Context-dependent chemical communication: Alarm pheromones of thrips larvae

de Bruijn, P.J.A.

Publication date
2015
Document Version
Final published version

Context-dependent chemical communication

Alarm pheromones of thrips larvae

Paulien de Bruijn

Context-dependent chemical communication

Alarm pheromones of thrips larvae

P.J.A. de Bruijn, 2015 *Context-dependent chemical communication – Alarm pheromones of thrips larvae*

PhD thesis, University of Amsterdam, The Netherlands

Publication of this thesis was financially supported by Koppert Biological Systems (www.koppert.nl)

ISBN: 978 94 91407 18 5

Cover + thrips drawings: Jan van Arkel (IBED)
Lay-out: Jan Bruin (www.bred.nl)

Context-dependent chemical communication

Alarm pheromones of thrips larvae

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
prof. dr. D.C. van den Boom
ten overstaan van een door het college voor promoties ingestelde commissie,
in het openbaar te verdedigen in de Agnietenkapel

op dinsdag 23 juni 2015, te 10:00 uur

door
Paulien Johanna Antonia de Bruijn

geboren te Maastricht

Promotiecommissie

Promotor:	Prof. dr. M.W. Sabelis (†) Prof. dr. S.B.J. Menken
Copromotor:	Dr. C.J.M. Egas
Overige leden:	Prof. dr. J.J.M. van Alphen, Universiteit van Amsterdam Dr. G.D. Broufas, Democritus University of Thrace Dr. A.T. Groot, Universiteit van Amsterdam Prof. dr. J.A. Pickett, Rothamsted Research Prof. dr. P.C. de Ruiter, Universiteit van Amsterdam Dr. I.M. Smallegange, Universiteit van Amsterdam Prof. dr. W.P. de Voogt, Universiteit van Amsterdam

Faculteit der Natuurwetenschappen, Wiskunde en Informatica

Contents

Author addresses

Institute for Biodiversity and Ecosystem Dynamics, Sections Population Biology and Evolutionary Biology, Science Park 904, 1098 XH Amsterdam, The Netherlands

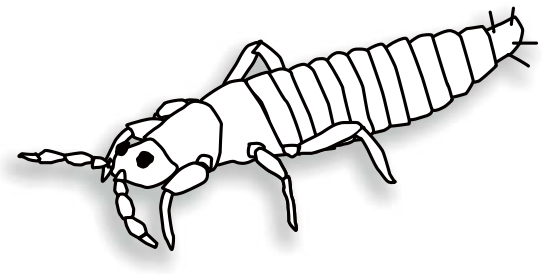
de Bruijn, Paulien J.A.
Egas, Martijn
Groot, Astrid T.
Janssen, Arne
Revynthi, Alexandra M.
Sabelis, Maurice W.
van Wijk, Michiel

Group of Arthropod Ecology and Behavior, Division of Plant Protection, Department of Crop Sciences, University of Natural Resources and Life Sciences, Peter Jordanstrasse 82, 1190 Vienna, Austria

Schausberger, Peter

1

General introduction



Alarm signals

Animals face the challenge to weigh the benefits of mating and foraging against the costs of being vigilant against predators. To deal with this challenge, they may have an alarm communication system, whereby conspecifics warn each other if danger is near. The best-studied alarm communication systems are those involving vocal alarm signals, such as the alarm calls of vervet monkeys (*Cercopithecus aethiops*, see Seyfarth *et al.* 1980a) and of ground squirrels (*Spermophilus beldingi* and *Xerus inauris*, see Sherman 1977; Robinson 1984; Furrer and Manser 2009). I will explore these three examples in more detail. First, vervet monkeys communicate alarm in a context-dependent fashion, meaning they produce different alarm calls when confronted with different predators: leopards, eagles or snakes, and the response to an alarm call depends on the type of alarm call given. When the call for leopard is voiced, vervet monkeys on the ground run into high trees where they are less vulnerable for leopard attacks; when the call for eagle is given, vervet monkeys look up and run into dense bush where eagles cannot reach them; and when the call for snake is given, vervet monkeys on the ground look down to spot and then avoid the ground predator (Seyfarth *et al.* 1980b). Given that these specific responses improve survival chances of vervet monkeys, these specific responses seem to represent adaptive strategies for coping with the hunting strategies of the predators involved (Seyfarth *et al.* 1980b). However, tests on survival of vervet monkeys when hearing specific calls and encountering one of three predators have, for practical reasons (for instance, permits to do such test on these animals would be hard to acquire), not been performed.

Second, Cape ground squirrels (*Xerus inauris*), live in a habitat where there is only one response to escape from predators; they run to their burrows. These squirrels emit urgency-dependent alarm calls; lower-urgency alarm calls for predators that are far away and higher-urgency calls for predators that are close by. The squirrels respond to playbacks depending on urgency, not predator type (Furrer and Manser 2009).

Third and last, Belding's ground squirrels also produce urgency-dependent alarm calls. Slow-moving predators evoke trills and fast-moving predators evoke chirps (Sherman 1977, 1985; Robinson 1984). Responses to trills are usually to stay put and assume an upright body posture, while chirps cause other squirrels to move away from their original position (Robinson 1984; Mateo 1996).

Although these examples show that there is a lot of knowledge on vocal alarm signals, knowledge on other alarm signals is scarce. These other alarm signals are visual signals (e.g., fin-flicking in glowlight tetras; Brown *et al.* 1999), chemical signals (pheromones, e.g., alarm pheromone of aphids; Pickett *et al.*

1992; Joachim *et al.* 2013), auditory signals other than calls (e.g., tail slapping by bottlenose dolphins; Würstig and Würstig 1979), or even mechanical signals (substrate-borne vibrations; Hill 2009). Chemical alarm signals are arguably the most common of all alarm signals, hence considering that knowledge on these signals is scarce, there is a bias in the amount of knowledge on the different types of alarm signals. Whereas many types of signals could convey an alarm message, different types of signals can have different costs to the sender, and costly signals can enforce honest communication (handicap principle; Zahavi 1975, 1977; Polnaszek and Stephens 2013). Therefore, reliable alarm signals will likely be those signals that are costly to the sender.

Evolution of alarm signals

Why do individuals alert others of impending danger? There are reasons not to call alarm, because alarm communication can be very costly. For instance, the signal may attract predators towards the calling individual or it may attract more predators towards the area where alarm was called (Sherman 1977; Klump and Shalter 1984; Teerling *et al.* 1993; Blumstein 2007; but see also Högstedt 1983; Chivers *et al.* 1996). Three common theories to explain the evolution of alarm communication are individual defence (Randall and Matocq 1997; Hasson 1991; Sherman 1985; Trivers 1971), reciprocal altruism (Trivers 1971) and kin selection (Hamilton 1964; Maynard Smith 1965).

Individual defence predicts that an individual calls alarm to defend itself (Trivers 1971; Sherman 1985). Alarm calling may help the individual in various ways, e.g., by warning the predator that it has been detected and hence the predator cannot attack the individual by surprise (Hasson 1991; Zuberbühler *et al.* 1999; Bergstrom and Lachmann 2001; Barbour and Clark 2012), by startling or confusing predators, causing them to abort the attack (Leger *et al.* 1980), by showing the predator that the prey is in good shape and hence pursuit will be difficult (FitzGibbon and Fanshawe 1988) or to avoid having a predator eat a nearby conspecific because the predator may then be more likely to stay in the vicinity and attack the alarm-calling individual at a later moment (Trivers 1971).

The second theory is **reciprocal altruism.** 'Altruistic behaviour can be defined as behaviour that benefits another organism, not closely related, while being apparently detrimental to the organism performing the behaviour, benefit and detriment being defined in terms of contribution to inclusive fitness.' (Trivers 1971). Altruism can be beneficial for an organism when *direct or indirect reciprocity* occurs. Direct reciprocity can occur when two individuals interact with each other multiple times. Then, in the long run it can be advantageous to coop-

erate (help each other), even though defecting (cheating) is more profitable at every single interaction (Trivers 1971; Axelrod and Hamilton 1981). Indirect reciprocity occurs either when an individual receives help because it offered help to another individual before (downstream reciprocity) or when an individual helps another individual because the former individual received help before (upstream reciprocity, also called generalized reciprocity).

Both direct reciprocity and downstream indirect reciprocity require that an individual is recognized by other individuals. However, upstream indirect reciprocity does not require recognition (Rutte and Taborsky 2007). An individual would only have to be more inclined to help because somebody else has helped him or her before. Which type of reciprocity is most applicable to alarm calling? When alarm signalling occurs in group-living species, direct reciprocity does not apply because there is no interaction between two individuals, but there is a signal from one individual to the others in his/her group. Downstream reciprocity is also unlikely, even if alerted group members might remember which individual sent the alarm signal, because the reciprocal action (sending an alarm signal at a later moment) would then not be directed towards this individual only but towards the entire group, giving cheaters an option to profit from the alarm signals. Upstream reciprocity is a more likely candidate to occur in alarm calling. Here, an individual is being warned by any individual from his/her group, and because he/she is being warned, he/she is more likely to warn others later, independent of who sent out the first alarm signal. Aphids produce less than half the amount of alarm pheromone when they are reared isolated from other individuals, compared to when they are reared alone, but could perceive odours from a colony of conspecifics including their alarm pheromone (Verheggen *et al.* 2009), this could be a possible example of upstream reciprocity.

Kin selection can explain the existence of alarm calling when the receivers of the alarm call are related by descent to the caller. In that case, by helping these related individuals, a caller increases his/her fitness and hence increases the chance that many copies of his/her own genes spread. In the case of kin selection, alarm calls are expected to be given only in the presence of close kin (Dunford 1977). Kin selection (described as inclusive fitness theory) is given by Hamilton's rule: C<r*B, where C is the reproductive cost to the individual performing the altruistic act, r is the genetic relatedness of the recipient to the actor, and B is the additional reproductive benefit gained by the recipient of the act (Hamilton 1964). So although alarm calling can be very costly, it can be adaptive if you save enough related individuals and thereby save copies of your own genes. Later on, a saved relative can spread its genes, including the one(s) that caused it to signal the presence of danger. If the alarm signalling appears to be

beneficial enough, the signalling gene(s) can become fixed in the population. There are a few examples where individuals call alarm more often when in the vicinity of kin (Dunford 1977; Sherman 1977). Of the species mentioned at the beginning of this introduction (vervet monkeys and ground squirrels), only Belding's ground squirrels produce alarm calls most frequently when relatives are closeby (Sherman 1977). Kin selection is expected to explain alarm signalling in species that show nepotism such as parental care.

Recently, a discussion has arisen on the usefulness of kin selection to explain social behaviour such as alarm calling. Nowak *et al.* (2010) argue that 'Inclusive fitness theory is a particular mathematical approach that has many limitations. It is not a general theory of evolution.' In their paper, they show that inclusive fitness theory is applicable under very limiting conditions (1- all interaction must be additive and pairwise and 2- populations are static or dynamic, but when dynamic global updating and binary interactions are required) and that then the inclusive fitness condition does not differ from the condition derived by standard natural selection theory for well-mixed populations. Furthermore, van Veelen (2009) claims that inclusive fitness can only be used in linear public goods games. When models allow for non-linear interactions, 'it is not possible to summarize their predictions on the basis of an evaluation of inclusive fitness'. On the contrary, Gardner *et al.* (2011) claim that inclusive fitness theory can be used because 'Hamilton (1964) made clear that inclusive fitness is defined by summing effects over potentially multiple recipients, and the rule is that natural selection will favour a trait when the total inclusive-fitness effect is positive.' However, they define that for the genetical theory of kin selection, relatedness is defined by similarity in phenotype rather than in genes. In my opinion, similar phenotypes can easily occur between two unrelated individuals, especially when different genes are evolved in a trait upon which selection acts. Whether kin selection is a useful addition to natural selection will be determined by a combination of theory and experimental work.

To test which of the theories mentioned above (individual defence, reciprocal altruism or kin selection) explains alarm signalling, it is necessary to test: (1) if an individual signalling alarm increases or decreases its own survival chance, (2) when alarm signalling increases survival chances for conspecifics, if alarm signalling can be reciprocal (hence an individual that received an alarm signal can return a signal at another point in time), and (3) if alarm signalling increases when siblings are in the vicinity. Hence, there is a need for an experimental system in which we can conduct all these tests. Only by conducting all these tests in a single system, we can properly explore the evolution of alarm signalling.

Context-dependent alarm signals

Danger can be encountered in many situations, such as an environment with predators and/or competitors. These predators and competitors can come in many forms, some being more harmful than others. Hence, a system with a single alarm signal seems insufficient to convey information about the nature of danger. As described in the examples at the beginning of this introduction, some species release a context-dependent alarm call which allows them to signal more specific information concerning the danger in the vicinity. Honest context-dependent alarm signals are required for any individual that has to effectively deal with various levels of danger. These various levels may involve different predators that vary in their capacity to kill prey, individuals within one prey species differing in vulnerability (e.g., because of a difference in size, see for instance Lima and Dill 1990; Tonn *et al.* 1992; Chase 1999) or predators being close by or further away. Hence, context-dependent alarm signals can have an impact for many, if not all, species, depending on the perception of their context.

The advantages of context-dependent communication do not depend on the type of alarm signal (calls, pheromones, etc.). So far, context-dependent signals have been shown to exist only for vocal alarm signals, for example in vervet monkeys (Seyfarth *et al.* 1980a) and Belding's ground squirrel (Sherman 1977). Little is known, however, about context-dependent alarm signalling in chemical communication (but see examples by Blum 1996). To the best of my knowledge, there are no examples of chemical alarm *signals* (pheromones) that vary depending on the danger imposed by the predator in the vicinity. What has been shown is that responses to predation-associated *cues* released by conspecifics or heterospecifics (even the predator itself can send cues) of the receiver can differ with the sender (Chivers and Smith 1998, who mistakenly call these cues pheromones; Kats and Dill 1998). Signals are primarily emitted to provide (either beneficial or misleading) information to the receiver (Hasson 1996), whereas cues are by-products, not primarily emitted for signal transfer. For example, in many fishes, chemicals that serve primarily as anti-biotics also act as cues in the sense that conspecifics respond to these chemicals when these cues are released upon attack by a predator (Cameron and Endean 1973). Hence, cues are not primarily emitted to provide information, they have initially limited possibilities to vary with context, although cues can evolve to become signals. Also, examples are known where responses to cues vary with the sender (Kats and Dill 1998). Many signals are known to contain information that depends on the context of the sender. Senders can adjust the signal they convey to optimize the benefit they obtain from sending the signal. Because pheromones are considered to be signals (Zahavi 2008), and natural selection will act upon signals to

provide information intended to benefit the receiver but should ultimately also benefit the sender, I expect that responses to alarm pheromones can vary with the sender. Moreover, I expect that senders of alarm pheromones can vary the signal as well, depending on the context.

There is a large body of literature on alarm pheromones (Pickett 1992; Blum 1996; Chivers and Smith 1998). Most of these studies on alarm pheromones, however, only analyse the chemicals composing the alarm pheromone in a single context, usually after an artificial attack by an experimenter. Hence, intraspecific variation in alarm pheromone composition is not reported in such studies. There are two examples where an alarm pheromone was shown to vary intraspecifically; (1) the alarm pheromone of Western flower thrips, *Frankliniella occidentalis* (Pergande) (Insecta: Thripidae), which is known to vary its composition of the pheromone with age (MacDonald *et al.* 2003) and (2) alarm chemicals in venom of the paper wasp *Polistes dominulus*, are excreted in different ratios by colony foundresses and workers (Bruschini *et al.* 2008), and workers have a different response to venom of workers and foundresses. Aphids cannot vary the composition of their alarm pheromone intraspecifically because they use a single chemical as alarm pheromone (Pickett *et al.* 1992). They do, however, vary the amount, frequency and duration of their pheromone release depending on the predator that is attacking (Joachim *et al.* 2013; Joachim and Weisser 2013). Furthermore, receivers of the alarm pheromone respond to the amount of the pheromone (Podjasek *et al.* 2005) as well as the frequency in which they perceive it (Kunert *et al.* 2005). Hence, chemical alarm signals, although largely overlooked, hold the promise of being context-dependent.

In this thesis, I study context-dependence in chemical alarm communication. To do so, I use Western flower thrips (see Box 1), a group-living species that has the advantage of being amenable to experiments because it produces its alarm pheromone in anal droplets that can be analysed individually for quantity and composition. The following sections will explain in more detail why Western flower thrips represent a more suitable model system to test why, when and how alarm communication occurs. Western flower thrips belong to the order of the Thysanoptera, where species occur ranging from solitary to eusocial (see Box 2), which gives possibilities for future studies to compare chemical alarm communication among species that differ in their social organisation.

Box 1: Western flower thrips

Western flower thrips are small herbivorous insects. It is a haplodiploid species, meaning that males are derived from unfertilized eggs and hence are haploid whereas females are from fertilized eggs and are therefore diploid. The life cycle of Western flower thrips consists of six stages: egg, first-instar larva, second-instar larva, prepupa, pupa and adult (FIGURE 1-1).

Originally a harmless inhabitant of Northern America, Western flower thrips became a major pest species in crops worldwide in the 1980's (Lewis 1997; Kirk 2002). What makes this species such a major pest is:

1. Fast development of insecticide resistance. Because of the high insecticide use, especially in glasshouses, a resistant strain was selected, which then spread over the world (Kirk 2003).
2. Polyphagy. Western flower thrips attack 244 plant species from 62 families (EPPO).
3. Fast population growth. Development time from egg to egg at 25 °C is approximately 14 days (van Rijn *et al.* 1995). On cucumber, the net reproduction per female ranges from 22.1 to 98 (van Rijn *et al.* 1995; Hulshof *et al.* 2003).

The damage caused by Western flower thrips on plants is either directly by feeding which leaves scars on the plant (FIGURE 1-2A,B) or indirectly, by transmission of plant viruses, such as the Tomato spotted wilt virus (FIGURE 1-2C) (van Rijn *et al.* 1995).

Thrips can be controlled chemically or by using mainly predators and entomopathogens (Lewis 1997). The success of predators used to control Western flower thrips varies for multiple reasons, among which: differences in vora-

FIGURE 1-1 Western flower thrips. From left to right: egg, first-instar larva, second-instar larva, prepupa, pupa, adult male and adult female (picture taken by J.K. Clark).

Figure 1-2 Typical damage caused by Western flower thrips. A: On a leaf of a sweet pepper plant (picture taken by PJAdB). B: On rose flower (picture taken by K. Muñoz Cárdenas). C: On a tomato, caused by Tomato Spotted Wilt Virus (picture from: http://hosting.caes. uga.edu/tswvramp/vectors/index.html)

ciousness of predator species (Sabelis and van Rijn 1997), the ability of predators to survive and reproduce on pollen as alternative food (van Rijn *et al.* 2002), the presence or absence of refuges for the Western flower thrips (Pallini *et al.* 1998), and the stage-dependent capacity of Western flower thrips to defend themselves (Bakker and Sabelis 1987). The defence of thrips consists of quickly moving their abdomen from side to side (swinging), trying to hit the predator and of producing an anal droplet and placing this droplet on the predator. Predators that are contaminated with this anal droplet retreat to groom (Bakker and Sabelis 1987, 1989). The droplet contains water, the alarm pheromone of thrips and probably other compounds (Teerling *et al.* 1993; MacDonald *et al.* 2003).

Next to alarm pheromone, other known pheromones of Western flower thrips are male sex pheromone (de Kogel and van Deventer 2003; Kirk and Hamilton 2004) and aggregation pheromone (Olaniran *et al.* 2013). Aggregation occurs also in the absence of any volatile, because both male and female Western flower thrips are attracted to specific colours, such as white, blue, violet and yellow (Terry 1997).

Outline of this thesis

The aim of this Thesis was threefold. First, I explored whether the alarm pheromone in anal excretions of Western flower thrips larvae indeed improves the defensive capacities of conspecifics (Chapters 2-4). Second, I investigated if the alarm pheromone of Western flower thrips is context-dependent (Chapters 5-6). Third, I carried out experiments to examine if thrips are amenable to research on

the evolution of alarm communication systems (Chapter 7). Chapter 8 of this thesis provides a general discussion on all three subjects mentioned above.

Pheromone as alarm signal?

Western flower thrips larvae exposed to alarm pheromone or other predation-related cues are known to respond to these stimuli by moving away from the source at some cost in terms of time and energy (Teerling *et al.* 1993; Venzon

Box 2: Alarm communication and sociality

There are many species of thrips, ranging from solitary to eusocial. Eusocial here is defined as having two distinct castes of which so-called soldiers are reduced fertile or infertile (Crespi and Mound 1997), and can usually be found in galls, where they live, eat and reproduce. The galls, and hence the colonies, are protected by soldiers.

Eusocial species that live in colonies may be more prolific producers of defensive secretions than solitary species (Terry 1997). This is very clear for alarm signals: without a conspecific receiver, the only reason to signal alarm is individual defence (as mentioned above in 'Evolution of alarm signals') whereas in large aggregations of conspecifics, an alarm signal might benefit the sender via reciprocal altruism or via kin selection as well. There is little information on alarm pheromone in solitary thrips species, although they are likely to produce anal droplets that deter predators, such as has been found in non-solitary thrips (Blum 1991). Wallen *et al.* (2014), found a component in the whole body extract of two solitary gall-inducing thrips that act as a repellent for second-instar larvae of these thrips species, however it is not known if individuals of these species can excrete this compound. On the contrary, in eusocial thrips we know of examples of an elaborate chemical alarm system (Terry 1997; de Facci *et al.* 2014). For instance, individuals of the eusocial *Kladothrips intermedius* produce anal droplets containing alarm pheromone, and soldiers produce larger droplets than dispersers, and these droplets also contain more pheromone (de Facci *et al.* 2013).

Many thrips species, such as Western flower thrips are not solitary or eusocial, but rather in-between, they are considered a communal species (Crespi and Yanega 1995). Here, communal means they do not show cooperative brood care, but they can live in large aggregations. These aggregations may consist of individuals that are related by descent, unrelated individuals, or a mixture of related and unrelated individuals. Hence, when an individual in such

an aggregation signals alarm, it may help kin or non-kin and because individuals can choose to signal alarm or not, they have the option to only signal alarm when kin individuals are nearby. Furthermore, individuals that have received a warning on the presence of a predator could perform the reciprocal action at another time, regardless of the relatedness between sender and receiver.

To conclude, alarm communication might have evolved for very different reasons in species with a different social organisation. Individual defence is a very likely reason for solitary species to excrete alarm pheromone, while kin selection or reciprocal altruism can be of more importance for eusocial species. Because thrips species range from solitary to eusocial, thrips lend themselves well for a comprehensive comparative research.

Alarm pheromone of Western flower thrips

Chemical alarm communication by thrips larvae is particularly accessible to experimentation for the following reasons; First, Western flower thrips excrete an alarm pheromone, consisting of two compounds: decyl acetate and dodecyl acetate (Teerling *et al.* 1993; MacDonald *et al.* 2003). They are both present in droplets of ca. 1 nl of rectal fluid (MacDonald *et al.* 2003). Upon excretion these so-called 'anal droplets' can be observed, counted and collected. Second, the release of an anal droplet can be triggered by prodding a larva with a fine brush. Third, because the chemicals constituting the alarm pheromone have been identified, synthetic mimics of the alarm pheromone can be made (Teerling *et al.* 1993; Chapters 2 & 4). Fourth, the amount and ratio of decyl acetate and dodecyl acetate are known to vary with the age of the larvae (MacDonald *et al.* 2003). Fifth, the age (and therefore the size) of thrips larvae matters to predation risk (Bakker and Sabelis 1987; Sabelis and van Rijn 1997). For example, predatory mites, which are ca. 0.5 mm in size, are much more successful in attacking first-instar thrips larvae (ca. 0.75 mm) than second-instar larvae (ca. 1.0 mm) (Bakker and Sabelis 1987; Sabelis and van Rijn 1997). Finally, thrips larvae exhibit easily observable defensive responses when exposed to the alarm pheromone, such as walking away (Teerling *et al.* 1993), retreating into refuges (Venzon *et al.* 2000), swinging their abdomen and producing an anal droplet in response to predator attack (Bakker and Sabelis 1989), and adults of Western flower thrips leave plants where alarm pheromone is present or avoid landing on these plants (MacDonald *et al.* 2002). Hence, Western flower trips can serve as a model species to study chemical alarm communication.

2000; MacDonald *et al.* 2002). I hypothesized that there is a less costly alternative if thrips larvae stay put and become more alert to a next exposure to danger. Therefore, in **chapter 2** I tested if thrips larvae exposed to alarm pheromone respond faster to contact (prodding with a small brush by the experimenter) than unexposed thrips larvae. Then, I hypothesized that if alarm pheromone exposure makes thrips larvae more alert to danger nearby, then they should be better able to survive an attack. Therefore, in **chapter 3**, I analysed the influence of alarm pheromone on the survival of thrips larvae.

Because the two larval instars of thrips produce an alarm pheromone that differs in the ratio of the two compounds and the total amount (ng) of these two compounds (MacDonald *et al.* 2003), and because the two larval instars of thrips do not suffer equal predation risk from predatory mites and predatory bugs (Bakker and Sabelis 1987; Sabelis and van Rijn 1997), the information value of an alarm signal may depend on the instar of the sender and the receiver. Therefore, in **chapter 4**, I tested if thrips larvae respond differently to natural alarm pheromone produced by first- or second-instar larvae and if so, whether this could be due to the difference in the total amount of pheromone or in the ratio of its two components.

Context-dependent alarm pheromone

Thrips larvae of a given instar may encounter predators that differ in the level of danger they impose, depending on the instar of the thrips larva. The composition of the alarm pheromone is known to vary with the age (instar) of the thrips larvae, but nothing is known on changes in alarm pheromone depending on the nature of danger, i.e., the predator a thrips larva encounters. In this thesis, I hypothesized that thrips larvae of a given instar change the composition of their alarm pheromone when exposed to predators imposing different levels of danger. I expected larvae to change the ratio and amount of decyl acetate and dodecyl acetate depending on the level of danger. To test this hypothesis, in **chapter 5** I analysed the chemical composition of anal droplets produced by thrips larvae in different predation contexts (i.e., nearby a very dangerous predator or a relatively harmless predator and under exposure of actual attack by these predators or not).

I hypothesized that if thrips communicate the type of danger via their alarm pheromone, then receiver thrips will differentiate their response to the composition of the alarm pheromone. To test this hypothesis, in **chapter 6** I analysed whether filter paper contaminated with anal droplets obtained in different predation contexts elicited a differential response in receiver larvae. In this way, the

focal larvae were exposed to the alarm pheromone produced in a given predation context, but not to the predator attacking the conspecific larvae that released the anal droplets in the first place. As a response variable of the thrips larvae under test I used movement into a refuge consisting of a silken web spun by spider mites (see Venzon *et al.* 2000).

Evolution of alarm communication

Alarm communication systems may evolve through various selection mechanisms (see section evolution of alarm signals). I tested in **chapter 7** whether kin selection could play a role in the evolution of thrips alarm pheromone and in particular if thrips larvae have a higher survival chance when they are surrounded by kin. My expectation was that if kin selection acts on thrips larvae, groups of kin larvae would survive better than groups of non-kin larvae. Subsequently, I conducted an experiment to analyse whether recognition of kin occurs through genotype-specific cues or through cues of the direct environment that correlate with relatedness, such as nest odour.

In the **chapter 8** of this thesis I discuss the results of my investigations into the alarm pheromone of thrips. First, I discuss what the results of this thesis add to the existing knowledge on chemical alarm communication. In conclusion, I explain why thrips may well be a promising model system to empirically test hypotheses on the evolution of alarm signals, especially in the light of the recent debate on kin selection vs. group selection (van Veelen 2009; Nowak *et al.* 2010; Gardner *et al.* 2011).

References

Bakker F.M. and Sabelis M.W. (1987) Attack success of *Amblyseius mckenziei* and the stage related defensive capacity of thrips larvae. Netherlands Journal of Zoology, 37: 437

Bakker F.M. and Sabelis M.W. (1989) How larvae of *Thrips tabaci* reduce the attack success of phytoseiid predators. Entomologia Experimentalis et Applicata, 50: 47-51

Barbour M.A and Clark R.W. (2012) Ground squirrel tail-flag displays alter both predatory strike and ambush site selection behaviours of rattlesnakes. Proceedings of the Royal Society B, 279: 3827–3833

Bergstrom C.T. and Lachmann M. (2001) Alarm calls as costly signals of antipredator vigilance: the watchful babbler game. Animal Behaviour, 61: 535–543

Blum M.S. (1991) Chemical ecology of the Thysanoptera, pp. 95–108, in B. L. Parker, M. Skinner and T. Lewis (eds.). Towards Understanding Thysanoptera, Gen. Tech. Report NE-147, USDA, Forest Service, Northeastern For. Expt. Sta., Radar, PA

Blum M.S. (1996) Semiochemical parsimony in the Arthropoda. Annual Reviews of Entomology, 41: 353-374

Blumstein D.T. (2007) The evolution, function, and meaning of marmot alarm communication. In: Advances in The Study of Behavior, Vol. 37 (Brockmann, H.J., Roper, T.J., Naguib, M., WynneEdwards, K.E., Barnard, C., Mitani, J., Eds), pp. 371-401. Elsevier Academic Press, San Diego, CA, USA.

Brown G.E., Godin J.J. and Pedersena J. (1999) Fin-flicking behaviour: a visual antipredator alarm signal in a characin fish, *Hemigrammus erythrozonus.* Animal Behaviour, 58: 469–475

Bruschini C., Cervo R., Protti I. and Turillazzi S. (2008) Caste differences in venom volatiles and their effect on alarm behaviour in the paper wasp *Polistes dominulus* (Christ). The Journal of Experimental Biology, 211: 2442-2449

Cameron A.M. and Endean R. (1973) Epidermal secretions and the evolution of venom glands in fishes. Toxicon 11: 401-410

Chase J.M. (1999) Food web effects of prey size refugia: Variable interactions and alternative stable equilibria. American Naturalist, 154: 559-570

Chivers D.P., Brown G.E. and Smith R.J.F. (1996) The evolution of chemical alarm signals: attracting predators benefits alarm signal senders. American Naturalist, 148: 649-659

Crespi B.J. and Yanega D. (1995) The definition of eusociality. Behavioral Ecology, 6: 109-115

Crepsi B.J. and Mound L.A. (1997) Ecology and evolution of social behavior among Australian gall thrips and their allies. In: The Evolution of Social Behaviour in Insects and Arachnids (ed. J.C. Choe, B.J. Crespi), pp. 166-180. Cambridge University Press, Cambridge, UK

de Facci M., Svensson G.P., Chapman T.W and Anderbrant O. (2013) Evidence for caste differences in anal droplet alarm pheromone production and responses in the eusocial thrips *Kladothrips intermedius*. Ethology, 119: 1118-1125

de Facci M., Wang H.-L., Yuvaraj J.K., Dublon I.A.N., Svensson G.P., Chapman T.W. and Anderbrant O. (2014) Chemical composition of anal droplets of the eusocial gall-inducing thrips *Kladothrips intermedius*. Chemoecology, 24: 85–94

de Kogel W.J. and van Deventer P. (2003) Intraspecific attraction in the western flower thrips, *Frankliniella occidentalis*; indications for a male sex pheromone. Entomologia Experimentalis et Applicata, 107: 87–89

Dunford C. (1977) Kin selection for ground squirrel alarm calls. American Naturalist, 111: 782-785

Eppo, http://www.eppo.int/QUARANTINE/insects/Frankliniella_occidentalis/FRANOC_ds.pdf?utm_source = www.eppo.org&utm_medium = int_redirect)

FitzGibbon C.D. and Fanshawe J.H. (1988) Stotting in Thomson's gazelles: an honest signal of condition. Behavioral Ecology and Sociobiology, 23: 69-74

Furrer R.D. and Manser M.B. (2009) The evolution of urgency-based and functionally referential alarm calls in ground-dwelling species. American Naturalist, 173: 400-410

Gardner A., West S.A. and Wild G. (2011) The genetical theory of kin selection. Journal of Evolutionary Biology, 24: 1020-1043

Griesser M. and Ekman J. (2004) Nepotistic alarm calling in the Siberian jay, *Perisoreus infaustus.* Animal Behaviour, 67: 933-939

Hamilton W.D. (1964) The genetical evolution of social behaviour. Journal of Theoretical Biology, 7: 1-52

Hamilton J.G.C., Hall D.R. and Kirk W.D.J. (2005) Identification of a male-produced aggregation pheromone in the western flower thrips *Frankliniella occidentalis.* Journal of Chemical Ecology, 31: 1369-1379

Hasson O. (1991) Pursuit-deterrent signals: Communication between prey and predator. Trends in Ecology and Evolution, 6: 325-329

Hasson O. (1996) Towards a general theory of biological signaling. Journal of Theoretical Biology, 185: 139-156

Hill P.S.M. (2009) How do animals use substrate-borne vibrations as an information source? Naturwissenschaften, 96: 1355–1371

Högstedt G. (1983) Adaptation unto death: function of fear screams. American Naturalist, 121: 562-570

Hoogland J.L. (1983) Nepotism and alarm calling in the black-tailed prairie dog (*Cynomys ludovicianus*). Animal Behaviour, 31: 472-479

Hulshof J., Ketoja E. and Vänninen I. (2003) Life history characteristics of *Frankliniella occidentalis* on cucumber leaves with and without supplemental food. Entomologia Experimentalis et Applicata, 108: 19-32

Joachim C., Hatano E., David A., Kunert M., Linse C. and Weisser W.W. (2013) Modulation of aphid alarm pheromone emission of pea aphid prey by predators. Journal of Chemical Ecology, 39: 773–782

Joachim C. and Weisser W.W. (2013) Real-time monitoring of (E)-β-farnesene emission in colonies of the pea aphid, *Acyrthosiphon pisum*, under lacewing and ladybird predation. Journal of Chemical Ecology, 39: 1254–1262

Kats L.B. and Dill L.M. (1998) The scent of death: Chemosensory assessment of predation risk by prey animals. Ecoscience, 5: 361–394

Kirk W.D.J. (2002) The pest and vector from the West: *Frankliniella occidentalis*. Thrips and Tospoviruses: Proceedings of the Seventh International Symposium on Thysanoptera (ed. by R. Marullo and L.A. Mound), pp. 33–44. Australian National Insect Collection, Canberra, Australia

Kirk W.D.J. and Terry L.I. (2003) The spread of the western flower thrips *Frankliniella occidentalis* (Pergande). Agricultural and Forest Entomology, 5: 301–310

Kirk W.D.J. and Hamilton J.G.C. (2004) Evidence for a male-produced sex pheromone in the western flower thrips *Frankliniella occidentalis*. Journal of Chemical Ecology, 30: 167-174

Klump G.M. and Shalter M.D. (1984) Acoustic behaviour of birds and mammals in the predator context; I. Factors affecting the structure of alarm signals. II. The functional significance and evolution of alarm signals. Zeitschrift für Tierpsychologie, 66: 189–226

Kunert G.S., Otto S., Röse U.S.R., Gershenzon J. and Weisser W.W. (2005) Alarm pheromone mediates production of winged dispersal morphs in aphids. Ecology Letters, 8: 596–603

Leger D.W., Owings D.H. and Gelfand D.L. (1980) Single-note vocalizations of California ground squirrels: graded signals and situation-specificity of predator and socially evoked calls. Zeitschrift für Tierpsychologie, 52: 227-246

Lewis T. (1997) Thrips as crop pests. CAB International, Wallingford, UK
Lihoreau M., Zimmer C. and Rivault C. (2007) Kin recognition and incest avoidance in a group-living insect. Behavioral Ecology, 18: 880-887
Lima S.L. and Dill L.M. (1990) Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology, 68: 619-640
MacDonald K.M., Hamilton J.G.C., Jacobson R. and Kirk W.D.J. (2002) Effects of alarm pheromone on landing and take-off by adult western flower thrips. Entomologia Experimentalis et Applicata, 103: 279–282
MacDonald K.M., Hamilton J.G.C., Jacobson R. and Kirk W.D.J. (2003) Analysis of anal droplets of the Western flower thrips *Frankliniella occidentalis.* Journal of Chemical Ecology, 29: 2385-2389
Mateo J.M. (1996) The development of alarm-call response behaviour in free-living juvenile Belding's ground squirrels. Animal Behaviour, 52: 489–505
Maynard Smith J. (1965) The evolution of alarm calls. American Naturalist, 99: 59-63
Nowak M.A., Tarnita C.E. and Wilson E.O. (2010) The evolution of eusociality. Nature, 446: 1057-1062
Olaniran O.A., Sudhakar A.V.S., Drijfhout F.P., Dublon I.A.N., Hall D.R., Hamilton J.G.C. and Kirk W.D.J. (2013) A male predominant cuticular hydrocarbon, 7-methyltricosane, is used as a contact pheromone in the Western flower thrips *Frankliniella occidentalis*. Journal of Chemical Ecology, 39: 559–568
Pallini A., Janssen A. and Sabelis M.W. (1998) Predators induce interspecific herbivore competition for food in refuge space. Ecology Letters, 1: 171–177
Pickett J.A., Wadhams l.J., Woodcoc C.M. and Hardie J. (1992) The chemical ecology of aphids. Annual Review of Entomology, 37: 67-90
Podjasek J.O., Bosnjak L.M., Brooker D.J. and Mondor E.B. (2005) Alarm pheromone induces a transgenerational wing polyphenism in the pea aphid, *Acyrthosiphon pisum.* Canadian Journal of Zoology, 83: 1138–1141
Polnaszek T.J. and Stephens D.W. (2013) Why not lie? Costs enforce honesty in an experimental signalling game. Proceedings of the Royal Society B, 281: 20132457
Randall J.A. and Matocq M.D. (1997) Why do kangaroo rats (*Dipodomys spectabilis*) footdrum at snakes? Behavioral Ecology, 8: 404-413
Robinson S.R. (1980) Antipredator behaviour and predator recognition in Belding's ground squirrels. Animal Behaviour, 28: 840-852
Rutte C. and Taborsky M. (2007) Generalized reciprocity in rats. Plos Biology, 5: 1421-1425
Sabelis M.W. and van Rijn P.C.J. (1997) Predation by insects and mites. In: Lewis T (ed) Thrips as crop pests, pp. 259–354. CAB International, Wallingford, UK
Seyfarth R.M., Cheney D.L. and Marler P. (1980a) Vervet monkey alarm calls: semantic communication in a free-ranging primate. Animal Behaviour, 28: 1070-1094
Seyfarth R.M., Cheney D.L. and Marler P. (1980b) Monkey responses to three different alarm calls: Evidence of predator classification and semantic communication. Science, 210: 801-803
Sherman P.W. (1977) Nepotism and the evolution of alarm calls. Science, 197: 1246-1253

Sherman P.W. (1985) Alarm calls of Belding's ground squirrels to aerial predators: nepotism or self-preservation? Behavioral Ecology and Sociobiology, 17: 313-323

Silk J.B. (2002) Kin selection in primate groups. International Journal of Primatology, 23: 849-875

Teerling C.R., Pierce H.D., Borden J.H. and Gillespie D.R. (1993) Identification and bioactivity of alarm pheromone in the western flower thrips, *Frankliniella occidentalis*. Journal of Chemical Ecology, 19: 681-697

Terry L.I. (1997) Host selection, communication and reproductive behaviour. In: Lewis T (ed) Thrips as crop pests, pp. 259–354. CAB International, Wallingford, UK

Tonn W.M., Paszkowski C.A. and Holopainen I.J. (1992) Piscivory and recruitment: mechanisms structuring prey populations in small lakes. Ecology, 73: 951-958

Trivers R.L. (1971) The evolution of reciprocal altruism. Quarterly Review of Biology, 46: 35-57

van Rijn P.C.J, Mollema C. and Steenhuis-Broers G.M. (1995) Comparative life-history studies of *Frankliniella occidentalis* and *Thrips tabaci* (Thysanoptera: Thripidae) on cucumber. Bulletin of Entomological Research, 85: 285-297.

van Rijn P.C.J., van Houten Y.M. and Sabelis M.W. (2002) How plants benefit from providing food to predators even when it is also edible to herbivores. Ecology, 83: 2664-2679.

van Veelen M. (2009) Group selection, kin selection, altruism and cooperation: When inclusive fitness is right and when it can be wrong. Journal of Theorectical Biology, 259: 589–600

Venzon M., Janssen A., Pallini A. and Sabelis M.W. (2000) Diet of a polyphagous arthropod predator affects refuge seeking of its thrips prey. Animal Behaviour, 60: 369-375

Verheggen F.J., Haubruge E., De Moraes C.M. and Mescher M.C. (2009) Social enviroment [sic-PJAdB] influences aphid production of alarm pheromone. Behavioral Ecology, 20: 283-288

Waterman J.M. (1995) The social organization of the Cape ground squirrel (*Xerus inauris*; Rodentia: Sciuridae). Ethology, 101: 130-147

Würstig B. and Würstig M. (1979) Behavior and ecology of the bottlenose dolphin, *Tursiops truncatus*, in the South Atlantic. Fishery Bulletin, 77: 399-442

Zahavi A. (1975) Mate selection: A selection for a handicap. Journal of Theorectical Biology, 53: 205-214

Zahavi A. (1977) The cost of honesty (further remarks on the handicap principle). Journal of Theoretical Biology, 67: 603-605

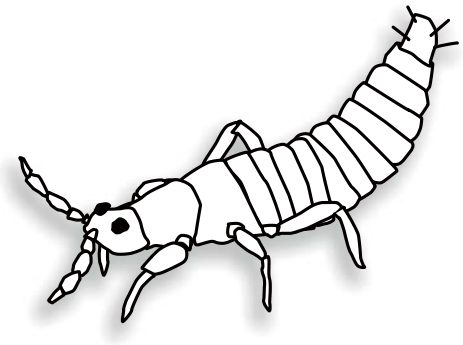
Zahavi Z. (2008) Communication in bacteria. In: Sociobiology of communication an interdisciplinary perspective (D'Ettorre, P. and Hughes, D. ed.), pp. 1-9. Oxford University Press, Oxford, UK

Zuberbühler K., Jenny D. and Bshary R. (1999) The predator deterrence function of primate alarm calls. Ethology, 105: 477-490

2

Pheromone-induced priming of a defensive response in Western flower thrips

Paulien J.A. de Bruijn, Martijn Egas, Arne Janssen & Maurice W. Sabelis



Abstract

The Western flower thrips, *Frankliniella occidentalis*, produces conspicuous anal droplets that function as a direct defence against various predators. These droplets also function in pheromonal communication in that they contain a mixture of decyl-acetate and dodecyl-acetate, which acts as an alarm. Exposure of thrips to synthetic pheromone is known to promote take-off or refuge-seeking, but the effect of the natural pheromone has not yet been studied. Here, we not only studied the response to natural pheromone, but also tested the new hypothesis that the alarm pheromone primes a defensive response in thrips. This test was carried out by measuring the reaction time to a simulated predator attack after exposure to synthetic or natural alarm pheromone (against a control with no pheromone at all). The reaction was quantified in terms of the time it takes a thrips larva to produce a droplet after attack. We found that thrips larvae produce droplets of alarm pheromone faster when cues associated with danger are present. There were no significant differences in reaction times of responses to synthetic pheromone, natural pheromone or odours from a patch with a predator attacking a thrips larva. This implies that the synthetic pheromone mimics the natural pheromone and that other cues emanating from the predator play a minor role. We conclude that the alarm pheromone increases the vigilance of the thrips and this may promote its survival.

Journal of Chemical Ecology 32: 1599-1603 (2006)

Introduction

Much intraspecific communication in insects involves chemical messengers, called pheromones, which elicit attraction of the other gender, aggregation or alarm. Alarm pheromones are animal-produced chemicals that intentionally or inadvertently communicate danger. Their release usually results in conspecifics showing anti-predator behaviour, such as fleeing, hiding, remaining motionless (e.g., in cryptic species), aggregating, changing group structure or even mobbing predators (Lima and Dill 1990). This behaviour is generally assumed to promote survival.

The Western flower thrips, *Frankliniella occidentalis* (Pergande), produces conspicuous droplets that are excreted anally. Upon encounter with a predator, thrips quickly move their abdomen from side to side (swing), trying to hit the predator, and they produce droplets when the threat of predation persists. Predators contaminated with such a droplet retreat to groom and in this way, the droplets serve as a defence (Bakker and Sabelis 1987, 1989). The droplets contain a solution of decyl-acetate and dodecyl-acetate (Teerling *et al.* 1993a,b) in a ratio that varies with the age of the larva (MacDonald *et al.* 2003). When a synthetic formulation of these compounds is present, thrips will increase their movements, reduce oviposition, decrease landing rates, increase take-off rates or hide (Teerling *et al.* 1993a,b; MacDonald *et al.* 2002), suggesting that the chemicals function as an alarm pheromone for the thrips.

Although the behaviour of thrips towards the synthetic alarm pheromone is well documented, there are no reports on the response of thrips to natural pheromone. It is also not known whether the alarm pheromone primes defensive responses. For instance, upon exposure to alarm pheromone, a thrips larva may become alerted and may produce an anal droplet faster upon actual attack. This hypothesis on priming of the defensive response was tested by assessing the effect of synthetic pheromone as well as natural alarm pheromone on the time elapsed from a simulated attack of a thrips larva until it produced an anal droplet. This effect is taken as a measure for the vigilance of thrips larvae, because it demonstrates the degree to which they are prepared to defend themselves.

Material and methods

Thrips. Thrips were collected from cucumber plants in a commercial greenhouse in Pijnacker, The Netherlands, in May 1994. They were reared in the laboratory under constant climatic conditions (25 °C and 60% RH at L16:D8) on a cucumber leaf provided with cattail pollen (*Typha latifolia*). The leaf was cut so as to fit in a Petri dish with a layer of wet cotton wool on the bottom. Cohorts of thrips were obtained by

introducing thrips pupae, allowing the emerging adults to oviposit and the larvae to develop until the pupal stage, after which this process was repeated.

Predatory bugs. Orius laevigatus (Fieber), obtained from Koppert BV, was reared using eggs of the flour moth *Ephestia kuehniella* (Zeller) as prey and bean pods as an oviposition substrate. Bean pods with *Orius* eggs were regularly collected from cages with adults in the reproductive phase, replaced by fresh ones and used to start new age cohorts (van den Meiracker 1994; Venzon *et al.* 1999).

Synthetic alarm pheromone. According to MacDonald *et al.* (2003), the alarm pheromone of Western flower thrips contains decyl-acetate and dodecyl-acetate in a molar ratio ranging from 0.4:1 (first instar larvae) to 1.5:1 (second instar larvae). Because we used second instar larvae in our behavioural experiments, we used 0.05 µl of the 1.5:1 mixture dissolved in 500 µl pentane following the procedure described by Teerling *et al.* (1993a). Decyl-acetate (98.1% pure), dodecyl-acetate (95+ % pure) and pentane (98% pure) were obtained from Sigma-Aldrich, Los Angeles, CA, USA.

Pheromone production after simulated attack. Five treatments were applied to test the response of thrips larvae to attacks simulated by the experimenter with the aid of a wooden toothpick. Four second-instar thrips larvae were transferred to a leaf disc (diameter 24 mm) cut from the cotyledons of a cucumber plant. Per replicate and per treatment, one focal individual larva was challenged repeatedly by contacting it with a toothpick until it responded by producing an anal droplet. In the first three treatments, the toothpick was dipped in synthetic alarm pheromone (dissolved in pentane) or solvents (water or pentane) as controls. Each focal thrips larva was challenged with a toothpick that had received one treatment only. Per treatment, 15 larvae on 15 different leaf discs were challenged. These tests were performed 'blind', i.e., without the observer knowing which liquid was applied to the toothpick. Therefore, variation in the way the thrips were challenged was independent of the treatment of the toothpick. In a fourth treatment, the toothpick was put in the anal droplet released by a thrips larva upon challenge with a toothpick. Subsequently, this toothpick was used to challenge another thrips larva, of which the response was recorded. For obvious reasons, this treatment could not be performed blind. In the fifth treatment, the focal thrips larva was challenged with a water-dipped toothpick, while one of the other three thrips was being attacked simultaneously by a predatory bug (*O. laevigatus*) and usually produced an anal droplet. Results of this treatment were compared with the controls with a toothpick dipped in water prior to challenge (see above).

All observations were done using a binocular microscope with magnification 6.3. The time elapsed between challenge with a toothpick and the production of a droplet by the focal thrips larva was measured and subjected to a one-way ANOVA. Multiple comparisons among treatments were made using a Tukey HSD post hoc test.

Results and discussion

The time elapsed between challenging thrips larvae with a toothpick and droplet production varied significantly among treatments (FIGURE 2-1; ANOVA: $F_{4,70}$ = 13.7, P<0.001). Compared to the controls (pentane, water), the response time was significantly shorter in treatments involving natural pheromone (pheromone droplet collected from another thrips larva), synthetic pheromone (decyl-acetate and dodecyl-acetate dissolved in pentane) and signals released nearby during the attack of another thrips larva by a predator (FIGURE 2-1). However, Tukey tests reveal no difference among these three treatments. Hence, other cues emanating from the predator appear to play a minor role in inducing vigilance in thrips larvae. Furthermore, the synthetic pheromone elicits responses similar to those elicited by the natural pheromone, indicating that the concentrations are in the same range. These results provide additional evidence that decyl-acetate and dodecyl-acetate act as an alarm pheromone. Our results also demonstrate that the response of a simulated attack with a toothpick is context-dependent; whereas attacks with a toothpick dipped in water elicits a slow

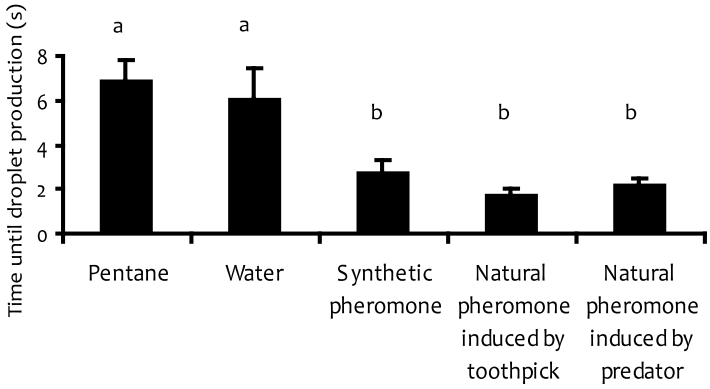


FIGURE 2-1 Mean (+ SE) response time of thrips larvae to a simulated attack, expressed as the number of seconds between attack and the production of an anal droplet containing the putative alarm pheromone. See text for explanation of the treatments; each treatment involves 15 replicates. Means with different letters differ significantly.

response, attacks with a similarly treated toothpick elicits a fast response when nearby thrips larvae produce a droplet.

If the anal droplet contains alarm pheromone, the response of the thrips larvae to the pheromone is expected to result in increased survival. We think this is the case for two reasons. First, thrips larvae with a fast response were observed to escape more often from an attack by the predatory mite *Iphiseius degenerans* (personal observation, PJAdB). Second, females of other predatory mite species have been reported to retreat upon contamination with the anal droplet (Bakker and Sabelis 1987, 1989). However, releasing a pheromone also makes the thrips larvae more conspicuous to predators since predatory bugs (*Orius tristicolor*) and predatory mites (*Neoseiulus cucumeris*) are attracted to a source of pheromone (Teerling *et al.* 1993a,b). Thus, we hypothesize that the decision to release pheromone-containing droplets upon predator attack depends on (1) the probability to escape due to increased alertness or predator retreat, on (2) the increased vigilance induced in conspecifics, but also on (3) the increased conspicuousness to other predators than the attacker. It remains to be investigated whether the balance of these costs and benefits explains the decisions of thrips larvae.

References

Bakker, F. M. and Sabelis, M. W. 1987. Attack success of *Amblyseius mckenziei* and the stage related defensive capacity of thrips larvae. *Neth. J. Zool.* 37: 437.

Bakker, F. M. and Sabelis, M. W. 1989. How larvae of *Thrips tabaci* reduce the attack success of phytoseiid predators. *Entomol. Exp. Appl.* 50: 47-51.

Lima, S. L. and Dill L. M. 1990. Behavioral decisions made under the risk of predation – a review and prospectus. *Can. J. Zool.* 68: 619-640.

MacDonald, K. M., Hamilton, J. G. C., Jacobson, R., and Kirk, W. D. J. 2002. Effects of alarm pheromone on landing and take-off by adult western flower thrips. *Entomol. Exp. Appl.* 103: 279-282.

MacDonald, K. M., Hamilton, J. G. C., Jacobson, R., and Kirk, W. D. J. 2003. Analysis of anal droplets of the Western flower thrips *Frankliniella occidentalis*. *J. Chem. Ecol.* 29: 2385-2389.

Teerling, C. R., Pierce, H. D., Borden, J. H., and Gillespie, D. R. 1993a. Identification and bioactivity of alarm pheromone in the Western flower thrips, *Frankliniella occidentalis*. *J. Chem. Ecol.* 19: 681-697.

Teerling, C. R., Gillespie, D. R., and Borden, J. H. 1993b. Utilization of Western flower thrips alarm pheromone as a prey-finding kairomone by predators. *Can. Entomol.* 125: 431-437.

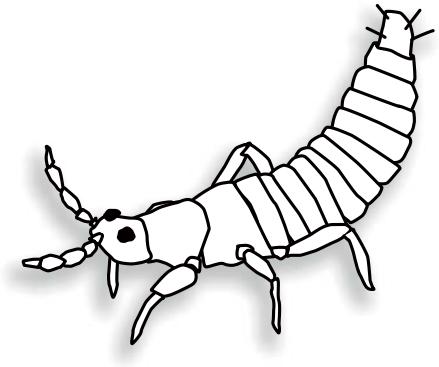
van den Meiracker, R. A. F. 1994. Life history evolution in mass rearing of *Orius insidiosus*. *Proc. Exp. Appl. Entomol.* 5: 25-30.

Venzon, M., Janssen, A., and Sabelis, M. W. 1999. Attraction of a generalist predator towards herbivore-infested plants. *Entomol. Exp. Appl.* 93: 305-314.

Increased survival of young thrips after exposure to alarm pheromone: Experiences with synthetic compounds

3

Paulien J.A. de Bruijn, Michiel van Wijk, Maurice W. Sabelis & Martijn Egas



Abstract

Animals may emit signals that make others aware of danger. Such alarm signals may only evolve if receivers of these signals benefit from it and if, ultimately, the sender also gains a selective benefit over others sharing the same gene pool (conspecifics). Although evidence for a response to these signals is widespread, evidence for a selective benefit (i.e., increased survival) for the receiver is rare. Here, we test whether exposure to alarm pheromone can increase survival in mixed-size (age) groups of Western flower thrips larvae (*Frankliniella occidentalis*) when a predatory mite (*Iphiseius degenerans*) is present. Young thrips larvae are most vulnerable to predation by predatory mites, whereas old larvae do not often suffer from predation from this predator. Indeed, we found that survival of old larvae is high irrespective of the presence of alarm pheromone. For young larvae, synthetic alarm pheromone did increase survival (but only in the treatment were thrips larvae received alarm pheromone 5 + 115 s every 2 min, but not in treatments where thrips larvae received alarm pheromone 5 or 0 s every 2 min). The increased survival of first-instar thrips larvae can be explained if larvae that are exposed to alarm pheromone respond faster to later contacts, which may be critical to survive attack by a predatory mite. Because we find that thrips alarm pheromone increases thrips survival, it can substantially promote thrips fitness.

Unpublished manuscript

Introduction

There are many animal species where, in response to danger, individuals emit alarm signals to warn conspecifics of danger (e.g., Caro 2005). Whereas the costs of producing alarm signals are often very clear (alerting a predator of your presence; Blumstein 2007; Sherman 1977; Klump and Shalter 1984; Teerling *et al.* 1993), the benefits of producing alarm signals are still subject to debate. Different theories explain such benefits, e.g., reciprocal altruism (Trivers 1971); warning kin (Hoogland 1983); individual defence (Randall and Matocq 1997); signalling to the predator that it has been spotted: (Hasson 1991; Caro 1995); attracting other predators which may disrupt the predation event (Chivers *et al.* 1996). Indeed, numerous studies show that animals respond to alarm signals (e.g., in amphibians: Rajchard 2006; in fish: Chivers and Smith 1998; in insects: Dicke and Grostal 2001), but only few studies have shown that individuals that respond to the alarm signal survive better than conspecifics that did not respond to it (as reviewed by Chivers and Smith 1998; Mirza and Chivers 2001).

In this paper, we study alarm communication in Western flower thrips (*Frankliniella occidentalis*) larvae. These thrips are known to send chemical alarm signals (alarm pheromones) consisting of decyl acetate and dodecyl acetate (Teerling *et al.* 1993; MacDonald 2003). Individuals perceiving alarm pheromone show anti-predator behaviour such as larvae walking away (Teerling *et al.* 1993) and adults flying away from the alarm pheromone source (MacDonald *et al.* 2002). Moreover, thrips larvae produce a defensive droplet earlier when alarm pheromone is present than when alarm pheromone is not present (a phenomenon called primed defence response by de Bruijn *et al.* 2006). The alarm pheromone of Western flower thrips can be synthetically produced and thrips larvae show anti-predator behaviour (increased take-off behaviour and priming) to this synthetic alarm pheromone as well (MacDonald *et al.* 2002; de Bruijn *et al.* 2006). Here, we test if the presence of synthetic alarm pheromone increases survival chance of thrips larvae under predation. Five young (first-instar) and five old (second-instar) thrips larvae were placed on a leaf arena together with a predatory mite, *Iphiseius degenerans*. These predatory mites are known to successfully attack first-instar larvae, but have difficulties attacking second-instar larvae (Sabelis and van Rijn 1997). Indeed, in a set-up similar to the one used in these experiments we previously showed that in 6 h, *I. degenerans* kills ca. two thrips larvae, almost exclusively first instars (de Bruijn *et al.* 2014). Thrips larvae can increase their chance to survive an encounter with this predator by showing defensive behaviour such as walking away, swinging their abdomen or producing a defensive anal droplet to stick it to the predator (Bakker and Sabelis 1989). The arena with thrips and *I. degenerans* was placed in an airflow containing only clean

air (control or '0 second alarm pheromone' treatment) only synthetic pheromone (referred to as '5 + 115 seconds alarm pheromone' treatment), or pulsed synthetic pheromone (at intervals of 5 s every 2 min, referred to as '5 second alarm pheromone' treatment). Pulsed presentation of alarm pheromone resembles the natural setting more closely, because thrips larvae will normally never be continuously exposed to alarm pheromone. After 6 h, larval survival was scored. We hypothesize that first-instar thrips larvae will have higher survival chances when (synthetic or natural) alarm pheromone is present.

Material and methods

Thrips

In March 2010, Western flower thrips were generously provided to us by G. Steenhuis-Broers and W.J. de Kogel from Wageningen University and Research Center, The Netherlands, who had reared the thrips on chrysanthemum. We reared the thrips in a climate room at a temperature of 25 °C and 60% RH at L16:D8 on cucumber leaves, cut to fit in a Petri dish on top of a layer of wet cotton wool that was put on the bottom of the Petri dish. Once a week, thrips pupae and adults from older leaves of the culture were put on the cucumber leaf whereas pollen of *Typha latifolia* was provided once a week on this leaf as additional food for the thrips. The next generation of pupae and adults were transferred to a new leaf in a new Petri dish to rear a next generation of thrips. At the time of the experiments reported here, we had maintained this culture in the laboratory for 2 years.

Experiments were done with 10 sibling thrips larvae per replicate. To establish such a sibling group, a single adult female was put on a leaf fragment to lay eggs and 4-8 days later, five first-instar larvae and five second-instar larvae were collected from this leaf fragment (i.e., all offspring from the same mother). Adult female thrips can lay 4-5 eggs per day (van Rijn *et al.* 1995). This enabled us to collect five first-instar larvae and five second-instar larvae from one leaf fragment.

Predatory mites

A strain of the predatory mite *Iphiseius degenerans,* originally collected in Rabat, Morocco, was reared on a diet of *Typha* pollen in a climate box at 25 °C, 60% RH and L16:D8. The rearing arenas consisted of a PVC sheet (6 × 15 cm) placed on a wet sponge in a water-containing tray. The edges of the PVC sheet were covered with paper tissue that absorbs water from the sponge underneath. The tissue served as a water source to the predatory mites and as a barrier to prevent

escape from the PVC arena. Short threads of cotton placed on the PVC sheet served as a substrate for oviposition by the predatory mites. For the experiments, we used adult females, 8-15 days old since hatching and 0.7 mm in length.

Experimental setup

A leaf disc (diameter 15 mm), excised from a cotyledon of a cucumber plant, was put on a layer of wet cotton wool in a plastic cup (height 70 mm, diameter 66 mm). One sibling group of thrips larvae was put on each leaf disc. Additionally, a single adult *I. degenerans* female was introduced to each leaf disc. Each leaf disc was kept in the laboratory (21 °C, daylight, in the period from March to May 2013) in an established airflow. Pressed air was guided through a splitter tube creating three air flows (see FIGURE 3-1). For 6 h, each of these three air flows were forced alternatingly through one glass bottle (Schott GL45, 100 ml, height 105 mm, diameter 56 mm) for 5 s and through another for 115 s. Some bottles contained alarm

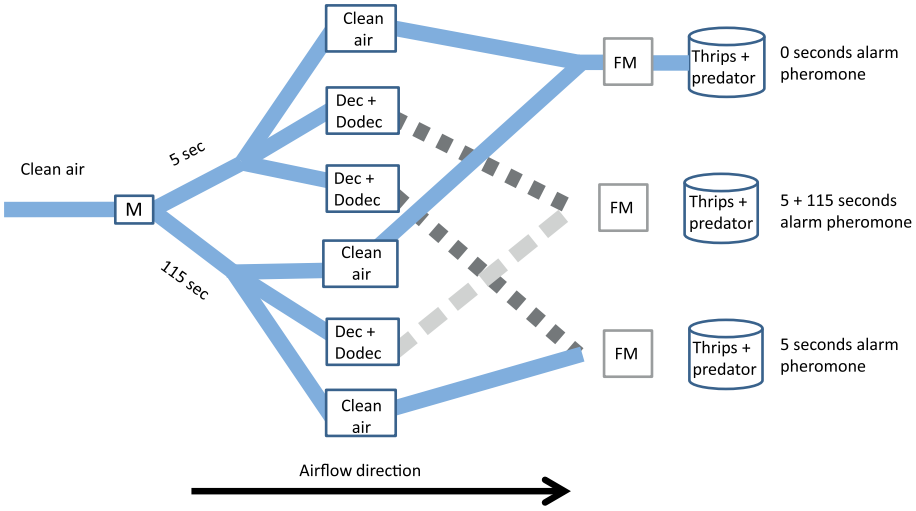


FIGURE 3-1 Experimental set-up. Clean air passes a solenoid valve (M) after which the air is either directed through the non-pulse side (115 s per 120 s) or through the pulse side (5 s per 120 s). Next, the airflow is split in three parts and let through a glass bottle that contains capillaries (empty or filled with decyl acetate and dodecyl acetate). Then, the air passes a flow meter and last, it passes a plastic cup containing the experimental arena. Clean air is presented as blue, solid lines, air with decyl acetate and dodecyl acetate during 5 s is presented as dark grey, dashed lines, and air with decyl acetate and dodecyl acetate during 115 s as light grey, dashed lines. Before reaching the experimental arena, the air passes a flow meter (FM), which allows us to determine the air speed.

pheromone, others did not. Bottles with alarm pheromone contained two capillaries (Vitrex, 32 mm long, diameter 0.6 mm, 9 µl) positioned at a 45° angle relative to the bottom of the bottle, allowing for constant pheromone evaporation. One capillary contained decyl acetate and one dodecyl acetate. The use of capillaries allowed us to refrain from using solvents (to which thrips may also respond) and ensures that the two pheromone compounds evaporate at a constant rate. Bottles without alarm pheromone contained two empty capillaries. Therefore, after passing through the bottles, the airflow consisted of clean air either with or without synthetic alarm pheromone (see FIGURE 3-2). Then, the airflow was guided through an air flow meter (Brooks Instruments, Veenendaal, The Netherlands), set at 0.1-0.15 m/s. We measured the evaporation rate of decyl acetate and dodecyl acetate from the capillaries in our setup. After 15 days, 3.5 mm of decyl acetate and 1.5 mm of dodecyl acetate had evaporated from the capillaries, corresponding to 0.98 and 0.42 µl, respectively. Hence, during each experiment (6 h), 14.2 µg decyl acetate and 6.1 µg dodecyl acetate evaporated freely from these capillaries. This translates into an evaporation rate of 3.2 ng decyl acetate and 1.4 ng dodecyl acetate per 5 s, to mimic the amount excreted as alarm pheromone by a second-instar thrips larva under artificial attack (Teerling *et al.* 1993; de Bruijn *et al.* 2006). This means that the mixture contained ca. 70% decyl acetate and ca. 30% dodecyl acetate.

The experiment consisted of three treatments: (1) larvae exposed to clean air only, referred to as '0 second alarm pheromone' treatment, (2) larvae exposed to synthetic alarm pheromone only, referred to as '5 + 115 seconds alarm

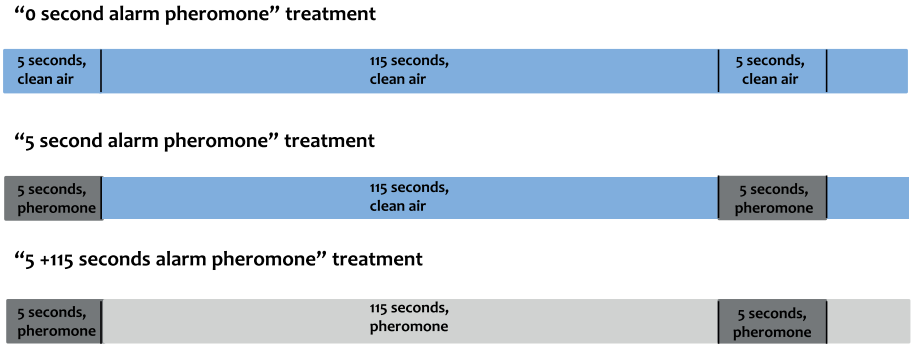


FIGURE 3-2 Schematic representation of the air content in the three treatments during more than 125 s. Clean air is presented as blue, solid lines, air with (relatively high concentrations of) decyl acetate and dodecyl acetate during 5 s is presented as dark grey, hatched lines, air with (relatively low concentrations of) decyl acetate and dodecyl acetate during 115 s is presented as light grey, hatched lines.

pheromone' treatment and (3) larvae perceiving alarm pheromone for 5 s every 2 min and clean air for the remaining 115 s, referred to as '5 second alarm pheromone' treatment (see FIGURES 3-1 and 3-2). In all three treatments the airflow passed through one glass bottle for 5 s and another glass bottle for 115 s. Hence, in the treatment where thrips larvae perceived alarm pheromone continuously, due to accumulation, the concentration of decyl acetate and dodecyl acetate was not equal during the 115 and 5 s. Note that predatory mites cause thrips larvae to excrete alarm pheromone during the experiment, and that this natural alarm pheromone can influence their survival. This factor, however, is present in all treatments including the control, and hence cannot explain possible differences in survival between treatments.

After 6 h, thrips larvae that were alive and present were counted. The instar of the larvae (first or second) was also noted. Any replicate where a predator died was discarded. In total, 25 replicates of each treatment were obtained. Because we were unable to observe the larvae in our experiment continuously, we do not know whether larvae that were missing were killed by the predator or drowned in the water barrier surrounding the leaf disc but we considered them to be killed by the predator. Indeed, in a prior study using a similar set-up (de Bruijn *et al.* 2014), 9.9 out of 10 thrips larvae survived 8 h without a predator present, which suggests that mortality not caused by predation is rare.

Statistical analyses

The number of dead individuals was analyzed using a GLM with Poisson distribution. Contrasts among treatments were assessed by pooling of treatments and comparing the model with the pooled treatments to that with all treatments separate (Crawley 2007) using ANOVA. Standard assumptions on residual variation were checked. All statistical treatments were conducted using R version 2.15.1.

Results

Thrips larval survival in the presence of a predatory mite was significantly higher in the '5 + 115 seconds alarm pheromone' treatment compared to the '0 second alarm pheromone' and '5 second alarm pheromone' treatment (FIGURE 3-1 and supplementary FIGURE S3-1, first- and second instar together; GLM: deviance = 13.1, d.f. = 2, $P<0.001$). We find no significant difference between the treatments where thrips are exposed to 0 or 5 s of alarm pheromone (supplementary FIGURE S3-1, first and second instar together; GLM: deviance = 87.7, d.f. = 1, $P<0.8$). Since predatory mites predominately prey on first-instar larvae, the observed overall improvement of larval survival as a result of continuous (5 + 115 s) exposure to

pheromone was hypothesized to result predominately from the increased survival of first-instar larvae. Indeed, we find that second-instar larvae do not benefit from any of the alarm pheromone treatments (FIGURE 3-3; GLM: deviance = 1.9, d.f. = 2, P = 0.39), whereas first-instar larval survival was higher in '5 + 115 seconds alarm pheromone' treatments but not in '5 second alarm pheromone' treatments (FIGURE 3-4; GLM: deviance = 12.8, d.f. = 1, P<0.001).

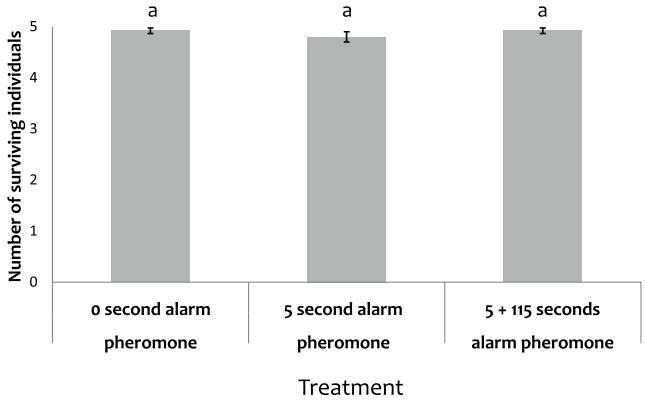


FIGURE 3-3 Survival of second-instar thrips larvae. The x-axis depicts treatments and the y-axis depicts the mean (± SEM) number of surviving thrips larvae. N = 25 for all treatments, each treatment contained five first-instar larvae and five second-instar larvae. Different letters above bars indicate significant differences between the treatments.

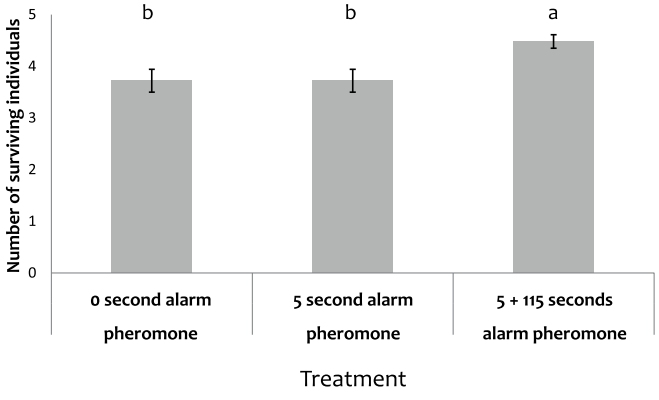


FIGURE 3-4 Survival of first-instar thrips larvae. The x-axis depicts treatment and the y-axis depicts the mean (± SEM) number of surviving thrips larvae. N = 25 for all treatments, each treatment contained five first instar-larvae and five second-instar larvae. Different letters above bars indicate significant differences between the treatments.

Discussion

Our results show that larvae in the '5 + 115 seconds alarm pheromone' treatment survive better than larvae in the '0 second alarm pheromone' treatment whereas larvae in the '5 second alarm pheromone' treatment did not. The '5 second alarm pheromone' treatment is thought to mimic natural alarm calling by thrips more realistically than the '5 + 115 seconds alarm pheromone' treatment, because thrips larvae produce alarm pheromone in discrete (anally secreted) droplets. One explanation of the increased survival only for thrips larvae that received alarm pheromone for 5 + 115 seconds is that either that thrips larvae need an exposure time above which they respond to alarm pheromone and that this time was not met in our '5 second alarm pheromone' treatment. However, another explanation of our results could be that thrips larvae in the '5 + 115 seconds alarm pheromone' treatment are sensitized by the continuous presence of synthetic alarm pheromone and then respond faster when attacked by a predator.

In contrast to these non-exclusive alternative explanations, the increased thrips survival may (also non-exclusively) result from decreased predator activity because predators confronted with alarm pheromone are deterred to attack. Hence attack rate would decrease the most during the '5 + 115 seconds alarm pheromone' treatment. Unravelling the role of these explanations is beyond the scope of this manuscript.

The percentage of decyl acetate in the synthetic pheromone blend in this manuscript is ca. 70% (i.e., 30% dodecyl acetate). For second-instar larvae, in Teerling *et al.* (1993) found 60% decyl acetate, but MacDonald *et al.* (2003), found 40% and we (de Bruijn *et al.* unpublished) found ca. 20% decyl acetate (under attack by a predatory mite). Because first-instar larvae produce alarm pheromone in such small quantities that both decyl acetate and dodecyl acetate are undetectable by GC, their pheromone has not been analysed. Hence we do not know what the concentration of decyl acetate is in the droplets of first-instar larvae. From previous experiments (de Bruijn *et al.* 2006) we know that second-instar larvae respond to alarm pheromone containing 60% decyl acetate, which we assume to be in the range of the 70% decyl acetate used in this manuscript. When we would have exposed thrips larvae to synthetic alarm pheromone containing a lower percentage of decyl acetate, the difference in survival may have been higher than found here.

Although it is usually assumed that the purpose of sending alarm signals is to increase the survival chance of conspecifics (Sherman 1977; Blumstein 2007; Vandermoten *et al.* 2012), to the best of our knowledge, our experiments is one of the first to test this assumption (Mirza and Chivers 2001) and to show an increase in survival of young (first-instar) larvae when alarm signals are present.

In our view, there are three (non-mutually exclusive) possibilities why these first-instar larvae can have an increased survival when alarm pheromone is present, irrespective of the nature of alarm pheromone (synthetic or natural). Firstly, when exposed to alarm pheromone, thrips larvae respond faster to contact by displaying anti-predator behaviour (de Bruijn *et al.* 2006). In the set-up used here, we expect thrips to show a faster response to predator contact in presence of alarm pheromone as well, which may then enhance their survival. Secondly, upon exposure to alarm pheromone, first-instar larvae have the option to move close to second-instar larvae, which would then make it more difficult for a predatory mite to attack such a first-instar larva. Thirdly, predatory mites may refrain from attacking thrips larvae when experiencing natural alarm pheromone and/or their synthetic mimic continuously. Which of these three possibilities explains our results, requires time-consuming and in-depth scrutiny.

Acknowledgements

PJAdB was supported by a grant from The Netherlands Organization for Scientific Research (NWO, project number 818.01.018).

References

Bakker FM & Sabelis MW (1989) How larvae of *Thrips tabaci* reduce the attack success of phytoseiid predators. Entomologia Experimentalis et Applicata 50: 47-51.

Blumstein DT (2007) The evolution, function, and meaning of marmot alarm communication. Advances in The Study of Behavior, Vol. 37 (ed. by HJ Brockmann et al.), pp. 371-401. Elsevier Academic Press, San Diego, CA, USA.

Caro TM (1995) Pursuit-deterrence revisited. Trends in Ecology and Evolution 10: 500-503.

Caro TM (2005) Antipredator defenses in birds and mammals. University of Chicago Press, Chicago, IL, USA.

Chivers DP, Brown GE & Smith RJF (1996) The evolution of chemical alarm signals: attracting predators benefits alarm signal senders. American Naturalist 148: 649-659.

Chivers DP & Smith RJF (1998) Chemical alarm signalling in aquatic predator-prey systems: A review and prospectus. Ecoscience 5: 338-352.

de Bruijn PJA, Egas M, Janssen A & Sabelis MW (2006) Pheromone-induced priming of a defensive response in western flower thrips. Journal of Chemical Ecology 32: 1599-1603. [Chapter 2 of this thesis]

de Bruijn PJA, Sabelis MW & Egas M (2014) Effects of kinship or familiarity? Small thrips larvae experience lower predation risk only in groups of mixed-size siblings. Journal of Behavioral Ecology and Sociobiology 68: 1029-1035. [Chapter 7 of this thesis]

de Bruijn PJA, Egas M, Sabelis MW & Groot AT (unpublished) Context-dependent alarm signalling in an insect. [Chapter 5 of this thesis]

Crawley MJ (2007) The R book. John Wiley and Sons, Chichester, UK.

Dicke M & Grostal P (2001) Chemical detection of natural enemies by arthropods: An ecological perspective. Annual Review of Ecology, Evolution, and Systematics 32: 1–23.

Hamilton WD (1964) The genetical evolution of social behaviour. Journal of Theoretical Biology, 7: 1-52

Hasson O (1991) Pursuit-deterrent signals: Communication between prey and predator. Trends in Ecology and Evolution 6: 325-329.

Hoogland JL (1983) Nepotism and alarm calling in the black-tailed prairie dog (*Cynomys ludovicianus*). Animal Behaviour, 31: 472-479

Klump GM & Shalter MD (1984) Acoustic behaviour of birds and mammals in the predator context. Zeitschrift für Tierpsychologie 66:189-226.

Kunert G, Otto S, Röse USR, Gershenzon J & Weisser WW (2005) Alarm pheromone mediates production of winged dispersal morphs in aphids. Ecology Letters 8: 596–603

MacDonald KM, Hamilton JGC, Jacobson R & Kirk WDJ (2002) Effects of alarm pheromone on landing and take-off by adult Western flower thrips. Entomologia Experimentalis et Applicata 103: 279–282.

MacDonald KM, Hamilton JGC, Jacobson R & Kirk WDJ (2003) Analysis of anal droplets of the Western flower thrips *Frankliniella occidentalis.* Journal of Chemical Ecology 29: 2385-2389.

Maynard Smith J (1965) The evolution of alarm calls. American Naturalist 99: 59-63.

Mirza RS & Chivers DP (2001) Do chemical alarm signals enhance survival in aquatic vertebrates? An analysis of the current research paradigm. Chemical Signals in Vertebrates, Vol. 9 (ed. by A Marchlewska-Koj, JJ Lepri & D Muller-Schwarze), pp. 19-26. Plenum Press, New York, NY, USA.

Pickett JA, Wadhams LJ & Woodcock CM (1992) The chemical ecology of aphids. Annual Reviews of Entomology 37:67-90.

Rajchard J (2006) Antipredator pheromones in amphibians: a review. Veterinarni Medicina 8: 409–413.

Randall JA and Matocq MD (1997) Why do kangaroo rats (*Dipodomys spectabilis*) footdrum at snakes? Behavioral Ecology, 8: 404-413

Sabelis MW & van Rijn PCJ (1997) Predation by insects and mites. Thrips as Crop Pests (ed. by T Lewis) CAB International, London, pp. 259–354.

Seyfarth RM, Cheney DL & Marler P (1980) Vervet monkey alarm calls: semantic communication in a free-ranging primate. Animal Behaviour 28: 1070-1094.

Sherman PW (1977) Nepotism and the evolution of alarm calls. Science 197: 1246-1253.

Sherman PW (1985) Alarm calls of Belding's ground squirrels to aerial predators: Nepotism or self-preservation? Behavioral Ecology and Sociobiology, 17: 313-323

Teerling CR, Pierce HD, Borden JH & Gillespie DR (1993) Identification and bioactivity of alarm pheromone in the western flower thrips, *Frankliniella occidentalis*. Journal of Chemical Ecology 19:681-697.

Trivers RL (1971) The evolution of reciprocal altruism. Quarterly Review of Biology 46: 35-57.

Vandermoten S, Mescher MC, Francis F, Haubruge E & Verheggen FJ (2012) Aphid alarm pheromone: An overview of current knowledge on biosynthesis and functions. Insect Biochemistry and Molecular Biology 42: 155-163.

van Rijn PCJ, Mollema C & Steenhuis-Broers GM (1995) Comparative life-history studies of *Frankliniella occidentalis* and *Thrips tabaci* (Thysanoptera: Thripidae) on cucumber. Bulletin of Entomological Research 85: 285-297.

Venzon M, Janssen A, Pallini A & Sabelis MW (2000) Diet of a polyphagous arthropod predator affects refuge seeking of its thrips prey. Animal Behaviour 60: 369-375.

Verheggen FJ, Haubruge E, De Moraes CM & Mescher MC (2009) Social enviroment [sic-PJAdB] influences aphid production of alarm pheromone. Behavioral Ecology 20: 283-288.

Supplementary figure

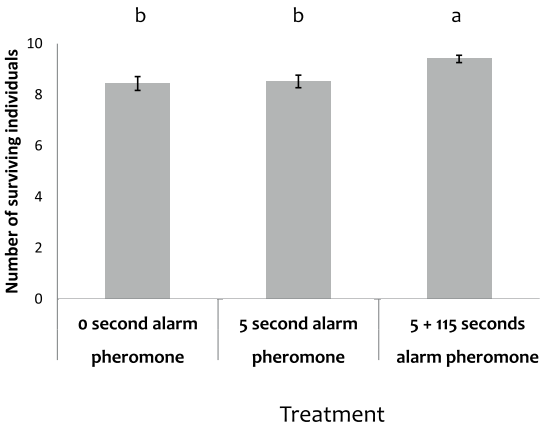
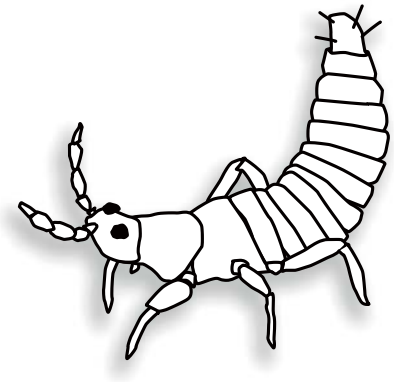


FIGURE S3-1 Survival of thrips larvae. On the x-axis is the treatment, on the y-axis the mean (± SEM) number of surviving thrips larvae. N = 25 for all treatments. Bars with different letters differ significantly.

Anti-predator responses to alarm pheromone in groups of young and/or old thrips larvae

Paulien J.A. de Bruijn, Maurice W. Sabelis, Arne Janssen & Martijn Egas

4



Abstract

Many prey species suffer from different predators in the course of their ontogeny. Hence, the alarm signal a small prey individual sends can have a different meaning than the signal a large prey individual sends, both for small and large receivers. Larvae of Western flower thrips are known to face predators that attack only small larvae (first instars), or predators that attack small larvae and large larvae (second instars). Furthermore, thrips larvae release a two-component alarm pheromone, which varies in composition with larval age. Here, we study if the response of Western flower thrips varies with their alarm pheromone. First, we confirmed that large and small larvae respond when nearby larvae of both sizes were prodded with a brush to induce alarm pheromone excretion. This suggests that larvae perceive the alarm pheromone of both small and large larvae. Subsequently, we tested whether thrips larvae of a given size respond differentially to alarm pheromone excreted by a small or large companion larva. We analyzed two types of behavior that are used in direct defense against a predator and one type of escape response. Only small (not large) larvae attempted to escape more frequently in response to excretions from a large larva. This difference in response could have been due to the alarm pheromone or to the companion larva in the vicinity. We subsequently tested for, but did not find an effect of size of the companion larva on the behavior of the test larva when exposed to synthetic pheromone mimicking that of a second-instar larva. Finally, we tested which aspects of pheromone composition affect anti-predator behavior by exposing thrips larvae to synthetic pheromones differing in amount and ratio of the two components. Only for small larvae, we found significant changes in escape behavior with pheromone amount, and a trend with the ratio. Overall, we conclude that small thrips larvae respond differentially to alarm pheromones excreted by small and large larvae, and that this differential response is due to differences in pheromone quantity, and possibly also quality. Our results suggest that responses to alarm signals can vary with the chemical composition of those alarm signals.

Unpublished manuscript

Introduction

The predation risk imposed by a predator on a prey individual often changes with prey size (e.g., Lima and Dill 1990; Tonn *et al.* 1992; Chase 1999). Larger individuals can be invulnerable to predators that effectively prey on smaller individuals of the same prey species and vice versa, whereas some predators are dangerous to prey of all possible sizes. Thus, if an alarm signal is sent by a small individual, it may convey information on a different danger level to another small individual than to a large one. So how can receivers of different sizes tell these differences in information apart if they have no clue of the sender's size? A possible solution to this problem may emerge if alarm signals vary consistently with prey size. Then, receivers may evolve an antipredator response that is balanced against other fitness-enhancing activities (Sih 1980; Lima and Dill 1990). Such context-dependent alarms and adaptive antipredator responses have been found for alarm cues in aquatic systems (e.g., Belden *et al.* 2000; Mirza and Chivers 2002), but to the best of our knowledge there are only two examples where alarm signals vary with ontogeny in terrestrial systems. First, colony foundresses and workers of the paper wasp *Polistes dominulus* (Christ) excrete alarm chemicals in different ratios in their venom, and workers respond differently to the pheromone of workers and that of foundresses (Bruschini *et al.* 2008). Second, the amount and ratio of the two components of the alarm pheromone of Western flower thrips, *Frankliniella occidentalis* (Pergande) (Insecta: Thripidae) varies with the age of the thrips larva emitting it (MacDonald *et al.* 2003). Here, we consider the second example in more depth by testing whether the response of thrips larvae to alarm pheromone varies with the age of the sender and receiver.

Thrips larvae have several features that make them suitable objects to study responses to chemical signals. First, the alarm pheromone is present in anal excretions that are released in the form of droplets of ca. 1 nl (MacDonald *et al.* 2003) and the release of these so-called 'anal droplets' can be observed. Second, the release of a droplet can be triggered by prodding a larva with a fine brush. Third, the chemicals constituting the alarm pheromone have been identified as decyl acetate and dodecyl acetate (Teerling *et al.* 1993), thus enabling the use of synthetic mimics of the alarm pheromone (Teerling *et al.* 1993; de Bruijn *et al.* 2006). Fourth, the variation in alarm pheromone described above concerns both the ratio and amount of decyl acetate and dodecyl acetate (MacDonald *et al.* 2003). Finally, thrips larvae exhibit easily observable antipredator responses when exposed to the alarm pheromone, such as walking away (Teerling *et al.* 1993), retreating into refuges (Venzon *et al.* 2000), swinging their abdomen and producing an anal droplet, which they try to bring into contact with the integument and extremities of the predator (Bakker and Sabelis 1987, 1989). These

droplets are thought to be acidic, and when predators become contaminated with it, they give up attacking and retreat to groom (Bakker and Sabelis 1989).

Thrips larvae commonly live in groups of mixed ages and – because their body size correlates well with age – also of mixed sizes. This is important because size matters to the predation risks that larvae experience (Bakker and Sabelis 1987, 1989; Sabelis and van Rijn 1997). For example, predatory mites, which are ca. 0.5 mm in size, are much more successful in attacking first-instar thrips larvae (ca. 0.75 mm, see suppl. data) than second-instar larvae (ca. 1.0 mm, see suppl. data) (Bakker and Sabelis 1987, 1989; Sabelis and van Rijn 1997), whereas predatory bugs (ca. 2 mm) attack both instars equally successfully (Sabelis and van Rijn 1997). Given the variation in pheromone composition with age and the size-dependent predation risk, the alarm pheromone excreted by small (first-instar) and large (second-instar) thrips larvae represents different information on the level of danger. However, to the best of our knowledge, nothing is known about responses of thrips to these different alarm signals.

We test the hypothesis that both small and large receiver larvae show differences in behavioral responses to alarm pheromone produced by a small or large companion larva. Because small larvae are more vulnerable to predation than large larvae, we expect small larvae to always respond to the alarm pheromone of both small and large larvae, whereas we expect large larvae to always respond to alarm pheromone of large larvae but less so to that of small larvae. We scored two types of behavior that thrips larvae use in direct defense against predators and one escape behavior. If larvae perceive alarm pheromone, this may indicate the presence of an attacking predator in the vicinity, but the receiving larva is not directly under attack. Hence, we expect that these larvae will not show an increase in defense behavior aimed at a predator, but will show an increase in escape behavior. Because small and large larvae release different amounts of alarm pheromones, we tested first whether alarm pheromone of larvae of different size invoked a response in all larvae, with a set-up that was previously used to show that larvae do respond to alarm pheromone from large larvae (de Bruijn *et al.* 2006). Subsequently, we tested behavior of small and large focal thrips larvae before and after the induced release of an anal droplet by a small or large companion larva present in the same experimental arena. To control for differences between companion larvae other than the alarm pheromone they excrete, we also observed responses of focal larvae to synthetic pheromone. Finally, we tested whether the total amount or the ratio of decyl acetate to dodecyl acetate affected the response of focal thrips larvae.

Materials and methods

Cultures

Cucumber plants, *Cucumis sativus* (var. Ventura RZ, Rijk Zwaan, De Lier, The Netherlands), were grown, free of herbivores, in a climate room at 25 °C, 70% RH, L16:D8 photoperiod. We had two cultures of Western flower thrips for our experiments. For the first culture, thrips were collected from cucumber plants in a commercial greenhouse in Pijnacker, The Netherlands, in February 2006. Thrips were subsequently reared in a climate box (25 °C, 60% RH, L16:D8) on cucumber leaves, cut to fit in a Petri dish on top of a layer of wet cotton wool that was put on the bottom of the Petri dish. Once a week, thrips pupae and adults from older leaves of the culture were put on such a cucumber leaf and pollen of *Typha latifolia* was provided on this leaf as additional food for the thrips. The adult females would lay eggs in this new leaf disc and after approximately a week this would result in new adults and pupae and the procedure was repeated. Unfortunately, this culture collapsed when our research group moved to a new building in 2010. For the second culture, thrips were generously sent to us by Greet Steenhuis-Broers and Willem Jan de Kogel from Wageningen University and Research Center in 2010. Before the thrips were sent to us, they had been kept on chrysanthemum. This new culture was reared in the same way as described above.

Synthetic alarm pheromone

Synthetic alarm pheromone was prepared by dissolving decyl acetate (Alfa Aesar, Germany) and dodecyl acetate (>99% pure, Sigma-Aldrich, USA) in cyclohexane (98% pure, Sigma-Aldrich, USA). Four different pheromone blends were prepared in such a way that 1 µl of such a blend corresponded to the amount and/or ratio of the two pheromone components present in the anal droplet of one first- or second-instar larva. In the first blend, the total amount and ratio of the two components corresponded to that of the alarm pheromone of one second-instar thrips larva (5 ng of each component in 1 µl; MacDonald *et al.* 2003). The second blend contained the total amount of pheromone released by one second-instar larva (10 ng), but in the ratio corresponding to the pheromone of first-instar larvae; 1:3 for decyl acetate:dodecyl acetate (MacDonald *et al.* 2003), hence, 1 µl contained 2.5 ng of decyl acetate and 7.5 ng of dodecyl acetate. The third blend contained the total amount of decyl acetate and dodecyl acetate as present in pheromone of a first-instar larva (0.6 ng; MacDonald *et al.* 2003), but the ratio of the two compounds was similar to the pheromone released by sec-

ond-instar larvae (1:1). Hence, 1 µl of the third blend contained 0.3 ng of each component. In the fourth blend, the total amount and ratio of the two compounds corresponded to that of pheromone of a first-instar larva, therefore 1 µl of the fourth blend contained 0.15 ng decyl acetate and 0.45 ng dodecyl acetate. In the experiments described below, either 1 µl of this solution of synthetic alarm pheromone was used or 1 µl of cyclohexane as a control.

Response to natural pheromone of small and large larvae

Adult female thrips from the Wageningen-culture were placed in groups of 3-5 on a rectangular leaf fragment of approximately 25 cm^2 and were allowed to oviposit for approximately 1 week. Subsequently, the females were removed. At this time, the leaf fragment harbored roughly 20 first- and second-instar larvae, of which we randomly selected up to five individuals for the experiment. We repeatedly prodded a first-instar (small) or second-instar (large) larva with a metal needle until it excreted an anal droplet, and then dipped the needle in this droplet. We immediately challenged a first- or second-instar larva (haphazardly chosen) on another leaf fragment with this needle by repeatedly prodding it until an anal droplet was excreted, and we measured the time it took for this induced response to occur. As control treatment, we also challenged first- or second-instar larvae with a clean needle. We chose not to isolate thrips larvae for this test, because that involves moving them with a brush which usually results in excretion of an anal droplet, and most thrips larvae do not excrete another droplet for at least several hours afterwards (PJAdB, personal observation). In our procedure, most thrips larvae on leaf fragments where we collected excreted droplets are challenged after other thrips larvae from the same fragment excreted droplets. The latter may affect their response, but this is the same for all treatments in the experiment. To otherwise minimize recent experience with anal droplets, larvae used to measure the time until droplet excretion were selected from different leaf fragments than larvae used to excrete an anal droplet in which the needle was dipped. We analyzed the data using a one-way ANOVA.

Responses to natural alarm pheromone and effect of companion larva

Small leaf discs (diameter 10 mm) were cut from cotyledons of cucumber plants and served as experimental arenas. Two thrips larvae from the Pijnacker-culture were placed on each experimental arena. One was designated as 'focal' larva and its behavior was observed during the experiment. The other larva was designated as 'companion' larva. To allow acclimatization of the larvae, the experi-

mental arena with both focal and companion larvae was incubated in a climate room (25 °C, 70% RH, and L16:D8 photoperiod) for 16 h. Approximately 5 min before the experiment, the experimental arena was placed on a larger leaf disc (diameter 24 mm), also cut from a cucumber cotyledon, which was placed in a Petri dish with a layer of wet cotton wool at the bottom (FIGURE 4-1). Five minutes appeared to be enough to allow thrips larvae to resume their feeding behavior (PJAdB, personal observation). The larger leaf disc served as alternative to which the thrips larvae could escape from the experimental arena.

For experiments on behavioral responses to alarm pheromone, we scored two types of defensive behavior: the excretion of an anal droplet and the execution of abdominal swings (i.e., a characteristic movement where the thrips larva jerks its abdomen from one side to another; Bakker and Sabelis 1987, 1989). In addition to these defensive behaviors, we also scored escape behavior, defined as thrips larvae moving off the experimental arena (smaller disc) onto the larger leaf disc. This escape behavior, however, was observed infrequently. Instead, we observed much more frequently that larvae move over the border of the experimental arena up to approximately half their body length, head first, yet move back to the experimental arena before they had fully moved off. We scored these partial crossings of the edge of the experimental arena (henceforth called 'partial crossings') because they arguably relate to a tendency to leave the experimental arena. If a focal thrips larva escaped the experimental arena (smaller leaf disc) before a treatment was applied, the replicate was discarded. In case a thrips larva escaped from the experimental arena within 2 min after applying a treatment, the observation was terminated. These replicates were included in the analyses after correcting for the shorter observation time by calculating the rate of the observed behaviors (number of scored behaviors divided by the observation time). Observations were made on 25 focal larvae per treatment. Thrips behavior was observed using a binocular microscope with a cold light source, and was recorded and timed using the

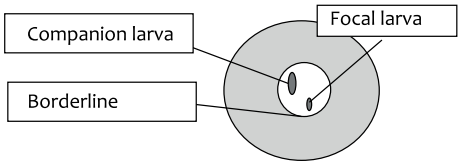


FIGURE 4-1 Experimental setup to test behavioral responses of a focal larva in the presence of a companion larva. We placed these two larvae on an experimental arena (white circle) made from a small leaf disc (diameter 10 mm) of a cucumber-plant cotyledon. The experimental arena was placed on a larger leaf disc (diameter 24 mm, grey circle), also cut from a cucumber-plant cotyledon.

freeware event recorder EthoLog version 2.2.5 (Ottoni 2000). This program is used to record the different types of behavior and the time at which they occurred.

We tested if and how the excretion of an anal droplet by a first-instar (small) or second-instar (large) companion larva affected thrips behavior. All four combinations of small and large focal and companion larvae were tested. We induced the production of alarm pheromone (hereafter called natural alarm pheromone) by gently prodding the head of the companion larvae once or twice with a fine brush. To assess the role of cues coming from the companion larva other than the alarm pheromone, we added a control where we tested the response of the focal larva, in the presence of first- or second-instar companion larvae, to synthetic pheromone mimicking that produced by a second-instar larva. If behavioral changes were induced by the pheromone alone, we would not expect a difference in response to the synthetic pheromone in the presence of a first- or second-instar companion larva. In contrast, if cues from the companion larva also affected the behavior of the focal larva, we would expect to find differences in behavior between these two treatments. Furthermore, as a control we added only the solvent of the synthetic control, cyclohexane. In these two controls, we used a Gilson pipette to apply 1 µl of pheromone solution or cyclohexane on the experimental arena, away from the thrips larvae. Thrips were randomly assigned to the natural pheromone treatment or one of the two controls.

Because the observed anti-predator behavior can also occur in the absence of alarm pheromone, we observed each focal larva for 2 min before and 2 min after application of a treatment. This enabled the detection of changes in droplet release by the thrips larva, which was subsequently used to test for the effects of the various treatments. To analyze behavioral differences due to companion larvae, changes in number of anal droplets released by individual larvae were analyzed using a Generalized Linear Model (GLM) assuming a Poisson error distribution. Contrasts among treatments were assessed through model simplification (Crawley 2007) and simplified models were compared with more extended models using the anova function in R. Furthermore, the standard assumptions on residual variation were checked.

The analysis of the change in behavior appeared to be possible only for the data on droplet release. Because the number of abdominal swings and partial crossings were zero-inflated, it was not possible to find an appropriate distribution to analyze the change in behavior. Therefore, we analyzed the number of swings and partial crossings before and after the treatment separately. Differences in the number of abdominal swings and partial crossings due to com-

panion larvae were analyzed using a non-parametric Kruskal-Wallis test (Siegel and Castellan 1988). Because the groups of thrips larvae with the same instar as companion were treated identically before applying one of the pheromone treatments, we pooled before-treatment data for each of these categories of focal and companion larvae. Data obtained after application of the treatments were first tested with an overall Kruskal-Wallis test in R, and if this showed a significant effect of treatment, we performed a post-hoc test correcting for multiple comparisons using the 'pgirmess' package (Giraudoux 2008).

We analyzed differences before and after applying treatments on the different behaviors separately, i.e., the excretion of anal droplets, abdominal swings and partial crossings of the edge of the arena. To do so, we used the non-parametric Wilcoxon rank sum test on the paired data before and after applying a treatment with pooled data from all treatments. With respect to the first occurrence of these behaviors, data were subjected to a time-to-event Kaplan-Meier analysis (Hosmer and Lemeshow 1999).

All statistical analyses were done using R (R Development Core Team 2010). To avoid the possibility that outliers dominated the average parameter values, we removed data points more than 3× the standard deviation away from the mean. In total, we removed 29 out of 1800 data points. Outliers in the data are marked red in the supporting information (Appendix TABLE S4-4).

Effect of amount and ratio of pheromone components on thrips behavior

Using only synthetic pheromone, we tested if and how differences in amount and ratio of the two pheromone components influenced thrips behavior. For this, we used the same set-up and tested the same behavior as described above (section *Responses to natural alarm pheromone*), except that we used the Wageningen thrips culture and we always used a second-instar larva as a companion. Focal thrips larvae (either first- or second-instar) were subjected to one of the following five treatments: four different synthetic pheromone blends as described above, and the solvent cyclohexane (all 1 µl). Assignment of thrips larvae to treatments was done using the Random() function in Excel (2003). The test was performed double blind, implying that the observer was unaware of the treatment applied. All statistical analyses were done as described above (section *Responses to natural alarm pheromone*), except that the differences in abdominal swings and partial crossings after application of the treatments were analyzed with a GLM (because these data were not zero-inflated) with a quasi-Gaussian error distribution.

Results

Response to natural alarm pheromone of first- and second-instar larvae

Small and large larvae released an anal droplet earlier when challenged with a needle containing pheromone than when challenged with a clean needle (FIGURE 4-2; one-way ANOVA: small larvae $F_{2,69} = 5.7$, $P = 0.005$; large larvae $F_{2,76} = 11.4$, $P<0.001$). For both types of larvae, there was no difference in response to a needle with alarm pheromone from a small larva or with alarm pheromone from a large larva (Tukey post-hoc test: small larvae $P = 0.92$, large larvae $P = 0.96$). Hence, thrips larvae respond to both types of alarm pheromone equally well.

Responses to natural alarm pheromone and effect of companion larva

For both small and large focal larvae, the change in droplet release (from before to after the alarm pheromone treatment) did not vary significantly with treatment or with companion larva (FIGURE 4-3, TABLE 4-1). Also, the number of abdominal swings after application of the treatment did not vary significantly with treatment or with companion larva (FIGURE 4-4; overall effect: small focal larvae KW = 7.09, d.f. = 5, P = 0.21; large focal larvae KW = 2.01, d.f. = 5, P = 0.85). For the number of times a focal larva partially crossed the border between the small and large leaf disc, the type of companion larva did not have a significant effect before treatments (small focal larvae KW = 0.35, d.f. = 1, P = 0.55; large focal lar-

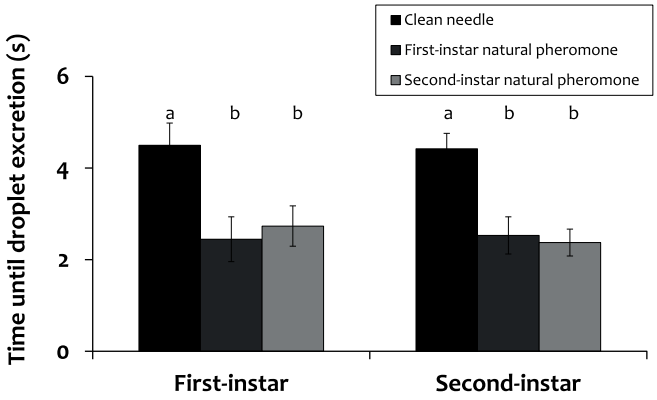


FIGURE 4-2 Response time of thrips larvae to a simulated attack. Shown is the mean (± SE) time (s) between attack with a needle and the release of an anal droplet by first- and second-instar larvae. The needle was either clean (N = 31), or had been dipped in first-instar (N = 21) or second-instar alarm pheromone (N = 20). For each instar of focal larva, different letters indicate significant differences.

vae KW = 0.05, d.f. = 1, P = 0.83), but after treatments, small larvae displayed significantly more partial crossings when exposed to natural pheromone from a large larva than to that from a small larva (FIGURE 4-5; overall effect: small larvae KW = 17.6, d.f. = 5, P<0.01; large larvae KW = 24.1, d.f. = 5, P<0.001; per treatment post-hoc effects in TABLE 4-2). If synthetic pheromone or only its solvent was released, there was no significant difference in partial crossings (TABLE 4-2). This shows that other cues from companion larvae play no role. Hence, thrips larvae

TABLE 4-1 Results from GLM-tests comparing the change in anal droplet release of a focal larva with treatment (natural pheromone, synthetic pheromone or cyclohexane) in the vicinity of a first-instar vs. second-instar companion thrips larva.

Focal larva	Factor	χ^2	d.f.	P
First instar	Companion	3e-15	1	1
	Treatment	1e-14	2	1
	Companion*treatment	6.97	4	0.14
Second instar	Companion	8.9e-15	1	1
	Treatment	6.2e-15	2	1
	Companion*treatment	4.16	4	0.38

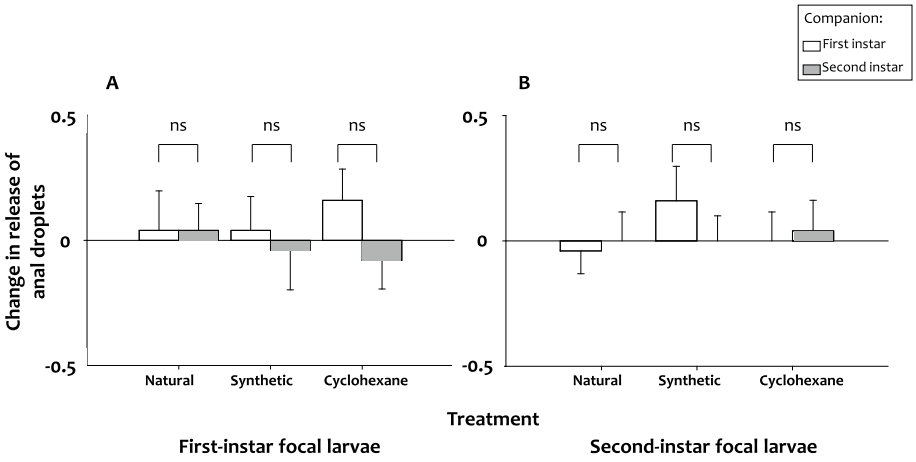


FIGURE 4-3 Changes in release of anal droplets (before and after treatment) in response to natural pheromone or control treatments. The treatments consisted of the triggered release of natural pheromone by a companion larva, the application of synthetic pheromone dissolved in cyclohexane or pure cyclohexane. N = 25 for all bars. Shown are the mean (+ SE) responses of first-instar (panel A) or second-instar larvae (panel B) that were in the company of either first-instar (white bars) or second-instar larvae (grey bars). The composition of the synthetic alarm pheromone corresponded to the natural pheromone of second-instar larvae. ns = not significant.

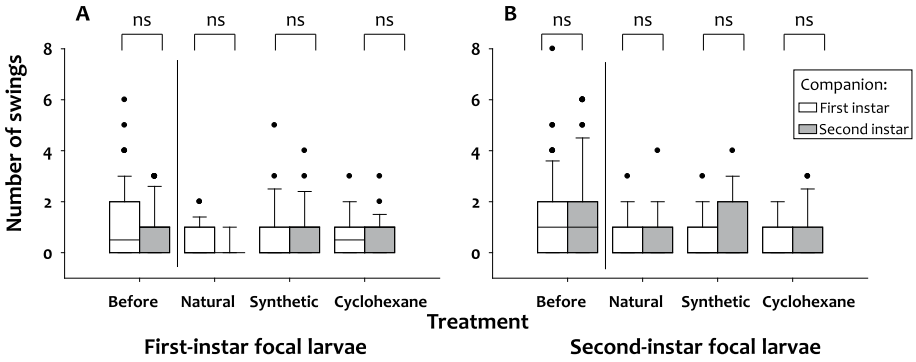


FIGURE 4-4 Number of abdominal swings in response to natural pheromone or control treatments. Shown are box plots of numbers of swings before treatment (pooled for all treatments, N = 75) and after treatment (triggered release of natural pheromone by the companion larva present, or controlled release of synthetic pheromone or cyclohexane by the experimenter, N = 25 each). Focal larvae were either first-instar (panel A) or second–instar larvae (panel B) and were in the company of either first-instar (white boxes) or second-instar larvae (grey boxes). Boxes indicate the second and the third quartile, horizontal lines separating the boxes indicate the medians, whiskers above the box indicate the 90th percentiles, dots indicate outliers; ns = not significant.

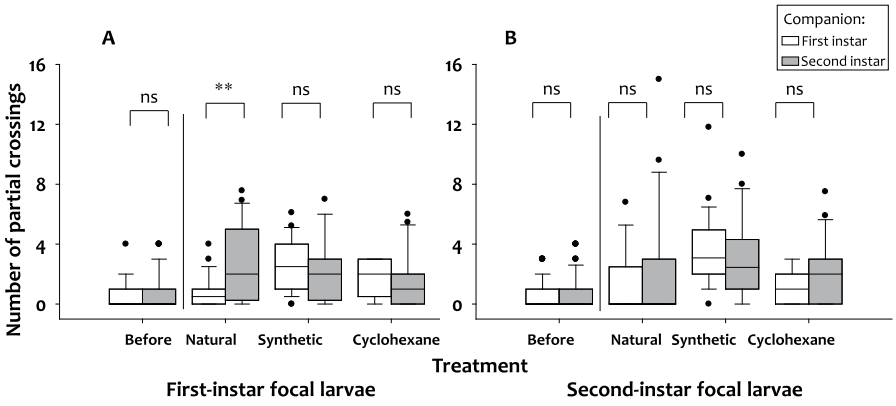


FIGURE 4-5 Number of partial crossings, in response to natural pheromone or control treatments. Shown are box plots of the numbers of crossings before treatment (pooled for all treatments, N = 75) and after treatment (release of natural pheromone, synthetic pheromone or cyclohexane, N = 25 each). Focal larvae were either first-instar (panel A) or second-instar larvae (panel B) and were in the company of either first-instar (white boxes) or second-instar larvae (grey boxes). Boxes indicate the second and the third quartile, horizontal lines separating the boxes indicate the medians, whiskers above and below the box indicate the 90th and 10th percentiles, dots indicate outliers; ns = not significant; ** indicates P<0.01.

Table 4-2 Results from Kruskal-Wallis tests comparing the number of partial crossings of a focal larva in the vicinity of a first-instar vs. second-instar companion thrips larva.

	Focal larva	Treatment	KW (d.f. = 1)	P
Before treatment	First instar	All treatments pooled	0.35	0.55
	Second instar	All treatments pooled	0.05	0.83
After treatment	First instar	Natural pheromone	7.70	<0.01
		Synthetic pheromone	1.15	0.28
		Cyclohexane	0.88	0.35
	Second instar	Natural pheromone	0.75	0.39
		Synthetic pheromone	1.61	0.20
		Cyclohexane	0.57	0.45

respond differentially to pheromones produced by small or large larvae. The number of partial crossings by large larvae after exposure to natural pheromone, synthetic pheromone or cyclohexane did not vary significantly with the type of companion larva (Table 4-2).

The number of anal droplets released, averaged over all treatments, was not significantly different before or after treatments (both instars; Table S4-2). The number of swings averaged over all treatments was lower after treatments than before treatments (bordering significance for first-instar larvae, significant for second-instar larvae; Table S4-2). The number of partial crossings averaged over all treatments was significantly higher after treatments than before treatments (both instars; Table S4-2).

For the timing of release of the first anal droplets or abdominal swings, no significant effect of treatment was detected (see Appendix Figure S4-A and S4-B). First-instar larvae partially crossed earlier when exposed to natural pheromone of large companion larvae than when exposed to that of small companion larvae ($\chi^2 = 4.2$, d.f. = 1, $P<0.05$) (Figure 4-6). Thus, partial crossings did not only occur more frequently, but also earlier.

Effect of amount and ratio of pheromone components on thrips behavior

For both small and large larvae, the change in anal droplet release did not significantly depend on the amount of pheromone offered or on the ratio of the two components (Figure 4-7; GLM: small larvae – amount: deviance = 0.01, d.f. = 2, P>0.99; ratio: deviance = 0.35, d.f. = 1, P = 0.55. Large larvae – amount: deviance <0.001, d.f. = 2, P = 1; ratio: deviance <0.001, d.f. = 1, P = 1). Moreover, the number of abdominal swings did not significantly depend on the amount of the pheromone offered or on the ratio of the two components in the pheromone offered (Figure 4-8; small larvae – amount: $F_{2,125} = 0.78$, P = 0.45; ratio: $F_{1,125} = 0.33$,

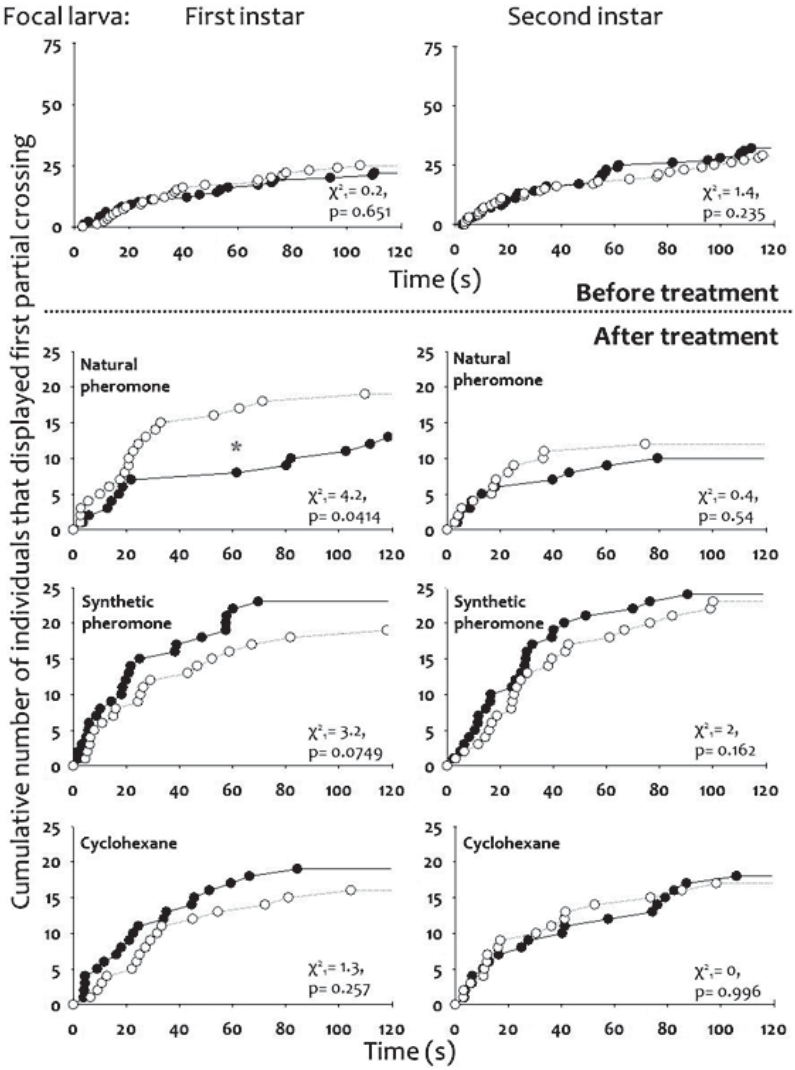


FIGURE 4-6 Timing of first partial crossing, in response to natural pheromone or control treatment. Shown is the increase of the number of individuals that has partially crossed the edge of the experimental disc over time (s) before treatment (pooled for all treatments, N = 75) and after treatment (release of natural pheromone, synthetic pheromone or cyclohexane, N = 25 each). Note that the y-axes are scaled to the maximum number of individuals that could have partially crossed (75 before treatments and 25 after treatments). Focal larvae were either first-instar (left column of graphs) or second-instar (right column of graphs). Companion larvae were either first-instar (black circles) or second-instar (white circles). * indicates $P<0.05$.

P = 0.57. Large larvae – amount: $F_{2,124}$ = 0.29, P = 0.75; ratio: $F_{1,124}$ = 0.81, P = 0.37). The amount of synthetic alarm pheromone had a significant effect on the partial crossings of small larvae (FIGURE 4-9; $F_{2,120}$ = 3.4, P = 0.04). The ratio of the two components in the alarm pheromones did not significantly affect this behavior of small larvae ($F_{1,119}$ = 1.8, P = 0.19), but there was a trend towards more partial crossings in response to mixtures where the ratio mimicked that of a large larva (FIGURE 4-9). For partial crossings of large larvae, we found no significant effect of amount or ratio (FIGURE 4-9; amount: $F_{2,119}$ = 0.14, P = 0.87; ratio: $F_{3,119}$ = 0.12, P = 0.73).

The number of anal droplets released, averaged over all treatments, was not significantly different before or after treatments (both instars; TABLE S4-3). The number of swings averaged over all treatments was significantly lower after treatments than before treatments (both instars; TABLE S4-3). The number of partial crossings averaged over all treatments was significantly higher after treatments than before treatments (both instars; TABLE S4-3). With respect to first occurrence of droplets, abdominal swings and partial crossings, no significant effects of concentration or ratio of components were detected (see Appendix FIGURE S4-C, S4-D and S4-E).

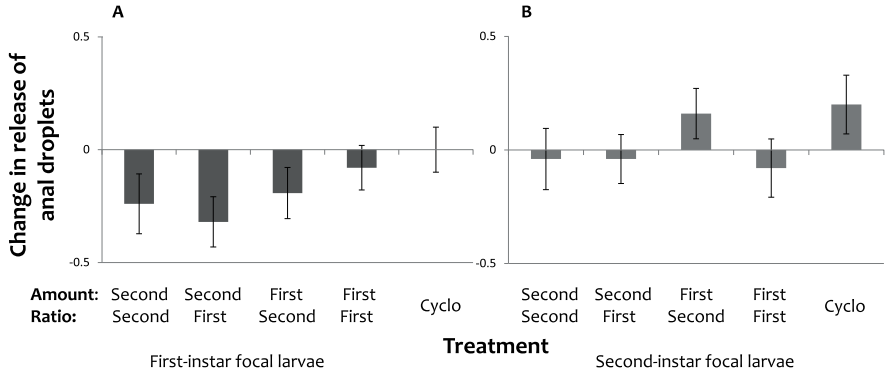


FIGURE 4-7 Change in release of anal droplets in response to different blends of synthetic pheromone. Shown are the means (± SE) of the difference in number of anal droplets produced after exposure to the pheromone minus before exposure per individual larva. Treatments consisted of various blends of synthetic pheromone or cyclohexane ('Cyclo') as the solvent control. Synthetic pheromone blends were prepared to mimic known amount and/or ratio of alarm pheromone components produced by first- or second-instar larvae (coded on the horizontal axis with 'First' and 'Second', respectively). Note that these blends include the mimics of first- and second-instar alarm pheromone. N = 25 for all bars. Focal larvae were either first-instar (panel A) or second-instar (panel B).

Discussion

We investigated alarm communication in Western flower thrips by addressing the following three questions: First, do both small and large larvae respond to alarm pheromones excreted by small and large larvae? Second, do thrips show differential behavioral responses to alarm pheromone produced by a small or a large companion larva? Third, does the amount of pheromone or the ratio of the two compounds affect anti-predator behavior? Below we discuss these three questions, compare our results with what is known about thrips and their defense behavior, and address the scope for context-dependent alarm signaling in thrips.

Evidence for the perception of natural alarm pheromone

Thrips larvae responded to an anal droplet excreted by a small or a large larva (FIGURE 4-2). We found a similar behavioral effect, called 'priming', in an earlier study using large larvae only and showed that this priming was caused by the alarm pheromone in the anal droplet (de Bruijn *et al.* 2006). For large larvae, the

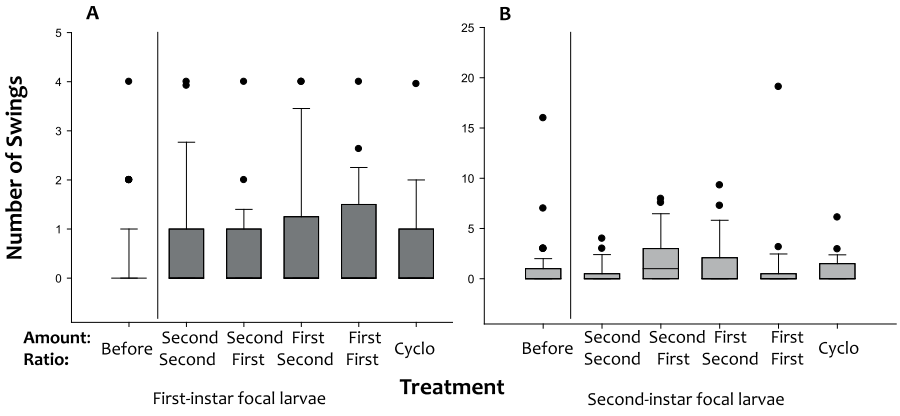


FIGURE 4-8 Number of abdominal swings in response to different blends of synthetic pheromone. Shown are box plots of numbers of swings before treatment ('Before'; pooled for all treatments, N = 125) and after treatment (various blends of synthetic pheromone or cyclohexane, 'Cyclo', N = 25 each). Synthetic pheromone blends were systematically varied to mimic known amounts and/or ratios of the alarm pheromone components produced by first- or second-instar larvae (coded on the horizontal axis with 'First' and 'Second', respectively). Note that these blends include the mimics of first- and second-instar alarm pheromone. Focal larvae were either first-instar (panel A) or second-instar (panel B). Boxes indicate the second and the third quartile, horizontal lines separating the boxes indicate the medians, whiskers above the box indicate the 90th percentiles, dots indicate outliers.

priming effect of anal droplets excreted by large larvae is similar in the previous and the present paper. The priming effect on large larvae and small larvae is also similar. Hence, the priming by droplets of small larvae suggests that large and small larvae can perceive alarm pheromone of small larvae.

Evidence for differential responses to alarm pheromone of small and large larvae

Small larvae show stronger responses when exposed to alarm pheromone from large larvae than to that from small larvae (FIGURE 4-5, TABLE 4-2). Large larvae do not show differential responses to alarm pheromone from small or large larvae (FIGURE 4-5, TABLE 4-2). Neither small nor large larvae seem to show increased partial crossings to natural alarm pheromone of a small companion larva (FIGURE 4-5). These results are in contrast with our expectation that small larvae would always respond to pheromone of small and large larvae, whereas large larvae would always respond to pheromone of large larvae, but only sometimes to that of small larvae. What could explain this stronger response of small larvae to an

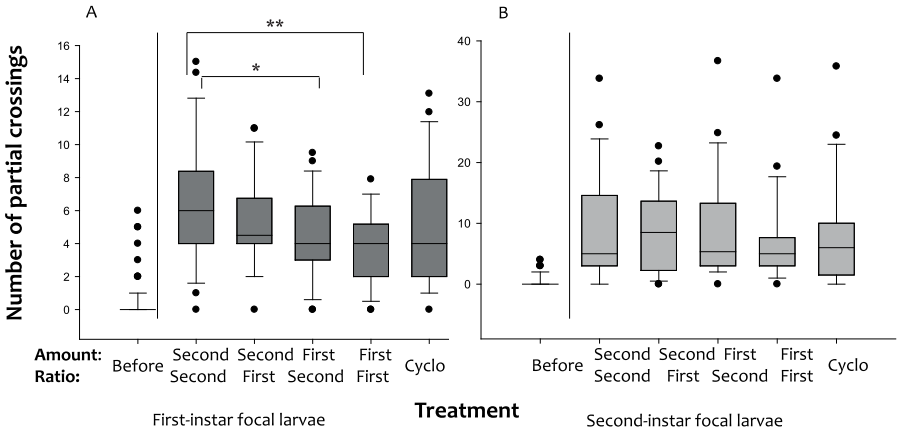


FIGURE 4-9 Number of partial crossings in response to different blends of synthetic pheromone. Shown are box plots of numbers of partial crossings before treatment ('Before'; pooled for all treatments, N = 125) and after treatment (various blends of synthetic pheromone or cyclohexane, 'Cyclo', N = 25 each). Synthetic pheromone blends were systematically varied to mimic known amounts and/or ratios of alarm pheromone components produced by first- or second-instar larvae (coded on the horizontal axis with 'First' and 'Second', respectively). Note that these blends include the mimics of first- and second-instar alarm pheromone. Focal larvae were either first-instar (panel A) or second-instar (panel B). Boxes indicate the second and the third quartile, horizontal lines separating the boxes indicate the medians, whiskers above and below the box indicate the 90th and 10th percentiles, dots indicate outliers; * P<0.05, ** P<0.01.

alarm pheromone of an instar other than their own? To the best of our knowledge, predators that form a threat to large larvae always form a threat to small larvae as well (but not always vice versa) and those predators are more voracious to small larvae than predators that attack only small larvae. Hence, small larvae should always respond to alarm pheromone of large larvae. Why large larvae do not differentiate between alarm pheromone from small and large larvae, remains unclear. The lack of response of small and large larvae to alarm pheromone excreted by small larvae recorded here suggests either that our set-up did not provide thrips larvae a chance to display the anti-predator behavior they would normally display when perceiving alarm pheromone, or that thrips larvae do not change their behavior when perceiving an alarm signal of a small larva under attack. In the latter case, a behavioral response may require additional cues of predation, such as cues elicited by the predator (as shown for thrips by Venzon *et al.* 2000) or cues from wounded conspecifics (this latter type of cue is commonly found in aquatic predator-prey systems; see, e.g., Chivers and Smith 1998).

We found no differential response to alarm pheromone in other aspects of anti-predator behavior (FIGURES 4-3 and 4-4, TABLE 4-1). Focal larvae also did not perform more partial crossings in the presence of a large companion larva than in the presence of a small companion larva before treatments, or after exposure to synthetic alarm pheromone of fixed composition (FIGURE 4-5, TABLE 4-2). Hence, the cue they responded to after treatments was the pheromone, and not any other cue related to the companion larva. To test if the presence of a companion larva has any effect on a focal larva, focal larvae should be presented with synthetic alarm pheromone in the presence or absence of a companion larva. We did not perform these tests, because we focused on the hypothesis that thrips larvae perceive a difference between natural alarm pheromone produced by small or large larvae.

Does response depend on ratio or amount of pheromonal components?

Given that small thrips larvae display more anti-predator behavior in response to alarm pheromone of large larvae than to that of small larvae, we also investigated whether this effect can be attributed to the difference in amount of pheromone or the difference in the ratio of the two components. We found that the total amount of the two components had a significant effect on the number of partial crossings small larvae make, but their ratio of the two compounds in the mixture did not. However, the strong response to the solvent cyclohexane may have masked subtle effects of the ratio of the components. Indeed, there is

a trend for small larvae to respond more strongly to mixtures with the ratio mimicking alarm pheromone of large larvae compared to mixtures with the ratio mimicking alarm pheromone of small larvae (as seen in FIGURE 4-9). Therefore, we suggest that the ratio of pheromone components does matter to the response of small thrips larvae.

Do responses to natural and synthetic pheromone correspond?

Throughout this article we assumed that the alarm pheromone consists of two components. However, we cannot exclude the presence of other components in the pheromone in concentrations below the detection threshold of analytical equipment, but which might cause a behavioral response of thrips larvae. To exclude that such components have a large effect on thrips behavior, we tested if the synthetic pheromone elicits a response mimicking that of natural pheromone. Small larvae made significantly more partial crossings when exposed to synthetic blends aimed to mimic alarm pheromone of large larvae than that of small larvae (one-way ANOVA: $F_{1,48} = 7.21$, $P<0.01$; FIGURE 4-9), which corresponds to our results using natural pheromone of these thrips larvae (FIGURE 4-4A). Large larvae did not make more partial crossings when exposed to synthetic blends mimicking alarm pheromone of large larvae than that of small larvae (one-way ANOVA: $F_{1,48} = 0.37$, $P = 0.55$; FIGURE 4-9), which again corresponds to our results found using natural pheromone (FIGURE 4-5B). Hence, the natural pheromone and its synthetic analog seem to have a similar effect on the response of thrips larvae.

Comparing results with known anti-predator behavior

Our results are in agreement with what is known of thrips anti-predator behavior. In an attempt to defend themselves, thrips larvae release anal droplets and swing their abdomen when contacted by a predator (Bakker and Sabelis 1989; Teerling *et al.* 1993). In the absence of contact with a predator, such anti-predator behavior is expected to occur at a lower frequency. Indeed, when thrips larvae were subjected to natural pheromone, we did not observe an increase in release of anal droplets (TABLE 4-1, FIGURE 4-3, TABLE S4-3), and a decrease in the number of abdominal swings (FIGURE 4-4, TABLE S4-3). However, we did observe an increase in the frequency of partial crossings (FIGURE 4-5, TABLE S4-3). We interpret the latter behavior as an increased tendency to avoid contact with a predator by leaving the area where alarm was raised.

Scope for context-dependent signals

Context-dependent alarm signals allow receivers to respond adaptively to predation risk (Blum 1996). In this article, we show that small thrips larvae respond differentially to alarm pheromone excreted by small larvae or large larvae and that this differential response could be explained by differences in amount of pheromone, and possibly its composition. If the amount of pheromone perceived by the receiver thrips would decrease with increasing distance from the sender, we would expect differential responses with increasing distance between sender and receiver. For a thrips larva, however, to be able to distinguish between two signals without knowing the distance between itself and a sender, the signals should not only differ in amount, but also in other aspects, such as the ratio of the two components. We did find a trend for first-instar larvae to respond more strongly to mixtures where the components had the ratio of second-instar alarm pheromone. Thrips larvae in this experiment responded not only to the synthetic pheromones, but also to the solvent used (FIGURE 4-9), which could have masked significant effects of the ratio of the components.

Context-dependent responses to alarm signals are known for vocal alarm calls (e.g., Sherman 1977; Seyfarth *et al.* 1980; Furrer and Manser 2009). Chemical alarm signals (alarm pheromones), however, have hardly been studied with respect to the extent to which conspecifics respond to intra-individual variation in pheromones. In invertebrates, we are aware of only one other example (of paper wasps) where the composition of alarm pheromone and the response to it varies (Bruschini *et al.* 2008). Our results add a second example of adjusted response to changes in alarm pheromone of an individual insect: the composition of alarm pheromone changes with the age of a thrips larva (MacDonald *et al.* 2003) and here we found that the response of small larvae changes with the composition of alarm pheromone. Moreover, sending thrips larvae are able to vary the ratio of decyl acetate and dodecyl acetate as well as the amount of pheromone with the level of danger they perceive (de Bruijn *et al.* 2014). Hence, together with these earlier findings, our results suggest that sender and receiver thrips change their behavior with the level of danger, and thereby display context-dependent alarm communication.

Acknowledgements

PJAdB was supported by a grant from The Netherlands Organization for Scientific Research (NWO, project number 818.01.018). We thank Tim Bulters for practical help with experiments.

Reference

Bakker FM, Sabelis MW (1987) Attack success of *Amblyseius mckenziei* and the stage related defensive capacity of thrips larvae. Neth J Zool 37:437.

Bakker FM, Sabelis MW (1989) How larvae of *Thrips tabaci* reduce the attack success of phytoseiid predators. Entomol Exp App 50:47-51.

Belliure B, Janssen A, Sabelis MW (2008) Herbivore benefits from vectoring plant virus through reduction of period of vulnerability to predation. Oecologia 156:797-806.

Blum MS (1996) Semiochemical parsimony in the Arthropoda. Annu Rev Entomol 41:353-374.

Borden JH (1989) Semiochemicals and bark beetle populations: Exploitation of natural phenomena by pest management strategists. Holarctic Ecol 12:501-510.

Bruschini C, Cervo R, Protti I, Turillazzi S (2008) Caste differences in venom volatiles and their effect on alarm behaviour in the paper wasp *Polistes dominulus* (Christ). J Exp Biol 211:2442-2449.

Cameron AM, Endean R (1973) Epidermal secretions and the evolution of venom glands in fishes. Toxicon ll:401-410.

Chase JM (1999) Food web effects of prey size refugia: Variable interactions and alternative stable equilibria. American Naturalist 154:559-570.

Chivers DP, Smith RJF (1998) Chemical alarm signalling in aquatic predator-prey systems: A review and prospectus. Ecoscience 5:338-352.

Crawley MJ (2007) The R book. John Wiley and Sons, Chichester, UK.

Diggle SP, West SA, Gardner A, Griffin AS (2008) Communication in bacteria. In: d'Ettorre P, Hughes D (ed) Sociobiology of communication an interdisciplinary perspective, pp. 11–31. Oxford University Press, Oxford, UK.

Dill LM, Frase, AHG, Roitberg BD (1990) The economics of escape behavior in the pea aphid, *Acrythosiphon pisum*. Oecologia 83:473-478.

de Bruijn PJA, Egas M, Janssen A, Sabelis MW (2006) Pheromone-induced priming of a defensive response in western flower thrips. J Chem Ecol 32:1599-1603. [Chapter 2 of this thesis]

de Bruijn PJA, Egas M, Sabelis MW, Groot AT (2014) Context-dependent alarm signalling in an insect. [Chapter 5 of this thesis]

Furrer RD, Manser MB (2009) The evolution of urgency-based and functionally referential alarm calls in ground-dwelling species. American Naturalist 173:400-410.

Giraudoux P (2008) pgirmess: data analysis in ecology. R package version 1.5.8. http://pagesperso-orange.fr/giraudoux. [Accessed 3 Oct 2014]

Hasson O (1996) Towards a general theory of biological signaling. J Theor Biol 185:139-156.

Kunert G, Otto S, Röse USR, Gershenzon J, Weisser WW (2005) Alarm pheromone mediates production of winged dispersal morphs in aphids. Ecology Letters 8:596-603.

Kats LB, Dill LM (1998) The scent of death: Chemosensory assessment of predation risk by prey animals. Ecoscience 5:361-394.

Lima SL, Dill LM (1990) Behavioural decisions made under the risk of predation: a review and prospectus. Can J Zool 68:619–640.

Lönnstedt OM, McCormick MI (2011) Chemical alarm cues inform prey of predation threat: the importance of ontogeny and concentration in a coral reef fish. Anim Behav 82:213-218.

MacDonald KM, Hamilton JGC, Jacobson R, Kirk WDJ (2003) Analysis of anal droplets of the western flower thrips *Frankliniella occidentalis*. J Chem Ecol 29:2385-2389.

Mirza RS, Chivers DP (2002) Brook char (*Salvelinus fontinalis*) can differentiate chemical alarm cues produced by different age/size classes of conspecifics. J Chem Ecol 28:555-564.

Nault LR, Montgomery ME (1976) Ant-aphid association: Role of aphid alarm pheromone. Science 192:1349-1351.

Ottoni EB (2000) Etholog 2.2 – a tool for the transcription and timing of behavior observation sessions. Behav Res Meth Instrum Comput 32:446-449.

Pickett JA, Wadhams LJ, Woodcock CM (1992) The chemical ecology of aphids. Annu Rev Entomol 37:67-90.

Podjasek JO, Bosnjak LM, Brooker DJ, Mondor EB (2005) Alarm pheromone induces a transgenerational wing polyphenism in the pea aphid, *Acyrthosiphon pisum*. Can J Zool 83:1138-1141.

R Development Core Team (2010) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Sabelis MW, van Rijn PCJ (1997) Predation by insects and mites. In: Lewis T (ed) Thrips as crop pests, pp. 259–354. CAB International, Wallingford, UK.

Seyfarth RM, Cheney DL, Marler P (1980) Vervet monkey alarm calls – semantic communication in a free-ranging primate. Anim Behav 28:1070-1094.

Sherman PW (1977) Nepotism and evolution of alarm calls. Science 197:1246-1253.

Svensson MGE, Bengtsson M, Löfqvist J (1997) Individual variation and repeatability of sex pheromone emission of female turnip moths *Agrotis segetum*. J Chem Ecol 23:1833-1850.

Siegel S, Castellan NJ (1988) Non-parametric statistics for the behavioural sciences, 2nd edn. McGraw-Hill International Editions, New York, NY, USA.

Sih A (1980) Optimal behavior: can foragers balance two conflicting demands? Science 210:1041–1043.

Teerling CR, Pierce HD, Borden JH, Gillespie DR (1993) Identification and bioactivity of alarm pheromone in the western flower thrips, *Frankliniella occidentalis*. J Chem Ecol 19:681-697.

Tonn WM, Paszkowski CA, Holopainen IJ (1992) Piscivory and recruitment: mechanisms structuring prey populations in small lakes. Ecology 73:951-958.

Venzon M, Janssen A, Pallini A, Sabelis MW (2000) Diet of a polyphagous arthropod predator affects refuge seeking of its thrips prey. Anim Behav 60:369-375.

Appendix

Table S4-1 Length, width and height of thrips larvae in mm (mean ± SE).

	First-instar thrips larvae		Second-instar thrips larvae	
	Freshly hatched	Before molting	After molting	Before pupation
Length	0.50 ± 0.004	0.92 ± 0.027	0.92 ± 0.03	1.16 ± 0.02
Width	0.11 ± 0.005	0.20 ± 0.009	0.21 ± 0.01	0.25 ± 0.01
Height	0.10 ± 0.003	0.13 ± 0.004	0.15 ± 0.01	0.22 ± 0.01

The size of thrips larvae was measured using a binocular microscope with an ocular graticule. We measured length, width and height of 10 small first-instar larvae (freshly hatched from eggs), 10 large first-instar larvae (about to molt into second-instar larvae), 10 small second-instar larvae (just molted from first instar) and 10 large second-instar larvae (about to molt into prepupae) Thrips larvae were measured on a cucumber leaf in a Petri dish (as described above for the thrips culture); length and width were measured with the Petri dish in a horizontal position, height was measured with the Petri dish in a vertical position.

Table S4-2 Statistical results from Wilcoxon signed rank tests comparing before and after data collected for the number of anal droplets releases, swings of the abdomen and partial crossings of a focal larva treatments in the '*Responses to natural alarm pheromone and effect of companion larva*' experiment.

Behavior	n	Before treatment	After treatment	Wilcoxon signed rank test
	First-instar larvae			
Excretion of anal droplet	150	0.45 ± 0.04	0.48 ± 0.04	V = 1039, P = 0.63
Swinging abdomen	150	1.06 ± 0.12	0.79 ± 0.10	V = 2658, P = 0.06
Partially crossing the edge of the arena	150	0.55 ± 0.08	1.94 ± 0.16	V = 521, P<0.001
	Second-instar larvae			
Excretion of anal droplet	150	0.24 ± 0.04	0.27 ± 0.04	V = 539, P = 0.57
Swinging abdomen	150	1.51 ± 0.19	0.90 ± 0.12	V = 2952, P<0.001
Partially crossing the edge of the arena	150	0.73 ± 0.09	2.22 ± 0.21	V = 1081, P<0.001

Table S4-3 Statistical results from Wilcoxon signed rank tests comparing before and after data collected for the number of anal droplets releases, swings of the abdomen and partial crossings of a focal larva treatments in the '*Effect of amount and ratio of pheromone components on thrips behavior*' experiment.

Behavior	n	Before treatment	After treatment	Wilcoxon signed rank test
	First-instar larvae			
Excretion of anal droplet	123	0.34 ± 0.04	0.51 ± 0.12	V = 656, P = 0.95
Swinging abdomen	126	0.30 ± 0.06	0.71 ± 0.01	V = 424, P<0.001
Partially crossing the edge of the arena	123	0.32 ± 0.09	4.94 ± 0.29	V = 102.5, P<0.001
	Second-instar larvae			
Excretion of anal droplet	123	0.2 ± 0.04	0.43 ± 0.11	V = 441, P = 0.16
Swinging abdomen	121	0.48 ± 0.07	0.97 ± 0.15	V = 559, P<0.01
Partially crossing the edge of the arena	125	0.42 ± 0.08	8.40 ± 0.74	V = 47.5, P<0.001

TABLE S4-4 Shown are all data. Outliers (values differ more than 3× the deviation from the mean) are marked red.

Responses to natural pheromone

droplets										
	focal larva: first-instar						focal larva: second-instar			
thrips	before	after (corrected for time on leaf disc)	companion	treatment		thrips	before	after (corrected for time on leaf disc)	companion	treatment
1	0	0	1	Natural pheromone		1	0	0	1	Natural pheromone
2	0	0	1	Natural pheromone		2	0	1	1	Natural pheromone
3	0	1	1	Natural pheromone		3	0	0	1	Natural pheromone
4	0	1	1	Natural pheromone		4	1	0	1	Natural pheromone
5	0	1	1	Natural pheromone		5	0	0	1	Natural pheromone
6	0	0	1	Natural pheromone		6	0	1	1	Natural pheromone
7	0	1	1	Natural pheromone		7	0	0	1	Natural pheromone
8	1	1	1	Natural pheromone		8	0	0	1	Natural pheromone
9	1	0	1	Natural pheromone		9	0	0	1	Natural pheromone
10	1	0	1	Natural pheromone		10	0	0	1	Natural pheromone
11	1	0	1	Natural pheromone		11	0	0	1	Natural pheromone
12	0	0	1	Natural pheromone		12	0	0	1	Natural pheromone
13	0	0	1	Natural pheromone		13	0	0	1	Natural pheromone
14	0	1	1	Natural pheromone		14	0	0	1	Natural pheromone
15	1	1	1	Natural pheromone		15	0	0	1	Natural pheromone
16	0	0	1	Natural pheromone		16	1	0	1	Natural pheromone
17	1	0	1	Natural pheromone		17	0	0	1	Natural pheromone
18	0	0	1	Natural pheromone		18	0	0	1	Natural pheromone
19	0	1	1	Natural pheromone		19	0	0	1	Natural pheromone
20	0	1	1	Natural pheromone		20	0	0	1	Natural pheromone
21	0	0	1	Natural pheromone		21	1	0	1	Natural pheromone
22	1	0	1	Natural pheromone		22	1	1	1	Natural pheromone
23	1	0	1	Natural pheromone		23	0	0	1	Natural pheromone
24	0	1	1	Natural pheromone		24	1	1	1	Natural pheromone
25	1	0	1	Natural pheromone		25	0	0	1	Natural pheromone
26	0	0	1	Synthetic pheromone		26	0	0	1	Synthetic pheromone
27	1	0	1	Synthetic pheromone		27	0	0	1	Synthetic pheromone
28	0	1	1	Synthetic pheromone		28	0	0	1	Synthetic pheromone
29	0	0	1	Synthetic pheromone		29	1	0	1	Synthetic pheromone
30	0	1	1	Synthetic pheromone		30	0	0	1	Synthetic pheromone
31	0	0	1	Synthetic pheromone		31	0	0	1	Synthetic pheromone
32	0	1	1	Synthetic pheromone		32	0	0	1	Synthetic pheromone
33	1	0	1	Synthetic pheromone		33	0	0	1	Synthetic pheromone
34	0	0	1	Synthetic pheromone		34	0	1	1	Synthetic pheromone
35	0	1	1	Synthetic pheromone		35	1	1	1	Synthetic pheromone
36	0	0	1	Synthetic pheromone		36	0	0	1	Synthetic pheromone
37	0	0	1	Synthetic pheromone		37	0	1	1	Synthetic pheromone
38	0	0	1	Synthetic pheromone		38	1	0	1	Synthetic pheromone
39	1	0	1	Synthetic pheromone		39	1	0	1	Synthetic pheromone
40	0	0	1	Synthetic pheromone		40	0	1	1	Synthetic pheromone
41	0	0	1	Synthetic pheromone		41	0	0	1	Synthetic pheromone
42	0	0	1	Synthetic pheromone		42	0	0	1	Synthetic pheromone
43	1	0	1	Synthetic pheromone		43	0	1	1	Synthetic pheromone
44	0	1	1	Synthetic pheromone		44	0	1	1	Synthetic pheromone
45	1	1	1	Synthetic pheromone		45	0	0	1	Synthetic pheromone
46	1	1	1	Synthetic pheromone		46	1	1	1	Synthetic pheromone
47	2	1	1	Synthetic pheromone		47	1	0	1	Synthetic pheromone
48	0	0	1	Synthetic pheromone		48	0	1	1	Synthetic pheromone
49	0	0	1	Synthetic pheromone		49	0	1	1	Synthetic pheromone
50	0	1	1	Synthetic pheromone		50	0	1	1	Synthetic pheromone
51	0	1	1	Cyclohexane		51	0	0	1	Cyclohexane
52	1	1	1	Cyclohexane		52	0	0	1	Cyclohexane
53	0	0	1	Cyclohexane		53	1	1	1	Cyclohexane
54	1	0	1	Cyclohexane		54	0	0	1	Cyclohexane
55	1	1	1	Cyclohexane		55	0	1	1	Cyclohexane
56	1	1	1	Cyclohexane		56	1	0	1	Cyclohexane
57	1	1	1	Cyclohexane		57	0	0	1	Cyclohexane
58	1	1	1	Cyclohexane		58	0	0	1	Cyclohexane
59	0	0	1	Cyclohexane		59	0	0	1	Cyclohexane
60	0	1	1	Cyclohexane		60	1	0	1	Cyclohexane
61	0	0	1	Cyclohexane		61	0	0	1	Cyclohexane
62	1	2	1	Cyclohexane		62	1	0	1	Cyclohexane
63	1	0	1	Cyclohexane		63	0	1	1	Cyclohexane
64	0	0	1	Cyclohexane		64	0	0	1	Cyclohexane
65	1	1	1	Cyclohexane		65	0	0	1	Cyclohexane
66	0	1	1	Cyclohexane		66	1	1	1	Cyclohexane
67	0	0	1	Cyclohexane		67	1	0	1	Cyclohexane
68	1	1	1	Cyclohexane		68	0	0	1	Cyclohexane
69	0	1	1	Cyclohexane		69	0	0	1	Cyclohexane
70	1	1	1	Cyclohexane		70	0	0	1	Cyclohexane
71	0	1	1	Cyclohexane		71	0	0	1	Cyclohexane
72	0	0	1	Cyclohexane		72	0	0	1	Cyclohexane
73	1	1	1	Cyclohexane		73	0	1	1	Cyclohexane
74	0	1	1	Cyclohexane		74	0	1	1	Cyclohexane
75	1	0	1	Cyclohexane		75	1	1	1	Cyclohexane

76	1	1	2	Natural pheromone	76	0	1	2	Natural pheromone
77	0	0	2	Natural pheromone	77	0	0	2	Natural pheromone
78	0	0	2	Natural pheromone	78	0	0	2	Natural pheromone
79	1	0	2	Natural pheromone	79	0	0	2	Natural pheromone
80	0	0	2	Natural pheromone	80	0	0	2	Natural pheromone
81	1	0	2	Natural pheromone	81	0	0	2	Natural pheromone
82	0	0	2	Natural pheromone	82	0	1	2	Natural pheromone
83	0	0	2	Natural pheromone	83	0	0	2	Natural pheromone
84	0	0	2	Natural pheromone	84	0	0	2	Natural pheromone
85	0	0	2	Natural pheromone	85	1	0	2	Natural pheromone
86	0	1	2	Natural pheromone	86	0	0	2	Natural pheromone
87	0	0	2	Natural pheromone	87	0	0	2	Natural pheromone
88	1	1	2	Natural pheromone	88	0	1	2	Natural pheromone
89	1	0	2	Natural pheromone	89	0	0	2	Natural pheromone
90	1	1	2	Natural pheromone	90	1	0	2	Natural pheromone
91	0	1	2	Natural pheromone	91	0	0	2	Natural pheromone
92	0	1	2	Natural pheromone	92	0	0	2	Natural pheromone
93	1	1	2	Natural pheromone	93	0	0	2	Natural pheromone
94	0	0	2	Natural pheromone	94	0	0	2	Natural pheromone
95	0	0	2	Natural pheromone	95	1	1	2	Natural pheromone
96	0	0	2	Natural pheromone	96	0	1	2	Natural pheromone
97	0	1	2	Natural pheromone	97	0	0	2	Natural pheromone
98	1	1	2	Natural pheromone	98	0	0	2	Natural pheromone
99	1	1	2	Natural pheromone	99	1	0	2	Natural pheromone
100	0	0	2	Natural pheromone	100	1	0	2	Natural pheromone
101	0	1	2	Synthetic pheromone	101	0	0	2	Synthetic pheromone
102	0	1	2	Synthetic pheromone	102	0	0	2	Synthetic pheromone
103	1	0	2	Synthetic pheromone	103	0	0	2	Synthetic pheromone
104	1	0	2	Synthetic pheromone	104	0	0	2	Synthetic pheromone
105	0	0	2	Synthetic pheromone	105	0	0	2	Synthetic pheromone
106	0	1	2	Synthetic pheromone	106	0	0	2	Synthetic pheromone
107	0	1	2	Synthetic pheromone	107	1	0	2	Synthetic pheromone
108	0	0	2	Synthetic pheromone	108	0	1	2	Synthetic pheromone
109	0	0	2	Synthetic pheromone	109	0	0	2	Synthetic pheromone
110	0	0	2	Synthetic pheromone	110	0	0	2	Synthetic pheromone
111	1	0	2	Synthetic pheromone	111	0	0	2	Synthetic pheromone
112	1	2	2	Synthetic pheromone	112	1	1	2	Synthetic pheromone
113	1	0	2	Synthetic pheromone	113	1	0	2	Synthetic pheromone
114	1	0	2	Synthetic pheromone	114	0	1	2	Synthetic pheromone
115	1	0	2	Synthetic pheromone	115	0	0	2	Synthetic pheromone
116	1	1	2	Synthetic pheromone	116	0	0	2	Synthetic pheromone
117	1	1	2	Synthetic pheromone	117	0	0	2	Synthetic pheromone
118	1	1	2	Synthetic pheromone	118	1	0	2	Synthetic pheromone
119	1	0	2	Synthetic pheromone	119	1	1	2	Synthetic pheromone
120	0	0	2	Synthetic pheromone	120	0	1	2	Synthetic pheromone
121	1	2	2	Synthetic pheromone	121	0	0	2	Synthetic pheromone
122	0	1	2	Synthetic pheromone	122	0	0	2	Synthetic pheromone
123	0	0	2	Synthetic pheromone	123	0	0	2	Synthetic pheromone
124	0	0	2	Synthetic pheromone	124	0	0	2	Synthetic pheromone
125	1	0	2	Synthetic pheromone	125	1	1	2	Synthetic pheromone
126	1	1	2	Cyclohexane	126	0	0	2	Cyclohexane
127	1	1	2	Cyclohexane	127	0	0	2	Cyclohexane
128	1	1	2	Cyclohexane	128	0	0	2	Cyclohexane
129	1	1	2	Cyclohexane	129	0	0	2	Cyclohexane
130	0	0	2	Cyclohexane	130	0	0	2	Cyclohexane
131	1	1	2	Cyclohexane	131	1	0	2	Cyclohexane
132	1	0	2	Cyclohexane	132	1	1	2	Cyclohexane
133	1	0	2	Cyclohexane	133	0	0	2	Cyclohexane
134	0	0	2	Cyclohexane	134	1	1	2	Cyclohexane
135	1	1	2	Cyclohexane	135	0	1	2	Cyclohexane
136	1	0	2	Cyclohexane	136	1	0	2	Cyclohexane
137	1	1	2	Cyclohexane	137	0	1	2	Cyclohexane
138	0	1	2	Cyclohexane	138	0	0	2	Cyclohexane
139	1	1	2	Cyclohexane	139	0	0	2	Cyclohexane
140	1	0	2	Cyclohexane	140	1	0	2	Cyclohexane
141	0	0	2	Cyclohexane	141	0	1	2	Cyclohexane
142	1	1	2	Cyclohexane	142	0	1	2	Cyclohexane
143	0	1	2	Cyclohexane	143	0	0	2	Cyclohexane
144	0	0	2	Cyclohexane	144	0	0	2	Cyclohexane
145	0	1	2	Cyclohexane	145	0	1	2	Cyclohexane
146	0	0	2	Cyclohexane	146	0	0	2	Cyclohexane
147	0	0	2	Cyclohexane	147	0	0	2	Cyclohexane
148	1	1	2	Cyclohexane	148	0	0	2	Cyclohexane
149	1	1	2	Cyclohexane	149	1	1	2	Cyclohexane
150	1	0	2	Cyclohexane	150	1	0	2	Cyclohexane

swings

	focal larva: first-instar					focal larva: second-instar				
thrips	before	after (corrected for time on leaf disc)	companion	treatment		thrips	before	after (corrected for time on leaf disc)	companion	treatment
1	1	1	1	Natural pheromone		1	1	0	1	Natural pheromone
2	2	0	1	Natural pheromone		2	0	1.153846154	1	Natural pheromone
3	1	0	1	Natural pheromone		3	0	2	1	Natural pheromone
4	0	1	1	Natural pheromone		4	3	3	1	Natural pheromone
5	1	0	1	Natural pheromone		5	0	0	1	Natural pheromone
6	4	3.93442623	1	Natural pheromone		6	1	0	1	Natural pheromone
7	0	1	1	Natural pheromone		7	2	0	1	Natural pheromone
8	0	0	1	Natural pheromone		8	15	9	1	Natural pheromone
9	1	0	1	Natural pheromone		9	8	1	1	Natural pheromone
10	1	2	1	Natural pheromone		10	0	0	1	Natural pheromone
11	4	0	1	Natural pheromone		11	1	0	1	Natural pheromone
12	1	1	1	Natural pheromone		12	2	0	1	Natural pheromone
13	9	0	1	Natural pheromone		13	0	0	1	Natural pheromone
14	1	0	1	Natural pheromone		14	4	1	1	Natural pheromone
15	3	1	1	Natural pheromone		15	0	0	1	Natural pheromone
16	0	1	1	Natural pheromone		16	0	0	1	Natural pheromone
17	2	0	1	Natural pheromone		17	1	0	1	Natural pheromone
18	0	0	1	Natural pheromone		18	0	0	1	Natural pheromone
19	0	1	1	Natural pheromone		19	3	2	1	Natural pheromone
20	0	1	1	Natural pheromone		20	0	0	1	Natural pheromone
21	0	0	1	Natural pheromone		21	0	1.132075472	1	Natural pheromone
22	0	0	1	Natural pheromone		22	0	0	1	Natural pheromone
23	3	1	1	Natural pheromone		23	0	0	1	Natural pheromone
24	0	0	1	Natural pheromone		24	3	1	1	Natural pheromone
25	0	0	1	Natural pheromone		25	0	0	1	Natural pheromone
26	1	1	1	Synthetic pheromone		26	3	0	1	Synthetic pheromone
27	1	0	1	Synthetic pheromone		27	1	1.621621622	1	Synthetic pheromone
28	0	1	1	Synthetic pheromone		28	0	0	1	Synthetic pheromone
29	2	6	1	Synthetic pheromone		29	2	0	1	Synthetic pheromone
30	3	1	1	Synthetic pheromone		30	3	0	1	Synthetic pheromone
31	0	2	1	Synthetic pheromone		31	5	2	1	Synthetic pheromone
32	0	3	1	Synthetic pheromone		32	0	0	1	Synthetic pheromone
33	2	0	1	Synthetic pheromone		33	1	2	1	Synthetic pheromone
34	0	0	1	Synthetic pheromone		34	0	3	1	Synthetic pheromone
35	0	0	1	Synthetic pheromone		35	4	1	1	Synthetic pheromone
36	6	2.033898305	1	Synthetic pheromone		36	1	0	1	Synthetic pheromone
37	1	0	1	Synthetic pheromone		37	0	0	1	Synthetic pheromone
38	0	5	1	Synthetic pheromone		38	1	0	1	Synthetic pheromone
39	1	2	1	Synthetic pheromone		39	1	1	1	Synthetic pheromone
40	0	0	1	Synthetic pheromone		40	0	0	1	Synthetic pheromone
41	0	0	1	Synthetic pheromone		41	0	0	1	Synthetic pheromone
42	0	1	1	Synthetic pheromone		42	13	6	1	Synthetic pheromone
43	2	0	1	Synthetic pheromone		43	0	0	1	Synthetic pheromone
44	0	0	1	Synthetic pheromone		44	1	0	1	Synthetic pheromone
45	0	0	1	Synthetic pheromone		45	0	0	1	Synthetic pheromone
46	1	0	1	Synthetic pheromone		46	1	0	1	Synthetic pheromone
47	3	0	1	Synthetic pheromone		47	1	0	1	Synthetic pheromone
48	0	1	1	Synthetic pheromone		48	0	0	1	Synthetic pheromone
49	0	1	1	Synthetic pheromone		49	0	0	1	Synthetic pheromone
50	1	0	1	Synthetic pheromone		50	4	2	1	Synthetic pheromone
51	2	1	1	Cyclohexane		51	2	0	1	Cyclohexane
52	0	0	1	Cyclohexane		52	0	2	1	Cyclohexane
53	5	1	1	Cyclohexane		53	1	1.714285714	1	Cyclohexane
54	0	1	1	Cyclohexane		54	0	0	1	Cyclohexane
55	0	0	1	Cyclohexane		55	0	0	1	Cyclohexane
56	0	3	1	Cyclohexane		56	0	0	1	Cyclohexane
57	0	0	1	Cyclohexane		57	2	1	1	Cyclohexane
58	4	1	1	Cyclohexane		58	0	0	1	Cyclohexane
59	0	0	1	Cyclohexane		59	0	2	1	Cyclohexane
60	3	1	1	Cyclohexane		60	5	5	1	Cyclohexane
61	3	1	1	Cyclohexane		61	2	0	1	Cyclohexane
62	0	0	1	Cyclohexane		62	1	0	1	Cyclohexane
63	1	1	1	Cyclohexane		63	0	1	1	Cyclohexane
64	2	2	1	Cyclohexane		64	3	0	1	Cyclohexane
65	1	2	1	Cyclohexane		65	1	0	1	Cyclohexane
66	0	0	1	Cyclohexane		66	1	0	1	Cyclohexane
67	0	4	1	Cyclohexane		67	2	0	1	Cyclohexane
68	1	1	1	Cyclohexane		68	1	1	1	Cyclohexane
69	2	0	1	Cyclohexane		69	0	0	1	Cyclohexane
70	0	0	1	Cyclohexane		70	1	2	1	Cyclohexane
71	0	0	1	Cyclohexane		71	0	2.5	1	Cyclohexane
72	0	1	1	Cyclohexane		72	0	0	1	Cyclohexane
73	0	0	1	Cyclohexane		73	0	2	1	Cyclohexane
74	1	0	1	Cyclohexane		74	0	0	1	Cyclohexane
75	1	0	1	Cyclohexane		75	4	2	1	Cyclohexane

76	0	0	2	Natural pheromone	76	0	1	2	Natural pheromone
77	0	0	2	Natural pheromone	77	0	0	2	Natural pheromone
78	0	0	2	Natural pheromone	78	0	0	2	Natural pheromone
79	1	1	2	Natural pheromone	79	0	0	2	Natural pheromone
80	0	0	2	Natural pheromone	80	6	7	2	Natural pheromone
81	1	0	2	Natural pheromone	81	1	0	2	Natural pheromone
82	0	0	2	Natural pheromone	82	0	2	2	Natural pheromone
83	0	0	2	Natural pheromone	83	3	2	2	Natural pheromone
84	3	2.181818182	2	Natural pheromone	84	3	0	2	Natural pheromone
85	1	0	2	Natural pheromone	85	3	1	2	Natural pheromone
86	0	0	2	Natural pheromone	86	0	0	2	Natural pheromone
87	0	0	2	Natural pheromone	87	2	0	2	Natural pheromone
88	3	0	2	Natural pheromone	88	0	0	2	Natural pheromone
89	1	0	2	Natural pheromone	89	0	0	2	Natural pheromone
90	3	0	2	Natural pheromone	90	6	4	2	Natural pheromone
91	3	0	2	Natural pheromone	91	8	2	2	Natural pheromone
92	0	0	2	Natural pheromone	92	0	0	2	Natural pheromone
93	2	1	2	Natural pheromone	93	2	0	2	Natural pheromone
94	0	0	2	Natural pheromone	94	0	0	2	Natural pheromone
95	0	0	2	Natural pheromone	95	3	1	2	Natural pheromone
96	1	0	2	Natural pheromone	96	1	1	2	Natural pheromone
97	1	1	2	Natural pheromone	97	0	0	2	Natural pheromone
98	3	3	2	Natural pheromone	98	0	0	2	Natural pheromone
99	1	0	2	Natural pheromone	99	1	1	2	Natural pheromone
100	0	1	2	Natural pheromone	100	0	0	2	Natural pheromone
101	3	3	2	Synthetic pheromone	101	0	1	2	Synthetic pheromone
102	3	0	2	Synthetic pheromone	102	2	1	2	Synthetic pheromone
103	1	1	2	Synthetic pheromone	103	1	0	2	Synthetic pheromone
104	2	1	2	Synthetic pheromone	104	4	3	2	Synthetic pheromone
105	0	2	2	Synthetic pheromone	105	1	0	2	Synthetic pheromone
106	1	1	2	Synthetic pheromone	106	1	2	2	Synthetic pheromone
107	0	1	2	Synthetic pheromone	107	1	0	2	Synthetic pheromone
108	0	0	2	Synthetic pheromone	108	1	4	2	Synthetic pheromone
109	0	0	2	Synthetic pheromone	109	0	0	2	Synthetic pheromone
110	1	4	2	Synthetic pheromone	110	0	0	2	Synthetic pheromone
111	1	0	2	Synthetic pheromone	111	6	0	2	Synthetic pheromone
112	2	0	2	Synthetic pheromone	112	0	2	2	Synthetic pheromone
113	0	1	2	Synthetic pheromone	113	2	0	2	Synthetic pheromone
114	6	0	2	Synthetic pheromone	114	2	8	2	Synthetic pheromone
115	2	1	2	Synthetic pheromone	115	0	0	2	Synthetic pheromone
116	2	1	2	Synthetic pheromone	116	0	3	2	Synthetic pheromone
117	0	0	2	Synthetic pheromone	117	0	0	2	Synthetic pheromone
118	1	0	2	Synthetic pheromone	118	6	0	2	Synthetic pheromone
119	0	2	2	Synthetic pheromone	119	4	1	2	Synthetic pheromone
120	0	1	2	Synthetic pheromone	120	1	1	2	Synthetic pheromone
121	1	2	2	Synthetic pheromone	121	2	0	2	Synthetic pheromone
122	0	0	2	Synthetic pheromone	122	3	2	2	Synthetic pheromone
123	0	1	2	Synthetic pheromone	123	0	0	2	Synthetic pheromone
124	0	0	2	Synthetic pheromone	124	2	0	2	Synthetic pheromone
125	0	0	2	Synthetic pheromone	125	1	3	2	Synthetic pheromone
126	2	1	2	Cyclohexane	126	1	3	2	Cyclohexane
127	1	0	2	Cyclohexane	127	1	0	2	Cyclohexane
128	1	1	2	Cyclohexane	128	0	1	2	Cyclohexane
129	7	0	2	Cyclohexane	129	5	3	2	Cyclohexane
130	0	1	2	Cyclohexane	130	0	0	2	Cyclohexane
131	1	0	2	Cyclohexane	131	3	0	2	Cyclohexane
132	1	0	2	Cyclohexane	132	1	2	2	Cyclohexane
133	1	1	2	Cyclohexane	133	0	0	2	Cyclohexane
134	1	0	2	Cyclohexane	134	5	2	2	Cyclohexane
135	1	1	2	Cyclohexane	135	0	0	2	Cyclohexane
136	0	0	2	Cyclohexane	136	1	0	2	Cyclohexane
137	0	1	2	Cyclohexane	137	2	1	2	Cyclohexane
138	0	6	2	Cyclohexane	138	0	0	2	Cyclohexane
139	2	0	2	Cyclohexane	139	2	0	2	Cyclohexane
140	1	0	2	Cyclohexane	140	0	0	2	Cyclohexane
141	2	0	2	Cyclohexane	141	0	0	2	Cyclohexane
142	1	1	2	Cyclohexane	142	2	5	2	Cyclohexane
143	1	5.454545455	2	Cyclohexane	143	0	0	2	Cyclohexane
144	0	1	2	Cyclohexane	144	0	0	2	Cyclohexane
145	0	0	2	Cyclohexane	145	0	0	2	Cyclohexane
146	0	1	2	Cyclohexane	146	2	0	2	Cyclohexane
147	0	0	2	Cyclohexane	147	6	0	2	Cyclohexane
148	0	1.034482759	2	Cyclohexane	148	2	1	2	Cyclohexane
149	1	2	2	Cyclohexane	149	0	0	2	Cyclohexane
150	1	3	2	Cyclohexane	150	2	2	2	Cyclohexane

partial crossings

	focal larva: first-instar						focal larva: second-instar				
thrips	before	after (corrected for time on leaf disc)	companion	treatment		thrips	before	after (corrected for time on leaf disc)	companion	treatment
1	1	1	1	Natural pheromone		1	1	0	1	Natural pheromone
2	5	0	1	Natural pheromone		2	5	2.307692308	1	Natural pheromone
3	2	0	1	Natural pheromone		3	1	0	1	Natural pheromone
4	0	0	1	Natural pheromone		4	2	0	1	Natural pheromone
5	1	0	1	Natural pheromone		5	2	2.666666667	1	Natural pheromone
6	7	5.901639344	1	Natural pheromone		6	3	2.696629213	1	Natural pheromone
7	1	3	1	Natural pheromone		7	2	5.274725275	1	Natural pheromone
8	0	0	1	Natural pheromone		8	0	0	1	Natural pheromone
9	2	1	1	Natural pheromone		9	2	0	1	Natural pheromone
10	0	0	1	Natural pheromone		10	3	4	1	Natural pheromone
11	0	4	1	Natural pheromone		11	3	0	1	Natural pheromone
12	0	0	1	Natural pheromone		12	2	0	1	Natural pheromone
13	1	0	1	Natural pheromone		13	1	0	1	Natural pheromone
14	0	0	1	Natural pheromone		14	0	1	1	Natural pheromone
15	0	0	1	Natural pheromone		15	0	0	1	Natural pheromone
16	2	1	1	Natural pheromone		16	1	1	1	Natural pheromone
17	1	1	1	Natural pheromone		17	0	0	1	Natural pheromone
18	0	1	1	Natural pheromone		18	0	0	1	Natural pheromone
19	0	1	1	Natural pheromone		19	0	2	1	Natural pheromone
20	0	1	1	Natural pheromone		20	0	0	1	Natural pheromone
21	0	0	1	Natural pheromone		21	1	6.79245283	1	Natural pheromone
22	0	1	1	Natural pheromone		22	1	5.274725275	1	Natural pheromone
23	0	2	1	Natural pheromone		23	0	0	1	Natural pheromone
24	0	2	1	Natural pheromone		24	0	0	1	Natural pheromone
25	0	0	1	Natural pheromone		25	0	0	1	Natural pheromone
26	0	4	1	Synthetic pheromone		26	0	3.333333333	1	Synthetic pheromone
27	0	4.736842105	1	Synthetic pheromone		27	0	3.243243243	1	Synthetic pheromone
28	0	4	1	Synthetic pheromone		28	0	3.870967742	1	Synthetic pheromone
29	0	2	1	Synthetic pheromone		29	0	4.8	1	Synthetic pheromone
30	0	1	1	Synthetic pheromone		30	0	2	1	Synthetic pheromone
31	0	1	1	Synthetic pheromone		31	3	2	1	Synthetic pheromone
32	1	4	1	Synthetic pheromone		32	1	2.857142857	1	Synthetic pheromone
33	0	1	1	Synthetic pheromone		33	1	2	1	Synthetic pheromone
34	0	10.90909091	1	Synthetic pheromone		34	3	5	1	Synthetic pheromone
35	1	4	1	Synthetic pheromone		35	0	1	1	Synthetic pheromone
36	0	6.101694915	1	Synthetic pheromone		36	0	5	1	Synthetic pheromone
37	0	0	1	Synthetic pheromone		37	2	3.157894737	1	Synthetic pheromone
38	0	1	1	Synthetic pheromone		38	0	3	1	Synthetic pheromone
39	0	4	1	Synthetic pheromone		39	2	2	1	Synthetic pheromone
40	2	1	1	Synthetic pheromone		40	0	2	1	Synthetic pheromone
41	4	5	1	Synthetic pheromone		41	0	11.80327869	1	Synthetic pheromone
42	0	1	1	Synthetic pheromone		42	0	1	1	Synthetic pheromone
43	1	5.217391304	1	Synthetic pheromone		43	0	5.357142857	1	Synthetic pheromone
44	1	4	1	Synthetic pheromone		44	1	0	1	Synthetic pheromone
45	0	2	1	Synthetic pheromone		45	1	7.058823529	1	Synthetic pheromone
46	0	2	1	Synthetic pheromone		46	0	24	1	Synthetic pheromone
47	0	3	1	Synthetic pheromone		47	0	1	1	Synthetic pheromone
48	2	0	1	Synthetic pheromone		48	0	4	1	Synthetic pheromone
49	0	1	1	Synthetic pheromone		49	0	5.901639344	1	Synthetic pheromone
50	0	3	1	Synthetic pheromone		50	1	3	1	Synthetic pheromone
51	2	2	1	Cyclohexane		51	0	3	1	Cyclohexane
52	0	3	1	Cyclohexane		52	0	0	1	Cyclohexane
53	2	1	1	Cyclohexane		53	0	1.714285714	1	Cyclohexane
54	0	1	1	Cyclohexane		54	1	0	1	Cyclohexane
55	0	1	1	Cyclohexane		55	0	0	1	Cyclohexane
56	0	2	1	Cyclohexane		56	1	2	1	Cyclohexane
57	0	0	1	Cyclohexane		57	3	1	1	Cyclohexane
58	0	3	1	Cyclohexane		58	2	2	1	Cyclohexane
59	0	0	1	Cyclohexane		59	2	1	1	Cyclohexane
60	0	3	1	Cyclohexane		60	0	2	1	Cyclohexane
61	0	0	1	Cyclohexane		61	0	3	1	Cyclohexane
62	0	0	1	Cyclohexane		62	0	8.181818182	1	Cyclohexane
63	0	0	1	Cyclohexane		63	0	0	1	Cyclohexane
64	1	2	1	Cyclohexane		64	0	1	1	Cyclohexane
65	1	3	1	Cyclohexane		65	0	1.5	1	Cyclohexane
66	5	2.580645161	1	Cyclohexane		66	1	0	1	Cyclohexane
67	2	0	1	Cyclohexane		67	1	3	1	Cyclohexane
68	0	3	1	Cyclohexane		68	0	1	1	Cyclohexane
69	0	3	1	Cyclohexane		69	0	0	1	Cyclohexane
70	0	3	1	Cyclohexane		70	1	0	1	Cyclohexane
71	0	1	1	Cyclohexane		71	0	2.5	1	Cyclohexane
72	0	3	1	Cyclohexane		72	0	1	1	Cyclohexane
73	0	2	1	Cyclohexane		73	0	1	1	Cyclohexane
74	0	2	1	Cyclohexane		74	0	1	1	Cyclohexane
75	0	1	1	Cyclohexane		75	2	1	1	Cyclohexane

76	0	3	2	Natural pheromone	76	0	3	2	Natural pheromone
77	2	15	2	Natural pheromone	77	2	0	2	Natural pheromone
78	2	1	2	Natural pheromone	78	2	20	2	Natural pheromone
79	1	0	2	Natural pheromone	79	1	0	2	Natural pheromone
80	2	6	2	Natural pheromone	80	1	0	2	Natural pheromone
81	9	1	2	Natural pheromone	81	4	0	2	Natural pheromone
82	0	0	2	Natural pheromone	82	0	0	2	Natural pheromone
83	3	6.923076923	2	Natural pheromone	83	1	1	2	Natural pheromone
84	2	6.545454545	2	Natural pheromone	84	0	0	2	Natural pheromone
85	3	6	2	Natural pheromone	85	4	3	2	Natural pheromone
86	4	4.444444444	2	Natural pheromone	86	1	8	2	Natural pheromone
87	4	0	2	Natural pheromone	87	2	0	2	Natural pheromone
88	1	5	2	Natural pheromone	88	0	4.705882353	2	Natural pheromone
89	0	2	2	Natural pheromone	89	4	2	2	Natural pheromone
90	0	7.567567568	2	Natural pheromone	90	0	0	2	Natural pheromone
91	0	0	2	Natural pheromone	91	0	0	2	Natural pheromone
92	0	2	2	Natural pheromone	92	0	0	2	Natural pheromone
93	0	2	2	Natural pheromone	93	0	1.538461538	2	Natural pheromone
94	0	2	2	Natural pheromone	94	0	15	2	Natural pheromone
95	1	3	2	Natural pheromone	95	1	0	2	Natural pheromone
96	0	1	2	Natural pheromone	96	0	0	2	Natural pheromone
97	0	0	2	Natural pheromone	97	1	9.6	2	Natural pheromone
98	0	5	2	Natural pheromone	98	0	1	2	Natural pheromone
99	0	0	2	Natural pheromone	99	0	7	2	Natural pheromone
100	0	1	2	Natural pheromone	100	0	0	2	Natural pheromone
101	3	2	2	Synthetic pheromone	101	0	0	2	Synthetic pheromone
102	1	5.217391304	2	Synthetic pheromone	102	0	3	2	Synthetic pheromone
103	0	2	2	Synthetic pheromone	103	4	2	2	Synthetic pheromone
104	0	6	2	Synthetic pheromone	104	0	3	2	Synthetic pheromone
105	0	2	2	Synthetic pheromone	105	2	3	2	Synthetic pheromone
106	0	2	2	Synthetic pheromone	106	2	0	2	Synthetic pheromone
107	0	0	2	Synthetic pheromone	107	5	3	2	Synthetic pheromone
108	1	3	2	Synthetic pheromone	108	1	3	2	Synthetic pheromone
109	0	3	2	Synthetic pheromone	109	1	7.5	2	Synthetic pheromone
110	0	3	2	Synthetic pheromone	110	5	2	2	Synthetic pheromone
111	0	12.85714286	2	Synthetic pheromone	111	0	5	2	Synthetic pheromone
112	1	1	2	Synthetic pheromone	112	3	2	2	Synthetic pheromone
113	0	1	2	Synthetic pheromone	113	0	10	2	Synthetic pheromone
114	4	7	2	Synthetic pheromone	114	0	8	2	Synthetic pheromone
115	0	2	2	Synthetic pheromone	115	0	7	2	Synthetic pheromone
116	0	1	2	Synthetic pheromone	116	0	1	2	Synthetic pheromone
117	0	0	2	Synthetic pheromone	117	1	4	2	Synthetic pheromone
118	1	6	2	Synthetic pheromone	118	1	1	2	Synthetic pheromone
119	0	0	2	Synthetic pheromone	119	0	1	2	Synthetic pheromone
120	0	0	2	Synthetic pheromone	120	2	2	2	Synthetic pheromone
121	0	1	2	Synthetic pheromone	121	1	4.615384615	2	Synthetic pheromone
122	1	2	2	Synthetic pheromone	122	0	1	2	Synthetic pheromone
123	0	0	2	Synthetic pheromone	123	1	2.448979592	2	Synthetic pheromone
124	0	2	2	Synthetic pheromone	124	0	0	2	Synthetic pheromone
125	1	0	2	Synthetic pheromone	125	0	1	2	Synthetic pheromone
126	0	0	2	Cyclohexane	126	0	0	2	Cyclohexane
127	0	0	2	Cyclohexane	127	0	5.454545455	2	Cyclohexane
128	1	1	2	Cyclohexane	128	0	0	2	Cyclohexane
129	0	2	2	Cyclohexane	129	0	0	2	Cyclohexane
130	0	1	2	Cyclohexane	130	3	0	2	Cyclohexane
131	0	0	2	Cyclohexane	131	0	0	2	Cyclohexane
132	0	6	2	Cyclohexane	132	0	3	2	Cyclohexane
133	1	1	2	Cyclohexane	133	0	0	2	Cyclohexane
134	2	1	2	Cyclohexane	134	0	0	2	Cyclohexane
135	0	1	2	Cyclohexane	135	0	1	2	Cyclohexane
136	0	1	2	Cyclohexane	136	1	2.857142857	2	Cyclohexane
137	0	0	2	Cyclohexane	137	0	1	2	Cyclohexane
138	0	0	2	Cyclohexane	138	0	2.448979592	2	Cyclohexane
139	0	0	2	Cyclohexane	139	0	5.901639344	2	Cyclohexane
140	0	2	2	Cyclohexane	140	0	1	2	Cyclohexane
141	1	4	2	Cyclohexane	141	1	7.5	2	Cyclohexane
142	0	0	2	Cyclohexane	142	0	0	2	Cyclohexane
143	0	5.454545455	2	Cyclohexane	143	0	1	2	Cyclohexane
144	0	0	2	Cyclohexane	144	3	3	2	Cyclohexane
145	0	0	2	Cyclohexane	145	0	3	2	Cyclohexane
146	0	2	2	Cyclohexane	146	0	2.448979592	2	Cyclohexane
147	4	2	2	Cyclohexane	147	0	2	2	Cyclohexane
148	3	5.172413793	2	Cyclohexane	148	0	2	2	Cyclohexane
149	0	1	2	Cyclohexane	149	1	3	2	Cyclohexane
150	0	4	2	Cyclohexane	150	1	2	2	Cyclohexane

Effect of amount and ratio

droplets																		
	focal larva: first-instar										focal larva: second-instar							
thrips	before	after (corrected for time on leaf disc)	treatment							thrips	before	after (corrected for time on leaf disc)	treatment					
1	1	0	Amount: second Ratio: second							1	1	0	Amount: second Ratio: second					
2	1	0	Amount: second Ratio: second							2	1	0	Amount: second Ratio: second					
3	1	0	Amount: second Ratio: second							3	1	0	Amount: second Ratio: second					
4	0	15.01877347	Amount: second Ratio: second							4	0	0	Amount: second Ratio: second					
5	1	0	Amount: second Ratio: second							5	1	5.82807188	Amount: second Ratio: second					
6	0	0	Amount: second Ratio: second							6	0	1	Amount: second Ratio: second					
7	0	5.749880211	Amount: second Ratio: second							7	0	0	Amount: second Ratio: second					
8	1	0	Amount: second Ratio: second							8	0	1.951219512	Amount: second Ratio: second					
9	0	3.927986907	Amount: second Ratio: second							9	0	0	Amount: second Ratio: second					
10	1	0	Amount: second Ratio: second							10	0	0	Amount: second Ratio: second					
11	0	0	Amount: second Ratio: second							11	0	0	Amount: second Ratio: second					
12	0	0	Amount: second Ratio: second							12	1	0	Amount: second Ratio: second					
13	0	0	Amount: second Ratio: second							13	0	0	Amount: second Ratio: second					
14	1	3.921568627	Amount: second Ratio: second							14	0	1	Amount: second Ratio: second					
15	0	0	Amount: second Ratio: second							15	0	0	Amount: second Ratio: second					
16	0	0	Amount: second Ratio: second							16	0	1	Amount: second Ratio: second					
17	1	3.589590188	Amount: second Ratio: second							17	0	1	Amount: second Ratio: second					
18	1	0	Amount: second Ratio: second							18	1	0	Amount: second Ratio: second					
19	0	0	Amount: second Ratio: second							19	0	0	Amount: second Ratio: second					
20	0	0	Amount: second Ratio: second							20	0	0	Amount: second Ratio: second					
21	1	1.754385965	Amount: second Ratio: second							21	0	0	Amount: second Ratio: second					
22	1	0	Amount: second Ratio: second							22	1	0	Amount: second Ratio: second					
23	1	0	Amount: second Ratio: second							23	0	0	Amount: second Ratio: second					
24	0	0	Amount: second Ratio: second							24	0	0	Amount: second Ratio: second					
25	0	0	Amount: second Ratio: second							25	0	0	Amount: second Ratio: first					
26	0	0	Amount: second Ratio: first							26	0	0	Amount: second Ratio: first					
27	1	0	Amount: second Ratio: first							27	0	1	Amount: second Ratio: first					
28	1	0	Amount: second Ratio: first							28	0	0	Amount: second Ratio: first					
29	0	0	Amount: second Ratio: first							29	0	0	Amount: second Ratio: first					
30	1	0	Amount: second Ratio: first							30	0	0	Amount: second Ratio: first					
31	0	0	Amount: second Ratio: first							31	1	0	Amount: second Ratio: first					
32	0	0	Amount: second Ratio: first							32	0	0	Amount: second Ratio: first					
33	1	1.870615744	Amount: second Ratio: first							33	1	0	Amount: second Ratio: first					
34	0	0	Amount: second Ratio: first							34	0	0	Amount: second Ratio: first					
35	1	5.484460695	Amount: second Ratio: first							35	1	0	Amount: second Ratio: first					
36	0	0	Amount: second Ratio: first							36	0	0	Amount: second Ratio: first					
37	1	0	Amount: second Ratio: first							37	0	0	Amount: second Ratio: first					
38	0	0	Amount: second Ratio: first							38	0	0	Amount: second Ratio: first					
39	0	0	Amount: second Ratio: first							39	0	0	Amount: second Ratio: first					
40	0	0	Amount: second Ratio: first							40	0	3.977461054	Amount: second Ratio: first					
41	1	0	Amount: second Ratio: first							41	0	0	Amount: second Ratio: first					
42	1	0	Amount: second Ratio: first							42	0	0	Amount: second Ratio: first					
43	0	0	Amount: second Ratio: first							43	0	7.566204288	Amount: second Ratio: first					
44	1	0	Amount: second Ratio: first							44	0	0	Amount: second Ratio: first					
45	0	7.599746675	Amount: second Ratio: first							45	0	0	Amount: second Ratio: first					
46	0	0	Amount: second Ratio: first							46	0	0	Amount: second Ratio: first					
47	0	0	Amount: second Ratio: first							47	0	0	Amount: second Ratio: first					
48	0	0	Amount: second Ratio: first							48	0	0	Amount: second Ratio: first					
49	1	0	Amount: second Ratio: first							49	1	0	Amount: second Ratio: first					
50	1	0	Amount: second Ratio: first							50	0	0	Amount:first Ratio: second					
51	1	0	Amount:first Ratio: second							51	0	0	Amount:first Ratio: second					
52	0	0	Amount:first Ratio: second							52	0	1	Amount:first Ratio: second					
53	0	0	Amount:first Ratio: second							53	0	0	Amount:first Ratio: second					
54	0	0	Amount:first Ratio: second							54	0	0	Amount:first Ratio: second					
55	0	0	Amount:first Ratio: second							55	0	0	Amount:first Ratio: second					
56	0	0	Amount:first Ratio: second							56	0	0	Amount:first Ratio: second					
57	0	0	Amount:first Ratio: second							57	1	1	Amount:first Ratio: second					
58	0	0	Amount:first Ratio: second							58	1	0	Amount:first Ratio: second					
59	1	0	Amount:first Ratio: second							59	0	0	Amount:first Ratio: second					
60	0	0	Amount:first Ratio: second							60	0	0	Amount:first Ratio: second					

61	0	0	Amount:first Ratio: second			61	0	0	Amount:first Ratio: second		
62	0	0	Amount:first Ratio: second			62	0	0	Amount:first Ratio: second		
63	0	0	Amount:first Ratio: second			63	0	1	Amount:first Ratio: second		
64	0	3.167898627	Amount:first Ratio: second			64	0	0	Amount:first Ratio: second		
65	1	0	Amount:first Ratio: second			65	1	0	Amount:first Ratio: second		
66	0	0	Amount:first Ratio: second			66	0	0	Amount:first Ratio: second		
67	0	0	Amount:first Ratio: second			67	0	0	Amount:first Ratio: second		
68	0	0	Amount:first Ratio: second			68	0	0	Amount:first Ratio: second		
69	0	0	Amount:first Ratio: second			69	0	0	Amount:first Ratio: second		
70	1	2.035278155	Amount:first Ratio: second			70	0	0	Amount:first Ratio: second		
71	1	0	Amount:first Ratio: second			71	0	21.62162162	Amount:first Ratio: second		
72	1	0	Amount:first Ratio: second			72	0	1	Amount:first Ratio: second		
73	1	0	Amount:first Ratio: second			73	0	0	Amount:first Ratio: second		
74	1	0	Amount:first Ratio: second			74	0	1	Amount:first Ratio: second		
75	0	14.49275362	Amount:first Ratio: second			75	0	1	Amount:first Ratio: second		
76	1	3.220611916	Amount:first Ratio: second			76	0	0	cyclohexane		
77	0	0	Amount: first Ratio: first			77	1	0	Amount: first Ratio: first		
78	1	0	Amount: first Ratio: first			78	0	1	Amount: first Ratio: first		
79	1	0	Amount: first Ratio: first			79	0	0	Amount: first Ratio: first		
80	1	0	Amount: first Ratio: first			80	0	0	Amount: first Ratio: first		
81	0	8.30449827	Amount: first Ratio: first			81	0	0	Amount: first Ratio: first		
82	0	0	Amount: first Ratio: first			82	1	0	Amount: first Ratio: first		
83	0	0	Amount: first Ratio: first			83	1	0	Amount: first Ratio: first		
84	0	0	Amount: first Ratio: first			84	0	0	Amount: first Ratio: first		
85	0	0	Amount: first Ratio: first			85	0	0	Amount: first Ratio: first		
86	0	0	Amount: first Ratio: first			86	0	0	Amount: first Ratio: first		
87	0	0	Amount: first Ratio: first			87	0	0	Amount: first Ratio: first		
88	0	0	Amount: first Ratio: first			88	0	0	Amount: first Ratio: first		
89	0	1.316655695	Amount: first Ratio: first			89	1	0	Amount: first Ratio: first		
90	0	0	Amount: first Ratio: first			90	0	0	Amount: first Ratio: first		
91	0	0	Amount: first Ratio: first			91	0	1	Amount: first Ratio: first		
92	0	0	Amount: first Ratio: first			92	0	0	Amount: first Ratio: first		
93	0	0	Amount: first Ratio: first			93	0	1	Amount: first Ratio: first		
94	1	1.048309601	Amount: first Ratio: first			94	0	0	Amount: first Ratio: first		
95	0	0	Amount: first Ratio: first			95	0	0	Amount: first Ratio: first		
96	0	0	Amount: first Ratio: first			96	0	0	Amount: first Ratio: first		
97	0	0	Amount: first Ratio: first			97	1	1.31061599	Amount: first Ratio: first		
98	1	0	Amount: first Ratio: first			98	1	0	Amount: first Ratio: first		
99	0	0	Amount: first Ratio: first			99	0	1	Amount: first Ratio: first		
100	0	0	Amount: first Ratio: first			100	1	0	Amount: first Ratio: first		
101	0	0	Amount: first Ratio: first			101	0	1	cyclohexane		
102	0	0	cyclohexane			102	0	4.484304933	cyclohexane		
103	0	0	cyclohexane			103	1	0	cyclohexane		
104	0	0	cyclohexane			104	0	0	cyclohexane		
105	0	0	cyclohexane			105	0	1.310759148	cyclohexane		
106	0	0	cyclohexane			106	0	0	cyclohexane		
107	1	0	cyclohexane			107	0	0	cyclohexane		
108	0	0	cyclohexane			108	0	1	cyclohexane		
109	0	0	cyclohexane			109	0	0	cyclohexane		
110	0	2.394253791	cyclohexane			110	0	0	cyclohexane		
111	1	0	cyclohexane			111	1	1	cyclohexane		
112	0	0	cyclohexane			112	0	0	cyclohexane		
113	1	6.546644845	cyclohexane			113	0	0	cyclohexane		
114	1	0	cyclohexane			114	0	0	cyclohexane		
115	0	0	cyclohexane			115	0	9.411764706	cyclohexane		
116	1	3.956478734	cyclohexane			116	0	6.116207951	cyclohexane		
117	0	0	cyclohexane			117	0	1	cyclohexane		
118	0	0	cyclohexane			118	1	0	cyclohexane		
119	0	0	cyclohexane			119	1	0	cyclohexane		
120	0	0	cyclohexane			120	0	1	cyclohexane		
121	0	0	cyclohexane			121	0	0	cyclohexane		
122	0	0	cyclohexane			122	0	0	cyclohexane		
123	1	1.014713343	cyclohexane			123	0	0	cyclohexane		
124	0	2.536997886	cyclohexane			124	0	0	cyclohexane		
125	0	1.312192455	cyclohexane			125	0	0	cyclohexane		
126	0	0	cyclohexane								

swings												
	focal larva: first-instar								focal larva: second-instar			
thrips	before	after (corrected for time on leaf disc)	treatment				thrips	before	after (corrected for time on leaf disc)	treatment		
1	2	0	Amount: second Ratio: second				1	0	0	Amount: second Ratio: second		
2	0	2	Amount: second Ratio: second				2	0	0	Amount: second Ratio: second		
3	0	0	Amount: second Ratio: second				3	1	0	Amount: second Ratio: second		
4	0	0	Amount: second Ratio: second				4	0	0	Amount: second Ratio: second		
5	1	1	Amount: second Ratio: second				5	0	0	Amount: second Ratio: second		
6	0	1	Amount: second Ratio: second				6	0	2	Amount: second Ratio: second		
7	0	0	Amount: second Ratio: second				7	0	0	Amount: second Ratio: second		
8	1	0	Amount: second Ratio: second				8	1	1.951219512	Amount: second Ratio: second		
9	0	0	Amount: second Ratio: second				9	1	1	Amount: second Ratio: second		
10	1	0	Amount: second Ratio: second				10	0	0	Amount: second Ratio: second		
11	0	0	Amount: second Ratio: second				11	1	0	Amount: second Ratio: second		
12	0	0	Amount: second Ratio: second				12	0	0	Amount: second Ratio: second		
13	0	0	Amount: second Ratio: second				13	0	0	Amount: second Ratio: second		
14	1	3.921568627	Amount: second Ratio: second				14	0	0	Amount: second Ratio: second		
15	0	0	Amount: second Ratio: second				15	0	0	Amount: second Ratio: second		
16	1	0	Amount: second Ratio: second				16	0	4	Amount: second Ratio: second		
17	1	0	Amount: second Ratio: second				17	0	0	Amount: second Ratio: second		
18	0	2	Amount: second Ratio: second				18	0	2	Amount: second Ratio: second		
19	0	0	Amount: second Ratio: second				19	0	0	Amount: second Ratio: second		
20	0	4	Amount: second Ratio: second				20	2	0	Amount: second Ratio: second		
21	2	1.754385965	Amount: second Ratio: second				21	2	0	Amount: second Ratio: second		
22	0	1	Amount: second Ratio: second				22	0	3	Amount: second Ratio: second		
23	1	0	Amount: second Ratio: second				23	0	0	Amount: second Ratio: second		
24	0	0	Amount: second Ratio: second				24	0	0	Amount: second Ratio: second		
25	0	0	Amount: second Ratio: second				25	0	0	Amount: second Ratio: first		
26	0	1	Amount: second Ratio: first				26	2	1	Amount: second Ratio: first		
27	0	0	Amount: second Ratio: first				27	0	4	Amount: second Ratio: first		
28	0	1	Amount: second Ratio: first				28	1	2	Amount: second Ratio: first		
29	0	0	Amount: second Ratio: first				29	2	4	Amount: second Ratio: first		
30	0	0	Amount: second Ratio: first				30	0	1	Amount: second Ratio: first		
31	0	1	Amount: second Ratio: first				31	0	0	Amount: second Ratio: first		
32	0	0	Amount: second Ratio: first				32	1	0	Amount: second Ratio: first		
33	0	0	Amount: second Ratio: first				33	0	0	Amount: second Ratio: first		
34	0	0	Amount: second Ratio: first				34	2	5.73431029	Amount: second Ratio: first		
35	1	0	Amount: second Ratio: first				35	1	0	Amount: second Ratio: first		
36	0	0	Amount: second Ratio: first				36	2	0	Amount: second Ratio: first		
37	1	1	Amount: second Ratio: first				37	3	0	Amount: second Ratio: first		
38	0	0	Amount: second Ratio: first				38	2	1	Amount: second Ratio: first		
39	0	0	Amount: second Ratio: first				39	0	0	Amount: second Ratio: first		
40	0	1	Amount: second Ratio: first				40	2	7.954922108	Amount: second Ratio: first		
41	4	1	Amount: second Ratio: first				41	0	0	Amount: second Ratio: first		
42	0	1	Amount: second Ratio: first				42	1	3	Amount: second Ratio: first		
43	0	0	Amount: second Ratio: first				43	1	7.566204288	Amount: second Ratio: first		
44	1	4	Amount: second Ratio: first				44	0	1.785714286	Amount: second Ratio: first		
45	2	0	Amount: second Ratio: first				45	0	2	Amount: second Ratio: first		
46	1	2	Amount: second Ratio: first				46	1	2.368732728	Amount: second Ratio: first		
47	0	0	Amount: second Ratio: first				47	0	0	Amount: second Ratio: first		
48	0	0	Amount: second Ratio: first				48	0	0	Amount: second Ratio: first		
49	1	0	Amount: second Ratio: first				49	1	0	Amount: second Ratio: first		
50	0	1	Amount: second Ratio: first				50	0	3	Amount:first Ratio: second		
51	0	0	Amount:first Ratio: second				51	0	0	Amount:first Ratio: second		
52	0	0	Amount:first Ratio: second				52	0	1	Amount:first Ratio: second		
53	0	0	Amount:first Ratio: second				53	0	0	Amount:first Ratio: second		
54	0	2	Amount:first Ratio: second				54	0	0	Amount:first Ratio: second		
55	0	0	Amount:first Ratio: second				55	0	9.309542281	Amount:first Ratio: second		
56	0	3	Amount:first Ratio: second				56	0	0	Amount:first Ratio: second		
57	0	0	Amount:first Ratio: second				57	0	0	Amount:first Ratio: second		
58	0	1	Amount:first Ratio: second				58	1	0	Amount:first Ratio: second		
59	1	1	Amount:first Ratio: second				59	0	0	Amount:first Ratio: second		
60	0	4	Amount:first Ratio: second				60	0	2	Amount:first Ratio: second		

61	0	1	Amount:first Ratio: second			61	1	7.271258332	Amount:first Ratio: second		
62	0	4	Amount:first Ratio: second			62	3	3	Amount:first Ratio: second		
63	0	0	Amount:first Ratio: second			63	0	4	Amount:first Ratio: second		
64	0	0	Amount:first Ratio: second			64	0	0	Amount:first Ratio: second		
65	0	0	Amount:first Ratio: second			65	1	2.180232558	Amount:first Ratio: second		
66	1	1	Amount:first Ratio: second			66	1	4.840661557	Amount:first Ratio: second		
67	0	0	Amount:first Ratio: second			67	1	0	Amount:first Ratio: second		
68	0	0	Amount:first Ratio: second			68	0	0	Amount:first Ratio: second		
69	0	0	Amount:first Ratio: second			69	0	0	Amount:first Ratio: second		
70	0	0	Amount:first Ratio: second			70	0	1	Amount:first Ratio: second		
71	0	1	Amount:first Ratio: second			71	1	0	Amount:first Ratio: second		
72	0	2	Amount:first Ratio: second			72	0	0	Amount:first Ratio: second		
73	1	0	Amount:first Ratio: second			73	2	0	Amount:first Ratio: second		
74	1	1	Amount:first Ratio: second			74	0	1	Amount:first Ratio: second		
75	0	0	Amount:first Ratio: second			75	0	2	Amount:first Ratio: second		
76	0	3.220611916	Amount:first Ratio: second			76	0	0	cyclohexane		
77	0	4	Amount: first Ratio: first			77	0	0	Amount: first Ratio: first		
78	0	2	Amount: first Ratio: first			78	1	0	Amount: first Ratio: first		
79	0	0	Amount: first Ratio: first			79	0	0	Amount: first Ratio: first		
80	0	0	Amount: first Ratio: first			80	0	2	Amount: first Ratio: first		
81	0	0	Amount: first Ratio: first			81	0	0	Amount: first Ratio: first		
82	0	0	Amount: first Ratio: first			82	0	0	Amount: first Ratio: first		
83	1	0	Amount: first Ratio: first			83	1	0	Amount: first Ratio: first		
84	0	1	Amount: first Ratio: first			84	0	0	Amount: first Ratio: first		
85	0	2	Amount: first Ratio: first			85	0	3.160944771	Amount: first Ratio: first		
86	0	0	Amount: first Ratio: first			86	0	1	Amount: first Ratio: first		
87	0	0	Amount: first Ratio: first			87	0	1	Amount: first Ratio: first		
88	1	1	Amount: first Ratio: first			88	0	0	Amount: first Ratio: first		
89	0	2.633311389	Amount: first Ratio: first			89	1	0	Amount: first Ratio: first		
90	0	0	Amount: first Ratio: first			90	0	0	Amount: first Ratio: first		
91	1	0	Amount: first Ratio: first			91	1	0	Amount: first Ratio: first		
92	0	0	Amount: first Ratio: first			92	0	0	Amount: first Ratio: first		
93	1	0	Amount: first Ratio: first			93	2	1	Amount: first Ratio: first		
94	2	0	Amount: first Ratio: first			94	0	0	Amount: first Ratio: first		
95	0	0	Amount: first Ratio: first			95	16	0	Amount: first Ratio: first		
96	0	0	Amount: first Ratio: first			96	0	19.10828025	Amount: first Ratio: first		
97	0	1	Amount: first Ratio: first			97	3	0	Amount: first Ratio: first		
98	0	2	Amount: first Ratio: first			98	0	0	Amount: first Ratio: first		
99	0	1	Amount: first Ratio: first			99	0	0	Amount: first Ratio: first		
100	0	2	Amount: first Ratio: first			100	1	0	Amount: first Ratio: first		
101	0	1	Amount: first Ratio: first			101	0	2	cyclohexane		
102	0	0	cyclohexane			102	0	0	cyclohexane		
103	0	1	cyclohexane			103	1	1	cyclohexane		
104	1	1	cyclohexane			104	7	0	cyclohexane		
105	0	2	cyclohexane			105	0	0	cyclohexane		
106	0	0	cyclohexane			106	1	1	cyclohexane		
107	0	0	cyclohexane			107	1	0	cyclohexane		
108	0	0	cyclohexane			108	1	1	cyclohexane		
109	1	2	cyclohexane			109	0	0	cyclohexane		
110	0	0	cyclohexane			110	0	0	cyclohexane		
111	1	0	cyclohexane			111	1	0	cyclohexane		
112	0	0	cyclohexane			112	0	2	cyclohexane		
113	0	0	cyclohexane			113	0	0	cyclohexane		
114	0	2	cyclohexane			114	0	0	cyclohexane		
115	0	0	cyclohexane			115	0	0	cyclohexane		
116	2	3.956478734	cyclohexane			116	0	6.116207951	cyclohexane		
117	0	0	cyclohexane			117	0	2	cyclohexane		
118	0	1	cyclohexane			118	2	1	cyclohexane		
119	0	0	cyclohexane			119	1	0	cyclohexane		
120	0	1	cyclohexane			120	0	2	cyclohexane		
121	0	1	cyclohexane			121	0	0	cyclohexane		
122	0	0	cyclohexane			122	0	0	cyclohexane		
123	1	0	cyclohexane			123	0	0	cyclohexane		
124	0	0	cyclohexane			124	0	2.941897524	cyclohexane		
125	0	0	cyclohexane			125	0	0	cyclohexane		
126	0	0	cyclohexane								

partial crossings

	focal larva: first-instar						focal larva: second-instar			
thrips	before	after (corrected for time on leaf disc)	treatment			thrips	before	after (corrected for time on leaf disc)	treatment	
1	1	0	Amount: second Ratio: second			1	2	7.606973059	Amount: second Ratio: second	
2	0	1	Amount: second Ratio: second			2	0	3.09544282	Amount: second Ratio: second	
3	0	6	Amount: second Ratio: second			3	0	18.1680545	Amount: second Ratio: second	
4	2	15.01877347	Amount: second Ratio: second			4	0	33.8028169	Amount: second Ratio: second	
5	0	8	Amount: second Ratio: second			5	4	17.48421564	Amount: second Ratio: second	
6	0	5	Amount: second Ratio: second			6	0	3	Amount: second Ratio: second	
7	0	11.49976042	Amount: second Ratio: second			7	1	2	Amount: second Ratio: second	
8	0	4	Amount: second Ratio: second			8	0	11.70731707	Amount: second Ratio: second	
9	0	11.78396072	Amount: second Ratio: second			9	0	0	Amount: second Ratio: second	
10	0	6	Amount: second Ratio: second			10	3	0	Amount: second Ratio: second	
11	0	4	Amount: second Ratio: second			11	0	5	Amount: second Ratio: second	
12	0	4	Amount: second Ratio: second			12	0	22.1266134	Amount: second Ratio: second	
13	0	2	Amount: second Ratio: second			13	1	3	Amount: second Ratio: second	
14	0	7.843137255	Amount: second Ratio: second			14	0	3	Amount: second Ratio: second	
15	0	2	Amount: second Ratio: second			15	1	4	Amount: second Ratio: second	
16	0	7	Amount: second Ratio: second			16	0	0	Amount: second Ratio: second	
17	0	14.35836075	Amount: second Ratio: second			17	0	4	Amount: second Ratio: second	
18	0	4	Amount: second Ratio: second			18	0	5	Amount: second Ratio: second	
19	0	9	Amount: second Ratio: second			19	0	2	Amount: second Ratio: second	
20	0	4	Amount: second Ratio: second			20	0	22.36719478	Amount: second Ratio: second	
21	0	8.771929825	Amount: second Ratio: second			21	0	26.1627907	Amount: second Ratio: second	
22	0	6	Amount: second Ratio: second			22	2	6	Amount: second Ratio: second	
23	3	6	Amount: second Ratio: second			23	0	8.149405772	Amount: second Ratio: second	
24	0	3	Amount: second Ratio: second			24	0	4	Amount: second Ratio: second	
25	0	5	Amount: second Ratio: second			25	2	8	Amount: second Ratio: first	
26	0	8	Amount: second Ratio: first			26	0	0	Amount: second Ratio: first	
27	0	4	Amount: second Ratio: first			27	0	0	Amount: second Ratio: first	
28	0	4	Amount: second Ratio: first			28	0	2	Amount: second Ratio: first	
29	0	6	Amount: second Ratio: first			29	0	3	Amount: second Ratio: first	
30	0	6	Amount: second Ratio: first			30	0	8	Amount: second Ratio: first	
31	5	2	Amount: second Ratio: first			31	0	17.10213777	Amount: second Ratio: first	
32	0	4	Amount: second Ratio: first			32	2	6.447568729	Amount: second Ratio: first	
33	0	9.353078722	Amount: second Ratio: first			33	2	15.55411536	Amount: second Ratio: first	
34	0	7	Amount: second Ratio: first			34	0	7.645747053	Amount: second Ratio: first	
35	0	10.96892139	Amount: second Ratio: first			35	0	13.25478645	Amount: second Ratio: first	
36	0	4	Amount: second Ratio: first			36	0	38.83495146	Amount: second Ratio: first	
37	0	4	Amount: second Ratio: first			37	0	2	Amount: second Ratio: first	
38	0	4	Amount: second Ratio: first			38	0	1	Amount: second Ratio: first	
39	0	4	Amount: second Ratio: first			39	0	14.37125749	Amount: second Ratio: first	
40	0	2	Amount: second Ratio: first			40	0	11.93238316	Amount: second Ratio: first	
41	0	11	Amount: second Ratio: first			41	0	13.78518093	Amount: second Ratio: first	
42	0	4	Amount: second Ratio: first			42	0	3	Amount: second Ratio: first	
43	0	6	Amount: second Ratio: first			43	0	22.69861286	Amount: second Ratio: first	
44	0	2	Amount: second Ratio: first			44	2	10.71428571	Amount: second Ratio: first	
45	0	22.79924003	Amount: second Ratio: first			45	1	2	Amount: second Ratio: first	
46	2	5	Amount: second Ratio: first			46	1	11.84366364	Amount: second Ratio: first	
47	0	7	Amount: second Ratio: first			47	1	11.76182308	Amount: second Ratio: first	
48	0	5	Amount: second Ratio: first			48	0	20.16806723	Amount: second Ratio: first	
49	0	0	Amount: second Ratio: first			49	0	9	Amount: second Ratio: first	
50	2	5	Amount: second Ratio: first			50	0	8	Amount:first Ratio: second	
51	0	4	Amount:first Ratio: second			51	0	0	Amount:first Ratio: second	
52	0	3	Amount:first Ratio: second			52	0	2	Amount:first Ratio: second	
53	0	4	Amount:first Ratio: second			53	0	7	Amount:first Ratio: second	
54	0	3	Amount:first Ratio: second			54	0	2	Amount:first Ratio: second	
55	0	1	Amount:first Ratio: second			55	0	18.61908456	Amount:first Ratio: second	
56	0	4	Amount:first Ratio: second			56	1	14.55721795	Amount:first Ratio: second	
57	0	1	Amount:first Ratio: second			57	0	3	Amount:first Ratio: second	
58	0	8	Amount:first Ratio: second			58	0	36.69724771	Amount:first Ratio: second	
59	0	4	Amount:first Ratio: second			59	0	6.364359586	Amount:first Ratio: second	
60	0	3	Amount:first Ratio: second			60	0	3	Amount:first Ratio: second	

61	0	4	Amount:first Ratio: second			61	0	9.695011109	Amount:first Ratio: second		
62	0	1	Amount:first Ratio: second			62	0	2	Amount:first Ratio: second		
63	0	8	Amount:first Ratio: second			63	2	4	Amount:first Ratio: second		
64	0	9.503695882	Amount:first Ratio: second			64	0	24.85758674	Amount:first Ratio: second		
65	0	9	Amount:first Ratio: second			65	0	4.360465116	Amount:first Ratio: second		
66	0	3	Amount:first Ratio: second			66	0	14.52198467	Amount:first Ratio: second		
67	0	0	Amount:first Ratio: second			67	0	3	Amount:first Ratio: second		
68	0	6	Amount:first Ratio: second			68	0	8	Amount:first Ratio: second		
69	0	0	Amount:first Ratio: second			69	4	5.667863216	Amount:first Ratio: second		
70	0	6.105834464	Amount:first Ratio: second			70	0	2	Amount:first Ratio: second		
71	1	4	Amount:first Ratio: second			71	1	21.62162162	Amount:first Ratio: second		
72	0	5	Amount:first Ratio: second			72	0	5	Amount:first Ratio: second		
73	0	6	Amount:first Ratio: second			73	2	40.6779661	Amount:first Ratio: second		
74	0	7	Amount:first Ratio: second			74	0	8	Amount:first Ratio: second		
75	0	28.98550725	Amount:first Ratio: second			75	0	3	Amount:first Ratio: second		
76	0	6.441223833	Amount:first Ratio: second			76	0	22.03856749	cyclohexane		
77	0	1	Amount: first Ratio: first			77	0	33.8028169	Amount: first Ratio: first		
78	0	1	Amount: first Ratio: first			78	0	5	Amount: first Ratio: first		
79	0	2	Amount: first Ratio: first			79	1	1	Amount: first Ratio: first		
80	0	3	Amount: first Ratio: first			80	0	3	Amount: first Ratio: first		
81	1	16.60899654	Amount: first Ratio: first			81	0	15.92568016	Amount: first Ratio: first		
82	0	3	Amount: first Ratio: first			82	0	3	Amount: first Ratio: first		
83	0	5	Amount: first Ratio: first			83	1	15.96806387	Amount: first Ratio: first		
84	0	4	Amount: first Ratio: first			84	0	6	Amount: first Ratio: first		
85	0	2	Amount: first Ratio: first			85	0	4.214593028	Amount: first Ratio: first		
86	1	7	Amount: first Ratio: first			86	0	3	Amount: first Ratio: first		
87	1	2	Amount: first Ratio: first			87	0	6	Amount: first Ratio: first		
88	0	3	Amount: first Ratio: first			88	0	19.36264623	Amount: first Ratio: first		
89	0	7.899934167	Amount: first Ratio: first			89	0	1	Amount: first Ratio: first		
90	4	5	Amount: first Ratio: first			90	0	4	Amount: first Ratio: first		
91	0	4	Amount: first Ratio: first			91	0	5	Amount: first Ratio: first		
92	0	0	Amount: first Ratio: first			92	0	0	Amount: first Ratio: first		
93	0	2	Amount: first Ratio: first			93	0	5	Amount: first Ratio: first		
94	0	5.241548004	Amount: first Ratio: first			94	0	15.49053356	Amount: first Ratio: first		
95	0	6	Amount: first Ratio: first			95	0	3	Amount: first Ratio: first		
96	0	4	Amount: first Ratio: first			96	0	38.21656051	Amount: first Ratio: first		
97	0	7	Amount: first Ratio: first			97	2	7.863695937	Amount: first Ratio: first		
98	0	5	Amount: first Ratio: first			98	0	7	Amount: first Ratio: first		
99	1	4	Amount: first Ratio: first			99	2	4	Amount: first Ratio: first		
100	0	7	Amount: first Ratio: first			100	7	5	Amount: first Ratio: first		
101	0	0	Amount: first Ratio: first			101	0	2	cyclohexane		
102	0	2	cyclohexane			102	0	17.93721973	cyclohexane		
103	0	2	cyclohexane			103	0	4	cyclohexane		
104	0	3	cyclohexane			104	1	0	cyclohexane		
105	0	3	cyclohexane			105	0	6.55379574	cyclohexane		
106	0	1	cyclohexane			106	0	1	cyclohexane		
107	0	0	cyclohexane			107	0	6.87994496	cyclohexane		
108	0	4	cyclohexane			108	2	1	cyclohexane		
109	0	9	cyclohexane			109	3	8	cyclohexane		
110	0	11.97126895	cyclohexane			110	0	6	cyclohexane		
111	1	2	cyclohexane			111	0	3	cyclohexane		
112	1	1	cyclohexane			112	0	0	cyclohexane		
113	0	13.09328969	cyclohexane			113	0	10.54945055	cyclohexane		
114	6	1	cyclohexane			114	0	35.82089552	cyclohexane		
115	0	5	cyclohexane			115	0	18.82352941	cyclohexane		
116	0	7.912957468	cyclohexane			116	3	24.4648318	cyclohexane		
117	6	6	cyclohexane			117	0	1	cyclohexane		
118	0	4	cyclohexane			118	0	7	cyclohexane		
119	0	6	cyclohexane			119	1	0	cyclohexane		
120	0	11	cyclohexane			120	0	6	cyclohexane		
121	0	4	cyclohexane			121	0	2	cyclohexane		
122	0	4	cyclohexane			122	0	9.484666456	cyclohexane		
123	0	7.102993404	cyclohexane			123	0	6.213860361	cyclohexane		
124	0	10.14799154	cyclohexane			124	1	5.883795048	cyclohexane		
125	1	7.873154729	cyclohexane			125	1	2	cyclohexane		
126	1	2	cyclohexane								

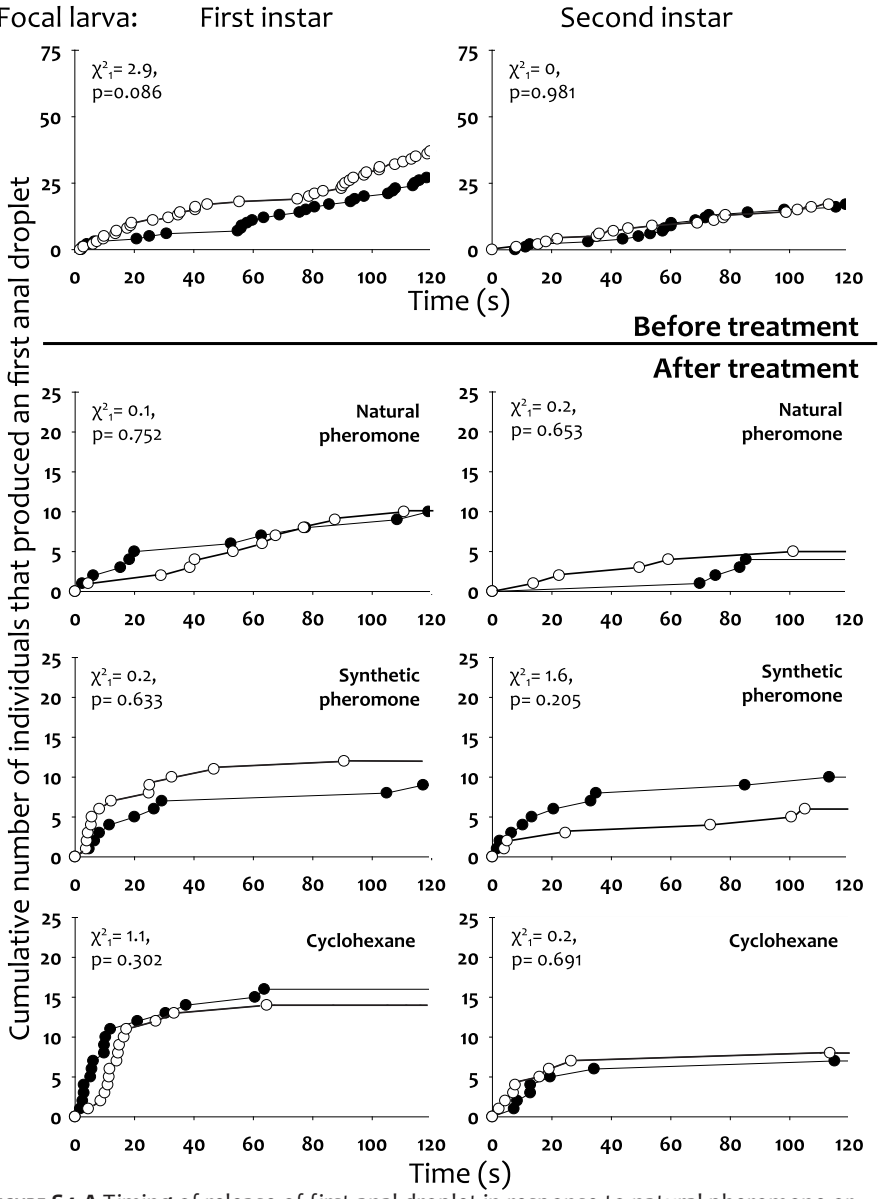


FIGURE S4-A Timing of release of first anal droplet in response to natural pheromone or control. Shown are the times (s) until first droplet was released before treatments (pooled for all treatments, N = 75) and after treatments (release of natural pheromone, synthetic pheromone or cyclohexane, N = 25 each). Focal larvae are either first-instar (upper graphs) or second-instar (lower graphs). Companion larvae were either first-instar (black circles) or second-instar (white circles). No significant differences were found.

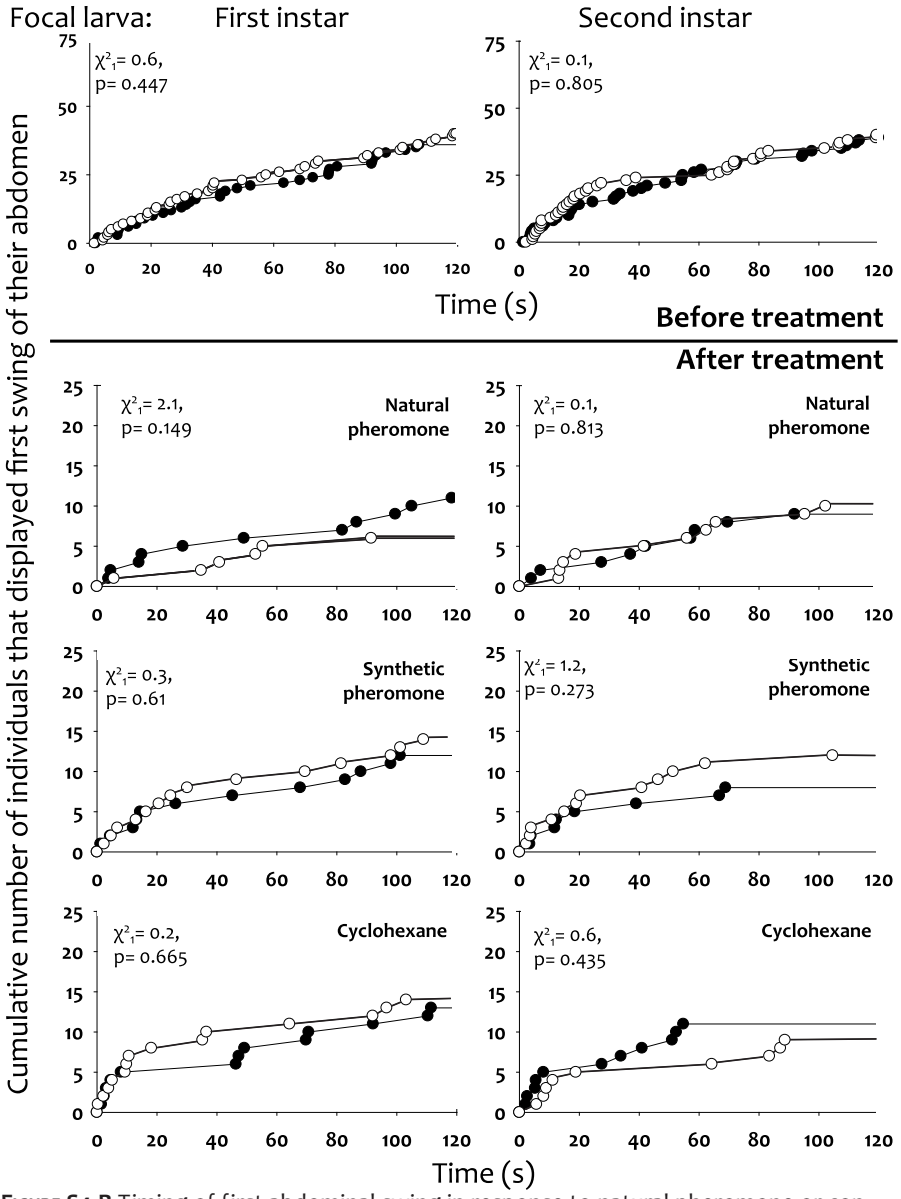


FIGURE S4-B Timing of first abdominal swing in response to natural pheromone or control. Shown are the times (s) until first swing before treatments (pooled for all treatments, N = 75) and after treatments (release of natural pheromone, synthetic pheromone or cyclohexane, N = 25 each). Focal larvae were either first-instar (upper graphs) or second-instar (lower graphs). Companion larvae were either first-instar (black circles) or second-instar (white circles). No significant differences were found.

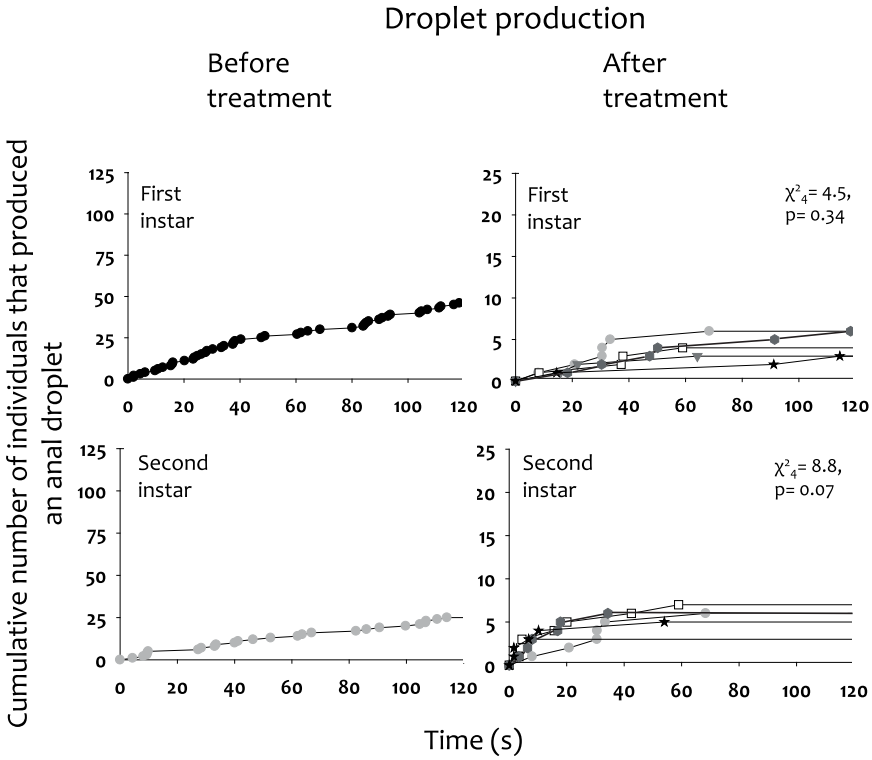


FIGURE S4-C Timing of release of first droplet. Shown are the time (s) until first droplet before treatment (pooled for all treatments, N = 125) and after treatment [blend of synthetic pheromone with amount and ratio of second-instar larvae (gray circles), amount of second-instar larvae and ratio of first-instar larvae (gray triangles), amount of first-instar larvae and ratio of second-instar larvae (white squares), amount and ratio of first-instar larvae (black stars) or cyclohexane as the solvent control (gray hexagons), N = 25 each]. Focal larvae are either first instar (upper graphs) or second instar (lower graphs). No significant differences were found.

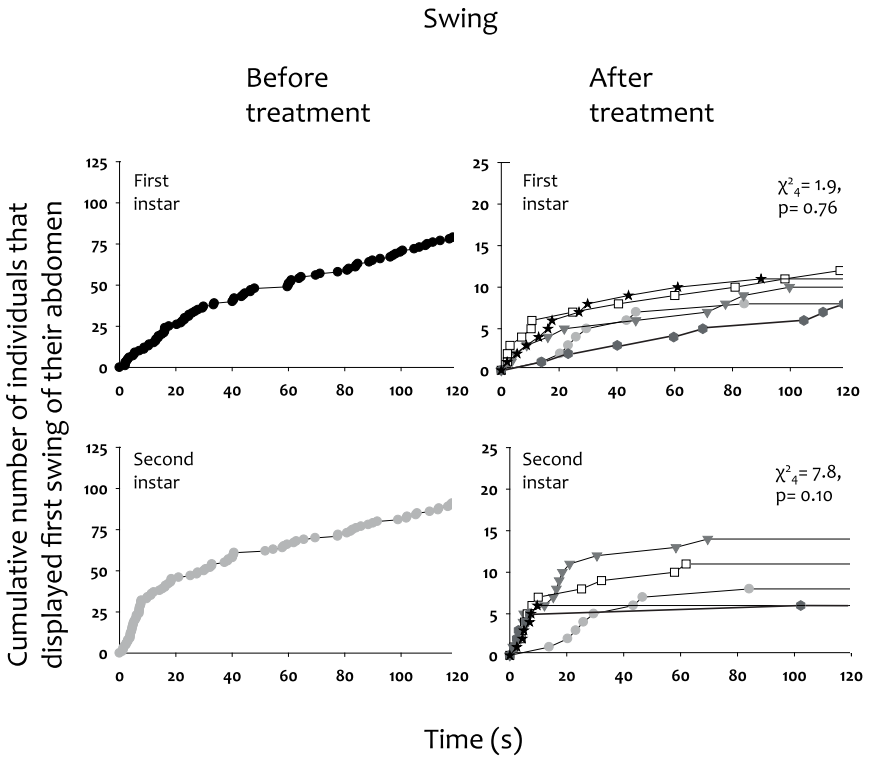


FIGURE S4-D Timing of first swing. Shown are the times (s) until first swings before treatment (pooled for all treatments, N = 125) and after treatment [blend of synthetic pheromone with amount and ratio of second-instar larvae (gray circles), amount of second-instar larvae and ratio of first-instar larvae (gray triangles), amount of first-instar larvae and ratio of second-instar larvae (white squares), amount and ratio of first-instar larvae (black stars) or cyclohexane as the solvent control (gray hexagons), N = 25 each]. Focal larvae are either first instar (upper graphs) or second instar (lower graphs). No significant differences were found.

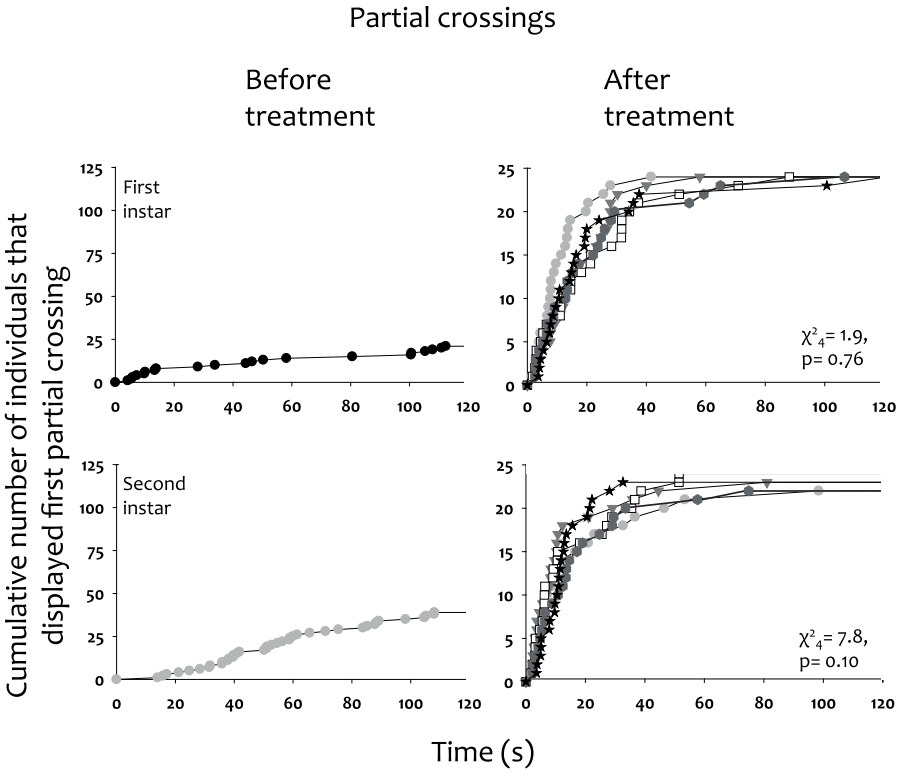
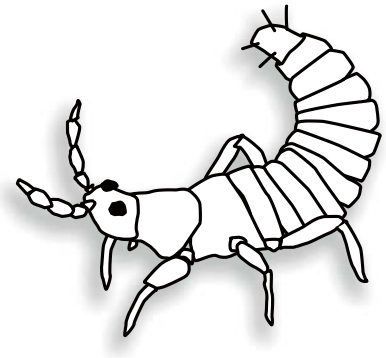


FIGURE S4-E Timing of first partial crossing. Shown are the times (s) until first partial crossings before treatment (pooled for all treatments, N = 125) and after treatment [blend of synthetic pheromone with amount and ratio of second-instar larvae (gray circles), amount of second-instar larvae and ratio of first instar larvae (gray triangles), amount of first-instar larvae and ratio of second-instar larvae (white squares), amount and ratio of first-instar larvae (black stars) or cyclohexane as the solvent control (gray hexagons), N = 25 each]. Focal larvae are either first instar (upper graphs) or second instar (lower graphs). No significant differences were found.

Context-dependent alarm signalling in an insect

Paulien J.A. de Bruijn, Martijn Egas, Maurice W. Sabelis & Astrid T. Groot

5



Abstract

Animals often respond to danger by raising alarm to inform others. Alarm signals come in many different forms, such as visual or mechanical display, sound or odour. Some animals produce vocal alarm signals that vary with the level of danger. For chemical alarm signals, virtually nothing is known about such context-dependent signalling due to a general notion that alarm pheromones have fixed compositions. Here, we show that larvae of the Western flower thrips (*Frankliniella occidentalis*) produce an alarm pheromone whose composition varies with the level of danger they face: the presence of a relatively harmless predator or a very dangerous predator, that is either actually attacking or not. The frequency of alarm pheromone excretion increases with the level of danger. Moreover, the composition of excreted alarm pheromone varies in the relationship between total and relative amount of the putative two components, decyl acetate (DAc) and dodecyl acetate (DDAc). When pheromone is excreted with a predator present but not attacking, the percentage DDAc increases with the total amount of pheromone. When a predator does attack, however, the relationship between percentage DDAc and total amount of pheromone is reversed. Taken together, the alarm signal of thrips larvae appears to be context-dependent.

Unpublished manuscript

Introduction

Many species are confronted with a variety of predators, some more dangerous than others. In order to successfully reproduce, individuals must avoid predation while simultaneously performing other activities such as foraging and mating (Lima and Dill 1990). Hence, individuals face a trade-off between engaging in survival-enhancing anti-predator behaviour and reproduction-enhancing behaviour (Lima and Dill 1990). To avoid unnecessary investment in anti-predator behaviour, an individual should be sensitive to the current level of predation risk (Lima and Dill 1990; Robinson 1980).

There are various ways in which a prey individual can detect that a predator is in the vicinity, such as the detection of cues from the predator (e.g., odours, sounds or vibrations) or the release of signals from conspecific prey individuals that can warn others of impending danger (e.g., vocal, visual or chemical signals). If predation risk is communicated by alarm signalling, natural selection tends to act on alarm signals so as to specify the type and level of danger in a context-dependent manner. Here, the context consists of sender, receiver and danger in the environment. There are examples of individual vertebrates that vary vocal alarm calls with context, such as vervet monkeys that make different calls for each of their main predators (Seyfarth *et al.* 1980) or ground squirrels that change their alarm call depending on the urgency of the situation (Robinson 1980; Furrer and Manser 2009). For chemical alarm signals (alarm pheromones), however, such examples are not known, which is striking because alarm pheromones are very common (Wyatt 2003; Verheggen *et al.* 2010).

We studied variation in the alarm pheromone of an insect, the Western flower thrips *Frankliniella occidentalis* (Pergande) (Insecta: Thripidae) in response to different types of danger. This thrips releases an alarm pheromone, consisting of two specific chemicals: decyl acetate (DAc) and dodecyl acetate (DDAc). These acetates are contained in droplets excreted from the rectum in response to artificial disturbance (Teerling *et al.* 1993; MacDonald *et al.* 2003) and – as we show in this article – it is also excreted in response to natural enemies. Amount of DAc plus DDAc (DAc+DDAc) and percentage of DDAc in the mixture of the two components (%DDAc) is known to vary with larval development: older thrips larvae release more DAc+DDAc and relatively more DAc than DDAc (MacDonald *et al.* 2003). This raises the question whether the insect varies its quantity (DAc+DDAc) and quality (%DDAc) to specify different types of danger.

Here, we tested if thrips larvae release different DAc+DDAc and/or %DDAc, depending on the type of predator they encountered or were attacked by. In nature, thrips larvae (0.5-1.2 mm in length) face different types of predators (Lewis 1973), each posing a different threat level depending on their size rela-

tive to that of the thrips larva (Sabelis and van Rijn 1997). To represent two very different levels of danger, we selected predatory mites (*Iphiseius degenerans*, ~0.7 mm long), and predatory bugs (*Orius laevigatus*, ~2 mm long). Predatory mites are successful in attacking young (first-instar) larvae, but are much less successful or even harmless to older (second-instar) larvae (Bakker and Sabelis 1987). Predatory bugs, however, are successful in attacking all developmental stages (Bakker and Sabelis 1987; Sabelis and van Rijn 1997). To test for context-dependent release of alarm pheromone, we used second-instar thrips larvae with predatory mites as relatively harmless predators and predatory bugs as very dangerous predators. Second-instar larvae release enough pheromone per individual to analyse its qualitative and quantitative composition (MacDonald *et al.* 2003).

To analyse pheromone composition in single, rectally released droplets, we first collected the droplet in an 8 cm glass capillary and then assessed DAc+DDAc, using Gas Chromatography (GC). The single droplets collected and analysed were released by individual larvae either when a predator was attacking the larva, or when the predator was present but not attacking. As a control for release of alarm pheromone upon contact with a predator, we prodded larvae with a brush and collected a single droplet per larva under 'attack' (brush contact). We recorded (a) which droplets contain DAc and DDAc, and if both were present, (b) DAc+DDAc, and (c) %DDAc of the droplets (cases where only DAc or DDAc were present did not occur).

Material and methods

Host plants

All experiments and the rearing of thrips were conducted on cucumber plants, *Cucumis sativus* (var. Ventura RZ, Rijk Zwaan, De Lier, The Netherlands) grown in a greenhouse at 25 °C, 70% RH, L16:D8 photoperiod. Plants were kept insect- and pathogen-free (as far as visible symptoms are concerned) until they were used for the experiments or cultures.

Thrips

Western flower thrips were collected from cucumber plants in a commercial greenhouse at Pijnacker, The Netherlands, in February 2006. Thrips were subsequently reared in a climate box (25 °C, 60% RH, L16:D8) on cucumber leaves, cut to fit in a Petri dish on top of a layer of cotton wool that was put on the bottom of the Petri dish. Once a week, thrips pupae and adults from older leaves of the

culture were put on the cucumber leaf and pollen of *Typha latifolia* was provided on this leaf as additional food for the thrips. From the eggs produced by the adult females, thrips larvae hatched. The emerging pupae and adults were then transferred to a new leaf in a new Petri dish to rear a next generation of thrips. This procedure was repeated to maintain a culture.

Predatory mites

A strain of the predatory mite *I. degenerans,* originally collected in Rabat, Morocco, was reared on a diet of *Typha* pollen in a climate box at 25 °C, 60% RH and L16:D8. The rearing arenas consisted of a PVC sheet (6 × 15 cm) placed on a wet sponge in a water-containing tray. The edges of the PVC sheet were covered with paper tissues that absorb water from the sponge underneath. These tissues served as a water source to the predatory mites and as a barrier to prevent escape from the PVC arena. Small threads of cotton placed on the PVC sheet served as a substrate for oviposition by the predatory mites. For the experiments, we used adult females, 8-15 days old since hatching and 0.7 mm in length.

Predatory bugs

Orius laevigatus were obtained from Koppert BV (The Netherlands) and reared in plastic boxes (40 × 25 × 25 cm) covered with fine nylon gauze. Twice a week, the bugs were fed eggs of the flour moth *Ephestia kuehniella* and provided with bean pods as an oviposition substrate and source of water supply. Boxes were lined with crumpled paper tissue to provide the juvenile bugs with places to hide from cannibalistic adults (Venzon *et al.* 2000). For the experiments, we used adult females of an unknown age and ca. 2 mm in length.

Experimental set-up

Leaf discs (diameter: 24 mm) with 5-10 second-instar thrips larvae were observed using a binocular microscope. These thrips larvae were presented with either a predatory mite or a predatory bug. We collected individual rectally excreted droplets with 80 mm capillary tubes (Hirschmann Laborgeräte). The droplets were released by thrips larvae when a predator was present but had not (yet) attacked, or while being attacked by a predator. As a control, we placed 5-10 thrips larvae on a leaf disc without a predator, prodded them with a small brush and collected the droplet that was produced in response to brush contact (which required up to three times prodding).

GC analysis

In the periods of November 2009 – March 2010 and February – June 2012, we collected and analysed a total of 612 individual excreted droplets for the presence and amount of the pheromone components decyl acetate and dodecyl acetate. Each droplet comprises approximately 10 nl liquid (MacDonald *et al.* 2003). By exerting a low level of air pressure, the droplet was removed from the capillary tube it was collected in and added to a solution of 3 µl internal standard (1 ng octyl acetate per µl hexane) and 2 µl n-octane in a 50-µl glass insert within a crimp-capped vial. Using a 7683 automatic injector, the entire volume of each extract was injected in a splitless inlet of a HP7890 gas chromatograph (GC) coupled with a high-resolution polar capillary column (DB-WAXetr [extended temperature range]; 30 m × 0.25 mm × 0.5 mm) and a flame ionization detector (FID). The gas chromatograph was temperature-programmed from 60 °C (2 min hold) to 180 °C at 30 °C/min, then to 230 °C at 5 °C/min, and finally to 250 °C at 20 °C/min, the FID detector was held at 250 °C. Helium was used as the carrier gas. To exclude the possibility that contaminants in the GC or solvents occur at the same retention time as the pheromone components, before each GC sequence we measured a blank sample, a sample containing only hexane and a sample containing only octane. To assess column performance as well as check the retention times of each of the components, we injected the authentic standards of octyl acetate (>99% pure, Sigma Aldrich, USA), decyl acetate (>99% pure, Alfa Aesar, Germany) and dodecyl acetate (>99% pure, Sigma Aldrich) before each GC sequence as well. The amount of each pheromone component in each rectal droplet was calculated relative to the 3 ng of internal standard in each sample. To exclude background noise, we only used those samples that contained at least 0.1 ng DAc+DDAc.

Statistical analysis

To test for differences in number of droplets produced that contained DAc and DDAc between the five treatments, we applied G-tests for frequencies in 2 × 2 tables (Table 5-1).

To test whether DAc+DDAc or %DDAc were the same in all treatments (excluding the treatment 'attack' by brush), we used a Generalized Linear Model with predator type and presence/absence of an attack as factors. To minimise residuals, we used a GLM assuming a quasi-Poisson distribution for DAc+DDAc, and a GLM assuming a normal distribution for %DDAc. This analysis was performed in R (R Development Core Team 2010).

To assess significant differences between two out of the five treatments, we first pooled the data obtained under these two treatments and then compared

the resulting model with the original model by calculating the contrast statistics (Crawley 2007). If these two models were significantly different, we concluded that the two treatments had a significantly different effect.

We checked for possible relationships between DAc+DDAc and %DDAc for each of the treatments, using regression analysis. To test if the slopes of regression lines differed from 0 (Zar 1999) and from each other (Sokal and Rohlf 1995), an ANOVA was applied.

Results

In total, we collected and analysed 612 droplets, 120 of which contained DAc and DDAc (Figure 5-1). The probability that a droplet contained DAc and DDAc was higher when the larva excreting the droplet was actually attacked by predators (see Figure 5-1A, Table 5-1). When a predator was not attacking, this probability was less than 1:30. Moreover, DAc and DDAc were found more often when the attacking predator was a dangerous predatory bug compared to a relatively harmless predatory mite.

The average DAc+DDAc released in the droplets differed depending on the type of attack (Figure 5-1B, Table 5-2; GLM, brush vs. mite attack: $F_{1,116} = 5.3$, $P = 0.02$; brush vs. bug attack: $F_{1,116} = 4.3$, $P = 0.04$). The DAc+DDAc was higher, although not significantly, in two cases: (1) when thrips were attacked by predatory bugs, as compared to when the predatory bugs were only present but not attacking (Figure 5-1B) and (2) when attacked by the predatory bug as compared to when attacked by the predatory mite (Figure 5-1B). Thus, thrips are able to release different DAc+DDAc depending on the context they experience.

Table 5-1 χ^2 analyses of frequencies of droplets containing DAc and DDAc (see Figure 5-1) (d.f. = 1).

		No attack		Attack		
		Predatory mite	Predatory bug	Predatory mite	Predatory bug	Brush
No attack	Predatory mite	–	G = 0.01, P = 0.91	G = 23.43, P<0.001	G = 142.76, P<0.001	G = 137.18, P<0.001
	Predatory bug		–	G = 22.40, P<0.001	G = 129.91, P<0.001	G = 124.56, P<0.001
Attack	Predatory mite			–	G = 18.20, P<0.001	G = 16.01, P<0.001
	Predatory bug				–	G = 0.13, P = 0.69
	Brush					–

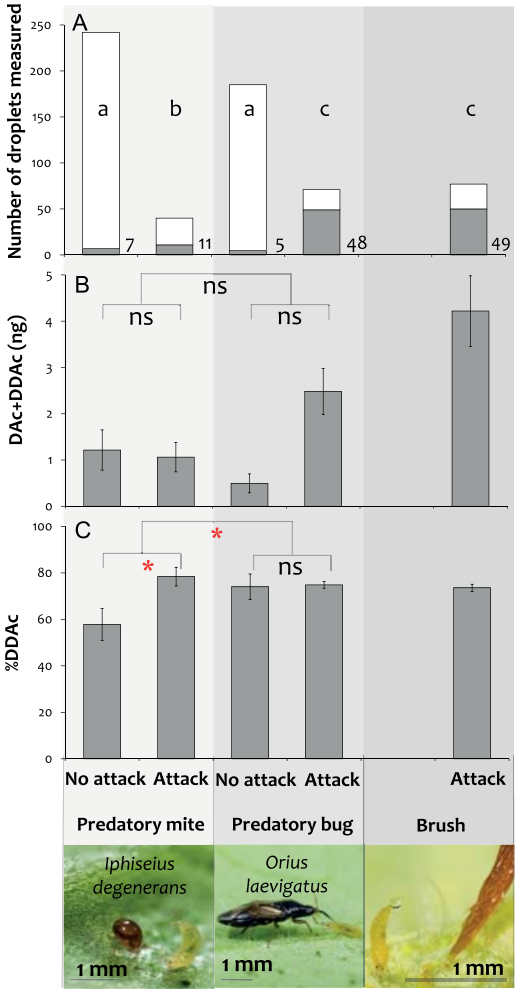


FIGURE 5-1 Presence of DAc and DDAc in rectal droplets excreted by Western flower thrips. **A**. Number of droplets containing the acetates (grey bars) and the number of droplets containing no acetates (white bars). **B**. Total amount of DAc and DDAc measured in the droplets containing these acetates (DAc+DDAc). **C**. Percentage DDAc of the total DAc and DDAc measured (%DDAc). The horizontal axis shows the different treatments (predatory mite attack/no attack, predatory bug attack/no attack, brush attack). The pictures under the horizontal axis illustrate thrips larvae attacked by predatory mite, predatory bug and brush. Note the droplet release of the larva under brush-attack. Bars in (**A**) with different letters display significantly different proportions ($P<0.05$) of droplets containing the acetates among treatments, and the numbers to the right side of these bars indicate the number of droplets in which acetates were found. Bars in (**B**) and (**C**) indicate mean values (± SEM); ns = not significant; * $P<0.05$.

Table 5-2 GLM analyses of effects of predator type, occurrence of an attack and their interaction on %DDAc and DAc+DDAc in all treatments, excluding brush 'attack'.

	%DDAc	Dac+DDAc
Predator	d.f. = 1,69, P = 0.20	d.f. = 1,69, P = 0.094
Attack	d.f. = 1,68, P = 0.004	d.f. = 1,68, P = 0.24
Predator*attack	d.f. = 1,67, P = 0.026	d.f. = 1,67, P = 0.21

When DAc and DDAc were present in excreted droplets, they consisted of ca. 75% DDAc in all treatments, except for the treatment where a relatively harmless predatory mite was present but not attacking. In that case, the %DDAc dropped to 55% (Figure 5-1C, Table 5-2). Thus, thrips larvae released DAc and DDAc in different proportions depending on whether a predatory mite attacked or not (Figure 5-1C, Table 5-2). In addition, thrips larvae released DAc and DDAc in different proportions depending on which of the two predators was present without attacking.

Using regression analysis, we checked for possible relationships between DAc+DDAc and %DDAc in each of the treatments (Figure 5-2). There was a reversal in the slope of regression line: when the predators were present but not attacking, the %DDAc increased with DAc+DDAc released, whereas the %DDAc decreased with DAc+DDAc released when the predators attacked ($F_{1,14}$ = 9.8, P<0.01 for the predatory mite; $F_{1,48}$ = 2.8, P = 0.1 for the predatory bug).

Discussion

Our experiments show that thrips larvae release a different quantity (DAc+DDAc) and/or quality (%DDAc) of the known compounds of alarm pheromone, DAc and DDAc, depending on the type of predator they are exposed to and depending on whether this predator actually attacked the thrips larva or not. These observations support our hypothesis that alarm signals of an insect can specify the level of danger and are thus context-dependent. To the best of our knowledge, this is the first report of context-dependent release and composition of an alarm pheromone.

We do not know how the thrips recognise the predator or estimate the level of danger. The recognition of a predator may be mediated by scent either from the prey consumed by the predator, from the predator itself or from both. Thrips larvae exposed to odours from predatory bugs have been shown to exhibit more frequent escape responses when the predatory bugs had previously been fed thrips larvae compared to predatory bugs fed on eggs of flour moths (Venzon *et al.* 2000). Hence, dietary cues may play a role in predator recognition by thrips

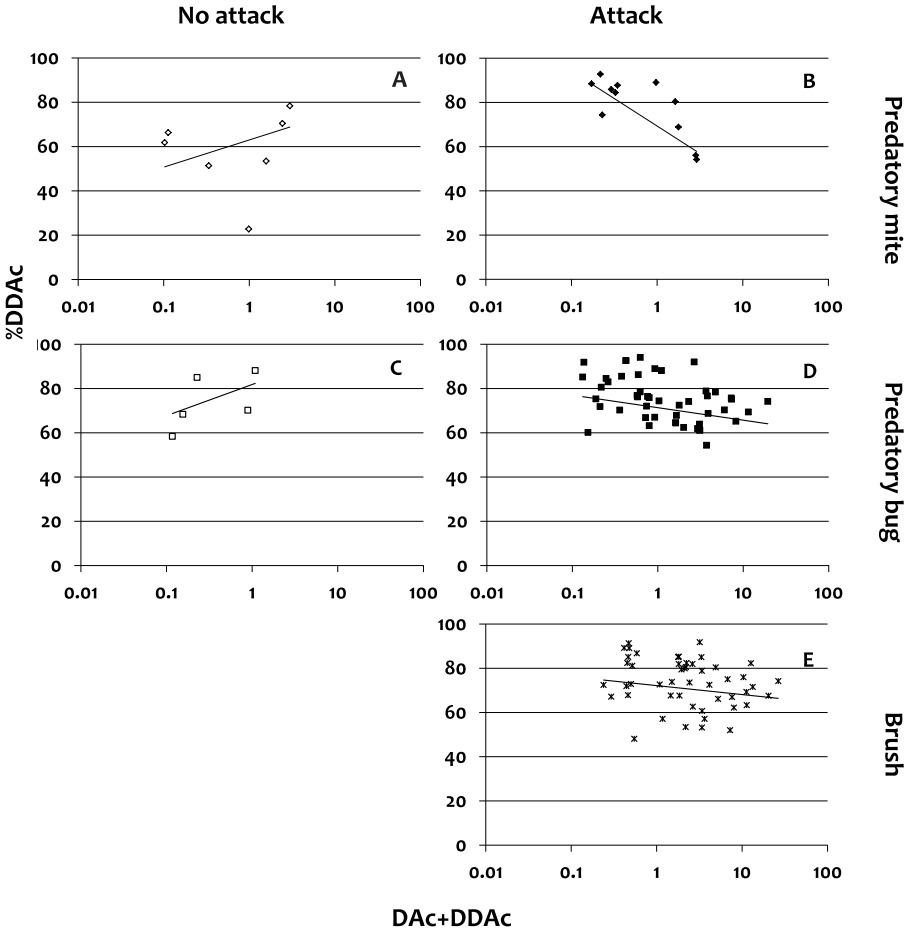


FIGURE 5-2 Regression analysis of quantity and quality of the two acetates DAc and DDAc. Scatter plots and linear regressions of %DDAc (y-axis) against DAc+DDAc (x-axis). Different panels relate to different treatments, indicated above and right of the panels. Each dot represents the results from a single droplet. The slopes of the regression lines for a predatory bug or mite without an attack do not differ significantly ($F_{1,8} = 0.4$, $P = 0.6$), but do differ significantly when a bug or mite is attacking ($F_{1,55} = 13.7$, $P<0.001$). The slopes also differ significantly between attack by a predatory mite and when thrips are prodded by a brush ($F_{1,56} = 12.2$, $P<0.001$). There is no significant difference in slopes for droplets produced under attack by a predatory bug or a brush ($F_{1,93} = 0.4$, $P = 0.5$). For other comparisons between slopes, see the main text.

larvae. The predators in our experiments had never eaten thrips larvae as prey in their lifetime, as the predatory bugs were reared on flour moth eggs and the predatory mites were reared on pollen. In our experiments, however, we cannot fully exclude a role of prey cues in predator recognition, since the diets of the predators differed. Apart from scent, touch may be another recognition cue for thrips larvae, not only to recognise the predator but also to recognise the level of danger. This is suggested by our finding that, in terms of DAc+DDAc and %DDAc, thrips larvae responded similarly to an attack by a predatory bug as to an 'attack' with a brush (FIGURE 5-1).

Our study adds to only few studies providing evidence for phenotypic plasticity in chemical communication processes, each of which requires its own time scale. For pheromone-mediated aggregation (Bashir *et al.* 2003) and sexual attraction of males (Groot *et al.* 2010) and females (Kent *et al.* 2008), changes in signal composition can take longer than those required for signalling danger via alarm pheromone, because the type of danger may change at such a short time scale. We found that, in response to either a relatively harmless or a very dangerous predator, actually attacking or not, thrips larvae vary the acetate composition of rectally excreted droplets in seconds or minutes after a thrips larva was exposed to its enemy. Thus, our results suggest that thrips have control over the composition of acetates they excrete.

We conclude that our experiments support the hypothesis that the thrips chemical alarm signal carries information on the level of danger, and suggest that an individual thrips can vary its pheromone composition depending on context, at a time scale similar to the examples of context-dependent signalling in vertebrate vocal alarm calls (Seyfarth *et al.* 1980; Furrer and Manser 2009). We propose that context-dependent alarm signalling by means of chemical alarm signals is widespread.

Acknowledgements

We thank Jan van Arkel for making the pictures included in FIGURE 5-1, and Arne Janssen for statistical advice. PJAdB was supported by a grant from The Netherlands Organization for Scientific Research (NWO, project number 818.01.018). This research was partly funded by the Max Planck Gesellschaft.

References

Bakker FM, Sabelis MW. 1987 Attack success of *Amblyseius mckenziei* and the stage related defensive capacity of thrips larvae. Neth. J. Zool. 37, 437.

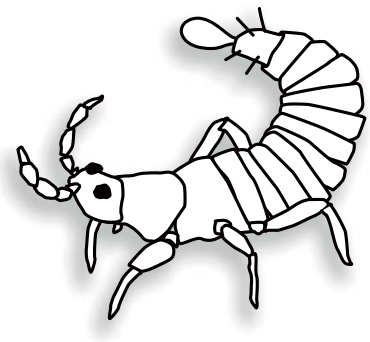
Bashir T, Hodges RJ, Birkinshaw LA, Hall DR, Farman DI. 2003 Phenotypic plasticity of *Rhyzopertha dominica* pheromone signaling: the effects of different hosts and pres-

ence of conspecific females on male produced aggregation pheromone. J. Chem. Ecol. 29, 945-959.
Crawley MJ. 2007 The R book. John Wiley & Sons, Chichester, UK.
Furrer RD, Manser MB. 2009 The evolution of urgency based and functionally referential alarm calls in ground-dwelling species. Am. Nat. 173, 400-410.
Groot AT, Claβen A, Staudacher H, Schal C, Heckel DG. 2010 Phenotypic plasticity in sexual communication signal of a noctuid moth. J. Evol. Biol. 23, 2731-2738.
Kent C, Azanchi R, Smith B, Formosa A, Levine JD. 2008 Social context influences chemical communication in *D. melanogaster* males. Curr. Biol. 18, 1384-1389.
Lewis T. 1973 Thrips: their biology, ecology and economic importance. Academic Press, London, UK.
Lima SL, Dill LM. 1990 Behavioral decisions made under the risk of predation: a review and prospectus. Can. J. Zool. 68, 619-640.
MacDonald KM, Hamilton JGC, Jacobson R, Kirk WDJ. 2003 Analysis of anal droplets of the western flower thrips *Frankliniella occidentalis*. J. Chem. Ecol. 29, 2385-2389.
Robinson SR. 1980 Antipredator behaviour and predator recognition in Belding's ground squirrels. Anim. Behav. 28, 840-852.
Sabelis MW, van Rijn PCJ. 1997 Predation by insects and mites. In: Thrips as crop pests (T. Lewis, ed.), pp. 259–354. CAB International, Wallingford, UK.
Seyfarth RM, Cheney DL, Marler P. 1980 Vervet monkey alarm calls – semantic communication in a free-ranging primate. Anim. Behav. 28, 1070-1094.
Sokal RR, Rohlf FJ. 1995 Biometry. WH Freeman, New York, NY, USA.
Teerling CR, Pierce HD Jr., Borden JH, Gillespie DR. 1993 Identification and bioactivity of alarm pheromone in the Western flower thrips, *Frankliniella occidentalis*. J. Chem. Ecol. 19, 681-697.
Venzon M, Janssen A, Pallini A, Sabelis MW. 2000 Diet of a polyphagous arthropod predator affects refuge seeking of its thrips prey. Anim. Behav. 60, 369-375.
Verheggen FJ, Haubruge E, Mescher MC. 2010 Alarm pheromones-chemical signaling in response to danger. In: Vitamins & hormones (G. Litwack, ed.), pp. 215–239. Academic Press, London, UK.
Wyatt TD. 2003 Pheromones and animal behaviour: communication by smell and taste. Cambridge University Press, Cambridge, UK.
Zar JH. 1999 Biostatistical analysis. Prentice Hall, Upper Saddle River, NJ, USA.

When alarm pheromones vary with the danger from predation: Differential refuge seeking by thrips larvae?

Paulien J.A. de Bruijn, Alexandra M. Revynthi, Maurice W. Sabelis & Martijn Egas

6



Abstract

Alarm signals are expected to vary with the level of danger, as shown for vocal alarm signals and recently also for chemical signals. Larvae of the Western flower thrips (*Frankliniella occidentalis*) are known to excrete anal droplets containing chemically well-defined alarm pheromone consisting of two compounds, decyl acetate and dodecyl acetate. With increasing levels of danger (predator species; encounter vs. attack), the chance that an excreted droplet contains alarm pheromone increases and moreover the two compounds increase in total and relative (% dodecyl acetate) amount. Here, we tested if the refuge-seeking response by receivers of the alarm signal reflects the context experienced by the sender, as defined by the attack of a predator species, even when those receivers do not experience any danger. Offering a silken spider-mite web on a leaf disc as a refuge, we analysed how often focal thrips larvae not exposed to predators nevertheless seek refuge after being presented with a filter paper containing alarm pheromone from another thrips larva attacked by a predatory mite or bug, or with a filter paper without pheromone. Because experience with predators might influence the response of thrips larvae to alarm pheromone, these experiments were done with thrips larvae that were naive or that had experience with predators. We found that thrips larvae rarely moved into the refuge when offered filter paper without anal droplets (negative control), but did so significantly more frequently when offered filter paper with anal droplets. Moreover, based on earlier measurement of the frequency of alarm pheromone in anal droplets, thrips larvae responded always to anal droplets excreted under attack by a predatory mite, but not always to anal droplets excreted under attack by a predatory bug. This suggests that thrips larvae respond to alarm pheromone in a context-dependent way.

Unpublished manuscript

Introduction

In many animal species, information about the presence of a predator in the vicinity is conveyed by alarm signals (Borden 1989; Ayasse *et al.* 2001). These signals can be vocal, chemical, visual or even mechanical (Lima and Dill 1990) and because danger may come in various forms, they are prone to be context-dependent (Blum 1996). For vocal alarm signals, there is ample evidence for context-dependent signals in that they vary with predator type or danger level (Sherman 1977; Seyfarth *et al.* 1980; Blumstein and Armitage 1997; Lima and Dill 1990; Furrer and Manser 2009). Whereas senders may adapt alarm to context, receivers adapt their response to the type of alarm call conveyed by the sender (Sih 1980; Lima and Dill 1990). For instance, Belding's ground squirrels (*Spermophilus beldingi*) release signals that denote different levels of response urgency (Robinson 1980). The signals vary with the speed and distance of the approaching predator but not specifically with the type of predator (Robinson 1981). As ground squirrels live in open habitats and run to their burrows in response to any predator type, information about urgency may be more important than that on predator type (Blumstein and Armitage 1997; Furrer and Manser 2009). Another example of context dependent alarm calls is that of ring-tailed lemurs (*Lemur catta*), which are known to contain information on predator type in their alarm calls (Macedonia 1993). This makes sense because they live in complex habitats, are hunted in ways depending on predator type and tune their escape strategy accordingly (Macedonia 1993; Macedonia and Evans 1993; Furrer and Manser 2009).

For chemical alarm signals, i.e., alarm pheromones, virtually nothing is known about context-dependent signalling (Verheggen *et al.* 2010; Blum 1996). There are examples of organisms that show inter-individual variation in chemical alarm pheromones, such as the pea aphid, *Acyrthosiphon pisum* (Pickett *et al.* 1992; Kunert *et al.* 2005; Podjasek *et al.* 2005), the paper wasp *Polistes domilus* (Bruschini *et al.* 2008) and the Western flower thrips (de Bruijn *et al.* 2014b, in prep.). Pea aphids are known to respond to the alarm pheromone (E)-β-farnesene (Pickett *et al.* 1992) by increasing the production of the winged offspring (Podjasek *et al.* 2005) and they show a stronger response to the release frequency of the pheromone than to the amount of pheromone perceived (Kunert *et al.* 2005). Paper wasps respond more strongly to the pheromone produced by workers – which differs quantitatively – than to the pheromone produced by the foundresses (Bruschini *et al.* 2008). To the best of our knowledge, there is only one example showing that conspecific larvae tune their response to variation in chemical alarm pheromone, namely Western flower thrips larvae. Recently, we found that the composition of alarm pheromone from second-instar larvae of

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), differs with the level of danger they experience (de Bruijn *et al.* 2014a, subm.). Thrips larvae may encounter different predators, varying in their voraciousness. For example, predatory bugs (*Orius laevigatus* (Fieber)) are successful in attacking all mobile stages (Sabelis and van Rijn 1997), whereas the much smaller predatory mites (*Iphiseius degenerans* (Berlese)) are more successful in attacking first-instar larvae and have little or no impact on all other stages. Despite the specialization of predatory mites on first-instar thrips larvae, both first- and second-instar larvae suffer most from predation by predatory bugs. The question we address here is whether Western flower thrips larval alarm pheromone produced under different levels of danger (attack by one or the other predator species) triggers different and adaptive antipredator responses in receiving larvae.

The alarm pheromone of Western flower thrips is present in anal excretions that are produced in the form of droplets – the so-called 'anal droplets' (MacDonald *et al.* 2003). Teerling *et al.* (1993) found that this pheromone consists of two compounds: decyl acetate (DAc) and dodecyl acetate (DDAc). De Bruijn *et al.* (2014a, subm.) found that anal droplets excreted by a second-instar larva during predator attack does not always contain alarm pheromone, and that alarm pheromone is released more frequently when the attacker is a predatory bug (69% of droplets contain pheromone) rather than a predatory mite (27.5% of droplets contain pheromone). Furthermore, when alarm pheromone is excreted, the ratio of decyl acetate and dodecyl acetate as well as the total amount of pheromone varies with the level of danger the sender encountered: dangerous vs. relatively harmless predator species, and encounter vs. attack. Alarm pheromone composition also varies with the instar of the sender (MacDonald *et al.* 2003), and first-instar larvae exhibit differential escape response to alarm pheromone of different instars (de Bruijn *et al.* 2014b, in prep.), in absence of a predator.

In this paper, we quantify the response of thrips larvae to anal droplets that are excreted by thrips larvae under attack by one or the other predator species, by scoring the frequency of refuge seeking. We use the silken web created by spider mites on a leaf as a refuge for the thrips (as in Venzon *et al.* 2000). Thrips and spider mites often co-occur on the same plants, and when threatened by predators, thrips larvae seek refuge in the web of spider mites where they are less vulnerable to predation by predatory bugs, as well as by predatory mites (Pallini *et al.* 1998). In absence of cues of these predators, thrips larvae prefer to stay outside of spider mite webs to avoid food competition and speed up development. In the presence of cues of these predators (but without a predator nearby) they

may move into the web refuge (Pallini *et al.* 1998; Venzon *et al.* 2000). We use the same setup to test whether thrips larvae seek refuge in response to anal droplets.

Unfortunately, the amount of pheromone (2-10 ng pheromone in a droplet of ca. 1 nl mostly containing water) excreted by individual thrips larvae is so small that we cannot simultaneously assess the presence of alarm pheromone in a droplet and the response to the droplet. Instead, to interpret our results, we assume that the probability that an anal droplet contains alarm pheromone and the composition of alarm pheromone are the same as we measured before (de Bruijn *et al.* 2014a). We hypothesize that thrips larvae always seek refuge when perceiving alarm pheromone, and hence more often when presented with anal droplets excreted by larvae under attack by predatory bugs rather than predatory mites.

Materials and methods

Cucumber plants

Cucumber plants (*Cucumis sativus* var. Ventura RZ, Rijk Zwaan, De Lier, The Netherlands) were grown from seeds, free of herbivores, in a climate room (25 °C, 70 ± 10% RH and L16:D8).

Thrips

Western flower thrips were collected from chrysanthemum plants in Wageningen, The Netherlands, in March 2010 and were reared in our laboratory on cucumber leaves in a climate room (25 °C, 60% RH, L16:D8) using the procedure described by de Bruijn *et al.* (2014c).

Spider mites

Spider mites (*Tetranychus urticae* Koch) were collected from cucumber plants in a commercial greenhouse in May 1994 and were reared in our laboratory on cucumber plants (Ventura RZ) in a climate room (25 °C, 60% RH, L16:D8).

Predators

Predatory bugs (*O. laevigatus*) were provided by Koppert Biological Systems and reared in the laboratory at 21 °C. The predators were kept in plastic boxes (40 × 25 × 25 cm^3) covered with fine nylon gauze. We maintained the culture by providing the predatory bugs with eggs of *Ephestia kuehniella* Zeller as food and bean

pods (*Phaseolus vulgaris* L.) as oviposition substrate and a source of moisture. For the experiments only adult females were used.

Predatory mites (*I. degenerans*) were reared on plastic arenas (8 × 15 cm), placed on wet sponges in a plastic tray with water (Nomikou *et al.* 2003). The trays were kept in a climate room (25 °C, 60% RH, L16:D8). The predatory mites had access to water surrounding the arena and were fed with pollen of *Typha latifolia*. For the experiments only adult females were used.

Response to alarm pheromone

We created leaf arenas with refuges consisting of a spider mite web using the following procedure (Pallini *et al.* 1998). A cucumber leaf disc (24 mm diameter) was cut in such a way that the main vein was in the middle and divided the disc in two halves. The leaf disc was placed on wet cotton wool in a Petri dish (30 mm diameter). The main vein was covered with wet cotton wool and on one half of the leaf disc 30 adult female spider mites were released. The spider mites were left for two days to feed, oviposit and produce web. After two days the cotton wool and the adult mites were removed carefully with the use of a thin needle. While removing the spider mites, care was taken to minimize damage to the web they had produced. The eggs produced by spider mites (ca. 500 spider mite eggs) were left on the leaf disc because it would damage the web too much if we would remove them. During the experiments, thrips larvae had the possibility to feed on spider mite eggs, but feeding on eggs was not observed during the experiments. Hence our arena consisted of a leaf disc, half of which was damaged and covered with spider mite eggs, faeces and web, whereas the other half remained intact and clean. On the clean half we introduced one first-instar thrips larva. In this experiment, we tested responses of first-instar larvae to alarm pheromone in absence of a predator. First-instar larvae were used because they have been shown to vary their response with the composition of alarm pheromone in a similar set-up (de Bruijn *et al.* 2014b). Subsequently, on a piece of filter paper (ca. 0.1 cm^2) we collected an anal droplet produced by a second-instar larva that was under attack and deposited the filter paper on the clean half of the leaf disc. Second-instar larvae were used to excrete alarm pheromone because they have been shown to vary their alarm pheromone with the level of danger (de Bruijn *et al.* 2014a). Filter paper without excretions served as a negative control, whereas filter paper with excretions induced by artificially prodding the larvae with a fine brush served as a positive control. Previously we found that 69% of the droplets excreted when a larva was prodded with a brush contained alarm pheromone (de Bruijn *et al.* 2014a) and when alarm pheromone was found, it was present in high amounts. Finally, we recorded the position of the first-instar focal larva (clean half or webbed half) after 1, 5 and 30 min.

We used first-instar larvae without experience with predators, or with experience with a predatory mite, with a predatory bug, or with both. In order to give thrips experience, we introduced one predatory bug, two predatory mites or one bug and two predatory mites on a leaf disc containing thrips, and allowed the predators to feed on thrips overnight (12 h). For experiments, we used first-instar larvae from these leaf discs that had survived this predation setting.

We collected anal droplets from second-instar larvae that were taken from the culture and hence had no experience with predators (cf. de Bruijn *et al.* 2014a). We collected the droplets on filter paper, while the second-instar larvae were under attack of a predator or were prodded by the experimenter using a fine brush. The predatory bug was released on the Petri dish and was allowed to feed on the second-instar larvae. As soon as the thrips larvae were under attack, they produced an anal droplet, which was then collected on a piece of filter paper by the experimenter. The predatory mite was not released in the Petri dish (the procedure used to collect droplets by de Bruijn *et al.* 2014a), but instead it was stuck on top of a fine brush. Using the fine brush the experimenter brought the predatory mite within reach of a second-instar thrips larvae, until the predator touched the thrips larva with its legs. As soon as the larva excreted an anal droplet, it was collected on a piece of filter paper. Attention was paid to avoid the brush contacting the thrips larva. This procedure was used here, because it takes quite some time before a predatory mite attacks a thrips larva and we intended to control the time between placing a first-instar larva on the leaf disc with the web refuge and collecting the excretion on filter paper. All predators used were starved for 24 h.

In total, we conducted four experiments, one for each experience treatment of the focal thrips larva (no experience, experience with predatory bugs, with mites or with both). Each experiment had four attack treatments (alarm pheromone excreted under attack by predatory mite or predatory bug, negative control and positive control), and in each attack treatment we observed the response of 30 independent thrips larvae (except in the positive control of predator-naive thrips where we tested 50 individuals).

Statistical analysis

Using the open source program R (R Development Core Team 2010), we applied a Kaplan-Meier survivorship analysis (Hosmer and Lemeshow 1999) on the number of larvae in the clean area to compare the groups that were exposed to filter paper with anal droplets collected under attack of a predatory mite or a predatory bug, by prodding with a brush (positive control) or without anal droplets. Contrasts among treatments were assessed through exclusion of

treatment that by eye appeared to be the most deviant from the others until the survivorship analysis did not show a significant difference among the remaining treatments.

To assess whether thrips larvae always seek refuge quickly when perceiving alarm pheromone, the frequency of thrips larvae that move into the web within 5 min was compared with the frequency of alarm pheromone in anal droplets we found previously (69% of all droplet contain pheromone when thrips are under attack by a predatory bug, 27.5% when under attack by a predatory mite; de Bruijn *et al.* 2014a). Using 95% confidence intervals of these expected frequencies from a binominal distribution of 30 individuals, between 16 and 25 individuals are expected to seek refuge when presented with a filter paper containing an anal droplet excreted under attack by a predatory bug and between 4 and 13 individuals when presented with a filter paper containing an anal droplet excreted under attack by a predatory mite.

Focal larvae that had experience with one or two predators, had survived ca. 16 h in the vicinity of that predator(s). Hence, we may have selected for larvae that are somehow better able to survive predators nearby. This selection could be severe, especially near a predatory bug, because they kill numerous larvae in one night. Naive focal larvae had not been subjected to such a selection. Therefore, we do not analyse comparisons of refuge use data among the different experience treatments.

Results

Naive thrips larvae

When exposed to a filter paper with an anal droplet excreted by a second-instar thrips larva under attack by one of the two predator species or prodded with a brush (positive control), roughly 20-30% of the naive thrips larvae moved into the webbed area within 5 min, as opposed to none when exposed to a clean filter paper (negative control). After 5 min, some thrips larvae still moved into the web refuge, but these numbers were always comparable among all treatments including the negative control (see supplementary information). We found a significant overall effect of treatment on the rate of decline of thrips individuals that were on the clean half of the leaf disc (Figure 6-1; $\chi^2 = 8.3$, d.f. = 3, $P = 0.040$), which was due to the negative control (there was no significant difference in refuge seeking among the other treatments; $\chi^2 = 0.5$, d.f. = 2, $P = 0.77$). We conclude that naive thrips seek refuge when presented with anal droplets on the filter paper and not when presented with the filter paper only.

After 5 min, eight thrips larvae moved into the web when presented with a droplet excreted under attack by a predatory bug, which is less than expected (16-25 thrips larvae). When presented with a droplet excreted under attack by a predatory mite, six larvae moved in the web which is within the 95% confidence interval (4-13 thrips larvae).

Experienced thrips larvae

When thrips larvae had survived on a leaf disc where a predatory bug, two predatory mites or both predator species had preyed on conspecific thrips larvae for one night, and subsequently tested for their response to pheromones, we found an overall effect of treatment (experience with predatory bug: $\chi^2 = 19.1$, d.f. = 3, $P<0.001$; experience with predatory mites: $\chi^2 = 14.3$, d.f. = 3, $P = 0.003$; experience with both predators: $\chi^2 = 10.2$, d.f. = 3, $P = 0.017$), that mainly emerged from the treatment where larvae were exposed to a filter paper without an anal droplet (FIGURES 6-2, 6-3 and 6-4). Larvae did not show significantly different refuge seeking behaviour when exposed to an anal droplet excreted at bug attack, positive control or mite attack (experience with predatory bug: $\chi^2 = 4.5$, d.f. = 2, $P = 0.10$; experience with predatory mites: $\chi^2 = 5.7$, d.f. = 2, $P = 0.059$; experience with both predators: $\chi^2 = 1.1$, d.f. = 2, $P = 0.58$).

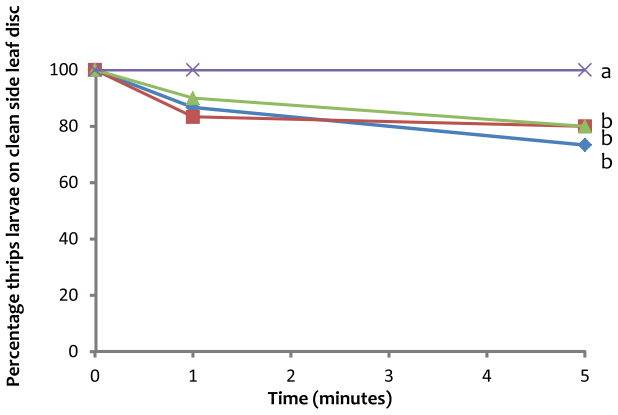


FIGURE 6-1 Percentage of predator-naive thrips larvae remaining outside the refuge after exposure to anal droplets. Different lines and symbols represent the four treatments: attack of a predatory bug (blue line with diamond markings), attack of a predatory mite (red line with squares), positive control (green line with triangles), negative control (purple line with crosses). N = 30 for all treatments, except when larvae were prodded with a brush, where N = 50. Treatments indicated with different letters exhibit significant difference in the frequency of refuge use.

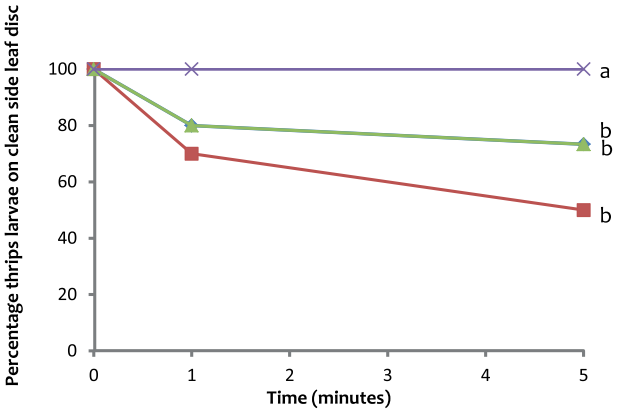


FIGURE 6-2 Percentage of thrips larvae remaining outside the refuge after exposure to anal droplets. Larvae had survived for 12 h in the vicinity of a predatory bug. Different lines and symbols represent the four treatments: attack of a predatory bug (blue line with diamond markings), attack of a predatory mite (red line with squares), positive control (green line with triangles), negative control (purple line with crosses). N = 30 for all treatments. Treatments indicated with different letters exhibit significant difference in the frequency of refuge use.

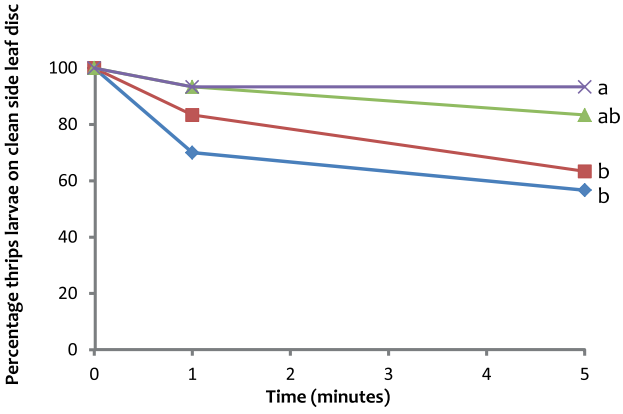


FIGURE 6-3 Percentage of thrips larvae remaining outside the refuge after exposure to anal droplets. Larvae had survived for 12 h in the vicinity of predatory mites. Different lines and symbols represent the four treatments: attack of a predatory bug (blue line with diamond markings), attack of a predatory mite (red line with squares), positive control (green line with triangles), negative control (purple line with crosses). N = 30 for all treatments. Treatments indicated with different letters exhibit significant difference in the frequency of refuge use.

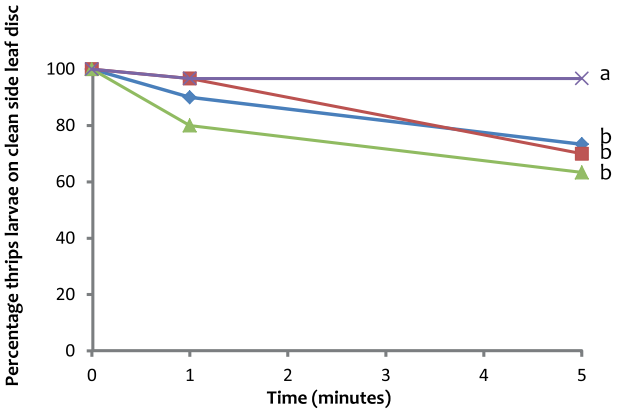


Figure 6-4 Percentage of thrips larvae remaining outside the refuge after exposure to anal droplets. Larvae had survived for 12 h in the vicinity of both predatory bug and mites. Different lines and symbols represent the four treatments: attack of a predatory bug (blue line with diamond markings), attack of a predatory mite (red line with squares), positive control (green line with triangles), negative control (purple line with crosses). N = 30 for all treatments. Treatments indicated with different letters exhibit significant difference in the frequency of refuge use.

After 5 min, the number of thrips larvae that moved into the web when presented with a droplet excreted under attack by a predatory bug was 8 (experience with predatory bug), 13 (experience with predatory mite), and 8 (experience with both predators). All three numbers are less than expected. When presented with a droplet excreted under attack by a predatory mite, 15 (experience with predatory bug), 11 (experience with predatory mite), and 9 (experience with both predators) moved into the web. These numbers are not different from what was expected, except for the predatory bug experience treatment, where it is more often than expected.

Discussion

By the experiments presented here, we investigated how thrips larvae respond to anal droplets putatively containing alarm pheromone in different frequencies and compositions, aiming to test whether they show differential refuge seeking behaviour. Since we evaluated the thrips response to anal droplets only and in absence of the agents (predators, brush) that induced their release, all information focal larvae receive must be contained within the anal droplets released by the sending thrips larvae and not by the predator or injured thrips. The results

showed that thrips larvae, irrespective of their experience, seek refuge in response to odours from anal droplets and do not seek refuge in the negative control. We found no difference in their refuge-seeking response among odours from anal droplets produced under the different types of attack (Figures 6-1 – 6-4). A simple explanation for the lack of differentiation in response to droplets excreted under attack by different predators is that thrips respond to the anal droplets, regardless of their content. However, it is known that thrips respond to alarm pheromone (MacDonald *et al.* 2002; de Bruijn *et al.* 2006) and that some anal droplets have a higher chance of containing alarm pheromone than others (de Bruijn *et al.* 2014a). Hence, it seems unlikely that our results can be explained exclusively by the presence of an anal droplet in absence of the alarm pheromone.

To interpret thrips refuge-seeking response to alarm pheromone, we had to make assumptions on pheromone presence in anal droplets, because we were unable to simultaneously measure pheromone in an anal droplet and the response of another thrips larva to that same droplet. We assumed that the proportions of anal droplets with pheromone is the same as measured earlier (de Bruijn *et al.* 2014a), i.e., 69% in the droplets derived from predatory bug attack and the positive control (brush attack), and 27.5% in droplets derived from predatory mite attack. Under this assumption, bug-induced droplets did not always trigger refuge-seeking behaviour to both naive and experienced thrips larvae, whereas all mite-induced droplets did. In the case where thrips have experience with a predatory bug and are presented with a droplet excreted under attack by a predatory mite, thrips larvae sought refuge significantly more often than 27.5% of the replicates. In this specific treatment therefore, our assumption on the percentage of excreted droplets containing pheromone appeared to be underestimated. Notwithstanding, our results suggest that thrips larvae seek refuge at different rates when presented with alarm pheromone excreted under attack by a predatory bug or predatory mite.

We found that thrips larvae move in the web less often than expected when they perceive droplets excreted under attack by a predatory bug. What could be the cause of this observation? When perceiving variation in alarm pheromone, thrips larvae might differentiate their response. Differentiation in anti-predator behaviour has been shown for various prey species that adapt their escape behaviour to the hunting strategies of the predator they face (Marler 1967; Cheney and Seyfarth 1990; Macedonia and Evans 1993; Furrer and Manser 2009). The predators used in our study have a different hunting strategy as well: predatory mites do not have eyes (although they can perceive light; Evans 1992) and hunt by smell and touch (personal observations, PJAdB), but predatory bugs

respond to movement while hunting (personal observations, PJAdB). Hence the response of first-instar thrips larvae not to move into the web when perceiving alarm pheromone excreted by a larva under attack of a predatory bug, might be adaptive when larvae are preyed upon earlier while moving when a predatory bug is nearby. Such an effect of movement on predation risk is not expected for thrips under attack of a predatory mite.

Varying an alarm signal with the context of the sender, enables receivers of this signal to respond adaptively. Adaptive responses are known for vocal alarm signals, but scarce for chemical alarm signals. Our results suggest that thrips larvae can distinguish between chemical alarm signals collected in different contexts and hence can tune their response to the signal they perceive.

References

Ayasse, M., Paxton, R.J. & Tengö, J., 2001. Mating behavior and chemical communication in the order Hymenoptera. Annual Review of Entomology 46: 31-78.

Blum, M.S., 1996. Semiochemical parsimony in the Arthropoda. Annual Review of Entomology 41: 353-374.

Blumstein, D.T. & Armitage, K., 1997. Alarm calling in yellow-bellied marmots: I. The meaning of situationally variable alarm calls. Animal Behaviour 53: 143-171.

Borden, J.H., 1989. Semiochemicals and bark beetle populations: Exploitation of natural phenomena by pest management strategists. Holarctic Ecology 12: 501-510.

de Bruijn, P.J.A., Egas, M., Janssen, A. & Sabelis, M.W., 2006. Pheromone-Induced priming of a defensive response in Western flower thrips. Journal of Chemical Ecology 32: 1599-1603. [Chapter 2 of this thesis]

de Bruijn, P.J.A., Egas, M., Sabelis, M.W. & Groot, A.T., 2014a. Context-dependent alarm signalling in an insect. [Chapter 5 of this thesis]

de Bruijn, P.J.A., Sabelis, M.W. & Egas, M., 2014c. Effects of kinship or familiarity? Small thrips larvae experience lower predation risk only in groups of mixed-size siblings. Behavioral Ecology and Sociobiology 68: 1029-1035. [Chapter 7 of this thesis]

de Bruijn, P.J.A., Sabelis, M.W., Janssen, A. & Egas, M., 2014b. Anti-predator responses to alarm pheromones in groups of mixed-size thrips larvae. [Chapter 4 of this thesis]

Bruschini, C., Cervo, R., Protti, I. & Turillazzi, S., 2008. Caste differences in venom volatiles and their effect on alarm behaviour in the paper wasp *Polistes dominulus* (Christ). Journal of Experimental Biology 211: 2442-2449.

Cheney, D.L. & Seyfarth, R.M., 1990. How monkeys see the world: Inside the mind of another species. University of Chicago Press, Chicago, IL, USA.

Chivers, D.P. & Smith, R.J.F., 1998. Chemical alarm signalling in aquatic predator-prey systems: A review and prospectus. Ecoscience 5: 338-352.

Davies, N.B., Madden, J.R. & Butchart, S.H.M., 2004. Learning fine-tunes a specific response of nestlings to the parental alarm calls of their own species. Proceedings of the Royal Society B 271: 2297–2304.

Dill, L.M. & Houtman, R., 1989. The influence of distance to refuge on flight initiation distance in the gray squirrel (*Sciurus carolinensis*). Canadian Journal of Zoology 67: 233-235.

Evans, G.O., 1992. Principles of acarology. CAB international, Wallingford, UK.
Furrer, R.D. & Manser, M.B., 2009. The evolution of urgency-based and functionally referential alarm calls in ground-dwelling species. American Naturalist 173: 400-410.
Griffin, A.S., 2004. Social learning about predator: A review and prospectus. Learning and Behaviour 32: 131-140.
Haff, T.M. & Magrath, R.D., 2012. Learning to listen? Nestling response to heterospecific alarm calls. Animal Behaviour 84: 1401-1410.
Hosmer, D.W. Jr. & Lemeshow, S., 1999. Applied survival analysis: regression modeling of time to event data. John Wiley and Sons, New York, NY, USA.
Kiriazis, J. & Slobodchikoff, C.N., 2006. Perceptual specificity in the alarm calls of Gunnison's prairie dogs. Behavioural Processes 73: 29-35.
Kunert, G., Otto, S., Röse, U.S.R., Gershenzon, J. & Weisser, W.W., 2005. Alarm pheromone mediates production of winged dispersal morphs in aphids. Ecology Letters 8: 596-603.
Lima, S.L. & Dill, L.M., 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology 68: 619-640.
van Maanen, R., 2012. One predator – two prey systems: Individual behaviour and population dynamics. PhD Thesis, University of Amsterdam, Amsterdam, The Netherlands.
MacDonald, K.M., Hamilton, J.G.C., Jacobson, R. & Kirk, W.D.J., 2003. Analysis of anal droplets of the Western flower thrips *Frankliniella occidentalis*. Journal of Chemical Ecology 29: 2385-2389.
Macedonia, J.M., 1993. The vocal repertoire of the Ringtailed Lemur (*Lemur catta*). Folia Primatologica 61: 186-217.
Macedonia, J.M. & Evans, C.S., 1993. Essay on contemporary issues in ethology: Variation among mammalian alarm call systems and the problem of meaning in animal signals. Ethology 93: 177-197.
Marler, P., 1967. Animal communication signals: We are beginning to understand how the structure of animal signals relates to the function they serve. Science 157: 769-784.
Napper, E. & Pickett, J.A., 2008. Alarm pheromones of insects. Encyclopedia of Entomology, 2nd edn (Ed. by Capinera, J.L.), pp. 85-90. Springer, Dordrecht, The Netherlands.
Nomikou, M., Janssen, A. & Sabelis, M.W., 2003. Phytoseiid predators of whiteflies feed and reproduce on non-prey food sources. Experimental and Applied Acarology 31: 15–26.
Pallini, A., Janssen, A. & Sabelis, M.W., 1998. Predators induce interspecific herbivore competition for food in refuge space. Ecology Letters 1: 171-177.
Pickett, J.A., Wadhams, L.J., Woodcock, C.M., Hardie, J., 1992. The chemical ecology of aphids. Annual Review of Entomology 37: 67-90.
Placer, J. & Slobodchikoff, C., 2000. A fuzzy-neural system for identification of species-specific alarm calls of Gunnison's prairie dogs. Behavioural Processes 52: 1-9.
Podjasek, J.O., Bosnjak, L.M., Brooker, D.J., Mondor, E.B., 2005. Alarm pheromone induces a transgenerational wing polyphenism in the pea aphid, *Acyrthosiphon pisum*. Canadian Journal of Zoology 83: 1138-1141.
R Development Core Team, 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project. org/.
Robinson, S.R., 1980. Antipredator behaviour and predator recognition in Belding's ground squirrels. Animal Behaviour 28: 840-852.

Robinson, S.R., 1981. Alarm communication in Belding's ground squirrels. Zeitschrift für Tierpsychologie 56: 150-168.
Sabelis, M.W. & van Rijn, P.C.J., 1997. Predation by insects and mites. Thrips as crop pests (Ed. by Lewis, T.), pp. 259-354. CAB International, Wallingford, UK.
Seyfarth, R.M., Cheney, D.L. & Marler, P., 1980. Vervet monkey alarm calls: Semantic communication in a free-ranging primate. Animal Behaviour 28: 1070-1094.
Sherman, P.W., 1977. Nepotism and the evolution of alarm calls. Science 197: 1246-1253.
Sih, A., 1980. Optimal behavior: can foragers balance two conflicting demands? Science 210: 1041–1043.
Teerling, C.R., Pierce, H.D., Borden, J.H. & Gillespie, D.R., 1993. Identification and bioactivity of alarm pheromone in the western flower thrips, *Frankliniella occidentalis*. Journal of Chemical Ecology 19: 681-697.
Venzon, M., Janssen, A., Pallini, A., Sabelis, M.W., 2000. Diet of a polyphagous arthropod predator affects refuge seeking of its thrips prey. Animal Behaviour 60: 369-375.
Verheggen, F.J., Haubruge, E., De Moraes, C.M and Mescher, M.C., 2009. Social enviroment influences aphid production of alarm pheromone. Behavioral Ecology 20: 283-286.
Ydenberg, R.C. & Dill, L.M., 1986. The economics of fleeing from predators. Advances in the Study of Behavior 16: 229-249.

Supplementary information

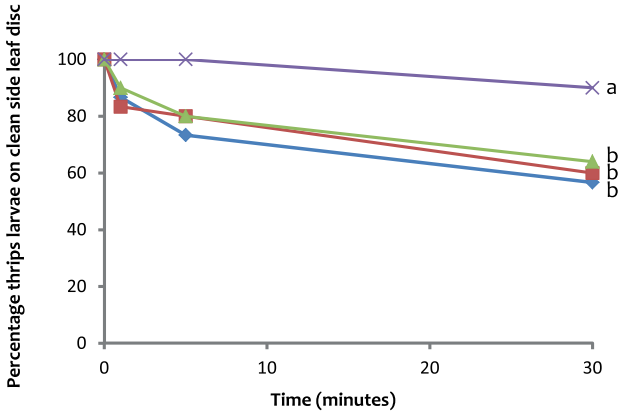


Figure S6-1 Percentage of predator-naive thrips larvae remaining outside the refuge 30 min after exposure to anal droplets. Different lines and symbols represent the four treatments: attack of a predatory bug (blue line with diamond markings), attack of a predatory mite (red line with squares), positive control (green line with triangles), negative control (purple line with crosses). N = 30 for all treatments, except when larvae were prodded with a brush, where N = 50. Treatments indicated with different letters exhibit significant difference in the frequency of refuge use.

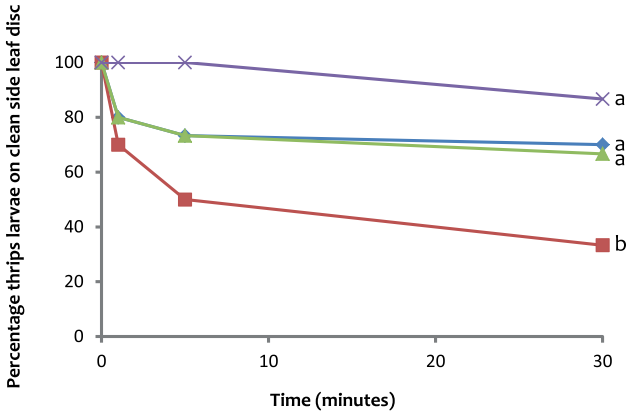


FIGURE S6-II Percentage of thrips larvae remaining outside the refuge 30 min after exposure to anal droplets. Larvae had survived for 12 h in the vicinity of a predatory bug. Different lines and symbols represent the four treatments: attack of a predatory bug (blue line with diamond markings), attack of a predatory mite (red line with squares), positive control (green line with triangles), negative control (purple line with crosses). N = 30 for all treatments. Treatments indicated with different letters exhibit significant difference in the frequency of refuge use.

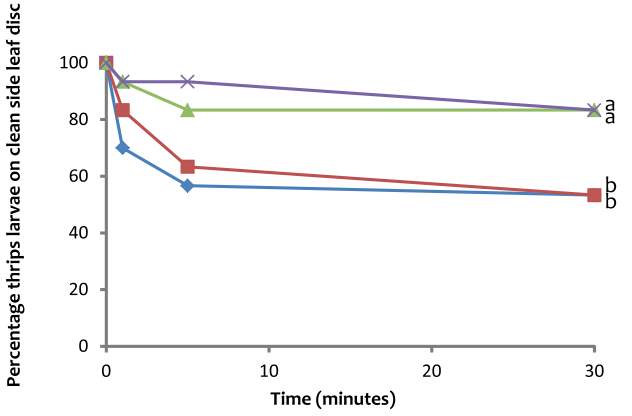


FIGURE S6-III Percentage of thrips larvae remaining outside the refuge 30 min after exposure to anal droplets. Larvae had survived for 12 h in the vicinity of predatory mites. Different lines and symbols represent the four treatments: attack of a predatory bug (blue line with diamond markings), attack of a predatory mite (red line with squares), positive control (green line with triangles), negative control (purple line with crosses). N = 30 for all treatments. Treatments indicated with different letters exhibit significant difference in the frequency of refuge use.

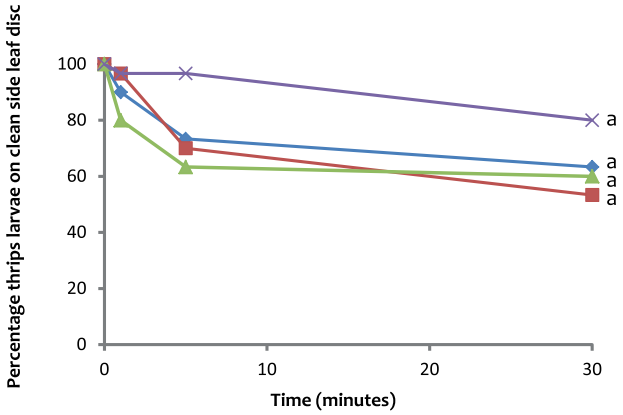
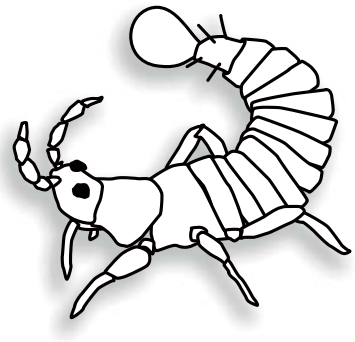


FIGURE S6-IV Percentage of thrips larvae remaining outside the refuge 30 min after exposure to anal droplets. Larvae had survived for 12 h in the vicinity of both predatory bug and mites. Different lines and symbols represent the four treatments: attack of a predatory bug (blue line with diamond markings), attack of a predatory mite (red line with squares), positive control (green line with triangles), negative control (purple line with crosses). N = 30 for all treatments, except when larvae were attacked by a predatory mite, where N = 29. No significant differences were found.

Effects of kinship or familiarity? Small thrips larvae experience lower predation risk only in groups of mixed-size siblings

Paulien J.A. de Bruijn, Maurice W. Sabelis & Martijn Egas



7

Abstract

In many species of insects, larvae are distributed in an aggregated fashion. As they may differ in size and size matters to predation risk, small larvae may be less likely to fall prey to predators when near large and therefore better-defended larvae. We hypothesize that the small larvae may profit even more when these large larvae are siblings. We tested this hypothesis on kinship-dependent survival in groups of larvae of the Western flower thrips (*Frankliniella occidentalis*) exposed to a predatory mite (*Iphiseius degenerans*). Our experiments showed that small larvae in sibling groups survive significantly better than in non-sibling groups, but only when such groups consisted of a mixture of small and large larvae. To test whether the survival effect we found is due to familiarity of thrips larvae growing up together (i.e., on one leaf), we also measured survival in sibling groups of larvae grown up on different leaves and in non-sibling groups of larvae grown up on the same leaf. These experiments showed increased survival of small thrips larvae only in groups of sibling larvae from the same leaf. Non-sibling larvae did not show an increased survival when they come from the same leaf. Our results indicated that the increased survival in sibling groups was only partly due to the familiarity effect we tested. Growing up together did not return the same survival effect for non-siblings as it did for siblings. We conclude that growing up together is a necessary but not sufficient condition for discrimination in thrips larvae.

Behavioral Ecology and Sociobiology 68: 1029-1035 (2014)

Introduction

Kin discrimination and its effects on fitness have been well studied in mammals (Silk 2002), birds (Komdeur and Hatchwell 1999), amphibians (Blaustein and Waldman 1992) and social insects (e.g., Queller and Strassmann 1998, but see also Keller 1997). For example, when presented with a predator model (stuffed badger), black-tailed prairie dogs call alarm more frequently when they are in groups with close genetic relatives than when they are in groups without (Hoogland 1983). In this way kin discrimination serves to direct potentially beneficial behaviour towards relatives, rather than towards unrelated individuals.

In non-social arthropods, kin discrimination received much less attention (Fellowes 1998, for specific examples see: Jasienski *et al.* 1988; Faraji *et al.* 2000; Magalhães *et al.* 2005; Schausberger 2007). Fellowes (1998) identified six behavioural elements in non-social arthropods that could be biased by relatedness: (1) resource exploitation, (2) sex allocation under local mate competition, (3) inbreeding, (4) cannibalism, (5) superparasitism, and (6) aggregation when exposed to predation risk, which is the element of interest in this study.

Here, we focus on effects of aggregation behaviour in response to predation risk. In such a situation, the composition of a group can be important for survival of individuals, especially when some individuals in a group are more vulnerable to predation than others. Vulnerability often depends on the size of an individual, because many predators attack prey of different sizes. For example, some predators attack only the smallest individuals (Lima and Dill 1990) or individuals from a certain size range (Tonn *et al.* 1992; Chase 1999). In the former case, smaller prey individuals can experience decreased predation risk in the vicinity of larger individuals because larger prey individuals may actively or inactively hinder the predator before or during an attack on smaller prey individuals. Such decreased predation risk for smaller individuals may be expected when the relatedness among prey individuals is high enough (conform kin selection, Hamilton 1964; however, see van Veelen 2009). We therefore hypothesize that small individuals (i.e., the preferred prey) will experience increased survival when near larger siblings.

To test this hypothesis, we use the Western flower thrips, *Frankliniella occidentalis* (Pergande). Thrips are suitable for an experimental approach to answer this question, for four reasons. First, the difference in size between first- and second-instar larvae is considerable (factor 1.5 in length and in width, and 1.6 in height; PJAdB, personal observation). Second, first- and second-instar thrips larvae occur together in groups on leaves. Third, thrips larvae are preyed upon by many different predators, differing in size, attack rate as well as attack success. Fourth, thrips have defensive traits that reduce the attack success of their pred-

ators. Upon encounter with a predator, thrips quickly move their abdomen to and fro (here referred to as abdominal swings), trying to hit the predator, and, when the threat of predation persists, they release anal droplets that contain an alarm pheromone and cause a predator to retreat and groom (Bakker and Sabelis 1989; de Bruijn *et al.* 2006). The effectiveness of these traits depends on the size of the predator encountered as well as on the size of the thrips larvae (Bakker and Sabelis 1987). In this study, we use *Iphiseius degenerans*, a blind predatory mite of ca. 0.5 mm that mostly attacks first-instar thrips larvae (ca. 0.75 mm; PJAdB, personal observation) (Faraji *et al.* 2001), and has difficulties in attacking second-instar larvae (ca. 1.0 mm; PJAdB, personal observation). When this predatory mite approaches a thrips larva at its flank, they may wrestle for some time, during which the predator usually tries to lift the thrips from the surface and to feed from the, then defenceless, thrips (PJAdB, personal observation). The chance that a first-instar larva survives an attack by a predatory mite is ca. 30%, while this chance is ca. 70% for second-instar larvae (PJAdB, personal observation). Given these characteristics of the predator-prey system under study, we predict that first-instar thrips larvae have a higher chance to survive predation by *I. degenerans* when living in groups with second-instar thrips larvae that are siblings.

To test this hypothesis, we measured survival of thrips larvae under predation in groups with same-sized individuals (all small or all large) and in mixed groups (half small and half large), both for groups where all individuals are siblings and groups where all individuals are non-siblings. To test how thrips discriminate kin, we added treatments with sibling groups where small and big larvae had never encountered each other before, and with non-sibling groups where larvae had grown up together on the same leaf.

Material and methods

Thrips

Western flower thrips were collected from cucumber plants in a commercial greenhouse near Pijnacker, The Netherlands, in February 2006. Thrips were subsequently reared in a climate box (25 °C, 60% RH, L16:D8) on cucumber leaves, cut to fit in a Petri dish on top of a layer of cotton wool that was put on the bottom of the Petri dish. Once a week, thrips pupae and adults from older leaves of the culture were put on the cucumber leaf and pollen of *Typha latifolia* was provided on this leaf as additional food for the thrips. From the eggs produced by the adult females, thrips larvae hatched. The emerging pupae and adults were then trans-

ferred to a new leaf in a new Petri dish to rear a next generation of thrips. This procedure was repeated to maintain a culture. The lab culture usually contained at least 500 individuals, with an occasional dip of ca. 200 individuals.

Predatory mites

The predatory mite *Iphiseius degenerans,* originating from Rabat, Morocco, was reared on a diet of *Typha* pollen in a climate box at 25 °C, 60% RH and L16:D8. The rearing arenas consisted of a PVC sheet (6 × 15 cm) placed on a wet sponge in a water-containing tray. The edges of the PVC sheet were covered with paper tissue that absorbs water from the sponge underneath. The tissue served as a water source to the predatory mites and as a barrier to prevent escape from the PVC arena. Short threads of cotton placed on the PVC sheet served as a substrate for oviposition by the predatory mites. For the experiments, we used adult females, 8-15 days old since hatching and 0.7 mm in length.

Experimental setup to measure survival under predation

Adult females were put each on a separate leaf fragment to lay eggs. Four to eight days later, larvae were collected from these leaf fragments. To establish sibling groups, 10 larvae were collected from a single leaf fragment (i.e., all offspring from the same mother), whereas to establish non-sibling groups, each of the 10 larvae was collected from a different leaf fragment. Because adult female thrips can lay 4-5 eggs per day (van Rijn *et al.* 1995), our setup enabled us to collect 10 similar-sized larvae from one leaf disc as well as from 10 different leaf fragments.

For the survival experiments, arenas were prepared in the following way. A leaf disc (diameter 24 mm), excised from the cotyledon of a cucumber plant, was put on a layer of wet cotton wool in a plastic cup (height 70 mm, diameter 66 mm). The cup had a lid with an opening covered with gauze, to prevent the arena from becoming too humid. Ten thrips larvae (either 10 first-instar larvae, or 10 second-instar larvae, or five first-instar larvae and five second-instar larvae), either all sibling or all non-sibling as described above, were put on the leaf disc and a single *I. degenerans* predator was added. From September 2006 until May 2007, for 5 days, twice per day (mornings between 10:00 and 11:00 AM, and afternoon between 6:00 and 7:00 PM), the thrips larvae that were present and alive were counted. Because we were unable to observe the larvae in our experiment continuously, we do not know whether larvae that were missing were killed by the predator or drowned in the water barrier surrounding the leaf disc but we considered them to be consumed by the predator. The instar of the larvae (first

or second) was also noted. In the groups of thrips larvae with mixed sizes, first-instar larvae that developed into the next instar were scored as second-instar larvae. When a thrips reached the pre-pupal state, it was removed and censored as a survivor. This was done because under natural conditions pre-pupal thrips leave the plant. Any replicate where a predator had died before the end of the experiment was discarded. In total, at least 20 replicates of each treatment were scored. As a control for causes of death other than predation, we performed the same experiments without a predatory mite. All survival experiments were performed in a climate room (25 °C, 60% RH, and L16:D8 photoperiod).

Experimental setup to test for kin discrimination

To test the possible mechanisms thrips larvae use to discriminate kin, we conducted a test similar to the one used for survival under predation, with a few modifications. These experiments were conducted in the laboratory (ca. 21 °C and ca. 50% RH, natural daylight), in the period from March to May 2013. Five first-instar thrips larvae and five second-instar thrips larvae were introduced on an arena as described above with an *I. degenerans* predator, and then the surviving first-instar and second-instar larvae were counted 6 h later. Compared to the 'survival under predation' set-up, we composed two more groups: 'sibling-different-leaf' (SDL) and 'non-sibling-same-leaf' (NSSL). For this, we put single adult females on separate leaf discs, and moved the females after 1, 2 and 3 days to new, clean, leaf discs. In this way, we established three groups of larvae, all siblings, that had never encountered a sibling of the other groups before. We created an SDL group by selecting five first-instar larvae from the youngest group of siblings and a total of five second-instar larvae from the two older groups of siblings. As before, we selected similar-sized larvae, but due to the different age of second-instar siblings on different leaf fragments, their variation in size was slightly higher than in the SSL treatment. An NSSL group was created by putting 10-15 females on a leaf fragment. To treat the NSSL group similar as the SDL group, we picked NSSL females up and put them back on the same leaf fragment on the same days as adult females for the SDL treatment were moved to different leaf fragments. After eight days we randomly took five similar-sized first-instar larvae and five similar-sized second-instar larvae from these leaf fragments. It was not possible for us to check the relatedness of the 10 larvae, but we can reasonably assume most larvae in each replicate were non-siblings. We composed groups with sibling larvae from one leaf fragment and non-sibling larvae from different leaf fragments as described above [henceforth called 'sibling-same-leaf (SSL) and 'non-sibling-different-leaf' (NSDL)], with one addition: thrips

females were picked up and put back on the leaf fragment on the same days as adult females for the SDL treatment were moved to different leaf fragments. Because the treatments SSL and NSSL differed in kinship of the thrips larvae as well as density of the thrips on the leaf fragment, and SDL and NSDL differed in kinship of the thrips larvae as well as the number of leaf fragments they were collected from, we compare only SSL with SDL and NSSL with NSDL to test for the effect of growing up on the same leaf.

Statistical analysis

To compare survival among treatments of each of the two experiments, we applied a GLM assuming a Poisson distribution of the number of dead larvae. Effects of treatment were tested in this GLM using a one-way ANOVA. All analyses were done using the open source program R (R Development Core Team 2010).

Results

Kin survival

When first- and second-instar larvae were put together on a leaf disc with a predatory mite, the difference in survival of larvae between sibling and non-sibling groups was significant after 8 h (Figure 7-1a, left panel; GLM: deviance = 4.2, d.f. = 1, $P<0.05$). The difference in survival between siblings and non-siblings in the groups starting with 10 first-instar larvae or 10 second-instar larvae was not significant (Figure 7-1b, c, left panels; GLM, first-instar larvae: deviance = 0.06, d.f. = 1, $P = 0.81$; second-instar larvae: deviance = 0.003, d.f. = 1, $P = 0.95$). In the treatment with first- and second-instar larvae and the treatment with exclusively first-instar larvae, most thrips did not survive up to the end of the experiment, but in the treatment with exclusively second-instar larvae, on average 4.6 of the 10 thrips larvae survived.

To further analyse the difference in survival of thrips larvae within mixed groups (as in Figure 7-1a, left panel), survival of first- and second-instar larvae is shown separately in Figure 7-2. The largest difference in survival between siblings and non-siblings was found in the first-instar larvae and this difference became manifest after ca. 8 h. Here, the difference in survival of first-instar larvae was significant (Figure 7-2; GLM: deviance = 4.3, d.f. = 1, $P<0.05$) but the difference in survival of second-instar larvae was not significant (Figure 7-2; GLM: deviance = 0.1, d.f. = 1, $P = 0.8$).

After 1 or 2 days, all first-instar larvae had developed into (and were therefore counted as) second-instar larvae. This explains the increase in the number of second-instar sibling-larvae after 1.3 days. FIGURE 7-2 shows that the difference in survival found for this mixed-size group, is mostly explained by the difference in survival of the first-instar larvae.

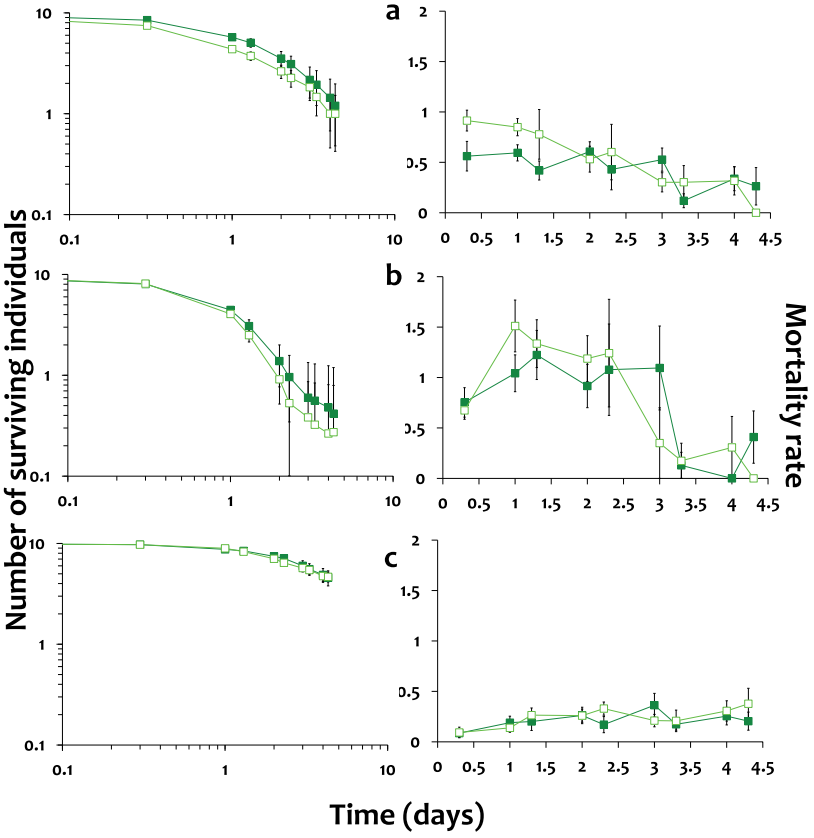


FIGURE 7-1 Survival (left panels) and mortality rate (right panels) of thrips larvae in presence of the predatory mite *I. degenerans*. On the x-axes is the time in days. On the y-axis of the left panels is the mean (± SE) number of surviving thrips larvae during 4.3 days in sibling groups (dark green filled boxes) or non-sibling groups (light green open boxes) that were composed of (**a**) five first-instar and five second-instar larvae (N = 20 for sibling groups, 19 for non-sibling groups), (**b**) 10 first-instar larvae (N = 31 for sibling groups, 35 for non-sibling groups), and (**c**) 10 second-instar larvae (N = 19 for sibling groups, 19 for non-sibling groups). To facilitate comparison of the survival data, the right panels show the corresponding mean (± SE) mortality rates (day^{-1}) calculated from the survival measurements. Note the difference in mortality rate at the start of the experiment in panel (**a**).

As a control for causes of death other than the presence of predator in the mixed-size groups, we repeated the experiment, but now without a predator (FIGURE 7-3). In this control experiment, there was no significant difference between sibling and non-sibling individuals after 8 h (GLM: deviance = 0.003, d.f. = 1, P = 0.95). We found that after 4.3 days, 6-7 individuals survived instead of less than two in the experiments with a predator.

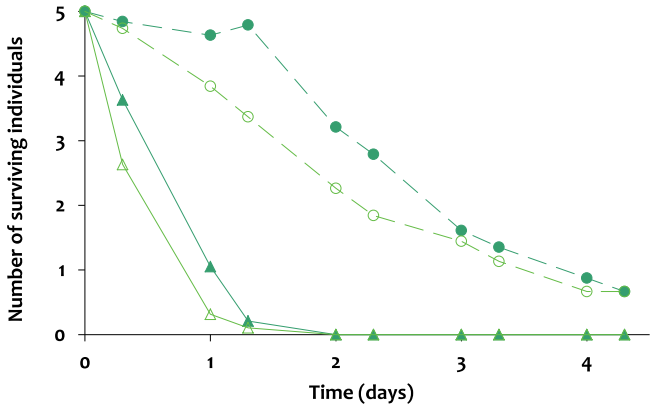


FIGURE 7-2 Survival of thrips larvae in mixed-size groups of siblings or non-siblings during 4.3 days. The data are the same as in FIGURE 7-1a, but displayed separately for first-instar larvae and second-instar larvae. On the x-axis is the time in days, on the y-axis the fraction of surviving individuals. First-instar larvae are presented with triangles and a solid line, second-instar larvae with circles and dotted line; sibling groups in dark green and non-sibling groups in light green.

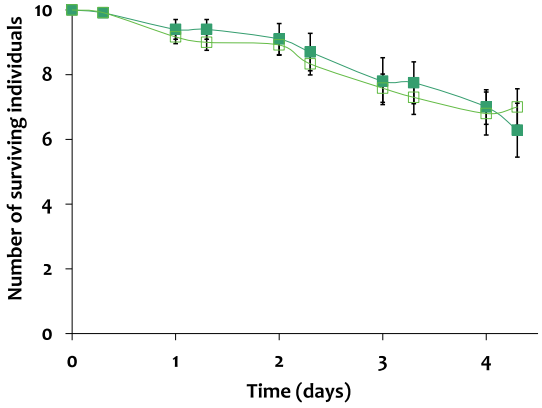


FIGURE 7-3 Survival of thrips larvae in mixed-size groups of siblings or non-siblings during 4.3 days in absence of predation. On the x-axis is the time in days, on the y-axis the mean (± SE) number of surviving individuals. N = 12 for both sibling groups (dark green filled boxes) and non-sibling groups (light green open boxes).

Kin discrimination

For sibling thrips larvae, we found a higher survival when the larvae have encountered each other before (FIGURE 7-4; GLM, first- and second-instar larvae together: deviance = 13.0, d.f. = 1, P<0.01). For non-sibling larvae, we did not find this difference (FIGURE 7-5; GLM, first- and second-instar larvae together: deviance = 2.6, d.f. = 1, P = 0.1).

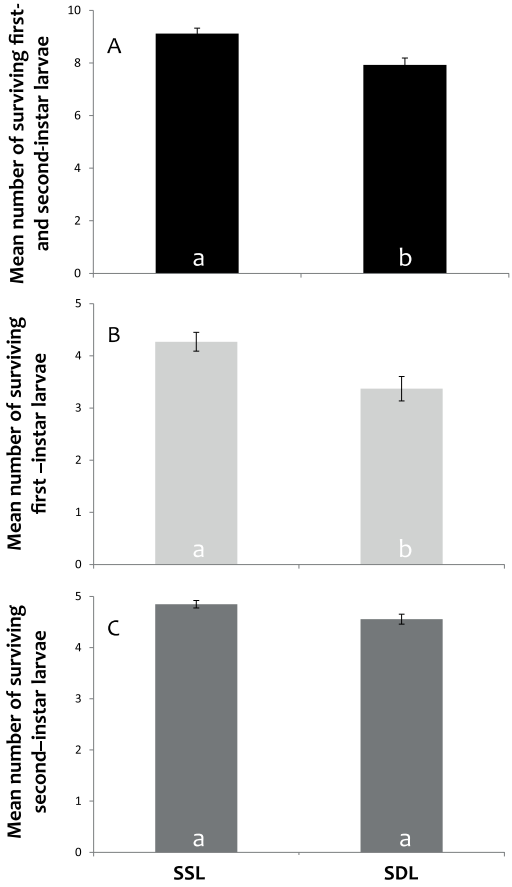


FIGURE 7-4 Survival of sibling thrips larvae in mixed-size groups after 6 h of exposure to predation. On the x-axis are the treatments, SSL refers to Sibling Same Leaf, SDL refers to Sibling Different Leaf. On the y-axis is the mean (± SE) number of surviving individuals. The number of replicates is 26 for SSL, 27 for SDL. (**A**) Survival of the first- and second-instar larvae together. (**B**) Survival of the first-instar larvae. (**C**) Survival of the second-instar larvae.

Discussion

When thrips larvae of different sizes occur in groups, small sibling larvae survive better than small non-sibling larvae. However, kinship does not influence larval survival in uniform-size groups. What causes the increased survival in mixed-size sibling groups? First-instar larvae are consumed more frequently by *I. degenerans* than second-instar larvae (FIGURE 7-1b, c). The difference in survival in the mixed-size groups is mainly due to increased survival of first-instar larvae in the sibling

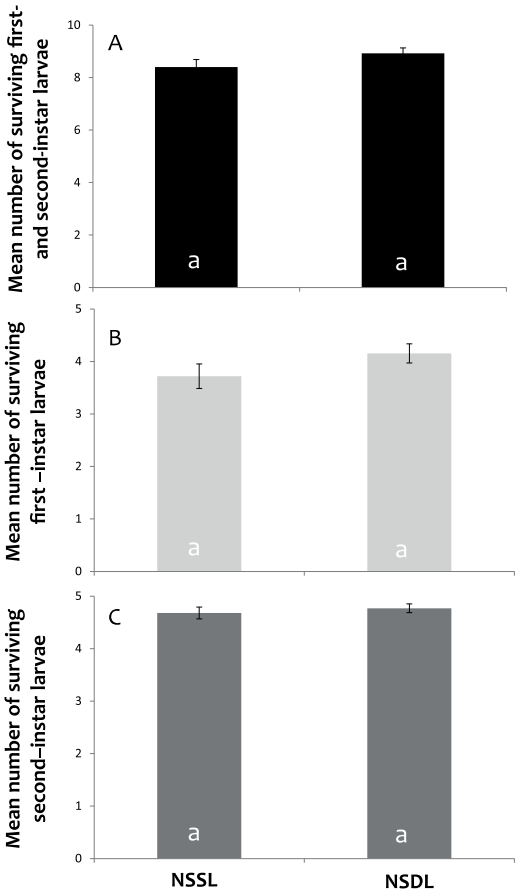


FIGURE 7-5 Survival of non-sibling thrips larvae in mixed-size groups after 6 h of exposure to predation. On the x-axis are the treatments, NSSL refers to Non-Sibling Same Leaf, NSDL refers to Non-Sibling Different Leaf. On the y-axis is the mean (± SE) number of surviving individuals. The number of replicates is 25 for NSSL, 26 for NSDL. (**A**) Survival of the first- and second-instar larvae together. (**B**) Survival of the first-instar larvae. (**C**) Survival of the second-instar larvae.

group (Figure 7-2). This difference becomes manifest after half a day. Thereafter, also a difference in second-instar larvae emerges, but this is because from day two on, first-instar larvae develop into second-instar larvae, and are subsequently scored as second-instar larvae. Because more first-instar larvae survive, we find more second-instar larvae from day two onwards. In absence of a predator, survival is high for both sibling and non-sibling groups (Figure 7-3). Hence, the data supports our hypothesis that the presence of second-instar larvae increases the survival of sibling first-instar larvae under predation by *I. degenerans*. We are not aware of other studies testing if vulnerable prey experience increased survival when in the vicinity of less vulnerable siblings. This kind of kinship effects, however, may occur generally in prey species with stages that vary in vulnerability to predators.

In these experiments, we find a very clear effect of kinship, despite the fact that the adult thrips females that are used to create groups of sibling thrips, but also non-sibling thrips, come from the same culture that we had maintained for multiple generations in our lab. This means that non-sibling thrips in our experiment are probably more related than non-sibling thrips in the field. Together, this leads to two, mutually non-exclusive predictions: (a) the effect of kinship would be even more pronounced with individuals from a natural population, or (b) thrips larvae recognise siblings when they have grown up with them, i.e., a familiarity effect.

We provide evidence that familiarity does contribute to the effect of kinship on larval survival. Sibling thrips larvae survive being near a predatory mite better when they all come from one leaf fragment than when the larvae have grown up on one of three different leaf fragments (Figure 7-4). This suggests that thrips larvae need to learn about the other sibling thrips before they can discriminate them as kin. For non-sibling larvae, we find no difference in survival between groups that come from one leaf fragment and groups that come from 10 leaf fragments (Figure 7-5) even though the groups of non-sibling larvae that come from one leaf fragment might contain some sibling individuals. This suggests that growing up on the same leaf fragment is not enough for thrips larvae to discriminate kin from non-kin.

Our results show that there is some form of kin discrimination in thrips larvae. This adds to the body of literature on kin recognition in non-social arthropods (Fellowes 1998; Gherardi *et al.* 2012). The way thrips recognise each other determines whether thrips help genuine kin or not. Individuals can recognise kin by cues that are determined by the genotype of this sibling, or by cues that come from the shared environment of the two siblings. There are many mechanisms that would allow recognition, both from genetic and environmental cues (for

example cuticular hydrocarbons (CHCs), see Singer 1998; for a specific example, see Weddle *et al.* 2013a). In ants, examples of cues from the environment that influence nest mate recognition include diet, ambient odours, and nest material (Obin 1986). If recognition occurs through environmentally determined cues, the cue is indirect and hence individuals could fail to recognise siblings from a different location or individuals could fail to distinguish between kin and non-kin from a common location. However, if recognition occurs through genotypically determined cues, individuals recognise kin by a direct cue, independent of a common environment (as shown for CHCs in Lihoreau *et al.* 2007). Gerlach *et al.* (2008) showed that for zebra fish, both genotypically and environmentally determined cues are necessary for kin recognition. Larvae are sensitive to olfactory cues of kin individuals in a specific time frame during development, in which imprinting on kin odours may occur. However, when larvae are exposed to non-kin cues in this time frame, imprinting did not occur. Our findings for thrips larvae are similar. Survival of sibling thrips larvae is higher when they have grown up together, but this effect is not found in non-sibling thrips larvae. These results suggest that kin recognition in thrips larvae requires environmentally as well as genotypically determined cues. In particular, it may be that the enhanced effect of growing up together is mediated by self-referent phenotype matching, i.e., an individual may only imprint on kin odours when these are sufficiently similar to those of itself. Self-referencing is a widespread mechanism in arthropods, often involving CHCs as cues (Weddle *et al.* 2013b), but it has not yet been studied in thrips. Several studies have characterized CHCs for the Western flower thrips (Gołębiowski *et al.* 2007; Zhao *et al.* 2011), and one of these CHCs is known to act as a male recognition pheromone (Olaniran *et al.* 2013). We therefore hypothesize that Western flower thrips are capable of kin recognition by self-referent phenotype matching using CHCs.

Acknowledgments

PJAdB was supported by a grant from The Netherlands Organization for Scientific Research (NWO, project number 818.01.018).

References

Bakker FM, Sabelis MW (1987) Attack success of *Amblyseius mckenziei* and the stage related defensive capacity of thrips larvae. Neth J Zool 37: 437.

Bakker FM, Sabelis MW (1989) How larvae of *Thrips tabaci* reduce the attack success of phytoseiid predators. Entomol Exp Appl 50: 47-51.

Blaustein AR, Waldman B (1992) Kin recognition in anuran amphibians. Anim Behav 44: 207-221.

Chase JM (1999) Food web effects of prey size refugia: Variable interactions and alternative stable equilibria. Am Nat 154: 559-570.
de Bruijn PJA, Egas M, Janssen A, Sabelis MW (2006) Pheromone-induced priming of a defensive response in western flower thrips. J Chem Ecol 32: 1599-1603. [Chapter 2 of this thesis]
de Roos AM, Persson L (2013) Population and community ecology of ontogenetic development. Monographs in Population Biology 51. Princeton University Press, Princeton, NJ, USA.
Faraji F, Janssen A, Sabelis MW (2001) Predatory mites avoid ovipositing near counterattacking prey. Exp Appl Acarol 25: 613-623.
Faraji F, Janssen A, van Rijn PCJ, Sabelis MW (2000) Kin recognition by the predatory mite *Iphiseius degenerans*: discrimination among own, conspecific, and heterospecific eggs. Ecol Entomol 25: 147-155.
Fellowes MDE (1998) Do non-social insects get the (kin) recognition they deserve? Ecol Entomol 23: 223-227.
Gerlach G, Hodgins-Davis A, Avolio C, Schunter C (2008) Kin recognition in zebrafish: a 24-hour window for olfactory imprinting. P Roy Soc B-Biol Sci 275: 2165-2170.
Gherardi F, Aquiloni L, Tricarico E (2012) Revisiting social recognition systems in vertebrates. Anim Cogn 15: 745-762.
Gołębiowski M, Maliński E, Nawrot J, Szafranek J, Stepnowski P (2007) Identification of the cuticular lipid composition of the western flower thrips *Frankliniella occidentalis*. Comp Biochem Physiol B 147: 288-292.
Hamilton WD (1971) Geometry for the selfish herd. J Theor Biol 31: 295-311.
Hoogland JL (1983) Nepotism and alarm calling in the black-tailed prairie dog (*Cynomys ludovicianus*). Anim Behav 31: 472-479.
Jasienski M, Korzeniak U, Lomnicki A (1988) Ecology of kin and nonkin larval interactions in *Tribolium* beetles. Behav Ecol Sociobiol 22: 277-284.
Keller L (1997) Indiscriminate altruism: Unduly nice parents and siblings. Trends Ecol Evol 12: 99-103.
Komdeur J, Hatchwell BJ (1999) Kin recognition: function and mechanism in avian societies. Trends Ecol Evol 14: 237-241.
Lihoreau M, Zimmer C, Rivault C (2007) Kin recognition and incest avoidance in a group-living insect. Behav Ecol 18: 880-887.
Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. Can J Zool 68: 619-640.
Lion S, van Baalen M (2008) Self-structuring in spatial evolutionary ecology. Ecol Lett 11: 277-295.
Magalhães S, Janssen A, Montserrat M, Sabelis MW (2005) Prey attack and predators defend: counterattacking prey trigger parental care in predators. P Roy Soc B-Biol Sci 272: 1929-1933.
Obin MS (1986) Nestmate recognition cues in laboratory and field colonies of *Solenopsis invicta* Buren (Hymenoptera, Formicidae) – Effect of environment and role of cuticular hydrocarbons. J Chem Ecol 12: 1965-1975.
Okamura B (1986) Group living and the effects of spatial position in aggregations of *Mytilus edulis*. Oecologia 69: 341-347.

Olaniran OA, Sudhakar AVS, Drijfhout FP, Dublon IAN, Hall DR, Hamilton JGC, Kirk WDJ (2013) A Male-predominant cuticular hydrocarbon, 7-methyltricosane, is used as a contact pheromone in the Western flower thrips *Frankliniella occidentalis*. J Chem Ecol 39: 559–568.

Queller DC, Strassmann JE (1998) Social insects provide the most surprising predictions and satisfying tests of kin selecton. Bioscience 48: 165-175.

R Development Core Team (2010) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

van Rijn PCJ, Mollema C, Steenhuis-Broers GM (1995) Comparative life-history studies of *Frankliniella occidentalis* and *Thrips tabaci* (Thysanoptera: Thripidae) on cucumber. B Entomol Res 85: 285-297.

van Rijn PCJ, Tanigoshi LK (1999) The contribution of extrafloral nectar to survival and reproduction of the predatory mite *Iphiseius degenerans* on *Ricinus communis*. Exp Appl Acarol 23: 281-296.

Shykoff JA, Schmid-Hempel P (1991) Genetic relatedness and eusociality- parasite-mediated selection on the genetic composition of groups. Behav Ecol Sociobiol 28: 371-376.

Schausberger P (2007) Kin recognition by juvenile predatory mites: prior association or phenotype matching? Behav Ecol Sociobiol 62: 119-125.

Silk JB (2002) Kin selection in primate groups. Int J Primatol 23: 849-875.

Singer TL (1998) Roles of hydrocarbons in the recognition systems of insects. Am Zool 38: 394-405.

Tonn WM, Paszkowski CA, Holopainen IJ (1992) Piscivory and recruitment: mechanisms structuring prey populations in small lakes. Ecology 73: 951-958.

van Veelen M (2009) Group selection, kin selection, altruism and cooperation: When inclusive fitness is right and when it can be wrong. J Theor Biol 259: 589–600.

Weddle CB, Hunt J, Sakaluk SK (2013a) Self-referent phenotype matching and its role in female mate choice in arthropods. Curr Zool 59: 239-248.

Weddle CB, Steiger S, Hamaker CG, Ower GD, Mitchell C, Sakaluk SK, Hunt J (2013b) Cuticular hydrocarbons as a basis for chemosensory self-referencing in crickets: a potentially universal mechanism facilitating polyandry in insects. Ecol Lett 16: 346-353.

West SA, Pen I, Griffin AS (2002) Conflict and cooperation – Cooperation and competition between relatives. Science 296: 72-75.

Zhao C-Y, He Y-R, Zhong F, Gao Y, Qi G-J, Shao X-Y, Lv L-H (2011) Analysis of cuticular hydrocarbons of *Frankliniella occidentalis*. Chin J Appl Entomol 48: 536–541.

Commentary

Taking care of group size and heterogeneity in social recognition systems

Peter Schausberger

Behavioral Ecology and Sociobiology 68: 1561-1562 (2014)

Scrutinizing the relative effects and inter-relations of genetic relatedness per se and familiarity (established independently, but maybe used as a proxy, of genetic relatedness) in social recognition systems is, owing to their fundamental evolutionary significance, an important but experimentally challenging task. De Bruijn *et al.* (2014) report that immature thrips *Frankliniella occidentalis* living in sibling groups have higher survival chances under predation risk when they are familiar than unfamiliar whereas this is not the case in non-sibling groups. The authors conclude that (1) direct familiarity enhances survival of thrips under predation risk and (2) thrips only familiarize with kin or kinship is needed for generating beneficial effects of familiarity under predation risk. Conclusion (1) is straightforward and clearly supported by the presented experiments; conclusion (2) is invalid.

Possible effects of prior association, leading to direct familiarity based recognition (e.g., Blaustein and Porter 1996; Mateo 2004), are commonly constrained by referent number and heterogeneity (label variability) during familiarization (e.g., Reeve 1989; Griffiths and Magurran 1997; Liebert and Starks 2004; Croney and Newberry 2007). Due to cognitive limitations, these constraints principally apply to both social recognition mechanisms based on familiarity via prior association, learning and memorizing distinct individual labels of the referents (direct familiarity), and its extension, generalizing on a shared feature of the referents (indirect familiarity, phenotype matching). Accordingly, the acceptance thresholds of the actors (the discriminating individuals) depend on and vary with the number and variability of referents (the label carrying individuals) encountered during the phase of neural template formation (Reeve 1989; Liebert and Starks

2004). The larger the group size, the higher the number of referents encountered, the less likely the chance of encountering every single possible referent, the more difficult forming and storing neural templates of every individual label, the less precise the individual neural templates formed, the more likely formation of a generalized template, the more liberal the acceptance threshold of the actor upon encountering conspecific individuals to be recognized (i.e., the recipients), the higher the chance of recognition errors. The tight linkage between acceptance thresholds and number and variability of referents/recipients is disregarded in experiment 2 of de Bruijn *et al.* (2014).

In experiment 2 of de Bruijn *et al.* (2014), sibling and nonsibling group size was the same during the experiment on survival under predation risk, but sibling and non-sibling group size and label heterogeneity differed decisively during the pre-experimental familiarization phase. Preexperimentally, siblings grew up in groups of 10 to 15 individuals, whereas non-siblings grew up in >10 times larger groups. De Bruijn *et al.* (2014) placed 1 vs. 10 to 15 ovipositing thrips females on the same leaf for 4 days giving rise to larvae used in sibling and non-sibling groups, respectively (at 21 °C, each thrips female deposits two to three eggs per day on cucumber—see Deligeorgidis *et al.* 2006). Following is that due to largely differing pre-experimental group sizes, the likelihood of mutual encounter between any pair of individuals, and with that the opportunity to mutually familiarize, was much lower in non-sibling than sibling groups. Additionally, due to the high number and associated variability of founder females of the non-sibling groups, referent label variability was much higher in non-sibling than sibling groups. While de Bruijn *et al.* (2014) acknowledge the difference in pre-experimental group size of siblings and nonsiblings and separately analyzed survival of familiar vs. unfamiliar thrips, they conclude from the separate analyses on genuine kinship effects, i.e., that familiarity is only established, or does only have an effect, in sibling but not non-siblings. This conclusion is invalid because confounded by pre-experimental group size (10 to 15 for siblings vs. >100 for non-siblings), casting doubt on that the observed familiarity effects have anything to do with kinship per se. The underlying recognition mechanism in the sibling group seems direct familiarity but, in general, both direct and indirect familiarization (leading to recognition via direct and indirect familiarity sensu Blaustein and Porter 1996 or via prior association and phenotype matching sensu Mateo 2004) are influenced by group size and heterogeneity and more likely in small and/or homogeneous than large and/or heterogeneous groups. Group sizes of >100 individuals, such as in the preexperimental non-sibling groups, are also far outside the range observed in *F. occidentalis* on wild plants (Chellemi *et al.* 1994), rendering unnecessary the evolution of cognitive abilities to familiarize with that many individuals.

Experiments adequately addressing the relative effects of genetic relatedness per se and social familiarity need to compare the survival of siblings and non-siblings that grew up in similarly sized groups and, to test for the effects of group heterogeneity (referent label variability), of individual siblings and non-siblings, both of which grew up in sibling groups. Only such rigorous protocols allow concluding on whether the effects of social familiarity indeed require close genetic relatedness, because familiarity is only established, or later only expressed, in groups consisting of genetically closely related individuals, or occur independently of genetic relatedness (e.g., Griffiths and Magurran 1999; Komdeur *et al.* 2004; Schausberger 2007; Strodl and Schausberger 2012).

References

Blaustein AR, Porter RH (1996) The ubiquitous concept of recognition with special reference to kin. In: Bekoff M, Jamieson D (eds) Readings in animal cognition. MIT, Cambridge, MA, USA, pp. 169–184.

Chellemi DO, Funderburk JE, Hall DW (1994) Seasonal abundance of flower-inhabiting *Frankliniella* species (Thysanoptera: Thripidae) on wild plant species. Environ Entomol 23: 337–343.

Croney CC, Newberry RC (2007) Group size and cognitive processes. Appl Anim Behav Sci 103: 215–228.

de Bruijn PJA, Sabelis MW, Egas M (2014) Effects of kinship or familiarity? Small thrips larvae experience lower predation risk only in mixed-size sibling groups. Behav Ecol Sociobiol 68: 1029–1035.

Deligeorgidis PN, Giakalis L, Sidiropoulos G, Vaiopoulou M, Kaltsoudas G, Ipsilandis CG (2006) Longevity and reproduction of *Frankliniella occidentalis* and *Thrips tabaci* on cucumber under controlled conditions. J Entomol 3: 61–69.

Griffiths SW, Magurran AE (1997) Schooling preferences for familiar fish vary with group size in a wild guppy population. Proc R Soc Lond B 264: 557–561.

Griffiths SW, Magurran AE (1999) Schooling decisions in guppies (*Poecilia reticulata*) are based on familiarity rather than kin recognition by phenotype matching. Behav Ecol Sociobiol 45: 437–443.

Komdeur J, Richardson DS, Burke T (2004) Experimental evidence that kin discrimination in the Seychelles warbler is based on association and not on genetic relatedness. Proc R Soc Lond B 271: 963–969.

Liebert AE, Starks PT (2004) The action component of recognition systems: a focus on the response. Ann Zool Fenn 41: 747–764.

Mateo JM (2004) Recognition systems and biological organization: the perception component of social recognition. Ann Zool Fenn 41: 729–745.

Reeve HK (1989) The evolution of conspecific acceptance thresholds. Am Nat 133: 407–435.

Schausberger P (2007) Kin recognition by juvenile predatory mites: prior association or phenotype matching? Behav Ecol Sociobiol 62: 119–125.

Strodl MA, Schausberger P (2012) Social familiarity reduces reaction times and enhances survival of group-living predatory mites under the risk of predation. PLoS ONE 7(8): e43590.

Reply

Alternative models of familiarity and false claims on social recognition systems

Paulien J.A. de Bruijn, Maurice W. Sabelis & Martijn Egas

Behavioral Ecology and Sociobiology 68: 1563 (2014)

Thrips represent an ideal biological system to investigate mechanisms of social recognition. In his commentary on our article introducing thrips for this purpose, Schausberger (2014) assumes a social recognition system in which individuals should memorize '*distinct individual labels of the referents*'. Clearly this imposes increasing cognitive capabilities with increasing group size. He states that due to cognitive limitations, familiarity-based recognition is constrained by referent number and heterogeneity (label variability) during familiarization. We agree that under these assumptions one should take care of group size and heterogeneity in experiments aiming to test this model of social recognition. However, there are alternative models. For example, ants groom, feed or contact nest mates and thereby contaminate them with their own blend of cuticular hydrocarbons (Lenoir *et al.* 2001; Bos and d'Ettorre 2012). Ultimately, all nest mates harbour the same mix of hydrocarbons that is perceived as a single label. This is the model of the so-called Gestalt colony odour (Crozier and Dix 1979). Under this assumption increasing group size does not pose a challenge to the cognitive capacities of group members. For the case of thrips we do not know the cue or the cues they use to familiarize, nor whether they originate from thrips individuals or from their environment. Hence it is premature to theorize about which social recognition model applies to thrips. However, from a selectionist point of view we expect the more simple (and less costly) social recognition system to evolve.

In addition, we cannot avoid emphasizing that Schausberger (2014) makes two false claims as to what we concluded from our experiments (de Bruijn *et al.* 2014). First, he rephrases our conclusion that '*growing up together is a necessary*

but not sufficient condition for discrimination in thrips larvae' into '*thrips only familiarize with kin or kinship is needed for generating beneficial effects of familiarity under predation risk*'. Then, he claims that this (rephrased) conclusion is invalid. However, we have never drawn this conclusion and instead found support for familiarization among thrips larvae in kin groups only. Second, Schausberger (2014) claims '*familiarity is only established, or does only have an effect, in sibling but not non-siblings*' as being our conclusion. However, we recognized the difference in pre-experimental group size of siblings and non-siblings and separately analysed survival of familiar vs. unfamiliar thrips. Therefore, we carefully refrained from comparing sibling and non-sibling survival, a point that Schausberger (2014) acknowledges but subsequently ignores for reasons unexplained.

References

Bos N, d'Ettorre P (2012) Recognition of social identity in ants. Front Psychol 3: 83.

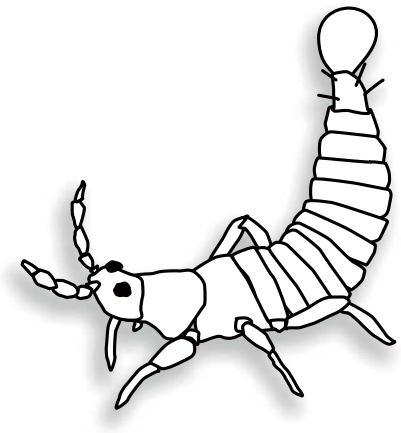
Crozier RH, Dix MW (1979) Analysis of two genetic models for the innate components of colony odor in social Hymenoptera. Behav Ecol Sociobiol 4: 217-224.

de Bruijn PJA, Sabelis MW, Egas M (2014) Effects of kinship or familiarity? Small thrips larvae experience lower predation risk only in mixed-size sibling groups. Behav Ecol Sociobiol 68: 1029-1035.

Lenoir A, d'Ettorre P, Errard C, Hefetz A (2001) Chemical ecology and social parasitism in ants. Annu Rev Entomol 46: 573-599.

Schausberger P (2014) Taking care of group size and heterogeneity in social recognition systems. Behav Ecol Sociobiol 68: 1561-1562.

Discussion



In this thesis I tested if Western flower thrips (in this discussion henceforth referred to as thrips) have an alarm communication system, implying that individuals can act alternatingly either as senders or receivers, and whether alarm communication is context-dependent. After discussing alarm communication among thrips, I discuss why alarm signalling has evolved in thrips.

Alarm communication

To test whether thrips possess a context-dependent alarm communication system I tested if individuals (1) respond to the alarm signal, (2) obtain higher survival chances, (3) change the composition of the alarm signal, depending on the context, and (4) respond differently to various signals. Question (1) is answered in chapters 2 and 4 where I show that thrips larvae perceive alarm pheromone and that they are alerted by it (primed) because at a simulated attack they excrete an anal droplet for defence faster when alarm pheromone is present than when absent. Question (2) is put to a test in chapter 3, showing that the presence of a synthetic mimic of the alarm pheromone increases the survival of thrips larvae. Question (3) is answered in chapter 5, in that thrips larvae change the composition of the pheromone they excrete when facing different levels of danger. The level of danger determines *if* alarm pheromone is present in the anally excreted droplets and *when* present, this level determines the composition of this alarm pheromone. Both the amount of decyl acetate and dodecyl acetate together as well as the ratio of decyl acetate and dodecyl acetate change depending on the predation context. Hence, thrips larvae can signal different levels of danger. The fourth (4) and last question is considered in chapters 4 and 6. In chapter 4, I tested if thrips larvae vary their response to the alarm signal depending on the instar of the sender larva. An earlier study indicated that the two larval instars have a different composition of the alarm pheromone they excrete in response to simulated attack (MacDonald *et al.* 2003). The results of chapter 4 show that first-instar thrips larvae attempt to escape more frequently (a behaviour inferred from partial borderline crossings in experiments described in chapter 4) when second-instar larvae excrete alarm pheromone than when first-instar larvae do that. This effect is not due to the presence of a first- or second-instar companion larva. I also show that the difference in amount of synthetic pheromone mimicking that of first- and second-instar larvae has a significant effect on the number of escape attempts (partial borderline crossings), and the ratio of the two components shows a trend towards more escape attempts (partial crossings) if the composition of the pheromone mimics that of a second-instar larva. Thus, thrips larvae vary their response to alarm pheromone, depend-

ing on the composition of the pheromone. Furthermore, in chapter 6 I show that thrips larvae appear to respond differently to an alarm pheromone excreted in different contexts, without having access to direct information on the context itself. When presented with a piece of filter paper contaminated with an anal droplet from a second-instar larva that suffered an attack from a predatory bug, first-instar larvae seek refuge in spider mite web less often than expected, but this was not the case when presented with a pheromone excreted under attack by a predatory mite.

The results described above show that thrips larvae are capable of context-dependent alarm communication. Senders can change the alarm pheromone depending on the context, and receivers respond differently to alarm pheromone produced by senders experiencing different contexts. In general, the ability of senders to tune their alarm signal to the context and the differential response of receivers to these signals have been demonstrated in vervet monkeys that communicate by vocal alarm calls (Seyfarth *et al.* 1980; Furrer and Manser 2009), but – to the best of my knowledge – not for animals that communicate by chemical alarm signals (alarm pheromones). I suspect that this lack of insight in chemical alarm systems is due to the dogma that pheromones have a fixed chemical composition. Indeed, one reason why context-dependent alarm pheromones may not have been reported in the literature is that alarm pheromones have often been tested in set-ups too simple to reveal context-dependence (e.g., Bowers *et al.* 1972; MacDonald *et al.* 2003; Chivers and Smith 1998). However, there are examples where pheromones change depending on the context. For example, *Rhyzopertha dominica* beetles change their aggregation pheromone depending on their host or the presence of females (Bashir *et al.* 2003), and the composition of the sex pheromone of the noctuid moth *Heliothis subflexa* depends on the experience of the females with other pheromones during the first three days of their adulthood (Groot *et al.* 2010). Hence, context-dependent pheromones do exist. Furthermore, many prey species may benefit from context-dependent alarm communication by pheromone, because prey species usually encounter many predators, differing in the level of danger they pose. For all the above reasons, I hypothesize that context-dependence of alarm pheromones is widespread in the animal kingdom.

Evolution of alarm signalling

Another aim of this thesis was to develop an experimental system amenable for research to understand *why* thrips larvae signal alarm. In sending an alarm signal there are several costs we can identify. First, natural enemies such as predatory

bugs (*Orius tristicolor*) are attracted to thrips alarm pheromone (Teerling *et al.* 1992a), so producing alarm pheromone before a predator has attacked will lure a predator to the vicinity of the sending individual. Second, it is known that at any time thrips larvae have enough decyl acetate and dodecyl acetate in their body to excrete just one or two anal droplets with alarm pheromone (MacDonald *et al.* 2003) and it takes at least several minutes before they can produce a new droplet (PJAdB, personal observations). The anal droplet itself is part of their defence once under attack (Bakker and Sabelis 1987, 1989), so producing a droplet with alarm pheromone before an attack makes a thrips larva lose future opportunities for defence. Given these costs, it is not immediately clear why and when individuals should release alarm. There are three general theories that may explain the evolution of alarm communication (Chapter 1): kin selection to promote the fitness of kin at the expense of one's own direct fitness (inclusive fitness or kin selection theory; Hamilton 1964), selection for reciprocal altruism (Trivers 1971) and selection to promote an individual's defence (e.g., via demonstrating alertness) (Randall and Matocq 1997; Sherman 1985; Trivers 1971). Here, I discuss which theory fits best to what we currently know of alarm signalling by thrips.

To empirically test if kin selection can play a role in the evolution of alarm calling one should test among others if alarm calling (1) increases survival chances for conspecifics and (2) increases when siblings are nearby. I tested these two requirements in this Thesis; in chapter 2 it is shown that when presented with alarm pheromone, thrips larvae indeed survive better. Yet, the results in chapter 5 show us that when the predator is nearby, but not yet attacking, the chance that an anal droplet contains pheromone is less than 5% and Table 8-1 shows that this chance did not increase when thrips larvae were on a leaf disc with siblings.

This suggests that alarm signalling in thrips did not evolve due to kin selection. Nevertheless, kin selection is important in other aspects of thrips defence, as shown in chapter 7 where I found that thrips larvae have an increased survival chance when in groups of kin. Which aspects of thrips defence are influenced by the presence of kin is unclear, but I suspect that larger siblings may tolerate small larvae to stay close to them, as long as these larvae are recognised as siblings. This would make it more difficult for a predator to reach them and moreover, larger siblings may actively chase a predator away from their siblings. Other papers have reported kin effects in survival as well. For instance, Strodl and Schausberger (2012) found that in the presence of an intraguild predator (*Amblyseius andersoni*), the predatory mite *Phytoseiulus persimilis* has increased survival when in groups of kin because then individuals shift their attention from group member assessment to other tasks such as anti-predator vigilance and response.

Table 8-1 Number of anal droplets containing alarm pheromone.

Predator	Attack	Kin		Non-kin	
		Pheromone	No pheromone	Pheromone	No pheromone
Mite	No	0	31	7204	
	Yes	1	2	10	27
Bug	No	1	18	4162	
	Yes	3	7	46	15

Can alarm signalling in thrips be explained by reciprocal altruism? If so, it should meet the following conditions: (A) individuals can choose to call alarm or not, (B) it is costly for individuals to call alarm, (C) individuals can expect to profit from alarm calling by other individuals at a later moment (downstream reciprocity) or have already profited from other individuals earlier (upstream reciprocity). Our results from chapter 2 and 5 show that thrips meet condition (A); not all anally excreted droplets contained alarm pheromone, before or during an attack and when larvae are primed by alarm pheromone, they produce an anal droplet (presumably containing alarm pheromone) faster than when they are not primed. Condition (B) is also met because excreting alarm pheromone is costly in terms of survival and in terms of future defence, as explained in the first paragraph of this section. Whether alarm calling can be reciprocal for thrips larvae (condition C) depends on the predator that is in the vicinity. When attacked by a predatory bug, the chance a thrips larva survives is very small (Sabelis and van Rijn 1997). In this case, a thrips larva cannot expect to profit from alarm calling by another individual later – but they may have profited from alarm calling earlier. When attacked by a predatory mite, the chance to survive is much higher (Bakker and Sabelis 1987), especially when a larva is already alerted that a predatory mite is present. Because the anal droplets larvae excrete are also part of their direct defence against a predator, it is important to distinguish between anal droplets containing alarm pheromone that are excreted during an attack or before an attack. The former droplets might be excreted only as self-defence, while the latter droplets will most likely serve to warn other individuals and are costly to excrete, as argued above. It is not known if – during an attack – the pheromone in the anal droplet increases the survival chance or if other chemicals in the droplet have this effect. If other chemicals cause the increased survival chance when a thrips larva is attacked, and adding alarm pheromone to the droplet does not influence this chance, then adding alarm pheromone to the droplet is a by-product mutualism rather than altruism. The excretion of anal droplets with alarm pheromone before an attack can be called an altruistic act and may yield reciprocal benefits as well. Thus, reciprocal altruism may have driv-

en the evolution of alarm pheromone communication in thrips, but this hypothesis requires testing, for example, for an effect of pheromone in the anal droplet on survival of the sender and especially for an effect of perceiving alarm pheromone earlier on the chance that an individual excretes alarm pheromone (upstream reciprocity).

The third theory why alarm calling might have evolved is individual defence. If individual defence is the main reason why thrips larvae release alarm pheromones, then the chance that they survive an attack should be higher when an anally excreted droplet contains alarm pheromone than when it does not. To the best of my knowledge, this has not yet been tested. What we do know from the results in chapter 5 is that when the level of danger for thrips larvae increases (due to a more dangerous predator species or due to an actual attack of the predator), so does the chance that a droplet contains pheromone. This suggests that thrips larvae produce alarm pheromone to defend themselves. However, with an increase in the level of danger, also the composition of the alarm pheromone changes and this change could only be explained by defensive behaviour is if some predators are more susceptible to decyl acetate and others to dodecyl acetate, which has never been tested. Hence, thrips larvae might excrete alarm pheromone as an individual defence, but when excreted, the alarm pheromone functions also as a context-dependent signal, which can be beneficial to conspecific larvae nearby. Thus, self-defence may have initially caused the evolution of alarm signals in thrips, but this does not preclude other selection mechanisms working in concert.

What causes alarm communication to evolve will depend on the ecology of the organism under study. I will give four examples of ecological determinants that need to be considered when studying the evolution of alarm communication and explain what is known for thrips in these examples. First, it is important to know whether individuals aggregate. Species that do not aggregate will have a smaller probability to evolve signals, because receivers may not be nearby. In that case, alarm signals may evolve because of self-defence. However, thrips do aggregate (Steiner 1990), often in large numbers and hence receivers are likely to be in the vicinity. Second, it is essential to know whether individuals aggregate in groups of kin. If aggregation occurs in groups of non-related individuals, then kin selection can be ruled out. Thrips occur often in greenhouses, where a few individuals invade a greenhouse to start a population (Higgins and Myers 1992). Hence in these populations relatedness by descent will be very high, thus enabling kin selection for alarm communication. A third ecological determinant for the evolution of alarm communication is the chance individuals survive an encounter with a specific predator and whether alarm signals promote this

chance for senders or receivers (or both). If individuals have a chance to survive an attack and the survival chances go up with the release of alarm signals by others, then there is room for reciprocal altruism to cause the evolution alarm communication. Indeed, thrips larvae may encounter different species of predators, and the chance it survives an attack depends on the type of predator attacking (in this thesis, predatory mite or predatory bug), the motivational state of the predator (Sabelis and van Rijn 1997) and the speed at which it responses to touch by a predator (personal observations). Thrips larvae are primed by the alarm pheromone and presence of the alarm pheromone improves the chance larvae survive being close to a predator (chapters 2 and 3). The fourth and final feature determining the evolution of alarm communication is the extent to which vulnerability to predator attack varies in the population. Predators often attack prey of a certain size range (Sabelis 1992; Tonn *et al.* 1992; Chase 1999). Hence prey individuals larger than that size range could help vulnerable conspecifics. Alarm calling might be less costly for non-vulnerable prey individuals. For thrips larvae it is known that for instance predatory mites have a higher success rate when attacking first-instar larvae than when attacking second-instar larvae. In chapter 7 I show that first-instar larvae have a higher survival chance near a predatory mite when second-instar siblings are nearby. However, the chance that an anally excreted droplet contained pheromone was not higher when second-instar larvae were among siblings (Table 8-1). All in all, the four determinants for the evolution of alarm communication lead me to conclude that the ecology of a species needs to be taken into account.

Summary

I conclude that thrips larvae possess a context-dependent alarm communication system, meaning that the alarm signal varies with the context and that the response to the signal varies with the signal. I hypothesize that because context-dependent alarm communication can increase survival chances of the receivers, many species that employ chemical alarm signals use context-dependent alarm communication. I want to stress that thrips are a good model system to test how alarm communication systems evolve by individual, kin or group selection. To test kin selection/group selection questions, it is advantageous to use an organism that lives aggregated, in either groups of siblings, or groups of unrelated individuals. Thrips larvae do this and the relatedness of the groups can be manipulated. Moreover, Western flower thrips are non-social insects, but social organization in other thrips species ranges from solitary to eusocial (Crespi and Yanega 1995) and hence I suggest that future studies can use thrips to test how commu-

nication varies with sociality. Finally, thrips are well suited to test chemical communication because the chemicals that thrips excrete are present in droplets that are visible under a binocular microscope and these droplets can be chemically analysed. Considering the advantages of thrips plus the knowledge gained in this thesis, I suggest testing the importance of sending the correct signal. This can be tested by analysing survival of thrips larvae in the vicinity of a predator with an alarm signal that matches this predator or with an alarm signal that does not match this predator.

References

Bashir T., Hodges R.J., Birkinshaw L.A., Hall D.R. and Farman D.I. (2003) Phenotypic plasticity of *Rhyzopertha dominica* pheromone signaling: the effects of different hosts and presence of conspecific females on male produced aggregation pheromone. Journal of Chemical Ecology, 29: 945-959.

Bakker F.M. and Sabelis M.W. (1987) Attack success of *Amblyseius mckenziei* and the stage related defensive capacity of thrips larvae. Netherlands Journal of Zoology, 37: 437.

Bakker F.M. and Sabelis M.W. (1989) How larvae of *Thrips tabaci* reduce the attack success of phytoseiid predators. Entomologia Experimentalis et Applicata, 50: 47-51.

Bowers W.S., Nault L.R., Webb R.E. and Dutky S.R. (1972) Aphid alarm pheromone: isolation, identification, synthesis. Science, 177: 1121-1122.

Chase J.M. (1999) Food web effects of prey size refugia: Variable interactions and alternative stable equilibria. American Naturalist, 154: 559-570.

Crespi B.J. and Yanega D. (1995) The definition of eusociality. Behavioral Ecology, 6: 109-115.

Furrer R.D. and Manser M.B. (2009) The evolution of urgency-based and functionally referential alarm calls in ground-dwelling species. American Naturalist, 173: 400-410.

Gardner A., West S.A. and Wild G. (2011) The genetical theory of kin selection. Journal of Evolutionary Biology, 24: 1020-1043.

Groot A.T., Claβen A., Staudacher H., Schal C. and Heckel D.G. (2010) Phenotypic plasticity in sexual communication signal of a noctuid moth. Journal of Evolutionary Biology, 23: 2731-2738.

Hamilton W.D. (1964) The genetical evolution of social behaviour. Journal of Theoretical Biology, 7: 1-52.

Higgins C.J. and Myers J.H. (1992) Sex ratio patterns and population dynamics of Western flower thrips (Thysanoptera: Thripidae). Environmental Entomology, 21: 322-330.

MacDonald K.M., Hamilton J.G.C., Jacobson R. and Kirk W.D.J. (2003) Analysis of anal droplets of the Western flower thrips *Frankliniella occidentalis.* Journal of Chemical Ecology, 29: 2385-2389.

Nowak M.A., Tarnita C.E. and Wilson E.O. (2010) The evolution of eusociality. Nature 446: 1057-1062.

Randall J.A. and Matocq M.D. (1997) Why do kangaroo rats (*Dipodomys spectabilis*) footdrum at snakes? Behavioral Ecology, 8: 404-413.

Sabelis M.W. (1992) Predatory arthropods. In: Crawley M.J. (ed) Natural enemies: the population biology of predators, parasites and diseases. Blackwell, Oxford, pp 225–264.

Sabelis M.W. and van Rijn P.C.J. (1997) Predation by insects and mites. In: Lewis T (ed) Thrips as crop pests, pp. 259–354. CAB International, Wallingford, UK.

Seyfarth R.M., Cheney D.L. and Marler P. (1980a) Vervet monkey alarm calls: semantic communication in a free-ranging primate. Animal Behaviour, 28: 1070-1094.

Sherman P.W. (1985) Alarm calls of Belding's ground squirrels to aerial predators: Nepotism or self-preservation? Behavioral Ecology and Sociobiology, 17: 313-323.

Steiner M.Y. (1990) Determining population characteristics and sampling procedures for the Western flower thrips (Thysanoptera: Thripidae) and the predatory mite *Amblyseius cucumeris* (Acari: Phytoseiidae) on Greenhouse Cucumber. Environmental Entomology, 19: 1605-1613.

Strodl M.A. and Schausberger P. (2012) Social familiarity reduces reaction times and enhances survival of group-living predatory mites under the risk of predation. PLoS ONE, 7: 1-5.

Teerling C.R., Borden J.H. and Gillespie D.R. (1993) Utilization of western flower thrips alarm pheromone as a prey-finding kairomone by predators. Canadian Entomologist, 125: 431-437.

Tonn W.M., Paszkowski C.A. and Holopainen I.J. (1992) Piscivory and recruitment: mechanisms structuring prey populations in small lakes. Ecology, 73: 951-958.

Trivers R.L. (1971) The evolution of reciprocal altruism. Quarterly Review of Biology, 46: 35-57.

van Veelen M. (2009) Group selection, kin selection, altruism and cooperation: When inclusive fitness is right and when it can be wrong. Journal of Theoretical Biology, 259: 589-600.

Summary

Animals of many species use alarm signals to warn conspecifics when danger is near. Such alarm signals can take many forms, including visual (for example, colour, move), mechanical, chemical (pheromone), or accoustic (call, yell, squeek) stimuli. The best studied alarm signals are vocal signals of mammals and birds, especially the alarm calls of vervet monkeys (*Chlorocebus pygerythrus*) and ground squirrels (*Xerus inauris* and *Spermophilus beldingi*). Vervet monkeys produce specific alarm calls for each of their three most important enemies: leopards, eagles and snakes. Conspecific monkeys that hear these enemy-specific calls respond differently to each of them and these specific responses appear to enlarge their indvidual chances of survival. Cape ground squirrels (*X. inauris*) emit urgency-dependent alarm calls, that tell whether a predator is nearby or far away. The squirrels always respond in the same way: they run into their burrows.

Why do individuals produce alarm signals? This is not self-evident, as an individual that gives off a signal also increases its own predation risk, because it may attract the attention of predators. For the receiver of an (honest) alarm signal the advantage is often clear, given that this animal is informed about the presence not only of a predator, but also of a competitor. Signal receivers can then respond in an adaptive manner, for instance by increasing their alertness, by fleeing or by hiding. Not all alarm signals are equally reliable – sometimes signals are being sent out without the presence of actual danger. If this is the case, the response of the signal-receiver may in fact benefit the signal-sender, for example by reducing local competition. The reliability of a signal is often linked to the costs for the signal-sender.

Although the advantages of alarm signal production may be less clear for the signal sender than for the receiver, on the whole the sender must experience a profit. After all, without such a profit selection would act against the production of alarm signals. Three common theories may explain the evolution of alarm signaling: individual defence, reciprocal altruism (signal receivers are not genetically related to signal senders), and kin selection (receivers and senders are related). The main goal of this thesis was to investigate whether all three of these theories can be tested in a single experimental system, based on the alarm signal of the Western flower thrips (*Frankliniella occidentalis*, from now on called 'thrips'). A suitable system would allow the testing of (1) increase or decrease of the chances of survival for the alarm signal sender, (2) reciprocity of alarm signalling

(if the signal increases survival chances of the receivers, do they return the favour and warn the initial sender later on?), and (3) kin-related signalling (are alarm signals more likely if the receivers are genetically related to the signal senders?).

Thrips have several advantages that make them particularly suitable for the study of the evolution of alarm signalling. When in danger, thrips larvae defend themselves by the excretion of 'anal droplets': a predator touched by such a droplet interrupts the attack and switches to cleaning. These droplets may contain an alarm pheromone, consisting of two coumpounds: decyl acetate and dodecyl actetate. The presence of alarm pheromone evokes anti-predator behaviour in thrips, such as elevated alertness and moving away from the scene, and this behaviour potentially improves the chances of survival of the signal receivers. Thrips larvae may encounter a range of predators, the one more dangerous than the other. If a thrips larva survives the presence of a predator, this larva may become a signal sender on a next occasion. Thrips live in groups, comprising both related and unrelated individuals. A practical advantage of the alarm pheromone of thrips is that synthetic mimics of its two components are available. The anal droplets can be observed, counted, collected and analysed for the presence and composition of pheromone. Furthermore, thrips larvae can be stimulated to produce droplets by prodding them with a fine brush. The combination of these advantages enables the manipulation of pheromone production as well as the determination of quality and quantity of the alarm pheromone in presence of various types of predator.

Three main questions are central to this thesis. First, does the alarm pheromone of thrips larvae indeed improve the defensive capacities of conspecific thrips? Second, is thrips alarm pheromone production context-dependent? And third, how does relatedness influence alarm communication?

Pheromone as alarm signal

In **chapters 2 and 4** I demonstrate that the alarm pheromone induces various forms of anti-predator behaviour in thrips (such as the production of anal drops). In addition, in **chapter 3** I show that thrips survival of predation is higher in presence of synthetic alarm pheromone.

Context-dependent alarm communication

Alarm signals that vary with the level of danger to the signal sender are called context-dependent alarm signals. If the receiver of a context-dependent signal adjusts its response to the variable signal, I call this context-dependent commu-

nication. So far, context-dependent alarm communication is predominantly known for accoustic signals (remember the vervet monkeys and the Cape ground squirrels). However, the advantages of context-specific communication (i.e., to optimize the response, in terms of nature and timing of the threat) are not limited to animals that use vocal alarm signals. Surprisingly, context-dependency has hardly been investigated for chemical alarm signals (pheromones); for chemical communication, the common opinion appears to be that alarm pheromones are the same in all circumstances. Possibly, this opinion is based on one of the best studied systems of chemical alarm signalling, that of aphids. Aphids have an alarm pheromone that consists of a single compound. This limits the possibilities to specify approaching danger – aphids can only change their alarm signal quantitatively (amount and frequency), but not qualitatively (ratio of components). An alarm pheromone that consists of two or more components allows for qualitative changes as well, thus enabling a much higher level of information specificity. The alarm pheromone of thrips consists of two components, so in principle thrips have the opportunity to adjust their pheromone both quantitatively and qualitatively to approaching danger. In **chapters 4, 5 and 6** I demonstrate that thrips indeed adjust their alarm communication to the level of danger.

It is known from earlier research that small and large thrips larvae differ in the amount of pheromone they secrete, and also the ratios of the two components are different. In **chapter 4** I compare the responses of thrips to alarm pheromone produced by either small or large thrips larvae. It turns out that thrips respond differently, possibly caused by the difference in amount of pheromone, but an effect of the difference in coumpound ratios cannot be ruled out. In **chapter 5** I chemically analyse the anal droplets produced by thrips when (1) near a relatively harmless predatory mite (*Iphiseius degenerans*), (2) near a very dangerous predatory bug (*Orius laevigatus*), or (3) triggered by a soft brush. In this experiment I differentiate between the mere presence of the various predators, and actual attacks. The results show that (i) the chance that the droplets contain alarm pheromone is positively related to the level of danger for the trips larva, (ii) the ratio of the two components changes when a trips is attacked by a predatory mite, compared to when the mite is only present, and (iii) the amount of pheromone secreted depends on the predator type. This indicates that thrips can adjust their alarm pheromone to the level of danger. In **chapter 6** I focus on the responses of signal-receiving thrips. In absence of a predator, to what degree do thrips hide in a refugium when exposed to droplets produced by thrips under actual attack by various predators? It appears that thrips hide as often when exposed to droplets produced when attacked by a predatory mite, as when exposed to droplets produced when under attack of a predatory bug, whereas

the chance that these droplets contain alarm pheromone differs much between the two predator types. This suggests that thrips enter a refugium more often when they smell pheromone secreted in response to a predatory mite attack.

Kinship and alarm communication

To test whether kin selection influences alarm communication in thrips, in **chapter 7** thrips survival in presence of a predatory mite is compared between groups of related vs. unrelated individuals. It turns out that small thrips larvae have a higher survival chance when surrounded by kin, but only if the groups comprise both small and large larvae. So, kin selection does play a role in thrips. But does kin selection also affect the production of alarm signal? To answer this, I did a preliminary experiment, comparing the secretion of alarm pheromone by thrips in groups of related vs. unrelated individuals (**chapter 8**). Thrips do not seem to give off more alarm pheromone when among kin.

With these last results, kin selection does not seem to hold as an immediate explanation for the evolution of alarm signalling in thrips. Direct individual defence appears to be the best candidate mechanism, as the droplets with the alarm pheromone are also used as defence against the predators. However, to confirm direct defence as the explanation for the evolution of alarm signalling in thrips, it remains to be demonstrated that droplets with pheromone are a better defence than droplets without pheromone. If reciprocal altruism were to explain alarm signalling in thrips, the secretion of alarm pheromone would have to meet with three conditions: (1) individuals can decide whether or not to release pheromone, (2) release of alarm pheromone has to be costly, and (3) signal senders benefit from the release of alarm pheromone by others at a later instance. This instance can be before or after the initial sender releases pheromone again. The results of chapters 2 and 5 show that thrips meet condition (1). Also condition (2) appears to be met: when thrips secrete a droplet with alarm pheromone, this may be at the cost of possible future defence, as they can produce not more than two pheromone-laden droplets at any moment. However, this cost remains to be quantified. If and how condition (3) is met, depends on the predator type. With certain predators, thrips survival chances are so low that the thrips cannot be expected to benefit later from the presence of pheromone released by conspecifics (although it may have benefited from earlier release of pheromone by others). Other predators may offer thrips a much higher chance of survival, especially if thrips have been alerted by the presence of pheromone. In such cases it is surely possible that the initial signal sender benefits from alarm signalling by a conspecific later on.

Follow-up research could focus on the costs (and thus the reliability) of alarm signalling for thrips. It will also be interesting to incorporate other thrips species with various social structures, and to investigate how social organisation relates to alarm communication. Some thrips species are solitary, whereas others are eusocial. *Frankliniella occidentalis* does live in aggregations, but parental care is lacking. One hypothesis would be that thrips species with more complex social structures require more sophisticated communication. Also alarm signalling in such species could be more elaborate. A comparative approach to alarm signalling among thrips species could provide insight in the role of communication in the evolution of sociality.

In conclusion, thrips are indeed a suitable experimental system to study the evolution of alarm signalling. Analysis of the anal droplets, the alarm pheromone and the kinship of thrips larvae allows for rigorous testing of individual defence, reciprocal altruism and kin selection in thrips. This thesis also demonstrates that thrips have a context-dependent alarm communication system, in which (1) the alarm signal varies with context and (2) the response varies with the alarm signal. Aphids, who can only modify the quantity of their alarm pheromone, are known to adjust the amount and frequency of pheromone to the level of danger. This suggests that also aphids display context-dependent alarm communication. I expect that context-dependent chemical alarm communication will be found in many more species with alarm pheromones. Especially species that display different defence strategies against different enemies will benefit from context-dependent communication.

Samenvatting

Veel diersoorten maken gebruik van alarmsignalen om soortgenoten te waarschuwen voor naderend gevaar. Alarmsignalen kunnen bestaan uit vele soorten stimuli, waaronder visuele (voorbeeld: kleur, beweging), mechanische, chemische (feromoon) of akoestische (kreet, roep). De best bestudeerde alarmsignalen zijn geluidsignalen van zoogdieren en vogels, in het bijzonder de kreten van de blauwaap (*Chlorocebus pygerythrus*) en van grondeekhoorns (*Xerus inauris* en *Spermophilus beldingi*). Blauwapen hebben specifieke kreten voor ieder van hun drie voornaamste vijanden: luipaard, arend en slang. Soortgenoten van de blauwaap die deze rover-specifieke kreten horen, reageren verschillend op elk van deze kreten en deze specifieke reacties lijken de overlevingskans van het reagerende individu te vergroten. De Kaapse grondeekhoorn (*X. inauris*) uit kreten die de mate van urgentie weergeven: het is te horen of een rover ver weg is of dichtbij. Deze dieren vertonen bij verschillende rovers altijd dezelfde respons - ze vluchten hun hol in.

Waarom geven individuen alarmsignalen af? Zo'n actie is niet vanzelfsprekend, omdat het individu dat het signaal verstuurt zijn eigen predatie-risico verhoogt doordat het ook de aandacht van de rover op zich vestigt. Daardoor is het voordeel van het versturen van een signaal voor de afzender twijfelachtig. Voor de ontvanger van een (betrouwbaar) alarmsignaal is het voordeel vaak wel duidelijk, want dit dier wordt gewaarschuwd voor predatoren maar bijvoorbeeld ook voor concurrenten. Ontvangers kunnen dan gedrag vertonen wat hun overlevingskans vergroot (bv. extra alert zijn, vluchten of zich verstoppen). Niet alle alarmsignalen zijn echter betrouwbaar, soms worden signalen verstuurd zonder de aanwezigheid van naderend gevaar. De afzender kan dan profijt hebben van de reactie van de ontvanger, bijvoorbeeld doordat een voedselplek vrijkomt. Voor het vertrouwen dat de ontvanger heeft in een signaal zijn de kosten die de afzender maakt bij het produceren van dat signaal veelal bepalend.

Hoewel de voordelen van het sturen van een alarmsignaal vaak minder duidelijk zijn voor de afzender dan voor de ontvanger van het signaal, zal de afzender, direct of indirect, profijt moeten hebben van het versturen van het alarmsignaal. Immers, als de afzender van het alarmsignaal geen voordeel heeft bij het versturen, dan zou het signaal weggeselecteerd worden. Er zijn drie theorieën die de evolutie van alarmsignalen kunnen verklaren: individuele verdediging, wederkerig altruïsme (ontvangers zijn niet genetisch verwant aan afzender) en selectie op verwanten (ontvangers zijn verwant aan afzender; zogenoemde *kin-*

selectie). Het hoofddoel van dit proefschrift was te onderzoeken of alledrie deze theorieën kunnen worden getoetst in één experimenteel systeem, gebaseerd op het alarmsignaal van de Californische trips, *Frankliniella occidentalis* (hierna 'trips' genoemd). In een geschikt systeem moet kunnen worden getoetst of (1) het versturen van een alarmsignaal de overlevingskans van een afzender doet toe- of afnemen, (2) als het alarmsignaal ervoor zorgt dat de gewaarschuwde ontvangers hogere overlevingskansen hebben, alarmsignalering ook wederkerig kan zijn (dus of de ontvanger van het signaal op een ander moment zelf een alarmsignaal zal versturen) en (3) de kans dat er alarmsignalen worden verstuurd groter is als individuen zich in de buurt van verwanten bevinden.

Tripsen hebben enkele eigenschappen die ze geschikt maken voor onderzoek naar de evolutie van alarmsignalen. Tripslarven scheiden bij gevaar 'anale' druppels uit die dienen voor de individuele verdediging van de tripsen: rovers die zo'n druppel op zich krijgen, onderbreken een aanval en proberen zich schoon te maken. De druppels bevatten een alarmferomoon, bestaande uit de chemische stoffen decylacetaat en dodecylacetaat. De aanwezigheid van alarmferomoon roept bij signaalontvangende tripsen anti-rovergedrag op, zoals verhoogde alertheid of vluchten, en dit gedrag vergroot potentieel de overlevingskans van de ontvangers. Tripslarven kunnen een scala aan predatoren tegenkomen, de een nog gevaarlijker dan de ander. Indien een tripslarve de nabijheid van een rover overleeft, kan deze larve op een ander moment zelf alarmferomoon uitscheiden. Tripsen leven in groepen, die kunnen bestaan uit zowel verwante als niet-verwante individuen. Een praktisch voordeel van het alarmferomoon van tripsen is dat de twee componenten synthetisch te verkrijgen zijn. De druppels die al of niet het feromoon bevatten zijn goed te zien en kunnen worden verzameld en individueel geanalyseerd op feromoonsamenstelling. Bovendien kunnen tripsen ertoe aangezet worden om deze druppels uit te scheiden door middel van aanraking. Deze combinatie van eigenschappen maakt het mogelijk om feromoonproductie te manipuleren, maar ook om de kwaliteit en kwantiteit van het alarmferomoon te bepalen in de aanwezigheid van verschillende predatoren. Drie hoofdvragen omtrent alarmsignalering bij tripsen staan centraal in dit proefschrift.

Vertonen tripsen in de aanwezigheid van het alarmferomoon (succesvol) anti-predatorgedrag?

In **hoofdstuk 2 en 4** toon ik aan dat het alarmferomoon verschillende vormen van anti-predatorgedrag oproept bij tripsen (zoals de productie van anale druppels). Bovendien laat ik in **hoofdstuk 3** zien dat tripsen de aanwezigheid van een predator beter overleven in aanwezigheid van synthetisch alarmferomoon.

Passen tripsen hun alarmcommunicatie aan aan de mate van gevaar?

Alarmsignalen die variëren met de mate van gevaar voor de afzender, heten context-afhankelijke alarmsignalen. Indien de ontvanger van een context-afhankelijk signaal zijn reactie afstemt op het variabele signaal, dan spreek ik van context-afhankelijke communicatie. Tot nu toe is context-afhankelijke alarmcommunicatie voornamelijk bekend van akoestische signalen (denk aan de blauwaap en de Kaapse grondeekhoorn). Echter, de voordelen die context-specifieke communicatie biedt (het optimaliseren van de respons, dat wil zeggen het afstellen van de reactie op de aard en het moment van de dreiging), beperken zich niet tot organismen die vocale alarmsignalen gebruiken. Opmerkelijk genoeg is nooit naar context-afhankelijkheid gezocht bij chemische alarmsignalen (alarmferomoon), maar is het heersende idee dat een alarmferomoon in iedere situatie gelijk is. Mogelijk heerst dit idee doordat een van de best bestudeerde chemische alarmsignaleringen dat van de bladluis is, en bladluizen hebben een alarmferomoon dat maar uit één component bestaat. Daardoor zijn bladluizen beperkt in hun mogelijkheden om naderend gevaar te specificeren, ze kunnen van hun alarmsignaal alleen de kwantiteit (hoeveelheid en frequentie) veranderen, maar niet de kwaliteit (verhoudingen van componenten). Een alarmferomoon dat uit ten minste twee componenten bestaat, kan ook kwalitatief veranderen en dit biedt organismen met zo'n alarmferomoon meer mogelijkheden om naderend gevaar te specificeren. Het alarmferomoon van tripsen bestaat uit twee componenten, dus tripsen hebben in beginsel de mogelijkheid om zowel kwantiteit als kwaliteit van hun feromoon aan te passen aan naderend gevaar. In **hoofdstukken 4, 5 en 6** laat ik zien dat tripsen hun alarmcommunicatie inderdaad aanpassen aan de mate van gevaar.

Uit eerder onderzoek was al bekend dat grote en kleine tripslarven verschillen in de hoeveelheid feromoon die ze uitscheiden en ook dat de verhouding van de twee componenten verschilt. In **hoofdstuk 4** vergelijk ik de respons van tripsen op alarmferomoon geproduceerd door een grote dan wel een kleine larve. Ik constateer dat tripsen verschillend reageren, mogelijk als gevolg van het verschil in de hoeveelheid feromoon, maar een effect van de verhouding van de twee componenten kan niet worden uitgesloten. In **hoofdstuk 5** analyseer ik de anale druppels die tripsen uitscheiden als ze zich bevinden in de buurt van een betrekkelijk ongevaarlijke roofmijt (*Iphiseius degenerans*), een zeer gevaarlijke roofwants (*Orius laevigatus*), of een zachte kwast, waarmee ik een kunstmatige 'aanval' uitvoer. Hierbij maak ik onderscheid tussen daadwerkelijke aanvallen door de verschillende rovers en alleen hun aanwezigheid. De resultaten laten zien dat (1) de kans dat er alarmferomoon in de uitgescheiden druppel zit, toeneemt met de

mate van gevaar voor de tripslarve, (2) de verhouding van de twee componenten verandert als een trips echt wordt aangevallen door een roofmijt, in vergelijking met alleen de aanwezigheid, en (3) de hoeveelheid uitgescheiden feromoon afhangt van de soort rover. Dit toont aan dat tripsen hun alarmferomoon kunnen aanpassen aan de mate van gevaar. In **hoofdstuk 6** concentreer ik me op de reactie van signaalontvangende tripsen. Ik ga na of tripsen in verschillende mate vluchten, wanneer ze – zonder dat er echt een predator aanwezig is – geconfronteerd worden met druppels die zijn uitgescheiden bij een aanval door verschillende rovers. Het blijkt dat tripsen even vaak naar een schuilplaats (refugium) gaan bij druppels die zijn uitgescheiden na een aanval door een roofmijt als bij druppels die zijn uitgescheiden na een aanval door een roofwants, terwijl de kans dat deze druppels alarmferomoon bevatten sterk verschilt tussen de twee rovers. Dit suggereert dat tripsen vaker het refugium in gaan als ze feromoon ruiken dat is uitgescheiden bij een aanval van een roofmijt.

Wat is de rol van verwantschap in alarmcommunicatie van trips?

Om te toetsen of *kin*-selectie van belang is voor de alarmcommunicatie van trips, onderzoek ik in **hoofdstuk 7** of tripsen de aanwezigheid van een roofmijt beter overleven als ze zich in groepen van verwante individuen bevinden dan wanneer ze zich in groepen van onverwante tripsen bevinden. Het blijkt dat kleine tripslarven de aanwezigheid van een roofmijt beter overleven in groepen verwante tripsen dan in groepen onverwante tripsen, maar alleen als deze groepen bestaan uit zowel kleine als grote individuen. *Kin*-selectie speelt dus wel degelijk een rol bij tripsen. Maar is *kin*-selectie ook van belang voor het afgeven van het alarmsignaal? Daartoe heb ik een kleine proef gedaan naar het uitscheiden van alarmferomoon door tripsen in groepen met verwante individuen en in groepen met onverwante individuen **(hoofdstuk 8)**. Tripsen lijken niet vaker alarmferomoon uit te scheiden als ze zich bevinden in een groep met verwante individuen.

Met deze laatste resultaten lijkt *kin*-selectie af te vallen als directe verklaring voor de evolutie van alarmsignalering bij trips. Directe verdediging lijkt een goede kandidaat, omdat de druppels met het alarmferomoon ook gebruikt kunnen worden als verdediging tegen rovers. Om de evolutie van alarmsignalering bij trips *echt* uit directe verdediging te verklaren, moet er echter nog getoetst worden of druppels met feromoon een betere verdediging voor tripsen vormen dan druppels zonder feromoon. Als wederkerig altruïsme de evolutie van alarmsignalering bij trips zou verklaren, dan moet het uitscheiden van alarmferomoon aan de volgende drie voorwaarden voldoen: (1) individuen kunnen ervoor kiezen om feromoon wel of niet uit te scheiden, (2) het uitscheiden van alarmferomoon

moet kostbaar zijn, en (3) afzenders profiteren op een ander moment zelf van het alarmferomoon dat een soortgenoot uitscheidt. Dit moment kan eerder of later zijn dan het moment waarop de afzender zelf opnieuw feromoon uitscheidt. De resultaten van hoofdstukken 2 en 5 tonen aan dat tripsen voldoen aan voorwaarde (1). Aan voorwaarde (2) lijkt ook te worden voldaan: indien tripsen een druppel met alarmferomoon uitscheiden, kan dit ten koste gaan van hun eventuele toekomstige verdediging, omdat ze op enig moment hoogstens maar twee zulke druppels kunnen produceren. Deze kosten dienen echter nog gekwantificeerd te worden. Of en hoe aan voorwaarde (3) voldaan kan worden, hangt af van de rover die aanvalt. De overlevingskans bij sommige rovers is zo klein, dat een trips niet kan verwachten dat het op een later moment zelf zal kunnen profiteren van de aanwezigheid van feromoon van een soortgenoot. De trips kan wel al eerder geprofiteerd hebben van de aanwezigheid van feromoon van een soortgenoot. Bij andere rovers is de kans dat een trips de aanval overleeft veel groter, zeker als de trips alert is gemaakt door reeds aanwezig alarmferomoon. In die gevallen is het zeker mogelijk dat een afzender later profiteert van alarmsignaal van een soortgenoot.

Vervolgonderzoek zou zich kunnen richten op de vraag of alarmsignalering kostbaar is voor tripsen, waarmee de betrouwbaarheid van het signaal kan worden nagegaan. Bovendien zou op langere termijn bij andere tripssoorten met verschillende sociale organisaties onderzocht kunnen worden hoe sociale organisatie zich verhoudt tot de alarmcommunicatie. Er zijn tripssoorten die solitair leven, maar ook eusociale tripsen komen voor. *Frankliniella occidentalis* leeft wel in groepen, maar ouders zorgen niet voor hun jongen. Eén hypothese is dat soorten met complexere sociale organisaties uitgebreidere communicatie behoeven. Ook de alarmsignalering in deze soorten zou uitgebreider kunnen zijn. Een vergelijking tussen alarmsignalering van verschillende tripssoorten zou inzicht kunnen opleveren in de rol van communicatie bij de evolutie van socialiteit.

Samenvattend concludeer ik dat tripsen inderdaad een geschikt experimenteel systeem vormen, om de evolutie van alarmsignalering te onderzoeken. Door bestudering van de anale druppels, het analyseren van het alarmferomoon en het bepalen van de verwantschap van tripslarven, zijn zowel individuele verdediging als wederkerig altruïsme en *kin*-selectie te toetsen bij tripslarven. Een andere conclusie van dit proefschrift is dat tripsen over een context-afhankelijk alarmcommunicatiesysteem beschikken, waarbij (1) het signaal verandert met de context en (2) de respons varieert met het signaal. We weten van bladluizen, die alleen de kwantiteit van hun feromoon kunnen veranderen, dat ze de frequentie en de hoeveelheid van hun feromoon kunnen aanpassen aan de mate van

gevaar. Dit suggereert dat ook bladluizen context-afhankelijke alarmcommunicatie vertonen. Mijn verwachting is dat context-afhankelijke alarmcommunicatie bij veel soorten met alarmferomonen gevonden zal worden. Met name soorten die bij verschillende rovers ook verschillende verdedigings-stratiegieën kunnen vertonen, zullen profiteren van context-afhankelijke communicatie.

Author contributions

2 – Pheromone-induced priming of a defensive response in Western flower thrips
Paulien J.A. de Bruijn, Martijn Egas, Arne Janssen & Maurice W. Sabelis
All authors designed the experiment and wrote the manuscript, PJAdB conducted the experiment, PJAdB and ME conducted the statistical analysis.
Published in: *Journal of Chemical Ecology* 32: 1599–1603 (2006)

3 – Increased survival of young thrips after exposure to alarm pheromone: Experiences with synthetic compounds
Paulien J.A. de Bruijn, Michiel van Wijk, Maurice W. Sabelis &Martijn Egas
All authors designed the experiment and wrote the manuscript, PJAdB conducted the experiment, MvW created software necessary for the experiments, PJAdB and ME conducted the statistical analysis.

4 – Anti-predator responses to alarm pheromone in groups of young and/or old thrips larvae
Paulien J.A. de Bruijn, Maurice W. Sabelis, Arne Janssen & Martijn Egas
All authors designed the experiment and wrote the manuscript, PJAdB conducted the experiment, AJ and PJAdB conducted the statistical analysis.

5 – Context-dependent alarm signalling in an insect
Paulien J.A. de Bruijn, Martijn Egas, Maurice W. Sabelis & Astrid T. Groot
All authors designed the experiment and wrote the manuscript, PJAdB conducted the experiment, PJAdB and ME conducted the statistical analysis.

6 – When alarm pheromones vary with the danger from predation: Differential refuge seeking by thrips larvae?
Paulien J.A. de Bruijn, Alexandra M. Revynthi, Maurice W. Sabelis & Martijn Egas
All authors designed the experiment and wrote the manuscript, PJAdB and AMR conducted the experiment, PJAdB and ME conducted the statistical analysis.

7 – Effects of kinship or familiarity? Small thrips larvae experience lower predation risk only in groups of mixed-size siblings
Paulien J.A. de Bruijn, Maurice W. Sabelis & Martijn Egas
All authors designed the experiment and wrote the manuscript, PJAdB conduct-

ed the experiment, PJAdB and ME conducted the statistical analysis.
Published in: *Behavioral Ecology and Sociobiology* 68: 1029–1035 (2014)

Commentary by Peter Schausberger: 'Taking care of group size and heterogeneity in social recognition systems.'
Published in: *Behavioral Ecology and Sociobiology* 68: 1561–1562 (2014)

Reply by authors: 'Alternative models of familiarity and false claims on social recognition systems.'
Published in: *Behavioral Ecology and Sociobiology* 68: 1563 (2014)

Curriculum vitae

Paulien Johanna Antonia was born on January 23rd 1980 in Maastricht. She attended the highschool Sint-Maartenscollege in Maastricht from 1992 until 1998, where she gained her 'VWO' diploma. Then she moved to Amsterdam to study biology at the Universiteit van Amsterdam. Here, she attained her 'propedeuse' in biology in 2000, and her Master degree in biology in 2004. Het master projects were on Yponomeuta, heath lands, dugongs and alarm communication in Western flower thrips. From 2004 until 2008 she worked as a technical assistant at the section Population Biology at the Universiteit van Amsterdam. And from 2008 until 2014 she worked as a PhD student on alarm communication in Western flower thrips at the section Population Biology of the Universiteit van Amsterdam. From 2011 through 2013 Paulien was the representative of IBED in the PhD council of the Faculty of Science. Furthermore, from 2007 through 2014 Paulien had been an Emergency Response Officer (Bedrijfshulpverlener) of the Faculty of Science and since 2009 she has been a group-leader in this team. Paulien is married to Steven Droge and has two children.

Dankwoord

Het schrijven van een dankwoord is niet makkelijk als je, zoals ik, lang ergens geweest bent, en heel veel hele fijne mensen om je heen gehad hebt. Ik word gelukkig als ik denk aan iedereen die mij in al die jaren heeft geholpen. Maar nu het bedanken, ik ga mijn best doen.

Maus, natuurlijk is het bij jou begonnen. Zodra ik je colleges volgde, wist ik dat ecologie was wat ik wilde. Ook tijdens mijn stage en promotietijd heb je me altijd weten te inspireren. Het feit dat jij nu net niet bij de afronding van ons project bent, maakt mij heel verdrietig. Wel ben ik nog dankbaarder geworden voor al het vertrouwen dat jij in mij hebt gehad. Het voelt voor mij heel bevoorrecht dat jij zo zeker wist dat ik dit aankan. Bedankt voor al je tijd, vertrouwen en inspiratie.

Martijn, minstens een paar keer per week loop ik je kamer binnen en bijna altijd weet je tijd voor me vrij te maken. Ik weet niet hoe je het doet, want je hebt het druk genoeg, maar voor mij is het geweldig, want ik krijg direct feedback van je, meteen als ik het nodig heb. Verder heb je nog een heel belangrijke eigenschap, en dat is dat je uitstraalt dat alles goed komt. Het is moeilijk om gestresst te blijven als je in de buurt bent, want jij bent altijd rustig.

Steph, je bent als tweede promotor in dit project gestapt toen we zonder Maus verder moesten. Ik ben heel blij dat ik hiervoor bij jou terecht kon en dat je meteen bereid was om mij te helpen.

Arne, tijdens mijn stage was je officieel mijn begeleider, maar ook tijdens mijn promotie heb je ook altijd klaar gestaan om me te helpen. Ik heb veel vragen kunnen stellen over opstellingen, controles en vooral natuurlijk statistiek. Dank voor al je energie en tijd.

Michiel, we hebben zoveel samengewerkt, eerst natuurlijk voor jouw proefschrift, maar je hebt mij ook tig keer geholpen met opstellingen bouwen, matlab (je hebt zelfs Paulien geschreven in matlab, met een Paulien-helpfunctie...), proefopzetten. Daarnaast ben je een van de eerste die ik opzoek als ik zomaar even iets kwijt moet, of als ik even wil filosoferen over het leven, de (Amerikaanse) politiek, carrières en bier. Je maakt wetenschap stukken leuker voor mij.

Astrid, ik vond het geweldig om een tijd in Jena met je te kunnen samenwerken, en wat was ik blij toen je daarna naar Amsterdam kwam. Bedankt voor al je hulp.

Zoals ik al zei, ik loop hier al heel lang rond, ik ga proberen om iedereen te noemen, maar dat wordt niet makkelijk... When I started at pop-bio, it seemed to

be a southern European colony, and I have been very happy about that! Marta, Sara and Belén, all your energy set the tone back then, thanks! Voor ieder met wie ik koffie heb gedronken op de bank in gebouw II, Tessa, Amir, Hans, Vera, Tom, Merijn (met een goede voorraad aan (sterke) verhalen voor tijdens de koffiepauze), Jan, Beata, Paul, Mathias, Joris, André, Maarten, Anieke, Tim, Marijn, Reinier, Hester, Karen, Tobias, Peter en Aletta, dank voor al die koffie-gezelligheid.

Iza, bedankt voor al je goede zorg en het heerlijke eten tijdens bezoeken aan Friesland.

Roos en Nicola, gelukkig drinken we af en toe nog wat, want we hebben toch te veel kleine en grote dingen gedeeld om zomaar elkaar niet meer te zien. Wat een geluk dat jullie er waren!

But also in the new building I've had some great colleagues; Floor, Zepeng, Juanma, Bart, Felipe, of course our Greek-Japanese diner producers Yuki, George and Maria, thank you for being so positive! Fernando, Heike, Dan-li, Karen, Bram (wat fijn dat jij ook zo graag praat over koken), Saioa, Nina, Jie, Christian.

Alexandra, you've helped me with tests that I would have never had time for by myself. Thank you, and thank you for being such a pleasant person to sit next to in the lab.

Tijdens mijn promotie heb ik nog 3 studenten mogen begeleiden die mij toch weer beter over mijn eigen werk hebben doen nadenken, Casper, Jeffrey en Tim, bedankt!

Ondanks mijn obsessie met beestjes van rond de 1 mm, zijn er toch nog genoeg mensen buiten de wetenschap overgebleven waar ik fijne tijden mee heb doorgemaakt.

Sas, vanaf de brugklas ben jij er al, en ik hoop dat je er altijd zult blijven. En Maarten, gelukkig hoor jij daar ook bij, gezellig.

Lieve Nel en Miek, ik noem jullie maar even samen, omdat we elkaar zo vaak samen zien. Maar ik ben heel blij met 2 vriendinnetjes waar ik me nooit anders of beter voor hoef te doen, maar waar ik goed genoeg ben zoals ik ben. Dat vind ik echt heel bijzonder.

Wetenschappers in crime: Mida en Jasper, wel ver weg, maar gelukkig is er skype. Rieneke, je bent er weer, hoera! Tim en Ingrid en Arne en Jeremy helemaal in Berkely, maar af en toe zien is veel beter dan niet zien.

Vriendjes voor wijn en bier, vriendjes voor spelletjes en films: Jessica en Onno, Lette en JW, Bart N. Mirek, de popjes, de kooltuiners (al weet ik niet eens of de kooltuin nog bestaat?). Bram, hoop dat we nooit contact zullen verliezen. Je bent er al zo lang.

Mijn hele Alkmaarse familie, waar ik vanaf dag 1 welkom ben geweest. John

en Tiny natuurlijk, bedankt voor alles, maar ook Martijn, Hellen, Lukas, Karin, Eef en grote Ruben, bedankt voor jullie heerlijke nuchtere gezelligheid. En de tuingroep, fijn dat we ons eens per 6 weken mogen uitleven op onschuldige boomstronkjes...

Pap en mam, jullie hebben mij nieuwsgierig gemaakt voor de wereld. Bovendien hebben jullie me ook altijd het gevoel gegeven dat ik alles kan als ik maar mijn best doe. Peet, ik was nooit zo hard mijn best gaan doen als ik geen slimme oudere zus had om tegen op te boksen. Lieve Remco, zorg je goed voor mijn zus?

Ruben en Tess, jullie zijn natuurlijk waar het echt om draait in het leven.

Steef, altijd alles. Je bent geweldig. Dat weet ik al heel lang, en toch bewijs je het elke dag opnieuw. Blij.