

Leaf Structure of *Zanthoxylum ailanthoides* SIEB. et ZUCC. (Rutales :
Rutaceae) Affecting the Mortality of a Swallowtail Butterfly,
Papilio xuthus L. (Lepidoptera : Papilionidae)

Mamoru WATANABE

*Laboratory of Forest Zoology, Faculty of Agriculture,
Tokyo University, Bunkyo-ku, Tokyo 113, Japan*

(Received March 3, 1981)

Effects of the leaf quality of the host tree, *Zanthoxylum ailanthoides*, on the larval development of a swallowtail butterfly, *Papilio xuthus*, were studied in the laboratory. The leaves collected from trees higher than 1 m (tall trees) were tough and thick while those from short (young) trees were tender and thin. The relation between the specific leaf area (SLA) and the tree height was not linear. Comparative observations of the transverse sections of the leaflets demonstrated that the thickness of a leaflet, the length of a cell and the number of cells in the palisade layer of tall trees were greater than those of short trees. In addition to these facts, the mortality rates of the populations reared on leaves from short trees were lower than those from tall ones, particularly in the 1st and the last instar stages, due to physiological death caused by diarrhoea, starvation or failure of ecdysis. The rate of larval development when reared on leaves from short trees was also greater than that from tall ones. These results supported the field observations that the mortality rates of populations on tall trees were higher than those on short ones due to leaf textures.

INTRODUCTION

It is well known that a certain variation in a leaf quality is a defensive agent against herbivorous insects. FEENY (1970) demonstrated that such defense mechanisms are secondary plant substances and that herbivores respond by evolutionary adjustments to circumvent the plants' defenses. Thus, the preference of an ovipositing insect for a particular host plant is a result of natural selection for behavior that avoids plants on which egg and larval survival is low (e.g. WIKLUND, 1975).

Since the larvae of many butterflies are relatively immobile and unable to move great distances searching for an appropriate food plant, the choice of the proper plant for larval growth and development is often made by females to ensure eggs being located sufficiently close to food. Although DETHIER (1959) mentioned that some butterflies fail in ovipositing directly on the proper plant, several authors have proposed that ovipositing females usually avoid plants on which larval growth and development is poor and search preferentially for plants on which growth and development is good (e.g. RAUSHER, 1978).

In the case of the swallowtail butterfly, *Papilio xuthus*, in a deforested area, the females oviposit by preference on the host tree, *Zanthoxylum ailanthoides*, which is shorter than its surrounding vegetation (WATANABE, 1976, 1979c, 1981). Generally,

the survival rates of *P. xuthus* on the shorter (=younger) host trees are higher than on the taller (=older) ones (WATANABE, 1976, 1981). The characteristic mortality factor of the natural population on the taller host trees was the physiological death of larvae, probably due to unsuitability of the leaves (WATANABE, 1981).

The short host trees have thin, tender leaves with plenty of thorns, whereas the tall ones have thick, tough leaves without thorns. Although the proximate cues used for host plant nutrient suitability are known for some species (e.g. FEENY, 1970), little is known about the differential larval survival associated with changes in the morphology of leaves. As has been reported by RAUSHER (1980), the mature leaves of a host plant of the swallowtail butterfly, *Battus philenor*, were tough and low in nutrients and were not eaten by larvae unless they had been starved.

In this paper, mortality of the swallowtail butterfly larvae due to differences in leaf structure will be reported, and the ecological relationship between the butterfly and its host plant will be discussed.

MATERIALS AND METHODS

Shoot elongation of *Z. ailanthoides* is supplied by the continuous flush which begins mid-April and ends mid-September. When the shoot is elongating, the rate of leaf expansion is very high. New leaves mature rapidly and a large part of the diet of *P. xuthus* larvae is composed of such mature leaves. Therefore, all leaves used for this experiment were those less than one-month old near the terminal buds. The height of the leaves collected largely concurred with that of the tree, because all leaves flush from terminal buds.

Since the morphology of the leaves of *Z. ailanthoides* seems to be an important indicator of their suitability as food, morphological changes in relation to tree height were examined. Leaves were collected randomly from host trees of various heights and weighed after drying at 80°C. Transverse sections prepared from leaflets removed randomly from trees of various heights were examined for texture in relation to tenderness.

Five groups with ten eggs of *P. xuthus* each were reared to pupal stage on leaves from either short or tall trees under four rearing regimes: 27°C-13L11D, 27°C-15L9D, 30°C-13L11D and room temperature in the summer season. The rearing experiment was repeated three times at each regime. In all, the number of eggs used exceeded 1200. In some rearing-experiments, in addition, the leaves from short trees were replaced by leaves from tall trees at an intermediate stage.

The females from which eggs were obtained were collected as young larvae from the vicinity of the experimental field plots in the deforested area of Kiyosumi, Chiba Prefecture, and reared under laboratory conditions. Eggs laid on the same day by different females were intermingled and used for rearing experiments in the summers of 1978 and 1979.

The leaves for larval food were collected from short (less than 1 m in height) and tall (more than 5 m in height) trees in the above mentioned area, where a great number of host trees of various heights were distributed at random. When new leaves were provided to larvae every second day, the remains of old leaves and faeces were removed. The larval instar was recorded every day to determine the 'mean instar' (Itô et al., 1970).

RESULTS

Leaf texture

Zanthoxylum ailanthoides is a deciduous tree which appears along the edge of forests or disturbed stands with more than 10% relative light intensity (WATANABE, 1979b). When the light intensity above a host tree is decreased by surrounding plants, the tree is easily killed. Therefore, each pinnately compound leaf of *Z. ailanthoides* can receive an ample light intensity on its surface.

It is generally known, however, that trees of most species have shade leaves only at their young stage (mainly seedlings). As the tree grows, such shade leaves are gradually transformed into sun leaves which have comparatively different characteristics: e.g. the leaf itself becomes thick, various substances become stuffed into mesophylls, vascular bundles become tough, and so on. Thus, there is a certain altitudinal trend in leaf conditions from shade leaves at understory to sun leaves at overstory. As has been expressed by YODA (1971), such a trend could be expressed in terms of the specific leaf area (SLA) as follows:

$$SLA = u/w,$$

where u is leaf area, and w is dry weight of the leaf. In other words, SLA is an index of the thickness of a leaf. The larger the value, the thinner the leaf is.

The relation between SLA and the height of *Z. ailanthoides* is shown in Fig. 1. An abrupt change in the trend is observed at a height of 0.5 m. Although the relation between SLA and tree height of many species could be fitted to a linear regression (YODA, 1971), the distribution of points was curvilinear in the case of *Z. ailanthoides* (Fig. 1). It can be said that the leaves of most trees taller than 1 m are tough and thick, while those of short (=young) ones are tender and thin.

Although the number of leaves per tree increased with growth (WATANABE, 1979a), there seems to be no relation between leaf area and tree height among tall trees (Table 1). Since the leaf area depends upon the length of a pinnately compound leaf (midrib), it is also independent of tree height. On the other hand, the leaf area is exclusively determined by the number of leaflets and the area of a leaflet. In tall trees the area of a leaflet remains almost the same irrespective of the length

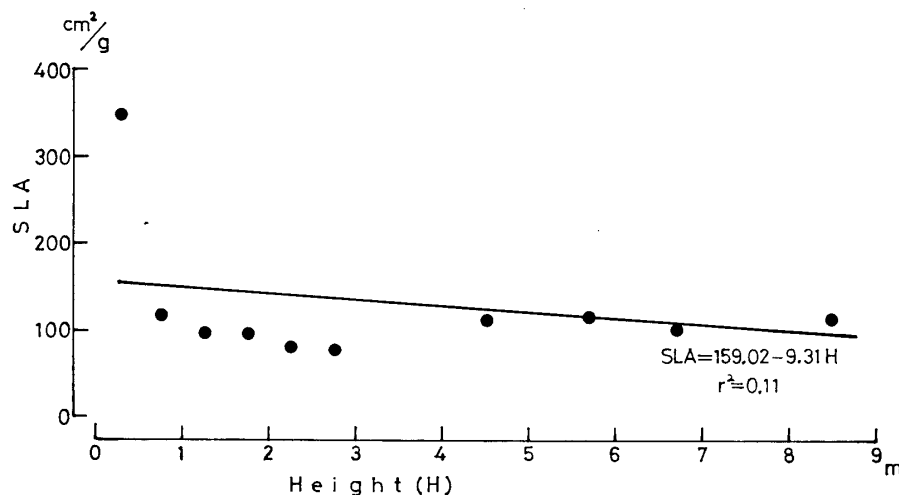


Fig. 1. The relation between SLA and the height of *Z. ailanthoides*.

le 1. Areas of leaflets collected from tall host trees, *Z. ailanthoides*, compared with those from short trees (with 95% confidence limit)

Height (cm)	850	670	570	450	:	50
No. leaves examined	100	24	32	36	:	10
Leaf area (cm ²)	189.1±10.2	315.0±46.3	296.0±47.1	454.1±48.2	:	44.2±10.3
Midrib length (cm)	24.6± 0.9	34.1± 2.8	30.6± 2.7	39.7± 2.3	:	9.8± 0.8
No. leaflets	11.3± 0.7	18.6± 1.4	15.9± 1.1	24.2± 0.8	:	12.7± 1.5
Leaflet area (cm ²)	17.7± 0.9	16.8± 1.9	18.5± 2.2	18.5± 1.5	:	3.6± 0.9

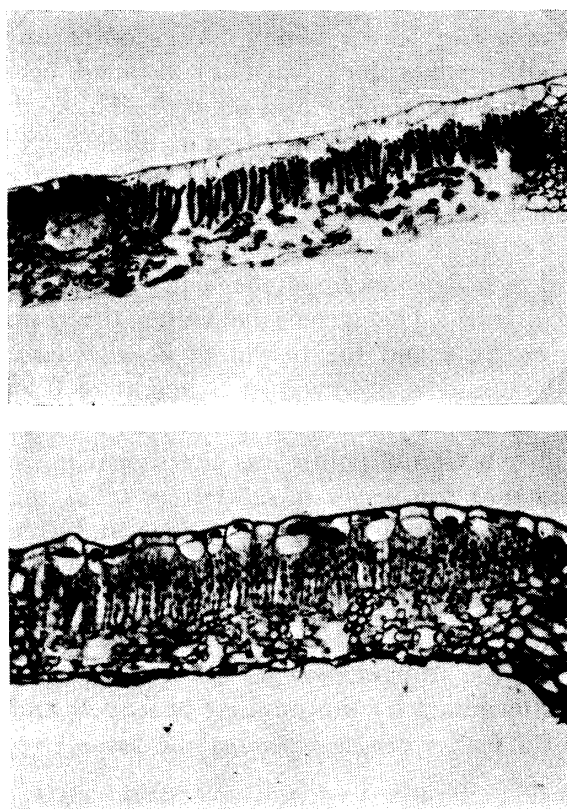


Fig. 2. Photographs of transverse sections of leaflets collected from short (*top*) and tall (*bottom*) trees of *Z. ailanthoides*. See Table 2 for the sizes of cells in palisade layers. ($\times 85$)

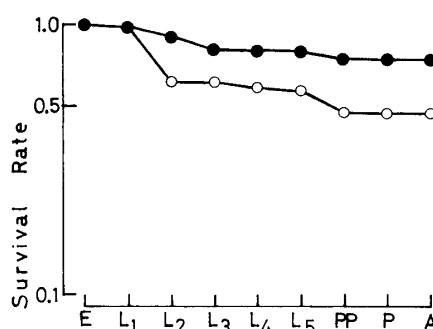
of midrib and tree height. Thus, tall trees increase the area of a leaflet and length of midrib instead of the number of leaflets as compared with short trees (Table 1).

Transverse sections of leaflets from short and tall trees are shown in Fig. 2. Although the epidermis and the spongy layer differed somewhat morphologically, the characteristics of the palisade layer were comparable because this was composed of only single cell stratum. Table 2 shows that the thickness of a leaflet, the length of a cell of the palisade layer and the number of cells in a unit length increase as the tree grows. Such texture of palisade layer may influence the feeding habit of young larvae of the swallowtail butterfly.

Mortalities of the laboratory-reared populations

Table 2. Sizes of cells in the palisade layer of leaflets collected from the host tree, *Z. ailanthoides*, of different heights (with 95% confidence limit)

	Short tree (<1 m)	Tall tree (>5 m)
Thickness of leaflet (μ)	159.6 \pm 4.6	200.6 \pm 6.9
Length of cell (μ)	63.6 \pm 2.7	82.3 \pm 4.6
Width of cell (μ)	9.7 \pm 1.1	9.8 \pm 0.9
No. cells in 1 mm	77.7 \pm 4.0	101.5 \pm 10.2

Fig. 3. Survivorship curves of *P. xuthus* reared on leaves of short host trees (●) and on those of tall trees (○). See Table 3 for the survival rates at respective stages.Table 3. Survival rates of *P. xuthus* larvae at each stage, reared on leaves of short and tall trees (\pm S.E.)

Stage	Short tree (%)	Tall tree (%)	
1st instar	90.74 \pm 6.69	62.16 \pm 9.87	P < 0.05
2nd instar	90.13 \pm 5.23	99.31 \pm 0.69	N.S.
3rd instar	98.89 \pm 1.11	96.37 \pm 2.20	N.S.
4th instar	99.42 \pm 0.58	97.16 \pm 1.76	N.S.
5th instar	93.52 \pm 3.64	83.69 \pm 10.39	N.S.
Total mortality	24.88 \pm 7.14	47.34 \pm 10.04	0.10 > P > 0.05

Basic features of the survivorship curves obtained from the populations reared on leaves from short and tall host trees did not differ greatly in temperature and/or photoperiod conditions (Fig. 3). As shown in Table 3, however, the total mortality rates of the population reared on leaves from short trees were lower than those reared on leaves from tall trees ($0.1 > P > 0.05$). The mortality of the first instar was due to physiological death caused by starvation or diarrhoea, and that of the last instar was also due to diarrhoea or failure of ecdysis. Particularly, there was a significant decrease in survival in the 1st instar when reared on leaves from tall trees ($P < 0.05$).

When the larvae were reared on leaves from short trees during the 1st instar and thereafter transferred to those from tall trees, the mortality of such larvae increased (Fig. 4). A slight increasing trend in mortality was also observed when the larvae were transferred to the leaves from tall trees after being reared on leaves from short trees up to the 2nd and 3rd instar stages, respectively. These results suggested that the food quality for the 1st instar larvae was critical for larval survival.

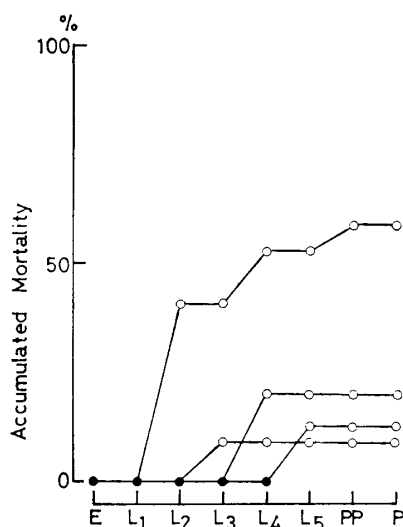


Fig. 4. Accumulated mortality rates of *P. xuthus*, when transferred from leaves of short (●) to those of tall (○) trees.

Since the more suitable the leaf as food the shorter the larval period is, the suitability of leaves for larvae was measured in terms of the length of larval period. Figure 5 shows an example of the progress of larval development expressed by the weighted mean instar (Itô et al., 1970).

As shown in this figure, there is no significant difference in the progress of development between populations on different food until the 3rd instar (2 weeks after hatching). After that, however, larvae reared on leaves from tall trees began to slow in development. It took 30 days from hatching to pupation on leaves from tall trees while it was only 26 days when the diet was leaves from short trees. This delay of 4 days was significant ($P < 0.01$). The other reared-populations also showed similar tendencies with the significantly protracted larval period (ca. 4 days) from the 1st instar ($P < 0.001$). Since the eggs used for these experiments were obtained randomly from several females by artificial oviposition, the difference in larval development may be mainly caused by the food itself, not by hereditary differences among the larvae. The coefficient of variation in the mean instar decreased as the larvae grew up (Fig. 5). Such a tendency was observed in all populations under different temperature and photoperiod conditions as mentioned above.

The experiment of changing leaves during larval development from short to tall trees showed that about 18 days was taken for larvae reared on leaves from tall trees from the *second* instar to the pupal stage. As suggested in Fig. 5, it took about 20 days for larvae feeding on leaves from only short trees to go from the *first* instar to the pupal stage. The duration of the 1st instar stage is 2–3 days. Thus, there is no significant difference in the length of the larval period between leaves from short and tall trees, if both 1st instar larvae were able to have access to leaves from short trees.

The size and weight of pupa were not significantly different among individuals of the two populations. Thus, a certain physical characteristic of the leaves seems to influence the larval survival rate and development.

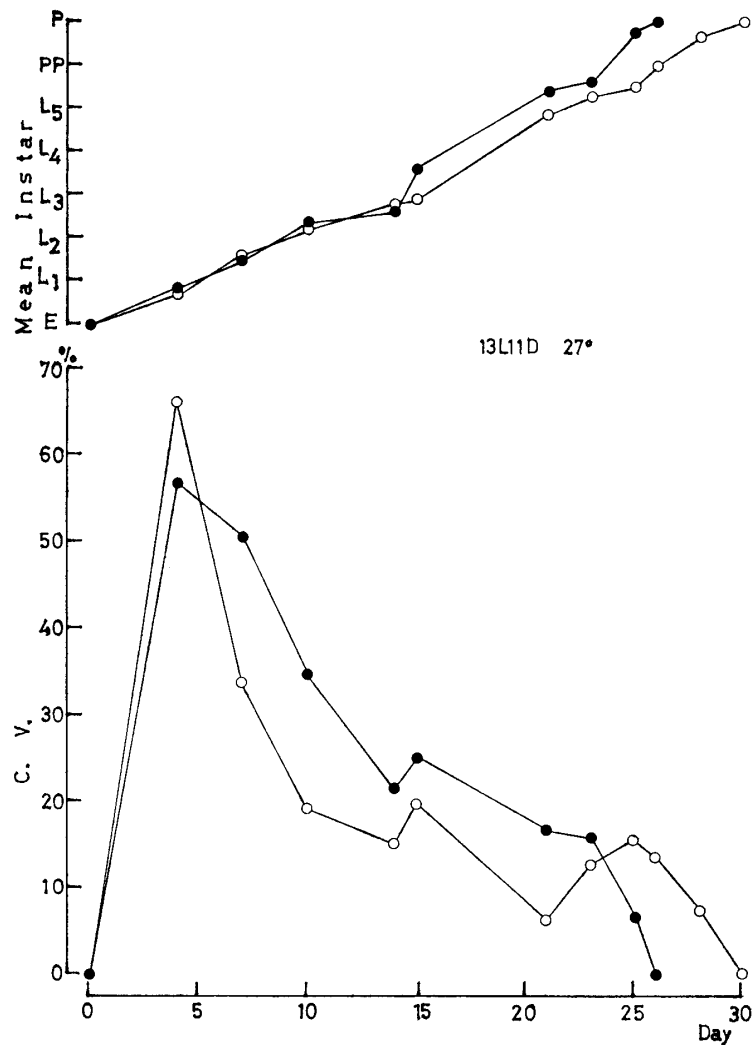


Fig. 5. Larval development of *P. xuthus* reared on leaves of short host trees (●) and tall trees (○). Development is measured by the mean instar and the coefficient of variation.

DISCUSSION

SUZUKI et al. (1976) reported that the spatial distribution of eggs of *P. xuthus* in a citrus grove was always contagious due to the variability in the quantity of new leaves among trees, because females preferentially oviposit on new leaves. Generally when newly hatched larvae were reared on two-year old citrus leaves, their larval stages were protracted or they became unhealthy and showed increased mortality.

Since *Z. ailanthoides* is a deciduous tree, it never has two-year old matured leaves and all leaves fully opened in the current year are sun leaves except in the young trees. During the growing season the growth of a tree with sun leaves continues until September when the hibernacles have already been formed. Thus, the variance of age in the matured leaves would be smaller in *Z. ailanthoides* than in citrus trees. A comparative rearing study on leaves from both *Z. ailanthoides* and citrus trees would be

an interesting subject. For this point, *Z. ailanthoides* is an ideal material to conduct a suitability experiment of leaves collected from trees of various heights (=ages) as food for larvae.

A protracted larval period is probably the first sign of impaired leaf quality, as suggested by HAUKIOJA et al. (1978). The longer larval period *per se* may likely result in a higher risk of predation and parasitism by natural enemies. Unhealthy larvae cannot keep away from even the small-sized predators (WATANABE, 1976, 1981). Strong wind also blows such larvae off of the tall trees in the field. In either case, consequently, the survival rates of larvae living on tall trees may become lower than those on short trees. The results of rearing experiments in the laboratory support field observations in which the survival rates of population on short trees were higher than those on tall ones (WATANABE, 1976, 1981). Thus, the oviposition behavior of female adults which have a preference for seedlings or short trees seems to be more advantageous for maintaining the *P. xuthus* population. However, the fact that larvae fed on the artificial diet which contains leaf powder derived from *Z. ailanthoides* grow normally irrespective of tree height (YAMASHITA, per. com.), indicates that the quantity and quality of nutrients for larval growth are not too different among trees of different heights.

FEENY (1970) reported that tannins of oak leaves tend to be concentrated in the palisade cells of parenchyma, and that one function of leaf tannins may be to inhibit insects from attack not only by reducing the nutritive value but also by influencing palatability. In general, the major function of tannins is to toughen the leaf by concentrating them with parenchyma. In the case of *Z. ailanthoides*, tannins seem to serve to defend leaves against the swallowtail butterfly.

The changes in SLA and texture of the palisade layer of the leaflet occur during the height growth (aging) of *Z. ailanthoides* after it becomes taller than 1 m. These changes make the tree unsuitable for *P. xuthus* females to oviposit (WATANABE, 1979c, 1981) and for larvae to feed on. The process of population decline with floral succession in the deforested area means that *P. xuthus* might relinquish tall trees and seek other habitats where younger trees grow (WATANABE, 1981). This view was also supported by this study.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to Dr. A. KOKUBO of Tokyo University for his invaluable advice and critical reading of this manuscript. Thanks are also due to Mr. T. KASUI of Nippon Kogaku & Co. Ltd., and Miss R. IMAICHI of Tamagawa University for their help in the observation of the transverse sections of leaflets. The staffs at Fudago of Tokyo University Forest in Chiba assisted me in many ways for which I am very grateful.

REFERENCES

- DETHIER, V. G. (1959) Egg-laying habits of lepidoptera in relation to available food. *Can. Ent.* **91** : 554-561.
- FEENY, P. (1970) Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology* **51** : 565-581.
- HAUKIOJA, E., P. NIEMELÄ, L. ISO-IIVARI, H. OJALA and E.-M. ARO (1978) Birch leaves as a resource for herbivores. I. Variation in the suitability of leaves. *Rep. Kevo Subarctic Res. Stat.* **14** : 5-12.

- ITÔ, Y., A. SHIBAZAKI and O. IWAHASHI (1970) Biology of *Hyphantria cunea* DRURY (Lepidoptera : Arctiidae) in Japan. XI. Results of road-survey. *Appl. Ent. Zool.* **5** : 133–144.
- RAUSHER, M. D. (1978) Search image for leaf shape in a butterfly. *Science* **200** : 1071–1073.
- RAUSHER, M. D. (1980) Host abundance, juvenile survival, and oviposition preference in *Battus philenor*. *Evolution* **34** : 342–355.
- SUZUKI, Y., K. YAMAGUCHI, M. IGA, Y. HIROSE and H. KIMOTO (1976) Spatial distribution of the eggs of *Papilio xuthus* LINNÉ (Lepidoptera, Papilionidae) in a citrus grove. *Jap. J. appl. Ent. Zool.* **20** : 177–183 (in Japanese with an English summary).
- WATANABE, M. (1976) A preliminary study on population dynamics of the swallowtail butterfly, *Papilio xuthus* L., in a deforested area. *Res. Popul. Ecol.* **17** : 200–210.
- WATANABE, M. (1979a) Population dynamics of a pioneer tree, *Zanthoxylum ailanthoides*, a host plant of the swallowtail butterfly, *Papilio xuthus*. *Res. Popul. Ecol.* **20** : 265–277.
- WATANABE, M. (1979b) Natural mortalities of the swallowtail butterfly, *Papilio xuthus* L., at patchy habitats along the flyways in a hilly region. *Jap. J. Ecol.* **29** : 85–93.
- WATANABE, M. (1979c) Oviposition of the swallowtail butterfly, *Papilio xuthus* L. (Lepidoptera : Papilionidae) in a deforested area. *Appl. Ent. Zool.* **14** : 484–487.
- WATANABE, M. (1981) Population dynamics of the swallowtail butterfly, *Papilio xuthus* L., in a deforested area. *Res. Popul. Ecol.* **23** : 74–93.
- WIKLUND, C. (1975) The evolutionary relationship between adult oviposition preferences and larval host plant range in *Papilio machaon* L. *Oecologia* **18** : 185–197.
- YODA, K. (1971) *Ecology of forests*. Tokyo: Tsukiji Shokan., 6+331 p. (in Japanese).