Toxicity of Five Insecticides Used to Control California Red Scale (Homoptera: Diaspididae) Against Susceptible Red Scale Strains

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ABSTRACT Toxicities of carbaryl, malathion, parathion, methidathion, and chlorpyrifos were tested against 1-d-old first instars of three strains of California red scale, *Aonidiella aurantii* (Maskell). On the basis of their history, two of these strains were presumed to be susceptible to insecticides. Probit lines for the third strain were slightly to the right of lines for the susceptible strains for malathion, methidathion, and chlorpyrifos, but the shifts were not large enough to suggest resistance. For all strains, chlorpyrifos was most toxic, closely followed by methidathion. Carbaryl was least toxic, and the toxicities of parathion and malathion were intermediate between those of carbaryl and methidathion. Use of different spreader–stickers with the insecticides affected the concentration–mortality responses of California red scale to some insecticides. Therefore, for comparative work, we suggest that a single spreader–sticker should be used. Diagnostic concentrations for testing populations of California red scale for resistance to each of these insecticides are recommended.

KEY WORDS Insecta, *Aonidiella aurantii*, insecticide toxicity, insecticide resistance

CALIFORNIA RED SCALE, *Aonidiella aurantii* (Maskell), is a major economic pest of citrus in most citrus-growing regions of the world (Talhouk 1975) and is the major arthropod citrus pest in California (Anonymous 1980). At high population densities, it is capable of causing death of large branches, permanently injuring citrus trees (Quayle 1911). At lower population densities, California red scale is a cosmetic contaminant on the fruit, resulting in the fruit being downgraded in the packinghouse (Moreno & Kennett 1985).

In the San Joaquin Valley, where more than half of California's citrus acreage is located, biological control of California red scale currently is ineffective, and growers rely heavily on insecticides to provide control (Kennett 1973). Therefore, the history of development of insecticide resistance by California red scale is a major concern to the California citrus industry. Resistance to hydrogen cyanide (HCN) developed in California when HCN fumigation was the principal means of its control (Quayle 1942). California red scale in the Union of South Africa developed organophosphate resistance in the mid-1970s (Georgala 1977), causing a disastrous economic effect on that country's citrus industry. More recently, resistance to insecticides has been reported in Israel (E. Cohen, Hebrew University, personal communication).

The ability to detect insecticide resistance in the early stage of development and to test for cross resistance to alternative insecticides would assist the California citrus industry in managing resistance. Early detection before resistance becomes widespread would allow more time to develop and implement alternative control strategies. Identification of cross resistance would assist in selecting insecticides that may be useful until alternatives to chemical control could be developed. Our objectives were to determine the baseline responses of susceptible California red scale to the five major scalicides used in California citrus (carbaryl, chlorpyrifos, malathion, methidathion, and parathion); and from these baselines, to select a diagnostic concentration for each chemical for screening populations for resistance to these pesticides.

Materials and Methods

Scale Colonies. Three colonies of California red scale were maintained in the laboratory by the method described by Tashiro (1966). Two of the colonies were presumed to be susceptible strains because they had not been subjected to an insecticide application in many years (although they probably have been exposed to field-weathered insecticide residues on fruit used to maintain the colonies). One of these (Lab colony) was brought into laboratory culture at the University of California, Riverside (UCR) in the 1920s before the development of modern insecticides. The other colony (BC colony) was established in 1984 with red scale collected from the biological control grove on the UCR campus, a grove that had never been treated with insecticides. The third colony (Stauffer colony) was established in 1983 with California red scale collected from the Stauffer Chemical Corporation experimental farm in Orangecove, Calif., an area of high insecticide exposure where control

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failures with parathion had been reported. Great effort was taken to keep the strains genetically isolated. However, because they were maintained on field-grown lemons and because California red scale is ubiquitous in California, feral individuals were probably introduced into the colonies on fruit. The main experiments were conducted from December 1985 through July 1987. Experiments with spreader–stickers were conducted from June 1988 through June 1990.

Scalicides. Commercial formulations of insecticide were used in these studies: carbaryl, 80% active ingredient (AI) (Sevin 80 Sprayable, Union Carbide, Research Triangle Park, N.C.); malathion, 80.5% AI (Malathion 8E [emulsifiable], FMC, Philadelphia); parathion, 44.85% AI (Parathion 4E, FMC); methidathion, 24.4% AI (Supracide 2E, CIBA–GEIGY, Greensboro, N.C.); and chlorpyrifos 40.7% AI (Lorsban 4E, Dow Chemical, Midland, Mich.). The same supply of formulated material was used throughout the main experiments and the percentage AI was determined periodically by gas chromatography. The percentage AI determined by gas chromatography was used to calculate concentrations and was always within 12% of the percentage AI on the label. For several of the scalicides in the spreader–sticker experiments, a different supply of formulated material was used as well as a different formulation of parathion (Parathion 8E; Puregro, W. Sacramento, Calif.) and malathion (Cythion 57% EC; American Cyanamid, Princeton, N.J).

Exposure to Scalicides. Clean lemons (nearly mature green fruit or yellow fruit) were half covered with wax by dipping them in molten paraffin while the long axis of the fruit was held horizontal so that the level of the paraffin came slightly above the stem and blossom ends. The resulting film of paraffin reduced the desiccation rate of these test fruit. In the morning, fruit infested with reproducing female California red scales were taken from a colony and were placed (blossom end up) under a fluorescent light to attract crawlers to the apex of the fruit. In late afternoon, these crawlers were transferred to the unwaxed surface of test fruit by gently brushing them onto the test fruit with a large, soft artist's brush or by blowing them onto the test fruit with a gentle stream of air. Test fruit were infested (each with a maximum of ≈50 crawlers) and were left at room temperature overnight. By the next morning, the crawlers had developed to the "whitecap" stage (Quayle 1938), and the positions of apparently healthy whitecaps were marked on the fruit by circling their locations with a fine-point pen. This ensured an initial homogeneous population of healthy scales and aided in locating scales for assessment of mortality (8–11 d later). In most bioassays, 35 test fruit were used (5 for each of six concentrations and a control). We attempted to test 25 healthy whitecaps per fruit, but this was not always possible when the availability of crawlers was low.

A stock solution (≈1 g [AI]/liter; exact concentration varied among chemicals) was prepared from the formulated material. This stock was used to prepare 500 ml of the desired concentrations in 1-liter glass beakers. The spreader–sticker Biofilm (Kalo Labs, Kansas City, Mo.) was added to each concentration (including the water control) at a rate of 0.5 ml/liter in most experiments. In earlier replicates, the concentration was 1 ml/liter (Stauffer colony: parathion, first five replicates; chlorpyrifos, first two replicates; BC colony: parathion, first two replicates; chlorpyrifos, first three replicates). One day after fruit were infested with crawlers, they were dipped in the test solutions (unwaxed [infested] side facing down) for about 10 s. The fruit then were removed from the solution and placed, infested side up, on plastic rings (≈1.3-cm-wide rings cut from 3.8-cm [inside diameter] plastic electrical conduit; rings were glued on a board and spaced 8–10 cm apart center to center; this prevented them from rolling. After the fruit air-dried, they were stored between 26 and 28°C until mortality was assessed 8–11 d later.

Mortality Assessment. Diaspidid scales are completely sessile except for the brief crawler stage and the adult male. They also do not produce honeydew. These characteristics eliminate traditional means of assessing mortality such as detection of body movements or production of honeydew (Melamed-Madjar et al. 1983). We estimated mortality by determining whether or not the scale had grown beyond the stage at which it was exposed to the insecticide. Those that grew enough to expand the margin of the scale cover beyond Stage 2 (Quayle 1938) and approached or exceeded Stage 3 were considered to have survived. In two tests for each scalicide, California red scales were evaluated again for mortality 22–28 d after treatment, by which time they should have molted twice (Tashiro & Beavers 1968). Those that still had not grown beyond Stage 2 (the first molt is Stage 4) were scored as dead. This second evaluation was done to determine if California red scale that had been scored as dead on the first count after treatment recovered and resumed growth by 22–28 d after treatment.

Data Analysis. For estimation of concentration–mortality regressions, all experiments in which control mortality was ≥30% were discarded. Because control mortality in this system was frequently high, we decided to use this relatively high value instead of the value more commonly used (10–20%) to avoid discarding an excessive number of experiments. Control mortality varied widely among replicates; consequently, we did not use a pooled estimate of control mortality in the probit analysis. Instead, before analysis, the number dead and the sample size for each dose within each replicate were adjusted for the control mortality that was observed in their respective replicate. Abbott's formula (Finney 1971) was used for this purpose. These adjusted values then were pooled among replicates and analyzed by the PROBIT procedure of SAS

(SAS Institute 1985, 639–645). We set control mortality at 0% and used a $\log_{10}$ transformation of concentration. In some colony and chemical combinations, the concentration–mortality relationship departed from linearity at lower rates. At these lower concentrations, the response became flat (e.g., Fig. 1, Lab colony, malathion). These concentrations were omitted before probit analysis to allow a better fit of the regression line in the region of greater interest (LC_{50}–LC_{99}).

Effect of Spreader–Stickers. The concentration–mortality response to each scalicide was compared between groups where different spreader–stickers were used. The materials compared were Triton B1956 (Rohm and Haas, Philadelphia) at a rate of 0.16 ml/liter versus Biofilm at a rate of 0.5 ml/liter. The laboratory colony was used in tests with carbaryl, parathion, methidathion, and chlorpyrifos. The BC colony was used in the test with malathion. For each replicate, 60 fruit were infested with California red scale as described earlier and were assigned randomly to the Triton group or the Biofilm group (30 fruit per group). Within each spreader–sticker group, five fruit (usually 25 scales per fruit) were used for each of five concentrations of the scalicide being tested and a control. The five concentrations were the same for both spreader–sticker groups; only the spreader–sticker that was added to each concentration and to the control differed between the two groups. The fruit were treated and then stored before assessment of mortality using methods similar to those described earlier, except that temperatures were more variable (average daily temperature ranged between 24 and 29°C).

Data for each spreader–sticker group were analyzed separately within each replicate. We used the PROBIT procedure of SAS with a $\log_{10}$ transformation of concentration and control mortality equal to mortality in the respective control group (SAS Institute 1985). Within each replicate, the slope and LC_{50} were compared between the two spreader–sticker groups. LC_{50}'s were considered to be significantly different if there was no overlap between their 95% fiducial limits; slopes were considered to be significantly different if there was no overlap between their standard errors.

Results and Discussion

In the tests in which California red scale were evaluated for mortality with each scalicide 8–11 d after treatment and again 22–28 d after treatment, the resultant probit lines were very similar for the two evaluations, indicating that our standard 8–11-d count provided a good measure of mortality. The probit statistics, estimates of LC_{50}, LC_{99}, their 95% fiducial limits, and recommended field rates (Atkins et al. 1984) are presented in Table 1. Comparison of LC_{50}'s and LC_{99}'s among the chemicals showed that chlorpyrifos was the most toxic, closely followed by methidathion; carbaryl was the least toxic, and parathion and malathion were of intermediate toxicity (Table 1). The LC_{99}'s for all chemicals were below the recommended field rate for dilute applications (which is most comparable with these dip tests) and, with the exception of carbaryl, the LC_{99}'s were usually $\geq$10 times below the field rate (Table 1).

In general, the positions of the probit lines for all chemicals were very similar among the three colonies. The LC_{50}'s for malathion, methidathion, and chlorpyrifos for the Stauffer colony were shifted slightly to the right of the two colonies that were presumed to be susceptible (Lab and BC). Although the LC_{50}'s of these insecticides were significantly higher for the Stauffer colony than for at least one of the two susceptible colonies, the magnitudes of these shifts ($\approx$2-fold) were not great enough to warrant concern about resistance. The 95% fiducial limits around the LC_{99} of these three compounds overlapped between the Stauffer colony and both colonies that were presumed to be susceptible. The relatively high LC_{99} for parathion against the Stauffer colony was estimated by extrapolation beyond maximum mortality observed and therefore may not be reliable.

The large number of replicates for each compound (Fig. 1–3) and the large number of individual used in the tests (Table 1) fixed the position of our concentration–mortality lines reliably. However, as can be seen in Fig. 1–3, there was considerable variation among replicates with some scalicides. At times, this variation appeared not to be randomly distributed in time. For example, in tests on the Lab colony from 2 April to 4 June 1987, five consecutive replicates for carbaryl and four consecutive replicates for malathion resulted in the five or four lines, respectively, farthest to the right for each compound in Fig. 1. The last replicate for these compounds shifted back to the left, closer in position to the earlier four or five replicates. A similar phenomenon was observed during the same time for methidathion but not for chlorpyrifos or parathion. Gas chromatographic analysis of the scalicides indicated that this variation was not the result of decomposition of active ingredients over time. We mention this variation to underscore the need for replicating concentration–mortality tests on unknown populations at least several times, preferably with the replications spaced at least 1 mo apart. Data for the replicates then can be pooled and compared with the toxicities of pesticides reported in Table 1. In the case of a single replicate, a concentration–mortality regression should be compared with the range of regressions illustrated in Fig. 1–3 because the LC_{50}'s of individual replicates often fell outside the 95% FL of the pooled replicates given in Table 1. These limits are rather narrow because of the large numbers of insects used for their estimation in the pooled analysis, and they do not reflect the large variation among replicates.

An additional source of variation is variation among test fruit. In control groups, we occasionally

Table 1. Responses of California red scale colonies and recommended field application rates for each insecticide

Insecticide	Colony	Slope ± SE	n^a	g (AI)/liter				
				LC_{50}	95% Fiducial limits	LC_{99}	95% Fiducial limits	Field rate[b]
Carbaryl	Lab	1.51 ± 0.09	8,331	0.0265	0.0221–0.0322	0.922	0.534–1.935	0.959–1.150
Carbaryl	BC	1.46 ± 0.09	3,874	0.0106	0.00854–0.0129	0.412	0.242–0.858	—
Carbaryl	Stauffer	1.89 ± 0.16	3,937	0.0211	0.0163–0.0277	0.360	0.198–0.918	—
Parathion	Lab	3.75 ± 0.10	6,154	0.00680	0.00659–0.00702	0.0284	0.0261–0.0310	0.419–0.719
Parathion	BC	2.95 ± 0.38	1,345	0.00567	0.00409–0.00968	0.0347	0.0160–0.451	—
Parathion	Stauffer	1.64 ± 0.28	2,467	0.00555	0.00361–0.0121	0.146	0.0401–4.888	—
Malathion	Lab	2.97 ± 0.13	8,052	0.00897	0.00806–0.00999	0.0545	0.0429–0.0744	0.719–1.019
Malathion	BC	2.35 ± 0.19	2,353	0.00536	0.00414–0.00672	0.0524	0.0328–0.113	—
Malathion	Stauffer	2.75 ± 0.30	3,595	0.0127	0.00951–0.0173	0.0886	0.0498–0.274	—
Methidathion	Lab	2.32 ± 0.20	10,359	0.00208	0.00169–0.00253	0.0209	0.0132–0.0433	0.300
Methidathion	BC	2.35 ± 0.10	2,274	0.00190	0.00177–0.00204	0.0185	0.0155–0.0229	—
Methidathion	Stauffer	2.45 ± 0.29	3,038	0.00321	0.00247–0.00432	0.0287	0.0154–0.0953	—
Chlorpyrifos	Lab	4.17 ± 0.32	7,516	0.00158	0.00138–0.00178	0.00570	0.00454–0.00799	0.449
Chlorpyrifos	BC	3.57 ± 0.41	2,773	0.00173	0.00130–0.00213	0.00776	0.00526–0.0170	—
Chlorpyrifos	Stauffer	3.79 ± 0.31	4,599	0.00260	0.00223–0.00302	0.0107	0.00783–0.0178	—

[a] Number of scale evaluated for mortality (includes controls and all concentrations shown in Fig. 1–3; some of these concentrations were omitted before probit analysis) (see Fig. 1–3 and *Materials and Methods*).
[b] Recommended concentrations for high-volume (dilute) spray applications (Atkins et al. 1984).

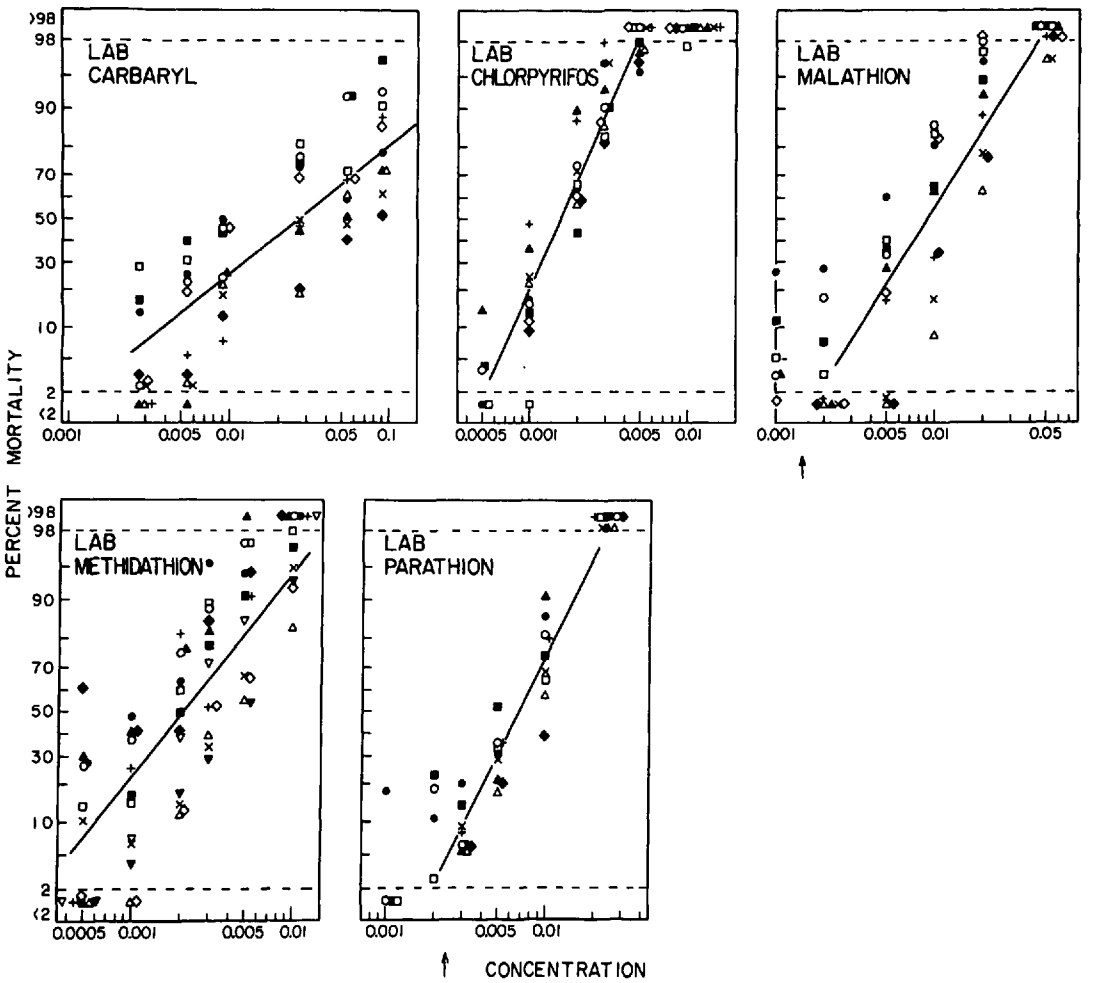

Fig. 1. Log concentration (g [AI]/liter)–probit mortality graphs for five insecticides against the Lab colony. Within each graph, different symbols indicate different replicates. Sequential order of replicates: ●○■□▲△×+◆◇▼▽. Regression lines are for pooled replicates. An arrow on the dose axis, when present, indicates that doses to the left of the arrow were omitted before calculation of the regression lines.

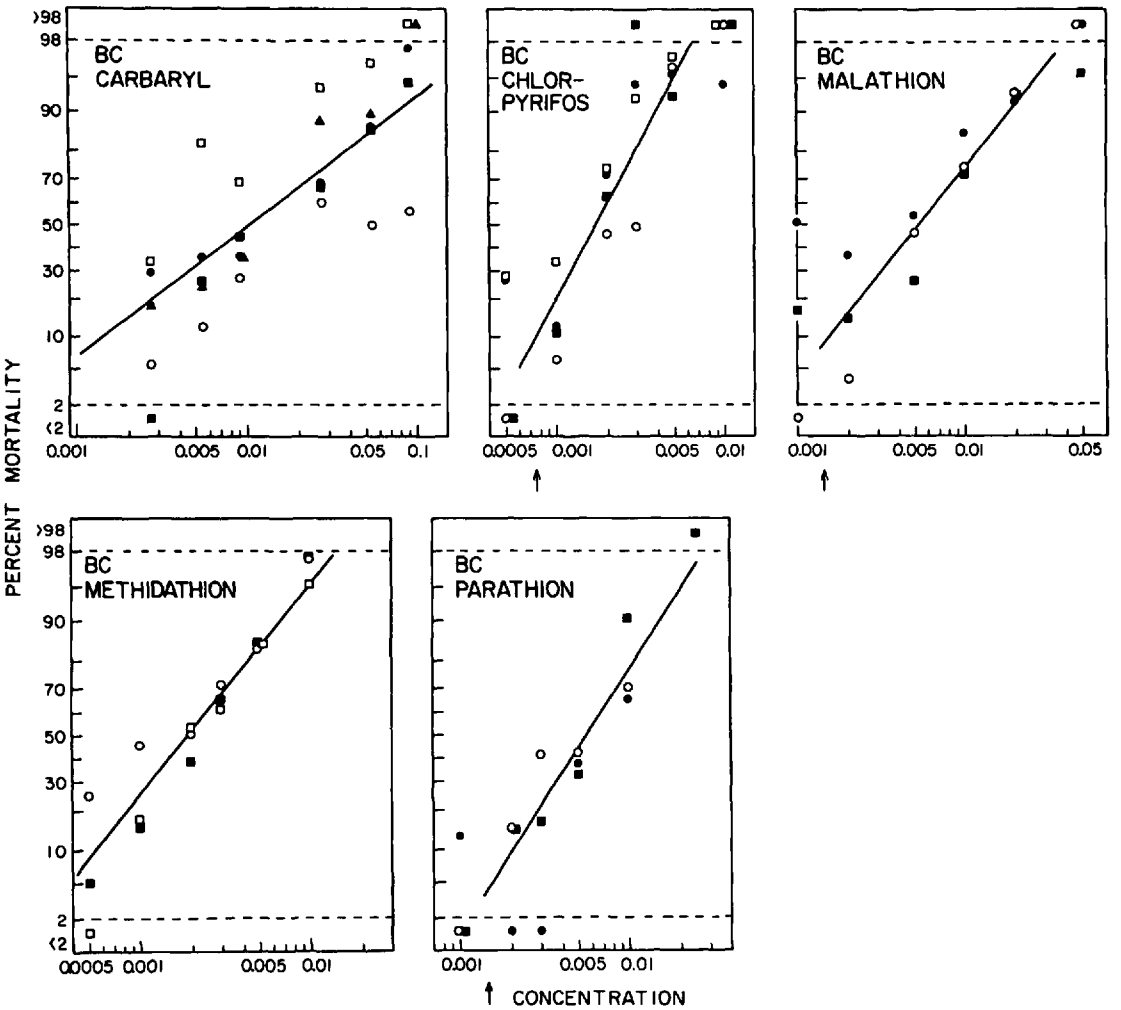

Fig. 2. Log concentration (g [AI]/liter)–probit mortality graphs for five insecticides against the BC colony. Interpretation as in Fig. 1.

observed that California red scale on four fruit had low mortality and scales on the fifth fruit had very high mortality. We suspect that the variation among fruit was caused by some fruit having residues from insecticide applications applied before the fruit were picked. This may have occurred despite our efforts to obtain fruit that had not been sprayed for 3 mo before picking. If only one fruit per concentration was used, variation in mortality among fruit could not be distinguished from variation among concentrations. We therefore recommend the use of at least five fruit per concentration to randomize the variation among fruit.

In tests in which the spreader-stickers Biofilm and Triton were compared, toxicities of carbaryl, parathion, malathion, and methidathion were only slightly affected or unaffected by the choice of spreader-sticker (Table 2). In one replicate each for carbaryl and methidathion, the LC_{50} was significantly lower in the Biofilm group than in the Triton group, and in one replicate for parathion, the LC_{50} was significantly lower in the Triton group than in the Biofilm group. However, the average LC_{50}'s of the Biofilm and Triton groups differed by <1.5-fold within each of these four insecticides. This difference is relatively small compared with the large variation among replicates when a single spreader–sticker is used (e.g., Fig. 1). The toxicity of chlorpyrifos, however, was affected greatly by the choice of spreader–sticker. The LC_{50} of the Biofilm groups averaged 2.5 times higher than the LC_{50} of the Triton groups. The average LC_{50} of chlorpyrifos in the Triton groups of the spreader–sticker experiments (Table 2) was similar to the LC_{50} of the earlier, main experiments where Biofilm was used (Table 1, Lab colony). The LC_{50} of the chlorpyrifos–Biofilm group in the spreader–sticker experiments (Table 2) differed by ≈2-fold from the LC_{50} in the main experiment. It is not clear why this occurred, but the last spreader–stick-

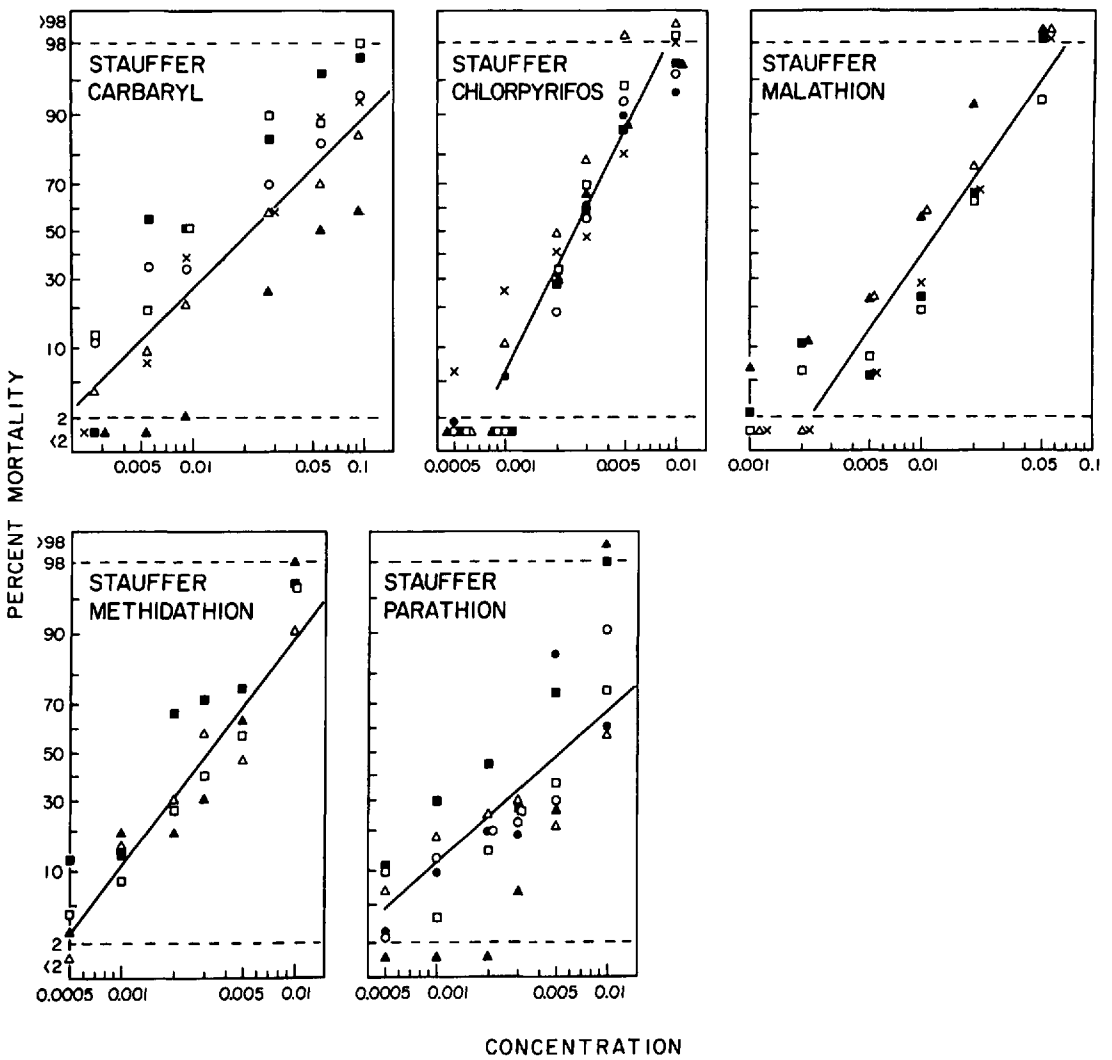

Fig. 3. Log concentration (g [AI]/liter)–probit mortality graphs for five insecticides against the Stauffer colony. Interpretation as in Fig. 1.

Table 2. Effects of Biofilm and Triton B1956 on toxicities of five insecticides against California red scale

Insecticide	Spreader–sticker	No. replicates for each insecticide	Avg LC_{50}, g (AI)/liter[a]	No. significant differences for LC_{50}[b]	Avg LC_{99}, g (AI)/liter[a]	Avg slope[a]	No. significant differences for slope[b]
Carbaryl	Biofilm	3	0.0206	1	0.169	2.57	1
Carbaryl	Triton	—	0.0294	—	0.431	2.01	—
Parathion	Biofilm	3	0.00594	1	0.0186	4.90	1
Parathion	Triton	—	0.00427	—	0.0149	4.29	—
Malathion	Biofilm	1	0.01069	0	0.0325	4.82	0
Malathion	Triton	—	0.00945	—	0.0425	3.56	—
Methidathion	Biofilm	4	0.00310	1	0.0115	4.22	1
Methidathion	Triton	—	0.00346	—	0.0181	3.26	—
Chlorpyrifos	Biofilm	5	0.00361	5	0.0186	3.86	0
Chlorpyrifos	Triton	—	0.00143	—	0.00664	4.06	—

[a] Average values of LC_{50}, LC_{99}, or slope for all replicates within each insecticide.
[b] Number of replicates where there was a significant difference (see *Materials and Methods*) between Biofilm and Triton.

er comparison using chlorpyrifos and Biofilm and more recent concentration–mortality studies with chlorpyrifos and Biofilm yielded LC_{50}'s closer to those in Table 1.

The pH of a solution of the Biofilm used in these studies was about 8.6 compared with ≈6.0 for the Triton that we used. The higher LC_{50} of chlorpyrifos in the Biofilm group could have resulted from alkaline hydrolysis, although chlorpyrifos (Lorsban 4E) is stable for up to 3 d at 23°C in water buffered at pH 9.0 (Drummond & Hemmer 1988). Drummond & Hemmer (1988) attributed the stability of Lorsban 4E in water buffered at pH 9.0 to chlorpyrifos remaining primarily in the oil phase of the emulsion and not being available for hydrolysis in the aqueous phase. However, the possible effects of a wetting agent like Biofilm on the emulsion are unknown. Consequently, the physical isolation of the chlorpyrifos in the oil phase from alkali in the aqueous phase may be disrupted. Regardless of the reason for the lower toxicity of chlorpyrifos in Biofilm solutions, Triton at 0.16 ml/liter appears to be a better spreader–sticker for Lorsban 4E than Biofilm at 0.5 ml/liter. Another advantage of Triton (0.16 ml/liter) over Biofilm (0.5 ml/liter) is that the control groups usually had lower mortality with Triton than with Biofilm.

For purposes of screening field populations for resistance, susceptibility of a population can be tested with a single diagnostic concentration (World Health Organization 1976). One such recommendation for a diagnostic concentration is three times the LC_{99} (G. P. Georghiou, personal communication). These values can be derived from the data in Table 1, but we stress that single-dose screening tests should be replicated because of the large variation among replicates (Fig. 1–3).

In the Union of South Africa, where resistance in California red scale is a problem, two comparisons of insecticide toxicity between susceptible and resistant red scale have been published (Nel et al. 1979, Schoonees & Giliomee 1982). Estimates of LC_{50} (g [AI]/liter) from unreplicated concentration–mortality studies for a susceptible laboratory strain, a susceptible field strain, and a resistant field strain were, respectively: parathion, 0.00125, 0.00641, 0.295; malathion, 0.00596, 0.00838, 0.520; methidathion, 0.00279, 0.00498, 0.0674; chlorpyrifos, 0.00147, 0.00303, 0.120 (Nel et al. 1979). Other estimates for LC_{50} for methidathion for a susceptible and resistant strain (Schoonees & Giliomee 1982) were 0.000816 and 0.0545, respectively. In each of these studies, a fruit-dipping assay similar to ours was used to test the responses of first instars. However, we dipped nymphs within 1 d of settling; Nel et al. (1979) dipped nymphs 2 d after settling, and Schoonees & Giliomee (1982) dipped nymphs 4 d after settling. Although our data is similar to their data for susceptible colonies, the difference in insect age makes it difficult to compare our data directly with theirs because age of the nymphs can affect their susceptibility to insecticides. For example, at single doses of malathion, parathion, carbaryl, and chlorpyrifos, percentage mortality of 3-d-old nymphs was, respectively, only 89, 67, 58, and 45% of the mortality of 1-d-old nymphs (when transformed to probits, probit mortality of 3-d-old nymphs was 94, 82, 86, and 79% of probit mortality of 1-d-old nymphs for the same four insecticides), whereas the toxicity of methidathion was similar between 1- and 3-d-old nymphs (G.P.W. & D.C.A., unpublished data). Nonetheless, the data of Nel et al. (1979) and Schoonees & Giliomee (1982) are relevant to our study because both suggest that resistance of the magnitude seen in South Africa would be easily detectable despite the relatively large variation in responses among replicates observed in our experiments.

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