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# *In vivo* metabolism of carbofuran in resistant and susceptible brown planthoppers, *Nilaparvata lugens* Stål

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**Abstract :** This study was conducted to find out the biochemical or metabolic resistance mechanism of brown planthopper (BPH) to carbofuran. Differences between resistant ($LD_{50}$; 20.3$\mu$g/g) and susceptible strains($LD_{50}$; 0.3$\mu$g/g) were shown. The amounts of carbofuran metabolite, benzofuranol, and the origin, not developed by Thin Layer Chromatography, were much more in the susceptible strain. But the mother compound, carbofuran, was much more in the resistant strain. The tendencies of metabolism one and three hours after treatment were similar in both strains except for the amounts of metabolites described above. From the study, it is supposed that hydrolytic enzyme, esterase, changes its role from cleaving the esteric bond of carbofuran to making conjugates with carbofuran. This seems to be the main resistance mechanism of BPH to carbofuran. Oxidase and transferase may play little or no role in resistance mechanism. Oxidative and transferring enzymes gave no effects on the metabolism of carbofuran in the resistant strain compared with the susceptible strain. (Received June 20, 1998, accepted July 30, 1998)

Key words : resistance, mechanism, *in vivo*, metabolism, carbofuran.

## Introduction

Rice is the most important crop because it is the main food in the Korean food. Rice plants are damaged by about 44 planthopper and leafhopper species (Choe, 1981). Among them, migrant brown planthopper (BPH) is the most economically important. BPH immigrates every year to Korea from foreign breeding sources such as the southern part of China (Ma, 1993). Control of the BPH population could be achieved using repeated or continued applications of insecticides. This control method inevitably brings out the resistance problem. Insecticide resistance is the major obstacle in controlling agriculturally and medically important pests. This worldwide problem has been documented for over 500 arthropod species by the 1980's, particularly on flies, caterpillars, beetles and mites (Georghiou, 1990). Pest resistance to insecticides result in increased pesticide application frequencies, increased dosage, decreased yields, environmental damage and outbreak of arthropod vectors of diseases. Given the tremendous difficulty and investment associated with development of new, safe and cost-effective insecticides, there is a grave need to preserve the efficacy of current and future insecticides. For this reason, it is essential to understand the mechanisms by which insects acquire resistance so that we can intelligently design strategies to delay their onset (Mullin and Scott, 1992). Carbofuran, a carbamate insecticide, is frequently used in controlling the BPH. It is known that acetylcholine esterase (AChE) inhibiting as insecticidal mechanism, and insensitive AChE is one of the resistance mechanism in BPH. But it is not known what the detoxifying mechanism of carbofuran is

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in BPH. In this paper, *in vivo* metabolism study was carried out to investigate the changes in the metabolic pathway of carbofuran in the resistant and susceptible BPH.

## Materials and Methods

### Insect strains

Two strains of the BPH were used in this study. The susceptible (S) strain has been maintained for 12 years without exposure to any insecticide in laboratory. Strain resistant to carbofuran was obtained by treating the S strain with carbofuran 10% wettable powder for 30 generations at 30~70% mortality. Hereafter, the strain was referred to as Rc-30. The selection was performed on the 3rd and 4th instar nymphs by spray method using the Potter's spay tower (Burkard, UK). Planthoppers which survived 24 hours after treatment were reared on 'Chucheong' rice plant (*Oryza sativa* L.) seedlings (7~10 days after germination) in acrylic cages (26×30×20 cm) at 26±1℃, 65±5% RH, and a photoperiod of 16:8(L:D) hours to produce the offspring. $LD_{50}$ of the resistant strain, RC-30, was 20.3 ㎍/g and that of the susceptible one, S, was 0.3 ㎍/g, respectively.

### Chemicals

$^{14}$C-Labelled carbofuran (2,3-dihydro-2,2-dimethyl-[ring -3-$^{14}$C] benzofuran-7-yl methyl carbamate) was used. The specific radioactivities of $^{14}$C-carbofuran with 99% chemical purity was 18.58 mCi/mmol. The solvents used in this studies were the highest grade and purchased from Sigma Chemical Company. Carbofuran and their authentic metabolites used in this study are shown in Table 1.

### Analysis of metabolites

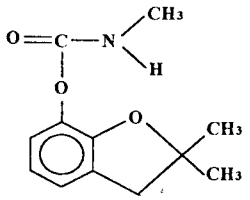
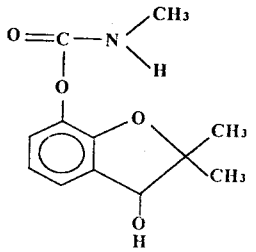
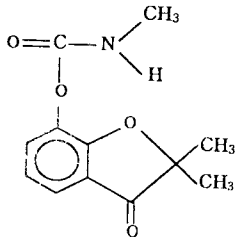
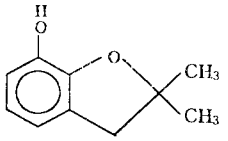
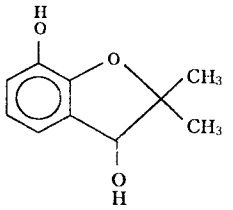
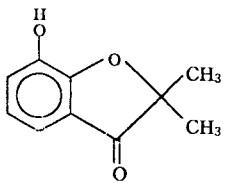
The susceptible and resistant BPHs treated with 70 dpm of $^{14}$C-carbofuran equivalent to $LD_{30}$ of susceptible ones were subsequently homogenized in the small ceramic bowl with 20 ㎖ dichloromethane three times and two times more with 20 ㎖ ethylacetate at one and three hours after treatment. These solutions were mixed as organic fractions. Then, the residues were washed three times with 20 ㎖ methanol and treated as water soluble fraction. These fractions were concentrated and separated by TLC. Precoated silica gel 60 $F_{254}$ thin layer chromatoplates (20×20 cm, 0.2 mm thickness, Merck, F.R.G.) and two dimensional chromatography were conducted using the following solvent systems for separation and identification of metabolites of carbofuran, hexane - chloroform - ethylacetate(3:6:1) and benzene - diethylether - ethanol(9:3:0.1). Identification of metabolites was carried out by co-chromatography with authentic compounds. Non labelled authentic compounds were detected under the UV light, while labelled metabolites were detected with RI image analyzer (Fuji, BAS 2500). Each detected spots by RI image analyzer were scraped into 6 ml scintillation bottles, and 5 ml of EP solution was added to each bottle. The radioactivities were measured with liquid scintillation counter (Hewlett Packard).

## Results

### Separation and identification of metabolites

Figs. 1~3 show RI images of the metabolites of $^{14}$C-carbofuran developed by TLC. The amount of metabolites were measured by scraping and counting the dpm of each spot. Tables 2 and 3 show the amount of metabolites of carbofuran in female BPH at one and three hours after treatment. Higher amounts of unmetabolized carbofuran were found in the resistant strain than the susceptible strain. At one hour after treatment, the amount of carbofuran left was 36% in susceptible strain and 76% in the resistant one. The amount of carbofuran in the susceptible strain exceeds two times more than the resistant one. The difference in carbofuran amount between the two strains was much larger three hours after treatment: 20% in susceptible, and 61% in resistant strain. It decreased from 36 to 20% in the susceptible and from 76 to 61% in the resistant one. The RI amounts of origin which were not developed in the TLC plate showed reverse tendency to that of carbofuran. Table 1 shows the formulas of carbofuran and its metabolites.

**Table 1. Structural formulas of carbofuran and its metabolites**

| Chemicals | Structural formula | Chemical name |
|---|---|---|
| Carbofuran |  | 2,3 - dihydro - 2,2 - dimethyl benzofuran - 7 - yl - methyl - carbofuran |
| 3 - Hydroxy carbofuran |  | 2,3 - dihydro - 2,2 - dimethyl 3 - hydroxy benzofuran - 7 - yl - methyl carbmate |
| 3 - Keto carbofuran |  | 2,3 - dihydro - 2,2 - dimethyl 3 - keto benzofuran - 7 - yl - methyl carbamate |
| Benzofuranol |  | 2,3 - dihydro - 2,2 - dimethyl -7-benzofuranol |
| 3 - Hydroxy benzofuranol |  | 2,3 -dihydro-2,2-dimethyl-3-hydroxy-7-benzofuran |
| 3 - Keto benzofuranol |  | 2,3 - dihydro - 2,2 - dimethyl - 3 - keto - 7 - benzofuran |

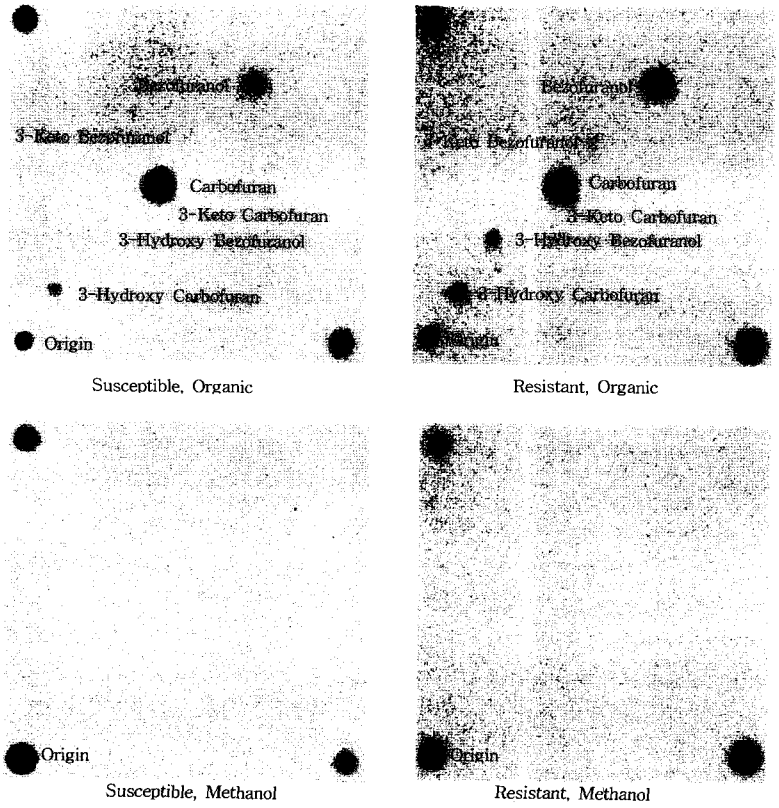



**Fig. 1. *In vivo* metabolites of carbofuran developed by TLC one hour after treatment.**

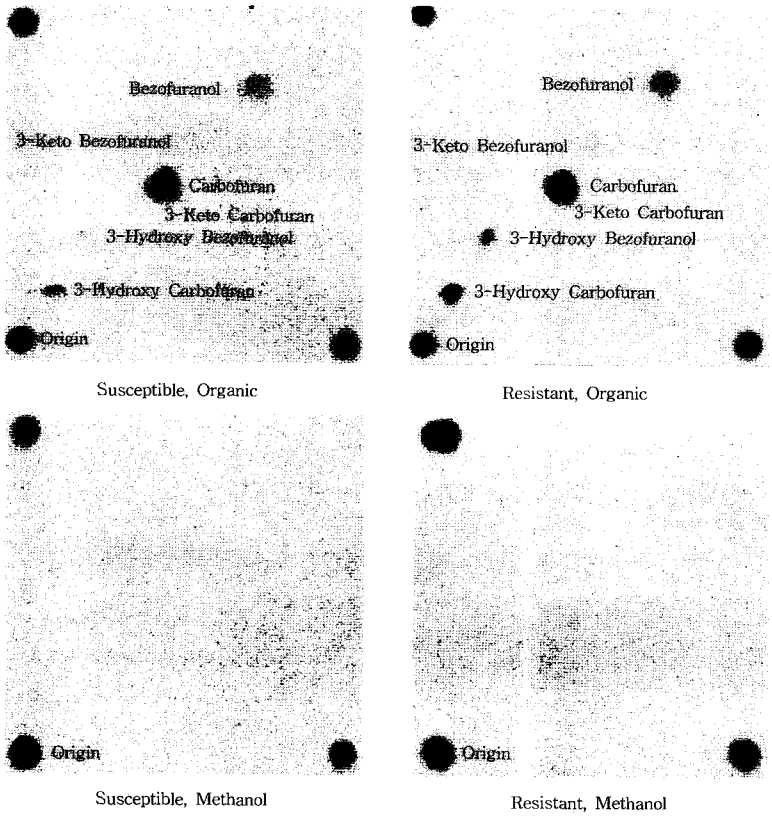



**Fig. 2. *In vivo* metabolites of carbofuran developed by TLC three hours after treatment.**

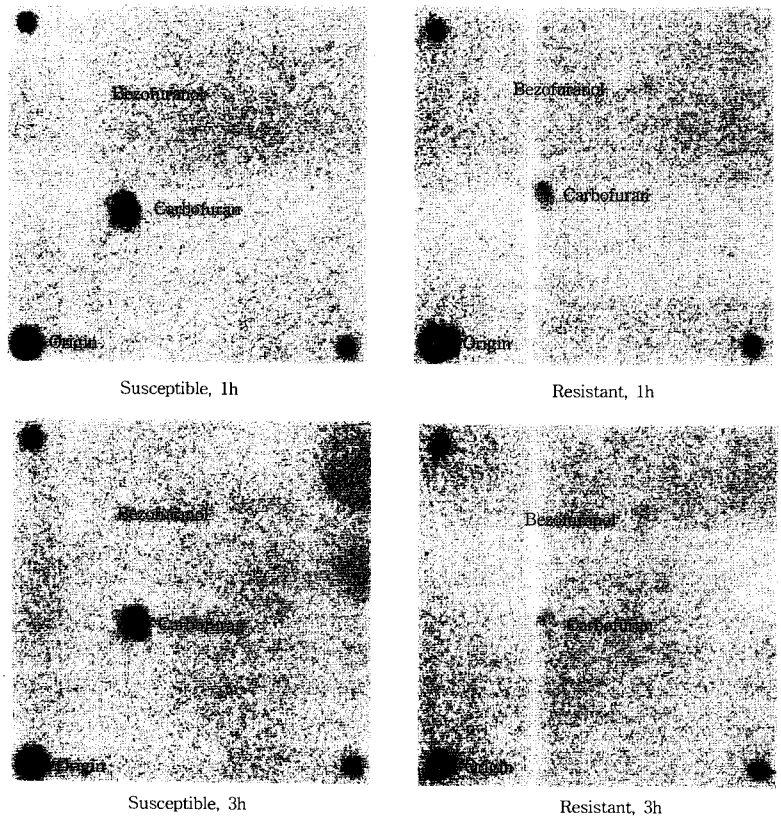



**Fig. 3. Metabolites contained in the origin developed by TLC.**

## Discussion

Metabolic detoxification is well known as a major factor of resistance in many insect pests (Khan *et al*, 1973; Plapp and Casida, 1969). Metabolic rate and percentage of its metabolites are very useful indicators to find out biochemical resistance mechanism by comparing them between the resistant and susceptible insect species. In general, detoxification make xenobiotics more polar by oxidizing or cleaving them. The major characteristic of the carbamates is that oxidative degradation activities through the mixed-function oxidase systems are responsible for their metabolism (Ahn *et al.*, 1993; Zhao *et al.*, 1995), Matsumura (1976) reported that the chemical nature of these insecticidal carbamic esters, hydrolytic products, such as naphthols and phenols, are often less polar than the parent compounds. This view disagrees with the intention that xenobiotic metabolism in animals is geared up to making them more polar so that they can be excreted (Willams, 1959).

It means that another metabolic process will be needed in secretion of carbamate metabolites. Structurally poor electrophilic moieties (e.g. substituted phenols and naphthols) are coupled with *n*-methyl or dimethyl carbamic acid in carbamates, whereas, in organophosphate insecticides strongly electrophilic substituents are used and, thus, OP (organophosphorus) insecticides are naturally susceptible to hydrolytic attack (O'Brien, 1967). Based upon these facts, it is expected that cleavage of esteric bond in carbofuran metabolism by enzymes will be more or less difficult. The opposite postulates have also been reported. However, hydrolysis plays a major role in the metabolism of many carbamates in rats. Schlagbauer and Schlagbauer (1972) showed an importance of hydrolysis responsible for metabolism of carbamates in rats and have documented the table of percent hydrolysis for various compounds. The portion of hydrolysis in total metabolism varied with compounds. In this study, furanol was the main metabolite. This means that hydrolase plays a major role in carbofuran

metabolism. Park and Choi (1991) reported that esterase and mixed function oxidase are responsible for carbofuran metabolism in BPH; about 90% of carbofuran and 80% of fenobucarb were in the water soluble fraction in nine hours. This suggests that carbofuran and fenobucarb are metabolized to more polar compounds. This results coincide with those of my metabolic study. In the metabolic studies, it seems that oxidative enzyme does not affect the metabolism of carbofuran. Only little oxidized metabolites were found in my metabolic study. But the role of oxidas is not clear.

In the present study, results were in disagreement with earlier findings that carbamic ester of carbofuran is relatively difficult to be hydrolyzed (O'Brien, 1967). Amouts of carbofuran were much more in the resistant strain than in the susceptible one. A decrease in the amount of carbofuran by time supports a metabolic contribution to detoxification of carbofuran. This suggests that another major metabolic resistance mechanism may be involved. The plausible factor as a major resistance mechanism might be binding proteins as following evidence: most metabolites were in the methanol fraction and any other metabolites were not detected, and origin part of RI compound might be mainly carbofuran conjugates because when origin parts were

**Table 2. *In vivo* metabolism of carbofuran in the resistant and susceptible female BPH one hour after treatment.**

| Metabolites | S | | | Rc-30 | | |
|---|---|---|---|---|---|---|
| | RI Amounts (dpm) | % of RI | S.E.[a] of % | RI Amounts (dpm) | % of RI | S.E. of % |
| Benzofuranol | 5,058 | 6.07 | 1.03 | 482 | 0.66 | 0.60 |
| 3-Keto benzofuranol | 620 | 0.75 | 1.34 | 611 | 0.84 | 0.46 |
| Carbofuran | 29,922 | 35.93 | 1.78 | 55,761 | 76.48 | 0.18 |
| 3-Keto carbofuran | 1,157 | 1.39 | 0.87 | 1,844 | 2.53 | 0.61 |
| 3-OH benzofuranol | 180 | 0.22 | 0.67 | 22 | 0.03 | 0.47 |
| 3-OH carbofuran | 3,288 | 3.95 | 1.01 | 2,918 | 4.00 | 0.20 |
| Origin | 35,373 | 42.48 | 1.98 | 9,249 | 12.69 | 0.13 |
| Residues | 7,676 | 9.22 | 0.12 | 2,026 | 2.78 | 0.09 |
| Total | 83,276 | 100 | | 72,916 | 100 | |
| Recovery rate(%) | 92.5 | | | 85.7 | | |

[a)]Standard error.

**Table 3. *In vivo* metabolism of carbofuran in the resistant and susceptible female BPH three hours after treatment by direct homogenizing method**

| Metabolites | S | | | Rc-30 | | |
|---|---|---|---|---|---|---|
| | RI Amounts (dpm) | % of RI | S.E.[a)] of % | RI Amounts (dpm) | % of RI | S.E. of % |
| Benzofuranol | 9,923 | 11.04 | 0.22 | 1,883 | 2.26 | 0.27 |
| 3-Keto benzofuranol | 4,486 | 4.99 | 0.67 | 481 | 0.58 | 0.48 |
| Carbofuran | 18,462 | 20.53 | 0.79 | 51,221 | 61.42 | 0.19 |
| 3-Keto carbofuran | 1,596 | 1.77 | 1.80 | 1,776 | 2.13 | 0.50 |
| 3-OH benzofuranol | 384 | 0.43 | 0.64 | 228 | 0.27 | 0.53 |
| 3-OH carbofuran | 2,923 | 3.25 | 0.59 | 3,086 | 3.70 | 0.31 |
| Origin | 46,313 | 51.50 | 0.66 | 19,982 | 23.96 | 0.08 |
| Residues | 5,833 | 6.49 | 0.05 | 4,735 | 5.68 | 0.22 |
| Total | 89,923 | 100 | | 83,395 | 100 | |
| Recovery rate(%) | 99.7 | | | 92.9 | | |

[a)]Standard error.

treated with protease only carbofuran was released. Esterase is thought to be the protein that takes charge of the conjugates with carbofuran. Because double roles of esterase as resistance mechanism and metabolic binding in aphids (Devonshire and Moores, 1982) and in green leafhoppers (Motoyama *et al.*, 1984) have been reported.

Carbofuran metabolite, bezofuranol may make conjugates such as glucose sulfate and glucuronic acid mainly in insects and in animals (Cool and Jankowski, 1985), but it was not certain in this study.

It is uncertain whether GSH transferases play some role in resistance mechanisms of the BPH to carbofuran, because there were no reports on the role of GSH-transferases in carbamate metabolism in insects and animals (Matsumura, 1976; Dauterman, 1980). More studies on GSH transferases are needed to elucidate their role in the resistance mechanism of the Rc-30 strains of the BPH to carbofuran. Although Zhao *et al.* (1995) reported the importance of microsomal monooxygenase in the metabolism of bendiocarb in flower thrips, microsomal oxidase does not seem to be a mechanism in resistance of BPH to carbofuran because the amounts of oxidized metabolites of carbofuran were almost same in the resistant and susceptible BPHs.

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**저항성 및 감수성 벼멸구 체내에서의 카보후란 대사**

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**요약 :** 벼멸구의 카보후란에 대한 저항성 기작을 구명하기 위해 실내에서 카보후란으로 30세대 도태하여 얻은 저항성 계통($LD_{50}$; 20.3$\mu$g/g)과 약제를 12년 동안 처리하지 않은 벼멸구 감수성 계통($LD_{50}$; 0.3$\mu$g/g)에 $^{14}$C-카보후란을 처리하여 계통 간 대사물 량의 차이를 조사한 바 카보후란의 대사물인 benzofuran과 TLC의 의해 전개되지 않은 원점 부분은 감수성 계통에서 많았으며, 대사되지 않은 카보후란은 저항성 계통에서 많았다. 이러한 경향은 처리 후 1, 3시간 대사에서도 같은 경향이었다. 이상의 결과로부터 저항성 벼멸구에서 가수 분해 효소의 일종인 에스테라제가 저항성이 발달함에 따라 에스터 결합을 끊는 역할에서 카보후란과의 결합체 역할로 그 기능이 전환되는 것으로 생각되며 이것이 저항성 기작 중 주요한 하나의 요인으로 사료된다. 산화효소와 전이효소는 저항성과 감수성 계통에서 대사물의 양에 차이가 없어 저항성과는 무관한 것으로 생각된다.

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