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Correspondence

Preliminary study on the potential of *Pyemotes zhonghuajia* (Acari: Pyemotidae) in biological control of *Aphis citricola* (Hemiptera: Aphididae)

LITAO LI[1], LIMIN HE[1], LICHEN YU[1,*], XIONG ZHAO HE[2], CHANGXIN XU[1], RUI JIAO[1], LINLIN ZHANG[1] & JINLI LIU[1]

[1]*Changli Institute of Pomology, Hebei Academy of Agriculture and Forestry Sciences, Changli 066600, Hebei, China.*

[2]*School of Agriculture and Environment, Massey University, Palmerston North, Private Bag 11222, New Zealand.*

* *Corresponding author. ylc825@hotmail.com*

Pyemotes zhonghuajia Yu, Zhang & He, first collected by Lichen Yu in early 1990s in China and initially identified as *P. tritici* by Dr John Moser (USA), was a ectoparasitic mite described by Yu *et al.* (2010) as a new species native to China, with a wide distribution in Northwest (Shanxi, Xinjiang and Ningxia) and North China (Hebei, Tianjin and Beijing). As other *Pyemotes* mites (Bruce 1989; Bruce & Wrensch 1990; Wrensch & Bruce 1991), *P. zhonghuajia* has a high reproductive potential (ca.100 offspring/female) with an extremely female-biased sex ratio (98%) and a very short life cycle (≈ 8 days at 25 ºC) (Yu *et al.* 2000). It has been mass-reared and released in the field, demonstrating a high efficiency for the biological control of Coleoptera as well as Lepidoptera, Hymenoptera, Homoptera and Isoptera. For example, field studies showed that *P. zhonghuajia* killed 89.7–98.4% larvae of the juniper bark borer, *Semanotus bifasciatus* (Motschulsky), 89.2% larvae of the cypress bark beetle, *Phloeosinus aubei* Perris and 67.8% larvae of the bostrichid beetle, *Sinoxylon japonicus* Lesne (Zhang *et al*. 2004, 2008a). He *et al.* (2009) further reported that *P. zhonghuajia* could reduce 91.7% overwintering larvae of the poplar and willow borer, *Cryptorhynchus lapathi* (L.) and 98.9% newly hatched larvae of the Asian longhorned beetle, *Anoplophora glabripennis* Motschulsky in the field. Moreover, *P. zhonghuajia* may also attack lepidopteran larvae causing high mortality of *Laspeyresia* sp. (94.2%) (He *et al.* 2009), *Dioryctria pryeri* Ragonot and *D. rubella* Hamposn (91.4%) (He *et al.* 2012) and *Zeuzera leuconotum* Butler (60.5%) (Zhang *et al.* 2008a). *P. zhonghuajia* has also been applied to control the hidden or aggregating pests, such as *Hyphantria cunea* Drury (Zhang *et al.* 2008b), *Yponomeuta sp*. (He *et al*. 2014), *Grapholitha molesta* Busck (Zhou *et al.* 2011), and *Reticulitermes sp*. (Dong *et al*. 2011; Duan *et al.* 2011a).

Spiraea aphid, *Aphis citricola* van der Goot, is one of the important pests in apple orchards in China. *A. citricola* has more than 10 generations a year (Chen *et al.* 2011). It prefers new growing shoots and young leaves for feeding (Figure 1A). Adults and nymphs aggregating on the newly formed leaves may result in twisting and curling of those leaves. High infestations by *A. citricola* may seriously delay the growth of new shoots, pollute the fruit and lead to early defoliation, which will significantly affect the yield and quality of apples (Chen *et al*. 2011). So far, the control of *A. citricola* mainly relies on spraying synthetic pesticides. Due to the frequent chemical applications, *A. citricola* has quickly developed resistance to most insecticides (Xu *et al*. 2002; Peng *et al*. 2010; Feng *et al*. 2012). Therefore, other strategies should be developed to reduce the use of chemical pesticides. Biological control using natural enemies provides one of the highest returns on investment available through integrated pest management in the world (Naranjo *et al*. 2015).

Duan (2011b) and Han (2016) investigated the efficiency of *P. zhonghuajia* in biological control of pests with soft body and piercing-sucking mouthpart, including *A. citricola*. They showed that *P. zhonghuajia* could cause high mortality of pests (usually > 90%) in both laboratory and field tests. However, they released superfluous mites to the target pests (50–100 and 10–20 times of pest density respectively in the laboratory and field tests), making it difficult to assess the real potential of *P. zhonghuajia* in biological control programmes. So far little is known about the effectiveness of *P. zhonghuajia* in biological control of *A. citricola* at 'low' mite release rates. In the present study, we observed the host searching and attacking behaviours of *P. zhonghuajia* and detected the mortality rate of *A. citricola* at relatively lower mite:aphid ratios (1:4, 1:2 and 1:1). Results of this study will improve our understanding of the mechanisms and potential of *P. zhonghuajia* in biological control of *A. citricola*.

TABLE 1. Mortality of *A. citricola* induced by *P. zhonghuajia* in 24 h and 48 h at different mite densities or mite:aphid ratios.

Mite density	Mortality (%)		$F_{1,10}$	P
(mite:aphid)	24 h	48 h		
0	1.2 ± 0.8 dα	1.3 ± 0.8 dα	0.02	0.8824
25 (1:4)	14.7 ± 1.9 cα	15.7 ± 1.8 cα	0.15	0.7022
50 (1:2)	50.5 ± 10.2 bα	51.8 ± 10.1 bα	0.01	0.9257
100 (1:1)	89.3 ± 2.3 aα	89.8 ± 2.3 aα	0.02	0.8796
$F_{3,20}$	67.10	67.81		
P	< 0.0001	< 0.0001		

Means (± SE) with different English letters in each column or with different Greek letters in each row are significantly different (ANOVA: $P < 0.05$).

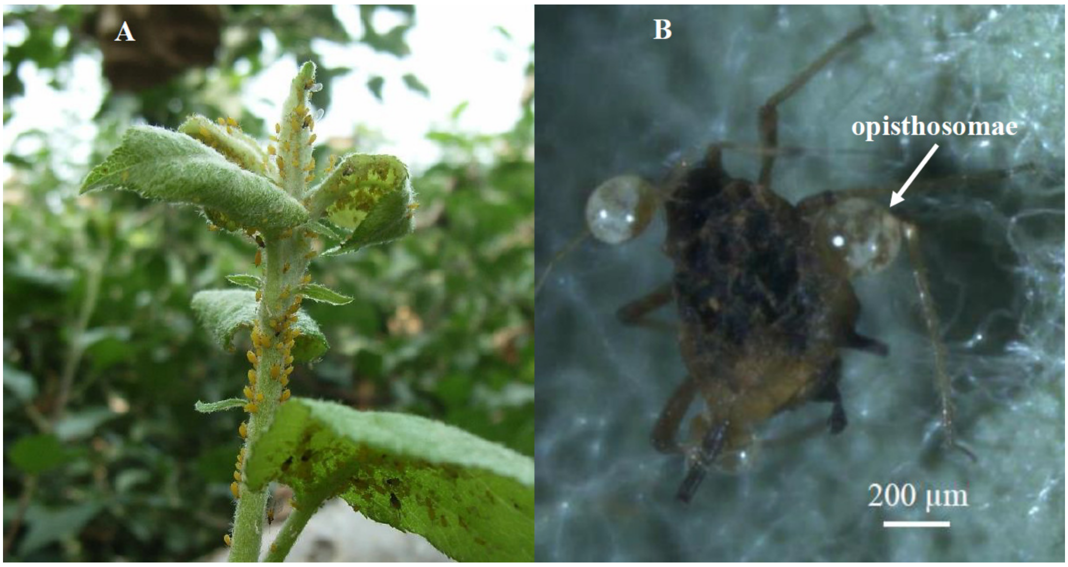

FIGURE 1. An apple shoot infested by *A. citricola* (**A**) and three *P. zhonghuajia* females feeding on an aphid and development of their posterior opisthosomae with their offspring developing inside seven days after the start of feeding (**B**).

Pyemotes zhonghuajia was reared on mature larvae of *Sitotroga cerealella* (Oliver) maintained on barley in the Technology Research Center of *Pyemotes* Production and Utilization, Changli

Institute of Pomology, Hebei Academy of Agriculture and Forestry Sciences. *A. citricola* specimens were collected from an apple orchard. Apple leaves each infested with 100 aphids of mixed life stages were individually placed upside down on a Petri dish. The leaves were kept fresh by wrapping the petioles with wet cotton. Ten newly emerged adult females of *P. zhonghuajia* were individually introduced on an aphid-infested leaf, and host searching and attacking behaviors were observed under a microscope (Leica EZ4, Germany) for one hour until the first aphid was located, stung and paralyzed. The host searching time (time period between mite introduction and host location), sting time (time period between the sting initiation and termination), and paralysis time (time period between sting initiation and aphid paralysis) were recorded, and the body parts stung were noted. After behaviorial observation, insects were maintained in darkness at 25 ± 1ºC and RH 60 ± 5% for 24 h, and after which time the number of paralyzed/dead aphids was then counted. To simulate the efficiency of field augmentative release of *P. zhonghuajia* in suppressing *A. citricola* population, 0 (control), 25, 50 or 100 newly emerged female adults were introduced on an apple leaf infested with 100 aphids in a Petri dish, and they were maintained at above mentioned environmental conditions. Aphid mortality was detected 24 h and 48 h later. There were six replicates for each mite density or mite:aphid ratio.

After being introduced onto an apple leaf, the *P. zhonghuajia* females randomly walked and searched for hosts. They located the first host in 9.2 ± 3.0 minutes (mean ± SE). First touching by the mites usually caused the aphids to move or escape. After climbing upon the aphids, the mites started to seek a suitable body part to sting and preferred the abdomen (80%) over the dorsum. After being stung, the aphids strongly struggled by swinging and sometimes lifting their abdomen tip. Stinging might last for 5.7 ± 1.1 minutes, and after 14.2 ± 3.2 minutes the aphids stopped struggling. Such cessation of struggling indicated a mite-induced paralysis. In *Pyemotes* mites, the females inject a neurotoxin-containing saliva into their hosts, paralyzing the hosts and enabling the females to feed on the hosts' hemolymph (Gerson *et al*. 2003). However, our results showed that the females did not immediately feed on the paralyzed aphids but left them and sought others. A female could paralyze and eventually kill 4.4 ± 0.5 aphids in 24 hours, and usually fed and reproduced on a larger host. These results indicate a high potential of *P. zhonghuajia* in biological control of *A. citricola*: firstly, as feeding on one host may allow a female mite to produce next generation, killing more aphids before breeding may quickly suppress an aphid population; and secondly, *P. zhonghuajia* preferring larger hosts for feeding is expected to produce more offspring, promoting the population size of next generation.

The aphid mortality rate significantly increased with the increase of mite density or ratio, but was not significant different between 24 h and 48 h (Table 1). These results have two implications: (1) *P. zhonghuajia* females will settle and feed on their hosts within 24 h; and (2) augmentative release of *P. zhonghuajia* with an even ratio of 1:1 (mite:aphid) may result in a similar control efficiency in suppressing *A. citricola* populations as that with superfluous releases (i.e., Duan 2011b; Han 2016). As reported above, a female could kill more than four aphids, it is expected that 100% mortality could be achieved in any of the three test densities or mite:aphid ratios; however, this was not observed, rather the number of aphids attacked per mite was less than one (Table 1). This result suggests an interference between the host searching mites, which may reduce their searching efficiency. We also found that more than one *P. zhonghuajia* female may feed on a host and breed their offspring (Figure 1B). However, the effect of such multiple parasitism on the reproductive output and offspring fitness of *P. zhonghuajia* is not clear.

Results of this preliminary study indicate that due to its high ability to suppress aphid populations, *P. zhonghuajia* may be an efficient biological control agent of *A. citricola*. Further laboratory and field investigations into parasite-host interactions (e.g. 1, the host stage preference of *P. zhonghuajia* and its impact on the host mortality and mite reproduction and offspring fitness, 2, the ability of *P. zhonghuajia* to respond to the increase of host densities, and 3, the interference between the host-searching females), will improve our knowledge to develop and implement the biological control programmes using *P. zhonghuajia*.

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