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Life Cycle and Host Preference of *Xylotrechus rusticus* (L., 1758) (Coleoptera: Cerambycidae) in the Heilongjiang Province, China

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ABSTRACT:

The gray tiger longicorn beetle, *Xylotrechus rusticus* (L., 1758) (Coleoptera: Cerambycidae), is a wood-boring forest insect species. An outbreak of this insect in the 2000s caused a great deal of damage to the Three North Shelter Forests in the western part of Northeast China. To control this beetle and provide data for risk analysis, the development timing, life cycle and host preference were investigated. *X. rusticus* completed one generation per year and overwintered as larva in its last instar in Harbin, Heilongjiang Province, China. The sex ratio was biased 2:1 toward males. The natural mortality was only 4%. The diameter at breast height (DBH) of the infested poplar in this study ranged from 10 to 42 cm. The larvae crowded in the trunks at a height of 3-5 m above the ground. The percentages of attack among various poplar lines were significantly different.

Key words: Gray tiger longicorn beetle, development timing, life history, host preference, sex ratio, poplar-boring longhorn beetle.

INTRODUCTION

The gray tiger longicorn beetle, *Xylotrechus rusticus* (L., 1758) (synonym: *Rusticoclytus rusticus* (L., 1758)) (Coleoptera: Cerambycidae: Cerambycinae), was distributed in East Palaearctic, Near East (including Asian Turkey, Caucasian Russian republics, Georgia, Armenia, Azerbaidjan, Lebanon, Syria, Israel, Jordan, Sinai Peninsula (Egypt), Arabian peninsula, Iran, Iraq), North Africa (Not including Sinai Peninsula) and Oriental region (Sama, 2013). It attacks dead standings or fallen tree trunks (Brelih *et al*., 2006; Danilevskaya *et al*., 2009); the exception is in China, where it mainly attacks living poplar trees (Meng *et al*.,1983; Xu, 1979). The larvae dig tunnels in poplars, which can lead either to the break of the tree by wind or to its death (Fig. 1). The earliest documented outbreak of *X. rusticus* in Northeast China was reported in 1979 (Meng *et al*.,1983; Xu, 1979). We have recorded its recent outbreaks in the Heilongjiang Province. In 2003, Daqing Hongqi farm (Daqing City, Heilongjiang Province) performed clear cutting on 178 hm^2 of forest that had been damaged by this beetle and additional selection cutting on another 186 hm^2. From

2000 to 2003, 129,798 trees (7,467 hm^2) were cut in Shuangcheng (a new district of Harbin, Heilongjiang Province), and 200 hm^2 of forest was cut in Zhaodong (Suihua City, Heilongjiang Province) due to the outbreak of this beetle. The outbreaks have also been reported in Changchun, the capital city of Jilin Province, in which the outbreak area spanned up to 1,300 hm^2, 460 hm^2 of which was seriously damaged (Meng, 2002).

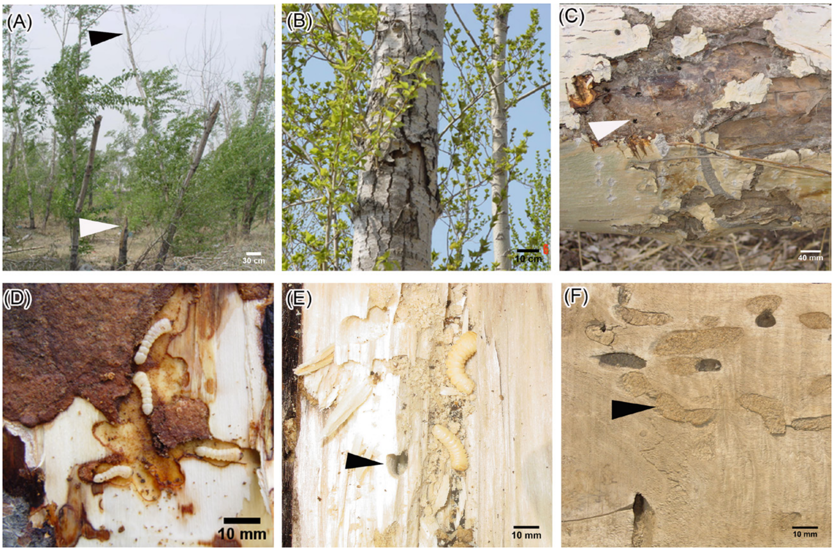

Fig. 1. Damages of *X. rusticus* on poplar. (A) Trees broken by wind at the location of the infestation (white arrow head) and dead trees (black arrow head). (B) Debarking at the infested location on the trunk. (C) Emergence pores on the trunk. (D) Young larvae boring beneath the bark. (E) Old larvae and galleries burrowed in the xylem of poplars (black arrow head). (F) Galleries being filled with frass and dust (black arrow head).

Though the serious damages caused by *X. rusticus* were only reported in China, this beetle is a potential threat to the rest of the world. Besides poplar, it also attacks species of *Betula* L., *Salix* L., *Acer* L., *Castanea* Mill., *Quercus* L., *Sorbus* L., *Tilia* L., and *Ulmus* L. (for review, see Han and Lyu, 2010) and even *Abies alba* L. (Szwalko and Krolik, 1990). Countries in which the tree taxa are distributed are potentially endangered areas, especially those in which *X. rusticus* is absent for now. For example, this beetle has been detected at the ports of USA (Berger, 2014), and is on the list of Indiana's 'most unwanted' invasive plant pests (Indiana Cooperative Agricultural Pest Survey (CAPS) Program, 2015).

The damaged sites in China are located in the central part of the Songnen Plain, and these forests are composed of single poplar lines. Poplar is distributed all over China (Chou *et al*., 1986). However, most poplar forests have been intentionally planted as wind breaking forest strips (shelterbelts). These strips of forests are known as the Three North Shelterbelt Program or the Green Great Wall. Poplar was selected to construct the Green Great Wall because it grows faster than other trees. Numerous hybrid poplar lines have been created in China to construct the Three North Shelter Forests.

An understanding of the biology and life cycle of this wood-boring beetle is important for determining appropriate control measures and their timing. However, the biology of this beetle is not very clear, even in China. In 2001-2002, we investigated the life history and other biological characters of this beetle in the Heilongjiang Province, China. These data will pave the way for the control of this beetle and provide information for risk analysis.

In this study, we intended to investigate the preference of this beetle to poplar lines, providing references for stand regeneration. In addition, preferences for Diameter at Breast Height (DBH) and the height of the infestation on the trunk were also investigated, which is helpful to monitor and control this beetle.

MATERIALS AND METHODS

Life history survey

The life history and habitat of the beetle were observed in the field in Shuangcheng (Fig. 2), now a district of Harbin, located in the central part of the Songnen Plain, China. The shelterbelts in Shuangcheng are composed of pure poplar stands. To determine the developmental timing of pupation and eclosion of *X. rusticus*, at least two infested trees (20-35 cm in DBH) (at least 60 insect specimens were collected) were cut into c.a. 100 cm long logs, and the logs were then chopped into pieces to record the numbers of larvae, pupae and adults in a survey. The insects from each log were also counted to plot the vertical distribution of *X. rusticus* on the trunk. The numbers of dead and live beetles were counted respectively to calculate the natural mortality which is equal to the number of total dead beetles divided by the sum of the numbers of total collected beetles. This survey started on May 23, 2001 and April 21, 2002 and was carried out every week until pupae were found. As soon as pupae were found, the investigation was conducted daily. All collected larvae and pupae were killed and buried after counting.

To study the developmental timing of the beetle, trees must be felled and hewed. In China, there is a strict procedure to apply for permission to fell a tree. We did not apply for this permission. Alternatively, we conducted this investigation when the forest manager was doing a clear cut. All felled trees should be fumigated with chemicals before the beetles' emergence in order to kill the beetles within the trunks. As a result, the dates we did the inspections were restricted to the timing of the clear cut. Since the dates for clear cut and fumigation varied from site to site, the complete investigations could not be carried out at all sites. With the permission and under the surveillance of the officials from the local forestry administrative organization, 30 logs were wrapped with wire cages (20 eye in mesh size) before emergence occurred, and the number and sex of the emerged beetle adults were recorded respectively every day to calculate the emergence rate and sex ratio. To avoid escaping, newly emerged beetles were killed by chemicals before they were collected out from the cage. After counting, the beetles were buried. This investigation lasted to another one week after no emergence occurred. Then all the logs were hewed and burned.

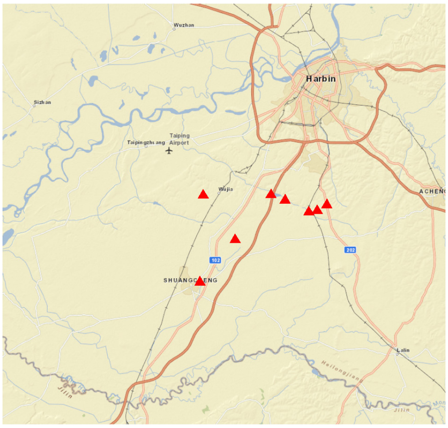

Fig. 2. Study sites. Triangles represent the sites in Shuangcheng district.

Generally speaking, the development of insects in any life stage can be sub-divided into starting period, peak period, and ending period when 16, 50, and 84% of the population reaches this stage, respectively (Xiao, 1992). There are two methods to determine these periods in the field. For emergence time, the chart of the cumulative emergence rate against date was plotted. However, this method is not suit for determination of the time of pupation and eclosion of this beetle since both the larvae and the pupae would die when they do not stay in the wood any longer. An alternative method was adopted to solve this problem. The daily percentages of pupation/ecolosion were plotted to make a normal-like curve. The date corresponding the vault of the bell curve is the peak period of pupation/eclosion.

The preference for poplar lines

To verify whether *X. rusticus* has a preference for certain poplar lines, we investigated the attack percentages of six poplar lines at eight sites of the Shuangcheng District (Fig. 2). Poplar trees with similar DBH (ca. 20-35cm) were selected for investigation to avoid the influence of poplar age. If obvious characteristics of attack (e.g., emergence pore) were observed on a tree, it was assigned as an attacked one.

Statistics

All statistical analyses and charts were designed using R (R Core Team, 2014). The study sites were mapped using the OpenStreetMap package (Fellows, 2013). Curve fit was carried out with nls() function and proportion test with prop.test() function. A series of z tests were adopted to perform multiple comparisons between the natural attack percentages of poplar lines, and Bonferroni-Holm adjustments were used to adjust the significance values (Holm, 1979). Shapiro test was used to determine the normality, and Kruskal-Wallis test was used to test one-way ANOVA by ranks. A series of Wilcox tests were used to perform multiple comparisons if null hypothesis in Kruskal-Wallis test was rejected, and the significance values were also adjusted with Bonferroni-Holm adjustments.

Abbreviations

PXH: *Populus* × *xiaohei* T.S. Hwang and Liang; PSI: *Populus simonii* Carrière; PBJ: *Populus beijingensis* W.Y. Hsu; SXZ: *Populus* × *xiaozhuanica* W.Y. Hsu and Liang; PSU: *Populus suaveolens* Fisch. ex Loudon; PPN: *Populus pseudosimonii* Kitag. × *Populus nigra* L.

RESULTS

Development timing

The pupation of *X. rusticus* in 2002 began between May 5 and May 10. The exact date was not determined for the data that were missing (Fig. 3 A). May 12 was observed as the ending point of larva and the starting point of pupa, at which the larval population was equal to that of pupa (Fig. 3A). Afterwards, the larval population being less abundant than that of pupa and ended at May 21, while the pupal population went to its summit around at May 19, then went down.

The peak period of pupa of *X. rusticus* fell in May 24 in 2001 (Fig. 3B), five days later than in 2002. May 31 was observed as the point when the larval population was equal to that of the adult (Fig. 3B). The eclosion began on May 26 in 2001, six days later than in 2002 (May 20) (Fig. 3A). The first appearance of emergence was observed on May 30 in 2001. It took four, six and seven days to reach its starting period (June 3 2001), peak period (June 5 2001) and ending period (June 6 2001), respectively (Fig. 5B). The cumulative emergence rate by date could be described as a Weibull curve: $y = 1-\exp(-0.0000452x^{5.31})$, where y is the cumulative emergence rate, and x is the number of days after May 29, with a range of (1, 8).

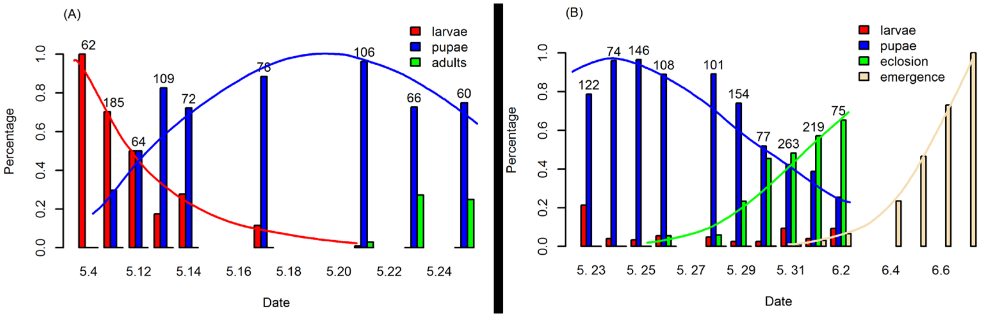

Fig. 3. Development time of *X. rusticus* in Shuangcheng in 2001 and 2002. (A) Pupation time in 2002. (B) Eclosion time and emergence time in 2001. The digits above bars show the numbers of insect specimens collected from wood per day. The data of emergence in (B) are cumulative emergence rates based on 676 insect specimens. Data from May 5-10, 15, 16, 18-20, 22, 24 in (A) and data from May 26 and June 3 in (B) are missing.

Life cycle

Based on the data from field survey, we summarized the life cycle of *X. rusticus* as follows. In the Shuangcheng District, *X. rusticus* completed one generation per year. The larvae in their final instar overwintered in the tunnels they had burrowed in the xylem of poplars. The overwintered larvae resumed boring in April of the following year. The tunnels were irregular, with many twists and turns. The larvae then entered the pupation stage in the middle of May. Before pupating, the larvae turned and made their way toward the exterior bark of the infested tree, and then made a pupal chamber in the outer sapwood, so that the adults could easily chew a round hole to emerge after the eclosion. Emergence occurred in late May. Adults mated as soon as they emerged from the trees. The eggs were laid into the bark crevices in clusters, which were comprised of ten to dozens of eggs. Larvae appeared in early June, initially burrowing into the bark, and entering the xylem in mid-August. The galleries were filled with a mixture of frass and shredded wood. In late October, larvae entered into the heartwood to overwinter. The life cycle is shown in Fig. 4.

Sex ratio

Of the 676 beetle specimens collected from 30 logs, 234 of them were females and 442 were males, fitting a ratio of 1:2 (female: male) (Chi-squared = 0.444, df = 1, p-value = 0.5052).

Natural mortality

Totally 1,393 beetle specimens were collected from hewed wood in the survey of 2001(May 23 to June 2), and 54 (40 larvae and 14 pupae) of them were dead and the rest were live (92 larvae, 863 pupae and 384 adults). Thus, the rate of mortality was 3.88%; proportion testing showed that the mortality equals 4% (Chi-squared = 0.0278, df = 1, p-value = 0.43), with a 95% confidence interval of [0.00, 4.86]. The mortalities of the larva and pupa were 43.48% and 1.62%, respectively. Proportion testing showed that the mortalities of the larva and pupa were equal to 50% and 2%, with 95% confidence intervals of [0.00, 52.58] and [0.00, 2.57], respectively. Thus, the mortality of the larva was 25 times higher than that of pupa. The mortality of the adults was not observed. The death of insects caused by diseases and parasitoids was not observed.

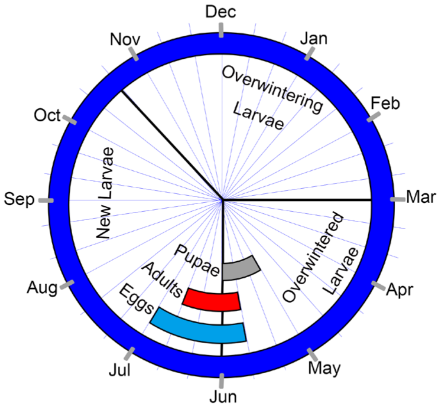

Fig. 4. Life history of *X. rusticus* in the Shuangcheng District.

Host preference

The DBH of the damaged trees in this study ranged from 10-42 cm, with a mean of 27.51±6.51 cm (mean±SD) (Fig. 5A). The median numbers of beetles per m on trunk from 1-9 m above the ground were 0, 0, 16, 15, 24, 0, 0, 0 and 0, respectively, indicating that the majority of the larvae were burrowed into the trunks at a height of 3-5 m above the ground (Fig. 5B). The numbers at 3 m, 4 m and 5 m were used to perform statistical analysis. Shapiro test showed that the numbers do not have a normal distribution (W = 0.8649, p-value = 9.057e-05). Kruskal-Wallis test showed that the numbers of beetles per m on trunk from 3-5 m above the ground were not significantly different (Kruskal-Wallis Chi-squared = 0.1651, df = 2, p-value = 0.9208). The attack percentages of six poplar lines (*Populus* × *xiaohei* T.S. Hwang and Liang; *Populus simonii* Carrière; *Populus beijingensis* W.Y. Hsu; *Populus* × *xiaozhuanica* W.Y. Hsu and Liang; *Populus suaveolens* Fisch. ex Loudon; *Populus pseudosimonii* Kitag. × *Populus nigra* L.) ranged from 36-60% (Fig. 5C). *P.* × *xiaohei* had the lowest attack percentage, followed by *P. simonii*, *P. beijingensis*, *P.* × *xiaozhuanica* , *P. suaveolens* and *P. pseudosimonii* × *nigra*. A proportion test (z test) showed that there were significant differences in the percentages of the attack among these lines (Chi-squared = 33.8152, df = 5, p-value = 2.591e-06). The results of a series of multiple comparisons with Bonferroni-Holm adjustments in significant levels showed that the attack percentage of *P.* × *xiaohei* is statistically different from *P.* × *xiaozhuanica* , *P. suaveolens* and *P. pseudosimonii* × *nigra*, *P. simonii* from *P. suaveolens* and *P. pseudosimonii* × *nigra*, and *P. beijingensis* from *P. suaveolens* and *P. pseudosimonii* × *nigra* (Fig. 5C).

DISCUSSION AND CONCLUSIONS

In this paper, we presented the life cycle (Fig. 4) and the timing of development of *X. rusticus* (Fig. 3), which are helpful to develop a control calendar. We started the survey on May 23 in 2001 since we did not have any information about the development time of this beetle. The results showed that the beetle was already in the peak period of pupa at that time (Fig. 3B). Therefore next year we started survey earlier (on April 21). As multiple sizes of the larvae were not observed during the investigation, it is reasonable to conclude that this beetle completes a generation in one year combined with the development time, as presented in Fig. 3. The peak period of pupa in 2002 was five days earlier than in 2001, indicating that the development time could be affected by the climate. The mean annual temperature (from previous July to current June) in 2001 was 0.527°C, while was 2.969°C in 2002. The mean annual temperature of 2001 was also far lower than that of 2000 (1.305°C). These data indicate that the extreme cold weather led to the development of *X. rusticus* postponed by five days. The peaks of the eclosion and emergence were five days apart (May 31 and June 5), suggesting that the adults do not emerge as soon as they eclose. According to the life cycle (Figs. 3 and 4), the best time to treat the adult beetles for the control should be in early June, and the best time to fumigate the larvae should be before May in the Shuangcheng District.

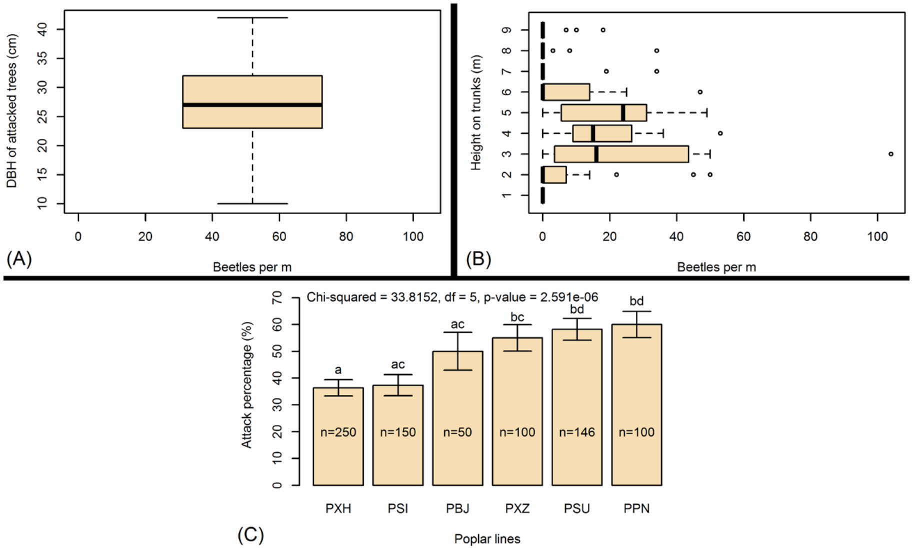

Fig. 5. Host preference of *X. rusticus*. (A) Box-plot of preference for diameter on breast height (DBH). (B) Box-plot of preference for height on the trunk. (C) Bar-plot of preference for poplar lines. “n” represents the total number of trees surveyed from each poplar line. The lowercase letters above bars in (C) show the significant differences. If two bars have a same letter, there are no significant differences in attack percentages between them and vice versa. The abbrevations of poplar line names see the Materials section in the text.

Sex ratio is a basic characteristic of an insect population. Manipulation of sex ratio can be a method to control pests. We collected all the specimens of *X. rusticus* from 30 logs to calculate the sex ratio, equivalent to collecting beetles from the whole flight season. Our results showed that the sex ratio of the population in Shuangcheng was biased 2:1 toward males. The natural mortality of this beetle (excluding the stages of egg and adult after emergence) was low (4%). Lower mortality is a primary characteristic of an insect with K-selected history strategy (Pianka, 1970). The natural mortality of *X. rusticus* is even lower than that of another poplar-boring longhorn beetle, *Anoplophora glabripennis* (Motschulsky, 1853) (28%) (Zhao *et al*., 1993). The reason for this difference may be due to the galleries of *X. rusticus* being filled with frass and dust, while the frass is extruded from the galleries of *A. glabripennis*. Blocked galleries prevent the entrance of natural enemies (especially parasitoids) and lead to difficulty in controlling insects. This reason also accounted for that none of parasitoids were observed in the field survey. Although the ground mortality was low for *X. rusticus*, the mortality of the larvae was as high as 50%. These results suggested that parasitoids are probably not an effective factor to control larvae or pupae of *X. rusticus*, while pathogens may be effective, especially for the larvae control. Unfortunately, the pathogens were not determined further.

Our investigation showed that the DBH of attacked poplar trees ranged from 10-42 cm, suggesting that the beetle does not attack young trees. In this study, the larvae crowded in tree trunks at the height of 3-5 m (Fig. 5B). We also cut 100 poplar trees into 50 cm-long logs, labeled the height on the trunk of each log, and then investigated the frequency of the attacked logs (Hu *et al*., 2004). The results showed that the height of 1-4 m above the ground on the trunk was attacked the most. These results showed that the larvae do not disperse to distribute uniformly on the trunk, even in high densities (the maximum was over 100 insect specimens per m; Fig. 5B). This habit led to the trees being broken by wind at the locations where the larvae were assembled. Meng *et al*. (1983) investigated the deposition heights on 62 trunks. In total, 186 egg clusters were found, 32% of which were on the lower parts of the trunks, 43% on the middle parts and 25% on the upper parts. These results suggest that the height of the infestation on the trunk is determined by the adults and the larvae disperse in quite a limited range.

Silviculture is an important part of an integrated pest management program. Both stand structures and tree species composition are two primary factors determining the forest susceptibility to a forest insect pest (Loreau *et al.*, 2001). The majority of poplar stands in China are single-species stands. It is well known that single-species stands are more susceptible to insects than mixed-species forests of more than 2-3 species (Loreau *et al.*, 2001). It seems a good idea to afforest single-species stands with a less susceptible poplar species to insects. It is readily apparent from Fig. 5C that the natural attack percentages among the poplar lines were significantly different. The percentages of attack on *P.* × *xiaohei* and *P. simonii* lines were below 40%, suggesting that they are less susceptible to the beetle than the other lines and could be candidate species to afforest or reforest cutting blank. However, the preference of the insect for the host is relative. Planting a species susceptible to the insect (*P. suaveolens* or *P. pseudosimonii* × *nigra*), as a bait, together with less susceptible species (*P.* × *xiaohei* or *P. simonii*) will also be reasonable afforesting strategy.

To sum up, *X. rusticus* has a potential expanding trend on the context of economic globalization, although it was not worldwide distributed for now. In this paper, we have presented data on the life history and host preference of this beetle, which are helpful to monitor and control this beetle globally.

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