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ORIGINAL ARTICLE

Distribution and survival of the predatory mite *Blattisocius mali* on cucumber leaves with the addition of bran, yeast and pollen

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Abstract

Biological control has a special position in sustainable agriculture that requires continuous exploration and diversification in bio-agents to cope with emerging crop pests. *Blattisocius mali* is a promising biological control agent against some acarid mites, nematodes and moth pests. This study aimed to examine factors that could increase survival and diminish dispersal of *B. mali* deprived of its prey, the mold mite *Tyrophagus putrescentiae*, from cucumber plants. The impact of the presence of males on starving females' lifespans and the influence of different substrates, i.e., wheat bran, dry yeast pellets, and cattail pollen, on the distribution and survival of starving females in groups with males were examined. Experiments were performed on cucumber leaf platforms in Petri dishes filled with water. The results showed that females lived longer and a lower percentage drowned in water when accompanied by males. On the platforms with the addition of a substrate, the mites mainly stayed within the substrate, and eggs were only recorded in the substrate. They clearly preferred bran over pollen or pollen + yeast pellet aggregations. However, the quality of the substrate deteriorated within the first days, and some mites died of entrapment in the substrates. On the 3rd day, the lowest mean percentage of live individuals was observed on platforms with yeast + pollen (54.4%) followed by pollen (68.9%) alone. At that time, females also stopped laying eggs, and cannibalism towards hatched larvae and adults was observed. By the end of the experiment, 54.67% of the mites had been found dead on the leaf surface, and none of the substrates had significantly influenced their lifespan. The females lived on average 8.19 days and the males 5.06 days. The obtained results are discussed in the context of potential application of *B. mali* in biological control strategies.

Keywords: biological control, cannibalism, starvation, suplementary diet, survival

Introduction

Biological control is playing an increasingly important role in sustainable agriculture, and there is a constant need to expand the range of biological agents, not only due to the appearance of new crop pests but also to the insufficient effectiveness of beneficial organisms already being used under all conditions (van Lenteren *et al.* 2018; Beretta *et al.* 2022).

An important step in the study of the effectiveness of natural enemies of pests is to examine their survival under conditions of scarcity or lack of target prey/host (Ghazy *et al.* 2016). As a rule, bioagents should be introduced into crops with a low density of pests (Yano 2021). Under such conditions, however, establishment of their population may not be possible. Therefore, a number of methods are used to support the development of populations of natural enemies, providing them with alternative prey/host, food, oviposition sites or shelter (Messelnik *et al.* 2014; Kumar *et al.* 2015; van Lenteren *et al.* 2018; Pasquier *et al.* 2021; Yano 2021). Moreover, providing predatory mites with an alternative prey or food makes it possible to apply them prophylactically to the crop before the target prey appears (so called Predator-in-First approach) (Kumar *et al.* 2015; McMurtry *et al.* 2015). For example, the soil-dwelling predatory mite *Geolaelaps aculeifer* (Canestrini) (Mesostigmata: Laelapidae) fed on sterilized eggs of the acarid mite *Aleuroglyphus ovatus* (Troupeau) (Astigmata: Acaridae) until larvae of the western corn root worm were present (Pasquier *et al.* 2021), while the phytoseiid mite *Amblyseilus swirskii* (Athias-Henriot) (Mesostigmata: Phytoseiidae) was prophylactically mass-released onto pepper seedlings and maintained on cattail pollen (Kumar *et al.* 2015).

Among predatory mites, phytoseiids are the predominantly used biocontrol agents (van Lenteren *et al.* 2018; Beretta *et al.* 2022). They are primarily above-ground and omnivorous predators that can obtain nutrients from both prey and plant sources (Palevsky 2016). In contrast to phytoseiids, predatory soil mites such as the two laelapids, *G. aculeifer* and *Stratiolaelaps scimitus* (Womersley), as well as the macrochelid mite, *Macrocheles robustulus* (Berlese) (Mesostigmata: Macrochelidae) are recommended against various edaphic pests or edaphic stages of above-ground pests (Beretta *et al.* 2022). Interestingly, there are also some generalist predatory insects and mites that can colonize both under and above- ground plant parts and effectively feed on pests in both habitats (Messelink and van Holstein-Saj 2006; von Berg *et al.* 2009; Muñoz-Cárdenas 2017; Beretta *et al.* 2022). Also, some 'typically' edaphic predatory mites such as *G. aculeifer* or *Blattisocius dendriticus* (Berlese) (Mesostigmata: Blattisociidae) have been observed on plants, from where they probably preyed upon pests (Waite and Gerson 1994; Kasuga *et al.* 2006; Wu *et al.* 2016; Esteca *et al.* 2018). According to Beretta *et al.* (2022), the general predators that form a link between the under- and above-ground food web could have significant advantages in biological control. Still, more research on these issues is needed.

Blattisocius mali (Oudemans) is a polyphagous, predatory mite that has been found in various habitats, mainly in soil and litter, grass seeds, fruit and vegetable storage, and also occasionally on plants, i.e., on the bark of apple trees and leaves of strawberries (Karg 1971; Çakmak *et al.* 2011; Amal *et al.* 2020; Gallego *et al.* 2020; Michalska *et al.* 2023a). Recent findings by Pirayeshfar *et al.* (2021, 2022) and Asgari *et al.* (2022) have confirmed its high effectiveness in biological control of the mold mite *Tyrophagus putrescentiae* (Schrank) (Astigmata: Acaridae). Mold mites are cosmopolitan and omnivorous pests of various stored food products, mushrooms as well as crop plants such as bulbs of ornamental plants, seedlings of cucumber, melon and pumpkin or in vitro cultures of anthurium (Hughes 1976; Nakao 1991; Zhang 2003; Itisha *et al.* 2017; Murillo *et al.* 2021). The method of *B. mali* application against mold mites that infest stored wheat grains was worked out by Dergachev (1998). As shown in a study by Payreshivar *et al.* (2021) on cucumber leaf platforms and by Asgari *et al.* (2022) in cages, the intrinsic rate of increase (r) of *B. mali* populations feeding on mold mites (an important life table parameter in assessing biological control agents) is much higher than that estimated for predatory mites *B. dendriticus* or *G. aculeifer*, both of which are recommended for biological control of mold mites. Although *B. mali* appears to be a promising biological agent, there are still many gaps in our knowledge about its biology and ecology as well its behavior in plant crops, including survival when faced with the lack of potential prey.

The aim of this study was to examine factors that could increase survival and diminish dispersal of *B. mali* deprived of its prey, the mold mite *T. putrescentiae*, from cucumber plants. First, we analyzed the effect of the presence of males in groups of starving females on the lifetime of the latter. Similar to *B. tarsalis* (Darst and King 1969), *B. mali* females copulate more than once in their lives (unpublished data). Therefore, it can be expected that apart from insufficient food, the lack of males may additionally increase exploration in starving females, which consequently may lead to increased mortality and dispersion rate of predatory females from cucumber plants. Secondly, we examined the effect of the presence of various substrates, i.e., wheat bran, dry yeast pellets and cattail pollen, on predators' distribution and survival on cucumber leaves. The wheat bran and yeast pellets were substrates familiar to *B. mali*, since the predator was previously mass-reared within the mixture of wheat bran and yeast together with the mold mites for which these substrates are food. Thus, the substrates might be cues for the presence of the prey and affect predator foraging and dispersal. Moreover, the aggregation of wheat bran may be used by a predator for shelter, while yeast or pollen might be alternative or supplementary food sources, as in the case of some phytoseiids (Sarwar 2016; Jin *et al.* 2023). Although there were no direct observations of *B. mali* (or other *Blattisociidae* species) feeding on pollen, Pirayeshfar *et al.* (2022) revealed that the addition of cattail pollen to frozen eggs of *T. putrescentiae* can have a positive effect on the development and fecundity of this predator.

Materials and Methods

Mites and general methods

The stock colony of *B. mali* came from the mite population of the Department of Plant Protection of Warsaw University of Life Sciences (WULS). The predatory mites were maintained on foam platforms with bran mixed with various stages of the mold mite *T. putrescentiae*, following the procedure of Michalska *et al.* (2023b). The mold mite cultures were kept in flasks with dry yeast pellets, in darkness, in an incubator at 26°C and 75–80% RH. They were added (together with yeast pellets fragmented by acarids) to the colonies of the predator at weekly intervals. Experiments and *B. mali* mass-rearing were conducted in a climatic room of the Department of Plant Protection (WULS), at 21–23°C, 65–70% RH and 16/8 h (D/N) photoperiod. The tests were conducted using gravid *B. mali* females and males randomly chosen from mass-rearings in which the predators were additionally fed mold mites *ad libitum* 1 day before the trials started. All manipulations with mites were made using a fine brush at 5–50 x magnification under a stereo microscope fitted to a cooled light source.

The predators were tested on leaf plaforms using a method which was similar to that of Pirayeshfar *et al.* (2021). Leaf rectangles (3 cm × 4 cm) were cut from cucumber leaves cv 'Skierniewicki' and put singly, underside up, on a water surface of the bottom plates of Petri dishes of 11 cm in diam. They were placed in the center of the plate, just above a piece of plasticine which was attached to the bottom of the plate in order to keep the leaf in place. The groups of mites were then released onto such prepared leaf platforms and inspected every 24 hours until no more live individuals were found on the leaves. The water barrier generally prevented the mites from escaping, although some 'accidental' deaths by drowning were noted.

Platform inspections were performed at the same time every day, during the light phase. During inspection, the number of eggs, as well as live and dead *B. mali* adults was counted on the surface of leaves, within a substrate or drowned in water. All relevant behavior of predators including cannibalistic behavior was recorded at this time. Also, water in the Petri dishes was replenished if necessary.

Experimental procedure

To examine factors that could increase survival and diminish dispersal of *B. mali* deprived of its prey from the cucumber leaves, three laboratory trials were conducted. First, we tested the effect of the presence of males on the survival and oviposition of starving females on leaf platforms (trial 1). Secondly, we examined the influence of various substrates on leaf platforms on the survival, oviposition and distribution of starving predators (trial 2), and thirdly, the ability of predators to feed on dry yeast pellets (trial 3) was determined. In trials 1 and 2, we also examined the number and distribution of predator eggs on platforms. However, the numbers of eggs that might have been killed as a result of cannibalism or the numbers of hatched larvae were not assessed.

Trial 1. To examine the effect of the presence of males on survival and oviposition of starving *B. mali* females, two combinations were carried out. On a 'clean' cucumber leaf platform (without any substrate), we released either a group of seven females or a group of seven females + two males. Both combinations were replicated N = 12 times. For each combination we calculated the mean lifetime of females that died on the leaf surface and the percentage of females that 'accidently' drowned in water. Since eggs were recorded on platforms only on the first and second days of the test, the average number of eggs that were found in each combination was calculated as the mean total number of eggs per platform during these 2 days of observations.

Trial 2. The influence of the presence of various substrates on cucumber leaves on survival, oviposition and distribution of starving *B. mali* was tested on leaf platforms using groups of seven females + two males**.** After preparation of a leaf platform, an aggregation (0.8–1 cm in diam) of a substrate was applied to the center of each leaf rectangle using a micro lab spatula. Finally, a group of predators was released onto each platform. In the test, we used 'familiar' substrates for *B. mali*, i.e., wheat bran or dry yeast pellets (Dr. Oetker), which were also used for predator mass-rearings. We also used a 'non-familiar' substrate, i.e., pollen collected from cattail *Typha* sp. grown in Moczydło Park (Warsaw, Poland). By providing them in the form of aggregations instead of fine granules evenly distributed over the leaf surface, we could examine whether these substrates would act as bait and /or predator shelters, at which they could gather to feed, or in which they could hide. The experiment was carried out in the following combinations: a leaf platform with an aggregation of (1) wheat bran, (2) cattail pollen, (3) yeast pellets, (4) cattail pollen and yeast pellets, (5) control – a 'clean' leaf platform without a substrate (Fig. 1). Each combination was repeated N = 10 times. As in the previous test, the lifetimes of starving females and males were estimated for mites that died 'naturally', i.e., did not undergo an accidental death, e.g., by being trapped in the mycelium growing on the substrate or by drowning in the water which surrounded the leaf rectangles. As in trial 1, the average number of eggs was determined for the first 2 days of observations when the eggs were still found on the leaf rectangles. On some leaf platforms, the quality of the substrates was already visibly deteriorated on the 3rd day of the experiment (e.g., the aggregation of moist pollen or bran formed a crusted cluster, fungi started to develop on some yeast pellets). A comparison of the percentage of mites visiting the substrates was carried out for the first 3 days of the experiment, while their survival was assessed on the 3rd inspection day.

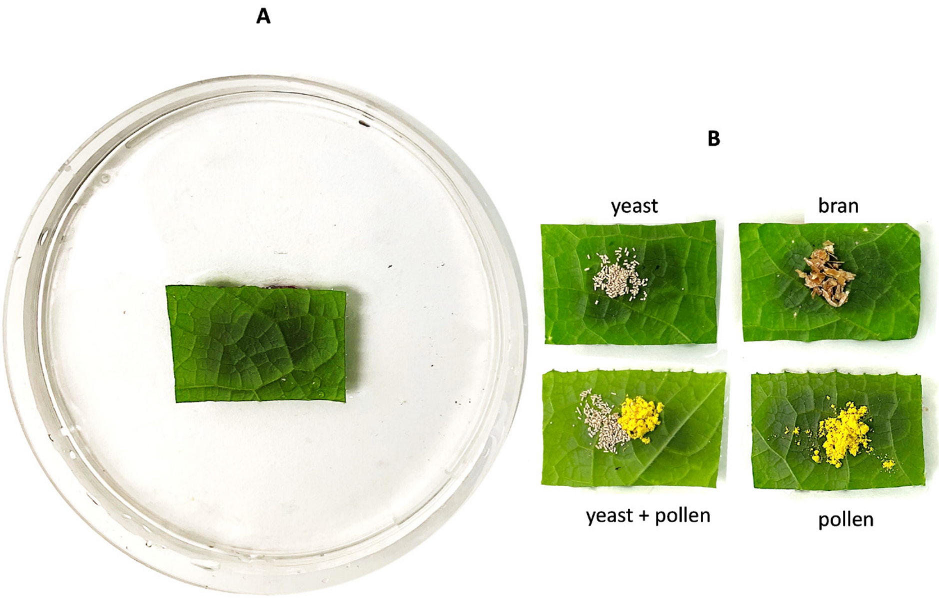

Fig. 1. A – a Petri dish with a cucumber leaf platform without substrate – control, B – platforms with different combinations of substrates

Trial 3. To examine whether *B. mali* could feed on dry yeast pellets, we prepared N = 20 leaf platforms, each with 10 evenly distributed yeast pellets. Then, we released a group of seven females + two males on each platform. As in the previous tests, the platforms were inspected every 24 hours for several days until the death of all mites. On each inspection day we carefully checked the 'state' of each pellet, examining for any signs of its damage, e.g., perforation or crumbling, indicating an attempt of a predatory mite to feed on it.

Statistical analysis

Statistical analysis was performed using R 4.2.1 software (R Core Team 2023). In order to compare the lifetime of *B. mali* females starved on cucumber leaf platforms in combination with males or without them, the one-factor Generalized Linear Model (GLM) with gamma distribution was applied. A one-factor GLM model with a binomial probability distribution was used to analyze the impact of the presence of males on the percentage of females that drowned in water, and the one-factor GLM model with a Poisson probability distribution was used to compare the number of predator eggs in combination with and without males.

To analyze the effect of the presence of the substrate on the lifetime of starving *B. mali*, the one-factor GLM with gamma distribution was applied for both females and males. The impact of the presence of the substrate on the number of predator eggs was analyzed using one-factor GLM with a Poisson probability distribution, while the effect of the substrate and time on the mean percentage of mites present in the substrate was assessed via a two-factor GLM model with a binomial probability distribution. We also analyzed the effect of the presence of the substrate on the percentage of live predators and those that died either on a leaf surface, in a substrate or in water, using one-factor GLM with binomial probability distribution. All pair-wise comparisons were carried out using Tukey's linear contrast method. The data is given as mean ±SE.

Results

The effect of the presence of males on survival and oviposition of starving *Blattisocius mali* females

The presence of males had a significant impact on the lifespan of females that died on cucumber leaf platforms (GLM: $\chi^2 = 38.826$; df = 1, $p = 0.00865$) and the percentage of females that drowned in water surrounding the platforms (GLM: $\chi^2 = 54.95$; df = 1, $p < 0.00001$). When accompanied by males, starved females lived their lives on average significantly longer (up to 15 days maximum) than females kept on platforms without male company (Fig. 2A; $p < 0.05$). In groups with males, the mean percentage of drowned females was 3.57 ± 2.02, significantly smaller than that in groups without males, in which it exceeded 50% (Fig. 2B; $p < 0.05$). On platforms without males, some females drowned on the first day. Others died in water on subsequent days up to the 13th day of inspection. Such females lived on average 6.26 ± 0.54 days (N = 43). Meanwhile, the mean lifetime of males that

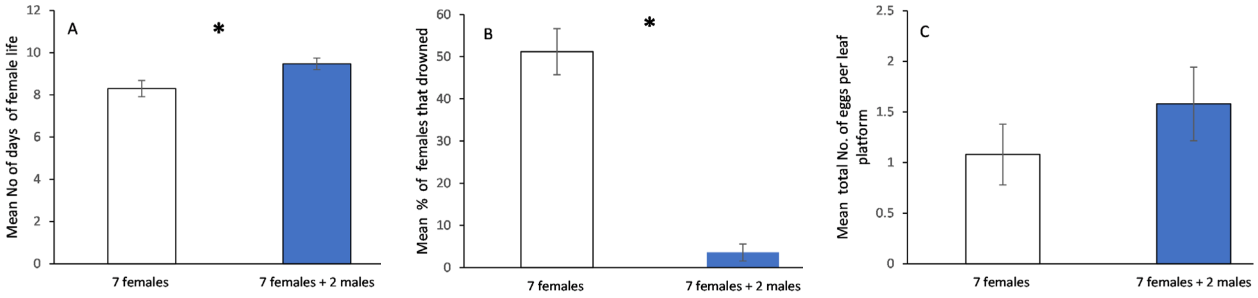

Fig. 2. Effect of the presence of males on mean ± SE. A – lifespan of starved *Blattisocius mali* females that died on cucumber leaf platforms, B – percentage of *B. mali* females that drowned in water, C – total number of *B. mali* eggs recorded per leaf platform. *$p < 0.05$

died on leaf platforms was 5.86 ± 0.67 (N = 22) days, and only 4.17 ± 2.991% of all tested males drowned.

During the first 2 days of the test, in both combinations, some starved females laid eggs. In groups of females with males, the mean total number of eggs per leaf platform was slightly higher than in the combination without males, although the difference between egg numbers was not significant (GLM: $\chi^2 = 1.1317$; df =1, $p = 0.2874$) (Fig. 2C).

The effect of the presence of substrates on cucumber leaf platforms on survival, oviposition and distribution of starving *Blattisocius mali*

As shown by analysis from the 3rd day of the experiment, the presence of the substrate had a significant impact on the survival of starved predators (GLM: $\chi^2 = 37.946$; df = 4, $p < 0.00001$) and on the predators' mortality, whether on the surface of a leaf, in a substrate or in water (GLM: $\chi^2 = 41.982$; df = 4, $p < 0.00001$). The lowest mean % of live individuals was observed on cucumber leaf platforms with yeast + pollen (54.40 ± 5.25%) and with pollen (68.9 ± 4.68%) (Fig. 3A). In these two combinations, the largest percentage of individuals, namely 42.22 ± 5.21% and 27.8 ± 4.72% for yeast + pollen and for pollen, respectively, died in a substrate (Fig. 3B). When the aggregation of pollen became wet, mites hidden inside often got stuck in it and died. Also, fungi gradually developed on wet pollen, yeast and bran, and mites would get tangled up in the growing mycelium. The mean percentage of mites that died on a leaf surface or drowned in water did not exceed 5%, except for mites in the combination with bran, for which the percentage of drowned mites was on average 16.67 ± 3.93 and was significantly higher than that of mites that died in the bran.

By the end of the experiment, 54.67%. of all mites had been found dead on the leaf surface. None of the substrates significantly influenced their lifespan, neither in females (GLM: $\chi^2 = 3.602$, df = 4, $p = 0.1877$), nor in males (GLM: $\chi^2 = 1.525$, df = 4, $p = 0.2064$) (Fig. 4). The females lived on average 8.19 days (min–max, 1–16 days), while the males lived for 5.06 days (min–max, 1–12 days).

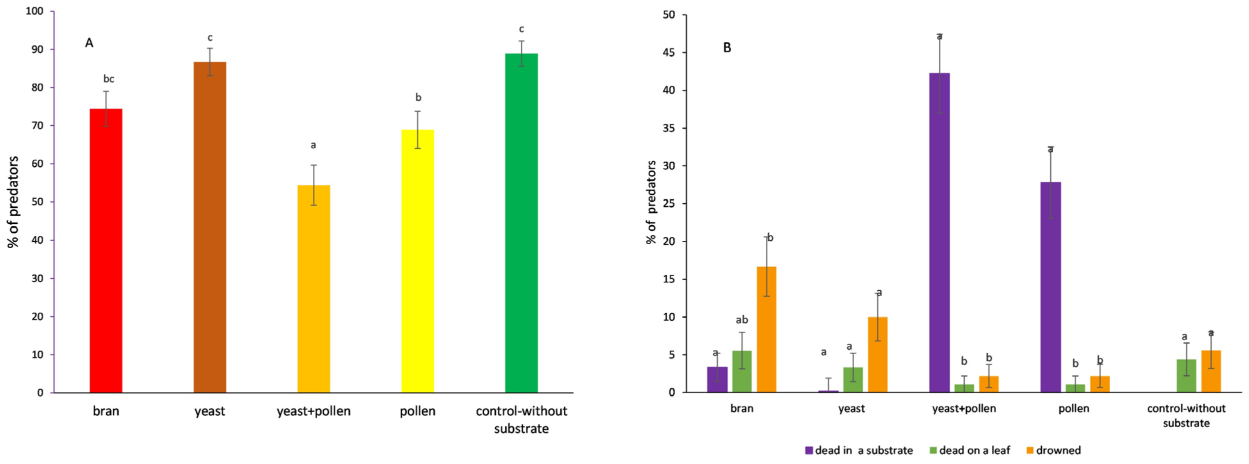

Fig. 3. Survival of *Blattisocius mali* females and males kept in groups on cucumber leaf platforms with various combinations of a substrate after the first 3 days of prey deprivation. A – mean ± SE percentage of live predators per platform, B – mean ± SE percentage of dead predators per platform either dead in a substrate, on a leaf or drowned in water. The letters a, b indicate significant differences between means ($p < 0.05$)

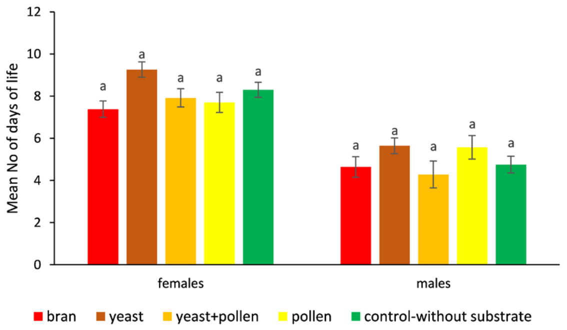

Fig. 4. Mean lifespan (±SE) of females and males of *Blattisocius mali* that died on a leaf surface when starved on platforms with or without substrate. ($p > 0.05$)

Predator eggs were recorded on the platforms during the first 2 days of the experiment, as were the larvae that later hatched from the eggs. When there was an aggregation of the substrate on a platform, eggs were recorded only in the substrate, never outside it. The presence of the substrate and its type had a significant effect on the number of eggs on the platforms both on the 1st (GLM: $\chi^2 = 16.998$, df = 3, $p = 0.0027$) and on the 2nd day (GLM: $\chi^2 = 2.549$, df = 3, $p = 0.0202$) (Fig. 5). On the 1st day, the highest number of eggs was found in bran. Significantly fewer eggs were noted in the aggregation of yeast pellets, and the fewest were found in the combinations with pollen and yeast + pollen. On the 2nd day, the number of eggs on the platforms decreased, but in bran and yeast aggregations it was still significantly higher than in the other combinations.

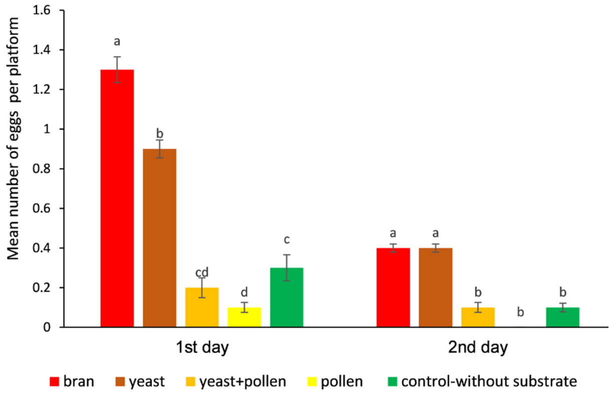

Fig. 5. Mean (± SE) number of eggs of *Blattisocius mali* on leaf platforms on the 1st and 2nd days of inspection. The letters a, b, c, d indicate significant differences between means ($p < 0.05$)

The mites willingly stayed in the substrate, not only on the 1st and 2nd, but also on the 3rd day of observation, when the females no longer laid eggs and the substrate had not yet deteriorated (Fig. 6). During this period, the total mean percentage of mites in a substrate was 61.21 ± 4.36. The frequency of female and male present in the substrate depended only on its type (GLM: $\chi^2 = 56.988$, df = 3;36, p >0.0001), and not on the day of observation (GLM: $\chi^2 = 87.029$, df = 2;36, $p = 0.9832$) or substrate x day interaction (GLM: $\chi^2 = 48.299$, df = 6;36, $p = 0.1736$). On the 1st and 2nd days, the mites clearly preferred bran over pollen. On the 3rd day, yeast and yeast with pollen were also preferable to pollen (Fig. 6).

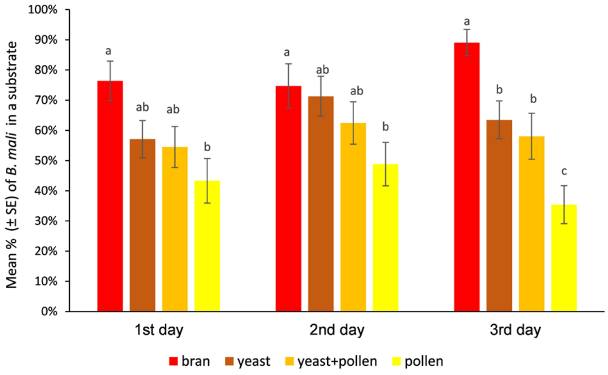

Fig. 6. Distribution of males and females of *Blattisocius mali* on leaf platforms with a substrate on the 1st, 2nd and 3rd inspection days. The letters a, b, c indicate significant differences between means ($p < 0.05$)

In the following days, cases of cannibalism were noticed. On the 4th day of the predators' starvation, on the platforms with yeast, a female attacked a larva and then consumed it. In turn, on the 9th day of starvation, consumption of a dead female by another female was observed.

Feeding of *Blattisocius mali* on dry yeast pellets

Our observations were conducted for 15 days until there was no live predator on the platforms. During this period none of the 200 tested yeast pellets showed any sign of damage indicating an attempt of predatory mites to feed on it. In the pilot tests, just after a few days, the pellets 'swelled', and even when touched gently with a preparation needle, the yeast fell apart. However, in our experiments, none of the particles fell apart or showed any perforation on their surfaces.

Discussion

In this study, the presence of males increased the survival of starved *B. mali* females but did not affect the number of predator eggs on cucumber leaf platforms. Seven females accompanied by two males lived longer and drowned in a much smaller percentage than those without males. Moreover, there was also a significant effect of the presence of substrate on the survival and distribution of starved *B. mali* males and females as well as the number of eggs and their distribution on platforms. During the first days of observation, most mites stayed in the substrate, but they clearly preferred wheat bran over cattail pollen aggregation or cattail pollen + wheat bran aggregation. The mean number of eggs found in each substrate combination decreased in the following order: bran > yeast > yeast + pollen or no substrate > pollen. The quality of the substrate already deteriorated in the first days of the experiment, and some of the mites died which had been trapped in the substrates; pollen and pollen + bran substrates were particularly unfavorable, with the highest mortality of predators observed on the 3rd day of the experiment. In addition to accidental deaths in the substrate or water, more than half of the predators tested were found dead on the surface of leaves. In this group of predators, however, the presence of the substrate did not affect the lifespan of males or females.

The observed drownings of *B. mali* predators can be interpreted as accidental deaths while exploring the edge of the leaf cucumber platform in the immediate vicinity of water. Such exploration may have been motivated by hunger and an attempt of the mites to escape from places where there was no prey. However, it is also possible that some of them died while attempting to drink water. The use of free water has been confirmed in phytoseiids (San *et al.* 2021), but it also seems to be important for predatory soil mites (Navarro-Campos *et al.* 2016). However, the significant increase in the percentage of drownings among starved *B. mali* females kept on platforms without males may have resulted primarily from intensive exploration of the platforms in search of a sexual partner. In *B. tarsalis* (Darst and King 1969), and also in *B. mali* (unpublished data) females copulate more than once in their lives. Multiple matings can be beneficial for females due to adequate sperm supply, with its delivery of extra nutrients with seminal fluids, or increased genetic diversity of offspring (Engqvist 2006). For these reasons, *B. mali* females may be highly motivated to seek males. However, this aspect of their reproduction requires further investigation.

During starvation of *B. mali* on leaf platforms, eggs were recorded only for the first 2 days, and the presence of males did not seem to increase their production. In phytoseiid mites, such as *Kampimodromus aberrans* Oudemans, multiple mating increases fecundity in females (Pappas *et al.* 2007). On the other hand, lack of access to food may inhibit egg production. For example, in the phytoseiid mite *Neoseiulus californicus* (McGregor), starved females laid no more than a total of three eggs and lived for an average of 4 days (maximum 16 days), while females with access to prey laid two eggs per day (Toyoshima *et al.* 2009). Interestingly, in our test, the lifespan of *B. mali* females staying with males was longer by an average of 1 day than females without male company. The extension of the female lifespan may have resulted from lower energy expenditure incurred in searching for males. However, one cannot exclude the possibility that repeated copulation conferred benefits to females in terms of nutrients delivered by males together with sperm transfer and induced their starvation resistance (Goenaga *et al.* 2012).

Research has shown that adding substrate directly to plants can have both a positive and negative effect on predators. On one hand, a substrate can be a hiding place for predatory soil mites and their eggs from unfavorable abiotic and biotic factors. On the other hand, however, it can become a death trap if it becomes moist, like the pollen they could get stuck in, or if mycelium develops, posing a risk of entanglement. During the first 3 days, bran worked best. Mites were eager to visit it, and most eggs were found here. It is worth noting that this substrate was familiar for *B. mali*, as it was used in its mass-rearing. Wheat bran is a commonly used substrate for phytoseiid application in a crop, either by sprinkling loose material on the leaves or in the form of slow-release sachets, to gradually release phytoseiids that previously multiplied in them with the addition of live or dead prey (Vila and Cabello 2014; Shimoda *et al.* 2017; Solano-Rojas 2022; Shimoda *et al.* 2023). Significantly, even in sachets, the quality of the substrate may deteriorate, e.g., due to moisture, which is why various types of sachet improvements are used, such as, e.g., 'sheltered sachets' (Shimoda *et al.* 2019). In the first days, mites also hid in yeast pellet aggregations, although significantly fewer eggs were found there, possibly due to its inferior 'hiding' properties. However, this substrate was also 'known' to the mites. Perhaps, in combination with pollen, the yeast pellets lured predators to the vicinity of the pollen, which resulted in a significantly higher mortality rate than in combination with pollen alone. The cattail pollen also completely failed as a substrate for laying eggs. Even fewer eggs were recorded in the pollen than in the combination without substrate.

As research by Pirayeshfar *et al.* (2021) has shown, *B. mali* females kept on cucumber leaf platforms similar to those in our experiment but fed a mixture of different developmental stages of *T. putrescentiae* laid nearly 22.5 eggs per female throughout their lives. In our experiments, the number of eggs per female was much lower. This could have been caused by a number of reasons, such as the lack of nutrients for egg production or possible egg retention or cannibalism in this predator. Sanderson and McMurtry (1984) found that when the substrate was not suitable for phytoseiid egg laying, i.e., instead of a leaf surface with veins and hairs, the surface was smooth and tilted, female *Phytoseius hawaiiensis* Prasad (Mesostigmata: Phytoseiidae) refrained from laying an egg until close to larvae hatching. Under conditions of high risk of predation and cannibalism, females of other phytoseiids laid fewer eggs, despite the availability of prey, which might also have been the result of egg retention and/or egg absorption (Abad-Moyanoe *et al.* 2010; Walzer and Schausberger 2011). Research by Croft *et al.* (1995) on several species of phytoseiids did not confirm egg absorption. However, these predators preserved egg resources in another, equally effective way, namely through egg cannibalism (Croft *et al.* 1995). Although we did not observe *B. mali* cannibalism on eggs during our rather quick platform inspections, we did note attacks by this predator on its larvae. Egg cannibalism in *B. mali* seems probable considering that it was also observed in another species of the genus *Blattisocius*, namely *B. dendriticus* (Rivard 1960). It is therefore likely that a substrate such as wheat bran or yeast pellets, in which we found most *B. mali* eggs, could have stimulated females to oviposit due to the possibility of hiding the eggs, while other substrates such as pollen or the lack of any substrate on a leaf platform could have caused egg retention in females. However, it is not excluded that some of the eggs could also have been cannibalized, especially in substrates with poorer hiding properties. Moreover, predators may have fed on larvae and then on weakened or freshly dead adult conspecifics, which would have increased their survival on leaf platforms and fecundity during the first 2 days of starvation. This can be seen in the phytoseiid mite *Neoseiulus barkeri* (Hughes), in which the nutritional benefits gained from cannibalism allowed it to reproduce and survive for longer periods (Momen 2010). Since cannibalism can significantly influence the structure and dynamics of a predator population and may also have an impact on efficacy of biological control (Collier and van Steenwyk 2004; Tsuchida *et al.* 2022), further research on this phenomenon in *B. mali* is undoubtedly needed.

Our research also showed that in the case of mites that did not drown or die in the substrate but on the leaf surface, none of the substrates influenced lifespan. This also applied to substrates such as pollen or yeast, which could have been potential foods. Recent studies by Jin *et al.* (2023) have indicated that powdered yeast supplementation with pollen could enhance reproduction in phytoseiid mites. Our test on *B. mali* using yeast pellets did not show, however, any predator attempts to feed on such a substrate. Neither *B. keegani* (Barker 1967) nor *B. tarsalis* (Haines 1981) survived longer when kept with yeast than when maintained without food. It seems that both yeast and pollen are not substrates that are intentionally fed on by *B. mali* under natural conditions, but rather consumed as contamination together with the prey body. After mixing the mold mite T. *putrescentiae* eggs with either cattail or olive pollen, *B. mali* females feeding on this food achieved higher fertility, but in subsequent generations, pollen as a supplement negatively affected life parameters of this mite (Pirayeshfar *et al.* 2022). In two other soil predatory mites, namely, *G. aculeifer* and *S. scimitus*, the presence of cattail pollen did not increase fecundity of these predators compared to the combination without food (Navarro-Campos *et al.* 2016). However, *G. aculeifer* can feed and develop only on pollen of various plants (Lobbes and Schotten 1980; Ragusa *et al.* 1989). Moreover, the addition of powdered yeast with mold mites to the diet of the phytoseiid *N. barkeri* enhanced its fecundity and efficiency towards other prey types (Huang *et al.* 2013; Yang *et al.* 2015). Therefore, further research on the role of various pollens and yeasts in the diet of *B. mali*, including ethological observation, seems to be required. It should also be noted that in our studies, all substrates lost their potential properties as shelter or food after just a few days, which could also significantly obscure their possible beneficial effect on the lifespan of *B. mali* females and males.

The average lifespan of starved *B. mali* females that died 'naturally' on the leaf surface both in the experiment with or without a substrate was comparable to that of starved females of some phytoseiid mites, i.e., *A. eharai* or *A. swirski* (Zhang *et al.* 2015). Other starved phytoseiids may have a shorter or longer survival time. *Typhlodromus bambusae* Ehara lived without prey for only an average of 4 days, while *Neoseiulus cucumeris* survived nearly an average of 14 days (Zhang *et al.* 2015). Interestingly, in a study by Pirayeshfar *et al.* (2021), *B. mali* females and males kept on cucumber leaf platforms with their prey, a mixture of different developmental stages of mold mites, survived only an average of 6 days, a clearly shorter time than that of *B. mali* females and males deprived of food in our study. However, in contrast to our predatory mites, they spent their entire lives on leaf platforms, both as juveniles and adults. Mold mites are known to produce alarm pheromones (Kuwahara *et al.* 1975; Midthassel *et al.* 2016), which, according to Pirayeshfar *et al.* (2021), could have caused constant disturbance of *B. mali* on platforms, limiting their foraging and ultimately shortening their lives. However, when frozen eggs of *T. putrescentiae* were used, *B. mali* lifespan increased to an average of 10 days for females and 13 days for males (Pirayeshfar *et al.* 2021). It should be stressed that, whether in our study or in that by Pirayeshfar *et al.* (2021), due to their small size, the cucumber leaf platforms used did not provide *B. mali* mites with many opportunities to hide or maintain individual distances, unlike whole cucumber plants on which, as our preliminary observations showed, these predators can remain for many days until there are no mold mites left.

Undoubtedly, further research is necessary, both on the survival rate of *B. mali* and on methods of reducing its dispersal from plants when prey density decreases or when the prey is absent. Our tests indicate the need to introduce both sexes of this mite into the crop and provide them and their offspring with an appropriate hiding place. A substrate such as wheat bran can be used for this purpose. As our studies have shown, it is readily inhabited by this mite. Further research is also necessary on dietary supplementation, the use of powdered yeast, plant pollen or frozen prey on which *B. mali* could temporarily multiply and which could also prevent its cannibalism. Finally, methods should be developed to secure predatory mites against worsening environmental conditions, and to secure substrate and food against deterioration of their quality on plants.

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