Classifying Resistance Severity in Field Populations: Sampling Inspection Plans for an Insecticide Resistance Monitoring Program

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ABSTRACT Detecting insecticide resistance before it increases above a critical level has been a driving force in developing tests to estimate resistance severity in field populations. Here, estimates of resistance severity (r) are classified into one of three categories. The classification is based on comparing r with two preselected levels of resistance severity (θ) to obtain a three decision sampling plan. Using the example of fenvalerate resistance in beet armyworm, *Spodoptera exigua* (Hübner), each θ was selected by comparing resistance test responses of populations that differed in resistance and exposure to insecticides. Test response was corrected for survival of moths after exposure to a 1,000 μg/g concentration of fenvalerate. This concentration was selected by comparing probit lines of susceptible and fenvalerate-selected strains. The determination that r was greater or less than each θ was made using sampling inspection plans based on the binomial distribution. A sequential sampling plan using the sequential probability ratio test (SPRT) required less sampling effort than single sampling plans of equal strength and was suited to our operational needs. Using the same susceptibility test, sequential analysis of survival proportions from the 1,000 μg/g concentration was also compared with probit analysis of survival proportions from multiple concentrations. Sampling effort required for the SPRT was substantially lower than that required for probit analysis. Despite differences in probit analysis and SPRT assumptions, probit estimates of resistance severity at 1,000 μg/g agreed with sequential classification of r using a 1,000 μg/g concentration.

KEY WORDS Insecta, sequential probability ratio test, resistance detection, sampling inspection

DETECTING INSECTICIDE RESISTANCE before it increases above a critical level has been a driving force in developing tests to estimate resistance severity in field populations. Susceptibility tests that consist of toxicant-impregnated or -coated surfaces have been used to detect resistance (e.g., Dennehy et al. 1987, Brewer & Trumble 1989, Plapp et al. 1990). In such tests, an estimate of resistance severity is expressed as a proportion of insects surviving single or multiple test concentrations. These tests assume that the survival rates of resistant and susceptible phenotypes differ at these concentrations. The specialized case of interpreting resistance severity as the frequency of a resistance allele may be possible if responses of isolated genotypes are known. Once an estimate of resistance severity is obtained, it can be compared with a preselected resistance severity. This value may serve as a threshold to implement or revise resistance management strategies.

Criteria for determining the number of individuals to sample to provide meaningful comparisons of estimates of resistance severity (r) with preselected resistance severity (θ) have been explored. Roush & Miller (1986) provided sample sizes required for detecting at least one resistant individual in populations differing in resistance frequencies when a single test concentration was used. Sampling a large number of insects was necessary to detect a chosen low resistance frequency defined here as θ (e.g., when $\theta = 0.01$, a random sample of 298 individuals was needed to detect with 95% probability at least one resistant individual). Mathematical modeling of resistance evolution has lent support to using a low θ when selected as a frequency of a resistant allele. For arguably common biological, operational, and genetic conditions, resistance can spread rapidly in a population under selection pressure (Georghiou & Taylor 1977a,b). Higher values of θ may be appropriate if resistance regresses rapidly when selection pressure is removed. Dennehy et al. (1987), using a toxicant-coated surface test, selected θ of 0.10 based on empirical data of spider mite (*Tetranychus* spp.) resistance to dicofol. Roush & Miller (1986) showed that sampling effort was much reduced at such higher frequencies. Other factors (e.g., immigration of susceptibles, overlap of the distributions of test responses of resistant and susceptible phenotypes) may also support selection of higher values of θ. Thus, selection of θ has great effect on sampling effort, yet must be based on the potential for resistance to evolve, the stability of resistance, and phenotypic responses to the toxicant.

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Unfortunately, the need to make decisions on whether to implement or revise resistance management strategies usually precedes understanding of genetic and nongenetic factors that influence resistance severity in a population. If effects of these factors on resistance risk are not understood, choosing θ with any specific well-defined meaning becomes difficult. As an empirical approach for choosing values of θ, Denholm et al. (1984) suggested comparing resistance detection test responses from poorly and well-controlled populations. Using this approach, values of θ may be selected by inspection of the variation of test responses and the associated pattern of control.

Once θ is selected, sampling inspection plans (Guenther 1977) provide efficient testing procedures for resistance monitoring when the intention of the program is to classify r into one of two categories separated by θ. Such plans extend basic sampling concerns of resistance monitoring that consider Type I error (Roush & Miller 1986) to obtain decision plans that also consider Type II error. Sampling inspection plans have been used extensively in quality control of industrial processes (Wadsworth et al. 1986). Beyond use of sampling plans with fixed sample size, sequential sampling plans based on Wald's (1947) sequential probability ratio test (SPRT) have been used in insect pest management to aid in monitoring insect populations and damage (Pieters 1978). Resistance severity can be classified using a fixed sample size or via sequential inspection of insects exposed to a test concentration assuming a binomial distribution of susceptible and resistant insects. Two sampling plans based on two values of θ may be operated simultaneously to classify resistance into one of three categories. A SPRT tends to reduce the average number of samples needed to make a classification compared with single or other sampling plans of equal strength, but increases administrative costs of the sampling program (Hald 1981, Wadsworth et al. 1986). Thus, operational conditions of specific resistance monitoring programs as well as sample size needs will govern the selection of a particular sampling plan.

Probit analysis has also been used in resistance monitoring (Brent 1986). Probit analysis estimates a concentration-mortality line by testing survival at multiple concentrations and fitting the data to the probit model. Roush & Miller (1986) have compared several aspects of efficiency of probit analysis with analysis of survival data from a single test concentration. The objectives of this study are to show that for our resistance monitoring program, resistance severity can be measured using a single test concentration bioassay, sampling inspection is an efficient approach to classify resistance severity using such a test, a SPRT is the most appropriate sampling plan, and two SPRTs operating simultaneously are a favorable alternative to probit analysis.

Materials and Methods

Resistance Data. Beet armyworm, *Spodoptera exigua* (Hübner), resistance to fenvalerate (E. I. du Pont de Nemours & Company, Wilmington, Del.) was evaluated using a susceptibility test that exposed insects caught in pheromone traps to insecticide-impregnated adhesive. Resistance to fenvalerate was monitored from 1987 to 1990 in field populations. Data were obtained from 21 sites in nine geographic regions bounded by Monterey County, Calif., in the north and the Guasave and Del Fuerte valleys of Sinaloa (Mexico) in the south. Multiple concentrations in the susceptibility test were used until differences in susceptibility of inbred lines of resistant and susceptible insects were documented. The procedures for preparing insecticide concentrations (μg [AI]/g of adhesive), trapping insects in pheromone traps, and collecting mortality data are documented elsewhere (Brewer et al. 1990). Briefly, serial dilutions of fenvalerate (94% purity) in 9:1 hexane/ethanol were mixed separately into insect adhesive (Tanglefoot Company, Grand Rapids, Mich.). Addition of 1 ml of the appropriate dilutions per 100 g of adhesive yielded concentrations of 0, 10, 40, 80, 160, 320, 500, 1,000, and 4,000 μg/g. Concentrations of 8,000 and 16,000 μg/g were made by adding the insecticide directly to the adhesive and heating the mixture at 37°C for 10 min. Treated adhesive (5.5 g) was spread evenly onto pressed cardboard inserts (325 cm^2) coated with wax and cut to fit into the base of a pheromone trap (Pherocon 1C, Trece, Salinas, Calif.). Seven to nine concentrations including an insecticide-free control were used in all tests. The traps were baited with beet armyworm pheromone lure (Trece, Salinas, Calif.), placed in the field in the late afternoon, and retrieved the next morning before the temperature exceeded 21°C. Inserts were removed from the traps and held at 21 ± 2°C and ≥95% RH. Mortality data were collected 38–40 h following sunset of the day the traps were placed in the field. Therefore, moths were inspected between 0800 and 1100 hours (PST) the day after trap inserts were removed from the field. A moth was considered dead if no movement of wings, legs, or head occurred after one wing was lifted from the adhesive. At least five concentrations resulted in mortality between 2 and 98%; an average of 442 insects were examined in each of 33 tests. Two to five tests were done in each region. Probit analysis (POLO-PC; LeOra Software 1987) was used to calculate probit regression statistics when multiple concentrations were used in the susceptibility test. The χ^2 statistic was used to test the hypothesis that the probit model adequately described the data. Detailed results from the 1987 to 1989 analyses have been reported (Brewer et al. 1990).

The regions represented a wide diversity of insecticide use (Brewer et al. 1990) and well- and

poorly controlled beet armyworm populations. Beet armyworm populations were broadly classified as well- or poorly controlled according to information provided by pest control advisors and without reference to a specific insecticide application. For all cases of poor control and most other cases, fenvalerate, permethrin, or both of these pyrethroids were components of the pest management strategy to control beet armyworm. Other insecticides were used including methomyl and the microbial *Bacillus thuringiensis* var. *kurstaki* (Berliner). The relative use rate of each of these materials has been reported (Brewer et al. 1990). This monitoring study was intended to provide empirical data on the diversity of resistance severity in field populations.

Selecting a Test Concentration and θ. The necessary criteria for using the survival proportion at a single test concentration as an estimate of resistance severity was considered by determining if θ was a function of the frequencies of resistant and susceptible phenotypes in the population, θ could be measured as the proportion of insects surviving exposure to a test concentration, and θ was related to beet armyworm control using pyrethroids in the field. Results from selection experiments were used to determine if one concentration could distinguish susceptible and resistant phenotypes (LC_{50} values from this experiment were reported by Brewer et al. 1990). Briefly, a laboratory strain was established from a field population that was known to be resistant to fenvalerate. Beginning at the F_1 generation, the strain was selected for 19 generations by topically applying fenvalerate to third instars. Mortality ranged from 45 to 85%. Selections were not done at generations 6, 11, 12, 15, and 16. A substrain partitioned from the original strain was not exposed to selection pressure. A laboratory (CA) strain was used as a susceptible control; it had been maintained continuously without insecticide pressure since establishment from collections made in Orange County, Calif., in 1982. The susceptibility of this colony corresponded closely to that of a 25-yr-old laboratory colony from the Western Cotton Research Laboratory (USDA–ARS, Phoenix, Ariz.). The susceptibility test described above was used with multiple concentrations to track resistance development in the adult stage at generations 1, 6, 11, and 20. Probit regression lines of the fenvalerate-selected and CA strains were compared at generation 20.

One of the concentrations previously used in the monitoring study was selected as the test concentration that best distinguished susceptible and resistant phenotypes (see *Results and Discussion*). A survival proportion at this concentration was chosen as a measure of resistance severity. Using data from the field study, the survival proportions of insects exposed to this concentration were correlated with history of beet armyworm control in the field. Two values of θ were selected from these data for use in developing sampling plans.

Sampling Inspection Plans Using the SPRT. Sampling inspection plans are applicable to a beet armyworm resistance monitoring program if the form of the distribution of resistant and susceptible individuals in the population is known, the unknown parameter of the distribution (θ) is estimable (r), and decision levels of the unknown parameter are selected. If two values of θ are selected, r is classified into one of three resistance severity categories by operating two sampling plans simultaneously. The hypothesis testing approach of sampling inspection assumes little practical consequence of making an incorrect decision as r approaches θ; therefore, upper (θ_1) and lower (θ_0) decision thresholds for each θ may be used where $\theta_1 \geq \theta > \theta_0$, or $\theta_1 > \theta \geq \theta_0$. The strength of a test is governed by selection of θ_1, θ_0, and Type I (α: the probability that action is taken when it is not warranted) and Type II (β: the probability that no action is taken when it is warranted) errors for each θ. A sampling plan can be denoted symbolically as a plan of strength (θ_0, α, θ_1, β).

A sequential sampling plan requires insects to be inspected one at a time or in groups. The results after each inspection determine whether a terminating decision is made or whether more sampling is necessary. A SPRT developed by Wald (1947) meets these criteria. Using a SPRT of strength (θ_0, α, θ_1, β) in our resistance monitoring program, the decision criteria are such that

$$\text{if } S_i \geq h_1 + bn_i \text{ then stop sampling and reject } r = \theta_0 \text{ (accept } r \geq \theta_1), \quad (1)$$

$$\text{if } S_i \leq h_0 + bn_i \text{ then stop sampling and accept } r \leq \theta_0, \quad (2)$$

$$\text{and if } h_0 + bn_i < S_i < h_1 + bn_i \text{ then continue sampling,} \quad (3)$$

where S_i is the corrected sum of insects surviving exposure to the test concentration for the ith group of insects inspected and n_i is the number of insects inspected through the ith group. The constants h_1, h_0, and b are based on the binomial distribution as defined by equations 5:14, 5:13, and 5:15, respectively from Wald (1947) and are unique to a test of specified strength (Wald's formulas are equivalent to those given in a review of sequential sampling applied to entomology once the following corrections to Table 2 [Fowler & Lynch 1987] are made: interchange h_1 and h_2 in the heading and take the negative of the value s for the binomial case). Operating two sampling plans simultaneously, a stop sampling decision must be made for both sets of decision lines before sampling is terminated.

Although a SPRT is based on taking single observations sequentially, a SPRT can be modified by sampling in groups. Group sampling has minimal effect on the protection against wrong decisions but increases the average number of observations required to reach a decision as group size increases (Wald 1947). In a second modification, a

definite upper bound for each pair of decision lines can be chosen to ensure a termination of the plan at a reasonable sample size. Wald (1947) proposed that this boundary be selected at 3 times the expected value of n at $\theta = b\,[3E_b(n)]$ where b is the common slope of the decision lines of each SPRT. The distribution of n across all values of θ given by the average sample number curve (based on single observations) can be used as an approximate distribution of n_i (based on grouped observations of 10 insects). Therefore, when $n_i \geq 3E_b(n)$ the decision criteria for each value of θ are truncated such that if $S_i \geq (h_1 + h_0)/2 + bn_i$ then accept $r \geq \theta_1$ and if $S_i < (h_1 + h_0)/2 + bn_i$ then accept $r \leq \theta_0$. A SPRT in which sampling is done in groups and the test is truncated at an upper boundary is also known as a multiple sampling plan (Wadsworth et al. 1986).

Application to the Susceptibility Test. The susceptibility test, using only a single test concentration and an insecticide-free concentration, was used in a sampling plan to classify r with respect to θ_1 and θ_0 for each preselected value of θ. We set θ_0 at $0.8 \times \theta$ and β at 0.05 because the consequences of accepting $r \leq \theta_0$, when the true value was greater than θ_1, were severe for the decision process presented here. This incorrect decision resulted in classifying resistance as lower than it actually was and possibly missing an opportunity to revise insecticide use strategies when needed. We selected θ_1 at each value of θ and α to 0.10 because the consequences of accepting $r \geq \theta_1$, when the true value was less than θ_0, were not as severe. This incorrect decision results in classifying resistance as higher than it actually was and prematurely implementing a change in strategy for managing resistance.

Expressed as a corrected proportion of insects surviving a test concentration, r was obtained by recording survival of insects caught in the pheromone traps. For sequential sampling, inspection was done in groups of 10 insects. Survival was corrected for mortality in an insecticide-free concentration such that

$$S_i = \sum_{k=1}^{i} c_k = 100 \sum_{k=1}^{i} s_k/x, \quad i = 1, 2, \ldots, \quad (4)$$

where $\sum_{k=1}^{i} c_k$ and $\sum_{k=1}^{i} s_k$ were the corrected and uncorrected sum of insects surviving exposure to the test concentration through the ith group of 10 insects, respectively, and x was the constant percent survival of insects in the insecticide-free concentration. This formula was an adaptation of Abbott's (1925) formula for the sequential process (*Appendix*). For comparison, in single sampling the number of survivors would be corrected for mortality in an insecticide-free concentration by applying equation 4, disregarding the group sampling notation, after inspection of the fixed sample size. Therefore, sequential sampling would incur some additional administrative costs because of the greater complexity of the procedure. The corrected sum was used instead of r_i to avoid the unnecessary calculation $r_i = S_i \div n_i$.

Choosing a Sampling Plan. Choice of a sampling plan was based on comparison of the statistical behavior of sampling plans and the operational needs of the resistance monitoring program described here. The statistical behavior of sampling inspection plans across all possible values of θ, $0 \leq \theta \leq 1$, were described by the operating characteristic (OC) and average sample number (ASN) curves (Guenther 1977, Hald 1981). The OC curve is a plot of the probability of accepting the null hypothesis, $r \leq \theta_0$, given θ. The ASN curve is a plot of the average number of observations needed to make a terminating decision given θ. The OC and ASN curves for the SPRT were approximated using equations 5:19, 5:20, and 5:23 from Wald (1947).

For comparison to the SPRT, single sampling plans of equal strength and their OC and ASN curves were developed using standard procedures (Guenther 1977, Chapter 1). The minimal values of the sample size n and the rejection number d were chosen to satisfy the conditions of the test (θ_0, α, θ_1, and β). Burstein's (1971) tables were used to obtain initial approximations of n and d. Beginning with these initial approximations, more precise values were found iteratively using the SAS function for the binomial distribution (SAS Institute 1985). The decision criteria of a single sampling plan were: if $S \geq d$ then stop sampling and accept $r \geq \theta_1$ and if $S < d$ stop sampling and accept $r \leq \theta_0$, where S was the corrected number of survivors after inspection of sampling size n. If during a single sampling inspection process done in groups as in the SPRT, $S_i \geq d$ or $n_i - S_i > n - d$ for the ith group, a decision could be made ($r \geq \theta_1$ and $r \leq \theta_0$, respectively). This curtailment of the sampling plan would not change the power of the test because the OC curves of single and fully curtailed single sampling plans are the same (Guenther 1977). This curtailed plan allowed comparison with a SPRT where in both tests sample size (n_i at the termination of the test) was a random variable. The OC curve for the constants n and d was determined (Guenther 1977) by implementing the SAS function for the binomial distribution (SAS Institute 1985) to find the OC values across θ in steps of 0.01. The procedure was truncated when the SAS function returned a probability of 0 or 1. The ASN curves of the fully curtailed sampling plans were approximated using equation 1.13 from Guenther (1977) and the SAS function for the binomial distribution to find the ASN values across θ in steps of 0.05 or smaller. The ASN for single sampling plans without curtailment was not applicable; sample size was fixed at n. Partly because of the effect of group sampling, estimates of the OC and ASN functions could be calculated with greater precision using Monte Carlo simulations (Fowler & Lynch 1987). The group sampling effect was not considered substantial for comparison of sequen-

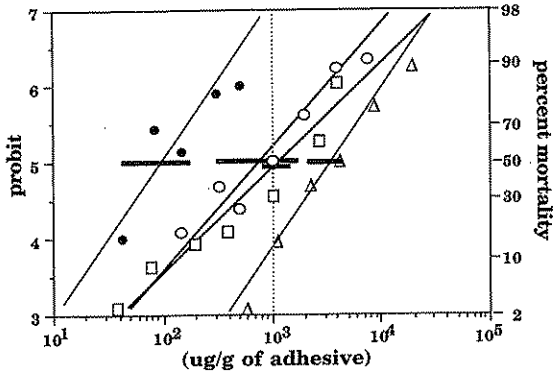

Fig. 1. Probit regression lines calculated from results of adult susceptibility tests. A susceptible strain (CA) was compared with the 20th generation of a fenvalerate-selected strain (Fen-R20) and an unselected strain (Fen-C20) originating from a field population (Fen-field). Horizontal bars are the 95% fiducial limits at the LC_{50}. The dotted vertical line is set at 1,000 μg/g. •, CA; △, Fen-R20; ○, Fen-C20; □, Fen-field.

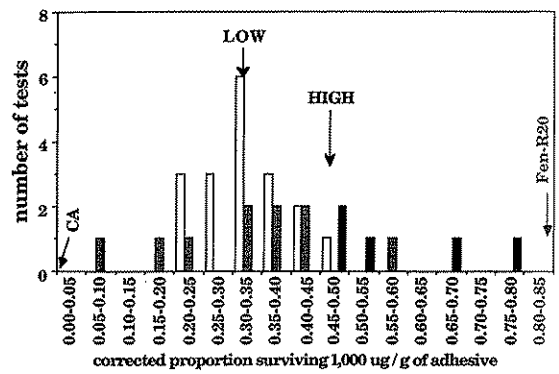

Fig. 2. Frequency histogram of corrected proportion of insects surviving exposure to 1,000 μg/g of adhesive calculated from 33 adult susceptibility tests done in nine regions, two to five tests per region. Beet armyworm populations were either well-controlled using one insecticide per application (unfilled bars), well-controlled using mixtures (checkered bars), or poorly-controlled using solid bars). Proportions selected as a threshold for high potential for control failure (HIGH) and a lower resistance severity (LOW) were used to classify resistance. The survival of the susceptible strain (CA) and fenvalerate-selected strain (Fen-R20) are shown for reference.

tial, single, and curtailed single sampling plans because the differences across most values of θ were greater than a group size of 10.

The operational needs of primary concern were the trapping procedure and the incubation time of the susceptibility test. They were the major time-consuming components of the resistance monitoring program.

Comparison of Sampling Inspection and Probit Analysis. Classification of a single proportion (using one concentration) and probit estimation of a concentration-mortality line (using multiple concentrations) could not be compared with ASN and OC curves because the basic assumptions and construction of the tests differ. The support for attempting such a comparison at all is the desire to compare a new methodology (the SPRT) with a standard (probit analysis) in common use (Brent 1986). Adequacy of a sampling plan using a SPRT and probit analysis to monitor for beet armyworm resistance was evaluated empirically. Specifically, sequential classification of r at the selected test concentration was compared with probit estimation of r at the same concentration using results from a field study. Beyond estimating r, we assumed that estimation of a concentration–mortality line was not an essential need of resistance monitoring (Roush & Miller 1986).

Sites in Monterey County, Calif., and the state of Sinaloa, Mexico, were monitored for beet armyworm resistance to fenvalerate. Two to four traps per concentration were used in the field. The test concentrations were 500, 1,000, 2,000, 4,000, and 16,000 μg/g. One of these concentrations was the concentration selected for the SPRT. The susceptibility test procedures were the same as used in the previous monitoring study. Fresh traps were placed in the field in subsequent days as needed to fulfill the sample size requirements of the SPRT and probit analysis. For probit analysis, approximately 60 insects per concentration were inspected, resulting in sample size (n) between 290 and 300 insects. This sampling criterion was expected to yield highly reliable LC_{50} values (Robertson et al. 1984); thus, sample size needs for probit analysis were considered fixed in our study. Using the SPRT, insects from the traps with the appropriate test concentration were inspected sequentially in groups of 10 until a terminating decision was made. Data from two to four insecticide-free traps were used to correct mortality for both analyses.

Results and Discussion

Selecting a Test Concentration and θ. In the laboratory selection experiment, the LC_{50} increased 3-fold in the fenvalerate-selected strain by the 20th generation and decreased in the unselected strain (Fig. 1). Resistance in the unselected strain was significantly lower than the selected strain at generations 11 and 20 (based on nonoverlap of 95% fiducial limits of the LC_{50} values; Fig. 1, Brewer et al. 1990). Resistance of the selected strain stabilized from generations 11 to 20 (Brewer et al. 1990). Therefore, the fenvalerate-selected strain at generation 20 was compared with the CA strain to determine which concentration best distinguished these susceptible and resistant phenotypes. A single test concentration of 1,000 μg/g of adhesive was selected (Fig. 1). The 1,000 μg concentration was estimated to kill >98% of the CA strain and 16% of the fenvalerate-selected strain. No insects tested

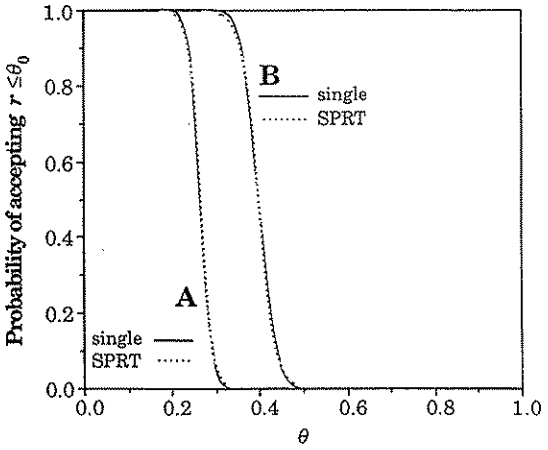

Fig. 3. Comparison of operating characteristic curves for single (with and without curtailment) sampling plans and SPRTs for tests of strength A (0.24, 0.10, 0.30, 0.05) and B (0.36, 0.10, 0.45, 0.05).

from the CA strain survived exposure to this concentration ($n = 55$). Results from this selection experiment validated the assumption that θ was a function of the frequencies of resistant and susceptible pheonotypes and could be estimated as a proportion of insects surviving exposure to a test concentration.

From results of the field study, two values of θ were selected based on the corrected proportion of insects surviving exposure to the 1,000 μg concentration. Survival was greatest in populations classified as poorly controlled, compared with all but one other population (Fig. 2). The poorly controlled populations were from sites in Sinaloa, Mexico. Pyrethroid use averaged 3.4 fenvalerate applications and 11.6 permethrin applications per tomato crop, three crops per year. Pyrethroid selection pressure for the populations classified as well controlled ranged from zero to five applications per year. Across all regions monitored, increasing LC_{50} values corresponded to increasing insecticide use (Brewer et al. 1990). This correspondence supported the assumption that θ was related to beet armyworm control using pyrethroids in the field. A proportion of 0.45 (corrected survival) was selected as a threshold of high potential for control failure. A proportion of 0.30 was selected for the lower θ to allow classification of resistance severity into one of three categories (Fig. 2).

The low θ was not necessarily intended to detect a low allelic frequency. The proportion of insects surviving this concentration could not be converted to a resistance allele frequency. The proportion probably overestimated resistance frequency because response of the heterozygote population was expected to be within the range of responses of the CA and fenvalerate-resistant strains. The heterozygote response for larvae was intermediate to semidominant for the resistant trait (M.J.B., unpublished data). The proportion could be converted into frequency of a resistance allele if the heterozygote response was known, the number of genes responsible for the trait was known, a concentration was known that completely discriminated one of the genotypes, and genotypic frequencies were in Hardy-Weinberg equilibrium.

Choosing a Sampling Plan. Using the 1,000 μg concentration, θ_0 and θ_1 were assigned for each value of θ and decision rules were constructed (Table 1). Differences in the OC curves for sampling plans of equal strength were slight (Fig. 3). Hald (1981) noted that differences in OC curves of sampling inspection plans of the same strength are

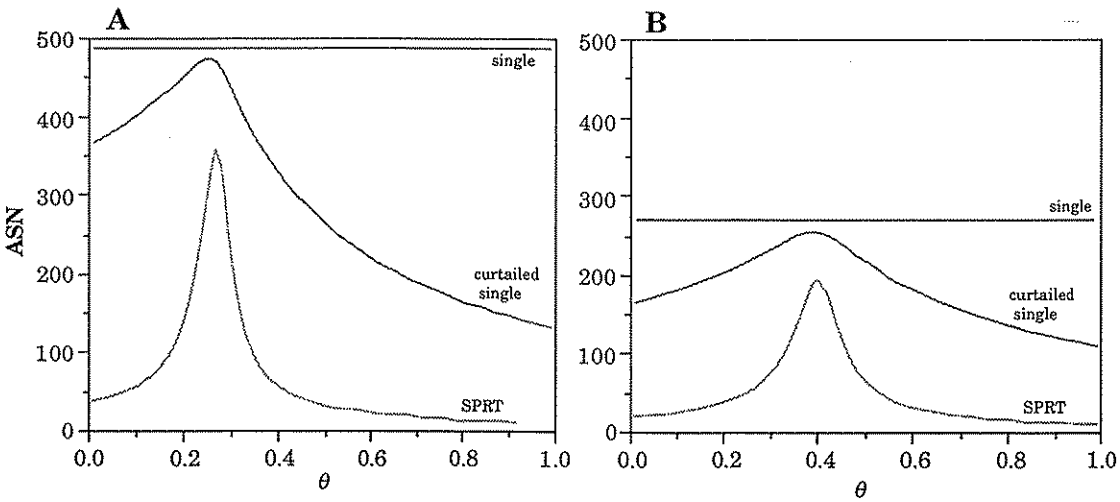

Fig. 4. Comparison of average sample number (ASN) curves for fully curtailed single sampling plans, and SPRTs for tests of strength A (0.24, 0.10, 0.30, 0.05) and B (0.36, 0.10, 0.45, 0.05). Fixed sample sizes of single sample plans of equal strength are plotted as horizontal lines.

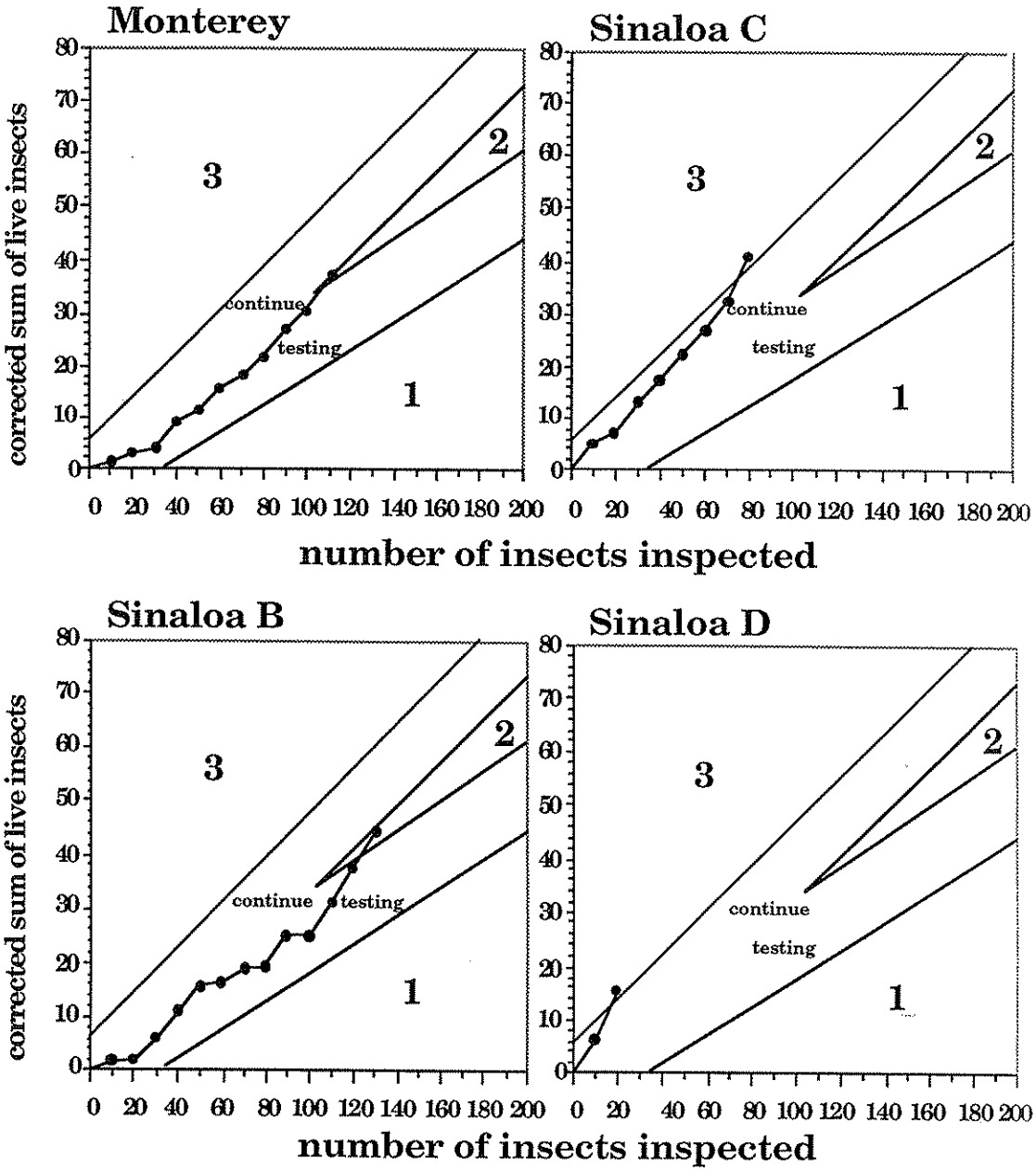

Fig. 5. SPRT graphs each showing two SPRTs operating simultaneously. The corrected cumulative sum of insects surviving the 1,000 μg concentration versus the number of insects inspected in groups of ten is plotted until one of three decisions is made: $r \leq 0.24$ (region 1), $0.30 \leq r \leq 0.36$ (region 2), or $r \geq 0.45$ (region 3). Tests were done at one site in Monterey County (Monterey) and three sites in Sinaloa, Mexico (Sinaloa B, C, D).

unimportant for most practical purposes. Comparing ASN values, decisions using the SPRTs required less sampling effort on average across all values of θ among the plans constructed (Fig. 4). In general, the SPRT leads to the smallest possible ASN at θ_0 and θ_1 among all sampling inspection plans of equal strength and reasonably small ASN at other values of θ (Hald 1981).

The operational needs of our resistance monitoring program were also relevant in choosing a plan. An unknown number of insects were lured into the pheromone traps. If enough insects were not caught to satisfy a fixed sample size requirement, more traps would have to be placed in the field before a decision could be made. With sequential sampling plans, a decision could be made

Table 1. Decision rules for single sampling plans, curtailed single sampling plans, and SPRTs for tests of equal strength (θ_0, α, θ_1, β)

(θ_0, α, θ_1, β)	Single		Curtailed single		SPRT	
	n^a	L_1	L_0	L_1	L_0	L_1	L_0
(0.24, 0.10, 0.30, 0.05)	491	$S \geq 131$	$S < 131$	$S_i \geq 131$	$n_i - S_i > 360$	$S_i \geqslant 7.37 + 0.27n_i$	$S_i \leqslant -9.46 + 0.27n_i$
(0.36, 0.10, 0.45, 0.05)	269	$S \geq 107$	$S < 107$	$S_i \geq 108$	$n_i - S_i > 161$	$S_i \geqslant 6.01 + 0.40n_i$	$S_i \leqslant -7.71 + 0.40n_i$

L_1, upper classification ($r \geq \theta_1$); L_0, lower classification ($r \leq \theta_0$).
[a] n is the fixed sample size for single sampling plans, it is also the maximum sample size for curtailed single sampling plans.

at any time during the inspection process and, on average, before reaching the fixed sample size (Fig. 4).

Sampling in small groups saved some of the additional administrative costs in data manipulation when sequentially sampling. For low or high values of θ, group sampling can have a noticeable effect on the sample size needed to terminate a test, but the increased efficiency in taking observations in groups was expected to offset this cost and rarely require additional trapping effort. Operationally, if an expected trap count was known, the average number of traps needed for a test could be estimated by calculating the maximum ASN of the two SPRTs operating simultaneously. For each plan, these values approximately occur at $\theta = b$ and can be easily calculated using equation 5:30 from Wald (1947). Additional trapping should rarely be necessary using the SPRT because the ASN across most values of θ was small relative to the maximum ASN. This attribute further increased the value of using the SPRT compared with the single sampling plans. If an additional night of collection is needed, additional insecticide-free traps also should be used to estimate a new control mortality.

Comparison of Sampling Inspection and Probit Analysis. The SPRT identified two regions (Sinaloa C and D, Fig. 5) where resistance to fenvalerate was known to occur (Brewer et al. 1990). For the probit procedure, exposure to five test concentrations resulted in mortality between 27 and 97% in four tests. With n between 290 and 300 insects per test, heterogeneity was high in two of four analyses (Table 2). This result occurred despite sample sizes within the range expected to yield reliable LC_{50} values from homogeneous populations (Robertson et al. 1984). Sampling from a mixed population of resistant and susceptible insects would explain the high heterogeneity. The probit model would be expected to decrease in adequacy for detecting resistance in mixed populations as the separation of concentration-mortality lines of susceptible and resistant phenotypes increased (Tsukamoto 1963). For the fenvalerate resistant trait, the probit model may have been useful in documenting resistance despite cases of high heterogeneity (Brewer et al. 1990) because the test responses of susceptible and resistant populations overlapped (Fig. 1). Therefore we assumed that probit analysis provided an adequate measure of resistance severity.

Sampling effort was substantially lower when using the SPRT than when using probit analysis. For probit analysis, sample sizes of approximately 300 insects (approximately 60 insects per concentration) were used. Using the 1,000 μg concentration, an average of 85 insects ($n = 4$ tests) were needed to terminate the SPRT (Fig. 3; Table 3).

Because probit analysis provided estimates of survival proportions across a concentration-mortality line, the SPRT classifications were compared with the corresponding probit estimate of survival at the 1,000 μg concentration. The analyses agreed

Table 2. Probit analysis of data from adult susceptibility tests using five test concentrations of fenvalerate, inspecting approximately 60 insects per concentration

Region Site	Date	% Control mortality	n	Slope ± SE	LC_{50} (90% FL)[a]
Monterey					
C	Nov. 89	4.0	290	1.31 ± 0.22	483[b]
Sinaloa					
B	Mar. 90	0.0	300	0.81 ± 0.17	328[b]
C	Mar. 90	0.0	300	1.39 ± 0.19	991 (747–1,247)
D	Mar. 90	0.0	295	1.21 ± 0.16	1,964 (1,203–3,173)

Beet armyworm sampled at Monterey County, Calif., and the Guasave and Del Fuerte Valleys of Sinaloa, Mexico. Sites were the same monitored in Brewer et al. (1990).

[a] Estimates given in μg/g of adhesive followed by 90% FL.

[b] Fiducial limits not computed because of high heterogeneity (LeOra Software 1987). The hypothesis that the probit model adequately described the data was rejected ($\chi^2 \geq 7.81$, df = 3, $P \leq 0.05$).

(Table 3). Although the main objective of the sampling plan was to make a decision, calculating r using data collected from the SPRTs after termination of the sampling plan yielded a good point estimate of θ although slightly biased (Phatak & Bhatt 1967, Guenther 1977). These estimates derived from the SPRT were near the probit estimates (Table 3). These data suggested that results from the two analyses did not differ to any substantial degree. The classification of resistance severity using one test concentration and an insecticide-free control in a SPRT appeared to be a favorable alternative to probit analysis because of reduced sample size needs as well as operational savings (fewer insecticide concentrations to prepare). Additional advantages of using one test concentration may also be pertinent (Roush & Miller 1986), especially when probit lines of susceptible and resistant phenotypes are widely separated. We assumed both tests were applicable for monitoring resistance in a population of unknown resistance severity.

General Considerations in Using Sampling Plans. These results support Roush & Miller's (1986) statement that use of a single test concentration is more efficient than use of multiple concentrations when detecting resistance in mixed populations of resistant and susceptible insects. Of course, if no information is available on the susceptibility of different phenotypes for a particular susceptibility test, probit analysis is a necessary prerequisite to using single test concentrations. Estimation of a survival proportion at one test concentration is based on the assumption that there are differences in tolerance of resistant and susceptible phenotypes, but no assumption is made about the tolerance distribution within phenotypes.

Of equal interest is the selection of the two values of θ for construction of two sampling plans to be operated simultaneously. These values can be based on empirical data of a resistance field study (Denholm et al. 1984). The diversity of resistance severity is a consequence of current and past insecticide selection, inbreeding, and migration. Changes in pest management practices may affect resistance risk to some degree; therefore, a specific well-defined meaning applied to an estimate of resistance severity may require extensive understanding of resistance risk in various pest management situations (Keiding 1986). But classification of resistance severity into categories, one of which has some relevance to field control, may be very useful. As results from additional monitoring efforts are analyzed, selection of a high θ associated with potential control failure may be fine-tuned. Combined with a high θ, a low θ can be selected to classify resistance severity into one of three categories. This classification scheme increases the ability to detect shifts in resistance severity in field populations by constructing an intermediate zone (Fig. 5). The actual methodology for construction of sampling inspection plans is independent of the selection of test concentration and θ. Once these values are selected, the predetermined decision process outlined here provide objective criteria for decision-making.

Sequential sampling plans using the SPRT may often be useful for monitoring resistance as part of a pest management program where extensive testing is not possible or is expensive. In resistance monitoring programs where sampling and assessment of resistance is inexpensive and sequential inspection is burdensome, single sampling plans may be more appropriate. If the measure of resis-

Table 3. Comparison of results from two SPRTs operating simultaneously (using a single 1,000 μg concentration) with the corresponding probit estimate of survival

Region Site	SPRT			Probit analysis	
	n	Decision	r[a]	n	r[b]
Monterey					
C	110	$0.30 \leq r \leq 0.36$	0.34	290	0.32
Sinaloa					
B	130	$0.30 \leq r \leq 0.36$	0.34	300	0.35
C	80	$r \geq 0.45$	0.52	300	0.50
D	20	$r \geq 0.45$	0.72	295	0.64

[a] SPRT $r = S \div n$ after termination of the test.

[b] Probit r, determined from the concentration–mortality line at 1,000 μg/g.

tance is a quantitative character, sampling inspection by variables based on the normal or other suitable distribution would be appropriate (Guenther 1977). Brown & Brogden (1987) reviewed resistance detection techniques that are available and are being developed. Choice of an appropriate sampling plan is based on its statistical behavior and the operational needs of a particular technique.

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Appendix

Abbott's (1925) formula is given as $z = (x - y)100/x$ where z is the corrected percent mortality in the treatment, x is the percent survival in the control, and y is the uncorrected percent survival in the treatment.

Defining $S_i = \sum_{k=1}^{i} c_k$, $\sum_{k=1}^{i} s_k$, and n_i as before, $n_i - \sum_{k=1}^{i} c_k$ is the corrected sum of insects dead after exposure to the test concentration through the ith group. Therefore

$$z_i = 100\left(n_i - \sum_{k=1}^{i} c_k\right) \Big/ n_i$$

and likewise,

$$y_i = 100 \sum_{k=1}^{i} s_k/n_i$$

where z_i and y_i, $i = 1, 2, \ldots$, are now determined sequentially for the ith group. By substitution,

$$100\left(n_i - \sum_{k=1}^{i} c_k\right) \Big/ n_i = \left(x - 100 \sum_{k=1}^{i} s_k/n_i\right)100/x$$

and simplifying

$$S_i = \sum_{k=1}^{i} c_k = 100 \sum_{k=1}^{i} s_k/x, \quad i = 1, 2, \ldots,$$

for n_i insects inspected where x is a constant. The same caveat given by Abbott (1925) applies (i.e., the reliability of the original data for treatment and control has been considered).