

Potential Vectors of Pierce's Disease in Taiwan: Ecology and Integrated Management

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ABSTRACT

Since Pierce's disease (PD) of grape was reported in Taiwan in 2002, *Kolla paulula* has been a common leafhopper found at PD infected areas. The study showed that *K. paulula* is a potential insect vector for both PD and pear leaf scorch. In order to establish the integrated management techniques for the environment of vineyards in Taiwan, the authors describe how to apply the data on the integrated management of the potential insect vector. Data represent the study results including life history of *K. paulula*, population dynamics, habitats and host ranges conducted from 2009 to 2012. In addition, this article also describes the effects of temperature and rainfall on the changes of the population of *K. paulula*.

Keywords: Taiwan, Pierce's disease, potential insect vector, *Kolla paulula*, host plant, population dynamics, integrated management.

INTRODUCTION

Pierce's disease (PD) of grapevines is an infectious disease caused by *Xylella*

fastidiosa, a xylem-limited bacterium. PD was found in all commercial grape cultivars, and has been an important limiting factor for the wine industry. PD is one of the typical insect-borne diseases. So far, it is known that crops including grapes, pears, almonds, peach, citrus, coffee, alfalfa and many others are infected with various *X. fastidiosa* species or subspecies. In addition to crops, more than 150 plant species are also infected^(6, 7, 8, 9, 13). PD was first discovered in California in 1892⁽¹⁵⁾. After that, PD was mainly reported from the American continent, such as U.S., Costa Rica, Mexico and Peru^(8, 9, 15).

Since 1998, PD has been found in other areas such as Kosovo⁽²⁾, China^(4, 5) and Taiwan⁽²²⁾. In 2002, suspected PD-like symptoms were reported at Nantou County in Taiwan. In 2013, the PD-like disorder was confirmed as the PD caused by *X. fastidiosa*. However, between 2002 and 2013, 12023 infected grapevines at Miaoli County, Taichung County and Nantou County were eradicated^(21, 22). The vineyards with infected grapevines are located in the hilly land (e.g., Tunghsiao Township in Miaoli County, Houli District and Waipu District at Taichung City), or near the ravines or rivers (e.g., Cholan Township in Miaoli County, Hsinshe District and Tunghshih District in Taichung City, and Tsaotun and Chushan areas in Nantou County). These vineyards are surrounded by shrubs or weeds. Some of the plant species are the host plants for *X. fastidiosa* or its potential vector- *Kolla paulula* (Walker, 1858)^(19, 20, 21). On the contrary, no PD was yet to be found in the vineyards at Changhua County. One possible reason was because these vineyards at Changhua County are relatively large, and the lands are generally flat. Moreover, the high level of underground water underneath the vineyards could damage grapevines after a long period of time. So the grapevines have to be replaced approximately every 7 years. The replacement could be the reason why PD-infected grapevines are not found in these vineyards⁽²¹⁾.

Grapevines in Taiwan are considered as high valued fruit trees. The average fruit price per kilogram is only lower than loquat, lychee, persimmon and Irwin mango. According to the AG Statistics Yearbook 2010, Agriculture and Food Agency, COA, Executive Yuan, Taiwan (<http://agrstat.coa.gov.tw/sdweb/public/book/Book.aspx>), the grape total harvest area was 3,054 hectares, and the output value of grapes was \$ 6 billion 730 million New Taiwan dollars. The main production regions for vineyards are located at Changhua County (1330 ha), Taichung (679 ha), Nantou County (518 ha) and Miaoli County (503 ha). The amounts of grapes produced in these four areas accounted for 99.54% of total production in Taiwan. The data shown above indicates

that the grapevines in Miaoli County, Taichung City and Nantou County are highly susceptible to PD infection. These vineyards produce 55.66% of total production. Therefore, if the integrated management of PD and insect vectors are not effectively established in these high-risk areas, PD would become a threat to other vineyards in Taiwan.

It has been understood that only phytophagous hemipteran insects are the potential vectors for the transmission of prokaryotic plant pathogens. Among these insects, the potential vectors capable of transmitting xylem-limited bacteria, *e.g.*, *X. fastidiosa*, only belong to the Aphrophoridae, Clastopteridae, Machaerotidae, and Cicadellinae (Hemiptera: Cicadomorpha) ^(1, 3, 9, 12, 14, 16, 17,). These vectors are known as xylem feeders or xylem-feeding insects ⁽¹²⁾. Su *et al.* ⁽²¹⁾ reported that among the xylem feeders, *Kolla paulula* is a dominate species found in the PD infection areas. Moreover, the DNA fragment of *X. fastidiosa* was detected in the *K. paulula* individuals, suggesting that *K. paulula* is a candidate vector for transmitting PD in Taiwan.

Currently, there are no chemicals for practical treatment of PD. Prevention of PD would focus on vector control, eradication of infected plants and alternative host plants of PD, breeding disease resistant vines, and cultivation of healthy seedlings. For vector control, the insecticides are used for emergency prevention or rapid suppression of population density of insect vectors. Other methods such as biological control, cultural control and physical control are also used to further reduce the density of vector insects. The goal of vector control is to effectively reduce the speed of the spread of PD. In fact, the development of integrated management for insect-borne diseases would consider the relationships between the pathogens, insect vectors, host plants and environmental factors. Multiple approaches should be used to prevent these insect-borne diseases and hence to reduce economic damages. The data in this context includes field surveys, research and a report regarding the promotion of practical prevention and treatment of insect vectors. The potential vectors of PD in Taiwan are briefly introduced. The ecology of the potential vectors such as host range, feeding habits, population dynamics and fly capacity, as well as the relationship between the potential vectors and environmental factors are included. This report could be used for the development of the integrated management strategy for prevention of the vectors transmitting PD in Taiwan.

Potential vectors of PD in Taiwan

During the fruit seasons, both summer and winter, between 2002-2012, the two corresponding authors preformed surveys of potential vectors using sweeping net,

yellow sticky paper, and D-vac at PD infected areas, including Cholan Township and Tungshiao Township in Miaoli County, Tungshih District, Houli District, Waipu District and Hsinshue District in Taichung City, and Tsaotun and Chushan areas in Nantou County. A total of 4 frog hopper and 32 leafhopper species were captured^(18,21). Among these insects, 6 cicadelline leafhoppers, 3 aphrophorid insects and 1 cercopid insect were identified as xylem feeders (potential vectors), including *Cicadella viridis* (Linnaeus, 1758), *Cofana spectra* (Distant, 1908), *Kolla paulula* (Walker, 1858), *Bothrogonia ferruginea* (Fabricius, 1787), *Anatkins horishana* (Matsunura, 1912), *Poophilus costalis* (Walker, 1851), *Clovia puncta* (Walker, 1851), *Ariptyelus auropilosus* (Matsumura, 1907)⁽²¹⁾, *Eoscarta zonalis* (Matsumura, 1907) and *Xyphon* sp. Among these xylem feeders, the DNA fragments of *X. fastidiosa* were detected in *K. paulula*^(21,22), *B. ferruginea*⁽²¹⁾, *A. horishana*⁽²¹⁾ and *P. costalis*⁽²¹⁾. These four insects are the candidate vectors for transmitting PD in Taiwan. Moreover, *K. paulula* is a common insect inhabiting in weeds or shrubs outside the vineyards in Taiwan⁽¹⁹⁾.

In order to understand whether foreign xylem feeders had been introduced to Taiwan, since 2002, the two corresponding authors not only surveyed insect vectors in fields every year, but also checked insect specimens perpetually persevered at several agencies and schools (such as the Taiwan Agricultural Research Institute, Insect and Mite Collection, Wufeng, Taichung, Taiwan (TARI); Taiwan Forestry Research Institute, Insect Collection, Taipei, Taiwan (TFRI); National Museum of Natural Science, Taichung, Taiwan (NMNS); National Taiwan University, Department of Entomology, Taipei, Taiwan (NTU); National Chung-Hsing University, Insect Collection, Department of Entomology, Taichung, Taiwan (NCHU)). The results showed that five confirmed vectors of Pierce's disease in United States of America *i.e.*, *Homalodisca vitripennis* (Germar) (GWSS), *Graphocephala atropunctata* (Signoret), *Draeculacephala minerva* (Ball), *Xyphon fulgida* (Nottingham) and *Philaenus spumarius* (Linnaeus), were not found in Taiwan⁽²¹⁾.

The ecology and habitats of leafhoppers at PD-infected areas- using *Kolla paulula* as an example

Excluding leafhoppers belonging to *Mileewa*, there are 18 cicadelline leafhoppers and sharpshooter leafhoppers^(10,23,24). Seven of them are commonly found in field, including *Anatkins horishana* (Matsunura, 1912), *Cicadella viridis* (Linnaeus, 1758), *Cofana spectra* (Distant, 1908), *Kolla atramentaria* (Motschulsky, 1859), *Kolla*

insignis (Distant, 1908), *Kolla paulula* (Walker, 1858), and *Bothrogonia ferruginea* (Fabricius, 1787). In addition to *Kolla atramentaria* (Motschulsky, 1859) and *Kolla insignis* (Distant, 1908), the other 5 species can be found in vineyards located at mountainsides.

In this study, *K. paulula* was used as a model insect to investigate the relationship between the ecology of potential insect vectors (xylem feeders) and their inhabitations at the PD-infected areas in Taiwan. Since 2009, four vineyards with different environments were chosen as studying sites. The first vineyard locates at Bai-mao-tai area of Hsinshe District in Taichung City. This vineyard was also assigned as a long-term studying site for the integrated management of potential insect vectors. The second vineyard was at Tsaotun area in Nantou County. No insect vectors control has been performed at this vineyard. The other two vineyards were at Houli District in Taichung City and Chushan area in Nantou County, respectively. The grapevines at these two vineyards were eradicated due to PD infestation in 2008. But the weeds outside the vineyards are still growing without any treatments. Therefore, the areas outside these two vineyards were chosen for monitoring the occurrences of potential insect vectors. In this study, yellow sticky papers were used for monitoring the species and occurrences of potential insect vectors year round. The survey and data collection was performed every two weeks. The data shown below are our recent studies on *K. paulula* and the research results of PD from foreign countries. Scholars who participate in the international symposium would discuss how to promote PD researches in Taiwan based on this information, as well as through the international cooperation in the future.

The geographic distribution and habitat of *Kolla paulula* in Taiwan

Kolla paulula distributes in Palearctic region and Oriental region, including the Indian subcontinent, Indochina, China, Taiwan, Malaya Peninsula and Indonesia⁽¹⁰⁾. Shih *et al.*⁽¹⁹⁾ indicated that *K. paulula* is a common xylem-feeder founded in the medium and low altitude areas in Taiwan. To understand the changes in the geographical distribution of *K. paulula* in Taiwan, since 2002, the first author not only performed field surveys, but also examined about 1,500 specimens of *K. paulula* (both dried and alcohol soaked specimens) stored at the TARI, NMNS, and NTU. The results indicated that most specimens of *K. paulula* were recorded at altitude of 500-1,300 meters mountainous area before 1990. However, in between 1990 and 2012, surveyed

records have included those collected from ground level to 800 meters mountains. In the meantime, two of *K. paulula*'s favorable host plants, mile-a-minute, *Mikania micrantha* Kunth, and spanish needles, *Bidens pilosa* L. var. *radiata* Sch. Bip, have been widely distributed in plains and in low to medium altitude mountains. These results suggest that *K. paulula* might follow their host plants to move down from the mountains. In addition, during the process of checking the specimens, the first author also found various marking and colors on the head, pronotum, and scutellum of *K. paulula* individuals captured from the same or different areas. The result of indoor subculture showed that leafhoppers feed on the same host plants also developed the same variance. Whether these phenotypes occurred resulted from chromosomal mutations warrants further study.

The results of field survey and collection conducted between 2002 and 2012 showed a few *K. paulula* individuals inhabiting inside the dense woods area. The characters of the habitat could be summarized as (1) before the sun rises and after 5 to 6:00 pm, *K. paulula* inhabits on wood and weeds at the edge areas of the orchards, and feeds on those host plants nearby and (2) most of the habitats are in cool and dry areas.

Identified host plants of *Kolla paulula*

The tests of host plants of *K. paulula* were performed according to the definitions made by Oman ⁽¹¹⁾: "Host plants" refers to that adults and nymphs can finish their life cycles on this plant; otherwise, "Food plant" refers to the adults or nymphs only feed on the plant. Based on this definition, the plants identified as the host plants of *K. paulula* mainly belong to the families of Compositae (e.g., *Mikania micrantha* Kunth, *Bidens pilosa* L. var. *radiata* Sch. Bip., and *Ageratum houstonianum* Mill.), Commelinaceae (e.g., *Commelina diffusa* Burm f.) ⁽¹⁹⁾, Moraceae, and Convolvulaceae (Shih, unpublished data).

The possible factors affecting the population dynamics of *Kolla paulula* throughout the year

In the period from 2009 to 2012, four PD-infected vineyards were selected as the studying sites, including Bai-mao-tai 60 (Bai-mao-tai, Hsinshe District, Taichung City), Houli-80 (Houli District, Taichung City), Pinglin-117 (Tsaotun, Nantou County), and Chushan-66 (Chushan, Nantou County). Monitoring of the population dynamics of *K. paulula* and other xylem feeders at the studying sites was performed using yellow

sticky papers throughout the year. The survey was conducted every two weeks. The results of four-year-survey demonstrated that the population of *K. paulula* was affected by several environmental factors, such as abundance of weed, temperature, rainfall and geographic location.

Integrated management of potential insect vectors of PD-using Bai-mao-tai 60 studying site (Hsinshe District, Taichung City) as an example

The Bai-mao-tai area (Hsinshe District, Taichung City) is located in the hillside tableland near Ta-Chia River. The altitude of Bai-mao-tai area is about 600 to 700 meters. Large temperature difference between day and night is recorded at Hsinshe District in Taichung City. It is an important area where high-quality grapes (Kyoho grapes, *Vitis vinifera* L. X *Vitis labruscana* B.) are produced. Annual production was unable to meet the domestic demand. Export markets include Japan, Singapore and Hong Kong. From 2002 to 2010, 368 grapevines (5 to 15 years old) at more than 20 vineyards at Bai-mao-tai area were infected with PD, then be eradicated⁽²¹⁾, resulting in huge economic losses. The vineyards at Bai-mao-tai area are located on sloping valleys covered with weeds and shrubs. Most of these plants are *K. paulula*'s host plants (such as *Mikania micrantha* Kunth, *Bidens pilosa* L. var. *radiata* Sch. Bip., and *Commelina diffusa* Burm f.). The environment and species of potential vectors are different from those identified in the United States. Therefore, it is necessary to establish the integrated management of potential insect vectors in Taiwan for reducing farmers' economic losses.

To do so, from 2009-2012, one of the infected vineyards, Bai-mao-tai 60, at Bai-mao-tai area was selected by Shih and Su to be a long-term studying site for investigation of the population density and the integrated prevention of *K. paulula* adults. In addition, the first author cultured *K. paulula* in a laboratory condition to establish the timing of chemical control of *K. paulula*. The results showed that the mean generation time (from eggs to adults) of *K. paulula* was about 62-94 days. The mean generation time in autumn and winter (October to February the following year) was 1.2 to 1.5 times longer than that in summer.

Preliminary results described below are based on the results of the integrated insect vector control at Bai-mao-tai 60 from 2009 to 2010 as well as the modified control strategies from 2011 to 2012.

1. Chemical control

Kolla paulula is one of the potential insect vectors of PD in Taiwan⁽²²⁾. The tests showed that in a laboratory condition, grapevines are only the food plants of *K. paulula*. Moreover, the life cycle of *K. paulula* is about 2 to 3 months. In order to reduce the population of *K. paulula*, the chemical control was tested in this study. Chemicals such as Imidacloprid (6,000 time dilutions of the 28.8% stock solution), Carbosulfan (2,500 time dilutions of the 48.34% stock solution), lambda-Cyhalothrin (1,000 time dilutions of the 2.8% stock solution) were applied at weeds outside the vineyard once every two weeks. Each chemical was administered three consecutive times. This study was conducted between March and August, two months before and after a period of germination to bursting of summer fruit and winter fruit from 2009 to 2010.

The results showed that from 2009 to 2011, the ratios of the populations of the first peak of *K. paulula* in each year were 1.7: 1: 1.5. It suggests that chemical control could not significantly reduce the population of *K. paulula*. Even though, before the confirmation of habitats and primary host plants of potential insect vectors, chemical control is still the best practical method to suppress the population density.

2. Weed control- prevention of the *K. paulula*'s host plants

Due to ineffective results of chemical control preformed at Bai-mao-tai 60 studying site, since March, 2011, Shih and Su changed the strategy and focused on weed control, especially against spanish needles, within and outside the vineyard. Prevention was conducted by the farmers. The spanish needles on roadsides and within the vineyard were removed by mower once every 1.5 months. The results showed that in 2011, the maximum population of *K. paulula* was only 6. In 2012, Three peaks of population of *K. paulula* were 7 (in mid-March), 6 (in mid-July) and 3 (late December). There is a correlation between the trends of disease and presence of large amount of *K. paulula* and its host plants (such as *Bidens pilosa* L. var. *radiata* Sch. Bip., *Ageratum houstonianum* Mill., and *Commelina diffusa* Burm f.) in other PD infected vineyards in Taiwan (such as Cholan Township and Tunghsiao Township in Miaoli County, Tungshih District, Houli District, Waipu District and Hsinshe District in Taichung City, and Tsaotun and Chushan areas in Nantou County). Therefore, the key to potential insect vector control at PD high risk areas is the eradication of *K. paulula*'s host plants.

In Taiwan, grapevines have high economic value. Although, using mower to remove weeds requires manpower and high costs, it can significantly reduce the

number of potential insect vectors. And it is also very safe for agricultural environments. Therefore, it is necessary to use mower to remove weeds for weed control.

3. Educational promotion

In order to increase farmers' awareness of the knowledge regarding PD symptoms and the favorable environment for potential insect vectors, Bureau of Animal and Plant Health Inspection and Quarantine (BAPHIQ) invites Su and Shih to hold informational meetings about the integrated management of PD and potential insect vector every year. So farmers can observe and cut down their infected grapevines, as well as prevent the host plants of potential insect vectors. At Bai-mao-tai 60 studying site, for example, after weed control by the farmers, the population of potential insect vectors was significantly reduced, and the numbers of PD-infected grapevines also continued to decline each year.

Future Prospects

PD was first reported in Taiwan in 2002. Since then, the researchers under the suggestion and support of the government performed immediate eradication of infected grapevines. Later, a cooperation between Taiwan Agricultural Chemicals and Toxic Substance Research Institute and Taiwan Agricultural Research Institute was established to study PD and the ecology of the leafhopper vectors, set up the management of potential insect vectors at different agriculture environments, investigate the hosts of pathogens and potential insect vectors, and reduce the population densities of pathogens and potential insect vectors in fields, thereby reducing economic losses.

Unfortunately, over the past decade, the researches on insect-mediated diseases and insect vectors received limited governmental funding. The studies of the potential insect vectors of PD in Taiwan, for example, require research works on technologies about monitoring migration of insect vectors and their feeding behavior, test of disease transmission, statistical population ecology, biological control, microbial control and new non-chemical materials. Therefore, it is urgent to incorporate domestic or international interdisciplinary collaborative researches, and to train research teams in this area which is one of the main purposes of this international symposium. With the publication of the papers presented at this symposium and the records of group discussions, it is expected to highlight the main scheme and bottlenecks of researches

on insect-mediated pathogens and insect vectors which can be a vital reference for the authorities in agriculture sector and researchers to develop related research plans. The scholars who participate in this international academic symposium are welcome to maintain cooperation with researchers in Taiwan. Together, the fundamental knowledge of insect-mediated pathogens and insect vectors would be built, and the technology that can fulfill the needs of the industry will be created in the future.

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Table 1. From January, 2009 to August, 2011, the monthly average temperature and total rainfall, and the populations of *Kolla paulula* at Bai-mao-tai area of Hsinshu District in Taichung City

	1	2	3	4	5	6	7	8	9	10	11	12
2009	The period of peak occurrence (every ten days)	※	※					○				□
	Monthly average temperature	12.16 °C	18.59 °C	17.71 °C	19.99 °C	25.48 °C	26.14 °C	26.32 °C	25.62 °C	23.75 °C	19.82 °C	15.23 °C
	Monthly total rainfall	0 mm	15 mm	189.7 mm	385.6 mm	22 mm	93.8 mm	111 mm	996.5 mm	27.5 mm	13.5 mm	23.5 mm
2010	The period of peak occurrence (every ten days)		※					○				□
	Monthly average temperature	14.95 °C	16.86 °C	19.34 °C	20.73 °C	25.22 °C	26.84 °C	26.28 °C	25.68 °C	23.43 °C	18.66 °C	15.46 °C
	Monthly total rainfall	47.9 mm	155.8 mm	32.5 mm	171.5 mm	795 mm	392 mm	376.5 mm	228.8 mm	6.5 mm	22.9 mm	37.3 mm
2011	The period of peak occurrence (every ten days)	※	※		○							□
	Monthly average temperature	-	15.04 °C	15.54 °C	20.71 °C	23.98 °C	27 °C	26.56 °C	27.01 °C	-	-	-
	Monthly total rainfall	-	33.5 mm	52.5 mm	2.5 mm	157.5 mm	185 mm	3542 mm	250 mm	-	-	-

Remarks: ※ represents the first peak of the population occurred every year; ○ represents the second peak of the population occurred every year; □ represents the third peak of the population occurred every year.

