Context-dependent chemical communication: Alarm pheromones of thrips larvae

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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.
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 anal 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-

**TABLE 8-1 Number of anal droplets containing alarm pheromone.**

<table>
<thead>
<tr>
<th>Predator</th>
<th>Attack</th>
<th>Kin Pheromone</th>
<th>No pheromone</th>
<th>Non-kin Pheromone</th>
<th>No pheromone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mite</td>
<td>No</td>
<td>0</td>
<td>34</td>
<td>7204</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1</td>
<td>2</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td>Bug</td>
<td>No</td>
<td>1</td>
<td>18</td>
<td>4162</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>3</td>
<td>7</td>
<td>46</td>
<td>15</td>
</tr>
</tbody>
</table>
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 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 of 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 analy 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-
Chapter 8

Chemical communication 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
Discussion


