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Seasonal abundance of *Agrilus planipennis* (Coleoptera: Buprestidae) and its natural enemies *Oobius agrili* (Hymenoptera: Encyrtidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in China

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Abstract

The seasonal abundance and population dynamics of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) and its natural enemies *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) and *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) were studied on ash (*Fraxinus* spp.) in northeastern China in 2004 and 2005. *A. planipennis* population density varied greatly between sites, trees, and heights in Manchurian ash (*Fraxinus mandshurica*) forests. At Benxi in Liaoning Province, *A. planipennis* completed a one-year life cycle on Manchurian ash, with eggs found from 2 to 24 June 2004. Further north at a mixed forest with plantings of Korean ash (*Fraxinus chinensis* var. *rhynchophylla*) and green ash (*Fraxinus pennsylvanica*) in Changchun of Jilin Province during 2005, *A. planipennis* demonstrated an asynchronous, one-year life cycle in green ash trees with larvae overwintering in all four instars. At least two generations of *O. agrili* were observed from *A. planipennis* in Changchun during the egg period in 2005, with parasitism of 56.3% and 61.5% for July and August, respectively. A portion of the *O. agrili* population diapaused within host eggs in the fall and winter months and emerged the following spring and summer, resulting in post-season parasitism of 28.6% in June 2004, 12.0% in May, and 43.8% in November 2005. Up to four generations of *T. planipennisi* emerged from host larvae at Changchun, with an average larval parasitism of 22.4%, ranging from 0 to 40.4%. These two parasitoids were important in the population dynamics of *A. planipennis* on green ash, with an estimated 73.6% reduction in host population densities during 2005 in Changchun. The characteristics of these parasitoids, such as high parasitism rates, short generation times, high reproduction rates, and life-cycle synchrony with host, suggest both species may prove useful in management of *A. planipennis* in North America as biocontrol agents.
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1. Introduction

Agrilus planipennis Fairmaire (Coleoptera: Buprestidae), a minor and periodic pest of ash trees (*Fraxinus* spp.) in its native range of northeastern Asia (CAS, 1986; Yu, 1992; Xu, 2003; Gao et al., 2004; Wei et al., 2004), was first discovered in Michigan and Ontario in 2002 following investigations of ash decline (Haack et al., 2002). *A. planipennis* has also spread to Ohio, Indiana, Maryland, Virginia, and Illinois due, in large part, to inadvertent human transport of infested ash materials (USDA APHIS,

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2006). As of 2006, over 25 million ash trees have been killed by this pest in Michigan alone (MDA, 2006). The potential impacts of *A. planipennis* on forest biodiversity, ash resources, and urban areas in North America will be profound since ash trees are widely distributed and planted throughout North America (MacFarlane and Meyer, 2005; Poland and McCullough, 2006).

Damage to ash trees is caused by feeding of *A. planipennis* larvae within the phloem and cambial region, resulting in disruption of host nutrient and water flow. Crown dieback and epicormic branching are two early symptoms of infestation, followed by mass attack of the main trunk resulting in above-ground death within one to three years (Poland and McCullough, 2006). Tree mortality, however, may take many more years due to aggressive stump sprouting in ash trees. In Michigan, *A. planipennis* has a one- or two-year life cycle, with larvae or prepupae overwintering in the outer sapwood or outer bark (Cappaert et al., 2005). Pupation begins as early as mid-April, and adults begin to emerge one month later, although peak emergence occurs from early to mid June. *A. planipennis* adults feed on ash foliage throughout their lives, and oviposit in bark crevices or between bark layers from June through August. Newly hatched larvae bore directly into the bark until reaching the cambial region and phloem where they feed, often forming serpentine galleries under the bark. There are four larval instars. In some areas of Michigan, most larvae reach the last instar by mid-October to early November (Cappaert et al., 2005). The biology and life cycle of *A. planipennis* in North America is similar to that reported for this species in China (CAS, 1986; Yu, 1992). Attempts to eradicate or contain this pest have been unsuccessful due to a lack of control and detection methods, the size of the infestation, and the difficulties and expense involved in quarantine enforcement. Clearly, research is needed to develop environmentally acceptable methods to reduce *A. planipennis* population densities and to slow its spread throughout North America.

Exotic insects may become invasive pests because natural enemies and/or host-plant resistance mechanisms are absent in their new environments. Classical biological control, or the introduction of natural enemies from a pest's native range to its country of introduction, has been used successfully to suppress populations of various invasive pests (Clausen, 1978; Hall et al., 1980; Luck, 1981; Greathead, 1986; Greathead and Greathead, 1992; Van Driesche and Bellows, 1996; Gurr and Wratten, 2000).

In 2003, we initiated such a project for *A. planipennis* by studying its population dynamics and natural enemy complex in China. During our initial exploratory surveys for *Fraxinus* spp. and *A. planipennis* in northeastern China, including Jilin and Liaoning Provinces, we found two parasitoid species attacking 3rd- and 4th-instar larvae on Manchurian ash (*Fraxinus mandshurica* Rupr.) (Liu et al., 2003). One of these parasitoids was a previously unknown gregarious larval endoparasitoid, *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), which we found in both provinces during the course of our study (Liu et al., 2003). Collections of this parasitoid were also made by Yang et al. (2006) from some of the same locations during 2004. In Jilin Province, we also found a second parasitoid in 2003: the gregarious larval ectoparasitoid, *Spathius agrili* Yang (Hymenoptera: Braconidae) (Liu et al., 2003). This finding expanded the known range for *S. agrili*, which was previously known only from the more southerly Tianjin City where it attacks *A. planipennis* larvae in stands of velvet ash (*Fraxinus velutina* Torr.) (Liu and Liu, 2002; Yang et al., 2005). In 2004 in Jilin Province, we discovered and later described *Oobius agrili* Zhang and Huang (Hymenoptera: Eulophidae), a previously unknown solitary parasitoid that attacks the eggs of *A. planipennis* (Zhang et al., 2005).

The overall objective of our research was to find parasitoids of *A. planipennis* in Asia for use in the possible biological control of this pest in North America. To meet this objective, we surveyed field populations of *A. planipennis* during 2004 and 2005 in northeastern China, and studied the (1) population density and seasonal dynamics of *A. planipennis*; (2) seasonal abundance of its egg parasitoid, *O. agrili*; (3) seasonal abundance of its larval endoparasitoid, *T. planipennisi*; and (4) impact of these two parasitoids on host populations in the field.

2. Materials and methods

2.1. Study sites in China

In 2004, we selected five study sites with *A. planipennis*-infested ash trees and varying site conditions (Table 1). The Changzui site was located in Jianchang Township (41°35′N, 124°40′E) of Benxi City in Liaoning Province, whereas the other four sites (Changbu, Dongfenqu, Dingjiagou, and Laoniujuan) were chosen from Jingyuetan Forest Park (43°52′N, 125°21′E) of Changchun City in Jilin Province.

The Changzui site in Liaoning Province consisted of 558 Manchurian ash trees planted in a single row along roadsides. These trees had been transplanted in spring 2000 from a nearby natural forest and appeared drought stressed. Over half of the trees were infested with *A. planipennis* as indicated by the weak crowns and epicormic branching. In addition, 21.3% trees had at least one adult exit hole from previous years.

A total of four sites were selected from Jingyuetan Forest Park in Changchun, a designated National Forest Park in China with an area over 9600 ha. Dominant tree species in the park are pines (*Pinus* spp.), oaks (*Quercus* spp.), elms (*Ulmus* spp.), birches (*Betula* spp.), and *Catalpa* spp. The Changbu, Dongfenqu, and Dingjiagou sites were selected from the edges of the park and were in similar condition. Each site contained approximately 150–200 30-year-old Manchurian ash trees with less than 10% of trees having old exit holes, epicormic branches, or visible crown dieback. These sites were separated from each other by at least

Table 1
Site conditions and host information for the study of *A. planipennis* and its natural enemies *O. agrili* and *T. planipennisi* in China 2004–2005

Province, city	Site/location	Host tree	Forest type	Size (ha)	Age (yr)	DBH (cm)	Height (m)	Description	Condition
Liaoning, Benxi	Changzui/Jianchang	*F. mandshurica*	Urban plantation	0.50	10	4–6	3–4	Roadside trees	Stressed
Jilin, Changchun	Changbu/Jingyuetan	*F. mandshurica*	Monoculture plantation	0.20	30	15–25	15–20	At the edge of a natural forest	Good
	Dongfenqu/ Jingyuetan	*F. mandshurica*	Monoculture plantation	0.20	30	15–25	15–20	At the edge of a natural forest	Fair
	Dingjiagou/ Jingyuetan	*F. mandshurica*	Monoculture plantation	0.20	30	15–25	15–20	At the edge of a natural forest	Fair
	Laoniujuan/ Jingyuetan	*F. pennsylvanica*	Mixed plantation	0.25	10	4–5	3–4	Mixed within a natural forest	Good
		F. chinensis var. *rhynchophylla*	Mixed plantation	0.20	10	4–5	3–4	Mixed within a natural forest	Very good

6 km. The fourth site in this park, located at Laoniujuan, was a mixed plantation contains two ash species: green ash (*Fraxinus pennsylvanica* Marsh) and Korean ash (*Fraxinus chinensis* var. *rhynchophylla* (Hance) Hemsley). Other tree species at this site were pines, larch (*Larix* sp.), box elder (*Acer negundo* L.), Amur cork tree (*Phellodendron amurense* Rupr.), and elms. Pines were the dominant tree species in this site. Green ash and Korean ash made up approximate 30% of the trees within the site and were found mainly on the east and west side.

2.2. Population density of *A. planipennis*

The population density of *A. planipennis* was estimated by sampling infested Manchurian ash trees at each of the four study sites (Changzui, Changbu, Dongfenqu, and Dingjiagou) from late May to early June 2004, before adult emergence (Table 2). After the trees were felled using a chainsaw, the main trunks were cut into 60-cm logs, from the tree base to the top with a diameter of 3 cm (Table 2). In the laboratory, log sections were placed individually in cardboard tubes (20–30 cm in diameter by 70 cm in length) capped on one end with a metal lid to exclude light, and on the other end with a metal lid modified by the addition of a translucent plastic collection cup. Each log was elevated 3–4 cm off the tube bottoms with two small wooden blocks to allow egress of adults from beneath the logs. The logs were then incubated at 20–26 °C, 20–40% RH, 16:8 (L:D) photoperiod, for adult emergence. *A. planipennis* adults are highly phototropic and readily enter the collection cups. The logs were held for four weeks after emergence of the last adult, at which time each log was dissected, and all *A. planipennis* adults, larvae, and pupae were removed and counted. *A. planipennis* density was calculated by dividing the total number of insects removed from the log section by the surface area (m^2) of the log section.

2.3. Seasonal distribution of *A. planipennis* oviposition

The seasonal distribution of *A. planipennis* oviposition was studied on Manchurian ash trees in Changbu and Changzui from 28 May to 2 July 2004. In Changbu, a total of five established ash trees and ten newly planted ash saplings were used during this period. The established ash trees were randomly selected within the plantation, whereas the ash saplings (3–4 cm in diameter and approx. 3 m in height) were transplanted in empty spaces within the plantation two weeks before the study began. The lower 200-cm trunk of each tree and sapling was monitored daily for *A. planipennis* eggs, which are about 1 mm in diameter. Newly laid eggs were counted and removed daily with a knife by excising each egg with a small patch of bark. In Changzui, egg deposition was monitored daily on five randomly selected established trees as described before. In addition, freshly cut Manchurian ash branches were used as “trap logs” for *A. planipennis* oviposition. The trap logs were ca. 5 cm in diameter by 200 cm in length and cut from

Table 2
Population density (means ± SEM) of *A. planipennis* in Manchurian ash stands in China 2004

Study site	Tree age (yr)	No. of trees sampled	Average DBH (cm)	Log sections examined	No. of insects found	Population density (insects/m^2)[a]
Changzui	10	20	5.4	100	223	27.8 ± 5.0a
Changbu	30	3	17.6	47	19	1.4 ± 0.4b
Dongfenqu	30	2	18.0	32	7	1.1 ± 0.4b
Dingjiagou	30	3	14.3	49	3	0.3 ± 0.2b

[a] Means followed by the same letter are not significantly different (Student–Neuman–Keul test, $\alpha = 0.05$).

Manchurian ash trees in a nearby forest. Each Friday, from 4 June to 25 June, we attached 10 trap logs to infested trees at the rate of one log per tree. Five of the logs were retrieved Monday and Friday of the following week and returned to the laboratory for removal and counting of *A. planipennis* eggs. A total of 40 trap logs were used during the 4-week observation period.

2.4. Host resistance

At the outset of this study, we compared the infestation level of *A. planipennis* on different ash species in Laoniujuan. Both green and Korean ash trees in this plantation were visually examined for signs and symptoms of *A. planipennis* infestation such as crown dieback, epicormic branching, longitudinal bark splits, and D-shaped adult exit holes. Bark removal was carried out on the lower 2-m trunk from symptomatic trees; infestation was confirmed by the presence of live *A. planipennis* in a tree. An estimate of ash resistance to *A. planipennis* was based on the infestation level for each species.

2.5. Seasonal development of A. planipennis larvae

We studied the seasonal development of *A. planipennis* larvae on green ash at Laoniujuan from April to August 2005; additional samples were collected in November 2005 to determine the overwintering stages. For each sample date, larvae were collected from 15 randomly selected ash trees by removing the bark from 2 m of lower trunk using knives and chisels. Larvae were separated into instars based on urogomphi length: 1st instar $\leqslant 0.2$ mm, 0.2 mm $<$ 2nd instar $\leqslant 0.4$ mm, 0.4 mm $<$ 3rd instar $\leqslant 0.7$, and 4th > 0.7 mm (Liu and Bauer, unpublished data). Larvae in the prepupal stage were identified by their characteristic short, cylindrical body shape. The number of larvae at each stage from each collection date was recorded and used for analysis of larval seasonal development.

2.6. Seasonal abundance of O. agrili

At the Laoniujuan site in Changchun, Jilin Province, the seasonal abundance of *O. agrili* was studied on green ash in June 2004, and monthly from April through August, and again in November 2005. This was done by collecting *A. planipennis* eggs from bark layers and crevices on the lower 2-m trunk from 20 randomly selected green ash trees. The eggs were placed directly into 35-mm Petri dishes, sealed with Parafilm®, returned to the laboratory, stored in the refrigerator at approx. 4 °C, and shipped monthly to the USDA Forest Service quarantine laboratory in East Lansing, Michigan, where they were sorted as empty or unhatched using a dissecting microscope. The empty eggs were further sorted into hatched eggs or eggs with *O. agrili* exit holes. Hatched eggs were identified by a crescent, or an occasional irregular-shaped, hole chewed by neonates during eclosion on the underside of the egg where it was attached to the bark. The exit hole chewed by *O. agrili* adult upon emergence from host eggs is a nearly circular hole on the upper egg surface. Unhatched parasitized eggs, which are usually black in color, were sorted from the other unhatched eggs, which ranged in color from white to brown depending on their age. All unhatched eggs were incubated in Parafilm-sealed 35-mm Petri dishes at 25 ± 1 °C and 16:8 (L:D) photoperiod for up to 8 months. *O. agrili* adults and *A. planipennis* larvae were recorded and removed daily. Estimates of *O. agrili* parasitism rates from our egg collections included (1) realized egg parasitism (P1) equals the number of adult *O. agrili* divided by the total number of unhatched eggs; (2) unrealized egg parasitism (P2) equals the number of black eggs divided by the total number of unhatched eggs; and (3) past parasitism (P3) equals the number of eggs with parasitoid exit holes divided by the total number of hatched eggs.

2.7. Seasonal abundance of T. planipennisi

The seasonal abundance of *T. planipennisi* was also studied at the Laoniujuan site with the same sampling frequency and dates described above in Section 2.6. *A. planipennis* larvae were removed from under the tree bark with chisels and knives from the lower 2-m trunk of 15 randomly selected green ash trees. In the field, larvae were maintained individually in a 35-mm Petri dish with ca. 10 g of weevil diet (Blossey et al., 2000), which was modified by adding insect cell-culture tested α-cellulose from Sigma (St. Louis, MO) instead of ground plant roots (Liu and Bauer, unpublished data). The insects were returned to the laboratory, stored in the refrigerator at approx. 4 °C in parafilm-sealed Petri dishes, and shipped monthly to Michigan as described above. In our quarantine laboratory, *A. planipennis* larvae were transferred to weevil diet modified by adding ground ash phloem instead of ground plant roots; previous research demonstrated second-, third-, and fourth-instar *A. planipennis* larvae complete development on this ash-based diet (Bauer and Liu, unpublished data). Plant materials such as ash phloem are not allowed in international shipments under current U.S. regulations. Ash phloem powder was made by peeling thin strips from the outer sapwood of healthy ash trees during the summer. The strips were air dried in the laboratory for several days, run through a compost shredder, dried in a Grieve high airflow drying oven (Grieve Corp., Round Lake, IL) at 120–130 °C for 3–4 d with periodic mixing, then ground in a Christie mill (Christie and Norris Ltd., Chelmsford, UK) using 2.0 and 0.8 mm screening. After transfer to the fresh ash-based diet, the larvae were incubated at 25 ± 1 °C and 16:8 (L:D) photoperiod, and examined daily for symptoms of parasitism. When confirmed, parasitized larvae were transferred to 35-mm Petri dish with a small cotton ball (approx. 0.3 cm in diameter) moistened with tap water, sealed with Parafilm, and incubated for exit of larval parasitoids. Parasitism rate was calculated by dividing the number of parasitized larvae by the total number of *A. planipennis* larvae collected.

2.8. Data analysis

Percent parasitism was first subjected to angular transformation before analyses. Analysis of variance (ANOVA) ($\alpha = 0.05$) was used to compare mean population densities of *A. planipennis* at different sites, seasonal distribution of *A. planipennis* oviposition, and production of *T. planipennisi* in host larvae. The Student–Neuman–Keul's test ($\alpha = 0.05$) was used to detect significant differences between sample dates, study sites, trees, or log sections (PROC GLM) (SAS Institute, 2004). The two-sample *t*-test was used to compare the average larval production of *T. planipennisi* from third- vs. fourth-instar *A. planipennis* larvae. Regression analysis (PROC REG) (SAS Institute, 2004) was used to evaluate the correlation between host egg density and parasitism rate of *O. agrili*, and host larval density and parasitism rate of *T. planipennisi.*

3. Results

3.1. Population density of A. planipennis

Significant differences in mean population density of *A. planipennis* infesting Manchurian ash trees during 2004 was found among the four study sites ($F = 18.75$, $df = 3, 205$, $P < 0.01$). The average density in Changzui was significantly higher than densities found in Changbu, Dongfenqu, and Dingjiagou, where densities of *A. planipennis* were similar (Table 2).

In Changzui, the average population density of *A. planipennis* was 27.8 insects/m^2, ranging from 1.7 to 186.7 insects/m^2, with significant differences observed among trees ($F = 10.99$, $df = 19, 99$, $P < 0.01$) and heights (log sections) ($F = 3.74$, $df = 4, 99$, $P = 0.01$). Significant differences in *A. planipennis* density among trees was also found in Changbu ($F = 9.11$, $df = 2, 46$, $P = 0.01$), but not among heights ($F = 0.77$, $df = 21, 46$, $P = 0.72$). No differences were found among trees or heights in Dongfenqu or Dingjiagou.

Our studies of *A. planipennis* within-tree distribution during 2004 in Changzui demonstrated larval concentrations were highest in the upper trunks, with 73.5% of larvae found 120 cm above the ground (Fig. 1). The lowest larval densities were found below 60 cm and highest from 120 to 240 cm; larval densities above 240 cm were similar to densities between 60 and 120 cm (Fig. 1).

3.2. Seasonal distribution of A. planipennis oviposition

Agrilus planipenis oviposition was confirmed by the presence of fresh eggs on established trees and trap logs studied in Changzui from 28 May to 2 July 2004. We found a total of 240 eggs on the five trees sampled during the collection period, with a range of 46–50 eggs per tree. *A. planipennis* oviposition began 2 June, at an average of 0.4 eggs per tree per day, with a peak of 5.2 eggs per tree on 9 June (Fig. 2). Oviposition decreased steadily, and the last new egg was found on 24 June. Significant differences in oviposition frequency were observed among sample dates ($F = 13.30$, $df = 22, 88$, $P < 0.01$), but not among trees ($F = 0.09$, $df = 4, 88$, $P = 0.984$). The trap logs were less attractive to *A. planipennis* females compared to live trees. A total of 23 eggs were found on five of the 40 traps logs, with an overall average of 0.6 ± 0.3 eggs per trap log. The first egg was found on a trap log on 14 June and the last on 25 June. No eggs were found on established ash trees or saplings in Changbu.

3.3. Host resistance

At the site in Laoniujuan, differences in host resistance to *A. planipennis* were observed between Korean ash and green ash, which is native to North America (Table 1). About 95% of the green ash trees at this site were moderately infested with *A. planipennis*, whereas no infestation was found in the Korean ash trees, although both species were of similar size and planted side by side (Table 1). Manchurian ash was also more susceptible than Korean ash to *A. planipennis* (Table 1).

3.4. Seasonal development of A. planipennis larvae

Stage-specific seasonal development of *A. planipennis* was evaluated using a total of 451 larvae and prepupae dissected from the cambial region and outer sapwood of green ash in Laoniujuan from April through November 2005 (Fig. 3). Based on the larval stages found, *A. planipennis* can overwinter at any larval stage. Except for larvae preparing to prepupate within a pupation chamber, all larval stages resumed feeding in April, resulting in a gradual

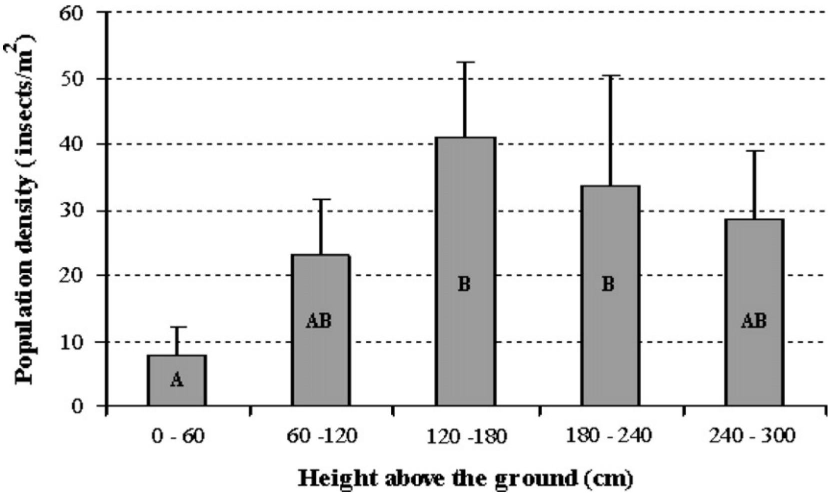

Fig. 1. Within-tree distribution of *A. planipennis* population (means ± SEM) in Changzui of Benxi, Liaoning Province in 2004. Each bar represents the average density of 20 samples. Bars with the same letters are not significantly different ($\alpha = 0.05$, Student–Neuman–Keul test).

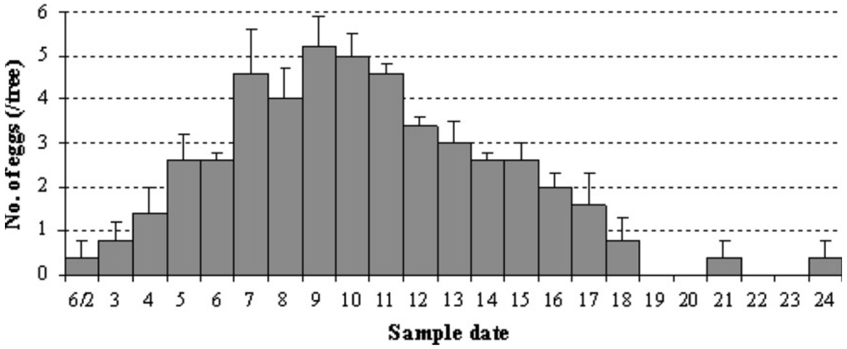

Fig. 2. Seasonal dynamics of *A. planipennis* eggs on live trees in Changzui of Benxi, Liaoning province in June 2004. Each data point represents the average number of eggs/tree (±SEM) based on five trees.

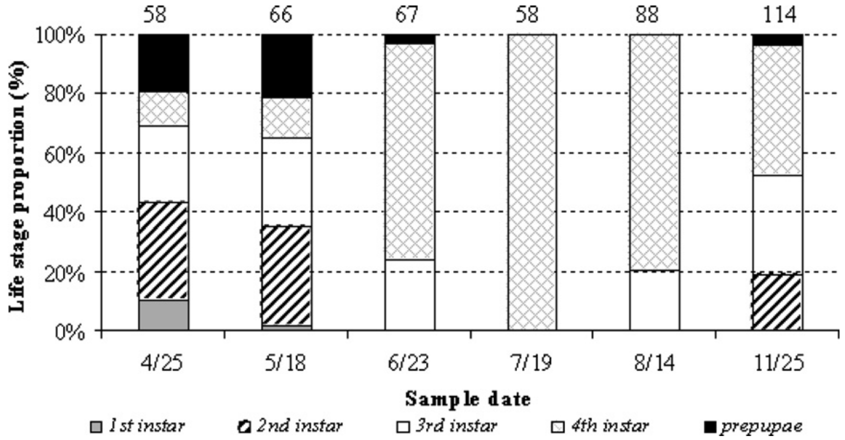

Fig. 3. Proportion of *A. planipennis* larvae by sample date at Laoniujuan of Changchun, Jilin province in 2005. Numbers on top of each bar represents the total sample size by date.

increase in numbers of 4th instars and prepupae and a corresponding decrease in 1st and 2nd instars from April to May. As the prepupae pupated and emerged as adults during June and early July, 4th instars dominated the sample by mid-July. By mid-August, only 3rd and 4th instars from the current generation were collected; the first two larval stages were not recovered in July and August, perhaps due to their small size or time spent traversing the outer bark, which was not dissected for larvae. By late November, *A. planipennis* were collected as overwintering 2nd, 3rd, and 4th instars, and prepupae (Fig. 3). These results support an unsynchronized one-year life cycle for *A. planipennis* on green ash in Laoniujuan.

3.5. Seasonal abundance of *O. agrili*

Egg parasitism by *O. agrili* was estimated from *A. planipennis* eggs collected from green ash in Laoniujuan during 2004 and 2005. Of the 105 *A. planipennis* eggs collected in June 2004, 87% of those eggs were empty with no content and 13% were unhatched. For the six egg collections made during April through November 2005, a total of 645 *A. planipennis* eggs were collected, of which 68.5% were empty and 31.5% unhatched. Although most *O. agrili* adults emerged within a month of collecting the host eggs, a portion diapaused within the host eggs, and emerged 6–8 months later. Based on numbers of *O. agrili* adults emerging from unhatched *A. planipennis* host eggs collected in June 2004, the realized percent egg parasitism (P1) for June was 28.6%; for the six collections in 2005, parasitism (P1) averaged 36.5% and ranged from 0 to 61.5% (Fig. 4). For host eggs laid during July and August 2005, parasitism (P1) was 56.3 and 61.5%, respectively (Fig. 4). The *O. agrili* that emerged from host eggs collected before July 2005 were presumably those that diapaused within host eggs laid during 2004, providing synchrony with *A. planipennis* adults, which began to emerge in early June at this site.

After dissection of the black, unhatched eggs, we found that 81.8% contained *O. agrili.* Therefore, we used the number of black eggs to estimate unrealized or potential parasitism (P2). As expected, unrealized parasitism (P2) was consistently higher than realized parasitism (P1) and showing similar seasonal trends (Fig. 4). Estimates of parasitism from previous generations (P3), as determined from the number of empty eggs with parasitoid vs. host exit holes, averaged 21.8% and ranged from 6.8 to 32.7% (Fig. 4).

3.6. Seasonal abundance of *T. planipennisi*

Overall, 15.2% of the *A. planipennis* larvae collected in 2004 and 22.4% in 2005 were parasitized by *T. planipennisi*. Parasitism rates increased steadily from 7.5% in May to 15.5% in July, and then increased significantly to 39.7% by August, when the majority of larval *A. planipennis* from the current generation (2005) were 4th instar (Figs. 3 and 5). *T. planipennisi* parasitism remained at a similar level thereafter, as indicated by the 40.4% rate in November (Fig. 5).

The external symptoms of parasitism by *T. planipennisi* in host larvae did not appear until parasitoid larvae were mature and ready to exit the host cadaver during their wandering phase prior to pupation. The symptoms of parasitism by this parasitoid include bulges on the surface of the host integument, reflecting the shape of parasitoid larvae within. A host cadaver containing large numbers of parasitoid larvae looks like a braided rope. On average, each host larva produced 35.2 parasitoid larvae, ranging from 5 to 122 per host larvae (Table 3). *T. planipennisi* attack both 3rd- and 4th-instar *A. planipennis* larvae, but clearly preferred 4th-instar. Of the 101 parasitized *A. planipennis* larvae collected, 89 were 4th instars and 12 were 3rd instars (Table 3). In general, 4th instar-host larvae also produced more parasitoids than the 3rd instars, although these differences were only significant for the August collections (Table 3). No differences in parasitoid production were observed in 3rd-instar host larvae between the August and the November collection; whereas 4th-instar larvae collected in May produced significantly fewer *T. planipennisi* compared with the August collection, with no significant difference observed among other collections (Table 3).

3.7. Impact of *O. agrili* and *T. planipennisi* on *A. planipennis* local populations

Positive density dependence was observed between the unrealized parasitism (P2) of *O. agrili* and unhatched host eggs collected ($F = 5.66$, $df = 1, 4$, $P = 0.08$, $R^2 = 0.586$).

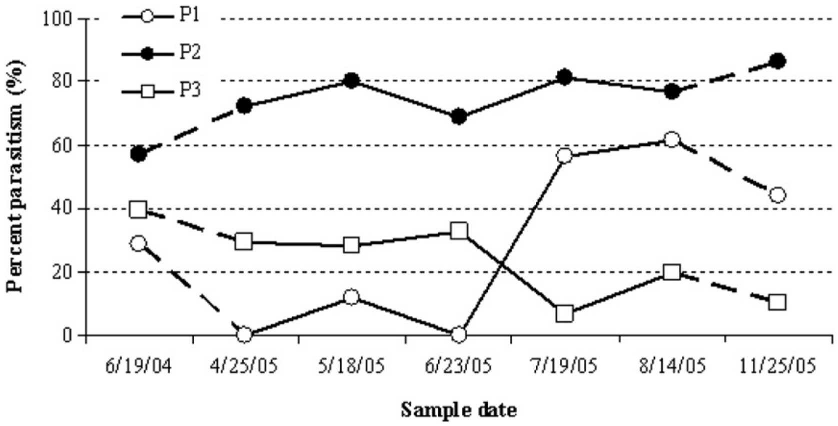

Fig. 4. Seasonal abundance of *O. agrili* parasitizing *A. planipennis* eggs at Laoniujuan of Changchun, Jilin province in 2004 and 2005. P1 = no. of parasitoid adults/no. of unhatched eggs; P2 = no. of black eggs/no. of unhatched eggs; P3 = no. of eggs with parasitoid exit holes/no. of hatched eggs. Broken line indicates sample gap.

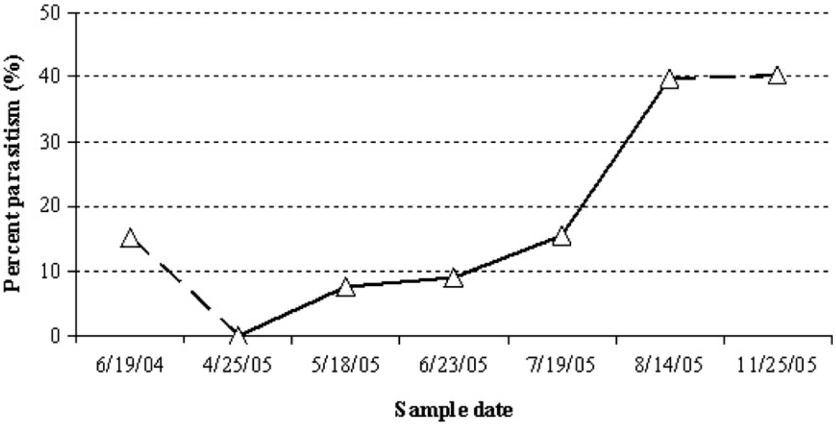

Fig. 5. Seasonal abundance of *T. planipennisi* parasitizing *A. planipennis* larvae at Laniujuan of Changchun, Jilin province in 2004 and 2005. Broken line indicates sample gap.

Table 3
Average number of *T. planipennisi* larvae produced by *A. planipennis* larvae collected from Laoniujuan of Changchun, Jilin Province in 2005

Sample date	No. host collected	Parasitized (*n*)		Parasitoids per host (Means ± SEM) (range)[a]	
		Third instar	Fourth instar	Third instar	Fourth instar
04/25/05	58	0	0	n/a	n/a
05/18/05	66	0	5	n/a	18.2 ± 2.9 (10–26)b
06/23/05	67	0	6	n/a	31.3 ± 8.1 (18–69)ab
07/19/05	58	0	9	n/a	24.7 ± 5.6 (5–57)ab
08/14/05	88	3	32	16.3 ± 2.7 (11–19)aA	42.7 ± 4.8 (7–122)aB
11/25/05	114	9	37	29.8 ± 5.4 (7–55)aA	35.2 ± 2.6 (7–87)abA

[a] Means followed by the same lower case letter within a column are not significantly different (Student–Neuman–Keul test, $\alpha = 0.05$); means followed by the same upper case letter within a row are not significantly different (T-test, $\alpha = 0.05$); n/a – not applicable.

The rate of parasitism increased with the increase of host egg density (Fig. 6).

Positive density dependence was also observed between *T. planipennisi* parasitism and the total number of host larvae collected over time ($F = 7.82$, $df = 1, 4$, $P = 0.05$, $R^2 = 0.662$). The higher the host larval density, the higher the parasitism rate (Fig. 7a). This correlation was strengthened when only 3rd- and 4th-instar host larvae were considered ($F = 64.03$, $df = 1, 4$, $P < 0.01$, $R^2 = 0.941$) (Fig. 7b). The slopes were similar for both regressions (Figs. 7a and b).

Both *O. agrili* and *T. planipennisi* played an important role in the population dynamics of *A. planipennis* at the Laoniujuan site in Changchun, Jilin. For 2004, *A. planipennis* population was reduced by 39.5% with these two parasitoids based on the parasitism in June. On the other hand, *O. agrili* and *T. planipennisi* were responsible for 73.6% host population mortality in 2005 when current year para-

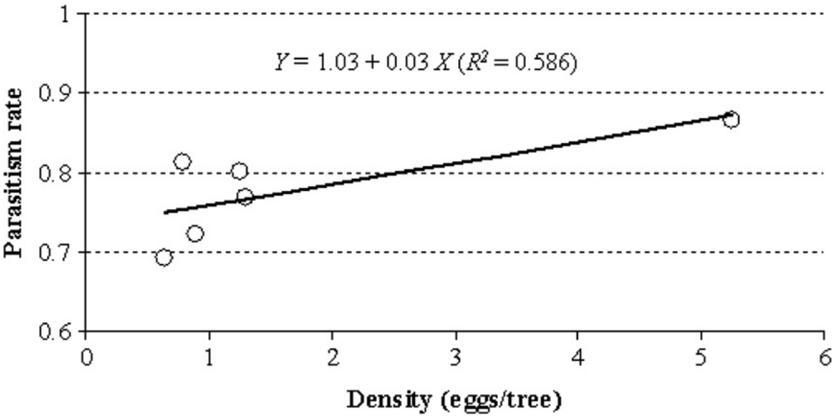

Fig. 6. Correlation between parasitism rate of *O. agrili* and *A. planipennis* egg density at Laoniujuan of Changchun, Jilin province in 2005. Each data point represents the parasitism rate (Y) in response to host egg density (X) of one collection. A total of six egg collections were made for the study.

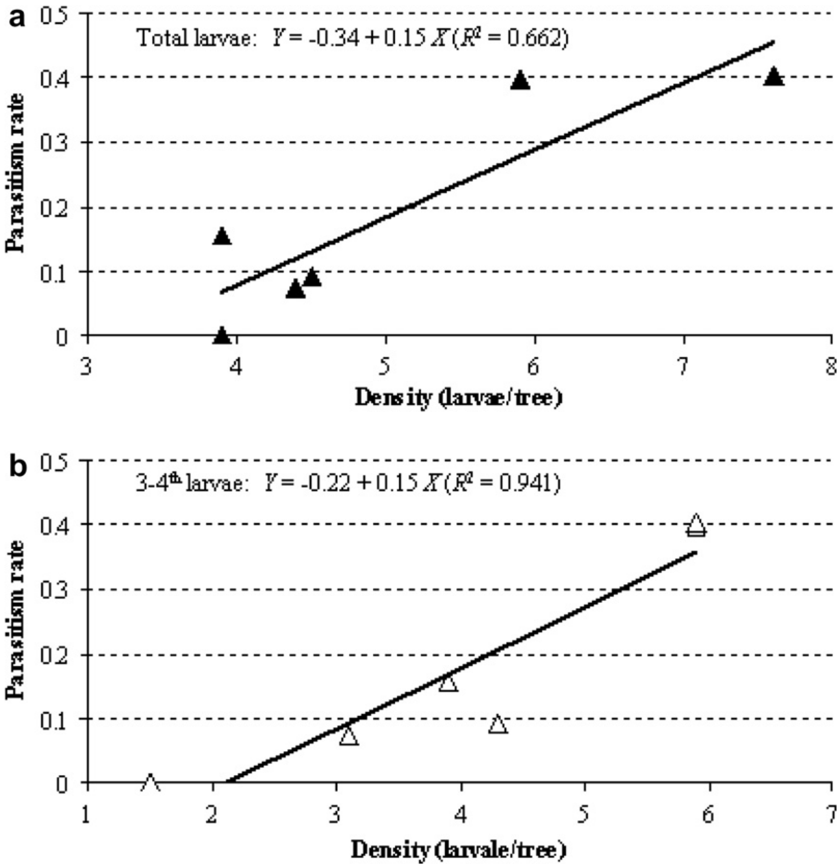

Fig. 7. Correlation between parasitism rate of *T. planipennisi* and (a) total or (b) 3–4th instar larval density of *A.planipennis* at Laoniujuan of Changchun, Jilin province in 2005. Each data point represents the parasitism rate (*Y*) in response to host larval density (*X*) of one collection. A total of six larval collections were made for the study.

sitism was used for *O. agrili* (56.3%) and *T. planipennisi* (39.7%). Both parasitoid species were synchronized well with *A. planipennis* under field conditions, with at least two generations for *O. agrili* attacking *A. planipennis* eggs between June and August (Fig. 4) and up to four generations of *T. planipennisi* attacking host larvae throughout the year (Figs. 3, 5). Diapause within host eggs after the field season allows for synchrony of *O. agrili* with its host population in the next year.

4. Discussion

The distribution of *A. planipennis* in China is patchy, with significant differences in density observed between sites, within a site, and vertically within a tree. The higher population densities found at Changzui in Benxi and Laoniujuan in Changchun than at Changbu, Dongfenqu, and Dingjiagou, reflected the difference in host species, infestation history, site conditions, and proximity to forests or parks. North American species such as green ash are more susceptible to *A. planipennis* than native ash species when planted at the same site. At our Changchun site, *A. planipennis* completed an unsynchronized one-year life cycle after overwintering at any larval stage. Better synchronization was observed at the more southerly sites such as Changzui in Benxi.

Oobius agrili was found in *A. planipennis* eggs throughout the year and was synchronized with the oviposition period of *A. planipennis* in Changchun, as indicated by the increase in parasitism rates from July to August 2005. Moderate correlation was found between unrealized parasitism of *O. agrili* and unhatched host egg density. However, this regression may be an artifact of parasitoid diapause and sampling frequency as egg samples made outside the oviposition period do not represent real time impact of *O. agrili* on the current generation of *A. planipennis*. It should rather be considered as a ‘‘residual effect’’ for either the current generation (samples after egg period) or the previous generation (samples before egg period). Persistence of *A. planipennis* unhatched eggs and egg shells on tree bark from the previous year further complicated this problem. More frequent sampling during the peak oviposition period may provide better correlation between parasitism and host density.

Oobius agrili is a newly described solitary egg parasitoid of *A. planipennis* from China with no other known hosts (Zhang et al., 2005). Other species within the genus also parasitize eggs of *Agrilus* spp. include *Oobius zahaikevistshi* Trjapitzin from *A. cuprescens* Menetries, *Agrilus lopatini* Alex., *Agrilus roscidus* (Kiesenwetter), *Agrilus shamyl* (Obenberger), and *Agrilus viridis* (L.) (Trjapitzin, 1963, 1989); and *Oobius abditus* Annecke and *Oobius funestus*

Annecke from *Agrilus gloriosulus* (Pringuey) (Annecke, 1967; Prinsloo, 1983). Although no egg parasitoids have been confirmed from *Agrilus planipennis* in Michigan (Bauer et al., 2005), species of *Ablerus*, *Avetianella*, *Ooencyrtus*, and *Thysanus* (Encyrtidae) were reared from eggs of the bronze birch borer, *Agrilus anxius* Gory, a common native *Agrilus* species in North America (Nash et al., 1951; Barter, 1957; Loerch and Cameron, 1983). *Avetianella longoi* Siscaro, an encyrtid species closely related to *Oobius* spp., was introduced from Australia to California for successful biological control of the eucalyptus longhorned borer, *Phoracantha semipunctata* (F.) (Coleoptera: Cerambycidae) (Hanks et al., 1995, 1996).

The results of our studies showed that parasitism of *T. planipennisi* on *A. planipennis* increased throughout the season in Changchun in 2005. Positive correlation was observed between parasitism rate and host density, and the presence of suitable host stages (3rd and 4th larvae) in all collections indicated the possibility for *T. planipennisi* to continue its generations throughout the year except winter. Its high productive rate per host supports rapid population increases and may enable *T. planipennisi* populations to respond to outbreaks of *A. planipennis* quickly.

We discovered *T. planipennisi* in Jilin and Liaoning Provinces during our early research on *A. planipennis* biological control in China (Liu et al., 2003); it was later found in Heilongjiang Province (Yang et al., 2006). *Tetrastichus* Haliday is a heterogeneous, diverse, and cosmopolitan genus with 482 species (Noyes, 2003), of which 21 are found in North America (LaSalle, 1994). A few species in this genus were found attacking *Agrilus* spp., including *T. rugglesi* Roh. from the bronze cane borer, *A. rubicola* Abeille, and the red-necked cane borer, *A. ruficollis* (F.) in New York (Mundinger, 1941); *Tetrastichus* sp. from the bronze birch borer in Pennsylvania (Loerch and Cameron, 1983). Although no *Tetrastichus* spp. have been found parasitizing *A. planipennis* in North America, <1% of larvae sampled from field populations in Michigan are parasitized by three native parasitoids: *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae), *Spathius floridanus* Ashmead (Hymenoptera: Braconidae), and *Atanycolus* spp. (Hymenoptera: Braconidae), and one exotic parasitoid, *Balcha indica* (Mani & Kaul) (Hymenoptera: Eupelmidae) (Bauer et al., 2004, 2005).

In northeastern China, *A. planipennis* is generally considered a sporadic pest of native ash species including Manchurian ash, Chinese ash (*F. c.* var. *chinensis*), and Korean ash (CAS, 1986; Yu, 1992; Liu et al., 2003; Xu, 2003; Gao et al., 2004; Wei et al., 2004). The introduction of North American ash such as green ash and white ash (*F. americana* L.) during the 1960s, however, increased the pest status of *A. planipennis* in China (Liu et al., 2003). A more recent outbreak of *A. planipennis* was reported in Tianjin City in 1989 when thousands of velvet ash trees became infested in city parks and along the streets (Zhang et al., 1995). The replanting of these North American ash species in coastal areas during recent decades likely facilitated the spread of *A. planipennis* in China.

The results from this study demonstrated that both *O. agrili* and *T. planipennisi* contribute significantly to *A. planipennis* population suppression on green ash in northeastern China. Previous studies showed that *T. planipennisi* was also an important mortality factor for *A. planipennis* in Manchurian ash in Changchun and Benxi (Liu et al., 2003). Although host resistance differs between native Chinese ash species and species introduced from North America, the ability of these parasitoids to locate and attack *A. planipennis* under the bark was not affected. This is confirmed by the presence of relatively healthy and reproducing green ash in Changchun where high populations of *A. planipennis* were suppressed by *O. agrili* and *T. planipennisi*. Coupled with their high parasitism rates, short generation times, high reproduction rates, and life-cycle synchronizations with their respective host stages, these parasitoids may prove useful for biological control of *A. planipennis* in North America. Geographical similarities between parasitoid ranges in China and *A. planipennis* distribution in Michigan may provide comparable parasitoid-host life cycle in the field. The state of Michigan stretches from 42°N in the south to 48°N latitude in the north, whereas *T. planipennisi* was found in Benxi (41°35'N) of Liaoning, Changchun (43°52'N) of Jilin (Liu et al., 2003), and Weihe (44°58'N) of Heilongjiang (Yang et al., 2006); *O. agrili* has been recorded in Changchun to date. Differences in host tree species, infestation levels, habitat and climatic conditions, and management approaches between China and North America may bring new challenges to their potential success as biocontrol agents of *A. planipennis*. We are continuing research in the laboratory on the biology, ecology, and host specificity of *O. agrili* and *T. planipennisi* as we evaluate their potential for the biological control of *A. planipennis* in North America.

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