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Biological traits and life history of *Pagiophloeus tsushimanus* (Coleoptera: Curculionidae), a weevil pest on camphor trees in China

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Abstract *Cinnamomum camphora* (L.) J. Presl. (Laurales: Lauraceae) is widely cultivated as an important landscape tree species in many urban areas in South China, especially in Shanghai City. *Pagiophloeus tsushimanus* Morimoto has become a destructive insect pest of *C. camphora* plantations in Shanghai, but the biological and ecological traits of this pest remain largely unknown. In this study, we investigated the damage and life history and determined the larval instar of *P. tsushimanus*. The results indicated that *P. tsushimanus* is a monophagous weevil pest, and *C. camphora* is the unique host tree species. *C. camphora* plantations in all administrative districts of Shanghai have been seriously damaged by *P. tsushimanus*. Adults often aggregate

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for feeding on the tender bark of twigs and occasionally on newly emerged buds. After experiencing damage, the twigs shrink and crack and the buds will shrink. Adults tend to repeatedly mate and oviposit, and all females lay single eggs at a time. Eggs will be covered with a mixture of secretions and wood chips by female adults. Larvae (1st-2nd instar) feed on the phloem, while 3rd-5th instar can bore into the phloem and the cambium. Massive tunnels, including three shapes (inverted "L", inverted "T", and inverted "Z"), were observed in the trunk of each tree, and resulted in swelling of the outer bark. P. tsushimanus has one life cycle per year in Shanghai. Both adults and larvae (3rd-5th instar) overwinter from early November to early April. Adults overwinter in grooves on the underside of branches or at branch nodes, and larvae overwinter in tunnels. Five larval instars of P. tsushimanus were determined according to Dyar's and Crosby's rules. The biological traits and life history of P. tsushimanus have been identified and can provide guidance in terms of pest control and plantation management.

Keywords Cinnamomum camphora · Damage traits · Life cycle · Dyar's rule · Head capsule width

Introduction

Camphor tree, *Cinnamomum camphora*, is an indigenous tree species of South China, Korea, Vietnam, and southern Japan (Li et al. 1982; Zhang et al. 2019). It is cultivated in numerous countries as an ornamental or as a medicinal plant for muscular strains, inflammation, and rheumatic treatment (Babu et al. 2003). In China, *C. camphora* is widely cultivated in the tropical and subtropical regions as an afforestation species in mountainous areas, and as a common and important landscape species in many urban areas in

south China, especially in Shanghai City (Fan 2018; Zhang et al. 2018). In addition, this species is a good resource for furniture manufacturing and for camphor and essential oil extraction (Guo et al. 2016). The essential oil has strong bioactivity against insect pests when applied as a fumigant (Nenaah and Ibrahim 2011), a contact agent (Guo et al. 2016), and as a repellent (Nenaah and Ibrahim 2011). However, *C. camphora* can be infected by some insect pests, such as *Pagiophloeus tsushimanus* (Chen et al. 2020), *Orthaga achatina* Butler (Lepidoptera: Pyralidae) (Yan et al. 2018), and *Orthaga olivacea* Warre (Wei et al. 2008).

P. tsushimanus was originally described by Morimoto (1982) and collected from Tsushima Island of Kyushu, Japan. Since the initial report of *P. tsushimanus* in China in 2014, it has become a destructive insect pest of *C. camphora* plantations in Shanghai (Huang et al. 2014), and is a potential threat in eastern urbanized areas (Gu et al. 2017; Chen et al. 2020). Therefore, an effective pest management strategy based on the biological traits of *P. tsushimanus* is extremely urgent.

A good understanding of the biological traits of this pest is fundamental for developing effective pest control strategies (Reznik et al. 2015; Qin et al. 2016; Shahid et al. 2017). To date, limited studies have been reported on *P. tsushimanus*, such as pest identification (Huang et al. 2014; Zhang et al. 2018), mating and oviposition behaviour (Zhang et al. 2017), development (Li et al. 2019), and stability of reference genes (Chen et al. 2020). A lack of information on the biological traits and life history has limited research on management strategies against this pest. In this study, we aimed to fill the gap by investigating the damage and life history, and by determining the larval instar of *P. tsushimanus*. These results will be valuable for both practical application and further studies.

Materials and methods

Study area

The research was carried out in Maogang, Songjiang District, Shanghai (30°56′6.15″N, 121°12′32.76″E). Annual mean temperature is 17 °C; there is approximately 125 rainy days per year, with an annual rainfall of 1157 mm; the annual sunlight is 1909.2 h. A 200-hectare plantation cultivated with 15-year-old *C. camphora* was selected where an outbreak of *P. tsushimanus* infestation was first discovered in 2014 (Huang et al. 2014). Mean height (HT) of the trees (n=60) was 5.0 ± 1.2 m (mean \pm standard error (SE)), and the diameter at breast height (DBH) was 21.1 ± 6.3 cm (n=60, mean \pm SE) (Table S1). The distance between trees was 3.0 m. A few other insects have occasionally infested *C. camphora* in this plantation such as *Orthaga olivacea*

Warren, *Thalassodes quadraria* Guenée, *Zeuzera coffeae* Neitner, and *Diaphania perspectalis* Walker.

Damage and life history

To study the occurrence and duration of the developmental stages of *P. tsushimanus* in the field, six 20 m \times 20 m sample plots were established. Ten trees that had been infested by *P. tsushimanus* were randomly selected, sampled in each plot and marked with red paint. From March 2016 to November 2018, surveys were carried out every ten days to collect insect samples at different stages of development. *C. camphora* plantations in other administrative districts of Shanghai were evaluated by random sampling.

Feeding, mating, and oviposition of adults after emergence were observed and recorded. Adult damage was investigated in the lower tree crowns in east, west, south, and north directions. Three fresh tunnels were dissected on each tree which were located by a cue of frass or powdery refuse accumulation at the entrance hole in the trunks (Cui et al. 2019). The trunks were peeled to reveal the complete structure of tunnels, and the development and associated damage of all immature larvae were recorded.

P. tsushimanus preparation before overwintering was observed; its distribution within the trunk was investigated to determine which stages overwintered, and the performance of the stages was recorded simultaneously. The surveyed adults and larvae were brought to the laboratory of Nanjing Forestry University and theadults were bred using fresh *C. camphora* twigs in plastic containers. Larvae were bred using an artificial diet of twig powder in a petri dish. The life cycle of *P. tsushimanus* was determined by both field investigations and laboratory breeding.

Larval instar determination and data analysis

Larvae (n = 461) were collected during field investigations and taken to the insect laboratory of Nanjing Forestry University. The highly ossified head capsules of the larvae were removed, cleaned, and fixed on a strip of adhesive tape (Gao et al. 2017). The head capsule width (HCW) and head capsule length (HCL) of each larva were measured under a Zeiss Discovery V20 stereomicroscope (Fig. 1).

According to their frequency distributions, 100-µm groups of HCWs and HCLs were established and normal multimodal fitting performed, each peak represented an instar (Panzavolta 2007). The intersection point of two adjacent normal curves was regarded as the point dividing larval instars (Panzavolta 2007). The number of larval instars obtained from the frequency distribution analysis was verified according to Dyar's and Crosby's (Brooks') rules (Dyar 1890; Crosby 1973; Loerch and Cameron 1983). A coefficient of variation less than 20% and a Crosby's index less

Fig. 1 Larval head capsule: **a** HCW, and **b** HCL





than 10% were used as criteria (Loerch and Cameron 1983; Dallara et al. 2012). Mean values, coefficients of variation, Brooks' index (Eq. 1), and Crosby's index (Eq. 2) were calculated by Microsoft Office Excel 2016 edition (Microsoft Corporation, Redmond, WA, USA). Pearson correlation analysis and linear regression analysis of the two morphological measurement data sets were performed via SPSS version 20.0 edition (IBM, GeoHack, NY, USA) (Qian and Song 2013). All figures were plotted with Origin version 8.5 edition (Origin Lab, Northampton, MA, USA).

Brooks' index =
$$\frac{X_n}{X_{n-1}}$$
 (1)

Crosby's index =
$$\frac{b_n - b_{n-1}}{b_{n-1}}$$
(2)

where X_n is the mean of each measurement index for larvae (n instar), X_{n-1} is the mean of each measurement index for larvae (n - 1 instar), b_n denotes Brooks' index of larvae (n instar), and b_{n-1} denotes Brooks' index of larvae (n - 1 instar).

Results

Infestation traits

According to field investigations, *P. tsushimanus* is a monophagous weevil pest and *C. camphora* is the unique host tree species. Areas infested with *P. tsushimanus* have increased yearly, and *C. camphora* plantations in all administrative districts of Shanghai have been seriously damaged by *P. tsushimanus*.

Both adults and larvae of *P. tsushimanus* can damage host trees and have seriously reduced the economic and aesthetic value of *C. camphora*. In particular, *P. tsushimanus* overwintered with adults and larvae, the adults in grooves on the underside of branches or branch nodes, the larvae in tunnels.

Adult damage

After adults emerge from the trunks of host trees, they climb up to the crown and feed on the tender bark of twigs (red arrows, Fig. 2a). Aggregation of adults were often observed during field investigations and indoor feeding (Fig. S1) with the most active period occurring from July to September. *C. camphora* twigs damaged by *P. tsushimanus* adults shrink and crack (Fig. 2b). Occasionally, adults feed on newly emerged buds and the buds shrink. The growth of host trees is seriously affected after severe damage by a large number of adults.

During feeding intervals, adults have the habit of putting their heads downwards near the node of the branch. Adults spread by crawling frequently, not flying, and thanatosis is common. When frightened by the noise around or attacked by other animals, adults immediately fell from the shoots to the ground. The colour and granular bulge on the adult integument are similar to the bark of *C. camphora*, indicating mimesis and camouflage (red arrow, Fig. 2c).

Mating frequently occurs on sunny sides of the crown. In this study, laboratory observations found that adults mate and oviposit repeatedly. When adults copulate, the male mounts the dorsum of the female, clasping with forelegs, controlling the female's elytra with the middle legs and clenching the end of the abdomen with the hind legs (Fig. S2).

After mating, females crawl along the trunk below the crown to find a suitable oviposition site. An egg chamber is created by the female with its rostrum and mouthparts into the cambium (Fig. 2d). The abdomen is bent downwards, the ovipositor extended and the egg laid. All females lay single eggs at a time, with one in each chamber. Once laid, the egg is covered with a mixture of secretions and wood chips (red arrows, Fig. 2e). Before overwintering, a groove which is slightly larger than the adult body is made on the underside of the branch or branch node (Fig. 2f).



Fig. 2 Adult damage: a tender bark damaged on twig; b aggregation for supplemental nutrition; c mimesis and camouflage of adults; d mating behaviour of adults; e cover of an egg; and f overwintering groove under a branch node

Larva damage

Larvae (1st-2nd instar) feed on the phloem and produce small, fine frass. Following this, 3rd-5th instar can bore into both phloem and cambium and produce large amounts of thick frass. After larval excavation, three shapes of tunnels (inverted "L", inverted "T", and inverted "Z") were recorded. Newly hatched larvae initially bore in a horizontal direction but when they develop into the last instar, they bore a perpendicular gallery to form a pupal chamber which is vertical to the horizontal galleries and finally form an inverted L-shaped tunnel (Fig. 3a). Some last-instar larvae created perpendicular galleries from the middle of the horizontal tunnel to finally form an inverted T-shaped tunnel (Fig. 3b). Sometimes the newly hatched larvae may bore in a vertical direction but feed in a horizontal direction. However, other last-instar larvae bore perpendicularly to the end of the horizontal tunnel and to eventually form a pupal chamber. These three sections formed an inverted Z-shaped tunnel (Fig. 3c).

Frass is pushed continuously by larvae so that frass near the hole of tunnel is expelled first and is scattered on the trunk surface (red arrow, Fig. 3d). The colour of a fresh tunnel is usually brown and darkened when the larva pupated within it. Larva at the pre-pupal stage stopped feeding and retreated to a vertical tunnel for pupation. During this period, a large amount of flocculent wood chips is made by the larva and piled beneath the larval head (Fig. 3b). Massive tunnels were observed in the trunk of each tree (red arrows, Fig. 3e) and resulted in swelling of the outer bark (red arrows, Fig. 3f), thus seriously affecting the growth of the tree, and sometimes resulting in death.

Life history

The life cycle of *P. tsushimanus* consists of four stages – egg, larva, pupa, and adult (Fig. 4a). According to field investigations, *P. tsushimanus* has one life cycle per year in Shanghai (Figs. 4b, 5). Both adults and larvae (3rd–5th instar) overwinter from early November to the following early April. Overwintering with eggs, larvae (1st–2nd instar) or pupae has not been found in the field.

Overwintering adults start activity in mid-April, crawling upwards to twigs for feeding. Mating is initiated in late April, with the oviposition period lasting from late April to early June. Eggs begin hatching in early June, and larvae start to pupate in late July and end in early September. Emergence of adults starts from early September to late September (Fig. 5).

Overwintering larval feeding is initiated in mid-April and ends in early July. Larvae pupate from mid-May to early July. Adults emerge from mid-June to early August, with mating and oviposition from mid-July to late September. The eggs begin hatching in late August (Fig. 5).





Fig. 3 Larva damage: a inverted "L"-shaped tunnel; b inverted "T"-shaped tunnel; c inverted "Z"-shaped tunnel; d frass discharged and scattered on the trunk; e massive tunnels in the trunk of each tree; and f the outer bark from tunnels swelling outward

Larval instar determination

The HCWs of *P. tsushimanus* larvae range from 480 to 3,100 μ m (n = 461), and HCLs from 620 to 4,130 μ m (n = 461) (Table S2). There are normally five peaks in the frequency distributions of both HCWs (Fig. 6a) and HCLs (Fig. 6b). Each peak represents an instar, and the intersection points of four normal distribution curves reveal four instar cut-off points. Therefore, these peaks indicate five larval instars of *P. tsushimanus*.

Crosby's indexes for the mean values of HCWs and HCLs measured for different instars were all less than 0.1, and the coefficients of variation of the two morphological measurement data for the different instars were all less than 15%, which suggests the determination of five larval instars (Table 1).

The natural logarithmic values of the mean HCW (r=0.985; n=5) and HCL (r=0.990; n=5) are positively correlated with instar. The linear regression between the natural logarithmic values of mean HCW and instars was highly significant $(R^2=0.970; F=96.044; df=1, 3; P=0.002)$ (Fig. 7a). The same linear regression was observed between the natural logarithmic values of mean HCL and instars $(R^2=0.981; F=153.527; df=1, 3; P=0.001)$ (Fig. 7b). These results strongly support the determination of larval instars.

In addition, the mean HCW was positively correlated with the mean HCL (r=0.983, n=461), and regression between HCW and HCL was highly significant ($R^2=0.966$; F=12,976.129; df=1, 459; P < 0.0001) (Fig. 8). Consequently, it is concluded that the number of larval instars of *P. tsushimanus* is five. The results suggest that either HCL or HCW could be a reliable index for larval instar determination.

Discussion

The results of the life history and larval instar of *P. tsu-shimanus* indicate that the weevil produces one generation per year in Shanghai and overwinters with adults and larvae (3rd–5th instar). Other wood borer weevils such as *Eucryp-torrhynchus chinensis* Oliver (McAvoy et al. 2014) and *E. brandti* Harold (Luo et al. 2016) also have similar life cycles and overwinter with larvae and adults (Yang et al. 2008; Yu et al. 2012). Overwintering with late-instar larvae and adults may be because eggs, young larvae, and pupae have less resistance to cold temperatures. *C. camphora* is an evergreen tree species, and branches and leaves naturally provide shelter for overwintering adults. However, the mechanism of staggered different stages during overwintering remains unclear. Overwintering adults mainly emerge in mid-April which is in line with the active growth period of host trees.

Fig. 4 Four stages: **a** egg, larva, pupa, and adult; and **b** the life cycle of *P*. *tsushimanus*



Therefore, new buds or young twigs of *C. camphora* are damaged by newly emerged adults, which negatively affects tree growth.

Larval boring in trunks can cut off vascular tissues and hinder the transport of water and nutrients. A heavy infestation of multiple larvae of P. tsushimanus will result in swelling of the outer bark or in the death of host trees. In addition, we observed that many flocculent wood chips, produced by last-instar larvae excavating, accumulated beneath the larval head in pupal chambers. These chips may prevent rainwater or natural enemies from entering, which should be verified in future studies. Although P. tsushimanus is currently identified as a specialist pest that infests only C. camphora in the field, a laboratory study showed that its larvae can complete their life cycle by feeding on an artificial diet of powdered twigs of Cinnamomum chekiangensis Nakai and Phoebe chekiangensis Shang (Laurales: Lauraceae). In addition, the developmental duration of larvae feeding on this artificial diet was significantly longer than those feeding on twigs of C. camphora (Li et al. 2019). C. chekiangensis and C. camphora are in the same genus, while P. chekiangensis does not, however, the three species belong to the same family. Specific nutritional acquisition from C. camphora or detoxifying enzyme activities of *P. tsushimanus* may be the reasons for its host specificity (Li et al. 2019). Assays on the growth and development of *P. tsushimanus* feeding on various species should be studied in the future, for a comprehensive evaluation of the risk of shifts in hosts.

Instar determination from field-collected larvae is a common problem in insect pest monitoring. Commonly, the morphological measurement data of different instars overlap to some extent and make it difficult to determine the accurate instar of a sample larva (Yazdani et al. 2015). Various techniques have been applied to estimate the overlap between adjacent instars in order to provide classification rules for field-collected larvae (Fox et al. 1972; Frampton 1986; Mcclellan and Logan 1994; Goldson et al. 2001; Hammack et al. 2003). Head capsule width is the most frequent measure used to determine larval instar and has been widely applied to other weevils, such as Eurhinus magnificus Gyllenhal (Coleoptera: Curculionidae), Cos*mopolites sordidus* Germar (Coleoptera: Curculionidae), and Sitophilus zeamais Motchulsky (Coleoptera: Curculionidae) (Logan et al. 1998; Merville et al. 2014). The method of frequency distributions of head capsule width (HCW) and head capsule length (HCL) has been widely



Fig. 5 Seasonal life cycle of *P. tsushimanus* (Songjiang District, Shanghai, 2016–2018). E, M, and L denote the early, middle, and late period (ten days) of a month, respectively



Fig. 6 Frequency histogram of a HCW and b HCL (b) in P. tsushimanus

applied in larval instar determination such as *Lobesia* botrana (Denis and Schiffermüller) (Lepidoptera: Tortricidae) and *Rhyssomatus subtilis* Fiedler (Coleoptera: Curculionidae) (Delbac et al. 2010; Cazado et al. 2014). There were five normal peaks in the frequency distributions of HCWs and HCLs of *P. tsushimanus* according to this method which followed the general standard that

morphological measurement data of individuals at the same developmental stage are distributed normally (Hunt and Chapman 2001).

The results of larval instar determination were generally verified by the application of Dyar's and Crosby's (Brooks') rules (Dyar 1890; Crosby 1973; Loerch and Cameron 1983). Thus, we used this method to verify larval instar
 Table 1
 Means, coefficients of variation, Brooks' indexes, and Crosby's indexes of the head capsule HCWs and HCLs from five instars of *P. tsushimanus* larvae

Head capsule	Larval instar	Mean \pm SE (μ m)	Coefficients of variation (%)	Brooks' index	Crosby's index
Width	1st	$653.6 \pm 10.9 (n = 59)$	12.8	_	_
	2nd	$1107.5 \pm 14.6 (n = 94)$	12.7	1.6945	_
	3rd	$1573.3 \pm 21.6 (n = 80)$	11.9	1.4658	-0.1350
	4th	$2490.5 \pm 19.2 (n = 128)$	8.7	1.5342	0.0467
	5th	$2832.6 \pm 15.9 (n = 100)$	5.6	1.1374	-0.2587
Length	1st	$784.6 \pm 11.1 (n = 59)$	10.9	_	_
	2nd	$1318.7 \pm 17.8 (n=94)$	13.1	1.6808	_
	3rd	$1969.9 \pm 22.9 (n = 80)$	10.4	1.4938	-0.1113
	4th	$3016.6 \pm 18.1 (n = 128)$	6.7	1.5314	0.0252
	5th	3688.3 + 18.8 (n = 100)	5.1	1.2227	-0.2016



Fig. 7 Regression between the natural logarithm of mean larval: a HCWs; and b HCLs with instars



Fig. 8 Linear regression analysis of the relationship between HCW and HCL $\,$

determination in *P. tsushimanus*. The increase in HCW of each larval instar was consistent with Dyar's rule. If the logarithmic head capsule measurements correspond to the number of larval instars, the perfect geometrical progression of the HCW is represented by a straight line (Gaines and Campbell 1935). Both HCWs and HCLs of *P. tsushimanus* corresponded to each larval instar and exhibited a straight line, which supported five instars of *P. tsushimanus* larvae. In addition, there is a highly significant linear relationship between HCW and HCL which confirms our conclusion convincingly.

To date, no effective strategies have been developed to control *P. tsushimanus*. The previous method to control larvae was injecting conventional insecticides into tree trunks (Fan 2018). However, the effect was poor because of the incorrect period of control. The results of our study suggest that the essential period is from July to September because larvae and adults are active during this period. Also, most

P. tsushimanus larvae are in the first instar which is highly sensitive to insecticides. The control of adults by prompting thanatosis or apparent death to reduce density could be performed soon after emergence when most adults disperse to the tree canopy for maturation feeding and mating.

The knowledge of biological traits of *P. tsushimanus* will provide guidance for managing this pest. However, there is still much to be studied such as its physiology (e.g., resistance to plant secondary metabolites), chemical ecology (e.g., attractants), and molecular biology (e.g., functional genes). Furthermore, investigations of natural enemies and development of semiochemical lures may be effective measures of control. Sex pheromones or host tree volatiles are used by many insects for mating or host location (Lee et al. 2005; Chung et al. 2013; Liu and Zhou 2016). The behavioural traits of P. tsushimanus adults suggested that aggregation pheromones of adults are produced by males (Zhang et al. 2017). Secretions produced by female adults that cover eggs might contain a host-marking or oviposition pheromone to deter other females from laying eggs (Rosi et al. 2001; Zhang et al. 2017). In addition, P. tsushimanus only infested C. camphora in the field, which implies that host tree volatiles are a potential attractants. Meanwhile, identification and application of sex pheromone attractants of P. tsushimanus could be conducive to the development of an alternative, eco-friendly method for controlling this pest.

Conclusions

P. tsushimanus is a monophagous weevil pest, feeding only on *C. camphora*. *C. camphora* plantations in all administrative districts of Shanghai have been seriously infected. Adults often aggregate for feeding on the tender bark of twigs, and occasionally on newly emerged buds. After damage, twigs will shrink and crack and buds will shrink. Adults tend to repeatedly mate and oviposit, and all females lay single eggs at a time. All eggs are covered with a mixture of secretions and wood chips by female adults.

P. tsushimanus has one life cycle per year in Shanghai. Both adults and larvae (3rd–5th instar) overwinter from early November to the following early April. Adults overwinter in grooves on the underside of branches or branch nodes and larvae in tunnels. Five larval instars of *P. tsushimanus* were identified.

The biological traits and life history of *P. tsushimanus* have been shown and can provide guidance in pest control and plantation management.

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Author's contribution CC and DH conceived and designed the experiments and wrote the manuscript. CC, CZ, SL, and HZ performed the experiments and analysed the data. BF, YW, PS, and YH collected the insects. All authors reviewed and approved the manuscript for publication.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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