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Activity of Mixed Function Oxidase in a few Insect Species in Relation to Their Food Source

먹이종류에 따른 몇가지 곤충의 MFO활성 비교

J. H. Lee and K. S. Boo
이 정 호 · 부 경 생

ABSTRACT Midgut tissues from 4 insect species were examined for the activity of cytochrome *P*-450 monooxygenases, a major enzyme involved in chemical detoxification. When *Helicoverpa assulta* larvae were reared on an artificial diet, the specific activity of the midgut cytochrome *P*-450 monooxygenases (MFO) was 3 times higher than that of the fat body. The specific activity of the midgut cytochrome *P*-450 monooxygenases was higher in *H. assulta* larvae when reared on *Nicotiana tabacum* leaves than when on *Capsicum annuum* fruits or an artificial diet. In the case of *Hyphantria cunea* larvae, *Tilia megaphyllo* leaves were the best in inducing midgut cytochrome *P*-450 monooxygenases activity. When larvae of *H. assulta*, *Spodoptera exigua*, *H. cunea* and *Spodoptera litura* were reared on their own artificial diet, the highest activity was seen in *S. exigua* larvae which is a polyphagous and insecticide-resistant strain.

KEY WORDS Cytochrome *P*-450 monooxygenases (MFO) activity, *Helicoverpa assulta*, *Hyphantria cunea*, *Spodoptera exigua*, *Spodoptera litura*, host plants

초 록 네가지 종류의 곤충 중장에 있는 주요 해독효소인 cytochrome *P*-450 monooxygenases의 활성을 그들의 먹이종류 및 섭식여부와 연관시켜 분석하였다. 담배나방유충을 인공사료로 사육하였을 때 전반적으로 중장의 cytochrome *P*-450 monooxygenases(MFO)활성이 지방체의 경우보다 3배가량 높게 나타났다. 고추열매나 인공사료를 공급하여 사육하였을 때보다 담배잎으로 사육하였을 때 담배나방유충의 중장MFO는 훨씬 높게 나타났으며, 흰불나방의 경우 염주나무잎이 MFO활성을 가장 높게 유기했다. 담배나방, 파밤나방, 흰불나방, 담배거세미나방 등의 유충들을 각각의 인공사료로 사육하였을 때 광식성이며 동시에 살충제저항성을 보유하고 있는 파밤나방의 MFO활성이 가장 높게 나타났다.

검 색 어 Cytochrome *P*-450 monooxygenases(MFO)활성, 담배나방, 흰불나방, 파밤나방, 담배거세미나방, 기주식물

Microsomal cytochrome *P*-450 monooxygenases (mixed-function oxidases: MFO or polysubstrate monooxygenases) system can be considered as an early development of biochemical evolution and it has many features in common between insects and vertebrates. Cytochrome *P*-450 monooxygenases are considered to be a family of isozymes of broad but possibly overlapping specificity involved in the oxidative metabolism of endogenous and exogenous sub-

서울대학교 농생대 농생물학과 및 농업생물신소재연구센터(Dept. of Agricultural Biology and the Research Center for New Biomaterials in Agriculture, College of Agriculture and Life Sciences, Seoul Natl. University, Suwon, 441-744, Korea)

strates (Hodgson 1983). A number of chemicals, including secondary plant substances, have been shown to induce or to inhibit insect cytochrome *P*-450 monooxygenases (Brattsten 1979b).

The importance of the cytochrome *P*-450 monooxygenases system in insect adaptation to variety of host plants was demonstrated by Krieger *et al.*(1971). Their results indicated that midgut microsomal monooxygenases activity was higher, on average, in polyphagous than in monophagous lepidopterous larvae and that varying levels of induction of the microsomal cytochrome *P*-450 monooxygenases system were brought about in insects by feeding on different host plants or on diets containing secondary plant substances.

In addition to these well documented exogenous influences, a number of other factors are known to affect the activity of insect cytochrome *P*-450 monooxygenases. These include strain, sex, age and hormonal status, etc. (Wilkinson & Brattsten 1972).

The oriental tobacco budworm, *Helicoverpa assulta*, having a oligophagous food pattern, utilizes *Capsicum annuum* fruits and *Nicotiana tabacum* leaves as a major food. The latter species produce poisonous nicotine. The tobacco cutworm, *Spodoptera litura*, has a similar food pattern like *H. assulta*. On the contrary, the beet armyworm, *Spodoptera exigua*, having a polyphagous food pattern, utilizes the herb as a food source and shows the insecticide resistance. The fall webworm, *Hyphantria cunea*, being polyphagous, utilizes the leaves of woody plants which contain a secondary plant substance, tannin, in relatively high concentration. This study was carried out to investigate the effects of host plants on the activity of microsomal cytochrome *P*-450 monooxygenases from *H. assulta*, *S. exigua*, *S. litura* and *H. cunea*.

Table 1. Composition of artificial diets for *Spodoptera litura*, *Spodoptera exigua*, *Helicoverpa assulta* and *Hyphantria cunea*

Ingredients	*S. litura*[a]	*S. exigua*[a]	*H. assulta*[b]	*H. cunea*[d]
Kidney bean(g)	100	95	–	–
Wheat germ(g)	100	95	–	–
Corn(g)	–	–	90	–
Soybean(g)	–	–	100	–
Morus alba leaf powder(g)[c]	–	28	24	300
Brewers' yeast(g)	40	–	6	–
Milk(g)	–	15	24	–
Agar(g)	15	–	4.3	–
Vitamin mixture(g)	–	6.0	4.1	–
Ascorbic acid(g)	4.0	2.2	2.2	–
Sorbic acid(g)	1.4	–	–	–
Propionic acid(ml)	–	3.0	2.4	3.6
Metyl-p-hydroxybenzoate(g)	2.8	–	4.0	–
Hot pepper seed oil(ml)	–	2.0	2.0	–
Formalin(35%)(ml)	2.0	800	1000	–
Distilled water(ml)	800			880

[a] Mochida and Miyahara (1974).
[b] Modified from Park (1991).
[c] Product of Dong Bang Yurayang Corp. Seoul.
[d] Formulated in the laboratory.

MATERIALS AND METHODS

Insects

Spodoptera litura, *Helicoverpa assulta* and *Hyphantria cunea* larvae were collected in the suburbs of Suwon and maintained on artificial diets. *Spodoptera exigua* having a resistance to several organophosphate insecticides were received from the Screening Research Center, Korea Research Institute of Chemical Technology(Table 1). *S. litura*, *S. exigua* and *H. assulta* larvae were reared first in groups of 20 in a 12 ×3cm(D×H) petridish until the 2nd instar and then in a 4×5cm petridish individually to the last instar. *H. cunea* larvae were reared in groups of 20 in a 12×20×6cm(L×B×H) plastic container throughout the larval stage. The insectary was maintained at 26±0.5℃ and a photoperiod of 16L/8D.

Effect of host plant species on cytochrome *P*-450 monooxygenases activity in different phytophagous type

H. assulta larvae were fed on *Nicotiana tabacum* or *Capsicum annuum* leaves and *H. cunea* were fed on *Morus alba*, *Salix babylonica* or *Tilia megaphyllo*. All experiments were repeated 4 times, each of which consisted of 20 larvae. From the beginning of the last instar, specific activity of the midgut cytochrome *P*-450 monooxygenases was measured everyday. In larvae of *H. assulta*, *S. exigua*, *S. litura* and *H. cunea* reared on an artificial diet, activity of cytochrome *P*-450 monooxygenases was also measured after the last molting.

Effect of starvation and feeding on cytochrome *P*-450 monooxygenases activity

H. assulta larvae were starved for 2 days from the 2nd to 4th day during the 5the instar and fed on its artificial diet again until pupation time. Content of midgut protein and activity of midgut cytochrome *P*-450 reductase were measured everyday until pupation time.

Preparation of the enzyme(cytochrome *P*-450 monooxygenases) extracts

Midgut and fat body microsomes were prepared by the method of Feyeresen *et al.* (1985). Midgut tissue was dissected in ice-cold 0.1M sodium phosphate buffer(SPB) (PH 7.2) containing 10% glycerol, 1mM ethylenediaminetetracetic acid (EDTA), 0.1mM 4,4-dithio-DL-threitol (DTT), 1mM 1-phenyl-2-thiourea (PTU) dissolved in ethylene glycol monoethyl ether and 1mM phenylmethylsulfonyl fluoride (PMSF) dissolved in ethylene glycol monoethyl ether (Lee & Scott 1989a). Contents were removed in the dissected tissues and tissues of 10 midguts were homogenized thoroughly in 10ml ice-cold 0.1M SPB (PH 7.2) in a teflon homogenizer. The homogenates were centrifuged at 10.000g for 15 min. At once, the supernatant fraction was utilized for cytochrome *P*-450 reductase activity assay. In this paper, the activity of NADPH-cytochrome *P*-450 reductase expresses that of cytochrome *P*-450 monooxygenases.

Activity assay of NADPH-cytochrome *P*-450 monooxygenases

NADPH-cytochrome *P*-450 monooxygenases activity was measured at 30℃ by Pye Unicam UV/VIS Spectrophotometer at 550 nm, (Williams & Kamin 1962). Compositions of the reaction mixture for NADPH-cytochrome *P*-450 monooxygenases activity assay are in Table 2. Proteins were determined by the Lowry method (Lowry *et al.* 1951) using bovine serum albu-

min(BSA) as standard.

Table 2. Composition of the reaction mixture for testing the activity of NADPH-cytochrome *P*-450 monooxygenases

Cytochrome *c* 2mM	150μl
NADPH 30mM	150μl
NaCN 40mM	20μl
SPB* 0.1M, PH7.2	200μl
Enzyme Extracts	20μl

*Sodium Phosphate Buffer

RESULTS

Midgut and fat body cytochrome *P*-450 monooxygenases during the last larval and pupal stage of *Helicoverpa assulta*

As a preliminary experiment, extraction and storage of midgut tissues in *H. assulta* have been tried for finding the best method. The specific activity of cytochrome *P*-450 monooxygenases was variable depending on the centrifugal force. The optimum relative centrifugal force (RCF) turned out to be 10,000g(Table 3). Fig. 1 clearly indicates the relative stability of enzyme activity for the period of up to 2 months when midgut tissues were immersed in sodium phosphate buffer containing 50% glycerol, without freezing or the tissues were frozen at -70℃. Therefore, all the subsequent experiments were carried out in the first way.

Table 3. Variation in cytochrome *P*-450 reductase activity from the midgut of the last larvae[a] of *H. assulta* depending on relative centrifugal force(RCF)

RCF[b]	Total protein (mg)	Total activity (△OD/insect/min)	Specific activity (△OD/mg protein/min)
600g	12.54±1.23	6.32±0.98	0.50±0.10
10,000g	9.72±0.90	6.13±0.72	0.63±0.09
25,000g	7.430.76	4.21±0.64	0.57±0.06

Each value represents the mean±SEM of 4 to 6 experments using 8 to 10 insects per experiment.
[a] 3 days after the last larval molt.
[b] Samples were taken from the supernatant after centrifugation of the gut homogenate at the RCF indicated.

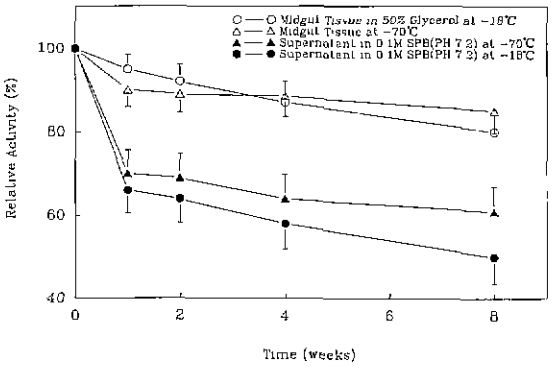

Fig. 1. Decrease of cytochrome *P*-450 reductase activity, depending on sample storage methods and periods, from the midgut of the last instar larvae in *H. assulta*. Each point represents the mean±SEM of 6 experiments using 10 insects per experiment. The absence of a vertical bar indicates that the SEM is smaller than the size of the datum point.

When *H. assulta* larvae were reared on an artificial diet, the specific activity of cytochrome *P*-450 monooxygenases peaked at the molt in the case of midgut, but that of the fat body gradually decreased from the last molting (Fig. 2). Overall, the specific activity of midgut cytochrome *P*-450 monooxygenases was 2.5 times higher than that of the fat body. The total activity of midgut *P*-450 monooxygenases was the highest on day 3.5 (mature stage) and it was due to the increase of protein content in the midgut. On day 3.5, total activity of the midgut was 5.7 times higher than that of the fat body. But, the total activity of fat body cytochrome *P*-450 monooxygenases showed its peak

at pupation, also due to the highest content of protein. Both the pattern in the change of midgut enzyme activity and midgut microsomal protein content differed from those observed in the fat body. Specially, in the fat body, change in the total activity well corresponded to the pattern in the protein content change (Fig. 2).

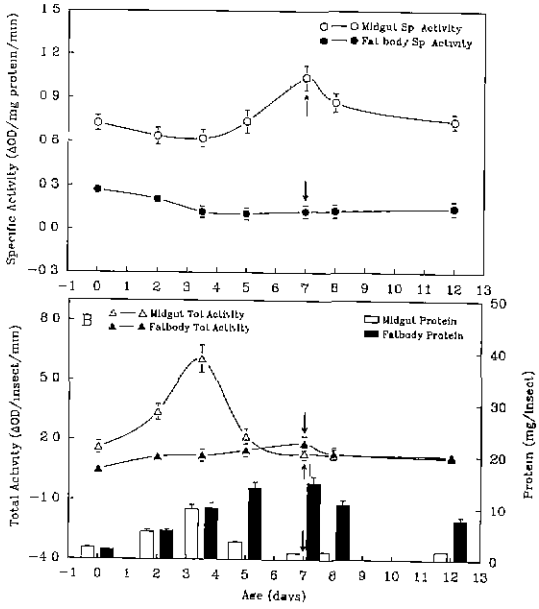

Fig. 2. Change in specific (A) and total (B) activity of cyt. *P*-450 reductase from the midgut and the fat body during the last larval and early pupal stage of *H. assulta* fed on the artificial diet (Pupation time is indicated by an arrow). Each point represents the mean ± SEM of 4 experiments using 10 insects per experiment. The absence of a vertical bar indicates that the SEM is smaller than the size of the datum point.

Effect of starvation and feeding on cytochrome *P*-450 monooxygenases activity in *H. assulta*

The specific activity of the midgut cytochrome *P*-450 monooxygenases and the midgut protein content decreased in the starved *H. assulta* larvae (Fig. 3). But the specific activity

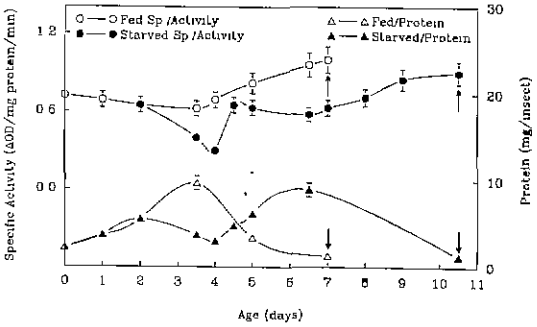

Fig. 3. Effect of starvation and feeding on midgut cyt. *P*-450 reductase activity in the last instar larvae of *H. assulta*. *H. assulta* larvae were fed on the artificial diet(○—○, △—△), or starved for 2 days from the 2nd to 4th day during the 5th larval instar and then given the artificial diet(●—●, ▲—▲) (Pupation time is indicated by an arrow). Each point represents the mean±SEM of 5 experiments using 8 insects per experiment. The absence of a vertical bar indicates that the SEM is smaller than the size of the datum point.

and protein content quickly started to increase when they were fed again. If the starved period were ignored, the pattern in the change of midgut enzyme activity and protein content in starved larvae well matched to that in normally fed larvae.

Effect of host plant species on cytochrome *P*-450 monooxygenases activity in different phytophagous type

The specific activity of the midgut cytochrome *P*-450 monooxygenases was higher in larvae of *H. assulta* when reared on *Nicotiana tabacum* than that on *Capsicum annuum* leaves or the artificial diet (Fig. 4A). When *Hyphantria cunea* larvae were reared on three different host plants, *Tilia megaphyllo* leaves were the best in inducing midgut cytochrome *P*-450 monooxygenases activity (Fig. 4B). But the overall pattern in the change of its activity was quite similar between the two insect species when reared on their host plant leaves. The

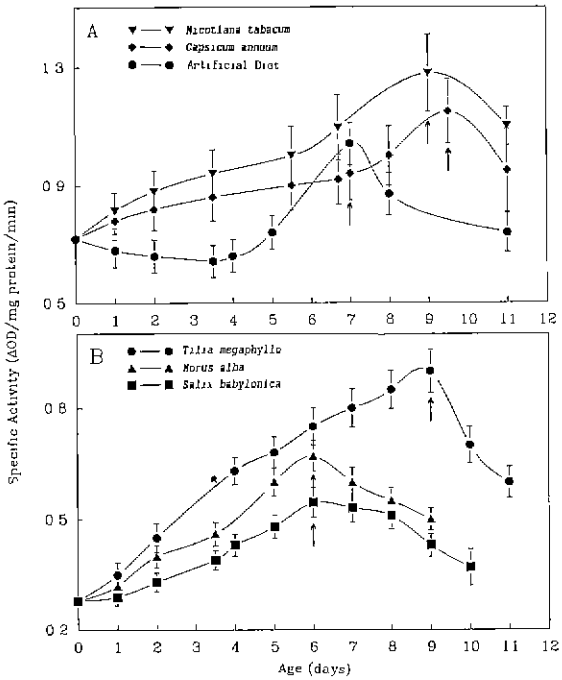

Fig. 4. Difference in specific activity of midgut cyt. *P*-450 reductase from the last instar larvae and early pupae of *H. assulta*(A) and *H. assulta*(B) reared with different host plants and the artificial diet (Pupation time is indicated by an arrow). Each point represents the mean±SEM of 4 experiments using 10 insects per experiment. The absence of a vertical bar indicates that the SEM is smaller than the sise of the datum point.

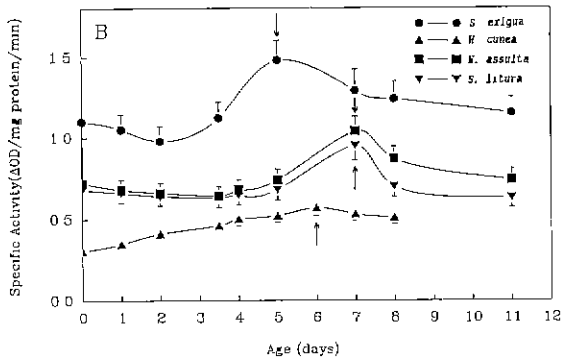

Fig. 5. Changes in specific activity of midgut cyt. *P*-450 reductase from the last instar and early pupae of *S. exigua, S. litura, H. cunea* and *H. assulta* reared on thier own artificial diet (Pupation time is indicated by an arrow), Each point represents the mean± SEM of 4 experiments using 11 insects per experiment. The absence of vartical bar indicates that the SEM is smaller than the size of the datum point.

highest activity seen during molting, regardless of the diet or developmental delay, was evident again in two other insect species, *Spodoptera exigua* and *Spodoptera litura* when they were reared on their own artificial diet (Fig. 5).

However, among four insect species tested, the number of possible host plant species does not seem to give much impact on the specific activity of midgut cytochrome *p*-450 monooxygenases since the highest and lowest activity were observed in two polyphagous species (Fig. 5). The highest level seen in *S. exigua* in this experiment seems to be related to their insecticide resistance.

DISCUSSION

The importance of the cytochrome *P*-450 monooxygenases system in insect adaptation to a variety of host plants was demonstrated by Krieger *et al.* (1971). Fraenkel (1959) suggested the evolutionary significance of a relationship between secondary plant substances and phytophagous insects.

We examined the cytochrome *P*-450 monooxygenases activities in both midgut and fatbody, and the relationship between cytochrome *P*-450 monooxygenases and feeding. Additionary, effect of host plant species on cytochromeqr *P*-450 monooxygenases activity in different phytophagous type was studied.

When *H. assulta* larvae were reared on an artificial diet, the specific activity of midgut cytochrome *P*-450 monooxygenases (MFO) was 3 times higher than that of the fat body. But Feyereisen & Farnsworth (1985) reported that the overall level of enzyme activity was also specific for each tissue during each development stage. For instance, during the last larval stage

of *Diploptera punctata*, the specific activities of aldrin epoxidase, 7-methoxy-4-methylcoumarin *O*-demethylase and NADPH-cytochrome *P*-450 reductase were higher in the midgut than in the fatbody, but the specific activity of methoxyresorufin *O*-demethylase was higher in the fatbody. Tate *et al.* (1982) have reported that the relative levels of *O*-demethylation of *P*-nitroanisole and *N*-demethylation of *P*-chloro-*N*-methylaniline are different in the midgut and the fatbody during the last larval stage of *Manduca sexta*. Caution should thus be exercised when interpreting the data on tissue distribution of cytochrome *P*-450 monooxygenases in the insects. Such distribution patterns are only valid for the particular enzyme activities studied and should not be generalized to other cytochrome *P*-450 monooxy-genases activities.

Among 4 species tested in this experiment, midgut cytochrome *P*-450 monooxygenases activity was the highest in *S. exigua* which is a polyphagous and insecticide-resistant strain when they were reared on their artificial diet. Generally speaking, the midgut cytochrome *P*-450 monooxygenases activity is known to be higher, on average, in polyphagous than in monophagous or oligophagous lepidopterous larvae (Krieger *et al.* 1971). For example, the polyphagous southern armyworm, *Spodoptera eridania*, larvae feeds on a number of plants from several different families. Many of these host plants cause significant induction of the midgut microsomal monooxygenases in *S. eridania* larvae feeding on them (Brattsten 1979a, b, Brattsten et al. 1977). Moreover, the very low cytochrome *P*-450 monooxygenases activity in the sucking insects, compared with chewing insects, may be due to their contact only with water-soluble materials in the host plant. For the sucking insects, lack of exposure to hydrophobic, toxic plant secondary substances has eliminated the need for this detoxifying enzyme system (Hung 1990). Therefore, it seems that the reason of the higher activity of midgut cytochrome *P*-450 monooxygenases in polyphagous species is that they may have more chance to be exposed to, as in the case of contrast between the chewing type- and sucking type-insects, hydrophobic toxic plant secondary substances in the host plant, and consequently has to develop this detoxifying enzyme system for survival. However, this does not fit in explaining the lowest activity of cytochrome *P*-450 monooxygenases from *H. cunea*, another polyphagous species, which showed the lowest value.

The specific activity of the midgut cytochrome *P*-450 monooxygenases was higher in larvae of *H. assulta* when reared on *Nicotiana tabacum* leaves than when on *Capsicum annuum* fruits or an artificial diet. In the case of *H. cunea* larvae, *Tilia megaphyllo* leaves were the best in inducing midgut cytochrome *P*-450 monoozygenases activity. Moreover, such induction can be also brought about by the addition of secondary plant substances to semi-defined artificial diets. The ecological significance of this induction is apparent from the observation that insects with its enzyme activity induced by feeding on *α*-pinene were more tolerant to the lethal effects of another secondary plant substance, nicotine, than those not so induced. This adaptation was seen not only with toxic plant substances such as nicotine, but also with synthetic insecticides such as carbaryl (Brattsten & Wilkinson 1973, Yu 1982). It has been shown that in the case of *S. eridania* feeding on either carrot foilage or carrot monoterpene, cytochrome *P*-450 monooxygenases activity was induced with an

increase of 134% (Brattsten 1983, Brattsten *et al.* 1984). Riskallah *et al.* (1986) reported that tobacco budworm larvae fed on wild tomato leaves had increased tolerance to the organophosphorus insecticide, diazinon. This was attributed to an increase in metabolism of the insecticide due to induction of the cytochrome *P*-450 monooxygenases. Therefore, it seems that the varying levels of induction of the cytochrome *P*-450 monooxygenases activity in *H. assulta* or *H. cunea* larvae by feeding on different host plants is due to the secondary substance in host plants.

H. cunea which utilizes the leaves of woody plants as a food source revealed the lowest level of cytochrome *P*-450 monooxygenases activity and when they were reared on their different host plant species, changes of the specific activity was not great. In order to repel insect feeding, a plant may not necessarily have to produce a substance that in highly toxic to the insect. It may be sufficient to produce a compound that is unpleasant or distasteful. Also, an effective barrier to most insect feeding can be erected by reducing the nutritional value of the plant. Thus it is apparent that the synthesis of some secondary compounds, especially of tannins, may have this effect on insect behaviour. Tannins combine with protein, often irreversibly, by forming bonds with the peptides and other functional groups. Such bonding prevents proteins from being attacked by trypsin and other digestive enzymes. Different from the toxin, tannin which occur very widely in relatively high concentration in the leaves of woody plants, does not induce detoxyfying enzymes (Harborne 1988). A similar response was reported by Feeny (1975) on insects feeding on oak trees. So, in contrast to the case of *S. exigua*, the lowest level of cytochrome *P*-450 monooxygenases activity in polyphagous *H. cunea* in this experiment seems to be related to the tannin in their woody host plant. additionally, it is assumed that the differential change in the induction of cytochrome *P*-450 monooxygenases activity when they were reared on their different host plant species may be related to other secondary compounds.

REFERENCES CITED

Brattsten, L.B. 1979a. Ecological significance of mixed-function oxidations. Drug Metab. Rev. 10: 35~58.

Brattsten, L.B. 1979b. Biochemical defence mechanisms in herbivores against plant allelochemicals. *In* Herbivores: their interactions with secondary plant metabolites, eds. by G.A. Rosenthal and D. H. Janzen, pp. 200~270. Academic Press. New York.

Brattsten, L.B., C.F. Wilkinson & T. Eisner. 1977. Herbivore-plant interactions: mixed-function oxidases and secondary plant substances. Science 196: 1349~1352.

Brattsten, L.B. & C.F. Wilkinson. 1973. Induction of microsomal enzymes in the sourthern armyworm(*Prodenia eridania*). Pest. Biochem. Physiol. 3: 393~407.

Brattsten, L.B. 1983. Cytochrome *P*-450 involvement in the interactions between plant terpenes and insect herbivores. In Plant Resistance to Insects(ed. by P.A. Hedin), PP. 173~198. Amer. Chem. Soc., Washington, DC.

Brattsten, L.B., C.K. Evans, S. Bonetti & L.H. Zalkow. 1984. Induction by carrot alleochemicals of insecticide-metabolizing enzymes in the southern armyworm, *Spodoptera eridania*. Comp. Biochem. Physiol. 77C: 29~37.

Feeny, P. 1975. Plant appearance and chemical defence. Recent Adv. Phytochem. 10: 1~40.

Feyereisen, R. & D.E. Farnsworth. 1985. Developmental changes of microsomal cytochrome *P*-450 monooxygenases in larval and adult *Diploptera punctata*. Insect Biochem. 15: 755~761.

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

Harborne, J.B. 1988. Introdution to ecological biochemisry : Insect feeding preferance. 3rd. ed., 356. pp. Academic Press, New York.

Hodgson, E. 1983. The significance of cytochrome *P* -450 in insects. Insect Biochem. 13: 237~246.

Hung, C.F., C.H. Kao, C.C. Liu, J.G. Lin & C.N. Sun. 1990. Detoxifying enzymes of selected insect species with chewing and sucking habits. J. Econ. Entomol. 83: 361~365.

Krieget, R.I., P.P. Feeny & C.F. Wilkinson. 1971. Detoxification enzymes in the guts of caterpillars: An evolutionary answer to plant defenses. Science 171: 579~581.

Lee, S.S.T. & J.S. Scott. 1989. An improved method for preparation, stabilization and storge of housefly(Diptera: Muscidae) microsomes. J. Econ. Ent. 82: 1559~1563.

Lowry, O.H., N.J. Rosebrough, A. L. Farr & R.J. Randall. 1951. Protein measurement with the folin-phenol reagent. J. Biol. Chem. 193 : 265~275.

Mochida, O. & Y. Miyahara. 1974. Effect of γ-radiation on male pupae and adults of *Spodoptera litura*(Lepidoptera: Noctuidae). Appl. Ent. Zoo. 9: 41~47.

Park, K.C. 1991. Composition and activity of female sex pheromone in the oriental tobacco budworm, *Helicoverpa assulta* (Guenee). A Ph. D. dissertation. Seoul. Natl. Univ.(in Korean).

Riskallah, M.R., W.C. Dauterman & E. Hodgson. 1986. Nutritional effects on the induction of cytochrome *P*-450 and glutathione transferase in larvage of the tobacco budworm, *Heliothis virenscens*. Insect Biochem. 16: 491~499.

Tate, L.G., S.S. Nakat & E. Hodgson. 1982. Comparison of detoxification activity in midgut and fat body during fifth instar development of the tobacco hornworm, *Manduca sexta*. Comp. Biochem. Physiol. 72C: 75~81.

Wiliams, C.H. & H. Kamin. 1962. Microsomal triphosphopyridine nucleotide-cytochrome *c* reductase of liver. J. Biol. Chem. 237 : 587~595.

Wilkinson, C.F. & L.B. Brattsten. 1972. Microsomal drug metabolizing enzymes in insects. Drug Metab. Rev. 1: 153~228.

Yu, S.J. 1982. Induction of microsomal oxidase by host plants in the fall armyworm, *Spodoptera frugiperda*. Pestic. Biochem. Physiol. 17: 59~67.

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