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Effect of Organophosphorus Synergists
on Fenitrothion Resistance in Rice
Stem Borer, *Chilo suppressalis* (WALKER)
(Lepidoptera: Pyralidae)¹

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(Received June 11, 1987)

The sudden outbreak of the rice stem borer, *Chilo suppressalis* (WALKER), in Okayama prefecture in 1978 and failure to control this insect by organophosphorus (OP) insecticides are closely related to resistance (TANAKA et al., 1982, 1983). To elucidate resistance mechanisms of the rice stem borer to OP insecticides or to overcome this resistance, synergism studies have been carried out by several researchers who have found that IBP, edifenphos and pirimicarb are able to enhance the efficacy of fenitrothion with which they are combined (TANAKA et al., 1982; MANABE et al., 1983; KONNO and KAJIHARA, 1985). In a preliminary report (KONNO and SHISHIDO, 1985), we demonstrated that the salioxon-analog K-2 is an effective synergist for

fenitrothion and fenitroxon against the OP-resistant rice stem borer, and that the synergism of fenitrothion by K-2 is due to the blocking of degradation of fenitroxon. The present study describes the synergistic effect of several noninsecticidal OP esters and metabolic inhibitors on OP resistance in the resistant strain of rice stem borer.

Two strains of rice stem borers were used in this study: an OP-resistant Hata strain collected at Hata in Soja, Okayama prefecture in 1983 and maintained in the laboratory without insecticidal selection pressure, and a susceptible strain (S) collected at Kawashimacho, Saitama prefecture in 1971 by the Institute of Physical and Chemical Research. The insects were reared on artificial diets (eggs—fourth instar larvae) and on rice seedlings (fourth instar larvae—pupae) by the modified methods of KAMANO (1974) and UCHIUMI (1974). Rearing conditions were kept at 26±1°C under a 16L–8D photoperiod regimen. LD₅₀ values of fenitrothion and fenitroxon topically applied to the Hata strain were 75.3 µg/g and 41.3 µg/g, respectively. Resistance factor (LD₅₀ of Hata strain/LD₅₀ of S strain) for fenitrothion and fenitroxon was 41.8 and 34.4, respectively (KONNO et al., 1986). Fifth instar larvae (60 to 70 mg of body weight) of the two strains were used in this study.

Fenitrothion and fenitroxon were 99% and 98% samples respectively, which were provided by Sumitomo Chemical Co., Osaka. The following organophosphorus compounds were synthesized by pub-

¹ Appl. Ent. Zool. **23** (1): 99–102 (1988)

lished procedures (ETO et al., 1962; MAEDA, 1970; MARKLEY, 1960) and purified by recrystallization, distillation or TLC: K-2 (2-phenoxy-4*H*-1,3,2-benzodioxaphosphorin-2-oxide), K-1 (2-phenyl-4*H*-1,3,2-benzodioxaphosphorin-2-oxide), DEF (*S,S,S*-tributyl phosphorotrithioate), IBP (*S*-benzyl *O,O*-diisopropyl phosphorothiolate), EBP (*S*-benzyl *O,O*-diethyl phosphorothiolate), (EtO)₂ P(*S*)OCH₂ Ph(*O*-benzyl *O,O*-diethyl phosphorothioate), and (EtO)₂ P(*O*)OCH₂ Ph(*O*-benzyl *O,O*-diethyl phosphate). TPP (triphenyl phosphate), TOCP (tri-*o*-cresyl phosphate) and PB (piperonyl butoxide) were purchased from Tokyo Kasei Kogyo Co., Tokyo. WARF anti-resistant (*N*-di-*n*-butyl-*p*-chlorobenzenesulfonamide) and S-421 (octachlorodipropyl ether) were a gift from Dr. J. Fukami of the Institute of Chemical and Physical Research.

Toxicity tests of OP insecticides were conducted according to the method described previously (KONO et al., 1986). Synergism tests were conducted as follows. A mixture of OP insecticide and test chemical at a ratio of 1:10 in 0.5 μ l of acetone was applied topically to the dorsal side of a larva's abdomen. Mortality was counted after 24 hr at

26°C. LD₅₀ values were computed using the method of FINNEY (1952) for probit analysis which was adapted to a program for the NEC N4700 computer. Since none of the test chemicals used as synergists is toxic at the test dose, the synergistic effect is expressed as the following ratio.

$$\text{Synergistic ratio} = \frac{\text{LD}_{50} \text{ of insecticide alone}}{\text{LD}_{50} \text{ of insecticide in mixture}}$$

The biological activity of various chemicals tested as synergists is presented in Table 1. In addition to K-2, four OP esters, i.e., K-1, DEF, IBP and EBP reduced the level of fenitrothion resistance from 8- to 2-fold against the Hata strain. DEF and K-2 were more effective synergists of fenitrothion than EBP, which was more effective than IBP and K-1. TPP was not synergistic and TOCP antagonized fenitrothion. EBP, the *O,O*-diethyl analog of IBP, was 1.7 times more synergistic than IBP. The synergism by EBP was completely lost by replacing the P(*O*)-*S*-benzyl group of EBP with a P(*O*)-*O*-benzyl group or with a P(*S*)-*O*-benzyl group. Therefore, the P-S-C bond in EBP and IBP appears to be important for synergistic action. Fenitrothion was antagonized strong-

Table 1. Synergism of fenitrothion by various chemicals against the Hata and susceptible strains of rice stem borers

Chemicals ^a	LD ₅₀ (μ g/g)	Synergistic ratio
Hata strain		
Fenitrothion alone	75.3	—
+ K-2	8.5	8.9
+ K-1	35.9	2.1
+ DEF	9.1	8.3
+ IBP	21.5	3.5
+ EBP	12.6	6.0
+ (EtO) ₂ P(<i>S</i>)OCH ₂ Ph	376.5	0.2
+ (EtO) ₂ P(<i>O</i>)OCH ₂ Ph	150.5	0.5
+ TPP	83.7	0.9
+ TOCP	188.3	0.4
+ PB	376.5	0.2
+ S-421	376.2	0.2
+ WARF anti-resistant	75.6	1.0
Susceptible strain		
Fenitrothion alone	1.8	—
+ K-2	1.8	1.0
+ DEF	2.0	0.9
+ IBP	1.8	1.0
+ EBP	1.8	1.0

^a Fenitrothion + synergist (1:10 ratio)

ly by PB and S-421. On the other hand, K-2, DEF, IBP and EBP had no effect on the toxicity of fenitrothion against the S strain.

The Hata strain showed resistance to phosphates and phosphonates such as fenitroxon, methyl paraoxon, diazoxon, pyridaphenthion-oxon and EPN-oxon, as reported previously (KONNO et al., 1986). Therefore, the effect of synergists on the toxicity of these oxons was examined. As shown in Table 2, K-2, DEF, IBP and EBP were all effective as syner-

gists for fenitroxon against the Hata strain, but the degree of the synergism of fenitroxon was lower than that of fenitrothion. K-2 and DEF also synergized various oxon analogs of phosphorothioates and phosphonothioate against the Hata strain. K-2 and DEF had no effect on the toxicity of fenitroxon and other oxons against the S strain.

It is now generally accepted that insecticide synergists act by blocking the enzymes effecting insecticide detoxication. Therefore, the mecha-

Table 2. Synergism of various oxon analogs of OP insecticides by several chemicals against the Hata and susceptible strains of rice stem borers

Chemicals ^a	LD ₅₀ (μ g/g)	Synergistic ratio
Hata strain		
Fenitroxon alone	41.3	—
+ K-2	6.7	6.2
+ DEF	8.4	4.9
+ IBP	27.5	1.5
+ EBP	25.8	1.6
Methyl paraoxon alone	7.2	—
+ K-2	1.8	4.0
+ DEF	2.3	3.1
Diazoxon alone	17.1	—
+ K-2	8.6	2.0
+ DEF	7.4	2.3
Pyridaphenthion-oxon alone	27.1	—
+ K-2	3.8	7.2
+ DEF	4.0	6.8
EPN-oxon alone	21.6	—
+ K-2	4.5	4.8
+ DEF	9.4	2.3
Susceptible strain		
Fenitroxon alone	1.2	—
+ K-2	1.3	0.9
+ DEF	1.2	1.0
Methyl paraoxon alone	0.5	—
+ K-2	0.5	1.0
+ DEF	0.6	0.8
Diazoxon alone	3.2	—
+ K-2	4.4	0.7
+ DEF	4.0	0.8
Pyridaphenthion-oxon alone	2.3	—
+ K-2	3.6	0.6
+ DEF	2.4	1.0
EPN-oxon alone	1.0	—
+ K-2	1.1	0.9
+ DEF	1.0	1.0

^a Oxon + synergist (1:10 ratio)

nism of resistance caused by detoxication can be presumed by the type of synergist. In this study, fenitrothion was indicated to be synergized by K-2, K-1, DEF, IBP and EBP, but antagonized by PB and S-421 in the Hata strain. Specific inhibitors of carboxylesterase such as TPP and TOCP were not synergistic. Synergists of fenitrothion were also effective for various oxons. These findings suggested that the resistance mechanism of the Hata strain is attributable not to oxidative, but to hydrolytic degradation of a phosphotriester bond. DEF and IBP have been shown to suppress the resistance of parathion, methyl parathion, methyl paraoxon, and temephos in the house fly and *Culex tarsalis* (PLAPP and TONG, 1966; APPERSON and GEORGHIOU, 1975; HEMINGWAY and GEORGHIOU, 1984). The synergistic effect by these synergists is normally considered as indicative of the involvement of esterases or phosphotriester hydrolases in the insecticide metabolism. K-2 has been shown to be an inhibitor of esterase-mediated parathion resistance in *Myzus persicae* (OPPENORTH and VOERMAN, 1975).

Among compounds tested as synergists, K-2 and DEF were the most effective for fenitrothion. However, neither synergist is capable of increasing the susceptibility of the Hata strain to the level of that in the S strain. In order to overcome the OP insecticide resistance of the Hata strain, a study to determine and synthesize new synergists is now in progress.

We thank Dr. J. FUKAMI, Dr. K. USUI and Mr. K. UCHIUMI in the Institute of Physical and Chemical Research for their valuable advice and supplying the susceptible strain of rice stem borer.

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