Acaricide Resistance in Northern Fowl Mite (*Ornithonyssus sylviarum*) Populations on Caged Layer Operations in Southern California[1]

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ABSTRACT Southern California caged layer operations were visited over 3 yr. Northern fowl mites from 26 field populations were tested for acaricide resistance using a capillary pipette and glass dish bioassay. One was a susceptible field population with no pesticide exposure for over 30 yr (reference site for resistance ratio calculation). Technical and commercial formulations of malathion, carbaryl (Sevin), permethrin, and a commercial formulation of tetrachlorvinphos/dichlorvos (Ravap) were tested. Malathion did not have high activity for mites relative to other materials, but resistance to both technical and commercial formulations was low (<5×). Resistance to other materials was moderate to extreme. Frequency of carbaryl resistance (>10×) was higher with the commercial (88%) than the technical material (41%); 19% of the populations had resistance >100× to commercial carbaryl. Frequency of Ravap resistance (>10×) was 68%; 8% of populations had resistance >100×. Frequency of permethrin resistance (>10×) was 72% for the technical material and 88% for the commercial formulation. Extreme permethrin resistance (>1,000×) was observed in 56 and 50% of mite populations assayed using the technical and commercial formulations, respectively. Among sites, resistance to permethrin was uncorrelated with resistance to other chemicals, suggesting a different resistance mechanism. Resistance to carbaryl and Ravap was highly correlated [r = 0.76 at the LC_{50} level (concentrations estimated to be lethal to 50% of the test population) and r = 0.99 at the LC_{95} level], suggesting a common resistance mechanism. Producers currently depend completely on pesticides to control mite infestations. Mite resistance to registered materials emphasizes the need for integrated control measures.

(*Key words*: northern fowl mite, resistance, acaricides, control, integrated pest management)

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INTRODUCTION

The northern fowl mite, *Ornithonyssus sylviarum* (Canestrini and Fanzago), is considered to be the most important ectoparasite of poultry in North America (Axtell and Arends, 1990; Hogsette et al., 1991). Mites complete the entire life cycle on the host (particularly the vent region) while feeding on blood, and the generation interval is only 5 to 12 d (Sikes and Chamberlain, 1954). Populations thus can become very high on birds kept for more than a few months, notably laying hens and breeders. Mites are problematic for producers either through potential direct effects on performance (e.g., weight gain, egg production, or sperm production in roosters; see Hogsette et al., 1991 for review) or as nuisance pests on workers, particularly people who handle hens or eggs. Once mites are established in a flock, control depends completely on pesticide applications, and relatively few synthetic pesticides are approved for this purpose.

As part of a larger survey of mite presence and sampling plan development in California, producers often report poor chemical control. The present study was designed to quantify levels of potential mite resistance in the field to registered materials.

MATERIALS AND METHODS

Field Sites

Caged layer facilities in the counties of Riverside, San Bernardino, and San Diego were visited in 1999, 2000, and 2001. Producers did not spray for mites for at least 1 mo prior to these visits. Details of interviews with producers about mite problems and control efforts will be reported elsewhere (B. A. Mullens, R. K. Velten, N. C. Hinkle, D. R. Kuney, and C. E. Szijj, unpublished). Sometimes details of past pesticide usage were unavailable.

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Abbreviation Key: LC_{50} = concentrations estimated to be lethal to 50% of the test population; RR = resistance ratio.

TABLE 1. Caged layer operations in southern California from which northern fowl mite populations were tested for acaricide resistance

Site	Year tested	County	Chemical (formulation) tested[1]
1[2]	1999, 2000	San Diego	P (t, c), C (t, c), R (c), M (t, c)
2	1999	San Diego	P (c), C (c), R (c), M (c)
3	1999	San Diego	P (c), C (c), R (c), M (c)
4	1999	San Diego	P (c), C (c), R (c), M (c)
5	1999	San Bernardino	P (c), C (c), R (c), M (c)
6	1999	San Bernardino	P (c), C (c), R (c), M (c)
7	1999	San Bernardino	P (c), C (c), R (c), M (c)
8	1999	San Diego	P (c), C (c), R (c), M (c)
9	2000	San Diego	P (c, t), C (t), R (c), M (t)
10	2000	San Diego	P (t), C (t), R (c), M(t)
11	2000	San Diego	P (c, t), C (t), R (c), M (c)
12	2000	San Bernardino	P (t), C (t), R (c), M (t)
13	2000	Riverside	P (t), C (t), R (c), M (t)
14	2000	Riverside	P (t), C (t), R (c), M (c)
15	2000	Riverside	C (t), R (c), M (t)
16	2001	Riverside	P (t, c), C (t, c), R (c), M (t, c)
17	2001	San Bernardino	P (t, c), C (t, c), R (c), M (t, c)
18	2001	Riverside	P (t), C (t, c), R (c), M (t, c)
19	2001	Riverside	P (t), C (t, c), R (c), M (t, c)
20	2001	San Bernardino	P (c), C (c), M (c)
21	2001	San Bernardino	P (t, c), C (t, c), R (c), M (t, c)
22	2001	Riverside	P (t, c), C (t, c), R (c), M (t, c)
23	2001	Riverside	P (t), C (t), R (c), M (t)
24	2001	San Bernardino	P (t), C (t), R (c), M (t)
25	2001	San Diego	P (t), M (t)
26	2001	San Bernardino	P (t, c), C (t, c), R (c), M (t, c)

[1]Chemicals (see text) included either technical (t) or commercial (c) formulations; P = permethrin, C = carbaryl, R = Ravap (tetrachlorvinphos/dichlorvos), and M = malathion.

[2]Site 1 was considered the susceptible (reference) mite population due to no pesticide use for over 30 yr.

When available, general information on past pesticide use relevant to northern fowl mite control was examined.

When large mite populations were encountered in flocks, heavily mite-infested feathers were plucked from at least 3 to 5 individual hens. The feathers were placed into Ziplock plastic bags and then transported to the laboratory in an insulated ice chest to confirm identification. Mites obtained from each population were mounted in Hoyer's medium on microscope slides and identified with the aid of a key developed by Baker (1999).

One producer (site 1) had not applied any insecticides for insect or mite control for over 30 yr. The mites from this site were designated as the susceptible reference population. The sampling sites, counties, year(s) tested, and materials tested are shown in Table 1.

Bioassay Preparation

Glass Pasteur pipettes (14.5 cm long and 0.5 cm inside diameter) were coated on the inside using a series of 5 dilutions of each pesticide (except tetrachlorvinphos/dichlorvos) according to the method of Foulk and Matthysse (1964). Concentration ranges were determined after preliminary testing on several field mite populations and a susceptible field population (see below). Commercial preparations were mixed in deionized water, whereas technical materials were mixed in acetone. Control tubes were coated with water or acetone alone. Pipettes were labeled by chemical and dose using an indelible marker. The liquid was drawn into the barrel of the tube and expelled, leaving a residue behind. Pipettes were first touched to dry tissue to remove remaining free liquid from the tip. The tubes then were placed overnight on an electronic shaker table, which rolled the open tubes constantly and ensured even spread of the residue on the interior of the pipette (acetone evaporated almost immediately). Once dried overnight, tubes were used in bioassays as early as the next day. If not used immediately, tubes were wrapped in aluminum foil (to exclude light), placed within tightly sealed plastic bags, and stored in an ultralow freezer (–80°C) pending use in bioassays.

The tetrachlorvinphos/dichlorvos mixture did not yield repeatable dose responses in the pipettes, probably due to the fumigating action of the dichlorvos in a confined space. For this material, open glass Petri dishes were used (9-cm diameter). The bottom of the dish was coated with a thin layer of the diluted pesticides (water for controls) by adding several milliliters to each dish. The dish was turned briefly to coat the entire bottom. Excess liquid was quickly poured off, and remaining liquid was wicked from the tilted dish with tissue. Dishes were allowed to dry overnight, wrapped in aluminum foil, sealed within tightly closed plastic bags, and frozen at –80°C until use in bioassays. Before use, the edges of the bottom of each dish were coated with a thin layer (2 to 3 mm wide) of sticky material,[4] by using a small wooden dowel, to prevent mites from crawling out of the dish.

[4]Tangle-Trap, Tanglefoot Co., Grand Rapids, MI.

The pesticides were those registered for use against northern fowl mites in caged layers in California. Technical materials were as follows: malathion 99.8%,[5] carbaryl 99%,[6] and permethrin 92%.[7] Commercial materials were purchased at a large local farm supply store and were as follows: malathion 57EC,[8] Sevin 80 WSP,[9] Permectrin II,[10] and Ravap EC.[11] Concentrated stock solutions of the technical materials were kept in dark bottles in a –20°C freezer pending dilution as needed to prepare treated tubes. Commercial mixtures were removed from the original containers, diluted, and applied to tubes or dishes.

Conducting Bioassays

Northern fowl mites survive for weeks away from a host (DeVaney and Beerwinkle, 1980). Mites were tested within 5 d of collection and usually within 1 to 2 days of collection. If the predetermined range of doses failed to yield an appropriate range of mortality (ideally about 10 to 90%), it was sometimes possible to conduct another bioassay the next day. Similarly, data for a small number of bioassays had severe problems, such as high control mortality, and had to be discarded.

Plastic bags containing mites were placed on ice in a plastic dishpan on a countertop. Mites crawled away from the cool feathers toward the open top of the bag, where they could easily be removed for the bioassays. Pipettes were plugged using clean cotton that blocked about 1 cm of the top (wide end). The pipette was then inserted into rubber tubing attached to a vacuum line under gentle airflow. The tubing had 2 crossed wires to prevent the cotton from being sucked out. Twenty large adult mites (mostly females) were gently sucked into a tube. The tube was removed from the vacuum line, and the tip (narrow end) was plunged promptly into a 3 to 4 mm thick layer of modeling clay. This effectively sealed the mites into the tubes, but some air exchange occurred through the cotton plug. Four tubes (80 mites) were used for each dose of each chemical, for a total of 480 mites per chemical per population (5 doses plus a water or acetone-treated control for commercial and technical preparations, respectively). Mites freely walked about the interior of the pipettes and were held on the countertop at 22 to 25°C. Live and dead mites (no movement even after disturbance) were counted after 24 h with the aid of a dissecting microscope.

For the tetrachlorvinphos/dichlorvos tests, mites were picked up using a small camel's hair brush and placed individually into each dish (20 mites per dish, 4 dishes per dose, 5 doses plus a water-treated control). The dishes were held open on the countertop along with the pipettes and counted as above.

Statistical Analysis

Concentrations for technical and commercial materials were expressed as parts of active ingredient per million (ppm; ppm tetrachlorvinphos was used for the Ravap). Mite mortality was subjected to $\log_{10}$ dose-probit analysis using the SAS equivalent option of PriProbit (Sakuma, 1998). This yielded LC_{50} values (concentrations estimated to be lethal to 50% of the test population), which were most robust (narrowest 95% confidence limits) in the analysis and were used for calculation of resistance ratios. Resistance ratios were calculated by dividing the LC_{50} of a test population for a particular chemical and formulation by the corresponding value for the susceptible reference population. The probit regression lines thus generated for the field mite populations were plotted by chemical and formulation together on graphs, which allowed an overview of the levels of resistance and slope of the lines for the different field populations (slopes reflect heterogeneity in susceptibility of the target population). Frequency distributions of resistance ratios were presented graphically. Correlation analysis was used to determine relationships among resistance estimates at the various sites and between chemical groups. When statistical comparisons between populations were presented for a mite population for a particular chemical and formulation, statistical significance was based on nonoverlap in the 95% confidence intervals. Paired *t*-tests were used to compare LC_{50} values for technical versus commercial preparations.

RESULTS

Mites were confirmed microscopically to be northern fowl mite, *Ornithonyssus sylviarum* (Canestrini and Fanzago), from 25 of 26 sites. At site 20, the mites appeared to be a mixed or possibly a hybrid population. Most mites were northern fowl mite, but a few mites matched the description of the tropical fowl mite, *Ornithonyssus bursa* (Berlese), which has a slightly different shape of the dorsal shield and pattern of setae placement on the ventral shield (Baker, 1999). The biology of the 2 species is very similar, but relatively little is known of the biology of *O. bursa* (Sikes and Chamberlain, 1954; Lancaster and Meisch, 1986).

Dose-mortality probit lines are shown graphically for the different chemicals and formulations in Figures 1 to 3. Malathion technical and commercial formulations (Figure 1, A and B) had relatively low mite activity. For the reference susceptible population (site 1), the LC_{50} and LC_{95} values for the technical formulation were 155.6 and 248.2 ppm, respectively. For the commercial formulation the LC_{50} and LC_{95} values were 65.1 and 283.1 ppm, respectively. Several mite populations actually had LC_{50} values less than the reference population. Only 1 mite population displayed a resistance ratio (RR at the LC_{50} level) of >5. This ranch had a chronic biting louse problem [*Menacan-*

[5]Cheminova Agro A/S, Lemvig, Denmark.
[6]Rhone-Poulenc Ag. Co., Research Triangle, NC.
[7]FMC Corp., Princeton, NJ.
[8]57% Malathion, Loveland Industries, Greeley, CO.
[9]80% carbaryl, Aventis, Montvale, NJ.
[10]10% permethrin, Boehringer Ingelheim Animal Health Inc., St. Joseph, MO.
[11]23% tetrachlorvinphos and 5.3% dichlorvos, Boehringer Ingelheim Animal Health, St. Joseph, MO.

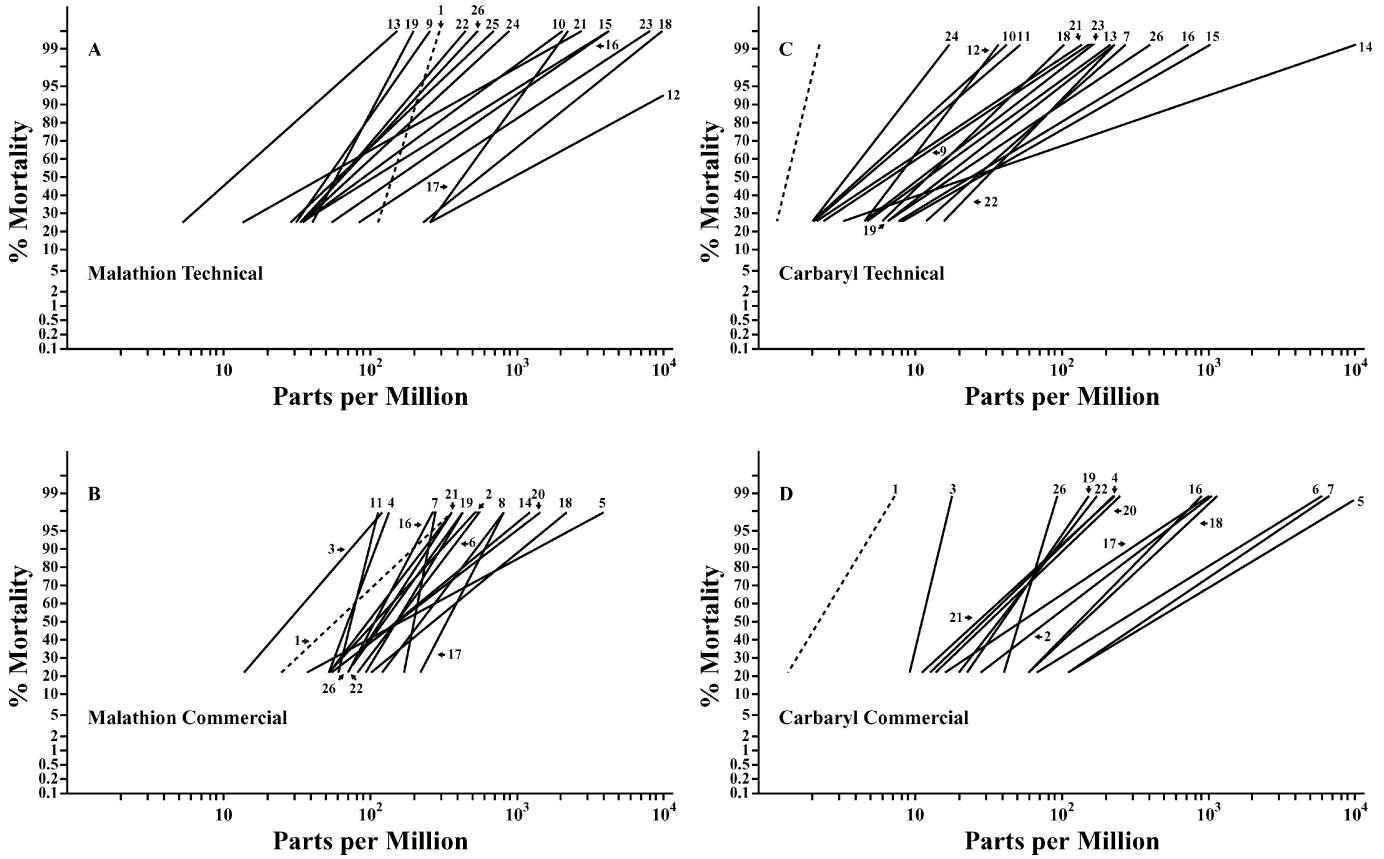

FIGURE 1. Log_{10} dose-probit mortality lines for malathion (A, B) and carbaryl (C, D) for northern fowl mites from southern California caged layer operations. Lines are grouped by chemical and formulation and are identified by site number (see Table 1). Site 1 is the susceptible reference population (dashed line).

thus stramineus (Nitzsch)] and was sprayed periodically with malathion for lice; the RR (LC_{50} level) was 7.2 for technical malathion.

Carbaryl dose-mortality lines are shown in Figure 1 (C and D). The susceptible reference population had LC_{50} and LC_{95} values for the technical formulation of 1.53 and 2.13 ppm, respectively. The LC_{50} and LC_{95} values for the commercial formulation were 2.59 and 6.13 ppm, respectively. Other mite populations for which 95% confidence intervals could be generated were significantly resistant to the commercial carbaryl formulation (7 out of 7), and 6 of 9 populations were significantly resistant to the technical formulation. The slopes of some lines (e.g., sites 5, 6, 7, and 14) were less steep, probably representing heterogeneity of that mite population for susceptibility to carbaryl. The most extreme resistance example (site 8) did use carbaryl periodically for mite control; resistance was extreme enough that a probit line could not be generated.

The dose-mortality lines for Ravap (tetrachlorvinphos/dichlorvos) are shown in Figure 2. The susceptible population had LC_{50} and LC_{95} values of 0.70 and 2.16 ppm tetrachlorvinphos, respectively. As was true for carbaryl, some mite populations showed relatively gentle line slopes, suggesting high heterogeneity (e.g., sites 6, 8, and 9). The most extreme resistance noted (site 8, RR at the LC_{50} level was 174.9) applied Ravap >3 times per year on every flock.

The dose-mortality lines for permethrin are shown in Figure 3. The susceptible reference population had LC_{50} and LC_{95} values for the technical formulation of 0.88 and 3.46 ppm, respectively. The LC_{50} and LC_{95} values for the commercial formulation were 0.27 and 0.84 ppm, respectively. Many mite populations had regression lines with relatively very gentle slopes, suggesting very high heterogeneity of field populations for susceptibility to this material. In fact, most populations exhibited mortality that was too low, even at the highest dose, to generate probit lines.

An overall presentation of resistance ratios is shown in Figure 4. For permethrin (Figure 4A), 72% of populations were >10× resistant to the technical material, and 88% were resistant at this level to the commercial material. Although resistance was often too high to measure in terms of a specific resistance ratio, permethrin resistance >1,000× was the single highest resistance category, noted in 56 and 50% of the mite populations tested using technical and commercial formulations, respectively. Resistance >10× to carbaryl (Figure 4B) was observed in 41% of mite populations tested with the technical formulation and 88% of mite populations tested with the commercial formulation. There were 18% of mite populations that had >100× resistance to carbaryl. Resistance to malathion rela-

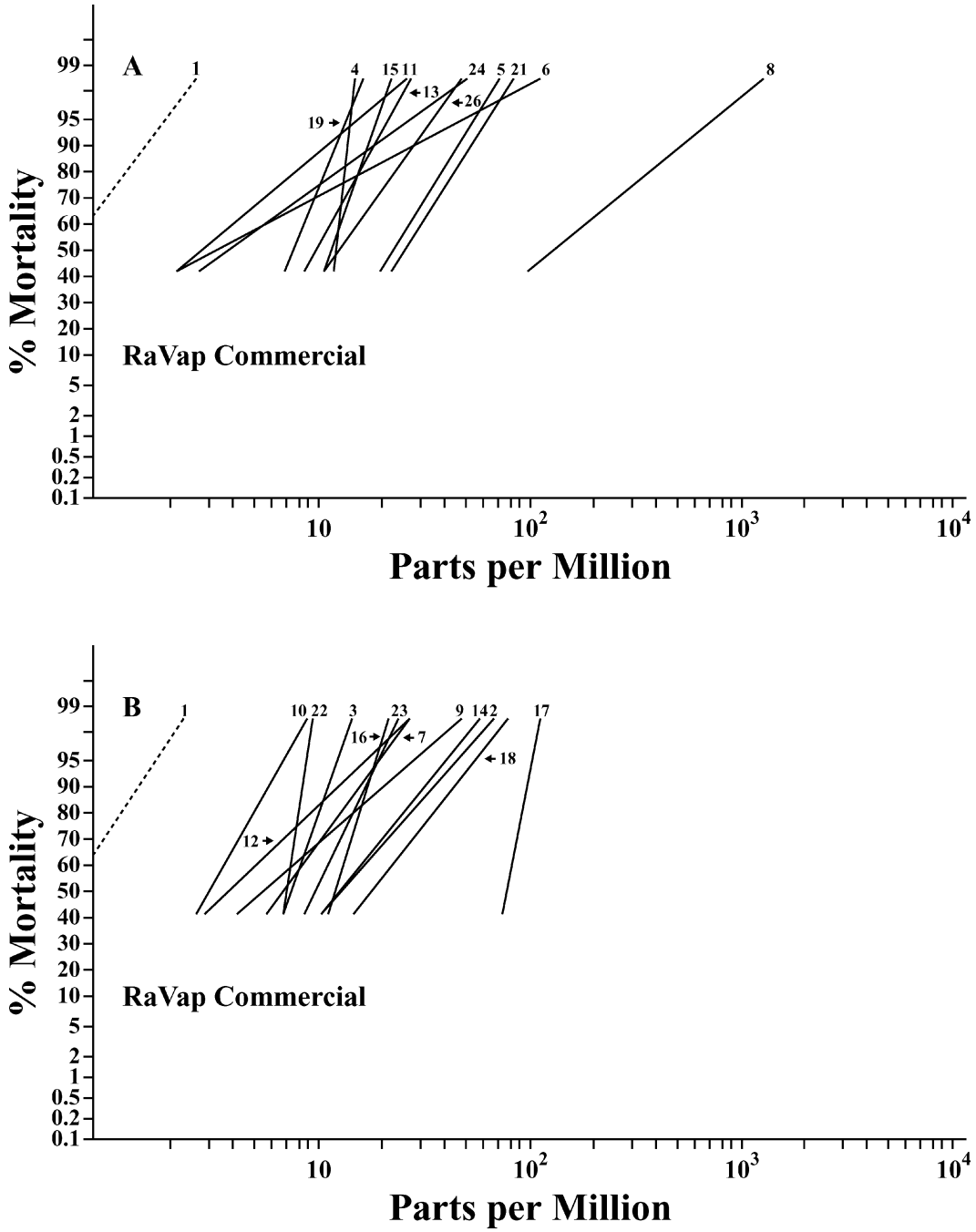

FIGURE 2. Log_{10} dose-probit mortality lines for commercial tetrachlorvinphos/dichlorvos (Ravap) for northern fowl mites from southern California caged layer operations. Lines are identified by site number (see Table 1). Site 1 is the susceptible reference population (dashed line).

tive to the susceptible population was very low overall (Figure 4C). Frequency of commercial Ravap resistance (>10×; Figure 4D) was 68%, and 8% of populations had resistance >100×.

An attempt was made to relate use patterns to resistance by categorizing the mite populations into 2 groups (higher resistance and lower resistance) for each of the materials Ravap, carbaryl, and permethrin. Resistance ratios were categorized as being greater or less than 20× for Ravap and carbaryl and greater or less than 1,000× for permethrin. These frequencies were then subjected to chi-squared analysis (Table 2). Somewhat higher levels of resistance to carbaryl were observed on sites where carbaryl was known to have been used, but this finding still was not significant using a chi-squared test ($P > 0.05$). Likewise the frequencies of higher or lower resistance did not differ significantly for facilities according to known use patterns of Ravap or permethrin.

Some populations were tested using both the technical and commercial formulations of a given chemical. When tests were made on the same population at the same time, the LC_{50} and LC_{95} values were subjected to correlation analysis (r). In general these correlations were positive. Because many populations were too resistant to

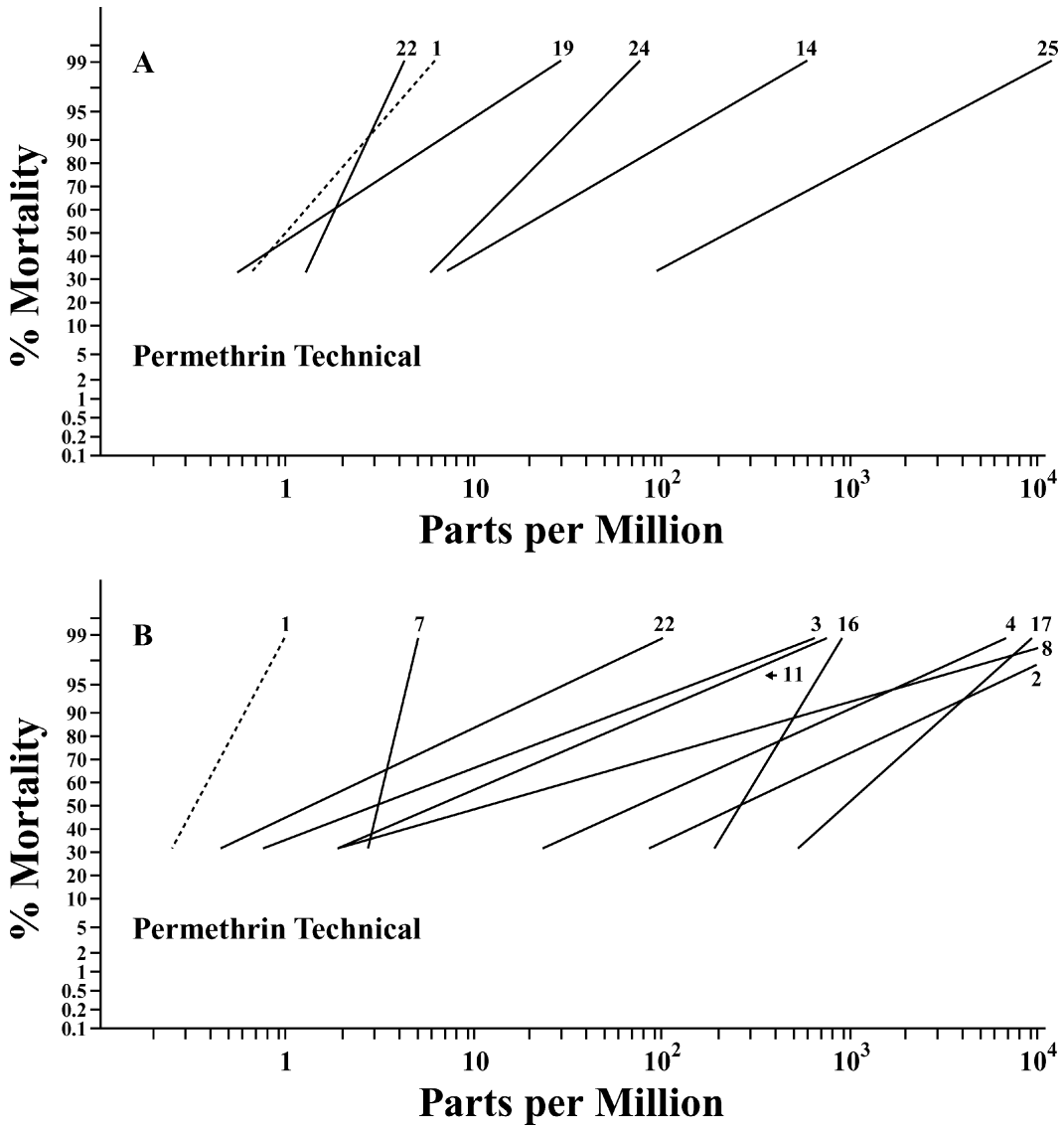

FIGURE 3. Log_{10} dose-probit mortality lines for technical (A) and commercial (B) formulations of permethrin for northern fowl mites from southern California caged layer operations. Lines are identified by site number (see Table 1). Site 1 is the susceptible reference population (dashed line). Many mite populations were too resistant to generate probit lines and are not shown on graph.

measure for permethrin, only 4 such pairs of data could be examined. The correlation at the LC_{50} level was –0.09 and was nonsignificant (one aberrant point) ($P > 0.05$). At the LC_{95} level the correlation was 0.99 ($P < 0.01$). Seven populations could be examined for carbaryl. The correlation at the LC_{50} level was 0.54 and the LC_{95} level was 0.39 (both $P > 0.05$). Seven populations could be examined for malathion. The correlation at the LC_{50} level was 0.87 ($P < 0.02$) and at the LC_{95} level was 0.92 ($P < 0.01$).

The LC_{50} values for technical versus commercial formulations of carbaryl and malathion were compared using a paired *t*-test on mite populations tested with both formulations at the same time. The differences were not significant ($P > 0.4$) for malathion, but commercial carbaryl had higher LC_{50} values relative to paired technical carbaryl values ($P < 0.05$).

Twenty-four populations were tested using all 4 chemicals. To correlate at least roughly the resistance profiles to the different materials among the populations, LC_{50} and LC_{95} levels for technical and commercial formulations were used. If data existed for resistance ratios for technical and commercial formulations of a particular chemical for a given mite population, those ratios were averaged to yield a single resistance ratio. The resistance ratio correlations are shown in a correlation matrix in Table 3. The r values for Ravap and carbaryl were highly significant ($P < 0.01$). All other correlations were not significant ($P > 0.05$).

DISCUSSION

It should be emphasized that the resistance data must be interpreted in the context of the bioassays. The assays

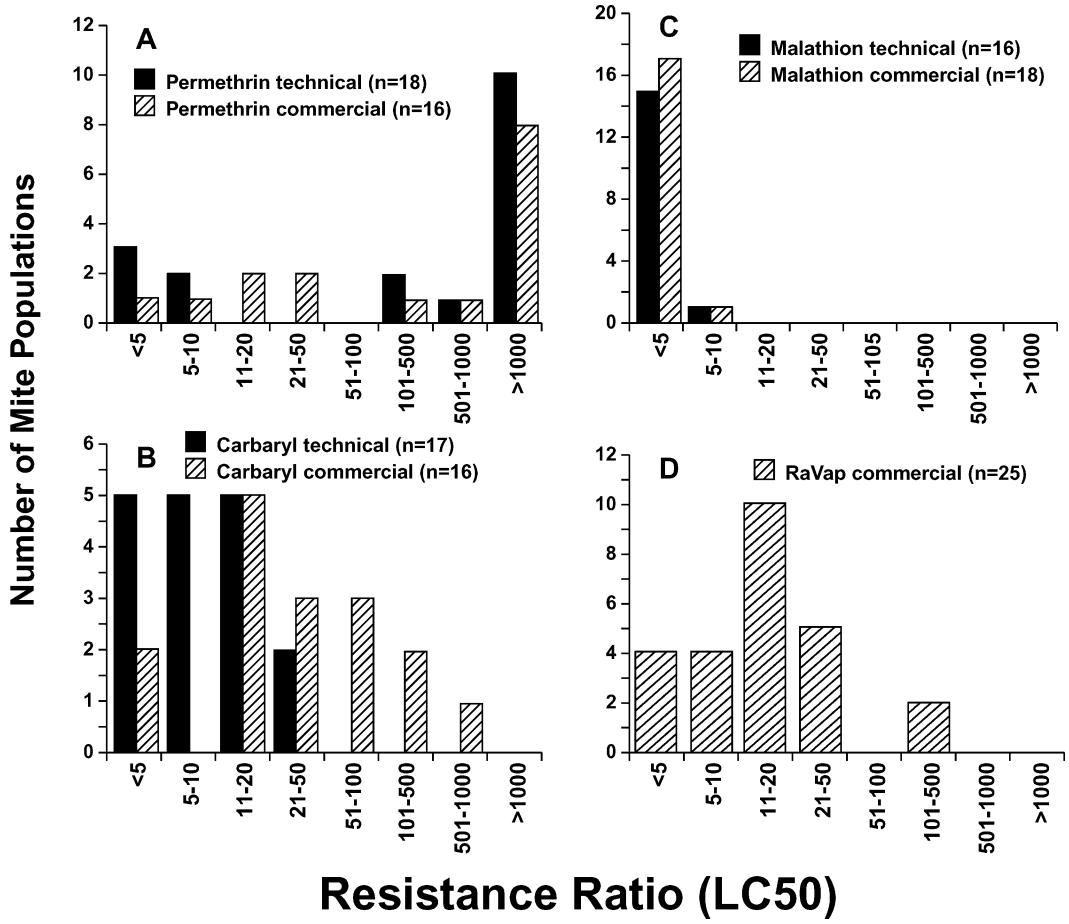

FIGURE 4. Frequency distributions of resistance ratios (LC_{50} level) of southern California northern fowl mites to registered acaricides. Number of mite populations assessed shown according to the acaricide and formulation.

are on a glass surface, rather than on a live host bird. While there is almost certainly some relationship between physiological resistance determined via bioassay and field control effectiveness, the two cannot be related directly. For example, mites in the bioassays contacted the residues only by walking on them. They were never sprayed or exposed in 3 dimensions on a porous surface (feathers and mite debris) in the confines of feathers enclosing the vent region. Many useful field tests have been done on mite susceptibility to acaricides applied to birds (e.g., Hall et al., 1978; Loomis et al., 1979; McKeen et al., 1983). Typically these tests are done on a single production or experimental facility. Poor mite control may suggest resistance, but it cannot be proven from such tests. Reports of resistance also may be due to poor application, rather than resistance per se (Axtell and Arends, 1990). Similarly, results from one field trial of a compound are difficult to compare directly with another, because environmental conditions and temperatures usually differ.

TABLE 2. Pesticide use patterns (based on producer interviews) relative to levels of northern fowl mite resistance to pesticides in southern California[1]

Use	High resistance	Low resistance
High Ravap use[2]	4	7
Low Ravap use	3	6
High carbaryl use	6	4
Low carbaryl use	3	8
High permethrin use	4	3
Low permethrin use	8	7

[1]For Ravap and carbaryl, high resistance (LC_{50} level) was arbitrarily set at >20× compared with a susceptible population. For permethrin, high resistance was >1,000×. Numbers of caged layer operations per category are shown.

[2]Pesticide use categories were as follows. High Ravap = >2 sprays per year; high carbaryl = ≥1 spray per year; high permethrin = ≥ 1 spray per year, including known use for fly control. Not all fly spray use could be documented (see text).

The glass pipette bioassay for northern fowl mite resistance (Foulk and Matthysse, 1964) has proven to be a useful tool for researchers and does allow for comparisons of mite resistance from different times or areas. With the exception of the reference population, which was a fortunate discovery, all mite populations in the present study were exposed periodically to pesticides in the field. The susceptible reference population (site 1) did compare well with mites from other published studies using the same bioassay technique. The LC_{50} value for technical permethrin (0.88 ppm) at site 1 compares well with published values of a mean of 0.53 ppm for mites temporarily colonized from 6 field sites in

TABLE 3. Correlation matrix (r values) for resistance ratios of pesticides tested against 24 field populations of northern fowl mites from southern California[1]

Pesticide	Permethrin	Carbaryl	Ravap	Malathion
Permethrin	N/A	–0.096/–0.199	–0.227/–0.187	0.245/0.296
Carbaryl	—	N/A	**0.763/0.989**	0.250/–0.049
Ravap	—	—	N/A	0.318/–0.083

[1]Statistically significant ($P < 0.05$) values are highlighted in bold. Values are shown for the LC_{50} level/LC_{95} level for each possible comparison

North Carolina (Arthur and Axtell, 1983) and 2.8 ppm for another colonized North Carolina population (Fletcher and Axtell, 1991). It is lower than the 21.9 ppm reported for a mite population in Virginia (Hall et al., 1978). The LC_{50} value for technical carbaryl at site 1 (2.59 ppm) compares with published values of 6.6 ppm (Hall et al., 1978), 4.11 ppm (Arthur and Axtell, 1983), 6.2 ppm (Fletcher and Axtell, 1991) but is higher than the 0.24 to 0.44 ppm reported for a northern fowl mite colony from New York by Matthysse et al. (1975). The LC_{50} value for technical malathion from site 1 (155.6 ppm) compares well with values of 119.4 (Arthur and Axtell, 1983) and 146 ppm (Hall et al., 1978) and is somewhat lower than 238.4 ppm (Fletcher and Axtell, 1991) and 230 to 800 ppm (Matthysse et al., 1975). Because we used a tetrachlorvinphos/dichlorvos mixture sold commercially (Ravap) and a different bioassay (Petri dish), it is difficult to compare our results directly with published values of tests done on the materials separately.

Most resistance studies use technical material for reasons of superior experimental control. We chose to use both technical and commercial materials, because the commercial materials are actually used in the field, and there frequently is a question of whether formulated materials differ in effectiveness due to carriers. The basic resistance picture for northern fowl mites was similar for technical versus commercial formulations, although data for carbaryl did differ between technical and commercial formulations. This result could have been caused by the fact that the commercial formulation of carbaryl, wettable powder mixed and suspended in water, might have somewhat more variability (both the concentration sold and its distribution within a pipette) than could be obtained by adding technical material to acetone in the laboratory.

Most prior resistance tests (Matthysse et al., 1975; Arthur and Axtell, 1983; Fletcher and Axtell, 1991) used mites derived from the field and then colonized for a period prior to testing. The advantages of this procedure are that mites can be tested repeatedly and are not subject to uncontrolled field conditions. Statistically problematic tests can be discarded and rerun. The disadvantage is that colonization (and lack of continued pesticide selection) might result in some change in mite resistance status over time, particularly if one assumes there is some fitness cost to mites associated with resistance. Colonization also requires space and facilities to hold live hosts, and thus testing of a large number of populations becomes quite difficult logistically. In the present study, mites were tested very soon after retrieval from field birds. Despite the inherent variability, and the inability to test populations repeatedly, the realism and ability to screen a far larger number of field populations made direct testing of field-collected mites worthwhile. The largest published survey of northern fowl mite resistance (Arthur and Axtell, 1983) involved mite populations from 5 farms in North Carolina. Mites in that study displayed less variability in resistance than was observed in the present survey examining 26 populations.

The probit analysis usually generated LC_{50} and LC_{95} values that appeared reasonable in light of the observed dose response. The program sometimes would not assign 95% confidence intervals, or those intervals would be extremely wide, if the data were rather heterogeneous. Nevertheless, plotting the probit lines by chemical and formulation resulted in an effective overview of resistance status among the large number of populations.

Results using malathion confirm that it has relatively low activity for northern fowl mites. Thus low frequencies of field resistance (relative to a reference population) do not necessarily mean malathion is superior to another material that is inherently more active but to which mite populations have some resistance. Since the study was completed, malathion is no longer available in California for poultry ectoparasite control. The remaining materials compose the widely available and used pesticides for this mite.

Carbaryl has been used for decades. In the case of technical and commercial materials, resistance in the field to carbaryl is significant. It is uncertain how high mite physiological resistance in the field, to carbaryl or other materials, would need to be before it resulted in obvious product failure. This is particularly true given that producers seldom actually look at birds for mites. Operators of caged layer facilities often base treatments on worker complaints of mites on eggs, and their estimate of control reflects lack of mites on eggs after treatment rather than actual lack of mites on hens (Mullens et al., 2000). However, it is likely that at least the 19% of sites with >100× resistance to commercial carbaryl might show poor control. Further, 41% of sites (technical) and 88% of sites (commercial) showed >10× resistance to carbaryl.

Of mite populations exposed to Ravap, 68% had >10× resistance, and 8% had >100× resistance. This finding is worrisome, because this material has been a favorite of producers for over 20 yr. Because only the commercial

mixture was tested, it is unclear whether the resistance was to tetrachlorvinphos (Rabon), dichlorvos (Vapona), or both.

To our knowledge this is the first documentation of widespread and extreme resistance to permethrin (technical or commercial) in northern fowl mite populations. It is highly probable that the cases of extreme resistance (>1,000×) would result in negligible field control; concentrations were so high that mites surviving in the test pipettes were walking on easily visible permethrin residues. In fact, for practical purposes, permethrin has probably lost effectiveness for mite control in this region. It was notable that many of these producers did not apply permethrin to control northern fowl mites, and the reasons for the extreme permethrin resistance were thus not immediately apparent. However, at least some, and perhaps most, of the producers used permethrin for fly control (*Musca* and *Fannia* spp.). Fly control sprays often are directed toward the inside rafter areas of the poultry houses, and residue can drift onto birds and cage wires. We hypothesize that this application may cause unintended resistance development in northern fowl mites exposed to those permethrin residues. If so, producers should be aware of the potential resistance of mites when fly control sprays are used. Similarly, it is possible that other materials used for fly control, such as the organophosphate insecticide naled (Dibrom) could contribute to unintended nontarget (mite) effects to related materials used for mites. Producers usually had a good concept of when mite treatments had been applied and applied these treatments themselves. Fly sprays, on the other hand, were applied much more frequently and sometimes by outside pest control operators on a regular spray schedule. It is quite possible that the regular but poorly documented (as far as our ability to track them) use of organophosphate and pyrethroid fly sprays helped obscure a relationship between pesticide use and mite resistance levels in the present study.

Toxicologically, it is interesting that there was no relationship between resistance to permethrin and resistance to other materials. Permethrin acts to block sodium ion movement along the axon of the nerve fiber (sodium channel blocker; Ware, 2000). This stimulates repetitive nerve discharges that lead to paralysis and death. Organophosphates (malathion, or the tetrachlorvinphos and dichlorvos in Ravap) and carbamates (carbaryl), on the other hand, act at the synapse. There they inhibit the activity of cholinesterase. Cholinesterase breaks down the neurotransmitter acetylcholine, which carries impulses across the synapse from one nerve cell to another. This activity can take several forms and may be somewhat specific to a particular material, as was perhaps seen in the present study with malathion versus Ravap or carbaryl. Cholinesterase inhibitors also result in continuous nerve discharges leading to paralysis and death, but the mode of action is very different from permethrin. Resistance to Ravap was well correlated with resistance to carbaryl, which may imply a similar resistance mechanism (for example enhanced levels of mixed function oxidases), although that remains to be elucidated. Resistance to malathion was not correlated with resistance to either Ravap or carbaryl, as mentioned above. The malathion resistance levels in general were quite low, and many probably were within the range of experimental error. The extreme resistance to permethrin, and its lack of correlation to resistance to Ravap or carbaryl, may suggest a different resistance mechanism, such as target site insensitivity. This also remains to be determined.

In summary, northern fowl mite resistance in California is very serious. As the number of effective materials declines, and costs of new pesticide registration rise, management of northern fowl mites is likely to become even more difficult. It behooves researchers and the industry to intensify the search for new control materials but also to explore integrated management options rather than relying completely on chemical control. These efforts are currently intensifying in Europe, where resistance of the chicken mite, *Dermanyssus gallinae* (DeGeer), coupled with loss of chemical options and changes in housing and production methods, have forced a resurgence of research on the biology of the pest (Chauve, 1998; Chirico and Tauson, 2002).

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