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Biotic Factors Affecting Overwintering Population of *Pissodes punctatus* (Coleoptera: Curculionidae) in Northeast Yunnan Province of China

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ABSTRACT

The mortality rate of overwintering population of the pine bark-weevil, *Pissodes punctatus* and causes of mortality were examined on newly infested host trees in Northeast Yunnan Province of China. We found an 80.76% mortality rate of *P. punctatus* that was greatly higher than the healthy (survival) rate. Life stages of overwintering population of this pest were overlapping including older larvae (3^{rd}-4^{th} instar) (71.20%), pupae (16.47%) and adults (12.32%). The causes of mortality were identified into different biotic factors, which included woodpecker predation, parasitism by insect parasitoids, tree resistance and undetermined diseases. Predation by woodpeckers (41.83%) was the dominant factor causing the mortality of immature stages of *P. punctatus* and significantly higher than the parasitism by insect parasitoids (18.99%), tree resistance (17.97%) and undetermined diseases (1.98%). Moreover, three hymenopteran ectoparasitoids (*Triaspis* sp., *Rhopalicus* sp. and *Eurytoma* sp.) were observed to attack immature stages of *P. punctatus*. *Triaspis* sp. was the dominant species amounting 70.59% of the parasitoids, indicating a high potential in the biological control programme of *P. punctatus*. Relevance of these findings to biological control associated with forest management of *P. punctatus* was discussed.

Key words:Pissodes punctatus, biological control, woodpeckers, parasitoids, wood borers.

INTRODUCTION

The pine bark-weevil, *Pissodes punctatus* Langor et Zhang, 1999, is a devastating stem borer of armand pines, *Pinus armandii* Franch. and has caused widespread tree mortalities in China's Yunnan Province (Chai and Liang, 1992; Duan *et al*., 1998; Liu *et al*., 2005a; Yao *et al*., 2003). *Pissodes punctatus* produces one generation per year. Larvae feed in the phloem and bore in sapwood before pupation. Adults start to emerge in late April and mature to oviposite after 15-20 days, and still can be found in November, but their oviposition occurs mainly in May to September. Thus, the life stages of overwintering population were overlapping, including older instar larvae, pupae and even some adults (Duan *et al*., 1998; Yao *et al*., 2003). Moreover, a female adult can survive 1-10 months with an average production of ~78 eggs (Lei *et al*., 2003). In addition, the pine bark-weevil can also attack some commonly planted forestry conifers such as *Pinus yunnanensis* Franch. and *P. massoniana* Lamb. (Duan *et al*., 1998; Lei *et al*. 2003; Yao *et al*., 2003), posing a threat to pine forests all round Yunnan Province.

Many approaches have been adopted to suppress population of this weevil. The common ways included spraying of chemical insecticides (Yao *et al*., 2003; Liu and Yang, 2005; Liu *et al*., 2005b), clearance of infested trees (Xie *et al*., 2002; Zhang, 2004) and application of pheromone etc. (Ze *et al*., 2010), but their effectiveness was limited due to covert damage, uneven life stages and high reproduction of *Pissodes punctatus*. Thus, it is necessary to explore other effective ways to control this weevil.

In natural forest stands, many biotic factors (e.g. tree resistance, natural enemies) can affect and may be potential to regulate pest population (Duan *et al*., 2012）. Natural enemies (e.g. woodpeckers, insect parasitoids or predators), as one of the key biotic factors, could play a critical role in suppression of pest population. Because natural enemies themselves can search and attack covert hosts over time, they could be more effective in control of wood borers than conventional methods (e.g. chemical control) once they were established or released. Use of natural enemies has aroused wide concerns amid researchers and been considered as an important way for sustainable management of wood borers (Yang, 2003; Yang, 2004). Therefore, understanding of biotic factors affecting pest population may provide more information on effects of natural enemies on pest population and be critical to develop an effective biological control programme.

It has been reported that there were many factors causing the death of immature *P. punctatus* (Liu *et al*., 2005c; Liu *et al*., 2008). Natural enemies (insect parasitoids, vertebrate predators or microbial parasites) and tree resistance can cause a high mortality of *P. punctatus* (Liu *et al*., 2008). Especially, parasitoids, which can attack different life stages of *P. punctatus*, led to ~33% parasitism rate (Liu *et al*., 2008), indicating their high potential for biological control of *P. punctatus*. However, the information provided in previous studies was inadequate (e.g. the parasitoids were not identified) and few studies on biological control were carried out.

In this study, we investigated the population density of *P. punctatus* at overwintering stage in Northeast Yunnan Province of China. The mortality rate of this weevil and cause of mortality was further identified, aiming to provide more information on biological control of *P. punctatus* in near future.

MATERIALS AND METHODS

Survey Methods

The field survey was conducted in Xinjie Forestry Region of Zhehai Forestry Farm (a State-owned farm) (26°25'31" N, 103°35'41" E, Altitude:2720 m), which is located in Huize County, Northeast Yunnan Province of China, in mid-March of 2013, when *P. punctatus* or associated natural enemies were still at overwintering stage. At this time, *P. punctatus* has developed to later immature stages (3^{rd}-4^{th} instar larvae, pupae) or some new adults (but still in the pupation cell). If any alive associated natural enemies could be found, these ones can be considered as potential biocontrol agents. This farm was dominated by 30-year old secondary forest of *Pinus armandii*. *Pissodes punctatus* has been found for >10 years and caused 1% damage rate of pine trees (mortality rate was below 0.1%). We chose this farm because a lower damage caused by pest may indicate a stronger control of biotic factors in the natural environment and more effective natural enemies may be found (e.g. the emerald ash borer biological control programme in the US. Yang *et al*., 2005, 2006; Liu *et al*., 2007; USDA APHIS, 2007). When sampling, five newly dead trees infested by *P. punctatus* (a DBH of 12-15cm, > 4m in height) were randomly selected and cut down. The main trunks were logged into 4 sections with each of them ~1 m long and then transported to laboratory for dissection. The trees logged were > 300 m apart from each other.

Methods for dissection were modified according to Duan *et al*. (2012). Before dissection, woodpecker predation was first examined based on visual destruction of bark or sapwood caused by woodpecker predation (Fig.1). Then, sections of main trunk for each tree were completely debarked with a wide-blade chisel. The exposed cambium tissues or pupation cells were examined for different stages of *P. punctatus* and the fate of each individual was noted as well. *P. punctatus* is the dominant wood borer in sampled forest and the immature stage can be clarified by its brown head part or the pupation cell in sapwood. Besides woodpecker predation, the mortality cause of individual *P. punctatus* was categorized as parasitism by insect parasitoids, undetermined diseases and tree resistance respectively. Otherwise, the individual was considered as a "healthy" one. Any dead individual of *P. punctatus* covered with mold was classified to mortality caused by undetermined diseases (Fig. 2). The *P. punctatus* cadavers surrounded with rosin or plant callus tissues were assigned to mortality caused by tree resistance (Fig. 3). Any associated parasitoids were carefully collected with soft forceps, put into ventilated plastic tubes and reared in a natural photoperiodic environment at 25 ± 5℃ and 60 ± 10% RH to obtain adults. Adult parasitoids were further identified and deposited at the Specimen Museum of Southwest Forestry University.

Data collection and analysis

Total number of dead or healthy *P. punctatus* of different life stage (3^{rd}-4^{th} instar larvae, pupae and adults respectively) was recorded for each tree. Moreover, the percentage of each life stage for healthy individuals was calculated (with the formula: number of each life stage/total number of healthy *P. punctatus* $\times$ 100%) and transformed by arcsine square root for analysis of variance (ANOVA) (Least Significant Difference Test (LSD), α=0.05). Furthermore, the percentage of each fate of *P. punctatus* for each tree was also calculated following the formula: number of each fate / (number of healthy ones + dead ones) $\times$100% and transformed by arcsine square root for ANOVA (LSD, α=0.05). However, untransformed means and standard errors (SE) are presented in figures. All statistical analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, US).

Fig. 1. Mortality of immature stages of *P. punctatus* caused by woodpecker predation: visual destruction of bark or sapwood by woodpecker.

Fig. 2. Mortality of immature stages of *P. punctatus* caused by undetermined diseases: cadaver covered with molds.

Fig. 3. Mortality of immature stages of *P. punctatus* caused by tree resistance: cadaver covered by plant callus.

RESULTS

Density and causes of mortality of *P. punctatus* on infested trees

Five infested (newly dead) trees were examined with a total number of 1 354 individuals of *P. punctatus* (both dead and alive, with an average of 270.8 individuals per tree). However, a high mortality rate of 80.76% was observed (Fig. 4). All dead *P. punctatus* belonged to immature stages (larvae or pupae). The healthy individuals bored in sapwood for overwintering. Among them, 3^{rd}-4^{th} instar larvae totaled up to 71.20%, which was significantly higher than the percentage of pupae (16.47%) (df = 1,8; F = 46.41; $P < 0.05$) and adults (12.32%) (df = 1,8; F = 43.00; $P < 0.05$) respectively (Fig. 5). Although some individuals have developed to adults, they were still in the pupation cell and not yet to emerge out of the bark.

The mortality rate of *P. punctatus* caused by each biotic factor is shown in Fig. 6. Regarding the dead individuals, a high rate of woodpecker predation (41.83%) was observed. The mortality rate caused by parasitism, tree resistance and undetermined diseases was 18.99%, 17.97% and 1.98% respectively, but each of them was significantly lower than the rate of woodpecker predation (woodpecker predation vs. parasitism: df = 1, 8; F = 9.40; $P < 0.05$; woodpecker predation vs. tree resistance: df = 1,8; F = 9.46; $P < 0.05$; woodpecker predation vs. undetermined diseases: df = 1,8; F = 33.44; $P < 0.01$). The other comparisons are shown in Fig. 6.

Hymenopteran parasitoids associated with *P. punctatus*

The larvae or cocoons of hymenopteran parasitoids attacking immature stages of *P. punctatus* were collected and kept in lab to obtain adults. Basically, one parasitoid was observed on one individual of *P. punctatus*. Totally, 85 adult parasitoids were collected and identified to three species, namely *Triaspis* sp. *Rhopalicus* sp. and *Eurytoma* sp. These species are ectoparasitoids. *Triaspis* sp. amounted 70.59% (60 of 85), *Rhopalicus* sp. 16.47% (14 of 85) and *Eurytoma* sp. 12.94% (11 of 85) respectively.

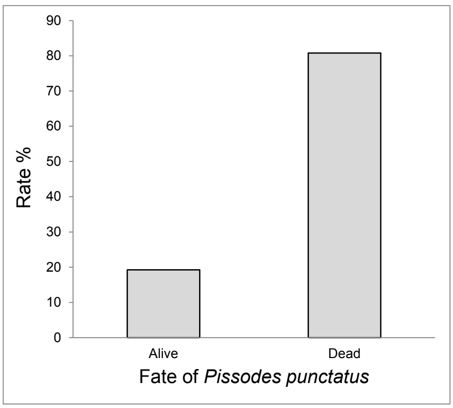

Fig. 4. Rate of alive (healthy) and dead *P. punctatus.*

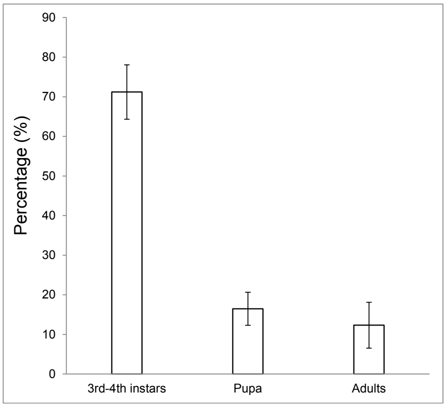

Fig. 5. The percentage for each life stages of healthy overwintering *P. punctatus* (± SE) (Different letters on the bars indicate significant differences, $P < 0.05$).

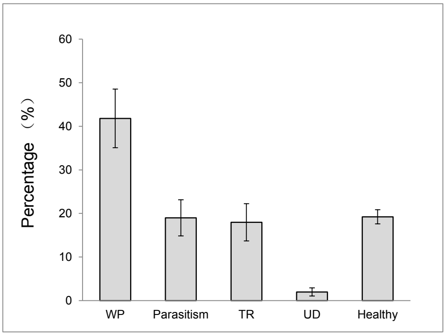

Fig. 6. Percentage of healthy *P. punctatus* and mortality of *P. punctatus* caused by different biotic factors (± SE) (WP: woodpecker predation; Parasitism: parasitized by insect parasitoids; TR: tree resistance; UD: undetermined diseases. Different letters on the bars indicate significant differences, $P < 0.05$).

DISCUSSION

Mortality causes

We found that the average density of *P. punctatus* was > 270 individuals per infested tree, but a high mortality rate of immature stages of this pest was observed (> 80%). The survivors of *P. punctatus* could develop to the stages of older instar larvae, pupae or adults before overwintering, but the immature stages were dominant (total percentage of older instar larvae and pupae was > 87%). These were similar to previous studies (Duan *et al*., 1998; Yao *et al*., 2003). The mortality of *P. punctatus* immature stages was caused by different biotic factors. In this study, only five infested trees were investigated, but these factors were observed on each sampled tree and the mortality pattern was very clear (Fig. 6). The results showed a highest rate (41.83%) of immature *P. punctatus* mortality caused by woodpecker predation, but a lowest rate (1.98%) mortality classified as undetermined diseases. Parasitism (18.99%) and tree resistance (17.97%) collectively caused > 36% of mortality. Three species of hymenopteran ectoparasitiods (*Triaspis* sp., *Rhopalicus* sp. and *Eurytoma* sp.) were collected. These species are larval ectoparasitoids attacking 3rd-4th instar larvae of *P. punctatus*. However, further studies will be conducted to confirm the species in near future.

Relevance to management of *P. punctatus*

Biological control

Conservation and utilization of beneficial birds (e.g. woodpeckers) could be essential to suppress the population of wood borers (Garmendia *et al.*, 2006; Gilberto, 2007; Hu, 2008). In this study, woodpecker predation was the dominant biotic factor affecting population of *P. punctatus.* This is different to previous study on life table of *P. punctatus*, which reported a much lower rate of vertebrate predation, but attributed this to rodent feeding (Liu *et al.*, 2008). It is the first report on the woodpecker predation of *P. punctatus*, and more information on species and biology of woodpeckers needs to be further investigated.

Parasitism by hymenopteran parasitoids was another key factor affecting population of immature stages of *P. punctatus*. It is worth noting that we only investigated the parasitoids on *P. punctatus* at overwintering stage in this study. There could be more parasitoids attacking earlier immature stages (e.g. egg stage or 1st-2nd instar larvae) (Liu *et al*., 2005b; Liu *et al*., 2008) and the parasitism rate by parasitoids would be higher in the entire generation of *P. punctatus*. The parasitoids observed in this study could successfully overwinter and survive to emerge, indicating their high ability of establishment and potential for biological control of *P. punctatus* in high-elevation (> 2700m) forests. Moreover, before the peak period of emergence (mid of June), it is possible to employ some biocontol approaches (e.g. release of larval or pupal parasitoids) to decrease the overwintering population of this bark weevil.

It was reported that *P. punctatus* is susceptible to microbial pathogens such as *Bacillus thuringiensis* and *Isaria farinosa* in experimental (confined) conditions (Wang *et al.*, 2007; Yang *et al.*, 2009), but these pathogens were not widely applied in the field practices. Immature stages of *P. punctatus* feed in host pines, and it is difficult for pathogens to infect them. Moreover, *P. punctatus* usually outbreaks in high-elevation (>1800m) forest stands in Yunnan Province of China where temperature and humidity are not quite suitable to development of microbial pathogens. Associated with our findings in this study (only ~2 % mortality rate caused by diseases), the control effect of microbial pathogens against *P. punctatus* appears to be very limited.

Forest management

In this study, we observed ~18% immature stages of *P. punctatus* were killed by tree themselves, indicating the important role of tree resistance in suppression of pest population. However, in Yunnan Province, *P. punctatus* usually outbreaks in high-elevation (>1800m) pine forests. Generally, these forests are in a weak growth and susceptible to *P. punctatus* due to poor management. Zhang *et al*. (1999) and Liu *et al*. (2005c) reported *P. punctatus* preferred to oviposite on weak-growing host pines and could cause higher damages in such forests. These suggested that also how to improve the growth condition and increase the self-resistance of forests is important to the management of *P. punctatus*.

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