Within plant migration as an alternative counter-measuring avoidance behavior in *Zetzellia mali* (Ewing) (Acari: Stigmaeidae)

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ABSTRACT

Predator species and intraguild predators can affect predation on important pest species. This predation response can influence pest consumption when multiple predators co-occur. In sour cherry orchards in Baraghan (Karaj, Iran), *Zetzellia mali* (Ewing) (Acari: Stigmaeidae) is a predatory mite that feeds on the plant pest *Amphitetranychus viennensis* (Zacher) (Acari: Tetranychidae) juveniles. Additionally, *Scolothrips longicornis* Priesner (Thysanoptera: Thripidae) is recorded as an intraguild predator of *Z. mali* juveniles. We have studied whether the oviposition behavior of predatory mites can change in the presence of intraguild predators through vertical migration within host plant. According to our results, vertical migration happened in a significant number of *Z. mali* females in presence of conspecific odors (P < 0.01). Odors associated with *A. viennensis* and the predatory mites triggered upward migration (P < 0.01) while the prey presence alone did not result in any significant movement (P > 0.05). Receiving odors related to the thrips, *Z. mali* showed no preference for a significant vertical movement, while the presence of thrips and *A. viennensis* on upwind plants led to an upward migration (P < 0.05).

Key words: Vertical migration, conspecific odors, intraguild predation, counter measuring, *Amphitetranychus viennensis*, *Scolothrips longicornis*.

INTRODUCTION

Plant volatile compounds send important information for arthropods in identifying potential host plants (Bell and Carde, 1994). Predatory mites can locate their prey through the volatiles produced by infested host plants (Sabelis and van de Baan, 1983). On the other hand, con- and heterospecific prey species may find plants according to odors (Dicke *et al.*, 1993). Several investigations have indicated that predators are attracted to plants with prey rather than clean ones (no prey present) or the ones with competitors (Dicke and Sabelis, 1988., Turlings *et al.*, 1995., Zahedi-Golpayegani *et al.*, 2007). Discriminating between clean plants and those occupied by prey or competitors could also depend on the cost of plant signalling and the individual experience of situations (Zahedi-Golpayegani *et al.*, 2007., De Boer and Dicke, 2006). There are also examples of predatory mites avoiding odors emanated from plants occupied by both herbivorous mites and conspecific predators.

Zahedi-Golpayegani *et al.* (2007) showed that female *Zetzellia mali* Ewing (Acari: Stigmaeidae) avoided odors related to leaves with both spider mites and conspecific predators. The predatory mite, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) is a good example of such a situation. The predator females avoid odors related to leaves occupied by two spotted spider mites accompanied with conspecific predators (Janssen *et al.*, 1997). These interactions not only play an important role in arthropod food web, but also are critical for organized biological control programs.

One of the interacting paths through which volatiles could be effective is making the pest arthropod avoid plants with predators, which is mostly known

as between plant migration (Magalhaes *et al*. 2002). But when predation risk is heterogeneously distributed within one host plant, that pest arthropod could also reduce the risk of predation by moving to a low-risk area within the same host plant (Magalhaes *et al*. 2002). The probability of such a small scale, within plant avoidance behavior in a predatory mite in response to these counter-measuring factors is discussed in this article.

The hawthorn spider mite, *Amphitetranychus viennensis* (Zacher) (Acari: Tetranychidae), *Zetzellia mali* (Ewing) (Acari: Stigmaeidae) and *Scolothrips longicornis* Priesner (Thysanoptera: Thripidae) are agriculturally important pest and biocontrol agents in fruit orchards in Iran respectively (Zahedi-Golpayegani *et al.* 2004). The predatory mite *Z. mali* feeds on various phytophagous mites in apple orchards (Jamali *et al*. 2001; White and Laing 1977a & b; Santos 1976). In Iran, *Z. mali* is considered the only biological control agent, feeding on eggs and juveniles of *A. viennensis* (Zahedi-Golpayegani *et al.* 2004).

The predatory thrips, *S. longicornis* is known to consume spider mites (Gerlach and Sengonca, 1985). Furthermore, its predation on the eggs of *Z. mali* has been observed in laboratory cultures (Zahedi-Golpayegani, Pers.Obs.).

Few papers are available on the ecological aspects of these species. In this article, we have presented the results of experiments in which adult females of *Z. mali* were offered a choice between remaining on or leaving the strata received volatiles from the leaves with different compositions of tetranychid mite, conspecifics and predatory thrips.

Material and Methods
Rearing units

Zetzellia mali, A. viennensis and *S. longicornis* were collected from the same host plants (non- sprayed sour cherry trees) in Baraghan, Alborz province, Iran.

Hawthorn spider mite was used as a common prey for short-time conserving predators in the green house we needed to rear it in plastic Cadogan and Laing (1977) transparent assembly. Six Petri dishes (6 cm in diameter) each contained a 3×3×1 cm sponge with a 3.1× 3.1 cm tissue on it. The Sour cherry leaf was placed on the tissue upside down. By covering the container a 70-75% RH was provided.

Zetzellia mali and *S. longicornis* were each transferred on an isolated seedling in order to provide sufficient predatory mites for the experiments. All predators were sustained by spider mites.

Seedlings of sour cherry were planted in a greenhouse compartment at 26 ± 5 °C and 70 ± 10% RH with the distance of one meter between the upwind and downwind ones at Department of Plant Protection, Faculty of Agriculture, University of Tehran, Karaj, Iran. Seedlings were planted in cement blocks and watered every other day. Care was taken to avoid seedlings infested by any other herbivorous agent. Same aged plants with approximately 14 leaves were used for experiments. Small fans with standard and wind speed (1 m/s, confirmed by Weather Forecasting Organization of Iran), in 20 cm distance fixed prior to upwind seedling. Each experimental unit (upwind and downwind plants with the wind source) was isolated from the other one (with plastic sheets) not to be interfered with its near experimental units (fig. 1).
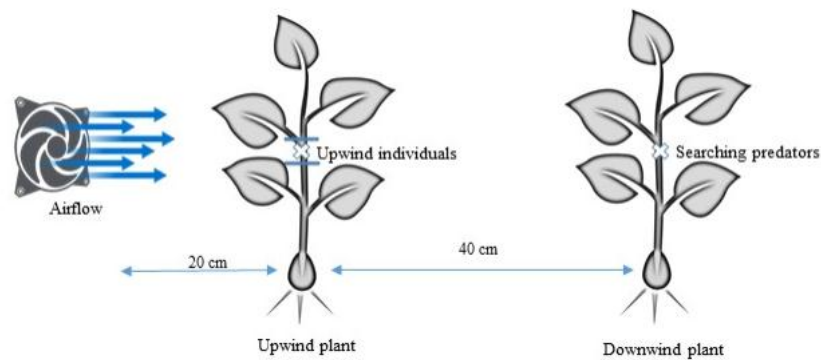
Figure 1. The upwind and downwind plant system and the source and searching individual locations

Experimental procedure
Experiments were performed in the greenhouse compartments. We introduced both the searching predators (downwind) and upwind individuals (source of odors) on the middle stratum of host plants and the rate of *Z. mali* migration to other strata (on downwind seedlings) was measured. The mites and thrips on upwind plants (middle stratum) were restricted to a 5 cm area (main stem and leaves) using two-sided tape glue in order to limit the source of inducing odors. To test whether *Z. mali* has the potential of migrating vertically, we exposed the individual predators on downwind plants receiving olfactory information related to upwind plants for 1 hour, with (1) 10 interacting conspecifics, (2) 10 interacting spider mites, (3) 10 conspecifics interacting with 10 *A. viennensis* females, (4) 10 heterospecific predators, (5) interacting prey and heterospecific predator. Upwind arthropods were placed in the middle stratum 5 hours prior to the experiment. Care was taken to replace the consumed individuals during this period of time. Predatory mites were not forbidden from choosing close leaves otherwise the probable migration could not be interpreted as predator self-choice. Each experiment was replicated 12–15 times, with new sets of inducing upwind arthropods and new unexperienced searching predator individuals. Under the null hypothesis, we expected 50% of the individuals to move either upwards or downwards. We tested for the significant deviations from this expectation. Statistical analysis was performed using a replicated G-test in Excel (Microsoft Corporation, Seattle Washington), which included a test for heterogeneity among replicate experiments (Sokal and Rohlf, 1995).

Results
Within plant migration in response to conspecifics
In counter-measuring trials a significant number (31 out of 40, $P < 0.01$) of the predators on downwind plants did migrate (Table 2b). No significant difference was observed between the number of predatory mites moved upwards (14 out of 31) and downwards (17 out of 31) ($P > 0.5$) (Table 1a). Approximately 30% of predators did not react through vertical migration (Tables 1a & b). They were sheltered mostly on the nearby leaf very close to the petiole. In the first 3–7 cm of vertical movements, *Z. mali* moved through a spiral fast running, the same behavior as was recorded in olfactometer experiments (Zahedi-Golpayegani *et al*., 2007). As *Z. mali* in downwind plant were not banned of

selecting leaves in spite of stems, the vertical escaping could be interpreted as a within plant migration.

Table 1. Results of three independent migration experiments (a) and Goodness of fit tests (b) when *Z. mali* received odors related to its conspecifics on upwind plants. Numbers of upward and downward migrations and no-migrations have been demonstrated as n (+), n (-) and n (0) respectively.

(a)

Replicated experiment	n (+)	n (-)	n (0)	n (total)
1	5	5	2	12
2	4	6	4	14
3	5	6	3	14

(b)

Replicated goodness of fit	d_f	G statistics	P value
G_h	2	0.185	0.9
G_p	1	0.3	0.5
G_t	3	0.48	0.9

Table 2. Integrated results of three independent migration experiments (a) and Goodness of fit tests when *Z. mali* received odors from its conspecifics (b).

(a)

Replicated experiment	n (+ & -)	n (0)	n (total)
1	10	2	12
2	10	4	14
3	11	3	14

(b)

Replicated goodness of fit	d_f	G statistics	P value
G_h	2	0.51	0.77
G_p	1	12.78	0.0003
G_t	3	13.29	0.0002

Migration in response to counter-measuring prey

When *A. viennensis* was on the upwind seedlings, 51% of predators (20 out of 39 individuals, P = 0.05) preferred migrating up or down. No significant directed migration was observed (P > 0.05) (Tables 3 & 4). The fraction in which migration happened, displayed a movement distance about 1.2–1.5 times less than that recorded in previous experiment.

Table 3. Results of three independent migration experiments (a) and Goodness of fit tests(b) when *Z. mali* received odors releted to *A. viennensis* on upwind plants. Numbers of upward and downward migrations and no-migrations have been demonstrated as n (+), n (-) and n(0) respectively.

(a)

Replicated experiment	n (+)	n (-)	n (0)	n (total)
1	3	4	5	12
2	4	2	6	12
3	4	3	8	15

(b)

Replicated goodness of fit	d_f	G statistics	P value
G_h	2	0.76	0.68
G_p	1	0.19	0.65
G_t	3	0.95	0.81

Table 4. Integrated results of three independent migration experiments (a) and Goodness of fit tests when *Z. mali* received odors from spider mites(b).

(a)

Replicated experiment	n (+ & -)	n (0)	n (total)
1	7	5	12
2	6	6	12
3	7	8	15

(b)

Replicated goodness of fit	d_f	G statistics	P value
G_h	2	0.38	0.82
G_p	1	0.32	0.05
G_t	3	0.41	0.93

Migration in response to receiving odors related to interacting *A. viennensis* and *Z. mali*

When *Z. mali* received odors related to interacting conspecifics and spider mites, a significant number of predators preferred to leave the releasing stratum (34 out of 41 individuals, 83%). A significant directed migration (P < 0.01) happened towards upper strata (26 out of 34 individuals, 76%) (Table 5).

Table 5. Results of three independent migration tests (a) and Goodness of fit experiment (b) when *Zetzellia mali* received odors related to interacting conspecific predators and spider mites. Number of migrating upwards, downwards and no migration have been demonstrated as n (+), n (-) and n (0) respectively.

(a)

Replicated experiment	n (+)	n (-)	n (0)	n (total)
1	10	3	2	15
2	8	3	3	14
3	8	2	2	12

(b)

Replicated goodness of fit	d_f	G statistics	P value
G_h	2	0.154	0.92
G_p	1	10.01	0.001
G_t	3	10.16	0.01

Migration in response to receiving odors related to thrips

When *Z. mali* received odors related to thrips, no significant difference was observed between the predators that left up and downwards (Table 6). Also the number of *Z. mali* females which preferred to stay at the releasing status did not differ with those preferred to leave (Table 7).

Migration in response to interacting prey and heterospecific predators

Receiving odors from leaves occupied by spider mites and the predatory thrips, 28 (82%) out of 34 predator individuals left their releasing strata, 20 (71%) of which moved upwards. In other word, *Z. mali* showed a significant upward migration (P < 0.05) (Table 8).

Discussion

Odors or chemical cues related to prey are factors tempting enough for directing predator choice toward it. Predatory mites can locate plants infested by their phytophagous prey, through the volatiles produced by plants in response to herbivore damage (Sabelis and Dicke, 1985). Hawthorn spider mite (*A. viennensis*) juveniles are considered as an obvious option for *Z. mali* females to feed on (Zahedi-

Golpayegani *et al.*, 2004). As a previous olfactory interaction has been reported among these mites (Zahedi-Golpayegani *et al.*, 2007), a significant

prey choosing response (through vertical migrating behavior) was expected.

Table 6. Results of three independent migration experiments (a) and Goodness of fit tests (b) when *Z. mali* received odors *S. longicornis* on upwind plants. Numbers of upward and downward migrations and no-migrations have been demonstrated as n (+), n (-) and n (0) respectively.

(a)

Replicated experiment	n (+)	n (-)	n (0)	n (total)
1	3	2	6	11
2	4	3	4	11
3	4	3	6	13

(b)

Replicated goodness of fit	d_f	G statistics	P value
G_h	2	3.58	0.16
G_p	1	1.05	0.3
G_t	3	4.63	0.98

Receiving odors from conspecifics, *Z. mali* females showed a significant vertical migration despite their accessibility to a closer, safe habitat (i.e. nearby leaves). Similar observations from olfactometer experiments in which *Z. mali* avoided arms ended to conspecifics (Zahedi-Golpayegani *et al.*, 2007) confirms that *Z. mali* is able to recognize and avoid patches occupied by conspecific competitors. Maleknia *et al.* (2012) also reported an avoidance behavior on behalf of *P. persimilis* from the plants occupied by interacting prey and conspecific predators. They discussed that this avoiding behavior would happen due to the predators previous experience (rearing background), and the information they received from the risky patches through odors. Here, no prey alarm pheromone seems to be the case as we did not introduce *T. urticae* on downwind seedlings. Our results contrasts with Pallini *et al.* (1997) who reported that *T. urticae* females preferred plants infested with conspecifics irrespective of the plant species through olfactory experiments.

Receiving odors related to prey

occupied patches, *Z. mali* did not migrate. This is in consistent with Zahedi-Golpayegani *et al.* (2007) who demonstrated a significant preference of the same predator species towards patches occupied by *A. viennensis* females. They interpreted such a behavior as either an avoidance from alternative arm (conspecifics) or the necessity of an interacting phase between prey and predator before any avoidance on behalf of predator from its counter-measuring prey females. Such a basis (interacting prey and predator) was not provided on our upwind plants, so that we interpret the predator sediment as its perception of the prey presence. This could be comparable with Magalhaes *et al.* (2002) who reported no significant vertical migration of Cassava Green mites in predator free trials. Although the difference between mites migrating upward or downward was not significant, it seems that the total significant migrating numbers refers to starved vagrant predators searching behavior receiving odors related to *A. viennensis*.

In response to odors from interacting

prey and conspecific predator, *Z. mali* reacted more restrained by an upward migrating movement. This could be interpreted as a probable counter-measuring behavior on behalf of prey so that makes predator alert its new coming conspecifics not to experience similar situation in parallel patch. These observations conform with olfactometer tests (Zahedi-Golpayegani *et al.* 2007) which showed a significant preference towards odors related to prey alone rather than interacting prey and conspecific predators. Avoiding from

patches occupied with conspecific competitors could be another explanation. Such an observation was previously recorded by Saito (1986) through counter attack behavior of *Schizotetranychus celarius* (Banks) (Acari: Tetranychidae) against its predator, *Typhlodromus bambusae* Ehara (Acari: Phytoseiidae). Magalhaes *et al.* (2005) also showed that female *Iphiseius degenerans* (Berlese) avoided patches which their juveniles would suffer from thrips prey (*Frankliniella ociidentalis* (Pergande) counter attack.

Table 7. Integrated results of three independent migration experiments (a) and Goodness of fit tests when *Z. mali* received odors related to thrips (b).

(a)

Replicated experiment	n (+ & -)	n (0)	n (total)
1	5	6	11
2	7	4	11
3	7	6	13

(b)

Replicated goodness of fit	d_f	G statistics	P value
G_h	2	1.43	0.48
G_p	1	0.12	0.72
G_t	3	1.55	0.67

Table ۸. Results of three independent migration experiments (a) and Goodness of fit tests(b) when *Z. mali* received odors releted to *A. viennensis* and *S. longicornis* on upwind plants. Numbers of upward and downward migrations and no-migrations have been demonstrated as n (+), n (-) and n (0) respectiveiy.

(a)

Replicated experiment	n (+)	n (-)	n (0)	n (total)
1	7	2	2	11
2	8	3	0	11
3	5	3	4	12

(b)

Replicated goodness of fit	d_f	G statistics	P value
G_h	2	0.49	0.7
G_p	1	5.31	0.02
G_t	3	5.8	0.12

When the predatory thrips was considered as the only odor source on upwind seedlings, *Z. mali* showed no dispersing reaction (Table 7). It seems that despite its voracity towards stigmaeid mites, thrips odors are not alarming enough to make the predatory mite choose a free patch to stay. This was more obvious when receiving

odors related to interacting predatory thrips and spider mites on upwind plastic cages, *Z. mali* mostly moved up. It seems that this reaction is due to spider mite alarm pheromone that reveals the risky position of the prey. This risk factor could be interpreted as a competitor for this conspecific receiver which could affect it not only

by competing but also through feeding on its offspring. This could be compared with Faraji et al. (2001) who reported that the predatory mite, *I. degenerans* put its eggs far away from the flowers which *F. occidentalis* was present on. They considered *F. occidentalis* as a counter attacking prey which could kill the predator eggs.

Our experiments showed that presence of *A. viennensis* and *S. longicornis* made the predatory mite leave the patch. The reduced predation rate of *Z. mali* in noted condition (Zahedi-Golpayegani pers. Obs.) was another confirmation on the necessity of a mediated odor source for another more dangerous source recognition.

This also was previously mentioned by Pallini *et al.* (1997). Conceiving alarms related to risky conditions reduces the arthropod foraging behavior (Faraji *et al.* 2001). This could intensify *Scolothrips* interference on spider mite population as the competition between heterospecific predators is reduced, but may also lead to reduction of predation on *Zetzellia* larvae on behalf of thrips. Although predatory mites avoided patches occupied by its competitor, its exclusive presence in leaves with spider mites eggs might increase its predation rate. The population dynamical consequences of these effects need further studies.

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