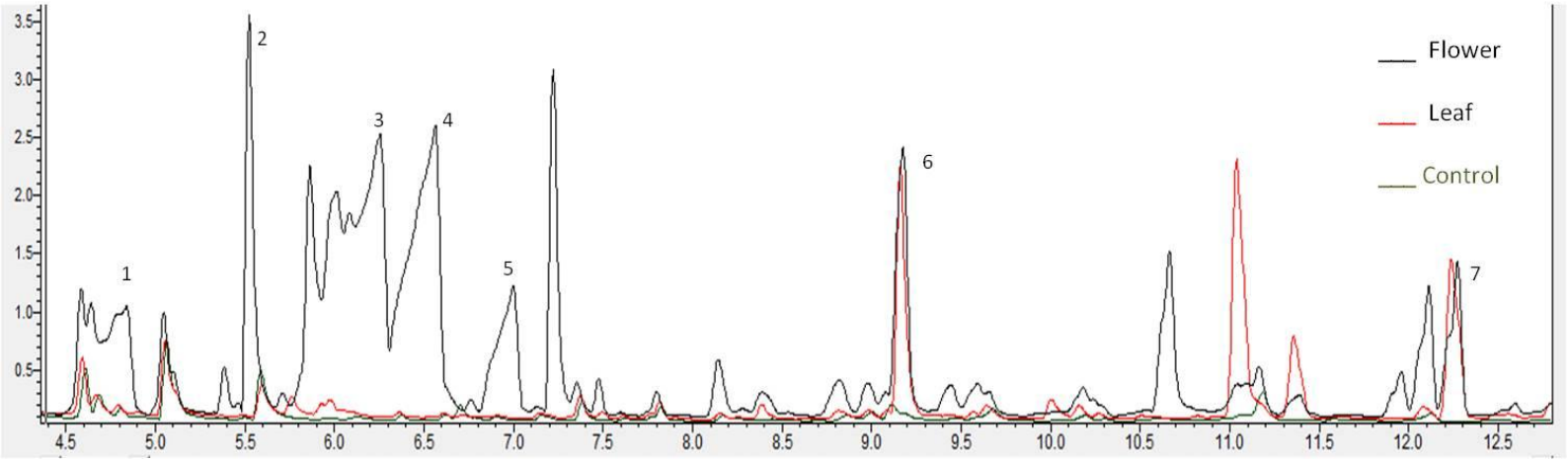


Fig. 21 Representative gas chromatograms of volatile organic compounds collected from flowers (black line) and leaves (red line) buckwheat (*Fagopyrum esculentum* Moench) plants. Green line represents the control. See Table 5 for peak identities and "Materials and Methods" for analysis conditions.

Tab. 1 Alyssum, (*Lobularia maritima* L.) volatile organic compounds identified and measured by gas chromatography–mass spectrometry. Average (±SE) amounts are reported as ng μl^{-1} of volatile organic compounds in methylene cloride solution; n = 4; RI Exp: experimentally determined retention index, RI Lit.: retention index in data bank. Peak numbers correspond to Fig. 17.

Peak	RT (min)	Compound	RI Exp.	RI Lit.	Amount (ng ± SE)
1	5.668	2-Pentanone	850	875	17.52 ± 3.01
2	8.215	Benzaldehyde	963	969	388.37 ± 20.79
3	10.029	Benzaldehyde, 2-hydroxy	1044	1049	95.17 ± 15.41
4	10.439	Acetophenone	1067	1065	2101.55 ± 360.53
5	11.485	Furan	1119	1090	47.13 ± 26.55
6	12.056	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	1149	1141	25.86 ± 5.76
7	18.260	α-Farnesene	1512	1506	24.84 ± 10.56

Tab. 2 Sweet basil (*Ocimum basilicum* L.) volatile organic compounds identified and measured by gas chromatography–mass spectrometry. Average (±SE) amounts are reported as ng μl^{-1} of volatile organic compounds in methylene cloride solution; n = 4; RI Exp: experimentally determined retention index, RI Lit.: retention index in data bank. Peak numbers correspond to Fig 18.

Peak	RT (min)	Compound	RI Exp.	RI Lit.	Amount (ng) ± SE
1	4.679	4-pentanal, 2-methyl	807	798	192.92 ± 122.36
2	5.871	2-hexanal	847	827	41.46 ± 14.61
3	5.930	3-hexen-1-ol (Z)	861	851	178.11 ± 147.43
4	7.557	α-pinene	934	936	31.46 ± 14.62
5	8.508	β-pinene	976	978	75.93 ± 41.40
6	9.274	3-hexen-1-ol, acetate (Z)	1011	1002	218,97 ± 43.65
7	9.733	1,8-cineol	1033	1024	633.42 ± 263.93
8	10.513	cis-sabinene hydrate	1071	1082	14.09 ± 4.55
9	10.943	cis-Linalool oxide	1092	1072	35.71 ± 8.22
10	11.162	Linalool	1102	1100	5924.64 ± 2236.30
11	11.482	4,8-Dimethylnona-1,3,7-triene	1117	1115	245.32 ± 128.16
12	12.691	(-)-Terpinen-4-ol	1182	1182	21.46 ± 14.62
13	13.080	Estragole	1203	1201	6183.47 ± 4088.75
14	16.214	α-copaene	1383	1379	32.71 ± 9.22
15	16.474	β-elemene	1398	1389	253.39 ± 50.51
16	17.162	Trans α-bergamottene	1442	1434	919.13 ± 442.33
17	17.228	α-guaiene	1445	1440	126.56 ± 39.27
18	17.496	α-humulene	1463	1452	95.01 ± 33.62
19	17.646	ɣ-muurolene	1473	1474	91.34 ± 38.96

20	17.931	ɣ-amorphene	1490	1492	735.43 ± 234.20
21	18.306	Guaia-9,11-diene	1515	1521	408.34 ± 104.38
22	18.433	β-copaene	1523	1565	402.47 ± 138.86
23	19.954	Cubenol	1627	1642	37.52 ± 10.56
24	20.312	Eudesm-3-en-7-ol	1651	1650	182.76 ± 59.62

Tab. 3 Buckwheat (*Fagopyrum esculentum* Moench) volatile organic compounds identified and measured by gas chromatography–mass spectrometry. Average (±SE) amounts are reported as ng µl^{-1} volatile organic compounds in methylene cloride solution; n = 4; RI Exp: experimentally determined retention index, RI Lit.: retention index in data bank. Peak numbers correspond to Fig. 19.

Peak	RT (min)	Compound	RI Exp.	RI Lit.	Amount (ng ± SE)
1	4.647	Butanoic acid	805	790	37.34 ± 14.72
2	5.913	Isovaleric acid	851	870	128.68 ± 30.24
3	6.144	2-methyl butanoic acid	871	854	114.34 ± 28.45
4	6.756	Hexanoic acid	898	951	14.42 ± 8.08
5	7.319	p-benzoquinone	923	905	19.89 ± 11.54
6	9.264	3-hexen-1-ol, acetate (Z)	1008	1002	79.28 ± 47.41
7	18.260	α-farnesene	1511	1506	81.46 ± 25.96

Tab. 4 French marigold (*Tagetes patula* L.) volatile organic compounds identified and measured by gas chromatography–mass spectrometry. Average (±SE) amounts are reported as ng μl^{-1} of volatile organic compounds in methylene cloride solution; n = 4; RI Exp: experimentally determined retention index, RI Lit.: retention index in data bank. Peak numbers correspond to Fig. 20.

Peak	RT (min)	Compound	RI Exp.	RI Lit.	Amount (ng) ± SE
1	5.916	3-hexen-1-ol (Z)	861	851	708.64 ± 368.84
2	7.563	α-pinene	934	936	29.21 ± 5.96
3	8.252	Benzaldehyde	963	970	233.19 ± 131.92
4	8.465	Sabinene	974	973	115.17 ± 32.11
5	8.895	β-myrcene	993	987	54.91 ± 14.76
6	9.141	α-phellandrene	1004	1002	62.88 ± 20.48
7	9.252	3-hexen-1-ol, acetate (Z)	1007	1002	1426.26 ± 537.06
8	9.663	Limonene	1029	1025	731.54 ± 148.80
9	9.887	(E)-β-ocimene	1041	1041	367.43 ± 243.40
10	10.052	Benzeneacetaldehyde	1043	1050	874.45 ± 476.23
11	10.200	Dihydrotagetone	1055	1047	230.14 ± 177.42
12	10.916	Terpinolene	1087	1082	1614.82 ± 582.93
13	12.049	(Z)-tagetone	1132	1134	55.91 ± 12.76
14	12.220	(E)-tagetone	1157	1152	249.11 ± 190.76
15	12.681	1,8-menthadien-4-ol	1174	1175	27.39 ± 10.73
16	12.849	p-cymen-8-ol	1190	1169	18.86 ± 7.79
17	13.728	n-valeric acid cis-3-hexenyl ester	1236	1239	232.91 ± 131.18
18	13.837	Verbenone	1245	1183	36.20 ± 28.64

19	15.348	7aH-silphiperfol-5-ene	1331	1329	45.72 ± 8.37
20	15.468	p-menthe-1,8-dien-4-hydroperoxide	1319	1333	29.39 ± 11.73
21	16.974	(E) β-Caryophyllene	1428	1421	324.96 ± 120.45
22	17.927	ɣ-amorphene	1490	1492	101.98 ± 23.75
23	18.172	Isogermacrene A	1506	1502	38.12 ± 9.48
24	18.258	α-farnesene	1512	1506	16.69 ± 7.86

Tab. 5. Buckwheat (*Fagopyrum esculentum* Moench) Main volatile organic compounds identified by gas chromatography–mass spectrometry from leaves and flowers. Peak numbers correspond to Fig. 19.

Peak	Compound
1	Butanoic acid
2	2-Pentanone 4 hydroxy 4 methyl
3	Isovaleric acid
4	2-methyl butanoic acid
5	Pentanoic acid
6	3-Hexen-1-ol, acetate (Z)
7	Internal standard

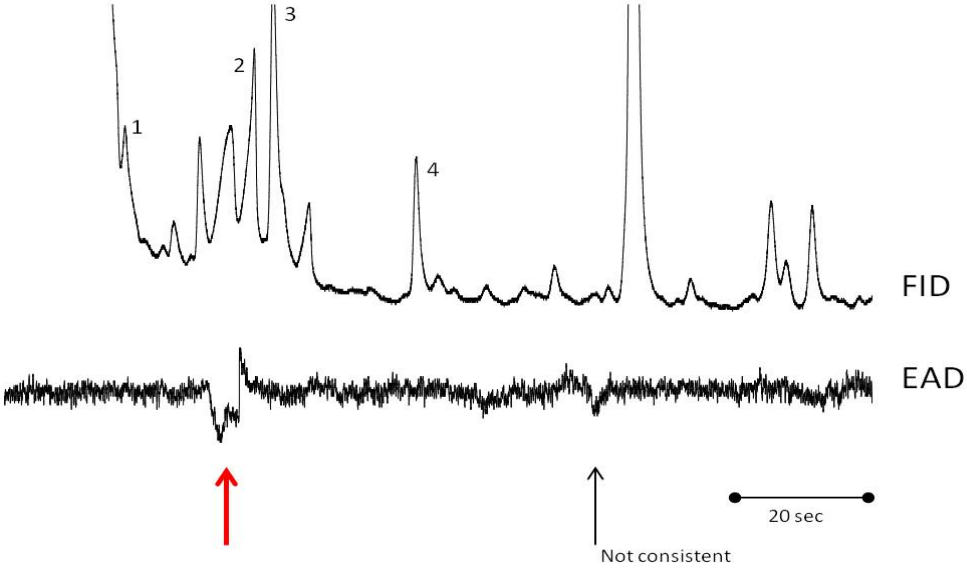
Electrophysiology experiments

Gas Chromatography-Electroantennogram Detection responses to buckwheat plant extracts are shown in Fig. 22. Among the identified compounds, GC-EAD analysis suggested that two EAD- active peaks, isovaleric acid and butanoic acid, 2 methyl, elicited consistent responses of *T. basalis* antennae.

EAG responses to the main compounds of buckwheat extract and to the carboxylic acids are shown in Fig. 23. Among the main compounds of buckwheat extract tested, butanoic acid, isovaleric acid, 3-hexen-1-ol,acetate (Z) and linalool elicited an antenna response significantly higher than the hexane. Moreover, 3-hexen-1-ol,acetate (Z) and linalool elicited the higher antenna response compared the other compounds. Among the carboxylic acids, butanoic acid, pentanoic acid, elicited a significantly higher response with respect to hexane. Neither of the tested compounds elicited an antenna response significantly different than caryophyllene, the standard used to normalize the antenna response.

Females of *T. basalis* adults showed dose-dependent EAG responses, with increasing responses to increase in doses of the 3 identified major buckwheat compounds, *i.e.*, isovaleric acid, 2-methyl butanoic acid and 3-hexen-1-ol,acetate (Z) (Fig. 24, Tab. 6). The dose of 1 µg elicited the weakest response for all the treatments. Significant statistical differences in the EAG responses were observed among the tested compounds. At the dose of 1 µg and 10 µg not significant difference was found among

the tested compounds ($p > 0.05$ Fisher's LSD test). At the dose of 100 µg the greater response was elicited by 3-hexen-1-ol, acetate (Z) ($p < 0.05$ Fisher's LSD test) whilst no significant difference was found between the response elicited by isovaleric acid and 2-methyl butanoic acid ($p > 0.05$ Fisher's LSD test).



Peak	Retention time	Compound
1	1.412	Butanoic acid
2	1.742	Isovaleric acid
3	1.784	Butanoic acid, 2 methyl
4	2.145	Pentanoic acid

Fig. 22. Flame ionization detector (FID) and electroantennographic detector (EAD; *Trissolcus basalis* antenna) responses to headspace volatiles from buckwheat plants. 2–3, EAD-active compounds. Peaks are identified in table.

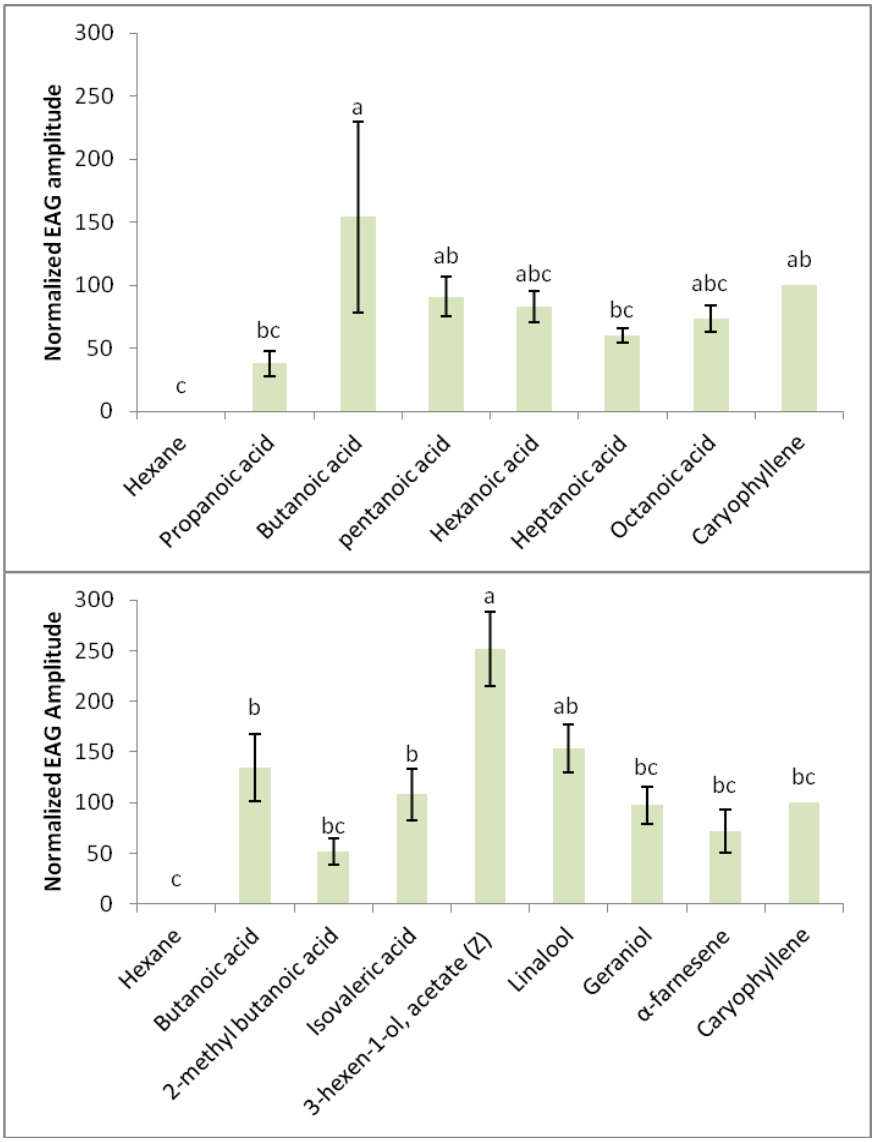


Fig. 23. EAG response of *Trissolcus basalis* to six carboxylic acids (above) and to the main 5 identified compounds of buckwheat extract, *i.e.* isovaleric acid, 2-methyl butanoic acid, 3-hexen-1-ol, acetate (Z), α-farnesene, and butanoic acid, and to 2 general plant volatiles, *i.e*. linalool and geraniol, (below). EAG amplitudes are control-adjusted and presented as proportional responses (mean±SE) to the standard caryophyllene.

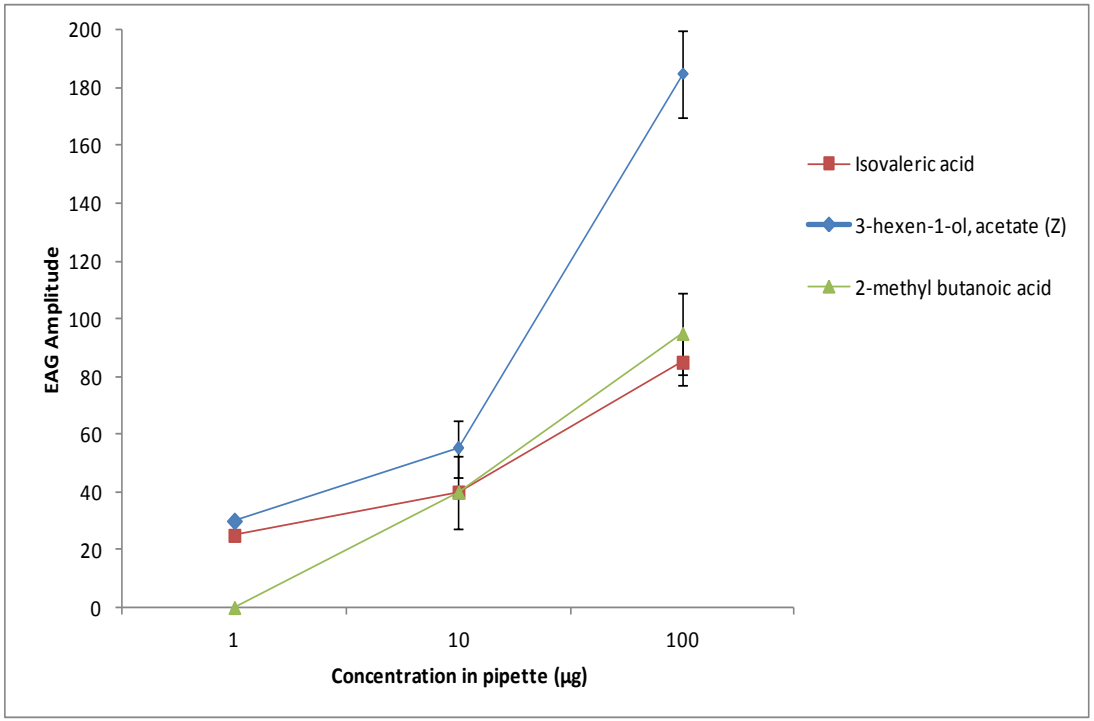


Fig. 24 EAG concentration–response curves of *Trissolcus basalis* to 3 major buckwheat volatile organic compounds. EAG amplitudes are control-adjusted and presented as proportional responses (mean±SE) to the standard caryophyllene. Error bars show the standard error of the mean. Significant differences are showed in Tab.6.

Tab. 6 Repeated-measures ANOVA of the EAG response of *Trissolcus basalis* females (SS = sum of squares; df = degree of freedom; MS = mean squares; F = F-test; asterisks indicate that values differ statistically for *P* < 0.05).

Effect	SS	Df	MS	*F*	*P*
Compound	14600	2	7300	6.96	0.01*
Dose	69066	2	34533	139.16	<0.01*
Dose*Compound	12333	4	3083	12.43	<0.01*

Discussion

This study shows the great importance of the flower nectar as nutritional resource for the parasitoid *T. basalis* since individuals with access to buckwheat and basil flowering plants significantly increased the number of their progeny.

On the total number of the emerged adults, no significant difference was found between buckwheat and basil, while a significant difference was found between buckwheat and alyssum, French marigold and water. The significant increase on the number on emerged adults was also found between basil and water while no statistic differences were found between basil, alyssum and French marigold. No difference was found comparing the data recorded for the treatments French marigold and alyssum comparing with water.

The importance of the nutrients for *T. basalis* was previously studied comparing two different diets: honeydew (from *Aphis fabae* reared on *Vicia fabae*), Safavi diet (Safavi, 1968) and water as control. Mattiacci et al., 1991 showed that the fecundity was significantly increased by Safavi diet, while no significant difference was found comparing the number of progenies from females provided with honeydew or water. The total number of the adult emerged by parasitized eggs by female with only water available was 88.60 ± 5.42 (mean ± SE). This number could represent the amount of mature eggs of emerged female wasps. In fact, *T. basalis* is classified as proovigenic (*i.e.*, females parasitoids complete oogenesis prior to eclosion and generally are able of lay their eggs soon after emergence) and its potential fecundity was registered as 61.55 ± 17.33 ovarian eggs (Mattiacci et al., 1991). Several are the authors that showed how the floral nectar type enhances parasitoid fecundity, increasing the reproductive lifespan and/or accelerating the rate of egg maturation (Baggen and Gurr, 1998; Schmale et al., 2001; Berndt and Wratten 2005; Winkler et al., 2006; Aduba et al., 2013). As well for proovigenic species, the food resources were found to increases the number of progeny by increasing oviposition rates. For example,

Wäckers in 2000 and Guren et al., 2004 showed respectively that *Heterospilus prosopidis* (Viereck) and *Trichogramma brassicae* Bezdenko benefited from the floral resources. Rahat et al., 2005 conducted laboratory studies and showed that *T. basalis* longevity was increased by access to flowers of the species French marigold, basil and buckwheat compared to others flowering plants, such as alyssum. The same authors identified the reason of this lack of performance on the architecture of the alyssum flower and the relatively large size of *T. basalis* adults so that the wasp may be prevented from reaching the nectaries within the small inflorescences of alyssum.

Conversely, the provided food type did not affect the sex ratio of *T. basalis* throughout all treatments. It was found that the sex ratio is influenced by other factors. The wasp *T. basalis* is haplodiploid and arrhenotokous (haploid males develop from unfertilized eggs and diploid females from fertilized ones), and thus, in direct control of their sex ratio by control of fertilization. Hence, mated females have a precise control over the sex of each offspring by choosing whether they fertilize an egg or not (Steiner and Ruther, 2009). Moreover, the number of host eggs per patch encountered elicits changes in sex ratio (Colazza et at, 1991). Mattiacci et al. in 1991 from laboratory observations of life time fecundity of females reared on a honey-water solution show that toward the end of the oviposition period, female wasps start to lay an increasing proportion of unfertilised eggs (and thus male progeny), suggesting that they did not receive a sufficient amount of sperm to fertilize all of their progeny (*i.e.*, sperm depletion). The availability of food was found to affect the offspring sex ratio of some parasitoid species (Leatemia et al., 1995; Khafagi, 1998). Berndt and Wratten in 2005 conducted laboratory experiment assessing the effect of floral food resources on the sex ratio of the Braconidae *Dolichogenidea tasmanica* (Cameron), a parasitoid of leafrollers (Lepidoptera: Tortricidae). The sex ratio of *D. tasmanica* was male-biased when parent female parasitoids had access to alyssum plants with flowers comparing with plants without flowers.

In the large view of the use of the flowering plants in conservation biological control prospective, the olfactory attraction turns certainly important to identify flowers that are attractive to the parasitoids. More detailed, the importance of the selection of the "right" flower resource is underlined by the results of the olfactometer bioassays that showed positive preference for flowers over the leaves only when buckwheat was the odour sources. Kugimiya et al., (2010) found a similar specific response to odours of mustard flowers *Brassica rapa* L. in the parasitoid *Cotesia vestalis* (Haliday). Indeed, *C. vestalis* showed a significant preference for the inflorescence over the inflorescence stem with flowers removed.

The preference of *T. basalis* females for the buckwheat flowers is strengthened by the GC-EAD results. Indeed, the results showed consistent GC-EAD responses in correspondence of two carboxylic acids, *i.e.* isovaleric acid and butanoic acid, 2 methyl, present on the chemical profile of volatiles collected from only buckwheat flowers. It is difficult to distinguish any specific pattern of response and the strength of perception from the parasitoids due to the not clear differences in sharpness of the GC peaks and the number of molecules that hit the antennae at the same time. The GC-EAD results were followed by olfactometer bioassays in order to assess the attractiveness of the buckwheat compounds using a synthetic blend based on the calculated amounts from buckwheat extract analysed by GS-MS. The bioassay showed attractive behaviour indicating that wasps are able to detect some of the compounds released by the buckwheat flowers. In this view, a more thorough identification of the GC peaks in the buckwheat extracts may reveal interesting prospects for application and should improve the understanding of the recognition by female parasitoids. The attractive behaviour of the wasps showed the innate preferences for the buckwheat flowers odours and it seems that *T. basalis* evolved mechanisms to respond to distinct plant volatiles and use these olfactory cues to locate available food sources efficiently. More detailed study should be done at the single sensillum level in order to determine the specificity of olfactory cells to the different compounds present in

buckwheat flowers. Moreover, the results show that *T. basalis* have a wide olfactory capability on detecting isovaleric acid and butanoic acid; further studies to understand the mechanisms mediating host finding by parasitic wasps may help in developing methods to optimize the efficiency of natural enemies as biological control agents. In conclusion, buckwheat plants, that have previously showed to be efficient for *T. basalis* in term of longevity (Rahat et al., 2005), show to increase also its fecundity. Moreover, *T. basalis* shows an interesting attractive behaviour for the buckwheat flowers. This highlights the importance of taking longevity, fertility and flower attractiveness into account in the choice of suitable flowering plants for conservation biological control.

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Chapter 4: Effect of exogenous and endogenous factors on host searching behaviour of *Trissolcus basalis*

Abstract

The potential for parasitoids to regulate host population depends on many factors such as reproductive potential, age, host-finding ability, temporal and spatial synchronism and environmental parameters. Several studies have carefully focused the interest on the effects of the endogenous and exogenous factors on the reproductive potential of the parasitoids as well as their life expectancy, focusing mainly on biological and physiological parameters. Here, it is investigated the effects of four factors, age (3days-old vs 20days-old), conspecific crowding (isolated or not for 20 days), the feeding status (feeding or not for 2 days) of wasps and environmental temperature (4°C vs 25°C) on the host location behaviour of an egg parasitoid, using the model of *Trissolcus basalis* (Wollaston) (Hymenoptera: Platygastridae) exploiting the footprints left by ith host *Nezara viridula* (L.) (Heteroptera: Pentatomidae).These cues represent a set of indirect host-related contact kairomones that induce arrestment and motivated searching behaviour, as they drive wasps in an area where there is a high probability of finding hosts but are not able to "promise" the presence of the suitable host stage. Bioassays were conducted on an open arena made by a filter paper sheet; in the middle, a circular area (4 cm diameter) was defined and exposed for 30 min to a single adult of *N. viridula*, while the surrounding area was left untreated. The results showed that the host location behaviour is influenced by age and feeding status of the wasps, whilst it did not affect by environmental temperature and wasps density. The potential significance of these results in the host location behaviour of *T. basalis* is discussed.

Keywords: *Trissolcus basalis, Nezara viridula*, host location, endogenous and exogenous factors.

Riassunto

L'efficienza dei parassitoidi nel regolare le popolazioni dei loro ospiti dipende da molti fattori, quali ad esempio il potenziale riproduttivo, l'età, l'abilità nella ricerca dell'ospite, il sincronismo spazio-temporale e i parametri ambientali. Molti studi hanno focalizzato la loro attenzione sugli effetti che i fattori endogeni ed esogeni possono avere sul potenziale riproduttivo del parassitoidi, come ad esempio la longevità, focalizzandosi, dunque, principalmente sui parametri biologici e fisiologici. In questo lavoro vengono studiati gli effetti di 4 fattori, età (3 giorni contro 20), densità d'allevamento (parassitoidi isolati singolarmente o non per 20 giorni), lo stato nutrizionale del parassitoide (alimentato o non per 2 giorni) e la temperatura ambientale (4°C o 25°C) sulle comportamento di ricerca dell'ospite da parte di un ooparassitoide, usando come strumento le tracce lasciate dall'ospite e come modello il sistema *Trissolcus basalis* (Wollaston) (Hymenoptera: Platygastridae)- *Nezara viridula.* (L.) (Heteroptera: Pentatomidae). Questi segnali, funzionando come cairomoni di contatto e segnali indiretti, guidano il parassitoide, inducendo una ricerca "motivata", verso un'area dove c'è una più alta possibilità di trovare il proprio target. Bioasaggi sono stati condotti su un'arena aperta costituita da un foglio di carta da filtro nel mezzo della quale è stata definita una superficie circolare (4 cm di diametro) ed esposta per 30 minuti ad un adulto di *N. viridula*; l'area circostante è stata lasciata non trattata. I risultati hanno mostrato che la ricerca dell'ospite da parte di *T. basalis* è influenzata dai fattori endogeni, quali età e stato nutrizionale, mentre i fattori esogeni, quali temperatura ambientale e la densità di allevamento, non hanno influenzato il comportamento del parassitoide. Il potenziale significato di questi risultati nel comportamento di ricerca dell'ospite da parte di *T. basalis* è discusso.

Parole chiave: *Trissolcus basalis, Nezara viridula*, localizzazione dell'ospite, fattori endogeni ed esogeni.

Introduction

Several studies have carefully focused the interest on the effects of the endogenous and exogenous factors on the reproductive potential of the parasitoids as well as their life expectancy. The age of the parasitoids and the nutritional sources are factors well known influencing their biology and fitness, such as the parasitism rate. For example, the egg parasitism by female *Ceratogramma etiennei* Delvare (Hymenoptera: Trichogrammatidae) is affected by its age, indeed the optimal age for successful parasitism by this parasitoid range from 1 to 2-d old (Amalin et al., 2005). Moreover, the age influences the flight activity of *Microplitis mediator* (Haliday) (Hymenoptera: Braconidae) females. Yu et al., in 2009 showed variability in flight capability at different ages: the mean flight distance gradually increased with age between 1 and 7 days. Many parasitoids require non-host food resources, for example floral nectar, honeydew or other sugar diets, such as honey-water solution, used often in laboratory conditions, to achieve maximum longevity and reproductive capability (Wäckers 2003). The survivorship of *Macrocentrus grandii* (Goidanich) (Hymenoptera: Braconidae) individuals, that were provided with sucrose-water, was found be higher than the individuals with only water available (Olson et al., 2000). Studies on insect cold-hardiness have been investigated in many insect species (Sømme, 1999) and are generally achieved by measuring the capacity to survive at constant (Bale 2002 and Renault et al., 2002) and/or fluctuating (Colinet 2006) low temperatures for extended periods. Experiments dealing with storage of *Telenomus busseole* Gahan (Hymenoptera: Scelionidae) adults indicated significant effect on its survival. At 8°C, the percentage of survival adults decreased sharply, while the ideal storage temperature is 12 ± 1°C (Bayram et al., 2005). Since the physiologic and biologic effects of the endogenous and exogenous factors are very well documented, less are the investigations on parasitoid behaviour impact. The environmental and

physiological conditions of the parasitoids may influence their host searching behaviour and, as a consequence, their efficiency as a biological control agent. The environmental factors include the leading stimuli from the host and/or the host habitat as well as abiotic factors, mainly climatic conditions (Harvey 2005). Moreover, the physiological state of the parasitoids influence their foraging motivation; age, egg load, hunger and mating status are among the parameters known to influence host selection behavior (Vinson 1998). The nutrient limitation might indirectly affect fitness traits such as host finding and dispersal efficiency (Bezemer et al., 2005). The flight activity of *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae), an egg-pupal parasitoid of many Tephritidae species, is strongly influenced by feeding status showing that the flight decreases after 48 h of starvation (Rousse et al., 2009). Parasitoid's performance is influenced by endogenous and exogenous factors in terms of parasitism ability, fecundity and host selection process. The study of these aspects has been evaluated using the generalist egg parasitoid *Trissolcus basalis* (Wollaston) (Hymenoptera: Platygastridae) that is commonly used as biological agent of the green vegetable bug *Nezara viridula* (L.) (Heteroptera: Pentatomidae). The potential effects of the age and of stress such as cold storage, hunger status and wasps density, so far, have not been considered on the walking behaviour of *T. basalis*. Its foraging behavior is mediated by semiochemicals associated with its hosts. The most reliable signal for egg parasitoids to optimize the host location is the footprints, chemicals remaining on the substrate where the host has walked (Vinson 1998). In response to these chemical signals, female of *T. basalis* displays a well known arrestment behaviour and a prolonged period of walking on contaminated areas with systematic return to the stimulus and characteristic sequences of walking changing in speed and in frequency of turning (Colazza et al., 1999; Peri et al., 2006; Rostàs et al., 2008). This behaviour has been remarkably studied. Naïve *T. basalis* females are able to discriminate between areas contaminated by chemical residues left by a host female or host male, with a clear preference for the first (Colazza et al., 1999). Furthermore, wasps'

arrestment response to chemical residues of host females became weaker when wasps were not rewarded by an oviposition experience, and stronger following successful oviposition (Peri et al., 2006). The contact kairomone that elicits foraging by *T. basalis* females is present in the cuticular lipids of *N. viridula*, and that the presence or absence of nC19 allows T. basalis females to distinguish between residues left by male or female hosts (Colazza et al., 2007). Moreover, studies demonstrated that *T. basalis* has some learning-forgetting capacities as far as the response to host footprints is concerned (Dauphin et al., 2009). In details, this study, using the host footprint exploitation as a tool, focus to the understanding how the endogenous and exogenous factors, such as (1) *Wasp age*, (2) *Wasp density*, (3) *Environmental temperature*, and (4) *Feeding status*, may influence the host searching ability of *T. basalis*.

Materials and methods

Insects

Nezara viridula and *Trissolcus basalis* colonies were started from materials collected during summer 2013 from fields located around Palermo, Italy. The *N. viridula* colony was reared in an environmental room (25±1°C, 50±10% relative humidity and L16:D8), inside wooden cages (50 x 30 x 35·cm) ventilated with mesh. Paper towels were placed inside each adult cage as an ovipositional substrate. All stages were fed with fresh cabbage, cauliflower, beens and sunflower seeds. Food was changed every 2 days and water was provided through cotton wool soaked in small containers.

Bugs used for bioassay preparation were females, mated, in pre-ovipositional physiological state (*i.e.*, with enlarged and slightly bloated abdomens) and approximately 10–14 days post-emergence. They were separated from males after mating and isolated individually for 24 h before the experiment.

The colony of *T. basalis*, established from around 140 wasp females emerged from *N. viridula* sentinel egg masses placed in cultivated fields nearby Palermo, was reared in an environmental room (25±1°C, 50±10% relative humidity and L16:D8), inside 85 ml glass tubes and fed with drops of honey-water solution (80:20 v/v). Three times per week, 1-2-day-old egg masse of *N. viridula* were removed from the oviposition substrate, glued on a strip of filter paper and singly exposed to 3 female wasps for 3 days in a 85 ml glass tube. The parasitized egg masses were held in the same environmental conditions until adult emergence. After emergence, males and females were kept together to allow mating and 24h later were differently reared according to experiments.

Effect of wasp age - wasps were individually isolated in 2-ml glass vials, fed with a drop of honey-water solution (80:20 v/v) and stored at 25° C for 3 (Young) or 25 days (Old). Each treatment was replicated 45 times.

Effect of wasp density - wasps were placed individually (Isolated) or in group of 40 (Crowded) inside a 85 ml glass tube provided of a drop of honey-water solution (80:20 v/v) for 20 days at 25° C. One hour before bioassay, wasps were isolated in 2-ml glass vial. Each treatment was replicated 25 times.

Effect of environmental temperature – wasps were individually isolated in 2-ml glass vials, provided of a drop of honey-water solution (80:20 v/v) and a piece of paper as provision of shelter. Vials were stored in climatic chamber in dark conditions for 5 days at 4°C (Cold) or 25°C (NoCold). Each treatment was replicated 45 times.

Effect of feeding status – wasps were individually isolated in 2-ml glass vials, and provided (Fed) or not (Unfed) of a drop of honey-water solution (80:20 v/v). Vials were stored at 25° C for 2 days. Each treatment was replicated 45 times.

Bioassay procedure

Bioassays were conducted in an open arena consisting of a quadrate sheet of filter paper (20x20·cm; wasp/arena ratio 0.002%), In the middle of each arena, a circular area (6·cm diameter; 7.1 % of the total area; wasp/arena ratio: 0.07%) was defined by a cardboard mask put on the filter paper, and exposed for 30 min to a single adult female of *N. viridula*, 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 device made from a transparent polyethylene Petri dish cover connected to a table watch. Open

arenas contaminated by bug's faeces were not used for bioassays. Sixty minutes prior to the experiments, female wasps were transferred to the bioassay room (25±1°C, 50 ± 10% RH) to acclimatize. After removing the bug, a female wasp was gently released in the middle of the circular area. All wasps used for the bioassays were naïve to oviposition experience, lacked previous contact with host chemical traces. The arena was illuminated from above by two 22-W cool white fluorescent tubes (Full spectrum 5900 K, 11W; Lival, Italy). Wasps that immediately displayed the typical arrestment posture, *i.e.* motionless with the antennae in contact with the leaf surface were scored as ''responding'' (Fig. 25). Wasps that did not show the arrestment response were recaptured and retested approximately 1 min later. After three unsuccessful trials, wasps were considered ''non-responding'' and excluded from the data analysis. Responding female behaviour was recorded using a monochrome CCD video camera (Sony SSC M370 CE) fitted with a 12.5–75 mm/F 1.8 zoom lens, until wasp flew away from or walked off the whole arena. Analog video signals from the camera were digitized by a video frame grabber (Studio PCTV–Pinnacle Systems, Mountain View, CA). Digitalized data were processed by XBug, a video tracking and motion analysis software. Wasp and arena were discarded after each successful bioassay. The walking behaviour of female wasps were measured as (1) residence time in the entire arena, *i.e.* pooling time spent by wasps inside and outside the circular contaminated area (Fig. 26), (2) average linear speed (mm s^{-1}) and (3) tortuosity index, *i.e.* a spatial index computed from the coordinates of the wasps (sample rate=15 points s^{-1}) calculated as 1–mp/tl where mp=maximum projection of the track in a general straight line of the plane, and tl=total length of the track. The value of this last parameter can range from 0.0 to 1.0, with higher values corresponding to more tortuous walking paths (Colazza et al., 1999). All experiments were carried out from 09:00 h to 13:00 h.

Fig. 25 "Responding" *Trissolcus basalis* females in arrestment position with antennae kept in touch with the substrate.

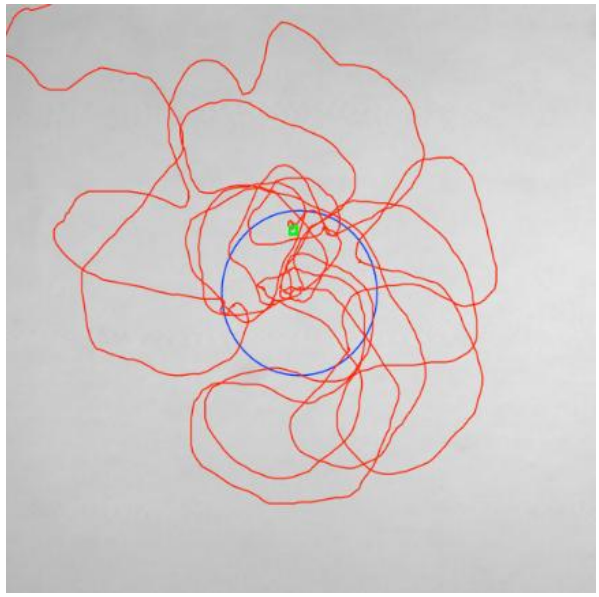

Fig. 26 Imagine taken by X-bug software. Red track represents walking path of *Trissolcus basalis* females on *Nezara viridula* females footprints. Inner circle was the treated area, remaining arena was untreated.

Statistical analysis

All parameters evaluated were analyzed using Student's t-test for independent samples. Statistical analyses were processed using Statistica7 software.

Results

Experiment 1: Effect of wasp age

The host searching behaviour of *T. basalis* old females, showed longer arena residence time compared to young wasps (t-value= -2.5654, df=88, *P*=0.012) (Fig. 27a). The mean linear speed of old wasps was lower (t-value= 2.4254, df=88, *P*=0.017) while no difference was found on the tortuosity index (t-value= -1.4460, df=88, *P*=0.1517) (Fig. 28a and 29a)

Experiment 2: Effect of wasp density

On the average, the arena residence time of responding wasps showed no statistical difference between isolated and crowded females (t-value= -0.6206, df=39, *P*=0.538) (Fig. 27b). In the same way, no statistical differences were found on linear speed (t-value= -1.5843, df=39, *P*= 0.121) and on tortuosity index (t-value= -1.0827, df=39, *P*= 0.285) (Fig. 28b and Fig. 29b).

Experiment 3: Effect of environmental temperature

Bioassays showed no statistic difference between cold and nocold wasps for all parameters taken into account (arena residence time: t-value= -0.2883, df=88, *P*=

0.774; linear speed: t-value= 0.9552, df=88, *P*= 0.3421; tortuosity index: t-value= -0.1550, df=88, *P*= 0.877) (Fig.27c, Fig.28c and Fig. 29c).

Experiment 4: Effect of feeding status

Fed wasps showed significantly a longer arena residence time (t-value= -2.7487, df=84, *P*= 0.007) (Fig. 27d) and lower linear speed (t-value= -3.2803, df=84, *P*= 0.001) (Fig. 28d) then unfed wasps. No statistical differences were found on tortuosity index (t-value= -1.1441, df=84, *P*= 0.256) (Fig. 29d).

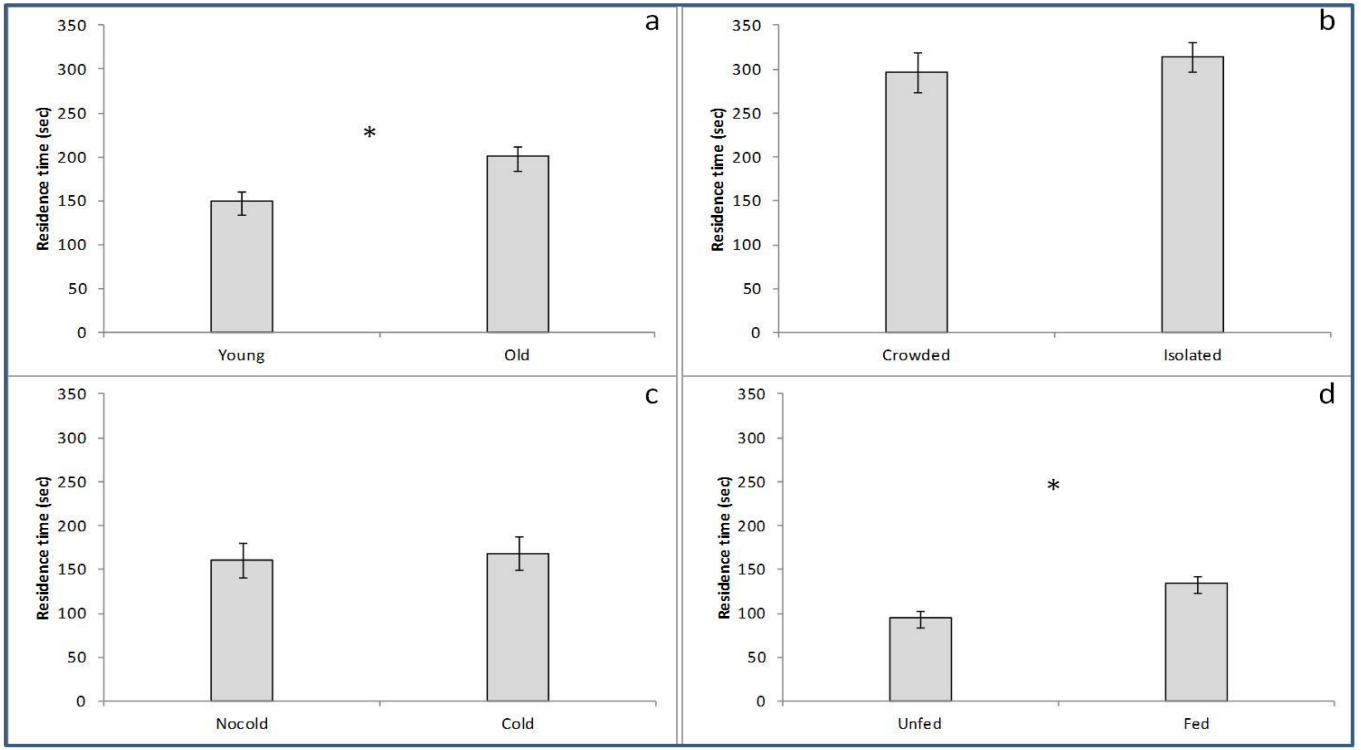

Fig. 27. Searching time expressed as Residence time (min) of females of *Trissolcus basalis* exploring an artificial substrate contaminated with footprints laid by adult female of the host *Nezara viridula*. Asterisks indicate values that differed significantly ($p<0.05$).

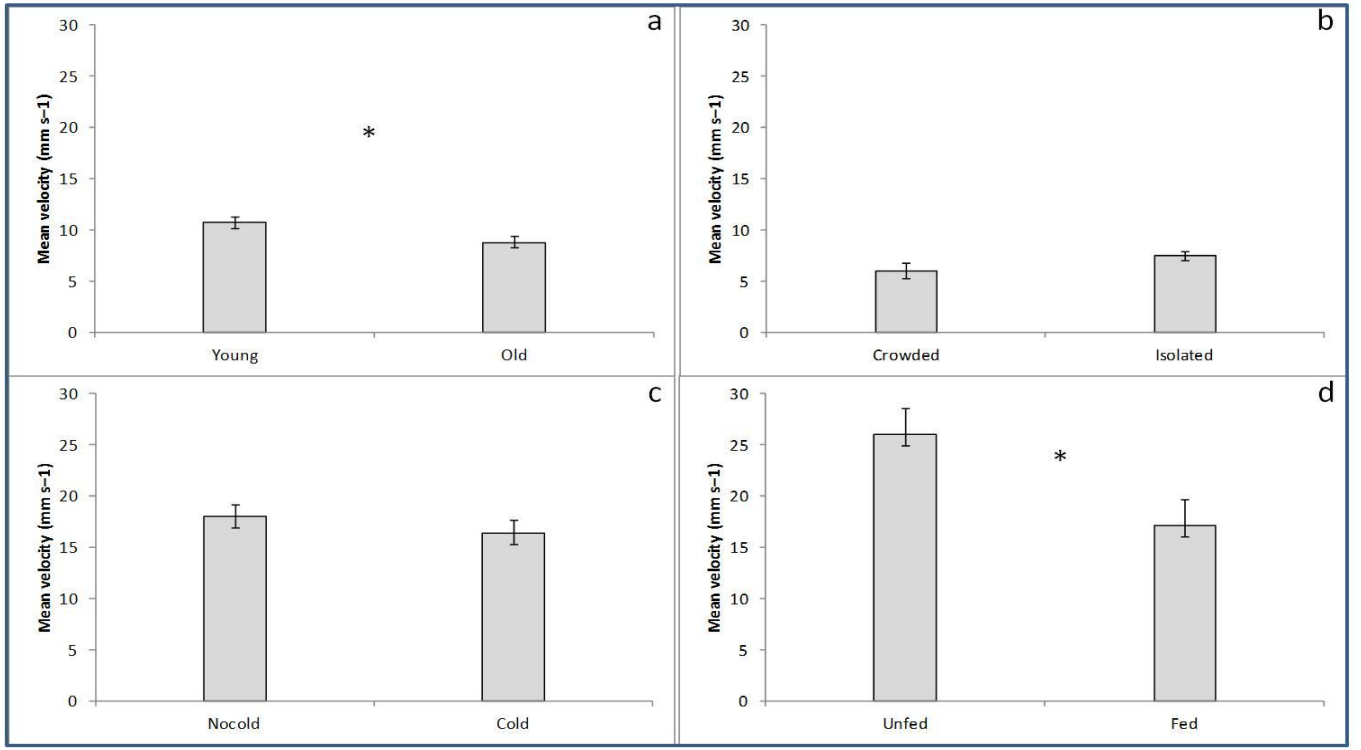

Fig. 28 Searching time expressed as men linear speed (mm sec^{-1}) of females of *Trissolcus basalis* exploring an artificial substrate contaminated with footprints laid by adult female of the host *Nezara viridula*. Asterisks indicate values that differed significantly ($p<0.05$).

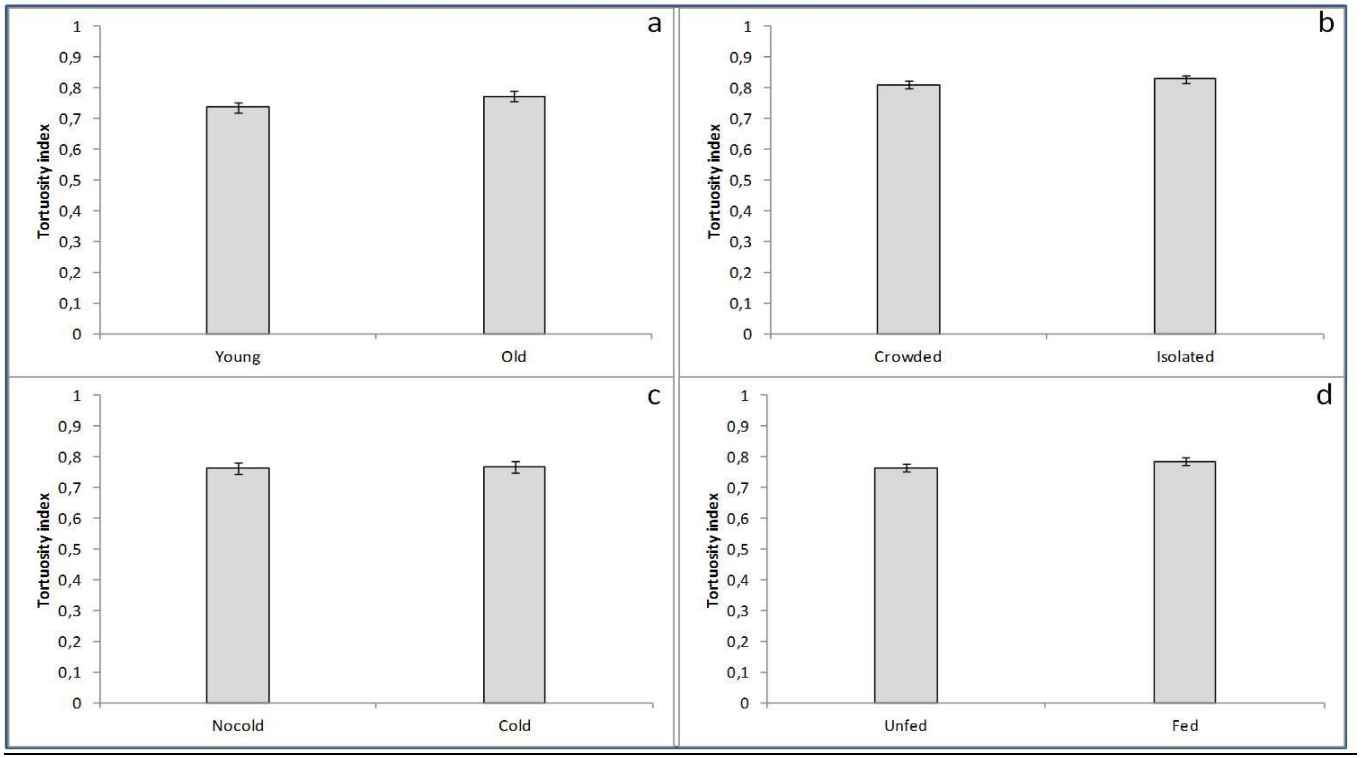

Fig. 29 Searching time expressed as Tortuosity index (see Bioassay procedure for definition) of females of *Trissolcus basalis* exploring an artificial substrate contaminated with footprints laid by adult female of the host *Nezara viridula*.

Discussion

The results showed that the searching ability is influenced by the endogenous factors, wasp age and feeding status, whilst it did not effect by the exogenous factors, environmental temperature and wasps density.

The process of host finding usually consists of some steps, *e.g.*, host habitat location, host location, host recognition and host acceptance (Vinsons 1998). Parasitoids of herbivorous insects use visual signals and chemical cues emitted by the infested plant or originated from the hosts to distinguish infested from uninfested areas and to locate the appropriate host stages (Turlings and Wäckers 2004; Vet and Dicke 1992). In particular, for egg parasitoids, once landed on a plant, host chemical footprints represent indirect host related contact cues used by the wasps to restrict the host searching to an area where host eggs are more likely to be found (Colazza et al. 2010). In this way, once on infested plants, parasitoid host searching behaviour is showed by lowered flight propensity, prolonged stay on the plant, reduced movement, and increased klinokinesis (Colazza et al., 1999). For T. basalis, this innate response to host chemical footprints is influenced by the reproductive success accumulated while foraging on plant surfaces contaminated by host residues. Indeed, the decision to remain on the path to search for hosts was influenced by oviposition experience. The arrestment responses to chemical residues of host females of *T. basalis* females became stronger following successful oviposition (Peri et al., 2006). The efficacy of using natural enemies to control pests under field conditions largely depends on their fitness, in term of mobility and, more specifically, capacity to quickly locate pest infestation. The environmental and physiological condition might affect these capacities (Boller 1972). In this study, the role of the host chemical footprints focused to improve the understanding how the endogenous and exogenous factors influence the host location behaviour of *T. basalis*.

The results showed that the age of the parasitoid is an endogenous factor affecting the host location; indeed, *T. basalis* females 25 days old showed longer arena residence time compared to younger parasitoids. To date, the effect of age of the parasitoids on their fitness has been documented from a biological and physiological point of view, for example in terms of ability to parasitize the host. Indeed, the optimum age for *Cotesia marginiventris* (Cresson), to successfully parasitize larvae of *Spodoptera frugiperda* (J.E. Smith) ranges from 48 to 96 h. *Cotesia marginiventris* younger or older than the above age were not able to parasitize a host (Rajapakse 1992).

Parasitoid survival and fecundity is generally enhanced with access to carbohydrate food sources. The feeding status is a crucial factor that affects the host location behaviour of parasitoids and not availability of food showed to change the fitness of unfed parasitoids. Lack of suitable food sources for adult wasps is recognized as primary cause of failure in biological control programs (McDougall and Mills 1997; Bautista et al., 2001). Indeed, the hunger status of the wasps might combine the food-patches research with host-patch exploitation. In this sense, the reduction in arena residence time and the increased linear speed showed by *T. basalis* unfed females may be related to accessible food research.

The tolerance to cold storage is another factor that might affect the host location ability of the biological control agents. The low temperature can strongly affect the insect locomotion (Kostál et al., 2006) or the flight capability (Luczynski 2007). The exposure time to cold treatment defined in this experimental protocol did not affect the post-storage ability of *T. basalis*, indeed no statistic difference was found on the host searching behaviour. Storage at low temperature may be a valuable method for increasing the shelf life of natural enemies such as insect eggs parasitoid (McDonald and Kok 1990 and Venkatesan et al. 2000). Although the outcome is influenced by a wide range of biotic and abiotic factors experienced before, or during, the cold exposure (Colinet and Boivin 2011), the development of cold storage techniques for *T.*

basalis may be considered of utmost importance to provide efficiency in mass production, as demonstrated by some studies focused on *Trichogramma* species. (Boivin, 1994; Li, 1994; Bayram et al., 2005).

From a biological control perspective, knowing the influence of endogenous and exogenous factors on the parasitoid searching ability is a crucial element to enhance their efficiency. Knowing the age of the parasitoids when they are "most efficient" might be very important in deciding the release in the field to obtain a meaningful level of parasitism.

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Concluding remarks

In assessing successful biological control programs by parasitoids, the knowledge of important traits, such as the host finding capability, *i.e*. the ability to find host and food resources, play the key role. Moreover, parasitoids in their natural environment parasitoids deal with a variable mixtures of natural cues. Some chemical cues are used by the wasps to locate their hosts, some ones drive wasps to feeding resources. The parasitoid response to these cues can fluctuate according to biotic factors and abiotic condition.

This dissertation focus in details on the role of two important tools, such as selective flowering plants as food resources to add within a crop area and the traces left behind by the hosts while moving on the plant used by parasitoids to locate their host. These tools are important in order to increase the natural enemy effectiveness and thus develop successful programmes of Conservation Biological Control.

In detail, the results of this 3-year period of research reported in this thesis focus on understanding the role of the flowering plants as food resources and the influence of endogenous and exogenous factors on the host finding behaviour using the egg parasitoid *Trissolcus basalis* (Wollaston) (Hymenoptera: Platygastridae) as a model.

On the first chapter is presented an overview on the Conservation Biological Control approch underlining the need to select the “right” diversity to add in agroecosystem.

The second chapter starts with a general introduction on the strategy of host searching behaviour of parasitoids and using the footprints as “indirect host-related cues”. The chapter is complemented by an overview of the studies on the behaviour of the egg parasitoid *Trissolcus basalis* (Wollaston) once in contact with the traces left by its adult host Nezara viridula (L.) (Heteroptera: Pentatomidae).

The third chapter tells the effect of different floral nectar diets on the fitness and on the attractiveness for the egg parasitoid *T. basalis*. The results showed that the floral resources of buckwheat plants (*Fagopyrum esculentum* Moench) were able to give the best performance in term of fitness in *T. basalis*. The attractiveness of buckwheat flowers for *T. basalis* females is demostrated by the olfactometer experiments, that

showed that the parasitoids spent significantly more time exploring the chambers connected to the flowers over the stems and leaves of the same plants only when the wasps are in contact with the buckwheat volatiles. Moreover, interesting results have arisen from electrophisiology experiments. Indeed, the attractiveness of some buckwheat compounds for *T. basalis* suggests a sort of peculiar ability to respond to distinct plant volatiles and use these olfactory cues to locate available food sources efficiently.

In the fourth chapter the influence of endogenous and exogenous factors affecting *T. basalis* host location behaviour and thus its efficiency as a biological control agent is discussed. By using the parasitoid exploitation of the chemical footprints left by its host *N. viridula* as a tool, it was found that only the endogenous factors wasps age and feeding status affect *T. basalis* host searching behaviour .

Further prospective

For survival and reproduction, it is well known that parasitoids are not only dependent on volatiles from the host or from the host plant in order to locate their hosts but also on floral odours to find plant nectar. Thus, improving floral resources within the crop can enhance recruitment and residency of beneficial arthropods.

Parasitic insects are known to rely on the detection of few specific volatile compounds to locate a resource successfully. The finding of the electrophisiology experiments, that is the consistent GC-EAD responses in correspondence of two carboxylic acids, *i.e*. isovaleric acid and butanoic acid, 2 methyl, present on the chemical profile of volatiles collected from only buckwheat flowers, may be an interesting scenario of *T. basalis* olfactory orientation to a resource by a match of resource-indicating key compounds.

Although positive results were obtained with buckwheat plants in the enclosed system used in this study, it is important to extend this findings to the field where the effects of the plant species on the wasps can be evaluated under natural conditions before they are deployed as farmscaping plants for management of *N. viridula*. Under field conditions the net benefit of these plants species for pest species can also be evaluated to ensure that the plants do not enhance pest risk.

Moreover, the use the use of wildflower strips, companion planting, trap cropping and intercropping, may be non-exogenous methods that might be used in "Attract and Reward" approach for the development of effective Conservation Biological Control strategies. Indeed, if "Attract and Reward" approach is based on the application of exogenous semiochemicals (for example synthetic HIPVs) as "attract" and non crop plants or floral resources as a "reward", may be intersting focus futher field experiments using buckwheat plants as rewarding elements. In this way may be possible to achieve the abundance and the establishment of *T. basalis*, with positive contribute to biological control of *N. viridula.*