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Article

***Amblydromalus limonicus*: a “new association” predatory mite against an invasive psyllid (*Bactericera cockerelli*) in New Zealand**

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Abstract

The tomato/potato psyllid, *Bactericera cockerelli*, has been a pest of crops such as potato and tomato in North America for nearly a century and recently invaded some parts of Central America. It invaded New Zealand in May 2006 and is now distributed in both the North and South Island. Before our study, there had been no reports of *Amblydromalus limonicus* feeding on the tomato/potato psyllid in the US or other areas. After the invasion of the tomato/potato psyllid into New Zealand, we have observed numerous populations of local *A. limonicus* attacking the tomato/potato psyllid on greenhouse capsicums and tomatoes in the greater Auckland region, providing a certain degree of natural control. In these areas, *A. limonicus* was common before the invasion of the tomato/potato psyllid. This new association between a local predatory mite and an invasive insect is now well established. *A. limonicus* cultures have been maintained on capsicums (and sometimes tomato) infested with psyllids in greenhouses for the last three years. Laboratory studies showed that *A. limonicus* adults could survive and reproduce on the eggs, and first, second, and third instar nymphs of *B. cockerelli*, as well as on sugar excreted by psyllids, while immature *A. limonicus* could complete development on the psyllid sugar and first instar nymphs of *B. cockerelli*. A female predator consumed on average 2.5 psyllid eggs, 2.1 nymph I, 0.5 nymph II, 0.03 nymph III and 1.6 psyllid sugar droplets per day. Given that *A. limonicus* can be mass-reared and is now commercialized, it offers a new opportunity for the biological control of this psyllid.

Key words: Invasive species, pest, insects, mites, predators, biological control

Introduction

When a pest species invades a new area and becomes established, it is exposed to local natural enemies, with the potential for forming new associations (Hokkanen & Pimentel 1984). Such is the case for the tomato/potato psyllid (TPP, *Bactericera cockerelli*), which was a pest of crops such as potato and tomato in North America for nearly a century and then has also been found in some parts of Central America (Butler and Trumble 2012). It was first detected in New Zealand in May 2006 and later became an invasive pest in both the North and South Island (Thomas *et al.* 2011). Potential new associations between naturalized coccinellid species in New Zealand and *B. cockerelli* were suggested by O’Connell *et al.* (2012). In this study, we report on a similar case between *B. cockerelli* and a predatory mite (*Amblydromalus limonicus*) in the North Island.

Bactericera cockerelli primarily attacks plants of the Solanaceae such as tomatoes and potatoes, although it is also found on plants of some other families (Martin 2008; Butler & Trumble 2012). TPP is able to vector a bacterium *Candidatus Liberibacter*, which can lead to a severe disease of potatoes (zebra chip), causing considerable crop losses in North America, Central America, and New Zealand (Al-Jabr & Cranshaw 2007; Butler and Trumble, 2012; Rondon *et al.* 2012).

Phytoseiid mites have been known to be effective in controlling greenhouse whiteflies, thrips, and plant mites (Nomikou *et al.* 2001; Zhang 2003; Messelink *et al.* 2006; McMurtry *et al.* 2013). *A. limonicus* is known in many countries of the Americas from the North to the South, Hawaii, and Australasia (Knapp *et al.* 2013). It is an important species, which became first known in the 1960s for its use against *Oligonychus punicae* and *Tetranychus cinnabarinus* (= *T. urticae* now) (McMurtry & Scriven 1970). In the early 1990s, it was successfully applied to the prevention and control of western flower thrips (Messelink *et al.* 2006). It is a generalist predator of phytophagous mites (e.g. Tetranychidae, Eriophyoidea, Tarsonemidae), immature insects (thrips, whitefly), and also the eggs of Hemiptera, Lepidoptera, and Diptera (Knapp *et al.* 2013). It can utilize pollens of various plants for population growth, can survive on plant nectar, and was recently shown to develop and reproduce well on factitious food such as the frozen eggs of flour moth (*Ephestia kuehniella*) and the cysts of brine shrimp (*Artemia franciscana*) (Vangansbeke *et al.* 2014b). In 2012, it became commercially available, being mass-reared on the mite *Carpoglyphus lactis* (Carpoglyphidae) (Knapp *et al.* 2013). A year before this, Z.-Q. Zhang was asked to identify mites found on greenhouse peppers in association with TPP in New Zealand and they were *A. limonicus*. However, there have been no published studies on the predator-prey relationships between *A. limonicus* and the psyllid, except a brief mention in a popular trade article stating “observations in New Zealand have found that it will also eat eggs of the new invasive potato/tomato psyllid” (Workman & Pedley 2007—mites identified by Z.Q. Zhang with voucher specimens in New Zealand Arthropod Collection). In this article, we demonstrate this new association between *A. limonicus* and TPP for the first time with experiments on the growth and development of *A. limonicus* on immature psyllids and other diets, as well as its predation on immature TPP and the associated reproductive performance.

Material and methods

Mite and insect cultures. The *A. limonicus* used in this study was sourced from greenhouse capsicums in south Auckland in 2012. Mite colonies were reared in the laboratory at 20°C, using a diet of TPP on potato or capsicum leaves. TPP colonies were started from a population associated with *A. limonicus* and were maintained on potato or capsicum plants in mesh-cages in a greenhouse at Landcare Research on the Tamaki Campus, the University of Auckland. All experiments were conducted in a laboratory at a temperature of 20°C, relative humidity ranging from 65% to 75%, and photoperiod 12 L: 12 D. All experiments were conducted in 2012.

A new rearing container. Because of the high mobility of *A. limonicus*, the typical leaf disc enclosed by strips of wet-cotton is not very effective in our experiments due to the relatively high rate of escape (especially when using unsuitable diet in some tests). We designed a new container with a mini-leaf disc to reduce the rate of mite escape. A centrifugal tube (0.5 ml) was used as a rearing container. A small hole (2 mm diameter) was drilled in the cap, which was covered with a small piece of black cloth, allowing air circulation but preventing mites from escaping. Before use, water (100 µl) was added to the bottom part of the tube and a piece of T-shaped filter paper with a square of 5 × 5 mm (hereafter, the head) connecting to a 1 × 20 mm strip (hereafter, the tail) was added to the tube. The tip of the tail was immersed in the water so that head area was also saturated with water. A mini potato leaf square (7 × 7 mm) was then added to the head area. The tail drew water to the head area under the leaf, keeping the leaf from desiccation. The relatively long tail allows separation of the head area and its associated leaf square from the source water, which can flood the leaf if they are too close. In experiments, individual adult mites or eggs were placed on leaves with food. Water was added daily when needed, and the leaves were changed at an interval of 4 days on average.

Experimental procedures. (1) *Immature development and survival using different diets.* Eggs laid by female *A. limonicus* were used in experiments within 24 hours. Individual predator eggs were subject to the following treatments to examine their effects on immature development and survival: 2 first instar nymphs per potato leaf (21 replicates); 4 psyllid sugar droplets per potato leaf (16 replicates); potato leaf only (13 replicates); potato leaf washed with 95% alcohol and dried before use (12 replicates); and 100 µl water only (12 replicates). Food items were replaced and the number of food items consumed was recorded daily for the first 2 treatments. Mite development and survival were also observed daily until death or completion of development to adults. (2) *Adult survival, predation and reproduction using different diets.* In this experiment, before being tested individual gravid females from the lab culture were kept in isolation without prey for 24 hours. The following treatments were tested for their effects on adult survival, predation, and oviposition: 10 potato psyllid eggs per potato leaf; 5 first instar nymphs per on potato leaf; 3 second instar nymphs per potato leaf; 1 third instar nymph per potato leaf; 6 psyllid sugar droplets per potato leaf; potato leaf only; potato leaf washed with 95% alcohol and dried before use; 100 µl 12.5% honey solution; 100 µl water only. There were 10 replicates per treatment in the first eight treatments and 9 replicates in the last treatment. Food items were replaced and eggs laid by *A. limonicus* were counted and removed daily for 16 consecutive days. Daily food consumption per female was calculated for the first 5 treatments and oviposition rates were calculated as the number of eggs laid per female per day. However, data for first 24-hour period were excluded in the calculation because oviposition during this period can be influenced by previous feeding.

Data analysis. All statistical tests were performed using GenStat Release 14.2. Log-linear regressions were used for immature survival (proportional) data and ANOVAs for other data (duration in days, number of eggs laid, and the number of food items consumed). The Kaplan-Meier test was used to check the equality of survival curves for adult mites fed on different diets. The significance level for statistical tests is set at 5%.

Results

Immature development and survival on different diets. The effects of diet treatments were not significant for survivals of *A. limonicus* into larvae ($P = 0.983$; Fig. 1 top left) and protonymphs ($P = 0.822$; Fig. 1 top right). However, the presence of the first instar nymphs of potato psyllid or psyllid sugar had significant effects on the development of *A. limonicus* further into deutonymphs ($P < 0.001$; Fig. 1 lower left), and then to adults ($P < 0.001$; Fig. 1 lower right); overall, 52.4% and 62.5% of individuals completed preimaginal development when feeding on the first instar nymph of TPP and psyllid sugar, respectively (Fig. 1). These mites completed development within 2 weeks at 20°C; the developmental time was significantly shorter feeding on the first instar nymph of TPP than on psyllid sugar (Fig. 2 left) and much shorter for males than females (Fig. 2 right).

The number of psyllid sugar droplets (12.4 ± 1.2) consumed by immature *A. limonicus* was significantly more than the first instar nymphs of TPP (5.6 ± 1.2) ($P < 0.001$; Fig. 3 left); there were no difference in food consumption by male (8.1 ± 1.5) and female (9.2 ± 1.0) ($P = 0.530$; Fig. 3 right).

Adult mite survival, predation and reproduction on different diets. Survival functions of adult mites under different diets are significantly different (Kaplan-Meier test: Log-rank = 46.430 df = 8; $P < 0.001$). With water only, the adults could survive approximately 10 days on average (Fig. 4); potato leaves, honey solution, and psyllid eggs did not significantly increase the number of days survived; however, psyllid nymphs I–III and psyllid sugar significantly increased the number of days of survival (Fig. 4).

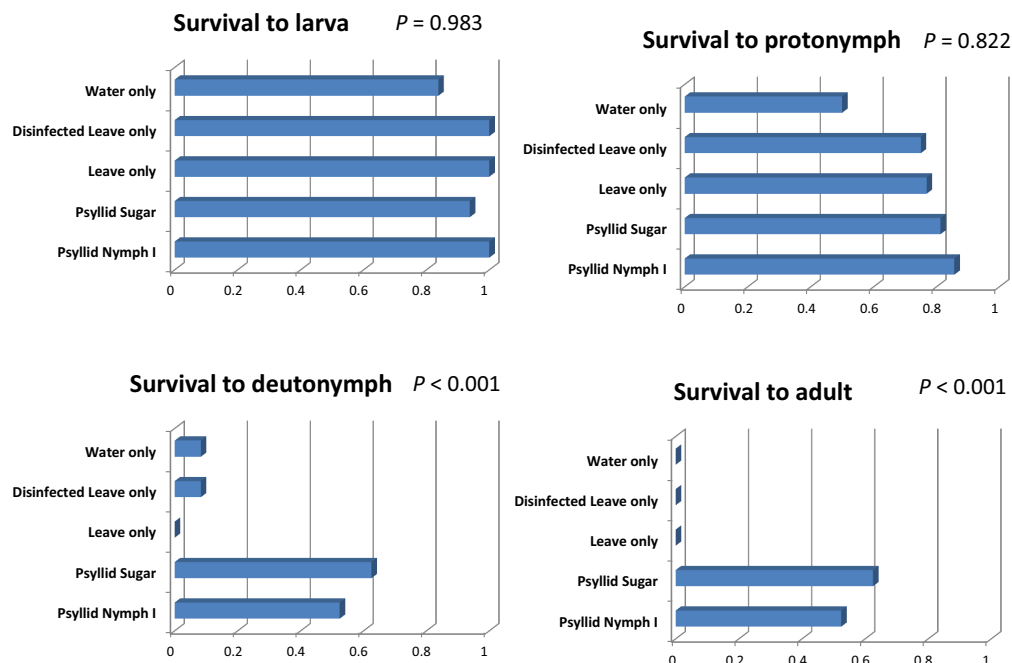


FIGURE 1. Immature survival rates of *Amblydromalus limonicus* when fed the first instar nymphs of the tomato/potato psyllid (*Bactericera cockerelli*) and psyllid sugar on potato leaves at 20°C, with leaves only or water only as controls.

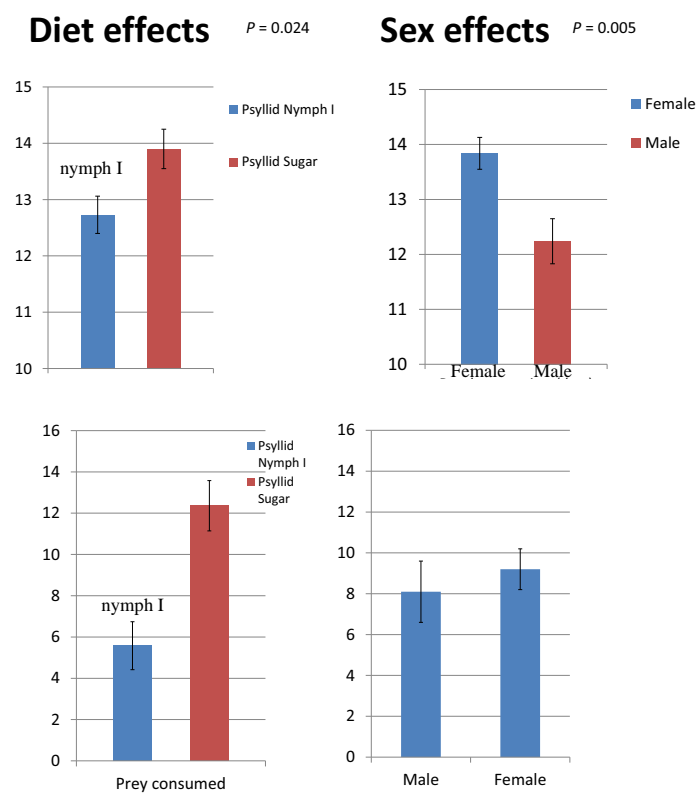


FIGURE 2. Developmental time (days) of *Amblydromalus limonicus* when fed the first instar nymphs of the tomato/potato psyllid (*Bactericera cockerelli*) and psyllid sugar on potato leaves at 20°C.

FIGURE 3. Food consumption by immature *Amblydromalus limonicus* when fed each day with two first instar nymphs of the tomato/potato psyllid (*Bactericera cockerelli*) or four droplets of psyllid sugar on potato leaves at 20°C.

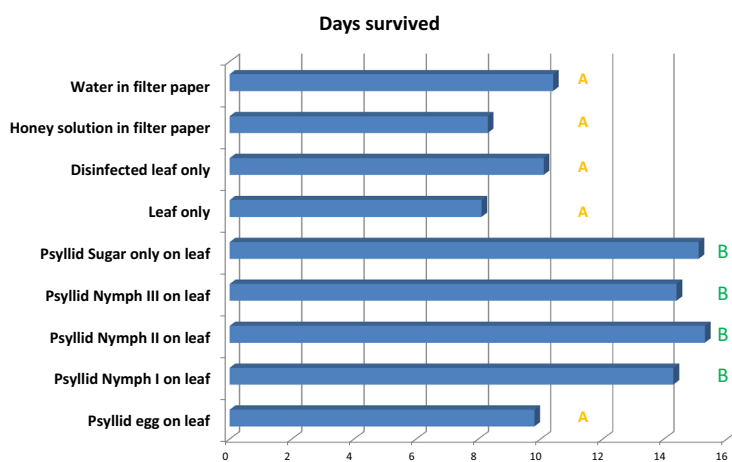


FIGURE 4. Survival of *Amblydromalus limonicus* adult females during a 15-day observation period when fed eggs or nymphs I–III of the tomato/potato psyllid (*Bactericera cockerelli*) or psyllid sugar on potato leaves at 20°C, with leaves only, honey solution or water only as controls (ANOVA: d.f.= 8; v.r. = 6.25; $P < 0.001$; means with the same letter not significantly different at $P = 0.05$).

Only psyllid eggs and nymphs I–II significantly increased the number of eggs laid per day, with nymph II and eggs slightly better (Fig. 5).

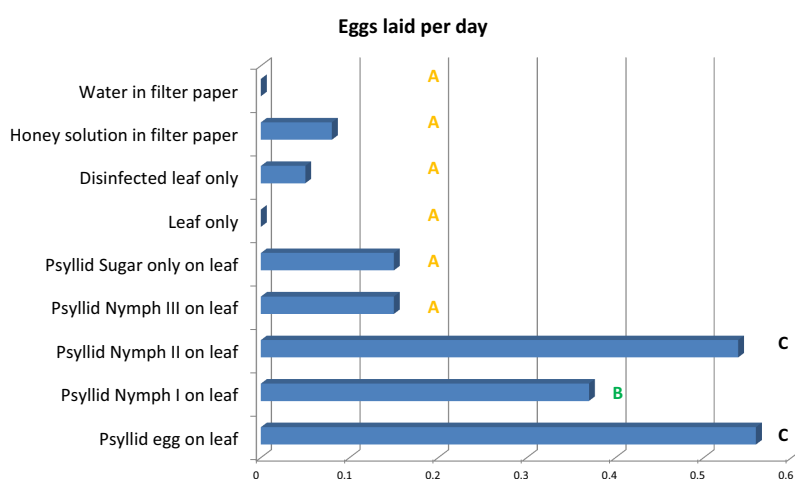


FIGURE 5. Survival of *Amblydromalus limonicus* adult females during a 15-day observation period when fed eggs or nymphs I–III of the tomato/potato psyllid (*Bactericera cockerelli*) or psyllid sugar on potato leaves at 20°C, with leaves only, honey solution or water only as controls (ANOVA: d.f.= 8; v.r. = 18.7; $P < 0.001$; means with the same letter not significantly different at $P = 0.05$).

Psyllid nymphs III were too big (their cuticle too hard) to feed for some predator individuals: 20% of the predators survived as long as predators fed water alone; only 30% predators were able to kill nymphs III. Of 10 potato psyllid eggs provided per day, only about a quarter was consumed, which was not significantly more than the number of psyllid nymph I consumed (Fig. 6). Of 6 psyllid sugar droplets provided per day, only a quarter was consumed, which was three times as much as psyllid nymph II consumed (Fig. 6).

Discussion

There is little doubt that TPP originated in the USA—it was restricted to some parts of the USA for four decades, then extended to Canada, with expansion to Mexico, Texas, Guatemala, Honduras, and

New Zealand occurring within the last two decades (Butler & Trumble 2012). The origin of *A. limonicus*, however, is not known, although this species has been reported from many countries with moderate temperature and fairly high relative humidity, with a wide range from North America to South America, Hawaii, and Australasia (De Moraes *et al.* 2004; Knapp *et al.* 2013; Demite *et al.* 2014). The distribution of *A. limonicus* overlaps with that of TPP in the USA, especially in California where there has been much research on both species, which also share Solanaceae as hosts; however, there have been no reports on the association between the two species (McMurtry & Scriven 1970; Butler & Trumble 2012; Knapp *et al.* 2013; McMurtry *et al.* 2013). *A. limonicus* was commonly found on Solanaceae in the areas around Auckland, New Zealand, before the invasion of TPP (NZAC specimen records, based on voucher specimens identified by Z.-Q. Zhang). After the TPP invasion around Auckland, the two have been newly associated—Z.-Q. Zhang has identified specimens of *A. limonicus* collected on Solanaceae in these areas from 2007 onwards, had personal observation of *A. limonicus* with TPP on capsicums and tomato in early 2012 based on a natural occurrence of mites on capsicum leaves, and has cultured *A. limonicus* with TPP as food on capsicums, tomato or potato since 2012.

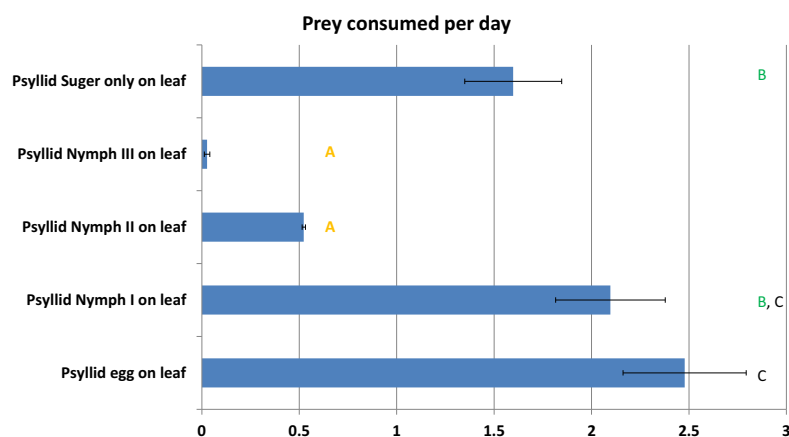


FIGURE 6. Food consumption of *Amblydromalus limonicus* adult females when fed eggs or nymphs I–III of the tomato/potato psyllid (*Bactericera cockerelli*) or psyllid sugar on potato leaves at 20°C (ANOVA: d.f. = 4; v.r. = 21.8; $P < 0.001$; means with the same letter not significantly different at $P = 0.05$).

Phytoseiid mites have been known as effective biocontrol agents of small insects such as thrips and whitefly (Zhang 2003; McMurtry *et al.* 2013; Gerson 2014). In the last few years, three phytoseiid species—*Neoseiulus cucumeris*, *N. barkeri*, and *Amblyseius swirskii* (all commercially available)—were tried against Asian citrus psyllid (*Diaphorina citri*) in the laboratory and greenhouses (Juan-Blasco *et al.* 2012; Fang *et al.* 2013; Zhang *et al.* 2013): they can attack eggs or the first instar nymphs of *D. citri* and the authors concluded that they might have potential as biocontrol agents against this new prey. While these three species represent a new “artificial” association of phytoseiids with a psyllid pest, our study is the first demonstrating a new natural association between a phytoseiid species and a psyllid pest.

Knapp *et al.* (2013) provided a comprehensive review of the various aspects of the biology and possible use of *A. limonicus* against phytophagous mites, thrips and whiteflies. Vangansbeke *et al.* (2014a,b) showed that *A. limonicus* performed as well on *E. kuehniella* and *C. capitata* eggs as on *Typha angustifolia* pollen and their adult females were cannibalistic on their own eggs when feeding on these three diets. Vangansbeke *et al.* (2014c) showed pollen enhanced thrips development but reduced thrips leaf feeding, anti-predator behavior, and their consumption by *A. limonicus*. Further studies using artificial diet enriched with extract of *A. franciscana* cysts resulted in higher reproductive success than natural or factitious foods (Nguyen *et al.* 2015). Pollen, factitious foods,

and artificial diets could enable food supplements in early crop seasons to support populations of generalist mites predators, which have been showed to be useful biocontrol agents of pests such as thrips and whitefly (Nomikou *et al.* 2001; Messelink *et al.* 2006; Knapp *et al.* 2013). Our studies in this paper showed that TPP third instar nymphs and lower stages (including psyllid sugar) could increase the survival of adult female predators by ca 50%; a female predator consumed 2.5 eggs, 2.0 nymph I, 0.5 nymph II, 0.03 nymph III, and 1.5 psyllid sugar droplets per day; predators fed psyllid eggs and nymphs I & II produced over 2–3 times as many eggs as those fed psyllid sugar and nymph III; psyllid sugar and nymph I could support predator egg to adult development. This is the first report on *A. limonicus* as a biocontrol agent for psyllids. Given that *A. limonicus* can be mass-reared and is now commercialized, we suggest that this species will be a promising new opportunity for preventive control of TPP by early releases of mass-reared predators, augmented using pollen, artificial diets, or other alternative foods.

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