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Combining foliar and soil-active predatory mites (*Amblyseius montdorensis* and *Hypoaspis sclerotarsa*) to improve thrips control

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ABSTRACT

In three separate greenhouse experiments we evaluated the effect of different densities of the mites Amblyseius montdorensis (foliar predator; AM at 0, 5, 10 or 15 per pot), different densities of Hypoaspis sclerotarsa (ground predator; HS at 0. 50, 100 or 150 per pot) or a combination of the two (0AM, 0HS; 15AM, 50HS; 15AM, 100HS; 15AM, 150HS) on emergence of western flower thrips (WFT), Frankliniella occidentalis Pergande (Thysanoptera: Thripidae) from soil; initial start populations of WFT were either small (10) or large (20). A completely randomized design was used and for each experiment there were three replicates per treatment and the experiment was repeated on two occasions. Single applications of A. montdorensis, H. sclerotarsa or a combination of both all had an impact on the number of WFT emerging compared with the control. There was a significant effect of A. montdorensis density on the number of WFT emerging from the soil (F=0.31, P= 0.420 df =1). There was no significant difference in the population densities of WFT emerging from soil in the control and following release of H. sclerotarsa when initial release densities of WFT at the two initial prey densities of 10 and 20. Combined use of A. montdorensis and H. sclerotarsa at a density of 150 with 15 A. montdorensis reduced adult WFT emergence at density of 20 WFT. These findings highlight the potential for a combined use of A. montdorensis with H. sclerotarsa for the control of soil-dwelling stages of thrips.

Key Words: *Hypoaspis sclerotarsa, Pupae, Biocontrol, Frankliniella occidentalis and Amblyseius montdorensis*

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I. Introduction

Western flower thrips (WFT), *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), are pests of economics importance on a wide range of crops throughout the world (Kirk & Terry, 2003). WFT are generally difficult to control because of their cryptic mode of life (Lewis, 1997; Michelakis and Amri,

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1997), the development of insecticide resistance (van Lenteren and Loomans, 1998) and because pupation occurs in the soil and not on the crop (Lewis, 1997; Berndt et al. 2004). A wide range of soil-dwelling predatory mites have potential to prey on the pupae of WFT in the soil (Karg 1993). However, *Amblyseius* species (Acarina: Phytoseiidae) and *Orius* species (Heteroptera: Anthocoridae) of predatory mites and bugs are most commonly used for biological control of WFT; both these groups of predators prey on the foliar-feeding life stages of WFT, i.e., the 1st (L1) and early 2nd (L2) larval instars and the adults, but not late L2 larvae that leave the canopy to pupate in the soil, or the prepupae and pupae which form in the soil (Ramakers 1995; Riudavets 1995; Sabelis and van Rijn, 1997). To date, augmentative releases of foliar-active predatory mites and bugs have had variable success and not always provided sufficient control of WFT, particularly on crops with low economic damage thresholds, such as ornamentals (Gillespie and Ramey, 1988; Frescata and Mexia, 1996). Thus, additional biological control agents are urgently needed for reliable management of WFT, particularly agents that target the predominantly soil-dwelling life stages.

While the majority of late L2 WFT leave the canopy to pupate in the soil (Varatharajan and Daniel, 1984; Tommasini and Maini, 1995), the actual proportion of thrips successfully pupating in the soil is influenced by host plant species. In general, thrips spend about one-third of their life cycle (mainly as prepupae and pupae) in the soil (Loomans and van Lenteren, 1995). One option for WFT control is the use of soil-inhabiting oligophagous predatory mites of the genus *Hypoaspis* (Acarina: Laelapidae). Recent studies have shown that *Hypoaspis aculeifer* Canestrini and *Hypoaspis miles* (Berlese) are promising predators against soil-dwelling stages of WFT (Gillespie and Quiring, 1990). At present, these two *Hypoaspis* species are commercially available for control of mushroom flies (Diptera: Sciaridae) (Wright and Chambers, 1994). The objective of the current study was to assess the effect of single applications of either the soil-active predatory mite, *Hypoaspis sclerotarsa* (Costa), the plant-active predatory mite, *Amblyseius montdorensis (*Schicha) or a combination of the two species together on WFT population development in the soil.

II. Materials and Methods

Rearing of WFT, *Hypoaspis sclerotarsa* and *Amblyseius montdorensis*

WFT colonies were originally sourced from the International Centre of Insect Physiology and Ecology (*icipe*) and reared in ventilated plastic containers (20cm high and with a diameter of 5 cm) at 24±3°C, 50-60% relative humidity and a L16:D8 photoperiod. For ventilation, a small hole (3cm in diameter) was cut in the lid of each container and covered with thrips-proof gauze with a mesh size of 215 µm. One hundred adult female and male WFT were introduced to each container and allowed to oviposit for 48 hours on ten fresh French bean pods (7-10cm long). To encourage oviposition the French beans pods were smeared with honey which provided a supplementary energy source. WFT development on the beans was monitored and, once the 2nd instar larvae had developed, they were carefully brushed off the beans, using a camel hair brush, into a new container with fresh beans and tissue paper at the base for pupation. Pupae obtained were collected and used in the experiment.

Hypoaspis sclerotarsa were collected from a rice-processing factory in the Thika region of Central Kenya (altitude 1,500m) in April and May 2014. The species was identified morphologically by Farid Faraji, Mitox Consultants/Eurofins, Amsterdam, based on mounted specimens (eight females and five males). Colonies of *H. sclerotarsa* were reared on the prey mite *Thyreophagus entomophagus*, at 20±1°C and >70% RH in small vials (7cm diameter, 7cm high). To achieve a sufficiently high humidity but avoid condensation, a layer of moistened plaster was placed in the base of the vial, and the lid was pierced with pinholes. A cover of mite-proof gauze beneath the lid prevented escape. Adults of *H. sclerotarsa* were collected and counted under a binocular microscope to ensure they were at the correct stage of development prior to use in experiments.

Amblyseius montdorensis were obtained from Real IPM, a commercial supplier of natural enemies. Adults of the same age were separated from substrate by sucking them into Pasteur pipettes where they were held briefly prior to release in defined numbers to meet the different treatment requirements. To prevent individuals from escaping one end of the pipette was covered with mite-proof gauze and the other sealed with plasticine.

Experimental set-up: In these separate experiments we investigated the effect on caged WFT populations of Exp. 1 release of A. montdorensis (foliar-active) alone at four densities: 0 (control), 5, 10, 15; Expt. 2) release of *H. sclerotarsa* (soil-active) alone at four densities: 0 (control), 50, 100, 150; or Expt. 3) release of a combination of both *A. montdorensis* (AM) and *H. sclerotarsa* (HS) at four densities: 0 AM and 0 HS (control); 15 AM and 50 HS; 15 AM and 100 HS; 15 AM and 150 HS). In each experiment, there were two initial start densities of WFT (10 or 20) and three replicate cages for each treatment combination and control in each experiment and each experiment was repeated on two occasions.

The cages had a transparent frame with fine insect gauze (diameter 30 cm, height 40 cm). Cages were placed in a greenhouse in a 12L: 12D light regime at a mean temperature of 21°C. Plastic planting pots (measuring 23cm in diameter, height 20cm) were each filled with sterilized soil into which French beans seeds var. Samantha (obtained from Amiran Kenya) were planted. Two seeds were sown in each replicate pot at a depth of 5cm and each pot was then placed into a cage and left to grow in the greenhouse. Thinning was done 1week after germination was apparent, leaving one plant per pot. The plants were left to grow to the 2-3 leaf stage.

In each experiment, *F. occidentalis* adults were collected from the laboratory culture, introduced individually onto the bean plant in each cage at start densities of either 10 or 20, and maintained in the greenhouse in a 12L: 12D light regime at a mean temperature of 21°C. A completely random design was used to position cages for each experiment. In Expt. 1, each density of *A. montdorensis* adults were introduced to replicate cages on day 6 after the WFT had been introduced. In Expt. 2 each density of *H. sclerotarsa* adults were introduced to replicate cages on day 9 after the WFT had been introduced. In Expt. 3, the *A. montdorensis* (at a constant density of 15) were introduced on day 6 and the different densities of *H. sclerotarsa* were introduced on day 9. At day 15, in all three experiments, all the bean foliage was removed and blue sticky traps placed in each cage for 7 days to capture and enumerate the WFT adults emerging from the soil.

Statistical Analysis: Data from each experiment were analyzed separately. Raw data on the number of WFT adults emerging from the soil in each cage were square root transformed to meet the assumption of normality and homogeneity of variance. In all experiments a repeated measures analysis of variance (PROC MIXED SAS institute 1999) using maximum likelihood estimation was done to test for differences amongst treatments on each sampling day. For pair-wise comparisons between treatments and to test the effects caused by the combined use of *A. montdorensis* and *H. sclerotarsa* a Tukey T-test was used. Significant differences between thrips population densities in the combined *A. montdorensis* and *H. sclerotarsa* treatment (Expt 3) compared with the sum of the mean thrips population densities when *H. sclerotarsa* and *A. montdorensis* were introduced separately (Expt 1 and 2) indicated whether there was an effect. Differences amongst treatment means were compared using Tukey's mean separation test, using $p<0.05$.

III. Results

Experiment 1. Effect of release of different densities of *A. montdorensis* on large and small populations of WFT.

There was a significant effect of *A. montdorensis* density on the number of WFT emerging from the soil ($F=0.31$, $P= 0.420$ df =1). In small WFT populations, significantly fewer WFT adults emerged following release of 15 *A. montdorensis* than in the control ($F = 0.42$, $P= 0.52$ df =1). There was no significant difference in the number of WFT emerging following release of either ten or 15 *A. montdorensis* ($P<0.05$) with mean values of 3.6 and 1.1 respectively (Figure 01A). In large WFT populations, significantly fewer WFT emerged following release of 15 *A. montdorensis* (3.5) compared with the control (13.1) ($P<0.05$). Also, significantly more WFT emerged following release of five or ten *A. montdorensis* than following release of 15 *A. montdorensis* ($P<0.05$) (Figure 01B).

Experiment 2. Effect of release of different densities of *H. sclerotarsa* on large and small populations of WFT

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In small WFT populations, there was no significant difference in the number of thrips emerging from soil in the control and following release of any density of the *H. sclerotarsa* (Figure 02A, 02B). In contrast, in large WFT populations the numbers emerging were significantly reduced in the treatment with 150 *H. sclerotarsa* (1.37 emerging).

Experiment 3. Effect of release of *A. montdorensis* (one density: 15) in combination with different densities of *H. sclerotarsa* on small and large populations of WFT

In small WFT populations there was no significant difference in the number of WFT emerging from the soil following release of a combination of *A. montdorensis* and either 50 or 100 *H. sclerotarsa* at P<0.001 (Figure 03A). However, when the release density of *H. sclerotarsa* was 150 the fewest WFT emerged (F= 123, P= 0.01, df= 2); significantly more WFT emerged from the control than either 50 or 150 *H. Sclerotarsa* P<0.01) (Figure 03A). In the large WFT populations, there was a significant difference amongst the treatments at P<0.05. When the treatments were compared to the control, release of *A. montdorensis* and *H. sclerotarsa* at a density of 150 achieved the greatest suppression of WFT adult emergence (P<0.01) (Figure 03B).

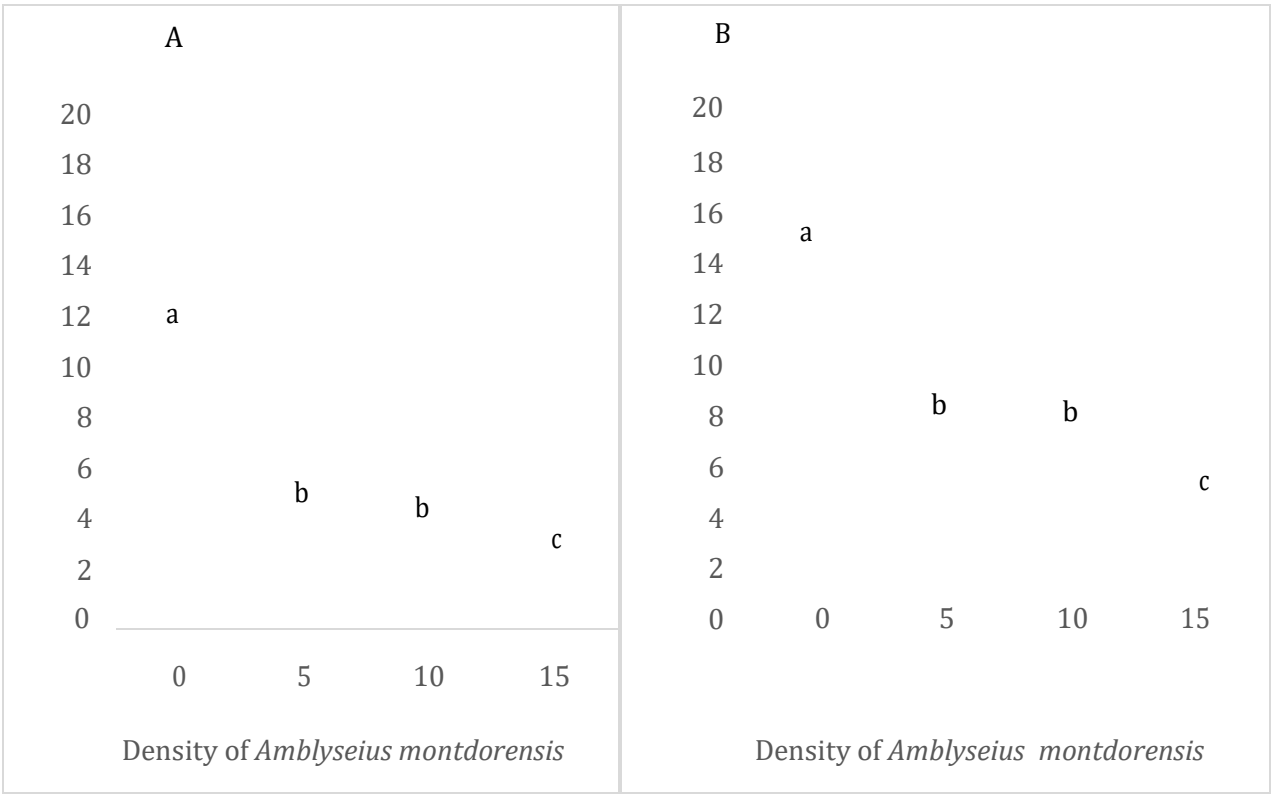

Figure 01. Mean (±SE) number of emerging, adult *F. occidentalis* (WFT) captured in blue sticky traps placed above bean plants between the 15th and 22nd sampling day after release of the foliar-active predator, *Amblyseius montdorensis*. Release densities of *A. montdorensis* were 0 (control), 5, 10 or 15. A: Initial starting density of WFT before release of predators = 10; B: Initial starting density of WFT before release of predators = 20. Vertical bars followed by the same letter are not significantly different from each other (P <0.05).

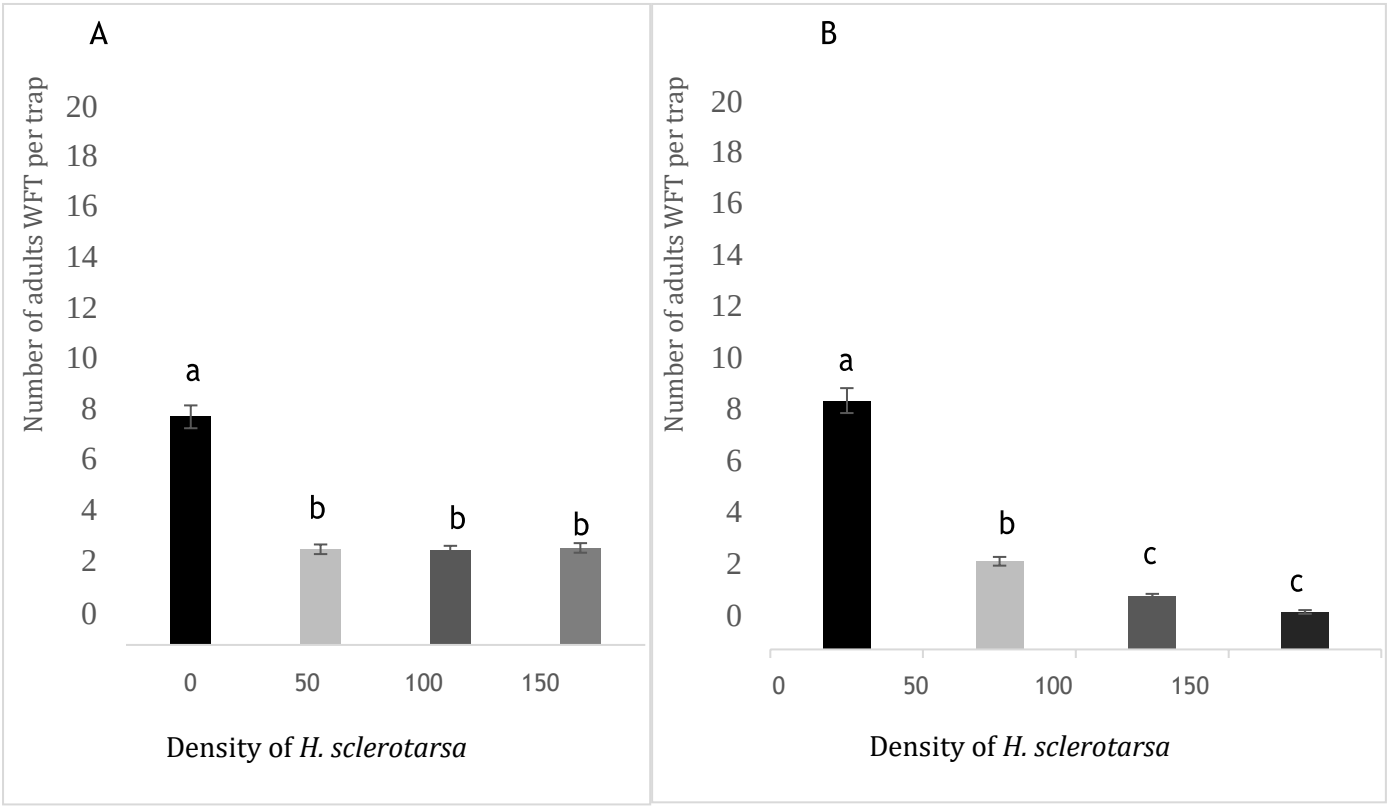

Figure 02. Mean (±SE) number of emerging, adult *F. occidentalis* (WFT) captured in blue sticky traps placed above bean plants between the 15th and 22nd sampling day after release of the soil-active predator, *Hypoaspis sclerotarsa*. Release densities of *H. sclerotarsa* were 0 (control), 50, 100 or 150. A: Initial starting density of WFT before release of predators = 10; B: Initial starting density of WFT before release of predators = 20. Vertical bars followed by the same letter are not significantly different (P <0.05).

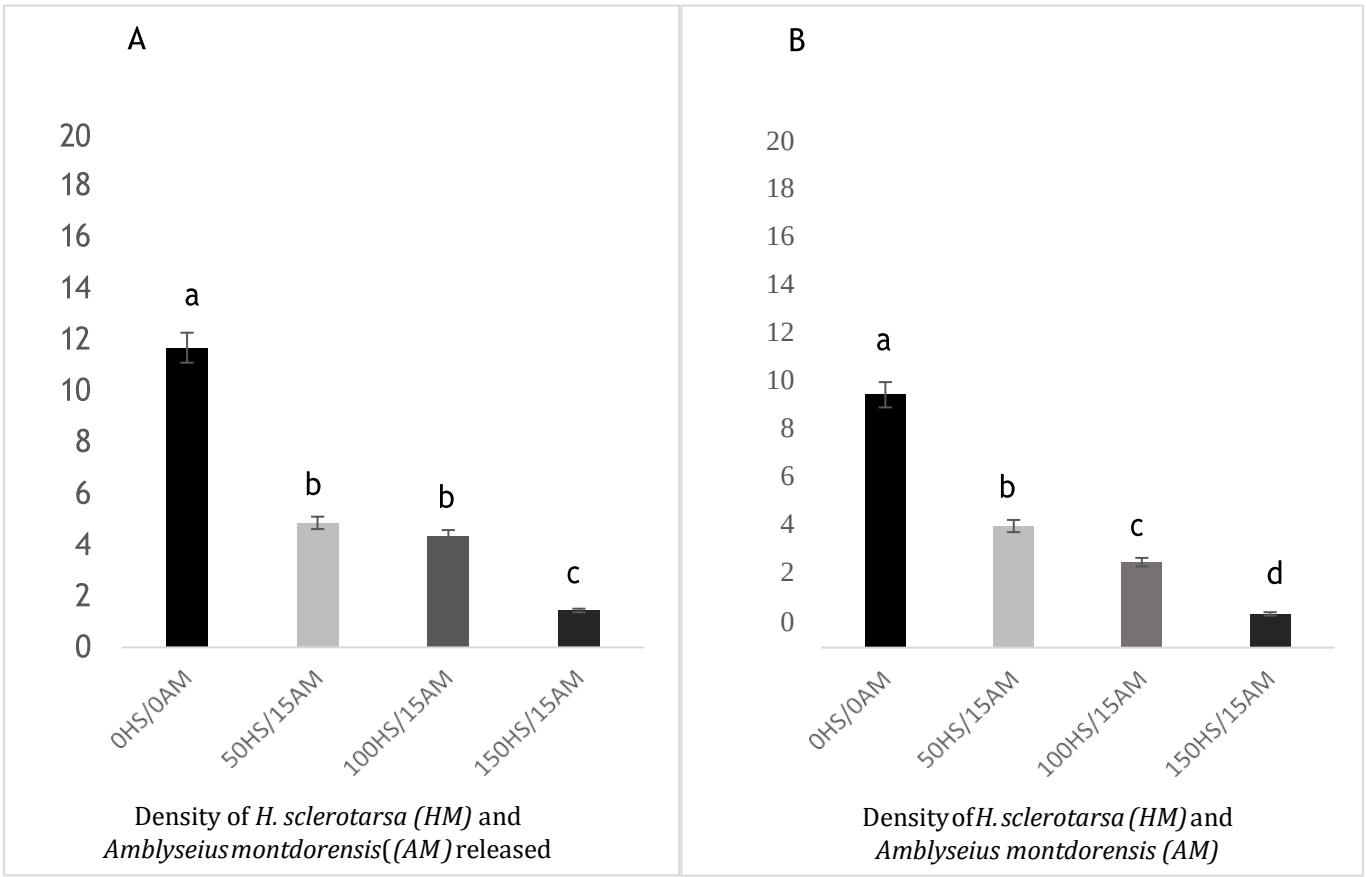

Figure 03. Mean (±SE) number of emerging, adult *F. occidentalis* (WFT) captured in blue sticky traps placed above bean plants between the 15th and 22nd sampling day after release of combinations of the foliar-active predator, *Amblyseius montdorensis (AM)* and the soil-active predator, *Hypoaspis sclerotarsa (HS)*. Release densities of *A. montdorensis* were 0 (control),15 while release densities of *H. sclerotarsa* were 0 (control), 50, 100 or 150. A: Initial starting density of WFT before release of predators = 10; B:

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Initial starting density of WFT before release of predators = 20. Vertical bars followed by the same letter are not significantly different (P <0.05).

IV. Discussion

Our results show that, in general, release of predatory mites (either *A. montdorensis* or *H. sclerotarsa*) has potential as a biological control strategy for WFT on French beans. From the first experiment a single application of *A. montdorensis* (at different densities) resulted in a reliable reduction in both small (initial release rate of 10) and large (initial release rate of 20) WFT populations (Figure 01A, 01B). This is likely to be because *A. montdorensis* reduced the number of WFT larvae on foliage and thus the number entering the soil to pupate and ultimately the number emerging as adults. *A. montdorensis* (and *A. limonicus*) had potential for biological control of thrips in some vegetable and ornamental crops. From the second experiment a single release of *H. sclerotarsa* (at different densities) also reduced both small and large WFT populations (Figure 02A, 02B). This is likely to be because *H. sclerotarsa* consumed WFT pupae in the soil, thereby reducing the number emerging as adults. In the third experiment, using a combination of *H. sclerotarsa* and *A. montdorensis* resulted in even more effective control of WFT. We hypothesize that this was because, when applied together, 1st instar WFT were consumed by *A. montdorensis* on the foliage stage and pupal stages of WFT were consumed by *H. sclerotarsa* in the soil; there was no competition between the two predators because they were spatially separated. Overall, our results indicate that while single applications of the foliar-active predatory mite *A. montdorensis* can deliver adequate control of WFT on bean, if it is combined with *H. sclerotarsa* it would have a greater impact on WFT populations. Other studies of the soil-dwelling predatory mite *H. aculeifer* showed that it was neither additive nor synergistic in suppressing soil-inhabiting thrips developmental stages when applied in combination with *Amblyseius cucumeris* (Berndt et al. 2004).

Additive/synergistic effects have been seen in other systems when foliar-active and ground-active predators are used together. For example, pea aphids (*Acyrthosiphon pisum* Harris) preyed on by foliar-active predators release alarm pheromones that make surrounding aphids attempt to escape by dropping off the plants and on to the ground where they become susceptible to ground-active carabid beetles (Losey and Denno 1998a; Losey and Denno 1998b). Although alarm pheromones have been identified for WFT, compared with aphid alarm pheromones, they only illicit weak behavioural responses in other WFT (Teerling et al. 1993; Teerling, 1995). Only a small percentage of L2 WFT drop off the plants in response to alarm pheromones. Nevertheless, a large proportion of L2 WFT naturally move to the soil for pupation (Bennison et al. 2002; Berndt et al. 2004).

Combination of predators that, together, are active in all the habitats that different life stages of the target prey occupy could be an ideal biological control strategy because there is potential for synergy to be achieved. However, host plant canopy density can influence the dropping rate of mites. Also, in some cases additive or synergistic effects have not been achieved. For example, when the two predators *Orius insidiosus* Say and *Amblyseius degenerans* (Berlese) were released together against WFT on cut roses, control levels were similar to those achieved using *O. insidiosus* alone.

IV. Conclusion

We found that the soil-active predatory mite *H. sclerotarsa* had a significant impact on WFT populations when released at a high density (150 *H. sclerotarsa*). At a lower density (50 *H. sclerotarsa*) it did still reduce the number of WFT emerging from the soil. According to our study, control of WFT may be enhanced by using a combination of *H. sclerotarsa* and *A. montdorensis*, although the outcomes in our study showed similar levels of control as that achieved when *A. montdorensis* was released alone. i.e there may have been a slightly greater reduction in WFT emerging but not a large difference hence the effect was more substantial hence more potential synergy due to spatial separation of the predators and them targeting a different life stage leading to avoidance of competition.

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APA

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