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Biology of a Gall-inducing Species of *Anselmella* (Hymenoptera: Eulophidae) within the Fruits of *Syzygium samarangense* (Myrtaceae)

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ABSTRACT

Gall-inducing insects develop within the galls they induce, and generally remain undetected. In recent years, many instances of gall-inducing insects turning into invasive organisms internationally have come to light. *Anselmella miltoni* induces seed-like galls within the fruits of *Syzygium samarangense* was found in Taiwan recently, thus impacting severely on the country's earnings made out of the export of fresh fruits of *S. samarangense*. Biological details of *A. miltoni* are sparsely known, thus limiting an effective management. We studied *A. miltoni* populations from an orchard in Jhongpu, Chiayi and report in this paper its biology, including notes on field populations, synchronization between fruit development in *S. samarangense* and gall development, emergence, and adult longevity. *Anselmella miltoni* lays eggs during the anthesis stage of fruit phenology of *S. samarangense*. The egg stage of *A. miltoni* overlaps with the petal fall stage in *S. samarangense*. Immature stage of *A. miltoni* develope through the rapid stage to middle fruit stage. *Anselmella miltoni* enters into pupal stage and adults emerge, when the fruits of *S. samarangense* in mature stage. A gall inside the fruit includes many compartments, each compartment with one individual of *A. miltoni* inside. Average number of gall compartments within a fruit is 20.08 ± 11.5. The emergent adults leave its fruit unsynchronized and the period of adult emergence from one gall last for 3~7 days. Adult longevity is 2.36 ± 1.16 days. Based on biological details, we offer suggestions for the management of *A. miltoni*.

Key words: gall, invasive species, ovule, phenology

Introduction

Gall-inducing insects such as *Orseolia oryzae* (Diptera: Cecidomyiidae) and *Leptocybe invasa* (Hymenoptera: Eulophidae) can damage economically useful plants, such as *Oryza sativa* (Poaceae) and different species of *Eucalyptus* (Myrtaceae), which are presently treated as 'invasive' organisms (Rajamani *et al*., 2004; Mendel *et al*., 2004). Gall-inducing insects remain concealed within galls, rendering their management difficult. Moreover, depending on the morphology of gall-bearing plant organs, some of the gall-inducing insects are hard to detect during early stages of growth. In such cases, spread and outbreaks occur easily through exporting and trading of host plants (Mendel *et al*., 2004; Li *et al*., 2006; Csóka *et al*., 2017).

Gall-inducing insects, such as *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) on various species of *Castanea* (Fagaceae), *Procontarinia robusta* (Diptera: Cecidomyiidae) on *Mangifera indica* (Anacardiaceae), *Litchiomyia chinensis* (Diptera: Cecidomyiidae) on *Litchi chinensis* (Sapindaceae), *Leptocybe invasa* (Hymenoptera: Eulophidae) on different species of *Eucalyptus*, *Quadrastichus erythrinae* (Hymenoptera: Eulophidae) on various species of *Erythrina* (Fabaceae) have been known as invasive organisms in Taiwan in recent years (Kim *et al*., 2004; Yang *et al*., 2004; Tang *et al*., 2015). They induce galls on either the foliage or young shoots and damage the plant by affecting either photosynthesis or bud growth.

A majority of the known gall-inducing Hymenoptera lives on stems, buds, and leaves, whereas those attacking fruits are few. However, a few instances of seed-gall-inducing Eulophidae, such as species of *Quadrastichodella*, *Leprosa*, and *Moona* are known (Ikeda, 1999; Doğanlar and Doğanlar, 2008; Kim and Salle, 2008). *Quadrastichodella nova* develops on *Eucalyptus resinifera* and *E. umbellatus* and induces galls within seed capsules (Doğanlar and Doğanlar, 2008). *Moona spermophaga* Kim & La Salle induces galls on the seeds of two *Corymbia* species (Myrtaceae) (Kim *et al*., 2005). *Anselmella miltoni* induces galls within the seed chamber of the fruits of *S. samarangense*. It has emerged as a serious quarantine problem (Fig. 1) (Xiao *et al*., 2006; Huang *et al*., 2008).

Anselmella includes four species (*A. kerrichi*, *A. malacia*, *A. miltoni*, and *A. oculata*) and mainly distributed in Malaysia, India, the Philippines, Fiji, and in the state of Queensland (Australia) (Bouček, 1988; Xiao *et al*., 2006). All the four species of *Anselmella* induce seed galls within the fruits of various *Syzygium* species (Xiao *et al*., 2006). Knowledge of *Anselmella* is limited to taxonomic reports only. Its status as an invasive was not known until it was trapped in port-quarantine stations. In 2003, at the airport quarantine control of Zhengzhou, China, galls within the fruits of *S. samarangense* from a passenger from Malaysia. The emergent adult from the gall was named three years later as *Anselmella malacia* (Xiao *et al*., 2006; Chao *et al*., 2007). Tsai *et al*. (2013) reported another similar instance in Hainan, China that the airport quarantine office intercepted *A. malacia* from fruits of *S. samarangense* carried by a passenger from Vietnam. Curiously, *A. malacia* was trapped only in the customs office of China, never known in its place of origin. Whether it is a pest of Malaysia and Vietnam presently remains unclear. Fruits of *S. samarangense* exported from Taiwan to China were examined by quarantine officials and found a species of the *Anselmella*, which was different from *A. malacia* and was determined as *A. miltoni* in 2005 and 2007 (Huang *et al*., 2008). The incidence of *A. miltoni* strongly affects export of *S. samarangense* from Taiwan to China. *Anselmella miltoni* are recognized as quarantine-restricted insects, since the Government of PR China claims that they have no record of *Anselmella* associated with the fruits of *S. samarangense* (Chao *et al*., 2007; Huang *et al*., 2008; Tsai *et al*. 2013).

Syzygium samarangense Merr. et Perry. (Myrtaceae), commonly known as wax apple tree, is indigenous to Malaysia and Andamans (Republic of India). This is often planted as crop, ornamental, and shade tree (Lin *et al*., 2004; Chen *et al*., 2012). In Taiwan, *S. samarangense* is an important crop tree, cultivated over 5,500 ha in southern and central Taiwan, with the average production exceeding 5,500 t/y (Chen *et al*., 2012). *Syzygium samarangense* flowers from January to April, and the fruit is usually harvested between May and July in Taiwan (Lan, 2004; Lin *et al*., 2004; Chen *et al*., 2012). Harvesting of the fruits would usually occur during wet season (May to September) in Taiwan, during when high humidity and temperature would induce cracking of fruit skin, which acts as a port for pathogens, such as *Pestalotiopsis eugeniae* (Melanconiales: Melanconiaceae), *Phytophthora palmivora* (Peronosporales: Peronosporaceae), and *Botryodiplodia theobromae* (Botryosphaeriales: Botryosphaeriaceae) (Tsai, 2012). Therefore, *S. samarangense* remains stressed and resulting in low-quality fruits during its natural season in Taiwan. Most farmers used various cultivation practices, such as pruning branches, covering the whole tree with black net or inducing stress by injuring root and trunk, which allows farmers to successfully adjust the time of harvesting *S. samarangense* whole year round according to the area condition (Lan, 2004; Chen *et al*., 2012), and highly promotes profit in price and quality (Huang, 1993; Lee, 2010).

The internal feeding habit with minimal external symptoms on the infested fruits of *S. samarangense* explain why little is known about this insect. Lack of detailed biological knowledge impede developing strategies for its effective management, especially when *A. miltoni* is a newly emerging invasive insect on *S. samarangense* in Taiwan. Not until August 2007, fruits of *S. samarangense* planted in Jhongpu, Chiayi of central Taiwan, were found attacked by *A. miltoni*. Many questions remain to be answered. How does *A. miltoni*'s life cycle match with host-plant phenology? How long do adults survive? Do they extend the longevity by consuming honey from the flowers of *S. samarangense*? At what stage do they initiate galls? How many individuals exis within one fruit? Do they reproduce sexually? If so, what is the sex ratio? Keeping these questions in full view, this study aimed at investigating the biology of *A. miltoni* on *S. samarangense* in Taiwan by answering the above questions with an intent to manage these populations.

Materials and Methods

Phenology of *S. samarangense* and *A. miltoni*

Our sampling orchard was at Jhongpu, Chiayi (23°24'47.2"N, 120°33'41.2"E), Taiwan where large areas suffer production loss because of *A. miltoni* infestation. Collections of *A. miltoni* were made weekly from May to August in 2014 and different developmental stages of the fruits of *S. samarangense* was obtained. Phenological stages was recorded according to the Manual of Plant Protection, Council of Agriculture, Government of Taiwan (Lin *et al*., 2004) which classified that into eight stages: middle bud stage (豆粒期), full white stage (白肚期), anthesis stage (盛花期), petal fall stag (胚仔期), rapid stage (合臍期), young fruit stage (幼果期), middle fruit stage (中果期), mature stage (熟果期) (Fig. 2). As the blooming of flowers on the tree is unsynchronized, quantitative recording of the presence of different phenological stages became necessary and we followed the sampling method of Peduzzi *et al*. (1996). In each field observation, we randomly selected different branches of sampling trees in the orchard to count the number of each developmental stages on each branch of *S. samarangense*. Phenological counting is completed when accumulated number of every developmental stage has reached ten. The ratio of each developmental stage to total number of sampling was recorded. In case all branches had been observed but some developmental stages remained less than ten, the ratio of developmental stages was counted directly using all the available samplings.

We collected adults of *A. miltoni* using both sticky traps and net sweeping. We suspended 20 yellow sticky traps in the sample site. Each trap was at least two *S. samarangense* trees apart (c. 10~30 m) and replaced every week. Collections of *A. miltoni* ceased when no flower and fruit could be observed for at least two weeks, which is around September. Samples from sticky traps and net sweeps were examined under stereo binocular microscope (Leica EZ4, Wetzlar, Germany). Adults of *A. miltoni* were separated and stored in 70% alcohol.

Gall development in relation to phenology of *S. samarangense*

Flowers and fruits of *S. samarangense* collected from the sample site were dissected to determine the developmental stages of the gall and *A. miltoni*. The *A. miltoni*-induced gall tissue of *S. samarangense* usually will include one gall, but may occasionally comprise up to three (Fig. 1), inside one single fruit chamber. Every gall consisted of several compartments, each with one individual larva of *A. miltoni*. Forty to 50 fruits of *S. samarangense* were collected weekly in March-September 2014 and were dissected in laboratory to determine the stage of insect development and corresponding phenology. The galls that included the pupae were placed in a circular plastic cage (diameter: 7.5 cm, height: 4 cm) to obtain adults. Emerging

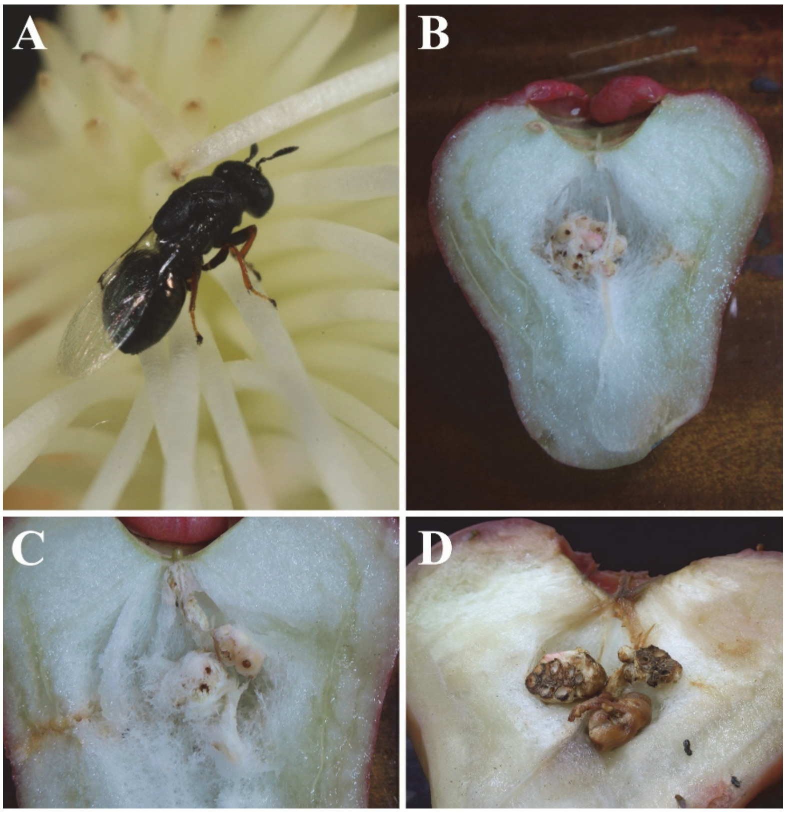

Fig. 1. A, Adult of *Anselmella miltoni* visiting flower of *Syzygium samarangense*. B, infected *S. samarangense* by *Anselmella miltoni* with one gall. C, D, two and three galls in one infected *S. samarangense* respectively. Adult emergence holes on the galls and a tunnel on the left of the fruit could be seen in C. The three galls in D had the upper two already being dissected the inside compartments could be seen while the bellow one remained intact.

adults were stored in 70% alcohol and all voucher specimens were deposited in the National Chung Hsing University.

Number of gall compartments and adult emergence span

Newly emerged *A. miltoni* adults were collected and counted daily from 10-12 am. We determined the sex and date of newly emerged adults. The period from first to the last adult emergence from the gall of a fruit was recorded as 'adult emergence span'. If no *A. miltoni* emerged from the gall for three days, we then dissected them to count their numbers and measure the gall-compartment diameter.

Galls with adult emergence > 50% were included for the analysis of relationship between number of compartments/gall and emergent adults using a simple linear regression. The linear regression model was performed using the program PAST 3 (Hammer *et al.*, 2001) and the tests of significance were verified at $P < 0.05$.

Adult longevity of *Anselmella miltoni*

We reared the newly emergent adults from the adult-emergence span experiment in round plastic cages for measuring adult longevity. Adults were reared individually in each of the following three treatments which provided either 10% honey solution (treatment 1) or water (treatment 2), or none (treatment 3). In treatments 1 and 2, cotton balls were soaked in appropriate solution (honey and water) and placed in a plastic dish (diameter 5 mm) which was placed within the plastic cage stored in a growth chamber at 25 ± 2℃. The soaked cotton balls were renewed daily. The dead adults were checked and removed, and stored in 70% alcohol daily. Data were analyzed using PAST 3, and nonparametric statistics were performed. Significant differences were accepted at $P < 0.05$.

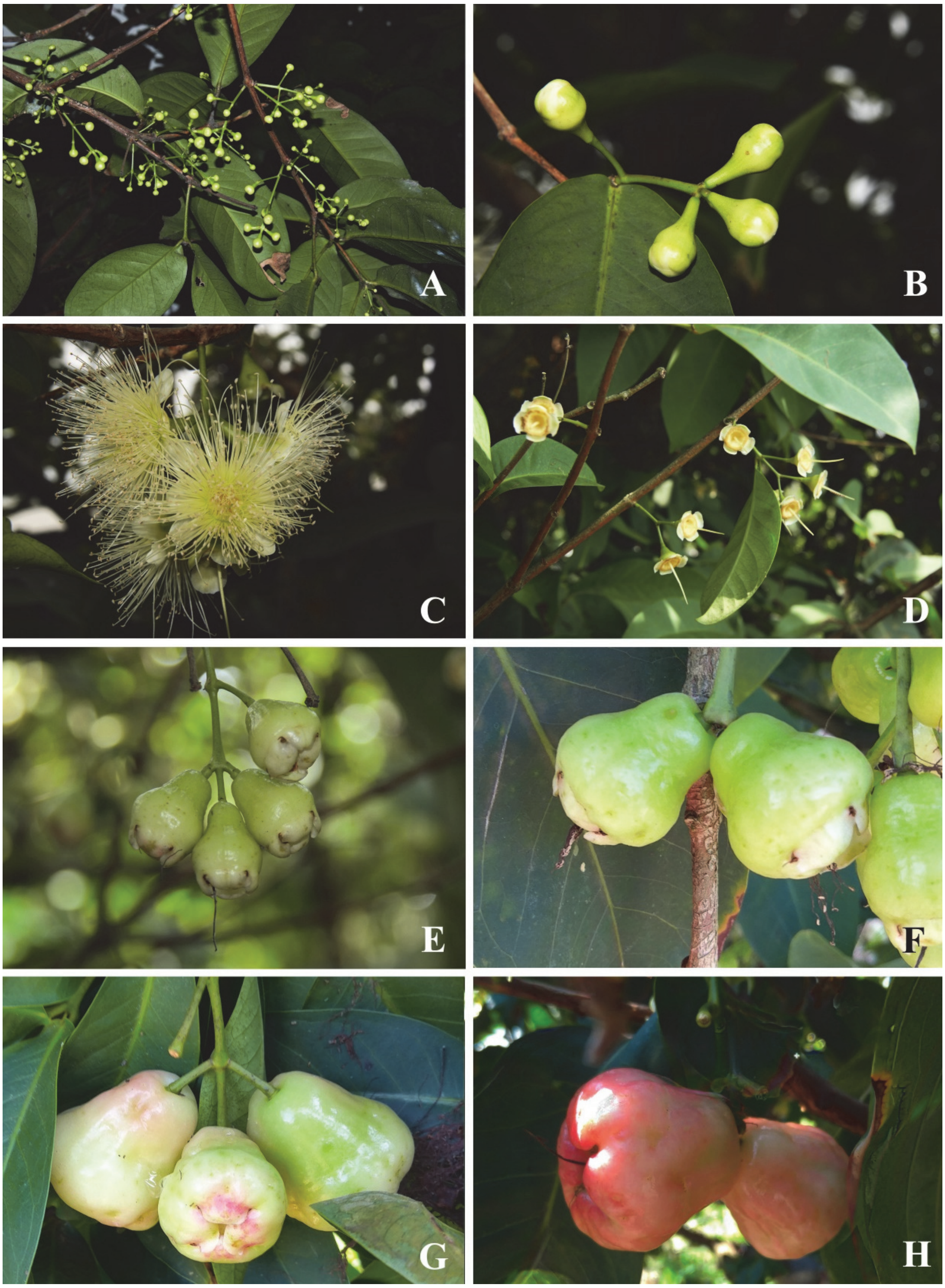

Fig. 2. Development stage of *Syzygium samarangense* based on Lin *et al*. (2004). A, Middle bud stage (豆粒期). B, Full white stage (白肚期). C, Anthesis stage (盛花期). D, Petal fall stage (胚仔期). E, Rapid stage (合膪期). F, Young fruit stage (幼果期). G, Middle fruit stage (中果期). H, Mature stage (熟果期).

Results

Phenology of *S. samarangense* and investigation of *A. miltoni* populations in orchard

The reproductive phase of fruits of *S. samarangense* in the sampling area lasted from May to August and two growing cycles occurred in the same year (Fig. 3). In mid-May, *S. samarangense* was in the middle bud stage. Fully blooming of flowers, at anthesis stage, were first observed on 19 May 2014, which

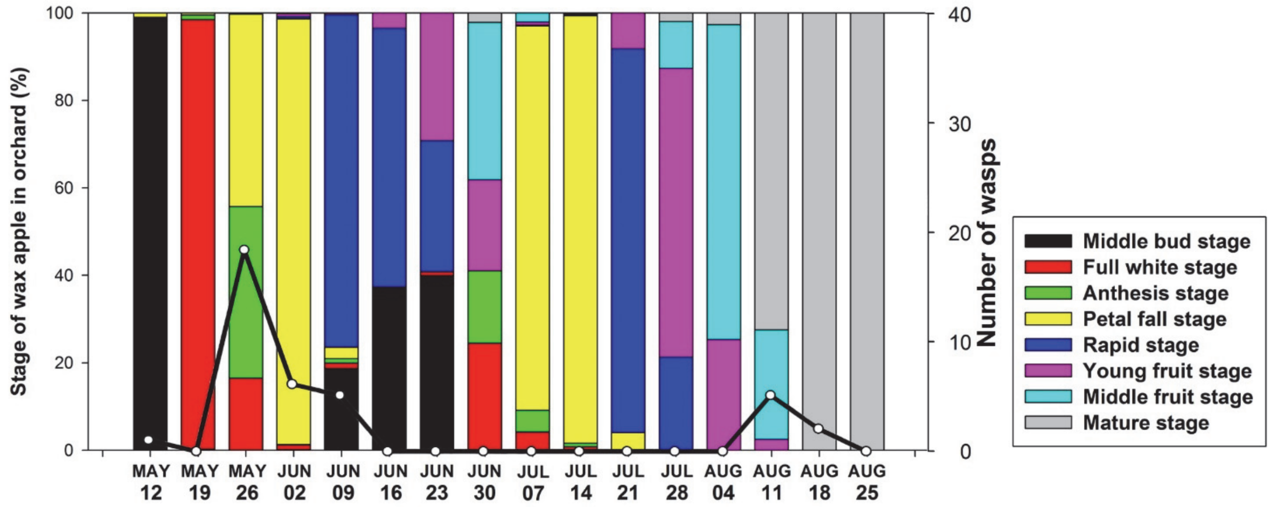

Fig. 3. The developmental stage of *Syzygium samarangense* fruit phenology and number of *Anselmella miltoni* adults collected by sweeping and sticky paper in orchard. Different colors indicate different growing stage of *S. samarangense*.

peaked at 39.2% of phenology in orchard in late May. Young fruits of the first growing cycle appeared in mid-June and by this time, the second middle bud stage also began. Mature stages of the first growing cycle did not occur because most of the fruits had dropped in late June. The second anthesis stage of *S. samarangense* also occurred in late June and through the next six weeks, mature stages occurred.

Using sweep nets and sticky papers, *A. miltoni* were collected when *S. samarangense* were at the anthesis stage and the petal fall stage during the first growth cycle, and a few individuals of *A. miltoni* were collected during mature stage of *S. samarangense* in their second growing cycle. The ratio of infestation in the two growing cycle were 28.6% (n = 406) and 7.5% (n = 268), respectively, and there were significant differences in the infestation rate between the two growing cycle (Chi-square test, $p < 0.001$).

Gall development in relation to phenology of *S. samarangense*

A fruit chamber with galls induced by *A. miltoni* included 20.8 ± 11.5 compartments (n = 57). Each compartment was circular (as in cross-sections) and the diameter was 2.55 ± 0.46 mm (n = 31). As the minute (< 0.1 mm) and transparent larvae of *A. miltoni* at the initial stage is generally hard to distinguish from the floral tissue of *S. samarangense*, characteristics of both insect and gall development were used to categorize their development. The six developmental stages (Fig. 4) were as follows: 'Ea' stage: eggs visible on host ovules with no obvious changes in the host-plant tissues; 'Eb' stage: sponge-like tissue developing around eggs, eggs still visible; 'La' stage: galls evident and the neonate larvae as well, inner wall of the gall moist and soft; 'Lb' stage: galls mature and the larvae obvious, inner wall of gall firm and no more moist; 'P' stage: larvae have turned into pupae; and 'PA' stage: both pupae and adults occur at the same time, one individual in one compartment, some adult may have chew a hole leaving the fruit

The life cycle of *A. miltoni* and developmental stage of *S. samarangense* were shown in Fig. 5. The Ea stage of *A. miltoni* occurs during petal fall stage of *S. samarangense*. During 'rapid stage', 21% and 38% *A. miltoni* develop to Eb and La stage. During young fruit stage, 82 *A. miltoni* (56.6%) were in La stage. Then, 46 individuals (46.9%) get into Lb stage and 33 individuals (33.7%) in P stage when *S. samarangense* reached the mid fruit stage. In mature stage of *S. samarangense*, 80% individual of *A. miltoni* grow to P stage and 20% have emerged. The *A. miltoni* adult stayed inside gall compartment for several days before exit the fruit (see next section). Overall, it takes about 35

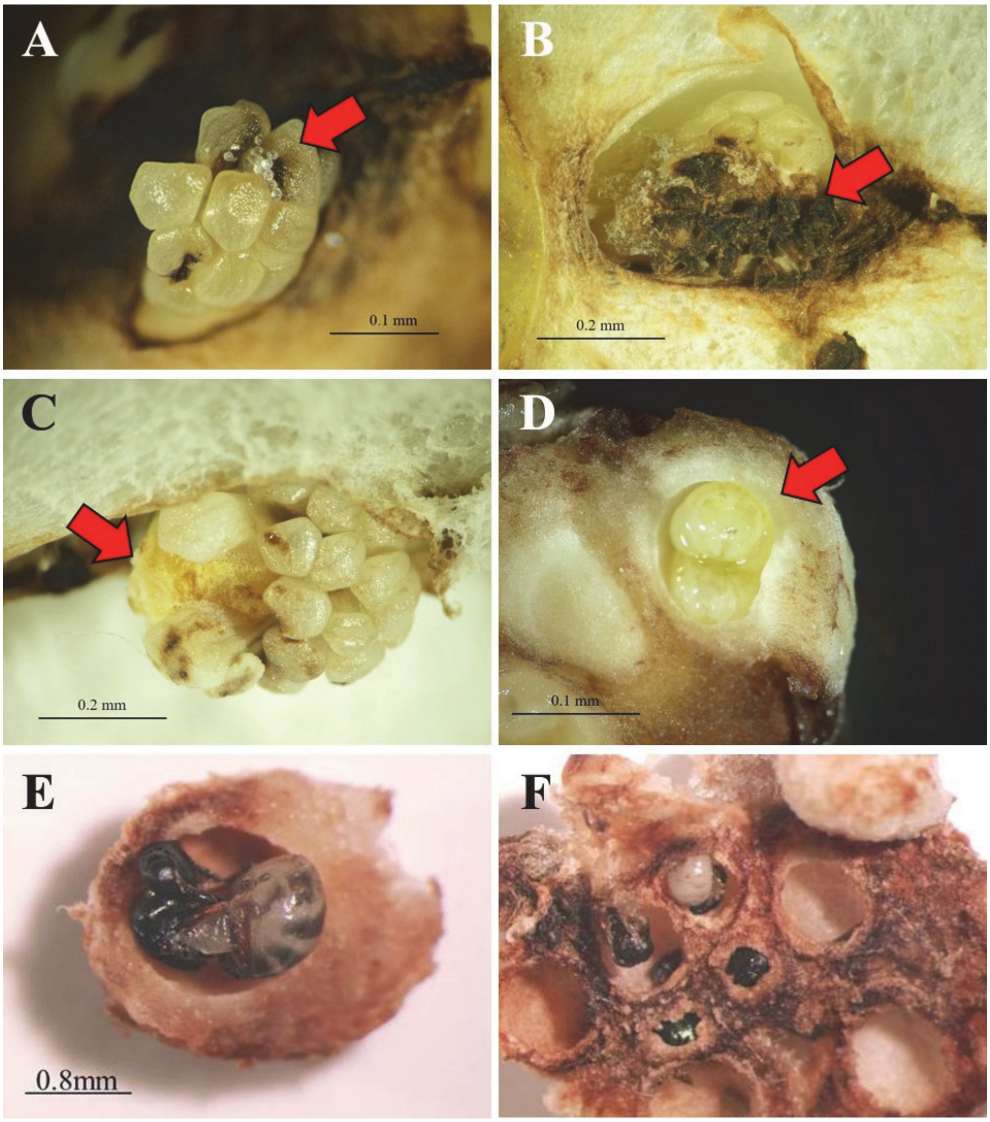

Fig. 4. Developmental stage of gall of *Anselmella miltoni*. A, Ea-eggs visible on ovules with no obvious changes in the host-plant tissues. B, Eb-sponge-like tissue developing around eggs, eggs still visible. C, La-galls evident and the neonate larvae as well, inner wall of the gall moist and soft. D, Lb-galls mature and the larvae obvious, inner wall of gall firm and no more moist. E, P-larvae have turned into pupae. F, PA-both pupae and adults occur at the same time; adults within gall compartments.

days from egg stage to adult.

Adult emergence span

Population of *A. miltoni* from galls did not emerge simultaneously. The time for all adults emerging from a gall may last from 3 to 7 days. The emergent period was spread over seven days and more males than females emerged in the first day (Fig. 6). The number of compartments positively correlated with adult emergence (y = 0.09153x + 0.2160, R^2 = 0.8178, P < 0.001) (Fig. 7). More the number of compartments in a galled fruit, the more number of days necessary for *A. miltoni* populations to emerge. The sex ratio (male/female) is 1: 0.66. Some galls included more of adult females than males.

Adult longevity of *A. miltoni*

The longevity of adult *A. miltoni* revealed no significant differences among 10% honey solution, water, and no food treatments (Kruskal-Wallis test, X^2 = 0. 0019, P= 0.99, Fig. 8), and also no significant differences between genders (Mann-Whitney test, U = 213, P = 0.8558). Longevity of adult *A. miltoni* reared by 10% honey solution was 2.21 ± 0.86 d (n = 56). Water treatment was 2.62 ± 1.67 d (n = 36) while

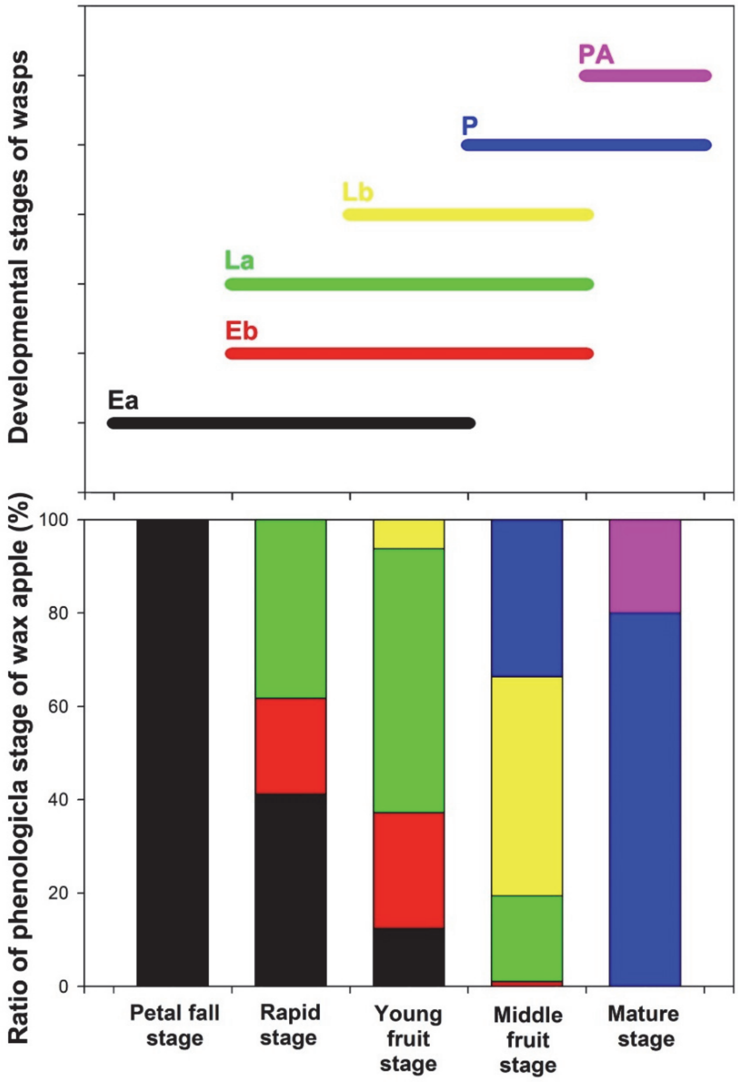

Fig. 5. The developmental stage of gall and *Anselmella miltoni* related to the phenological stage of *Syzygium samarangense*. Colors indicate different developmental stage of *A. miltoni*. Ea, eggs visible on ovules with no obvious changes in the host-plant tissues. Eb, sponge-like tissue developing around eggs, eggs still visible. La, galls evident and the neonate larvae as well, inner wall of the gall moist and soft. Lb, galls mature and the larvae obvious, inner wall of gall firm and no more moist. P, larvae have turned into pupae. PA, both pupae and adults occur at the same time; adults within gall compartments.

no food treatment was 2.36 ± 0.96 d (n = 34). Adult longevity of male and female were 2.7 ± 1.3 d (n = 73) and 2.3 ± 1.3 d (n = 53), respectively (Fig. 8), while the average of total individuals was 2.36 ± 1.16 d (n = 126).

Discussion

Anselmella miltoni is a newly emerging invasive living on the fruits of *S. samarangense* in Taiwan. Although this species does not induce either an immature fruit drop or decay in pulp in *S. samarangense* as would populations of *Bactrocera dorsalis* (Diptera: Tephritidae) do, the Taiwanese horticultural industry sees *A. miltoni* as an issue of deep concern. Traditionally farmers growing *S. samarangense* wrap the branches bearing young fruits with a paper bag to avoid infestations by *B. dorsalis* and other pestiferous arthropods (Lin *et al.*, 2004). However, this practice does not work for *A. miltoni* because *A. miltoni* lay eggs much earlier, during anthesis and petal fall stages in flowers, much before bagging done by farmers.

Agriculturally relevant gall-inducing insects are often neglected because of two reasons. First is that the host usually does not display obvious symptoms during early stages. Second, gall-inducing insects live most of life endophytically, i.e., remain embedded, concealed within host-plant tissues, which makes it all the more difficult to manage using contact insecticides (Xu *et al*., 2009; Csóka *et al*., 2017). These reasons also caused barriers in the present study. Attack by populations of *A. miltoni* did not result in apparent damage to fruits. Most likely *A. miltoni* will not emerge as a key pest of *S. samarangense* compared with *B. dorsalis*. However, the export of *S. samarangense* fruits from Taiwan to countries such as People's Republic of China are declared pest-free areas which made it a matter of deep concern. The *A. miltoni* is listed as a quarantine pest by the Chinese Quarantine Authority, and therefore, any inspected fruits including the infestation at the harbor will result in the destruction of the commodity and the estimated overall damage would cost USD 8.6 million annually to Taiwan (COA, 2017). An effective management, therefore, is necessary to prevent this economic loss. Because of the embedded nature of *A. miltoni*, an integrated pest management would be the practice of choice, since the biology and bionomics of *A. miltoni* supplied in this paper would be useful.

As adults of *A. miltoni* emergence from a mature fruit of *S. samarangense* would take ca. 7 days (Fig. 6), some of the early emerging individuals can flag early signs of insect emergence to farmers. If the paper bags tied around young fruit-bearing branches are endowed with transparent windows, periodical inspection for the *A. miltoni* could be made easily. For reducing the export loss, we propose that farmers should inspect early emerging population using the transparent window of paper bags during cultivation. In spite of *A. miltoni* population, no obvious symptom may occur on the infested fruits of *S. samarangense*. Once an individual adult found inside a certain bag, all fruits in that paper bag should be excluded from exporting and inspection on other bags should be done more carefully.

Gall-inducing insects require particular host stage to induce galls. Therefore, they always synchronize with specific phenological stages in their host plants (Yang and Tung, 1998; Yukawa, 2000). Adults of *A. miltoni* do not emerge

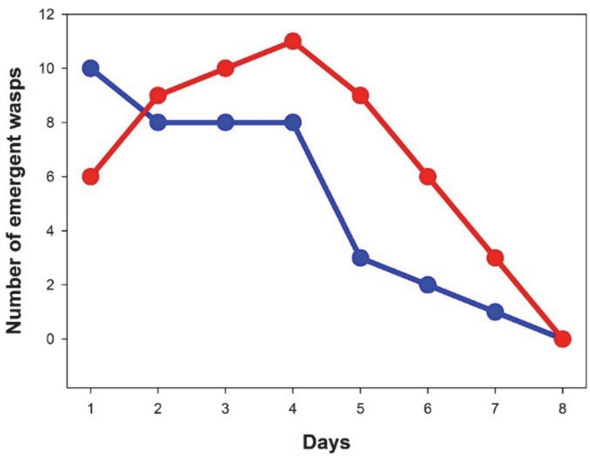

Fig. 6. The emergence frequency of two genders of *Anselmella miltoni* from 15 galls. Blue: male; red: female.

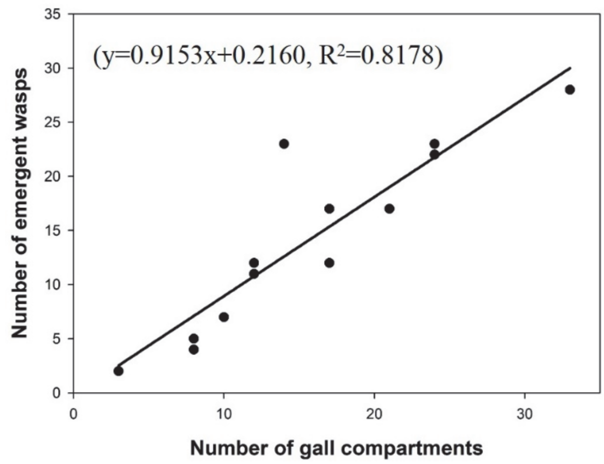

Fig. 7. The relationship of number of gall compartments and emergent adults of *Anselmella miltoni*.

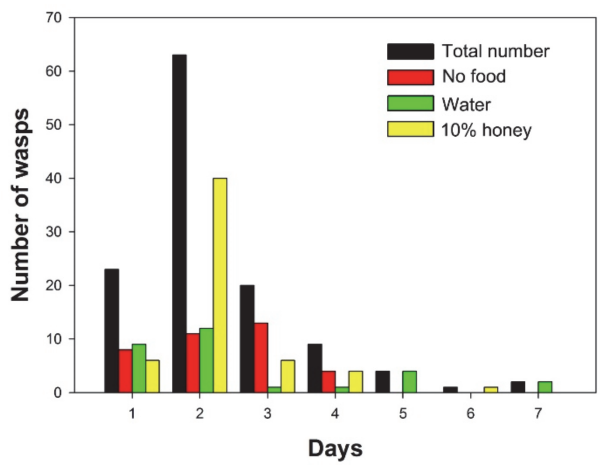

Fig. 8. Adult longevity of *Anselmella miltoni* of different rearing treatments. Black: total number in three treatments; red: no food; green: water; yellow: 10 % honey.

simultaneously. Flowering time in *S. samarangense* lasts for 7~10 days in January-May each year. Reproductive period of *S. samarangense* extends between 90 and 160 days, the remaining is vegetative period. Adults of *A. miltoni* live only for 2~3 days. However, the unsynchronized emergence time of adults from a gall, usually last for 7 days, may extend the appearance of the population in the field. *Syzygium jambos* (L.) Alston (Myrtaceae) is also reported as host of *A. miltoni* in Taiwan forming seed-like galls similar to those known in *S. samarangense* (Yang and Lin, 2016). Although both of the hosts bloom in March to April, however, the time span of flowering is not quite the same and varies with different cultivation areas. How this short-lived insect completes its life cycle needs further research.

The adult emerging trait of *A. milton*i also emphasizes the importance of field sanitation. According to this study, one seed gall includes 20.8 ± 11.5 compartments. The maximum emerging period for *A. miltoni* could up to seven days and the more gall compartments the longer emerging period. No matter whether the emerging holes appear, the dropped infested fruits must be removed and burned, since they could be source of new infestations.

There is no previous record of *A. miltoni* of Taiwan until Huang *et al*. (2008) reported it from the fruits of *S. samarangense* imported at the Fu-Chien Harbor (China) during routine quarantine inspection. However, examination of the specimens will find that some head characters do not fit the original description of *A. miltoni* Girault 1926 and the species status appears to be dubious. As the known hosts of this species are exotic to Taiwan, when and where this species of *Anselmella* wasp get into Taiwan and how it becomes an invasive pest remained to be answered.

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於蓮霧（桃金孃科）果實內部造癭的 ***Anselmella*** 釉小蜂（膜翅目：釉小蜂科）生物學

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摘　要

造癭昆蟲生活於植物組織形成的癭內，隱匿式的習性不易被偵測，近年來不乏成為國際入侵性害蟲的例子。危害蓮霧的米爾頓釉小蜂在果實空腔內形成類種子癭，近年於台灣發現，嚴重影響台灣蓮霧的外銷收益，然而關於此物種的生物學資料仍相當缺乏，影響防治效率。因此本研究以嘉義中埔之蓮霧園作為樣區，針對米爾頓釉小蜂進行基礎生物學研究，包含田間發生、蓮霧物候及蟲癭發育之同步性、羽化形式以及成蟲壽命，結果顯示小蜂在田間主要於蓮霧盛花期前來產卵，小蜂卵期與蓮霧胚仔期同步，幼期發育則歷經合臍期至中果期且主要於熟果期化蛹並羽化為成蟲，單一果實蟲癭具有 20.8 ± 11.5 個癭室，一蟲一癭室，成蟲不取食，且壽命為 2.36 ± 1.16 天，本研究也依據其生物學特性提供相關的防治建議。

關鍵詞：蟲癭、入侵物種、胚珠、物候學