WALYSSON MENDES GOMES

MATING BEHAVIOUR IN THE TWO-SPOTTED SPIDER MITE *Tetranychus urticae* KOCH (ACARI: TETRANYCHIDAE)

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Magister Scientiae*.

Orientador: Simon Luke Elliot

Coorientadores: Robert Weingart Barreto
Verônica Saraiva Fialho

VIÇOSA - MINAS GERAIS
2021

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T
Gomes, Walysson Mendes, 1995-
G633m Mating behaviour in the two-spotted spider mite
2021 *Tetranychus urticae* Koch (Acari: Tetranychidae) / Walysson
Mendes Gomes. – Viçosa, MG, 2021.
1 dissertação eletrônica (35 f.): il. (algumas color.).

Texto em inglês.
Orientador: Simon Luke Elliot.
Dissertação (mestrado) - Universidade Federal de Viçosa.
Referências bibliográficas: f. 31-35.
DOI: https://doi.org/10.47328/ufvbbt.2021.160
Modo de acesso: World Wide Web.

1. Ácaros rajados - Comportamento sexual.
2. Acasalamento de animais. 3. Ácaros rajados - Reprodução.
I. Universidade Federal de Viçosa. Departamento de
Entomologia. Programa de Pós-Graduação em Entomologia.
II. Título.

CDD 22. ed. 595.42

Bibliotecário(a) responsável: Alice Regina Pinto CRB6

WALYSSON MENDES GOMES

MATING BEHAVIOUR IN THE TWO-SPOTTED SPIDER MITE *Tetranychus urticae* KOCH (ACARI: TETRANYCHIDAE)

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Magister Scientiae*.

APROVADA: 26 de julho de 2021.

Assentimento:

Walysson Mendes Gomes

Walysson Mendes Gomes
Autor

Simon Luke Elliot
Orientador

Aos meus pais, família e professores.

AGRADECIMENTOS

Agradeço primeiramente meus pais José e Ana, base de tudo que hoje sou, servindo como maior exemplo de vida, onde sempre me incentivaram, ajudaram e estiveram presente durante toda minha caminhada.

Agradeço também meu irmão Welysson, por toda companhia, apoio e conversas.

Agradeço a minha família, principalmente minhas avós Inácia e Maria, ao qual sou eternamente grato por tudo que fizeram por mim. Agradeço também minhas tias e tios, em especial minhas tias Maria, Ivanilda, Dalva e Romilda, as quais sempre estiveram presente em minha vida, sendo eternamente grato por todo o apoio e incentivo.

Ao meu orientador Sam, por ter me aceitado como orientado, por ter me ajudado a crescer, a melhoras meus pontos críticos e discussões acadêmicas. Agradeço também por toda a confiança envolvida durante a realização do meu projeto.

Agradeço a Verônica Saraiva, amiga e Técnica do laboratório, por todos os ensinamentos, todas as conversas e por todo o apoio oferecido durante meu mestrado.

Agradeço ao Pablo Fernandes, estagiário do laboratório, que me ajudou durante toda minha metodologia.

Aos meus amigos e colegas de laboratório, por toda ajuda e troca de conhecimento.

A minhas amigas Keminy e Elenir, por todo afeto, apoio, estando ao meu lado em diversos momentos.

Aos meus amigos Lorena e Jose, que tive o prazer de conhecer em Viçosa e me proporcionaram ótimos momentos e risadas.

Às minhas amigas Karen e Carol, pela amizade construída em Viçosa, pelos conselhos, conversas e momentos de descontrações.

A Vanessa Farias pela ajuda na estatística e escrita da minha dissertação, por todo apoio, conselhos e abraços oferecidos durante esta fase.

A Ítalo, por se colocar à disposição de me ajudar, por ter me ajudado a compreender e interpretar minha estatística.

Ao INSECTUM, minha terceira família aqui em Viçosa, pela troca de conhecimento, acolhimento, me permitir conhecer várias pessoas do programa e crescer no profissional e pessoal.

À Eliane e Cheyane, secretárias do Departamento de Entomologia por todo auxílio prestado

A equipe de segurança da UFV, por todo auxílio e disposição prestadas durante meu projeto.

Aos professores, em especial Prof. Angelo Pallini e Prof. Gustavo, pelas oportunidades de aprender e adquirir mais conhecimento.

À Universidade Federal de Viçosa e ao Programa de Pós-graduação em Entomologia pela oportunidade de adquirir conhecimento e contribuir com meu crescimento durante o mestrado.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), pela concessão da bolsa de estudos.

À Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), pela concessão da bolsa de estudos.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pela concessão da bolsa de estudos.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

"Uma pessoa nunca repara o que foi feito, mas sim o que ainda precisa ser feito."

(Marie Curie)

RESUMO

GOMES, Walysson Mendes, M.Sc, Universidade Federal de Viçosa, julho de 2021. **Mating behaviour in the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae)**. Orientador: Simon Luke Elliot. Coorientadores: Robert Weingart Barreto e Verônica Saraiva Fialho.

Para obter sucesso no acasalamento, os machos e fêmeas realizam diferentes comportamentos pré-copulatórios. No comportamento sexual, machos investe tempo e energia para realizar uma série de padrões de comportamentos para atrair ou ter acesso a fêmea. Em algumas espécies de ácaros, os machos realizam uma série de comportamentos antes de acasalar com a fêmea. Machos de *T. urticae* permanecem em cima de fêmeas em estágio quiescente, até que elas cheguem à idade adulta. Este comportamento pode durar várias horas e saber reconhecer entre as diferentes fases quiescentes pode influenciar na performance sexual do macho. Deste modo, este trabalho buscou observar a preferência do macho de *T. urticae* a dois estágios quiescentes e entre deutoninfas fêmeas vivas e mortas. Além disto, descrevemos e classificamos os comportamentos sexuais de machos de *T. urticae* e criar um ethograma que represente este comportamento nesta espécie. Machos e fêmeas virgens foram criados separadamente em discos de folha até a idade adulta. Testes de escolhas foram realizados com machos virgens, em arenas compostas por discos de folhas, contendo cada um dos tratamentos. As filmagens dos comportamentos foram realizadas durante vinte minutos, e a montagem do ethograma foi realizada de maneira manual, apresentando todas os padrões de comportamentos realizados pelos machos. Machos de *T. urtica* é capaz de reconhecer deutoninfas quiescentes e diferenciar entre deutoninfas vivas e mortas. No total, dezessete dos vinte machos copularam com as fêmeas. Machos realizaram um total de seis comportamentos. As ordens de ocorrência dos comportamentos variaram entre as amostragens. A diferenciação do macho entre as diferentes fases e estados (vivas e mortas) de indivíduos quiescentes pode ser explicada através de pistas químicas reconhecidas pelo macho. Os comportamentos realizados pelos machos de *T. urticae* são semelhantes ao encontrado em ouras espécies de ácaros e carrapatos. Em *T. urticae,* densidade de machos e o desenvolvimento sem a presença sugeriu que estes indivíduos apresentam comportamento estereotipado.

Palavras-chave: Seleção sexual. Comportamento de cópula. Comportamento pré-copulatório. Preferência do macho.

ABSTRACT

GOMES, Walysson Mendes, M.Sc, Universidade Federal de Viçosa, July, 2021. **Mating behaviour in the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae)**. Adviser: Simon Luke Elliot. Co-advisers: Robert Weingart Barreto and Verônica Saraiva Fialho.

To achieve mating success, males and females perform different precopulatory behaviours. In sexual behaviour, males invest time and energy to carry out a series of behaviours patterns to attract or access the female. In some mite species, males perform a series of behaviours before mating with the female. Males of *T. urticae* remain on top of females in the quiescent stage until they reach adulthood. This behaviour can last for several hours and, knowing how to recognize the different quiescent phases can influence the male's sexual performance. Thus, this work sought to observe the male preference of *T. urticae* to two quiescent stages and between living and dead female deutonymphs. In addition, we describe and classify the sexual behaviour of males of *T. urticae* and create an ethogram that represents this behaviour in this species. Virgin males and females were reared separately on leaf discs until adulthood. Male preference experiments were carried out with virgin males in arenas composed of leaf discs containing each one of the treatments. The male courtship behaviours were recorded for twenty minutes. The ethogram was assembled manually, showing all the behaviour patterns performed by the males. Males of *T. urtica*e can recognize quiescent deutonymphs and differentiate between living and dead deutonymphs. In total, seventeen out of twenty males copulated with females. Males performed a total of six behaviours. The order of occurrence of the behaviours varied between the samples. The male choice between quiescent protonymph and deutonymph and quiescent live and dead individuals are made through chemical cues. The behaviours performed by males of *T. urticae* are like those found in other species of mites and ticks. In *T. urticae*, male density and development without the presence suggested that these individuals exhibit stereotyped behaviour.

Keywords: Sexual selection. Copulatory behaviour. Precopulatory behaviour. Male choice

LISTA DE ILUSTRAÇÕES

SUMÁRIO

GENERAL INTRODUCTION

Sexual selection is a natural force between females and males, involving morphological and behavioural adaptations that increase mating success (Jones and Ratterman, 2009; Kuijper et al., 2012). When looking for a sexual partner, male can be perform set (Clutton-Brock, 2007). Males of diverse species perform various behaviours to locate the female, attract female attention and have access to female for achieve mating success (Clutton-Brock, 2007). The attempt for the chance to leave descendants can increase intrasexual competition and male behaviours, as a courtship behaviour, there is then a strong selection among males (Tripet et al., 2006).

On the other hand, while males compete for access to females, females also can choose males according to morphological and behavioural traits, thus obtaining direct or indirect benefits (Bleu et al., 2012; Lwasa, 1996) In some examples nonetheless, in some species, females are previously chosen by the male before reaching reproductive age (Jarzebowska and Radwan, 2010) . In Precopulatory mate choice, male guarding a juvenile female, waiting for it to reach adulthood, to then have a chance to calm down (Kuijper et al., 2012). In this type of behaviours, competition between males for a female can occur, where the female can indirectly select the male (Kuijper et al., 2012; Oku, 2009a).

The Chelicerata (Cheliceriformes) present a series of distinct strategies of mating behaviour, involving female and male movements and positions. The mating behaviour of *Aponomma hydrosauri* and *Amblyomma albolimbatum* (Acari: Ixodidae), a reptile tick, occurs in six phases. Before mating, the male goes beneath the female and mating can occur (Andrews, 1982; Andrews and Bull, 1980). Along with this, the female tilts the final part of her body, allowing mating to take place (Andrews, 1982).

Mites (Acari) present a series of interesting sexual behaviours and strategies (Boudreaux, 1963). Various mite species adopt different mating strategies to achieve mating success. Mite reproduction can occur indirectly or directly (Morais and Flechtman, 2008). Males of some groups of mites leave their spermatophores on a substrate and, when this is found by a female, sperm transfer occurs (Evans, 1992). Spermatophores are small structures and when they are placed in (spatially intricate) soil, female mites can have difficulty locating them (Michalska et al., 2010). Therefore, males have several strategies incorporating pheromones, secretions, and behaviours to increase the chances of spermatophores being found by a female (Proctor, 1998).

The two spotted spider mite *Tetranychus urticae* is a serious world-wide phytophagous pest in several plant cultures (Migeon et al., 2010). They reproduce by arrhenotokous parthenogenesis, and virgin females produce only haplodiploid unfertilized eggs and mated females produce fertilized diploid eggs as female offspring. (Oku, 2014). They life cycle during around 15 days and passes through egg, larval, protonymph, deutonymph, and adult stages (Kumral et al., 2019; Laing, 1969). Between nymphs stages a quiescent stage is present, where the duration of the quiescent phase is usually half the duration of the nymph stage (Laing, 1969). The first mate is responsible to fecund female, thar mating only in her live (Cone, 1985; Oku, 2008; Potter et al., 1972). Male also presents a precopulatory and postcopulatory behaviours for grant female mate and avoided sperm competition (Oku, 2014; Potter. et al., 1972). Because of spider mites present this characteristic, this species is a good model study for observe the male preference between quiescent stages and precopulatory mite behaviour.

1. INTRODUCTION

Courtship behaviours plays a crucial role in male preferences and female choice (Darwin, n.d.). Male courtship can be characterized by a series of stereotyped behaviours targeted at the female prior to mating (IWASA, 1996). The courtship behaviour can involve a series of component as a time investment, male performance, and behaviour sequence (Lasbleiz et al., 2006). In arthropods, it can be found a series of courtship behaviour strategies that male can be used for calling female attention, as a pheromone, sounds, and elaborated dances (Scharf et al., 2013).Nonetheless, it also can be found examples that has an absent of courtship behaviours (Matthews and Matthews, 2009).

Some arthropods use a different strategy for grant mate chance, as a territoriality, intermale aggression and female mating guard (Parker, 1978). In mating guard behaviour, male guard a female that was close to get adult age. This behaviour is present in trick, crabs, and mite species. In the spider mite *Tetranichus urticae,* the first mating results in female fertilization (Helle, 1967; Oku, 2008; Satoh et al., 2001). Male of spider mites realise the guard behaviour in females quiescent deutonymph, and shortly after moving into adult female, the mate can happen (Oku, 2014). During mate guard behaviour, a second male may appear (Satoh et al., 2001). In a presence of a secund male, an intense intermale fight can occur, that may cause injury or lead to death. Contrasting, males can also guard the same quiescent deutonymph, where the male who occupies a better position manages to mate the female first (Potter et al., 1972).

Quiescent deutonymph phase may vary with temperature and humidity, where the duration of this phase is half the time of the complete phase of the deutonymph (Ikegami et al., 2000). Male can remain in guard behaviour for several hours, and early studies have pointed out that males prefer near quiescent deutonymphs to become adults(Potter et al., 1972; Sato et al., 2014). Nonetheless, males show preferences only if the time difference between the quiescent deutonymphs is 23 hours (Oku and Saito, 2014). However, how do males find and identify deutonymphs, are doubts have been studied for some time. Spider mites do not have well-developed eyes and consequently good vision(Oku, 2014). Thus, male is able to use physical cues, as female silk

(Penman and Cone, 1974), and new findings appoint for chemical cues, realised by quiescent and adults mite (Oku et al., 2015; Rodrigues et al., 2017).

During guard behaviour, both males and females can be exposed to predators. Female deutonymphs present strategies to protect themselves during the quiescent period(Oku et al., 2003). The females, quiescence in a web of web, so that they are suspended in the leaf. Despite this, if it is the male itself, knowing how to identify the status of the deutonymph (alive or dead) is crucial both for being less exposed and for its fitness (since the "guarded" organism may not be viable for reproduction).

In mites, the genetical material can be transfer from a male to a female by a spermatophores (Evans, 1992), or direct, by sperm transference. In both cases, a male can perform a series of behaviour for have female acceptance. In water mite *Arrenurus manubriator* (Acari: Arrenuridae), the male and female perform a sequence of seventeen behaviours before mating. In this example, the female is responsible to perform most of the behaviour. She climbs on the male and, the male produces a viscous adhesive substance that holds the female fixed above him during mating (Proctor, 1991; PROCTOR, 1992). After a set of behaviours, the male can lay the spermatophore on the ground or transfer the sperm to the female (Proctor and Smith, 1994).

Individuals that transfer the genetic material via sperm transference also realise a stereotyped series of courtship behaviour. In the predatory mites *Phytoseiulus persimilis* and *Amblyseius andersoni*, mating behaviour lasts an average of 5 to 10 minutes (Amano and Chant, 1977). In this species, male perform a series of behaviour, included touch and climb on female before copulation. (Amano and Chant, 1977). In tetranychid mites, mating behaviour occurs in six phases. In general, the male touches the female body, moves to the ventral part of the female and mating occurs (Cone, 1985). Mating lasts an average of 3 to 5 minutes, while 40 seconds is the minimum time needed to ensure fecundation of the female (Satoh et al., 2001).

For the best we know, the literature involves mite courtship behaviour is scarce, bringing information only on some species or groups of mites. Furthermore, some of the observations about the copulation behaviours of these individuals are found in a shallow way in the literature (Cone, 1985). This study objectives are: Observe if the *Tetranychus urticae* male can differentiate between quiescent protonymph and quiescent deutonymph, and between live and dead quiescent female deutonymph. Also, we characterized and bring more detailed aspects of male mating behaviour of *Tetranychus urticae*. Along with this, we want to observe the different possibilities and successions between courtship behaviours presented in an ethogram.

2. Materials and methods

2.1 Plant culture

Jack bean plants (*Canavalia ensiformis* L.; Sementes Caiçara®, Brazil) were produced in 3 L plastic pots, with a commercial potting soil (Bioplant, Bioplant Misturadora Agrícola Ltda, Minas Gerais, Brazil), maintained in a greenhouse (25 ± 7°C, 70 ± 20% RH). Eight plastic pots containing five seeds per pot were planted weekly and watered automatically (250ml) twice a day. Plants did not receive any pesticides or fertilizers. Two-week-old plants were used for the rearing of spider mites and experiments.

2.2 Mite rearing

The two-spotted spider mite, *Tetranychus urticae* Koch was reared on jack bean plants in the Laboratory of Acarology of Universidade Federal de Viçosa (Minas Gerais, Brazil), in a climate-controlled room (25±3 °C, 70–90% relative humidity and a photoperiod of 12 Light: 12 Dark). The infested plants remained in smaller trays (45 × 30 × 9 cm), placed under the larger trays (53 × 38 × 9 cm) containing water to prevent the escape of mites. Once a week infested old leaves were cut and placed on uninfested jack bean plants, allowing passage of spider mites to a new leaf.

2.3 Production of quiescent *T. urticae* protonymphs and deutonymphs.

Spider mite development consists of stationary quiescence phases between nymph stages. For the male preference experiment, we use a stationary quiescent protonymph and quiescent female deutonymph. Female quiescent deutonymphs differ from their male quiescent deutonymph counterparts in having a rounded opisthosoma and, a more robust body (Potter. et al., 1972).

For rearing quiescent stages, adult female *T. urticae* provided by mite culture were placed individually on jack bean leaf discs (15 mm diameter), with the adaxial surface up, on foam beds soaked with water in 90 × 15mm glass Petri dishes (five-leaf disc per dish). Females were kept on the discs for 24 hours to lay eggs and then were removed. The Petri dish with female *T. urticae* was placed on a plastic tray (43 × 29 ×

7cm), kept in the Laboratory of Insect-Microbe Interactions of Universidade Federal de Viçosa, under controlled conditions (23 ± 2°C, 60% relative humidity and a photoperiod of 12:12 L:D).

To observe spider-mite development, a single egg was left on each leaf disc. Daily observation was made to monitor spider-mite development. Pilot studies were conducted to establish the time needed for eggs to develop into protonymphs (8-9 days) or quiescent female deutonymphs (12-13 days). The leaf discs were changed every 5 days or when the presence of saprobic fungi was observed.

2.4. Production of virgin males *T. urticae*

Tetranychus urticae females are haplodiploid organisms. Thus, adult virgin females will produce only male offspring (Laing, 1969). For virgin male production, 1–3-day old virgin females (from quiescent production), were placed individually on bean leaf discs (15 mm diameter) maintained under the same conditions described in section 2.3. Females were kept on the discs for 24 hours to lay eggs and, then the females were removed. Daily observations were made to monitor spider-mite development. Pilot studies were conducted to establish the time needed for eggs to develop into adult males (12-13 days). The leaf discs were changed every 5 days or when the presence of saprobic fungi was observed.

2.5 Male preference experiments

2.5.1 Male preference between quiescent *T. urticae* protonymphs and deutonymphs

A two-choice test was conducted for observe male preference between quiescent protonymphs and quiescent female deutonymphs. A two-choice test was conducted on arenas consisting of two jack bean leaf discs (15 mm diameter) connected by a smaller jack bean leaf disc (7 mm diameter). The leaf discs were placed on 1.5% water agar in a 90×15mm plastic Petri dish. Before each assay, a few drops of water were added to the water-agar to prevent male mites from leaving the leaf discs. The position of quiescent protonymphs and quiescent female deutonymphs was reversed in half of the replicates to allow us to determine if there was any directional bias in male choice. With the aid of a brush, a quiescent *T. urticae* protonymph or quiescent female deutonymphs was gently placed in the centre of one of the leaf discs. A 1–3-day old virgin male was released in the centre of the smaller jack bean leaf disc of each arena and observations were made, for six hours of experiment. The observation time was extended, since males raised alone take longer to find females (Oku, 2009b). Males that did not move after five minutes were replaced (Vacacela Ajila et al., 2020). The choice assay had fifteen replicated (n=15) and was repeated three times (n=45) on the 21st, 23rd and 23rd of June 2021 in Laboratory of Insect-Microbe Interactions under ambient laboratory conditions (20± 3°C 50-75% RH) during the day between 12:00 and 6:00pm.

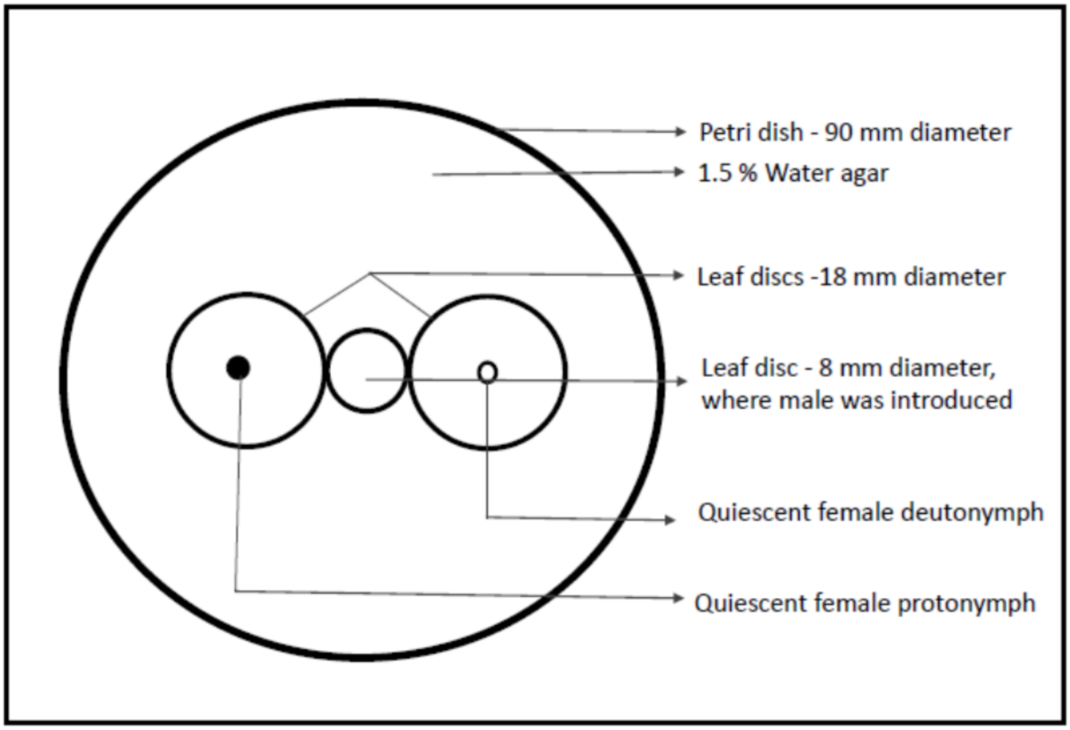

Figure 1: Schematic representation of the set up for the male preference experiment. The arena consists in a Petri dish (90 mm) with 1.5% water-agar. Inside was putted two leaf disc (18 mm) where was placed a *Tetranychus urticae* quiescent protonymph or quiescent female deutonymph. The male was introduced on the smaller leaf disc (8 mm). All analyses were observed between 12:00 and 6 pm.

2.5.2 Male preference between live and dead quiescent female deutonymphs

For observe if male *T. urticae* can differentiate between a live and dead quiescent individual, we realise a choice experiment with quiescent female deutonymphs live and dead. Quiescent female deutonymph was killed by frozen, kept in a freezer (3° C), for five hours before the experiment. The choice arena was set up as described in section 2.5.1. The choice assay had twenty replicated (n=20) and, the experiment was repeated three times (n=60) on the 5th, 7th, and 9th of July 2021, under ambient laboratory conditions (20± 3°C 50-75% RH) during the day between 10:00 am and 6:00pm.

2.5.3 Behavioural analysis of male mate-guarding

To examine the male preference between quiescent treatments (quiescent protonymph x quiescent female deutonymph and, quiescent female deutonymph live x death) we observed the male mating-guarding behaviour. The mate-guarding behaviour (Fig. 2) was considered if the male remained motionless for 30 seconds or longer while mounted upon or very close to (one body length) the quiescent female (Potter. et al., 1972). Once an hour, we carry out observations in each arena, individually for 1 minute and record the male choice through mate-guard behaviour. In addition, the side on which the treatment chosen by the male was positioned (left and right) was noted. Males that did not choice any quiescent treatment was excluded from samples. Each Petri dish was observed through a stereo microscope (80× magnification). To make sure that no quiescent females were killed during the experiment, quiescent development from live and dead protonymph and deutonymph was observed the following day.

2.6 Mating behaviour of *Tetranychus urticae*

Tetranychus urticae females were placed individually on bean leaf discs (15 mm diameter), with the adaxial surface up, on foam beds soaked with water in 90×15mm glass Petri dishes (five-leaf disc per dish). The Petri dish with female *T. urticae* was placed on a plastic tray (43 × 29 × 7cm) kept in the laboratory under controlled conditions (23 ± 2°C, 60% RH and 12/12h light/darkness). For virgin male production, 1–3-day old adult virgin females from the development experiment were placed individually on bean leaf discs (15 mm diameter), maintained in the same room condition.

. Mating trials were conducted using 1–3-day old virgin male and virgin female adult *Tetranychus urticae*. The spider mites were selected and placed in a small jack bean leaf disc (7 mm diameter). We use small leaf discs to decrease the foraging time and increase the chance of encounter between individuals. Furthermore, using small leaf discs allowed us to capture images of the entire discs while recording. The leaf disc was placed onto a plastic Petri disc (50mm diameter) with 1.5% water agar. The spider mite courtship behaviour was recorded with a Nikon D3400 digital camera with a Zeiss STEMI 2000-c stereoscopic microscope. The recorder was taken place for 30 minutes. All trials took place from the 2nd to the 9th of July between 10:00am and 5:00pm in the Laboratory of Acarology in ambient conditions (22± 3°C 50-65% RH).

2.7 Behaviour sequences and ethogram

The videos were analysed individually, manually. For each video, the observed behaviours, the duration of each behaviour and the possibilities between the sequences of changes between the behaviours were recorded. After observing all the videos, we created a manually ethogram, representing the main sequence of copula behaviours performed by *T. urticae* males.

2.8 Statistic analyses

The preference of *T. urticae* male to quiescent protonymph or quiescent female deutonymph and quiescent female deutonymph live or quiescent female deutonymph dead were verified using Generalized Linear Models (GLM). The data presented normal distribution and analyses were carried out by binomial models. The models were compared using a Chi-square-test ($p>0.05$). Mites that did not choice any quiescent treatment in sex hours of observation, was exclude of analyses. All analysis was done using the Software R (version 4.1.0).

3. Results

3.1 Male preference experiments

To determine male preference between quiescent protonymphs and quiescent female deutonymphs, virgin males were allowed to choose between leaf discs containing each for six hours. Virgin males would prefer and performed guard behaviour in quiescent female deutonymph than quiescent protonymph (χ^2= 4, df= 10.835, *p*< 0.001) (Fig. 2a). The occurrence of guarding behaviour performed by males was observed with both live and dead deutonymphs. In quiescent female deutonymph live and quiescent female deutonymph dead, males showed preference and performed guard behaviour to live quiescent deutonymphs than dead quiescent deutonymph (χ^2= 4, df= 4.7664, *p*= 0.024) (Fig 2b). Once males started guarding deutonymphs, they remained until the end of the experiment, or in the case of live females, until they emerged as adults.

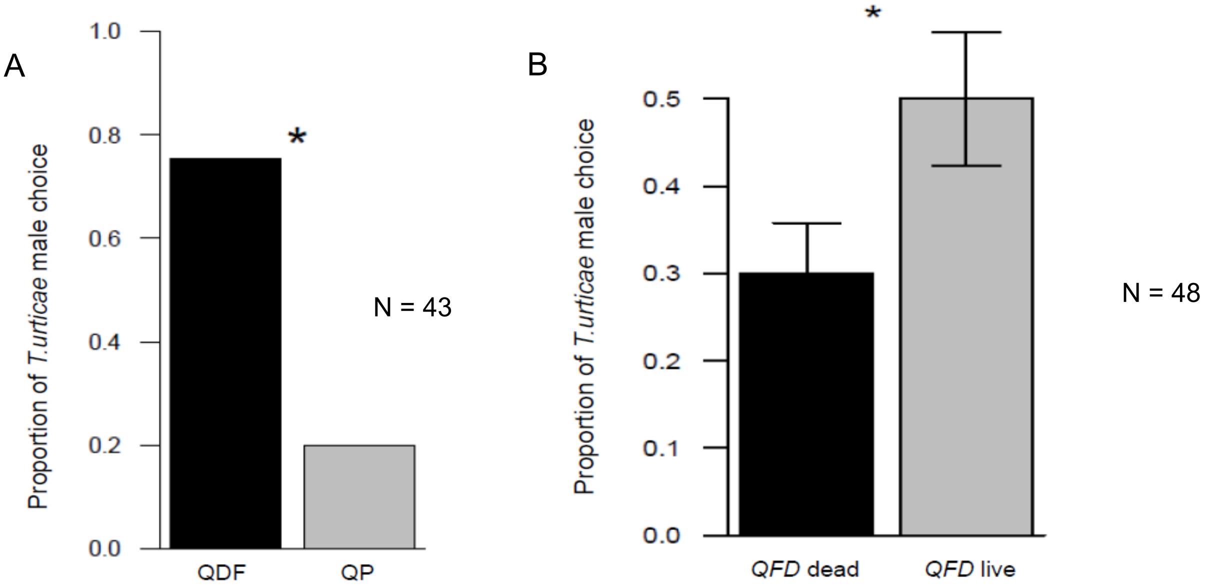

Fig 2: A 1–3-day old virgin male *T. urticae* was used for chosen experiment between (A): quiescent female deutonymph and quiescent protonymph (*p*< 0.001), and (B) frozen killed quiescent female deutonymph and live quiescent female deutonymph (*p*= 0.024). Male preference and guard behaviour was measured after 6 hours of experiment. * Symbols represent difference between treatments.

3.2 Descriptions of *Tetranychus urticae* male courtship behaviour

We discerned eight distinct behaviours in *Tetranychus urticae* adult male. For six of these, copulation was observed, and a representative sequence can be seen in video form: https://youtu.be/PenQ3qFWFfE

The courtship behaviour of *T. urticae* males was characterized and described as the follow sequence (Fig 3):

1) *Touching*: Male use his forelegs, palps, and chelicerae to touch the female body. Normally, touching occurs when the female is stationary. Males can approach females and start this behaviour in three different locations: (a) from the front, the male starts to lick the female gnathostome and continues circling and touching the female's body until it reaches the end of the opisthosoma, (b) from the side of the female, the male start touching the female dorsal surface, also circling and touching the female's body until it reaches the end of the opisthosoma, and (c) from the opisthosoma: the male approaches from behind the female and touches the dorsal surface of the female opisthosoma.

2) *Holding female legs*: After touching the female body, the male uses the first pair of legs to hold the female. The male may try to hold the female by grabbing her third or fourth legs or holding the female opisthosoma. Some males also use their first two pairs of legs, using the first to hold the female legs and the second to hold the female opisthosoma. This behaviour is very quick, and it can occur while the male tries to position himself under the female or starts to raise his opisthosoma

3) *Male ventral positioning*: When the male can hold the female, it starts to move under the female's body. through small ‘zig-zag’ movements the male positions himself, placing the initial part of his gnathostomes (up to eye level) under the female’s body This behaviour can occur while the male starts to raise his opisthosoma.

4) *Opisthosoma curvature*: While the male positions himself under the female’s body, he gradually elevates and bends the opisthosoma region. This behaviour can occur together with the others, or even before the male moves under the female. However, copulation only occurs after the male bends his opisthosoma

5) *Mating*: The male positions its opisthosoma connecting to the female opisthosoma. At this point, the male connects his aedeagus to the female genital opening. During the mating period, the male's third and the fourth pairs of legs remain suspended. In some cases, the entire final part of the male body may remain suspended. During copulation, the female may try to walk or take a few steps. However, males that apparently had difficulty holding the female were carried while the female walked.

6) *Cessation of mating*: At the end of mating, there is separation of the genitalia, and the male body gradually returns to its starting position. The male releases the female legs and opisthosoma and emerges from under the female's body still with his opisthosoma raised, lowering this gradually until it returns to its original position.

In addition to these behaviours, the male can make a quick and small change of position, ceasing copulation and returning seconds later, without leaving the mating position. During the *Touching* behaviour, the female can reject the male. In the first moments of the *touching* behaviour, the female quickly moves away from the male. However, the male can follow the female, and return to the *touching* behaviour later. Even after the female had refused the male, the second mating attempt always resulted in copulation. We also observed *Male quitting behaviour*. The male begins to perform the behaviour of touching the female, yet this does not continue in the sequence of behaviours. When this happened, the male did not train to copulate with the female again.

Tetranychus urticae male can perform different sequences of behaviours, which lead to mate or rejection by female/male desistence. The flow ethogram (Fig. 4) represents the totality of possibilities of behaviours observed, suggesting a model of *T. urtica*e male mating behaviour. In total, the average duration of *Mating* was 268.8 seconds. The other behaviours had an average duration of: *Touching* (24.2 sec.), *Holding female legs* (5.5 sec.), *Male ventral position* (5.11 sec.), *Opisthosoma curvature* (10.4 sec.) and *Secession of mating* (12.7 sec).

Figure 3: Main sequence of male *Tetranichus urticae* male behaviour: Touching (A); Holding female legs (B); Male ventral positioning (C); Opisthosoma curvature (D); Mating (E) and Cessation of mating (F). The recorder of courtship behaviour of 1-3 old virgin female and male was made for 20 minutes. The arrows point to male anterior legs, holding the female posterior leg (B) and, male opisthosoma curvature (D).

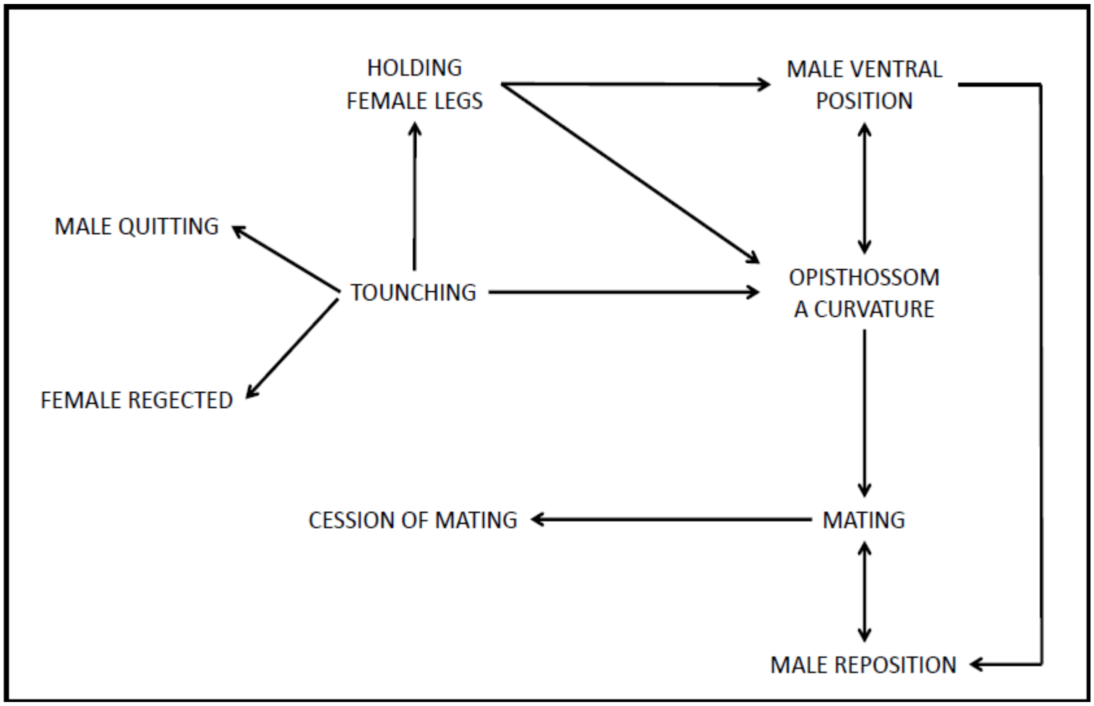

Figure 4: Ethogram of male *Tetranychus urticae* courtship behaviour, derived from 20 mating recorder from 1-3 days old female and male spider mite. For manually ethogram construction, we analysed the sequences of 17 mating and 03 unsuccessful mating, resulting in male quitting of female rejected. The directions of the arrows represent transitions in which one behaviour was followed by the next.

4.Discussion

We showed that male *T. urticae* prefer female quiescent deutonymphs over quiescent protonymphs. Male *T. urticae* use cues to locate quiescent deutonymphs (Yoshioka and Yano, 2014). Females deutonymph produces strands of silk before quiescence (Penman and Cone, 1974), and the male can use these strands to recognize and find the quiescent female (Penman and Cone, 1974; Yoshioka and Yano, 2014). In our experiment, the deutonymphs were handled and moved to new leaf discs after entering a quiescent state, therefore, males were not able to find females through these cues. The lack of this cue might have caused males to spend more time searching the arena for a female. Also, males that develop without the presence of other males and females require a longer time to find quiescent females (Oku, 2009b).

Quiescent female deutonymphs can also release sex pheromones to attract males. Oku (2009) showed that female quiescent deutonymphs can control the release of these pheromones. Furthermore, when a female is already being guarded by a male, there is a greater release of these pheromones (Oku, 2009a; Oku et al., 2015). We already know that males use chemical cues to differentiate between virgin and mated adult females (Rodrigues et al., 2017). Thus, it is possible to hypothesize that males may have used chemical cues to locate deutonymph females and go towards the leaf discs on which they are located.

We observed that, despite remaining on the same disc as the quiescent protonymph, guard behaviours performed by males in these individuals were not observed. Penman and Cone (1974) considered that touching behaviour is an important stimulus for the male to recognize the female in quiescence. Following the touching behaviour, males tended to perform the mate guarding behaviour with the female quiescent deutonymphs but not with the female quiescent protonymphs. In *T. urticae*, mating may occur soon after the emergence of an adult female from the quiescent deutonymph phase (Potter et al., 1972). This explains the strategy used by *T. urticae* males of guarding these quiescent individuals (Oku and Saito, 2014; Potter et al., 1972; Satoh et al., 2001).

We observed that males differentiate between live female quiescent deutonymphs and dead female quiescent deutonymphs. However, some males performed the mate-guarding behaviour with dead quiescent deutonymphs. Males are also known to perform the touching and mating-guard behaviours with female mummies infected with *N. floridana* (Trandem et al., 2015). Further studies are needed to understand the occurrence of these behaviours in dead mites and the effect of this behaviour in male sexual reproduction.

Mating behaviour has been studied in vary fill mite species, and the present literature of mite behaviour does not bring a complete information of behaviour sequences in spider mite. In this study, we found that *Tetranychus urticae* males performed six types of behaviours that lead to mating. Male of *T. urticae* perform a simple sequence of behaviour before mate with female, which may vary in the order of occurrence of these behaviours.

The courtship behaviour of *T. urticae* males share a similar sequence that is also observed in other mite species (Andrews, 1982; Eickwort, 1992; Proctor and Smith, 1994), and ticks (Andrews, 1982; Andrews and Bull, 1980; Gladney and Drummond, 1970; Yuval et al., 1990). The similarity between these behaviours in other mites may indicate that copulation behaviour cold be stereotyped, where the general characteristics of these behaviours are found in other members of the Chelicerata group. In this study, only the male behaviours were recorded, nonetheless, we did not focus on female behaviour or positions. In other mite species, as *Arrenurus manubriator,* the courtship behaviour is performed by male and female. Booth remade a contact in the first behaviour and, the female mount on the male for mating (Andrews, 1982)

Tetranychus kanzawai males can recognise the mate status of female (Oku et al., 2005). As a *T. kanzawai*, *T. urticae* males can differentiate between virgin and mated female, using chemical compounds (Macke et al., 2012), however they mate with mated females in the absence of virgin females (Oku, 2010). In our observation, some males start to copulation with the same females shortly after the first mating. Some males repeated all the courtship behaviours, or started in *Holding female legs* (Gomes, 2021 – personal observation). Hire, only one couple was maintained in leaf disc, and due to the lack of other females on the disc, the second mating may have occurred. Furthermore, mating and the presence of male can affect female fecundity (Macke et al., 2012), and egg production (Oku, 2010), but not affect the male fitness.

For behaviour recorder, we used only virgin females and males, thar developed alone in a leaf disc. Nevertheless, the mite used hire, only they only encountered another organism at the time of the observations. In this species, male present plasticity and learning when they are in the presence of females with other males nearby (Oku and van den Beuken, 2017). Hire, all male performs a set of stereotypical behaviours, implying that density does not influence courtship behaviour, but could be an implication on time needed to find a female and time of mating itself.

The poor performance of a behaviour can cause the female to refuse, or even the male cannot copulate with the female (Jones et al., 2017). In our observations, we notice that males thar did have difficulty to hold the leg of female, present a less time in mate or copulate with the female while she moved. This also could be observed in couples where males were much smaller than females (Enders, 1993; Li and Zhang, 2018). To better understand if male and female size influence in male behaviour of male mating, its necessary to measure they size in next experiments.

The *T. urticae*, the first copulation can grant paternity, but if other male copulation with the female until 24 hours interval, there is a chance for both males leave descendants (Helle, 1967). Some behaviours and strategies can be done by males for improve their sexual behaviour. Males of *T. urticae* can disrupt copulation and interrupt mating (Helle, 1967) and perform a post-copulatory mating guard (a male guard a recent mated female) can be done for grant male paternity. In our observation, no male performed the post-copulatory mating guard (Potter et al., 1972; Sato et al., 2014).

For better explore the courtship behaviour of *Tetranichus urticae*, our next step it will be evaluated the influence of male and female density on courtship behaviour and male-female interaction. Along with that, we will observe if the female performs a behaviour or movement thar can influence the courtship. Lastly, the courtship behaviour needs to be availed in other mite population, to see if the courtship behaviours will follow the same patter.

While carrying out the experiments, we observed that our methodology could be improved. For a better observation of male preference, we suggest carrying out the experiments in a single arena and the observation was crazy within an hour of starting the experiment. In conclusion, our study brings new information about *Tetranichus urticae* male mating guard courtship behaviour. Also, we study contribute with information already observed by other authors and can help to elucidate the precopulatory behaviours performed by these organisms in females killed for other reasons, as fungi. Together with ethogram, we have recorder the individual behaviour, bring new materials, that to our knowledge are absent in literature. Also, we could identify some new observation thar cold be done for more explore the sexual behaviour in *Tetranichus urticae,* that can be used for new behavioural studies.

REFERENCE

AMANO, H.; CHANT, D. A. Life history and reproduction of two species of predacious mites, Phytoseiulus persimilis Athias-Henriot and Amblyseius andersoni (Chant) (Acarina: Phytoseiidae) . **Canadian Journal of Zoology**, v. 55, n. 12, p. 1978–1983, 1977.

ANDREWS, R. H. Mating behaviour and reproductive isolation of three species of reptile tick. **Animal Behaviour**, v. 30, n. 2, p. 514–524, 1982.

ANDREWS, R. H.; BULL, C. M. Mating behaviour in the Australian reptile tick Aponomma hydrosauri. **Animal Behaviour**, v. 28, n. 4, p. 1280–1286, 1980.

BLEU, J.; BESSA-GOMES, C.; LALOI, D. Evolution of female choosiness and mating frequency: Effects of mating cost, density and sex ratio. **Animal Behaviour**, v. 83, n. 1, p. 131–136, 2012.

BOUDREAUX, H. B. Biological Aspects of Some Phytophagous Mites. **Annual Review of Entomology**, v. 8, n. 1, p. 137–154, 1963.

CLUTTON-BROCK, T. Sexual selection in males and females. **Science**, v. 318, n. 5858, p. 1882–1885, 2007.

CONE, W. W. Mating and chimical comunication. In: HELLE, W.; SABELIS, M. W. (Eds.). . **Spider mites: their biology, natural enimies and control, Vol I**. ElsevierAm ed. [s.l: s.n.]. p. 243–251.

DARWIN, C. **1889_Descent_F969.pdf**, [s.d.].

DE MORAIS, G. J.; FLECHTMAN, C. H. W. **Manual de Acarologia: Acarologia Básica e**. Holos Edit ed. [s.l: s.n.].

EICKWORT, G. C. **The Acari: Reproduction, Development and Life-History Strategies**. [s.l: s.n.]. v. 85

ENDERS, M. M. **The effect of male size and operational sex ratio on male mating success in the common spider mite, Tetranychus urticae koch (acari: Tetranychidae)Animal Behaviour**, 1993.

EVANS, G. O. **Principles of Acarology**. CAB Intern ed. [s.l: s.n.].

GLADNEY, W. J.; DRUMMOND, R. O. Mating behavior and reproduction of the lone star tick, Amblyomma americanum. **Annals of the Entomological Society of**

America, v. 63, n. 4, p. 1036–1039, 1970.

HELLE, W. Fertilization in the two-spotted spider mite (Tetranychus urticae: Acari). **Entomologia Experimentalis et Applicata**, v. 10, n. 1, p. 103–110, 1967.

IKEGAMI, Y. et al. **Function of quiescence of Tetranychus kanzawai (Acari: Tetranychidae), as a defense mechanism against rainApplied Entomology and Zoology**, 2000.

JARZEBOWSKA, M.; RADWAN, J. Sexual selection counteracts extinction of small populations of the bulb mites. **Evolution**, v. 64, n. 5, p. 1283–1289, 2010.

JONES, A. G.; RATTERMAN, N. L. Mate choice and sexual selection: What have we learned since darwin? **In the Light of Evolution**, v. 3, p. 169–190, 2009.

JONES, S. D.; BYRNE, P. G.; WALLMAN, J. F. Exploring the influence of individual courtship behaviors on male mating success in a blow fly. **Journal of Insect Behavior**, v. 30, n. 5, p. 528–543, 2017.

KUIJPER, B.; PEN, I.; WEISSING, F. J. A guide to sexual selection theory. **Annual Review of Ecology, Evolution, and Systematics**, v. 43, p. 287–311, 2012.

KUMRAL, N. A. et al. Life table of tetranychus urticae (Koch) (acari: Tetranycidae) on different turkish eggplant cultivars under controlled conditions. **Acarologia**, v. 59, n. 1, p. 12–20, 2019.

LAING, J. E. Life history and life table of Tetranychus urticae Koch. **Acarologia**, v. 11, n. 1, p. 32–42, 1969.

LASBLEIZ, C.; FERVEUR, J. F.; EVERAERTS, C. Courtship behaviour of Drosophila melanogaster revisited. **Animal Behaviour**, v. 72, n. 5, p. 1001–1012, 2006.

LI, G. Y.; ZHANG, Z. Q. Does size matter? Fecundity and longevity of spider mites (Tetranychus urticae) in relation to mating and food availability. **Systematic and Applied Acarology**, v. 23, n. 9, p. 1796–1808, 2018.

LWASA, M. A. AND Y. Sexual selection. **Trends in Ecology and Evolution**, v. 10024, n. 2, p. 100242S, 1996.

MACKE, E. et al. Mating modifies female life history in a haplodiploid spider mite. **American Naturalist**, v. 179, n. 5, 2012.

MATTHEWS, R. W.; MATTHEWS, J. R. Nesting behavior of Abispa ephippium (Fabricius) (Hymenoptera: Vespidae: Eumeninae): Extended parental care in an

Australian Mason Wasp. **Psyche (London)**, v. 2009, 2009.

MICHALSKA, K. et al. Behavioural studies on eriophyoid mites: An overview. **Experimental and Applied Acarology**, v. 51, n. 1, p. 31–59, 2010.

MIGEON, A.; NOUGUIER, E.; DORKELD, F. Trends in Acarology. **Trends in Acarology**, n. December, 2010.

OKU, K. et al. Mating strategies of Tetranychus kanzawai (Acari: Tetranychidae) in relation to mating status of females. **Annals of the Entomological Society of America**, v. 98, n. 4, p. 625–628, 2005.

OKU, K. Is only the first mating effective for females in the Kanzawa spider mite, Tetranychus kanzawai (Acari: Tetranychidae)? **Experimental and Applied Acarology**, v. 45, n. 1–2, p. 53–57, 2008.

OKU, K. Female mating strategy during precopulatory mate guarding in spider mites. **Animal Behaviour**, v. 77, n. 1, p. 207–211, 2009a.

OKU, K. Effects of density experience on mate guarding behavior by adult male Kanzawa spider mites. **Journal of Ethology**, v. 27, n. 2, p. 279–283, 2009b.

OKU, K. Males of the two-spotted spider mite attempt to copulate with mated females: Effects of double mating on fitness of either sex. **Experimental and Applied Acarology**, v. 50, n. 2, p. 107–113, 2010.

OKU, K. Sexual selection and mating behavior in spider mites of the genus Tetranychus (Acari: Tetranychidae). **Applied Entomology and Zoology**, v. 49, n. 1, p. 1–9, 2014.

OKU, K. et al. Altered Volatile Profile Associated with Precopulatory Mate Guarding Attracts Spider Mite Males. **Journal of Chemical Ecology**, v. 41, n. 2, p. 187–193, 2015.

OKU, K.; SAITO, Y. Do males evaluate female age for precopulatory mate guarding in the two-spotted spider mite? **Journal of Ethology**, v. 32, n. 1, p. 1–6, 2014.

OKU, K.; VAN DEN BEUKEN, T. P. G. Male behavioural plasticity depends on maternal mating status in the two-spotted spider mite. **Experimental and Applied Acarology**, v. 71, n. 4, p. 319–327, 2017.

OKU, K.; YANO, S.; TAKAFUJI, A. Spider mite's use of a refuge during the quiescent stage in the presence of a predator. **Eisei kagaku**, v. 21, n. 6, p. 301–306, 2003.

PARKER, G. A. Evolution of Competitive Mate Searching. **Annual Review of Entomology**, v. 23, n. 1, p. 173–196, 1978.

PENMAN, D. R.; CONE, W. W. Role of web, tactile stimuli, and female sex pheromone in attraction of male twospotted spider mites to quiescent female deutonymphs. **Annals of the Entomological Society of America**, v. 67, n. 2, p. 179–182, 1974.

POTTER., A. D.; WRENSCH, D. L.; E, J. D. Guarding, Aggressive Behavior, and Mating Success in Male Twospotted Spider Mites 12. n. Laing 1969, p. 707–711, 1972.

POTTER, D. A.; WRENSCH, D. L.; JOHNSTON, D. E. Guarding, Aggressive Behavior, and Mating Success in Male Twospotted Spider Mites 12. n. Laing 1969, p. 707–711, 1972.

PROCTOR, H. C. The evolution of copulation in water mites: a comparative test for nonreversing characters. **Evolution**, v. 45, n. 3, p. 558–567, 1991.

PROCTOR, H. C. Mating and spermatophore morphology of water mites (Acari: Parasitengona). **Zoological Journal of the Linnean Society**, v. 106, n. 4, p. 341–384, 1992.

PROCTOR, H. C. Indirect sperm transfer in arthropods: Behavioral and evolutionary trends. **Annual Review of Entomology**, v. 43, n. 77, p. 153–174, 1998.

PROCTOR, H. C.; SMITH, B. P. Mating behaviour of the water mite Arrenurus manubriator (Acari: Arrenuridae). **Journal of Zoology**, v. 232, n. 3, p. 473–483, 1994.

RODRIGUES, L. R. et al. Male spider mites use chemical cues, but not the female mating interval, to choose between mates. **Experimental and Applied Acarology**, v. 71, n. 1, p. 1–13, 2017.

SATO, Y.; SABELIS, M. W.; EGAS, M. Alternative male mating behaviour in the two-spotted spider mite: Dependence on age and density. **Animal Behaviour**, v. 92, p. 125–131, 2014.

SATOH, Y.; YANO, S.; TAKAFUJI, A. Mating strategy of spider mite, Tetranychus urticae (Acari: Tetranychidae) males: Postcopulatory guarding to assure paternity. **Applied Entomology and Zoology**, v. 36, n. 1, p. 41–45, 2001.

SCHARF, I.; PETER, F.; MARTIN, O. Y. Reproductive Trade-Offs and Direct

Costs for Males in Arthropods. **Evolutionary Biology**, v. 40, n. 2, p. 169–184, 2013.

TRANDEM, N. et al. Fatal attraction: Male spider mites prefer females killed by the mite-pathogenic fungus Neozygites floridana. **Journal of Invertebrate Pathology**, v. 128, p. 6–13, 2015.

TRIPET, F. et al. Sperm Competition and Its Evolutionary Consequences in the Insects. **Annals of the Entomological Society of America**, v. 96, n. 2, p. 169–170, 2006.

VACACELA AJILA, H. E. et al. Effects of lime sulfur on Neoseiulus californicus and Phytoseiulus macropilis, two naturally occurring enemies of the two-spotted spider mite Tetranychus urticae. **Pest Management Science**, v. 76, n. 3, p. 996–1003, 2020.

YOSHIOKA, T.; YANO, S. Do Tetranychus urticae males avoid mating with familiar females? **Journal of Experimental Biology**, v. 217, n. 13, p. 2297–2300, 2014.

YUVAL, B.; DEBLINGER, R. D.; SPIELMAN, A. Mating behavior of male deer ticks Ixodes dammini (Acari: Ixodidae). **Journal of Insect Behavior**, v. 3, n. 6, p. 765–772, 1990.