PREDATION OF CITRUS RED MITE (*PANONYCHUS CITRI*) BY *STETHORUS* SP. AND *AGISTEMUS LONGISETUS*

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ABSTRACT

The ladybird *Stethorus* sp. and the predatory mite *Agistemus longisetus* are the most abundant natural enemies found with high populations of citrus red mite (CRM) (*Panonychus citri*). To enable the continued use of pesticides that may be toxic to one or both of these predators, knowledge of their roles in regulating CRM populations is required. In laboratory bioassays, both natural enemies alone and in combination reduced the number of eggs and immature CRM. However, *A. longisetus* alone did not reduce the number of CRM adults as effectively as *Stethorus* sp. alone or in combination. In a glasshouse trial, predation by a combination of *Stethorus* sp. and *A. longisetus* reduced CRM populations faster than predation by *Stethorus* sp. alone. *Stethorus* sp. is the more effective of the two common CRM natural enemies at reducing high populations of CRM, but the combined predation of the two predators may be complementary in highly dispersed populations where there are a large number of leaves with few eggs.

Keywords: citrus red mite, *Panonychus citri*, *Stethorus* sp., *Agistemus longisetus*, biological control.

INTRODUCTION

Citrus red mite (CRM) (*Panonychus citri* McGregor) (Acarina: Tetranychidae) is considered to be a 'pesticide induced' pest of citrus (Pyle & Stevens 2004). The wide-spectrum activity of some insecticides targeting other pests disrupts the activity of the natural enemies that usually regulate CRM populations. The two most common predators of CRM in New Zealand are the ladybird *Stethorus* sp. (Coleoptera: Coccinellidae) and the mite *Agistemus longisetus* (Acarina: Stigmaeidae) and their presence has been associated with the decline of CRM populations to 'trace' levels within orchards in 4-5 weeks (Jamieson et al. 2005). The optimum long-term solution to CRM control may be to eliminate the use of pesticides that disrupt the activity of its effective natural enemies. In the meantime, the necessary use of pesticides that may be toxic to one or both of these predators must be optimised. This requires the knowledge of their respective roles in regulating CRM populations.

METHODS

Laboratory predation bioassays

CRM-infested leaves were collected from a lemon orchard in Auckland and all stages of naturally occurring predators removed. *Stethorus* sp. and *A. longisetus* individuals were collected from the same orchard and a citrus orchard in Kerikeri. The four treatments applied to single leaves with mixed CRM lifestages were: no predators (control), a single adult *Stethorus* sp., five *A. longisetus* mobile life stages, or a combination of both. Treated leaves were placed individually into minigrip™ bags and stored at 23°C, 16:8 h light:dark. Seven replicates of each treatment were established. The numbers of CRM eggs, immature stages (larvae and nymphs) and adults, and the number and life stage of

predators on each leaf were recorded daily for 8 days. Voucher specimens of *Stethorus* sp. have been kept in the HortResearch IPM insect collection.

Greenhouse predation trial

Twelve small 'Genoa' lemon plants (ca 1 m high) were infested with 100 CRM adults (5 females and 5 males on each of 10 leaves) and left for 5 weeks to allow the mites to establish and multiply. Single trees were enclosed in a terylene material bag and treated with one of three treatments: no predators (control), 10 *Stethorus* sp. (ca five adults or pupae and five larvae) or a combination of 10 *Stethorus* sp. and 10 *A. longisetus* (ca five adults and five immature). Each treatment was replicated four times. Three leaves from each replicate were picked on days 0, 7, 14, 21, 28 and 32 following the treatment. The numbers of CRM eggs, immature stages (larvae and nymphs) and adults on each leaf were recorded. Where predators were found on sample leaves they were placed back on to the plant.

Statistical analysis

Generalised estimating equations (GEE) were fitted to the numbers of eggs, immature stages (larvae and nymphs), adults and total CRM (eggs, immature stages and adults) per leaf (laboratory bioassay) and three-leaf sample (greenhouse trial) with Poisson distribution, log link function and order 1 autoregressive covariance pattern, using initial counts of eggs (immature stages, as appropriate) as offsets to adjust for the initial difference between leaves (laboratory bioassay) or trees (greenhouse trial).

GEE are methods of parameter estimation for correlated data. When data are collected on the same units across successive points in time, these repeated observations are correlated over time. Regression analysis with the GEE methodology is a common choice when the outcome measure of interest is discrete (e.g. binary or count data, possibly from a binomial or Poisson distribution) rather than continuous. GEE are appropriate for the citrus red mite data, as there are repeated counts of various outcomes (eggs, immature stages, etc.) on leaves.

RESULTS

Laboratory predation bioassays

Leaves with *Stethorus* sp. or *A. longisetus* or a combination of both predators had fewer CRM on them than the control leaves (no predators), 1 day after predators were introduced and thereon after (Fig. 1a, $P < 0.05$). The total number of CRM remained lower than the control throughout the 8 days of the trial ($P < 0.05$). Predation by *Stethorus* sp. and the combined predation by *Stethorus* sp. and *A. longisetus* reduced the number of eggs, immatures and adults compared with those on the control at most assessment times ($P < 0.05$) (Figs 1b–d). Predation by *A. longisetus* alone significantly reduced the numbers of eggs and immature stages. However, the number of adult CRM remained similar to those on leaves with no predators. The number of eggs on control leaves declined during the trial indicating that the experimental setup was less than optimal for egg laying.

The mean number of *Stethorus* sp. eggs on leaves peaked after 5 days, with an average of 12.7 and 6.7 eggs/leaf on foliage with *Stethorus* sp. alone and a combination of *Stethorus* sp. and *A. longisetus*, respectively. The mean number of *Stethorus* sp. larvae was greatest 8 days after predators were introduced. An average of 7.1 larvae/leaf was recorded on leaves with *Stethorus* sp. compared with 3.1 larvae/leaf on foliage with both predators. On leaves with *A. longisetus* only, the mean number of *A. longisetus* eggs peaked 7 days after predators were introduced (10.1 eggs/leaf). The mean number of *A. longisetus* eggs on leaves with both predators peaked 7 days after predator introduction at 3 eggs/leaf. On average, 4.7–5.8 *A. longisetus* mobile life stages (immature stages and adults)/leaf were observed for both predator treatments.

FIGURE 1: Mean numbers ($\pm$ SEM) of (a) total citrus red mite (CRM) (eggs, immatures and adults), (b) CRM eggs, (c) immature CRM (larvae and nymphs) and (d) CRM adults on lemon leaves, 0-8 days after addition of treatments: (1) no predators (control), (2) one *Stethorus* sp. adult (St), (3) five *A. longisetus* mobile life stages (Ag) or (4) one *Stethorus* sp. and five *Agistemus longisetus* (St + Ag). Predation treatments with the same letter on the same assessment day are not statistically different ($P>0.05$).

Greenhouse predation trial

Trees with *Stethorus* sp. alone and a combination of both predators had significantly fewer total CRM on them than control trees 3 days after the introduction of predators and this difference remained throughout the trial (Figs 2a-d). The combination of predators reduced the number of CRM more rapidly than *Stethorus* sp. alone. Unfortunately the phytoseiid mite *Phytoseiulus persimilis* was found on trees 7 days after *Stethorus* sp. and *A. longisetus* predators were introduced, and this resulted in a reduction of CRM in control and treatment trees. The mean number of *P. persimilis* mites found on leaf samples (three leaves) was 3.2, 1.3 and 0.7 for control leaves, leaves with *Stethorus* sp., and leaves with the combination of *Stethorus* sp. and *A. longisetus*, respectively.

FIGURE 2: Mean numbers ($\pm$ SEM) of (a) total citrus red mite (CRM) (eggs, immatures and adults), (b) CRM eggs, (c) immature CRM (larvae and nymphs) and (d) CRM adults on three mandarin leaves, 0-32 days after addition of treatments: (1) no predators (control), (2) 10 *Stethorus* sp. ladybirds (St) or (3) 10 *Stethorus* sp. and 10 *A. longisetus* (St + Ag), to small trees. Predation treatments with the same letter on the same assessment day are not statistically different ($P > 0.05$).

DISCUSSION

The coccinellid ladybird *Stethorus* sp. and the stigmæid mite *A. longisetus* are the most abundant natural enemies found in association with CRM populations (Jamieson et al. 2005). On single-leaves, 8 days of predation by *Stethorus* sp. reduced the number of CRM on leaves significantly more than *A. longisetus*. The combined predation of *Stethorus* sp. and *A. longisetus* reduced the number of CRM faster than *Stethorus* sp. alone on trees in a glasshouse, but this enhanced effect of combined predation was not apparent on single leaves in a bioassay.

The difference in the predation abilities of *Stethorus* sp. and *A. longisetus* is probably explained by their feeding preferences (prey lifestage), different predation rates, ability to disperse and rates of natural increase. While *Stethorus* sp. were observed consuming all life stages of CRM, *A. longisetus* were most commonly observed consuming CRM eggs, occasionally larvae and on one occasion a protonymph. Overseas studies show that *Agistemus* species also prefer CRM eggs (Inoue & Tanaka 1983; Yue & Tsai 1995). In the present study, *A. longisetus* had no impact on the number of adult CRM mites while *Stethorus* sp. reduced the number CRM adults.

Preliminary investigations into the predation rates of each predator suggest that a single *Stethorus* sp. adult consumes 7-20 CRM adults (increasing predation rates with increasing prey density) over a 24 h period and a *Stethorus* sp. larva consumes 8-18 CRM eggs per day, while *A. longisetus* adults consume only 2-3 CRM eggs over the same period (L.E. Jamieson, unpubl. data). Overseas studies have reported that a single

Stethorus punctum adult can kill 41 CRM/day (Lui 2002) or 9 CRM/h (Hull et al. 1977) and Peterson et al. (2000) also reported that *Stethorus bifidus* became more efficient predators when prey density increases.

In the laboratory bioassay, the number of *Stethorus* sp. increased more than the number of *A. longisetus*. The rates of natural increase of other *Agistemus* species in other countries are reported to be very similar to that of CRM (Inoue & Tanaka 1983). Therefore, it has been suggested that *Agistemus* spp. are less efficient at controlling CRM than other predators. However, the ability of *Agistemus* spp. at locating CRM colonies when populations are low, therefore preventing outbreaks, is unknown.

Although predation of CRM by *P. persimilis* was not in the scope of this study, the unplanned invasion of this mite into the caged control trees demonstrated that *P. persimilis* does have the ability to reduce CRM populations. However, Jamieson et al. (2005) found *P. persimilis* only on a few occasions when CRM were present. Because of their relatively small size, phytoseiid mites can survive on small populations of prey and achieve regulation at low host densities (DeBach & Rosen 1991). Inundate releases of *P. persimilis* to control CRM where biological control by *Stethorus* sp. is unreliable warrants investigation.

Stethorus sp. seems to be the principal regulator of CRM when populations are high. It is therefore important to minimise the use of pesticides that are toxic to *Stethorus* sp. to avoid CRM outbreaks in New Zealand citrus orchards. *Agistemus longisetus* alone does not seem to be a key regulator of high CRM populations, but the combined predation of the two predators, *Stethorus* sp. and *A. longisetus*, may be complementary in highly dispersed populations where there are a large number of leaves with few eggs.

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