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# Pest characteristics of *Trigonorhinus* sp. (Coleoptera: Anthribidae) and the developmental process of its galls in *Caragana liouana* Zhao Y. Chang and Yakovlev

Yanru Zhang<sup>1\*</sup>, Yaru Hu<sup>1</sup>, Haiyan Jiang<sup>1</sup>, Shengguo Zhao<sup>2</sup>, Jingwen Lei<sup>1</sup>, Ruigang Wang<sup>3</sup>, and Yuhong Chen<sup>4</sup>

<sup>1</sup>Department of Forest Conservation, Forestry College, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, 010018, People's Republic of China, <sup>2</sup>Forestry and Grassland Pest Control and Quarantine Station of the Inner Mongolia Autonomous Region, Hohhot, Inner Mongolia, 010010, People's Republic of China, <sup>3</sup>Key Laboratory of Plant Stress Physiology and Molecular Biology, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, 010018, People's Republic of China, and <sup>4</sup>Tongliao Forestry and Grassland Science Institute, Tongliao, Inner Mongolia, 028399, People's Republic of China

\*Corresponding author. Email: [zhangyanru4479@126.com](mailto:zhangyanru4479@126.com)

(Received 27 September 2022; accepted 23 January 2023)

## Abstract

In recent years, a *Trigonorhinus* sp. (Coleoptera) has caused serious damage to *Caragana liouana* Zhao Y. Chang and Yakovlev, a major ecological restoration shrub in China. Here, we survey the occurrence and damage pattern of *Trigonorhinus* sp. and its galls and compare the growth of affected and unaffected plants. *Trigonorhinus* sp. larvae usually infest the main trunk and lateral branches of the plant, causing the affected branches to become partially swollen and verrucose. Galls develop in stages depending on whether eggs are laid in May or July and proceed in sequence over three years from egg-laying to formation, expansion, dormancy, maturity, dormancy, maturity, and death. Galls inhibit plant development to some extent. On average, six (at least one, and no more than 18; standard error of the mean = 3) larvae occupy each gall, and the number of larvae within a gall did not significantly affect gall size. Gall size significantly affected branch dieback, and large-diameter infested branches had larger galls. This study clarifies the growth dynamics of *Trigonorhinus* sp. galls and provides a basis for further research into the growth mechanism of the species' galls.

## Introduction

There are more than 200 000 flowering plant species, and at least 500 000 insect species interact with them (Metcalf and Kogan 1987). Over their 400-million-year coexistence, insects and plants have developed extremely diverse and complex interactions (Samways 1993). More specifically, they have developed nutrition, reproduction, protection, and defence relationships (Qi *et al.* 2020).

A gall is any enlargement of plant cells, tissues, or organs that was induced by the stimulus of a parasitic organism as a regular incident in the life history of the parasite (Cosens 1913). Galls can develop on any plant tissue, but the majority occur on leaves (Dreger-Jauffret and Shorthouse 1992). Although plant galls can be formed by bacteria, fungi, mites, nematodes, parasitic plants, viruses, and insects, gall induction is mainly perpetrated by insects. Worldwide, the number of gall-producing insect species is estimated to be between 21 000 and 211 000 species, many of which are yet to be described (William 2011).

Subject editor: Jeremy deWaard

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Fig. 1. Typical hazards of *Trigonorhinus* sp.

*Caragana liouana* Zhao Y. Chang and Yakovlev, a leguminous shrub, is distributed in Ningxia, Inner Mongolia, Shanxi, and Shaanxi in China. It occurs naturally in semifixed and fixed sandy areas and loess hills in grasslands and desert grasslands, and it forms building blocks on fixed sand dunes or flat sandy areas. In this way, it plays an important role in revegetating fragile ecosystems in the arid and semiarid regions of China (Luo *et al.* 2007). In recent years and with widespread planting of *C. liouana*, gall tumour damage to the branches and trunks of plants in the middle of the *C. liouana* desert forest has broken out, and the damage is spreading (Song *et al.* 2010).

*Trigonorhinus* sp. is a newly discovered stem-boring pest that we discovered through indoor dissection of galls on the main and lateral branch stems of *C. liouana* (Fig. 1). The pest was first discovered in Wuqi County, Shaanxi Province, China (Song *et al.* 2010) but has neither been scientifically named nor been recorded in Inner Mongolia, China. We have no information about the pest's damage characteristics, growth, and development. It was identified by Russian weevil expert Boris A. Korotyaev, who identified the insect as Coleoptera, family Anthribidae, genus *Trigonorhinus*. Further identification work is in progress. A similar species, *Trigonorhinus dolgovi*, was described by Korotyaev (1977). Before that, *Trigonorhinus alternatus* and *Trigonorhinus tomentosus tomentosus* were discovered and reported by Say in 1826, and *Trigonorhinus areolatus* was discovered by Boheman in 1845 (Hume *et al.* 2013), with its description published by Antonio *et al.* (2013).

Anthribid beetles are a rare and ancient group with peculiar habits. Larval habits vary, with some living in woody fungi (Legalov *et al.* 2018), others feeding on seeds (Legalov *et al.* 2018)

or the eggs of their hosts (Dervišević *et al.* 2019), and some preying on the eggs of scale insects (Dervisevic and Graora 2019). Although a few records report Anthribids causing and inhabiting plant galls, there are no descriptions of how the insects actually form the galls (Valentine 1998). For instance, the weevil *Urodontus scholtzi* Louw (Anthribidae) induces oval–cylindrical galls on the stems of host plant *Galenia africana* Linnaeus (Aizoaceae) (Price and Louw 1996), and Oberprieler and Scholtz (2018) described the unusual gall-forming habit and life history of *Urodontidius enigmaticus* Louw (Anthribidae) larvae, which form galls on the leaves of *Ruschia versicolor* Linnaeus Bolus and then feed on the gelatinous cells inside the galls.

*Trigonorhinus* sp. females lay eggs in the central stem of *C. liouana* and, after hatching, the larvae feed on stem tissue, which then induces gall production. Unfortunately, the swelling and tuberculation of *C. liouana* branches caused by *Trigonorhinus* sp. result in severely decreased fruit set, and multiple gall tumours lead to conductive tissue damage and death, thereby posing a serious threat to plant growth. Few studies have been undertaken to investigate the frequency and kind of impacts caused by galling insects on their host plants (Sacchi *et al.* 1988). Because of this, we investigated both the growth of infected *C. liouana* during the *Trigonorhinus* sp. gall-forming process and the relationship between gall size and *Trigonorhinus* sp. population density. Our aim was to examine these questions: (1) What are the specific growth dynamics of the galls? (2) What are the changes in *C. liouana* growth potential after it has been affected? and (3) What factors are associated with gall size?

## Methods

### Study site description

The study site, which covered an area of about 3000 m<sup>2</sup> in the *C. liouana* Forest in Usutu National Forest Park (60° 26' 30" N, 111° 9' 20" E) in Hohhot, China, was located on a semifixed sandy area halfway up a mountain slope. The forest's average plant height was 112.87 cm, average annual temperature was 10 °C, average annual rainfall was 770 mm, annual sunshine was 2077 hours, and sunshine rate was 46%.

### Gall and plant growth

Thirty current-year galls were randomly marked, and their lengths and widths, as well as the width of each infested branch and the ground diameters and heights of the infested plants, were measured using Vernier calipers every 7 days from late March to early November 2019. The galls were obtained from 30 different host plants, and the host plants received different degrees of damage. In the same plot, five healthy plants were randomly selected as controls, and their heights and ground diameters were measured at 7-day intervals.

### Relationships between galls and each insect development stage

In 2019, we used branch cutters to randomly collect 20–30 galls every 7 days and every 30 days from 2020 to 2021. We then measured the length and width of each gall and the width of the affected branch, and then we dissected the collected galls to determine the development stages of the insects. To facilitate identification, insect developmental stages were divided into eggs, larvae, prepupae, pupae, and adult. The dissected galls and the different stages of the *Trigonorhinus* sp. individuals found within the galls were observed and photographed using a DVM6 digital microscope (Leica Microsystems, Wetzlar, Germany). In this experiment, each emergence hole in a gall was considered an individual because only one adult will emerge from each hole.

### Statistical analyses

Because the galls are approximately cylindrical, their volumes ( $V$ ) and relative volumes ( $RV$ ) were calculated as:

$$V = 3.14 \times (GD/2)^2 \times GL$$

and

$$RV = 3.14 \times [(GD/2)^2 - (HD/2)^2] \times GL,$$

where  $GD$  represents gall width,  $GL$  represents gall length, and  $HD$  is the width of ungalled tissue (Qi *et al.* 2020).

To test whether the gall volume formula provided a statistically reliable estimate of gall size, we randomly resampled 50 galls by immersing each gall in a graduated tube filled with water and recording the volume of displaced water. Because the relationship between the two measurements was linear ( $y = 1.0328x + 0.1047$ ,  $R^2 = 1$ ), we used the gall volume formula to estimate gall size throughout the study.

The differences among the effects of growth period and branch size on gall length, width, relative width, volume, and relative volume were tested using one-way analysis of variance, as were the effect of gall size on branch dieback and the effects of the number of larvae in the galls on gall length, width, volume, relative volume, and whether the branches died. Statistical analyses were performed using SPSS 25.0 (IBM, Chicago, Illinois, United States of America).

## Results

### The growth of insects, galls, and host plants

***Trigonorhinus* sp. life cycle.** *Trigonorhinus* sp. produce one generation a year in Inner Mongolia. Each *Trigonorhinus* sp. larva is hidden in a single chamber, and there were 3–15 larvae per gall. While overwintering, the larvae feed in the gall and then stop feeding to pupate (Fig. 2C, D, H) in April. The grey–brown adults (Fig. 2E, I, J) emerge in early May and seek mating opportunities. After mating, females lay eggs in mid to late May on new branches of the year or on branches without galls (usually located in the middle or at the bottom of the branch; Fig. 2A, F). Their eggs hatch in late May, and the larvae (Fig. 2B, G) feed inside galls they form on the branches. In late July, adults mate for the second time and lay eggs on new branches of the year or on branches without galls. The larvae from those eggs feed, go dormant, and overwinter (August–March) inside galls on branches, and then the cycle begins again.

**Gall development dynamics.** When galls begin to swell in March and April, the overwintered *Trigonorhinus* sp. larvae are now active and gnawing on branches. After the adult females lays eggs on new branches in mid to late May, the subsequent newly hatched larvae form new, tender, green, juicy, oval–cylindrical galls with much fibrous and watery internal tissue. In July, the galls are 1.8–5.5 times the diameter of a normal branch. Meanwhile, as galls develop and mature by larval feeding, they enlarge and their tissues become dry, woody, and brown-coloured. Gall stages include (1) winter dormancy (from August of year 1 to March of year 2), (2) maturity (from April to July in year 2), (3) winter dormancy (from August in year 2 to March of year 3), and (4) maturity (from April to July of year 3), followed by death (Fig. 3). Galls vary in size from 5.2 to 205.4 mm ( $n = 876$ ) long, 0.8 to 37.6 mm ( $n = 876$ ) wide, and 0.003 to 106.5 cm<sup>3</sup> ( $n = 876$ ) in volume (Fig. 3).

The average monthly length, width, relative width, volume, and relative volume of galls during the growth period (April–July) differed significantly among the months (length:  $F_{(8,22)} = 136.553$ ,  $P = 0.000$ ; width:  $F_{(8,22)} = 129.171$ ,  $P = 0.000$ ; relative width:  $F_{(8,22)} = 34.384$ ,  $P = 0.000$ ; volume:

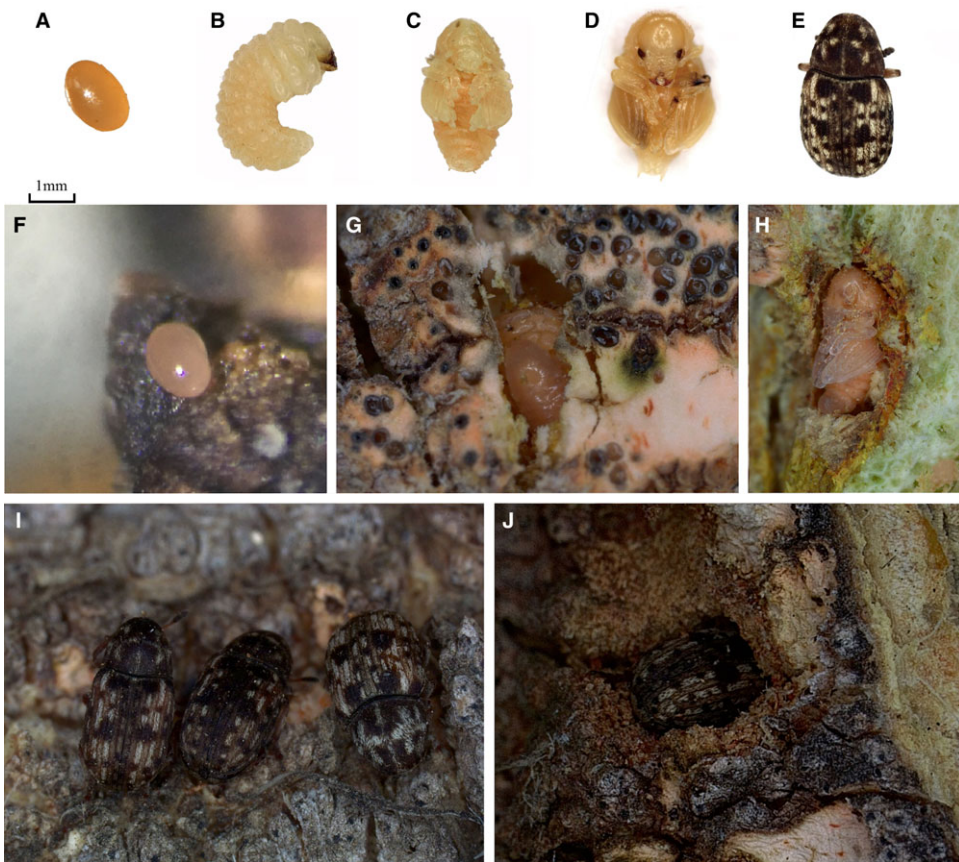


Fig. 2. *Trigonorhinus* sp. morphology: A and F, egg; B and G, larva; C and H, prepupa; D, pupa; and E, I, and J, adult.

$F_{(8,22)} = 183.613$ ,  $P = 0.000$ ; relative volume:  $F_{(8,22)} = 157.421$ ,  $P = 0.000$ ; Table 1), thus indicating that the galls increased in size as the plants did.

***Caragana liouana* growth.** Beginning in April, the average monthly diameter at ground level of *C. liouana* with galls was considerably less than that of healthy *C. liouana* (Fig. 4). Although there was some error in the shrub height measurements, the average monthly growth rates of plants with galls were generally lower than those of healthy plants (Fig. 5), indicating that galls can inhibit *C. liouana* growth and development to some extent.

#### Relationships between the number of larvae and galls

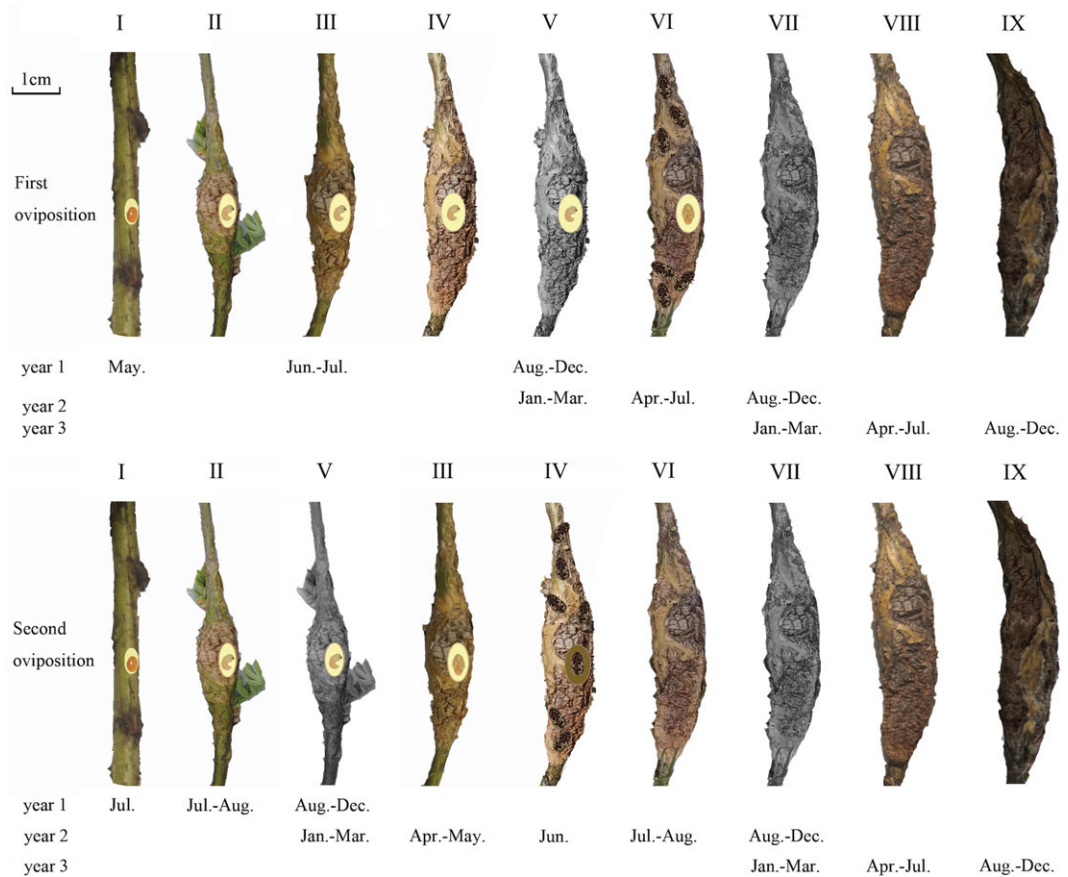
**Quantitative characteristics of *Trigonorhinus* sp. larvae in galls.** Usually, we found 1–4 galls per *C. liouana* branch, and each gall contained an average  $6.84 \pm 3.227$  *Trigonorhinus* sp. larvae, with a maximum of 18 and a minimum of 1 (Fig. 6). The usual number of *Trigonorhinus* sp. in a gall was 6–9, which accounted for 44.86% of all galls.

**Relationship between the number of *Trigonorhinus* sp. and gall size.** The number of larvae within a gall had no significant effect on gall length, width, volume, or relative volume ( $n = 876$ ; length:  $F_{(17,858)} = 0.860$ ,  $P = 0.622$ ; width:  $F_{(17,858)} = 0.921$ ,  $P = 0.548$ ; volume:

**Table 1.** Average gall measurements during the *Trigonorhinus* sp. gall growth period, April–July 2019.

Month	Gall length (mm)	Gall width (mm)	Relative gall width †(mm)	Gall volume (cm <sup>3</sup> )	Relative gall volume (cm <sup>3</sup> )
April	46.42	10.77	7.96	42.29	39.41
May	53.48	12.15	8.89	61.94	57.48
June	59.65	12.94	9.51	77.98	72.52
July	65.56	13.68	10.14	96.33	89.86

† Relative gall width = width of galled tissues – width of ungalled tissue.



**Fig. 3.** Dynamic diagram of *Trigonorhinus* sp. gall development: **I**, spawning; **II**, formation; **III**, initial expansion; **IV**, expansion; **V**, dormancy; **VI**, maturity; **VII**, dormancy; **VIII**, maturity; and **IX**, death.

$F_{(17,858)} = 0.512, P = 0.947$ ; relative gall volume:  $F_{(17,858)} = 0.532, P = 0.938$ ). This indicates that the effects of gall formation are the same regardless of the number of larvae infesting a *C. liouana* shrub.

### Relationships between galls and host plants

**Number and volume of *Trigonorhinus* sp. galls in relation to *C. liouana* branch dieback.** Of the 876 galls collected, 293 died, accounting for 33.45% of the total, and the branches on which the dead galls were located also died. Analysis of variance showed that, although the number of *Trigonorhinus* sp. within the galls did not significantly affect *C. liouana* branch dieback ( $F_{(1,874)} = 0.428$ ,  $P = 0.513$ ), gall volume was significantly related to branch dieback ( $F_{(1,874)} = 279.836$ ,  $P = 0.000$ ). Therefore, the number of *Trigonorhinus* sp. larvae does not cause branch dieback, but the size of the galls does: the larger the galls are, the more likely *C. liouana* branches will die.

**Relationships between the branch on which galls were located and the gall.** The diameter of the *C. liouana* branches on which galls were located differed significantly with respect to the length, width, relative width, volume, and relative volume of the galls (gall length:  $F_{(434,441)} = 1.487$ ,  $P = 0.000$ ; width:  $F_{(434,441)} = 2.461$ ,  $P = 0.000$ ; relative width:  $F_{(434,441)} = 2.232$ ,  $P = 0.000$ ; volume:  $F_{(434,441)} = 1.773$ ,  $P = 0.000$ ; relative volume:  $F_{(434,441)} = 1.567$ ,  $P = 0.000$ ). These results show that larger-diameter infested branches had larger galls.

### Other insects in the gall

We found several other insect species within the dissected galls ( $n = 876$ ). First, *Pyemotes* spp. (Fig. 7A, B) parasitises *Trigonorhinus* sp. larvae in galls and causes larval mortality, but that species was found only on one larva. The larvae of Buprestidae species (Fig. 7C) were found in the gall, accounting for only 3% of larvae of other species. Larvae of *Xylotrechus* spp. (Fig. 7D) were also found; they accounted for 4.5% of the total number of insects. How these insects are related to *Trigonorhinus* sp. and how they are related to each other warrant further study.

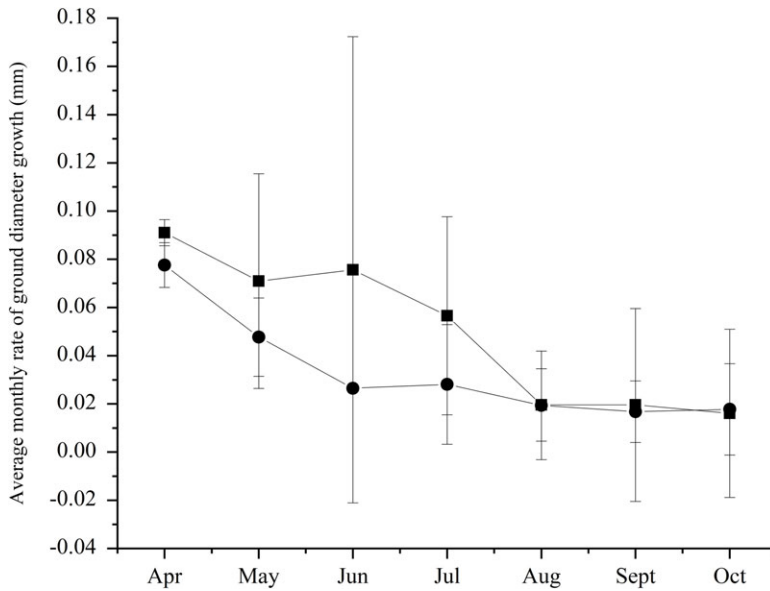
## Discussion

### *Trigonorhinus* sp. damage to *Caragana liouana*

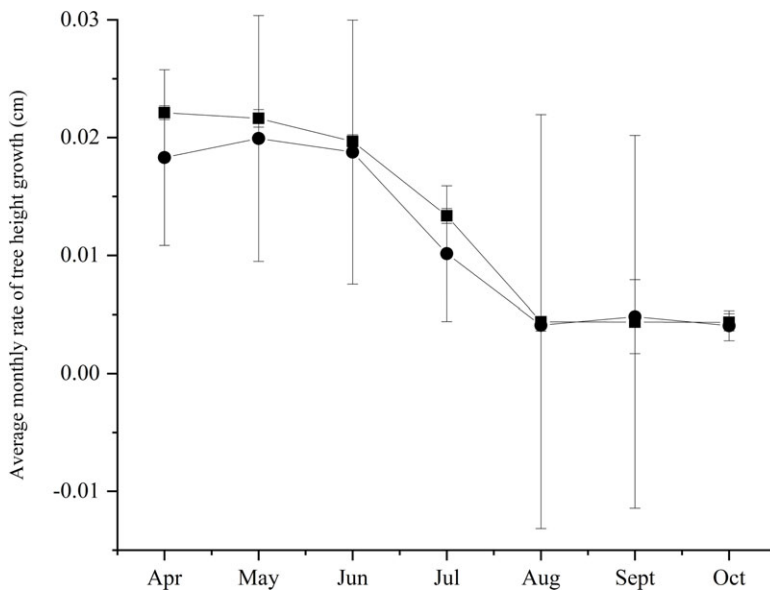
The growth of *C. liouana* infested with galls was obviously worse than that of healthy *C. liouana*, especially when *Trigonorhinus* sp. are active (May–July). These results are not unexpected: gall-inducing insects develop and complete their life cycles either within or on plants, deriving nutrition from the plant as they do so (Stone and Schönrogge 2003; Raman 2011). Fay *et al.* (1993) showed that the formation of galls can prevent photosynthesis, inhibit growth, and divert sufficient energy to gall tissue, thereby ensuring the growth and development of both the galls and the gall-causing insects. According to Miller and Raman (2018), the type of symbiosis (exploitative, mutual, and commensal) between two interacting organisms is based on the net gains or losses to the interactants. In our study, the affected host plants (*C. liouana*) obviously suffered net energy drains, as shown by stunting and death, while the gall-inducing insect (*Trigonorhinus* sp.) gained and usually successfully reproduced. This demonstrates an exploitative (parasitic) symbiotic relationship.

### Gall response to damage by *Trigonorhinus* sp.

There are three hypotheses as to what factors influence gall growth and development. The first hypothesis states that the relationship between gall size and the number of pests in a gall is positively correlated – that is, the larger the number of pests in the gall, the larger the gall (Wool and Ben 1998; Zhang *et al.* 2013). Leggo and Shorthouse (2006) showed that the overall size of mature galls is determined by the number of larval chambers. The second hypothesis views galls as phenotypic entities that develop under the influences of both plant



**Fig. 4.** Monthly growth rates, measured as diameter at ground level, of *Caragana liouana* Zhao Y. Chang and Yakovlev. Solid squares indicate healthy *C. liouana*, and solid circles indicate *C. liouana* with galls. Data are means; bars represent the standard error of the mean.



**Fig. 5.** Monthly growth rates, measured as shrub height, of *Caragana liouana*. Solid squares indicate healthy *C. liouana*, and solid circles indicate *C. liouana* with galls. Data are means; bars represent the standard error of the mean.

and insect genotypes (Weis *et al.* 1988), and it considers gall size to be the result of natural selection that depends on a balance between the causes of mortality for a given population. Thus, the net selective pressure may favour either an increase or a decrease in gall size (Qi *et al.* 2020). The third hypothesis states that gall development is independent of the number



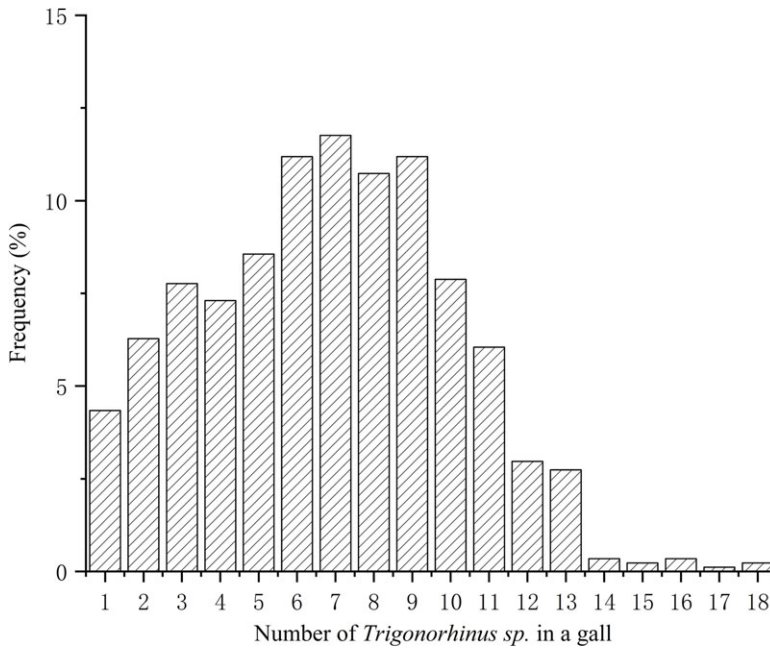


Fig. 6. Frequency distribution of the number of *Trigonorhinus* sp. larvae in individual *Caragana liouana* galls ( $n = 867$ ).

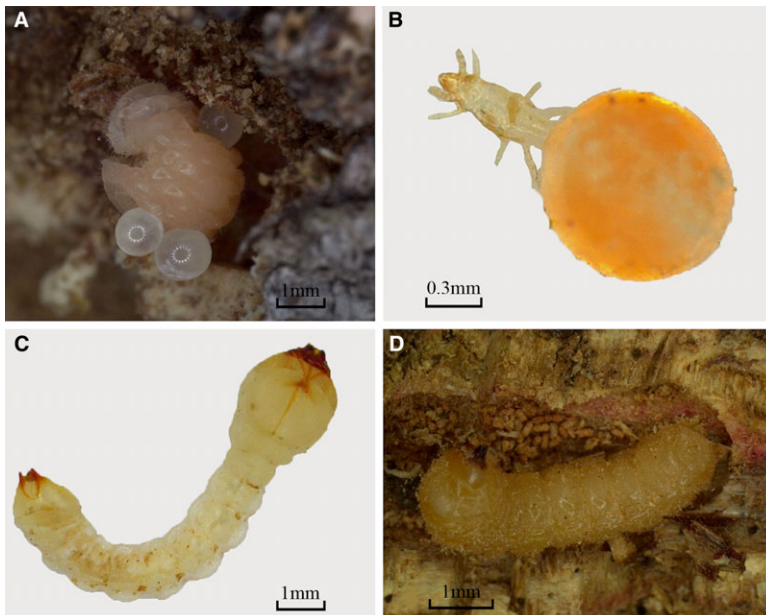


Fig. 7. Other insects found within the galls: **A** and **B**, *Pyemotes* spp.; **C**, the larvae of Buprestidae species; and **D**, the larvae of *Xylotrechus* spp.

of pests in the galls and that the gall-causing insects serve only to initiate gall formation (Wool and Manheim 1986; Wool and Burstein 1991; Wool and Bar-el 1995; Stone *et al.* 2002; Qi *et al.* 2020). Our observations support the third view: the galls formed by *Trigonorhinus* sp. in the present

study grew constantly during the *C. liouana* growing season, regardless of whether they were occupied, and we found no significant correlation between gall size and the number of insects in individual galls.

### Harmful characteristics of *Trigonorhinus* sp.

Although most *Trigonorhinus* sp. galls contained multiple individuals, each larva within a gall occupied its own separate chamber. When the larvae were dissected from the galls and placed together, they killed each other. The presence of multiple insects within a gall may be due to the female laying multiple eggs at close intervals on the same branch, possibly making the most of available resources. *Trigonorhinus* sp. females select egg-laying sites in attempts to maximise their productivity and their offspring's survival (Thompson 1988; Craig *et al.* 1989; Price 1991). Stone and Schönrogge (2003) showed that insect egg-laying behaviour occurs before gall formation and that the physical or chemical action of the host plant influences the gall-causing insect's egg-laying, which in turn determines the insect's development and survival. Because gall-causing insect larvae obtain nutrients from the host plant, females may select more vigorous host plants on which to lay their eggs (Tooker and Helms 2014). Gall-forming coleopteran larvae employ many mechanisms during their life cycles. For instance, *P. affinis* (Coleoptera), a major pest of *Gossypium* spp., has larvae that usually survive by first forming galls at the base of the stem near the soil and then tunnelling around the stem, creating spiral channels that destroy vascular tissue and cause plant death (Dharajothi *et al.* 2004). After hatching, *H. truncatulus* larvae gnaw on host stem tissues to induce galls and begin feeding on plant tissues by cutting longitudinal feeding channels (Agarwal 1985). *Alcides bubo* females lay eggs on the stem, and the hatching larvae bore into the stem, inducing thickening (Govindan 1975; Jalaluddin 1999). Likewise, larval *Trigonorhinus* sp. feeding on stem tissue induce gall production. These coleopteran larvae all feed on stem tissue, which induces galls. The present study enriches this understanding.

**Acknowledgements.** This research was supported by the National Natural Science Foundation of China (32160372), the Inner Mongolia Autonomous Region Natural Science Foundation (2020BS03014), the Inner Mongolia Autonomous Region Higher Education Research Project (NJZZ18047), the Inner Mongolia Autonomous Region Linxue “Double First-class” Construction Project (DC2100001171), the Inner Mongolia Autonomous Region Science and Technology Plan Project (2019GG007), the Inner Mongolia Agricultural University experimental teaching equipment development and specimen making project (YZ2019007), and the Inner Mongolia Agricultural University High-level Talent Research Startup Plan (203206038). Yanru Zhang and Yaru Hu should be considered joint first authors of this paper.

**Competing interests.** The authors declare they have no competing interest.

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