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Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES

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SUPERINDUSTRIA Y COMERCIO
Radicación: 0001/310 0000000/ Folios: 22
Fecha (AMD): 2005-08-31 17:27:39
Trámite: 0002 PATENTES D 1 REGISTRO/ 444 REPUESTA
Dependencia: 2002 DIVISION DE NUEVAS CREACIONES

E. S. D.

Re: P1.-WYETH HOLDINGS CORPORATION.-Solicitud de
Patente de Invención para "COMPOSICIONES
INSECTICIDAS SINERGICAS QUE COMPRENDEN UN
ANTAGONISTA DEL CANAL DE SODIO NEURONAL Y UN
INSECTICIDA".-Clase A.01N.-Expediente número
00-017.310.-

1. Me refiero al auto dictado por ese Despacho notificado por fijación en lista el 25 de Agosto de 2005.
2. De acuerdo con el Concepto Técnico No. **696** se tiene que:

"DE LA SOLICITUD DE LA OFICINA

"A efectos del examen de patentabilidad y como requerimiento de esta oficina, el solicitante deberá proporcionar, en un plazo que no excederá de 3 meses, el siguiente documento relativo a la solicitud extranjera correspondiente a la misma invención que se examina:

- 1- Harder, H. H ET AL.: "DPX-MPO62: A novel broad-spectrum environmentally soft, insect control compound" Brighton Crop Prot Pests Dis (1996) Vol-2, 449-454.
- 2- JOURNAL OF ECONOMIC ENTOMOLOGY, Vol 90 No. 5, October 1997, pages 1170-1180.

TO BE RESEARCHED

5A-1

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DPX-MP062: A NOVEL BROAD-SPECTRUM, ENVIRONMENTALLY SOFT, INSECT CONTROL COMPOUND

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ABSTRACT

DPX-MP062 [Indeno [1, 2-e] [1, 3, 4] oxadiazine-4a (3H)-carboxylic acid, 7-chloro-2,5-dihydro-2-[[[(methoxycarbonyl) [(4-trifluoromethoxy) phenyl] amino] carbonyl]-, methyl ester] is a highly efficacious new insect control compound demonstrating broad spectrum control of lepidoptera target pests at rates of 12.5-70 g a.i./ha. Field studies, conducted over several years around the world, demonstrate that DPX-MP062 is highly effective in controlling populations of *Heliothis*, *Helicoverpa*, *Spodoptera*, *Plutella*, *Trichoplusia*, *Lobesia*, *Cydia* and other lepidopteran target pests in various crops such as cotton, vegetable and fruit. The product demonstrates good efficacy on target insect pests while preserving beneficial insects and mites. DPX-MP062 is an enriched active isomer (75% DPX-KN128 insecticidal component). Most of the data here have been developed on the racemic material DPX-JW062.

DPX-MP062 has a very favorable environmental profile with a relatively short half life, low environmental loading, and large margins of safety to mammalian, avian, aquatic, and non-target organisms. DPX-MP062 has a novel mode-of-action, resulting in lack of cross resistance to standard insect control compounds such as pyrethroids, O.P.s, and carbamates and consequently will fit well into resistance management programs, and integrated control situations.

INTRODUCTION

The future of agriculture in a modern society inundated with environmental and toxicological concerns is dependent on the discovery of novel solutions to insect control. Although a number of recent products have been introduced, many suffer from disadvantages in bioefficacy (eg. narrow spectrum, high use rates) or pose threats to the environment (eg. impact on beneficials, impact on aquatic systems). This leaves a clear need of growers for effective low use rate, novel mode-of-action, environmentally sound products that fit into current and future IPM programs. DPX-MP062 insect control agent is the result of an extensive discovery and development program within DuPont to address these specific areas of concern and create a product for the future that meets many of the standards established by both growers and environmental/regulatory agencies.

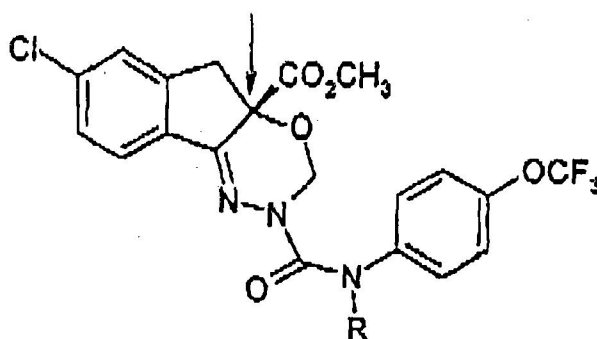
CHEMICAL AND PHYSICAL PROPERTIES

DuPont is currently developing two insecticidal compounds, both of which contain the same active ingredient; these are referred to as DPX-JW062 and DPX-MP062. Both DPX-JW062 and DPX-MP062 contain two optically active isomers, only one of which is insecticidally active. These optical isomers are called DPX-KN128 and DPX-KN127. DPX-KN128 is the insecticidally active isomer and the active ingredient which is common to both DPX-JW062 and DPX-MP062. DPX-KN127 has no insecticidal activity and from a toxicological and environmental perspective can be considered an inert. The chief difference then between DPX-JW062 and DPX-MP062 is in the level of DPX-KN128 and DPX-KN127 they contain. Table 1 describes the different isomer ratios in each compound.

Table 1. Isomer ratios for DPX-JW062 and DPX-MP062.

Compound code	Isomer code	Ratio of isomers (%)
DPX-JW062	DPX-KN128	50
	DPX-KN127	50
DPX-MP062	DPX-KN128	75
	DPX-KN127	25

Structure of DPX-MP062, some physico-chemical properties



R = COCH₃, = MP062

R- = DPX-KN127, THE INACTIVE ISOMER

S- = DPX-KN128, THE ACTIVE ISOMER

→ = Site of the chiral carbon

Chemical Name

Indeno [1,2-e] [1,3,4] oxadiazine-4a (3H)-
carboxylic acid, 7-chloro-2,5-dihydro-2-[[
(methoxycarbonyl)[4-trifluoromethoxy]
phenyl] amino]carbonyl]-, methyl ester

CAS Registry No.

144171-61-9

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1. Molecular weight: 527.87 g/mole
 2. Melting point (for solids only): 140-141° C
 3. Solubility (in water, organic solvents, lipids and fats):

Water	<0.5 mg/l
1-Octanol	480 mg/l
Methanol	390 mg/l
Acetonitrile	76000 mg/l
Acetone	140000 mg/l
 4. Partition coefficient in octanol/water: Approx. 40000
 5. Vapour Pressure: Less than 10^{-3} Pascals at 20-25° C

Physical chemical properties cited here refer to the racemic compound (DPX-JW062)

MAMMALIAN TOXICOLOGY

Technical (based on DPX-JW062)

Acute oral LC50, (rat):	> 5000 mg/kg (EPA Tox. Cat. IV)
Acute dermal LD50, (rabbit):	> 2000 mg/kg (EPA Tox. Cat. III)
Acute inhalation LC50, (rat):	> 2 mg/l (EPA Tox. Cat. III)
Eye, skin irritation, (rabbit):	None
Dermal sensitization, (guinea pig):	No evidence
Ames Test:	Negative

ENVIRONMENTAL SAFETY

Avian toxicity (based on DPX-JW062)

Bobwhite quail and Mallard duck, acute oral LD ₅₀ :	> 2250 mg/kg
Bobwhite quail and Mallard duck, 5-day dietary LC ₅₀ :	> 5620 mg/kg diet

Aquatic toxicology

Bluegill sunfish 96 hr. LC ₅₀ :	> 1.0 mg/l
Rainbow trout 96 hr. LC ₅₀ :	> 0.5 mg/l

Environmental Fate

Soil half-life:	4-5 days tams silt loam soil
Aqueous Hydrolysis:	pH 5 > 30 days, pH 7 ~ 30 days, pH 9 ~ 2 days
Aquatic Photolysis:	1-2 days at pH 5.0

Beneficial Arthropod Evaluations

Following 4-6 applications of 30-50 g a.i./ha DPX-KN128, little or no adverse effects were reported on the parasitic wasp *Aphidius rhopalosiphii*, predatory mite *Typhlodromus pyri*, ground dwelling predator *Aleochara bilineata* and aphid predator *Ephedrus* *balteatus* (Metz-Briggs et al., 1996).

BIOLOGICAL PROPERTIES - FIELD STUDIES

Table 2. Control of *Loebesia botrana* on vine in Europe (1995)

Treatment	Rate g a.i./ha	France		Italy		Germany
		L*	D**	L*	D**	L*
DPX-KN128	30	89	87	88	85	96
DPX-KN128	37.5	98	91	92	96	97
DPX-KN128	45	97	91	96	97	-
chlorpyrifos	285	84	82	48	71	-
l-cyhalothrin	17.5	79	64	-	-	-
m-parathion	120	-	-	-	-	72
Untreated		(323)	(728)	(33)	(52)	(89)

* L = % control (based on larvae/100 bunches)

** D = % reduction (based on holes/100 bunches)

() = No. larvae or holes/100 bunches

Table 3. Control of *Plutella xylostella* and *Trichoplusia ni* on head cabbage (Florida, USA, 1996)

Treatment	Rate g a.i./ha	% Control
DPX-KN128	25	98
DPX-KN128	30	96
l-cyhalothrin	40	90
tebufenozid	50	30
tebufenozid	100	69
Untreated	-	(240)

() = No. of larvae/100 plants

Table 4. Control of pests on tomato and sweet pepper (1995)

Treatment	Rate g a.i./ha	Spain*	Spain**	Australia***	USA****
		(pepper)	(tomato)	(tomato)	(tomato)
DPX-KN128	25	96	96	-	85
DPX-KN128	37.5	97	100	89	-
DPX-KN128	50	-	-	95	91
DPX-KN128	62.5	-	-	99	100
deltamethrin	12.5	93	48	-	-
sulprofos	720	-	-	95	-
chlorfenvapyr	225	-	-	-	67
Untreated		(28)	(68)		

* *Spodoptera exigua*

** *Plusia gamma*

*** *Helicoverpa armigera*

**** *S. eridania*

() = No. of larvae

Table 5. Control of various pests on apple (Pennsylvania, USA, 1995)

Treatment	Interval (days)	Rate g a.i./ha	% Clean Fruit (at harvest)	% infest <i>Cydia pomonella</i>	% control <i>Typhlocyba pomaria</i>	% control <i>Phyllonorycter blancardella</i>
DPX-KN128	10	50	83	4	68	58
DPX-KN128	10	75	88	4	69	46
DPX-KN128	14	50	87	6	66	36
DPX-KN128 + azinphos-methyl	14	75 + 250	93	1	70	47
azinphos-methyl	14	500	87	2	66	47
Untreated	-	-	48	29	0	0

Table 6. Control of various pests on apples in Europe (1995)

Treatment	Rate (g a.i./hl)	France*	Germany**	Spain*	Hungary*
DPX-KN128	2.5	-	71	-	-
DPX-KN128	3.8	-	100	-	-
DPX-KN128	4.0	65	-	80	92
DPX-KN128	5.0	88	-	100	98
azinphos-methyl	43.7	88	-	100	94
phosalone	60	82	46	-	95
Untreated	-	17	12	3	15

* % control of *Cydia pomonella*

** % control of *Adoxophyes orana*, *Archips podana*

Table 7. Control of cotton pests (1995)

Treatment	Rate g a.i./ha	USA*	Spain**
DPX-KN128	38	93	89
DPX-KN128	50	90	91
DPX-KN128	63	-	100
DPX-KN128	75	100	-
thiodicarb	840	93	-
l-cyhalothrin	34	83	-
deltamethrin	19	-	60
Untreated	-	(29)	(12)

* % control of *H. virescens* (based on No. larvae/100 terminals)

** % control of *Helicoverpa armigera* (based on No. larvae/100 fruit)

() = No. larvae/100 terminals

MODE OF ACTION

DPX-KN128 offers a totally novel mode-of-action compared to other insect control products. Biochemical studies have demonstrated that DPX-KN128 (Wing, personal commun.) and related chemistry (Salgado, 1990) blocks sodium channels in nerve cells. The blockage of these sodium channels in insects leads to poor coordination, paralysis and ultimately death of the target insect.

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The routes of entry into insects is via both contact and ingestion. Insect behaviour is rapidly altered following exposure to a toxic dose of DPX-KN128, resulting in a rapid cessation of feeding and consequently excellent plant protection of the target crop. Extensive laboratory and field studies on strains resistant to a broad range of commercially available products demonstrate a lack of cross resistance to DPX-KN128, thus offering a valuable tool for IPM and resistance management programs.

SUMMARY

DPX-MP062 is a novel insect control agent containing the active ingredient DPX-KN128. Tests on a range of crops and pests worldwide have shown outstanding larval control in the range 12.5-70 g a.i./ha. The low toxicity to non-target organisms and short persistence in the environment indicate that DPX-KN128 is surprisingly environmentally benign for such an effective control agent. The novel mode of action of this molecule raises the probability of effective control within the framework of IPM and resistance management strategies.

ACKNOWLEDGEMENTS

Product development involves extensive teamwork across disciplines. We would like to express our gratitude to all our colleagues who have contributed to our understanding of the value of DPX-MP062.

REFERENCES

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Interactions of Recombinant and Wild-Type Baculoviruses with Classical Insecticides and Pyrethroid-Resistant Tobacco Budworm (Lepidoptera: Noctuidae)

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J. Econ. Entomol. 90(5): 1170-1180 (1997)

ABSTRACT In tests with neonate *Heliothis virescens* (F.), we characterized interactions of all combinations of a recombinant *Autographa californica* (Speyer) nuclear polyhedrosis virus (AcAaIT) that expresses an insect-selective neurotoxin (AaIT) and wild-type AcNPV when combined with low concentrations of several conventional insecticides. All combinations of the recombinant virus AcAaIT and insecticides showed a positive interaction (decrease in the median lethal time (LT₅₀) compared with the LT₅₀ for either component alone). A type II pyrethroid (cypermethrin, which modifies currents of sodium channels) and a carbamate (methomyl, an inhibitor of acetylcholinesterase) were synergistic in combination with AcAaIT. Other insecticides also showed a positive interaction when tested in combination with the recombinant virus, but joint activity was slightly antagonistic (i.e., less than predicted activity when combined) with wild-type AcNPV. We also characterized the effectiveness of AcAaIT against pyrethroid-resistant *H. virescens* larvae. Our results show that a resistant strain of *H. virescens* is more sensitive to the recombinant virus compared with a susceptible strain. Results of these studies should be useful in planning of future field trials to increase the effectiveness of nuclear polyhedrosis viruses and to manage resistance to pyrethroids and other insecticides.

KEY WORDS *Heliothis virescens*, baculovirus, insecticide, pyrethroid, recombinant microorganism, nuclear polyhedrosis virus

SYNTHETIC ORGANIC CHEMICALS are important components of modern agriculture (Mellor and Adams 1984, Croft 1990). However, their use is increasingly criticized because of problems associated with environmental contamination and toxicity to nontarget organisms. These problems are compounded by the increasing costs of discovery, development, and registration of new insecticides. In addition, an increasing number of insect species have become resistant to the available insecticides.

Consequently, use of alternative control agents (including insect pathogens) is being explored. However, many of these pathogens lack the inherent properties of synthetic organic insecticides. Recombinant DNA technology may provide a tool to engineer microbes to yield increased insecticidal properties (Miller et al. 1983, Keeley and Hayes 1987). Recently, the pathogenic virus *Autographa californica* (Speyer) nuclear polyhedrosis virus (AcNPV) (Baculoviridae) has been genetically modified to increase its speed of kill. The introduction of toxins selective to insects (Maeda et al.

1991, McCutchen et al. 1991; Stewart et al. 1991; Tomalski and Miller 1991, 1992), the enzyme juvenile hormone esterase derived from insects (Hammock et al. 1990, Bonning and Hammock 1994), and a maize mitochondrial protein, URF13 (Korth and Levings 1993), has resulted in ~20–30% reduction in time to death.

Although recombinant NPVs are becoming more competitive with synthetic insecticides, these modified viruses will encounter other problems before they are used for insect control (Hammock et al. 1993, McCutchen and Hammock 1994). Besides formulation, registration, and production problems, recombinant NPVs must effectively compete with classical insecticides to gain a share of the commercial market. Currently, biological agents account for <1% of total insecticide sales; *Bacillus thuringiensis* Berliner accounts for most of this percentage (Jutsum 1988).

Interactions Between Classical Insecticides and AcNPV. One strategy for use of natural or recombinant NPVs may be the simultaneous application of low rates of conventional insecticides. Although several investigators have documented a positive interaction between classical insecticides and microorganisms, a few insecticides have been shown

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Table 1. Insecticides combined with wild-type and recombinant baculoviruses

Compound	Type	Mode of action	Source
Allethrin	Pyrethroid type I	Sodium channel agonist	Roussel Uclaf, Agrovotensaire, Paris
Cypermethrin	Pyrethroid type II	Sodium channel agonist	ICI Americas (Zeneca), Goldsboro, NC
DDT	Chlorinated hydrocarbon	Sodium channel agonist	Synthesis by Hammock, UC Davis
Endosulfan	Cyclodiene	GABA channel agonist	FMC, Ag Chemical Group, Middleport, NY
Methomyl	Carbamate	AChE inhibitor	DuPont, Wilmington, DE
Profenofos	Organophosphate	AChE inhibitor	Ciba, Greensboro, NC

GABA, γ -aminobutyric acid; AChE, acetylcholinesterase.

to improve the activity of wild-type NPVs (Ignoffo and Montoya 1966, Benz 1971).

The 1st objective of our study was to identify combinations of insecticides and NPVs that hold promise for field trials by demonstrating potentiation. To achieve this objective, we characterized the interaction of a representative recombinant NPV (AcAaIT) expressing an insect-selective neurotoxin (AaIT), and wild-type AcNPV with representative compounds from different classes of synthetic organic insecticides.

Efficacy of a Recombinant NPV in Pyrethroid-Resistant Larvae. Another concern associated with the use of classical insecticides is increased tolerance and resistance of pest populations to the compound. For example, pyrethroids are the most commonly used insecticides for control of *Heliothis virescens* (F.), tobacco budworm, and *Helicoverpa zea* (Boddie), cotton bollworm, 2 of the most economically destructive pests in North America. Resistance of *H. virescens* to pyrethroids has been a widespread problem in cotton in the southeastern United States for more than a decade. Control failures and monitoring studies in the late 1970s and early 1980s revealed that resistance levels to pyrethroids (primarily permethrin and fenvalerate) were increasing rapidly (Harding et al. 1977, Plapp 1981, Martinez-Carillo and Reynolds 1983). The increased occurrence of pyrethroid resistance in the tobacco budworm has raised interest in the search for strategies to prolong the use of pyrethroids (e.g., Luttrell and Roush 1987, Campanhola and Plapp 1988, McCutchen et al. 1989, Plapp et al. 1990).

Therefore, our 2nd objective was to investigate the effectiveness of the recombinant virus, AcAaIT, against tobacco budworm resistant to pyrethroids. Both pyrethroids and AaIT bind to receptors in the sodium channel of the insect nerve and modify the influx of sodium through the channel, resulting in paralysis of muscle tissue. Because AaIT has a similar mode of action to that of the pyrethroids, we investigated cross-resistance to assess whether resistant *H. virescens* larvae were more or less tolerant to AcAaIT than the susceptible strain.

Materials and Methods

Interactions Between Classical Insecticides and AcNPV. A susceptible strain (Stoneville) of tobacco budworm was provided by the Southern Field Crop Insect Management Laboratory, USDA-ARS, Stoneville, MS; this strain has been reared for >5 yr without exposure to insecticides. Laboratory colonies of tobacco budworm were maintained on artificial diet as described by Vanderzant et al. (1962).

Six synthetic organic insecticides were tested alone and in combination with wild-type or recombinant NPVs (Table 1). All chemicals were supplied as technical grade materials. In addition, phenobarbital (maleate salt, Sigma, St. Louis, MO) was tested as an antidote to the neurotoxic effects induced by AcAaIT. For this study, phenobarbital was dissolved in double-distilled water to produce a 10% solution. Second-instar *H. virescens* that had been infected with a >LC₅₀ (unpublished data) of AcAaIT 24 h earlier were placed on a 2-mm³ cube of diet saturated with 5 μ l of the phenobarbital solution. Polyhedron occlusion bodies (POBs) of AcAaIT, AcJHE.KK (another recombinant NPV that expresses a modified form of juvenile hormone esterase; Bonning and Hammock 1994), and wild-type AcNPV were prepared from cell culture as described by O'Reilly et al. (1992).

Neonate tobacco budworm were exposed to chemicals films on the inner surfaces of 20-ml glass liquid scintillation vials as described in Plapp and Campanhola (1986) and Campanhola and Plapp (1989). Concentration-response curves were then estimated for each insecticide. Interactions of the NPVs and each insecticide were determined. Initially, larvae infected with NPVs were exposed to 2,000 POBs (>LC₅₀) using the modified droplet feeding method (Hughes et al. 1986). Three NPVs were used: wild-type AcNPV, AcAaIT, and AcJHE.KK. AcJHE.KK was used as a control for the recombinant NPV AcAaIT to demonstrate that synthetic insecticides do not indiscriminately synergize recombinant NPVs. Larvae were transferred immediately to vials treated with the LC₁₀-LC₂₀ of the insecticide (except for controls). The LC₁₀-

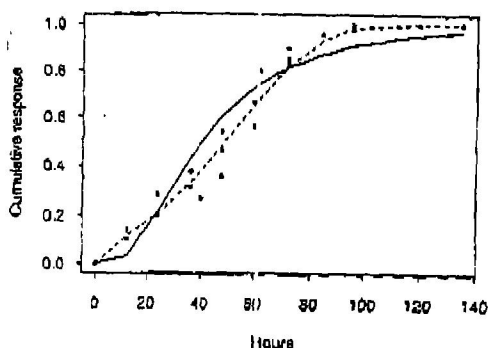


Fig. 1. Probit curve (solid curve) and a nonparametric smooth curve (dashed line) fitted to cumulative mortalities over time. Probit analysis is not appropriate for bioassays over time because the probit curve often gives a bad fit, as illustrated here.

LC₂₀ for each insecticide was estimated from the percentage mortalities of larvae that were exposed to the insecticide only at 24 h after initial exposure. Five neonate larvae were placed in each vial with ~500 mg of artificial diet (Campanhola and Plapp 1989). All treatments were replicated 3 times using at least 25–30 larvae per treatment. NPV only and insecticide only controls were run concurrently with each replicate of NPV/insecticide combinations.

During the synergism and antidotal bioassays, insects were kept in an incubator maintained at 25 ± 1°C and a photoperiod of 14:10 (L:D) h. Larvae that moved when probed with a small brush were considered alive. Mortality was recorded at 8- to 12-h intervals.

Efficacy of a Recombinant NPV in Pyrethroid-Resistant Larvae. A strain of tobacco budworm resistant to pyrethroids (P_{EG}) was obtained from Zeneca (Richmond, CA). The resistant strain was prepared from a mixture of 10 different populations collected from cotton fields in different states. Selection for resistance has been continuous since 1983 by exposure to permethrin and cypermethrin. The presence of target-site resistance has been identified in the P_{EG} strain (Campanhola and Plapp 1989, McCaffery et al. 1989). We obtained eggs from Zeneca, and a population of neonate larvae (3 replicates of at least 25–30 insects per replicate) were tested for resistance with a discriminating concentration (0.5 µg cypermethrin per vial) as described in McCutchen et al. (1989). In addition, for each replicate a sample of the population (10 insects per replicate) was reared to 3rd instar and exposed to vials containing a concentration of 75 µg cypermethrin per vial (Campanhola and Plapp 1989) to confirm that resistance levels were ≥50 times that of the Stoneville strain.

Resistant and susceptible neonate larvae were tested with wild-type AcNPV and AcAaIT by using the same conditions and parameters described previously by McCutchen et al. (1991). Treatments

were replicated 10 times using 24 larvae per treatment for each replicate.

Statistical Methods. Probit analyses with standard statistical programs (e.g., POLO-PC [LeOra 1987]) or PROBIT procedure in SAS (SAS Institute 1988) are not appropriate for bioassays over time for several reasons. First, observations on the same group of larvae at different times are not independent. Second, the probit curve often gives a bad fit when used to model concentration-responses over time or responses to mixtures of compounds over time (Fig. 1). This problem is demonstrated by a plot of the observed responses, the estimated log-probit curve, and an estimated nonparametric smooth curve for insects exposed to a mixture of insecticide and NPV (Fig. 1).

Therefore, we used an alternative procedure. We let the expected probability of an insect in the j th replicate dying by time t_j be characterized by

$$p_j = 1 - \exp[-\exp(\alpha + \beta\tau_j)], \quad (1)$$

where α and β are unknown parameters and τ_j is a transformation of the time point t_j . We calculated estimates of the parameters α and β by maximizing the conditional likelihood function

$$\prod_{j=1}^J \prod_{i=1}^{s_j} \left[\frac{p_{(i+j)} - p_j}{1 - p_j} \right]^{d_j} \left[1 - \frac{p_{(i+j)} - p_j}{1 - p_j} \right]^{s_j - d_j} \quad (2)$$

where s_j is the number of insects in replicate j that are still alive at time t_j , and d_j is the number of insects that have died in time interval $(t_j, t_{(i+j)}]$.

All calculations were done with the S-PLUS statistical package (Statistical Sciences 1993). Estimation was done in 2 steps. First, we evaluated the transformations τ_j by fitting the cumulative mortalities over time with the loess smoothing routine within the generalized additive fitting function `gam()` (Hastie 1992). Next, we used the transformed values τ_j and the likelihood function in equation 2 to calculate estimates of parameters and appropriate standard errors. We used the general minimizing routine `ms()` to minimize minus the log-likelihood. Estimates of LT₅₀ and plots of the estimated mortality probabilities were used to study the effects of mixtures of small amounts of conventional insecticides and virus on the speed of kill. LT₅₀s for different treatments were considered significantly different when the 95% CI did not overlap.

We tested 2 hypotheses regarding the mode of action of the mixtures. We tested the independent action model of 2 compounds in a mixture by comparison of estimated probabilities of response of the observed mixture with expected probabilities of response. The expected probability of response for a given time and assuming independence was estimated by the equation

$$p_{12} = p_1 + p_2 - p_1 p_2 \quad (3)$$

Table 2. Lethal concentrations of classical insecticides used in combination with wild-type or recombinant NPVs against larvae of tobacco budworm.

Compound	n	µg insecticide/ vin	% mortality at 24 h
methrin	121	0.05	14.0
cypermethrin	110	0.008	11.8
DT	80	0.02	18.8
indosulfan	80	0.002	17.5
methomyl	125	0.02	20.8
carbenclofos	150	0.06	10.3

Lethal concentrations of insecticides (LC_{50} - LC_{90}) were chosen based on results from Campanholo and Plapp (1989).

where p_1 and p_2 are estimated probabilities of mortality for a given time for insects exposed to the single compounds, respectively. The model for independent action assumes that the probabilities of effects of 2 compounds are additive (equation 3). Biologically, additive means that the insect dies when the amount of at least 1 of the compounds exceeds the threshold of tolerance of the insect.

The 2nd hypothesis, similar action additive interaction, assumes that both compounds act on the same site (or have the same mode of action), and the compounds are additive at the dose level. The insect dies when the sum of the doses at the site of action exceeds the threshold of mortality of the insect. In bioassays in which only one concentration of each compound is used, this model assumes that the insect dies when the sum of the doses at the site of action exceeds insect tolerance level by time t . Although doses at the site of action at a given time are not measured, values proportional to these amounts can be estimated as follows: let $D_1(t)$ and $D_2(t)$ be the dose of the single compound at the site of action at time t ; and

$$p_1 = 1 - \exp[-\exp[\alpha + \beta \log(D_1(t))]] \quad (4)$$

$$p_2 = 1 - \exp[-\exp[\alpha + \beta \log(D_2(t))]] \quad (5)$$

be the expected cumulative mortality probabilities for single compounds. Then the expected cumulative mortality probability for the mixture, assuming similar action additive model, is

$$p_{12} = 1 - \exp[-\exp[\alpha + \beta \log(D_1(t) + D_2(t))]]. \quad (6)$$

We tested the similar action additive model by first estimating α , β , $D_1(t)$, $D_2(t)$ in equations 4 and 5 with data from the single compound bioassays. Next, we substituted these estimates into equation 6 to calculate expected cumulative response probabilities of mixtures, assuming the similar action model.

Thus, we define a combination to be synergistic when the probabilities estimated from the observed mortalities are significantly greater ($P < 0.05$) than those calculated assuming the indepen-

dent or additive models. We further define an interaction to be antagonistic ($P < 0.05$) when the probabilities are less than those calculated from the independent or additive models. For all hypotheses, curves were considered significantly different ($P < 0.05$) when the expected curve was outside the 95% CI of observed responses.

Results

Interactions Between Classical Insecticides and AcNPV. When we examined the interaction of low rates (Table 2) of several insecticides in combination with wild-type AcNPV or the recombinant NPVs, AcAaIT and AcJHE.KK ($>LC_{50}$) in neonate *H. virescens*, plots of the observed mortalities for bioassays with single compounds indicated that the 2 recombinant NPVs and the wild-type NPV produced no significant mortality in the first 50 h after infection (Fig. 2). Mortality resulting from conventional insecticides, however, occurred much earlier. Almost all the insects that died by the end of the experiment from the insecticides did so in the first 40–60 h.

Many researchers use a comparison of LT_{50} s (the time required to kill 50% of the sample population) to evaluate the efficacy of baculoviruses (McCutchen et al. 1991, O'Reilly and Miller 1991, Tomalski and Miller 1991, Cory et al. 1994). Estimated LT_{50} s for insects treated with the mixture of NPV and a conventional insecticide were shorter than those obtained with the NPV alone (Fig. 3). However, the decrease in LT_{50} was significant ($P < 0.05$) only in bioassays in which conventional insecticides were added to AcAaIT (i.e., the LT_{50} for all combinations of AcAaIT and an insecticide were significantly shorter than those with the virus alone).

Decreases in LT_{50} s for combinations of wild-type NPV (Fig. 3) or the recombinant AcJHE.KK (data not shown) with an insecticide were not significantly different (i.e., $P > 0.05$) from the LT_{50} s with the virus alone (Fig. 3) for all but one of the insecticides (methomyl). Only methomyl significantly decreased ($P < 0.05$) the median time to death when added to the wild-type NPV.

Lethal Times of Mixtures of Conventional Insecticides and Recombinant NPVs. Although median times to death by the virus expressing the neurotoxin AaIT in combination with any of the insecticides tested were shorter than the corresponding time for the virus alone, only the type II pyrethroid (cypermethrin) and the carbamate (methomyl) interacted synergistically with the virus. Fig. 4 shows plots of observed responses (and 95% confidence regions for the estimated response probabilities) for insects treated with mixtures of conventional insecticides and the recombinant AcAaIT. These plots also indicate the expected response probabilities assuming the independent action model and the similar action additive model, suggesting synergism between AcAaIT and both cypermethrin and methomyl. With AcAaIT, cypermethrin and metho-

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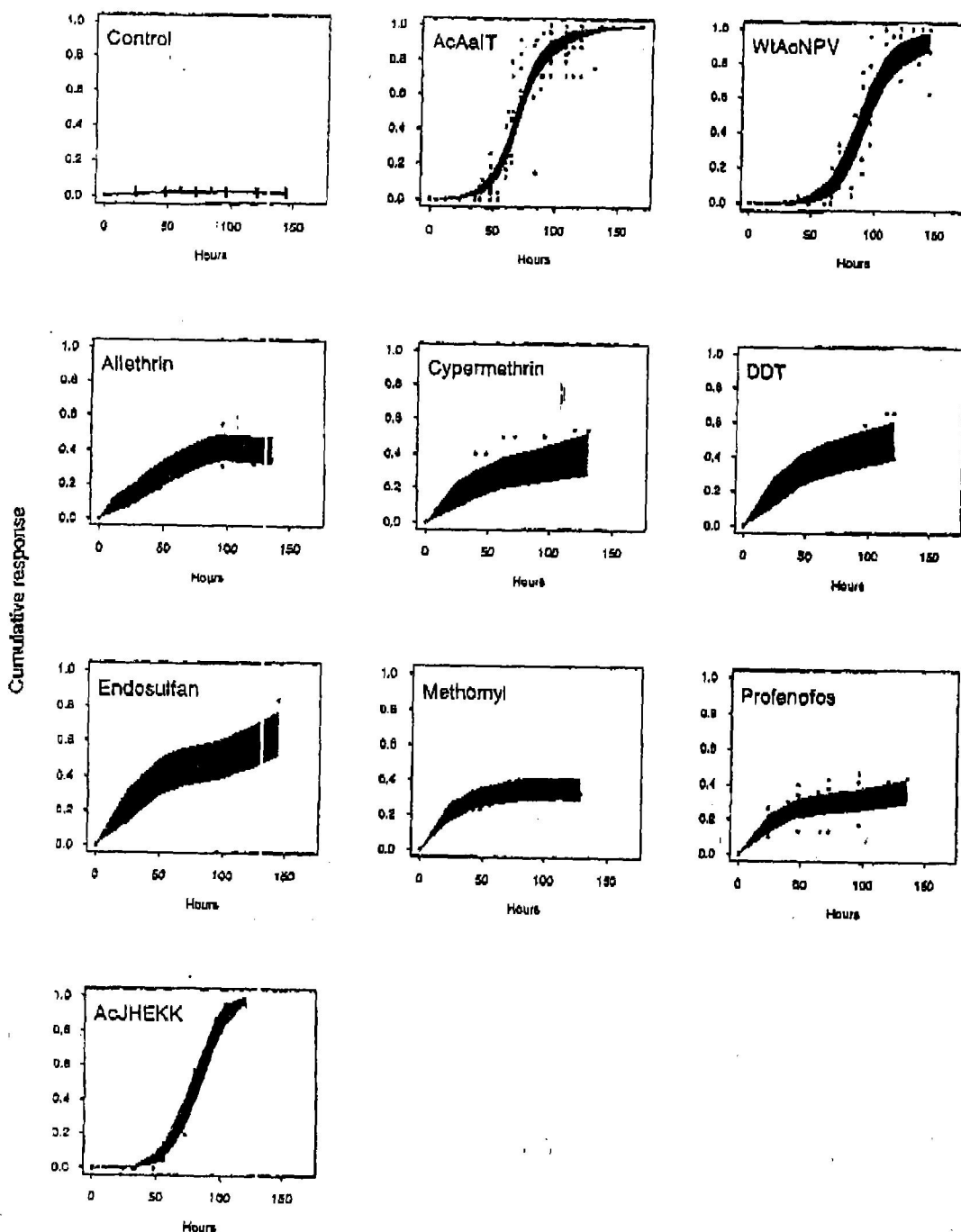


Fig. 2. Observed cumulative mortalities over time for the bioassays with single preparations. Dashed lines through the points are the fitted curves. Shaded areas are approximate point-wise 95% CL of the fitted curve. The control was treated with acetone (solvent for insecticides) only. AcAaIT is the recombinant form of *A. californica* nuclear polyhedrosis virus (AcNPV) that expresses a scorpion toxin. Wt AcNPV is the wild-type of AcNPV; AcJHEKK is the recombinant form of AcNPV that expresses a modified form of juvenile hormone esterase.

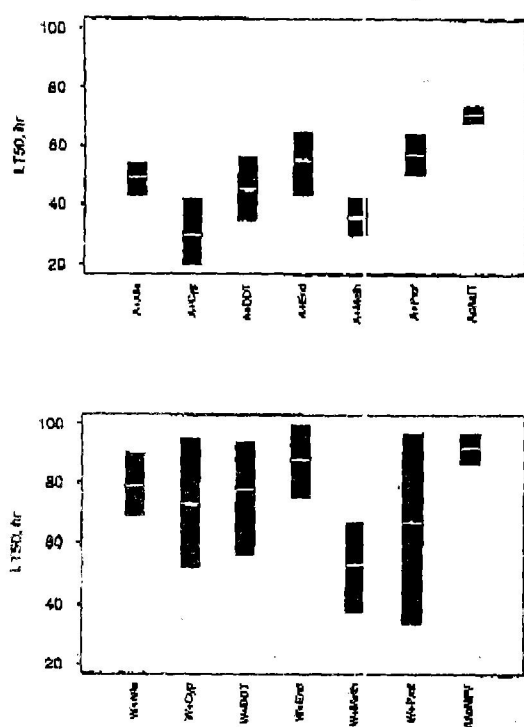


Fig. 3. Upper panel shows estimates (and 95% CL) for 50% LTs of insects treated with the mixture of AcAaIT and synthetic organic insecticides; estimates for wild-type NPV (WtAcNPV) with conventional insecticides are shown in the lower panel. A is NPV (AcAaIT); W is NPV (WtAcNPV); Alle is allethrin; Cyp is cypermethrin; End is endosulfan; Meth is methomyl; Prof is profenofos. The LT₅₀ for all combinations of AcAaIT and an insecticide were significantly shorter ($P < 0.05$) than those with the virus alone. Decreases in LT₅₀ for combinations of wild-type NPV and an insecticide were not significantly different (i.e., $P > 0.05$) from the LT₅₀s with the virus alone, except for methomyl ($P < 0.05$).

myl had an LT₅₀ of 30 h after infection (a reduction of 58% compared with the virus alone).

Although interactions of allethrin, DDT, endosulfan, or profenofos with AcAaIT were not synergistic by our definitions (Fig. 4), these mixtures significantly reduced their respective median times to death compared with the corresponding values for the virus alone (Fig. 3) and the insecticide alone (Fig. 2). The rates of mortality for these combinations were consistent with either the independent or the similar action additive models. The LT₅₀s of profenofos and DDT were reduced by 37%, whereas LT₅₀s of allethrin and endosulfan were reduced 31% compared with the virus alone.

With the exception of methomyl, responses observed when insects were treated with the wild-type NPV plus conventional insecticides suggest a slight antagonism between the 2 agents. Estimated response curves for the combinations of insecti-

cides and wild-type NPV were marginally higher than the expected responses assuming the hypotheses of independent or similar action additive (Fig. 4). Although estimates of LT₅₀s from bioassays with the wild-type NPV alone were not significantly different from those of the mixtures of wild-type NPV and conventional insecticides (Fig. 4), the total percentage mortalities at 120 h were significantly lower than those expected assuming models of independent or similar additive action (see Fig. 4 and Table 3).

The combination of some recombinant viruses with insecticides will not enhance activity, as shown by the combination of cypermethrin with the recombinant virus AcJHE.KK. We found no evidence of synergism or antagonism when cypermethrin was combined with AcJHE.KK (Fig. 5).

Activity of AcAaIT with Phenobarbital. Because phenobarbital is an anticonvulsant (Katzung 1995), we hypothesized that alleviation of symptoms of neurotoxicity may occur in larvae infected with AcAaIT, thus increasing the lethal times. However, we observed no significant difference in LT₅₀. The LT₅₀ for AcAaIT ($n = 79$) and for the AcAaIT/phenobarbital combination were 94.3 h (90.5–98.1, 95% CL) and 94.5 h (90.7–98.3, 95% CL), respectively. In summary, we found no significant differences in the mortality curves for these 2 treatments.

Efficacy of a Recombinant NPV in Pyrethroid-Resistant Larvae. In comparing the lethal times of larvae infected with wild-type AcNPV or AcAaIT against pyrethroid-susceptible and -resistant (PEG) larvae of *H. virescens*, no significant differences in the LT₅₀ or LT₉₀ for the 2 strains treated with the wild-type virus were found (Fig. 6; Table 4). However, when the recombinant virus was compared for effectiveness against susceptible and pyrethroid-resistant larvae, the results were surprising. Analysis of these data indicated a significant difference both at the LT₅₀ and LT₉₀ between the 2 strains treated with AcAaIT. The resistant strain died from infection by AcAaIT significantly faster. The LT₅₀ and LT₉₀ for the PEG strain were faster by 11 and 25%, respectively, compared with the susceptible Stoneville strain (Table 4).

Discussion

Interactions Between Classical Insecticides and AcNPV. Evaluation of the interactions between the baculovirus and conventional insecticides based on examination of the effect of our treatment combinations on the entire survival curve for each agent and each combination (Fig. 2) suggest that decreases in lethal times resulting from a combination of an NPV and an insecticide might occur assuming several different models. For example, decreases in median times to death would be expected if the insecticide and the NPV acted independently. Assuming the model for independent action, the insecticides might cause most deaths in the first 50 h

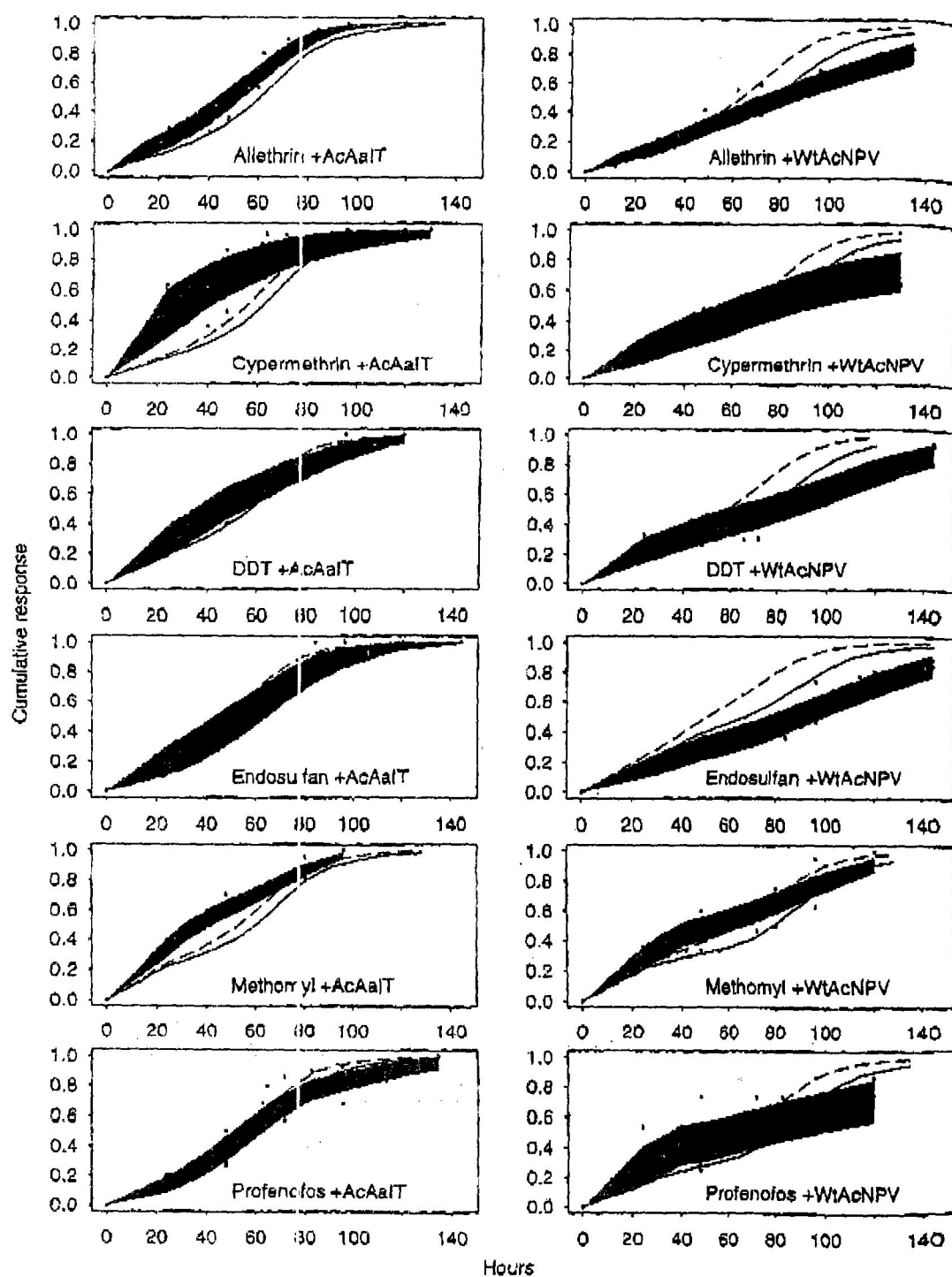


Fig. 4. Observed responses and 95% confidence regions (gray bands) of the estimated response curve (dotted lines) for insects treated with mixtures of conventional insecticides and NPV. Solid curves are expected response probabilities assuming independent action model. Dashed curves are the expected response curves assuming similar action additive model.

Table 3. Estimated percentage of mortality at 120 h postinfection of neonate tobacco budworm to ϕ 61-type AcNPV with low concentrations of classical insecticides

WT AcNPV +	% mortality at 120 h postinfection		
	Observations (95% CL)	Independent model	Additive model
Allethrin	74 (66-81)	91	98
Cypermethrin	70 (57-83)	91	97
DDT	76 (66-84)	93	90
Endosulfan	72 (64-80)	94	99
Methomyl	92 (86-96)	91	97
Profenofos	72 (57-85)	90	97

(i.e., during the period when the NPV levels at the site of action are not lethal). Thus, 50% mortality would occur more quickly in bioassays with insecticides and NPV, rather than with the NPV alone. Alternatively, shorter lethal times might also occur assuming the interactive model. Both models would produce shorter lethal times although the models do not assume synergistic or antagonistic interactions between the 2 agents.

Another model that could predict decreased lethal times involves synergism between insecticide and NPV. Synergism might have a number of biological explanations based on site of action: the same target site/tissue (e.g., insect nerve, same ionic channel); the same target site/tissue, but a different location (e.g., different ionic channels in an insect nerve; the same target site/tissue, same location in the tissue, but different binding site

(e.g., insect nerve, same sodium channel, but different binding sites on the sodium channel); or other physiological interactions (e.g., NPV infection might facilitate absorption or distribution of insecticide, suppress detoxication mechanisms, infection of nerve cells results in increased sensitivity of nervous tissue to insecticides).

Despite the fact that AcJHE.KK kills insects at a rate comparable with that of AcAaIT, lack of increased speed of kill by AcJHE.KK combined with conventional insecticides suggests that enhanced speed of kill will not necessarily occur when other recombinant baculoviruses are combined with conventional insecticides. AcJHE.KK expresses a modified version of juvenile hormone esterase, which is an insect-derived enzyme important in the regulatory development of many lepidopteran insects. The modified JHE is lethal to several lepidopteran species (Bonning and Hammock 1994). Although the mode of action of JHE.KK is not yet fully understood, it probably does not interfere with the insect nervous system. Thus, classical insecticides may not indiscriminately synergize any recombinant baculovirus.

Our results do suggest, however, that the best predictions of positive (=synergistic) interaction among recombinant biological and synthetic pesticides might be based on the potential for interaction between the pesticide and the recombinant product at a receptor (in this case, an ion channel). More subtle interactions may occur at a tissue or physiological level. AaIT and cypermethrin both bind at the insect sodium channel. In support of this interpretation, Herrmann et al. (1995) showed that different scorpion peptide toxins that bind to different sites on the sodium channel can interact

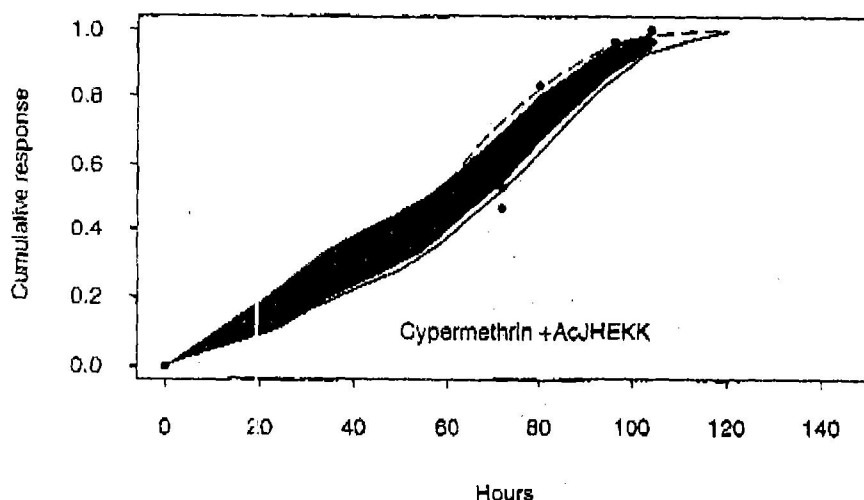


Fig. 5. Observed responses and 95% confidence regions (gray bands) of the estimated response curve (dotted lines) for insects treated with a mixture of cypermethrin and AcJHEKK. Solid curves are expected response probabilities assuming independent action model. Dashed curves are the expected response curves assuming similar action additive model.

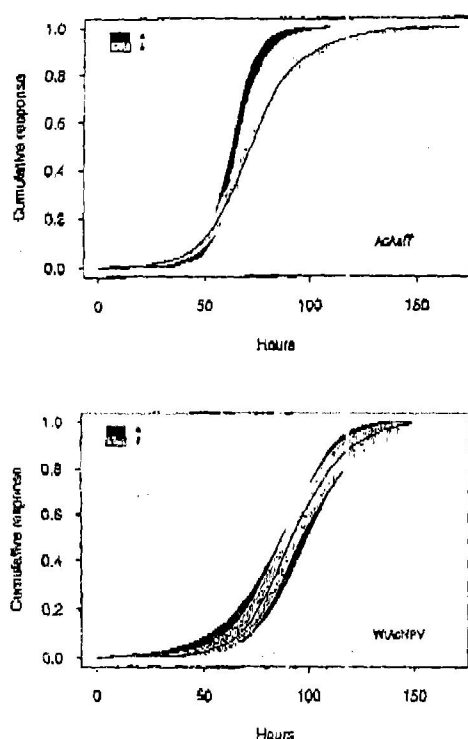


Fig. 6. Estimated cumulative mortality probabilities (and 95% confidence regions) for resistant and susceptible strains treated with AcAaIT (upper panel) and wild-type AcNPV (lower panel).

synergistically, whereas toxins that displace each other from the same binding do not.

Another type of synergistic interaction is illustrated by the carbamate and organophosphate insecticides that act as acetylcholinesterase inhibitors. Accumulation of acetylcholine produces a series of neurological responses that result in paralysis. In the case of synergism between methomyl and AaIT, methomyl probably produces a generalized hyperexcitability of the nervous system, reducing the amount of AaIT needed to cause paralysis. Thus, the materials act at different sites, but a synergistic interaction may be predictable based upon the known pharmacology of each agent.

Although we reported on temporal synergism between a recombinant organism and classical insecticides, synergism may also occur between conventional insecticides and other baculoviruses expressing neurotoxins; other microbials (especially those expressing toxins such as certain fungi and *B. thuringiensis*) and with transgenic plants, especially those expressing *B. thuringiensis* toxins. In many cases, transgenic organisms will be used in pest management programs with synthetic pesticides and other biological agents. We suggest that, as researchers develop these agents for pest con-

Table 4. Time-response of pyrethroid-resistant and -susceptible neonate larvae of *H. virescens*.

Treatment ^a	n	LT ₅₀ (h) (95% CL)	LT ₉₀ h (95% CL)
S AcNPV	306	98 (87-96)	121 (113-132)
PEG AcNPV	168	94 (87-99)	118 (111-128)
S AcAaIT	411	71 (68-74)	103 (95-112)
PEG AcAaIT	240	63 (62-65)	77 (74-84)

PEG, pyrethroid resistant strain; S, pyrethroid susceptible strain.

^a Neonate larvae of PEG and S strains of *H. virescens* were infected with an >LC₅₀ of wild-type AcNPV, or AcAaIT. Infected larvae were monitored for mortality at 8- to 12-h intervals.

trol, they test them with a series of commonly used classical pesticides and other pest management agents for potential synergistic interactions.

Efficacy of a Recombinant NPV in Pyrethroid-Resistant Larvae. These results indicate that the recombinant virus AcAaIT may be more effective against pyrethroid-resistant larvae than pyrethroid-susceptible larvae in field situations, and might be useful combatting pyrethroid resistance. A possible explanation for our results may include a fitness deficit associated with the pyrethroid-resistant strain of *H. virescens*. Campanhola et al. (1991) indicated that this resistant strain has several biological characteristics including slower developmental rates, reduced fecundity and lowered mating efficiency. However, our data obtained with larvae of the PEG strain infected with wild-type AcNPV indicate that the biological constraints identified by Campanhola et al. (1991) are not major factors in time-response. In another study, *H. virescens* populations with an increased tolerance to an organophosphate or pyrethroid insecticide did not show increased susceptibility to infection by NPV (Ignoffo and Roush 1986). Possibly, the pyrethroid-resistant larvae are more sensitive to the neurotoxin AaIT, indicating negatively correlated cross-resistance. Only 1 study to date has confirmed the presence of negative correlation of cross-resistance in insects (Yamamoto et al. 1993).

Pyrethroids now dominate the insecticide market and have accounted for a larger percentage of the total market for insecticides over several years both in the United States and worldwide. However, sales are declining caused in part by the wide-scale presence of pyrethroid resistance. AcAaIT or other recombinant and nonrecombinant baculoviruses with selectivity for resistant insects might provide a means to deter the onset of insecticide resistance. Such viruses might be used to drive resistant populations toward susceptibility.

Slow mortality and resultant crop damage is often cited as a major limitation to the commercial success of viral insecticides. Twenty-four- to 48-h mortality is a common target for conventional in-

secticides, but recombinant baculoviruses designed for enhanced speed of kill have yet to reach this target (Maeda et al. 1991; McCutchen et al. 1991; Stewart et al. 1991; Tomalski and Miller 1991, 1992; Korth and Levings 1993; Bonning and Hammock 1994). Given the emphasis on developing agents that kill target pests more quickly, we concentrated on studying speed of kill expressed as lethal times. Analogous approaches might also emphasize factors likely to be of major economic importance such as lethal doses or concentrations.

In summary, our data indicate that conventional insecticides, especially those that act on the insect nervous system (e.g., pyrethroids and ACHE inhibitors), may provide the greatest chance of synergizing the recombinant viruses in the field. For the pyrethroids, our data suggest that a simultaneous exposure of AaIT and Type II pyrethroids result in a synergistic interaction at the insect sodium channel. Based on these results, we recommend that varying rates of insecticides, in combination with AaAIT and other recombinant baculoviruses that express insect neurotoxins, be tested for efficacy in the field. Recently, several studies have documented that the specificity of recombinant NPVs has not been affected (Heinz et al. 1995; McNitt et al. 1995; McCutchen et al. 1996). We used an approximate LC_{10} - LC_{20} (24 h) of the pesticide to minimize symptoms and mortality resulting directly from the conventional pesticide. In practice, various factors would influence the rates used, including the cost and availability of the biological and synthetic chemical pesticide, environmental concerns, level of resistant populations in the field, and other pest management considerations. The combination of low rates of insecticides and recombinant viruses may also be useful in resistance management strategies.

Acknowledgments

We thank Min Su for his diligent help with the bioassays. We also thank the numerous chemical companies that provided us with technical grade materials. This work was supported by USDA grants 90-37240-5239 and 94-37302-0567, US/Israel BARD #1S-2530-95C, University of California Systemwide Biotechnology Research and Education Program, E. I. DuPont DeNemours & Company, and USDA Forest Service grant 23-696. B.F.M. was supported by a fellowship from National Institutes of Health Environmental Health Sciences. K.H. is a Novo-Nordisk Entotech fellow.

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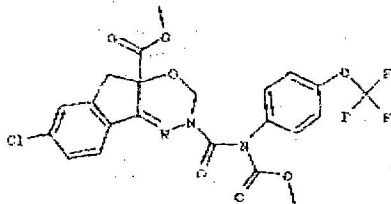
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Received for publication 26 September 1996; accepted 12 March 1997.

Mixtures of Arthropodicides and Fungicides

The compound indeno[1,2-e][1,3,4]oxadiazine-4a(3H)-carboxylic acid, 7-chloro-2,5-dihydro-2-[(methoxycarbonyl)[4-(trifluoromethoxy)phenyl]amino]carbonyl-, methyl ester which has Formula I:



is described in US 5,462,918, together with its preparation, use as an arthropodicide and arthropodical compositions containing it. Other methods for the preparation of the compound of Formula I are described in WO 9529171.

The compound of Formula I may be used as an arthropodicide in its own right or it may be used as an arthropodicide in the form of a composition comprising a solid or liquid carrier or diluent and optionally a wetting, dispersing or emulsifying agent. It may also be used with one or more other arthropodicides and/or with one or more fungicides.

The compound of Formula I may be used in any proportion with other arthropodicides. By including another arthropodicide, the resulting composition can have a broader spectrum of activity, a greater level of intrinsic activity than the compound alone, or a greater level of activity than the additive control of the individual components of the composition. Examples of insecticidal compounds which may be included are: abamectin, chlorfenvinphos, beta-cyfluthrin, emamectin, fenpyroximate, metaldehyde, phosmichlor, spinosad (spinosyns), tebufenozide, tebufenpyrad, thiodicarb, Acephate, Acrinathrin, Alanycarb, Aldicarb, Aldoxycarb, Allethrin, Azadirachtin, Amitraz, Azamethiphos, Azinphos-ethyl, Azinphos-methyl, Borax, Bendiocarb, Benfuracarb, Bensultap, RU 15525, Bifenthrin [(Z)-(1R)-cis isomer], Bioallethrin, Biolallethrin [(S)-cyclopentenyl isomer], Bioresmethrin, Buprofezin, Bifenthrin [(Z)-(1S)-cis isomer], Abamectin, Aluminium phosphide, Butocarbonyl, Butoxyacarbonyl, Cadusafos, Carbaryl, Carbofuran, CGA 183893, Carbosulfan, Cartap, Cartap hydrochloride, Chlordane, Pyrethrin II, Chlorfenvinphos- (Z) isomer, Chlorfenvinphos- (E) isomer, Chlorfluazuron, Chlorfenvinphos, Chloropicrin, CL 26 691, Chlorpyrifos, Chlorpyrifos-methyl, Cloethocarb, Coumaphos, Cyanophos, Cycloprothrin, Cyfluthrin, Cythothrin, Lambda-Cyhalothrin, Cypermethrin, alpha-Cypermethrin, Cyphenothrin, Dazomet, DDT, Deltamethrin, Demeton-S-methyl, Diafenthiuron, Diazinon, Dichlorvos, Dicrotophos,

Dimethylnaphthos, Difenolol, Diflubenzuron, Pyridaphenthion, Dimethoate, Eipronil, Dioxabenzofos, Disulfoton, DNOC, Emperthrin, Endosulfan, EPN, Esfenvalerate, Ethiofencarb, Ethion, Ethoprophos, Ethylene dihydride, Etofenphos, Fenphur, Fenitrothion, Fenobucarb, Fenoxycarb, Fenpropathrin, Fenitrothion, Fenvalerate, Flucyclozuron (E-isomer), Flucythrinate, Flufenoxuron, tau-fluvalinate, Fonofos, Formetanate, Formothion, Furathiocarb, GY-81, Halfeuprox, HCH or BHC or Lindane, Heptachlor, Heptenophos, Hexafluoruron, Hydramethyluron, Hydrogen cyanide, Hydroperene, Imidacloprid, Isazofos, Isafenphos, Isoprocarb, Isoxathion, Malathion, Mecarbam, Mephosfolan, Mercurous chloride, Metam, Metam-sodium, Methacifos (E-isomer), Methacifos, Methamidophos, Methidathion, Methiocarb, Methomyl, Methoprene, Metborychlor, Methyl isothiocyanate, Metolcarb, Mevinphos, Monocrotophos, Naled, Nicotine, Nitenpyram, Omethoate, Oxamyl, Oxydemeton-methyl, Parathion, Parathion-methyl, Pentachlorophenol, Phosphine, Permethrin, Phenothrin, Phenothate, Phorate, Phosalone, Zinc phosphide, Phosmet, Phosphamidon (Z isomer), Phoxim, Pirimicarb, Pirimiphos-ethyl, Pirimiphos-methyl, Prallethrin, Profenofos, Propaphos, Propetamphos, Propoxur, Prothiofos, Pymetrozine, Pyraclofos, Pyridaben, Pyriproxifen, Quinalphos, Resmethrin, Rotenone, Sodium fluoride, Sulcofuron, Sulfuramid, Sulfotep, Sulfuryl fluoride, Sulfonates, Tefluthrin (Z) (1R)-cis, Tefluthrin, Tefluthrin (Z) (1S)-cis, Tefluthrin, Temephos, Terbufos, Tetrachlorvinphos, Tetramethrin, Thiocyclam hydrogen oxalate, Thiofanox, Thiometon, Transluthrin, Tralometrin, Triazamate, Triazophos, Trichlorfon, Triflumuron, Vamidothion, Xyltylcarb, XMC, Trimethacarb (3,4,5 isomer), Cinerin I, Mevinphos (Z-isomer), Mevinphos (E isomer), Tetramethrin [(1R)-isomers], Thiocyclam, AC 303,630, Calcium polysulfide, Chlorofenoxys, Beta-cyfluthrin, beta-Cypermethrin, zeta-Cypermethrin, Sodium cyanide, Lufenuron, NI-25, Nithiazine, Pyrethrin I, Pyrimidifen, Silafluofen, Sulcofuron-sodium, Trimethacarb, Cinerin II, Phenothrin [(1R)-trans-isomer], Phosphamidon (E isomer), Jasmolin I, Jasmolin II, Aldrin, Aminocarb, Bromocyclen, Bromophos, Bufencarb, Bulencarb, Carbon disulfide, Carbon tetrachloride, Carbophenothion, Chlordimeform, Chlorphoxim, Chlorthiophos, Cusumate, Crotoxyphos, Cyanafenphos, Demephion, Demephion, Demeton-O, Demeton-S, Dialifos, Demeton-S-methylsulphon, Dichlorfenthion, 1,1-Dichloro-2,2-bis(4-ethylphenyl)ethane, O-2,4-Dichlorophenyl O-ethyl phosylophosphonothioate, 1,2-Dichloropropane, Dimefox, Dioxathion, Dioxacarb, Endrin, Ethiofencarb, Fenchlorphos, Estrifos, Flucifuron, Fosmethilan, Fosthiolan, IPSP, Isothioate, Jodfenphos, Kinoprene, Leptophos, Naphthalene, Oxydeprofos, Phosfolan, 5-Methyl-m-cumenylbutyl(methyl)carbamate, Promecarb, Prothoate, Ryanodine, Schradan, Sodium hexafluorosilicate, Thionazin, O,O,O',O'-Tetraptopyl dithiopyrophosphate, Triamphos, 2,2,2-Trichloro-1-(3,4-dichlorophenyl)ethyl acetate, Trichloronat, Allylcarb, 1,1-Bis(4-chlorophenyl)-2-nitropropane, 1,1-Bis(4-chlorophenyl)-2-nitrobutane, "1,1-Bis(4-chlorophenyl)-2-nitropropane and -butane mixture", (1R,2R,4R)-bornyl thiocyanacetate, Bis(2-chloroethyl) ether, 1-Bromo-2-chloroethane, 2-(2-Butoxyethoxy)ethyl thiocyanate, Chlordecone, Cyanthoate, Endothion, Ethoate-methyl, Fensulfathion, 2-Isovalerylindane-1,3-dione, Mecarphon, Mexacarbate, Mirex, Nitrilacarb, Nitrilacarb zinc chloride complex, Oxydisulfoton, Thioquinox, DAEP, Acrylonitrile, Amidithion, Amiton hydrogen oxalate, Amiton, Anabasine, Athidathion, Azothoate, Bis(dithoxyphosphinothioyl) disulfide,

Bis(di-isopropoxyphosphinothiyl) disulfide, 3-Bromo-1-chloroprop-1-ene, Camphechlor, Carbamate, Chlorbicyclen, O-3-Chloro-4-nitrophenyl O,O-dimethyl phosphorothioate, 2-Chlorovinyl diethyl phosphate, 2-(4-Chloro-3,5-xylyloxy)ethanol, α -Cumenyl methylcarbamate, Dicapthion, O-2,5-Dichloro-4-iodophenyl O-ethyl ethylphosphonothioate, 1,1-Dichloro-1-nitroethane, 2,2-Dichlorovinyl 2-ethylsulfinylethyl methyl phosphate, Dieldrin, O,O-Diethyl O-4-methyl-2-oxo-2H-chromen-7-yl phosphorothioate, O,O-Diethyl O-6-methyl-2-propylpyrimidin-4-yl phosphorothioate, Diethyl 5-methylpyrazole-3-yl phosphate, Dimethrin, 5,5-Dimethyl-3-oxocyclohex-1-enyl dimethylcarbamate, Dinex, Fluvalinate, Fosphate, Isobenzan, Isodrin, Isolare, Lirafos, Mesulfenfos, Methanesulfonyl fluoride, 3-Methyl-1-phenylpyrazol-5-yl dimethylcarbamate, 2-Methyl(prop-2-ynyl)aminophenyl methylcarbamate, "4-Methyl(prop-2-ynyl)amino-, 3,5-xylyl methylcarbamate", Mipafos, Morphothion, Normicotine, Phenkapton, TDE, Barium polysulfide, Bipernemethrin, Bromfenvinofos, Butathiofos, (E)-2-Chlorobenzoyl-(2,3-dihydro-4-phenyl-1,3-thiazol-2-ylidene)acetonitrile, Chlorprazophos, Coumthioate, (S)-Cyano-3-phenoxycarbonyl (1R)-trans-3-(4(RS)-1,2-dibromo-2,2-dichloroethyl)-2,2-, Decarbofuran, O,O-Diethyl naphthalene-1,8-dicarbonyl-oxophosphorothioate, 2-(4,5-Dimethyl-1,3-dioxolan-2-yl)phenyl methylcarbamate, O-4-Dimethylsulfamoylphenyl O,O-diethyl phosphorothioate, Dioprop, Dithicofos, 2-(1,3-Dithiolan-2-yl)phenyl dimethylcarbamate, EMPC, Fenethcarb, Fenethrin, Fenoxacrin, Fenpirithrin, Fluencil, Flumethrin, Formparanate, Kelevan, Lythidathion, Mazidox, Methocrotophos, Methoquin-butyl, S-Methyl N-(carbamoyloxy)thioacetimidate, Metoxadiazone, Nifluride, Phoxim-methyl, Pirimethaphos, Primidophos, Pyresmethrin, Pyrimitate, Quinalphos-methyl, Quinofos, Quintofos, Sodium selenate, Tazimcarb, Tebupirinfos, Terafethrin, Thicofos, Thiocarbonyl, 2-Thiocyanatoethyl laurate, Transpermethrin, Trifenofos, Zolaprosfos, Phosnichlor and Sophamide.

Suitable acaricides which may be incorporated in a composition with compound of Formula I include: Amidothioate, amitraz, Aramile, Azobenzene, azocyclotin, benzoximate, Bis(4-chlorophenoxy)methane, 1,1-Bis(4-chlorophenyl)-2-ethoxyethanol, bromopropylate, Butacarb, chinomethionat, chlorfenithal, chlorfenson, Chlorfensulphide, Chloromebutham, 4-Chlorophenyl phenyl sulfone, S-4-Chlorophenylthiomethyl O,O-dimethyl phosphorodithioate, chlorobenzilate, Chloropropylate, cyhexatin, 2,4-Dichlorophenyl benzenesulphonate, dicofol, dienechlor, dimocap, Dinopenton, Diphenyl sulfone, Dofenapyn, etoxazole, Fenazafos, fenazaguan, fenbutatin oxide, Fenson, fenothiocarb, fenprothrin, fenpyroximate, Fenrifanil, flucycloxyuron, Fluorbenside, formetanate hydrochloride, Hexadecyl cyclopropanecarboxylate, hexythiazox, Milbemectin A3, Milbemectin A4, MNAP, Pmclonol, propargic, Prothidathion, pyridaben, RA-17, SSI-121, tebufenpyrad, tetradifon, Tezasul and Triarathene.

Suitable biological insecticidal agents which may be incorporated in a composition with compound of Formula I include: *Bacillus thuringiensis*, *Bacillus*

thuringiensis delta endotoxin, baculovirus, and entomopathogenic bacteria, virus and fungi.

Suitable fungicides which may be incorporated in a composition with compound of Formula I include: acypetacs, anilazine, azarconazole, azoxystrobin, benalaxyl, benomyl, biphenyl, bitertanol, blastidicin-S, borax, Bordeaux mixture (tribasic copper sulfate), bromuconazole, bupirimate, butylamine, calcium polysulfide, captafol, captan, carbendazim, carboxin, chinomethionat, chloroneb, chlorothalonil, chlorzoxinate, copper hydroxide, copper oxychloride, copper salts, copper sulfate, cuprous oxide, cymoxanil, cyproconazole, cyprodinil (CGA 219417), dazomet, debacarb, dichlofluanid, diclomezine, dichlone, dichlorophen, diclomezine, dictoran, diethofencarb, difluoconazole, dimethirimol, dimethomorph, diniconazole, diniconazole-M, dinocap, diphenylamine, dithiazon, DNO, dodemorph, dodine, edifenphos, epoxiconazole (BAS 480F), ethirimol, ethoxyquin, etridiazole, famoxadone, fenarimol, fenbuconazole, fenfuram, fenpiclonil, fenpropidin, fenpropimorph, fentin, ferbam, ferimzone, fluazinam, fludioxonil, fluoromide, fluquinconazole, flusilazole, flusulfamide, flutolanil, flutriafol, folpet, formaldehyde, fosetyl-aluminum, fuberidazole, furalaxyl, guazatine, GY-81, hexachlorobenzene, hexaconazole, 8-hydroxyquinoline sulfate, hymexazol, ICI 164502, imazalil, imibenconazole, iminocyclodiol, ipconazole, iprobenfos, iprodione, isopamphos, isoprothiolane, kasugamycin, kresoxim-methyl, mancozeb, mancozeb, maneb, mepanipyrin, mepronil, mercuric chloride, mercuric oxide, mercurous chloride, metalaxyl, metam, metconazole, methasulfocarb, methyl isothiocyanate, 5-methyl-5-(4-phenoxycyclohexyl)-3-phenylamino-2,4-oxazolidinedione (DPX-TE874), metiram, S-methyl 7-benzothiazolecarbothioate (CGA 245704), myclobutanil, nabam, naphtheneic acid, natamycin, neo-asozin (feric methanearsonate), nickel bis(dimethyldithiocarbamate), nitrothal-isopropyl, nuarimol, octhilinone, ofurace, oxadixyl, oxine-copper, oxycarboxin, pefurazate, penconazole, pencycuron, pentachlorophenol, phenylmercury acetate, 2-phenylphenol, phosdiphen, phthalide, pipetalin, polyoxins, probenazole, prochloraz, procymidone, propamocarb, propiconazole, propineb, pyrazophos, pyrifentox, pyrimethanil, pyroquilon, quintozene, SSF-109, spiroxamine (KWG4168), sulfur, tar oils, tebuconazole, tecnazene, tetraconazole, thiabendazole, thifluzamide, thiophanate-methyl, thiram, tolclofos-methyl, tolyfluanid, triadimenol, triadimenon, triadimenol, triazoxazole, tridemorph, triflumizole, triflorine, triticonazole, validamycin, vinclozolin, zineb and ziram.

The compositions containing the compound of Formula I may be used to control one or more of the arthropods listed in US 5,462,938 and others, including southern corn rootworm (*Diabrotica undecimpunctata howardi*), aster leafhopper (*Mastrotyle fasciatus*), boll weevil (*Anthonomus grandis*), two-spotted spider mite (*Tetranychus urticae*), fall armyworm (*Spodoptera frugiperda*), black bean aphid (*Aphis fabae*), green peach aphid (*Myzus persicae*), cotton aphid (*Aphis gossypii*), Russian wheat aphid (*Diuraphis noxia*), English grain aphid (*Sitobion avenae*), tobacco budworm (*Heliothis virescens*), rice water weevil (*Lissorhynchus oryzaephilus*), rice leaf beetle (*Oulema oryzae*), whitebacked planthopper (*Sogatella furcifera*), green leafhopper (*Nephotettix*

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cincticeps), brown planthopper (*Nilaparvata lugens*), small brown planthopper (*Laodelphax striatellus*), rice stem borer (*Chilo suppressalis*), rice leafroller (*Cnuphalocroceis medialis*), black rice stink bug (*Scotinophara lurida*), rice stink bug (*Oebolus pugnax*), rice bug (*Leptocoris chinensis*), slender rice bug (*Cleus puniger*), southern green stink bug (*Nezara viridula*), *Tetranychus urticae*, *Tetranychus cinnabarinus*, *Tetranychus modanii*, *Tetranychus pacificus*, *Tetranychus turkestanii*, *Byrbia rubriculus*, *Panonychus ulmi*, *Panonychus citri*, *Eotetranychus carpini borealis*, *Eotetranychus hirsutus*, *Eotetranychus sexmaculatus*, *Eotetranychus yuensis*, *Eotetranychus banksi* and *Oligonychus pratensis*, Tenuipalpidae including *Brevipalpus lewisii*, *Brevipalpus phoenicis*, *Brevipalpus californicus* and *Brevipalpus obovatus*; Eriophyidae including *Phyllocoptruta oleivora*, *Eriophyes sheldoni*, *Acidus cornutus*, *Ephritomerus pyri* and *Eriophyes mangiferae*.

The compound of Formula I or a composition containing it as already described may be applied by land or aerial application systems, mixed with soil, peat or other rooting media for the protection of plants against seed-borne or foliar arthropod pests.

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Multi Connector Clip Attachment

Traditional designs incorporate 1 clip per connector and then the connector is mounted in a hole. With the features incorporated in the multi connector clip attachment, 2 connectors can be attached to each other (Fig. 1). A single clip can then be attached to the multi connector clip attachment (Fig. 2). The entire array can then be mounted in a single hole. This is beneficial when only 1 clip hole is available for attachment of a connector.

Another advantage of the multi connector clip attachment is 2 connectors can be strapped to the wiring harness with a tie strap by sliding it through the hole feature in the multi connector clip attachment (Fig. 3). The array can then be attached to the harness bundle. This is beneficial when there is no attachment holes to mount the connectors.

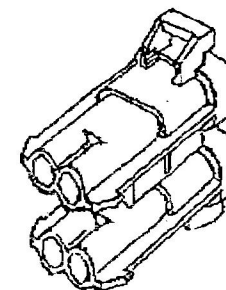


Fig. 1

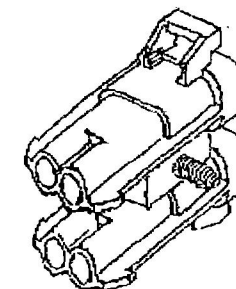


Fig. 2

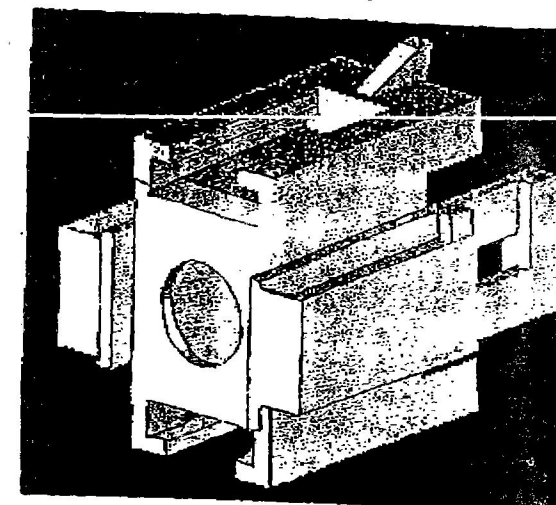


Fig. 3

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A B O G A D O S

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SUPERINDUSTRIA Y COMERCIO

Radicación : 00017310 00000000 Folios: 11
Fecha (AMD): 2005-09-07 16:54:36
Trámite : 002 PATENTES D 1 REGISTRO/ 444 REPUESTA
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Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

DIVISION DE NUEVAS CREACIONES

E. S. D.

Re: P1.-WYETH HOLDINGS CORPORATION.-Solicitud de Patente de Invención para "COMPOSICIONES INSECTICIDAS SINERGICAS QUE COMPRENEN UN ANTAGONISTA DEL CANAL DE SODIO NEURONAL Y UN INSECTICIDA".-Clase A.01N.- Expediente número 00-017.310.-

1. Me refiero al auto dictado por ese Despacho y notificado por fijación en lista de 16 de Junio de 2005.
2. De acuerdo con el Concepto Técnico No. **682**, el señor Examinador objeta la claridad del capítulo reivindicatorio actualmente presentado, afirmando que: *"La reivindicación 1, es muy general y no delimita el alcance de la invención puesto que abarca en forma general grupos de compuestos que corresponden a funciones químicas diferentes y de otro lado no se especifican los compuestos. Así como tampoco esta suficientemente sustentado que todas las mezclas posibles de acuerdo a lo reivindicado presenten efecto sinérgico"* (resaltado fuera de texto).

Sobre este particular, me permito manifestar que el solicitante ha decidido modificar las reivindicaciones 1, 2 y 3 estudiadas por el señor Examinador, con el fin de superar las objeciones anteriormente expuestas. En este sentido, **se ha limitado considerablemente** el alcance de la reivindicación 1 al restringir la definición de los compuestos antagonistas del canal neuronal de sodio únicamente a los compuestos de Fórmula I, eliminando de esta forma los compuestos de Fórmula II, III, IV y V y los significados de sus respectivas variables. Igualmente, se ha reemplazado la expresión "toxina" por "neurotoxina", existiendo el sustento del anterior cambio en el numeral 3 de la página 10 de la descripción.

En relación con lo anterior, se ha modificado tanto la reivindicación 2 para eliminar la expresión "**ó III**", como la reivindicación 3 para eliminar la expresión "**Z' es Cl; R₃₃ y G**

son cada uno CO_2CH_3 ; Q es p-(trifluorometoxi)fenilo", correspondientes a la materia que ha sido excluida de la reivindicación 1 ahora presentada.

De acuerdo con las modificaciones expuestas, me permito manifestar al señor Examinador que el alcance de la invención ha sido significativamente delimitado, tal como lo había solicitado en su Concepto Técnico No. **682**, toda vez que el número de posibles combinaciones ha sido drásticamente reducido y enfocado únicamente a aquellas que se puedan realizar únicamente con el compuesto de Fórmula I.

En consecuencia, me permito presentar a esa Oficina un nuevo capítulo reivindicatorio en el cual se han realizado las modificaciones arriba mencionadas, y el cual reemplaza el juego de reivindicaciones que se encuentra actualmente bajo estudio. Adicionalmente, me permito manifestar que el nuevo capítulo reivindicatorio presentado permite superar dichas objeciones de claridad, toda vez que se define de manera más clara y completa la materia objeto de la presente invención.

Ahora bien, frente a la afirmación según la cual *"tampoco esta suficientemente sustentado que todas las mezclas posibles de acuerdo a lo reivindicado presenten efecto sinérgico"*, atentamente me permito dirigir la atención del señor Examinador a los ejemplos enseñados en la descripción de la presente solicitud, **específicamente a los Ejemplos 2 y 3 (páginas 16 a 20), en los cuales se demuestra el comportamiento sinérgico de las mezclas de los compuestos de Fórmula I y diferentes exponentes de los compuestos del Grupo I, lo que permite afirmar que el efecto sinérgico de las posibles mezclas, contrario a la opinión del señor Examinador, sí se encuentra suficientemente sustentado dentro de la presente invención**, máxime si se tiene en cuenta la limitación realizada con respecto a los antagonistas del canal neuronal de sodio manifestada mediante el presente escrito.

Adicionalmente, el señor Examinador objeta la claridad de la solicitud argumentando que *"Las reivindicaciones de método para el control de insectos, así como la protección de plantas de la infestación de insectos, tal como se reivindican son muy generales y no define una forma de realización específica. De otra parte es el método no se caracteriza por el tipo de producto o compuesto que se aplique"*.

Frente a estas objeciones, me permito manifestar al señor Examinador que de acuerdo con el contexto de la invención, los métodos han sido claramente definidos y pueden ser realizados por cualquier persona versada en la materia que desee aplicar los conocimientos revelados por el solicitante en la presente invención, es decir, las reivindicaciones son completamente claras y cumplen con la finalidad de delimitar el alcance de la invención. En este sentido, resulta de la mayor importancia resaltar que al tratarse de composiciones insecticidas, los métodos para la protección de plantas y control

de plagas deben corresponder a la aplicación de la novedosa e inventiva composición reivindicada sobre aquellos objetos que la requieren.

Adicionalmente, el señor Examinador debe tener en cuenta que los métodos reivindicados no corresponden a cualquier tipo de método general y sin limitación, sino que comprenden la etapa de aplicación de una composición insecticida **única y específica**, tal como ha sido enseñada por el solicitante, sobre unos objetos también específicos con el fin de alcanzar un resultado técnico bien definido, como lo es la protección de plantas de la infestación por insectos o el mismo control de insectos.

Por lo tanto, me permito manifestar que las reivindicaciones de método sí cumplen con el requisito de claridad, toda vez que están definiendo en forma clara y precisa el alcance de la invención y enseñan a la persona versada en la materia (a quien va dirigida la invención), cómo realizar la misma dentro del campo técnico relacionado.

Finalmente, me permito manifestar a esa Oficina que las modificaciones realizadas en el nuevo capítulo reivindicatorio **no amplían** de ninguna manera el alcance de la presente invención, toda vez que las mismas corresponden a significativas limitaciones de la materia descrita en la solicitud, dirigidas a superar las objeciones de claridad, establecidas en el Concepto Técnico No. **682**, así como también a resaltar las características novedosas e inventivas de la presente invención.

3. Por otra parte, el señor Examinador objeta la unidad de invención de la presente solicitud argumentando que *"La invención comprende una amplia gama de mezclas diferentes"* entre los compuestos de fórmulas I a V y los compuestos del grupo I, complementando su discusión al manifestar que ***"los compuestos son conocidos en el arte previo de manera que no existe un único concepto común inventivo en todas las mezclas. El hecho de que los compuestos de fórmulas I a V sean antagonistas del canal de sodio neuronal, corresponde a una propiedad de dichos compuestos que es inherente a los mismos"*** (resaltado fuera de texto).

Sobre este particular, me permito llamar la atención del señor Examinador a los siguientes argumentos técnicos, por medio de los cuales se superan plenamente sus objeciones con respecto a la falta de unidad de invención. Como primera medida, me permito resaltar nuevamente que la reivindicación 1 de la presente solicitud ha sido considerablemente modificada, con el fin de limitar aún más el alcance de la misma, **por lo cual la "amplia gama de mezclas" posible señalada por el señor Examinador se ha visto reducida en forma significativa a aquellas mezclas que solamente incluyen los compuestos de fórmula I**, mezclas que cuentan con el sustento proporcionado mediante los ejemplos de la memoria descriptiva, tal como se manifestó anteriormente en este mismo escrito.

Adicionalmente, me permito resaltar al señor Examinador que el concepto único inventivo de la presente solicitud no yace en el hecho de que el compuesto de fórmula I o cualquiera de los compuestos del Grupo I sean conocidos en el estado de la técnica *per se*, toda vez que el solicitante no está buscando protección para cualquiera de estos compuestos. Por el contrario, la unidad inventiva radica en la **novedosa e inventiva composición insecticida sinérgica que comprende una mezcla de estos componentes**, de tal forma que el hecho de que los compuestos sean conocidos en el estado de la técnica no afecta de ninguna forma la unidad de la presente invención, ya que los compuestos individuales en sí **no son el objeto de la solicitud. El objeto de la invención es la composición insecticida y los métodos de control de plagas y protección de plantas que involucran a dicha composición.**

Finalmente, me permito señalar que el solicitante no comprende el argumento del señor Examinador relacionado con la objeción a la unidad de invención, dirigido a resaltar que *"El hecho de que los compuestos de fórmulas I a V sean antagonistas del canal de sodio neuronal, corresponde a una propiedad de dichos compuestos que es inherente a los mismos"*. Con respecto a lo anterior, si bien se trata de una propiedad de estos compuestos, esta no ha sido desconocida por el solicitante, ni corresponde al objeto a reivindicar, **sino que es una característica de estos compuestos que contribuye al efecto sinérgico enseñado por las composiciones que constituyen el verdadero objeto de la invención.**

En conclusión, dada la significativa limitación hecha sobre el alcance de la invención mediante la restricción a los compuestos de fórmula I en el capítulo reivindicatorio tal como ha sido modificado, y considerando que el objeto de la invención es la **composición insecticida sinérgica** y no los compuestos de fórmulas I a V o del Grupo I *per se* (o algunas de sus propiedades), se ha demostrado que la presente solicitud sí cumple con los requisitos de claridad y unidad de invención exigidos por la Decisión 486.

4. Anexo me permito presentar:

- (i) Recibo número 05 - 64,180 expedido por la Superintendencia de Industria y Comercio, que acredita el pago de las modificaciones mencionadas; y
- (ii) Copia de las páginas 21 a 25, que corresponden al nuevo capítulo reivindicatorio, en el cual se han realizado las modificaciones anteriormente expuestas y que reemplaza el capítulo que se encuentra actualmente bajo estudio.

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5) formamidine compounds which are known to be insecticidally active such as amitraz, chlordimeform, hydramethylnon, chlorfenamidine, or the like;

6) macrocyclic lactone compounds which are known to be insecticidally active such as spinosad, avermectin, emamectin, milbemectin, nemadectin, moxidectin or the like;

7) amidinohydrazone compounds which are known to be insecticidally active such as hydramethylnon;

8) GABA antagonist compounds which are known to be insecticidally effective such as fipronil, endosulfan, or the like;

9) acetylcholine receptor ligand compounds which are known to be insecticidally effective such as imidacloprid, acetamiprid, nitenpyram, thiamethoxam, or the like.

Descriptions of the above-listed commercially available compounds may be found in The Pesticide Manual, 11th Edition, British Crop Protection Council (1997) among other publications.

Preferred compositions of the invention are those compositions having a neuronal sodium channel antagonist compound of formula I or formula III in combination with one or more compounds selected from Group A.

More preferred compositions of the invention are those compositions having a formula I or formula III compound wherein W is O; X is trifluoromethoxy and is in the 4-position; Y is trifluoromethyl and is in the 3-position; Z is CN and is in the 4-position; A is CH₃; n is 0; m, p and q are each independently 1; R and R₁ are each independently H; Z' is Cl; R₂ and G are each independently CO₂CH₃; Q is p-(trifluoromethoxy)phenyl; and the dotted line configuration C=N represents a double bond in combination with one or more compounds selected from Group A.

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5. En vista de lo anterior, atentamente me permito solicitar a ese Despacho, proceder a estudiar nuevamente la solicitud a la luz de las modificaciones realizadas y de los argumentos aquí expuestos y con base en ellos conceder el privilegio de patente solicitado.

Señor Superintendente,


ALICIA LLORED A RICAURTE
T.P. 53.215
Código 231

ESTA CERTIFICACION DE EXCEDENTE DE PAGO SOLO PODRA SER UTILIZADA
COMO PARTE DE LA CANCELACION DE UNA TARIFA VIGENTE Y NO PODRA SER
SUSTITUIDA POR NINGUN RECIBO.

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

SUPERINDUSTRIA Y COMERCIO

Radicacion : 00017310 00000000

Folios: 11

Fecha (AMD): 2005-09-07 16:54:36

Tramite : 002 PATENTES D 1 REGISTRO/ 444 REPUESTA

Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

RECIBO OFICIAL DE CAJA : 05 - 64,180
FECHA : AGOSTO 24 DE 2005

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	Vr. PAGO
LLOREDA Y CIA S. A	CONSIGNACION	BANCO POPULAR	050-00110-6	39307495	24/08/2005	1,908,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-03	CAMBIO DE MODALIDAD Y MODIFICA	
		12 MARCAS Y LEMAS COMERCIALES	91,000.00
		TOTAL :	\$ 91,000.00

SON: NOVENTA Y UNO MIL PESOS

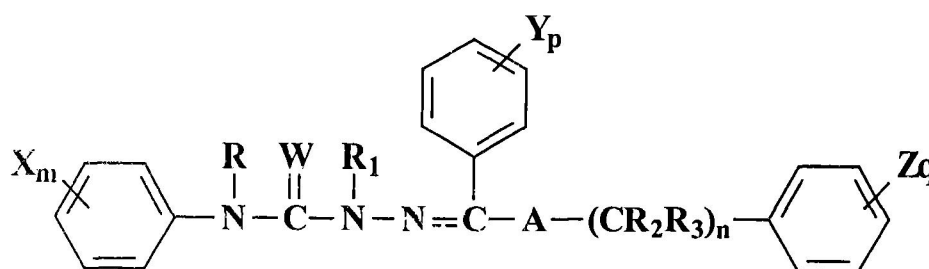
RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

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REIVINDICACIONES

1. Una composición insecticida que comprende un antagonista del canal de sodio neuronal de la fórmula I:



(I)

en donde:

A es CR^4R^5 o NR^6 ;

W es O o S;

X, Y y Z son independientemente H; halógeno; OH; CN; NO_2 ; alquilo C_1-C_6 no sustituido o sustituido con uno o más grupos halógeno, alcoxi C_1-C_3 , haloalcoxi C_1-C_3 , cicloalquilo C_3-C_6 , alquenilo C_2-C_6 o sulfonilo;

alcoxi C_1-C_6 no sustituido o sustituido con uno o más grupos halógeno, alcoxi C_1-C_3 o cicloalquilo C_3-C_6 ;

alcoxicarbonilo C_1-C_6 , cicloalquilcarbonilo C_3-C_6 , fenilo no sustituido o sustituido con uno o más grupos halógeno, alquilo C_1-C_4 , o alcoxi C_1-C_4 ;

aminocarbonilo no sustituido o sustituido con uno o más grupos alquilo C_1-C_3 ;

alcoxicarbonilo C_1-C_6 ; alquilsulfonilo C_1-C_6 ; alqueno C_2-C_6 ; o $NR^{12}R^{13}$;

m, p y q son cada uno independientemente un entero de 1, 2, 3, 4 ó 5;

n es un entero de 0, 1 ó 2;

R, R¹, R², R³, R⁴ y R⁵ son cada uno independientemente H o alquilo C₁-C₄;

R⁶ es H, alquilo C₁-C₆, haloalquilo C₁-C₆, alcoxialquilo C₁-C₆, alcoxi C₁-C₆, haloalcoxi C₁-C₆, alqueno C₂-C₆, alquino C₂-C₆, alquilcarbonil C₁-C₆, alcóxicarbonil C₁-C₆, alquiltio C₁-C₆, o haloalquiltio C₁-C₆;

R¹² y R¹³ son cada uno independientemente H o alquilo C₁-C₆;

y la configuración de línea punteada C===N representa un enlace doble o un enlace simple;

o un estereoisómero del mismo;

y uno o más compuestos seleccionados del Grupo I:

- 1) compuestos piretroides;
- 2) compuestos de tipo peritroide;
- 3) nucleopolihedrovirus recombinantes capaces de expresar una neurotoxina de insecto;
- 4) compuestos de organofosfato;
- 5) compuestos de carbamato;
- 6) compuestos de formamidina;
- 7) compuestos de lactona macrocíclica;
- 8) compuestos de amidinohidrazona;
- 9) compuestos antagonistas de GABA; y
- 10) compuestos de ligando de receptor de acetilcolina;

en una cantidad sinérgicamente activa del antagonista del canal de sodio neuronal en combinación con uno o más compuestos seleccionados entre el Grupo I en una relación de 1 parte en peso del antagonista del canal de sodio neuronal a 0,01-100 partes en peso de los compuestos seleccionados del Grupo I.

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2. La composición de acuerdo con la reivindicación 1, en donde el antagonista del canal de sodio neuronal es un compuesto de fórmula I y la configuración de línea punteada $C \cdots N$ representa un enlace doble.

3. La composición de acuerdo con la reivindicación 2, en donde W es O; X es trifluorometoxi, y está en la posición 4; Y es trifluorometilo y está en la posición 3; Z es CN y está en la posición 4; A es CH_2 ; n es 0; m, p y q son cada uno 1; R y R_1 son cada uno H.

4. La composición de acuerdo con la reivindicación 3, en donde el o más compuestos seleccionados entre el "Grupo (I)" son (RS)- α -ciano-3-fenoxibenzil (1RS,3RS;1RS,3SR)-3-(2,2-diclorovinil)-2,2-dimetilciclopropanocarboxilato (cipermetrina), (RS)- α -ciano-3-fenoxibenzil (Z)-(1RS,3RS)-(2-cloro-3,3,3-trifluoropropenil)-2,2-dimetilciclopropanocarboxilato (cihalotrina), (RS)- α -ciano-4-fluoro-3-fenoxibenzil (1RS,3RS;1RS,3SR)-3-(2,2-diclorovinil)-2,2-dimetilciclopropanocarboxilato (ciflutrina), 3-fenoxibencil (1RS,3RS;1RS,3SR)-3-(2,2-diclorovinil)-2,2-dimetilciclopropanocarboxilato (permetrina), 2-(4-etoxifenil)-2-metilpropil 3-fenoxibencil eter (etofenprox), (4-etoxifenil)[3-(4-fluoro-3-fenoxifenil)propil](dimetil)silano (silafluofén), (RS)- α -ciano-4-fluoro-3-fenoxibenzil (1RS,3RS;1RS,3SR)-3-(2,2-diclorovinil)-2,2-dimetilciclopropanocarboxilato (fipronil), (1,4,5,6,7,7-hexaclaro-8,9,10-trinorborn-5-en-2,3-ilenobismetileno) sulfito (endosulfón), 1-(6-cloro-3-piridilmetil)-N-nitroimidazolidin-2-ilidenoamina (imidacloprid), (E)- N^1 -[(6-cloro-3-piridil)metil]- N^2 -ciano- N^1 -metilacetamidina (acetamiprid), (E)-N-(6-cloro-3-piridilmetil)-N-etil-N'-metil-2-nitrovinilidenodiamina (nitenpiram), 3-(2-cloro-1,3-tiazol-5-ilmetil)-5-metil-1,3,5-oxadiazinan-4-ilideno(nitro)amina (tiametoxam), O-4-bromo-2-clorofenil O-etil S-propil fosforotioato (profenofos), O,S-dimetil acetilfosforamidotioato (acefato), O-etil O-4-(metiltio)fenil S-propil fosforoditioato (sulprofos), dietil (dimetoxitiofosforiltio)succinato (malatión), O,O-dietil O-2-isopropil-6-metilpirimidin-4-il fosforotioato (diazinón), O,O-dimetil O-4-nitrofenil fosforotioato (metil paratión), S-tert-butiltiometil O,O-dietil fosforoditioato (terbufós), S-metil N-(metilcarbamoiloxi)tioacetimidato (metomil), 3,7,9,13-tetrametil-5,11-dioxa-2,8,14-tritia-4,7,9,12-tetra-azapentadeca-3,12-dieno-6,10-

diona (tiodicarb), S-4-fenoxibutil dimetiltiocarbamato (fenotiocarb), N-metilbis(2,4-xililiminometil)amina (amitraz), N²-(4-cloro-o-tolil)-N¹,N¹-dimetilformamidina (clordimeform), N'-(4-cloro-2-metilfenil)-N,N-dimetilmetanimidamida (clorfenamidina), mezcla de (10E,14E,16E,22Z)-(1R,4S,5'S,6S,6'R,8R,12S,13S,20R,21R,24S)-6'-[(S)-sec-butil]-21,24-dihidroxi-5',11,13,22-tetrametil-2-oxo-(3,7,19-trioxatetraciclo[15.6.1.1^{4,8}.0^{20,24}])pentacosa-10,14,16,22-tetraeno)-6-espiro-2'-(5',6'-dihidro-2'H-piran)-12-il 2,6-dideoxi-4-O-(2,6-dideoxi-3-O-metil-α-L-arabino-hexopiranosil)-3-O-metil-α-L-arabino-hexopiranosido y (10E,14E,16E,22Z)-(1R,4S,5'S,6S,6'R,8R,12S,13S,20R,21R,24S)-21,22-dihidroxi-6'-isopropil-5',11,13,22-tetrametil-2-oxo-(3,7,19-trioxatetraciclo[15.6.1.1^{4,8}.0^{20,24}])pentacosa-10,14,16,22-tetraeno)-6-espiro-2'-(5',6'-dihidro-2'H-piran)-12-il 2,6-dideoxi-4-O-(2,6-dideoxi-3-O-metil-α-L-arabino-hexopiranosil)-3-O-metil-α-L-arabino-hexopiranosido (avermectina), mezcla de (10E,14E,16E,22Z)-(1R,4S,5'S,6S,6'R,8R,12S,13S,20R,21R,24S)-6'-[(S)-sec-butil]-21,24-dihidroxi-5',11,13,22-tetrametil-2-oxo-(3,7,19-trioxatetraciclo[15.6.1.1^{4,8}.0^{20,24}])pentacosa-10,14,16,22-tetraeno)-6-espiro-2'-(5',6'-dihidro-2'H-piran)-12-il 2,6-dideoxi-3-O-metil-4-O-(2,4,6-trideoxi-3-O-metil-4-metilamino-α-L-lixo-hexapiranosil)-α-L-arabino-hexapiranosido y (10E,14E,16E,22Z)-(1R,4S,5'S,6S,6'R,8R,12S,13S,20R,21R,24S)-21,24-dihidroxi-6'-isopropil-5',11,13,22-tetrametil-2-oxo-(3,7,19-trioxatetracyclo[15.6.1.1^{4,8}.0^{20,24}])pentacosa-10,14,16,22-tetraeno)-6-espiro-2'-(5',6'-dihidro-2'H-piran)-12-il 2,6-dideoxi-3-O-metil-4-O-(2,4,6-trideoxi-3-O-metil-4-metilamino-α-L-lixo-hexapiranosil)-α-L-arabino-hexapiranosido (emamectina), mezcla de: (10E,14E,16E,22Z)-(1R,4S,5'S,6R,6'R,8R,13R,20R,21R,24S)-6'-etil-21,24-dihidroxi-5',11,13,22-tetrametil-3,7,19-trioxatetraciclo[15.6.1.1^{4,8}.0^{20,24}])pentacosa-10,14,16,22-tetraeno-6-espiro-2'-tetrahidropiran-2-ona y (10E,14E,16E,22Z)-(1R,4S,5'S,6R,6'R,8R,13R,20R,21R,24S)-21,24-dihidroxi-5',6',11,13,22-pentametil-3,7,19-trioxatetraciclo[15.6.1.1^{4,8}.0^{20,24}])pentacosa-10,14,16,22-tetraeno-6-espiro-2'-tetrahidropiran-2-ona en la proporción 7 a 3 (milbemectina), [6R,23E,25S,(E)]-5-O-demetil-28-deoxi-25-(1,3-dimetil-1-butenil)-6,28-epoxi-23-hidroxi-milbemicina B. (nemadectina), o [6R,23E,25S,(E)]-5-O-demetil-28-deoxi-25-(1,3-dimetil-1-butenil)-6,28-epoxi-23-(metoxiimino)milbemicina B. (moxidectina).

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5. La composición de acuerdo con la reivindicación 3, en donde el o más compuestos seleccionados entre el "Grupo (I)" es un nucleopolihedrovirus recombinante capaz de expresar toxina de insecto, el cual es el HzNPV-AaIT, Helicoverpa Nucleopolihedrovirus que expresa toxina del insecto Androctonus australis.
6. La composición de acuerdo con la reivindicación 3, en donde el o más compuestos seleccionados entre el "Grupo (I)" son **hidrametilnón** (5,5-dimetilperhidropirimidin-2-ona
4-trifluorometil- α -(4-trifluorometilstilil)cinamilidenohidrazona).
7. Un método para el control sinérgico de insectos, que comprende poner en contacto dicho insecto con una composición de cualquiera de las reivindicaciones 1-6.
8. El método de acuerdo con la reivindicación 7, en donde el insecto se selecciona entre el grupo que consiste de Blatarios, Isópteros, Dípteros e himenópteros.
9. El método de acuerdo con la reivindicación 8, en donde los insectos son lepidópteros o cleópteros.
10. Un método para la protección de una planta contra la infestación y ataque de insectos, que comprende aplicar al follaje o tallo de dicha planta, una cantidad sinérgicamente efectiva de una composición de acuerdo con cualquiera de las reivindicaciones 1-6.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES

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2006/03/11

2020

Bogotá, D.C.:

Doctora
Alicia Lloreda Ricaurte
Apoderada

Oficio 3094

REFERENCIA: Radicación N° 00-17310
Trámite 002
Evento 001
Actuación 430
Folios 004

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. DOCUMENTOS ESTUDIADOS:

- Capítulo descriptivo folios 5 a 25, 188 a 199
- Capítulo reivindicatorio reivindicaciones 1 a 10 folios 261 a 265,; enviadas como respuesta, junto con memorial de 07 de septiembre de 2005, folios 255 a 260.

El artículo 9 de la Decisión 486 de la Comisión del Acuerdo de Cartagena establece que:

"La primera solicitud de patente de invención o de modelo de utilidad, o de registro de diseño industrial o de marca, válidamente presentada en otro País Miembro o de una autoridad nacional, regional o internacional con la cual el País Miembro estuviera vinculado por algún tratado que establezca el derecho de prioridad análogo al que establece la presente Decisión, conferirá al solicitante o a su causahabiente un derecho de prioridad para solicitar en el País Miembro una patente o un registro respecto de la misma materia. El alcance y los efectos del derecho de prioridad serán los previstos en el Convenio de París para la protección de la Propiedad Industrial..."

"...Para beneficiarse del derecho de prioridad, la solicitud que la invoca deberá presentarse dentro de los siguientes plazos improrrogables contados desde la fecha de presentación de la solicitud cuya prioridad se invoca:

- a) doce meses para las patentes de invención y de modelo de utilidad; y
- b) seis meses para los registros de diseños industriales y de marcas"

La solicitud fue presentada el 29 de marzo de 2000 y se reivindicó prioridad de las solicitudes US 60/124306 de 12 de marzo de 1999 y US 60/158201 de 7 de octubre de 1999, de las cuales se presentó la copia respectiva. Por lo tanto, de acuerdo a los artículos 9 a 11 de la decisión 486, la prioridad reivindicada es válida.

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2. OBJETO DE LA INVENCION:

La invención comprende:

- Una composición insecticida que comprende un antagonista del canal de sodio neuronal de fórmula I y uno o más compuestos seleccionados del grupo I: (1) compuestos piretroides, (2) compuestos tipo piretroide, (3) nucleopolihedrovirus recombinantes capaces de expresar una toxina de insecto, (4) compuestos de organofosfato; (5) compuestos de carbamato, (6) compuestos de formamidina, (7) compuestos de lactona macrocíclica, (8) compuestos de amidinohidrazona; (9) compuestos antagonistas de GABA; y (10) compuestos de ligando de receptor de acetilcolina; en una cantidad sinérgicamente activa del antagonista del canal de sodio neuronal en combinación con uno o más compuestos seleccionados entre el grupo I en una relación de una parte en peso del antagonista del canal de sodio neuronal a 0,01-100 partes en peso de los compuestos seleccionados del grupo (I).
- Método para el control de insectos que comprende poner en contacto dicho insecto con una de las composiciones reivindicadas..
- Método para proteger una planta contra la infestación y ataque de insectos que comprende aplicar al follaje o al tallo de dicha planta una cantidad sinérgicamente eficaz de la composición.
- Clasificación Internacional de Patentes A01N47/28.

3. CLARIDAD DE LA SOLICITUD:

Conforme a lo establecido en el artículo 30 de la decisión 486 de la comisión de la comunidad andina las reivindicaciones deben ser claras, concisas y estar enteramente sustentadas en la descripción.

No obstante se haya modificado el capítulo reivindicatorio, la reivindicación 1, continua siendo muy general por cuanto o se delimita el alcance de la invención puesto que abarca diez grupos de compuestos que corresponden a funciones químicas diferentes y donde no se especifican los compuestos. Tampoco esta suficientemente sustentado en el capítulo descriptivo que toda esa amplia gama de mezclas posibles de acuerdo a lo reivindicado presenten efecto sinérgico.

Con respecto a la fórmula estructural no es claro la forma como se indica que "la configuración de línea punteada C===N representa un enlace doble o un enlace simple" pero en la fórmula no aparece dicho enlace representado así, lo que allí se muestra es un enlace formado por una línea continua y una punteada, lo cual de acuerdo a lo contemplado en la reivindicación N° 2 representa un enlace doble entonces no es congruente la forma de representarlo en la estructura con lo que se indica dentro de la reivindicación N°1 y con lo indicado en la reivindicación N°3. De otra parte tampoco se indica cuando dicho enlace corresponde a un enlace sencillo como queda la fórmula estructural con respecto a los átomos de C y N que conforman dicho enlace, puesto que debe entonces completarse la valencia de cada uno de ellos con algún sustituyente.

Las reivindicaciones de método para el control de insectos, así como para la protección de plantas de la infestación de insectos, tal como se reivindican son muy generales y no define una forma de realización específica. De otra parte es el método no se caracteriza por el tipo de producto o compuesto que se aplique.

El encabezado de la reivindicación N° 7 "un método para el control sinérgico de insectos.." no es claro por cuanto un "control sinérgico de insectos" no significa nada.

Es de anotar que si bien la memoria descriptiva sirve para una mejor comprensión de la invención, las reivindicaciones deben ser claras por si mismas y delimitar perfectamente el alcance de la misma.

4. DETERMINACIÓN DE UNIDAD DE INVENCION

La invención no comprende un grupo de invenciones relacionadas entre sí, de manera que se integre un único concepto inventivo conforme al artículo 25 de la decisión 486 de la comisión de la comunidad andina, puesto que:

De acuerdo con el nuevo capítulo reivindicatorio la invención aún comprende una amplia gama de mezclas diferentes, no obstante se haya restringido la composición con respecto a los compuestos antagonistas del canal de sodio a los compuestos de fórmula I, los compuestos del grupo I con los cuales se combinan corresponden a grupos con funciones diferentes como piretroides, carbamatos, nucleopolihedrovirus etc, eso sin contemplar que dentro de cada uno de estos se tienen igualmente un sinnúmero de compuestos con estructura química muy diferente, tal como se puede determinar de acuerdo con la reivindicación N° 4, por lo tanto resulta que se tiene una infinidad de mezclas diferentes.

Ahora bien los compuestos de fórmula I son conocidos en el arte previo, de manera que no existe un único concepto común e inventivo, en todas las posibles mezclas.

5- ARGUMENTOS DEL INTERESADO:

Con respecto a lo expresado por el interesado con relación a la objeción de que "no encuentra suficientemente sustentado que todas las mezclas posibles de acuerdo a lo reivindicado presenten efecto sinérgico", si bien es cierto como lo afirma el interesado en su memorial que en las páginas 16 a 20, ejemplos 2 y 3 se demuestra el comportamiento sinérgico de las mezclas de los compuestos de fórmula I, es de anotar que allí solo se muestra el comportamiento de un compuesto de fórmula I en combinación con un compuesto específico que corresponde a "hidrametilnón" y con un nucleopolihedrovirus que expresa la toxina de insecto *Androctonus australis*, lo cual no es representativo frente a la amplia gama de mezclas que se dan de un lado por los compuestos de fórmula I, donde el significado de sus sustituyentes en amplio y contempla grupos funcionales diferentes y de otra parte el hecho de que entre los compuestos del grupo I se tienen diez diferentes tipos de compuestos químicos como piretroides, organofosforado, carbamato etc y dentro de cada uno de ellos existe una gran variedad de compuestos lo cual se puede ver fácilmente en la reivindicación N°4, de manera que en la descripción por lo menos debería contemplarse ejemplos de mezclas de los compuestos de fórmula I con los compuestos específicos comprendidos en dicha reivindicación.

Con respecto a que los métodos reivindicados han sido claramente definidos y que comprenden la etapa de aplicación de una composición insecticida única y específica es de anotar nuevamente que la mezcla insecticida no es única y específica como ya se indico anteriormente y de otra parte el método no se caracteriza por el producto que se aplique, si bien se esta indicando que se pone en contacto la composición ya sea con el insecto o con la planta, esto constituye una etapa obvia muy general que no presenta ninguna característica específica, por lo tanto la objeción se hace con el propósito de que se defina y delimite el método reivindicado de manera que se pueda diferenciar del los métodos convencionales.

Ahora con relación a la falta de unidad de invención ya se indico en el numeral 4, de acuerdo al nuevo capítulo reivindicatorio por qué razón aún existe falta de unidad de invención, ahora cabe anotar que como es conocido en materia de patentes para que exista un único concepto común e inventivo, el elemento novedoso e inventivo debe estar presente en todas las reivindicaciones, así como también como en el caso de las mezclas en cada una de ellas, de manera que debería existir dicho elemento novedoso e inventivo en todas las mezclas, así que los compuestos de fórmula I que corresponden al elemento común para todas las mezclas no son nuevos ni inventivos, por lo tanto no se está tomando cada compuesto independientemente, justamente se hace está referencia en razón a se trata de mezclas muy diferentes con compuestos estructuralmente muy diferentes y aunque cada una podría ser nueva, la combinación de los compuestos que es lo nuevo e inventivo no es la misma para todas las mezclas, de otra parte no se puede afirmar como lo hace el interesado que el efecto sinérgico se constituya en el concepto común inventivo, porque si así fuera toda mezcla sinérgica podría ser contemplada en una única solicitud, es de anotar nuevamente que el efecto sinérgico es el resultado de la mezcla específica de dos o más componentes activos y lo que se protege como invención es la composición más no sus propiedades que son inherentes a la misma.

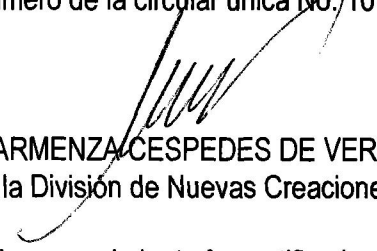
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Es de anotar que una cosa es la novedad de la invención y otra el concepto común inventivo, el hecho de que la combinación de dos o más principios activos sea nueva no implica necesariamente que una pluralidad de mezclas que puedan llegar a ser nuevas cada una independientemente, conformen todas un único concepto común inventivo, por esta razón se hace la anotación de que los compuestos son conocidos en el arte previo, de manera que como los compuestos comunes a todas las mezclas son conocidos no existe un único concepto común inventivo, y además porque como ya se indico se trata de compuestos estructuralmente muy diferentes.

Hasta tanto el interesado no ajuste su solicitud, de acuerdo a las observaciones antes anotadas no se podrá continuar con la búsqueda y estudio de patentabilidad.

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta días contados a partir de la fecha de la notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento **o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.**

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la circular única No. 10 de 2001.


ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones

16 MAR. 2006

El anterior requerimiento fue notificado por fijación en lista, de fecha _____

CONCEPTO TÉCNICO Número 381	EXAMINADOR TÉCNICO Código 202013
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