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Life history and mortality factors of *Agrilus mali* Matsumura (Coleoptera: Buprestidae) in wild apples in Northwestern China

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Abstract

1 Wild apple *Malus sieversii* (Ledeb.) M. Roem. (Rosales: Rosaceae), the ancestor of cultivated apples, is widely distributed in Central Asia and is recognized as an important germplasm bank. Recently, the invasive pest *Agrilus mali* Matsumura (Coleoptera: Buprestidae), originally distributed in eastern Asia, has damaged endemic apple forests in the Yili River valley, Xinjiang, China, and has spread rapidly, infesting more than 80% of wild apple trees in this region.

2 We investigated the life-history traits and native natural enemies in the recently invaded range during 2016 and 2017. *Agrilus mali* has a univoltine life cycle and overwinters as young larvae in galleries in the cambium. Adults emerged from early June to mid-August and their density peaked in mid-July.

3 Several native natural enemies were identified from *Agrilus mali* larvae, including *Atanycolus denigrator* (L.) (Hymenoptera: Braconidae), the mite *Pyemotes moseri* Yu et Liang (Acari: Pyemotidae) and fungal entomopathogens.

4 Combined, these natural enemies were responsible for mortality rates ranging from 20% to 80% during the summer and autumn. The most abundant and important natural enemy was *A. denigrator*, which was responsible for up to 15% mortality of *A. mali*.

5 The results of the present study suggest that augmentation and conservation of *A. denigrator* and *P. moseri* should be considered with respect to biological control against this devastating pest.

Keywords ancestral apples, biological control, braconid parasitoid, natural enemy, wood borer.

Introduction

There are more than 7500 apple cultivars derived originally from wild apple species, principally *Malus sieversii* (Ledeb.) M. Roem. (Rosales: Rosaceae), the key ancestor of cultivated apples (Harris *et al.* 2002; Cornille *et al.* 2012). This particular wild apple species is found only in the mountains of Central Asia, including southern Kazakhstan, eastern Uzbekistan, Kyrgyzstan, Tajikistan, Turkmenistan and Xinjiang province, China (Volk *et al.* 2013). This important germplasm resource (Chen *et al.* 2007; Richards *et al.* 2009) has been severely impacted by overgrazing, pest insects, diseases, timber industries and climate change (Liu *et al.* 2014b). Recently, the wild apple forests of northwestern China have been damaged by an invasive beetle, the apple buprestid *Agrilus mali* Matsumura (Coleoptera: Buprestidae) (Liu *et al.* 2014a).

After its initial detection in 1995 in the Yili River valley of Xinjiang Uyghur Autonomous Region (hereinafter referred to as Xinjiang), *A. mali* spread rapidly across the western regions of Xinjiang and now poses a significant threat to the stability, biodiversity and function of the local and regional

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forest ecosystem (Wang 2013; Cui *et al.* 2015). This buprestid has caused serious damage to the endemic wild apple species *M. sieversii* (Wang *et al.* 2005; Wang 2013). Subsequent to its introduction, *A. mali* has killed millions of wild apple trees and infested over 80% of the total area of wild apple forests in the Yili River valley (Cui *et al.* 2015). Moreover, in other parts of its range in central Asia, *M. sieversii* is at high risk of invasion by *A. mali* as a result of a suitable climate, geographical proximity and the local prevalence of this host.

The apple buprestid is native to the Russian Far East, Japan and the Korean Peninsula and is currently widely distributed in Heilongjiang, Jilin, Liaoning, Shaanxi, Gansu, Xinjiang and Qinghai provinces/regions of northern China (Wang 2013). Its congener, the emerald ash borer *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), comprising a serious invasive wood boring pest that also originated in eastern Asia, is similar to *A. mali* both morphologically and ecologically in that it has a univoltine life cycle and overwinters as larvae. The emerald ash borer has killed hundreds of millions of ash trees (*Fraxinus* sp., Oleaceae) in North America (Haack *et al.* 2002; Poland & McCullough 2006; Crook & Mastro 2010). It attacks branches in the canopy and the main bole of ash trees (Cappaert *et al.* 2005). Similar to other *Agrilus* species, it feeds in the cambial region on phloem and outer sapwood, forming S-shaped or irregular galleries that disrupt nutrient and water flow (Haack & Benjamin 1982; Poland *et al.*, 2015). Despite the significant pest status of *A. mali* in cultivated apples in northeastern and northern China subsequent to the 1950s, basic knowledge of its life history and population dynamics in its native range is lacking (Wang 2013). Historically, few studies have been conducted with respect to this species, and most are occurrence records (Cui *et al.* 2015), reports of damage caused (Liu *et al.* 2014a, 2014b) and management options (Niu 2000; Liu *et al.* 2016).

Research has been conducted to develop and implement integrated area-wide management for other invasive *Agrilus* species, including *A. planipennis* (McCullough *et al.* 2011; Mercader *et al.* 2011) and the gold spotted oak borer, *Agrilus auroguttatus* Schaeffer (Coleman *et al.* 2012; Lopez & Hoddle 2013). Currently, there are limited approaches to the management of *A. mali* in cultivated apple orchards, as well as in forest ecosystems. Options for controlling *A. mali* in wild apple forests mainly involve aerial insecticide spraying and pruning of infested branches. The use of insecticide has been largely unsuccessful, probably because of the mismatched timing of sprays and peak adult emergence (Liu *et al.* 2016). Spraying large infested areas could be harmful to the forest ecosystem and may result in a loss of biodiversity and ecological functions (Poland & McCullough 2006; Zhong *et al.* 2010). Thus, aerial spraying without a prudent design is not recommended for future management programmes (Liu *et al.* 2016). Moreover, pruning is both labour-intensive and expensive, and is not considered practical for large forested areas or steep mountain terrain. Therefore, alternative management options need to be explored. Natural enemies may be key agents for controlling this pest and preventing its spread, as reported for other invasive forest pests (Yang *et al.* 2014; Duan *et al.* 2015). However, natural enemies of *A. mali* have been rarely investigated where this pest occurred in China and other regions subsequent to the 1940s because it was not a serious pest in orchards (Wang 2013). After its invasion to wild apple forests in Xinjiang in the 1990s, 11 species of natural enemies on *A. mali* were reported, including Braconidae and a limited abundance of Raphidiidae, Eupelmidae, *Xorides* sp. (feeding on fourth- and fifth-instar larvae and pupae) and *Pyemotes moseri* Yu et Liang. Nevertheless, the biological control efficacy of those natural enemies in the laboratory, as well as in the field, was not clarified. This restrained the implementation of biological control in nature.

The present study aimed (i) to gain basic knowledge of the life-history traits of *A. mali* in the Yili River valley, Xinjiang, China; (ii) to investigate mortality factors for *A. mali* in infested branches; and (iii) to determine whether native natural enemies are utilizing this invasive buprestid in the invaded region. This report on the life-history traits of the pest and associated native natural enemies will provide guidance for pest and forest management options.

Materials and methods

Survey sites

Four study sites separated by at least 10 km (Jiaolesai, Saha, Balian and Alemasai) in Gongliu County, Yili River valley, Xinjiang, China, were chosen for the present study (Table 1). These four sites were representative of the dominant forest type in this area. *Malus sieversii* was the dominant tree species in these forest stands. Other tree and shrub species present at these sites included *Armeniaca vulgaris* L. (Rosales: Rosaceae), *Lonicera hispida* Pallas (Rubiales: Caprifoliaceae), *Crataegus sanguinea* Pallas (Rosales: Rosaceae) and *Berberis nummularia* Bunge (Ranales: Berberidaceae). At each site, wild apple tree density was estimated by counting trees in three randomly chosen 20-m radius plots and then converting to tree numbers per ha (Table 1). Wild apple forests are located between 900–1700 m in altitude, and are distributed mostly on shady slopes of mountain ranges (Tao *et al.* 2016).

Life history of A. mali *and the composition of its natural enemies at Jiaolesai in 2016 and 2017*

The life history of *A. mali* infesting wild apple trees in Jiaolesai was monitored over two consecutive seasons in 2016 and 2017. Jiaolesai has a large area of wild apple forest that stretches alongside the mountain range for 10 km and is less disturbed than other wild apple forest patches. Detailed surveys were conducted on a weekly basis, from late May to late September in 2016, and from late May to late October in 2017. On each sampling date, 50–100 trees with similar health status (received moderate damage by *A. mali* larvae feeding, visibly 10–20% branches dying) were sampled, and two infested branches with visible scars and brown liquid exudates presumably caused by pest infestation were cut. As a result, 100–200 infested branches were cut and transported to a nearby field station. These branches were then dissected to locate and record *A. mali* larvae, pupae and pharate (pre-emergence) adults. Larvae were recorded as young (first, second and third instar) or old (fourth and fifth instar) according to body size (head capsule width ≤ 0.35 mm for young larvae, and > 0.35 for old larvae) based on Wang (2013).

Table 1 The four sites used for surveying *Agrilus mali* and its native natural enemies in wild apple forest in Gongliu county, Yili River valley, Xinjiang, China

Site	Altitude (m a.s.l.)	Location	Average wild apple tree density per one ha
Saha	1291	43.258°N, 82.854°E	120
Jiaolesai	1325	43.232°N, 82.778°E	180
Balian	1316	43.250°N, 82.854°E	230
Alemasai	1302	43.241°N, 82.840°E	280

For each branch, the length, basal diameter and diameter of the cross-section where infestation occurred were measured.

Mortality of larvae and pupae were recorded at the same time when branches were dissected. We aimed to identify the biological agents causing mortality. Mortality factors were classified into four categories: (i) killed by unknown microorganisms based on soft and watery body (see Supporting information, Fig. 1A); (ii) parasitized by Braconidae species (see Supporting information, Fig. 1B); (iii) parasitized by mites (Pyemontidae) (see Supporting information, Fig. 1C); and (iv) killed by unknown biological factors (see Supporting information, Fig. 1D). We then calculated the cumulative larval mortality caused by each factor. We also compared the percentage of developmental stages and mortality factors between 2016 and 2017.

To investigate the parasitoids of *A. mali*, another 150 infested branches were sampled each year and enclosed in net cages ($10 \times 10 \times 10$ cm; mesh size of 1 mm) in a shaded place at the field station to avoid excessive heat as a result of direct sunshine until the parasitoids emerged. These cages were visually checked weekly, and the emerging parasitoids were then collected and identified to species by taxonomists in China (individuals that impossible to identify to species were identified to genus level). (Sheng 1990; Wang *et al.* 2009; Petersen-Silva *et al.* 2012). No natural enemy species have been introduced for *A. mali* in this region and, as a result, any natural enemies recorded are considered native to this area, although it is possible that some may have been introduced together with *A. mali*.

To determine the life span of adult *A. mali*, pupae dissected from branches were placed in cages ($10 \times 10 \times 10$ cm; mesh size of 1 mm) in mid-July. Newly-emerged adults were gathered, and sexual distinction was achieved by reference to males having a longer and erect pubescence on the medial part of the prosternum and prosternal process. Then, all adults were transferred to cages immediately. Each cage contained five adults of the same sex. To address the importance of adult nutrition from supplementary feeding, caged adults were either provided with fresh wild apple leaves daily or with no foliage (controls). An unpaired *t*-test was used to compare the difference between males and females, as well as fed and unfed groups. There were six replicates of each treatment for each sex. All of the cages were held under natural field conditions, as well as in shade to moderate temperature (lower than 30 °C) inside the cages. The lifespan of each adult (time to death) was recorded for each treatment. Additionally,

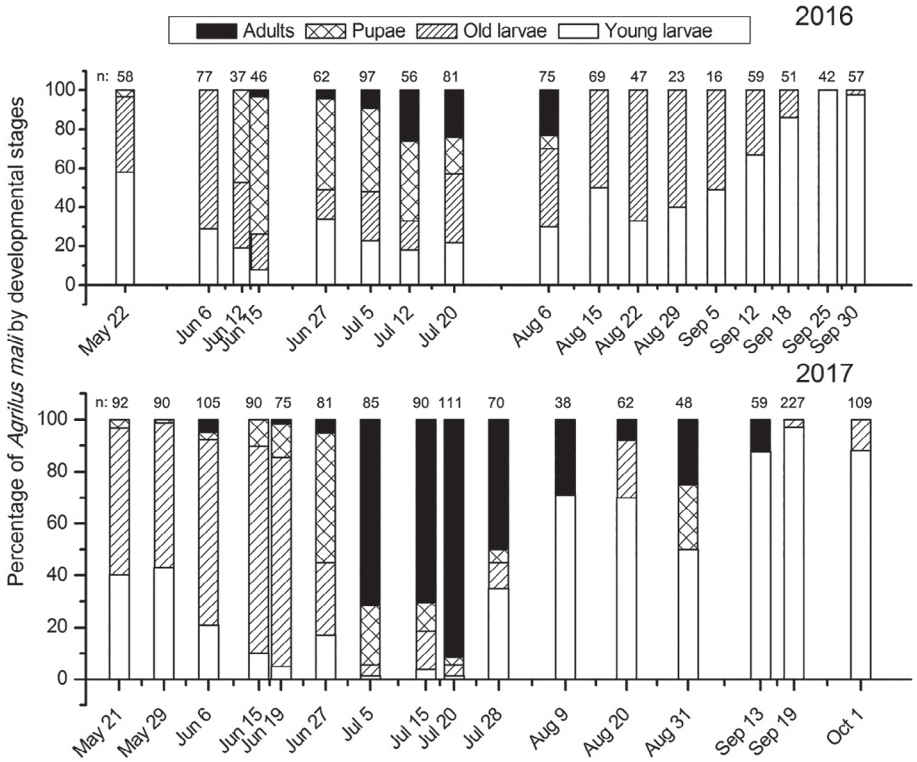

Figure 1 Age structure of *Agrilus mali* (young larvae, old larvae, pupae and adults) recorded on each sampling date at Jiaolesai, Yili River valley, Xinjiang China (2016–2017). The numbers of *A. mali* sampled on each date are shown above each bar.

adult behaviours were observed visually in the field from 09.00 h to 17.00 h daily in the natural forest from 5 July to 15 July in 2017 because the adults are most active during this period (Sun *et al.* 1979). Five trees that were frequently observed to have adults moving along branches and flying around were chosen as the observation units. One observer stood under each tree to record adult feeding, flying, and occasionally mating and oviposition for approximately 1 min at intervals of 15 min.

Comparative survey of A. mali *development and the composition of natural enemies at four sites (Jiaolesai, Saha, Balian and Alemasai) in 2017*

To investigate differences in the development of *A. mali* and associated biological mortality factors in different forest stands, a comparative survey was carried out at Jiaolesai, Saha, Balian and Alemasai. Surveys were conducted in summer (5 and 20 June, 5 and 20 July) and autumn (3 October) of 2017. The survey at these four sites followed the same protocols for sampling and dissecting infested branches as described above, with 50–100 trees being sampled and 100–200 infested branches being collected at each site on each date. The development of *A. mali* was calculated with respect to the percentage of developmental stages, and the composition of natural enemies was represented by the percentage of mortality that they caused. Then, we compared these results among four sites using one-way analysis of variance. All of the analysis performed were using ORIGINPRO, version 9.0 (OriginLab Corporation, Northampton, Massachusetts).

Emergence phenology of A. mali *adults and its parasitoid* Atanycolus denigrator *at Jiaolesai in 2016*

The emergence of adult *A. mali* and natural enemies was assessed using field cages. Infested branches ($n = 158$) were pruned randomly from an unmanaged orchard of over 200 domesticated apple trees at the end of May, 2016. This orchard (43°27′06.8″N, 82°09′26.93″E, altitude 806 m a.s.l.) was adjacent to the wild apple forest and had been abandoned subsequent to 2001 because of the high levels of damage by *A. mali*. The infested branches were cut into short sections with approximately the same curve length (less than 90 cm) and kept in nylon screen cages (1 × 1 × 1 m) at a field station near Gongliu County (43°15′16.26″N, 82°49′20.40″E, altitude 1193 m a.s.l.) under natural conditions until no *A. mali* or parasitoid adults emerged for 10 consecutive days. Emerged adult *A. mali* and natural enemies were collected and recorded daily. After emergence was complete, branches were dissected and checked for dead adults on 7 September 2016.

Results

Detailed life history of A. mali *in Jiaolesai*

Adults were observed feeding on wild apple leaves and flying between branches in the tree canopies. Females deposited eggs inside bark cracks and crevices of tree branches. When beetles were provided with fresh foliage, adult females lived significantly longer ($t = -2.46$, d.f. = 8, $P < 0.05$), on average 56.8 ± 4.5 days (mean ± SE, $n = 5$), whereas male lifespan was 32.7 ± 8.7 days (mean ± SE, $n = 5$). Conversely, without the provision of fresh leaves, the life span of both females and males was less than 5 days on average (males: 3.3 ± 0.6 ays; females: 4.9 ± 1.1 days, mean ± SE, $n = 5$ for males and females, respectively) and there was no significant difference between females and males ($t = -1.32$, d.f. = 8, $P > 0.05$). The adult life span of *A. mali* (average of females and males) was significantly different between fed and unfed groups ($t = 6.58$, d.f. = 18, $P < 0.001$).

In 2016 and 2017, after hatching, larvae developed through five instars and each larva created a gallery. Larvae developed within branches with diameters of 1–10 cm. Young larvae (first, second and third instar) fed on the outer bark and phloem. Old larvae (fourth and fifth instar) burrowed into the sapwood, became J-shaped larvae and pupated there during May to June. Adults emerged through D-shaped exit holes in late June until the end of August. Larvae of the next generation were observed in early August (Fig. 1) and they were heavily attacked by natural enemies (Fig. 2). Most larvae remained as first- and second-instar larvae by late-October, and overwintered as young larvae after the end of October (Table 2). A few old larvae were found after mid-September. By contrast, old larvae were dominant during the next spring, and the percentage of old larvae increased to 40–60% as the temperature increased in the spring and early summer (Fig. 1).

Native natural enemies for A. mali *larvae in Jiaolesai*

In total, eight species of parasitic wasps were recorded, including *A. denigrator* (L.) (Hymenoptera: Braconidae), *Eupelmus* sp. (Hymenoptera: Eupelmidae), two species of *Doryctes* spp. (Hymenoptera: Braconidae), *Pareucorystes varinervis* Tobias (Hymenoptera: Braconidae), *Polystenus rugosus* Foerster (Hymenoptera: Braconidae), *Spathius generosus* (Wilkinson) (Hymenoptera: Braconidae) and an unidentified species of *Spathius*. Parasitism of *A. mali* by *A. denigrator*, a larval and pupal ectoparasitoid, accounted for 95% of all braconid parasitism in the field based on our cage experiment for identifying insects. In total, 107 individuals out of 2385 *A. mali* were parasitized by a mite identified as *P. moseri* Yu et Liang (Acari: Pyemotidae), with typically 10–30 individual mites per host. In addition, some unknown microorganisms that presumed to be fungal entomopathogens were found causing mortality of *A. mali* larvae and pupae, especially in autumn (Fig. 2).

Based on the dissection of branches in two consecutive years, larval mortality caused by braconids became obvious in early June and increased gradually to high levels in August and September (Fig. 2). Cumulative larval mortality caused by natural enemies ranged from 20% to 80% from early-mid July to the end of September. The braconid species *A. denigrator*, together with an unknown fungal entomopathogen, was responsible for 30–40% larval mortality during mid-June to the end of September. Mortality from the parasitic mite (*P. moseri*) peaked at 21% in mid-August 2016 and 13% in 2017. The average larval mortality of *A. mali* for all factors pooled was over 30% each year (Table 2).

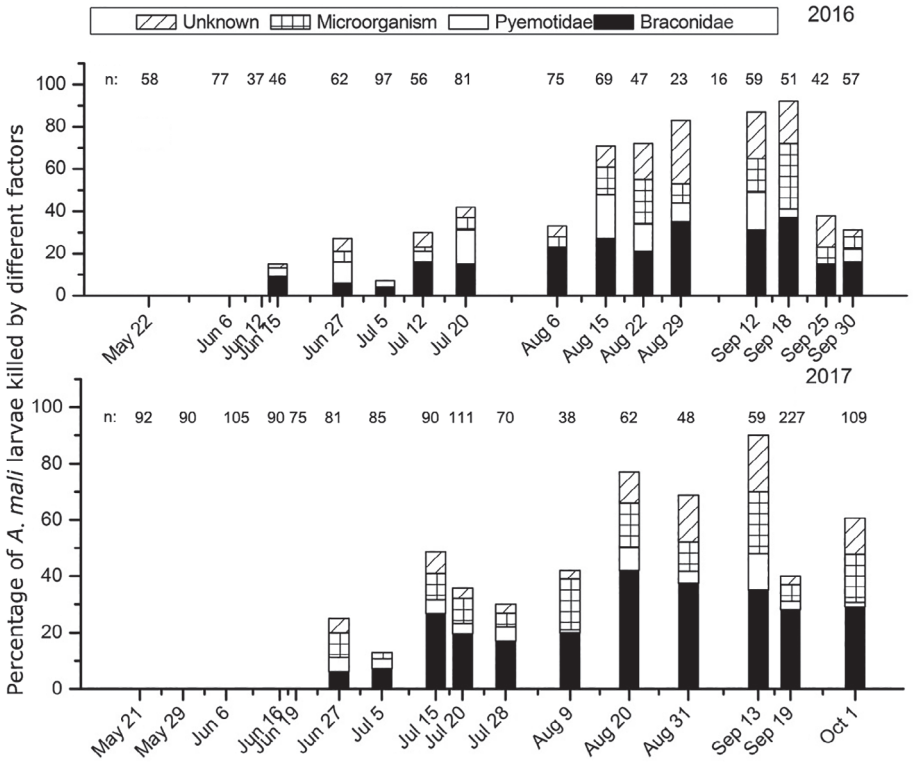

Figure 2 Larval mortality of *Agrilus mali* caused by different factors in wild apple forest at Jiaolesai, Yili River valley, Xinjiang, China (2016–2017). The numbers of *A. mali* sampled on each date are shown above each bar.

Table 2 Annual-mortality (%) of *Agriuls mali* caused by biotic agents across sample sites in Jiaolesai, Yili River valley, Xinjiang, China (2016–2017)

Mortality agent	*Agrilus mali* mortality in 2016 (%)	*Agrilus mali* mortality in 2017(%)
Braconidae	13.6	18.4
Pyemotidae	6.3	3.7
Microorganism	6.1	7.3
Unknown	6.8	5
Total	32.8	34.4

The number of insects sampled was 953 and 1432 individuals in 2016 and 2017, respectively.

Comparison of life-history traits of A. mali *and natural enemies in different locations*

Life history and larval mortality of *A. mali* were similar among the four sites in 2017 ($F = 0.06$, d.f. $= 3$, $P > 0.05$) (Fig. 3). *Atanycolus denigrator* was the dominant natural enemy at all four sites from early July to early October, with an average parasitism rate of $20.26\% \pm 3.01\%$ (mean $\pm$ SE, $n = 12$).

Emergence phenology of A. mali *adults and native natural enemies*

In 2016, *Agrilus mali* adults in the caged branches began to emerge in early June, and were present until 15 August (Fig. 4), peaking at the end of June (85 adults on 22 June) (Fig. 4). Overall, 1116 *A. mali* adults emerged and were captured in the cages, and 209 dead adults were dissected from the branches on 7 September 2016 after emergence was complete. Adults of the parasitic wasp *A. denigrator* were first collected on 25 June, peaked on 9 July and persisted until 6 August. Overall, only 41 *A. denigrator* adults emerged in our cage experiment.

Discussion

We found that *A. mali* has a univoltine life cycle in the Yili River valley, Xinjiang and overwinters as young larvae in galleries beneath the bark of wild apple branches. The staggered presence of developmental stages of *A. mali* throughout the year is likely a result of the extended period of adult activity and the oviposition behaviour of females. Adult females can lay 60–70 eggs intermittently over a period greater than 1 month (Wang 2013) and such prolonged oviposition shapes the pattern of progeny development. Similarly, Haavik *et al.* (2013) reported staggered developmental stages throughout the year, as well as a corresponding prolonged adult flight period from May to mid-October for a sibling species, the gold-spotted oak borer. Another notorious invasive species, the emerald ash borer *A. planipennis*, also has a prolonged adult emergence period from early May to early August and staggered life stages throughout the year (Herms & McCullough 2014), although it can require 2 years to complete development (Cappaert *et al.* 2005). In the present study, the peak emergence of adults in cages was earlier

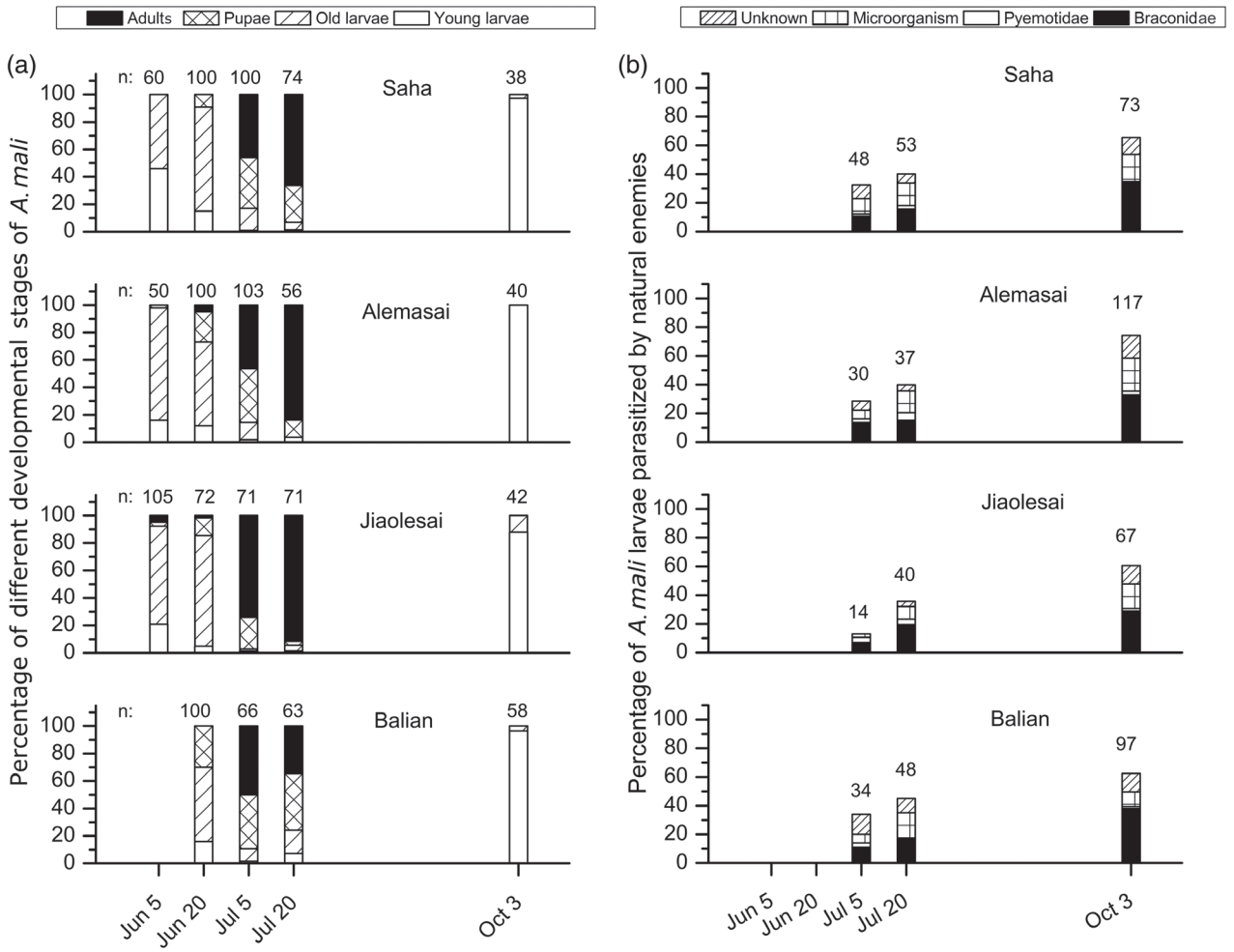

Figure 3 (A) Comparative survey of *Agrilus mali* age structure at four sites: Jiaolesai, Saha, Balian and Alemasai in the Yili River valley, Xinjiang, China (2017). The numbers of *A. mali* sampled on each date are shown above each bar. No samples were collected on 5 June in Balian because of rain. (B) Comparative survey of mortality by natural enemies at four sites: Jiaolesai, Saha, Balian and Alemasai in the Yili River valley, Xinjiang, China (2017). The numbers of *Agrilus mali* sampled on each date are shown above each bar. No samples were collected on 5 June in Balian because of rain.

than peak emergence based on branch dissection. This difference might be a result of the microclimate within cages compared with ambient conditions at the field site. Microenvironment can influence insect development and population dynamics (Willmer 1982), as shown by the emerald ash borer (Duan *et al.* 2013).

Provision of fresh host leaves for adult feeding is crucial for the lifespan of *A. mali*. Adults lived for 45 days (average lifespan of males and females) when provided host foliage, which significantly differs from the adult lifespan without access to leaves (less than 5 days for both sexes). The results of the present study are in accordance those reported by Li *et al.* (2017), who found that *A. mali* adults required nutrition for survival and persisted for up to 20 days in Shaanxi province, China, when provided fresh leaves, although the difference in adult longevity under natural conditions between the two studies conducted in Xinjiang and Shaanxi is not clear.

The performance of *A. mali* is complex in mountainous area. The temperature in different altitudes will determine the development of *A. mali*. The infestation of *A. mali* reduced the water availability of *Malus sieversii* tree (Z.Z.Lu, unpublished data). Low water availability increased larval abundance and mean larval mass of emerald ash borer, especially in Manchurian ash compared with black ash *Fraxinus nigra* Marshall, a more susceptible ash native to North America (Chakraborty *et al.* 2014). The water content change after *A. mali* infestation may also disturb the performance of an insect. Accordingly, temperature differentiation in altitude and water availability might influence the performance of *A. mali*. Consequently, this will influence the life-history traits of *A. mali* in wild apple forest, and so pesticide spraying at different altitudes should be considered carefully and practically.

Latitude determines insect voltinism (Coates *et al.* 2004). Emerald ash borer generally has a 2-year life cycle in Harbin, China, and Moscow, Russia, whereas, it is typically univoltine further south in Tianjing, China, and in many northern states in the U.S.A. (Wang *et al.* 2005; Orlova-Bienkowskaja & Bienkowski 2016). However, the life cycle of *A. mali* reportedly has different patterns in various locations of China, although

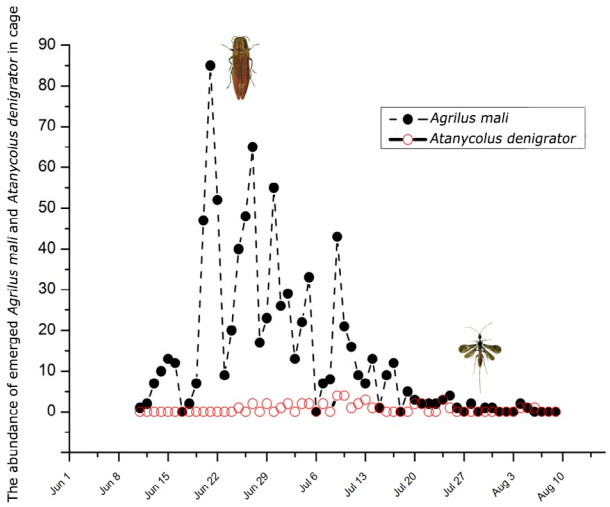

Figure 4 Emergence phenology of *Agrilus mali* adults and the parasitoid *Atanycolus denigrator* adults from infested wild apple branch sections held within a cage under natural field conditions in Jiaolesai, Yili River valley, Xinjiang, China (2016). [Colour figure can be viewed at wileyonlinelibrary.com].

controversial issues remain among studies (Li & Zhang 2017). Although our preliminary results indicated that *A. mali* overwinter as young larvae in their galleries, it is unknown whether *A. mali* pupae can overwinter in Xinjiang. It is also unknown whether *A. mali* requires 2 years to complete development in colder climates such as in northeast or northwest China. Whether the univoltine and semivoltine population of *A. mali* in China can co-occur also remains to be clarified.

Mortality rates of *A. mali* larvae from natural enemies averaged more than 30% per year, with most mortality being observed from mid-June to October in both years of the present study (Fig. 2 and Table 2). During this time, larval mortality of *A. mali* from natural enemies reached almost 60% in both years in Jiaolesai (Fig. 2) and was similar across the other three sites in 2017 (Fig. 3). Similarly, Wang (2013) reported that mortality of *A. mali* by natural enemies was more than 65% in the field in different years and different locations in the Yili River valley, Xinjiang. However, Wang (2013) neither considered parasitic mites, nor annual mortality fluctuation. Native natural enemies may serve as effective biological control agents for this invasive beetle species in wild apple forests. The outbreak of *Spathius polonicus* Niezabitowski (Hymenoptera: Braconidae), a native natural enemy of *A. planipennis* in European Russia, has significantly suppressed *A. planipennis* (Orlova-Bienkowskaja & Belokobylskij 2014). Parasitoids have been released for the management of emerald ash borers in the U.S.A. (Duan *et al.* 2017). A promising candidate for controlling *A. planipennis* in North America is the native parasitoid *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae) (Roscoe *et al.* 2016a, 2016b) and it has been suggested that *P. sulcata* should be released as pupae near the pest-infested trees to achieve a better efficacy and potential biological control success (Gaudon *et al.* 2018). Of the various natural enemies in our study area, *A. denigrator* was the dominant parasitoid from early May to mid-October in both 2016 and 2017. This wasp is widely distributed in Europe, Mongolia, Russia, central Asia and China (Wang *et al.* 2009; Wang, 2013); hence, it may be a good candidate for augmentative biological control of *A. mali*. However, *A. denigrator* is an opportunistic, generalist parasitoid of wood-boring insects and may not be effective for regulating populations of *A. mali*, especially when densities are low. Application of this braconid to control the apple buprestid could be challenging because it is difficult to rear in mass production as a result of the lack of commercially-available alternative hosts. The mite *P. moseri* may be an alternative option for augmentative biological control of *A. mali* because of its broad host range, allowing it to be mass-reared with good fitness on many alternative hosts (Kim *et al.* 2015). However, a broad host range also makes the natural enemy ineffective when attacking one specific pest species. Furthermore, natural enemies with broad host ranges may pose threats to nontarget species; therefore, suitable host-range testing would be necessary. Mass production and augmentative releases of natural enemies could potentially provide biological control of *A. mali* and contribute to the integrated management of this pest.

Little is known about the natural enemies of *A. mali* in its original distribution range of the Russian Far East, Japan and Korea. Exploration for co-evolved or specialized natural enemies for biological control may improve the management of *A. mali*, especially at early stages of invasion or at low population levels. In the U.S.A., emerald ash borer larval mortality as a result of parasitism by native natural enemies, including *Atanycolus* spp., was high during outbreak periods (Duan *et al.* 2014, 2015); however, the effectiveness of parasitoids fluctuated with host population densities. When emerald ash borer infestation levels declined, parasitism by generalist native natural enemies that opportunistically parasitized emerald ash borer also declined. By contrast to generalist parasitoids, generalist predators such as woodpeckers contributed a steady predation rate (Duan *et al.* 2015). A specialist parasitoid *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) was introduced from China as a biological control agent when densities of emerald ash borer were increasing. This parasitoid soon became widely established, killing 36–85% of late-instar larvae (Liu *et al.* 2016). The mortality of emerald ash borer caused by *T. planipennisi* increased even after its population collapsed, which suggested a long-lasting effect of this specialist natural enemy (Duan *et al.* 2017).

The present study has several shortcomings. First, we did not examine eggs of *A. mali* because of difficulties relating to screening for eggs in the field. Some egg parasitoids, including *Ooencyrtus* sp., *Coccidencyrtus* sp. and *Oobius* sp. (Hymenoptera: Encyrtidae), can cause high rates of mortality to eggs of *Agrilus* spp. (Taylor *et al.* 2012; Abell *et al.* 2014; Triapitsyn *et al.* 2015). Future studies on egg parasitoids are planned because they have potential application in the management of *A. mali*, which will help the recovery of wild apple forest ecosystem. Second, we included *A. mali* mortality caused by host tree resistance in the 'unknown biotic factors' category in the present study, which is one of the most important biotic mortality factors of emerald ash borer larvae and this should be quantified independently in further studies (Duan *et al.* 2010). Therefore, germplasm of *Malus* sp., which is potentially resistant to the apple buprestid, should be assessed and implemented in apple breeding programmes to provide alternative methods to meet the future challenges of this invasive pest.

Concerning the efficacy of management for such a devastating pest, key information lies within the life history traits of *A. mali*. In two consecutive years, larvae developed from early August to mid-June during the next year. Therefore, environmentally-safe insecticides could be injected into the trunks of apple trees from mid-June to mid-August, enabling the insecticides to be translocated to branches before larval development is initiated in early August. Moreover, infested branches can be pruned and destroyed in late autumn or early winter to reduce pest populations. Aerial insecticide sprays that target apple buprestid adults may be challenging as a result of the long duration of adult emergence (more than 2 months), resulting in a broad treatment window requiring multiple sprays, higher costs and negative impacts on nontarget species. Aerial insecticide sprays should be implemented during the peak of adult flight based on phenological models or growing degree-days, and the spray timing should match the climatic conditions of different regions. The results of the present study provide information on the life history and development of *A. mali* and its native natural enemies that will aid in the development of integrated management programmes for domesticated apple orchards and the conservation of wild apple forests.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. The mortality factors for *Agrilus mali*: (A) killed by microorganisms based on a soft and watery body, (B) parasitized by Braconidae species, (C) parasitized by mites and (D) killed by an unknown factor.

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