



Harmfulness and biological characters of a leaf beetle *Gastrolina depressa* (Coleoptera: Chrysomelidae) to its host plant *Pterocarya stenoptera* (Fagales: Juglandaceae)

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Abstract

Gastrolina depressa (Coleoptera: Chrysomelidae) has been found to damage many walnut plants in China. It prefers to feed on the annual leaves of an important economic plant, *Pterocarya stenoptera* (Fagales: Juglandaceae), and causes extensive cosmetic destruction, even plant death. Nevertheless, its biological characteristics are mostly unknown. In this study, we investigate the harmful and biological characteristics of *G. depressa* with different developmental stages. The results showed that *G. depressa* had four developmental stages: egg, larva (1st–3rd instar), pupa, and adult, the life history lasted nine days, with obvious overlapping generations. The food intake of the larvae increased with the increase of the instar, and the maximum food intake of the 3rd instar larvae was 448.19 mm². The abdomen of the females was enlarged before oviposition, and the average number of eggs laid was 47.63 ± 13.53. Bioassay showed that there was no difference in the search time for the young (2.03, 1.25, 1.15, and 0.91 min) and old leaves (1.68, 1.15, 0.90, and 1.93 min) of *P. stenoptera* between 1st–3rd instar larvae and adults; the feeding preference of 1st–3rd instar larvae and adults for young leaves was higher than that to old leaves (23.33%, 26.67%, 33.33%, and 21.67%, respectively). The successful selection rates of 1st–3rd instar larvae and adults to young leaves (81.67%, 80%, 85.00%, and 70.00%, respectively) was higher than to old leaves (8.35%, 11.67%, 3.34%, and 11.67%, respectively).

Keywords *Gastrolina depressa* · *Pterocarya stenoptera* · Biological characteristics · Bioassay

Introduction

The walnut leaf beetle, *Gastrolina depressa* (Baly, 1859), is a notorious insect pest of walnut trees (*Juglans regia*) and Chinese Wingnut trees (*Pterocarya stenoptera*) in Asia (Zhao et al. 2010). It is mainly distributed in southern Gansu, Shanxi, Henan, Jiangsu, Zhejiang, Hubei, Hunan, Fujian, Guangdong, Guangxi, Sichuan, and Guizhou provinces in

China (Zhang et al. 2006). Both larvae and adults feed on walnut leaves and seriously influence walnut growth and yield (Wang and Tang 2018). *Gastrolina depressa* is a holometabolous insect, a female can lay about 200 eggs, demonstrating a strong reproductive capability, which also hints at the devastating damage that the pest may cause to host plants (Ma et al. 2023). *Gastrolina depressa* was discovered not only to destroy walnuts but also to badly impact the growth of *P. stenoptera* in Tongren, Guizhou province (Yang et al. 2014).

Pterocarya stenoptera belongs to Juglandaceae and is frequently planted as one of the principal landscape plants in South China due to its wide application (Zhang et al. 2023). Previous studies have shown that *P. stenoptera* volatiles and extracts have therapeutic potential, the volatiles can be utilized to capture *Helicoverpa armigera* (Hübner, 1808), and the extracts can kill storage pests like *Sitophilus zeamais* (Motschulsky, 1855) (Xiao et al. 2008). Meanwhile, researchers have discovered that *P. stenoptera* has

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antibacterial, antioxidant, antiviral, and anticancer properties (Yu et al. 2018). With the exploration and application of its medicinal value and greening function, *P. stenoptera* is planted on a large scale across the country (Zhang et al. 2015). Not surprisingly, plant pests become a serious issue. At present, the main pests of *P. stenoptera* include *G. depressa* and *Redoa leucosccla* (Collenette, 1934) (Zhang et al. 2018). The outbreak of *G. depressa* in scenic forests in Taishan Mountain, Shandong Province, not only destroyed the landscape but also caused the death of ancient trees (Meng et al. 2008). In recent years, the pest insect issue has become increasingly serious in Fanjing Mountain's world heritage area. As an ornamental plant in the Fanjing Mountain Scenic Area, *P. stenoptera* is suffering from the damage of *G. depressa*. However, there are few studies about the biological characteristics of the leaf beetle *G. depressa* on the host-plant *P. stenoptera*. Therefore, in the test, the feeding behavioral and life history of *G. depressa* on *P. stenoptera* were carried out in order to improve its effective prevention and control in the future.

Materials and methods

Insects and host plants

The walnut leaf beetles were collected from Zhaisha Dong Village (108°45'59"E, 27°49'36" N) and Pingnan Town (108°46'40"E, 27°45'56"N) in Tongren City, Guizhou Province, China. We conducted continuous breeding (> 5 generations) in the laboratory to establish a stable experimental population for the test. *Pterocarya stenoptera* leaves were gathered from Huaxi Wetland Park, Huaxi District, Guiyang City, Guizhou Province, and the leaves were kept at -4 °C.

Feeding method. The insects were bred in transparent plastic tanks (height: 15 cm; diameter: 10 cm) with the mouths of the tanks sealed with gauze (300 mesh), avoiding insect escape; fresh *P. stenoptera* leaves were inserted into the cement (3×3×3 cm) mounted in the plastic cans for feeding purposes, which were refreshed every day, at a temperature of 25 ± 1 °C and humidity of $75 \pm 1\%$ (Fig. 1a). Following oviposition, the leaves with eggs were cut off

and placed separately in glass tubes to facilitate observation of larval development and determination of food intake; excreta and food residues were cleaned regularly throughout the feeding process to maintain the suitable environment.

Field observation of *G. depressa*

Fourteen sample trees of *P. stenoptera* were selected in certain spots in Fanjing Mountain scenic area (Zhaisha Dong Village and Pingnan Town, Tongren, Guizhou Province, respectively). For the observation of *G. depressa* abundance in different developmental stages, four branches were cut from each sample tree in the East, West, South, and North directions. The feeding damage of *G. depressa* to *P. stenoptera* was observed and quantitatively detected.

Observation of the biological characteristics of *G. depressa* on *P. stenoptera*

To observe the biological characteristics of *G. depressa* in different developmental stages, eggs were deposited in 32 glass tubes. Fresh leaves of *P. stenoptera* were inserted into the cement (0.5×0.5×0.5 cm) (Fig. 1b). The young leaves were replaced in time to keep the tube clean. The morphological characteristics of *G. depressa* eggs, larvae, and pupae were observed with a dissecting microscope. Simultaneously, the biomass of *G. depressa* in immature stages was monitored each day till the emergence of adults, including body size (length and width, mm), body weight (g), and the mean developmental period of the females (d).

Fitness and fecundity of *G. depressa*

We chose 100 pupae to be individually reared in the above-mentioned glass tube (Fig. 1b). After adult eclosion, sufficient fresh food was supplied; The body weight, food intake, and abdominal changes of adults were recorded daily. After mating, female (1) and male (1) were reared in tanks, a total of 20 replicates (Fig. 1a). The performance parameters of *G. depressa* adult and its offspring were observed and recorded, including female fecundity (eggs/female), the body weight of offspring (g), and food intake (mm²/d). The fresh leaves were replaced every day till the end of the oviposition or the death of beetles.

Choice test of larvae and adults of *G. depressa*

A circular filter paper (D=150 mm) was placed into the bottom of a glass Petri dish with a diameter (D=120 mm) and divided into four equal areas, and two leaf discs (D=20 mm) of the host plants - young leaves (light green) and old leaves (deep green) of *P. stenoptera* were placed in

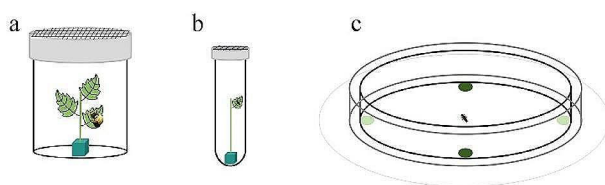


Fig. 1 **a** Devices for feeding adults; **b** devices for feeding larvae; **c** device for selective experiments.

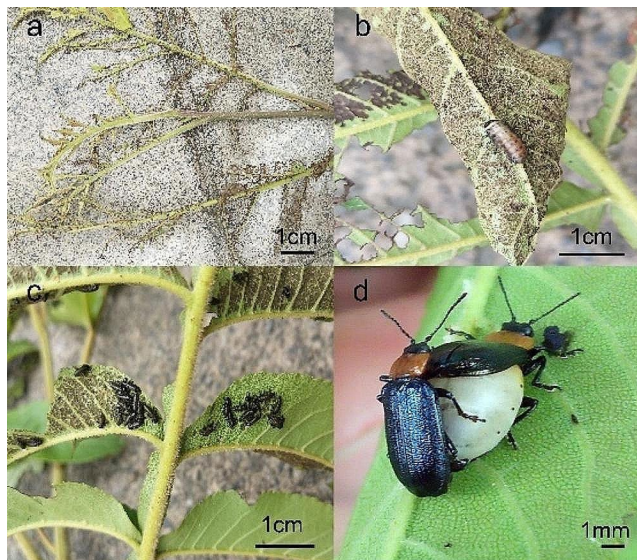


Fig. 2 The field research on *G. depressa* resulted in the discovery of 2nd instar larvae after molting. **a** *G. depressa* causes significant harm to *P. stenoptera*; **b** 2nd instar larvae after molting; **c** 2nd instar larvae actively feeding; **d** *G. depressa* that are mating.

the clockwise direction at equal intervals in an alternating manner, respectively (Fig. 1c). Then, one test individual of *G. depressa* of different developmental stages (1–3 instar larvae and adults) starved for 2 h and were placed in the center of the filter paper. The lid was quickly closed and the initial time was marked. Each stage experiment was repeated sixty times to observe the behavior of *G. depressa* searching and feeding on leaf discs and the time (min) that *G. depressa* searched for a leaf disc was recorded; after 2 h of supplying, the selection of leaf disc in the same supplying field by the test insects was recorded: preference selection rates (%), successful selection rates (%), and food intake (mm²). The measurement of leaf area refers to the method of Tang et al. (2007).

Data analysis

Excel was used to sort out the data, and SPSS 26.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The least significant difference method of one-way ANOVA and t-test were run on parameters, such as the body length, body width, body weight, food intake, fecundity, oviposition duration, search time, preference selection rates, successful selection rates, and searching for significant differences of *G. depressa* among different developmental stages. Photo-shop 2023 and Origin 2021 were used to make drawings.

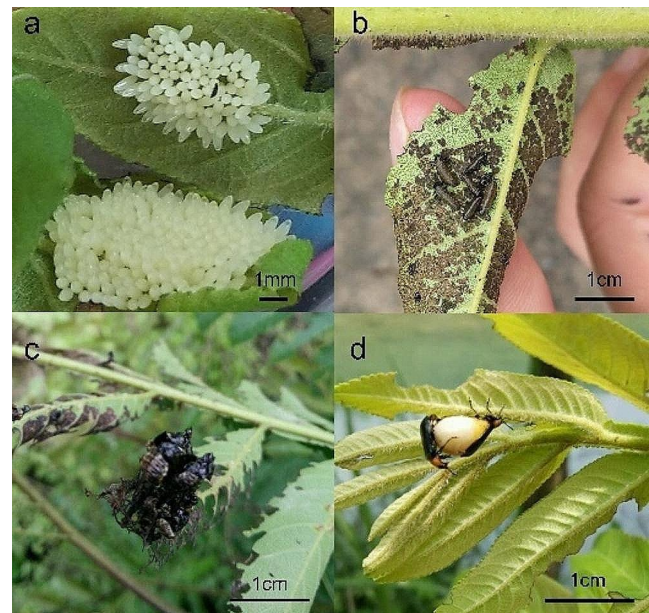


Fig. 3 Multiple stages of *G. depressa* were found to coexist through the field survey: **a** eggs, **b** larvae, **c** pupae, **d** adults.

Results

Field occurrence of *G. depressa*

Feeding behavior

The occurrence of the leaf beetle *G. depressa* on the host-plant *P. stenoptera* was compared in two survey locations: Zhaisha Dong Village and Pingnan Town. Both adults and larvae of *G. depressa* were more likely found on the young branches and leaves of *P. stenoptera*, and the leaves were bitten into a mesh (Fig. 2). The newly hatched 1st instar larvae mainly fed on leaf epidermis and mesophyll; the 2nd instar larvae consumed leaf epidermis and mesophyll; the 3rd instar larvae consumed veins and petioles when food was insufficient, and mature larvae stop feeding in the early stage of pupae.

In addition, surveys revealed the coexistence of multiple stages of *G. depressa*, such as eggs, larvae, pupae, and adults (Fig. 3). In the Zhaisha Dongzhai, mostly larvae were present, with an average of 2.378 ± 0.733 individual/branch (Fig. 4a-d). In contrast, egg masses (0.021 ± 0.008 individual/branch), larvae (0.31 ± 0.135 individual/branch), pupae (0.05 ± 0.031 individual/branch), and adults (0.125 ± 0.056 /branch) of *G. depressa* were found to occur in the Pinnan Village. This shows that *G. depressa* has overlapping generations (Fig. 4e-f).

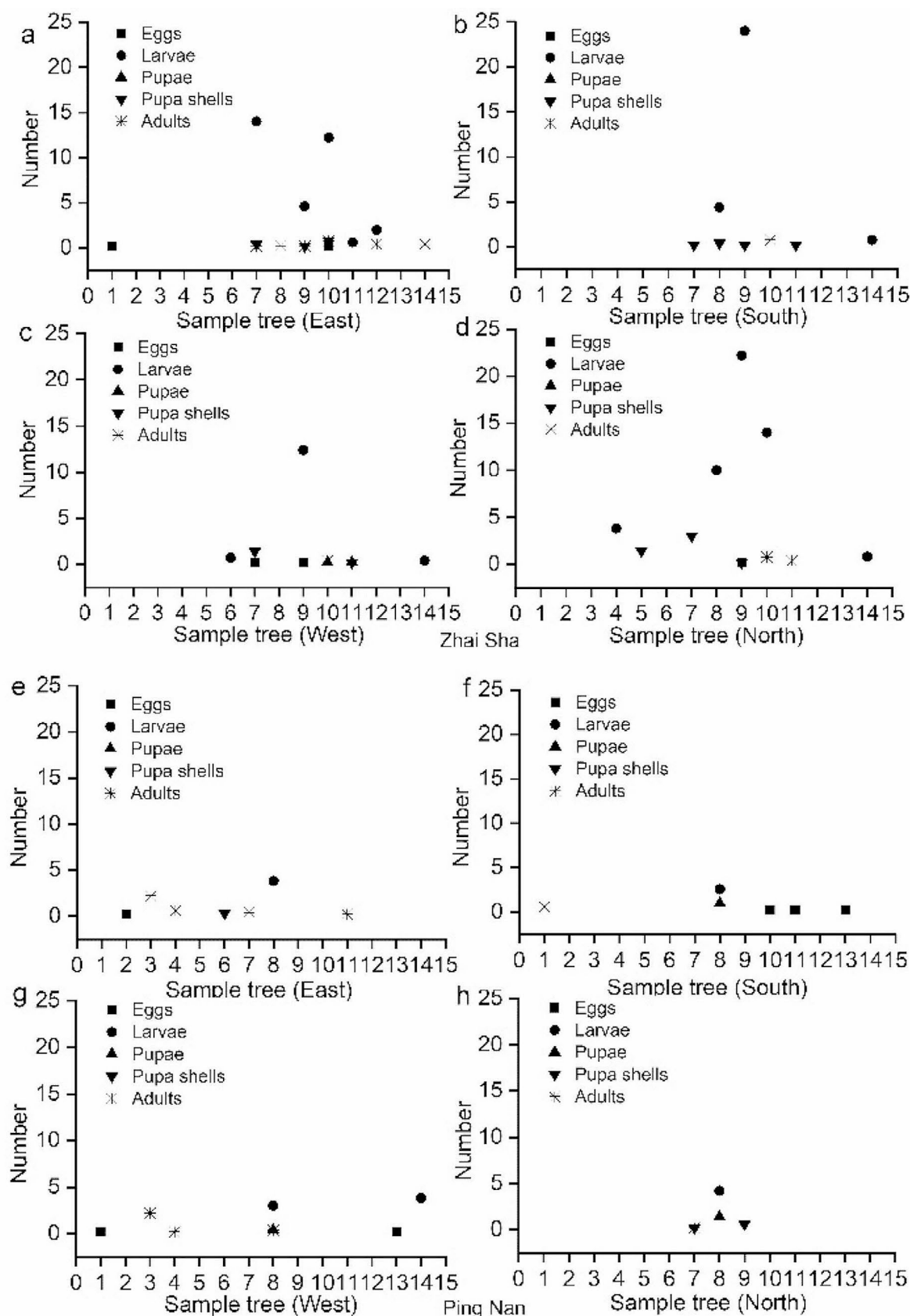


Fig. 4 The dispersion of *G. depressa* at Zhaisha Dong Village in the East (a), West (b), South (c) and North (d) and Pingnan Town in the East (e), West (f), South (g), and North (h) from each sample tree.

Morphological characteristics and life history of *G. depressa*

Four developmental stages of *G. depressa* were recorded: egg, larva, pupa, and adult. The mean developmental duration of *G. depressa* to complete a generation was 9.31 ± 0.22 days ($t=41.122$, $df=1,32$, $p<0.001$, $n=32$) (Fig. 5). Among them, the eggs duration was 2.69 ± 0.08 days ($t=32.283$, $df=1,32$, $p<0.001$, $n=32$), the 1st instar larvae duration was 1.69 ± 0.08 days ($t=20.270$, $df=1,32$, $p<0.001$, $n=32$), the 2nd instar larvae duration was 1.65 ± 0.10 days ($t=17.181$, $df=1,32$, $p<0.001$, $n=32$), and the 3rd instar larvae duration was 2.37 ± 0.09 days ($t=27.314$, $df=1,32$, $p<0.001$, $n=32$); after 0.859 ± 0.04 days later ($t=21.284$, $df=1,32$, $p<0.001$, $n=32$), it entered into a pupae duration, and 2.40 ± 0.08 days later ($t=27.279$, $df=1,32$, $p<0.001$, $n=32$), the adults emerged.

The morphological characteristics of each developmental stages were as follows:

Eggs: 1.5–2.0 mm in length, oblong, light yellow, with a tapered top (Fig. 6a).

First instar larvae: 1–3 mm in length and 0.5 mm in width, featuring three pairs of legs, and emerging with a black head. The body starts as translucent and light gray, but soon turns black (Table 1; Fig. 6b).

Second instar larvae: 4–6 mm in length and 1 mm in width, featuring three pairs of legs. Upon molting, it takes on a black head and a striking yellow body but soon shifts to a muted gray and eventually black (Table 1; Fig. 6c).

Third instar larvae: 7–10 mm in length and 2–3 mm in width, featuring three pairs of legs. Upon molting, its head is dark and its body is a striking yellow (Table 1; Fig. 6d).

Pupae: 5–6 mm in length and 3–4 mm in width, and has a light black color. It then shrinks and attaches itself to the tube wall or leaf. The pupa is completely inverted, displaying visible segments of the head, thorax, and abdomen (Table 1; Fig. 6e).

Adults: 5–7 mm in length and 3–4 mm in width. The filamentous antennae are short and with 11 articles each, the

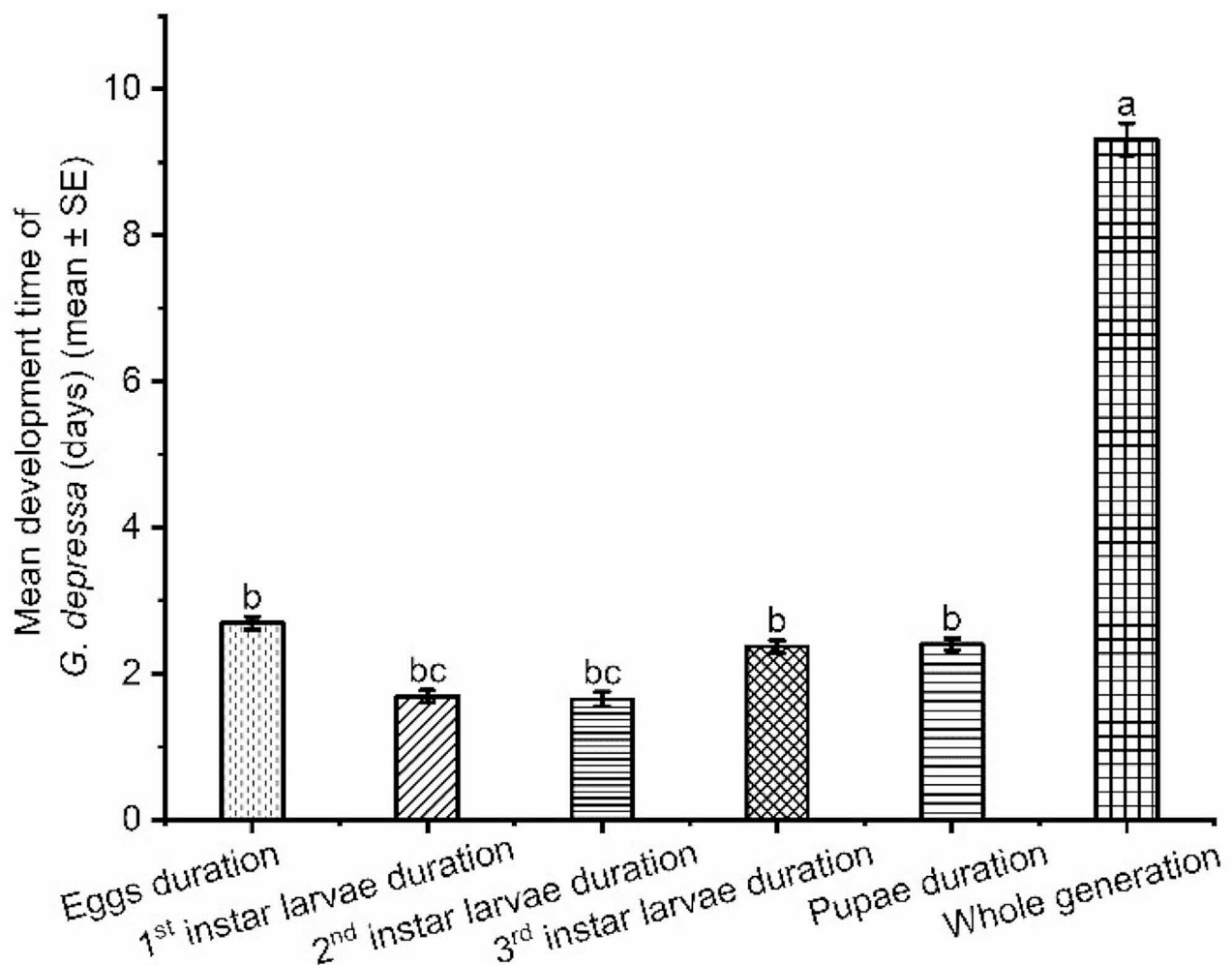


Fig. 5 The mean duration of developmental stages of *G. depressa*.

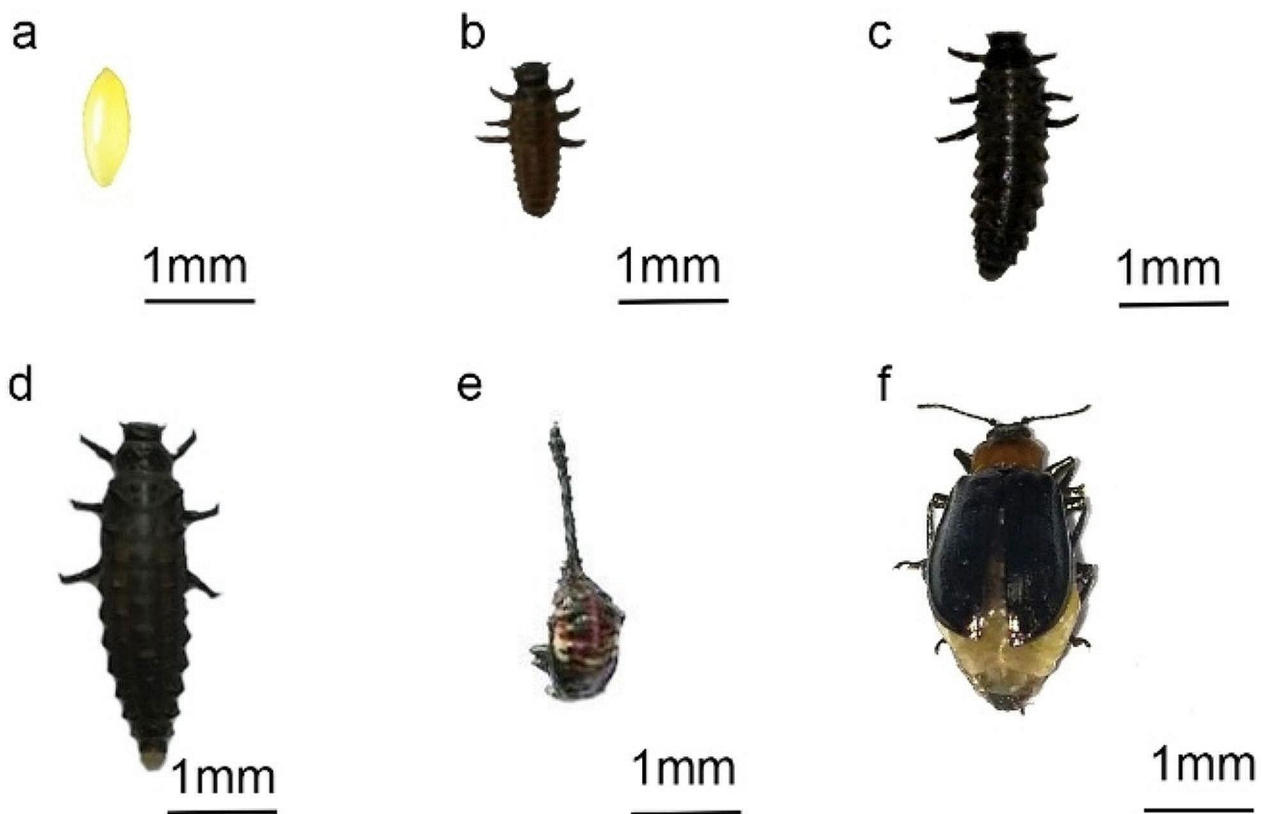


Fig. 6 Different developmental stages of *G. depressa*. **a** Egg; **b** 1st instar larva; **c** 2nd instar larva; **d** 3rd instar larva; **e** pupa; **f** adult.

body has a slightly rectangular shape and a flat back, and the thorax, antennae, and legs are all black (Table 1; Fig. 6f). There are differences in the size of males and females, but the characteristics are not obvious. In the early stage of mating, the abdomen of females expands sharply. During mating, the females lie down, the males cling to the end of the back of the females, and the tails of the males and females are connected for mating and fertilization.

Fitness of *G. depressa* female on *P. stenoptera*

Changes in food intake and body weight of larvae

As shown in Table 2, there was a strong positive correlation between the food intake and the body weight of the larvae ($r=0.927$, $p<0.001$, $n=100$), i.e., the body weight of larvae increased with the increase of food intake. The body weight and food intake of developmental stages exhibited a significant variation (Fig. 7b). The mean body weight of the 1st and 2nd instar larvae was 0.006 ± 0.002 g, while that of the 3rd instar larvae was 0.014 ± 0.005 g ($F_{1,63} = 279.936$, $p<0.001$, $n=100$) (Fig. 7a).

The mean food intake for 1st instar larvae was 49.03 ± 2.86 mm² ($t=17.151$, $df=1,32$, $p<0.001$, $n=100$). For the 2nd

instar larvae, it was slightly higher at 60.88 ± 4.44 mm² ($t=13.699$, $df=1,32$, $p<0.001$, $n=100$). As for the 3rd instar larvae, their food intake reached 448.19 ± 18.90 mm² ($t=23.714$, $df=1,32$, $p<0.001$, $n=100$) (Fig. 7a).

Oviposition behavior and fecundity of *G. depressa*

Experimental observation showed that the abdomen of pre-pregnant females (abdomen is bulged by unfertilized oocytes) was swollen before and during the spawning period. After seven days of supplementary nutrition, the female's weight reached 0.014 g and mated with the males. After 3–4 days of supplementary nutrition, the female's weight increased from 0.004 g to 0.081 g. After the females oviposited, the body weight gradually decreased (average weight: 0.06 g). The oviposition duration by females was 13.89 ± 1.55 days (Fig. 8a). The number of eggs laid per female was 47.63 ± 13.53 (Fig. 8b), the average number of eggs laid by females was 40.68 ± 10.69 (Fig. 8c). Eggs were usually deposited in clusters on the bottom of the young leaves, occasionally on the surface of the leaves, neatly arranged and vertically attached to the surface or bottom of the leaves. Females continuing to feed young leaves will accelerate abdominal swelling and spawning. On the

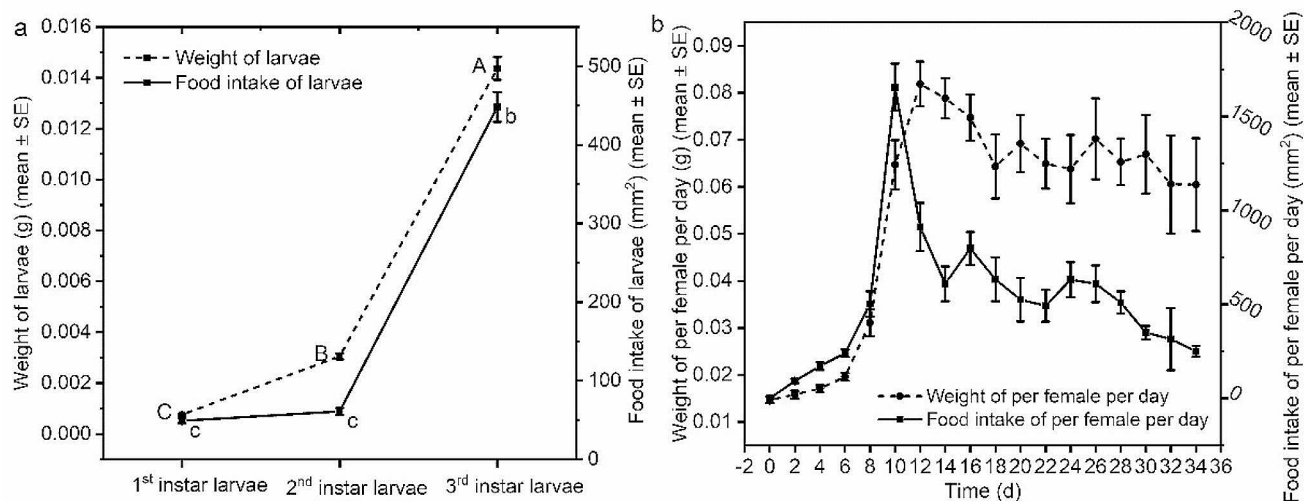


Fig. 7 **a** The weight and food intake of *G. depressa* larvae; **b** weight and food intake per *G. depressa* female per day.

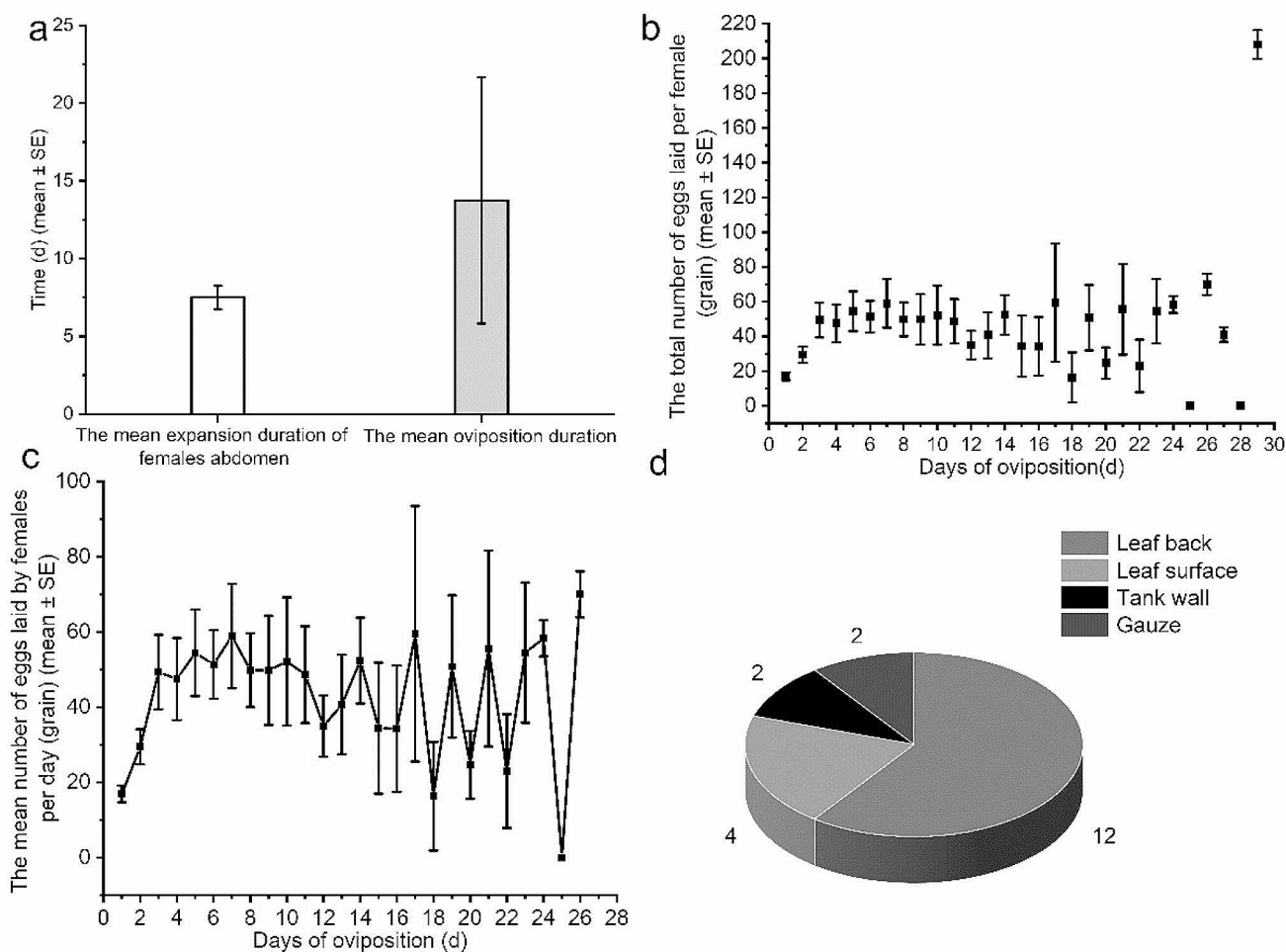


Fig. 8 **a** The mean oviposition duration of the female of *G. depressa*; **b** the total number of eggs laid per female; **c** the number of eggs laid per female per day; **d** location of egg deposition on leaves and number of females of *G. depressa* in laboratory.

contrary, feeding on older leaves delays spawning. Replacing old leaves with young leaves can stimulate females to

lay eggs. After the completion of female oviposition, the abdomen shrank until death (Fig. 8d).

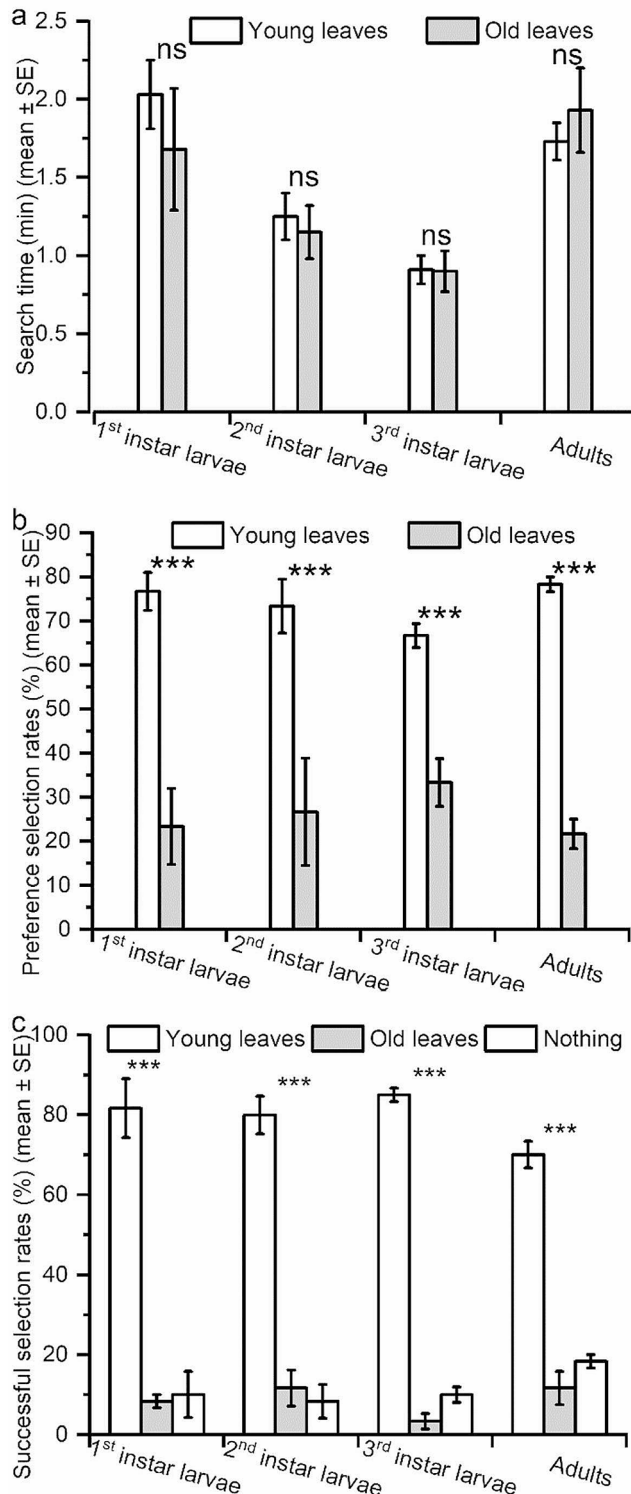


Fig. 9 **a** The search time of 1st–3rd instar larvae and adults of *G. depressa* for *P. stenoptera*; **b** the preferential selection rates of 1st–3rd instar larvae and adults of *G. depressa* to *P. stenoptera*; **c** the successful selection rates of 1st–3rd instar larvae and adults of *G. depressa* to *P. stenoptera*.

The search and selection of *G. depressa* on *P. stenoptera*

For *G. depressa*, in terms of the search time for young and old leaves of *P. stenoptera*, there were no difference in the 1st instar larvae (2.03 ± 0.22 min; 1.68 ± 0.39 min) ($Z = -1.066$, $p > 0.05$, $n = 60$), 2nd instar larvae (1.25 ± 0.15 min; 1.15 ± 0.17 min) ($Z = -0.008$, $p > 0.05$, $n = 60$), 3rd instar larvae (0.91 ± 0.09 min; 0.90 ± 0.13 min) ($Z = -1.414$, $p > 0.05$, $n = 60$) and adults (1.73 ± 0.12 min; 1.93 ± 0.27 min) ($Z = -0.826$, $p > 0.05$, $n = 60$) ($Z = -0.826$, $p > 0.05$, $n = 60$) (Fig. 9a) (“–” for direction, just focus on the absolute Z value).

There were significant differences in the preference selection rates of 1st–3rd instar larvae and adults for young and old leaves (Fig. 9b). The preference of 1st–3rd instar larvae and adults for young leaves (76.67%, 73.35%, 66.67%, and 78.33%, respectively) was higher than that to old leaves (23.33%, 26.67%, 33.33%, and 21.67%, respectively). The results showed that the selection tendency of 1st–3rd larvae ($t_1 = 8.211$, $df = 2, 60$, $p < 0.01$; $t_2 = 7.185$, $df = 2, 60$, $p < 0.01$; $t_3 = 5.636$, $df = 2, 60$, $p < 0.01$, respectively, $n = 60$) and adults ($t = 8.784$, $df = 2, 60$, $p < 0.01$, $n = 60$) on the young leaves was greater than on the old ones.

The results showed that the successful selection rates of 1st–3rd instar larvae and adults for young leaves and old leaves were different (Fig. 9c). The successful selection rates of 1st–3rd instar larvae and adults to young leaves (81.67%, 80%, 85.00%, and 70.00%, respectively) was higher than to old leaves (8.35%, 11.67%, 3.34%, and 11.67%, respectively); no selection to leaves was observed in 10.00%, 8.34%, 13.33%, and 18.33% larvae and adults, respectively). It indicates that the successful selection rates of 1st–3rd instar larvae ($F_{2,177} = 105.245$, $df = 3, 60$, $p < 0.001$; $F_{2,177} = 79.982$, $df = 33, 60$, $p < 0.01$; $F_{2,177} = 145.761$, $df = 33, 60$, $p < 0.01$, respectively, $n = 60$) and adults ($F_{2,177} = 31.899$, $df = 3, 60$, $p < 0.01$, $n = 60$) to leaves were affected by leaves.

Discussion

Combined with field observation and indoor bioassay, the phytophagous insect *G. depressa* in Fanjing Mountain Scenic Area revealed four developmental stages - egg, larvae (1st–3rd), pupa, and adult. And it takes nine days to complete a generation so there was an obvious phenomenon of overlapping generations. The food intake of larvae of 1st–2nd instar was low, while the food intake of 3rd instar larvae was the largest (about 448.19 mm²). The adult beetles started

to mate and feed after their eclosion. Female fecundity is high, the number of eggs laid per female was 47.63 grain, the average number of eggs laid by females was 40.68 grain.

Previous studies have reported that *G. depressa* mainly fed on leaves of two economic walnut tree species, *P. stenoptera* and *J. regia*, and the harm to the plant is explosive and destructive (Meng et al. 2008). In the early stage of the damage, the leaves were bitten into a mesh or a notch, and all the leaves could be eaten in a short time during the outbreak (Wang et al. 2018), which was conducive to the invasion of secondary pests such as longicorns (Liu 2019). In the experiment, it was found that the 3rd instar larvae would enter the gluttony period, while the adults had a certain migration ability and host transfer would occur. When generations overlap, the damage to plants is incalculable. Therefore, to reduce the risk of cross-infestation of *G. depressa*, we should carry out prevention and control before the 3rd instar larvae, which can minimize the population density and reduce the risk.

For another, the economic plant *P. stenoptera* has been found to have antitumor and antibacterial activities in early studies (Liu et al. 2005), and it is also applied to agriculture as an “insecticide”. However, *G. depressa* was able to live and reproduce in large numbers on the host-plant *P. stenoptera*, we speculate that the leaf beetle would physiologically detoxify, digest or tolerate to those insecticidal active substances of *P. stenoptera*, which is worthy of further study in the future. Ma et al. (2023) results showed that the diversity of the gut bacterial community in larvae was generally higher than that in adults, and young larvae (1st – 2nd larvae) possessed the most diversified and abundant community. The gut bacteria of *G. depressa* were mostly enriched in metabolic pathways (carbohydrate metabolism and amino acid metabolism) as revealed by functional prediction. The next step is to screen the key microflora of gut bacteria to reveal the host preference and adaptive mechanism of *G. depressa* to *P. stenoptera* (Zhang et al. 2023).

Author contributions WY and LL conceptualized and designed the experimental work. WY, LQ and HY collected material. WY performed the experiments. LQ and HY collected and analyzed the data, under guidance of LL, and advice of ZX. LL was responsible for research funding. WY and LL wrote the manuscript together with input of all other authors. All authors contributed to and approved the submission of the manuscript.

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Data availability All data is contained within the manuscript.

Declarations

Ethical approval Not applicable.

Informed consent Not applicable.

Conflict of interest Authors declare that they have no conflict of interests.

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