Pesticide Biochemistry and Physiology 69, 153–165 (2001)
doi:10.1006/pest.2000.2527, available online at http://www.idealibrary.com on IDEAL®

Evidence for Direct Neural Toxicity of a "Light" Oil on the Peripheral Nerves of Lightbrown Apple Moth

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Received June 21, 2000; accepted November 3, 2000

The mode of action of petroleum oils on insects is usually assumed to be suffocation due to blocked spiracles. However, Citrus Postharvest Dip, a formulated C15 alkane used by Australian citrus packers to control surface pests, can also affect the neural activity of lightbrown apple moth (LBAM), *Epiphyas postvittana* Walker, (Lepidoptera: Tortricidae). The alkane penetrates deep into the tracheoles and absorbs onto nerve membranes, apparently causing direct nervous disruption. In electrophysiological experiments, Citrus Postharvest Dip in the ganglia induced a rapid onset of multiple nerve firing in peripheral nerves of LBAM larvae. Repetitive firing after exposure to the C15 alkane or surfactants used in the formulation showed that either of the components of Citrus Postharvest Dip can similarly affect the nerves. Nervous disruption by the oil is unlikely to be due to specific chemical binding. Assays with bovine acetylcholine esterase showed no specific inhibition of that enzyme using high oil doses (1%) and long incubation times (15 h). It is proposed that oils displace the protective lipids by their solvent action, affecting nerve activity by increasing membrane permeability to ion exchange. One of the major mechanisms of pyrethroid resistance in insects is reduced neuronal sensitivity. A role for alkanes in overcoming insecticide kdr-like resistance to pyrethroids is proposed.

INTRODUCTION

Petroleum spray oils have been used to control insect pests for over 100 years. Spray oils are considered to act directly on insects by blocking the spiracles and causing suffocation (1). More recent studies, using an alkane on lightbrown apple moth, *Epiphyas postvittana* Walker (LBAM),[1] indicate that some oils may have modes of action other than suffocation (2). Ampol Citrus Postharvest Dip (CPD), a formulated C15 alkane, is used by citrus packing sheds in Australia to control surface pests of quarantine significance. CPD is more efficacious than petroleum spray oils when applied as a dip (2) and causes a rapid "knockdown" of LBAM larvae within minutes of exposure (3). Kerosene vapors have a "knockdown" effect on many insects (4) after the vapors, consisting of alkanes below decane, enter the respiratory system (5), but higher analogues, such as CPD (C15), do not have sufficient volatility to show fumigant action (6, 7). Once oils have penetrated into the tracheal system they may diffuse into the hemolymph through the walls of the tracheae. Diffusion of dyed oils through tracheal walls and into the hemolymph has been observed in a number of insects (8, 9). Oil entering the hemolymph preferentially lodges in lipid-containing tissues in close connection with the tracheoles, including the nerve sheaths (10). Petroleum oils may absorb onto lipoprotein membranes and cause the disruption of critical nerve processes.

[1] *Abbreviations used:* LBAM, lightbrown apple moth; CPD, Citrus Postharvest Dip; C*n*, oil with *n*-paraffin carbon number; AChE, acetylcholine esterase; ATC, acetylthiocholine; mOD, mean optical density; LAR, larval activity rating; kdr, knockdown resistance.

0048-3575/01 $35.00

This study investigated the penetration and action of CPD on LBAM larval nervous tissue. Oil penetration through the tracheal system and nervous tissue were recorded using confocal microscopy. Nervous disruption due to oils was examined by measuring changes in spontaneous electrophysiological activity from peripheral nerves of larvae exposed to oil.

MATERIALS AND METHODS

Lightbrown Apple Moth Colony and Oil Formulations

The LBAM larvae used in the experiments were collected from a laboratory culture maintained at the South Australian Research and Development Institute, Waite Precinct (Adelaide). Singh, Clare, and Ashby (11) described the rearing procedure.

Ampol Research and Development Laboratories, Brisbane, Queensland supplied Citrus Postharvest Dip, Ampol CPD (an alkane with a carbon number of 15, i.e., C15; paraffin content i.e., %Cp $>$ 99%), and a commercial spray oil, Ampol DC-Tron NR (a narrow-range oil with mean equivalent *n*-paraffin carbon number of a C23 alkane (12)); (%Cp $<$ 70%). General specifications of CPD and DC-Tron can be found in Taverner *et al.* (2).

Ace Chemical Company (Camden Park, SA, Australia) supplied *n*-pentane (Cas No. 109-66-0; molecular weight, 72.15; density, $\approx$ 0.62; distillation range $\approx$ 34–37°C) which was used to assess the fumigant effect of a low-molecular-weight paraffin on LBAM larvae.

Symptomology of Oil-Dipped LBAM Larvae

LBAM larvae (5th instar) were dipped in either CPD or DC-Tron and the activity and coordination were assessed during a 4-h period. Larvae were dipped in 10,000 ppm oil emulsions as described in Taverner *et al.* (2). Briefly, groups of 10 larvae were immersed for 30 s in well-agitated oil/water emulsions. Control larvae were dipped in water. After dipping, larvae were placed in rearing containers in a controlled environment room at 20 $\pm$ 3°C, 55 $\pm$ 5% RH, and 14-h photoperiod (natural light). Symptoms were classified by the behavioral and physical changes following oil treatment and compared to controls. Larvae were also rated for their responsiveness and the ability to right themselves at predetermined intervals of 30 min and 1, 2, 3, and 4 h using criteria developed by Firko and Hayes (13). Larvae were counted as dead (at 24 h) if they did not move after repeated prodding with a needle.

Statistix 4.1 (14) was used for analysis of variance (ANOVA) and estimation of the standard error of the mean (SE). Mean separation was determined using the least significant difference method.

LBAM Larval Symptomology in Saturated Oil Atmospheres

Petroleum oils applied as contact insecticides may actually kill by the vapors entering the respiratory system. The lower range in the paraffin series, up to decane, shows moderate fumigant action and acts as narcotics (7). LBAM larvae (5th instar) were exposed to a saturated atmosphere of either *n*-pentane or CPD to determine symptomology during 4-h exposure. Groups of five larvae were placed in gauze cages and arranged on racks in plastic containers (260 $\times$ 190 $\times$ 60 mm) above 50 ml of oil. Control larvae were placed in containers without oil. The treatments were replicated three times. All containers with larvae were placed in incubators at 20°C. After exposure for 4 h the larvae were removed and placed in a room at 20 $\pm$ 3°C, 55 $\pm$ 5% RH, and 14-h photoperiod (natural light). The activity of the larvae was assessed at 30 min and 4 h exposure and 24 h after exposure. Larvae were rated for their responsiveness and the ability to right themselves using criteria developed by Firko and Hayes (13).

Staining and Microscopy of Tracheal and Nervous Tissue

Confocal microscopy was used to determine the location of fluorescent oil in larval structures, particularly the tracheal system and ganglia. Paraffins have negligible autofluorescence, so an

oil-soluble fluorescent dye, Fluorescent Yellow FG (Morton Chemical Co., Chicago, IL), was added at a rate of 1 and 10 ml/L to label oils used to study the tracheae and nerve ganglia, respectively. Emulsions were made using the fluorescent stock solution and deionized water. Larvae were dipped in labeled oil, emulsions, or water only as described in Taverner *et al.* (2). Larvae dipped in labeled oil emulsions were held in air for predetermined exposure times of 10 min and 2 h before being mounted on slides. To examine tracheae and cuticle, larvae were rinsed thoroughly after dipping and then mounted laterally on glass slides within a plasticine well. Glycerol was added and a glass coverslip pressed on the sides of the plasticine well until it rested against the cuticle. To view interactions of nervous tissue and oil, dissected ganglia were mounted on a glass slide in immersion oil with negligible fluorescence (Leitz) before adding a coverslip. Mounting in the immersion oil inhibited the desiccation of the ganglia.

A Bio-Rad MRC-1000 laser Scanning Confocal Microscope System in combination with a Nikon Diaphot 300 inverted microscope in fluorescence mode with excitation at 488/10 nm and emission at 522/32 nm was used. The images of the larvae and nerve ganglia were collected using a 20× NA 0.40 dry objective lens and 40× water lens. The confocal intensity settings used to capture an image of fluorescently labeled oil produced a faint image of tracheae of control larvae.

Electrophysiology of Oil-Dipped Larvae

Spontaneous activity from peripheral nerves was measured in muscles of the larval body walls using methods described by Gunning *et al.* (15). Briefly, 5th-instar LBAM larvae were pinned to a plasticine-coated dish and eviscerated by dorsal dissection, and the ventral body wall muscles were flooded with saline. A suction electrode picked up activity from the peripheral nerves and the preparation was grounded using a stainless steel insect pin. The recording electrode was connected to a preamplifier (Ilesworth, UK 101A). The signal was fed into a MacLAb System (ADInstruments, U.S.A.). Nerve action potentials were recorded and displayed using MacLab Scope v3.5 Software (ADInstruments) on an Apple Macintosh computer.

Initially, CPD was perfused directly over dissected larvae to allow direct contact with nerves. Subsequently, larvae were dipped in oil/water emulsions before dissection to determine whether oil could effectively translocate into the nervous tissue and elicit a response. The dipping method for oil emulsions was as described by Taverner *et al.* (2). At least four larvae were dipped per treatment and controls were dipped in water only. Oils without emulsifiers were highly agitated in water to ensure adequate mixing during dipping. At 10–20 min after dipping, larvae were dissected and spontaneous nerve activity was recorded over a 15-min interval. The number of action potentials (firings per min) were counted for at least two periods after each dissected larvae regained a stable resting state (>5 min after dissection). The frequency of nerve firing of all treated and untreated larvae were recorded and mean frequency (six recordings per treatment) were analyzed using one-way analysis of variance to determine the effects of oil on spontaneous nerve activity.

Sensitivity of AchE Activity to Oil

A number of insecticides, such as organophosphates, exhibit their toxic action by inhibiting certain important enzymes of the nervous system, such as cholinesterases. Although alkanes do not mimic the molecular shape of neurotransmitters or other enzyme substrates, it seems possible that they might inhibit key enzymes in some other way. Acetylcholine esterase (AchE) activity against a substrate, acetylthiocholine (ATC), was used to test the enzyme sensitivity to CPD. AchE solutions were prepared by adding 200 μl of 0.1 M, pH 7.5, sodium phosphate buffer, 0.01% egg albumen (0.1 mg/ml), and 0.4 units of pure bovine AchE source (0.2 mg/50 μl) to sterilized 1.5-ml microtubes. The microtubes were agitated and placed in ice until required. CPD (0.5 μl) was added to 50 μl of the AchE solution and incubated for 0, 0.5, 1, 1.5, 3, and 15 h. Control solutions contained no oil. Substrate

solutions consisted of 0.1 ml of 2.25 M ATC (substrate) solution, 0.5 ml of an indicator, dithionitrobenzene (DTNB) (Sigma Melbourne, Australia), and 9.4 ml phosphate buffer. After predetermined incubation periods, 50 μl of each AchE solution was added to separate rows of a 96-well microplate using a multichannel pipettor. Then 100 μl of substrate solution was added to each well and the microplate was placed in a Kinetic UV Max Microplate Reader set to 405 nm, Kinetic L1 mode, 10-s read interval, and 2-min run-time. The enzyme activity of AchE on ATC led to a reaction in DTNB, which produced a color change. The strength of the color change over time indicated enzyme activity.

AchE activity was measured by the mean optical density (mOD). Blanks using oil only + substrate and egg albumin only + substrate showed no significant change in optical density. Therefore, a comparison between treatments was made using the raw mOD/min data and analyzed using ANOVA.

RESULTS

Symptomology of Oil-Dipped LBAM Larvae

The larval activity rating (LAR) of LBAM larvae dipped in CPD was greatly reduced compared to that in DC-Tron (Table 1). DC-Tron-dipped larvae had slightly reduced coordination associated with a physical restriction of movement by the oil, whereas CPD-dipped larvae showed a very rapid loss of coordination and reduced activity more consistent with narcosis.

Symptomology of CPD-dipped larvae was as follows: larvae on removal from the oil emulsion were very flaccid and showed no spontaneous movement. During the next 30 min the abdominal segments became swollen and paralyzed. The anterior portion of the larva exhibited slow writhing when prodded and rapid twitching of prolegs. The cuticle began to darken after 2 h exposure. Dehydration was associated with the large spiracular openings of the 1st thoracic and 8th abdominal segments, which became pronounced 3 h after application. By 4 h, the hemolymph in some segments became blackened and there was no response to stimulation.

LBAM Larval Symptomology in Saturated Oil Atmospheres

Volatile components of oil, which are lipsoluble, are potentially narcotic. LBAM larvae held in a saturated atmosphere of *n*-pentane appeared very agitated, followed by ataxia and eventually paralysis, which is consistent with the succession of symptoms associated with narcotic vapors. All larvae held for 4 h in a saturated atmosphere were moribund and showed no recovery after being held for 24 h in air (Table 2). In contrast, LBAM larvae held in a sealed container with CPD for up to 24 h showed no loss of coordination or mortality, suggesting no direct fumigant action on the nerves.

Microscopy of Dyed Oil in Tracheal and Nervous Tissue

The autofluorescence of the tracheae and nervous tissue was faint when observed under the

TABLE 1
Progressive Larval Activity Rating (LAR) of LBAM Larvae (5th Instar) after Dipping in 10,000 ppm Oil Emulsions of DC-Tron or CPD

	Mean LAR[a] (SE)				
Trearment	30 min	1 h	2 h	3 h	4 h
Water	10.0 (0.0)a	10.0 (0.0)a	10.0 (0.0)a	10.0 (0.0)a	10.0 (0.0)a
DC-Tron	6.2 (0.9)b	5.5 (0.9)b	4.8 (0.9)b	4.5 (0.9)b	4.1 (0.9)b
CPD	1.9 (0.8)c	2.2 (0.6)c	1.5 (0.6)c	1.1 (0.7)c	0.6 (0.6)c

[a] Values are the mean larval activity rating of three replicates of 10 larvae. Values within a column followed by the same letter are not significantly different according to analysis of variance of the data ($P > 0.05$, least significant difference).

TABLE 2
Larval Activity Rating of LBAM Larvae (5th Instar) Exposed to *n*-Pentane and CPD Atmospheres at 30 min and 4 h and 24 h after Removal from 4-h Exposure

	Mean LAR[a] (SE)		
	Exposure		
Treatment	30 min	4 h	Recovery 24 h
n-Pentane	1.33 (0.33)a	0.00 (0.00)a	0.00 (0.00)a
CPD	10.00 (0.00)b	10.00 (0.00)b	10.00 (0.00)b

[a] Values are the mean larval activity rating of three replicates of five larvae. Values within a column followed by the same letter are not significantly different according to analysis of variance of the data ($P > 0.05$, least significant difference).

same sensitivity settings of the confocal microscope used to observe fluorescently labeled oils. Imaging of intact larvae dipped in labeled DC-Tron showed strong fluorescence confined to the main tracheal branches associated with spiracles (Fig. 1). Labeled CPD appeared to penetrate more extensively into the tracheal system (Fig. 2) than DC-Tron. Optical cross sections of tracheae dipped in CPD revealed that, rather than blocking the upper tracheae, the oil coated them (Fig. 3) and fluorescence associated with very small tracheoles (1–2 μm) suggested that oil flowed deep into the tracheal system (Fig. 4).

Tracheoles have a very strong association to certain tissues, including nerve tissue. Nerve ganglia removed from 5th-instar LBAM larvae dipped in 15 ml/L CPD emulsions revealed strong fluorescence in the tracheoles leading to ganglia and inside the ganglia themselves (Fig. 5). Penetration into the nervous tissue was very rapid, with fluorescence detected in ganglia 10 min after exposure to oil dips.

Electrophysiology of Oil-Dipped Larvae

Spontaneous nerve activity was measured using the body wall tissue of oil-dipped and water-dipped LBAM larvae. Nerve activity in untreated larvae was initially erratic, but became more stable after 5 min. Superfusion of CPD directly over the body wall muscles of larvae induced an increase in activity 5 min after exposure (Fig. 6).

A comparison of larvae dipped in various concentrations of CPD shows an increase in the frequency of action potentials for concentrations above 200 ppm compared to control larvae (Table 3). The frequency of the action potentials in larvae dipped in CPD changes, with rapid multiple nerve firings and long trains of high-amplitude spikes lasting many seconds (Fig. 7). This was recorded in the peripheral nerves 20 min after intact larvae were dipped in CPD, demonstrating that oil rapidly translocated into nervous tissue to alter the pattern of activity.

CPD is predominantly a C15 alkane, but it also contains small volumes (<10% vol/vol) of nonionic surfactants. To isolate the effects of each component, larvae were dipped in either C15 oil alone or surfactants alone to assess their individual effects. The deposit of the C15 alkane was difficult to control as emulsification could be achieved only by rapid agitation of the solution. Electrophysiological recordings suggested that larvae treated with the C15 alkane increased the frequency of action potentials compared to control larvae (Figs. 8A and 8B). High doses of surfactants (10,000 ppm) also induced a response demonstrating that surfactants can reach and disrupt nervous tissue in dipped larvae (Fig. 8C). However, the surfactant levels in CPD are less than 10% of the total volume. Lower concentrations of surfactant (1000 ppm) that reflect the proportion of surfactant found in an efficacious dose of CPD had no effect on the frequency of the action potentials (Fig. 8D). It is, therefore, unlikely that the levels of surfactant in efficacious concentrations of CPD (10,000 ppm) are primarily responsible for effects on the nervous tissue. Any synergistic interaction of

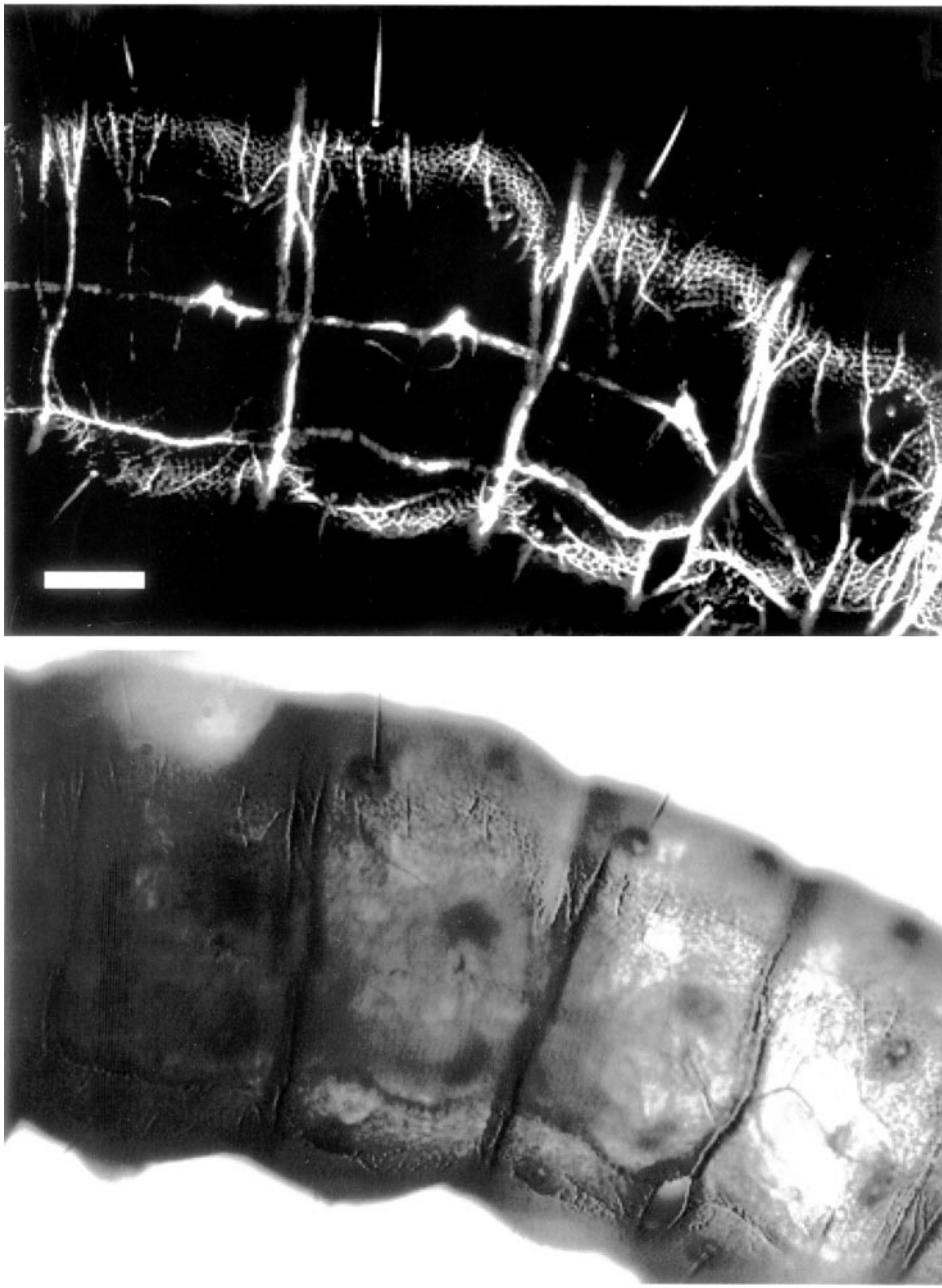

FIG. 1. Lateral view of LBAM larvae dipped in fluorescently labeled DC-Tron showing fluorescence of main tracheal branches associated with the spiracles (top) and transmission image (bottom). Scale bar, 200 μm.

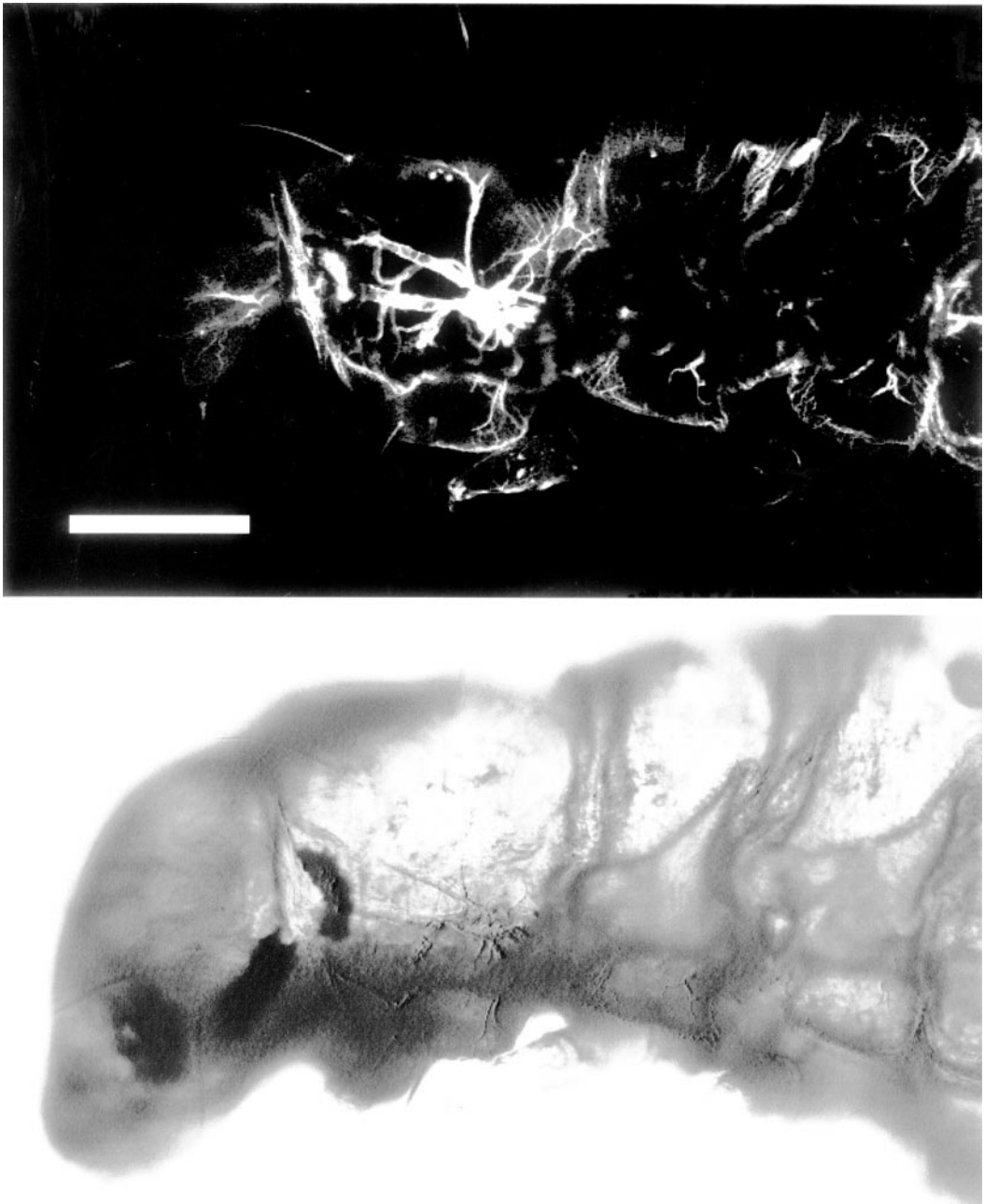

FIG. 2. Lateral view of LBAM larvae dipped in fluorescent CPD showing extensive fluorescence of tracheae of head and thorax (top) and transmission image (bottom). Scale bar, 100 μm.

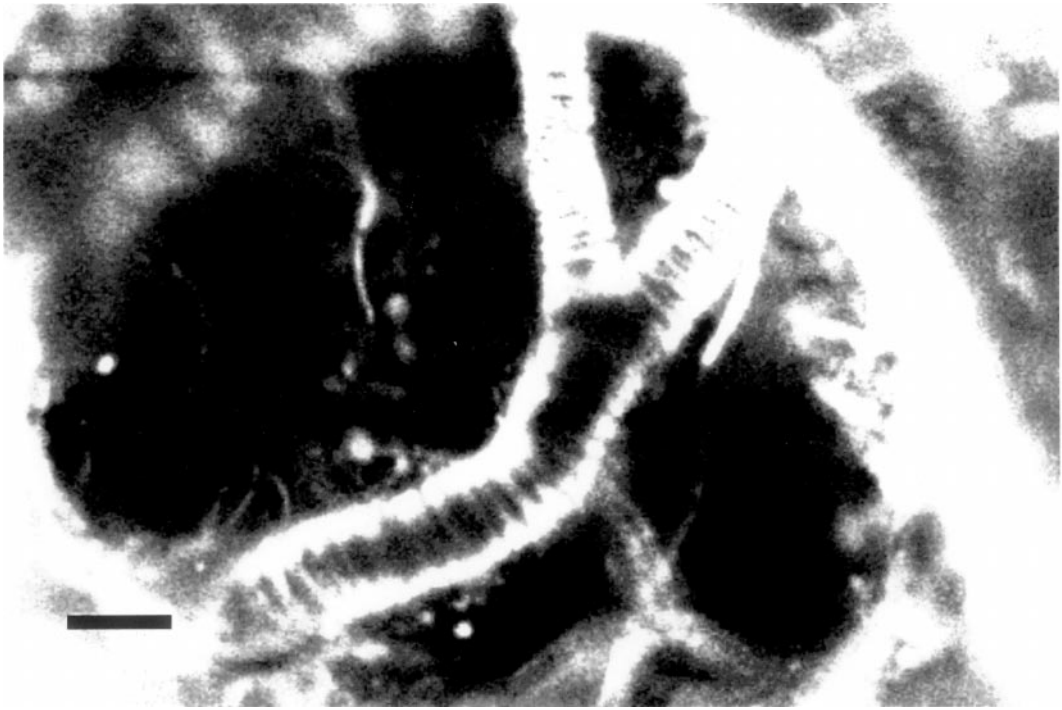

FIG. 3. Confocal image of LBAM larva dipped in CPD showing optical cross section of fluorescent tracheae. Strong fluorescence on the surface of the trachea indicates oil coating rather than filling the interior of the trachea. Scale bar, 10 μm.

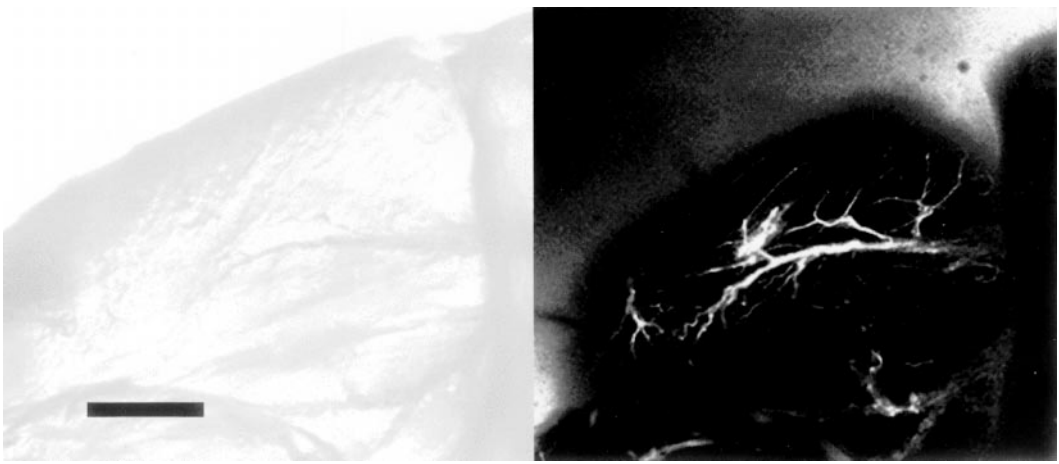

FIG. 4. LBAM larva dipped in CPD showing strong fluorescence of fine tracheoles (right) and respective transmission image (left). Scale bar, 100 μm.

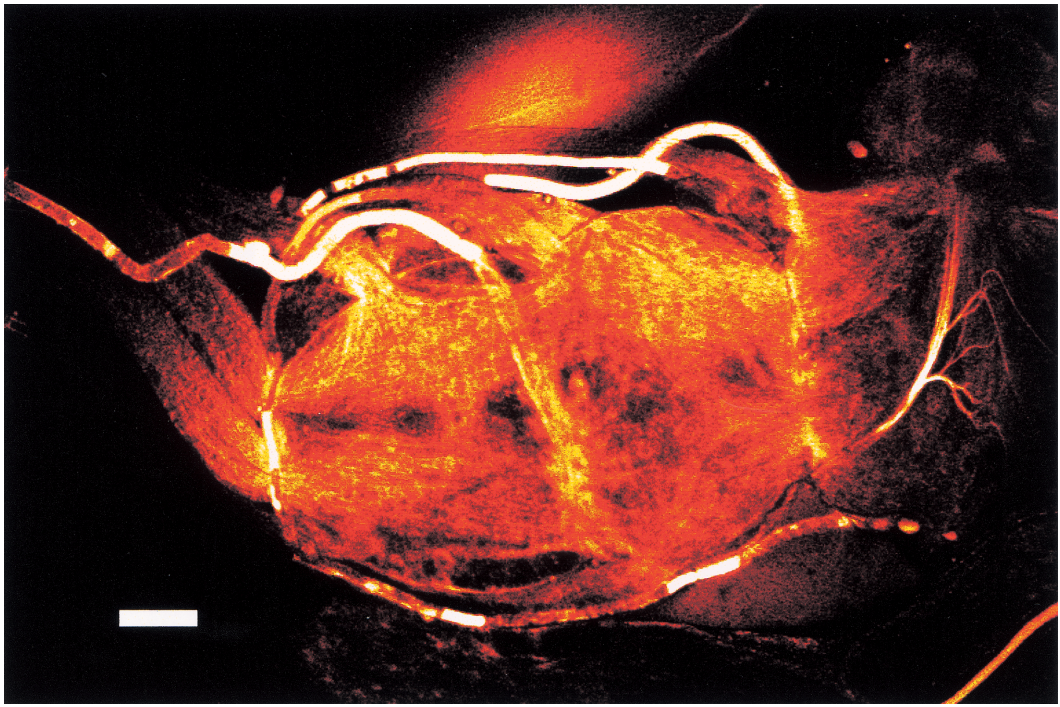

FIG. 5. Nerve ganglion dissected from a 5th-instar LBAM larva dipped in CPD. Ganglion and associated tracheoles show strong fluorescence related to the presence of fluorescent oil. Scale bar, 100 μm.

the C15 alkane and surfactants has not been determined in these experiments.

AchE Sensitivity to Oil

Alkanes do not mimic the molecular shape of neurotransmitters, as do organophosphates, but perhaps they inhibit cholinesterases in some other way. Acetylcholine esterase activity against a substrate, acetylthiocholine, was used to test the enzyme sensitivity to CPD. CPD produced no inhibition of AchE for incubation times of 0 h ($F = 1.79$, $df = 1$, $P > 0.5$), 0.5 h ($F =$

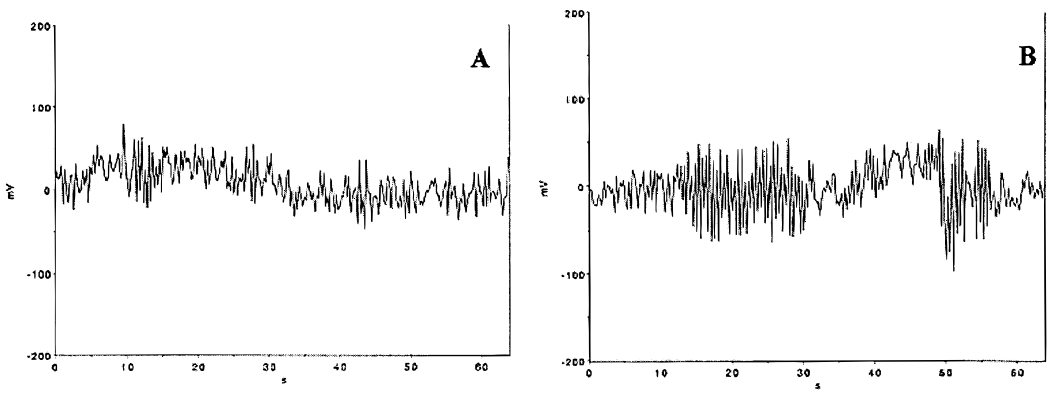

FIG. 6. Spontaneous nerve activity of a dissected LBAM larvae resting and untreated (A) and after 5 min of exposure of CPD (B). Recordings show electrophysiological responses over a 65-s period.

TABLE 3
Frequency of Nerve Activity (Action Potentials min^{-1}) in Peripheral Nerves of 5th-Instar LBAM Larvae Dipped in Four Concentrations of CPD and Water Only

Treatment	Frequency of nerve activity (action potentials min^{-1})[SE]
Water	88.33 [2.36] a
CPD 200 ppm	94.00 [1.95] ab
CPD 1000 ppm	102.00 [2.14] c
CPD 5000 ppm	101.17 [3.06] bc
CPD 10,000 ppm	100.83 [3.60] bc

Note. Mean of six recordings. Means within a column followed by the same letter are not significantly different according to one-way analysis of variance ($P > 0.05$, least significant difference).

0.28, $df = 1$, $P > 0.5$), 1 h ($F = 0.22$, $df = 1$, $P > 0.5$), 1.5 h ($F = 5.25$, $df = 1$, $P > 0.5$), 3 h ($F = 5.25$, $df = 1$, $P > 0.5$), and 15 h ($F = 3.34$, $df = 1$, $P > 0.5$) (Table 4).

DISCUSSION

The mode of action of petroleum oils on insects is usually considered to be suffocation due to blocked spiracles. However, the symptomology of larvae dipped in oil CPD is more consistent with a rapid narcosis or neurotoxicity. Narcosis due to oil vapor invasion is possible for very volatile oil fractions (16). In this study, *n*-pentane vapors induced rapid narcosis in LBAM larvae, but CPD did not produce any fumigant effects when larvae were exposed at

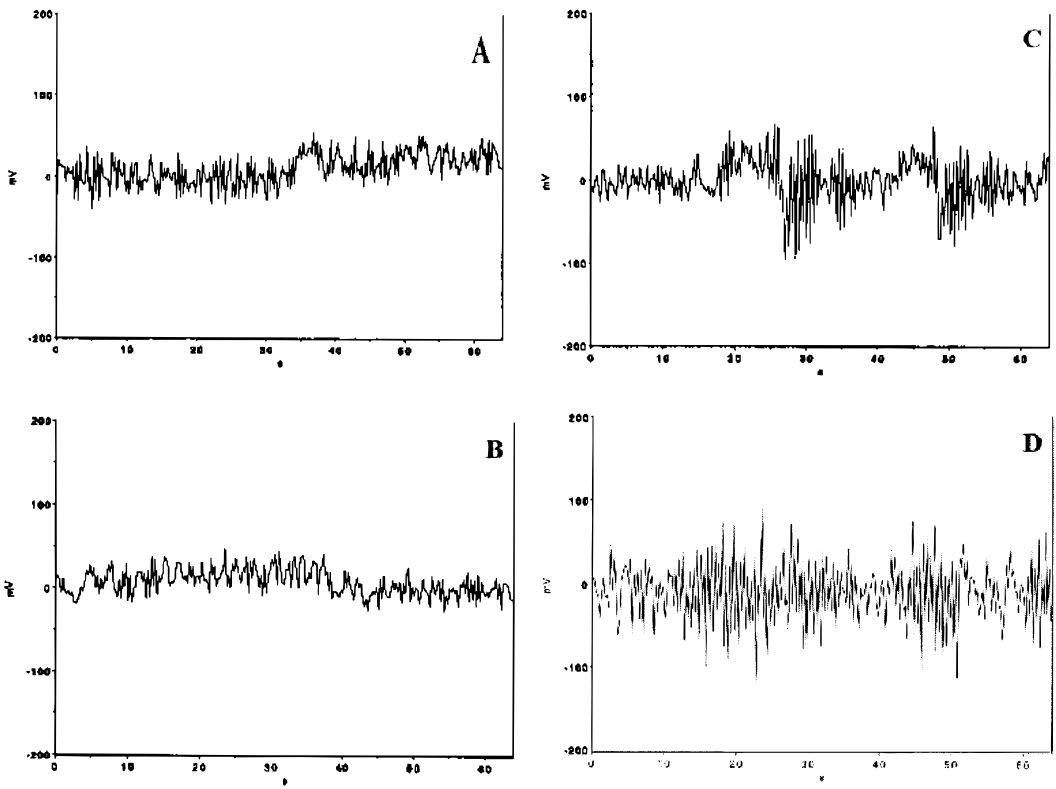

FIG. 7. Spontaneous nerve activity of a dissected LBAM larvae resting and untreated (A) and 15–20 min after larvae were dipped in 200 ppm (B), 1000 ppm (C), and 10,000 ppm of CPD (D). Recordings show electrophysiological responses over a 65-s period.

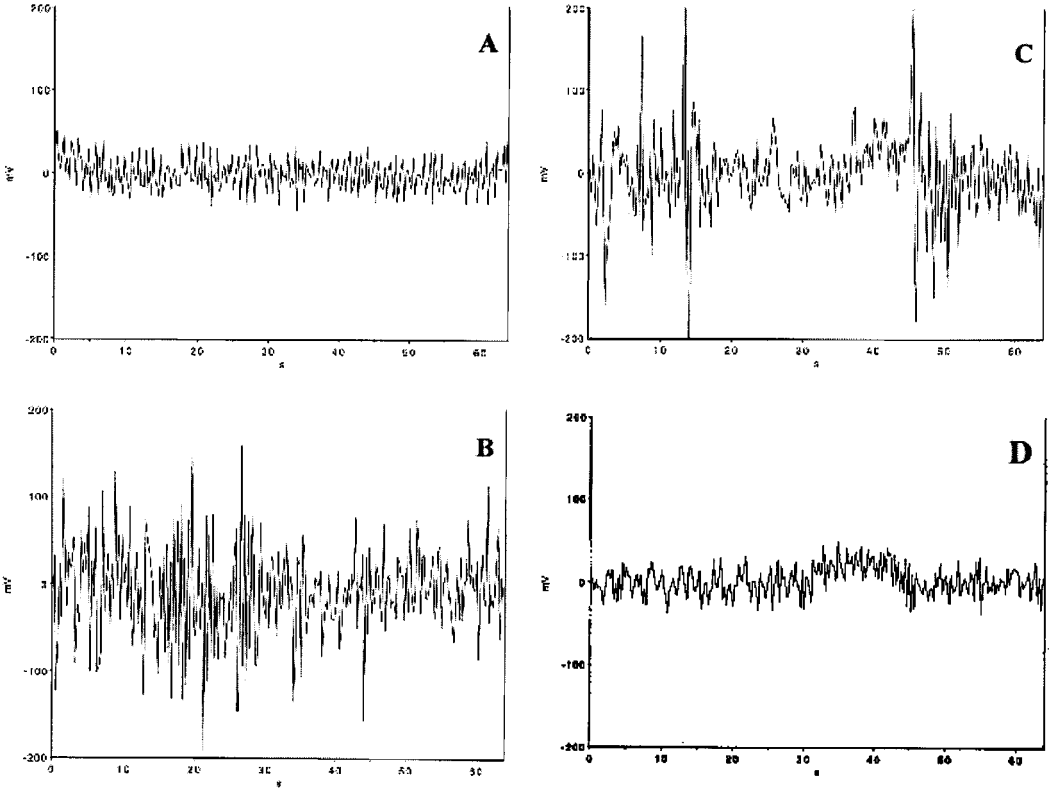

FIG. 8. Spontaneous nerve activity of a dissected LBAM larvae resting and untreated (A), and 15–20 min after larvae were dipped in components of CPD: 10,000 ppm C15 alkane (B), 10,000 ppm surfactant blend (C), and 1000 ppm of surfactant blend (D). Recordings show electrophysiological responses over a 65-s period.

ambient temperatures. This supports earlier work showing that only the lower range in the paraffin series, up to decane, produced fumigant action in insects (6, 7).

"Knockdown" could be induced if the oil blocked the tracheae, causing an excess of carbon dioxide. Confocal microscopy showed that CPD penetrated the tracheal system extensively and "knockdown" may be associated with rapid CO_2 accumulation. Exposure of high concentrations of CO_2 can affect the coordination of LBAM larvae, but the process is completely reversible even after several hours exposure (3). It was not possible to remove the liquid oil from

TABLE 4
Bovine AChE Activity (mOD/min) after Incubation with CPD over Six Different Periods (0–15 h)

	Bovine AchE Activity (mOD/min) [SD]					
Treatment	0 min	0.5 h	1 h	1.5 h	3 h	15 h
Control	273.57	257.57	248.27	253.43	265.33	246.43
	[9.19]	[31.12]	[2.10]	[20.50]	[3.35]	[6.76]
CPD	243.33	251.97	241.63	258.23	236.90	258.73
	[44.61]	[15.96]	[26.38]	[17.09]	[21.00]	[10.30]

the tracheal system after dipping to verify reversibility but it seems unlikely that complete recovery would occur. Additional symptoms, such as twitching of the prolegs, dehydration, and darkening of the hemolymph, suggested that other systems were disrupted due to contact with the oil.

CPD has an effect on the neuronal activity of LBAM larvae. Gerolt (17) proposed that certain toxic substances are more likely to gain access to the central nervous system of insects via the tracheal system. Direct nervous disruption would require deep penetration of oil into the tracheoles and absorption onto nerve membranes. Confocal microscopy shows that if larvae are dipped in oil and then exposed to the air, the oil can invade the tracheal system, but the extent of penetration varies with oils. CPD, an emulsified C15 alkane, can penetrate much deeper into the tracheal system than DC-Tron (a narrow-range oil with mean equivalent *n*-paraffin carbon number of a C23 alkane (12)), presumably due to a lower interfacial tension between CPD and the tracheal lining. CPD appears to have the physical properties necessary to rapidly move down into the nerve ganglia via the tracheal system, but this should be substantiated by contact angle measurements on tissue surfaces.

The presence of CPD in the larvae affects the activity of the peripheral nerves of larvae. The response of intact larvae dipped in CPD supported confocal observations that the oil rapidly moves down the tracheae into nervous tissue. It is possible that oil deep in the terminal branches of the tracheae would block gas exchange and induce oxygen starvation. The narcotic action of oils is generally associated with decreased activity through anoxia (18). However, exposure to CPD did not produce decreased activity, but induced a rapid onset of multiple nerve firing in peripheral nerves of LBAM larvae. This increased activity suggests an effect on nerves by the oil that is contrary to the symptoms of anoxia.

Surfactants disrupt plant tissue due to their surface activity (19), and cell disruption of nerve membranes may also occur due to this property. CPD is formulated as a mixture of a C15 alkane with low levels of surfactants ($<$10% vol/vol) to aid in emulsification. Exposure to the C15 alkane and surfactants separately induced repetitive firing, demonstrating that oil and surfactants both contribute to a nervous response. Surfactants have traditionally been used by formulators to control oil deposit on sprayed surfaces, but they may also be important in achieving translocation of the oil into the nervous tissue of insects. This may involve a complex synergy, as the surfactants may aid entry into spiracles by controlling the deposit, whereas the oil may equally be assisting the translocation of the surfactants to nervous tissue.

The pharmacological effect of the absorption of hydrocarbons into phospholipid membranes is not clear, but is probably not due to a specific site, as is true for most insecticides (20). Thus, nervous disruption would not involve the formation of specific chemical binding to receptors or the active sites of enzymes, which is consistent with the lack of any apparent structural complexity or stereo isometry of the oils, especially compared to other insecticides. Assays using bovine AChE support this by showing no specific inhibition of that enzyme using high oil concentrations (1%) and long incubation periods (up to 15 h). Further evaluation with insect AChE and other enzyme targets is required to better substantiate whether alkanes have a nonspecific action. However, it is likely that alkanes have a direct effect on the neural lipid membranes. The anesthetic potency of alkanes is related to their adsorption by lipid bilayers. The alkane adsorbed increases membrane thickness and tension, which reduces bilayer conductance (21). The anesthetic potency of alkanes also declines with increasing chain length, with only *n*-octane or smaller alkanes affecting ion channel stability (22). In this study, exposure to CPD, an emulsified C15 alkane, increased activity, which is contrary to the anesthetic potency of *n*-octane and smaller alkanes. The larger alkane may achieve the displacement of protective neural lipids by solvent action (23) and affect nerve activity by increasing membrane permeability to ion exchange.

The effect of oils on arthropod nervous activity has important implications for the use of oils as insecticides. The formulation of insecticidal oils has been focused on using their physical characteristics to achieve efficacy by anoxia. Special-purpose oils, such as CPD, use other physical characteristics of oils to achieve efficacy by an alternative mode of action. The increased excitability of nerves exposed to oils may also have a role in overcoming insecticide resistance to neurotoxins. One of the major mechanisms for pyrethroid resistance in insects is reduced neuronal sensitivity. A combination of a pyrethoid and a light alkane might be part of a resistance management strategy for kdr-like traits in insects. It is possible that an alkane could improve efficacy by increasing the sensitivity of the nerves and assist in translocation of the pyrethroid into nervous tissue via the tracheal system. Further evaluation is required, but a greater understanding of the range of symptoms caused by oils should lead to products that are more effective in this fashion.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the research assistance of Nancy Cunningham, of the South Australian Research and Development Institute, and David Johnson, of Caltex Australia Research and Development Laboratories, for supplying oil formulations. This study was supported by the Horticultural Research and Development Corp. and the University of Adelaide.

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