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Improving the Control of Tetranychus urticae on Edible Glasshouse Crops Using a Specialist Coccinellid (Stethorus punctillum Weise) and a Generalist Mite (Amblyseius californicus McGregor) as Biocontrol Agents

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Improving the Control of *Tetranychus urticae* on Edible Glasshouse Crops Using a Specialist Coccinellid (*Stethorus punctillum* Weise) and a Generalist Mite (*Amblyseius californicus* McGregor) as Biocontrol Agents

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Current glasshouse biological control practice relies on regular prophylactic introductions of one or two 'best' species of natural enemy. Whilst this is effective for much of the time, occasional failures occur due to factors such as differences in response to seasonal changes in environmental conditions and/or host plant effects. This study looks at the predatory behaviour of a specialist coccinellid, Stethorus punctillum *Weise, and a generalist mite,* Amblyseius californicus *McGregor (which predate on the two-spotted spider mite,* Tetranychus urticae*) in order to assess how they responded to temperatures and relative humidities typical of glasshouse conditions on four edible crop plant species. Activity (distance covered, time spent walking, walking speed, angular velocity, and turning rate) was recorded at 20, 25 and 30°C and at relative humidity (RH) levels of 33, 65 and 90%, on tomato, pepper, aubergine and cucumber leaves, and analysed using video-computer techniques. The results show that the activity of* S. punctillum *significantly increased at higher temperature levels. Host plant species also strongly influenced the performance of the predator, with it being most active on pepper and tomato and least active on aubergines. RH had no significant influence. The activity and predation by* A. californicus *increased at low humidity levels, especially in terms of time spent moving and number of prey killed. Temperature levels had no significant influence, but host plant species strongly influenced the performance of the predator, which was most active on pepper, and least active on aubergines. Further research was conducted with semi-field trials to investigate the efficacy in controlling TSSM with different combination of predators. When contrasting the commercially available predatory mite* Phytoseiulus persimilis, *used alone, compared with its use in a treatment with a combination of predator species, there was a stronger decrease in TSSM numbers on the crop plants in the latter treatment.*

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INTRODUCTION

To date, advances in the biological control of pests in glasshouses have relied on the so-called magic bullet approach, with the use of just one or two 'best' species of natural enemy as biocontrol agents. For example, current glasshouse biological control of the two-spotted spider mite (TSSM), *Tetranychus urticae* Koch, relies on regular prophylactic introductions of *Phytoseiulus persimilis* Athias-Henriot. Whilst this is effective most of the time, occasional failures occur due to factors which are often poorly understood. These may include differences in the response to seasonal changes in temperature, humidity and light levels between the natural enemy and pest species and/or host plant effects (Nihoul, 1992, 1993a b). The indications are therefore, that biocontrol agents in addition to *P. persimilis* may be required to successfully manage TSSM in glasshouses.

Population studies of pest species in their native environment have shown that natural control is often achieved, not by one major species alone, but by a guild of several species of parasitoid and/or predator each occupying a particular niche in the reproductive cycle of the prey, and not necessarily competing with each other (Hussey & Huffaker, 1976; Debach & Rosen 1991; Ehler, 1992). Indeed, even where close competition is apparently occurring, host/prey population regulation may be enhanced due to differences in environmental preferences amongst natural enemies. For example, TSSM was found to be rare on orchards in Australia (except where disturbances caused by pesticide use had occurred) due to predation by a guild of mites, thrips and coccinellids (Bailey & Caon, 1986). Studies of a number of target pest species indicate that there are interactions among predator species (Ehler, 1992; MacRae & Croft, 1993, 1997; Lucas *et al*., 1998; Roy *et al*., 1999), and there is growing evidence that the use of a single 'best' species as a biocontrol agent is no longer the most efficient means of pest control.

All known species of the genus *Stethorus* Weise (Coleoptera: Coccinellidae) are predators of spider mites (McMurtry *et al*., 1970; Hoy & Smith, 1982; Chazeau, 1983; Charles *et al*., 1985; Bailey & Caon, 1986), and several species have been suggested to have potential as biological control agents of spider mites in agricultural crops (Readshaw, 1975; Hull *et al*., 1976, 1977). *Stethorus* species are relatively small and remarkably well-adapted to live and search for prey in the habitats of plant-feeding mites. Therefore, the addition of a winged predator in biocontrol practices, that could move rapidly to spider mite 'hot spots' would be useful. In comparison, *Amblyseius californicus* is a more generalist predator, feeding readily on pollen and other mites as alternative food sources (McMurtry & Rodriquez, 1987; McMurtry & Croft, 1997; Monetti & Croft, 1997; Croft *et al*., 1998). Reports in the literature suggest that the survival rate of *A. californicus* species under low TSSM densities is higher than that of similarly-stressed *P. persimilis* (Friese & Gilstrap, 1982; Gilstrap & Friese, 1985).

This study is part of a MAFF project aiming to improve the control of *T. urticae* by using a complex of predatory species, each optimally adapted to a particular niche in the life cycle of the pest and to differing climatic conditions. This paper examines the predatory behaviour of a specialist: *Stethorus punctillum* Weise, and a generalist: *Amblyseius californicus* McGregor on TSSM, to assess how each species responded to environmental factors and host plant species typical of glasshouse conditions. While there have been numerous studies on various aspects of TSSM predators under different temperature, relative humidity (RH) and prey conditions (MacRae & Croft 1993; Croft *et al*., 1993; Monetti & Croft, 1997), most of these studies concentrate on one predator species, and one aspect of humidity (Castagnoli & Simoni, 1994; Monetti & Croft, 1997) or temperature (Castagnoli & Simoni, 1992). Furthermore, little work has been done on *S. punctillum* and its response to varying environmental factors (Putman, 1955). In this study, the activity and level of predation for both predator species was first tested individually in the laboratory. Then their efficacy was observed under semi-field trials in multi-species predator releases. For this, two commercially available predators *P. persimilis* (Acari: Phytoseiidae) and the predatory gall midge *Feltiella acarisuga* Vallot (Diptera: Cecidomyiidae) were tested along with *S. punctillum* and *A. californicus*.

MATERIALS AND METHODS

The behavioural studies were conducted from March 1998 to March 1999. TSSM colonies were reared on broad beans (*Vicia faba* L.). The TSSM were reared in a glasshouse between 25 and 30°C with a 16:8 L:D photoperiod. The RH fluctuated between 40 and 70%. Additional TSSM colonies were also reared on tomato (*Lycopersicon esculentum* Miller: cvs. SOLAIRO and AILSA CRAIG), pepper (*Capsicum annuum* L.: cv. BELL BOY), aubergine (*Solanun melongena* L.: cv. MONEYMAKER) and cucumber (*Cucumis sativus* L.: cv. TYRIA) to ensure that the TSSM used for the experiments were acclimated to the host plants. The *S. punctillum* and *A. californicus* cultures were reared on TSSM-infested broad beans in a controlled temperature room at 25°C (± 1°C) and an RH of 50–60%.

The behaviour of individual predators was observed on 1 cm^2 discs cut from non-infested leaves of the host plant species being tested. For each predator observed, a freshly cut leaf disc was laid, bottom-side up, in a Petri dish covered with a wet filter paper. This kept the leaf disc from drying out too quickly and inhibited mite and predator emigration from the leaf disc (leaf-island method; Sengonca & Gerlach, 1983). Experiments were conducted in incubators kept at three different temperature levels (20, 25 and 30°C). Humidity chambers were constructed from transparent plastic boxes measuring 270 × 160 × 100 mm. Saturated salt solutions of $MgCl_2 6H_2O$, $NaNO_2$ and KNO_3 were used within individual boxes to maintain a constant RH of 33, 65 and 90%, respectively (Winston & Bates, 1960). Within each box, four small glass-tubes held a 15 × 20 cm piece of vinyl tile above the salt solutions, and the Petri dishes containing the leaf discs were placed on these tiles. Predator behaviour was tested on TSSM infested leaf discs. Ten immature TSSM deutonymphs were taken from the leaves of the colony and placed on the leaf disc 24 h prior to the trial observations. This ensured that all leaf discs had approximately the same conditions at the beginning of the observations. Freshly emerged third instar *S. punctillum* larvae and adult female *A. californicus*, which had just started ovipositing, were used for the trials. Each larva and adult female tested, was starved for 24 h prior to the experiments to standardize the condition of the predator. Each larva/female was placed on the leaf disc and allowed to acclimatize for 30 min to the arena and the temperature/humidity conditions before recording data. Each predator was observed individually for a period of 10 min using a high resolution video camera. The following activity parameters were measured using Micromeasure V3 (a computer/video measurement package; Varley *et al.*, 1994); distance covered, time spent walking, walking speed (distance walked/time spent walking), angular velocity (degrees s^{-1}), and turning rate (degrees turned mm^{-1} calculated as an average over a 5 mm sampling distance). Observations were also made on general behaviour, and attack behaviour on encountering prey, attempts to disperse). For each plant species, 15 replicates per predator species were carried out at each temperature and RH using a factorial design, with no individual being tested more than once. Unless otherwise stated, all results were tested using a factorial analysis of variance. Differences between means of performances were calculated using Tukey's honest significance test at the 5% level.

The semi-field trials were conducted from May 1999 to September 1999 in large cylindrical insect cages (diameter 30 cm; height 42 cm), with netting in the top of the cage for ventilation, under greenhouse conditions using three combinations of predators. To ensure sufficient air circulation, air was also pumped into each cage via an air-pump. One potted plant was placed per cage. Two crop plant species were tested: tomato and pepper, each infested with two levels of TSSM density (high and low). The tomato plants were roughly the same age as equivalent tomato plants of commercial growers (i.e. they had been planted in December 1998 and were cut to fit the cage), while the pepper plants were one month old at the beginning of each trial. Temperature and humidity were monitored inside a cage with a potted plant on a continuous basis throughout the summer. Initial TSSM densities were measured at the beginning of each trial using the damage index (Hussey & Scopes, 1985), and thereafter monitored weekly, in order to assess the efficacy of the various predator combinations. Trials were replicated three times at each level in a randomized complete block design and repeated on a monthly basis.

The following three predator combinations were used:

Treatment 1: TSSM + *P. persimilis* (control)
Treatment 2: TSSM + *P. persimilis* + *S. punctillum* + *F. acarisuga*
Treatment 3: TSSM + *P. persimilis* + *S. punctillum* + *A. californicus*

Depending on the treatment, the number of each predator species introduced per cage, was as follows: three *F. acarisuga* pupae, six adult female *P. persimilis*, six adult female *A. californicus* (adult female phytoseiids were introduced, which had just started ovipositing) and three *S. punctillum* first instar larvae. At the end of each monthly trial, the number of remaining TSSM and predators (all developmental stages) were scored from the whole plant. Data were analysed using analysis of variance with plant type nested in date of trial, and density nested in date of trial and plant type. All other factors were analysed in a crossed (factorial) design.

RESULTS

In the behavioural trials, analysis of the main effects showed that, in *S. punctillum*, activity (i.e. distance moved, turning rate etc.) increased at temperatures above 25°C ($P < 0.001$), but this did not result in significant increase in the number of prey encountered or prey killed (see Table 1). Humidity (Table 2) caused a significant influence only on the walking speed, with it being lowest at 65% RH ($P < 0.01$), while crop plant species (i.e. leaf surface) strongly influenced activity ($P < 0.05$) (see Table 3 and below). The interaction between plant species and temperature showed significant increase in activity such as turning rate, walking speed, and number of prey encountered ($P < 0.01$), whilst only distance moved was significant in the three-way interaction ($P < 0.05$). All other interactions were not significant.

In *A. californicus*, activity was strongly influenced by RH (see Table 2). It influence the likelihood of *A. californicus* finding prey, with the number of TSSM encountered ($P < 0.01$) and time spent moving ($P < 0.05$) being highest at 33% RH. The crop plant species also significantly affected activity ($P < 0.001$) (see Table 3). Temperature did not seem to affect the behaviour. The only significant interaction was between crop plant and relative humidity, which strongly influenced the walking speed ($P < 0.005$).

A comparison of the predatory behaviour of *S. punctillum* and *A. californicus* shows that *S. punctillum* and *A. californicus* had slightly different environmental preferences, with *S. punctillum* moving significantly faster ($P < 0.01$) and showing higher angular velocity ($P < 0.001$) at nearly all measured temperature and RH conditions. The only difference that was not significant could be seen for the walking speed at 20°C (Table 1). However, *A. californicus* encountered significantly more TSSM ($P < 0.001$) compared to *S. punctillum*. Most of the other activities did not differ significantly from each other.

The activity of both predators depended strongly on the crop plants they were tested on (see Table 3). *S. punctillum* showed significantly higher activity (such as distance moved, time spent moving and number of TSSM encountered; $P < 0.001$) on pepper and tomato plants when compared to aubergine and cucumber. However, there were no significant differences in distance covered, and turning rate between tomato and pepper and between aubergine and cucumber, but it did result in significant differences for no. of prey killed ($P < 0.001$).

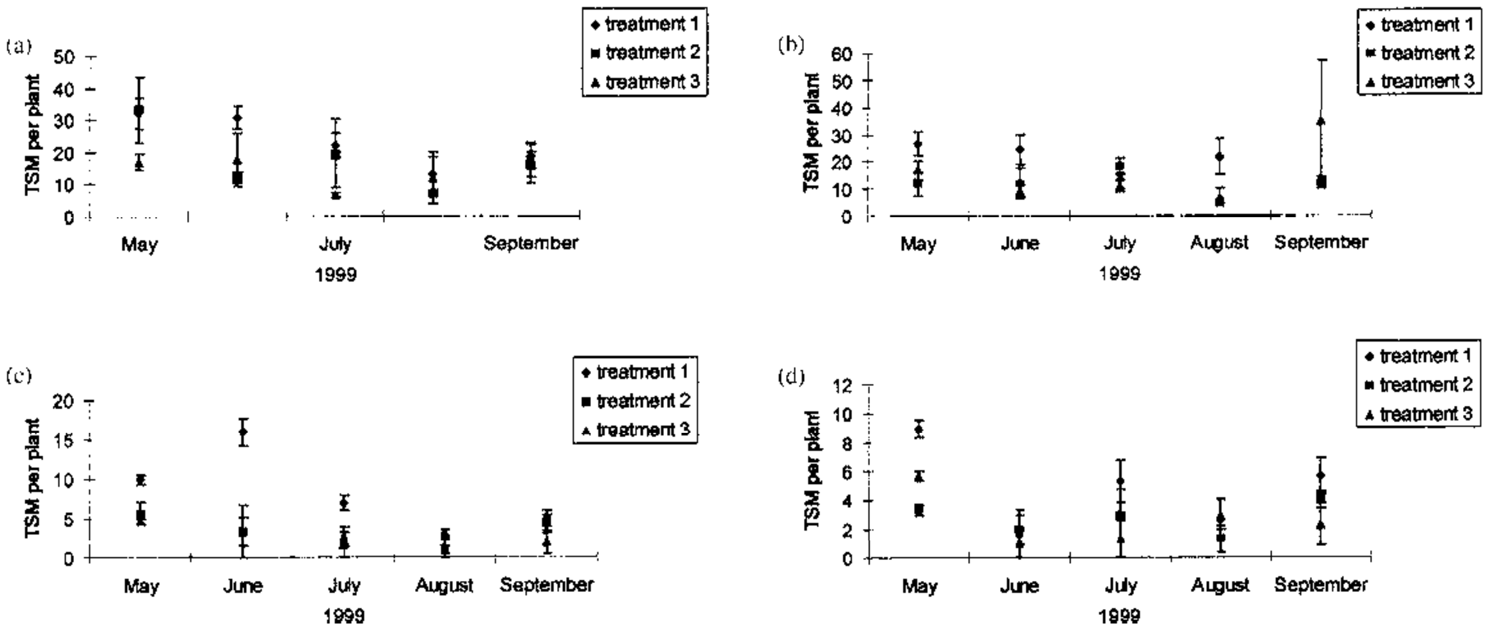

FIGURE 1. Semi-field trials showing final TSSM density (±SE) per plant with the three different predator releases (treatment 1 = TSSM + *P. persimilis* (control); treatment 2 = TSSM + *P. persimilis* + *S. punctillum* + *F. acarisuga*; treatment 3 = TSSM + *P. persimilis* + *S. punctillum* + *A. californicus*). (a) Tomato with high TSSM infestation; (b) pepper with high TSSM infestation; (c) tomato with low TSSM infestation; (d) pepper with low TSSM infestation.

TABLE 1. Effect of temperature on *S. punctillum* and *A. californicus* (± SE) on leaf discs with TSSM as prey (pooled data for four plant species and three RHs)

	Distance (mm) moved in 10 min		Angular velocity (°s^{-1})		Turning rate (°mm^{-1})		Walking speed (mm s^{-1})		Time moving (%)		Prey encountered		Prey killed	
	S. punctillum	*A. californicus*	*S. punctillum*	*A. californicus*	*S. punctillum*	*A. californicus*	*S. punctillum*	*A. californicus*	*S. punctillum*	*A. californicus*	*S. punctillum*	*A. californicus*	*S. punctillum*	*A. californicus*
20°C	12.9 ± 1.1 a	16.1 ± 1.7 a	10.1 ± 0.7 a*	7.0 ± 0.5 a*	3.5 ± 0.4 a*	5.0 ± 0.6 a*	0.47 ± 0.01 a	0.42 ± 0.01 a	6.0 ± 0.5 a	6.7 ± 0.7 a	0.68 ± 0.06 a*	2.62 ± 0.27 a*	0.41 ± 0.04 a	0.33 ± 0.04 a
25°C	22.6 ± 2.1 b*	15.8 ± 1.5 a*	12.9 ± 0.9 ab*	7.10 ± 0.5 a*	5.5 ± 0.5 b	5.9 ± 0.6 a	0.52 ± 0.01 b*	0.43 ± 0.01 a*	8.0 ± 1.0 b	6.4 ± 0.6 a	0.73 ± 0.08 a*	2.62 ± 0.25 a*	0.39 ± 0.04 a	0.42 ± 0.04 a
30C	21.2 ± 1.9 b	19.0 ± 1.9 a	13.9 ± 0.9 b*	7.94 ± 0.5 a*	6.3 ± 0.5 b	6.1 ± 0.6 a	0.53 ± 0.01 b*	0.44 ± 0.01a*	7.0 ± 1.0 ab	7.2 ± 0.7 a	0.72 ± 0.06 a*	2.97 ± 0.28 a*	0.46 ± 0.04 a	0.41 ± 0.04 a

Means within any column followed by the same letter are *not* significantly different at $P = 0.05$ (Tukey's Test; range = 3.34; 540 d.f.). Means followed by * within a row at any temperature level and within each variable indicate significant differences between the two predators ($P = 0.05$, Tukey's Test; range = 3.34; 540 d.f.).

TABLE 2. Effect of relative humidity on *S. punctillum* and *A. californicus* (± SE) on leaf discs with TSSM as prey (pooled data for four plant species and three temperatures)

	Distance (mm) moved in 10 min		Angular velocity (°s^{-1})		Turning rate (°mm^{-1})		Walking speed (mm s^{-1})		Time moving (%)		Prey encountered		Prey killed	
	S. punctillum	*A. californicus*	*S. punctillum*	*A. californicus*	*S. punctillum*	*A. californicus*	*S. punctillum*	*A. californicus*	*S. punctillum*	*A. californicus*	*S. punctillum*	*A. californicus*	*S. punctillum*	*A. californicus*
33% RH	21.4 ± 2.0 a	19.3 ± 2.1 a	13.3 ± 0.9 a*	8.2 ± 0.6 a*	5.5 ± 0.5 a	5.8 ± 0.6 a	0.52 ± 0.01 ab*	0.43 ± 0.01 a*	8.0 ± 0.8 a	7.6 ± 0.7 a	0.79 ± 0.08 a*	3.11 ± 0.31 a*	0.47 ± 0.04 a	0.47 ± 0.04 a
65% RH	18.7 ± 1.8 a	16.4 ± 1.5 a	12.6 ± 0.9 a*	7.3 ± 0.5 a*	5.6 ± 0.5 a	6.1 ± 0.6 a	0.48 ± 0.01 a	0.39 ± 0.01 b*	7.0 ± 0.6 a	7.4 ± 0.7 a	0.69 ± 0.07 a*	3.04 ± 0.26 a*	0.41 ± 0.04 a	0.33 ± 0.04 b
90% RH	16.5 ± 1.5 a	15.2 ± 1.5 a	10.9 ± 0.8 a*	6.5 ± 0.5 b*	4.3 ± 0.4 a	5.3 ± 0.6 a	0.53 ± 0.01 b*	0.47 ± 0.01 c*	6.0 ± 0.6 a	5.4 ± 0.5 b	0.64 ± 0.06 a*	2.05 ± 0.22 b*	0.38 ± 0.04 a	0.36 ± 0.04 b

Means within any column followed by the same letter are *not* significantly different at $P = 0.05$ (Tukey's Test; range = 3.34; 540 d.f.). Means followed by * within a row at any temperature level and within each variable indicate significant differences between the two predators ($P = 0.05$, Tukey's Test; range = 3.34; 540 d.f.).

TABLE 3. Effect of crop plant species on *S. punctillum* and *A. californicus* (± SE) on leaf discs with TSSM as prey (pooled data for three plant species and three RHs)

	Distance (mm) moved in 10 min		Angular velocity (°s^{-1})		Turning rate (°mm^{-1})		Walking speed (mm s^{-1})		Time moving (%)		Prey encountered		Prey killed	
	S. punctillum	*A. californicus*	*S. punctillum*	*A. californicus*	*S. punctillum*	*A. californicus*	*S. punctillum*	*A. californicus*	*S. punctillum*	*A. californicus*	*S. punctillum*	*A. californicus*	*S. punctillum*	*A. californicus*
Tomato	24.3 ± 2.7 a*	14.7 ± 1.6 b*	15.1 ± 1.2 a*	7.2 ± 0.7 b*	5.2 ± 0.5 a	5.0 ± 0.6 b	0.40 ± 0.01 a	0.38 ± 0.01 b	11.0 ± 1.0 a*	7.5 ± 0.9 b*	1.44 ± 0.11 a*	2.41 ± 0.2*	0.68 ± 0.05 a*	0.37 ± 0.04 a*
Pepper	24.7 ± 2.5 a*	33.4 ± 2.8 a*	12.8 ± 1.0 a*	10.7 ± 0.7 a*	5.6 ± 0.5 a*	10.3 ± 0.7 a*	0.54 ± 0.01 b	0.56 ± 0.02 a	8.0 ± 0.8 a	10.3 ± 0.8 a	0.85 ± 0.07b*	5.01 ± 0.41 a*	0.55 ± 0.04 a*	0.39 ± 0.05 a*
Aubergine	10.9 ± 0.8 b*	8.1 ± 0.9 c*	9.0 ± 0.8 b*	4.9 ± 0.4 c*	3.9 ± 0.5 a	3.0 ± 0.5 c	0.53 ± 0.01 b*	0.38 ± 0.01 b*	4.0 ± 0.4 b	3.9 ± 0.5 c	0.30 ± 0.04 c*	1.59 ± 0.21 c*	0.26 ± 0.04 b	0.33 ± 0.04 a
Cucumber	15.7 ± 1.2 b*	11.7 ± 1.1 bc*	12.3 ± 0.9 ab*	6.7 ± 0.5 b*	5.7 ± 0.6 a	4.5 ± 0.6 c	0.57 ± 0.01 ab*	0.40 ± 0.01 b*	5.0 ± 0.4 b	5.3 ± 0.6 c	0.24 ± 0.04 c*	1.92 ± 0.22 c*	0.19 ± 0.03 b*	0.46 ± 0.05 a*

Means within any column followed by the same letter are *not* significantly different at $P = 0.05$ (Tukey's Test; range = 3.65; 540 d.f.). Means followed by * within a row at any temperature level and within each variable indicate significant differences between the two predators ($P = 0.05$, Tukey's Test; range = 3.65; 540 d.f.).

TABLE 4. Mean number of TSSM remaining per plant at the end of each semi- field trial (± SE)

		Treatment		
Initial Mite Density	*n*	1. *P. persimilis* only	2. *P. persimilis* ± *S. punctillum* ± *F. acarisuga*	3. *P. persimilis* ± *A. californicus* + *S. punctillum*
Low	30	6.5 (1.73) a	3.0 (0.41) a	2.7 (0.47) a
High	30	21.5 (1.73) a	14.7 (2.04) b	14.9 (2.63) b
Pooled data	60	14.0 (1.36) a	8.8 (1.28) b	8.8 (1.54) b

Means within any row followed by the same letter are *not* significantly different at $P = 0.05$ (least significant difference test).

A. californicus on the other hand not only walked faster on pepper plants ($P < 0.001$), but also covered a larger distance ($P < 0.001$) and spent more time moving ($P < 0.001$) than on the other plant species. This resulted in more TSSM being encountered ($P < 0.001$) on pepper, although the number of prey killed during the trials did not differ significantly between plant species. In the semi-field trials clear treatment differences were detected (Table 4, 1) showing control to be improved on the plants that had high densities of TSSM and with more than one predator species predating on them ($P < 0.001$). Effects of time and density were highly significant ($P = 0.001$ and < 0.001, respectively). Treatment 3 (combination of *P. persimilis*, *A. californicus* and *S. punctillum*) improved TSSM control in all months except August (pepper: low infestation) and September (pepper: high infestation).

Predator survival was generally very low for all treatments. However, significantly more *S. punctillum* and *F. acarisuga* survived than either of the mite species ($P < 0.001$, one-way analysis of variance). Mean survivorship was 0.23 (SD = 0.47) and 0.10 (SD = 0.35) immatures per cage for *S. punctillum* and *F. acarisuga* whilst that of adult and immature *P. persimilis* and *A. californicus* was 0.008 (SD = 0.09) and 0.00 per cage, respectively.

Under laboratory conditions, *S. punctillum* was observed to survive long periods with little or no food as an adult. The larvae, however, needed many TSSM to complete their development. In this study, only a mean of 0.15 (SD = 0.2) larvae per cage (i.e. 5% of the total) managed to pupate successfully.

DISCUSSION

The results of this study suggested that there were differences in environmental requirements for the two predators in the individual behavioural trials. *S. punctillum* was strongly influenced by temperature and host plant species (i.e. the leaf surface) whilst *A. californicus* was strongly influenced by the RH levels (e.g. killing more TSSM at 33% RH) and the crop plant species. Coccinellid feeding behaviour is characterized by sweeping head movements whilst in search of prey (Putman, 1955). This behaviour accounts for the high angular velocity and walking speed measured with *S. punctillum* compared to the activity of *A. californicus*.

Relatively few studies have examined temperature and humidity preferences of *Stethorus* species, and of these most only describe rearing conditions (Putman, 1955, Tanigoshi & McMurtry, 1977; Houck, 1986, 1991) or field situations (Felland & Hull, 1996). However, while temperatures were kept around 25–29°C, RH varied greatly, indicating that other factors override any effect relative humidity may have on the development of *Stethorus* spp. This agrees with the current study where *S. punctillum* showed increased activity at 25–30°C, but not with change of humidity.

With *A. californicus*, past research on temperature and RH effects showed that *A. californicus* demonstrated a great tolerance to high (above 30°C) and low (below 20°C) temperatures (Castagnoli & Simoni, 1992), with high temperatures and high humidities minimizing egg mortality and shortening egg developmental times (Castagnoli & Simoni, 1994). These results are contrary to the present study which showed increased predator activity under low humidity conditions. However, this study observed active adult females while most research on *A. californicus* was conducted on the various egg and larval stages. Other studies indicate that *Amblyseius* species generally prefer hot dry conditions (Beard & Walter, 1998; Pringle, 1998), so the lack of any temperature preference in this study must be due to overriding factors such as crop plant and relative humidities. Also, even though the RH level was measured inside the humidity chamber, the predators were observed on a leaf disc placed on wet filter paper. Therefore the humidity experienced by these small organisms (i.e. RH level on the boundary layer of the leaf) could have been different from that in the airspace of the chamber, thus influencing the activity of the predators. However, since *A. californicus* showed significantly higher activity at low RH levels, the method is accurate enough to show a response of the predator to changes in the humidity of the environment.

Host-plant effects strongly influenced predatory behaviour, with pepper being the most favourable in terms of prey location. However, despite increased prey location on crop plants such as pepper, the overall number of prey killed showed no significant difference across the crop plants. This can partly be explained by increased dispersal of TSSM once initially disturbed by the predators. It was furthermore observed that, occasionally, individual predators took longer than the allocated 30 min to acclimatize to the trial conditions and the leaf disc, showing predatory activity only at the end of the 10 min trial. Leaf surface appears to be an important factor when assessing predator activity (Southwood, 1986; Nihoul, 1993b; Walter 1996; Drukker *et al*., 1997; Bottrell *et al*., 1998). Both tomato and aubergine leaves considerably slowed predatory behaviour of *A. californicus* due to presence of trichomes. The trichomes can result in the entrapment of TSSM as well as the predators. Such entrapment usually results in mortality due to starvation (Synder & Carter, 1984). Putman (1955) reported that leaves with trichomes damage the integument of the coccinellids, thus decreasing their mobility. In the current study, aubergine plants, and to a lesser extent, cucumber leaves appear to have the least favourable leaf surface for *S. punctillum*, while both tomato and aubergine plants considerably slowed predatory behaviour for *A. californicus.* Other studies on host plant effects have shown that when TSM feeds on different food plants, they not only differ in their biological parameters but also in their nutrient quality which in turn affects the predator. For example *P. persimilis* populations increased more rapidly when feeding on TSSM on Soya plants compared to carnation plants (Popov & Khudyakova, 1989).

However, since laboratory findings can often be completely different to the situation in the field (Force, 1974; Ehler & Hall, 1982), the results of this study were followed up by semi-field trials. With these, we aimed to add more insight into the efficacy of *S. punctillum* and *A. californicus* as biocontrol agents in greenhouses, as well as looking at multi-species predator releases. Other multi-species release studies (Laing & Huffaker, 1969; Oatman *et al*., 1977; Ehler, 1992; Croft & Slone, 1997) suggest that this kind of treatment is more efficacious than single predator releases and that interspecific competition only occurs after the TSSM population has crashed. The findings in this study support this, in that more *S. punctillum* and *F. acarisuga* managed to survive under extremely low prey densities in contrast to *P. persimilis* and *A. californicus* which died out under such conditions. However, these findings contrast with the results of earlier semi-field trials carried out by the authors in 1998 (unpublished data) which used the same methodology as that described above but in which combinations of *P. persimilis*, *A. californicus Galendromus occidentalis* (Nesbitt) and *F. acarisuga* were used. Under these conditions, significantly more *A. californicus* survived than other mite species *viz*. 0.33 ± 0.9 adults and immatures per cage compared with 0.12 (± 0.5) *P. persimilis* and 0.05 (± 0.2) *G. occidentalis*. Significantly more *P. persimilis* and *F. acarisuga* (0.27 ± 0.5) also survived in the absence of *S. punctillum*. The most likely interpretation of the data is that *S. punctillum* predates on the predatory mite species (and *F. acarisuga* to a lesser extent) when prey is scarce although further laboratory work and full crop trials are indicated in order to demonstrate this. These data therefore suggest that careful consideration should be given to the combinations of predators used in multi-species releases when pest densities are at very low levels.

In general the results of the semi-field trials show that multi-species predator releases have the potential for greatly improving the control of TSSM in greenhouses. Other examples in the literature showed similar results: Croft and Slone (1997) reported better control of apple mites when three different species of predatory mites were present in the orchard. In another study, Roy *et al*. (1999), looking at different raspberry field systems in Canada, showed higher spider mite densities and lower predator diversity in pesticide treated fields compared to untreated and wild fields. However, in our study the predators were confined to one plant per cage. Therefore, once the TSSM numbers went below a certain threshold level, the predators would die off rather than disperse. Our findings also indicate that factors such as timing of releases and number of predators released as well as combinations of predator species are all major determinants of ecological impact. Glasshouse trials are now needed in order to test and redefine these findings in a crop situation.

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