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ABSTRACT Baseline carbaryl susceptibility and potential resistance mechanisms were identified in U.S. populations of western corn rootworm, *Diabrotica virgifera virgifera* LeConte, using lethal concentration, diagnostic concentration, and synergism bioassays. Twenty-two adult rootworm populations were examined from the states of Indiana, Illinois, Iowa, Kansas, Nebraska, Ohio, Pennsylvania, South Dakota, Texas, and Virginia. Lethal concentration bioassays, conducted within carbaryl-coated glass vials on 12 populations, identified resistance in only 2 Nebraska populations. A diagnostic concentration of carbaryl was subsequently identified which caused 100 and 40% mortality in standard susceptible and known resistant populations, respectively. In diagnostic concentration bioassays on all 22 populations, substantial decreases in mortality were observed only in 3 populations from outside of Nebraska. Synergism bioassays on standard susceptible and resistant Nebraska populations indicated significant reductions in resistance levels with inhibitors of both cytochrome P450 monooxygenases and esterases, suggesting an involvement in resistance by both these classes of detoxification enzymes. Results are discussed with respect to the validation of diagnostic concentration bioassays as a resistance monitoring tool for existing corn rootworm areawide management programs, and the potential roles of cytochromes P450 and esterases in carbaryl resistance.

KEY WORDS *Diabrotica virgifera virgifera*, insecticide resistance, carbaryl, areawide management, cytochrome P450, esterase

THE WESTERN CORN rootworm, *Diabrotica virgifera virgifera* LeConte, is an important pest of field corn, *Zea mays* (L.) (Sutter et al. 1990; Spike and Tollefson 1991; Godfrey et al. 1993a, b). Because of its pest status in continuous corn production, the western corn rootworm has traditionally been treated during its larval stages with soil insecticides (Mayo and Peters 1978) or during adult stages with spray-based contact insecticides which primarily suppress egg-laying and reduce root feeding in the subsequent growing season (Pruess et al. 1974).

Extensive use of soil-applied organochlorine insecticides in the late 1950s and early 1960s led to the development of resistance in the western corn rootworm in Nebraska (Ball and Weekman 1963). Following this development of organochlorine resistance, adult beetles from Nebraska migrated to other areas of the U.S. Corn Belt (Metcalf 1986). In the 1960s-1980s, organophosphate and carbamate insecticides were heavily relied upon for both adult and larval rootworm suppression. Extensive use of the organophosphate methyl parathion and the carbamate carbaryl for adult suppression in Nebraska has since led to the development of resistance (Meinke et al. 1998, Miota et al. 1998) and associated control failures (Wright et al. 1996). To our knowledge, no resistance to these insecticides has been documented in western corn rootworm populations outside of Nebraska.

Areawide management is an approach to agricultural pest management that suppresses a key pest below economic thresholds over large geographical areas (Knipling 1980, Kogan 1995, Calkins et al. 1997). Additional characteristics of areawide management include central coordination, program managers being principal decision makers, and adherance of all participants to specified program guidelines. Areawide management pilot studies for the western corn rootworm are currently in progress in 4 areas of the U.S. Corn Belt (Comis 1997). Bait formulations containing carbaryl as the active ingredient are mainly being used in these pilot studies to suppress adult corn rootworm populations. The bait formulation (SLAM, Microflow, Lakeland, FL) is semiochemical in nature and consists of cucurbitacin-containing buffalo gourd root powder as a movement arrestant-feeding stimulant, carbaryl

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Table 1. Collection sites of western corn rootworm populations in 1997

State	County	Site No.	Mo. collected	Areawide collection[a]
Indiana–Illinois[b]	Benton (IN)	1	Aug.	Treated
	Iroquois (IL)	2	Aug.	Untreated
Iowa	Clinton	1	July	Treated
		2	July	Untreated
Kansas	Finney	1	Aug.	—
	Republic	1	July	Treated
		2	July	Untreated
Nebraska	Cherry	1	Aug.	—
	Clay	1	Aug.	—
	Dixon	1	Aug.	—
	Perkins	1	Aug.	—
		2	Aug.	—
	Phelps	1	July	—
	Saunders	1	July	—
	Scotts Bluff	1	Aug.	—
	York	1	July	—
Ohio	Clark	1	Sept.	—
Pennsylvania	Centre	1	Aug.	—
South Dakota	Brookings	1	Aug.	Treated
		2	Aug.	Untreated
Texas	Swisher	1	July	—
Virginia	Montgomery	1	Aug.	—

[a] Some collections were made from corn rootworm areawide pilot management program sites, which comprised carbaryl-treated and untreated zones. Collections were initiated before carbaryl field applications began.

[b] Collections were made from bordering counties on the Indiana–Illinois state line.

as the toxicant, and binding agents which allow it to adhere to leaf surfaces (Comis 1997). A legitimate concern with this large-scale use of SLAM is the development of resistance to the active ingredient carbaryl.

This research was undertaken as a companion study to existing areawide management pilot studies in Kansas, Iowa, South Dakota, and on the Indiana–Illinois border. Additionally, we examined populations from Nebraska, Kansas, Texas, Ohio, Pennsylvania, and Virginia. The objectives of this investigation were as follows: (1) to examine baseline carbaryl susceptibility in U.S. populations of the western corn rootworm, (2) to determine a diagnostic concentration of carbaryl which could be used to rapidly assess evolving resistance in field-collected populations from areawide management sites, and (3) to identify potential carbaryl resistance mechanisms by conducting synergism bioassays on resistant populations from Nebraska.

Materials and Methods

Rootworm Populations. Adult beetles were collected and used in all studies. Descriptions of collection locations and dates are provided in Table 1. Populations derived from areawide management sites were collected from both treated and untreated (control) areas, providing a mechanism to study the effects of carbaryl treatments in subsequent years. Areawide collections were initiated before insecticide applications were made. All populations except Saunders, York, Phelps, and Clay counties (Nebraska), and Republic County 1 and 2 (Kansas) were collected by cooperators and shipped by overnight express to the University of Nebraska–Lincoln. The Saunders, York, Phelps, and Clay, and Republic county populations were collected by University of Nebraska and Kansas State University technicians, respectively, and directly transported to laboratories at the University of Nebraska–Lincoln. All beetles, regardless of origin, received corn tassels and ears during transport and were maintained in the laboratory in plastic cages at 25–27°C, using a 14:10 (L:D) h photoperiod, on a standard diet of lettuce, ground corn, and bee pollen (supplemented with corn ears), and agar as a water source. All bioassays were conducted within 7 d of beetle collections.

Chemicals. Technical grade carbaryl (99.8% ([AI]) was provided by Rhone-Poulenc (Research Triangle Park, NC). The esterase inhibitor *S,S,S,-tributyl-phosphorotrithioate* (DEF; 96% ([AI]) was purchased from Chem Services (West Chester, PA). The cytochrome P450 monooxygenase inhibitor piperonyl butoxide (PBO; 96% [AI]) was purchased from Crescent Chemical (Hauppage, NY). All insecticide and synergist stock solutions and dilutions were in Baxter Diagnostic S/P acetone (>99.5% purity; Deerfield, IL).

Lethal Concentration and Synergism Bioassays. Carbaryl toxicity in the presence and absence of synergists was assayed by confinement of adult rootworms within 20-ml glass scintillation vials coated with ranges of carbaryl concentrations. At least 4 concentrations that produced between 1 and 100% mortality were used, and 6 replicates were conducted per concentration. Vials were treated with either 500 μl of technical insecticide-in-acetone solutions or 500 μl of acetone for controls, rolled continuously at room temperature until dry within a fume hood (≈0.5 h), and immediately used in bioassays. Mixed-sex beetles were anesthetized on ice, placed in vials (10 per vial), and held for 24 h at 22°C with minimal exposure to light. Mortality was scored as an inability by the beetles to move when prodded.

Synergism experiments were conducted by placing a 0.5-μl droplet of synergist-in-acetone solutions onto the ventral abdomen of adult rootworms 1.5 h before placement in carbaryl-treated vials. Concentrations of the synergists PBO (piperonyl butoxide) and DEF (S,S,S,-tributyl-phos-phorotrithioate) were used that did not produce mortality in susceptible Saunders County beetles at 24 h after treatment (PBO = 2.0 μg per beetle; DEF = 0.2 μg per beetle), and were determined based on preliminary investigations of several synergist concentrations.

Lethal concentration data were analyzed by probit analysis (Finney 1971) as adapted for PC use (LeOra Software 1987). This software also was used to statistically compare slope values of probit mortality curves. Synergism and resistance ratios were calculated using the conventional approach of dividing tolerant by standard susceptible LC_{50} and LC_{90} values. Confidence intervals for resistance and synergist ratios were calculated by the method of Robertson and Preisler (1992) and were used to test the significance of synergist and resistance ratios at the 95% level of confidence. With this test, if the 95% confidence interval

Table 2. **Results of probit analysis of carbaryl toxicity as assessed by vial-lethal concentration bioassays at 24 h, for various U.S. populations of *D. virgifera virgifera***

State	Population	n^a	χ^b	Slope (±SE)[c]	LC_{50} (95% CI)[d]	RR_{50} (95% CI)[e]	LC_{90} (95% CI)[d]	RR_{90} (95% CI)[e]
Nebraska	Saunders Co.	150	1.9	4.1 (0.8)c	1.67 (1.33-2.03)	—	3.42 (2.72-5.07)	—
	York Co.	150	0.8	2.4 (0.4)a	7.50 (5.80-9.76)	4.5 (2.6-7.9)	26.26 (17.75-54.75)	7.7 (3.0-20.5)
	Phelps Co.	210	10.0*	2.2 (0.3)a	9.40 (5.16-18.88)	5.6 (3.2-9.8)	36.94 (18.52-281.73)	10.8 (4.5-26.0)
Iowa	Clinton Co. (#1)	300	1.0	5.2 (0.6)c	1.55 (1.39-1.72)	0.9 (0.6-1.5)	2.72 (2.36-3.31)	0.8 (0.4-1.6)
	Clinton Co. (#2)	360	6.6*	2.5 (0.3)a	2.11 (1.17-3.28)	1.3 (0.8-2.1)	6.86 (4.19-23.27)	2.0 (0.9-4.2)
Kansas	Republic Co. (#1)	420	6.8*	2.3 (0.2)a	1.79 (1.25-2.45)	1.1 (0.7-1.7)	6.58 (4.45-12.92)	1.9 (0.9-3.9)
	Republic Co. (#2)	420	2.7	3.4 (0.3)b	1.82 (1.59-2.07)	1.1 (0.6-1.5)	4.31 (3.61-5.45)	1.3 (0.6-2.2)
Indiana-Illinois	Benton Co. (#1)	300	5.4*	3.4 (0.4)b	1.20 (0.99-1.43)	0.7 (0.4-1.2)	2.89 (2.38-3.65)	0.8 (0.4-1.7)
	Iroquois Co. (#2)	300	2.1	3.3 (0.4)b	1.18 (0.68-1.71)	0.7 (0.4-1.2)	2.92 (1.98-7.92)	0.9 (0.4-1.7)
South Dakota	Brookings Co. (#2)	240	5.5*	4.4 (0.6)c	0.95 (0.52-1.35)	0.6 (0.3-1.1)	1.87 (1.32-5.97)	0.5 (0.3-1.1)
Ohio	Clark Co.	240	2.2	3.2 (0.4)b	1.12 (0.71-1.58)	0.7 (0.4-1.1)	2.81 (1.92-7.38)	0.8 (0.4-1.7)
Texas	Swisher Co.	361	1.5	3.9 (0.4)c	1.76 (1.55-1.99)	1.1 (0.7-1.7)	3.76 (3.19-4.69)	1.1 (0.6-2.2)

[a] The number of insects on which each probit analysis is based.
[b] Chi-square goodness-of-fit statistics as determined using POLO-PC software (LeOra Software 1987). Degrees of freedom ranged from 2 to 4; *, departures from an expected model based on heterogeneity factors > 1.0 (i.e., ratio of χ^2/df > 1.0).
[c] Values followed by the same letter are not significantly different, as determined using POLO-PC software.
[d] Lethal concentrations of carbaryl (in μg/ml) with 95% confidence intervals (CIs) at the 50% (LC_{50}) and 90% (LC_{90}) levels of probit mortality.
[e] Resistance ratios with 95% confidence intervals indicating the fold-difference for each population in comparison with the standard susceptible Saunders Co. (Nebraska) population at LC_{50} and LC_{90}. Confidence intervals which include 1.0 indicate no significant difference from the Saunders Co. population ($P \leq 0.05$; Robertson and Preisler 1992).

calculated for a ratio does not include 1.0, a significant difference exists between the values being compared (Robertson and Preisler 1992, Scharf et al. 1995).

Diagnostic Concentration Bioassays. A concentration of carbaryl 1.2 times larger than the LC_{99} value of a standard susceptible rootworm population (Saunders Co., NE; Meinke et al. 1998) was used as a diagnostic concentration (5.0 μg/ml; 2.5 μg/vial). We used 6–10 replicate vials for each population, each containing 10 unsexed beetles. Insects were anesthetized on ice, placed in vials, and held for 24 h before scoring mortality as described above. Percentage mortality data were arcsine transformed and analyzed by analysis of variance (ANOVA) using a general linear model (PROC GLM, SAS Institute 1990). The Ryan-Einot-Gabriel-Walsh multiple range test (Ryan Q-test; $P \leq 0.05$; SAS Institute 1990) was used to determine if significant differences occurred in percentage mortality among populations.

Results

Lethal Concentration Bioassays of Carbaryl Toxicity. Twelve western corn rootworm populations were tested to estimate carbaryl toxicity and variability in response across large geographic distances (Table 2). Trends were identical at both the LC_{50} and LC_{90} levels of probit mortality, and all populations were statistically identical ($P \leq 0.05$) with the exception of the York and Phelps county populations (Nebraska). Resistance ratios for the York and Phelps populations were 4.5 and 5.6 at LC_{50} and 7.7 and 10.8 at LC_{90}, respectively; however, these lethal concentration estimates were not significantly different ($P \leq 0.05$) at each respective probit mortality level. Significantly smaller slope values of probit mortality lines for the York and Phelps populations indicate that increased heterogeneity is present (ffrench-Constant and Roush 1991). Additionally, 2 populations with nonsignificant elevated tolerance (Iowa, Clinton County, 2; Kansas, Republic County, 1) also showed this smaller slope trend (Table 2).

Diagnostic Concentration Determination and Bioassays. The carbaryl LC_{99} (and 95% CI) for the susceptible Saunders Co. population is 4.26 μg/ml (3.22–12.13). This LC_{99} concentration (4.26 μg/ml) is associated with ≈35–45% mortality in the resistant York and Phelps populations (Fig. 1). Three candidate diagnostic concentrations were subsequently evaluated on these 3 populations (5.0, 10.0, and 20.0 μg/ml; Fig. 2), and mortality in the resistant populations increased significantly ($P \leq 0.05$) in a concentration-

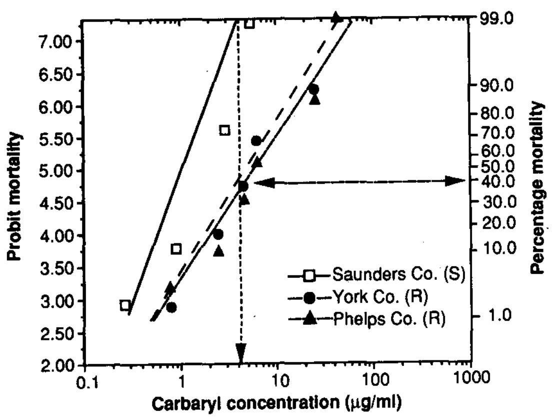

Fig. 1. Probit mortality plots of carbaryl toxicity, as determined by lethal concentration bioassays for the Saunders (susceptible), York and Phelps (resistant) County, NE, populations. Lines represent probit-transformed results of raw mortality data (shown as individual points). The identified concentration of 4.26 μg/ml represents the LC_{99} of the susceptible Saunders population.

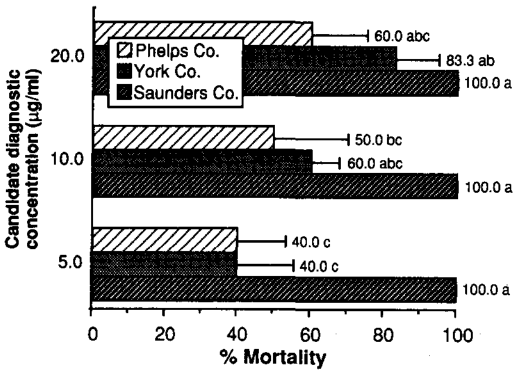

Fig. 2. Mean ± SEM mortality for the Saunders (susceptible), York and Phelps (resistant) County, NE, western corn rootworm populations, as determined using 3 carbaryl concentrations. Means followed by the same letter are not significantly different by the Ryan Q-test (n = 60–100 beetles; $P \leq 0.05$; SAS Institute 1990). For GLM: $F = 7.46$; df = 8,2; $P = 0.0002$.

dependent fashion. To assure that a concentration was selected that would provide 100% mortality in the susceptible Saunders population and significantly less mortality in the resistant populations (see Fig. 2), a value 1.2 times larger than the LC_{99} (5.0 μg/ml; 2.5 μg per vial) was chosen as a final diagnostic concentration.

Twenty-two corn rootworm populations were examined using the diagnostic concentration of 5.0 μg/ml (Fig. 3). As predicted by the probit mortality plot shown in Fig. 1, the Saunders Co. population had 100% mortality, and the York and Phelps populations had 40% mortality at the concentration of 5.0 μg/ml (Fig. 3). The majority (18 of 22) of the populations had average percentage mortalities which were not significantly different from the standard susceptible population (i.e., ≥85%). Only 6 populations had significantly lower mortality than the standard susceptible population (Finney County [Kansas]; and Clay, Cherry, Perkins County, 2; York, and Phelps counties [Nebraska]). It also should be emphasized that no populations which are part of the areawide pilot management program had average mortality <90%.

Synergism of Carbaryl Toxicity. Toxicity of carbaryl to the Saunders (susceptible), York and Phelps (resistant) populations following synergist pretreatment, as assessed by the vial-lethal concentration bioassay, is shown in Table 3. Following pretreatment with either cytochrome P450 monooxygenase or esterase inhibitors (PBO piperonyl butoxide or DEF; S,S,S,-tributyl-phosphorotrithioate, respectively), resistance ratios decreased in comparison with the nonsynergized resistance ratios shown in Table 2. However, neither PBO nor DEF completely lowered toxicity in the resistant populations to the level of the synergized-susceptible Saunders population. Synergist ratios, calculated by comparing LC_{50} values for each population in the presence and absence of either synergist, indicate significant reductions ($P \leq 0.05$) for all synergized LC_{50} estimates except the Saunders population following PBO pretreatment (Fig. 4). Slope values for probit-mortality lines, in comparison with nonsynergized results, indicate differing trends between PBO and DEF (Tables 2 and 3). Following PBO pretreatment, slope did not change significantly in the susceptible Saunders population, but increased significantly for both resistant populations. Alternatively, reductions occurred in slope values following DEF pretreatment for all populations, but were only significant for the Phelps population.

Discussion

General Carbaryl Susceptibility. Based on both lethal (i.e., LC_{50} and LC_{90}) and diagnostic concentration bioassay results, there are no significantly decreased levels of carbaryl susceptibility in any populations from outside of Nebraska with the exception of Finney County, KS. Although not significant, 2 non-Nebraska populations also appear to be segregating for resistance, or at a static level of decreased susceptibility (Kansas, Republic County, 1; and Iowa, Clinton County, 2).

The 2 most resistant Nebraska populations identified in the lethal concentration investigations were not significantly different in terms of their carbaryl resistance levels, contrasting results for methyl parathion (Miota et al. 1998), which showed the Phelps to be 3.3 times more tolerant than the York population in an identical bioassay. It was previously observed that Nebraska rootworm populations had become highly resistant to organochlorine insecticides in the late 1950s and early 1960s (Ball and Weekman 1962). Our own inquiries also indicate a history of substantial methyl parathion use, with lower but nonetheless substantial use of carbaryl across Nebraska as well (Wright et al. 1996). Thus, it remains unclear if previous selection by carbaryl, organochlorine, or other carbamate or organophosphate insecticides are responsible for the resistance observed in the York and Phelps populations.

Significantly smaller slope values obtained for probit-mortality lines suggest that greater genetic variability is present in the resistant York and Phelps populations. Such slope trends are suggestive of potential qualitative adaptations (Robertson and Preisler 1992) in these Nebraska populations, and similar significant trends also were observed in 2 populations from Kansas and Iowa with nonsignificantly elevated levels of tolerance. Although the differences are not as substantial for these Kansas and Iowa populations, the slope values suggest that these 2 populations are not in a static condition and have potential to progress to a less susceptible level with selection. Current levels of tolerance in these Kansas and Iowa populations, however, still do not exceed levels of the resistant Nebraska populations (York and Phelps) following synergist pretreatment.

Diagnostic Concentration Validation. Topical application (lethal dose) bioassays conducted previously (Meinke et al. 1998) produced carbaryl resistance ratios very similar to those identified in the

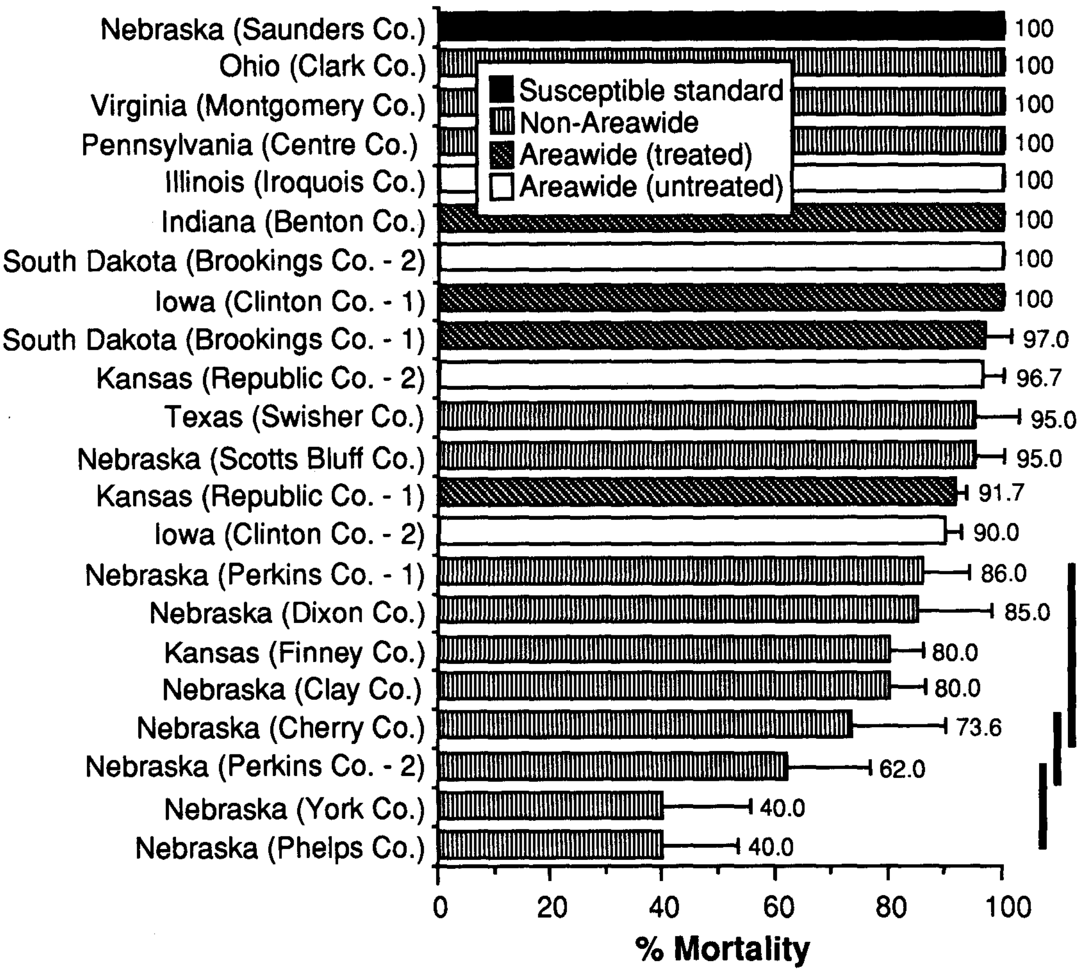

Fig. 3. Mean ± SEM mortality for U.S. populations of western corn rootworm, as determined by diagnostic concentration bioassays using 5.0 μg/ml carbaryl (2.5 μg per glass scintillation vial). Populations followed by the numbers 1 or 2 indicate areawide pilot program-derived populations (see Table 1 for details). Population averages encompassed by the same solid vertical bars are not significantly different by the Ryan Q-test ($n = 60$–100 beetles; $P \leq 0.05$; SAS Institute 1990). For GLM: $F = 17.11$; df = 21,9; $P = 0.01$.

current vial bioassays at 50% probit mortality. These results indicate that the scintillation vial method of exposure provides a similar measure of toxicity to lethal dose bioassays with the advantages of not having to correct for body weight or to dedicate excessive effort to treatment of insects. Slope values obtained using the scintillation vial technique also are larger (i.e., steeper) than those obtained using a topical application procedure, thus providing a greater degree of discrimination between populations (ffrench-Constant and Roush 1991). Furthermore, the scintillation vial format may be suited better for field use by cooperators and/or growers because it minimizes the handling of insecticide solutions and can be prepared at a central location under controlled conditions.

The concentration chosen for use in diagnostic concentration bioassays (5.0 μg/ml; 2.5 μg per vial) is 1.2 times larger than the LC_{99} of the susceptible Saunders Co. population. The Saunders population was collected at a University of Nebraska experimental farm, has a well-documented history of insecticide exposure, has never been associated with control failures, and can be obtained easily in the future as a positive control. The York and Phelps populations were collected from areas of continuous commercial corn production, and also have a history of control failures with both methyl parathion and carbaryl (Wright et al. 1996). Approximately 50% of the York and Phelps lethal concentration probit-mortality curves overlap with the susceptible Saunders population, and similar results were obtained when using the diagnostic concentration technique (susceptible = 100%; resistant = 40.0 ± about 10% mortality).

The more conventional approach of relying upon the upper 95% confidence limit of the LC_{99} (12.13 μg/ml) as a diagnostic concentration may be excessive in this situation. The concentration of 5.0 μg/ml is suited better for the identification of evolving resis-

Table 3. Results of probit analysis of 24-h carbaryl toxicity following synergist pretreatment, as assessed by vial-lethal concentration bioassays, for insecticide susceptible and resistant populations of *D. virgifera virgifera* from Nebraska

Treatment[a]	Population	n^b	χ^{2c}	Slope (±SE)	LC_{50} (95% CI)[d]	RR_{50} (95% CI)[e]	LC_{90} (95% CI)[d]	RR_{90} (95% CI)[e]
Carbaryl + PBO	Saunders (S)	120	2.6	4.0 (1.0)	0.63 (0.43–0.78)	—	1.31 (1.04–2.13)	—
	York (R)	209	3.1*	2.7 (0.3)	2.20 (1.45–3.28)	3.5 (1.9–6.6)	6.50 (4.16–16.22)	5.0 (2.2–11.2)
	Phelps (R)	210	4.7	4.0 (0.9)	3.21 (2.52–4.10)	5.1 (2.7–9.6)	12.47 (8.86–20.81)	9.5 (4.0–22.4)
Carbaryl + DEF	Saunders (S)	150	0.4*	3.0 (0.5)	1.12 (0.58–1.48)	—	3.66 (2.52–10.13)	—
	York (R)	240	6.2	1.7 (0.2)	3.30 (2.44–4.38)	2.9 (1.7–5.0)	19.75 (13.18–36.02)	5.4 (2.7–15.2)
	Phelps (R)	240	3.7	1.4 (0.2)	3.59 (2.56–4.92)	3.2 (1.8–5.4)	28.89 (17.87–60.86)	7.9 (3.7–23.6)

[a] Treatments consisted of carbaryl + the synergist PBO (2.0 μg/insect) or DEF (0.2 μg/insect). Synergists were topically applied to the ventral abdomen 1.5 hr before initiation of 24-hr carbaryl exposure. See Table 2 for nonsynergized (baseline) results.

[b] Number of insects on which each probit analysis is based.

[c] Chi-square goodness-of-fit statistics as determined using POLO-PC software (LeOra Software 1987). Degrees of freedom ranged from 2 to 4; *, departures from an expected model based on heterogeneity factors > 1.0 (i.e., ratio of χ^2/df > 1.0).

[d] Lethal concentrations of carbaryl (in μg/ml) with 95% confidence intervals (CIs) at the 50% (LC_{50}) and 90% (LC_{90}) levels of probit mortality.

[e] Resistance ratios with 95% confidence intervals indicating the fold-difference for each population in comparison with the standard susceptible Saunders Co. (Nebraska) population at LC_{50} and LC_{90}. No confidence intervals include 1.0, indicating significant differences from the Saunders Co. population ($P \leq 0.05$; Robertson and Preisler 1992).

tance as is required by areawide management pilot studies. It is commonly agreed that a diagnostic dose (or concentration) which provides 100% susceptible mortality is most useful as a decision-making tool (ffrench-Constant and Roush 1991). In this regard, the carbaryl concentration of 5.0 μg/ml causes 100% susceptible mortality and provides a significant degree of discrimination between existing resistant and susceptible populations. Furthermore, this concentration has identified slightly tolerant populations which may be segregating for resistance (Kansas, Republic County, 1 [91.7%]; Iowa, Clinton County, 2 [90.0%]; and Nebraska, Perkins County, 1 [86.0%] and Dixon County [85.0%]), which may not have been possible if using the upper 95% confidence limit of the LC_{99}. Future research efforts, however, will be necessary to identify the relationship between percentage mortality in the diagnostic concentration bioassay and level of control in the field, and sample sizes necessary to provide a consistently high degree of statistical confidence.

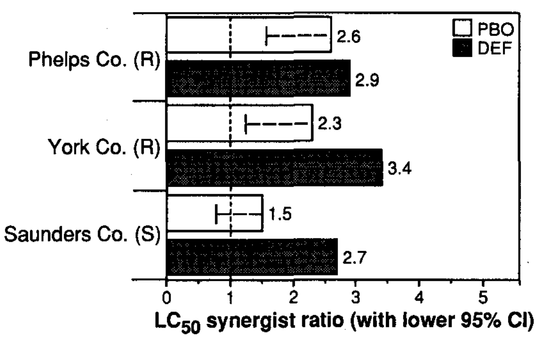

Fig. 4. LC_{50} synergist ratios with lower 95% confidence intervals, calculated in comparison with bioassay results for trials without synergist pretreatment, for the Saunders (susceptible), York and Phelps (resistant) County, NE, populations. The synergists PBO (piperonyl butoxide) or DEF (S,S,S,-tributyl-phosphorotrithioate) were applied to beetles 1.5 h in advance of 24-h carbaryl exposure. Confidence intervals (shown by error bars) which do not span 1.0 indicate significance of synergistic effects ($P \leq 0.05$; Robertson and Preisler 1992).

Potential Carbaryl Resistance Mechanisms. Synergism bioassays using cytochrome P450 monooxygenase (piperonyl butoxide) and esterase (S,S,S,-tributylphosphorotrithioate) inhibitors identified significant reductions in carbaryl resistance levels for both the York and Phelps populations. These results suggest the involvement of both esterases and cytochromes P450 in carbaryl resistance. Similar synergism results also were obtained in studies of methyl parathion toxicity in these populations (Miota et al. 1998). In addition, results of methyl parathion metabolism and in vitro esterase and cytochrome P450 studies were consistent with patterns identified in methyl parathion synergism bioassays for these populations (Miota et al. 1998). Earlier studies have determined that carbaryl is sensitive to esterase-based hydrolysis and cytochrome P450-based oxidation (Matsumura 1985 and references therein); and recently completed investigations of [^{14}C] carbaryl metabolism have confirmed the involvement of both esterases and cytochromes P450 as carbaryl resistance mechanisms in the York and Phelps populations (M.E.S., unpublished data). It also will be of particular interest to determine if these same mechanisms are elevated in more tolerant populations from outside of Nebraska, and/or if they occur following continued carbaryl selection as a result of areawide management efforts.

Implications of Findings to Areawide Rootworm Management. Areawide pilot studies in Kansas, Iowa, South Dakota, and on the Indiana–Illinois border were initiated in the summer of 1997, and will proceed until the summer of 2000. Both a semiochemical-based bait formulation of carbaryl (SLAM) and a spray-based formulation with contact activity (Sevin XLR) are being used for primary suppression of rootworm populations in these areas. A legitimate concern with such large-scale selection over large geographic areas is the rapid development of insecticide resistance. As documented previously (Meinke et al. 1998, Miota et al.

1998), resistance has developed in and around York and Phelps counties, Nebraska, because of the widespread use of methyl parathion, continuous corn production, and potentially other unidentified factors. Only 2 populations currently being studied as part of the corn rootworm areawide management program are showing reduced levels of carbaryl susceptibility (Kansas, Republic County, 1; Iowa, Clinton County, 2); however, these levels are presently not significantly different from the standard susceptible population.

Here, we have reported the results of studies which examined carbaryl susceptibility and potential resistance mechanisms in U.S. populations of the western corn rootworm using lethal concentration, diagnostic concentration, and synergism bioassays. The diagnostic concentration identified here (5.0 μg/ml; 2.5 μg per vial) will be highly valuable as a decision-making tool for existing rootworm areawide management programs. If resistance levels begin to increase in these populations as a result of areawide management efforts, this assay technique will allow its detection and implementation of resistance management strategies. Continuing research, however, will be necessary to identify the correlation between mortality in diagnostic concentration bioassays and level of control in the field, mechanisms of carbaryl resistance, and viable insecticide alternatives to carbaryl.

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