A Study on the Occurrence and Fecundity of White Spotted Longicorn, Anoplophora malasiaca (Coleoptera: Cerambycidae)

Ren Iwaizumi 1), Makoto Arimoto 2) and Tamotsu Kurauchi 3)

Research Division, Yokohama Plant Protection Station. 1-16-10 Shin-yamashita, Naka-ku, Yokohama, 231-0801, Japan.

Abstract: A study to clarify the occurrence and fecundity of *Anoplophora malasiaca* (Cerambycidae) adults was conducted to prevent the attack on Bonsai and nursery trees for export effectively. In Citrus orchards and urban areas (Host: *Platanus*, *Salix* etc.) of Kanagawa prefecture, the beetles were collected by hand, then, the fecundity and longevity were examined by rearing them in net cages. In the fields, the beetles were observed in the period from mid-June to early October, and the peak of occurrence was considered as early July. Females laid eggs on host branches (above 1cm in diameter) of *Citrus*, *Pyrus*, *Platanus* and *Acer*. Most females laid eggs until the end of August, then died in September. From these results, effective control was recommended for plant quarantine, especially in the period between mid-June and late August in Kanagawa prefecture.

Key words: pest control, host plant, fecundity, plant quarantine, Cerambycidae

Introduction

The white spotted longicorn, Anoplophora malasiaca (Cerambycidae) is distributed in Japan and Korean peninsula. The adult is usually observed from May to October. This species is well known as an important pest of fruit trees (Citrus, Pyrus etc.) and ornamental trees (Platanus, Acer etc.) in Japan, and more than 50 tree species were recorded as hosts (Kojima and Nakamura, 2011). In Japan, Four Anoplophora species except A. malasiaca are recorded, A. oshimana, A. ryukyuensis, A. macularia and A. ogasawaraensis. While in China, a related species, A. chinensis is distributed extensively. The border of distribution between A. malasiaca and A. chinensis is thought to be the north part of the Korean peninsula (Makihara, 2000).

Recently, U. S. researchers revised the genus *Anoplophora*, and they consider *A. malasiaca* to be a synonym for *A chinensis* and only *A. chinensis* is distributed in Japan because they share common morphology (Lingaefelter and Hoebeke, 2002). However, Japanese researchers still hold that these two species

exist independently because they have different morphological characters, e.g. whether or not a pair of white spots is present on the pronotum, and can be discriminated with the sequence of CO I region of mitochondria DNA (Makihara, 2007; Ohbayashi et al., 2009). While, according to a report that examined Mitochondria DNA on Japanese Anoplophora spp. except for A. ogasawaraensis, variations that implied local populations or species were recognized and it was expected to contribute to the clarification of movement of them in Japan and interspecific relationships of them (Muraji et al., 2011). Recently, especially in 2000s, A. malasiaca and A. chinensis were detected by EU, U. S. and Canada in the import plant quarantine of Bonsai and nursery trees. They were also recognized in the field in EU (Gaag et al., 2008; Haack et al., 2010). Thus, the countries have requested emergency actions on the host plant. Now, it is necessary to conduct strict quarantine actions (isolated cultivation in net house, annual inspection at growing sites before export etc.) to satisfy their request in Japan (Plant Protection Station, 2009; European Union, 2012).

¹⁾ Yokohama Plant Protection Station

²⁾ Research Division of Yokohama Plant Protection Station

³⁾ Yokohama Plant Protection Station

In order to control A. malasiaca population with high reliability in the production areas of Bonsai and nursery trees for export, it is fundamentally important to grasp the exact period of adult occurrence and oviposition. Therefore, we have conducted the field survey in Citrus orchards and urban areas in Kanagawa Prefecture, as well as examinations on the fecundity and longevity of collected individuals. From these studies, we have obtained new information on their reproductive behavior and verified the existing information on fecundity and longevity. Thus, we report the results and discuss reproduction in this paper.

Materials and Methods

1. A survey on the occurrence of adults in the field and the longevity of collected individuals

1) Survey in Citrus orchards

The survey in Citrus orchards (3ha) was conducted in July-October, 2011 in Odawara City, Kanagawa Prefecture. The annual mean temperature of the city is 15.3°C, and the region is well known as a Citrus cultivation area because of the warm climate. The survey was done in two orchards (2ha, 1ha) owned by Kanagawa Prefectural Agricultural Research Center. Three or four persons walked around the whole orchard in the period from 9 am to 11 am. The adults dropped on the ground by shaking trees, inhabited at the trunk base etc. were collected and kept alive. They were reared in net cages installed in the outdoors (W1 \times D1 \times H2m) or a greenhouse (W1.3 \times D2.9 \times H1.9m) in Research Division of Yokohama Plant Protection Station. Several pieces of Citrus trees planted in flower pots were supplied as food. The temperature in the rearing period was 20-33 $^{\circ}$ C (outdoors and greenhouse).

2) Survey in urban areas

Most of the survey was done in urban areas of Yokohama City, in June-November, 2010, 2011 and 2012. The adults observed around host trees (*Citrus, Platanus, Salix* etc.) were collected and examined for the fecundity and longevity test mentioned below.

2. Examination on the fecundity

Both males and females collected by the above survey were reared in a net cage (W0.3 \times D0.3 \times H0.3m), one by one, in the greenhouse or laboratory. The thin branches of *Citrus* were supplied as food. For females, host branches of *Citrus*, *Pyrus*, *Acer* and *Platanus* (diameter 0.6-4cm, length 20-30cm) were supplied additionally as oviposition materials. To compare the preference of oviposition by female, the branches of different diameter were prepared by putting on wet vermiculite in a plastic cap (ca. 1000cc). 4-10 days after insertion, the branches were removed from the cage, then the number of eggs were counted under the binocular microscope. The temperature in the rearing period was 20-33 $^{\circ}$ C (greenhouse) and 24-28 $^{\circ}$ C (laboratory).

Results

1. Survey on the occurrence of adults in the field and the longevity of collected individuals

1) Survey in Citrus orchards (Table 1)

Nearly equal numbers of adult were collected in July (23ex. 15 females, 8 males) and August (26ex. 10 females, 16 males), while no adults were collected in October. In September, the survey was not done because of typhoon.

2) Survey in urban areas (Table 2)

In the three year period of 2010-2012, adults were collected or observed between June 18 and October 2. Most of them were recorded in June and July, but small numbers of them were still recorded in August-October.

Table 1. Collection and longevity records of A. malasiaca in Kanagawa prefecture (Citrus orchards, 2011)

Date	Site	Female	Male	Total	Death record
Jul.13, 2011	Odawara 1 (2ha)	15	8	23	Female:Jul.22-Sep.21; Male:Aug.1-Oct.6
Aug.18, 2011	Odawara 2 (1ha)	10	16	26	Female:Aug.22-Sep.16; Male:Aug.24-Sep.29
Oct.5, 2011	Odawara 1 (2ha)	0	0	0	
Oct.26, 2011	Odawara 2 (1ha)	0	0	0	
Total		25	24	49	

Table 2. Collection, observation and longevity records of A. malasiaca in Kanagawa prefecture (Urban areas, 2010-2012)

Date	Site	Female	Male	Total	Death record
Jul.8, 2010	Kanagawa	1	2	3	Female: Sep.13; Male: Oct.6
Jul.21, 2010	Naka	0	1	1	Male: Sep.8
Jul.22, 2010	Naka	1	0	1	Female: Sep.10
Jun.19, 2011	Naka 1	1	1	2	Female: Aug.5; Male: Sep.25
Jun.28, 2011	Naka 2	1	0	1	Female: Jul.29
Jul.3, 2011	Sakae 1	1	0	1	Female: Sep.5
Jul.4, 2011	Naka 2	1	0	1	Female: Jul.23
Jul.7, 2011	Naka 2	1	0	1	Female: Oct.9
Jul.15,2011	Naka 2	1	0	1	Female: Jul.31
Aug.5, 2011	Naka 2	1	0	1	Female: Aug.29
Aug.6, 2011	Sakae 2	1	0	1	Female: Aug.26
Sep.10, 2011	Odawara	0	1	1	Male: Oct.18
Jun.18, 2012	Naka 1	1	0	1	Female: Sep.5
Jun.20, 2012	Naka 2	1	0	1	Female: Sep.2
Jun.21, 2012	Naka 2	1	1	2	Female: Aug.16; Male: Oct.8
Jun.26, 2012	Naka 3	1	0	1	Female: Jul.14
Jun.27, 2012	Naka 1	0	1	1	Male: Aug.8
Jun.27, 2012	Naka 3	4	0	4	no record (not examined)
Jun.28, 2012	Naka 3	1	0	1	Female: Jul.19
Jun.29, 2012	Naka 3	1	1	2	Female:Sep.23; Male: Oct.2
Jul.1, 2012	Naka 2	1	0	1	Female: Aug.21
Jul.2, 2012	Naka 3	0	1	1	Male: Sep.20
Jul.6, 2012	Naka 2	0	1	1	Male: Sep.7
Jul.8, 2012	Midori	2	3	5	Female: Aug.25; Male: Jul.30
Jul.9, 2012	Naka 2	4	2	6	Female: Sep.5, 10; Male: Jul.13, Sep.10
Jul.11, 2012	Naka 2	3	1	4	no record (not collected)
Jul.19, 2012	Naka 2	1	3	4	no record (not collected)
Jul.28, 2012	Midori	2	0	2	no record (not collected)
Oct.2, 2012	Naka 3	0	1	1	Male: Oct.5
Total		33	20	53	

Figure 1 shows the collection and observation records of adults in total, both Citrus orchards and urban areas in 2010-2012. Both males and females were collected from July 1-15 in the maximum number, then the number declined and none were collected from October 16-31.

Figure 2 shows the death records of adults in total, both Citrus orchards and urban areas in 2010-2012. Both males and females died between Sep.1-15 in the maximum number, then the number of dead declined and none survived after the start of November.

2. Examination on the fecundity

Table 3-a, b shows the number of eggs laid by females in each period, host plant, branch respectively. Numbers in parentheses just after the number of eggs indicate the diameter of branch (mm).

Table 3-a shows the oviposition records of two females in 2010. The two females laid eggs constantly until the end of August. In September, the eggs laid by them were very few and they died on September 10 and 13. The total fecundity was 60 (No.1) and 85(No.2). The three males reared in the same conditions died on September 8(1 ex.), October 6(2 exs.).

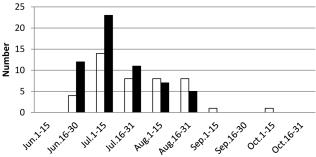


Fig. 1. Collection and observation records of *A. malasiaca* in Kanagawa prefecture in 2010-2012

□ Male
■ Female

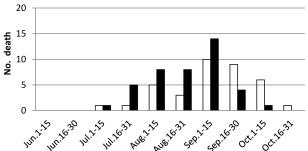


Fig. 2. Death records of *A. malasiaca* collected in Kanagawa prefecture in 2010-2012

 Table 3-b shows the oviposition records of three females in 2012. The three females also laid eggs constantly until the end of August. In September, they died on September 2, 5 and 23. The total fecundity was 89 (No.1), 106(No.2) and 140(No.3). The three males reared in the same conditions died on August 8, September 20 and October 2. The fecundity increased in 2012 as compared to 2010 and the result might be due to

the longer period of examination (June-September) in 2012

Table 4-a, b shows the fecundity in different hosts in 2010 and 2012. In any kind of host, the number of eggs per branch was highest in diameters above 20cm. By contrast, no eggs were laid in diameters below 10mm. Thus, it was verified that females choose relatively thick branches or trunks as oviposition sites as was already

Table 3-a. The fecundity of A. malasiaca on host branch (2010)

		F	emale 1 (8th July Collec	ted)	Female 2 (22nd July Collected)			
Date of Observation*	Host	No. of No. of eggs branches on each branch tested (Diameter of branch, mm)		No. of eggs laid	No. of branche tested	No. of eggs s on each branch (Diameter of branch, mm)	No. of eggs laid	
29-Jul	Citrus				2	8 (25), 5 (23)	13	
2-Aug	Citrus	4	5 (28), 1 (19), 0 (18), 0 (16)	6	3	7 (28), 2 (18), 0 (5)	9	
5-Aug	Citrus	4	5 (30), 0 (18), 0 (9), 0 (8)	5	4	5 (30), 4 (22), 3 (15), 0 (10)	12	
12-Aug	Acer	8	0 (23), 1(18), 0 (17), 0 (13), 0 (10), 0 (9), 0 (7), 0 (7)	1	7	3 (28), 1(22), 0 (14), 0 (12), 0 (8), 0 (7), 0 (7)	4	
19-Aug	Citrus	10	13 (24), 2 (24), 2 (18), 1 (15), 2 (12), 0 (12), 0 (11), 0 (10), 0 (10), 0 (9)	20	10	8 (20), 7 (20), 5 (15), 2 (15), 1 (15), 1 (12), 1 (10), 0 (9), 0 (8), 0 (7)	25	
23-Aug	Pyrus	4	6 (24), 3(18), 0 (8), 0 (6)	9	4	4 (35), 4(18), 0 (8), 0 (7)	8	
27-Aug	Pyrus	6	5 (17), 4(14), 5 (13), 0 (11), 0 (8), 0 (7)	14	6	6 (20), 2(16), 0 (12), 0 (10), 0 (8), 0 (7)	8	
30-Aug	Pyrus	4	5 (25), 0 (17), 0 (13), 0 (7)	5	4	4 (22), 2 (22), 0 (10), 0 (7)	6	
	Total			60			85	

 $[\]boldsymbol{*}$: Observation started 28th July, 2010.

Table 3-b. The fecundity of A. malasiaca on host branch (2012)

		I	Female 1 (18th June Collec	eted)		Female 2 (20th June Collec	ted)		Female 3 (29th June Collect	ted)
Date of Observation*	Host	No. of branches tested	No. of eggs on each branch (Diameter of branch, mm)	No. of eggs laid	No. of branches tested	No. of eggs on each branch (Diameter of branch, mm)	No. of eggs laid	No. of branche tested	No. of eggs s on each branch (Diameter of branch, mm)	No. of eggs laid
1-Jul	Citrus	1	12 (40)	12	1	10 (40)	10			
6-Jul	Citrus	4	9 (20), 2 (15), 2 (15), 0 (12)	13	4	4 (17), 3 (15), 3 (13), 3 (13)	13			
10-Jul	Acer	2	0 (18), 0 (14)	0	2	0 (15), 0 (11)	0	1	9 (35) (Citrus)	9
14-Jul	Platanus	3	13 (21), 0 (12), 1 (11)	14	3	9 (25), 0 (13), 0 (10)	9	1	12 (30) (Citrus)	12
20-Jul	Platanus	: 1	10 (30)	10	1	6 (30)	6	1	12 (30)	12
31-Jul	Platanus	1	22 (33)	22	2	16 (27), 0 (12)	16	4	9 (30), 15 (25), 1 (15), 1 (14)	26
10-Aug	Citrus	4	1 (22), 2 (20), 0 (10), 0 (9)	3	3	16 (25), 2 (16), 0 (9)	18	2	20 (40), 0 (9)	20
15-Aug	Acer	3	0 (30), 0 (16), 0 (11)	0	5	9 (30), 0 (15), 0 (12), 0 (9), 0 (9)	9	5	3 (25), 0 (15), 0 (12), 0 (12), 0 (10)	3
20-Aug	Citrus	2	4 (25), 8 (17)	12	2	9 (30), 3 (20)	12	2	13 (32), 2 (18)	15
25-Aug	Citrus	1	2 (40)	2	1	5 (40)	5	1	17 (45)	17
30-Aug	Citrus	2	1 (20), 0 (17)	1	2	3 (20), 5 (16)	8	3	3 (18), 2 (18), 4 (16)	9
5-Sep	Citrus	2	0 (35), 0 (14)	0	2	0 (17), 0 (13)	0	3	4 (25), 3 (18), 2 (16)	9
10-Sep	Citrus							2	5 (40), 0 (12)	5
15-Sep	Citrus							2	2 (21), 1 (17)	3
20-Sep	Citrus							2	0 (18), 0 (13)	0
	Total			89			106			140

^{* :} Observation started 26th June, 2012.

noticed by many authors.

For the comparison of female preference of oviposition as hosts, the fecundity in diameters above 10mm per branch were compared among host trees. In 2010, Citrus, Pyrus and Acer were examined for the test. The fecundity of Acer was apparently low in small numbers (0.56). The hardness of Acer branches might be the reason for low fecundity. By contrast, females laid more eggs on both Citrus and Pyrus. Nearly equal numbers

per branch were recorded, 2.90(Citrus) and 2.78(Pyrus) (No significance in t test).

In 2012, *Citrus*, *Acer* and *Platanus* were examined for the test. The fecundity of Acer was also low in small numbers (0.80). By contrast, females laid more eggs on both *Citrus* and *Platanus*, resulting in nearly equal numbers, 5.07(*Citrus*) and 7.19(*Platanus*) (No significance in *t* test). The fact that the fecundity on *Citrus* in 2012 was higher than in 2010 might be due to the earlier start of testing in 2012 when females had high ability.

Table 4-a. The total fecundity of A. malasiaca in each host (2010)

Host]	Diameter of branc	:h	Total No.	No. of eggs per branch (> 10mm)
	5-9mm	10-19mm	20-35mm	of eggs	$(Mean \pm SE)$
Pyrus	0(10)	23(12)	27(6)	50	2.78 ± 0.54
Citrus	0(6)	20(20)	69(11)	89	2.90 ± 0.58
Acer	0(6)	1(6)	4(3)	5	0.56 ± 0.34

Note: Number of eggs (Number of branches)

Table 4-b. The total fecundity of A. malasiaca in each host (2012)

Host]	Diameter of branc	eh.	Total No.	No. of eggs per branch (> 10mm)
_	5-9mm	10-19mm	20-45mm	of eggs	$(Mean \pm SE)$
Platanus	0(0)	3(7)	112(9)	115	7.19 ± 1.77
Citrus	0(3)	49(20)	159(21)	208	5.07 ± 0.78
Acer	0(2)	0(12)	12(3)	12	0.80 ± 0.62

Note: Number of eggs (Number of branches)

Discussions

Adachi (1988) investigated the fecundity and longevity of A. malasiaca. He collected individuals from Citrus orchard in early-mid June, the early season of occurrence, and reared them by one pair of sex in grass cylinders kept in the outdoors, giving them Citrus branch in the diameter about 4cm. As results, the total number of eggs per female was about 200, and the longevity of females in days was about 80. He also reported that the peak of oviposition was realized about one month after emergence (4.5 eggs /day /female), then the number gradually decreased to almost zero after September. In

this study, the total number of eggs and the number of eggs per day per female were slightly lower than those of Adachi (1988). The reasons might be two, the start of testing was later, while *Acer* was examined one or two times in this study. While longevity almost coincided with Adachi (1988), in that both males and females were dead by the end of October.

The results in this study were consistent with those of previous studies that were undertaken in Citrus orchards (Kawamura, 1980; Komazaki and Sakagami, 1989; Mitomi et al., 1990) or in the laboratory (Adachi, 1988) on occurrence, fecundity and longevity. Therefore, similar trends of seasonal prevalence will be expected regardless of hosts they depend on. In Kanagawa

Prefecture, located in the flat areas of Kanto region, the high reproductive season of *A. malasiaca* is considered to be mid-June to late August. Thus, control actions such as isolated cultivation by net house or chemical spray around the field are necessary on host plants for export, especially during this period to satisfy the quarantine requirements.

Acknowledgements

We thank Dr. Hiroshi Makihara of Forestry and Forest Products Research Institute for his support on this study. We also thank Mr. Takayoshi Nodomi of Kanagawa Prefectural Agricultural Research Center for his support on the survey in Citrus orchards.

References

Adachi, I. (1988) Reproductive biology of the White Spotted Longicorn Beetle, *Anoplophora malasiaca* Thomson (Coleoptera: Cerambycidae) in citrus trees. *Appl. Enomol. Zool.* **23:** 256-264.

European Union (2012) Commission implementing decision of 1 March 2012 as regards emergency measures to prevent the introduction into and the spread whithin the Union of *Anoplophora chinensis* (Forster).

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:064:0038:0047:EN:PDF

Gaag, D. J., M. Ciampitti, B. Cavagna and F. Herard (2008) Pest risk analysis *Anoplophora chinensis*. Plant Protection Service, Wageningen, The Netherlands. 49pp. Haack, R. A., F. Herard, J. H. Sun and J. J. Turgeon (2010) Managing Invasive Populations of Asian Longhorned Beetle and Citrus Longhorned Beetle: A Worldwide Perspective. *Ann. Rev. Entomol.* **55**:521-546. Kawamura, M. (1980) Studies on the seasonal prevalence of eggs and adults of the White spotted longicorn, *Anoplophora malasiaca* Thomson. *Bull. Kochi Inst. Agri & Forest Sci.* **12**: 35-45. (in Japanese)

Kojima, K. and S. Nakamura (2011) Food Plants of Cerambycid Beetles (Cerambycidae, Coleoptera) in Japan (Revised and Enlarged Edition). Hiba Society of Natural History. Shobara, 505pp.

Komazaki, S. and Y. Sakagami (1989) Capture-recapture study on the adult population of the White Spotted Longicorn Beetle, *Anoplophora malasiaca* (Thomson) (Coleoptera: Cerambycidae), in a citrus orchard. *Appl. Entomol. Zool.* **24**: 78-84.

Lingaefelter, S. W. and E. R. Hoebeke (2002) Revision of Anoplophora (Coleoptera: Cerambycidae). Entomological Society of Washington, Washington D.C. 236pp.

Makihara, H. (2000) True taxonomy and distribution of *Anoplophora* cerambycid beetles in East Asia. *Forest pests* **49**: 180-194.

Makihara, H. (2007) Geneus Anoplophora in Longicorn Beetles of Japan. Tokai University Press, Tokyo, pp.583-585.

Mitomi, M, E. Kuroda and H. Okamoto (1990) Ecological study of the White spotted longicorn beetle, *Anoplophora malasiaca* Thomson (Coleoptera: Cerambycidae) Investigation of adult emergence holes in Citrus orchards in Kagawa Prefecture. *Jpn. J. Appl. Ent. Zool.* **34**: 7-13. (in Japanese with English summary)

Muraji, M., S Wakamura, H. Yasui, N. Arakaki, Y. Sadoyama, S. Ohno and K. Matsuhira (2011) Genetic variation of the white-spotted longicorn beetle *Anoplophora* spp. (Coleoptera, Cerambycidae) in Japan detected by mitochondrial DNA sequence. *Appl. Entomol. Zool.* 46: 363-373.

Ohbayashi, N., J. Ogawa and Zhi-Hui Su (2009) Phylogenetic analysis of the Lamiine Genus *Anoplophora* and its relatives (Coleoptera, Cerambycidae) based on the mitochondrial CO I gene. *Spec. Bull. Jpn. Soc. Coleopterol.*, *Tokyo* (7): 309-324.

Plant Protection Station (2009) Current status of the export plant quarantine of Bonsai and Planting trees. *Shokubutubouekisho Byougaichujohou* 88:1-2. (in Japanese)

和文摘要

ゴマダラカミキリの発生時期と産卵に関する調査 (英文)

岩泉 連1)・有本 誠2)・倉内 保3)

横浜植物防疫所調査研究部

輸出盆栽・苗木を加害するゴマダラカミキリの産卵を効果的に防止するため、成虫の発生と産卵に関する調査を行った。神奈川県内の柑橘園と都市部の発生地(寄主:プラタナス、ヤナギ等)で成虫の採集を行うとともに、同成虫を飼育して産卵と寿命を調査した。野外で成虫は6月中旬から10月上旬まで発見され、7月上旬が発生ピークと考えられた。成虫の飼育の

結果、カンキツ、ナシ、プラタナス、モミジ切り枝直径lcm以上)で産卵が認められた。雌は概ね8月下旬まで産卵し、9月に入ると死亡した。調査結果から、神奈川県においては特に6月中旬から8月下旬までの間、寄主に対する効果的な防除が推奨された。

¹⁾横浜植物防疫所

型横浜植物防疫所調査研究部

³⁾横浜植物防疫所