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Abstract

The two-spotted spider mite, *Tetranychus urticae,* is a serious pest in a wide variety of crops worldwide, and can negatively affect the production of strawberries. Males of *T. urticae* approach and guard quiescent deutonymph females to increase their chances of fathering offspring, because only the first mate results in fertilization. Male mating behavior is a key step in spider mite reproduction.

This study investigated the behavior of *T. urticae* males towards females killed by entomopathogenic fungal species in two different taxons: *Neozygites floridana* (Division Entomophthoromycota, Class Neozygitomycetes, Order Neozygitales) and *Beauveria bassiana s.s* (Division Ascomycota, Class Sordariomycetes Order Hypocreales). Single males were exposed to one of the following choice situations (treatments), each with two different types of non-moving females present: (A) healthy quiescent deutonymph *vs. T. urticae* cadaver killed by *N. floridana*; (B) healthy quiescent deutonymph *vs. T. urticae* cadaver killed by *B. bassiana*; (C) *T. urticae* cadaver killed by *N. floridana vs. T. urticae* cadaver killed by *B. bassiana;* and (D) healthy quiescent deutonymph *vs.* healthy adult freeze-killed. The females were placed individually on two partly overlapping leaf discs. Male behavior was observed and analysed during two minutes every hour for 6 hours. An analog CCTV camera was used to record the experiment, and the second observation in treatments C and D were analysed using Ethovision® XT8 software to observe the distance moved by the males.

Results showed that males visited leaf discs with cadavers killed by fungi significantly more often than discs with healthy quiescent females. Female cadavers killed by fungi were also more touched and guarded by males than the healthy quiescent female. When males were exposed to two disease-free females (Treatment D) touching and guarding towards the freeze-killed female was less commonly observed than towards quiescent female. Further, males moved a longer distance in the presence of two fungal cadavers (C) than in the presence of two disease-free females (D).

The results confirm the earlier finding that males prefer females killed by *N. floridana* and demonstrate that males exhibit similar behavior towards *B. bassiana* cadavers. This behavior could compromise the mating goal (chances of fathering offspring) and in addition promote the spread of entomopathogens. Understanding this phenomenon could be important to improve biological control of spider mites, and more research is needed to investigate the mechanism behind the attraction of *T. urticae* males to fungally killed female.

1. Introduction

The two-spotted spider mite, Koch (Acari: Tetranychidae), is a serious pest of many crops throughout the world (Jeppson et al. 1975; Greco et al. 2005), including strawberries (Cross et al. 2001) and can lead to 25 % reduction in yield (Walsh, 1998). *T. urticae* feeds on chlorophyll, water, and nutrients present in leaf cells using a piercing–sucking process resulting in reduced photosynthesis, foliar damage (van der Geest, 1985; Martinez-Ferrer et al. 2006) and also lead a decrease in fruit size and fruit set (Polk, 1994).

The control of *T. urticae* represents a challenge for the worldwide crop industry and to the pest management approach. Exposing spider mites to chemical pesticides is one of the most common strategies to control *T. urticae* population. However, the development of resistance it has been shown a problem (van Leeuwen et al. 2010). Its rapid developmental rate, short generation time, and high net reproductive rate combined with favorable climatic conditions, allows *T. urticae* populations to increase to damaging levels and seem to facilitate the evolution of pesticides resistance (Van Leeuwen et al. 2010), making chemical control of this species difficult (Helle & Sabelis, 1985a; James & Price, 2002; Marcic, 2003). Moreover the use of chemicals to control *T. urticae* creates negative effects on the environment and facilitates further population growth by disrupting the natural enemies of spider mites (van der Geest, et al. 2000; Bostanian et al. 2003). Biological control using predatory mites is another method to control *T. urticae* and can provide good levels of control (Wysoki, 1985; Fitzgerald et al. 2007**).** According to St Leger, Wang, & Fang (2011) entomopathogens are becoming an important tool to control pests, and they are being developed as alternatives to chemical pesticides.

The life cycle of *T. urticae* consists of five life stages, three of them ending in a quiescent stage (Fig. 1) [Quiescent stages are periods of female inactivity with slow metamorphosis (Crooker, 1985)]: egg, larva, quiescent larva, protonymph, quiescent protonymph, deutonymph, quiescent deutonymph and adult stages (Crooker, 1985).

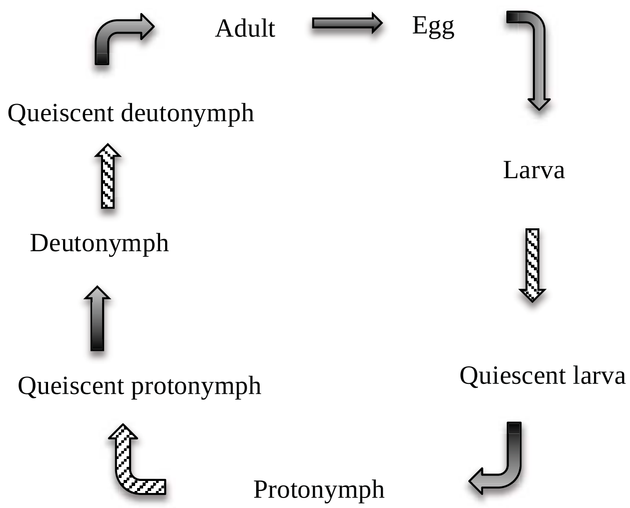

Fig. 1: *Tetranychus urticae* life cycle. Black arrows indicate a life stage and striped arrows indicate quiescent stages.

T. urticae is a species that guards its mate prior to copulation (Sathoh & Takafuji, 2001). Touching and hovering is also an important stage on mating process of *T. urticae* (Sonenshine, 1985). *T. urticae* males guard quiescent deutonymph females, the stage immediately before adult emergence and sexual maturation (Potter et al. 1976), because only the first male to mate the quiescent deutonymph will fertilize the eggs (Potter and Wrensch, 1978). Guarding can be risky for males, because, they are exposed to predators, diseases and competitors, and also lose opportunities for other activities, such as searching for females that are closer to becoming receptive (Parker, 1974; Oku, 2013).

Entomopathogenic fungi are also promising biocontrol agents for *T. urticae* **(**Chandler et al. 2000, van der Geest, et al. 2000)**.**
Neozygites floridana (Division Entomophthoromycota, Class Neozygitomycetes, Order Neozygitales) is an obligate fungal pathogen to species of mites in the family

Tetranychidae (Keller, 1997) especially *T. urticae* (Westrum et al. 2014), as a natural enemy (Elliot, 1998; Klingen and Westrum, 2007). Capilliconidia of *N. floridana* attach to the legs of *T. urticae*, germinates and penetrates the cuticle. Then the fungus develops inside the host as hyphal bodies, kills the host, penetrates the cuticle and produces primary conidia. Primary conidia are ejected from swollen cadavers and then germinate to form capilliconidia that can infect new mites (Carner, 1976; Elliot, 1998; Delalibera et al. 2006).

Beauveria bassiana s.s. (Division Ascomycota, Class Sordariomycetes Order Hypocreales) is a generalist entomopathogenic fungus that is widely distributed throughout the world (St. Leger et al. 1992; Boucias & Pendland, 1998, Meyling et al. 2009). For example it has been isolated from insects, mites, and soil, it is a part of the normal microbial flora, and other substrates (MacLeod, 1954; Goettel et al. 1990, Boucias & Pendland, 1998). As *N. floridana*, *B. bassiana* has also a great potential in the regulation of two-spotted spider mite (Wekesa et al. 2006; Seiedy et al. 2010). Conidia of *B. bassiana* land on its host, germinate and penetrate the cuticle. Then the fungus develops inside the host body, kills the host, penetrates the cuticle and produces conidia that can infect new mites (Boucias & Pendland, 1998; Zimmermann, 2007). During the infection process, *B. bassiana* produces proteolytic enzymes and toxins. These fungal compounds e.g. Oxalic acid (Roberts, 1981), beauvericin, bassianin, bassianolide, beauverolides, beauveriolides, tenellin, oosporein (Strasser et al. 2000; Vey et al. 2001), and bassiacridin (Quesada-Moraga & Vey, 2004) can either directly damage the principal functions of the hemolymph or cause damage indirectly by producing a toxic by-product in the host (Kučera & Samšiňáková, 1968).

Few studies have investigated how these fungal pathogens affect mating behaviour of T. urticae and thus resulting in dispersion of the fungi over the population of T. urticae. Trandem et al. (2015) found that the fungal pathogen *Neozygites floridana* affected *T. urticae* male mating behavior. Males were significantly more often observed near non-sporulating and primary conidia sporulating cadavers killed by *N. floridana* than near healthy *T. urticae* females. In the present study, the aim is to find out whether another fungal pathogen, *B. bassiana s.s*. that is taxonomically very different from *N. floridana* also affects *T. uricae* male mating behavior and whether there is an effect of live versus dead without the fungus.

To investigate the effect of fungal species and female state (live versus dead) on mating behavior of *T. urticae* males, we observed males given the following choices of females: (A) healthy quiescent deutonymph vs. *T. urticae* cadaver killed by *N. floridana*; (B) healthy quiescent deutonymph vs. *T. urticae* cadaver killed by *B. bassiana;* (C) *T. urticae* cadaver killed by *N. floridana* vs. *T. urticae* cadaver killed by *B. bassiana*, and (D) healthy quiescent deutonymph and vs. freeze-killed *T. urticae* female cadaver (healthy before freezing). Unlike in Trandem et al., (2015), sporulating cadavers were not included in the study.

2. Material and methods

2.1. *T. urticae* stock culture

The *T. urticae* was collected in a strawberry field at Kirkejordet, Ås, Norway (59º 42” N, 10º 44” E) in 2015. *T. urticae* stock culture was maintained on strawberry plants, *Fragaria x ananassa* (Duch), in a climatic chamber at 21 ± 1ºC, 60 % Relative Humidity and photoperiod of 16 ligth: 8 darkness.

2.2. *N. floridana* isolate

The *N. floridana* isolate NCRI 271/04 used in these experiments was collected from its natural host *T. urticae* found on strawberry plants at Stensrud, Vassum, Ås, Norway (59º 42” N, 10º 44” E) in 2004. This isolate was chosen because it is well adapted to the temperatures in northern temperate regions (Klingen and Nilsen, 2009). The isolate was stored as *N. floridana* killed *T. urticae* cadavers in NUNC Cryo Tube™ (1.8 ml) in a fridge at 5 °C prior to the production of new cadavers for the experiment.

2.3. *B. bassiana* isolate

The *B. bassiana* isolate (NCRI 2/01/Bb) used in this study was isolated from an infected bark beetle *Ips typographus* (L.) (Coleoptera, Scolytidae) at Asker, Norway (59°51′ N 10°25′ E) in 2001 and the species identification has been confirmed molecularly (Klingen et al. 2015). This isolate was chosen because it has shown promising results in tests of properties that are important for a biocontrol agent used under northern temperate regions (Klingen et al. 2015). The isolate was stored in 86% glycerol and 10% skimmed milk solution inside a NUNC Cryo Tube™ (1.8 ml) and storage in the freezer at -80 ºC prior to the production of new cadavers for the experiment.

2.4. Production of *N. floridana*-killed cadavers

Three non-sporulating *N. floridana* (NCRI 271/04) killed *T. urticae* cadavers were taken out of the fridge and placed with their dorsal side up on a strawberry leaf disc (15 mm diameter) with the adaxial side down onto 1.5% water agar in a Petri dish (5 cm diameter and 2 cm high). Six such Petri dishes with water agar, leaf discs and cadavers were placed in a plastic box (22x16x7 cm) with the lid slightly open, to provide the right RH, and wrapped with aluminium foil for darkness. The box was kept in a climatic chamber at 23 ±1ºC, 60% RH for 24 h, for the cadavers to sporulate. Thirty healthy adult females were then transferred to each leaf disc with sporulating cadavers and placed at the conditions described above for 24 h for *N. floridana* inoculation. The next day the leaf discs with *N. floridana* inoculated *T. urticae* were transferred to a 3-week old strawberry plant at ambient laboratory conditions (21-25 ºC, 20-35% RH and photoperiod of 24 h of light). As the leaf disc with inoculated mites started to wilt, mites walked on to the strawberry plant and established there. After 8-9 days, infected *T. urticae* died and dry non-sporulating cadavers were collected and stored in a cotton cloth in a NUNC Cryo Tube™ (1.8

ml) and stored in the refrigerator at 3-4 ºC for 15 days before used in the experiment (Fig. 6D) following the method used by Trandem et al. (2015).

2.5. Production of *B. bassiana*-killed cadavers

The *B. bassiana* isolate NCRI 2/01Bb was taken out of the freezer and transferred to potato dextrose agar (PDA) and cultured for 19-25 days at ambient laboratory conditions (21-25 ºC; 20-35% RH). Conidia of *B. bassiana* isolate from the potato dextrose agar were then harvested in 0.05% Tween 20 by using a sterile spatula. The conidia solution was filtered through a cotton cloth. Conidia suspension was counted using a hemocytometer and adjusted to 1 x 10^7 conidia/ml. Four *T. urticae* were placed on a strawberry leaf disc (15 mm diameter) with the adaxial side down onto 1.5% water agar in a Petri dish (5 cm diameter and 2 cm high). Ten petri dishes with water agar, leaf discs and mites were placed in a plastic box (30x24x15 cm) and petri dishes were sprayed with the conidia suspension of *B. bassiana*. The box was kept in a climatic chamber at 25±1ºC, 70% RH and photoperiod of 12L: 12D for 7 days. During the production of *B. bassiana* cadaver daily observation was made to record any mortality of *T. urticae* before the 7th day of infection. Infected *T. urticae* dry non-sporulating cadavers were collected and stored in a cotton cloth in a NUNC Cryo Tube™ (1.8 ml) and stored in the refrigerator at 3-4 ºC for 15 days before used in the experiment (Fig. 6A).

2.6. Production of healthy quiescent female deutonymphs

Two adult *T. urticae* females were placed individually on strawberry leaf discs (15 mm diameter) with the adaxial side down onto 1.5% water agar in a 30 ml plastic vial with lid. Fifteen holes were made in the lid with insect pin no. 4 for aeration. Vials with leaves and *T. urticae* females were placed in a climatic chamber at 23 ±1ºC, 60% RH, photoperiod of 16L: 8D for photoperiod of 24 h for mites to lay eggs. *T. urticae* females were then removed and eggs left to hatch and develop into quiescent female deutonymphs at the same climatic conditions as mentioned above for 7-8 days. The quiescent *T. urticae* female deutonymph, hereafter referred to as '♀QD', were used in the choice experiment immediately after they were observed (Fig. 6G).

2.7. Production of freeze-killed female cadavers

Live adult *T. urticae* females from the stock culture were placed individually on strawberry leaf discs (15 mm diameter) with the adaxial surface down onto 1.5% water agar in 30 ml plastic vials with lid. Each vial had four females and leaf discs. To kill the adult *T. urticae* females, vials were then placed into an -80 ºC freezer for 12 hours to insure that females were dead (Fig. 6H).

2.8. Collecting males

Sixteen males of *T. urticae* were collected from the culture 24 h before the experiment started. The males were placed on strawberry leaf discs (15 mm diameter) with the adaxial surface down onto 1.5% water agar in a Petri dish (5 cm diameter and 2 cm high) and the dishes were kept in a climatic chamber at 23 ±1ºC, 60% RH, 16L: 8D until used in the experiment.

2.9. Choice treatments

Two strawberry leaf discs (12 mm diameter) were placed with the adaxial surface down on top of 1.5% water agar in a Petri dish (5 cm diameter and 2 cm high) they were slightly overlapping allowing the males to walk freely on both discs. A few drops of water were added to the agar to prevent males from walking off the discs. Males should choose between two different types of *T. urticae* females. The male of *T. urticae* was then introduced where the two leaf discs overlapped. The experiment was run four times at ambient laboratory conditions (21-25 ºC, 20-35% RH) during daytime from 10:00am – 17:00pm involving N= 16 Petri dishes. The sequence of the treatments was randomized and the position of the Petri dish (e.g. cadaver to the right or to the left) was randomized at each recording. The Petri dishes were without lids throughout the experiment, the low RH in the laboratory inhibited sporulation. The experiment was divided into three temporal blocks (I: 8, 9, 10 and 11 December 2015; II: 8, 9, 10 and 11 February 2016 and III: 15, 16, 17 and 18 February 2016).

2.9.1. Choice treatment A: ♀QD *vs. N. floridana*- ♀cadaver.

A non-sporulating *N. floridana*-killed *T. urticae* cadaver was placed in the center of one of the leaf discs and a ♀QD was placed in the center of the other leaf disc that males could choose between a healthy live female and a *N. floridana*-killed female cadaver (Fig. 2).

2.9.2. Choice Treatment B: ♀QD *vs. B. bassiana* ♀cadaver

This experiment was conducted in the same way as the experiment described in Section 2.9.1), except that *B. bassiana*-killed *T. urticae* cadavers were used instead of *N. floridana*-killed *T. urticae* cadavers.

2.9.3. Choice treatment C: *B. bassiana* ♀cadaver *vs. N. floridana* ♀cadaver

This experiment was conducted in the same way as the experiment described in Section 2.9.1), except that males were given a choice between a *B. bassiana*-killed *T. urticae* cadavers and a *N. floridana*-killed *T. urticae* cadavers.

2.9.4. Choice treatment D: ♀QD *vs.* freeze-killed female cadaver

This experiment was conducted in the same way as the experiment described in Section 2.9.1.), except that males were given a choice between non-inoculated, freeze killed cadavers and ♀QD. Freeze-killed cadavers were collected one hour before the experiment started.

2.10. Observation of male behaviour

The two leaf discs in each Petri dish were observed and recorded during two minutes every hour for 6 h, starting immediately after male introduction.

An analog CCTV camera (model Nº WV-CP 460/6 Panasonic® with lens 3.5-8 mm) was used to record the experiment. On all six occasions, the leaf discs were monitored for three male behaviours: (1) Leaf disc choice, (2) Touching (3) Guarding.

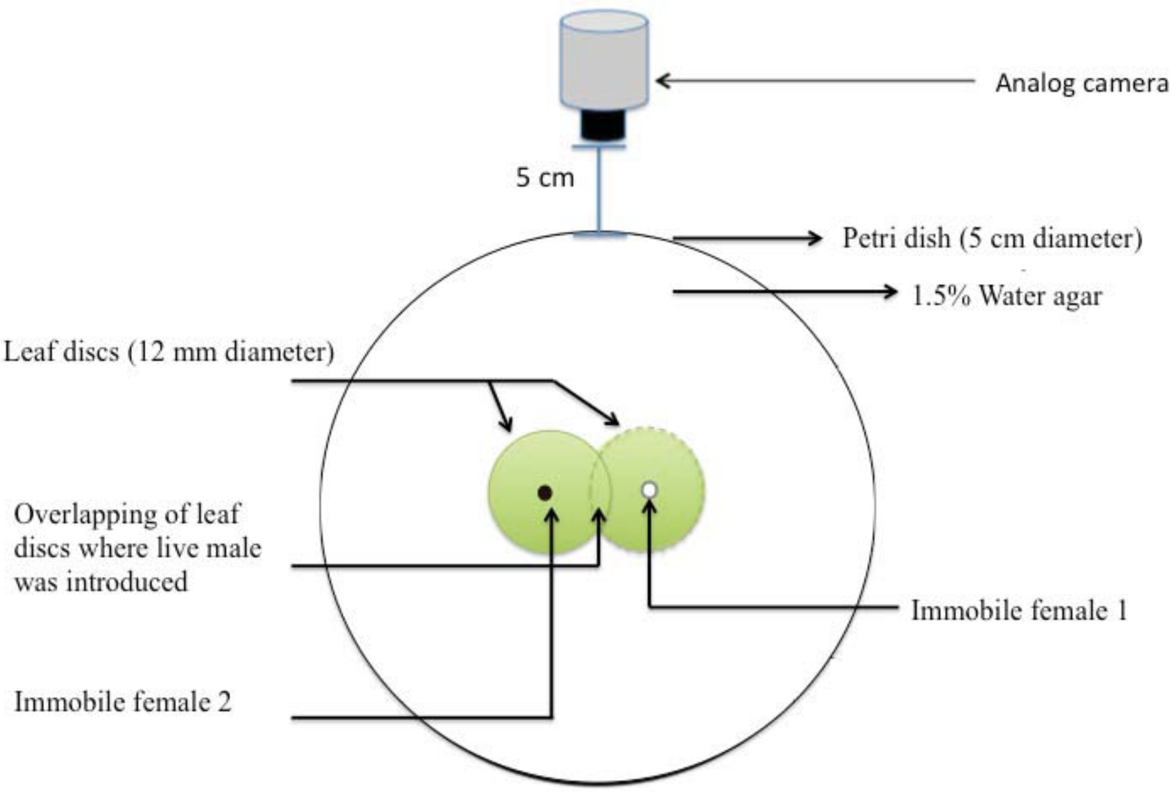

Fig. 2- Experimental set up for the four choice experiments. Immobile females: *N. floridana* cadavers, *B. bassiana* cadavers, healthy quiescent females and freeze-killed females.

The recordings made during the second observation of each Petri dish in treatment C and D were also analysed using Ethovision® XT8 software (Noldus Information Technology, Wageningen, the Netherlands) (Noldus et al. 2002). The distance moved by the male during the two minutes was recorded with 25 video frames per second. Tracking was made using one of the detections methods that Ethovision® XT8 software offers (e.g. Dynamic subtraction). In this method, each sampled image is compered with the reference image that is updated regularly. The program processes the image using algorithms to detect the mite against the background. During data acquisition, Ethovision® displays the live video image, tracking statistics (elapsed time, number of samples) and the x, y co-ordinates of the T. urticae males on the arena (Noldus et al. 2002). To track the same side of the leaf discs, X co-ordinates from half of the total number of replicates in treatments C and D were multiplied by -1. The total time spent on the arena (in seconds) and the mean speed (mm/s) was recorded (Fig. 8 and Fig. 9).

2.10.1. Leaf disc choice

Each petri dish was observed for 10 s to register which leaf disc the male was on, using the first 10 s of each recording, following the method used by Trandem et al. (2015).

2.10.2. Touching behaviour

A method modified after Trandem et al. (2015) was used: A male was considered to be touching if he touched the quiescent female or cadaver in any way and the contact last less than 30s at the time of observation. Males were observed for 2 min if seen moving towards healthy female or cadaver, to check if touching occurred.

2.10.3. Guarding behaviour

A *T. urticae* male is considered to be guarding if he remained mounted upon or within one body length of the quiescent female or cadaver and did not leave it for 30 s or longer (Collins et al. 1993). This was also the criterium used in this study.

2.11. Statistical analysis

All data were analyzed with Minitab® Statistical Software version 17.1 using Petri dish as the experimental unit and a significance level of 0.05.
Data were analyzed with each Petri dish as a single unit to avoid pseudo-replication. Following the method used by Trandem (2015), "an index of the total leaf disc choice in each Petri dish was calculated by summing the number of times the male was seen on the disc with the female cadaver during the six observations and dividing by the number of observations". In eight dishes the quiescent female developed into an adult towards the end of experiment and in seven dishes the male died on the water agar.
The hourly observations before ecdysis/death were keeped, but observations after these events were not indexed.

"The index thus ranged between 0 and 1, with 0.5 representing a Petri dish with equal number of observations of males on the two leaf discs, and 1.0 a Petri dish with male present on the disc with a female cadaver in all 6 observations" (Trandem, 2015). The indexed data on leaf disc choice were then analyzed with the general linear model (GLM). Treatments A, B and D were analyzed together, using block, day and treatment as explanatory factors. Block was nested in day within treatments A, B and D. Treatment C was analysed separately, using block and day as explanatory factors. When a factor was significant, post-hoc comparison among means were carried out using Tukey tests at 95.0% confident interval. For each treatment a two-tailed 1-sample *t*-test was used to test if the index mean was significantly different from 0.5.

"Data on guarding and touching behavior were not normally distributed. These behaviors were therefore analyzed with the binary logistic regression tool (BLR), letting the response '1' signify at least one occurrence of the behavior during the six observations, and the response '0' an absence of the behavior during all six observations" (Trandem, 2015). For each petri dish there were four such binary responses: male touching a female cadaver at least once, male touching a healthy ♀QD (or cadaver in treatment C) at least once, and the corresponding two responses for guarding behavior.

Following the method used by Trandem (2015), in the binary logistic regression of each behavior, both relevant binary responses (one for healthy and one for cadaver) were included, meaning that each Petri dish was entered twice. The four choice experiments were analyzed separately, the explanatory factors were: female type (*N. floridana* cadaver, *B. bassiana* cadaver, healthy quiescent female and freeze-killed cadaver).

The data on distance moved by males, obtained from the video tracking, were analyzed with the general linear model using block, day and treatment as explanatory factor.

3. Results

3.1. Leaf disc choice

Treatment had an effect on male choice (GLM, $F_{2,130}$= 4.7, *P*= 0.011, treatments included in the analysis were A, B and D. (Fig. 3A)). Block (GLM, $F_{2, 130}$= 1.10, *P*= 0.374) and day $_{(block)}$ (GLM, $F_{2, 130}$= 0.74, *P*= 0.675) had no significant effect on male choice.

On treatments A (*N. floridana* vs. healthy ♀QD) and treatment B (*B. bassiana vs.* ♀QD) despite a living healthy ♀QD being present, males were most often observed on the leaf disc with cadaver. In a given leaf disc choice between two cadaver types, *N. floridana* ♀cadavers and *B. bassiana* ♀cadavers, no preference was observed (Fig. 3B). In the leaf disc choice between non-inoculated freeze-killed female and healthy ♀QD, males had no preference (Fig. 3A, Tab.1).

Choice treatment	N	T	P
A (*N. floridana* vs. healthy ♀QD)	48	2.69	0.010
B (*B. bassiana vs.* ♀ healthy QD)	48	3.36	0.002
C (*N. floridana* vs. *B. bassiana*)	48	1.92	0.060
D (freeze-killed healthy female *vs.* healthy ♀QD)	48	-0.76	0.452

Tab. 1) A two-tailed 1-sample *t*-test was used to test if the index mean on the leaf choice was significantly different from 0.5.

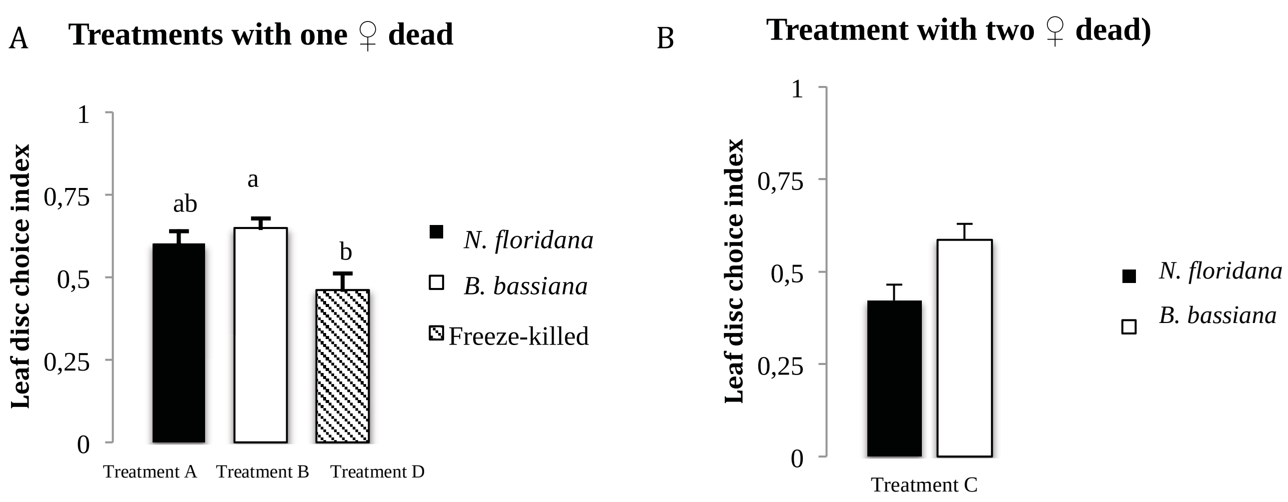

Fig. 3: Leaf disc choice index (mean+ SE) in the choice experiments indicating males leaf disc preference. Index > 0.5 indicates a preference for the leaf disc with cadaver. Fig. 3A: Treatments with one healthy ♀QD and one dead female, the cause of death shown. Fig. 3B: Leaf disc choice index in the experiment with two ♀ cadavers (*N. floridana* cadaver *vs*. *B. bassiana* cadaver Different letters above a column denote significant differences using Tukey test at 95% CI.

3.2. Touching and Guarding

In the choice between *N. floridana* cadaver and healthy ♀QD, touching behaviour was more frequently directed towards the cadaver than the healthy ♀QD (Fig. 4, Fig. 6E; BRL, Odds Ratio= 0.21; 95% CI: 0.0846; 0.5326; *P*= 0.001). Males also guarded cadavers more frequently than healthy ♀QD (Fig 4, Fig. 6F; OR= 0.1818, 95% CI: 0.0555; 0.5956; *P*= 0.005).

In the choice between *B. bassiana* and ♀QD**,** touching and guarding behaviour followed the same pattern, with more cadavers being touched (Fig 4; BRL, OR= 0.3522; 95% CI: 0.1393; 0.8904; *P*= 0.027) and guarded (Fig. 4, Fig. 6B and 6C; BRL, OR= 0.2824; 95% CI: 0.0925; 0.8619; *P*= 0.026).

When choosing between two cadaver types, *N. floridana* ♀cadavers and *B. bassiana* ♀cadavers, touching behaviour was more frequently observed towards *B. bassiana* cadavers than *N. floridana* cadavers (Fig 4; BRL, OR= 0.4231, 95% CI: 0.1852; 0.9665; *P*= 0.041). No difference was found in guarding behaviour between the two cadaver types (Fig. 4; OR= 0.3455; 95% CI: 0.1002; 1.1915; *P*= 0.092).

In the control treatment with non-inoculated freeze-killed female *vs*. healthy ♀QD, males touched healthy ♀QD significantly more often than freeze-killed females (Fig. 4; OR= 5.5714, 95% CI: 1.4723; 21.0831; *P*= 0.011). Male touching non-inoculated freeze-killed females was uncommon and was observed just 3 times. With respect to guarding, males showed a preference towards healthy ♀QD compared to freeze-killed females (Fig. 4; OR= 6.0526, 95% CI: 1.2494; 29.3209; *P*= 0.025).

I also tested whether *N. floridana* cadavers from treatment A (cadaver vs. healthy) and treatment C (cadaver vs. cadaver) were equally attended by males. No difference was found for touching (BLR, OR= 0.5520 95% CI: 0.2446; 1.2455; *P*= 0.150). However, males more frequently guarded *N. floridana* cadaver in treatment A than *N. floridana* cadaver in treatment C (BRL, OR= 0.1818; 05%CI: 0.0555; 0.5956; *P*= 0.005).
The same comparisons were made for *B. bassiana* cadavers from treatment B (cadaver vs. healthy) and C (*B. bassiana* cadaver vs. *N. floridana* cadaver). No significant difference between the two treatments in male touching (BLR, OR= 1.2915, 95% CI: 0.5742; 2.9048; *P*= 0.536) or guarding (BLR, OR= 0.6391, 95% CI: 0.2511; 1. 6265; *P*= 0.348) was found.

3.3. Tracking distance

Males moved a longer distance in Petri dishes with two fungal infected ♀cadavers (treatment C) than in dishes with two disease-free females on treatment D (GLM; $F_{1, 83}$ = 8.48, P= 0.005). Block (GLM; $F_{2, 83}$= 0.67, P= 0,536) and day $_{(block)}$ (GLM; $F_{9, 83}$=0.20, P=, 0.977) had no significant effect on distance moved by males (fig. 5).

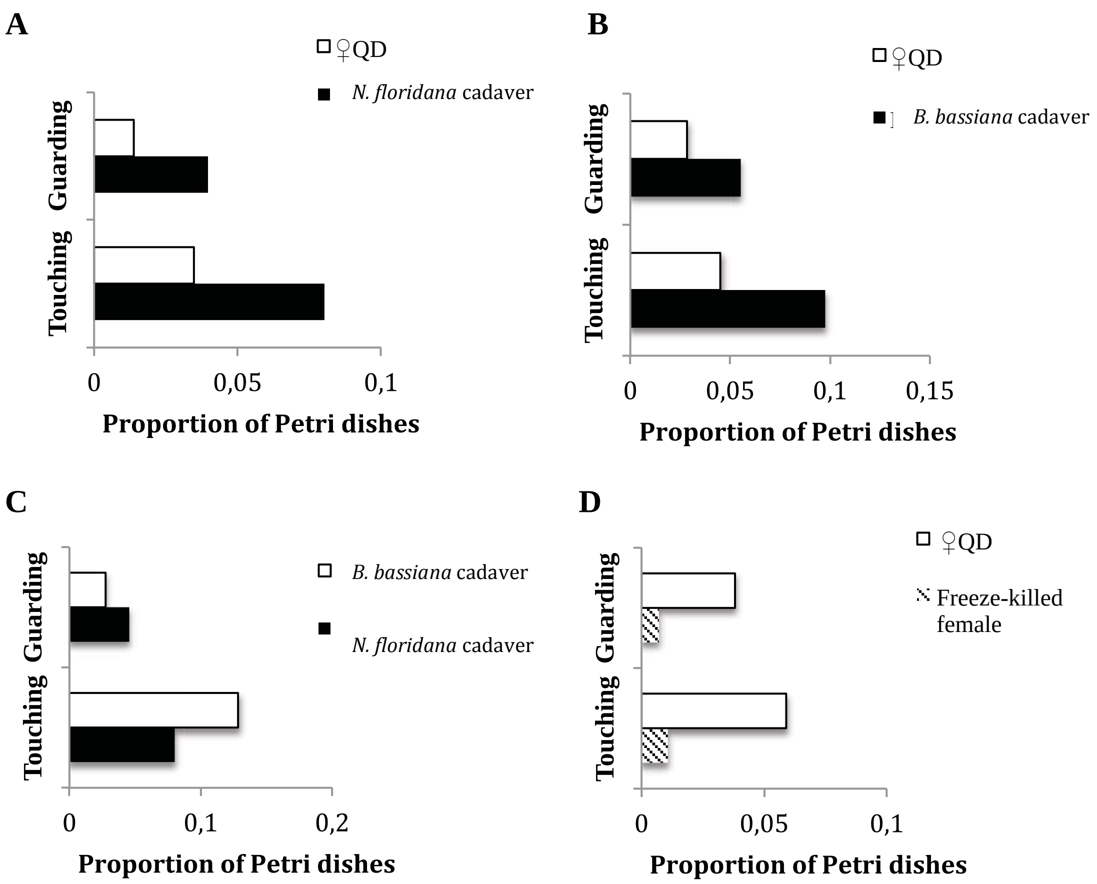

Fig. 4: Proportion of Petri dishes observed with a male touching or guarding the two females at least once.. Treatments: A) Healthy quiescent vs. *N. floridana* cadaver; B) Healthy quiescent *vs*. *B. bassiana* cadaver; C) *N. floridana* cadaver *vs*. *B. bassiana* cadaver D) Healthy quiescent *vs*. freeze-killed cadaver

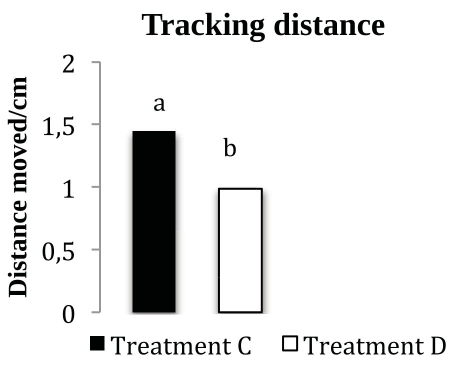

Fig. 5: Distance moved by males exposed to two female cadavers (black bar) and to two disease-free females (white bar). Different letters above a column denote significant differences using Tukey test at 95% CI.

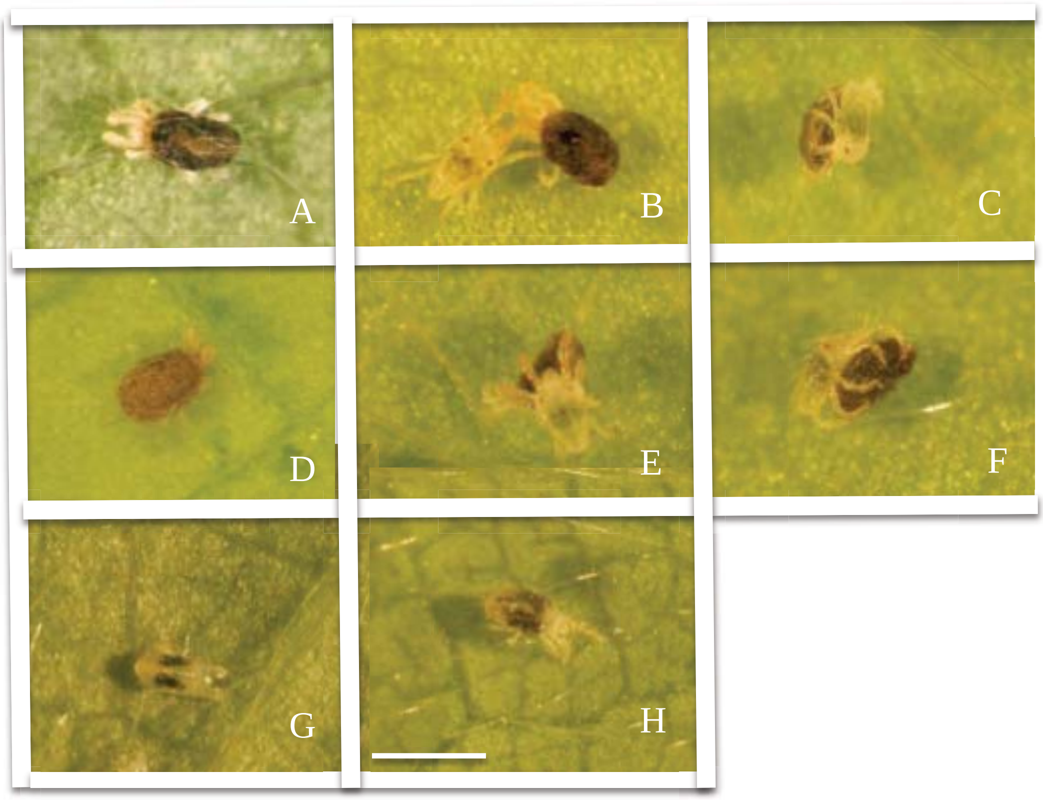

Fig. 6. A) *Tetranychus urticae* killed by *Beauveria bassiana* B) *T. urticae* male touching a *B. bassiana* cadaver C) *T. urticae* male guarding a *B. bassiana* cadaver D) *T. urticae* killed by *Neozygites floridana* E) *T. urticae* male touching *N. floridana* cadaver F) *T. urticae* male guarding *N. floridana* cadaver G) *T. urticae* quiescent female deutonymph H) Freeze-killed female. (Photos A and D, Karin Westrum, NIBIO, photos B, C, E, F, G and H, Dirce Silva, NMBU). Scale bar (white) = 0.5mm.

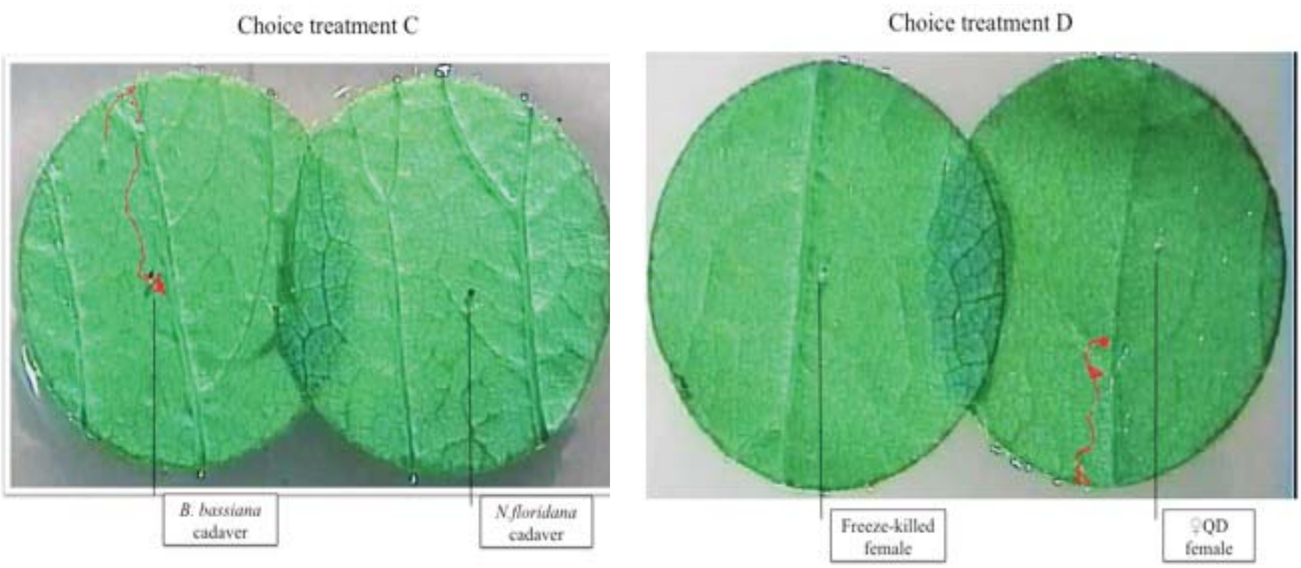

Fig. 7A. Example of locomotory track of male walking in arena on treatment C (male exposed to two female cadavers). Fig. 7B. Example of locomotory track of male walking in arena on treatment D (male exposed to two disease-free females).

Treatment C

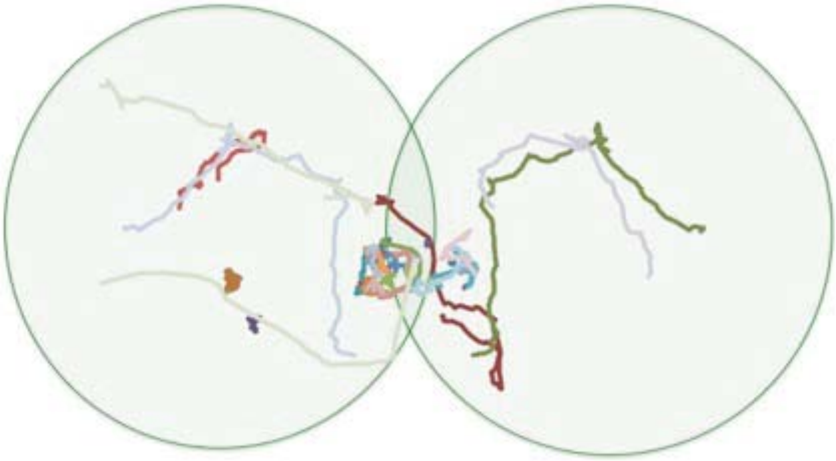

Fig. 8: Locomotory tracking of *T. urticae* males exposed to two females cadavers (treatment C). Tracks were extracted from Ethovision® XT8 software using the x, y co-ordinates of the males on the arena. *B. bassiana* cadavers were placed on the left side and *N. floridana* cadavers were placed on the right side of the arena. Tracks belong to the second hour observation, involving 46 replications.

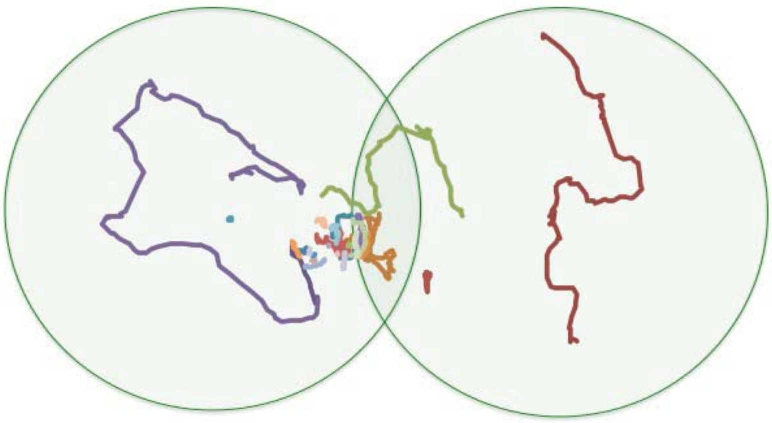

Fig. 9: Locomotory tracking of *T. urticae* males exposed to two disease-free females (Treatment D). Tracks were extracted from Ethovision® XT8 software using the x, y co-ordinates of the males on the arena. Freeze-killed females were placed on the left side and healthy quiescent females were placed on the right side of the arena. Tracks belong to the second hour observation, involving 45 replications.

4. Discussion

The present results suggest that males preferred leaf discs with dead fungal killed female to discs with healthy quiescent females or females killed by freezing. Except for the treatment with the healthy quiescent and the freeze-killed female, males touched and guarded female cadavers more often than healthy ones. These results indicate that males are unable to discriminate between fungal infected and healthy quiescent female .In contrast, males seem to avoid females killed by freezing. *T. urticae* males inability to detect fungal killed cadavers demonstrated when they prefer female cadavers instead of healthy quiescent females might be explained by a host-pathogen association. This might imply in a manipulation of the fungi on male behaviour defence.

T. urticae males should be able to distinguish between females killed by entomopathogenic fungi and healthy quiescent females although the latter is as immobile as the former (Trandem et al. 2015). Males should be able to detect the risk of mating with infected conspecifics by avoiding and/or rejecting the fungally-killed female. According to De Rood & Lefevre (2012), the most effective defence against disease may be behavioural avoidance of pathogens.

Regarding touching and guarding behaviour, when males were exposed to both disease-free females (healthy vs. healthy) simultaneously, reveals that healthy quiescent female were more attractive than freeze-killed female. This indicates that males were able to perceive that the freeze-killed females were not worth guarding. Thus seems that males were not attracted to the deadness *per se.*

Spider mites are known to perceive volatiles (Oku, 2015). Males of *T. urticae* use chemical, visual and tactile cues to recognize the surrounding information and also to encountering females that are worth guarding (Oku et al. 2003). The presence of setae in spider mites acts as sensory organs for touch and odor (Bostanian & Morrison, 1973). Sex attractants compounds emitted by *T. urticae* quiescent females like:

farnesol, nerolidol, geraniol, and citronellol have been reported to attract males, and these compounds are related to sexual communication in spider mites population (Regev & Cone, 1980; Sonenshine, 1985).

This study suggests that olfactory stimuli can be one of the factors that triggered male behaviour response. The pathogens might manipulate the infected female inducing an alteration on pheromone production, making the odour that exudes from fungal killed cadavers to be more attractive compare to the natural odour produced from the healthy quiescent female. Most likely, this is the reason that active males choose the leaf disc with fungal killed female cadavers, in adittion, males not being able to detect and avoid the fungal pathogen and being indifferent to killed female might present restrictions in their sensory capabilities.

Results present here, confirm that males are attracted to *N. floridana* female cadavers (Trandem et al. 2015). Notably, results also show that *B. bassiana* cadavers can also influence male mating behavior as *N. floridana*. No difference was observed in touching behaviour between *N. floridana* cadavers compared to healthy ♀QD (treatment A) and *N. floridana* cadavers compared to *B. bassiana* cadavers (treatment C). Regarding guarding behaviour, when *N. floridana* cadavers were in the same choice with *B. bassiana* cadavers on treatment C, *N. floridana* cadavers were less solicited by males than when present in the choice with healthy ♀QD on treatment A. When males were exposed to both entomopathogenic fungi, touching behaviour was more often observed towards *B. bassiana* cadavers. However, *N. floridana* cadavers were more preferred for the guarding behaviour.

Entomopathogenic fungi can induce different behavioral responses in arthropods. The ectoparasitoid *Cephalonomia tartsalis* is not able to detect the presence of *B. bassiana* or *B. bassiana* infected conspecifics, this behavior can result in mortality of infected parasitoids and can lead in a negative impact on *C. tartsalis* population (Lord, 2001). Hountondji et al. (2009) showed that the cassava green mites, *Mononychellus tanajoa*, avoided leaf discs with spores of *Neozygites tanajoa*. However, mites showed to be indifferent to leaf discs with its fungal pathogen as long no spores were produced (Hountondji et al. 2009). Alternatively, Mburu (2009) tested the effect of different fungal isolates of *Metarhizium anisopliae* on the termites *Macrotermes michaelseni* and confirming that termites can detect the presence of *M. anisopliae* through olfaction and avoid direct physical contact with the fungus. Mburu (2009) also founded that spores of *M. anisopliae* contain compounds that are repellent to termites and these compunds seemed to be detected from a distance.

In the present study males were exposed to non-sporulating cadavers. However, cadavers were full of hyphal bodies that also produce fungal compounds (Trandem et al. 2015). Results suggest that either *B. bassiana* or *N. floridana* use the non-infective form to attract males. According to Hountondji et al (2009), entopathogens have the ability to stay unnoticed in a non-infective form called as 'Trojan horse behaviour'. Cadavers killed by the fungi might produce infective spores when exposed to favorable condictions. Indeed, the cues involved in the male attraction and the 'Trojan horse behaviour' may enhance transmission and profit the fungal pathogen.

Visual cues can also be involved in male preference for cadavers. Oku et al. (2003) reported that *T. uticae* have limited visual capacity. In this study, female cadavers were usually bigger, round and had a different colour compared to quiescent females. However, it seems that chemical cues play the most important role in this attraction regarding male preference towards fungally dead females. When males were exposed to two disease-free females (healthy vs. freeze-killed), they walked around in both leaf discs, including the leaf disc with the freeze-killed female. Freeze-killed female was bigger, round and yellowish than the healthy ♀QD. Visually, freeze-killed females could have been more attractive to males. Nevertheless, males showed almost none interest in touching or guarding the freeze-killed female.

The use of a tracking system like Ethovision®, helped us to have a better understanding on how active males move and hence also the performing in touching and guarding behaviour in the presence of a female infected by entomopathogenic fungus. Etovision results suggest that when exposed to two fungally-killed females two-spotted spider mite male dispersal is influenced by the presence of fungus. When female cadavers are absent, the dispersion seems to be triggered by the demand for resources or more suitable paths. In addition, when fungally killed female cadavers are present, males spend more time walking around the leaf disc suggesting that fungal presence can work as a motivator for males being close to fungally-killed females. Otsuchi & Yano, (2014) demonstrate that the greater dispersal distance of *Tetranychus kanzawai* was induced by the presence of its predator *Neoseiulus womersleyi*.

Based on results presented here, male lethal behaviour can play an important ecologic role in the epizootic of these fungi and may dramatically enhance the efficacy of biological control on *T. urticae* and also the bio pesticide approach. This will further enhance the efficacy of fungi applied as biocontrol agents. In order to gain a better control of *T. urticae* population and to have a greater performance of entomopathogenic fungi, abiotic factors are important. Environmental temperature, RH and light are strongly related with the infective ability of fungal pathogens and their success in spread infection within the hosts. (Meyling & Eilenberg, 2007; Klingen et al. 2016). Pathogens performance under optimal conditions may offer advantages in controlling its pest (Wekesa et al. 2015).

The correct choice for mate is one way to avoid pathogens. By choosing healthy mates individual males can increase their success in fathering offspring. Results confirm that entomopathogenic fungi affect male response on mating choice. A host-pathogen interaction involves an evolutionary arms race in which detection and avoidance between the pathogen and host affects the life history, behavioural strategies and fitness of both hosts and pathogens (Roy et al. 2006). Unless males have an immune resistance to avoid infection, pathogens seem ahead of its host.

Why *T. urticae* males are attracted to a lethal pathogenic fungus is unclear. Fungal compounds produced by *B. bassiana* and *N. floridana* might have influence in *T. urticae* male sexual behaviour affecting and increasing male attractiveness for the cadavers. Therefore, prospect research should investigate the effect of these compounds on male preference for fungally killed females, and it should consider how these findings would perform in the environment. Prospect studies are needed to reveal the mechanisms involved on male dispersion when exposed to a fungal pathogen.

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6. Appendix

Appendix I: Data indexing for leaf disc choice, touching and guarding on treatment A: N. floridana cadaver *vs.* Healthy female quiescent deutonymph.

Appendix II: Data indexing for leaf disc choice, touching and guarding on treatment B: *B. bassiana* cadaver *vs.* Healthy female quiescent deutonymph.

Appendix III: Data indexing for leaf disc choice, touching and guarding on treatment C: *N. floridana* cadaver *vs. B. bassiana* cadaver.

Appendix IV: Data indexing for leaf disc choice, touching and guarding on treatment D: Healthy female quiescent deutonymph *vs.* Freeze-killed female.

Appendix V: Data indexing for distance moved on treatments C: *N. floridana* cadaver vs. *B. bassiana* cadaver and on treatment D: Healthy female quiescent deutonymph vs. Freeze-killed female.

Here,

Female type: type of cadaver.

Block: temporal block where experiment was conduct.

Day: day where the experiment was conduct.

Petri dish: Petri dish number

Leaf disc choice mean: summing the number of times the male was seen on the disc with the female cadaver during the six observations and divided by the number of observations made.

Touching and guarding: 1's for occurrence of the behaviour and 0's absence of the behaviour.

Appendix: I

Data indexing for leaf disc choice, touching and guarding on treatment A: N. floridana cadaver vs. Healthy female quiescent deutonymph.

Female type	Block	Day	Petri dish	Treatment	Leaf disc choice Mean	Touching	Guarding
N. floridana cadaver	1	1	1	A	0,83	1	0
N. floridana cadaver	1	1	2	A	0,33	1	0
N. floridana cadaver	1	1	3	A	0,5	1	0
N. floridana cadaver	1	1	4	A	0,17	1	0
N. floridana cadaver	1	2	1	A	0,33	0	0
N. floridana cadaver	1	2	2	A	0,67	1	1
N. floridana cadaver	1	2	3	A	0,5	1	0
N. floridana cadaver	1	2	4	A	0,83	0	0
N. floridana cadaver	1	3	1	A	1	1	1
N. floridana cadaver	1	3	2	A	0,17	0	0
N. floridana cadaver	1	3	3	A	1	1	1
N. floridana cadaver	1	3	4	A	0,5	0	0
N. floridana cadaver	1	4	1	A	0,67	1	0
N. floridana cadaver	1	4	2	A	0	0	0
N. floridana cadaver	1	4	3	A	0,33	0	0
N. floridana cadaver	1	4	4	A	0,67	1	1
N. floridana cadaver	2	5	1	A	0,8	0	0
N. floridana cadaver	2	5	2	A	0,83	0	0
N. floridana cadaver	2	5	3	A	0,5	0	0
N. floridana cadaver	2	5	4	A	1	1	0
N. floridana cadaver	2	6	1	A	0,83	1	1
N. floridana cadaver	2	6	2	A	0,67	1	1
N. floridana cadaver	2	6	3	A	0,83	1	1
N. floridana cadaver	2	6	4	A	0,33	0	0
N. floridana cadaver	2	7	1	A	1	1	1
N. floridana cadaver	2	7	2	A	0,5	1	1
N. floridana cadaver	2	7	3	A	0,33	0	0

N. floridana cadaver	2	7	4	A	0,67	0	0
N. floridana cadaver	2	8	1	A	0,8	0	0
N. floridana cadaver	2	8	2	A	0,67	0	0
N. floridana cadaver	2	8	3	A	0,83	1	1
N. floridana cadaver	2	8	4	A	0,33	0	0
N. floridana cadaver	3	9	1	A	0,83	1	1
N. floridana cadaver	3	9	2	A	0,83	1	1
N. floridana cadaver	3	9	3	A	0,67	0	0
N. floridana cadaver	3	9	4	A	0,5	0	0
N. floridana cadaver	3	10	1	A	0,83	1	1
N. floridana cadaver	3	10	2	A	0,83	0	0
N. floridana cadaver	3	10	3	A	0,83	1	1
N. floridana cadaver	3	10	4	A	0	0	0
N. floridana cadaver	3	11	1	A	0,67	1	0
N. floridana cadaver	3	11	2	A	0,33333	0	0
N. floridana cadaver	3	11	3	A	0,67	0	0
N. floridana cadaver	3	11	4	A	0,5	0	0
N. floridana cadaver	3	12	1	A	0,83	1	1
N. floridana cadaver	3	12	2	A	0,17	0	0
N. floridana cadaver	3	12	3	A	0,5	1	1
N. floridana cadaver	3	12	4	A	0,5	1	0
♀QD	1	1	1	A	0,16667	0	0
♀QD	1	1	2	A	0,66667	0	0
♀QD	1	1	3	A	0,5	0	0
♀QD	1	1	4	A	0,83333	0	0
♀QD	1	2	1	A	0,66667	0	0
♀QD	1	2	2	A	0,33333	0	0
♀QD	1	2	3	A	0,5	0	0
♀QD	1	2	4	A	0,16667	0	0
♀QD	1	3	1	A	0	0	0
♀QD	1	3	2	A	0,83333	0	0
♀QD	1	3	3	A	0	0	0
♀QD	1	3	4	A	0,5	0	0
♀QD	1	4	1	A	0,33333	0	0
♀QD	1	4	2	A	1	1	0
♀QD	1	4	3	A	0,66667	0	0
♀QD	1	4	4	A	0,33333	1	1
♀QD	2	5	1	A	0,2	0	0

♀QD	2	5	2	A	0,16667	0	0
♀QD	2	5	3	A	0,5	0	0
♀QD	2	5	4	A	0	0	0
♀QD	2	6	1	A	0,16667	0	0
♀QD	2	6	2	A	0,33333	0	0
♀QD	2	6	3	A	0,16667	0	0
♀QD	2	6	4	A	0,66667	0	0
♀QD	2	7	1	A	0	0	0
♀QD	2	7	2	A	0,5	1	0
♀QD	2	7	3	A	0,66667	0	0
♀QD	2	7	4	A	0,33333	0	0
♀QD	2	8	1	A	0,2	0	0
♀QD	2	8	2	A	0,33333	0	0
♀QD	2	8	3	A	0,16667	0	0
♀QD	2	8	4	A	0,66667	0	0
♀QD	3	9	1	A	0,16667	0	0
♀QD	3	9	2	A	0,16667	0	0
♀QD	3	9	3	A	0,3333	0	0
♀QD	3	9	4	A	0,5	1	1
♀QD	3	10	1	A	0,16667	0	0
♀QD	3	10	2	A	0,16667	0	0
♀QD	3	10	3	A	0,16667	0	0
♀QD	3	10	4	A	1	1	0
♀QD	3	11	1	A	0,33333	1	0
♀QD	3	11	2	A	0,66667	1	0
♀QD	3	11	3	A	0,3333	1	1
♀QD	3	11	4	A	0,5	1	0
♀QD	3	12	1	A	0,16667	0	0
♀QD	3	12	2	A	0,83333	0	1
♀QD	3	12	3	A	0,5	0	0
♀QD	3	12	4	A	0,5	0	0

Appendix: II

Data indexing for leaf disc choice, touching and guarding on treatment B: B. bassiana cadaver vs. Healthy female quiescent deutonymph.

Female type	**Block**	**Day**	**Petri dish**	**Treatment**	**Leaf disc choice Mean**	**Touching**	**Guarding**
B. bassiana cadaver	1	1	5	B	0,33	0	0
B. bassiana cadaver	1	1	6	B	0,67	1	1
B. bassiana cadaver	1	1	7	B	1	1	1
B. bassiana cadaver	1	1	8	B	0	0	0
B. bassiana cadaver	1	2	5	B	1	1	1
B. bassiana cadaver	1	2	6	B	0,5	0	0
B. bassiana cadaver	1	2	7	B	0,5	0	0
B. bassiana cadaver	1	2	8	B	0,2	1	1
B. bassiana cadaver	1	3	5	B	1	1	1
B. bassiana cadaver	1	3	6	B	0,83	0	0
B. bassiana cadaver	1	3	7	B	0,5	1	1
B. bassiana cadaver	1	3	8	B	0,67	1	1
B. bassiana cadaver	1	4	5	B	0,83	0	0
B. bassiana cadaver	1	4	6	B	0,25	0	0
B. bassiana cadaver	1	4	7	B	0,67	0	0
B. bassiana cadaver	1	4	8	B	1	0	0
B. bassiana cadaver	2	5	5	B	0,17	0	0
B. bassiana cadaver	2	5	6	B	0,67	0	0
B. bassiana cadaver	2	5	7	B	0,83	0	0

B. bassiana cadaver	2	5	8	B	0,5	1	1
B. bassiana cadaver	2	6	5	B	1	1	1
B. bassiana cadaver	2	6	6	B	0,83	1	1
B. bassiana cadaver	2	6	7	B	0,67	0	0
B. bassiana cadaver	2	6	8	B	0,17	0	0
B. bassiana cadaver	2	7	5	B	0,33	0	0
B. bassiana cadaver	2	7	6	B	0	0	0
B. bassiana cadaver	2	7	7	B	1	0	0
B. bassiana cadaver	2	7	8	B	0,67	0	0
B. bassiana cadaver	2	8	5	B	0,83	1	0
B. bassiana cadaver	2	8	6	B	1	1	1
B. bassiana cadaver	2	8	7	B	1	0	0
B. bassiana cadaver	2	8	8	B	0,5	0	0
B. bassiana cadaver	3	9	5	B	0,67	0	0
B. bassiana cadaver	3	9	6	B	0,17	0	0
B. bassiana cadaver	3	9	7	B	0,5	1	0
B. bassiana cadaver	3	9	8	B	0,33	0	0
B. bassiana cadaver	3	10	5	B	1	0	0
B. bassiana cadaver	3	10	6	B	1	0	0
B. bassiana cadaver	3	10	7	B	1	0	0
B. bassiana cadaver	3	10	8	B	1	0	0
B. bassiana cadaver	3	11	5	B	0,67	1	1
B. bassiana cadaver	3	11	6	B	0,67	1	0
B. bassiana cadaver	3	11	7	B	1	1	0
B. bassiana cadaver	3	11	8	B	0,67	1	0
B. bassiana cadaver	3	12	5	B	0,5	1	0
B. bassiana cadaver	3	12	6	B	0,17	0	0
B. bassiana cadaver	3	12	7	B	0,83	0	1
B. bassiana cadaver	3	12	8	B	0,83	1	1
♀QD	1	1	5	B	0,66667	0	0
♀QD	1	1	6	B	0,3333	0	0
♀QD	1	1	7	B	0	0	0
♀QD	1	1	8	B	1	1	1
♀QD	1	2	5	B	0	0	0
♀QD	1	2	6	B	0,5	0	0
♀QD	1	2	7	B	0,5	0	0
♀QD	1	2	8	B	0,8	1	1
♀QD	1	3	5	B	0	0	0

♀QD	1	3	6	B	0,16667	0	0
♀QD	1	3	7	B	0,5	0	0
♀QD	1	3	8	B	0,33333	1	1
♀QD	1	4	5	B	0,16667	0	0
♀QD	1	4	6	B	0,75	1	0
♀QD	1	4	7	B	0,33333	1	0
♀QD	1	4	8	B	0	0	0
♀QD	2	5	5	B	0,83333	0	0
♀QD	2	5	6	B	0,33333	0	0
♀QD	2	5	7	B	0,16667	0	0
♀QD	2	5	8	B	0,5	1	1
♀QD	2	6	5	B	0	0	0
♀QD	2	6	6	B	0,16667	0	0
♀QD	2	6	7	B	0,33333	0	0
♀QD	2	6	8	B	0,83333	0	0
♀QD	2	7	5	B	0,67	1	1
♀QD	2	7	6	B	1	0	0
♀QD	2	7	7	B	0	0	0
♀QD	2	7	8	B	0,33333	0	0
♀QD	2	8	5	B	0,16667	0	0
♀QD	2	8	6	B	0	0	0
♀QD	2	8	7	B	0	0	0
♀QD	2	8	8	B	0,5	0	0
♀QD	3	9	5	B	0,33333	0	0
♀QD	3	9	6	B	0,83333	0	0
♀QD	3	9	7	B	0,5	0	0
♀QD	3	9	8	B	0,66667	0	0
♀QD	3	10	5	B	0	0	0
♀QD	3	10	6	B	0	0	0
♀QD	3	10	7	B	0	0	0
♀QD	3	10	8	B	0	0	0
♀QD	3	11	5	B	0,3333	0	0
♀QD	3	11	6	B	0,3333	0	0
♀QD	3	11	7	B	0	0	0
♀QD	3	11	8	B	0,33333	0	0
♀QD	3	12	5	B	0,5	1	0
♀QD	3	12	6	B	0,83333	1	0
♀QD	3	12	7	B	0,16667	0	0

♀QD	3	12	8	B	0,16667	0	0

Appendix: III

Data indexing for leaf disc choice, touching and guarding on treatment C: N. floridana cadaver vs. B. bassiana cadaver.

Female type	**Block**	**Day**	**Petri dish**	**Treatment**	**Leaf disc choice Mean**	**Touching**	**Guarding**
B. bassiana cadaver	1	1	9	C	0,33333	1	1
B. bassiana cadaver	1	1	10	C	0,5	0	0
B. bassiana cadaver	1	1	11	C	0,16667	0	0
B. bassiana cadaver	1	1	12	C	0,33333	0	0
B. bassiana cadaver	1	2	9	C	0,83333	0	0
B. bassiana cadaver	1	2	10	C	0,16667	0	0
B. bassiana cadaver	1	2	11	C	0	0	0
B. bassiana cadaver	1	2	12	C	0,33333	0	0
B. bassiana cadaver	1	3	9	C	0,66667	1	1
B. bassiana cadaver	1	3	10	C	0,66667	0	0
B. bassiana cadaver	1	3	11	C	0,83333	0	0
B. bassiana cadaver	1	3	12	C	0,75	0	0

B. bassiana cadaver	1	4	9	C	0,83333	1	0
B. bassiana cadaver	1	4	10	C	0,66667	0	0
B. bassiana cadaver	1	4	11	C	0,83333	1	0
B. bassiana cadaver	1	4	12	C	1	0	0
B. bassiana cadaver	2	5	9	C	0,16667	0	0
B. bassiana cadaver	2	5	10	C	0	0	0
B. bassiana cadaver	2	5	11	C	0,33333	0	0
B. bassiana cadaver	2	5	12	C	0,66667	1	0
B. bassiana cadaver	2	6	9	C	1	1	1
B. bassiana cadaver	2	6	10	C	1	1	0
B. bassiana cadaver	2	6	11	C	0,5	1	0
B. bassiana cadaver	2	6	12	C	0,33333	0	0
B. bassiana cadaver	2	7	9	C	0,66667	0	0
B. bassiana cadaver	2	7	10	C	1	1	0
B. bassiana cadaver	2	7	11	C	0,83333	0	0
B. bassiana cadaver	2	7	12	C	0,16667	0	0
B. bassiana cadaver	2	8	9	C	0,66667	0	0
B. bassiana cadaver	2	8	10	C	0,83333	1	1
B. bassiana cadaver	2	8	11	C	1	1	0
B. bassiana cadaver	2	8	12	C	0,66667	1	1
B. bassiana cadaver	3	9	9	C	0,66667	1	1
B. bassiana cadaver	3	9	10	C	0,5	1	0
B. bassiana cadaver	3	9	11	C	0,83333	1	0
B. bassiana cadaver	3	9	12	C	0,5	0	0
B. bassiana cadaver	3	10	9	C	1	0	0
B. bassiana cadaver	3	10	10	C	0,83333	1	1
B. bassiana cadaver	3	10	11	C	0,16667	0	0
B. bassiana cadaver	3	10	12	C	0,16667	0	0
B. bassiana cadaver	3	11	9	C	1	1	0
B. bassiana cadaver	3	11	10	C	0	0	0
B. bassiana cadaver	3	11	11	C	0,83333	1	0
B. bassiana cadaver	3	11	12	C	0,5	1	0
B. bassiana cadaver	3	12	9	C	0,83333	1	1
B. bassiana cadaver	3	12	10	C	0,66667	1	1
B. bassiana cadaver	3	12	11	C	0,33333	1	0
B. bassiana cadaver	3	12	12	C	0,5	0	1
N. floridana cadaver	1	1	9	C	0,67	0	0
N. floridana cadaver	1	1	10	C	0,5	1	0

N. floridana cadaver	1	1	11	C	0,83	0	0
N. floridana cadaver	1	1	12	C	0,67	1	1
N. floridana cadaver	1	2	9	C	0,17	0	0
N. floridana cadaver	1	2	10	C	0,83	0	0
N. floridana cadaver	1	2	11	C	1	0	0
N. floridana cadaver	1	2	12	C	0,67	1	0
N. floridana cadaver	1	3	9	C	0,33	0	0
N. floridana cadaver	1	3	10	C	0,33	0	0
N. floridana cadaver	1	3	11	C	0,17	0	0
N. floridana cadaver	1	3	12	C	0,25	0	0
N. floridana cadaver	1	4	9	C	0,17	1	0
N. floridana cadaver	1	4	10	C	0,33	1	0
N. floridana cadaver	1	4	11	C	0,17	0	0
N. floridana cadaver	1	4	12	C	0	1	0
N. floridana cadaver	2	5	9	C	0,83	0	0
N. floridana cadaver	2	5	10	C	1	0	0
N. floridana cadaver	2	5	11	C	0,67	0	0
N. floridana cadaver	2	5	12	C	0,33	0	0
N. floridana cadaver	2	6	9	C	0	0	0
N. floridana cadaver	2	6	10	C	0	0	0
N. floridana cadaver	2	6	11	C	0,5	0	0
N. floridana cadaver	2	6	12	C	0,67	1	0
N. floridana cadaver	2	7	9	C	0,33	0	0
N. floridana cadaver	2	7	10	C	0	1	0
N. floridana cadaver	2	7	11	C	0,17	0	0
N. floridana cadaver	2	7	12	C	0,83	1	0
N. floridana cadaver	2	8	9	C	0,33	0	0
N. floridana cadaver	2	8	10	C	0,17	1	0
N. floridana cadaver	2	8	11	C	0	0	0
N. floridana cadaver	2	8	12	C	0,67	0	0
N. floridana cadaver	3	9	9	C	0,33	0	0
N. floridana cadaver	3	9	10	C	0,5	1	0
N. floridana cadaver	3	9	11	C	0,17	0	1
N. floridana cadaver	3	9	12	C	0,5	0	0
N. floridana cadaver	3	10	9	C	0	0	0
N. floridana cadaver	3	10	10	C	0,17	0	0
N. floridana cadaver	3	10	11	C	0,83	1	0
N. floridana cadaver	3	10	12	C	0,83	1	0

N. floridana cadaver	3	11	9	C	0	0	0
N. floridana cadaver	3	11	10	C	1	1	1
N. floridana cadaver	3	11	11	C	0,17	0	0
N. floridana cadaver	3	11	12	C	0,5	1	0
N. floridana cadaver	3	12	9	C	0,17	1	0
N. floridana cadaver	3	12	10	C	0,33	1	1
N. floridana cadaver	3	12	11	C	0,67	1	0
N. floridana cadaver	3	12	12	C	0,5	0	0

Appendix: IV

Data indexing for leaf disc choice, touching and guarding on treatment D: Healthy female quiescent deutonymph vs. Freeze-killed female.

Female type	Block	Day	petri dish	Treatment	Leaf disc choice mean	Touching	Guarding
Freeze-killed female	1	1	13	D	0,17	0	0
Freeze-killed female	1	1	14	D	0,67	0	0
Freeze-killed female	1	1	15	D	0,17	0	0
Freeze-killed female	1	1	16	D	0,83	0	0
Freeze-killed female	1	2	13	D	0,17	0	0
Freeze-killed female	1	2	14	D	0,17	0	0

Freeze-killed female	1	2	15	D	0,17	0	0
Freeze-killed female	1	2	16	D	1	0	0
Freeze-killed female	1	3	13	D	0,5	0	0
Freeze-killed female	1	3	14	D	0,67	0	0
Freeze-killed female	1	3	15	D	0	0	0
Freeze-killed female	1	3	16	D	1	0	0
Freeze-killed female	1	4	13	D	0	0	0
Freeze-killed female	1	4	14	D	0,17	0	0
Freeze-killed female	1	4	15	D	0,83	0	0
Freeze-killed female	1	4	16	D	0,33	0	0
Freeze-killed female	2	5	13	D	1	0	0
Freeze-killed female	2	5	14	D	0,33	0	0
Freeze-killed female	2	5	15	D	0,5	0	0
Freeze-killed female	2	5	16	D	0,17	1	1
Freeze-killed female	2	6	13	D	1	0	0
Freeze-killed female	2	6	14	D	0	0	0
Freeze-killed female	2	6	15	D	0,17	0	0
Freeze-killed female	2	6	16	D	0,33	0	0
Freeze-killed female	2	7	13	D	0,5	1	1
Freeze-killed female	2	7	14	D	0,67	0	0
Freeze-killed female	2	7	15	D	0,33	0	0
Freeze-killed female	2	7	16	D	1	0	0
Freeze-killed female	2	8	13	D	0,83	0	0
Freeze-killed female	2	8	14	D	0,83	0	0
Freeze-killed female	2	8	15	D	0,17	0	0
Freeze-killed female	2	8	16	D	0,17	0	0
Freeze-killed female	3	9	13	D	0,67	0	0
Freeze-killed female	3	9	14	D	0,17	1	0
Freeze-killed female	3	9	15	D	0,33	0	0
Freeze-killed female	3	9	16	D	1	0	0
Freeze-killed female	3	10	13	D	0,17	0	0
Freeze-killed female	3	10	14	D	1	0	0
Freeze-killed female	3	10	15	D	0	0	0
Freeze-killed female	3	10	16	D	0,17	0	0
Freeze-killed female	3	11	13	D	0,83	0	0
Freeze-killed female	3	11	14	D	0,83	0	0
Freeze-killed female	3	11	15	D	0,5	0	0
Freeze-killed female	3	11	16	D	0,5	0	0

Freeze-killed female	3	12	13	D	0,17	0	0
Freeze-killed female	3	12	14	D	0,17	0	0
Freeze-killed female	3	12	15	D	0	0	0
Freeze-killed female	3	12	16	D	0,83	0	0
♀QD	1	1	13	D	0,83333	0	0
♀QD	1	1	14	D	0,33333	0	0
♀QD	1	1	15	D	0,83333	0	0
♀QD	1	1	16	D	0,16667	0	0
♀QD	1	2	13	D	0,83333	0	0
♀QD	1	2	14	D	0,83333	0	0
♀QD	1	2	15	D	0,83333	1	1
♀QD	1	2	16	D	0	0	0
♀QD	1	3	13	D	0,5	0	0
♀QD	1	3	14	D	0,33333	0	0
♀QD	1	3	15	D	1	0	0
♀QD	1	3	16	D	0	0	0
♀QD	1	4	13	D	1	0	0
♀QD	1	4	14	D	0,83333	0	0
♀QD	1	4	15	D	0,16667	0	0
♀QD	1	4	16	D	0,66667	0	0
♀QD	2	5	13	D	0	0	0
♀QD	2	5	14	D	0,66667	0	0
♀QD	2	5	15	D	0,5	0	0
♀QD	2	5	16	D	0,83333	1	1
♀QD	2	6	13	D	0	0	0
♀QD	2	6	14	D	1	0	0
♀QD	2	6	15	D	0,83333	1	0
♀QD	2	6	16	D	0,66667	1	0
♀QD	2	7	13	D	0,5	0	0
♀QD	2	7	14	D	0,33333	0	0
♀QD	2	7	15	D	0,66667	0	0
♀QD	2	7	16	D	0	0	0
♀QD	2	8	13	D	0,16667	0	0
♀QD	2	8	14	D	0,16667	0	0
♀QD	2	8	15	D	0,83333	0	0
♀QD	2	8	16	D	0,83333	1	1
♀QD	3	9	13	D	0,33333	1	1
♀QD	3	9	14	D	0,83333	1	1

♀QD	3	9	15	D	0,66667	0	0
♀QD	3	9	16	D	0	0	0
♀QD	3	10	13	D	0,83333	1	0
♀QD	3	10	14	D	0	0	0
♀QD	3	10	15	D	1	0	0
♀QD	3	10	16	D	0,83333	1	1
♀QD	3	11	13	D	0,16667	0	0
♀QD	3	11	14	D	0,16667	1	1
♀QD	3	11	15	D	0,5	0	0
♀QD	3	11	16	D	0,5	0	0
♀QD	3	12	13	D	0,83333	1	1
♀QD	3	12	14	D	0,83333	0	0
♀QD	3	12	15	D	1	1	1
♀QD	3	12	16	D	0,16667	1	1

Appendix: V

Data indexing for distance moved on treatments C: N. floridana cadaver vs. B. bassiana cadaver and on treatment D: Healthy female quiescent deutonymph vs. Freeze-killed female.

Block	**Day**	**Treatment**	**Petri dish**	**Distance cm**	**Velocity cm/s**
1	1	C	9	1,24	0,04

1	1	C	10	1,10	0,09
1	1	C	11	0,80	0,10
1	1	C	12	0,24	0,02
1	1	D	13	0,96	0,03
1	1	D	14	1,54	0,14
1	1	D	15	0,24	0,02
1	1	D	16	1,37	0,05
1	2	C	9	2,13	0,02
1	2	C	10	1,81	0,02
1	2	C	11		
1	2	C	12	0,24	0,02
1	2	D	13	2,84	0,03
1	2	D	14	1,34	0,01
1	2	D	15	0,11	0,01
1	2	D	16	1,06	0,01
1	3	C	9	0,90	0,01
1	3	C	10	2,24	0,02
1	3	C	11	0,78	0,01
1	3	C	12	1,30	0,01
1	3	D	13	1,44	0,01
1	3	D	14	1,54	0,02
1	3	D	15	0,24	0,02
1	3	D	16		
1	4	C	9	1,75	0,01
1	4	C	10	1,44	0,01
1	4	C	11	1,71	0,02
1	4	C	12	0,41	0,00
1	4	D	13	2,24	0,02
1	4	D	14	0,79	0,01
1	4	D	15	0,99	0,01
1	4	D	16	1,54	0,02
2	5	C	9	1,58	0,01
2	5	C	10	0,66	0,01
2	5	C	11	1,03	0,01
2	5	C	12	1,86	0,02
2	5	D	13	0,73	0,01
2	5	D	14	3,34	0,03
2	5	D	15	0,22	0,01

2	5	D	16	1,45	0,01
2	6	C	9	0,79	0,01
2	6	C	10	1,38	0,01
2	6	C	11	0,10	0,01
2	6	C	12	4,40	0,04
2	6	D	13		
2	6	D	14	2,24	0,02
2	6	D	15	0,24	0,02
2	6	D	16	0,11	0,01
2	7	C	9	0,65	0,01
2	7	C	10	1,58	0,01
2	7	C	11	2,24	0,02
2	7	C	12		
2	7	D	13		
2	7	D	14	0,55	0,01
2	7	D	15	0,74	0,01
2	7	D	16	1,07	0,01
2	8	C	9	0,88	0,01
2	8	C	10	1,21	0,01
2	8	C	11	2,95	0,03
2	8	C	12	2,13	0,02
2	8	D	13	0,18	0,01
2	8	D	14	0,74	0,01
2	8	D	15	1,90	0,02
2	8	D	16	0,45	0,01
3	9	C	9	1,24	0,04
3	9	C	10	2,42	0,03
3	9	C	11	1,13	0,01
3	9	C	12	0,64	0,01
3	9	D	13	0,74	0,01
3	9	D	14	0,55	0,01
3	9	D	15	0,79	0,01
3	9	D	16	1,78	0,02
3	10	C	9	1,89	0,02
3	10	C	10	1,32	0,01
3	10	C	11	1,49	0,01
3	10	C	12	0,40	0,03
3	10	D	13	0,54	0,01

3	10	D	14	1,32	0,02
3	10	D	15	1,10	0,01
3	10	D	16	0,10	0,01
3	11	C	9	1,80	0,02
3	11	C	10	2,19	0,02
3	11	C	11	2,40	0,02
3	11	C	12	1,90	0,02
3	11	D	13	0,74	0,01
3	11	D	14	1,46	0,01
3	11	D	15	0,22	0,01
3	11	D	16	0,40	0,01
3	12	C	9	2,19	0,02
3	12	C	10		
3	12	C	11	0,80	0,01
3	12	C	12	1,59	0,02
3	12	D	13	0,73	0,01
3	12	D	14	1,14	0,01
3	12	D	15	1,02	0,01
3	12	D	16	0,32	0,01

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