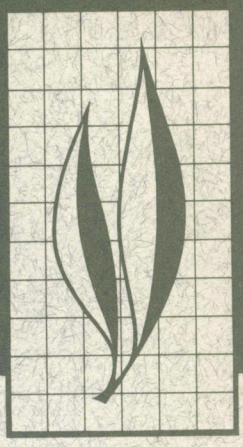
HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE PUBLISHED BY THE CALIFORNIA AGRICULTURAL EXPERIMENT STATION



Volume 53 • *Number 6* • *October 1985*

Intraspecific Variability in Response to Pesticides in the Common Green Lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae)

Elizabeth E. Grafton-Cardwell and Marjorie A. Hoy

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Adult common green lacewings, *Chrysoperla carnea* (Stephens) (formerly known as *Chrysopa carnea),* were collected from alfalfa fields, each separated by approximately 200 to 500 km, in four California counties. Adults, as well as larvae and eggs reared from those adults, were screened with six pesticides: two carbamates (carbaryl and methomyl), two pyrethroids (permethrin and fenvalerate), and two organophosphorus insecticides (diazinon and phosmet), to detect intraspecific variability in their responses to pesticides. Methods for screening adults, larvae, and eggs were developed. Adults and larvae were highly tolerant of field rates of the pyrethroids. Larvae also tolerated phosmet, probably due to hydrolysis by esterase enzymes. Lacewings from the four locations responded significantly differently to all six pesticides. In general, San Joaquin County lacewings exhibited the highest and Imperial County lacewings the lowest mortality, with Kern and Fresno County lacewings variable in their responses. These results correspond with pesticide usage in California. Egg hatch was not affected by any of the pesticides tested. Reciprocal crosses of San Joaquin and Imperial County adults were successful, indicating that reproductive isolation does not exist.

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Elizabeth E. Grafton-Cardwell and Marjorie A. Hoy

Intraspecific Variability in Response to Pesticides in the Common Green Lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae)[1,2]

INTRODUCTION

As interest in integrated pest management increases, more attention is being directed toward qualities of pesticides and the impact pesticides have on beneficial insects. In general, pesticides are more toxic to beneficial insects than to their hosts or prey. This may be due to differences in physiology, such as enzymes (Mullin and Croft 1985), or indirectly due to starvation from loss of prey or hosts after sprays are applied (Huffaker 1971; Newsom 1974; Croft and Morse 1979; Croft and Strickler 1983). If selective pesticides can be used that favor natural enemies, then lower doses or fewer applications of pesticides, or both, may be required. Many of the pesticides that are commonly used, however, are broad spectrum in effect. One solution is to collect or to select pesticide-resistant strains of beneficial arthropods to release where the broad spectrum pesticides must be used (Hoy 1979). At this time, phytoseiid mites are the only natural enemies that have developed high enough levels of pesticide resistance, either through field or laboratory selection, to be successfully mass-reared and used in integrated pest management programs (Croft and Brown 1975; Croft and Strickler 1983; Hoy 1985).

The common green lacewing, *Chrysoperla carnea* (Stephens) (formerly known as *Chrysopa carnea)* has undergone extensive pesticide screening because it is distributed worldwide; has a voracious appetite; has a wide prey range; is important as a predator in orchards, greenhouses, and field crops (Ridgway and Kinzer 1974; Ridgway and Murphy 1984; Tulisalo 1984); and it tolerates certain pesticides (Bigler 1984; New 1975). The results of some laboratory programs that have screened *C. carnea* for its response to various pesticides are compiled in table 1. Effects of the pesticides are difficult to categorize because many different methods were used for testing, including LT_{50}, LC_{50}, and single field doses. Many different stages were also tested. Therefore, a simple 0 to 4 rating is used, adapted after Bigler (1984) in which 0 = no effect, 1 = a low detrimental effect, 2 = a medium detrimental effect, 3 = a high detrimental effect and 4 = an extremely high detrimental effect. The detrimental effect includes both lethal and sublethal effects such as; immediate mortality, long-term reduction in survival, lowered fecundity, lowered fertility, and reduced predation by larvae.

In general, eggs and pupae are the most resistant stages and larvae are more tolerant of pesticides than adults (table 1). Only oils caused significant mortality of *C. carnea* eggs (Bartlett 1964). Several of the organophosphorus insecticides (Wiackowski 1968) and diflubenzuron (Ables et al. 1977) are reported to have a detrimental effect on pupation. Larvae have shown a general tolerance to some of the chlorinated hydrocarbons including chlorobenzilate, DDT, dilor, endosulfan, methoxychlor, TDE, and toxaphene (table 1). All stages of *C. carnea* are highly tolerant of many synthetic pyrethroids, botanicals, microbial insecticides *(Bacillus thuringiensis* and nuclear polyhedrosis viruses), insect growth regulators,

[1]Based on a thesis submitted in partial fulfillment of the Ph.D. degree, University of California, Berkeley, January 1985.

[2]Accepted for publication May 12, 1985.

TABLE 1. SUMMARY OF RESULTS OF LABORATORY SCREENING PROGRAMS TO DETERMINE THE RESPONSE* OF VARIOUS STAGES OF *C. CARNEA* TO PESTICIDES

	Egg	Larval		Pupal	Adult		Egg	
Pesticide	Mortality	Mortality	Predation	Mortality	Mortality	Fecundity	Fertility	References
Chlorinated hydrocarbons								
Aldrin	0	4	—	—	4	—	—	Bartlett 1964
Benzene hexachloride	0	3	—	—	4	—	—	Bartlett 1964
Chlordane	0	4	—	—	4	—	—	Bartlett 1964
DDT	0	1	—	—	4	—	—	Bartlett 1964
	—	0	—	—	—	—	—	Plapp & Bull 1978
	—	2	—	—	—	—	—	Sukhoruchenko et al. 1977
Dieldrin	0	4	—	—	4	—	—	Bartlett 1964
	—	3	—	—	—	—	—	Plapp & Bull 1978
Dilor	—	1	—	—	—	—	—	Sukhoruchenko et al. 1977
Endosulfan	0	4	—	—	4	—	—	Bartlett 1964
	1	—	—	—	—	—	—	Franz et al. 1980
	—	1	—	—	—	—	—	Plapp & Bull 1978
	—	1	—	1	2	—	—	Wiackowski 1968
Endrin	1	4	—	—	4	—	—	Bartlett 1964
Heptachlor	0	4	—	—	4	—	—	Bartlett 1964
Lindane	0	2	—	—	4	—	—	Bartlett 1964
	—	1	—	—	—	—	—	Hassan et al. 1983
Methoxychlor	0	1	—	—	4	—	—	Bartlett 1964
Mirex	—	2	—	—	—	—	—	Sukhoruchenko et al 1977
TDE	0	1	—	—	3	—	—	Bartlett 1964
Toxaphene	0	3	—	—	4	—	—	Bartlett 1964
	—	0	—	—	—	—	—	Plapp & Bull 1978
	—	—	1	—	—	2	—	Suter 1978
	—	1	—	—	3	—	—	Wilkinson et al. 1975

Carbamates								
Carbaryl	0	4	—	—	4	—	—	Bartlett 1964
	—	1	—	—	—	—	—	Plapp & Bull 1978
	—	4	—	—	—	—	—	Sukhoruchenko et al. 1977
	—	1	—	—	4	—	—	Wilkinson et al. 1975
Ethiofencarb	—	—	4	—	—	—	—	Grapel 1982
	—	—	1	—	—	1	—	Suter 1978
Methomyl	1	—	—	—	—	—	—	David et al. 1980
	—	4	—	—	—	—	—	Plapp and Bull 1978
	—	3	—	—	—	—	—	Hassan et al. 1983
	—	3	—	—	—	—	—	Pitts & Pieters 1982
Pirimicarb	—	1	—	—	—	—	—	Franz et al. 1980
	—	—	1	—	—	—	1	Grapel 1982
	0	1	—	1	1	—	—	Helgesen & Tauber 1974
Propoxur	—	4	—	—	—	—	—	Hassan et al. 1983
Organophosphates— phosphoric acid prototypes								
Dicrotophos	0	3	—	—	4	—	—	Bartlett 1964
	—	3	—	—	—	—	—	Lingren & Ridgway 1967
Heptenophos	—	—	4	—	—	—	—	Grapel 1982
	—	—	4	—	—	—	—	Suter 1978
Monocrotophos	—	4	—	—	—	—	—	Plapp & Bull 1978
Naled	0	1	—	—	2	—	—	Bartlett 1964
Phosphamidon	0	4	—	—	—	—	—	Bartlett 1964
	—	3	—	—	—	—	—	Lingren & Ridgway 1967
	—	—	4	—	—	—	—	Suter 1978
Profenofos	—	4	—	—	—	—	—	Plapp & Bull 1978
Schraden	—	0	—	—	—	—	—	Ahmed 1955
	0	1	—	—	2	—	—	Bartlett 1964
Tepp	0	0	—	—	0	—	—	Bartlett 1964
Tetrachlorvinphos	—	0	—	—	—	—	—	Sukhoruchenko et al. 1977
	—	—	1	—	—	1	—	Suter 1978

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TABLE 1. CONTINUED

Pesticide	Egg Mortality	Larval Mortality	Larval Predation	Pupal Mortality	Adult Mortality	Adult Fecundity	Egg Fertility	References
Organophosphates-phosphoric acid prototypes (Cont.)								
Trichlorofon	0	1	—	—	1	—	—	Bartlett 1964
	—	1	—	—	—	—	—	Hassan et al. 1983
	—	1	—	—	—	—	—	Lingren & Ridgway 1967
	—	1	—	—	—	—	—	Plapp & Bull 1978
	—	3	—	—	—	—	—	Sukhoruchenko & Tolstova 1981
Organophosphates— thiophosphoric acid prototypes								
Acephate	—	3	—	—	—	—	—	Plapp & Bull 1978
	—	—	4	—	—	—	—	Suter 1978
Bromophos	—	1	—	1	2	—	—	Wiackowski 1968
Cyanophos	—	—	—	—	1	—	—	Sukhoruchenko & Tolstova 1981
Demeton	—	1	—	—	—	—	—	Ahmed 1955
	0	1	—	—	3	—	—	Bartlett 1964
	—	2	—	—	—	—	—	Lingren & Ridgway 1967
	—	3	—	—	—	—	—	Sukhoruchenko et al. 1977
	—	1	—	1	1	—	—	Wiackowski 1968
Diazinon	0	4	—	—	4	—	—	Bartlett 1964
	—	—	4	—	—	—	—	Suter 1978
Duraphos	—	1	—	1	2	—	—	Wiakowski 1968
	0	4	—	—	4	—	—	Bartlett 1964
	—	—	4	—	—	—	—	Suter 1978
Epn	—	3	—	—	—	—	—	Plapp & Bull 1978
Ethyl parathion	—	4	—	—	—	—	—	Plapp & Bull 1978
Etrimphos	—	—	4	—	—	—	—	Suter 1978
Fenitrothion	—	—	—	—	0	—	—	Sukhoruchenko & Tolstova 1981
	—	2	—	2	3	—	—	Wiakowski 1968
Fenthion	1	3	—	—	4	—	—	Bartlett 1964

Methyl parathion	—	2	—	—	—	—	—	Lingren & Ridgway 1967
	—	4	—	—	—	—	—	Plapp & Bull 1978
	—	2	—	—	4	—	—	Wilkinson et al. 1975
Oxydemeton-methyl	—	4	—	—	—	—	—	Franz et al. 1980
	—	—	4	—	—	—	—	Grapel 1982
	—	—	4	—	—	—	—	Suter 1978
	—	4	—	3	4	—	—	Wiackowski 1968
Parathion	1	4	—	—	4	—	—	Bartlett 1964
	—	3	—	—	—	—	—	Hamilton & Kieckhefer 1969
	—	—	4	—	—	—	—	Suter 1978
Pirimiphos-methyl	—	4	—	—	—	—	—	Hassan et al. 1983
Triazophos	—	—	1	—	—	2	—	Suter 1978
Vamidothion	—	—	1	—	—	2	—	Suter 1978
Organophosphates — dithiophosphate prototypes								
Azinphosmethyl	0	3	—	—	4	—	—	Bartlett 1964
	—	—	4	—	—	—	—	Suter 1978
Bromophos	—	1	—	1	2	—	—	Wiackowski 1968
Carbophenothion	0	4	—	—	4	—	—	Bartlett 1964
Dimethoate	0	1	—	—	4	—	—	Bartlett 1964
	—	3	—	—	—	—	—	Sukhoruchenko et al. 1977
	—	—	4	—	—	—	—	Suter 1978
	—	4	—	3	4	—	—	Wiackowski 1968
Dioxathion	0	1	—	—	3	—	—	Bartlett 1964
Disulfoton	—	2	—	—	—	—	—	Feese & Wilde 1975
Ethion	0	3	—	—	4	—	—	Bartlett 1964
Formothion	—	1	—	1	2	—	—	Wiackowski 1968
Malathion	0	3	—	—	4	—	—	Bartlett 1964
	—	3	—	—	—	—	—	Hamilton & Kieckhefer 1969
	—	0	—	—	2	—	—	Wilkinson et al. 1975
Menazon	—	1	—	1	1	—	—	Wiackowski 1968
Methidathion	—	4	—	—	—	—	—	Hassan et al. 1983

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TABLE 1. CONTINUED

Pesticide	Egg Mortality	Larval Mortality	Larval Predation	Pupal Mortality	Adult Mortality	Adult Fecundity	Egg Fertility	References
Organophosphates-dithiophosphate prototypes (Cont.)								
Phosalon	—	1	—	—	—	—	—	Franz et al. 1980
	—	3	—	—	—	—	—	Sukhoruchenko et al. 1977
	—	—	1	—	—	2	—	Suter 1978
Phosmet	—	3	—	—	—	—	—	Sukhoruchenko et al. 1977
Sulprofos	—	3	—	—	—	—	—	Plapp & Bull 1978
Thiometon	—	4	—	3	3	—	—	Wiackowski 1968
Synthetic pyrethroids								
Cypermethrin	—	0	—	—	—	—	—	Ishaaya & Casida 1981
	—	2	—	—	—	—	—	Rajakulendran & Plapp 1982a
	—	1	—	—	—	—	—	Rajakulendran & Plapp 1982b
Deltamethrin	—	0	—	—	—	—	—	Ishaaya & Casida 1981
Fenvalerate	—	3	—	—	—	—	—	Hassan et al. 1983
	—	1	—	—	—	—	—	Plapp & Bull 1978
	—	1	—	—	—	—	—	Shour & Crowder 1980
Flucythrinate	—	1	—	—	—	—	—	Rajakulendran & Plapp 1982b
Fluvalinate	—	1	—	—	—	—	—	Rajakulendran & Plapp 1982a
	—	1	—	—	—	—	—	Rajakulendran & Plapp 1982b
Permethrin	—	4	—	—	—	—	—	Hassan et al. 1983
	—	0	—	—	—	—	—	Ishaaya & Casida 1981
	—	1	—	—	—	—	—	Plapp & Bull 1978
	—	1	—	—	—	—	—	Shour & Crowder 1980
Phenothrin	—	1	—	—	—	—	—	Rajakulendran & Plapp 1982a
	—	1	—	—	—	—	—	Rajakulendran & Plapp 1982b
Tralomethrin	—	1	—	—	—	—	—	Rajakulendran & Plapp 1982a
	—	1	—	—	—	—	—	Rajakulendran & Plapp 1982b
Formamidine								
Chlordimeform	2	—	—	—	—	—	—	Bull & House 1978
	—	1	—	—	—	—	—	Pitts & Pieters 1982

	—	1	—	—	—	—	—	Plapp & Bull 1978
	—	1	—	—	—	—	—	Rajakulendran & Plapp 1982b
	—	2	—	—	—	—	—	Sukhoruchenko et al. 1977
Botanicals								
Garlic	—	2	—	1	—	—	—	Nasseh 1982b
Nicotine	0	0	—	—	0	—	—	Bartlett 1964
	—	1	—	1	2	—	—	Wiackowski 1968
Pyrethrin	—	1	—	—	—	—	—	Franz et al. 1980
	—	0	—	—	—	—	—	Plapp & Bull 1978
	—	—	1	—	—	2	—	Suter 1978
	—	0	—	—	1	—	—	Wilkinson et al. 1975
Rotenone	0	1	—	—	3	—	—	Bartlett 1964
Rotenone & pyrethrin	—	—	2	—	—	2	—	Suter 1978
Ryania	0	0	—	—	1	—	—	Bartlett 1964
Sabadilla	0	1	—	—	0	—	—	Bartlett 1964
Microbials								
Bacillus	—	0	—	—	—	—	—	Franz et al. 1980
thuringiensis	—	—	1	—	—	—	—	Salama et al. 1982
	—	—	1	—	—	1	—	Suter 1978
	—	0	—	—	1	—	—	Wilkinson et al. 1975
Mamestra NPV	0	—	0	—	0	—	—	Hassan & Groner 1977
Heliothis NPV	—	0	—	—	0	—	—	Wilkinson et al. 1975
Fungicides								
Benomyl	—	—	1	—	—	1	—	Suter 1978
Bordeaux	0	0	—	—	2	—	—	Bartlett 1964
Bupirimat	—	1	—	—	—	—	—	Franz et al. 1980
Captafol	—	1	—	—	—	—	—	Franz et al. 1980
	—	—	1	—	—	1	—	Suter 1978
Captan	0	0	—	—	0	—	—	Bartlett 1964
	—	1	—	—	—	—	—	Hassan et al. 1983
Carbendazim	—	1	—	—	—	—	—	Hassan et al. 1983
Dichlofluanid	—	2	—	—	—	—	—	Franz et al. 1980

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TABLE 1. CONTINUED

Pesticide	Egg Mortality	Larval Mortality	Larval Predation	Pupal Mortality	Adult Mortality	Adult Fecundity	Egg Fertility	References
Fungicides (Cont.)								
Ditalimfos	—	1	—	—	—	—	—	Hassan et al. 1983
Fentinacetat	—	—	1	—	—	2	—	Suter 1978
Kupferoxychlorid	—	—	1	—	—	1	—	Suter 1978
Mancozeb	—	3	—	—	—	—	—	Franz et al. 1980
	—	—	1	—	—	1	—	Suter 1978
Maneb	—	—	1	—	—	1	—	Suter 1978
Metiram	—	—	1	—	—	1	—	Suter 1978
Netzschwefel	—	—	1	—	—	1	—	Suter 1978
Propineb	—	—	1	—	—	1	—	Suter 1978
Pyrazophos	—	3	—	—	—	—	—	Franz et al. 1980
Sulphur	0	0	—	—	1	—	—	Bartlett 1964
	—	1	—	—	—	—	—	Hassan et al. 1983
Thiophanat-methyl	—	1	—	—	—	—	—	Franz et al. 1980
	—	—	1	—	—	1	—	Suter 1978
Triadimefon	—	1	—	—	—	—	—	Hassan et al. 1983
Vinclozolin	—	1	—	—	—	—	—	Hassan et al. 1983
Zineb	0	0	—	—	0	—	—	Bartlett 1964
	—	—	1	—	—	2	—	Suter 1978
Growth regulators								
CGA 13353	—	0	—	0	—	—	—	Nasseh 1982a
Diflubenzuron	—	3	—	3	3	—	—	Ables et al. 1977
	—	4	—	—	—	—	—	Franz et al. 1980
	—	0	—	1	0	—	—	Wilkinson et al. 1978
Epophenone	—	1	—	1	—	—	—	Nasseh 1982a
Kinoprene	—	1	—	1	—	—	—	Nasseh 1982a
Ro 10-3108	—	—	1	—	—	4	—	Suter 1978

ZR 777	—	—	1	—	—	1	—	Suter 1978
Herbicides								
2,4-DB	—	0	—	—	0	—	—	Wilkinson et al. 1975
Desmetryne	—	1	—	—	—	—	—	Hassan et al. 1983
Diclofop-methyl	—	1	—	—	—	—	—	Franz et al. 1980
Difenzoquat	—	1	—	—	—	—	—	Hassan et al. 1983
Dinoseb	—	4	—	—	—	—	—	Franz et al. 1980
HOE	—	—	3	—	—	—	2	Grapel 1982
Monlinuron	—	2	—	—	—	—	—	Hassan et al. 1983
Phenmedipham	—	2	—	—	—	—	—	Franz et al. 1980
Propachlor	—	1	—	—	—	—	—	Hassan et al. 1983
Propyzamid	—	1	—	—	—	—	—	Franz et al. 1980
Acaricides								
Azocyclotin	—	3	—	—	—	—	—	Hassan et al. 1983
Benzoximate	—	1	—	—	—	—	—	Hassan et al. 1983
Bromopropylate	—	0	—	—	—	—	—	Sukhoruchenko et al. 1977
Chlorobenzilate	0	0	—	—	1	—	—	Bartlett 1964
Cyhexatin	—	4	—	—	—	—	—	Franz et al. 1980
	—	2	—	—	—	—	—	Sukhoruchenko et al. 1977
Dicofol	0	0	—	—	0	—	—	Bartlett 1964
	—	1	—	—	—	—	—	Franz et al. 1980
	—	0	—	—	—	—	—	Sukhoruchenko et al. 1977
Dinobuton	—	0	—	—	—	—	—	Sukhoruchenko et al. 1977
Fenbutatin-oxid	—	1	—	—	—	—	—	Franz et al. 1980
Propargite	—	0	—	—	—	—	—	Sukhoruchenko et al. 1977
Tetradifon	0	0	—	—	0	—	—	Bartlett 1964
Zectran	0	4	—	—	4	—	—	Bartlett 1964

*0 = no effect, 1 = low detrimental effect, 2 = medium detrimental effect, 3 = high detrimental effect, 4 = extremely high detrimental effect, and — = no information.

fungicides, herbicides, acaricides, and the formamidine insecticide, chlordimeform (table 1). The carbamate and organophosphorus insecticides seem to be generally toxic to *C. carnea* at field doses, with a few exceptions that include pirimicarb, naled, schraden, TEPP, tetrachlorvinphos, trichlorfon, demeton, duraphos, triazophos, vamidothion, and bromophos (table 1).

Variability in response of *C. carnea* larvae to pesticide screening was evident for endosulfan, toxaphene, carbaryl, trichlorphon, demeton, dimethoate, malathion, phosalon, fenvalerate, permethrin, diflubenzuron (table 1) and may be due to the fact that different test methods were utilized. Another possible explanation, which has not been previously examined for *C. carnea,* is that different populations of *C. carnea* are responding to local pesticide pressures resulting in geographical and temporal variation in pesticide tolerances.

Although tolerant of many pesticides, *C. carnea* is highly susceptible to field rates of most carbamate and organophosphorus insecticides. Since these pesticides are commonly used in agriculture today, the ultimate goal of this project was to select *C. carnea* in the laboratory for resistance to one or more pesticides. A common step, however, in a genetic selection program is to screen populations from various geographical locations with several pesticides to determine if intraspecific variability exists (Hoy 1979). If variability exists, but complete resistance has not developed yet, then the most tolerant population can be used for laboratory selection, possibly speeding up the selection process. Intraspecific variability in resistance or tolerance to pesticides has been demonstrated for many phytoseiid mites (Hoy 1985). Predatory mites separated by distances of only a few miles varied significantly in their responses to pesticides and so appear to be responding to local selection pressures (Hoy 1985). This paper discusses the methods and results of a screening program to detect intraspecific variability in the response of *C. carnea* populations to pesticides.

METHODS AND MATERIALS

Field Collection of Adults

Adults were collected from alfalfa during 1981 and 1982, one county at a time, in seven counties (fig. 1); near Yreka in Siskiyou County, near Willows in Glenn County, east of Stockton and West of Tracy in San Joaquin County, near Los Banos in Fresno County, between Wasco, Shafter, and Lost Hills in Kern County, between Lancaster and Palmdale in Los Angeles County, and in the vicinity of Brawley and El Centro in Imperial County. Eight to 15 alfalfa fields per county (depending on availability) were visited and swept with a net to find one or two fields that contained adult lacewings.

A 100 meter strip of one or more alfalfa fields containing adult *C. carnea* was sprayed in the late afternoon with an attractive bait (450 g Formula 57 + 450 g sucrose in 3.785 L of water) with a 3.785 L garden spray can (Tassan et al. 1979). Formula 57 is a spray-dried *Saccharomyces fragilis* yeast product (Hagen and Tassan 1970). Lacewing adults fly primarily at night (Duelli 1980) and are attracted to and arrested by the bait (Hagen et al. 1971; Tassan et al. 1979). The following morning the fields were swept with an insect net until 400 to 500 lacewing adults were captured.

The lacewing adults were brought into the laboratory and screened with six insecticides (table 2) using four concentrations per insecticide and 20 adults per concentration. Preliminary trials were conducted to determine concentrations such that mortality ranged from 10 to 90 percent. Carbaryl, methomyl, diazinon, and phosmet were chosen for screening

Fig. 1. Distribution of alfalfa in California. Each *dot* represents 1,000 acres (=405 hectares). *Closed circles,* counties where sufficient numbers of adults of *Chrysoperla carnea* were collected for pesticide screening. *Open circles,* counties that were sampled but did not produce sufficient *C. carnea* for testing.

TABLE 2. INSECTICIDES USED IN SCREENING *C. CARNEA* COLLECTED FROM SAN JOAQUIN, FRESNO, KERN, AND IMPERIAL COUNTIES IN CALIFORNIA

Pesticide	Formulation	Field rate	
		lb/acre*	g AI/L H_2O
Carbamates			
carbaryl	(Sevin, 50% WP)	1.5	18.0
methomyl	(Lannate, WS liquid)	0.45	5.4
Organophosphorus insecticide			
diazinon	(50% WP)	0.5	6.0
phosmet	(Imidan, 50% WP)	1.0	12.0
Pyrethroid†			
permethrin	(Ambush, Pounce, EC)	0.2	2.4
fenvalerate	(Pydrin, EC)	0.1	1.2

*Rate recommended for control of pests in alfalfa, mixed in 10 gal of water (University of California Extension Leaflet 2763).
†Not registered for use in alfalfa.

because they are frequently used to control pests in hay alfalfa fields in California (C.D.F.A. 1978). The pyrethroids were not registered for alfalfa, but have a unique action and so were included in the study. Sufficient lacewings for complete testing were collected from one site each in San Joaquin, Fresno, and Kern counties (fig. 1). Imperial County lacewings were collected and reared until there were sufficient numbers for testing. No lacewings were found in the Siskiyou County sites sampled and insufficient lacewings for rearing and complete testing were found in Glenn or Los Angeles County sites sampled.

Rearing Methods

Cages for adults consisted of plastic boxes (17.3 cm x 12.0 cm x 6.5 cm) with removable lids. A hole (5 cm in diameter) was cut in each lid and replaced with fine polyester cloth mesh. Up to 20 adults in approximately a 50:50 female:male ratio were placed in each cage. Water-soaked cotton was placed inside and food was spread on the side of the box. The food consisted of a mixture of 1 g yeast hydrolysate, 6 g Formula 57, 10 g of honey, and enough water to make the food into a moist paste. A sheet of paper toweling (22 cm x 6 cm) was placed in the lid for females to deposit eggs on. The adults were moved to fresh cages every 48 to 72 hours. Cages were kept in a rearing room at ambient conditions; 16L:8D, 24°C to 28°C, and 40 to 65 percent relative humidity.

After the adults were placed in a fresh cage, the eggs that had been deposited during the previous 48 to 72 hours were placed in a growth chamber (25°C, 16L:8D, 40% relative humidity) for 4 days. After the second day, eggs of the Mediterranean flour moth, *Anagasta kuehniella* (Zeller), were added to the cages in preparation for emergence of the first instar larvae. During the first 1½ years of the project, *A. kuehniella* eggs were simply sprinkled into the box. Subsequently, cannibalism was reduced when the eggs were sprinkled onto a 10.2 cm by 15.3 cm index card that had been thinly coated on one side with honey. The card could be placed in the box honey-side down and larvae rested and fed on the underside of the card. During the first year of rearing, plastic light diffuser squares were placed in the boxes to provide refuges and reduce cannibalism. Later, 2 cm wide strips of plastic sheeting loosely twisted and set in the box were found to control cannibalism better. In addition, the plastic strips could be thrown away, reducing the incidence of disease as well as the time spent washing. When the larvae reached the second instar, they were fed, depending on availability, pea aphids, *Acyrthosiphon pisum* (Harris), *A. kuehniella* eggs, and heat-treated larvae of the potato tuberworm, *Phthorimaea operculella* (Zeller) (Finney 1948), every 48 to 72 hours until pupation. Pupae were removed from the larval boxes every 24 to 48 hours to reduce cannibalism and placed in a screened 480 mL jar.

During 3 years of laboratory rearing, the vitality of the colony depended primarily on successful development of the larvae. Successful larval development depended on the quantity of food received, which affected the level of cannibalism, and the variety of food received, which affected nutrition. Larvae pupated more quickly and more successfully when they received food at least every 48 hours and when they received more than one type of prey. Tulisalo (1984) also noted that production of lacewing larvae is the critical stage in mass-rearing.

Screening Methods

Adults

Before screening with the six pesticides (table 2), a test cage for adults was developed to measure contact toxicity with a minimal influence of fumigant effects. The bottom half of a glass petri dish, closed with a square of porous, disposable paper facial tissue, was used to screen adult *C. carnea* (fig. 2). The inside of the dish was sprayed for 1 second with a solution of formulated insecticide in distilled water or distilled water alone using a Crown Spra-Tool aerosol can held 30 cm from the dishes, then air dried for 1 hour. The dish was placed upside down on the tissue. Test concentrations for carbaryl were 0.18 g, 0.9 g, 1.8 g, and 3.6 active ingredient/liter (AI/L); for methomyl 0.0675 g, 0.135 g, 0.27 g, and 0.54 g

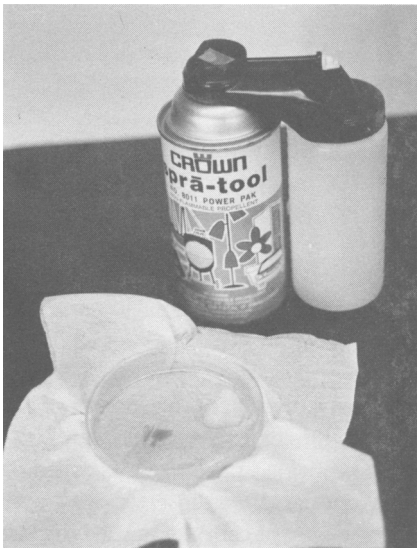

Fig. 2. Petri dish test cage used to screen adult *Chrysoperla carnea.*

AI/L; for diazinon 0.03 g, 0.06 g, 0.12 g, and 0.6 g AI/L; for phosmet 0.3 g, 0.6 g, 1.2 g, and 2.4 g AI/L; for permethrin 1.2 g, 2.4 g, 4.8 g, and 9.6 g AI/L; and for fenvalerate 2.4 g, 4.8 g, 9.6 g, and 48 g AI/L. Inside each dish two waxed paper squares (2 cm^2) were placed, one with food streaked on it and one with cotton containing distilled water. The food consisted of a mixture of 4 g yeast hydrolysate, 7 g sucrose, and 10 mL distilled water (Hagen and Tassan 1970).

Five adult *C. carnea,* 3 females:2 males, were placed in each dish and the tissue was secured with a rubber band. There were four dishes per concentration. Dishes were placed on a rack so that air could circulate through the tissue on the bottom. Adult lacewings generally prefer to hang upside down on the petri dish and so were exposed to the test pesticide most of the time. The test animals remained in the dish for 48 hours at ambient conditions. After 48 hours the number of surviving adults and the number and position of eggs deposited in the test dish were recorded. Mortality of adults was analyzed using the POLO computer program (Robertson et al. 1980). Adults from San Joaquin, Fresno, Kern, and Imperial counties were tested on separate dates directly after collection from the field. Adults from San Joaquin and Imperial counties were retested simultaneously after 2 years in laboratory culture.

Larvae

Larvae were more difficult to screen than adults since they were very active and cannibalistic. In addition, some chemicals (permethrin, fenvalerate, and diazinon) were repellent and the larvae ran off the treated surface into Stikem barriers and died. Two different methods were developed to test larvae with the six pesticides in this project. A brief summary of previous methods for screening *C. carnea* larvae is presented to point out some of the problems and to justify developing additional methods:

1. Cage spray. Cages were sprayed with formulated materials to runoff, allowed to dry 1 hour, and individual lacewings were introduced (Helgesen and Tauber 1974). Advantages of this method are that the entire surface is treated and escape is impossible.

Disadvantages are that if the cage is plastic or cloth, it cannot be readily cleaned for reuse; if it is glass, then there is no ventilation.

2. Munger cell cage. Mist atomization of formulated material on wax paper was used as the base of a Munger cell cage (plexiglass with holes sandwiched between two plates of plexiglass). Twenty first-instar larvae were placed in each cell with a diet of honey and moth eggs. Cells were ventilated with an air pump (Bartlett 1964). Advantages are that ventilation is provided and escape is difficult. Disadvantages are that only part of the cage is treated, the air pump setup is complicated and time-consuming, thereby limiting the number of larvae that can be tested, and the plexiglass may absorb the pesticides.
3. Sprayed leaf placed in petri dish. Leaves sprayed with formulated material were allowed to dry and then were placed in a closed, glass petri dish with 10 lacewing larvae (Sukhoruchenko et al. 1977). Advantages are that testing is fast, dishes can be cleaned for reuse, and larvae find it difficult to escape. Disadvantages are that not all of the surfaces are treated, cannibalism is not prevented, and there is no ventilation.
4. Pesticide film in glass tube. A film of formulated pesticide in acetone was rolled on the inside of a liquid scintillation counting vial, one larva was inserted with a 10 percent honey solution and moth eggs for food, and a plug of cotton was used to close it (Plapp and Bull 1978, Rajakulendran and Plapp 1982a, 1982b). Advantages are that a known amount of pesticide is applied and escape is impossible. Disadvantages are that cotton plugs are not treated, ventilation is poor, and it is time-consuming to set up many of these test cages.
5. Treated filter paper placed in petri dish. Glass rings were glued to plastic petri dishes to form cells and so to contain individual larvae. Two holes were cut at the top of each cell, one plugged with a cork for entry of larvae and one covered with organdy for ventilation. Treated filter paper was inserted in the bottom (Wilkinson et al. 1975). Advantages are that escape is impossible and a known amount of pesticide is applied to the paper. Disadvantages are that not all of the surfaces are treated and plastic is difficult to clean and reuse.
6. Plexiglass with holes on treated glass plate. Rows of holes were drilled in plexiglass and then encircled with a slippery material called Fluon to which the larvae cannot hold. The plexiglass was placed on top of a glass plate treated with formulated pesticide (Hassan and Groner 1977, Suter 1978; Franz et al. 1980; Grapel 1981; Hassan et al. 1983). Advantages are that larvae are individually caged, ventilation is good, all the surfaces are treated, and the setup is fast. The only disadvantage is that the plexiglass may absorb pesticides.

A critical problem was that all the previously described methods were time-consuming to set up and so the number of larvae that could be tested per day was limited. Most previous work had been done using large third instar larvae with an average of 30 individuals per dose. We wanted to test 30 individuals per dose, five doses per insecticide, and four colonies: adding up to 600 individuals per test date. Two methods were developed for this project. The plate method is modified after Suter (1978). The cup method was developed for use with chemicals that were repellent.

Plate method. Plastic, disposable Microtiter plates (12.4 cm x 8.2 cm) manufactured by Dynatech Laboratories and containing 96 wells (diam. = 0.6 cm, depth = 1.0 cm) were used (fig. 3). The wells were filled with a solution of formulated insecticide in distilled water with 0.1 percent wetting agent (Triton B) to help the insecticide adhere to the plastic. Four concentrations of carbaryl (0.018 g, 0.036 g, 0.072 g, and 0.36 g AI/L), methomyl (0.014 g, 0.027 g, 0.054 g, and 0.108 g AI/L), and phosmet (6 g, 12 g, 60 g, and 120 g

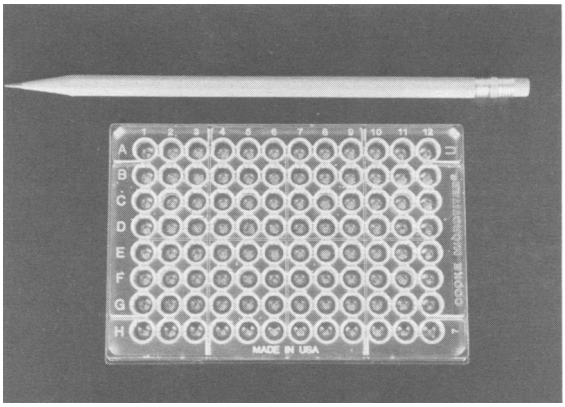

Fig. 3. Microtiter plate used to screen first instar larvae of *Chrysoperla carnea* with carbaryl, methomyl, and phosmet.

AI/L) were tested. The solution was emptied after 5 seconds and the plate was tapped four times upside down on paper towels and dried in front of a fan for 30 minutes. Approximately 100 *A. kuehniella* eggs were poured into each of the wells to serve as food for the lacewing larvae. Each well was encircled with a sticky material (Stickem Special) to act as a barrier using a disposable glass pipet. Larvae were fed *A. kuehniella* eggs for 24 to 48 hours after hatch and then were placed individually in wells of the treated test plates. The larvae remained in the test plates for 72 hours and then survival, mortality, and runoff into the Stickem were recorded. Individuals that could not right themselves when turned on their backs were considered dead since experience showed that they would not survive to adulthood. Mortality of the larvae was analyzed using the POLO computer program (Robertson et al. 1980). During the experiment, plates were placed in a 3.75 L stainless steel cabinet inside a Percival growth chamber at 25°C and 16L:8D. The desiccator cabinet contained a plastic tray with a saturated sodium nitrite salt solution that maintained relative humidity at approximately 64 percent (Winston and Bates 1960).

Effects of humidity. The effects of three humidity levels on the response of lacewing larvae to four concentrations of carbaryl (0.018 g, 0.036 g, 0.072 g, 0.36 g AI/L), methomyl (0.014 g, 0.027 g, 0.054 g, 0.108 g AI/L), and phosmet (6, g, 12 g, 60 g, and 120 g AI/L) were examined using the plate method. The effects of humidity on the response of lacewing larvae to permethrin, fenvalerate, and diazinon were not examined since these pesticides were tested using closed cups and humidity could not be controlled. Plates were placed in one of three stainless steel desiccator cabinets that contained trays of either calcium chloride, sodium nitrite, or potassium nitrate saturated salt solutions. These salt solutions maintained relative humidities at approximately 29.5 percent, 64.0 percent, and 92.5 percent, respectively (Winston and Bates 1960). Mortality of the larvae for the three humidity levels was analyzed and compared using the POLO computer program (Robertson et al. 1980).

Cup method. The cup method was used to test the effects of diazinon (0.015 g, 0.03 g, 0.06 g, and 0.12 g AI/L), permethrin (1.2 g, 2.4 g, 4.8 g, and 9.6 g AI/L), and fenvalerate (1.2 g, 6.0 g, 12.0 g, and 24.0 AI/L). In this method, 30 mL disposable plastic cups and lids were dipped in formulated insecticide mixed with distilled water and 0.1 percent wetting agent for 5 seconds, drained on paper towels, and allowed to dry in front of a fan for 30 minutes. Approximately 100 *A. kuehniella* eggs and one first instar lacewing larva were placed in each cup with a lid. The cups were placed in a growth chamber held at 25°C and 16L:8D. Survival and mortality of the larvae were determined after 72 hours. There was no runoff since the cups were covered with lids. Mortality of the larvae was analyzed using the POLO computer method (Robertson et al. 1980).

The cup method had two disadvantages. First, cups and lids were individually dipped in the insecticide and so preparation was time-consuming. Only 300 larvae could be tested in 1 day using the cup method vs. 1000 larvae using the plate method. Second, since lids were used, a fumigant effect could not be avoided. This method was necessary, however, because permethrin, fenvalerate, and diazinon repelled the larvae and caused such a high level of runoff into the Stikem that the test results of these pesticides using Microtiter plates were obscured.

Mechanism of phosmet tolerance. First instar lacewing larvae were pretreated with the oxidase inhibitor, piperonyl butoxide (PB), or the esterase inhibitor, phenyl saligenin cyclic phosphonate (PSCP), to see if these inhibitors affect the response of larvae to phosmet. The dorsal thorax of each larva was pretreated with a 0.5 mL droplet of PB in acetone (250 μg/g insect), PSCP in acetone (90 μg/g insect), or acetone alone. The larvae were then placed individually in the wells of plates that had been treated with formulated phosmet in the manner described previously. The concentrations of phosmet used were 0.0 g, 0.12 g, 1.2 g, 12 g, and 120 g AI/L. The plates were placed in the stainless steel desiccator cabinet within the Percival growth chamber (64% relative humidity, 25°C, 16L:8D) for 72 hours and mortality of the larvae was recorded.

Eggs

Eggs for pesticide screening were obtained by allowing 15 to 20 female lacewings to deposit eggs on a 17 cm by 11 cm area of paper toweling for 24 hours. Each paper towel was cut in half. Half was sprayed for 1 second with formulated insecticide in distilled water while the other half was sprayed with distilled water alone to act as a control. The spraying was done using a Crown Spra-tool aerosol can held 30 cm from the eggs. Field rates of formulated material recommended for use in alfalfa mixed in 10 gallons of water were used (table 2). Eggs were allowed to air dry then placed in a growth chamber for 72 hours at 25°C, 16L:8D and 40 percent relative humidity. After 72 hours the eggs were clipped off the egg stalks and placed individually into wells of the Microtiter plates. Each well was encircled with Stikem and after 72 hours larval emergence was recorded.

Reproductive Compatibility

Imperial and San Joaquin strains

Fifty 3-day-old larvae from each of the Imperial and San Joaquin County colonies were placed individually in 30 mL plastic cups and reared to adult stage on a diet of *A. kuehniella* eggs and *P. operculella* larvae. To ensure comparable vigor the two colonies were fed the same quantity and types of food while reared from egg to adult. As adults emerged they were sexed and placed as pairs in rearing cages consisting of a 480 mL plastic cup with lid. A hole approximately 6 cm in diameter was cut in the lid for ventilation and a square of paper tissue was placed in the lid to prevent escape of the adults. Adult diet was streaked on the side of the cage and water soaked cotton was placed inside. Crosses included four each of an Imperial female and an Imperial male (I x I), an Imperial female and a San Joaquin male (I x S), a San Joaquin female and an Imperial male (S x I), and a San Joaquin female and a San Joaquin male (S x S). Each pair was moved to a fresh cage and all eggs were counted every 48 to 72 hours until the female died. For the first 6 weeks of oviposition, eggs were clipped

off their stalks and placed individually in wells of the Microtiter plates. Wells were ringed with Stikem and eggs were allowed to develop. After 72 hours, the percentage of eggs that had hatched was recorded. Differences in daily fecundity and egg hatch between Imperial and San Joaquin County strains were analyzed by ANOVA.

RESULTS AND DISCUSSION

Response of adult *C. carnea* to two pyrethroids

Adult *C. carnea* were extremely tolerant of both pyrethroids tested (table 3) (permethrin and fenvalerate), easily tolerating the field rates, which are 2.4 g and 1.2 g AI/L in 37.8 L of water, respectively (table 2). In fact, it was difficult to mix the formulated materials in sufficiently high concentrations to obtain concentration mortality lines. This low mortality coupled with a high level of variability between tests did not allow 95 percent confidence intervals to be computed for the LC_{50}s and LC_{90}s. Probit analysis showed no differences between the LC_{50} values or slopes of the four adult lacewing colonies when exposed to permethrin or fenvalerate.

After 2 years of laboratory culture, adult lacewings from San Joaquin County and Imperial County, retested simultaneously, still exhibited high tolerance to both pyrethroids (fig. 4). No significant difference in response to fenvalerate between the two colonies was found. However, a significant difference between the slopes and intercepts ($p \leq 0.05$) was found for the San Joaquin County lacewings and the Imperial County lacewings tested with permethrin.

Permethrin and fenvalerate were not registered for use in alfalfa when the lacewing adults were collected. Therefore, this high tolerance by adult lacewings to pyrethroids is probably not due to exposure in the field, but is a natural tolerance. Ishaaya and Casida (1981) demonstrated high natural esterase activity in larvae of *C. carnea* and suggested that this is an important biochemical mechanism for resistance to pyrethroids. Although these high levels of esterase enzymes have not been looked for in *C. carnea* adults, it is possible that the adults also possess them.

Response of adult *C. carnea* to two carbamates

Adult lacewings were more susceptible to the carbamates than to the pyrethroids tested (table 3). The LC_{50} values ranged from 0.552 g to 1.440 g AI/L for carbaryl and 0.108 g to 0.308 g AI/L for methomyl. These values are well below the field rates used for these chemicals in alfalfa (18.0 g and 5.4 g AI/L, respectively, see table 2). For carbaryl, Imperial County adults exhibited significantly lower mortality ($p \leq 0.05$) than Kern and Fresno County adults and San Joaquin County adults exhibited the highest mortality of the four colonies. For methomyl, Fresno County adults exhibited the lowest mortality and San Joaquin County adults the highest. Imperial and Kern counties had intermediate mortality.

Table 4 presents data for pesticide usage in California alfalfa fields in total pounds active ingredient for the years 1976, 1979, 1981, and 1982. San Joaquin and Kern County alfalfa received the lowest amounts of carbaryl and methomyl during those years, which corresponds to the low tolerance lacewing colonies exhibited to these insecticides. Fresno and Imperial County lacewing adults exhibited highest tolerance to carbaryl and methomyl,

TABLE 3. DIFFERENTIAL RESPONSES TO SIX PESTICIDES EXHIBITED BY ADULT *C. CARNEA* COLLECTED FROM FOUR CALIFORNIA COUNTIES IN 1981

Pesticide	LC_{50}*	LC_{90}	Slope of dosage
Colony tested†	g AI/L (95% confidence limits)‡	g AI/L (95% confidence limits)	Mortality line ± standard error
Permethrin			
San Joaquin	5.376a (−)	24.96 (−)	3.29 ± 1.53
Fresno	11.232a (−)	390.34 (−)	1.43 ± 0.78
Kern	2.401a (−)	354.00 (−)	1.44 ± 1.77
Imperial	12.816a (−)	345.32 (−)	1.94 ± 1.18
Fenvalerate			
San Joaquin	10.431a (−)	11.28 (−)	11.11 ± 0.24
Fresno	10.644a (−)	32.00 (−)	4.60 ± 1.92
Kern	17.496a (−)	233.47 (−)	1.95 ± 1.51
Imperial	13.812a (−)	45.37 (−)	2.25 ± 0.92
Carbaryl			
San Joaquin	0.552a (0.180-0.864)	1.674 (0.990-5.346)	4.34 ± 1.03
Fresno	0.990b (0.648-1.314)	2.070 (1.530-4.950)	6.89 ± 2.02
Kern	0.774b (0.324-1.206)	2.844 (1.728-10.390)	3.87 ± 0.98
Imperial	1.440c (0.972-2.160)	3.618 (2.340-13.320)	5.44 ± 1.16
Methomyl			
San Joaquin	0.108a (0.032-0.135)	0.205 (0.167-0.416)	7.82 ± 3.36
Fresno	0.308c (0.198-0.631)	1.193 (0.887-2.420)	3.71 ± 1.52
Kern	0.130ab (0.114-0.528)	0.313 (0.227-0.664)	5.81 ± 1.49
Imperial	0.151b (0.121-0.332)	0.529 (0.259-0.631)	3.98 ± 1.44
Diazinon			
San Joaquin	0.084a (0.066-0.114)	0.132 (0.102-0.240)	7.21 ± 1.70
Fresno	0.096a (0.060-0.426)	0.564 (0.204-107.22)	2.87 ± 1.01
Kern	0.210b (0.114-0.528)	0.444 (0.246-3.864)	6.71 ± 1.70
Imperial	0.150b (0.108-0.408)	0.270 (0.180-3.590)	4.42 ± 1.25

Continued

TABLE 3. CONTINUED

Pesticide	LC_{50}*	LC_{90}	Slope of dosage
Colony tested†	g AI/L (95% confidence limits)‡	g AI/L (95% confidence limits)	Mortality line ± standard error
Phosmet			
San Joaquin	0.588a (0.318-0.699)	0.648 (0.453-0.819)	17.87 ± 8.20
Fresno	0.588a (0.318-0.698)	0.636 (0.412-0.999)	17.00 ± 8.00
Kern	0.312a (0.211-0.598)	0.576 (0.286-0.718)	8.06 ± 4.26
Imperial	0.588a (0.264-0.614)	0.636 (0.484-0.715)	16.89 ± 8.90

*LC_{50} values for each pesticide in each column followed by the same letter indicate that the slopes and intercepts are not significantly different at the p = 0.05 level according to the maximum likelihood method.

†Twenty adults (collected from the field) were tested at each dose.

‡(−) indicates that 95% confidence limits could not be computed.

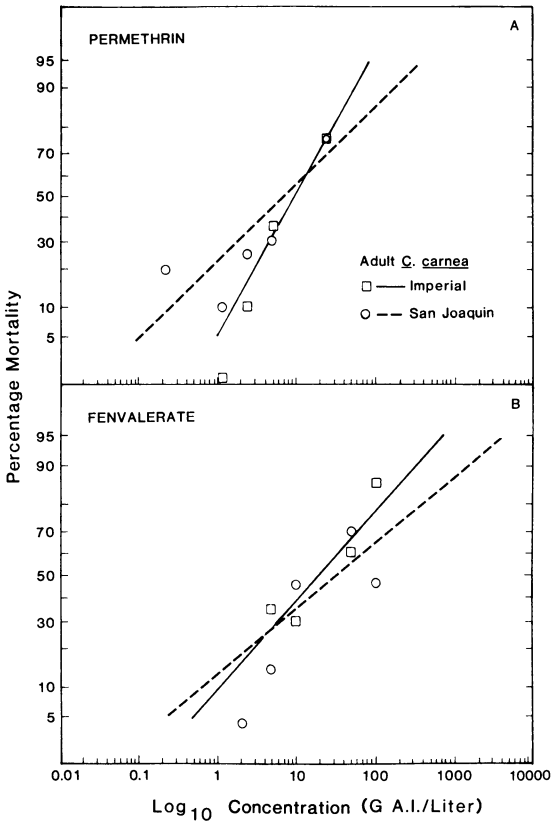

Fig. 4. Concentration-mortality relationships of adult *C. carnea* from Imperial and San Joaquin counties *(A)* exposed to permethrin and *(B)* exposed to fenvalerate.

TABLE 4. TOTAL INSECTICIDE USAGE IN CALIFORNIA ALFALFA FIELDS

Insecticide	Total pounds active ingredient used in alfalfa during 1976, 1979, 1981, and 1982*			
	San Joaquin	Fresno	Kern	Imperial
Carbaryl	1,693.0	33,520.4	2,901.0	23,024.8
Methomyl	12,202.2	100,094.3	27,353.0	262,676.1
Diazinon	32,923.8	48,595.4	59,489.6	100,116.4
Phosmet	4,664.1	41,698.4	21,916.9	98,905.2

*Source: California Department of Agriculture Pesticide Usage Data Bank.

which again corresponds to the higher amounts of carbaryl and methomyl used there. Thus, higher pesticide use appears to correspond with higher tolerance of adult *C. carnea* to carbaryl and methomyl.

After 2 years of laboratory rearing, the San Joaquin and Imperial County lacewings were retested simultaneously with carbaryl and methomyl (fig. 5). The resulting LC_{50} and LC_{90} values were similar to the previous testing. Once again, significant differences ($p \leq 0.05$) in responses to both carbaryl and methomyl were found between these two colonies.

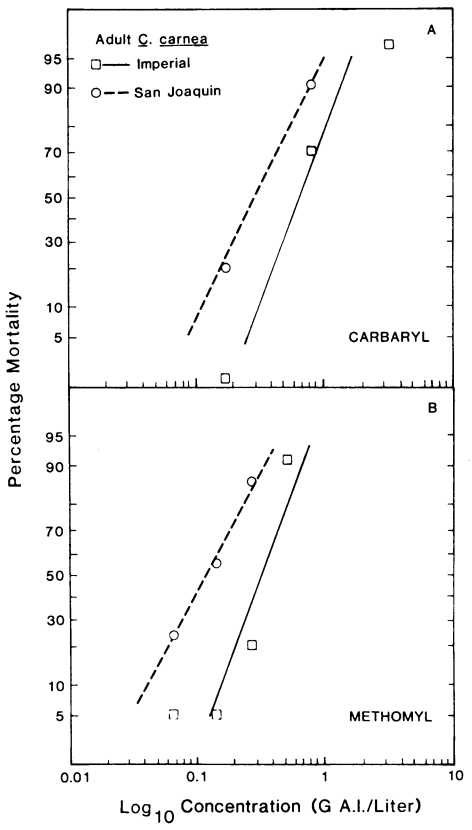

Fig. 5. Concentration-mortality relationships of adult *C. carnea* from Imperial and San Joaquin counties *(A)* exposed to carbaryl and *(B)* exposed to methomyl.

Response of adult *C. carnea* to two organophosphorus insecticides

Adult lacewings were highly susceptible to the organophosphorus insecticides (table 3). The LC_{50} values ranged from 0.084 g to 0.210 g AI/L for diazinon, and 0.312 g to 0.588 g AI/L for phosmet. These values are well below the field rates used for these chemicals in alfalfa (6.0 g and 12.0 g AI/L, respectively, see table 2). For diazinon, Kern and Imperial County adults exhibited significantly lower mortality ($p \leq 0.05$) than Fresno and San Joaquin County adults. There were no significant differences between the slopes and intercepts for any of the adults tested with phosmet.

After two years of laboratory rearing, the San Joaquin and Imperial County lacewings were retested simultaneously with diazinon and phosmet (fig. 6). The results were similar to those previously obtained. Significant differences ($p \leq 0.05$) in response to diazinon were found between these two colonies. No significant differences in concentration responses existed between the colonies tested with phosmet.

Again, higher exposure to diazinon in Kern and Imperial counties (table 4) corresponds to increased tolerance of diazinon by adult *C. carnea* from these counties (table 3). However, higher exposure to phosmet did not improve the tolerance of adult *C. carnea* to this chemical.

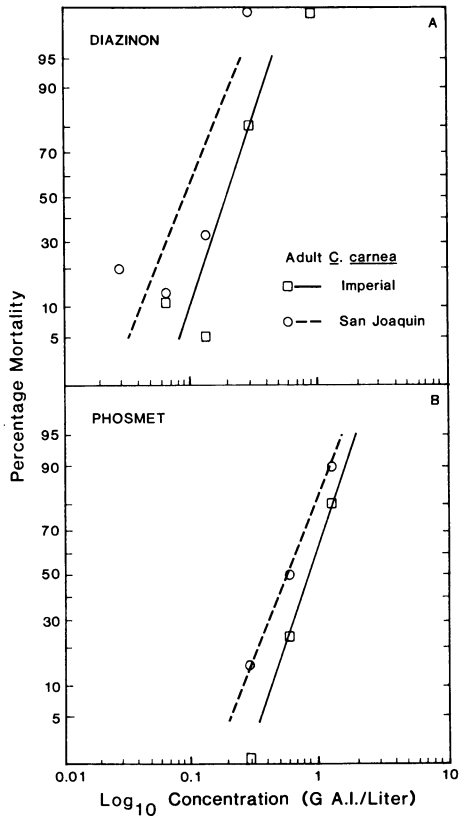

Fig. 6. Concentration-mortality relationships of adult *C. carnea* from Imperial and San Joaquin counties *(A)* exposed to diazinon and *(B)* exposed to phosmet.

Effects of relative humidity on larval response to carbaryl, methomyl, and phosmet

Humidity had no significant effect on lacewing responses to carbaryl (table 5). First instar larvae exposed to methomyl exhibited significantly lower mortality ($p \leq 0.05$) at 92.5 percent relative humidity than at 29.5 percent or 64 percent relative humidity, as demonstrated by the higher LC_{50} value. First instar larvae exhibited higher mortality on phosmet residues as humidity increased and this difference was significant ($p \leq 0.05$) between all three humidity levels.

TABLE 5. DIFFERENTIAL EFFECTS OF HUMIDITY ON THE RESPONSE OF *C. CARNEA* LARVAE TO CARBARYL, METHOMYL, AND PHOSMET

	LC_{50} values obtained at three different relative humidities*†		
Insecticide	29.5%	64.0%	92.5%
Carbaryl			
LC_{50}	0.0389a	0.0272a	0.0443a
Slope ± S.E.	1.96 ± .28	1.63 ± .26	1.73 ± .30
Methomyl			
LC_{50}	0.0108a	0.0151a	0.0278b
Slope ± S.E.	5.05 ± 1.01	5.12 ± .81	6.53 ± .91
Phosmet			
LC_{50}	354.0a	166.8b	52.8c
Slope ± S.E.	3.09 ± 1.43	2.67 ± .83	2.18 ± .57

*LC_{50} values for each pesticide in each column followed by the same letter indicate that the slopes and intercepts are not significantly different at the $p = 0.05$ level according to the maximum likelihood method.
†g AI/L.

Response of *C. carnea* larvae to two pyrethroids

Lacewing larvae, like the adults, were tolerant of the pyrethroids, permethrin and fenvalerate (table 6). LC_{50} values ranged from 2.75 g to 29.81 g AI/L for permethrin and 16.75 g to 26.6 g AI/L for fenvalerate. These rates are well above the field rates recommended for field crops (2.4 g and 1.2 g AI/L, respectively). It was difficult to obtain LC_{50} values with 95 percent confidence intervals for larval response to these chemicals since there was very little mortality even at field rates for all four colonies. This tolerance is apparently natural. It is thought to be primarily due to high esterase enzyme activity in the larvae (Ishaaya and Casida 1981).

Interestingly, even though the larvae were extremely tolerant of both pyrethroids, significant variability in response to these pesticides occurred between the four colonies (table 6). For permethrin, Fresno and Imperial County larvae exhibited significantly lower mortalities ($p \leq 0.05$) than Kern and San Joaquin County larvae. For fenvalerate, Imperial County larvae exhibited significantly lower mortality ($p \leq 0.05$) than San Joaquin and Fresno County larvae. It is not likely that the variability in tolerance to permethrin and fenvalerate is due to variability in exposure to these two pesticides because they have been registered for less than 5 years on only a few crops in California. It is more likely that many pesticides have contributed, through cross-resistance, to the improved tolerance of the Imperial strain of *C. carnea* Although it is impossible to determine specifically which pesticides have con-

tributed to lacewing tolerance, we can look at total pounds and total pounds/crop acre of nonfumigant pesticides used in the four counties in California in 1976 (table 7). Imperial County applied three to four times the amount of pesticides per crop acre than the other counties. This information supports the hypothesis that the higher tolerance of Imperial County lacewings is due to higher exposure to pesticides in general.

TABLE 6. DIFFERENTIAL RESPONSES TO SIX PESTICIDES EXHIBITED BY FIRST INSTAR *C. CARNEA* LARVAE REARED FROM ADULTS COLLECTED FROM FOUR COUNTIES IN CALIFORNIA

Pesticide		LC_{50}*	LC_{90}	Slope of dosage
Colony tested	No. larvae tested per dose	g AI/L (95% confidence limits)†	g AI/L (95% confidence limits)	Mortality line ± standard error
Permethrin				
San Joaquin	55	7.78a (3.68-11.44)	22.56 (15.29-48.81)	2.77 ± 0.41
Fresno	30	29.81c (−)	244.02 (−)	1.40 ± 1.04
Kern	30	2.75a (−)	134.42 (−)	0.76 ± 0.76
Imperial	65	20.64b (14.75-27.48)	53.38 (37.11-131.57)	3.11 ± 0.45
Fenvalerate				
San Joaquin	70	18.01a (11.78-28.90)	64.69 (37.01-309.18)	2.31 ± 0.31
Fresno	35	16.75a (8.91-24.63)	34.26 (21.22-120.63)	4.12 ± 0.94
Kern	35	19.64ab (15.21-38.91)	97.89 (40.30-220.92)	1.84 ± 0.56
Imperial	95	26.60b (17.58-52.10)	208.72 (87.03-2080.56)	1.43 ± 0.19
Carbaryl				
San Joaquin	288	0.0164a (0.0058-0.0259)	0.064 (0.0407-0.1528)	2.17 ± 0.15
Fresno	72	0.0284b (0.0221-0.0628)	0.082 (0.0731-0.5110)	2.79 ± 0.38
Kern	144	0.0355b (0.0245-0.0850)	0.075 (0.0680-0.4318)	3.93 ± 0.34
Imperial	576	0.0378b (0.0171-0.0623)	0.189 (0.1066-0.7045)	1.84 ± 0.89
Methomyl				
San Joaquin	180	0.0110ab (0.0052-0.0151)	0.028 (0.0207-0.0591)	3.12 ± 0.28
Fresno	88	0.0141b ((0.0068-0.0193)	0.054 (0.0354-0.0921)	2.20 ± 0.30
Kern	168	0.0047a (0.0012-0.0083)	0.042 (0.0318-0.0511)	1.34 ± 0.26
Imperial	276	0.0146b (0.0082-0.0203)	0.050 (0.0337-0.1183)	2.42 ± 0.17
Diazinon				
San Joaquin	196	0.0276a (0.0115-0.0403)	0.102 (0.0680-0.2682)	2.25 ± 0.22

Continued on next page

TABLE 6. CONTINUED

Pesticide		LC_{50}*	LC_{90}	Slope of dosage
Colony tested	No. larvae tested per dose	g AI/L (95% confidence limits)†	g AI/L (95% confidence limits)	Mortality line ± standard error
Fresno	40	0.0292a (−)	0.051 (−)	5.33 ± 1.23
Kern	40	0.0132a (−)	0.054 (−)	2.09 ± 0.83
Imperial	312	0.0524b (0.0246-0.839)	0.131 (0.0822-0.7675)	3.23 ± 0.20
Phosmet				
San Joaquin	48	120.0a (−)	244.2 (−)	1.70 ± 0.85
Fresno	48	209.6a (−)	863.4 (−)	2.09 ± 0.68
Kern	96	439.2b (−)	4750.1 (−)	1.24 ± 0.28
Imperial	96	1902.0c (−)	38475.5 (−)	0.98 ± 0.36

*LC_{50} values in the same column for each pesticide followed by the same letter indicate that the slopes and intercepts are not significantly different at the $p = 0.05$ level according to the maximum likelihood method.

†(−) indicates that 95% confidence intervals could not be computed.

Response of *C. carnea* larvae to two carbamates

Larvae from all four counties were susceptible to field rates of carbaryl ($LC_{50} = 0.0164$ g to 0.0378 g AI/L) and methomyl ($LC_{50} = 0.0047$ g to 0.0146 g AI/L) (table 6). For carbaryl, San Joaquin County larvae exhibited significantly higher mortality ($p \leq 0.05$) than Fresno, Kern, and Imperial County larvae. For methomyl, Kern County larvae showed significantly higher mortality ($p \leq 0.05$) than Fresno and Imperial County larvae. Fresno and Imperial County larvae exhibited the highest tolerances, which is consistent with the data showing that these two counties have the highest carbaryl and methomyl usage in alfalfa (table 4).

TABLE 7. NONFUMIGANT PESTICIDE LOAD IN THE COMMODITIES USING 98.9% OF THE RESTRICTED PESTICIDES APPLIED IN SAN JOAQUIN, FRESNO, KERN, AND IMPERIAL COUNTIES DURING 1976*

	San Joaquin	Fresno	Kern	Imperial
Total lb active ingredient used	423,945	982,283	477,727	1,523,161
Total active ingredient/ crop acre	1.00	0.97	0.61	2.82

*Source: California Department of Food and Agriculture (1978).

Response of *C. carnea* larvae to two organophosphorus insecticides

Exposure to diazinon resulted in LC_{50} values of 0.0132 g to 0.0524 g AI/L (table 6).

Larvae from Imperial County exhibited significantly lower mortality ($p \leq 0.05$) than the other three colonies. Once again, this result is consistent with higher pesticide exposure in Imperial County alfalfa fields (table 4).

Lacewing larvae, unlike adults, were extremely tolerant of the organophosphorus pesticide phosmet (LC_{50} = 120 g to 1902 g AI/L) (table 6). All four colonies exhibited a high tolerance. The low mortality did not allow 95 percent confidence intervals to be computed. Piperonyl butoxide (PB), a mixed-function oxidase inhibitor, had little or no effect on the response of *C. carnea* larvae to phosmet (fig. 7). The esterase inhibitor, phenyl saligenin cyclic phosphonate (PSCP), however, increased the mortality of both Imperial and San Joaquin larvae significantly ($p \leq 0.05$) (fig. 7). This suggests that esterase enzymes are being utilized in the detoxification of phosmet, an organophosphorus insecticide. This is an interesting result as organophosphorus insecticides are more frequently detoxified oxidatively (Hodgson 1968).

Even though *C. carnea* larvae were extremely tolerant of phosmet, the four colonies of lacewings responded significantly differently to this chemical (table 6). Imperial County larvae exhibited the lowest mortality and San Joaquin and Fresno counties exhibited the highest mortality. Again, the improved tolerance of Imperial lacewings may be due not just to phosmet exposure but rather to the total pesticide exposure, which is three to four times higher in this county than in the other counties (table 7).

Effects of pesticides on egg hatch

There was no significant difference ($p \leq 0.05$) between percentage hatch of the pesticide-treated eggs as compared to their water controls for any of the six pesticides tested. Egg hatch varied from 81.1 percent to 97.4 percent (table 8). These results agreed with the tests conducted with carbaryl (Bartlett 1964), methomyl (David et al. 1980), diazinon (Bartlett 1964), and other pesticides (Bartlett 1964, Helgesen and Tauber 1974, Hassan and Groner 1977, Bull and House 1978). Only oils have been found to have a significantly detrimental effect on egg hatch (Bartlett 1964).

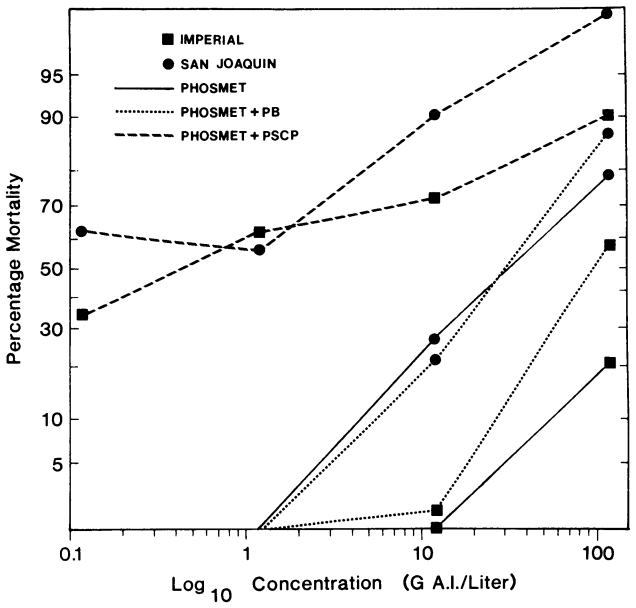

Fig. 7. Response of San Joaquin and Imperial County first instar larvae to phosmet when pretreated with phenyl saligenin cyclic phosphate *(PSCP)* in acetone, piperonyl butoxide *(PB)* in acetone, or acetone alone.

TABLE 8. NONSIGNIFICANT EFFECT OF SIX PESTICIDES ON EGG HATCH OF *C. CARNEA* COLONIES FROM IMPERIAL AND SAN JOAQUIN COUNTIES IN CALIFORNIA

Colony	Treated		Control	
Pesticide tested	Tested (No.)	Egg hatch (%)	Tested (No.)	Egg hatch (%)
Imperial County				
Carbaryl	38	97.4	56	94.6
Methomyl	53	88.7	36	83.3
Diazinon	80	82.5	100	92.0
Phosmet	88	93.2	62	87.1
Permethrin	112	83.9	97	88.6
Fenvalerate	82	89.0	75	93.9
San Joaquin County				
Carbaryl	48	89.6	33	84.8
Methomyl	60	85.0	29	86.2
Diazinon	90	81.1	61	83.6
Phosmet	73	82.2	29	86.2
Permethrin	49	81.6	27	88.8
Fenvalerate	49	93.9	31	93.6

Reproductive compatibility between Imperial and San Joaquin strains

One possible explanation for the large difference in pesticide tolerances exhibited by Imperial and San Joaquin County lacewings is that they are different species or subspecies. Adult and larval specimens were sent to C. A. Tauber (Department of Entomology, Cornell University), who was unable to detect any morphological evidence to reject the hypothesis that they are the same species.

A breeding experiment was conducted to determine whether the two colonies could interbreed and produce fertile offspring. The San Joaquin colony females deposited a lower cumulative mean number of eggs than the Imperial females; the reciprocal crosses responded in an intermediate manner (fig. 8). However, the slopes of the lines for cumulative oviposition were not significantly different ($p \leq 0.05$). Daily oviposition ranged from 10.4 to 14.7 eggs per day per female but was not significantly different between the four groups ($p \leq 0.05$) (table 9) and the total fecundity was greater than 500 eggs for all hybrid females. Thus, the fecundity of hybrid females from S x I and I x S crosses appeared to be normal. Moreover, percentage egg hatch was not significantly different ($p \leq 0.05$) between the four crosses (table 9). Therefore, Imperial and San Joaquin County lacewings appear to be conspecific and differences in their response to pesticides appear to be due to differences in pesticide use in the different counties.

TABLE 9. COMPARISONS OF DAILY OVIPOSITION AND PERCENTAGE EGG HATCH OF THE IMPERIAL (I) AND SAN JOAQUIN COUNTY (S) STRAINS OF *C. CARNEA* AND THEIR RECIPROCAL CROSSES (S x I) AND (I x S)

Cross	No. eggs deposited per female per day*†	Percentage egg hatch
S x S	10.4a (1.9)	83.0a (7.8)
S x I	11.3a (1.4)	89.6a (2.8)
I x S	14.7a (3.2)	88.7a (16.5)
I x I	14.1a (2.4)	75.9a (17.7)

*Standard deviation is in parentheses.

†Means followed by the same letter in each column are not significantly different at the $p = 0.05$ level according to ANOVA.

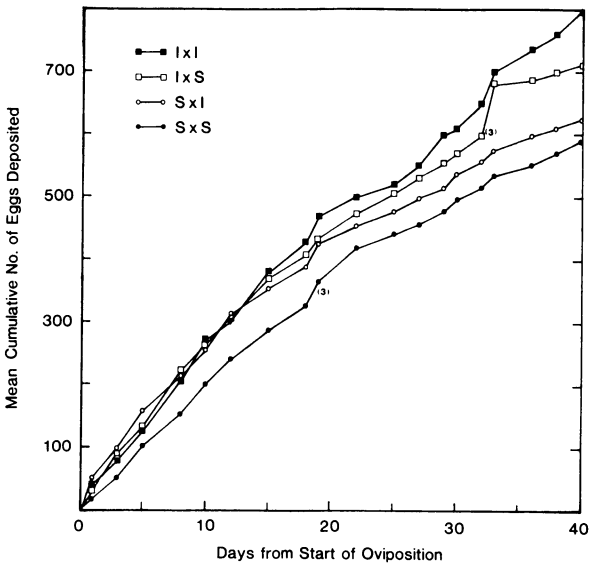

Fig. 8. Mean cumulative oviposition by interbreeding pairs of *C. carnea* from Imperial *(I)* and San Joaquin *(S)* counties. The *(3)* signifies point at which the initial four pairs were reduced to three.

CONCLUSIONS

Test results obtained with carbaryl and diazinon for adult *C. carnea* agreed with the literature that reports these pesticides to be highly toxic to adults (Bartlett 1964). The effects of permethrin, fenvalerate, methomyl, and phosmet on adult *C. carnea* had not been previously reported.

The effects of methomyl, diazinon, permethrin, and fenvalerate on larval mortality generally agreed with previous data. Methomyl was highly toxic to larvae (Plapp and Bull 1978; Pitts and Pieters 1982; Hassan et al. 1983), as was diazinon (Bartlett 1964; Suter 1978). With the exception of the work by Hassan et al. (1983), permethrin and fenvalerate have been shown to exert little direct effect on *C. carnea* larvae (Shour and Crowder 1980; Ishaaya and Casida 1981; Plapp and Bull 1978). Hassan et al. (1983) combined mortality of larvae and effects on adult fecundity into one rating. Thus, it is difficult to know exactly which stage was affected. In addition, tests by Hassan et al. (1983) were long-term tests, not short-term measures of mortality.

Our results differed with the only published report on the effects of phosmet on larval mortality. Sukhoruchenko et al. (1977) reported a highly toxic effect of phosmet on larvae of *C. carnea.* Our tests demonstrated that *C. carnea* larvae in California are highly tolerant of phosmet.

Reports in the literature on the effects of carbaryl on *C. carnea* larvae are variable. Bartlett (1964) and Sukhoruchenko et al. (1977) reported a highly toxic effect while Wilkinson et al. (1975) and Plapp and Bull (1978) reported a very low toxic effect on larvae. The results of the present study indicate that carbaryl is moderately toxic to lacewing larvae in California, and that the response varies geographically. The differences reported in the literature may be explained by the fact that Bartlett (1964) and Sukhoruchenko et al. (1977) used first instar larvae and the other workers used the more tolerant third instar larvae. This variability in results points out the need for uniform testing methods so that results can be compared. Another explanation for the variability in results is that some strains could have become partially resistant to carbaryl through field selection.

Larvae or adult *C. carnea* from different counties in California exhibited significantly different responses to most chemicals tested. The fact that Imperial County lacewings were more tolerant of pesticides in general suggests that they are gradually developing resistance to these insecticides due to a higher pesticide pressure (although the resistance level has not attained field spray rates yet). Although levels of exposure of individual pesticides in alfalfa bear out the trend of higher tolerance with higher exposure, it may be more important to look at exposure to all pesticides in all major crops. Female *C. carnea* have a two to three day period of adaptive dispersal before mating (Duelli 1980). Moreover, adults are highly mobile in their search for honeydew and females may deposit their eggs on a crop different from the one they inhabited as immatures. Thus, they may experience more than one pesticide during their lifetime. Finally, development of resistance to one pesticide may increase the level of tolerance to another (cross-resistance) or decrease tolerance to another (negatively-correlated cross-resistance). For example, exposure to one organophosphorus insecticide may induce enzyme levels that aid in detoxification of another pesticide. At this time there is no way to determine how much or which types of pesticides are affecting the tolerance of Imperial lacewings.

This is the first documentation of intraspecific variability of pesticide tolerance in *C. carnea.* Shour and Crowder (1980) tested two colonies of *C. carnea* larvae from widely separated geographical locations that had different pesticide treatment histories and did not find significant differences between the colonies. However, they tested permethrin and fenvalerate, which *C. carnea* naturally tolerate. They also used third instar larvae, which are more tolerant of pesticides than first instar larvae. Our work with the same chemicals, using first instar larvae, demonstrated significant variation in larval response to these chemicals.

Generalizations about the tolerance of green lacewings for many pesticides are frequently made after comparing data from different countries. Tolerance comparisons may be valid for chemicals for which *C. carnea* has a natural tolerance. However, our data (tables 3, 6; figs. 4, 5, 6) suggest that lacewings respond to local pesticide regimes and lacewing response to a particular chemical in one locality may not be the same as in another geographically isolated area. As is the case with phytoseiid mites (Hoy 1985), *C. carnea* appears to be responding to local levels of pesticide exposure. Although selection has not caused *C. carnea* to develop field rate resistance levels, the existence of more tolerant strains gives encouragement that field or laboratory selection for full resistance may one day be successful.

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ACKNOWLEDGMENTS

Partial support for this project was provided by the California Department of Food and Agriculture; Environmental Monitoring and Pest Managment, and Biological Control Services Program. Pesticides for screening were supplied by Shell Development, Ceiba-Geigy, Stauffer, Union Carbide, and Dupont chemical companies.

2½m-pr-10/85-SHG/ALS