台灣昆蟲 23: 49-57 (2003)
Formosan Entomol. 23: 49-57 (2003)

Host-Plant Utilization of Two Luna Moths (*Actias* spp.) on *Liquidambar formosana* and *Cinnamomum camphora*

Chih-Wei Wang, Li Lin, Jen-Wei Liu, and Shaw-Yhi Hwang*
Department of Entomology, National Chung Hsing University, 250 Kuo-Kuang Road, Taichung, Taiwan 402, R.O.C.

ABSTRACT

The effects of host plants on larval performance were investigated in larvae of the luna moths, *Actias selene ningpoana* Felder & Felder and *Actias heterogyna subaurea* Kishida. Neonate larvae were fed leaves of *Liquidambar formosana* Hance and *Cinnamomum camphora* Presl. Larval survival, weight, duration, food processing efficiencies, pupal weight, and pupal duration were monitored as indices of food quality. To evaluate the effects of foliage quality on insect feeding performance, leaf tissues were collected from test plants and assayed for water and nitrogen contents. Results showed substantial variation in insect performance between these two host plant species. Larvae of both luna species survived and grew well on foliage of *L. formosana*; but all larvae died when fed foliage of *C. camphora*. Chemical analysis, however, revealed that foliar water and nitrogen contents were similar between these two host plant species. Results of this study suggest that host plant utilization of luna moths is more specialized at the individual or population level than at the species level and that foliar allelochemicals may play an important role in such specialization.

Key words: luna moths, *Liquidambar formosana*, *Cinnamomum camphora,* plant-insect interaction.

Introduction

Luna moths, *Actias* spp., belonging to the family Saturniidae, are well represented in the Oriental region (Barlow, 1982). In Taiwan, three species of luna moth have been identified, *A. selene ningpoana*, *A. heterogyna subaurea*, and *A. neidhoeferi*. All these species are widely distributed in forests (800 to 2000 m in elevation) of this island (Wang, 1994). According to Wang (1994), among these three, *A. selene ningpoana* is the largest, and inhabits most of the Oriental region. *A. heterogyna subaurea* is intermediate in size and is only found in southern China and Taiwan. The third species , *A. neidhoeferi*, is the smallest and exists only in Taiwan. As forest defoliators, luna moths are generally considered moderately polyphagous, feeding on trees from at least five plant

*Correspondence address
e-mail:oleander@dragon.nchu.edu.tw

families including the Betulaceae, Hamamelidaceae, Lauraceae, Lythraceae, and Rosaceae (Heppner *et al*., 1988; Wang, 1994). Relative little is known about the relationship between luna moths and their host plants, especially the suitability of various tree species as larval food plants.

The research reported herein was conducted to assess the influence of documented host plants on the performance (feeding and growth) of *A. selene ningpoana* and *A. heterogyna subaurea* larvae. A second objective of this research was to investigate the host plant utilization efficiency of these two luna moths. These studies provided the opportunity to discover differential responses of these luna moths to various host plants.

Materials and Methods

Insects

Luna moth eggs were collected from female moths captured at An-Ma Mountain (24.16°0.7'N, 121.0°49.8'E) and the Qing-Jing Farm (24.3°50.4'N, 121.10° 11.8'E) for *A. selene ningpoana* and *A. heterogyna subaurea*, respectively. Female moths were enclosed in brown paper bags (25 x 13 x 7 cm), whereupon approximately 80 and 90 eggs were laid by *A. selene ningpoana* and *A. heterogyna subaurea*, respectively. Newly hatched, first instar larvae were used for the insect bioassays.

Plants

Two plant species were used in this study, *L. formosana* and *C. camphora*, which are abundant and easily found on the campus of National Chung Hsing University, Taichung, west-central Taiwan. Both plant species have previously been recognized as host plants of *A. selene ningpoana* and *A. heterogyna subaurea* (Wang, 1994). All leaves used in this study were of similar phenological age. New and fully expanded leaves were randomly collected from trees located throughout the campus during the bioassay.

Feeding Trials

Two types of insect bioassays (long-term feeding trials and short-term feeding trials) were conducted to evaluate the feeding performance of *A. selene ningpoana* and *A. heterogyna subaurea* on foliage of different plant species. For experimental bioassays, insects were reared in a Percival growth chamber (14 : 10-h light : dark photoperiod) at a constant 25°C (day) and 20°C (night).

Long-term feeding trails were conducted to assess the effects of foliage quality on insect development and growth over the entire larval feeding and pupal stages. Feeding trails began on 6 July 2001, when eggs hatched. One newly hatched larva of each insect species was weighed and put into a plastic rearing cup (250 ml) with leaves from one of the test plant species. Eight replicates were conducted for each plant species for *A. selene ningpoana*, and fifteen replicates were conducted for *A. heterogyna subaurea*. All larvae were reared separately in cups until pupation. Leaf materials were changed every day to ensure freshness. To monitor the growth rates of insects over the entire larval period, larval weights were taken daily. Upon pupation, pupal weights were recorded, and larval durations were calculated as the elapsed time from egg hatching to pupation. Each pupa was weighed 3 days after pupation and enclosed in a rearing cup until adult emergence. Pupal duration was calculated as the time between pupation and adult emergence. Mean and standard errors were calculated for larval weights, pupal weights, larval durations, and pupal durations for insects fed on foliage of different plant species. Moreover, additional leaf materials from the test

plants were collected during the bioassay to measure leaf water and nitrogen content.

Short-term feeding trials were conducted to evaluate the foliage quality effects of *L. formosana* and *C. camphora* on growth rates, food consumption rates, and food processing efficiencies of fourth instar larvae (of *A. selene ningpoana* and *A. heterogyna subaurea*). Fifty newly hatched larvae from each insect species were grown on *L. formosana* foliage until molting to fourth instars. Each assay consisted of a newly molted and weighed larva placed into a rearing cup (250 ml) containing a leaf from either one of the test plant species (n = 15 replicates per insect species per plant species). Leaves were changed every 1-2 days or as necessary during the bioassay. Upon molting to fifth instars, larvae were frozen, oven dried at 50°C for 1 week, and reweighed. Nutritional indices were calculated to evaluate insect growth, consumption, and food utilization efficiency (Haynes and Millar, 1998). These indices were calculated from standard formulas for approximate digestibility (AD), efficiency of conversion of digested food (ECD), and efficiency of conversion of ingested food (ECI) as described by Waldbauer (1968) and Haynes and Millar (1998). Initial rather than average weights of larvae were used to calculate the relative growth rate (RGR) and relative consumption rate (RCR) (Farrar *et al.*, 1989). Initial dry weights of test insects were estimated based on a wet-to-dry weight conversion factor determined from 10 newly molted fourth instars for each insect species. Similarly, initial dry weights of leaves fed to insects were estimated by a dry weight conversion using foliage collected from each plant species at the time of the bioassay. Means and standard errors were calculated for the duration, relative growth rate (RGR), relative consumption rate (RCR), total consumption (TC), approximate digestibility (AD), efficiency of conversion of digested food (ECD), and efficiency of conversion of ingested food (ECI) for insects fed on foliage from different plant species. Statistical analysis was used to compare the performance of the two insect species within each of the plant species, and the insect performance between the two plant species. As with the long-term feeding study, additional leaf materials from test plants were also collected during the bioassay to measure leaf water and nitrogen contents.

Foliar Chemistry of Plant Materials

Concurrent with the insect feeding trials, additional foliage (about 10 leaves) were collected from plants used in bioassays (8 plants/species), flash frozen in liquid nitrogen, freeze-dried, ground, and stored in a freezer. Water content and total nitrogen were quantified for each foliar sample. Differences between wet and dry weights of leaf samples were used to determine water contents. Foliar nitrogen contents were determined by standard micro-Kjeldahl assays. Leaf samples were first digested in acid (Parkinson and Allen, 1975), and nitrogen contents were quantified by a micro-Nesslerization technique (Lang, 1958). Glycine *p*-toluenesulfonate (5.665% N) was used as the standard. Means and standard errors for foliar water and nitrogen concentrations for each of the plant species were calculated.

Results

Long-term Feeding Trials

Within the first few days of the feeding trial, almost all larvae of both *A. selene ningpoana* and *A. heterogyna subaurea* fed on *C. camphora* died, therefore, no data were reported for insects fed on this plant species. Mean larval weights, larval duration, pupal weights, and pupal duration were only

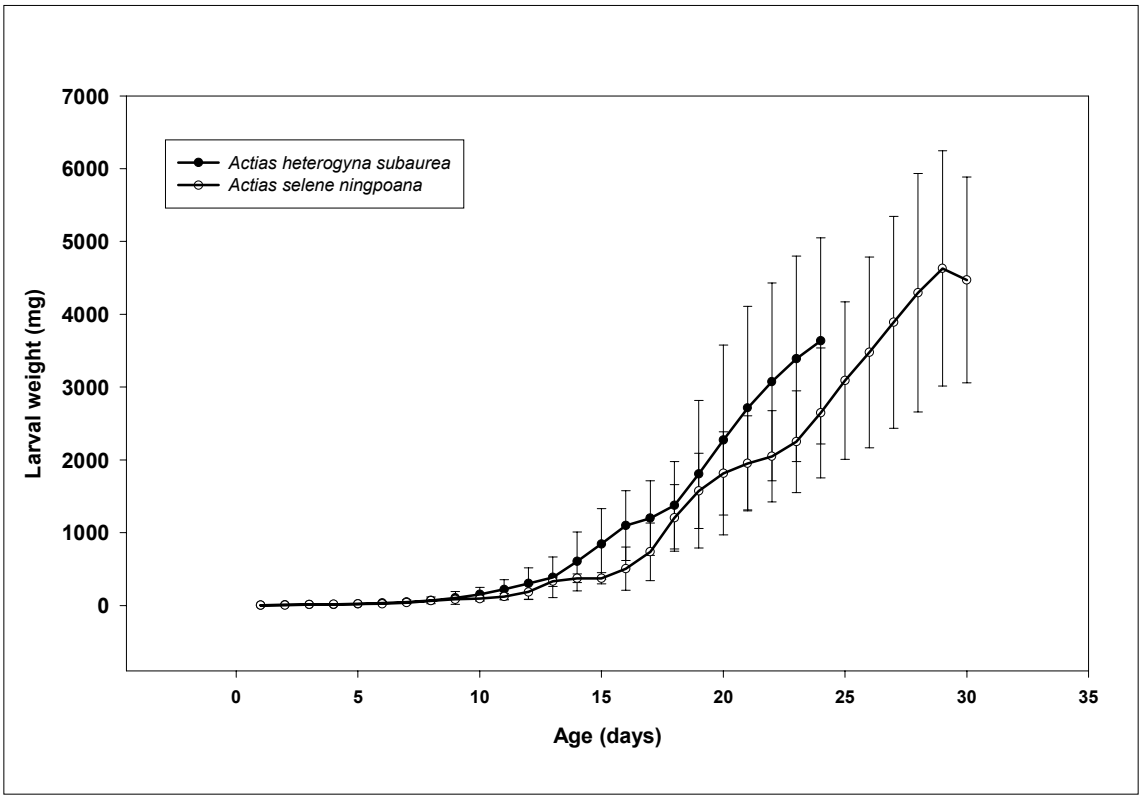

Fig. 1. Growth of *A. selene ningpoana* and *A. heterogyna subaurea* on foliage of *L. formosana*. Each point represents the mean value (± SE) for weight of insects on foliage of *L. formosana* (8 and 15 replicates for *A. selene ningpoana* and *A. heterogyna subaurea*, respectively).

calculated for insects fed on leaves of *L. formosana*. Student *t*-tests (PROC TTEST; SAS Institute, 1988) were conducted to compare larval duration, pupal weights, and pupal duration of these two insect species. Larval weights varied markedly between the two insect species reared on *L. formosana* foliage, with the range of variation being more than 1.5-fold from 10 days of age onward (Fig. 1). Generally, larvae of both insect species grew well on foliage of *L. formosana*, with mortality rates of 25% and 13% for *A. selene ningpoana* and *A. heterogyna subaurea*, respectively. Larval duration varied significantly between these two insect species by a factor of 1.3 (Table 1). Pupal weights of insect species reared on *L. formosana* varied by a factor of 1.4 and the variation in pupal duration was about 1.3-fold (Table 1).

Short-term Feeding Trials

Similar to the long-term feeding trial, all larvae of both insect species fed on *C. camphora* died within the first few days of the bioassays, and therefore, no data were reported for insects fed on this plant species. Larvae of both *A. selene ningpoana* and *A. heterogyna subaurea* performed well on foliage of *L. formosana* (Table 2). Mean performance parameters (growth rates, consumption rates, food processing efficiencies, etc.) were only calculated for insects fed on foliage of *L. formosana* (Table 2). Student *t*-tests (PROC TTEST) were used to compare feeding performance parameters between

Table 1. Performance of two luna moth species on *Liquidambar formosana* foliage (mean ± SE)

	Survival (%)	Larval duration (Days)	Pupal weight (mg)	Pupal duration (Days)
Actias heterogyna subaurea	86.7	31.17 ± 0.78	2125.7 ± 114.9	12.77 ± 0.30
Actias selene ningpoana	75	39.00 ± 1.71	2922.2 ± 50.7	16.33 ± 0.95
t		-4.8396	-6.3389	-4.61
df		16	10	17
*p**		0.0002	0.0001	0.0002

*Significant difference (*t*-test, $p < 0.005$)

Table 2. Fourth instar feeding performance of two luna moth species on *Liquidambar formosana* foliage (mean ± SE)

Species	Survival (%)	Duration (Days)	RGR (mg/mg/days)	RCR (mg/mg/days)	AD (%)	ECD (%)	ECI (%)	TC (mg)
Actias heterogyna subaurea	100	5.66 ± 0.21	0.45 ± 0.02	6.45 ± 0.20	30.35 ± 1.36	23.62 ± 1.35	6.95 ± 0.21	1363.0 ± 72.8
Actias selene ningpoana	53.3	8.94 ± 1.48	0.46 ± 0.06	6.31 ± 0.77	15.96 ± 1.89	51.63 ± 7.57	7.35 ± 0.32	2044.7 ± 104.4
t		-2.1946	-0.1463	0.1811	6.2224	-3.6437	-1.0974	-5.4388
df		7	8	7	21	7	21	21
*p**		0.069	0.8875	0.8608	< 0.0001	0.0075	0.2849	< 0.0001

RGR, relative growth rate; RCR, relative consumption rate; AD, approximate digestibility; ECD, efficiency of conversion of digested food; ECI, efficiency of conversion of ingested food; TC, total consumption.
* Significant difference (*t*-test, $p < 0.005$)

these two insect species. Generally, performance on *L. formosana* foliage was similar for these two insect species (Table 2). The relative growth rate (RGR), relative consumption rate (RCR), and efficiency of conversion of ingested food (ECI) were similar for the two insect species (Table 2). However, larvae of *A. selene ningpoana* had a longer duration for each instar than larvae of *A. heterogyna subaurea* (1.4-fold) (Table 2). Larvae of *A. selene ningpoana* had lower approximate digestibility (AD) than larvae of *A. heterogyna subaurea* (1.9-fold). In contrast, the efficiency of conversion of digested food (ECD) and total consumption (TC) were higher for larvae of *A. selene ningpoana* than for larvae of *A. heterogyna subaurea* (as much as 54% and 33% for ECD and TC, respectively).

Foliar Chemistry of Plant Materials

Water contents varied significantly between *L. formosana* and *C. camphora* (Table 3). However, levels of water varied by only 1.1-fold between the two plant species. In addition, nitrogen concentrations were similar between these two plant species. Foliage of *C. camphora* had slightly higher water content (58% versus 53%) than foliage of *L. formosana*, and also higher nitrogen content (2.59% versus 2.52% dry weight).

Discussion

This study clearly demonstrates that the performance of *A. selene ningpoana* and *A. heterogyna subaurea* varied significantly between two documented host plants, *L. formosana* and *C.*

Table 3. Concentrations of nitrogen and water in the two host-plant species (mean ± SE)

	Nitrogen (%)	Water (%)
Cinnamomum camphora	2.59 ± 0.48	57.72 ± 0.01
Liquidambar formosana	2.52 ± 0.79	52.52 ± 0.01
t	0.2821	3.1799
df	30	14
*p**	0.7798	0.0067

* Significant difference (*t*-test, $p < 0.005$)

camphora. Both insect species survived and grew well on foliage of *L. formosana*, but performed extremely poorly on foliage of *C. camphora*.

The literature on butterfly and moth food plants has documented that both *A. selene ningpoana* and *A. heterogyna subaurea* feed on plants from several plant families (Heppner *et al.*, 1988; Wang, 1994). In Taiwan, these two luna moths are easily found in forest areas, and many of the known food plants, such as *L. formosana* and *C. camphora*, are also very common in their geographic range. However, little work has experimentally evaluated the relationship between these luna moths and their host plants. This study is the first documented experiment in Taiwan to assess the host-plant use of these two luna moths. The results of this study showed that the performance of these two insect species on test plants differed significantly from that predicted on the basis of published host plant records (Heppner *et al.*, 1988; Wang, 1994). Survivorship values of 0% on *C. camphora* for both insect species were particularly surprising. Larvae of both species performed quite well, and fairly similarly, on *L. formosana*. Lindroth (1989) also found a similar result for *Actias luna* which performed differently on several of its general host plants, and it was suggested that these conflicting results may be explained by the low genetic variability in the laboratory population. Likewise, a variety of plant families may be available throughout the geographic range of both *A. selene ningpoana* and *A. heterogyna subaurea*, but some localized populations or individuals may specialize on particular plant families. Other research has also shown similar results for other species of saturniids (Scriber and Feeny, 1979). In summary, results of our study and others (Scriber and Feeny, 1979; Lindroth, 1989) tend to support the idea of Fox and Morrow (1981) that insects displaying generalized diets at the species level are likely to have specialized diets at the population level.

Although our results showed that *L. formosana* is an ideal food plant for both *A. selene ningpoana* and *A. heterogyna subaurea*, some degree of variation in larval performance could still be found. Survival rates varied markedly between these two insect species fed on *L. formosana*. We found some *A. selene ningpoana* larvae infested with fungal diseases during the feeding trials, and those larvae died because of the disease. Although growth rates and consumption rates were similar for these two insect species, *A. selene ningpoana* grew bigger than *A. heterogyna subaurea*. The reason is probably because *A. selene ningpoana* had a higher efficiency of conversion of digested food (ECD) and total consumption (TC) than did *A. heterogyna subaurea*. In addition, *A. selene ningpoana* also fed for a longer time than did *A. heterogyna subaurea*. This is consistent with field observation results that *A. selene ningpoana* is usually larger

than *A. heterogyna subaurea*.

In contrast to the results from *L. formosana*, almost all larvae died after feeding on foliage of *C. camphora*. Neonate larvae ate small amount of leaves, stopped feeding, and died within 1~2 days. The cause of death was not clear, but may have been related to the quality of host plants. Growth and reproduction of insects are usually very closely related to foliar water and nitrogen contents (Scriber and Feeny, 1979; Scriber, 1984; Schoonhoven *et al*., 1998). However, in this study foliar water and nitrogen contents were very similar between *L. formosana* and *C. camphora* (Table 3), and this small difference is not likely to cause such dramatic variation in insect performance. In addition to foliar nutritional content, non-nutritional factors (allelochemicals) can also affect host plant suitability to phytophagous insects (Fraenkel, 1959; Schoonhoven *et al*., 1998). Allelochemicals may indirectly affect insect performance by blocking the bioavailability of nutrients due to reductions in food processing efficiencies (Broadway and Duffy, 1986). In addition, plant secondary compounds may affect insect performance directly because of their toxicity (Schoonhoven *et al*., 1998). Several secondary chemicals such as hydrolyzable tannins and terpenoids have been identified in *L. formosana* (Hatano *et al*., 1986; Okuda *et al.,* 1987), but these compounds do not seem to have significant negative effects on luna moths. Although many essential oils have been identified in *C. camphora* (Mishra *et al*., 1991; Dung *et al*., 1993; Pelissier *et al*., 1995; Rajapakse and Emden, 1997; Pino and Fuentes, 1998), none has been reported to have negative effects on luna moths. However, one chemical, cinnamomin, was found to be toxic to the bollworm (*Helicoverpa armigera*) and mosquito (*Culex pipines pallens*) (Zhou *et al*., 2000). This chemical and other allelochemicals in *C. camphora* may play an important role in its defense against insect herbivores.

In summary, we found that both *A. selene ningpoana* and *A. heterogyna subaurea* had significantly different performances on two documented host plants, *L. formosana* and *C. camphora*. Foliar water and nitrogen contents are not likely explanations for the variation in performances of luna moths on *L. formosana* and *C. camphora*. Allelochemicals may play an important role in affecting insect performance; however, no experimental data has yet verified this effect. Additional and more-comprehensive studies are needed to understand the actual host range and the role of phytochemistry on the performance of these two insect species.

Acknowledgments

We thank Dr. C. Papa for comments on the manuscript. Two anonymous reviewers provided helpful comments. This research was supported by NSC-89-2316-B-005-008 to SYH.

References

Barlow, H. S. 1982. An introduction to the moths of South East Asia. The Malayan Nature Society, Faringdon, U.K. 305 pp.

Broadway, R. M., and S. S. Duffey. 1986. Plant proteinase inhibitors; mechanism of action and effect on the growth and digestive physiology of larvae *Heliothis zea* and *Spodoptera exigua*. J. Insect Physiol. 32: 827-833.

Dung, N. X., P. V. Khien, H. T. Chien, and P. A. Leclercq. 1993. The essential oil of *Cinnamomum camphora* (L.) Sieb. var. *linaloolifera* from Vietnam. J. Essent. Oil Res. 5: 451-453.

Farrar, R. R. Jr., J. D. Barbour, and G. G. Kennedy. 1989. Quantifying food consumption and growth in insects. Ann. Entomol. Soc. Am. 82: 593-598.

Fox, L. R., and P. A. Morrow. 1981. Specialization: species property or local phenomenon? Science 211: 887-893.

Fraenkel, G. S. 1959. The raison d'etre of secondary plant substances. Science 129: 1466-1470.

Hatano, T., R. Kira, M. Yoshizaki, and T. Okuda. 1986. Seasonal changes in the tannins of *Liquidambar formosana* reflecting their biogenesis. Phytochemistry 25: 2787-2789.

Haynes, K. F., and J. G. Millar. 1998. Methods in chemical ecology. Vol 2. Chapman & Hall, New York. 406 pp.

Heppner, J. B., H. Y. Wang, and Y. C. Chang. 1988. Larval morphology of Taiwan Saturniidae (Lepidoptera): *Actias selene ningpoana* Felder. J. Taiwan Mus. 41: 107-114.

Lang, C. A. 1958. Simple microdetermination of Kjeldahl nitrogen in biological materials. Anal. Chem. 30: 1692-1694.

Lindroth, R. L. 1989. Chemical ecology of the luna moth: effects of host plant on detoxification enzyme activity. J. Chem. Ecol. 15: 2019-2029.

Mishra, A. K., S. K. Dwivedi, N. Kishore, and N. K. Dubey. 1991. Fungistatic properties of essential oil of *Cinnamomum camphora*. Int. J. Pharm. 29: 259-262.

Okuda, T., T. Hatano, T. Kameda, M. Yoshizaki, and T. Shingu. 1987. Liquidambin, an ellagitannin from *Liquidambar formosana*. Phytochemistry 26: 2053-2055.

Parkinson, J. A., and S. E. Allen. 1975. A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. Comm. Soil Sci. Plant Anal. 6: 1-11.

Pelissier, Y., C. Marion, S. Prunac, and J. M. Bessiere. 1995. Volatile components of leaves, stems and bark of *Cinnamomum camphora* Nees et Ebermaier. J. Essent. Oil Res. 7: 313-315.

Pino, J. A., and V. Fuentes. 1998. Leaf oil of *Cinnamomum camphora* (L.) J. Presl. J. Essent. Oil Res. 10: 531-532.

Rajapakse, R., and H. F. Van Emden. 1997. Potential of four vegetable oils and ten botanical powders for reducing infestation of cowpeas by *Callosobruchus maculatus*, *C. chinese* and *C. rhodesianus*. J. Stored Prod. Res. 33: 59-68.

SAS Institute. 1988. SAS user's guide: statistics. SAS Institute, Cary, NC.

Schoonhoven, L. M., T. Jermy, and J. J. A. Van Loon. 1998. Insect-plant biology. Chapman & Hall, New York. 409 pp.

Scriber, J. M. 1984. Host-plant suitability. pp. 159-202. *In*: W. J. Bell, and R. T. Carde, eds. Chemical Ecology of Insects. Chapman & Hall, New York.

Scriber, J. M., and P. Feeny. 1979. Growth of herbivorous caterpillars in relation to feeding specialization and to the growth form of their food plants. Ecology 60: 829-850.

Waldbauer, G. P. 1968. The consumption and utilization of food by insects. Adv. Insect Physiol. 5: 229-288.

Wang, Y. C. 1994. Illustrations of giant silkmoth and carpenter moths in Taiwan. ShuShing Press, Taipei. 137 pp.

Zhou, X., X. D. Li, J. Z. Yuan, Z. H. Tang, and W. Y. Liu. 2000. Toxicity of cinnamomin-a new type II ribosome-inactivating protein to bollworm and mosquito. Insect Biochem. Mol. Biol. 30: 259-264.

Received Nov. 11, 2002
Accepted Dec. 24, 2002

兩種水青蛾（*Actias* spp.）對寄主植物楓香及樟樹的取食情形

王至偉　林　立　劉人瑋　黃紹毅*　國立中興大學昆蟲學系　台中市國光路 250 號

摘　要

本研究探討在台灣常見的兩種水青蛾與其寄主植物間之關係。本研究目的在探討這兩種水青蛾對楓香及樟樹的取食及利用之情形。在餵食實驗方面，以楓香和樟樹的葉片餵食兩種水青蛾的幼蟲，直到幼蟲化蛹爲止。於期間，觀察及紀錄幼蟲的存活率、體重、幼蟲時間、食物利用效率、蛹重、及蛹期等數據。同時爲了瞭解植物葉片對水青蛾在寄主植物上表現之影響，於餵食分析的同時，也採集葉片以備化學成分分析。餵食結果卻顯示這兩種蛾類完全無法取食樟樹；而楓香對這兩種蛾類而言則是一非常好的食草。總之，本研究發現所用的兩個水青蛾族群僅以楓香爲食，是否所有族群或整個物種均是如此，還需採集及驗證其他族群才可證明。另外，是否有其他更多食草可供其食用，也需進一步的實驗證明。

關鍵詞：長尾水青，楓香，樟樹，昆蟲–植物交互作用