First description of the life cycle of the emerald ash borer *Agrilus planipennis* in Europe and its comparison with the life cycle of the pest in Asia and North America

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Running title: Life cycle of the emerald ash borer in Europe

Abstract

1. *Agrilus planipennis* (Coleoptera: Buprestidae), native to Asia, is a destructive invasive ash pest in USA, Canada and European Russia. It is spreading quickly and is likely to be detected in other European countries soon.
2. Information about the life cycle of the pest is needed for detection and survey efforts, development of control options and predicting the potential range in Europe. The life cycle has been examined in North America and Asia, but has not been studied in European Russia before.
3. The aim is to define the number of larval instars and duration of development of the emerald ash borer in European Russia. Distributions of width of epistome and length of urogomphi indicate four larval instars. Development in most of the specimens took two years.
4. The life cycle is flexible. In the warm regions, the majority of specimens finish development in one year, while it takes two years in the cold regions. In intermediate regions, the ratio between one-year and two-year life cycle depends on additional factors. Flexibility of the life cycle allows *A. planipennis* to establish in regions where climate differs from the climate in its native range.

Keywords Emerald ash borer, EAB, life cycle, Europe, *Agrilus planipennis*, Coleoptera, Buprestidae, *Fraxinus*, pest.

Introduction

Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is one of the most destructive forest pests in the world (Herms & McCullough, 2014). In Europe, it is a quarantined pest included in the NAPPO Alert List, the EPPO A2 list of pests recommended for regulation, and the EU Annex II/A1 (EPPO, 2014).

This ash pest originating from East Asia was first detected in North America in 2002 (Haack *et al*., 2002) and in Europe in 2003 (Mozolevskaya & Izhevskiy, 2007). Since that time it has spread quickly in both continents and destroyed ashes (Cappaert *et al*., 2005; Orlova-Bienkowskaja, 2014). The epicenter of invasion in Europe is Moscow. Almost all ashes in the city are dead or dying (Orlova-Bienkowskaja, 2013) and the outbreak of *A. planipennis* has facilitated the outbreak of some other xylophagous beetles (Orlova-Bienkowskaja & Volkovitsh, 2014). There is little doubt that *A. planipennis* will cross the border of Russia and be found in other countries soon (Straw *et al*., 2013). Native European species of ash *Fraxinus excelsior* L. is highly susceptible to the pest (Majorov *et al*., 2012). The ecological and economic consequences of *A. planipennis* establishment could be tremendous since ashes are common and play an important role in the biodiversity of European forests (FRAXIGEN, 2005).

Efforts to contain the spread of *A. planipennis* and to manage populations in the infested area will require a broad understanding of its biology, so it is very important to know its life history. First, basic information on phenological events (adult activity, larval development, and overwintering behavior) is needed for detection and survey efforts and development of control options. Second, longevity of larval development strongly influences *A. planipennis* population dynamics and mechanisms and rate of spread. Third, knowledge of the life cycle provides the basis for studying interactions with natural enemies, such as the recently discovered parasitoid *Spathius polonicus* Niezabitowski (Hymenoptera: Braconidae: Doryctinae) (Orlova-Bienkowskaja & Belokobylskij, 2014). In addition, understanding the life cycle should help elucidate key evolutionary adaptations which allow *A. planipennis* to become established in regions where the climate significantly differs from the climate in its native range.

The life cycle of *A. planipennis* in European Russia has not been studied before. It is known that adults begin to emerge in late May or early June, peak flight occurs between 5–15 June, and some full-grown larvae overwinter in pupal cells (Mozolevskaya & Izhevskiy, 2007). Duration of development and number of instars in species of Buprestidae can be different in different regions (Beer, 1949). The number of instars and duration of development in European Russia was unknown. The aim of the present study is to fill this gap in knowledge.

The life cycle of *A. planipennis* has been previously examined in southeastern Michigan (USA) (Cappaert *et al*., 2005), and in Tianjin, Benxi, Harbin and Changchun (China) (Wang *et al*., 2005; Liu *et al*., 2007; Wei *et al*., 2007; Wang *et al*., 2010). In the present study, the life cycles in different regions are compared and summarized in a series of phenological diagrams. This summary suggests that the life cycle is flexible. Usually in one part of a cohort (i.e. the generation of specimens hatched from eggs at the same year), the life cycle is one-year, but two-year for another part of the same cohort. In the warm regions, the majority of specimens complete development in one year, while in the cold regions it takes two years. In intermediate regions, the ratio between one-year and two-year life cycle depends on additional factors.

Material and methods

For determination of the period of adult activity, approximately 100 *F. pennsylvanica* trees were surveyed for adults twice a week from 15.05.2014 to 19.07.2014. Adults were also collected in July 2011 and in June and July 2013. Immature stages of *A. planipennis* were collected each month from the beginning August 2013 to the end of July 2014, i.e. the whole year from the hatching of one cohort of larvae to the hatching of another cohort. Larvae were collected from about 100 heavily infested *Fraxinus pennsylvanica* Marsh. in Moscow. The trees were 10–30 years old and grew near the street. Their diameter at breast height was 20–50 cm. All trees were alive with foliage density markedly reduced. We have no permission and possibility to fell the trees. So we collected larvae from under the bark in the lower part of stems (up to 1.5 m). For this purpose, the lower 1.5 m of trunks of standing trees were debarked by chisel and hammer.

Two hundred and nine larvae and prepupae were collected from the cambial region and outer sapwood. The methods of collection, duration of collection, and the number of collected larvae correspond to studies made by other researchers in USA and China, so the results are comparable (200 larvae were collected during the study of the life cycle in 2004 Michigan USA (Cappaert *et al*., 2005), and 700 larvae were collected from August 2003 to August 2004 in Tianjin (Wang *et al*., 2005)).

Six characters were used for distinguishing of larval instars: (1) width of epistome (i.e. exposed sclerotized portion of the head capsule, sometimes erroneously referred to as peristome (Wang *et al.*, 2005)); (2) width of prothorax; (3) length of urogomphi (i.e. paired terminal processes); (4) width of the last abdominal segment (Fig. 1); (5) number of excretory ducts on the urogomphi; and (6) presence or absence of ledges (i.e. numerous secondary subdivisions of excretory ducts). These characters have been already used for determining of number of instars of *A. planipennis* in the USA (Cappaert *et al*., 2005; Chamorro *et al*., 2012) and China (Wang *et al.*, 2005).

Fig. 1

Results and discussion

Larval instars

Distribution of measurements of sclerotized parts (width of epistome and length of urogomphi) clearly indicate that there are four distinct size classes (Fig. 2). The measurements of the smallest larvae correspond to those of first instars reared in the laboratory (Cappaert *et al*., 2005; N.W. Siegert, unpublished data). Measurements of the largest larvae correspond to those of prepupae (i.e. non-feeding terminal phase of the fourth instar lying in a pupal cell). Therefore, we confirm that there are four larval instars.

Fig. 2

Width of prothorax and last abdominal segment are not appropriate characters for distinguishing instars because they do not effectively divide larvae into distinct size classes (Table 1). Furthermore, these soft parts of the larvae gradually increase as it grows and develops over time.

Table 1

Chamorro *et al*. (2012) proposed distinguishing developmental stages by the number of excretory ducts of the urogomphi and the presence/absence of ledges. Our data indicate that these characters change with larval development, but are variable and do not provide a clear differentiation between instars (Table 1).

It seems that fourth instars *A. planipennis* in Moscow are smaller than those in Tianjin or Michigan. Mean width of epistome in Moscow is significantly less than in Tianjin or Michigan (t-Student's, $P<0.05$), and the mean length of urogomphi is significantly less in Moscow than in Tianjin (t-Student's, $P<0.05$) (urogomphi were not measured in the Michigan study) (Fig. 3). Mean values of width of prothorax and width of last abdominal segment in Moscow and Tianjin do not differ significantly, but maximal values are higher in Tianjin. It is well known that the insects are smaller if larvae do not get enough nutrients (Nijhout, 2003). The larvae in Moscow may be smaller because the warm period is shorter and they have less time to feed and develop. This hypothesis remains to be tested though.

Fig. 3

Life history

The flying period of *A. planipennis* adults begins in early June in Moscow. Adult beetles were captured from 8 June to 5 July in 2013 and from 2 June to 9 July in 2014. Adults feed on ash foliage and oviposit in bark crevices of stems. In early June, there are many third and fourth instars present under the bark (Fig. 4). These larvae cannot belong to the cohort of beetles which emerge as adults the same year because no prepupae occur until August. They also cannot belong to the cohort that hatched from eggs during the current year because they were present before oviposition took place or during the very beginning of the oviposition period. Therefore, they belong to a separate cohort, indicating that the life cycle lasts more than one year. Most larvae develop to fourth instar by the end of July. In August, some excavate pupal cells and become prepupae. The new cohort of current-year first instars are also present at this time.

Fig. 4

From late August to early May, the distribution of larval instars does not change significantly. Many larvae remain in the first instar from autumn to the beginning of May. Larvae do not grow much if at all in this cold period. Larvae of all instars are present in winter. The rate of the fourth instar larvae and prepupae decreases in winter, and are rare by early May. For instance, in early May, we found only one fourth instar larva and no prepupae, though 40 larvae of other instars were collected. This decrease probably reflects a high level of mortality caused by insectivorous birds, since the bird damage are usual on the ash stems in Moscow (Mozolevskaya & Izhevskiy, 2007; Orlova-Bienkowskaja, 2013).

The potential for *A. planipennis* development in Moscow to last for three or more years remains a possibility. Delay in development occurs in some Buprestidae (Beer, 1949) and no direct laboratory or field observations on the duration of development of *A. planipennis* in Moscow have occurred to date. It appears that all larvae collected in June and July belong to the same cohort. In early June, about half of them are third instars and the other half are fourth instars. By the end of July, almost all the larvae reach the fourth instar.

Comparison of life cycle in different regions

Comparison of the emerald ash borer life cycle in Moscow with data on its life cycle in southeastern Michigan (USA) (Cappaert *et al*., 2005) and in Tianjin, Harbin and Changchun (China) (Wang *et al*., 2005; Liu *et al*., 2007; Wei *et al*., 2007; Wang *et al*., 2010) has confirmed that there are four larval instars in all these locations, but that the duration of development vary (Table 2).

Table 2

In the southern part of the native range in Tianjin and Benxi, the life cycle is one-year in almost all specimens and larvae overwinter once as prepupae in pupal cells (Wang *et al.*, 2005; Liu *et al*., 2007). Few larvae of younger instars (<1%) overwinter in their galleries and continue development the following year, taking two years to complete development (Wang *et al*., 2010). In Harbin, which is located far north, the life cycle is two-year and larvae overwinter twice; the first time in larval galleries and the second time in the pupal cells (Wei *et al.*, 2007). It was supposed that in Changchun, which is north to Tianjin but south to Harbin, the life cycle of *A. planipennis* is unsynchronized one-year (Liu *et al.*, 2007), but the seasonal distribution of larval stages in Changchun indicates that the life cycle in most specimens is two-year (Liu *et al*., 2007; Wei *et al.*, 2007). Development is synchronized because all pupae appear from overwintered prepupae. Adults emerge in June and early July, though there are third and fourth instars at the same time, which cannot belong to the same cohort as adults. In Michigan, one part of population has a one-year life cycle, though another part exhibits a two-year cycle (Cappaert *et al.*, 2005). Some larvae hatched in summer reach prepupal stage in autumn of the same year and become adults next spring. When larvae fail to reach the fourth instar before winter, pupation appeared to be delayed until the second autumn. Delayed development also appears to be more common in low-density *A. planipennis* populations. In stressed trees, larvae develop faster than in healthy trees (Tluczek *et al*., 2011). In Moscow, the life cycle of *A. planipennis* is two-year. Development lasts for two years even on heavily stressed trees.

The duration of the life cycle could be theoretically different in different host plants. The studies in Changchun carried out on *Fraxinus pennsylvanica* (Liu *et al.*, 2007) and *F. mandshurica* Rupr. (Wei *et al.*, 2007), however, have not revealed significant differences in the life cycle. The life cycle in Moscow and Michigan though appears to differ, despite larvae developing on *F. pennsylvanica* in both regions.

Speed of larval development may be primarily determined by climate, first of all duration of the warm period and the amount of heat, which larvae receive in one season. Number of months with mean temperature above 10° is seven in Tianjin, six in Michigan, and five in Moscow, Harbin and Changchun (Hijmans *et al*., 2005). In many insect species that survive in both warm and cold regions or habitats, the duration of the life cycle varies according to conditions; larval development lasts for an additional year or more at lower temperatures (Danks, 1992). If the life cycle is two-year, the population consists of two cohorts, which reach maturity in subsequent years. In this case, the cohorts are not isolated from each other because a small portion of the each cohort finishes development faster or slower than the main part. This phenomenon is called cohort-splitting. Life cycle flexibility is typical for some buprestid beetles. For example, development of *Agrilus anxius* Gory native to North America varies with latitude and host condition. In the north part of the range, larvae require at least two years to develop and, in the south, development lasts one or two years depending on host vigor (Beer, 1949).

Conclusions

Though the emerald ash borer has four larval instars in all regions, where larvae were studied, duration of development vary from one to two years according to climate condition. In regions with warm climate specimens overwinter once: as prepupae; in the regions with cold climate the specimens overwinter twice: first time as larvae and the second time as prepupae; in regions with intermediate climate a part of the population has one-year life cycle, though another part has two-year life cycle. Flexibility of the life cycle allows *A. planipennis* to establish in regions where climate significantly differs from the climate in its native range.

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Figure captions

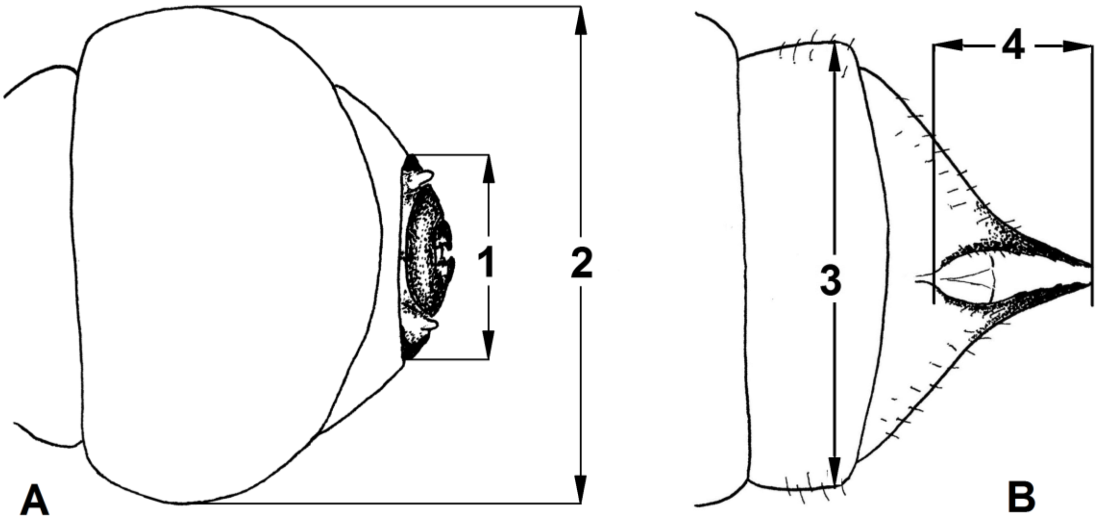
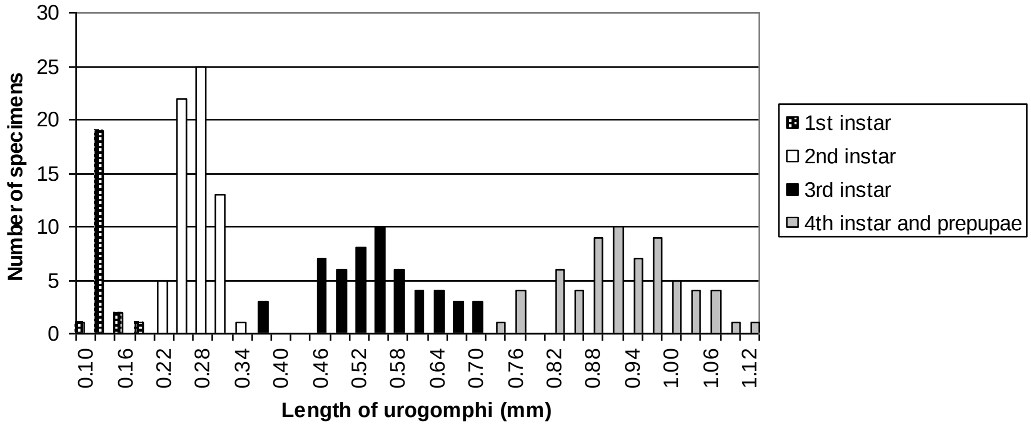
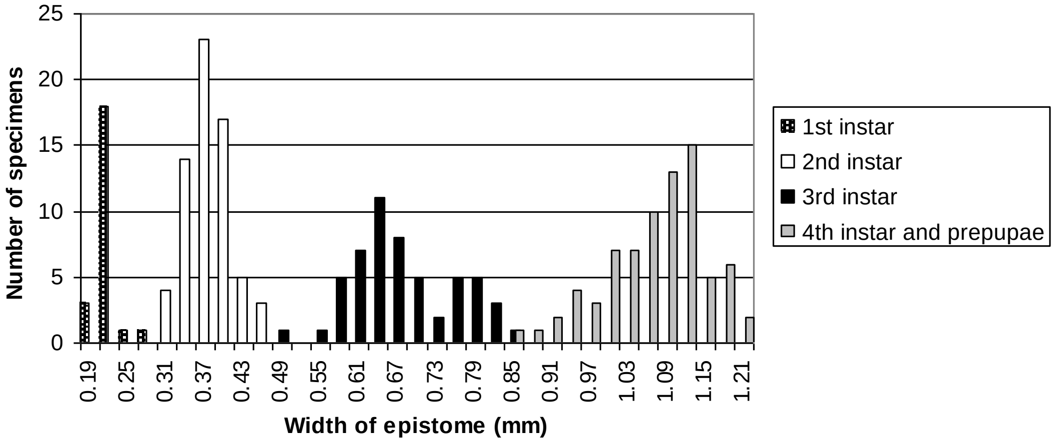


Figure 1 Measurements used for distinguishing between larval instars of *Agrilis planipennis*. A – anterior part of the body, B – posterior part of the body. 1 – width of epistome, 2 – width of prothorax, 3 – width of last abdominal segment, 4 – length of urogomphi.





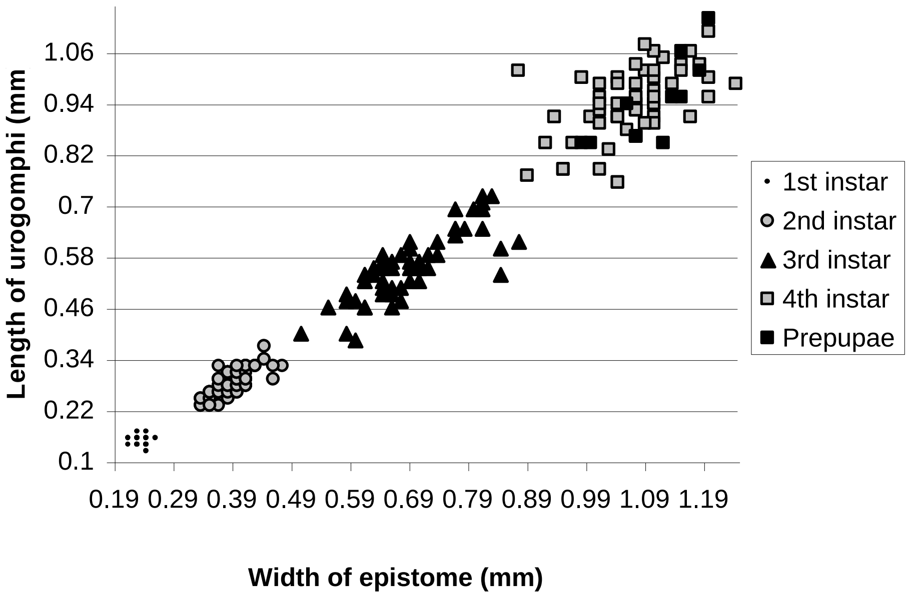
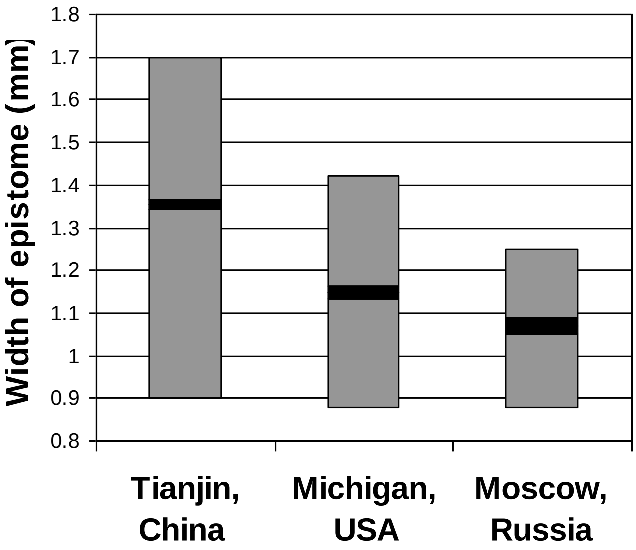


Figure 2 Distribution of measurements of sclerotized parts.



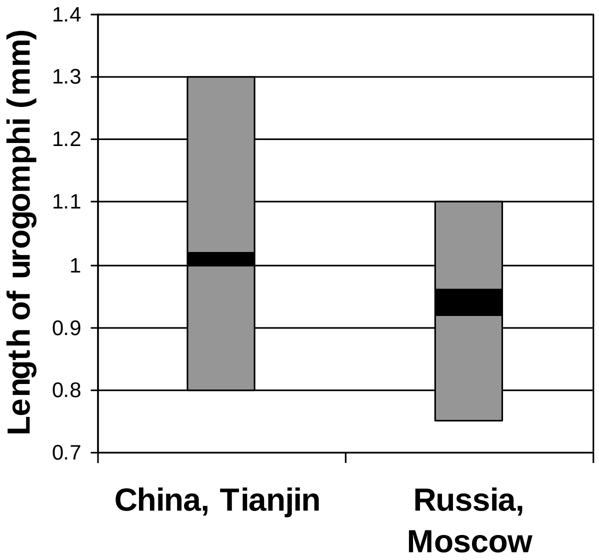


Figure 3 The width of epistome and length of urogomphi of fourth instar larvae in different regions. The interval between minimal and maximal values is shaded gray. The confidence interval of mean value is shaded black (P=0.05).

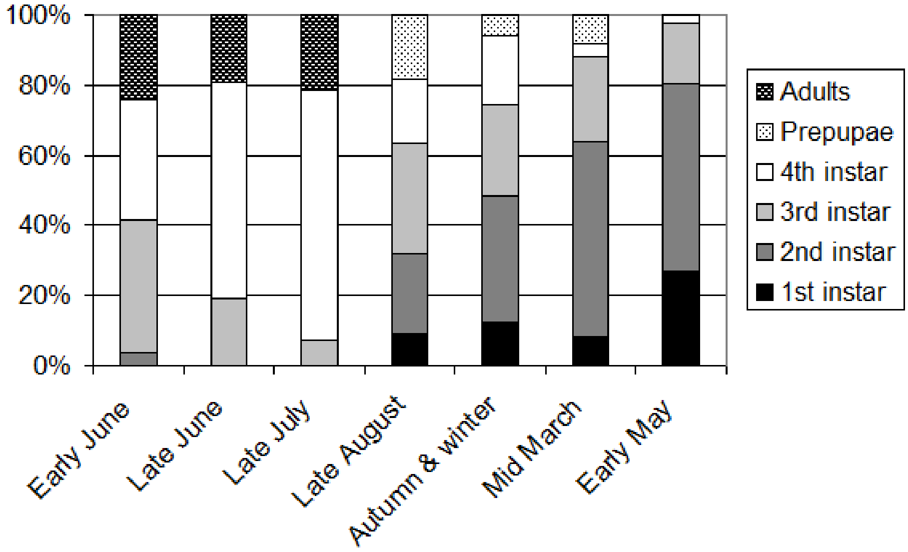


Figure 4 Rate of different development stages in different seasons.

Table 1 Measurements of the immature stages (for each metric character the interval between minimal and maximal value and mean value with confidence interval are indicated).

Stage	Width of epistome (mm)	Length of urogomphi (mm)	Width of last segment (mm)	Width of prothorax (mm)	Number of excretory ducts	Rate of specimens with ledges (%)
1st instar	0.22–0.29	0.12–0.20	0.23–0.40	0.52–0.8	1–2	0
	0.24±0.01	0.15±0.01	0.28±0.02	0.64±0.03		
2nd instar	0.34–0.48	0.23–0.37	0.38–0.71	0.62–1.38	1–3	0
	0.39±0.01	0.29±0.01	0.57±0.02	1±0.04		
3rd instar	0.51–0.88	0.38–0.72	0.68–1.65	1.03–2.29	2–3	24
	0.69±0.02	0.56±0.02	1.19±0.05	1.65±0.08		
4th instar	0.88–1.25	0.75–1.10	1.63–2.77	1.97–3.38	3	78
	1.07±0.02	0.94±0.02	2.08±0.05	2.52±0.09		
Prepupa	0.98–1.20	0.85–1.14	1.86–2.49	2.26–3.54	3	90
	1.11±0.02	0.95±0.02	2.21±0.05	2.94±0.09		

Table 2 Life cycle of *Agrilus planipennis* in different parts of its range: China (after Wang *et al*., 2005; Liu *et al.*, 2007 and Wei *et al.*, 2007), USA (after Cappaert *et al.*, 2005) and European Russia (original data). ◇– larvae, ■ – prepupae, ● – adults. Period of active feeding of larvae is shaded gray. Egg and pupa stages are not shown.

Region	Duration of life cycle	1st year												2nd year												3rd year			Host plant
		May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	
Tianjin, China	1 year	◇	◇	◇	◇	◇	◇	◇																					*Fraxinus velutina*
				■	■	■	■	■	■	■	■	■	■																
														●	●	●													
Michigan, USA	1 year			◇	◇	◇	◇	◇																					*Fraxinus pennsylvanica*
							■	■	■	■	■	■	■																
														●	●	●	●												
	2 year				◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇										*Fraxinus pennsylvanica*
																			■	■	■	■	■	■	■				
																										●	●	●	
Harbin, China	2 year		◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇												*Fraxinus mandshurica*
																	■	■	■	■	■	■	■	■	■	■			
																										●	●	●	
Changchun, China	2 year			◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇											*Fraxinus pennsylvanica*
																			■	■	■	■	■	■	■	■	■		
																											●	●	
	2 year		◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇													*Fraxinus mandshurica*
																■	■	■	■	■	■	■	■	■	■	■			
																								●	●	●		
Moscow, Russia	2 year				◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇	◇													*Fraxinus pennsylvanica*
																	■	■	■	■	■	■	■	■	■	■			
																											●	●	