Research Article

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Performance of *Metarhizium anisopliae*-treated foam in combination with *Phytoseiulus longipes* Evans against *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae)†

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Abstract

BACKGROUND: *Tetranychus evansi* (*Te*) is an exotic pest of solanaceous crops in Africa. The predatory mite *Phytoseiulus longipes* (*Pl*) and the fungus *Metarhizium anisopliae* (*Ma*) are potential biocontrol agents of *Te*. The present study investigated the efficacy of fungus-treated foam placed above or below the third *Te*-infested tomato leaf. The persistence of fungus-treated foam and the performance of *Pl* with and without fungus-treated foam were evaluated.

RESULTS: The fungus-treated foam was effective when *Te* infestation was below the third tomato leaf as no damage was recorded on any of the upper tomato leaves up to 30 days post-treatment. However, in the control treatments, the infestation increased considerably from 9 ± 0.3% to 100 ± 0% (mean ± standard error) at 15 days post-treatment. The reuse of the fungus-treated foam at 15, 30 and 45 days post-treatment resulted in 19 ± 1.4%, 25 ± 1.2% and 54 ± 2.1%, respectively, infestation by *Te*. The fungus-treated foam and *Pl* alone were efficient, but there was no benefit to combining them for use against *Te*.

CONCLUSION: The fungus-treated foam is an effective method to optimise the use of *Ma* in screenhouse conditions. These two control agents could be integrated in an integrated pest management strategy for crop protection. However, these results need to be confirmed in large field trials.

Keywords: fungus-treated foam; red spider mites; emulsifiable formulation; tomato plant; predatory mites

1 INTRODUCTION

The tomato spider mite (TSM) is among the key pests affecting tomato production in sub-Saharan Africa.[1–5] Yield losses of between 65 and 90% have been reported in small-holder production in East and West Africa.[6,7] TSM is an invasive species that originated from South America[8,9] and its management is mainly based on frequent applications of synthetic chemical acaricides. Up to 12 applications per month have been reported on tomato crops.[7–10] However, use of these chemicals is problematic as they are ineffective and costly,[6–8] and mites have developed resistance to various classes of these chemical acaricides.[11,12] They are also harmful to the environment and residues lead to food contamination. This has prompted the search for environmentally friendly alternatives such as biological control using predators and/or entomopathogenic fungi.[13]

Entomopathogenic fungi have been reported to be virulent against *Tetranychus evansi* and *Tetranychus urticae* Koch (Acari: Tetranychidae) in the screenhouse and in the field.[14,15] An isolate of *Metarhizium anisopliae* (Sorok.) Vuill. is commercially available under the trade name of Achieve® from Real IPM (Nairobi, Kenya) for the control of TSM. Use of entomopathogenic fungi for the inundative biological control of spider mites faces challenges, including high cost as a consequence of the large amount of inoculum used in spraying and the frequent applications necessitated by the short persistence of the inoculum in the crop and vulnerability to ultraviolet (UV) photodegradation.[16] As an alternative, a Brazilian predatory mite *Phytoseiulus longipes* Evans has been found in

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association with *T. evansi*[17] and has been reported to be effective against *T. evansi* in the laboratory and greenhouse.[17–19] These two biological control agents could therefore be integrated as components of a tomato spider mite management strategy. Furthermore, young females of *T. evansi* during the day shows gregarious behaviour and spreads on the tomato plant when its resources become limited[20]. This behaviour could therefore be exploited to develop a control strategy based on fungus-impregnated devices placed along the stalk for dissemination of conidia among migrant females which can in turn contaminate other stages on the leaves. This strategy would allow for longer persistence of the inoculum and save on cost. As *M. anisopliae* has been shown not to be pathogenic to *P. longipes,* the two control agents could be combined for greater efficacy.

The present study evaluated (i) the performance of the fungus-treated foam according to the location of mites on the tomato leaf plant; (ii) the persistence of the fungus-treated foam, and (iii) the the effect on red spider mite infestations of tomato plants of applications of both fungus-treated foam and the release of the predatory mite *P. longipes*.

2 MATERIALS AND METHODS

2.1 Plants

Seeds of *Solanum lycopersicum* L. var. 'Money Maker' from the East African Seed Company (Nairobi, Kenya) were sown in soil enriched with compost in plastic seed trays. Plants (21 days old) were transplanted into plastic pots 20.5 cm in diameter and 20 cm in height, each containing a mixture of red soil plus bovine manure (3:1), and placed in a greenhouse until they were 45 days old and had at least four completely developed leaves. The plants were watered daily and each pot was top dressed with 3 g of calcium ammonium nitrate [CAN (26% N) from Jumbo Agrovet, Nairobi, Kenya] 2 weeks after transplanting. These were then used for the rearing of spider mites and for experiments. Subsequently, 45-day-old plants with at least five completely developed leaves were used in the experiments, with the unfolded primary leaves being used either for the experiments or for the rearing of spider mites.

2.2 Mites

The red spider mites, *T. evansi,* used in this study were obtained from a regularly regenerated colony maintained at the International Centre of Insect Physiology and Ecology (ICIPE) on potted tomato plants of the variety 'Money Maker'. They were maintained at a temperature of 25 ± 1 °C at 50–70% relative humidity (RH) and with a 12- h photoperiod. Quiescent deutonymphs were collected from the mite culture using a fine camel hair brush and placed on tomato leaf discs. Two days later, newly emerged adult female mites were selected and used in the experiments.

2.3 Predatory mites

Phytoseiulus longipes was imported into Kenya in September 2005 from a Brazilian colony and maintained at ICIPE at 25 ± 1 °C and 60 ± 10% RH and with a photoperiod of 12:12 h light:dark. *Phytoseiulus longipes* had access to all developmental stages of *T. evansi* on infested tomato plants.

2.4 Fungus

The *M. anisopliae* isolate ICIPE 78 used in this experiment was obtained from the ICIPE Arthropod Germplasm Centre. It was selected for its virulence against *T. evansi*[14–22] and is commercially available as Achieve (Metarhizium78). Conidia were formulated in an emulsifiable formulation (Tween 80, corn oil, and sterile distilled water in a ratio of 0.25:6:93.75). A concentration of 1×10^8 conidia mL^{-1} was used in all the experiments according to Bugueme *et al.*[15] Conidia were harvested by scraping the surface of 3-week-old sporulating cultures grown on Sabouraud dextrose agar (SDA) in Petri dishes at 26 ± 2 °C. Conidia were suspended in 20 mL of sterile distilled water containing 0.05% Triton X-100. The suspension was vortexed for 5 min to produce a homogenous conidial suspension. The viability of conidia was then determined by spread-plating 0.1 mL of the suspension (titrated to 3.0×10^6 conidia mL^{-1}) on SDA plates. A sterile microscope cover slip was placed on each plate. Plates were incubated at 26 ± 2 °C and the percentage germination was determined for 100 spores in each plate after 24 h using a compound microscope at 400× magnification. Conidial germination was 90 ± 0.5%.

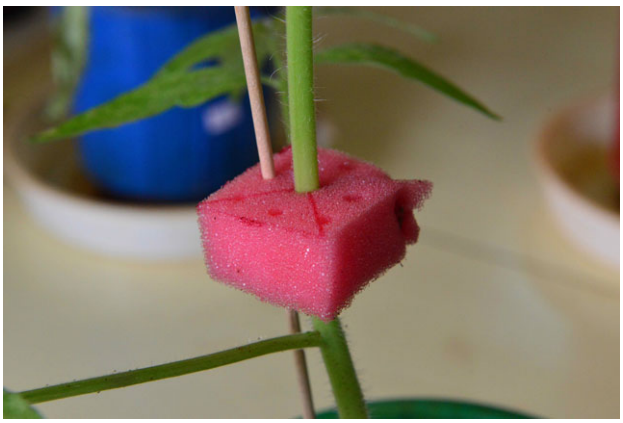

Figure 1. Bioassay setup.

2.5 Bioassays

2.5.1 *Inoculation of foams and treatments*

The foam (2 cm in diameter and 2 cm thick) used in this experiment was purchased from a local supermarket in Nairobi. A hole 5 mm in diameter was created in the middle in order to fit the foam around the stem of the tomato plant (Fig. 1). The foam was impregnated with 2 mL of emulsifiable formulation of the fungal conidia as described above and then affixed to the stem. In the control treatments, foam was impregnated with emulsifiable formulation without fungal conidia. The foam was fixed around the tomato stem in such a way that leaves L1 and L2 were below the foam and leaves L3, L4 and L5 above the foam (Fig. 2, experiment 1). The tomato plants were artificially infested with 100 *T. evansi* females on L2 or L3. The following treatments were applied: T1, untreated control with *T. evansi* infestation on leaf L2 below the foam; T2, untreated control with *T. evansi* infestation on leaf L3 above the foam; T3: fungus-treated foam with *T. evansi* infestation on leaf L2 below the foam; and T4, fungus-treated foam with *T. evansi* infestation on leaf L3 above the foam (Fig. 2, experiment 1). Treatments were replicated four times.

2.5.2 *Interaction between* M. anisopliae*-treated foam and the predatory mite* P. longipes *on* T. evansi

Another experiment was carried out whereby predatory mites were included, with the following treatments being applied (Fig. 2, experiment 2): T5, untreated foam with *T. evansi* and *P. longipes*

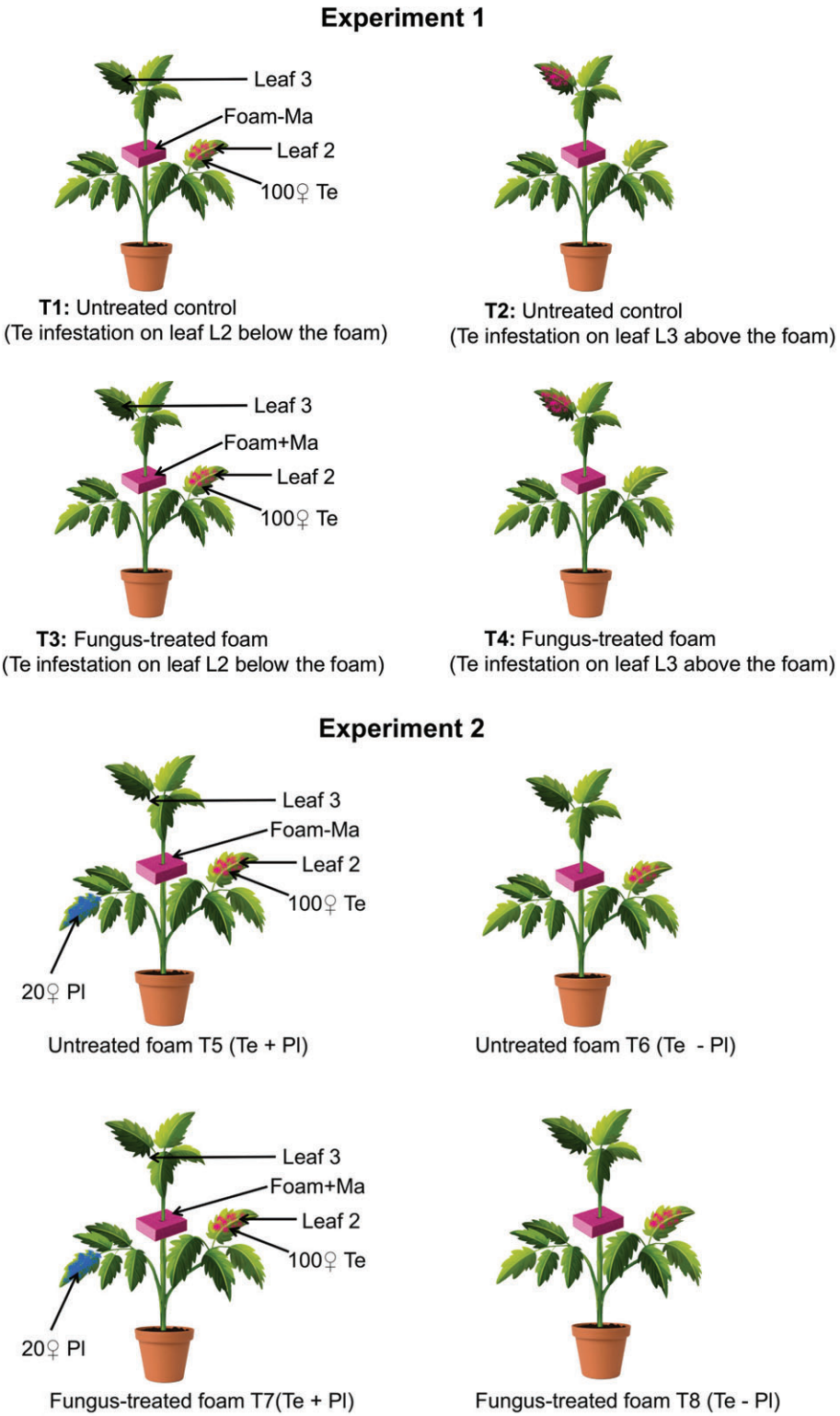

Figure 2. Experiment 1: experimental devices for testing the efficacy of a fungus-treated foam disc placed below or above the disc to control *T. evansi* (Te). Experiment 2: experimental devices for testing the efficacy of a fungus-treated foam disc placed below or above infested leaves to control *T. evansi*. The second experiment combined the fungus-treated foam disc with the release of *P. longipes* (PI). Ma, *Metarhizium anisopliae*.

(*Te* + *Pl*); T6, untreated foam with *T. evansi* but without *P. longipes* (*Te* – *Pl*); T7, fungus-treated foam with *T. evansi* and *P. longipes* (*Te* + *Pl*); and T8, fungus-treated foam with *T. evansi* but without *P. longipes* (*Te* – *Pl*). Twenty female predatory mites were starved for 24 h and released on leaflet L1, 3 days after the infestation of tomato plants with 100 *T. evansi* females. Treatments were randomised and repeated four times.

2.5.3 *Assessment of the efficacy of treatments*

For 15 days after treatment application, the percentage of leaflet infestation by *T. evansi* was determined every 3 days by recording the number of red spider mites alive per leaflet per plant. In the experiment on the interaction between the fungus-treated foam and predatory mites, the number of mobile stages of spider mites and predatory mites was counted on the foam and above the foam at days 6 and 15 post-treatment. The leaf mean damage index (LMDI) or damage index (ID) was determined using a scale from 0 (no damage) to 5 (the leaf beginning to shrivel), following a previously described method.[23] To determine the persistence of conidia on the foam, the fungus-treated foam was reused in successive trials without re-impregnation. The foam was removed at 15 days post-treatment and placed on 45-day-old non-infested plants. Leaf L2 was then infested with 100 *T. evansi* females as described above. The protocol was repeated for the third time after 15 days. In the control treatment, foam was not impregnated with the fungus. In the three successive assays, leaf infestation was determined every 3 days for 15 days post-treatment following the application of treatments as described above.

2.6 Data analysis

A non-parametric test, the Kruskal – Wallis test, was used to analyse the effects of the treatments on mite infestation and leaf damage. The response variable was the average infestation or LMDI per plant. The first time-point (day 0) was excluded from the calculation of these values because it was too early for treatments to have had an effect. Post hoc pairwise tests were performed with the Wilcoxon method. All the analyses were carried out using the statistical software JMP 12.[24]

3 RESULTS

3.1 Tomato leaf infestation

The mean percentage of leaflet infestation by mites increased from 9 ± 0.3% at day 0 to 100 ± 0.1% (mean ± Standard error) at day 15 post-treatment in the control (T1 and T2) and in the treatment with fungus-treated foam placed below the mite-infested leaflet (T4) (Fig. 3). Leaves in these treatments were completely damaged by mites and died off. At the same time, leaf infestation was significantly lower ($\chi^2 = 13.06$; df = 3; $P = 0.0045$) when fungus-treated foam was placed above the mite-infested leaf (T3) (Fig. 3).

3.2 Leaf mean damage index

Regardless of the location of the foam in treatments T1 (untreated control with *T. evansi* infestation on leaf L2 below the foam), T2 (untreated control with *T. evansi* infestation on leaf L3 above the foam) and T4 (fungus-treated foam with *T. evansi* infestation on leaf L3 above the foam), the ID was ≥1. Leaves in these treatments were completely damaged by mite infestation. However, in treatment T3 (fungus-treated foam with *T. evansi* infestation on leaf L2 below the foam), the ID of leaves located above the fungus-treated foam was practically nil and the ID of leaves below the fungus-treated foam was 2. The Kruskal – Wallis test showed a significant difference ($\chi^2 = 5.46$; df = 1; $P = 0.02$) between the treatments (Fig. 4). These results showed that the infestation level was linked to the position of the fungus-treated foam; the results are congruent with the results on infestation intensity (Fig. 3).

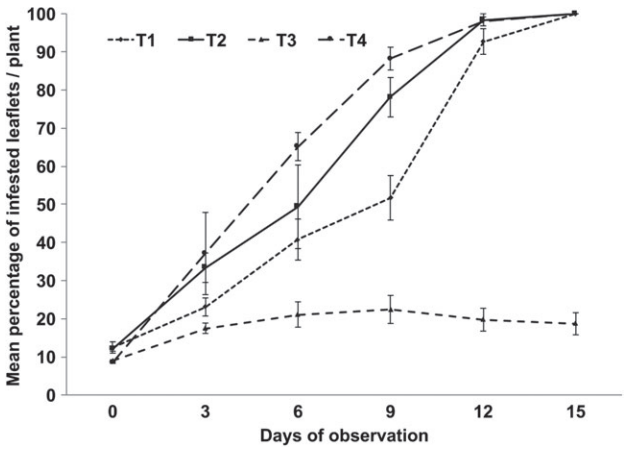

Figure 3. Mean percentage of tomato leaflets infested by *T. evansi* per plant and per observation date. The following treatments were applied: T1, untreated control with *T. evansi* infestation on leaf L2 below the foam; T2, untreated control with *T. evansi* infestation on leaf L3 above the foam; T3, fungus-treated foam with *T. evansi* infestation on leaf L2 below the foam; and T4, fungus-treated foam with *T. evansi* infestation on leaf L3 above the foam. On the first day (T0), 100 females of *T. evansi* were deposited on leaf L2 just below the foam or on leaf L3 just above the foam. The treatments were replicated four times and bars indicate the standard errors.

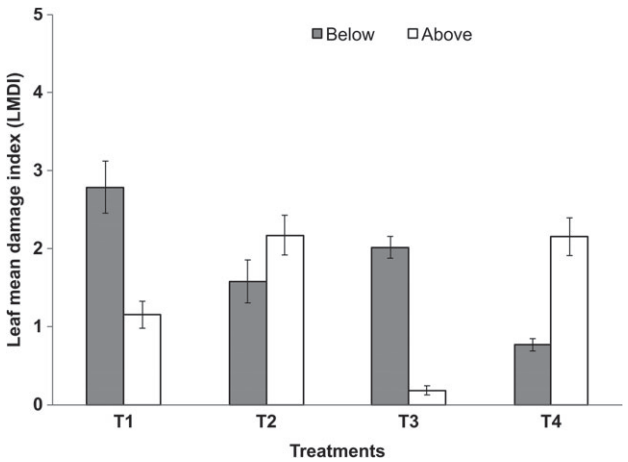

Figure 4. Tomato leaf mean damage index (LMDI) for damage by *T. evansi* recorded on the leaves below and above the fungus-treated foam. The following treatments were applied: T1, untreated control with *T. evansi* infestation on leaf L2 below the foam; T2, untreated control with *T. evansi* infestation on leaf L3 above the foam; T3, fungus-treated foam with *T. evansi* infestation on leaf L2 below the foam; and T4, fungus-treated foam with *T. evansi* infestation on leaf L3 above the foam. The treatments were replicated four times and bars indicate the standard errors.

3.3 Persistence of fungus-treated foam

The persistence of fungus-treated foam was evaluated in terms of percentage infestation of tomato leaflets by *T. evansi*. In the control treatments, the infestation of tomato leaflets by mites increased considerably from the initial deposit to reach 100 ± 0% at day 15 post-treatment in all the three experimental sets

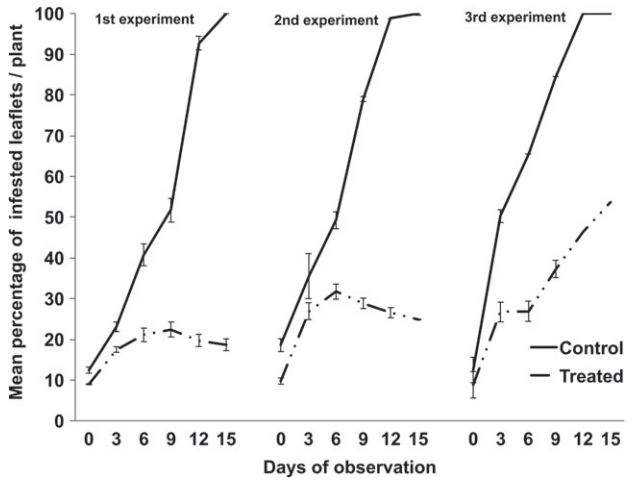

Figure 5. Mean percentage of tomato leaflets infested by *T. evansi* per plant and per observation date. The following treatments were applied: T1, untreated control with *T. evansi* infestation on leaf L2 below the foam; T3, fungus-treated foam with *T. evansi* infestation on leaf L2 below the foam. The fungus-treated foam was reused in two subsequent experiments. On the first day of each experiment (T0), 100 females of *T. evansi* were deposited on leaf L2 just below the foam. The arrows indicate the dates of mite infestation. The treatments were replicated four times and bars indicate the standard errors.

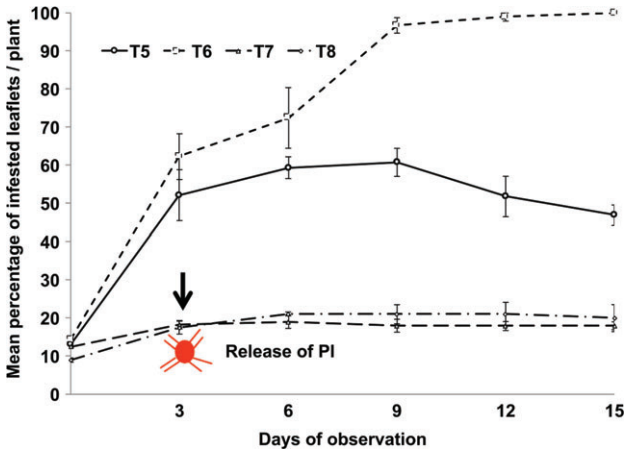

Figure 6. Mean percentage of tomato leaflets infested by *T. evansi* per plant and per observation date. The following treatments were applied: T5, untreated foam with *T. evansi* and *P. longipes* (*Te* + *Pl*); T6, untreated foam with *T. evansi* but without *P. longipes* (*Te* − *Pl*); T7, fungus-treated foam with *T. evansi* and *P. longipes* (*Te* + *Pl*); T8, fungus-treated foam with *T. evansi* but without *P. longipes* (*Te* − *Pl*). On the first day (T0), 100 females of *T. evansi* were deposited on leaf L2 just below the foam or on leaf L3 just above the foam. On day T0 + 3, 20 females of *P. longipes* were released on leaf L1. The treatments were replicated four times and bars indicate the standard errors. The solid arrow indicates the date of mite infestation. The dashed arrow indicates the date of predatory mite release.

(Fig. 5). In the fungus treatments, the percentage of mite-infested leaflets was significantly lower ($\chi^2 = 5.33$; df = 1; $P = 0.02$). For example, when the foam was used for the first time (first 15 days post-treatment), 19 ± 1.44% leaflet infestation was recorded. When the same fungus-treated foam was used for the second time (i.e. 30 days post-treatment), leaflet infestation was 25 ± 1.24% while it increased to 54 ± 2.12% following the third use (i.e. 45 days post-treatment) (Fig. 5). The leaflet under the fungus-treated foam was highly infested while there were no mites on the leaflet above the foam. Overall, fungus-treated foam could still protect tomato plants for at least 45 days post-treatment under our experimental conditions.

3.4 Interaction between fungus-treated foam and predatory mites on *T. evansi* infestation

The percentage infestation by mites on tomato leaflets increased from 14 ± 0.8% to 100 ± 0% at day 15 post-treatment in T6 (untreated foam; *Te* − *Pl*) while it increased from 13 ± 0.7% to 47 ± 2.7% after 15 days in treatment T5 where predatory mites were released (untreated foam; *Te* + *Pl*) (Fig. 6). In T8 (fungus-treated foam; *Te* − *Pl*), the percentage of tomato leaflet infestation by mites was 20 ± 3.5% after 15 days of observation while it was 18 ± 1.03% in the treatment T7 (fungus-treated foam; *Te* + *Pl*) (Fig. 6). The differences were significant ($\chi^2 = 13.5000$; df = 3; $P = 0.0037$) between T5 (untreated foam; *Te* + *Pl*), T6 (untreated foam; *Te* − *Pl*), T7 (fungus-treated foam; *Te* + *Pl*) and T8 (fungus-treated foam; *Te* − *Pl*). However, the non-parametric comparisons for each pair using the Wilcoxon method did not show any statistically significant difference between the two treatments T7 and T8 ($P = 0.11$).

Regardless of the treatment, the LMDI was ≥ 1 below the foam (Fig. 7) while it was nil above the foam in treatment T5 (untreated foam; *Te* + *Pl*), T7 (fungus-treated foam; *Te* + *Pl*) and T8 (fungus-treated foam; *Te* − *Pl*), which is consistent with the results on mite infestation (Fig. 6). There was a significant difference between treatments T5, T7, T8 and T6 ($\chi^2 = 3.94$; df = 1; $P = 0.04$).

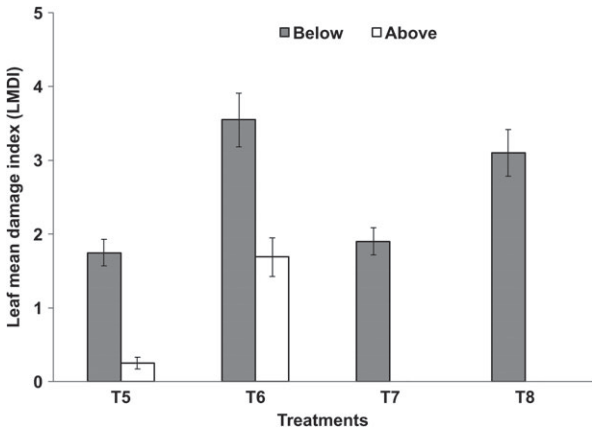

Figure 7. Leaf mean damage index (LMDI) for damage by *T. evansi* on tomato plants recorded for the leaves below and above the foam. The following treatments were applied: T5, untreated foam with *T. evansi* and *P. longipes* (*Te* + *Pl*); T6, untreated foam with *T. evansi* but without *P. longipes* (*Te* − *Pl*); T7, fungus-treated foam with *T. evansi* and *P. longipes* (*Te* + *Pl*), and T8, fungus-treated foam with *T. evansi* but without *P. longipes* (*Te* − *Pl*). The treatments were replicated four times and bars indicate the standard errors.

The predatory mite and the fungus-treated foam seemed to have the same effect on mite infestation.

The mean number of *T. evansi* recorded on leaf 3 was significantly higher in treatment T6 (untreated foam; *Te* − *Pl*) (465 *T. evansi*) than in treatment T5 (untreated foam; *Te* + *Pl*) (235 *T. evansi*), while it was almost the same in treatments T7 (fungus-treated foam; *Te* + *Pl*) and T8 (fungus-treated foam; *Te* − *Pl*) (0 and 1 *T. evansi*, respectively) (Table 1). These results confirmed the previous observations for LMDI (Fig. 7).

The mean number of *P. longipes* recorded on the foam was not significantly different in T5 and T7 (86 and 81 *P. longipes*, respectively). The fungus-treated foam seemed not to affect the predatory mites.

Table 1. Mean number of *T. evansi* and *P. longipes* on the foam and on leaf 3 (L3), 15 days post-treatment. The following treatments were applied: T5, untreated foam with *T. evansi* and *P. longipes* (*Te* + *Pl*); T6, untreated foam with *T. evansi* but without *P. longipes* (*Te* − *Pl*); T7, fungus-treated foam with *T. evansi* and *P. longipes* (*Te* + *Pl*), and T8, fungus-treated foam with *T. evansi* but without *P. longipes* (*Te* − *Pl*)

Treatment	*T. evansi* on leaf 3	*P. longipes* on foam
T5, untreated foam; *Te* + *Pl*	235 ± 1 b	86 ± 4
T6, untreated foam; *Te* − *Pl*	465 ± 50 a	-
T7, fungus-treated foam; *Te* + *Pl*	0 ± 0 c	81 ± 2
T8, fungus-treated foam; *Te* − *Pl*	1 ± 1 c	-
F	86.56	-
P-value	<0.0001	0.57

[a, b, c] Post hoc pairwise tests with the Wilcoxon method.

4 DISCUSSION

The present study showed that tomato plants could be protected against *T. evansi* when fungus-treated foam was placed above mite-infested leaves. Moreover, protection could last for 30–45 days post-treatment, resulting in a significant reduction in the infestation rate. These results can be explained by the mite behaviour of moving along the plant stem in a circadian fashion.[20,25,26] Subsequently, mites would be contaminated by the fungus and unable to colonise new leaves, except those under the fungus-treated foam.

One of the main challenges of using entomopathogenic fungi in the field is their short persistence in the environment as a result of their vulnerability to UV radiation. In the present study, the fungus-treated foam could protect the tomato plant for up to 30 days post-treatment in laboratory conditions with only 25 ± 1.2% leaflet infestation (54 ± 2.1% leaflet infestation 45 days post-treatment) (Fig. 5). These results suggest that the fungus-treated foam approach could be a sustainable strategy against this pest and an alternative to expensive inundative applications, as suggested by Mfuti *et al.*[27]

The fungus-treated foam and the release of *P. longipes* did not provide additional benefits to the fungus alone in terms of mite infestation, the LMDI and the number of *T. evansi* recorded on leaf 3. The treated foam used alone or combined with the predatory mites had the same effect on mite infestation in treatments T5 and T7. The effectiveness of *P. longipes* in controlling *T. evansi* populations has been reported elsewhere under laboratory conditions.[17,19,21] Therefore, there might not be benefits in combining *M. anisopliae*-treated foam and *P. longipes* for *T. evansi* control on a tomato crop. Similar results were reported by Maniania *et al.*,[13] who did not find any additional effect of combining *M. anisopliae* and *P. longipes* in the control of *T. evansi* populations in the screenhouse and under field conditions. No dead predatory mites were observed on the foam and their numbers were similar in treatments T5 (untreated foam) (86 predatory mites) and T7 (fungus-treated foam) (81 predatory mites). These results confirm observations by Maniania, who reported compatibility between *P. longipes* and *M. anisopliae*, as exposure of *P. longipes* to a conidial suspension of *M. anisopliae* did not cause mortality of predatory mites in the laboratory (Maniania, unpubl. data).

The new approach proposed here could reduce the number of inocula applied and improve fungal persistence. As linalool or methyl salicylate are known to attract predatory mites in the field,[28] it might be possible to combine fungus-treated foam with these compounds. However, their compatibility must first be tested. Furthermore, open field experiments are needed to confirm the effectiveness of the fungus-treated foam applied alone or in combination with the release of *P. longipes* in controlling spider mites on roses and tomatoes in greenhouses. We also suggest carrying out the compatibility test of *M. anisopliae* with *P. longipes* in the laboratory at high humidity to simulate greenhouse conditions.

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AUTHOR CONTRIBUTIONS

Thibaud Martin and **Komi K. Fiaboe** made substantial contributions to the idea of using *Metarhyzium anisopliae* fixed on the foam.

Ginette Y. Azandémè Hounmalon carried out the experimentation, the acquisition of data, and analysis and interpretation of data, and wrote the manuscript.

Nguya K. Maniania assisted in the use of entomopathogenic fungi, and was involved in drafting the manuscript and reviewing it critically for important intellectual content.

Niassy Saliou and **Simon Fellous** carried out the statistical analysis and made comments on the manuscript.

Serge Kreiter and **Emilie Delétré** also made comments on the manuscript for its improvement.

All authors have agreed on the order in which their names appear in the manuscript.

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