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Seasonal abundance and development of the Asian longhorned beetle and natural enemy prevalence in different forest types in China

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HIGHLIGHTS

- ALB abundance was significantly lower in urban forest than in rural forest.
- Prevalence of *D. helophoroides* did not differ between Anci and Tangerli.
- *D. helophoroides* parasitism differed among sampling dates and peaked in June.
- Mean *D. helophoroides* clutch size was 5.67 ± 0.37 parasitoids/host.
- Local epizootic of *B. bassiana* at Tangerli resulted in 2.4% infection rate.

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ABSTRACT

Seasonal abundance and population development of the Asian longhorned beetle (ALB), *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), and prevalence of its natural enemies were studied on Hankow willow (*Salix matsudana* Koidz.) at an urban forest site (Anci) and a rural forest site (Tangerli) in Hebei province in northern China from June to October, 2002. Overall, ALB abundance was significantly lower in the urban forest in Anci than in the rural forest in Tangerli. Population density ranged from 0 to 1.0 pupae/branch, 0.1 to 0.7 adults/tree, 0.1 to 9.2 eggs/branch, and 5.9 to 18.4 larvae/branch at Anci, and 0.1 to 0.9 pupae/branch, 0.1 to 1.6 adults/tree, 0 to 22.9 eggs/branch, and 5.0 to 34.1 larvae/branch at Tangerli, respectively. A significant effect of sampling date was observed for all ALB life stages. Significantly fewer 3rd and 4th size class larvae were found in the urban forest at Anci compared to the rural forest at Tangerli. Prevalence of the gregarious larval-pupal ectoparasitoid *Dastarcus helophoroides* (Fairmaire) (= *D. longulus*) (Coleoptera: Bothrideridae) did not differ between Anci and Tangerli, but percent parasitism differed among sampling dates and peaked in June at 32.8 ± 7.4% at Anci and 12.0 ± 3.5% at Tangerli. Parasitism was lowest in August in Tangerli and September in Anci when most ALB larvae were at an early stage of development. The mean *D. helophoroides* clutch size was 5.67 ± 0.37 parasitoids per ALB larva or pupa and did not differ between the two sites or among sample dates. Similar ALB population densities and *D. helophoroides* parasitism were also found at two supplemental study sites in Tianjin city (Yangcun and Xiaoxitian) where samples were only taken in June 2002. A localized epizootic of *B. bassiana* was observed on immature stages of ALB at Tangerli in July, resulting in an average infection rate of 2.4%. Differences in ALB population development and natural enemy prevalence between urban and rural forest site, and the potential of *D. helophoroides* and other natural enemies in the management of *A. glabripennis* are discussed.

1. Introduction

Urban trees tend to harbor more herbivorous arthropods than rural trees (Hanks and Denno, 1993; Speight et al., 1998; Raupp et al., 2010). Both abiotic (e.g., temperature) and biotic (e.g., predation and parasitism) factors are thought to contribute to elevated urban pest abundance (Dale and Frank, 2014; Frankie and Ehler,

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1978; Hairston et al., 1960; Kahn and Cornell, 1989; Meineke et al., 2013; Raupp et al., 2010). Warmer temperatures in urban habitats as a result of the "heat-island" effect increases survival and fitness of arthropods through the decrease in developmental time (Kozlowski, 1992; Berger et al., 2008). In addition, reduced vegetation complexity in urban areas generally leads to decreased regulation by natural enemies (Root, 1973; Letourneau, 1987).

The Asian longhorned beetle (ALB), *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) is a wood-boring pest of poplars (*Populus* spp.), willows (*Salix* spp.), elms (*Ulmus* spp.) and other deciduous trees in its native range of China and Korea (Lingafelter and Hoebeke, 2002; Yan, 1985; Yan and Qin, 1992). In China, its pest status was not established until the early 1980s when the first outbreaks were reported in the eastern part of the country. Those outbreaks were linked to major afforestation and reforestation efforts in the rural areas during the 1960s when a single poplar species, *Populus dakuanensis* Hsu was used for most plantings (Yan, 1985). The initiation and implementation of the Three-North Shelterbelt Forest Program in 1978 in China to combat desertification and reduce soil erosion led ALB to further expand its range to include north, northeast, and northwest China. Widespread tree mortality was reported from several northern provinces (e.g., Ningxia and Inner Mongolia) where susceptible poplar species were planted, with millions of trees being infested and destroyed (Gao and Li, 2001; Huang and Luo, 1991; Luo and Li, 1999; Pan, 2005). By the early 2000s, ALB was reported in all provinces on mainland China (SFA-GFPCS, 2008). See review by Haack et al. (2010) for a worldwide perspective on the management of this pest.

ALB was first discovered in North America on urban shade trees in New York in 1996 (Cavey et al., 1998; Haack et al., 1996, 1997). Since then, additional infestations were found in Illinois (1998), New Jersey (2002), Ontario (2003), Massachusetts (2008), and Ohio (2011) (CFIA, 2016; Dodds and Orwig, 2011; Haack, 2006, 2016; Hu et al., 2009; Meng et al., 2015; Poland et al., 1998; USDA-APHIS, 2016a). ALB also found its way to Europe, with the first record from Austria in 2001, and is now also known in Belgium, Finland, France, Germany, Italy, the Netherlands, Switzerland, and the United Kingdom (EPPO, 2013; Eyre and Haack, 2016; Meng et al., 2015). Regulatory agencies continue in their efforts to eradicate ALB outside its native range. A typical eradication program consists of pest survey (preliminary and secondary), regulation, eradication (tree removal and chemical treatment), and public education (Haack et al., 2010; USDA-APHIS, 2014).

ALB is a large, polyphagous cerambycid with more than 100 host species. Major tree hosts in China include species of poplar (*Populus* spp.), willow (*Salix* spp.), elm (*Ulmus* spp.), and maple (*Acer* spp.) (Hu et al., 2009). In North America, it prefers maples (*Acer* spp.), horse chestnut (*Aesculus hippocastanum* L.), birches (*Betula* spp.), willows, and elms (American, Siberian, and Chinese) (Haack et al., 1997; Wang, 2015). Tunneling by its larvae under the bark and inside wood girdles the trunks and large branches. Repeated attacks lead to crown dieback, structural weakness of trunks, stems and branches, and eventual death of the trees. Left unchecked, its potential impact to lumber, maple syrup, nursery, commercial fruits, and tourism industries in the U. S. could reach $669 billion, with a tree mortality of 30% and a total canopy reduction of 35% (Nowak et al., 2001).

In China, ALB has a 1- or 2-yr life cycle depending on host condition, geographic location, and time of egg deposition. Adults typically begin emerging in May and peak in early July, and females deposit their eggs in oviposition pits chewed in the outer bark of branches and main trunks of host trees between June and July (Yan and Qin, 1992). Eggs hatch in ca. 10 d. Newly hatched larvae begin feeding on bark and phloem, excreting brown frass from the feeding galleries through the oviposition sites. Second-instar larvae begin feeding on healthy phloem and outer sapwood and expel brown frass. By the third- and fourth instar, larvae enter the inner sapwood and heartwood and produce white frass. Larvae overwinter in their galleries in the sapwood and heartwood, and pupate inside the wood in spring and early summer. In the field, adults may survive >50 d, and each female deposits an average of 32 eggs in her lifetime (Yan and Qin, 1992). Due to the overlapping and asynchronous nature of its life cycle, ALB can overwinter as eggs, larvae, or pupae in the field (Yan and Qin, 1992).

No ALB egg parasitoids have been reported from China. However, several species of ectoparasitoid attack ALB larvae or pupae in the field in China, including *Dastarcus helophoroides* (Fairmaire) (Coleoptera: Bothrideridae) (= *Dastarcus longulus* Sharp (Coleoptera: Colydiidae)) (Gao and Qin, 1992; Huang et al., 2008; Li et al., 2007; Ogura et al., 1999; Qin and Gao, 1988; Tang et al., 1996), *Scleroderma guani* Xiao et Wu (Hymenoptera: Bethylidae) (Cheng et al., 2003; Wang et al., 1999a,b; Zhang and Tian, 1992), and *Iphiaulax impostor* (Scopoli) (Hymenoptera: Braconidae) (Tang et al., 1996). Fungal pathogens such as *Beauveria bassiana* (Balsamo) Vuilemin, *Isaria farinosa* (Holmskjold) Fries (= *Paecilomyces farinosus* (Holm ex SF Gary) Brown & Smith) (Hypocreales: Clavicipitaceae) were also found infecting ALB larvae and pupae (Wang et al., 1990, 1997; Zhang et al., 1999a).

Dastarcus helophoroides, a gregarious ectoparasitoid of ALB larvae and pupae, is the most abundant parasitoid of ALB in the field in China (Qin and Gao, 1988; Wei et al., 2007; Yan and Qin, 1992). Eggs of this parasitoid are deposited in rows or clusters in the frass of ALB larvae inside their galleries, or directly on larval gallery walls. Newly hatched *D. helophoroides* larvae actively seek hosts in the galleries. When successful, they chew through the host cuticle at the intersegmental membrane or beneath the elytra and insert their heads inside the host body to feed inside. ALB late-instar larvae and pupae are usually attacked. Feeding *D. helophoroides* larvae become legless later as they develop (Gao and Qin, 1992; Qin and Gao, 1988).

Other potential parasitoids of ALB include *Scleroderma sichuanensis* Xiao, a parasitoid primarily of the citrus longhorned borer, *Anoplophora chinensis* (Forester) in China (Kaishu, 2006). In Italy, a gregarious endoparasitoid, *Aprostocetus anoplophorae* Delvare (Hymenoptera: Eulophidae), was identified from field-collected *A. chinensis* eggs (Delvare et al., 2004). However, results from one experiment showed that it failed to attack ALB eggs in the field (Hérard et al., 2005). Hymenopteran parasitoids such as *Ontsira palliates* (Enderlein) (Braconidae) and *Zombrus bicolor* Cameron (Braconidae) from China, and *Spathius erythrocephalus* Wesmael (Braconidae) and *Trigonoderus princeps* (Westwood) (Pteromalidae) from Italy may also have potential in future biological control of ALB because they attack closely related hosts in the field (Smith et al., 2008; Hérard et al., 2013).

Trees in urban areas, especially street trees, endure additional environmental stresses compared to those in rural forests, including soil compaction, limited root volume, confined surface area, reduced water potential, and air pollution (Quigley, 2004). These urban conditions are known to have negative effects on tree health as they promote outbreaks of phytophagous insects and fungi (Mattson and Haack, 1987; Wargo, 1996). Urbanization in China is more rapid than in other countries due to rapid economic growth (United Nations, 2014). In addition, urban forests in China are usually less diverse than rural forests with fewer species. In a study of three major urban forest types in Southern China, roadside trees were found to have the lowest species diversity compared to urban parks and institutional plantings. Roadside trees also had the most significant dominance by a small subset of common species with 75.6% of the trees belonging to the dominant group of only 12 species (Jim and Liu, 2001). Similarly, in Beijing, the urban forest was found to be dominated by a few common species with only 10 species comprising 77% of all trees, and the top two species, *Sophora japonica* and *Populus tomentosa*, comprising 37% of urban trees (Yang et al., 2005).

The objectives of this study were to examine the seasonal abundance and development of ALB in different forest types (urban vs. rural), prevalence of its natural enemies, and document the development of the larval-pupal ectoparasitoid *D. helophoroides* at study sites in both urban and rural forests in northern China. We hypothesized that ALB population levels would be higher whereas prevalence of natural enemies and parasitism rates would be lower at the urban site compared to the rural site. Implications for the potential of the natural enemies in the suppression of ALB populations in North American forests are discussed.

2. Materials and methods

2.1. Study sites

A total of four sites with ALB-infested Hankow willows (*Salix matsudana* Koidz.) were selected for this study, including two primary sites in Hebei province: Anci an urban site in Langfang city (39.52N, 116.70E) and Tangerli a rural site in Bazhou county (39.07N, 115.22E), and two supplemental sites in Tianjin city: Xiaoxitian an urban site (39.22N, 117.40E) and Yangcun a rural site (39.38N, 117.10E) (Fig. 1, Table 1). The average annual temperature at Anci is 11.9 °C based on data from 1971 to 2000 (baike.baidu.com/view/14861.htm), and the average annual temperature for the same time period at Tangerli is 11.4 °C (baike.baidu.com/view/68142.htm#3). The trees sampled in this study were planted 15–30 years earlier and varied in diameter, height, and health (Table 1). Sample trees at the primary study sites were of similar age (20 yr), however, those at Anci were planted along city streets and displayed symptoms of stress, whereas those at Tangerli were planted as a windbreak along agricultural fields and showed no signs of stress (Table 1). At the supplemental study sites, we sampled older stressed trees from an urban forest belt at Xiaoxitian and healthy younger trees from a monoculture plantation at Yangcun (Table 1). The stressed trees showed signs of reduced growth (both in diameter and height), crown thinning, and branch dieback (Table 1).

2.2. Seasonal abundance of ALB

The population density of ALB was estimated by sampling infested willow trees at the two primary sites in Hebei province (Anci and Tangerli) and the two supplemental sites in Tianjin City

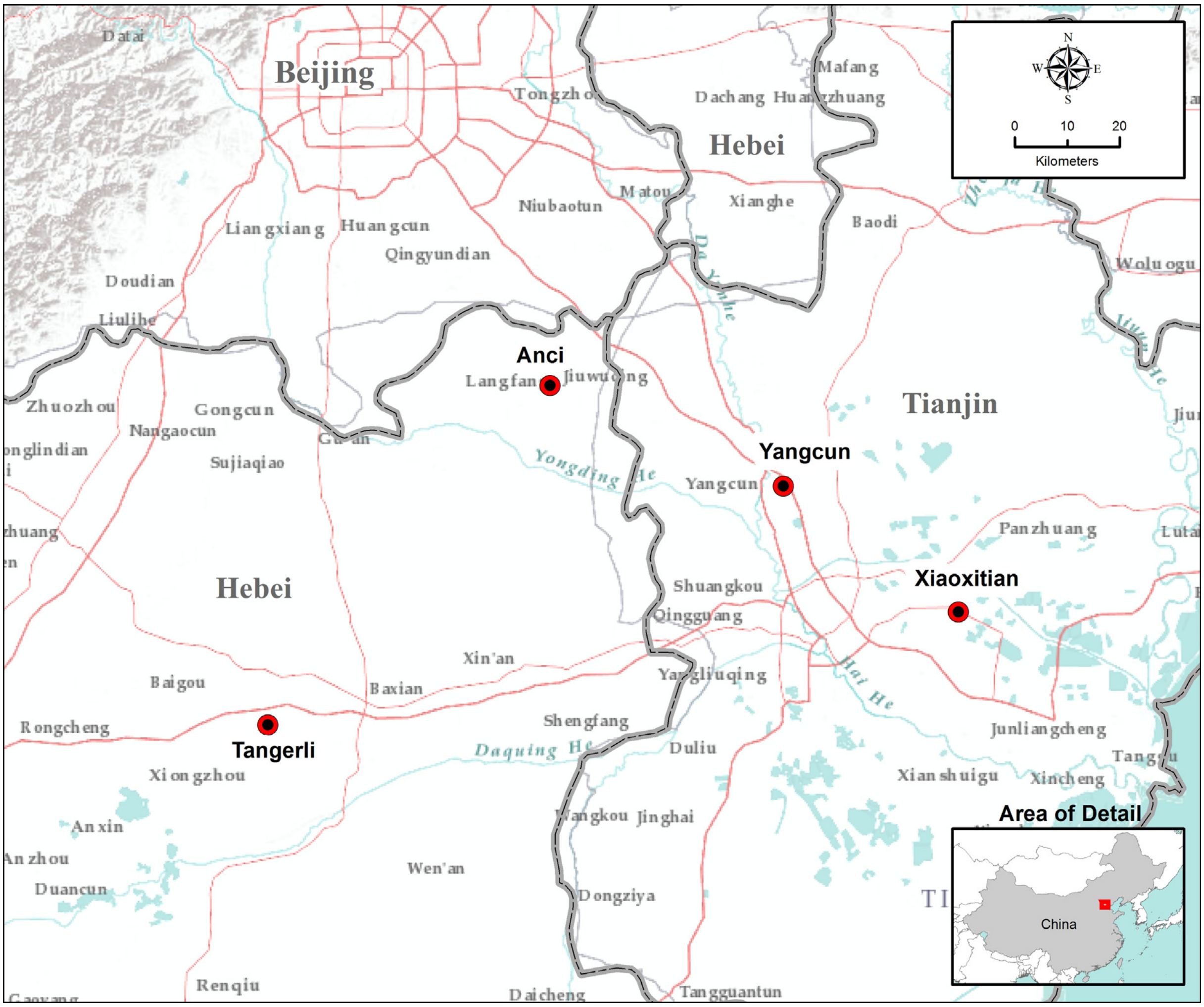

Fig. 1. The location of study sites in northern China where Hankow willow were sampled for ALB in 2002 are shown as red and black dots. The two primary sites are located in Hebei province (Anci and Tangerli) and two supplemental sites in Tianjin city (Yangcun and Xiaoxitian). Service layer credits: United States National Park Service. Map created by United States Department of Agriculture, Forest Service, Northeastern Area State and Private Forestry, Office of Knowledge Management (Durham, New Hampshire, United States of America). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Study sites in Hankow willow forests in Hebei and Tianjin in China, 2002.

Location	Forest type	Age (yr)	DBH (cm)	Height (m)	Description	Tree health [a]
Anci, Langfang, Hebei province	Urban	20	20	4.0	Street trees planted along paved sidewalks, no grass or bushes underneath	Stressed
Tangerli, Bazhou, Hebei province	Rural	20	25	5.0	Agricultural windbreaks, a mixture of Hankow willow and poplars, *Populus* spp. About 50 m wide and several kilometers long within a large area of farmland	Healthy
Yangcun, Wuqin, Tianjin city	Rural	15	20	5.0	Monoculture Hankow willow plantation along a railroad	Healthy
Xiaoxitian, Wuqin, Tianjin city	Urban	30	25	6.0	Urban forest belt. A mixture of willow, poplars, and other perennial bushes	Stressed

[a] The stressed trees showed signs of reduced growth (both in diameter and height), crown thinning, and branch dieback.

(Yangcun and Xiaoxitian) in 2002. A total of 20 ALB-infested willow trees were sampled monthly at Anci and Tangerli from June to October, whereas 10 trees were sampled at Yangcun and Xiaoxitian in June only. At each site, willow trees with similar infestation levels of ALB were selected based on the degree of external ALB signs and symptoms such as oviposition pits on the bark and frass accumulation at branch crotches or base of the tree.

ALB egg density was estimated based on numbers of actual eggs found on willow branches from each selected tree at each site. Every month from June to October 2002, a main branch that was about 2-m-long and 6–8 cm in diameter at the base was randomly selected from each of the 20 sample trees at the two primary sites in Hebei province (Anci and Tangerli). Similar samples were taken from 10 willow trees at the two supplemental sites in Tianjin city (Yangcun and Xiaoxitian) in June 2002. All branches were brought back to laboratories in Langfang of Hebei province (for samples from Anci), Bazhou of Hebei province (for samples from Tangerli), and Wuqin of Tianjin city (for samples from Yangcun and Xiaoxitian) to examine for ALB eggs, which are elongated ellipses, 5.5–7.0 mm long, and slightly curved at both ends. They are milky white at first, and change to yellow just before hatching. Eggs are deposited under the bark of trees from the base up to small branches with a diameter of $\geqslant 3$ cm. The adult ALB female chews an oviposition pit on the bark with its mandibles and deposits a single egg in it. The pit is then sealed with a female secretion. Egg deposition results in discoloration of approximately one square centimeter of bark surface area (Yan and Qin, 1992). ALB oviposition pits on each willow branch were examined, and eggs were removed using knives and forceps within two days after being brought back from the field. Eggs from the same branch were placed directly into a 35 mm × 10 mm (diameter × height) petri dish (Corning, NY), sealed with Parafilm M (Bemis, PA), and held in a growth chamber at 25 ± 1 °C and 16:8 (L:D) photoperiod for 7 d for further confirmation under a dissection scope.

Larval and pupal densities were also estimated from these branch samples by dissecting each branch with hammers, chisels, and axes in the laboratory within two days after being brought back from the field. ALB larvae and pupae found in the wood were removed, counted, killed individually in water-filled 5-ml self-standing sample tubes (Global Scientific, NJ), stored in the refrigerator at 4 °C, and shipped monthly to the USDA Forest Service laboratory in East Lansing, MI for further examination.

For ALB adults, the same selected trees at each study site were visually examined including the crown, foliage, branches, and the trunk for adult presence. Live adults found on each tree were then counted and collected by hand. The relatively large body size (20–40 mm in length), white-banded antennae, and the characteristic black body decorated with a dozen or so white blotches on the elytra make ALB adults easily visible against the green willow foliage from the ground. ALB adults were collected with ease as they are weak fliers with no phototaxis and often play dead when disturbed (Yan and Qin, 1992). Collected adults were sealed individually in water-filled 10-ml self-standing sample tubes (Global Scientific, NJ) for at least a week to be killed before being prepared and shipped monthly as described above.

We also examined the seasonal development of ALB larvae at the two primary sites in Hebei province (Anci and Tangerli) from June to October 2002. For each sample date, ALB larvae were separated into five developmental size classes based on head-capsule width: 1st size class $\leqslant$ 1.62 mm, 1.62 mm < 2nd size class $\leqslant$ 2.50 mm, 2.50 < 3rd size class $\leqslant$ 3.27, 3.27 < 4th size class $\leqslant$ 4.15, and 5th size class > 4.15 (He and Huang, 1992). Size classes may not correspond to instars (number of molts) because supernumerary molting occurs when larvae develop more slowly at cooler temperatures or less favorable conditions and pass through more instars (Keena and Moore, 2010). The total number of each larval size class was recorded.

2.3. Prevalence of *D. helophoroides*

Dastarcus helophoroides parasitism on ALB was studied at the two primary sites in Hebei province (Anci and Tangerli) with the same sampling frequency and dates as described above for seasonal abundance of ALB. Additional samples were collected in June at the two supplemental sites in Tianjin city (Yangcun and Xiaoxitian). For each sample date, the number of ALB larvae and pupae parasitized by *D. helophoroides* was recorded for each willow branch after dissection. Parasitized ALB larvae and pupae were easily recognized in the field as they were consumed or being consumed externally in their galleries by *D. helophoroides* larvae. Parasitism rates were calculated by dividing the number of parasitized larvae and pupae by the total number of ALB collected on each sample date.

Dastarcus helophoroides clutch size was determined based on the number of each life stage per parasitized ALB larva or pupa. Larvae, pupae, and adults of *D. helophoroides* found in ALB larval galleries during the dissection of willow branches were recorded and collected. Those from the same gallery were treated as a single clutch, which were later removed, counted, and killed in water-filled 5-ml self-standing sample tubes (Global Scientific, NJ) before being prepared and shipped monthly as described above.

Dastarcus helophoroides seasonal development was examined at the two primary sites in Hebei province (Anci and Tangerli) from June to October 2002. For each sample date, *D. helophoroides* found in ALB larval galleries were separated to larvae, pupae, and adults. The total number of larvae, pupae, and adults was recorded for each sample date.

2.4. Prevalence of fungal pathogens

ALB larvae, pupae, and adults obtained from the population density study were also examined for fungal pathogens. Cadavers with evidence of fungal infection were first examined under a dissecting microscope for rhizoids, cystidia, mycelia, or conidia before isolation, whereas those without external symptoms were surface sterilized through the following procedures: 70% ethyl alcohol 5 s, sterile distilled water (SDW) 5 s, 1% sodium hypochlorite 10 s, and a series of three washes in SDW for 5 s each (Lacey and Brooks, 1997). Surface sterilized cadavers were then placed individually in sterile CellBIND® 24-well culture plates (Corning, NY). The bottom of each well was lined with a moist paper disc and each plate was sealed with Parafilm M along the edges to maintain high humidity inside. All plates were incubated at 24 ± 1 °C for 4 wk or until sporulation. Individuals with external symptoms of fungal infection were subjected to isolation. Isolation of fungi was carried out on infected individuals using Sabouraud dextrose agar with 0.25% w/v yeast extract (SDAY) media (Goettel and Inglis, 1997). A small quantity of mycelia or conidia was scraped from the cadaver with a sterile needle and transferred to the medium inside a 60 mm × 15 mm (diameter × height) disposable petri dish (VWR International, PA). All dishes were cultured at 24 ± 1 °C in the dark for 10 d before identification.

2.5. Specimen identification

The identity of ALB and *D. helophoroides* was determined by H.-P. Liu, and voucher specimens were deposited at the Insect Collection of the Chinese Academy of Forestry in Beijing, China, and the USDA Forest Service, Northern Research Station, Lansing, MI. Fungal pathogens were identified by H.-P. Liu and Dr. Richard A. Humber of USDA ARS, Collection of Entomopathogenic Fungal Cultures (ARSEF), Ithaca, NY, where voucher cultures were deposited.

2.6. Data analyses

The densities of ALB of each life stage (eggs, larvae, pupae, adults), of ALB larvae of each developmental size class, and of each *D. helophoroides* life stage (larvae, pupae, adults) obtained from parasitized ALB larvae or pupae were compared among the primary sites (Anci and Tangerli), dates, and site × date interaction by a general linear mixed model (PROC GLIMMIX) because assumptions of analysis of variance were violated (PROC UNIVARIATE). Site, date, and their interaction were fixed effects in the model. Branch (or tree) within site was included as a random effect to prevent pseudoreplication. The response distribution was negative binomial with the log link function. Degrees of freedom were determined using the Kenward Roger method. Differences among sites, dates, or site × date were tested with the Tukey-Kramer means comparison procedure. Months during which no individuals of a given life stage were found at one or both sites were omitted from the analysis. *D. helophoroides* clutch size (number of *D. helophoroides* of all life stages per parasitized ALB larva or pupa) was analyzed by a similar general linear mixed model (PROC GLIMMIX) with negative binomial distribution and log link function. Differences among sites, dates, or site × date interaction were tested with the Tukey-Kramer means comparison procedure.

The percentage of ALB larvae and pupae parasitized by *Dastarcus helophoroides* was also compared among sites, dates, and site × date by a general linear mixed model (PROC GLIMMIX) with site, date, and their interaction as fixed effects in the model and branch within site as a random effect. The beta response distribution was used with the logit link function. Degrees of freedom were determined using the Kenward Roger method. Differences among sites, dates, or site × date were tested with the Tukey-Kramer means comparison procedure. Data for the month of August when no parasitized ALB were found at Tangerli were omitted from the analysis.

Densities of ALB life stages (egg, larvae, pupae, adults), *D. helophoroides* life stages, percent parasitism, and clutch size at the two supplemental sites (Yangcun and Xiaoxitian) sampled only in June were compared to data collected in June at the primary sites (Anci and Tangerli) using similar general linear mixed models (PROC GLIMMIX) with site as a fixed effect and branch (or tree) within site as a random effect, negative binomial response distribution and log link function for density data and beta response distribution and logit link function for percent parasitism .

All analyses were conducted using the SAS 9.4 for Windows statistical package (SAS Institute, 2012) with an α-level of 0.05. No analysis was performed on fungal pathogens although multiple isolates of a single species were recovered on one sample date.

3. Results

3.1. Seasonal abundance of ALB

Overall, 4689 ALB were collected from the two primary study sites in Hebei between June and October 2002, including 1681 (467 eggs, 1170 larvae, 21 pupae, and 23 adults) from Anci, and 3008 (674 eggs, 2228 larvae, 21 adults, and 85 adults) from Tangerli. By comparison, 140 ALB were collected from the two supplemental sites in Tianjin in June 2002, including 82 (32 eggs, 32 larvae, 10 pupae, and 8 adults) from Yangcun, and 58 (24 eggs, 24 larvae, 7 pupae, and 3 adults) from Xiaoxitian.

ALB egg density did not differ by site ($F = 0.12$; df = 1, 39; $P = 0.73$), but differed significantly by date ($F = 15.81$; df = 3, 121; $P < 0.0001$), and by site × date interaction ($F = 5.41$; df = 3, 121; $P = 0.002$). ALB egg density was significantly higher in July 2002 on healthy rural trees at Tangerli (mean 22.9 ± 4.2 eggs/branch) compared with stressed street trees at Anci (mean 6.7 ± 2.1 eggs/branch) in July or trees at either site in June or September. Egg densities at both sites were intermediate in August. Egg densities increased at both sites from June to July, reached their peak in July at Tangerli and in August at Anci, then declined at both sites in September and disappeared toward the end of October (Table 2).

No significant difference in ALB egg density was found among the four sites (Anci, Tangerli, Yangcun, and Xiaoxitian) for the June 2002 sampling ($F = 0.69$, $df = 3, 56$, $P = 0.563$) (Table 2).

ALB larvae were the most encountered stage in all sample dates for both sites except the July collection at Anci when eggs were more abundant (Table 2). While larvae were present on all dates, their numbers varied by site ($F = 22.44$; df = 1, 39; $P < 0.0001$), date ($F = 35.53$; df = 4, 151; $P < 0.0001$), and their interaction ($F = 6.64$; df = 4, 151; $P < 0.0001$). Larval density was significantly higher in healthy trees at the rural Tangerli site (34.1 ± 5.2 larvae/branch) in September 2002, compared to stressed urban trees in Anci in September (16.8 ± 3.6 larvae/branch). Larval densities were lowest at both sites in June, increased to a peak in August at Anci and in September at Tangerli, then decreased at both sites in October (Table 2). No significant differences in larval densities were found among all four sites (Anci, Tangerli, Yangcun, and Xiaoxitian) for samples taken in June 2002, ($F = 1.20$; $df = 3, 56$; $P = 0.317$), indicating comparable overwintering populations of ALB at these study sites (Table 2).

ALB pupae were only observed in June and July. Pupal density was significantly higher in June than in July at both Anci and Tangerli ($F = 17.18$; df = 1, 76; $P < 0.0001$) but there was no significant difference between the Anci and Tangerli sites ($F = 0.35$; df = 1, 76; $P = 0.63$) or by site × date interaction ($F = 0.22$; df = 1, 76; $P = 0.55$). There was also no significant difference in pupal density among all four sites (Anci, Tangerli, Yangcun, and Xiaoxitian) in June ($F = 0.14$, $df = 3, 56$, $P = 0.948$) (Table 2).

ALB adults were found active on willow trees from June into October. Adult density varied by site ($F = 17.29$; df = 1, 114; $P < 0.0001$), date ($F = 5.02$; df = 2, 114; $P = 0.008$), and by site × date interaction ($F = 5.16$; df = 2, 114; $P = 0.0072$). There were signifi-

Table 2
Mean density (± SE) of *A. glabripennis* eggs, larvae, and pupae per branch and adults per tree on Hankow willow at different sites in China in 2002. ($N = 20$ main branches (2-m long × 6–8 cm in diameter) per tree sampled at the primary study sites and $N = 10$ at the supplemental study sites).

Life stage	Sample date	Density (Mean ± SE) by site[a] Primary Sites: Hebei Province Supplemental Sites: Tianjin City			
		Anci	Tangerli	Yangcun	Xiaoxitian
Eggs	06/27	5.7 ± 2.1 BC	3.1 ± 1.3 BC	3.2 ± 1.4	2.4 ± 1.5
	07/27	6.7 ± 2.1 AB	22.9 ± 4.2 A	n/a[b]	n/a
	08/26	9.2 ± 2.4 AB	5.9 ± 1.4 BC	n/a	n/a
	09/23	1.8 ± 0.5 C	1.8 ± 0.9 C	n/a	n/a
	10/28	0.1 ± 0.1	0	n/a	n/a
Larvae	06/27	5.9 ± 1.0 C	5.0 ± 1.3 C	4.2 ± 1.4	2.5 ± 0.5
	07/27	6.3 ± 1.0 C	26.1 ± 2.6 AB	n/a	n/a
	08/26	18.4 ± 2.7 AB	29.9 ± 5.1 AB	n/a	n/a
	09/23	16.8 ± 3.6 B	34.1 ± 5.2 A	n/a	n/a
	10/28	16.3 ± 2.6 B	19.5 ± 3.8 AB	n/a	n/a
Pupae	06/27	1.0 ± 0.4 A	0.9 ± 0.3 A	1.0 ± 0.3	0.7 ± 0.3
	07/27	0.1 ± 0.1 B	0.1 ± 0.1 B	n/a	n/a
	08/26	0	0	n/a	n/a
	09/23	0	0	n/a	n/a
	10/28	0	0	n/a	n/a
Adults	06/27	0.7 ± 0.3 BC	1.6 ± 0.4 AB	0.8 ± 0.2	0.3 ± 0.3
	07/27	0.1 ± 0.1 C	2.3 ± 0.2 A	n/a	n/a
	08/26	0.3 ± 0.1 C	0.4 ± 0.1 C	n/a	n/a
	09/23	0.1 ± 0.1	0	n/a	n/a
	10/28	0	0.1 ± 0.1	n/a	n/a

[a] Within each life stage for eggs, larvae, and adults, means followed by different letters across rows and columns (site × date interaction) are significantly different. For pupae, effects of site and site × date interaction were not significant so different letters indicate differences across dates only. Data were analyzed by general linear mixed model (GLIMMIX) with negative binomial distribution and log link function, followed by the Tukey-Kramer means separation test ($P < 0.05$).

[b] n/a – not applicable, no samples collected.

cantly more ALB adults on healthy trees at Tangerli in July (2.3 ± 0.2 adults/tree) than on stressed street trees at Anci in July (0.1 ± 0.1 adult/tree) or any other date. No significant differences in adult densities were found among the four sites for samples taken in June ($F = 2.23$, $df = 3, 56$, $P = 0.095$) (Table 2). At Anci, adult density was highest in June, then declined in July and August. At Tangerli, adult numbers increased from June to a peak in July, then declined in August. By late October, all ALB found were larvae except for a single adult at Tangerli. In general, although ALB densities were higher, development was slower at Tangerli than at Anci.

Overall, 1170 (180 first, 200 second, 173 third, 273 fourth, and 344 fifth size class) larvae were found at Anci and 2228 (378 first, 382 second, 483 third, 637 fourth, and 348 fifth size class) larvae were found at Tangerli. There was no significant difference in the number of first size class larvae between sites ($F = 3.04$, $df = 1, 152$, $P = 0.08$); but numbers differed among dates ($F = 178$, $df = 3, 152$, $P < 0.0001$) and by site × date interaction ($F = 7.53$, $df = 3, 152$, $P < 0.0001$). Density of first size class larvae was significantly higher in July at Tangerli than at Anci in July or at either site in October. At Anci, first size class larvae increased in numbers from June to August, then declined in September and October. At Tangerli, no first size class larvae were found in June, they peaked in July and declined from August to October (Fig. 2).

Similarly the number of second size class larvae did not differ between the two sites ($F = 3.34$, $df = 1, 55$, $P = 0.07$), but differed among dates ($F = 24.9$, $df = 3, 112$, $P < 0.0001$) and by site × date interaction ($F = 3.41$, $df = 3, 112$, $P = 0.02$). Density of second size class larvae was significantly higher in July at Tangerli than at Anci in July or at either site on any other date. Density of second size class larvae was lowest at both sites in October. Second size class larvae were found on all dates at Anci but none were found in June at Tangerli. They increased from June to July and peaked in August at Anci then decreased in September and October; whereas, they peaked in July at Tangerli then declined from August to October (Fig. 2).

Third through fifth size class larvae were present on all dates at both sites. The numbers of third size class larvae differed between sites ($F = 12.55$; $df = 1, 190$; $P = 0.0005$), among dates ($F = 15.51$; $df = 4, 190$; $P < 0.0001$) and by site × date interaction ($F = 4.63$; $df = 4, 190$; $P = 0.0014$). The density of third size class larvae was significantly lower at both sites in June and higher in August, September and October. At Anci, the density of third size class larvae was lowest in June, increased in July and August, peaked in September then declined in October. At Tangerli, third size class larval density was lowest in June and increased to a peak in August then decreased thereafter (Fig. 2). Fourth size class larvae differed between sites ($F = 11.6$; $df = 1, 63$; $P = 0.001$) and among dates ($F = 23.26$; $df = 4, 184$; $P < 0.0001$) but not by site × date interaction ($F = 3.04$; $df = 4, 184$; $P = 0.29$). The numbers of fifth size class larvae also differed among dates ($F = 23.34$; $df = 4, 190$; $P < 0.0001$), but not between sites ($F = 0.0$; $df = 1, 190$; $P = 0.86$) or by site × date interaction ($F = 1.28$; $df = 4, 190$; $P = 0.28$). The numbers of fourth and fifth size class larvae decreased from June to July then increased in August in Anci and Tangerli, however, both size classes peaked in September at Tangerli but in October at Anci (Fig. 2). Based on the larval stages found in October, ALB overwintered as various development size classes. In spring, as larval development resumed, fourth and fifth size class larvae became the dominant stages in the June collection, which was then followed by pupation, adult emergence, and egg-deposition, resulting in an increase in the proportion of first and second size class larvae during July and August. By September, all larval stages were ready for overwintering. The asynchronous nature of larval development is the direct result of the prolonged egg-deposition period for ALB females.

3.2. Prevalence of *D. helophoroides*

Dastarcus helophoroides was found parasitizing ALB at the four study sites in 2002 with an average parasitism of 10.7% for Anci and 4.3% for Tangerli over the sampling year. A total of 173 mature

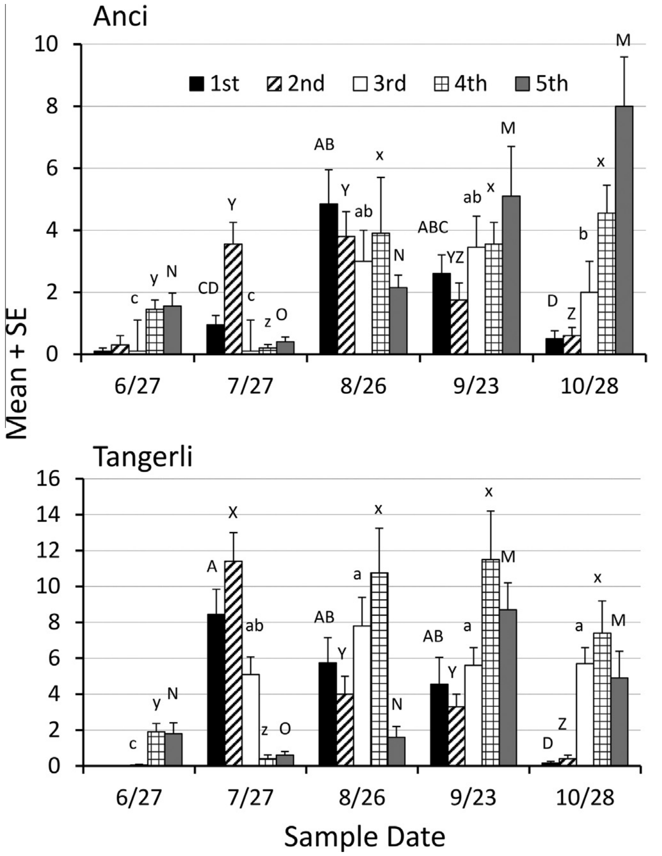

Fig. 2. Mean (+ SE) number of *A. glabripennis* larvae of each developmental size class per branch by sample date collected from infested Hankow willow at two sites in Hebei province, China in 2002. Within each developmental size class, means across sites and dates (site × date interaction) followed by the same letter (1st – ABCD, 2nd – XYZ, 3rd – abc, 4th – xyz, 5th – MNO) are not significantly different, general linear mixed model (GLIMMIX) with negative binomial distribution and log link function followed by Tukey-Kramer means separation test, $P < 0.05$ (N = 20). Bars without letters are for months that were omitted from the analysis because no individuals of a given developmental stage were found at one site.

ALB larvae or pupae were found parasitized by *D. helophoroides* at the four study sites, including 162 from Hebei province from monthly sampling between June and October (101 from Anci, 61 from Tangerli), and 11 from Tianjin city from samples taken in June (10 from Yangcun, and 1 from Xiaoxitian). There were no significant differences in *D. helophoroides* percent parasitism between the two primary sites (Anci and Tangerli) in Hebei province ($F = 1.43$; $df = 1$, 105; $P = 0.23$), but percent parasitism differed among sample dates ($F = 6.85$; $df = 3$, 120; $P = 0.0003$) and by site × date interaction ($F = 5.87$; df = 3, 120; $P = 0.0009$) (Fig. 3). At Anci where all host street trees were stressed, *D. helophoroides* parasitism was highest in June, and decreased thereafter from July to September, and increased again by October, with significantly higher parasitism found in June and July compared with all other sample dates (Fig. 3). A similar trend was also found at the rural site at Tangerli where all host trees were healthy, with higher parasitism for *D. helophoroides* found in June and October compared with other sample dates (Fig. 3) although no *D. helophoroides* were found for the sample date of August. By comparison, *D. helophoroides* parasitism was 16.1% at Yangcun (rural site) and 2.0% at Xiaoxitian (urban site) for the collections made in June 2002.

The clutch size for *D. helophoroides* ranged from 1 to 23 *D. helophoroides* per ALB larva or pupa, with an overall mean across all dates and sites of 5.67 ± 0.37. There was no significant difference in *D. helophoroides* clutch size among different sites ($F = 0.54$; $df = 3$, 161; $P = 0.65$), sample dates ($F = 2.54$; $df = 4$, 161; $P = 0.06$), but there was a significant effect of site × date interaction ($F = 2.95$; $df = 4$, 161; $P = 0.02$); however, means separation failed to reveal any differences among site and date combinations. Mean clutch size ranged from 3.3 ± 1.0 *D. helophoroides* per ALB host in October to 8.3 ± 2.4 in September at Anci, 2.5 ± 1.5 in July to 7.0 ± 1.0 in June at Tangerli, 5.2 ± 1.6 in June at Yangcun, and 2.0 ± 0 at Xiaoxitian.

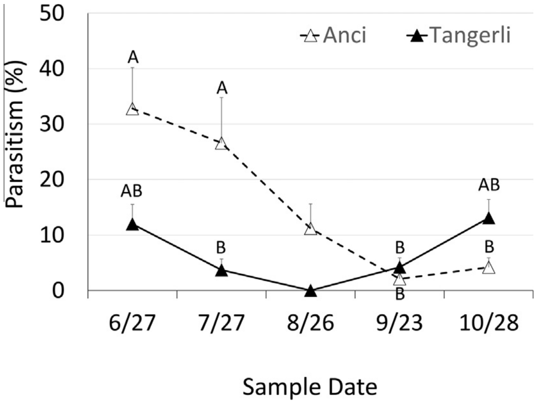

Fig. 3. Mean (+ SE) percent parasitism of *A. glabripennis* larvae and pupae by *D. helophoroides* at two sites in Hebei province, China in 2002. Means across sites and dates (site × date interaction) followed by the same letter are not significantly different, general linear mixed model (GLIMMIX) with beta distribution and logit link function followed by Tukey-Kramer means separation test, $P < 0.05$. (N = 20).

Of the 162 parasitized ALB larvae at the two primary sites (Anci and Tangerli) in Hebei province, 826 *D. helophoroides* were recovered, including 67 larvae, 386 pupae and 373 adults. Analysis of the seasonal development of this parasitoid suggests that *D. helophoroides* overwintered as adults in Hebei province. Adults were the most frequently encountered stage for all sample dates except in June, when pupae dominated the collection (Fig. 4). Except for a single recovery of a larva in August at Anci, *D. helophoroides* larvae were only found in samples made in June at Anci and Tangerli (Fig. 4) and there was no significant difference in number of larvae between the two sites ($F = 0.01$; df = 1, 71; $P = 0.93$). The number of *D. helophoroides* pupae did not differ between the sites ($F = 0.26$; df = 1, 104; $P = 0.61$) or by site × date interaction ($F = 2.63$; df = 1, 104; $P = 0.11$), but varied among dates ($F = 48.31$; df = 1, 104; $P < 0.0001$) with significantly more pupae present in June than in October at both sites (Fig. 4). On the other hand, the number of *D. helophoroides* adults increased from June through September and remained high in October (Fig. 4). Numbers of adults differed among dates ($F = 20.66$; df = 3, 65; $P < 0.0001$) and by site × date interaction ($F = 3.21$; df = 3, 65; $P = 0.02$), but not between sites ($F = 0.3$; df = 1, 108; $P = 0.58$). The increase in proportion of *D. helophoroides* adults coincided with the decrease in larvae and pupae as the latter matured and emerged as adults through July and August, which resulted in 100% adults in the collection of September (Fig. 4). By October, most *D. helophoroides* became adults, preparing themselves for overwintering; with a small portion of pupae possibly originated from eggs laid in the late summer (Fig. 4).

3.3. Prevalence of fungal pathogens

A total of 13 *Beauveria bassiana* (Balsamo) Vuillemin isolates were recovered from ALB sampled at Tangerli on July 2002, including 12 isolates from first-size class larvae, and one isolated from a pupa. Of the 20 trees sampled on this sample date, eight of them contained ALB infected with *B. bassiana*. The average infection rate of *B. bassiana* was 2.4%, ranging from 0 to 18.2% among different

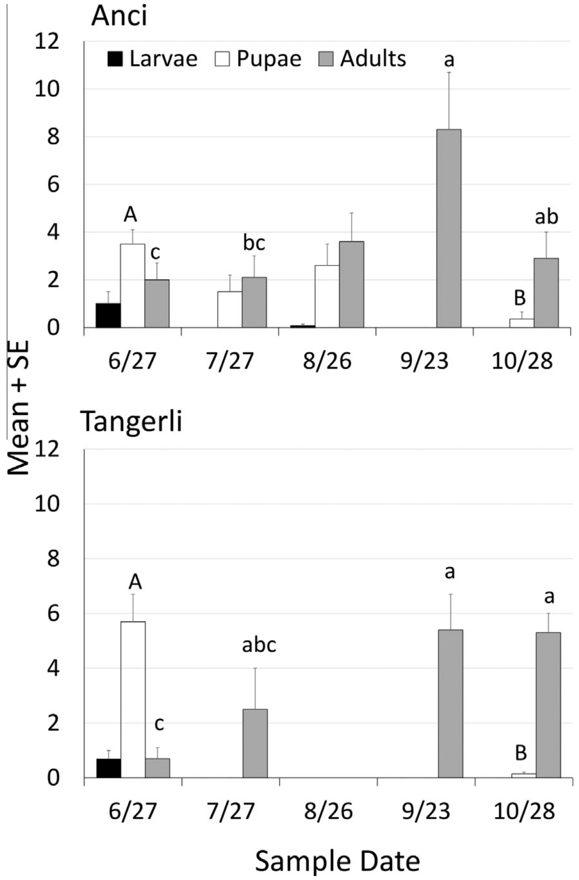

Fig. 4. Mean (+ SE) number of *D. helophoroides* larvae, pupae, and adults per clutch in ALB larva or pupa collected by sample date collected from infested Hankow willow at two sites in Hebei province, China in 2002. Within each life stage, means across sites and dates (site x date interaction) followed by the same letter (pupae – AB, adults – abc) are not significantly different, general linear mixed model (GLIMMIX) with negative binomial distribution and long link function followed by Tukey-Kramer means separation test, $P < 0.05$ (N = 4–26 parasitized ALB hosts per site and date). Bars without letters are for months that were omitted from the analysis because no individuals of a given life stage were found at one site.

trees. No fungal pathogens were recovered from other samples at Tangerli, or samples from Anci, Yangcun, and Xiaoxitian throughout the season.

4. Discussion

ALB larval and adult densities were significantly higher in Hankow willow trees grown at the rural forest compared to the urban forest. Although ALB egg densities did not differ between Tangerli and Anci during most of the season, significantly higher egg densities were observed in July at Tangerli compared to Anci, coinciding with the peak egg period in the field. The number of ALB pupae found was too low to detect differences between sites. Our results do not support the general hypothesis that pest populations tend to be higher in urban forests where trees may be stressed and lack of ecosystem complexity limits natural enemies compared to rural forests. However, our sample size is small with only one primary urban site and one primary rural site. Differences in several site factors between the two specific sites, including other pest species, ALB attack patterns, infestation history, and host conditions likely explain the higher ALB population density at the rural site in Tangerli compared to the urban site at Anci and the discrepancy with other studies that have found higher pest populations in urban forests. ALB larvae tended to develop faster at Anci compared to Tangerli as indicated by the stage composition on each sample date (Fig. 2). Generally warmer temperatures in Anci may have contributed to the reduced development time for all instars. Many previous studies have compared pests that feed on their hosts externally (e.g., scale insects) (Dale and Frank, 2014; Meineke et al., 2013). As a woodboring insect that spends most of its life cycle as larvae or pupae inside the host, ALB is better protected than external feeding insects from many abiotic or biotic factors. It is also likely that the infestation at Anci was near its end and more advanced than the infestation at Tangerli which was just getting started with building populations. Severely stressed or dying trees may be less suitable for new ALB attacks while healthy hosts can support higher populations.

D. helophoroides parasitism on ALB larvae and pupae was not significantly different between the rural forest in Tangerli and the urban forest in Anci, with higher parasitism rates observed from June to August samples and lower rates observed for the September and October samples at Anci compared to Tangerli. It is likely that the ALB infestation occurred earlier and was more advanced at the Anci than the Tangerli site, which provided time for *D. helophoroides* population and parasitism level to increase over time at Anci. Vegetation complexity might have little to do with the prevalence of *D. helophoroides* at both sites as adults can survive long periods of time even without food (Gao and Qin, 1992; Qin and Gao, 1988).

Li et al. (2007) reported 50–70% parasitism by *D. helophoroides* on late stage ALB larvae in Shaanxi, with parasitism increasing from April to July, and peaking again in late September. They also obtained 1–4 *D. helophoroides* larvae per host from 80% of the parasitized ALB larvae. Similar results in field parasitism were also reported by Gao and Qin (1992) and Qin and Gao (1988) in Beijing, and Wei et al. (2009a) in Shaanxi. By comparison, the overall parasitism rates were lower in the current study although clutch size for *D. helophoroides* was larger.

Dastarcus helophoroides has potential as a biological control agent for ALB. The lower ALB larval density at Anci in June and July corresponded with higher *D. helophoroides* parasitism. *D. helophoroides*, which prefers to parasitize large larvae and pupae (Gao and Qin, 1992; Qin and Gao, 1988), was also well synchronized with ALB larval development; parasitism rates were highest in June, July, and October when higher proportions of late instar larvae were present. *D. helophoroides* overwinters as adults in leaf litter, bark crevices, and host galleries (Wei et al., 2007). Adults may survive for up to four years, and each female can deposit up to 400 eggs in her lifetime (Gao and Qin, 1992; Qin and Gao, 1988; Tang et al., 1996; Wang et al., 1996; Wei et al., 2009b). The prolonged lifespan and high fecundity provide support for rapid population increases in response to increasing host density. The moderate to high parasitism rates observed in this and other studies support its potential as an important natural enemy of ALB (Li et al., 2007, 2009b). Up to 90% parasitism was observed in the field when *D. helophoroides* eggs were released at a rate of 15–20 eggs/host larva (Li et al., 2009a). Other studies on this parasitoid focused on its biology (Lei et al., 2003; Zhou et al., 1985), behavior (Wei and Jiang, 2011; Wei et al., 2008, 2013), and mass-rearing (Ogura et al., 1999).

Dastarcus helophoroides also parasitizes other cerambycid larvae such as *Monochamus alternatus* Hope, *Massicus raddei* (Thomson), *Apriona swainsoni* (Hope), *A. germari* Hope, and *Batocera horsfieldi* (Hope) (Wei et al., 2009a). Since the 1980s, it has been used to control *B. horsfieldi*, *A. swainsoni*, *M. raddei*, and *M. alternatus* in China, South Korean, and Japan (Li et al., 2009a; Lim et al., 2012; Lu et al., 2012; Tadahisa, 2003, 2006; Yang et al., 2012). This apparent lack of host specificity for *D. helophoroides* may compromise its potential as a classical biological control agent in the U.S. as it could potentially attack other non-target species in the field after introduction.

A localized fungal epizootic was observed at Tangerli in July 2002. Field infection by *B. bassiana* and *P. farinosus* on ALB was also observed by Wang et al. (1990, 1997) and Zhang et al. (1999a). Several fungal pathogens were evaluated for their efficacy against ALB (Hajek and Bauer, 2009; Hajek and Tobin, 2010). Both *B. bassiana* and *P. farinosus* were capable of infecting ALB fourth and fifth size class larvae under laboratory conditions (Wang et al., 1997; Zhang et al., 1999a). *Beauveria brongniartii* (Saccardo), *B. bassiana,* and *Metarhizium anisopliae* (Metchnikoff) Sorokin have all been shown to increase mortality and decrease fecundity of ALB (Dubois et al., 2004a,b; Hajek et al., 2006; Zhang et al., 1999b). Goble et al. (2014) recently found that ALB adult longevity was significantly reduced after exposure to *Metarizium brunneum* Petch or *B. asiatica* S.A. Rehner & Humber. Given its high virulence and registration status, *M. brunneum* was selected as the candidate for further development in the microbial control of ALB in the U.S. (Ugine et al., 2013a,b).

Although we compared only two primary sites (one urban and one rural) with different tree health conditions, our results suggest that variation in forest type, tree health, and ALB density have little effect on parasitism success, since parasitism rates, development of life stages, and clutch size of *D. helophoroides* did not vary among sites. The lack of a significant site effect on natural enemy prevalence may have more to do with infestation history of the two sites rather than differences in vegetation complexity between the different habitats. This finding may provide valuable information for considering biological control of ALB in China and in North America.

Effective management of ALB in North America will require a combination of regulatory, cultural, mechanical, chemical, and biological approaches due to its unique biology and dispersal patterns. While successful eradication of several urban infestations of ALB through tree removal and chemical treatment in the past two decades is encouraging, ongoing battles in woodlots in Worcester, MA and Clermont, OH may present a new set of challenges to forest health managers because of the location and the number of trees infested. Both infestations are found near continuous hardwood forests with numerous host tree species. A total of 24,404 infested trees were cut so far in MA and 16,446 tress in OH (OALBCEP, 2016; USDA APHIS, 2016b; Haack, 2016). An integrated pest management option is needed should eradication and containment measures fail. Results from this study demonstrate that natural enemies such as *D. helophoroides* and *B. bassiana* may play an important role in the population dynamics of ALB in the field. Biological control of ALB in North America will depend on continued research on the natural enemies of ALB in North America and host-specificity testing of *D. helophoroides* and other parasitoids from ALB's native range in Asia.

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