ificity inherent in enzyme-substrate reactions and should therefore be useful as a cytochemical technique.

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1 April 1968

Propanil Hydrolysis: Inhibition in Rice Plants by Insecticides

Abstract. *Hydrolysis of the herbicide propanil (3',4'-dichloropropionanilide) by rice plants is inhibited by insecticides. The inhibitory activity of an organophosphate such as paraoxon in vivo and in vitro is significantly stronger than that of an organothiophosphate such as parathion. The injury to rice plants by insecticides sprayed on them with propanil seems to be caused by the inhibition of the propanil detoxifying enzyme.*

Propanil (3',4'-dichloropropionanilide) is a highly selective herbicide which discriminates between rice and certain weed plants, especially barnyard grass (*Echinochloa crusgalli*). There is reason to believe that the tolerance of rice plants to propanil, and hence its selectivity, rests on the ability of the rice plant to metabolize or detoxify the chemical. McRae *et al.* (*1*), Adachi *et al.* (*2*), and Ishizuka *et al.* (*3*) reported the existence of an enzyme capable of hydrolyzing the anilide into 3,4-dichloroaniline and propionic acid in rice plant homogenates. Still and Kuzirian (*4*) have described the metabolism in more detail. The purification and properties of a rice aryl acylamidase (aryl-acylamine amidohydrolase, E.C. 3.5.1.13) which could hydrolyze the anilide have been reported recently (*5*).

In paddy fields the combination of certain insecticides with propanil causes injury to rice plants (*6*). Both organophosphorus insecticides and carbamate insecticides extremely enhance the herbicidal activity of propanil against rice. Since propanil is used as a selective herbicide on rice plants, the enhancement of toxicity by insecticides used to protect the rice is very important in crop management. However, the synergistic action of the insecticides with propanil is being utilized in a combination of propanil and carbaryl for the control of crabgrass (*Digitaria adscendens*) in citrus orchards in Japan. This combination takes advantage of the fact that the insecticide inhibits the high enzymatic activity of the crabgrass, thus permitting action of the propanil.

Two different types of experiments were performed to investigate the mechanism of the interaction of the organophosphorus and the carbamate insecticides in the phytotoxicity of propanil.

A homogenate was prepared from rice plants at the five-leaf stage (Variety: Nôrin 29) by grinding 5 g of fresh tissue with a small amount of 0.05*M* phosphate buffer (*p*H 7.0) with 10 g of quartz sand for 15 minutes. The resultant paste was diluted with additional buffer to 50 ml, filtered through two layers of cheesecloth, and centrifuged at 1000*g* for 10 minutes. The supernatant was then used as the enzyme solution. The reaction was carried out with 5 ml of enzyme solution, 5 ml of 0.1*M* phosphate buffer (*p*H 8.3), and 5 ml of $1.5 \times 10^{-4}M$ propanil and inhibitors. Initially both propanil and insecticides were dissolved in ethanol. The final concentration of ethanol in the reaction mixture was lower than 0.33 percent. At the end of 2 hours at 30°C, the reaction was stopped by the addition of 1.5 ml of cold 50 percent trichloroacetic acid, the mixture was centrifuged, and the supernatant was used for determination of 3,4-dichloroaniline by the method of Goto and Sato (*7*). The inhibitors used in this study included parathion, paraoxon, sumithion (fenitrothion), and sumioxon (Fig. 1). The concentrations required to produce 50 percent inhibition were calculated (Table 1).

The inhibitory patterns for both the propanil-hydrolyzing enzyme in rice plants and the acetylcholinesterase in insects are very similar. It is speculated, therefore, that the propanil detoxifying enzyme and acetylcholinesterase may resemble each other. Carbaryl (1-naphthyl-*N*-methylcarbamate), an insecticide which inhibits acetylcholinesterase, is also a strong inhibitor of the propanil detoxifying enzyme (*5*). On the other hand, BHC (benzene hexachloride), an insecticide having no effect on acetylcholinesterase also had no effect on the rice enzyme. In the

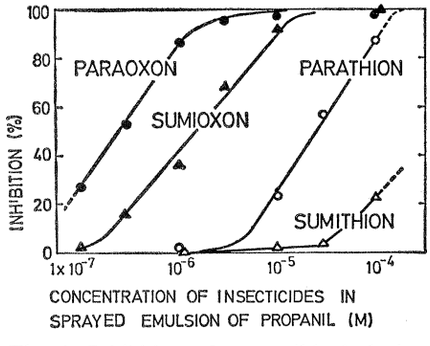

Fig. 1. Inhibition of propanil-hydrolyzing enzyme in rice plants by insecticides.

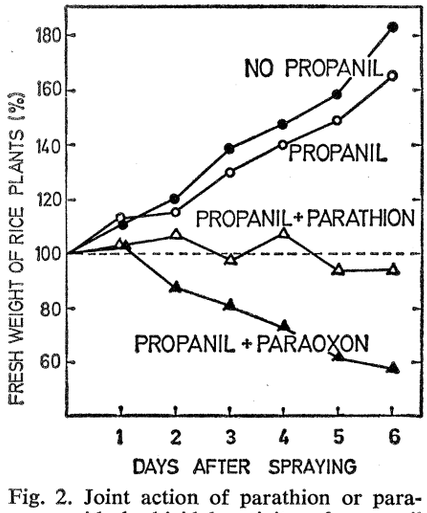

Fig. 2. Joint action of parathion or paraoxon with herbicidal activity of propanil in rice plants.

Table 1. Concentrations of organic phosphate insecticides required for 50 percent inhibition of propanil-hydrolyzing enzyme in rice plants (PHE) and acetylcholinesterase (AChE.).

Compound	Concentration (mole/liter) for 50 percent inhibition of	
	PHE	AChE
Parathion	2.4×10^{-5}	2.0×10^{-5} (*9*)
Paraoxon	2.4×10^{-7}	2.0×10^{-9} (*9*)
Sumithion	3.4×10^{-4}	2.3×10^{-4} (*10*)
Sumioxon	1.5×10^{-6}	1.17×10^{-7} (*10*)

case of insects, the conversion of parathion to the more toxic paraoxon is inhibited by such materials as piperonyl butoxide, sulfoxide, and others (*8*). In the case of the rice enzyme, piperonyl butoxide had no significant effect on the inhibitory activity by parathion to the propanil detoxifying enzyme.

In the second experiment, rice plants were cultivated in a 500-ml plastic beaker filled with rice paddy soil, treated wtih 100 mg of ammonium sulfate per pot. Fifteen rice seeds were sown in each pot, covered with 2 mm of fine soil, and then incubated in a growth chamber at 30°C for 12 hours and 20°C during the night. To prevent flowering, two fluorescent lamps were maintained even at night. When rice reached the 2½-leaf stage, an emulsion of propanil at 0.3 percent was applied. Before dilution of the herbicide the insecticides were added to the emulsifiable concentrate of propanil at one-tenth the concentration of the propanil itself. As a further check, plants were treated with a diluted solution of the emulsifying agent and solvents used in formulating the propanil itself. Daily measurements were made of the fresh weight of the rice plants (Fig. 2).

The fresh weight of the rice plants was expressed as a percentage of the initial fresh weight at the time of treatment. Both the parathion and paraoxon used in this experiment enhanced the phytotoxicity of propanil toward rice plants. The paraoxon was significantly greater in its effect than parathion. At the termination of the experiment, plants treated with a combination of propanil and parathion still showed evidence of growth in that they formed new leaves, whereas the plot with propanil plus paraoxon was nearly completely dead.

Sumioxon, the oxygen analog, also had greater activity than sumithion, the parent compound. On the other hand, insecticides having no activity against acetylcholinesterase did not enhance phytotoxicity (*6*).

The two experiments described above may suggest some similarities between acetylcholinesterase and the propanil-hydrolyzing enzyme in rice plants. The inhibition of the propanil hydrolyzing enzyme by the organophosphorus and carbamate insecticides would appear to account for the phytotoxicity of the combination of propanil and insecticide to rice, since the tolerance of rice plants to the herbicide appears dependent upon hydrolysis.

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4 March 1968

Sleep Patterns of the Monkey and Brain Serotonin Concentration: Effect of p-Chlorophenylalanine

Abstract. *The amount of time that monkeys* (Macaca mulatta) *slept was reduced after they were given* p-*chlorophenylalanine, a selective depletor of serotonin in animal tissues. The time spent in the rapid eye movement stage of sleep was unchanged, but the time in other sleep stages decreased. Seven regions of the brain had a 31 to 46 percent decrease in serotonin content; the concentration of cerebellar serotonin increased by 44 percent.*

Advances in the understanding of the physiology of sleep have led to the idea that there are two alternating, physiologically distinct sleep patterns during the normal sleep period of mammals (*1*). By behavioral, physiologic, electroencephalographic, and autonomic measurements, a short-term cycle of recurring patterns can be defined. Because of the general occurrence of this sleep cycle of rapid eye movement and nonrapid eye movement (REM-NREM) and because of studies implicating chemical control in the central nervous system (CNS) of these sleep states, a variety of naturally occurring substances, particularly presumed CNS transmitters, have been investigated (*2*). The demonstration that major alterations of the sleep patterns of man and animals occur when drugs are administered which affect the monoaminergic neurons has led investigators to postulate that serotonin and norepinephrine participate in the control of the sleep-waking cycle (*3*).

p-Chlorophenylalanine, which has been shown to be a potent and selective depletor of serotonin in animal tissues including the brain, acts by inhibiting hydroxylation of tryptophan, the first and rate-limiting reaction in the biosynthesis of serotonin (*4*). We now report the effects of administering *p*-chlorophenylalanine on sleep patterns (*5*) and on the regional concentrations of serotonin in brain tissue in monkeys

Silver disk electrodes for recording electroencephalographic (EEG) activity were placed on the dura mater overlying the brain of each monkey at six sites (through burr holes in the skull) and cemented in place with acrylic dental cement. Three additional electrodes were placed subcutaneously just lateral to the orbit overlying the temporalis muscle, bilaterally and above one eye. These recorded eye movements and activity of the temporalis muscle. The wires from the electrodes were attached to a connecting plug cemented to the skull. The monkeys, maintained in a restraining unit, were kept awake during the day and allowed to sleep from 10:00 p.m. to 6:00 a.m. During this sleep period, continuous EEG activity, eye movements, and temporalis muscle activity were recorded on an Offner model T machine. Direct observations of facial and eye movements were also