

# New Research on *Matsucoccus matsumurae* (Kuwana) (Hemiptera: Matsucoccidae) in China

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**Abstract:** The Japanese pine bast scale, *Matsucoccus matsumurae* (Kuwana) is the type species of the genus *Matsucoccus* Cockerell, 1909 (Hemiptera: Coccoidea: Matsucoccidae). *M. matsumurae* was first found on *Pinus thunbergii* Parlatores in Tokyo, Japan, and described as a new species by Kuwana in 1903. *Matsucoccus matsumurae* is mainly found in Japan, North and South Korea and especially in China, where the most significant economic losses occur. In this article, we review its invasion and dispersal in China, discuss its occurrence and control, and outline new results from our research on this pest, including comparing its life cycle in two different climatic regions, Fushun in Liaoning province, northeast China, and Jinhua in Zhejiang province, southeast China. We also studied: the genetic differences between three geographic populations, namely from Fushun in Liaoning, Qingdao in Shandong and Jinhua in Zhejiang province, using molecular techniques; looked at variation in the wax-secreting pores and types of wax on the body surface during development, using both light microscopic and electron microscopic techniques; investigated fatty acid synthase and fatty acid elongase in relation to wax synthesis within the body at different developmental stages; studied the natural enemies and their application for biological control, and considered the effect of pathogenic fungi infecting *M. matsumurae* and their potential in biological control.

**Keywords:** Pine bast scale; *Matsucoccus matsumurae*; Matsucoccidae; geographic populations; biological control

## Introduction

The pine bast scales are a group of the most destructive pests of pine forests. They belong to the family Matsucoccidae (Coccoidea), which contains just one extant genus, *Matsucoccus* Cockerell (1909), in which about 39 species have been recorded. The Japanese pine bast scale, *Matsucoccus matsumurae* (Kuwana) (PBS), is the type species and was first recorded on Japanese black pine, *Pinus thunbergii* Parlatores, in 1903 by Kuwana in Tokyo, Japan. It then spread within Japan, to the Korean Peninsula, and then to China in the 1940s. Since then, it has become widespread and infested pine forests in the eastern regions of China, badly damaging two Chinese pine

species *Pinus tabulaeformis* Carrière and *P. massoniana* D. Don. Here we review the infestation and control practices for this species during the past 60 years in China and report on our new research.

## Invasion, infestation and control of PBS in China

In the past 60 years, the infestation and control of PBS in China has gone through three stages (Fig. 1). The first was the invasion and population explosion stage. In the middle of 1940s, PBS was first discovered at Lushun, a port city in Liaoning Province, northeastern China, and then at Yantai, another port

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city in Shandong Province. It mainly attacked *P. tabuliformis*, *P. densiflora* Siebold & Zucc. and *P. thunbergii* Parl. By the 1960s, PBS had spread from Lushun to the borders of Fushun, Yingkou, Dandong, Dalian, in Liaoning Province, and had spread from Yantai northwards to Qingdao, another port city in Shandong Province, and southwards to neighbouring regions. Thus PBS successfully established itself in China during this period.

The second stage was the chemical control period which occurred in 1970s-1980s. By this time, PBS was found throughout Liaoning Province in the northeast, and had spread to Shanghai, Nanjing, and Hangzhou in eastern region, where the major pine forests are composed of *P. massoniana*. In these new territories, PBS has adapted well to its new host and was not controlled by its natural enemies, so that its population increased very quickly, causing serious damage. The damaged trees showed bark dehiscence, needle defoliation, twig wilt, treetop droop, and even death. Such heavy infestations attracted much attention from the forest managers, scientists and governments. The State Forestry Department of China promulgated PBS to be a quarantine pest and the worst destructive exotic forest pest. A lot of chemical insecticides were used to control PBS. However, the effectiveness was limited because the scale insects conceal themselves under or in bark crevices and possess wax coatings that cover the body surface. In many areas, damaged pine trees were cut down to check the spread of the scale (SUN, LI 1982). In spite of these control measures, PBS have still damaged over 70,000 km<sup>2</sup> of pine forest each year in recent years.

In this period, many surveys and studies on PBS were conducted regarding its morphology, taxonomy, biology, ecology and natural enemies. With regard to morphology and taxonomy, an interesting controversy occurred between Professor YOUNG and Professor TANG, regarding the identity of the PBS and distribution. TANG (1978) described a new species *M. liaoningensis* Tang based on the specimens collected from Liaoning province. However, YOUNG (1979) considered that *M. liaoningensis* was a synonym of *M. matsumurae*, and that all pine bast scales throughout China were the same species, *M. matsumurae*. This is discussed further below and is generally accepted today. In addition, YOUNG, YAO (1986) studied the ultrastructure of the epidermal glands of adult female PBS, and found two glands (bilocular tubular ducts & multilocular disc pores) that secreted wax plus another type of non-wax secreting gland with a smooth surface, often with numerous, obvious fine micro-pores. In addition, the sex pheromone of PBS has been studied since 1983, but only “6R,10R-4,6,10,12-tetramethyl-ZE,4E-trideadien-7-one” has elicited strong attraction to adult males and is believed to be the structure of the naturally occurring sex pheromone of PBS (QI *et al.* 1983, 1984, 1990, 1994; YOUNG *et al.* 1984,). In addition, a series of surveys were carried out looking at the natural enemies of PBS in China, and YAN *et al.* (1989) listed 35 species in 8 orders.

The third stage commenced in the 1990s, when the spread of PBS was extended from Liaoning to Changchun, the capital of Jilin province, in the northeast in 2001, and PBS has the potential distribution to extend to Heilongjiang province in

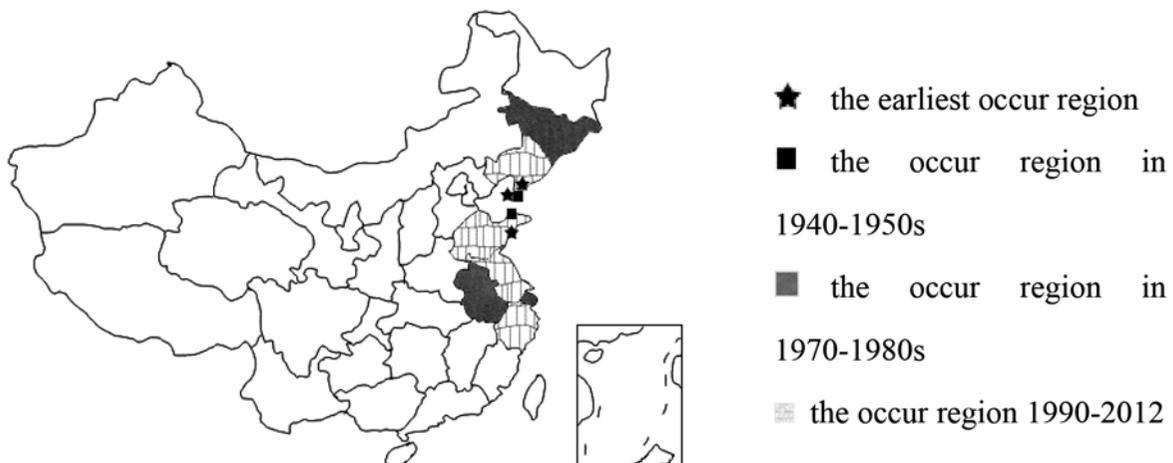


Fig. 1. Spread and distribution of *M. matsumurae* in China

the near future (WANG *et al.* 2009). In Zhejiang Province, there was an outbreak of PBS in 2005, and this has spread to Anhui Province. Thus, PBS is now found in over nine provinces in China. During this time, the risk of damage to the pine forests in

other provinces was also evaluated (LI *et al.* 2005). To implement IPM, some stations for monitoring PBS were established, and such natural enemies as the ladybeetle, *Harmonia axyridis* (Pallas), are gradually introduced as a biocontrol agent of PBS.

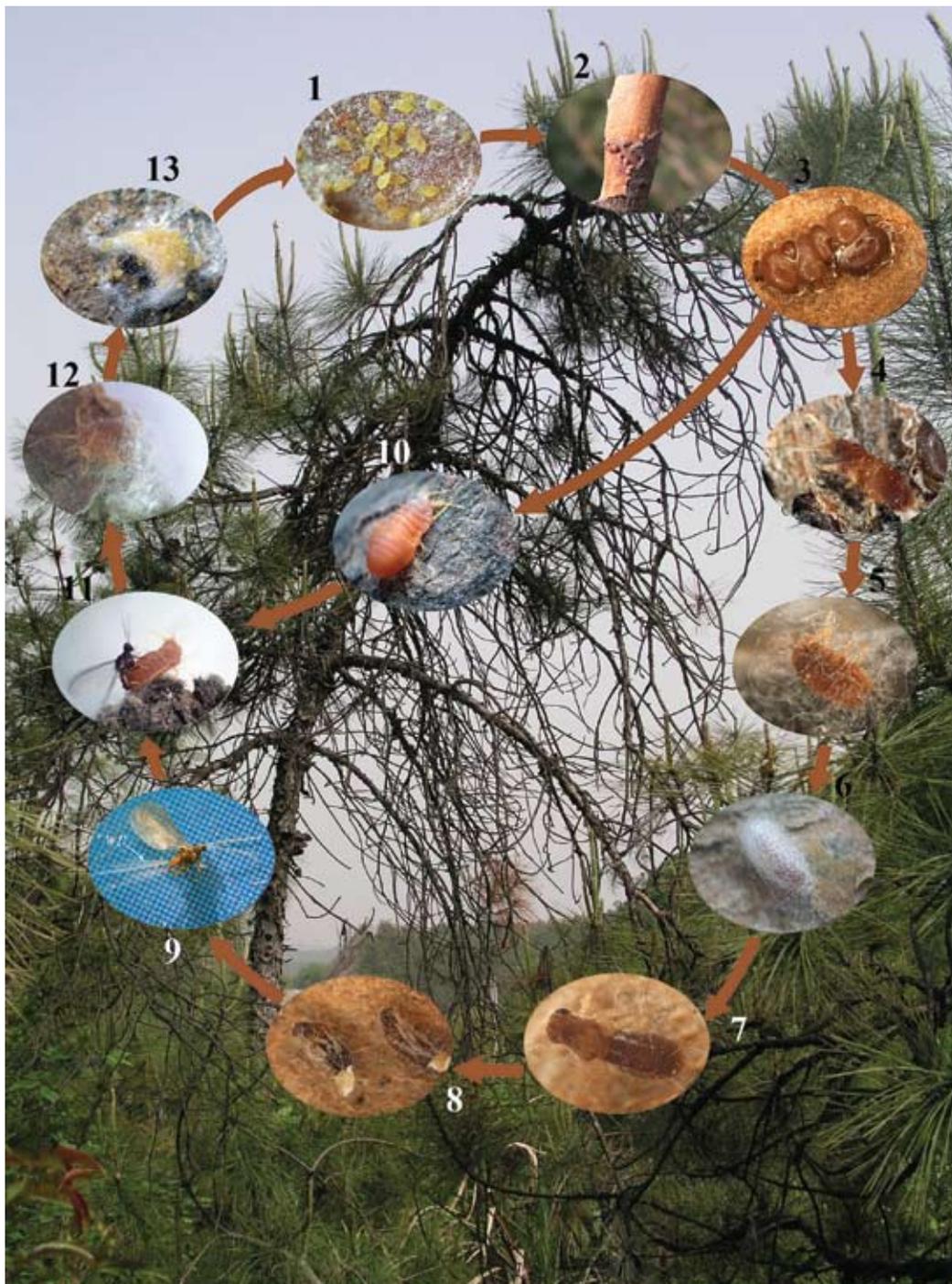


Fig. 2. Symptom of the *Pinus massoniana* infected by *M. matsumurae* and life cycle of *M. matsumurae*. 1. 1st-instar nymph; 2. 2nd-instar nymph of overwintering; 3. 2nd-instar nymph of exposure; 4. New molting male 3rd-instar nymph; 5. Male 3rd-instar nymph of secreting wax; 6. Male 3rd-instar nymph with cocoon; 7. Male prepupa; 8. Male pupa; 9. Adult male; 10. Female adult; 11. The adult female and male are mating; 12. Female adult is secreting wax to form eggsac; 13. oviposition

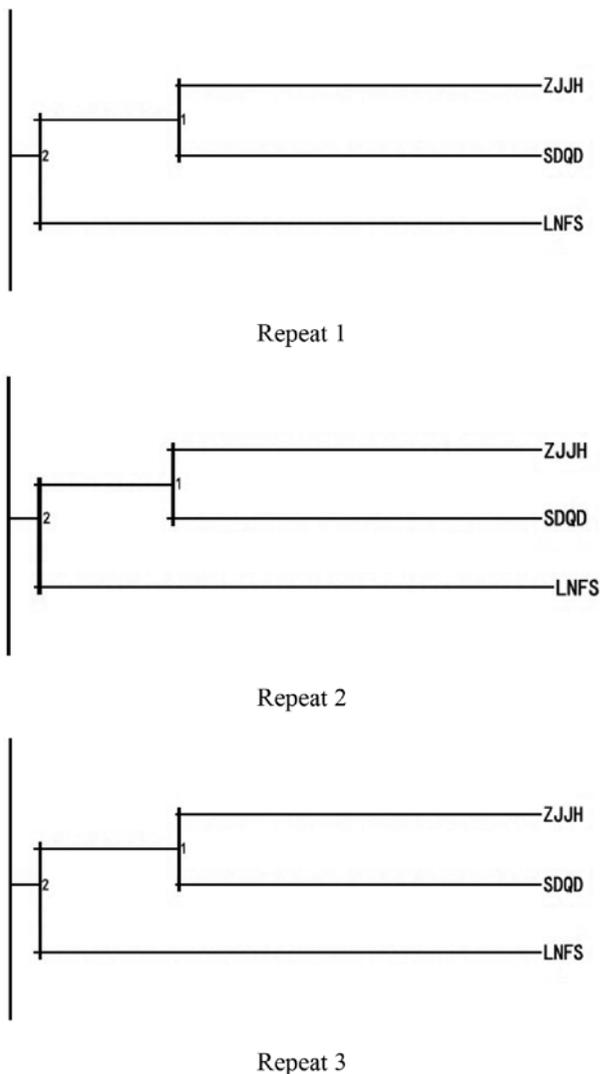


Fig. 3. UPGMA dendrograms of the three geographical populations of *Matsucoccus matsumurae*

## New research on *Matsucoccus matsumurae* (Kuwana)

### Difference between the life cycles of PBS in the northeast and southeast

PBS completes two generations annually in China. In each generation, female development usually involves 4 stages: egg, first- and second-instar nymphs and the adult female. Male development involves 6 stages: egg, first-, second- and third-instar nymphs, prepupa, pupa and adult male (Fig. 2).

However, the climatic conditions in the northeast and southeastern regions are very different, and this has resulted in an obvious change in instar development and life cycle. In northeastern China, the winter is usually much longer and colder than in the south and this has caused the overwintering

stage to take longer in the north. In the southern areas, such as Jinhua (Zhejiang), spring comes earlier and the overwintered first-instar nymphs develop into the pearl-shaped 2<sup>nd</sup>-instar nymphs in early March. From the middle to late March, 2<sup>nd</sup>-instar male nymphs moult into the 3<sup>rd</sup>-instar stage, which searches for a site on the branches or twigs to pupate. This stage then secretes wax filaments to form a cocoon in which it pupates. The adult males emerge in the late March and early April. The adult females also moult from the pearl-shaped 2<sup>nd</sup>-instar nymphs to emerge as the adult female at this time. Copulation and egg deposition occurs in April and early May.

However, in such northern areas as Fushun, Liaoning, spring is one and a half months later. Here oviposition occurs in late May and early June, and the first generation is completed between July and October. The nymphs of the 2<sup>nd</sup> generation appear in October and start to overwinter in late October and early November. In contrast, the southern populations in Jinhua, Zhejiang, do not enter the overwintering stage until December (Table 1).

### Genetic differences between populations

In the late 1970s, Professor YOUNG believed that, based on morphological characters, all pine bast scales throughout China were *M. matsumurae* (Kuwana), whether from the northeastern areas, Shandong, Jiangsu or Zhejiang, while Professor TANG (1978) considered the species in Liaoning province to be a new species, *M. liaoningensis*. This uncertainty has caused problems in the management of PBS, because accurate species identification is important in the application and use of sex pheromones and in population monitoring. In order to clarify the genetic variation of geographic populations, we collected samples in 2011 and 2012 from Fushun (Liaoning Province (LNFS)), Qingdao (Shandong Province (SDQD)) and Jinhua (Zhejiang Province (ZJJH)) and applied RAPD-PCR technology with 4 random primers to investigate genetic variation in these populations. The results indicated that DNA extracted from single individual adult females was enough for RAPD-PCR. The genome size of the species was 9416 bp; at the species level, the coefficient of genetic differentiation ( $G_{st}$ ) of the three repeats was 0.2078, 0.1919 and 0.2075 respectively, indicating that 20.78%, 19.19% and 20.75% of genetic diversity was be-

**Table 1.** Nei’s measures of genetic identity and genetic distance between populations

Population	Repeat 1			Repeat 2			Repeat 3		
	ZJJH	SDQD	LNFS	ZJJH	SDQD	LNFS	ZJJH	SDQD	LNFS
ZJJH	****	0.9601	0.9537	****	0.9651	0.9606	****	0.9561	0.9499
SDQD	0.0407	****	0.9371	0.0355	****	0.9453	0.0449	****	0.9349
LNFS	0.0474	0.0650	****	0.0402	0.0562	****	0.0514	0.0673	****

**Table 2.** Life history of *Matsucoccus matsumurae* in Jinhua, Zhejiang Province

Generation	Month									
	1-2	3	4	5	6	7-8	9	10	11	12
Overwinter generation	(--)	---	---	---						
		Δ	ΔΔΔ	ΔΔΔ						
		+	+++	+++						
First generation			●●●	●●●	●					
			-	---	---	---	---	---		
							ΔΔΔ	ΔΔΔ		
							+	+++	++	
Second generation								●●●	●●●	
								--	---	
Overwinter generation										(--)

● Eggs, - Nymph, Δ Male pupae, + Imago, (-) Overwintering parasitic nymphs.

tween inter-populations, and 79.22%, 80.81% and 79.25% of genetic diversity was within populations; the minimum genetic distance of the three repeats was 0.0407, 0.0355 and 0.0449 between ZJJH and SDQD, respectively, and the maximum was 0.0650, 0.0562 and 0.0673 between SDQD and LNFS (Table 2). These are all below 0.1. We therefore conclude that the three populations are genetically very close and all refer to *M. matsumurae*. According to the UPGMA dendrograms, the 3 populations could be grouped into two clades, one containing the ZJJH and SDQD populations and the other LNFS only, suggesting a closer genetic relationship between ZJJH and SDQD (Fig. 3).

We also found the three geographic populations to be almost identical morphologically, the only small difference appeared to be that, in SDQD and ZJJH populations, the claw digitules do not exceed the end of claw whereas they do in the LNFS populations.

We consider that the genetic and morphological differentiation between LNFS population and the SDQD and ZJJH populations was probably caused by the different climatic conditions in the northeastern and southeastern regions in China. These results

provide a significant basis for the use of sex pheromones and natural enemies in the pest management of this scale.

**Characteristics of wax secretion by PBS and the role of FAS and FAE in wax synthesis**

The characteristics of the wax secreted at different stages during the life cycle of *M. matsumurae* were observed and the changes in fatty acid synthase (FAS) and fatty acid elongase (FAE) were studied. The results (Table 3) showed that the males secreted wax mainly during the 2<sup>nd</sup>-and 3<sup>rd</sup>-nymphal stages. The 2<sup>nd</sup>-instar nymph, with a “pearl-shaped body”, secreted a thin layer of wet wax on the body surface, plus long wax filaments from the segmentally arranged spiracles. The 3<sup>rd</sup>-instar male nymphs secreted a large number of wax filaments, forming a wax cocoon. The females secreted wax mainly during the 2<sup>nd</sup>-instar and adult female stages. The changes of FAS contents in the body were closely related to the wax-secreting activity of the scale insect. In the nymphal males, the highest content of FAS was in the 3<sup>rd</sup>-instar, intermediate in the 2<sup>nd</sup>-instar, and lowest in the pupal stage. For the females, the highest FAS occurred whilst the adult female was constructing the ovisac and the low-

est was after egg laying was complete. The 2<sup>nd</sup>-instar stage was intermediate. The changes in FAE contents were similar to those of FAS, but were relatively higher in the 2<sup>nd</sup>-instar nymph. These results indicate that FAS and FAE play an important role in the synthesis and secretion of wax.

### Natural enemies

A survey of the natural enemies found about 32 species (Table 4). All are predators - no parasitoids have been found to date.

### Effectiveness of entomogenous fungi as a biocontrol agent

We also investigated the effectiveness of fungal infection by four strains of fungi: *Lecanicillium lecanii* strain V3.4504, originally isolated from

brown plant hopper, *Lecanicillium lecanii* strain V3.4505, originally isolated from a species of scale insect (both strains purchased from China General Microbiological Culture Collection Center), and *Fusarium incarnatum-equiseti* strains HEB01 and HEB02, both originally isolated by us from the brown soft scale *Coccus hesperidum* L. (Hemiptera: Coccidae). Older 2<sup>nd</sup>-instar nymphs (with “pearl-shaped body”) of PBS were infected with one or other of the four strains (conidial suspension  $1 \times 10^8$  spores / mL in concentration). All four strains were virulent to PBS, with *L. lecanii* V3.4505 having the greatest virulence (Table 5).

We also inoculated other instars (young 2<sup>nd</sup>-instar nymphs, young adult females, male 3<sup>rd</sup>-instar nymphs and male pupae with wax cocoon) with *L.*

**Table 3.** The contents of FAS and FAE in the body of *Matsucoccus matsumurae* in different development stages

Development stages	FAS contents (nmol/L)	FAE contents (nmol/L)
2nd instar male nymphs	1.642±0.107 a	3.542±0.831 a
3rd instar male nymphs	2.117±0.150 b	2.556±0.207 b
male pupae	0.995±0.046 c	2.375±0.133 b
2nd instar female nymphs	1.642±0.107 a	3.542±0.831 a
Female adults before secreting waxy ovisac	2.286±0.158 b	3.370±0.580 a
Female adults secreting wax to form ovisac	2.203±0.104 b	3.490±0.704 a
Female adults after finished secreting waxy ovisac	1.143±0.225 c	2.039±0.040 b

**Table 4.** Natural enemies of *Matsucoccus matsumurae* in China

Order	Family	species	species
Hemiptera	Anthocoridae	<i>Anthocoris chibi</i> Hiura	<i>Anthocoris japonicus</i> Poppius
		<i>Dufouriella ater</i> (Dufour)	<i>Elatophilus nipponensis</i> Hiura
	Miridae	<i>Cimidaeorus nigrorufus</i> Hsiao et Ren	
Rhaphidioptera	Inocelliidae	<i>Inoceuia crassicornis</i> Schummel	<i>Inoceuia sinensis</i> Navás
Neuroptera	Chrysopidae	<i>Chrysopa jormosa</i> Brauer	<i>Chrysopa phyllochroma</i> Wesmael
		<i>Chrysopa intima</i> MacLachlan	<i>Chrysopa shansiensis</i> Kuwayama
		<i>Chrysopa kulingensis</i> Navás	<i>Chrysopa sinica</i> Tjeder
	Hemerobiidae	<i>Symphorobius amicus</i> Navás	<i>Symphorobius weisong</i> Yang
		<i>Symphorobius matsucocciphagus</i> Yang	
Coleoptera	Coccinellidae	<i>Coccinula quatuordecimpustulata</i> (L.)	<i>Amatis ocellata</i> (L.)
		<i>Coelophora biplagiata</i> (Swartz)	<i>Chilocorus kuwanae</i> Silvestri
		<i>Cryptogonus horishanus</i> (Ohta)	<i>Propylaea japonica</i> (Thunberg)
		<i>Harmonia axyridis</i> (Pallas)	<i>Sticholotis punctata</i> Crotch
		<i>Harmonia obscurusignata</i> (Liu)	<i>Synharmonia bissexnotata</i> (Mulsant)
		<i>Scymnus(Scymnus) frontalis</i> Fabricius	
Diptera	Cecidomyiidae	<i>Lestodiplosis</i> sp.	<i>Oligotrophus</i> sp.
Hymenoptera	Formicidae	<i>Formica rufibarbis</i> ??Emery	<i>Pheidole</i> sp.
		<i>Polyrhachis affinis</i> Smith	

*lecanii* V3.4505. The results (Table 6) showed that 3<sup>rd</sup>-instar males and adult females were the most susceptible stages, with mortality reaching 100% in 8 days. The adult females died quickest, whereas male pupae, protected beneath their waxy cocoon, had the lowest mortality.

Our studies showed that adult female PBS became inactive 24h after inoculation, followed by emergence of the hyphae, and the body color changing from a brownish red colour to dark brown. After 4 days, almost all the insects were dead (Fig. 4). Invasion by the fungus was mainly by integument penetration, especially along inter-segmental folds, the body margin and through the vulva.

## Conclusion

The pine bast scales are oligophagous insects and parasitize only pine trees (*Pinus* spp., Pinaceae). Fossil data suggest that the pine bast scales are an ancient group with a co-evolutionary relationship with their host pine trees (KOTEJA 1984). Their complex metamorphosis, innate wax-secreting

behavior and strong adaptability to various ecological environments have resulted in major control problems. Although *M. matsumurae* is found

**Table 5.** Infectivity of 4 strains to the 2nd-instar nymph of *M. matsumurae*

Fungi	No. 2 <sup>nd</sup> -instar	No. infected	Infection rate
V3.4504	100	64	64%
V3.4505	100	70	70%
HEB01	100	56	56%
HEB02	100	37	37%
Ck	100	0	0

**Table 6.** Infectivity of *L. lecanii* strain No. V3.4505 to different developmental stages of *M. matsumurae*

Instar	No. treatment	No. infected	Infection rate
2nd-instar	50	35	70%
3rd-instar	50	50	100%
pupae with cocoon	50	12	24%
adult female	50	50	100%



**Fig. 4.** Infected symptoms of *M. matsumurae*. A: 2nd-instar nymph; B: female adults; C: female adults; D: male adult

in Japan, Korea and China, damage to pine forests is greatest in China. Here we have reviewed its invasion history and spread within China. The use of chemical insecticides against PBS has been inefficient and so biocontrol is the main sustainable control measure. Our surveys on natural enemies have shown significant control by predators, such as Anthorcoridae and Coccinellidae. We are also trialing the use of entomopathogenic fungi and these appear to have potential as a biocontrol agent. Recently, European scientists have been studying the use of sex pheromones of *M. josephi*, *M. feytaudi* and *M. matsumurae* to trap the adult

males for population monitoring (BRANCO *et al.* 2004, 2006) and this should be tried in China. Our studies on the population genetics, life cycles and morphological characteristics of three geographic populations in China indicate the presence of only one *Matsucoccus* species, suggesting that the use of sex pheromones and other natural enemies should be effective in the future.

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