ORGANOPHOSPHATE PESTICIDES AND DMSO AFFECT MYCELIAL GROWTH AND PHOSPHATIDYLCHOLINE BIOSYNTHESIS IN AN ENTOMOPATHIC FUNGUS

David C. Ross and U. E. Brady
Entomology Department
University of Georgia
Athens, GA 30602
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ABSTRACT

The effect of three organophosphate pesticides, dichlorvos, edifenphos, and methyl parathion on growth of the entomopathic fungus, *Nomuraea rileyi* (Farlow) Samson was examined under laboratory conditions. Each of the 3 pesticides with dimethyl sulfoxide (DMSO) carrier reduced weight gain of mycelia by up to 54% of controls. Dichlorvos without DMSO solvent decreased weight gain by only 18%. These pesticides were also tested with and without DMSO (0.5 to 5%) for their effect on the biosynthesis of phosphatidylcholine (PC) in *N. rileyi* by monitoring transmethylation of ^{14}C-methyl from methionine into phosphatidylcholine (PC). Edifenphos had no effect at 1 ppm, but caused a 45% decrease in ^{14}C incorporation into PC at 10 PPM, and an 89% decrease at 100 PPM. Methyl parathion and dichlorvos decreased incorporation of ^{14}C only at 100 ppm. DMSO at a 5% concentration decreased weight gain of mycelia by 53% but increased ^{14}C labelling of PC by 70%. Methyl parathion and dichlorvos insecticides in combination with DMSO share a mode of action similar to that of the fungicide edifenphos in *N. rileyi* although expressed to a lesser degree. The action of DMSO, on both mycelial growth and biosynthesis of PC appears different from that of these organophosphates.

Key Words: *Nomuraea rileyi,* organophosphate pesticide, phosphatidylcholine.

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INTRODUCTION

The entomopathic fungus, *Nomuraea rileyi* (Farlow) Samson, causes natural epizootics in at least 6 Lepidopterous species of soybean pests (Puttler et al. 1975). However, the use of chemical pesticides may inhibit the effectiveness of this pathogen (Ignoffo et al. 1975). It is well known that some insecticides and acaricides have adverse effects on entomogenous fungi (Wilding 1972; Ignoffo et al. 1975; Roberts and Campbell 1977; Hall 1981) with the organophosphate pesticides being particularly deleterious. Ignoffo et al. (1975) found that of 9 OP insecticides tested at recommended rates, all inhibited growth of *N. rileyi* to some extent, with methyl parathion being most active.

In studies on their mode of action, Kodama et al. (1979 and 1980) showed that the OP fungicides IBP and edifenphos inhibit phosphatidylcholine (PC) biosynthesis in the rice blast fungi, *Pyricularia oryzae.* The point of inhibition is the transmethylation to phosphatidylethanolamine (PE) of the methyl group of S-adenosyl methionine (SAM) to form PC, resulting in non-selective permeability of cell walls.

Our tests were designed to determine the effect of methyl parathion, dichlorvos, and edifenphos in combination with dimethyl sulfoxide (DMSO) carrier on mycelial growth and the biosynthesis of PC in *N. rileyi.*

MATERIALS AND METHODS

Chemicals

Pesticides listed below were provided gratis by the producer: Dichlorvos (2,2-dichlorovinyl dimethyl phosphate, 99%), Shell Chemical Co., Houston, TX; edifenphos (O-ethyl S,S-diphenyl phosphorodithiolate, 88.6%), Mobay Chemical Co., Kansas City, MO; methyl parathion (O,O-dimethyl O-*p*-nitrophenyl phosphorothioate, 99.5%), Stauffer Chemicals, Mountain View, CA. Dimethyl sulfoxide (DMSO) and DL-β-phosphatidyl choline dipalmitoyl (PC) were purchased from Mallinckrodt Chemical Co., and Sigma Chemical Co. Maltose, neo-peptone, Sabouraud's maltose agar (SMA), and yeast extract (YE) were purchased from Difco Laboratories, Detroit, MI. L-[methyl-^{14}C] methionine (57.6 mCi/mmol) was from Amersham International (Amersham, UK). All water was distilled.

Fungal Culture and Preparation

Nomuraea rileyi spores originated from the Missouri strain (Ignoffo 1978). Spores were inoculated on SMA + 1% YE (Bell 1975) in 15 × 100 mm plastic petri plates and cultured at 25°C for 4 days. Microbial contaminated plates were discarded. Hyphi and adhering spores from pure cultures were inoculated into 200 ml nutrient broth (maltose — 40g, neo-peptone — 10g, water — to 1 liter water, Bell 1975) using sterile technique. Broth cultures were kept at 25°C and agitated daily for 1 week. Mycelia from 2 - 3 mats were rinsed in water, chopped with a razor blade to ca. 0.5 mm size, rinsed in water, and filtered prior to use (Kodama et al. 1979).

Experimental

Experimental procedures followed those used by Kodama et al. (1979).

Growth Inhibition

Mycelia (120 mg wet wt.; 19.9 mg dry wt.) were suspended in 5 ml 0.05 M phosphate buffer (pH = 7.0) + 1, 10, or 100 ppm organophosphate pesticide dissolved in 0.05, 0.5 or 5% DMSO (Draughon and Ayres 1981), respectively. Increasing levels of DMSO were utilized to dissolve increasing concentrations of pesticides. Controls contained 0, 0.05, 0.5, or 5% DMSO in 5 ml buffer with no pesticide. Only dichlorvos was tested with and without DMSO carrier. Mycelia were incubated in a Dubanoff metabolic incubator for 24 hours at 27°C and 100 motions/min, rinsed in water, filtered, dried at 145°C for 20 min, and weighed. Samples dried for more than 7.5 hours lost no additional weight.

Labelling of Phosphatidylcholine With ^{14}C-Methionine

Conditions were identical to those above with the following exceptions. L-[methyl-^{14}C] methionine (2 μCi) was added to 5 ml buffer prior to mycelial suspension. Mycelia were incubated for 2 hours, rinsed in water, filtered, and homogenized in 12 ml methanol: chloroform (2:1) for 30 sec on ice.

Extraction of Lipids

The lipid extraction procedure followed that of Bligh and Dyer (1959). Lipids were extracted by adding 4 ml chloroform and homogenizing for 15 sec. Homogenates were centrifuged for 5 min, and the pellets were rehomogenized in 6 ml chloroform:methanol (1:1) for 15 sec, centrifuged, and the extracting liquids combined. The extract was separated into 2 phases by centrifugation following the addition of 8 ml water. One ml each of the aqueous and chloroform phases and incubation media was added to 10 ml Beckman "Universal" scintillation fluid (2,5-diphenyloxazole-5g/l, naphthalene-100g/l, dioxane-balance of liter), and counted for ^{14}C by liquid scintillation counting (LSC).

Isolation of Phosphatidylcholine

PC was isolated by thin layer chromatography (TLC) on silica gel (Whatman LK5D, 250 μ thickness) in chloroform: methanol:10% ammonium hydroxide (70:35:5) (Kodama et al. 1979). PC was visualized by exposure to I_2 vapor and autoradiography, and identified by co-chromatography with 20 μg of PC standard, All PC spots were collected and counted in 10 ml of scintillation fluid. Counts per min (CPM) were converted to disintegrations per min (DPM) for the total sample with a computer program (written by D. C. Ross) using the external standard procedure for determining % counting efficiency.

RESULTS AND DISCUSSION

Growth Inhibition

Dichlorvos without DMSO did not cause statistically significant inhibition of growth of *N. rileyi* at the three rates tested compared to controls with neither dichlorvos nor DMSO (Table 1). Dichlorvos in combination with DMSO significantly inhibited growth compared to DMSO controls and DMSO-free controls. We conclude that the effect on fungal growth by dichlorvos + DMSO is due primarily to the solvent.

Both 100 ppm methyl parathion and edifenphos in 5% DMSO caused a highly significant degree of inhibition of growth compared to 5% DMSO alone. Dry weight of *N. rileyi* mycelia increased in 24 hrs by $9.4 \pm 0.9\%$ and $18.9 \pm 2.9\%$ when treated with 100 ppm methyl parathion and edifenphos respectively in 5% DMSO, compared to $47.7 \pm 6.3\%$ dry matter increase with 5% DMSO alone. However, in the absence of data for the effect of methyl parathion and edifenphos without DMSO, we conclude that this growth inhibitory effect is due to an interaction of DMSO and pesticide.

Draughon and Ayres (1981) showed that 100 ppm dichlorvos inhibited growth of *A. parasiticus* by 29% while inhibiting aflatoxin production by 99% compared to DMSO solvent controls. Naled, at 100 ppm in the same system, inhibited mycelial growth by 100% while malathion and diazinon at 100 ppm demonstrated no inhibition. Naled also inhibited mycelial growth and zearalenone production in *Fusarium graminearum* (Berisford and Ayres 1976). Ignoffo et al. (1975) found methyl parathion applied at recommended rates was an effective inhibitor of *N. rileyi* growth, but not as effective as 6 fungicides. Hall (1981) showed that diazinon and malathion applied at recommended rates would inhibit *Verticillium lecanii* (Zimm) Viegas germination by 100%, and mycelial growth by 65%. The effect of OPs on

Table 1. Percent increase in weight of *Nomuraea rileyi* treated with dichlorvos and DMSO.*

Treatment	Concentration of dichlorvos (ppm) and DMSO (%)			
Dichlorvos	0	1.0	10.0	100.0
DMSO	0	0.05	0.5	5.0
	% increase in dry weight of *N. rileyi*			
Control	101.3 ± 13.2 a†	119.9 ± 9.5 a	131.6 ± 12.0 a	47.7 ± 6.3 b
(+ DMSO)	a	a	a	a
Dichlorvos	101.3 ± 13.2 a	85.1 ± 12.3 b	82.1 ± 10.7 b	31.3 ± 3.1 c
(+ DMSO)	a	b	b	c
Dichlorvos	101.3 ± 13.2 a	85.8 ± 5.1 a	79.9 ± 3.5 a	83.4 ± 8.3 a
(− DMSO)	a	b	b	b

* Data expressed as % increase in weight at 24 hrs post treatment. Means of 3 replications ± SEM. 120 mg (wet wt.) (19.9 mg dry wt.) mycelia incubated in 5 ml 0.05 M phosphate buffer, pH = 7.0 (Kodama et al. 1979) + 0, 1, 10, or 100 ppm pesticide and 0, 0.05, 0.5, or 5% DMSO (0, 2.5, 25, and 250 μl respectively); held for 24 hours at 27°C and 60 motions/min.; mycelia dryed at 145°C for 20 min.

† Means with same letter are not significantly different from each other (upper letter — rows, lower letter — columns); Duncan's new multiple range test, $p = 0.05$.

growth of mycelia of *N. rileyi* as well as the efficacy of OP treated fungi to infect insects warrant further investigation.

Inhibition of Phosphatidylcholine Synthesis

No significant inhibition of ^{14}C-methionine incorporation into the chloroform or aqueous soluble fractions or into PC was observed at 1 ppm OP and 0.05% DMSO (Table 2). At 10 ppm OP and 0.5% DMSO, only edifenphos caused a significant inhibition of incorporation of ^{14}C into PC and into the chloroform and aqueous soluble fractions (Table 3). Dichlorvos without DMSO stimulated incorporation of ^{14}C into PC as well as increased levels of ^{14}C in chloroform

Table 2. Effect of 1 ppm organophosphate pesticides and 0.05% DMSO on incorporation of [^{14}C-methyl] methionine in the fungus, *Nomuraea rileyi*.*

Treatment	Chloroform Phase	Aqueous Phase	Incubation Media	PC†
	% of control DPMs			
Edifenphos	86 ± 9.1‡	106 ± 11.2	102 ± 0.5	102 ± 7.5
(+ DMSO)				
M. parathion	98 ± 4.4	112 ± 9.4	103 ± 1.1	84 ± 9.0
(+ DMSO)				
Dichlorvos	101 ± 5.1	111 ± 6.5	102 ± 0.5	96 ± 2.5
(+ DMSO)				
	DPM's (× 1000)			
Control	80 ± 16	81 ± 10.5	3,087 ± 121	36 ± 2.0
(+ DMSO)				

* 120 mg (wet wt) washed mycelia incubated in 5 ml 0.05 M phosphate buffer, pH = 7.0 (Kodama et al. 1979) + 2 μCi [methyl-^{14}C] methionine + 10 ppm OP pesticide in with or without (−) 0.5% DMSO; held for 2 hours at 27°C and 60 rpm; extracted in chloroform: methanol (1:1).

† PC (phosphatidylcholine) isolated by TLC on silica gel in chloroform: methanol:10% ammonium hydroxide (70:35:5). DPM = disintegrations per minute.

‡ Means of 3 replications ± SEM; no means were significantly different from others, ANOVA, $p = 0.05$.

Table 3. Effect of 10 ppm organophosphate pesticides and 0.5% DMSO on incorporation of [^{14}C methyl] methionine in the fungus, *Nomuraea rileyi*.*

Treatment	Chloroform Phase	Aqueous Phase	Incubation Media	PC†
	% of control DPMs			
Edifenphos (+ DMSO)	38 ± 1.4 b‡	69 ± 1.8 b	98 ± 2.0 a	55 ± 3.9 b
M. parathion (+ DMSO)	92 ± 4.0 a	83 ± 0.7 ab	102 ± 1.0 a	97 ± 2.1 a
Dichlorvos (+ DMSO)	113 ± 1.7 a	127 ± 4.5 c	97 ± 0.1 a	99 ± 3.6 a
Dichlorvos (− DMSO)	159 ± 13.5 c	174 ± 5.1 c	101 ± 1.4 a	123 ± 3.0 c
	DPM's (× 1000)			
Control (+ DMSO)	115 ± 6.1 a	134 ± 3.2 a	3,391 ± 17.0 a	58 ± 1.9 a
Control (− DMSO)	106 ± 17.2 a	106 ± 18.3 b	3,249 ± 80.4 a	58 ± 2.3 a

* 120 mg (wet wt) washed mycelia incubated in 5 ml 0.05 M phosphate buffer, pH = 7.0 (Kodama et al. 1979) + 2 μCi [methyl-^{14}C] methionine + 10 ppm OP pesticide in with or without (−) 0.5% DMSO; held for 2 hours at 27°C and 60 rpm; extracted in chloroform: methanol (1:1).

† PC (phosphatidylcholine) isolated by TLC on silica gel in chloroform: methanol:10% ammonium hydroxide (70:35:5). DPM = disintegrations per minute.

‡ Means of 3 replications ± SEM. Means in columns with the same letter are not significantly different from each other, Duncan's multiple range test, $p = 0.05$.

and aqueous fractions (Tables 3 and 4). Dichlorvos at 100 ppm with DMSO decreased the amount of ^{14}C-methyl transfer compared to appropriate controls (Table 4).

Results of this study and those reviewed by Roberts and Campbell (1977) demonstrate the adverse effect many OP insecticides have on entomopathic fungi, but their mode of action is not known. Research on OP mode of action in fungi has previously concerned mainly fungicides. Maeda et al. (1970) showed IBP inhibits incorporation of ^{14}C-glucosamine into cell walls of *P. oryzae*, and de Waard (1972) showed that edifenphos inhibits incorporation of ^{14}C-glucosamine into mycellium of the same fungus. Leighton et al. (1981) demonstrated that IBP inhibits chitin synthetase in a cell free *Phycomyces* system ($I_{50} = 4.7 \times 10^{-4}$ moles/liter).

The action of edifenphos and IBP on chitin synthesis, however, may be a secondary effect. De Waard (1972) stated that the fungitoxicity of edifenphos was due to its effect on cell membrane permeability, while its inhibition of chitin synthesis was not primary to toxicity. Kodama et al. (1979 and 1980) showed that IBP and edifenphos inhibit incorporation of N-methyl from SAM into PC of *P. oryzae*. The 50% inhibitory concentration (I_{50}) of edifenphos for chitin synthetase inhibition was 146 ppm, while the I_{50} for inhibition of both mycelial growth and N-methyl transferase was 13 ppm.

The mode of action of edifenphos in *N. rileyi* appears to be similar to its effect in *P. oryzae*. It inhibits N-methyl transfer from S-adenosyl methionine in *N. rileyi* with an I_{50} of 14.6 ppm (Tables 3 and 4). Methyl parathion inhibits *N. rileyi* mycelial growth as well as edifenphos, but inhibits methyl transfer only about half

Table 4. Effect of 100 ppm organophosphate pesticides and 5% DMSO on incorporation of [^{14}C methyl] methionine in the fungus, *Nomuraea rileyi*.*

Treatment	Incubation Media	PC†
	% of control DPMs	
Edifenphos (+ DMSO)	111 ± 0.5 bc‡	11 ± 0.8 d§
M. parathion (+ DMSO)	114 ± 1.3 c	52 ± 1.9 c
Dichlorvos (+ DMSO)	104 ± 2.8 a	80 ± 10.1 ab
Dichlorvos (− DMSO)	100 ± 0.7 b	120 ± 7.0 bc
	DPM's (× 1000)	
Control (+ DMSO)	1,625 ± 57 a	102 ± 11 a
Control (− DMSO)	1,716 ± 25 bc	60 ± 7 bc

* 120 mg (wet wt) washed mycelia incubated in 5 ml 0.05 M phosphate buffer, pH = 7.0 (Kodama et al. 1979) + 2 μCi [methyl-^{14}C] methionine + 100 ppm OP pesticide in with or without (−) 5% DMSO; held for 2 hours at 27°C and 60 rpm; extracted in chloroform: methanol (1:1).

† PC (Phosphatidylcholine) isolated by TLC on silica gel in chloroform:methanol:10% ammonium hydroxide (70:35:5). DPM = disintegrations per minute.

‡ Means of 3 replications ± SEM. Means in columns with the same letter are not significantly different from each other, Duncan's multiple range test, $p = 0.05$.

§ Significantly different at $p = 0.01$.

as well. Another mechanism of action such as inhibition of chitin synthesis by OP (Leighton et al. 1981) may play a role in the fungitoxicity of methyl parathion.

These results support earlier reports by Ignoffo et al. (1975), Kodama et al. (1979 and 1980), and Roberts and Campbell (1977), that some insecticides exhibit a degree of fungicidal activity. Results of this study suggest that toxic action may be due in part to inhibition of PC synthesis.

DMSO Effects

DMSO at 5% is an effective inhibitor of growth of mycelia, but contrary to the effect of edifenphos and methyl parathion, DMSO increases N-methyl transfer in the formation of PC (Table 4).

Draughon and Ayres (1981) reported no DMSO effect when used to solubilize insecticides in studies on their effect on aflatoxin production in *Aspergillus parasiticus*. Conversely, de la Torre (1983) cites many effects of DMSO on phospholipid and prostaglandin metabolism in mammals. In particular, DMSO inhibits phosphodiesterases and appears to aid in membrane stabilization. Tapiero et al. 1983 showed that 20% DMSO increased the level of PC by 10.8% in Friend leukemia cells, while decreasing the level of phosphatidylethanolamine by 7.7%. These factors may explain the increased labelling of PC in the present investigation following DMSO treatment, by reduced phospholipid turnover and increased biosynthesis.

Prospectus for Pest Management

OP insecticides such as methyl parathion have in many cases been applied without regard to effects on beneficial organisms and may negate potential benefits (Ignoffo et al. 1975) if applied during periods of elevated fungal activity. The present study indicates that low concentrations of OPs are compatible with *N. rileyi* and some, like dichlorvos may increase growth of mycelia. However, our results with higher concentrations of methyl parathion and edifenphos, and the results of previously published experiments (Ignoffo et al. 1975 and Hall 1981) indicate a need for caution in choosing chemicals in pest control.

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