

STRATEGIES AND STATISTICS OF SAMPLING FOR RARE INDIVIDUALS^{1*}

Robert C. Venette,^{1,2,3} Roger D. Moon,²
and William D. Hutchison^{2,3}

¹USDA-APHIS, ²Department of Entomology, and ³Midwest Ecological Risk Assessment Center, 1980 Folwell Ave., University of Minnesota, St. Paul, Minnesota 55108; e-mail: venet001@umn.edu, rdmoon@umn.edu, hutch002@umn.edu

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■ **Abstract** Diverse subdisciplines within entomology recognize the detection of rare individuals as the precursor to effective management of these individuals. Unfortunately, detection methods have often developed on a case-by-case basis, and advances in one subdiscipline have not carried over to similarly related fields. The biology of a particular organism will certainly affect sampling methods, but the underlying principles governing the power of a sampling strategy to detect rare individuals will apply across taxa. Our review of the sampling literature demonstrates the common problem of detecting rare individuals, reviews the fundamentals of probability theory as a foundation for any monitoring program, and discusses the inferences that can be drawn from samples, especially when resources limit sampling efforts. Particular emphasis is placed on binomial-, beta-binomial-, and hypergeometric-based sampling strategies as they pertain to quarantine inspections for exotic pests, veterinary/medical entomology, and insecticide resistance monitoring.

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INTRODUCTION

Rare individuals can initiate significant ecological changes. Invasions of ecosystems by nonindigenous species begin with the arrival of a few individuals. Equally, the arrival of disease-bearing arthropods may start life-threatening epidemics, and experience with traditional insecticides demonstrates that selection for rare, resistant individuals will undermine initially effective management tactics. Diverse subdisciplines within entomology are well aware of such concerns and have devoted considerable effort to improving detection methods.

From a biological perspective, rarity of a species is related to the abundance and range of a population (49, 50). Although numerous definitions of rarity exist, we concur that "... rarity is merely the current status of an extant organism which, by any combination of biological or physical factors, is restricted either in numbers or area to a level that is demonstrably less than the majority of other organisms of comparable taxonomic entities" (105). Numerous operational definitions of rarity exist for arthropods and large invertebrates (e.g., 10, 13, 22, 45, 49, 53, 110, 137). Definitions vary depending on the scope and purpose of a study. Within specific management arenas, rare individuals are those organisms within a spatially defined area (e.g., commodity shipment, field, herd, farm, or county) that represent <25% of the total abundance of a species (50) or are associated with <10% of potential hosts, (e.g., 30, 59, 96). Specification of sampling space and effort will have a significant impact on what might be considered rare at the moment or in the future (50, 77, 92).

The common problem of detecting rare individuals has been addressed in entomology, but often on a pest-by-pest basis. In many cases, advances within one subdiscipline have not carried over to related fields. The biology of a particular pest certainly affects sampling methods (120). However, the underlying principles governing the power of a sampling strategy to detect scarce individuals (and the inferences that can be drawn from those samples) apply to multiple pest species in several scenarios. The objectives of this review are to (a) discuss common challenges surrounding the detection of rare individuals; (b) apply elements of probability theory, through multiple areas of application, to the design of detection and monitoring programs; and (c) describe inferences that can be drawn from samples

when resources are limited. This review does not attempt to describe the breadth of technologies (e.g., insect traps, elutriation, polymerase chain reaction, or enzyme linked immunosorbent assays) that may be used to detect rare individuals.

DETECTION OR ESTIMATION

Two questions are of general interest to practitioners who are concerned with rare organisms: Does a certain type of individual occur within a defined area, and if it does, how many individuals are there? The first question deals with classification of an area (e.g., infested or not) or a population (e.g., resistant or susceptible), and the second question addresses the issue of magnitude. Magnitude may be expressed in absolute numbers or in proportion to some readily observed feature (e.g., the proportion of host plants or animals infested or infected with a particular pest or the proportion of a population with resistance to a pesticide). The initial question of classification, although seemingly simple and straightforward, can be exceptionally difficult to answer.

The search for rare individuals can be accomplished by census or sampling. For a census, one must know all of the locations (e.g., hosts and habitats) where individuals might reside, examine each location, and recognize an individual when it is encountered. In theory, a census provides perfect information about a population, but in practice the conditions for a census can be difficult to satisfy. Only a census can prove that a rare species does not exist within a defined space and time (91).

The search for rare individuals is more commonly based on sampling. Sampling is done to draw an inference about a population that exists at a specified place and time. A focal population may reside in units of a commodity in a cargo shipment, plants within a field, individuals in a herd or flock, human residents in a census tract or municipal area, or a population of vectors in a circumscribed area. Focal populations may range in size (N) from small and finite to large and effectively infinite. In this review we are concerned with the X individuals that have a given "trait" of interest and the trait's individual-level frequency, $f = X/N$, in the focal population. Needs for disease control and certification for international trade have directed attention to aggregates of populations, such as fields or herds within agricultural production regions. In these cases frequency for these higher levels (e.g., field- or herd-level frequency) is defined as the probability that an aggregate contains one or more positive individuals.

However the focal population is defined, the fundamental observational unit is the individual, which can be positive or negative for the trait. Ideally, individuals should be drawn with a probability-based sampling design, because such designs have the desirable property that estimated proportions or means and associated variances are unbiased (33). Unfortunately, the logistically easier haphazard method of selecting individuals can yield markedly biased estimates, and if individuals are selected with a systematic procedure, care must be taken that the period of selection does not coincide with an underlying pattern in the population

(see 74). Commonly used probability designs are simple (unrestricted) random sampling, stratified random sampling, cluster sampling, and multi-stage (nested) sampling. Where individuals are arranged in subgroups, individuals can be chosen with probability proportional to group size.²

Another form of sampling to detect rare individuals involves the selection and inspection of sample units based on additional information that may not be documented. In some cases this additional information may consist of personal beliefs or judgments that are used to determine whether a particular sample unit ought to be inspected. For example, in Britain a targeted sampling approach is used to survey for rhizomania, a disease of sugar beets (*Beta vulgaris*) caused by the soil-borne pathogen, *Polymyxa betae* (135). Farms that have a history of dealing with imported material (e.g., seed potatoes) or producing poor-quality sugar beets are surveyed for symptoms (e.g., patches of stunted growth) from the air. Suspect fields are photographed, and these photographs guide field scouts to potentially diseased areas within a field. Within these patches, only those plants with leaf symptoms are dug to inspect roots, and of the dug plants, only a small subset are tested by a diagnostic laboratory to confirm the presence of the pathogen. This approach relies on purposely biased sampling, and experience suggests this sampling approach is likely to detect low levels of the pathogen without the demands of processing an excessive number of samples (135). Because the information that was used to guide the sampling effort is not quantified, inferences about the frequency of affected plants or severity (degree of infection per affected plant) of the disease cannot be drawn from such samples (135). Such samples simply confirm the presence of the disease.

ERRORS IN DETECTION

Table 1 illustrates the possible outcomes of sampling for rare individuals (28). Symptoms, in the broad context used here, may also include the results of diagnostic tests. True positives (A) occur when the presence of symptoms corresponds with the presence of a pest. False positives (B) occur when symptoms are present, but a pest is not. False negatives result when symptoms are not apparent, but a pest is present (C). True negatives occur when neither symptom nor pest is present (D). If perfect correspondence exists between organism and symptoms, only A and D will result. However, the association between organism and symptom is not perfect.

²A brief note on terminology: Throughout this review we use “frequency” to indicate the proportion of a population that has a particular trait (e.g., infested, diseased, or resistant) at a specific time. Rare individuals occur infrequently. Among veterinary entomologists this measure is known as “point prevalence” (28), whereas plant pathologists refer to this measure as “incidence” (27). A sample unit is some measure of space (e.g., leaf, chicken, m²-quadrat) in which the trait of interest might occur. The sample universe is the population of units from which a sample can be drawn.

TABLE 1 Relationship between presence of an individual and evidence of its presence (i.e., symptoms) that might be used to guide the search for rare individuals

Results from sampling (symptoms)	Actual condition	
	Present	Absent
Present	(A) Correct	(B) Incorrect (false positives)
Absent	(C) Incorrect (false negatives)	(D) Correct

Few if any tests are thought to be perfectly sensitive and specific (see 57). Sensitivity (Se) is the probability that an individual with the trait will be judged by the test to be positive, relative to a cut-off value, T , such as a critical titer of a reactant or number of visible organisms [“tally threshold” (72, 76 cited in 12)]. From Table 1, Se equals $A/(A + C)$. Specificity (Sp) is the probability that an individual without the trait will be judged negative and equals $D/(B + D)$. Where $Se < 1$, true positives will be incorrectly classified as negative, and apparent individual-level frequency will be an underestimate of the true frequency. If $Sp < 1$, true negatives will be incorrectly diagnosed as positive, causing an overestimate of the true frequency. Errors may be in either direction if both Se and $Sp < 1$ (32, 57, 89). When seeking rare traits, lack of specificity is most problematic, because a few false positives will make the apparent frequency substantially greater than true frequency (32). Where Se and Sp can be characterized with point estimates or empirical distributions, apparent individual-level frequencies can be corrected (57, 74, 109).

At higher-level groupings of individuals (e.g., commodity lots or herds), higher-level sensitivity (HSe) and higher-level specificity (HSp) are the probabilities that a given test will correctly identify positive and negative lots, respectively, depending on whether the number of reactors in a sample of size n from each lot exceeds a critical cut-off number of sample units ($1 \leq T \leq n$). Imperfect test sensitivity and specificity will distort apparent higher-level frequency in the same way as occurs in populations of individuals (32, 57, 89). However, the effect of sample size on HSp where frequency is low can be overcome by raising T and thereby reducing the risk of false positives. Clustering of true positives among truly positive lots increases HSe and reduces underestimation of frequency. Donald et al. (43) and Jordan & McEwen (74) outlined approaches to choosing the best values n and T for an anticipated level of frequency that maximize HSe and HSp . A DOS-based program [AGG.EXE (80)] for accomplishing this task is available on the Internet (http://city.vetmed.fu-berlin.de/~mgreiner/validation_course/Agg.zip).

Regardless of the sampling approach, a sample can demonstrate only that rare individuals or traits are present. Sampling can never provide sufficient evidence to prove that a rare individual or trait is truly absent (91). Additional sampling

efforts may reveal the presence of rare individuals or traits. However, samples can demonstrate that the number of individuals is below a specified level or within a specified range with a known degree of confidence.

Detection of rare individuals can be critical if we wish to effectively manage the ecological or economic changes that these individuals may bring (19, 40, 69, 70). As in medicine, early detection of pests increases the amount of information about a potential problem and extends the window of opportunity to take effective action (99). For example, eradication may be proposed as the management strategy of choice when dealing with exotic pests (38). Eradication is likely to succeed (i.e., eliminate every potentially reproducing individual of a species) only if populations of the pest are small and spatially confined (98).

The statistical foundations for strategies to detect rare individuals are reviewed in three areas of application below. The first area addresses sampling during quarantine inspections for rare exotic species. The second area deals with sampling animals and arthropod vectors for pathogens, and the third area focuses on the detection of arthropods with resistance to insecticides. The three areas are provided for illustrative purposes only. General sampling objectives and statistical methods in each area apply to multiple subdisciplines within entomology. As seen below, successive areas of application build on statistics and strategies presented earlier in this review.

SAMPLING FOR EXOTIC PESTS DURING QUARANTINE INSPECTIONS

Goals

This area of application addresses three common questions: (a) What is the likelihood of finding one or more rare individuals with a defined sampling strategy? (b) What sample size is needed to achieve a desired probability of detecting a rare individual? (c) How should a researcher interpret a sample that fails to detect a rare individual?

Inspection of commodities at ports of entry is a prominent component of the safeguarding system used by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS) to protect U.S. agriculture and ecosystems from quarantine pests (100). Quarantine pests are those organisms “of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled” (46). Under Quarantine 56 (Title 7 Code of Federal Regulations §319.56) and the Plant Protection Act Title IV of 2000, USDA-APHIS Plant Protection and Quarantine has broad authority to regulate the importation of fruits and vegetables into the United States.

For example, *Copitarsia* spp. (Lepidoptera: Noctuidae) are quarantine pests of concern to the United States. *Copitarsia* occurs from central Mexico to the southern tip of Argentina along the western edges of North and South America

(56). The genus is not known to occur in the United States. In its native range it has been reported to feed on 39 crop plants in 19 different families (56). Reported hosts include asparagus (*Asparagus officinalis*), cabbage (*Brassica oleracea*), peas (*Pisum* spp.), and lily of the Incas (*Alstroemeria* spp.) (56).

Two sampling strategies are typically used to inspect imports of potentially infested commodities for *Copitarsia*. The first sampling protocol, Agriculture Quarantine Inspection Monitoring (AQIM), adjusts sampling effort based on the size of the shipment with the goal of maintaining a 95% probability of detecting pests at a frequency of infestation of 10% or greater (133). The sample unit may either be an individual unit of the commodity or a container (e.g., box or tray) with several units of the commodity. AQIM samples are collected at random, and the data provide a statistically valid basis to estimate a mean and confidence interval for the frequency of infestation in a shipment.

A second, more common approach for inspection relies on targeted sampling in which approximately 2% of a shipment is examined. The goal of the inspection effort is simply to determine if *Copitarsia* or other pests of quarantine significance are present. An inspector may adjust her sampling effort around the 2% rule of thumb based on the perceived likelihood that a shipment contains pests. Owing to the volume of produce that may arrive at a port at a given time and the demand to move produce to market as quickly as possible, sample units may not be selected at random from the entire shipment. Under high-pressure situations, tailgate inspections can be common (133). For a tailgate inspection, only those boxes of produce that are quickly accessible near the end of the shipment are examined.

Under both approaches inspection involves offloading the commodity, removing individual items from boxes (if applicable), and employing a combination of plant dissection, beat techniques, and visual inspections to look for *Copitarsia* larvae or noctuid eggs. An item is considered infested if it has ≥ 1 egg or larva. In this case, the sampling universe is the shipment (e.g., the bed of a semi-trailer truck), and the shipment is subject to regulatory action if one or more units is detected.

Statistics and Strategies

HYPERGEOMETRIC-BASED SAMPLING AQIM sampling is based on the hypergeometric distribution, which is appropriate to describe the probability of finding a pest when the size of the sample universe is relatively small (i.e., more than 5% of the shipment will be inspected) and sample units are examined without replacement (60, 97). In this case as each unit of the commodity is inspected, the likelihood of finding an infested unit in the next sample changes. Thus, the outcome of each sample unit inspected is dependent on the outcome of other sample units taken from the shipment (119). A shipment is composed of infested and noninfested commodity units. If a shipment of N units has X infested units, then the shipment has $N - X$ noninfested units. Throughout this review, we use upper case symbols to refer to variables in the target populations and lower case symbols to refer to variables for a sample.

When a sample of size n is taken (without replacement) from the shipment, a defined probability of detecting x infested units can be calculated. This probability is given by

$$P(x = i) = \frac{\binom{X}{i} \binom{N-X}{n-i}}{\binom{N}{n}}, \quad (1)$$

where $P(x = i)$ is the probability of observing i infested units, $\binom{a}{b} = \frac{a!}{b!(a-b)!}$, $a! = a \cdot (a-1) \cdot (a-2) \cdot \dots \cdot 1$, and $0! = 1$ (60, 94, 97). The probability of finding no infested units, $P(x = 0)$, is the complement to finding one or more infested units, $1 - P(x = 0)$. To illustrate, if a shipment of 50 heads of cabbage ($N = 50$) arrived at a port and two randomly selected heads were inspected ($n = 2$), assuming 10% of the commodity was infested ($X = 0.1 \cdot 50 = 5$), the probability of finding no pests in the sample is 80.8%. Sampling effort can be adjusted to raise the probability of observing a pest. Solving Equation 1 for n is difficult arithmetically, but can be done with approximation (28) or through maximum likelihood estimation (97).

Table 2 provides examples of USDA's sampling recommendations for cargo on trucks (133). To maintain a 95% probability of finding one or more infested commodity units when 10% of the shipment is infested, 22 of the 50 commodity units should be selected at random and examined (Table 2). Similarly, McInnis & Cunningham (93) used the hypergeometric distribution during an eradication program with the sterile insect technique to estimate the number of sterile marked flies that must be counted from a trap to be 95% or 99% confident that no more than 1, 2, or 3 rare, wild flies would also be in the trap.

BINOMIAL-BASED SAMPLING If individual items of the commodity (e.g., heads of cabbage) were mixed sufficiently as the commodity was harvested and packed in an enormously large shipment and items were selected at random from the shipment, the likelihood of finding *Copitarsia* may be approximated by simple binomial statistics. Binomial statistics apply only when the size of the sample is relatively small compared with the size of the shipment [i.e., $n/N < 0.05$ (78)]. The probability of observing exactly x infested items out of the n units in a sample is given by the binomial distribution

$$P(x = i) = \binom{n}{i} f^i (1-f)^{n-i}, \quad (2)$$

where f is the average proportion of infested items in a shipment (34, 94). An estimate of f is provided by x/n or $\{\sum x\}/\{\sum n\}$ if summed over multiple subsamples. This estimate for f will be wrong in the sense that another sample of n individuals is unlikely to produce exactly x infested units (34). The probability of not observing an infested unit in a sample of n plants is $P(x = 0) = (1-f)^n$, and the probability of observing one or more infested units is $P(x > 0) = 1 - (1-f)^n$ (91). The likelihood of finding at least one infested item increases as either the

TABLE 2 Sampling strategies used to examine commodities imported on trucks for quarantine pests, assuming 10% of the boxes have an infested commodity unit

Boxes on truck	Hypergeometric-based sampling (Agriculture Quarantine Inspection Monitoring)		Binomial-based sampling (“2% Rule”)	
	Boxes to inspect	Probability of detecting infested cargo	Boxes to inspect	Probability of detecting infested cargo
10	10	1.0	1	0.100
50	22	0.954	1	0.100
100	25	0.952	2	0.190
200	27	0.953	4	0.344
300	28	0.955	6	0.469
400	28	0.953	8	0.570
500	28	0.952	10	0.651
1000	29	0.955	20	0.878
1500	29	0.954	30	0.958
3000	29	0.954	60	0.998

proportion of infested items in a shipment or the number of sample units that are collected increases. For example, in a shipment of 1000 heads of cabbage an inspector would examine 20 cabbages (i.e., 2%). If we presume that the average level of infestation is 10% (i.e., $f = 0.1$), the probability of observing one or more infested units is 88% (Table 2). Figure 1 illustrates how the probability of detecting one or more pests would change if the true frequency of infestation was $<10\%$.

Equation 2 has also been used to a limited extent to estimate the probability of detecting exotic pests, notably *Ceratitis capitata*, within a trapping network (79a). In this application, f is the frequency with which males are captured in a trap, and n is the number of traps in a specified area. However, f is not constant but varies as a function of trap efficiency, insect activity, and distance between a population and a trap. Thus, the design of a trapping network depends significantly on the interaction between pest and trap. Evaluating the performance of a trapping network in the field may require data that are not readily available.

As indicated previously, sampling cannot prove that a pest is truly absent from a shipment (91). As a result, for quarantine inspections, we may be satisfied to demonstrate that the frequency of infestation is below some level (34). Thus, the question becomes, What level of infestation could a sample of n units detect with

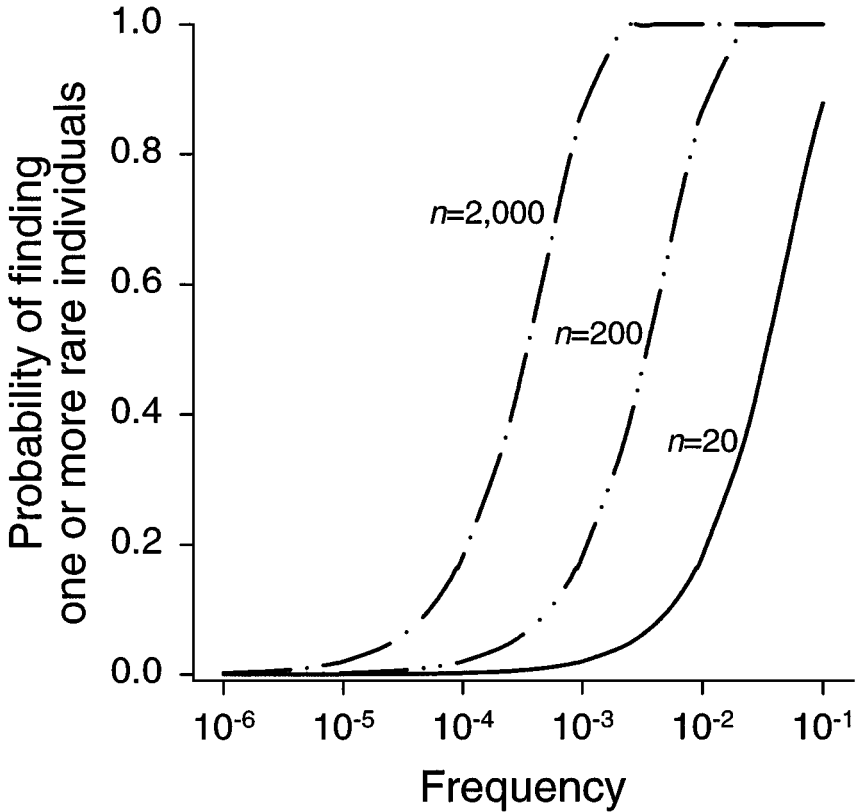


Figure 1 Probability of detecting one or more individuals [$P(X \geq 1)$] as affected by sample size (n) and the frequency of those individuals in the sampling universe, assuming binomial statistics apply.

a specified level of confidence? Or, to put it another way, what is the maximum level of infestation in a shipment if no infested items are found after sampling n units? In this case, rearrangement of Equation 2 yields

$$f_{max} = 1 - [1 - P(x > 0)]^{1/n}, \quad (3)$$

where f_{max} is the maximum frequency of the pest in the shipment and $P(x > 0)$ is the desired probability of observing an infested unit (34, 91). The true frequency ranges between 0 and f_{max} with $P(x > 0) \cdot 100\%$ confidence. If we apply Equation 3 to our previous example, our sample of 20 cabbages could detect a level of infestation at 14% or greater with 95% confidence. Curtis et al. (37) used this method to estimate with 95% confidence that the frequency of codling moth, *Cydia pomonella*, in packed nectarines from the San Joaquin Valley in 1985 was $< 2.66 \times 10^{-5}$.

Rather than basing the size of the sample on the size of the shipment, an inspector could choose to modify her sampling effort to detect a defined level of infestation with a specific degree of confidence. By rearranging Equation 3 to solve for n , we find that

$$n = \frac{\ln[1 - P(x > 0)]}{\ln(1 - f)} \quad (4)$$

(34, 91). As an example, if an inspector desires to detect an infestation of 10% (i.e., $f = 0.10$) with 95% confidence, he would need to inspect 28 sample units. In general, requisite sampling effort increases as the desired probability of detection increases or as the degree of infestation decreases (Figure 1).

As demonstrated in Table 2, sampling a fixed proportion of a shipment may provide too many or too few observations to achieve a desired probability of detection. In general, fixed-proportion sampling calls for too few sample units from smaller shipments (i.e., less than ~ 1500 containers) and too many units from larger shipments (i.e., more than 3000 containers) to achieve $>95\%$ confidence of detection. As demonstrated in Table 2, when 10% of a shipment is infested and ~ 30 sample units have already been collected, collection of an additional 30 sample units doubles the cost of sampling the shipment, but only increases the probability of detecting one or more pests from 95% to 99.8%.

The previous calculations assume that f is constant over all sampling units and that sampling observations are independent. These assumptions could be violated in three situations. First, the assumptions would not hold if the number of pests or the distribution of pests on plants changed in the time required for sampling, which seems unlikely. Second, insect populations are often aggregated or overdispersed in the field (i.e., the chances of a plant being infested are greater if a neighboring plant is infested than if neighbors are not infested). Because many commodities are harvested and immediately packed in the field, the distribution of infested units in a shipment may not be independent. Finally, unscrupulous exporters may realize that only the most accessible portion of a shipment is likely to be inspected and may deliberately place pest-free produce where inspectors are most likely to look.

BETA-BINOMIAL-BASED SAMPLING Sampling plans to detect rare pests can be adjusted to compensate for aggregated spatial distributions (64). For these adjustments to apply, we must assume that the commodity is sampled in batches (e.g., boxes) and that each item in a chosen batch is examined (cluster sampling) (67). When infested units of a commodity occur in clusters rather than at random, f is no longer constant across all clusters but will follow a beta density function (117), which can be combined with the binomial distribution to yield

$$P(x = i) = \binom{n}{i} \frac{\prod_{j=0}^{i-1} (f + j\theta) \prod_{j=0}^{n-i-1} (1 - f + j\theta)}{\prod_{j=0}^{n-1} (1 + j\theta)}, \quad (5)$$

where $P(x = i)$ is the probability of observing x infested units of the commodity in a batch, n is the number of commodity units (e.g., heads of cabbage) in a batch, x and f are defined as before, Π is the product function, and θ provides a measure of aggregation for the j th batch (87). The variable θ is $0 \leq \theta \leq 1$; if θ is 0, Equation 5 reduces to the binomial distribution (65). If x_j is the number of infested units found in batch j and n_j is the number of items examined in j , then f can be estimated by $\hat{f} = \sum x_{ij} / \sum n_{ij}$. An initial estimate of θ is provided by $\hat{\theta} = [s^2 - n\hat{f}(1 - \hat{f})] / [n^2\hat{f}(1 - \hat{f}) - s^2]$, where s^2 is the variance in the observed number of infested plants per batch (58, 66). Parameter values and associated standard errors for the beta-binomial distribution can be estimated by maximum likelihood (84, 118). For those instances in which no rare species are detected in quarantine samples, θ cannot be estimated from previously collected samples (86). Madden et al. (87) report estimates of θ between 0.016 and 0.090 for grape plants infected with the fungus *Eutypa lata*; Hughes & Madden (65) give estimates of θ between 0.0056 and 0.123 for tobacco infected with tobacco etch virus and tobacco vein mottling virus.

For quarantine sampling, we are often more concerned with the probability of not observing any infested plant material after inspecting several batches. For a single batch, the probability that $x = 0$ is $P(x = 0) = 1 - \prod_{j=0}^{n-1} (1 - f + j\theta) / (1 + j\theta)$ and the probability that each of several batches has no infested material, $\Pr(x = 0)$, equals $P(x = 0)^m$, where m is the number of batches. Note $P(x = 0)$ designates one batch and $\Pr(x = 0)$ reflects inspection of several batches. When f is low, Equation 5 can be approximated by the negative binomial distribution (85). Consequently, as demonstrated by Madden et al. (87), Equation 5 becomes

$$P(x = 0) \approx (1 + n\theta)^{-(f/\theta)} \quad (6)$$

and

$$\Pr(X = 0) \approx (1 + n\theta)^{-(mf/\theta)}. \quad (7)$$

The probability of observing one or more pests ($x > 0$) in a batch is simply $1 - P(x = 0)$, and the probability of observing one or more pests after sampling several batches is $1 - \Pr(x = 0)$. To apply Equation 6 to our previous example of a shipment of 1000 cabbages, if 10 cabbages are packed per box, an inspector might examine 2 boxes. If we assume a 10% level of infestation ($f = 0.1$) and a high degree of aggregation ($\theta = 0.15$), the probability of observing an infested head in an individual box is $1 - 0.543$, or 45.7%. The probability of observing an infested head after sampling two boxes, calculated through Equation 7, is $1 - 0.295$, or 70.5%, compared with the 88% probability we observed when infested heads were distributed at random. Aggregation of infested units of a commodity will always lower the likelihood of finding infestation.

Because aggregation lowers the probability of detecting an infested unit, we should expect that aggregation will raise the number of sample units required to

maintain a desired probability of pest detection (e.g., 95%). We can rearrange $1 - \Pr(x = 0)$ to solve for m (86). This rearrangement yields

$$m = \frac{-\theta}{f} \left[\frac{\ln(1 - P(x > 0))}{\ln(1 + n\theta)} \right]. \tag{8}$$

Figure 2 illustrates the influence of n , θ , and f on the requisite sample size, m , when the desired probability of detecting one or more pests is 0.95 (i.e., 95%). Sample requirements decrease as the proportion of commodity units that are infested rises or as the degree of aggregation declines. Requirements will also decline as the number of commodity units in a batch increases, but the reader should keep in mind that the total number of units to be processed is nm . As a result, a greater number of commodity units in a batch may not reduce total sampling effort.

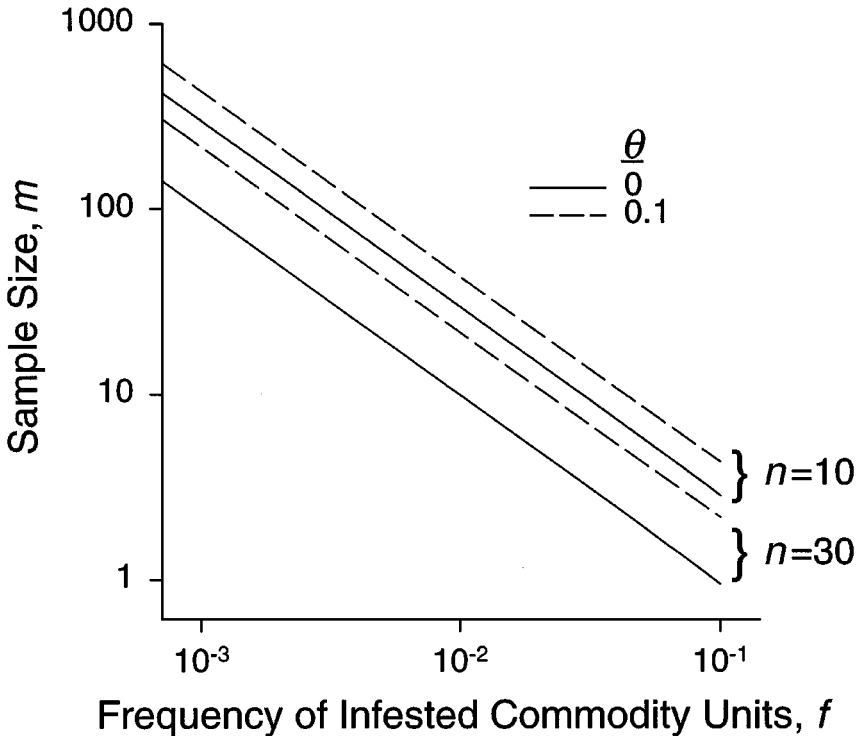


Figure 2 Requisite sample size (m) to detect one or more individuals with 95% confidence as affected by the proportion of a commodity that is infested, batch size (n), and the degree of aggregation (θ ; if $\theta = 0$, no aggregation), assuming beta-binomial statistics apply.

SAMPLING FOR PATHOGENS IN VETERINARY AND MEDICAL ENTOMOLOGY

Goals

This area of application addresses two common questions: (a) What sample size provides a specified level of confidence that a rare individual is not present in a population? (b) To what extent can sampling efficiency be improved by group testing?

Methods for sampling rare traits have been used extensively in veterinary medicine. Common applications involve chronic diseases in animal agriculture caused by arthropod, helminth, microbial, and viral pathogens of livestock and poultry (14, 130), and some of the diseases are subject to regulatory control. These disease-causing organisms are generally host-specific and spread mainly through contact between infected (positive) and uninfected (negative) animals. Consequently, it is feasible with closed flocks or herds to eradicate the pathogens, either locally or regionally, and prevent reestablishment indefinitely. Where isolation is incomplete, positive cases must be detected and identified, and then be removed or treated effectively to prevent spread. Small herds and flocks can be inspected completely, but sampling is required to detect diseases in larger populations of animals. Positive individuals would likely be rare at time of initial establishment or during reestablishment.

Sampling for rare individuals also arises in public health, in which results of monitoring for pathogen transmission are used to anticipate and prevent cases of human disease. For example, West Nile encephalitis was first detected in New York in 1999, when 62 cases and 7 deaths were reported in the New York City area (29). This disease, new to the Western Hemisphere, is caused by West Nile virus, which is transmitted biologically among birds and other vertebrate hosts by culicine mosquitoes. In response, the U.S. Centers for Disease Control and Prevention worked with public health agencies in the eastern United States to conduct active field surveillance to monitor the geographic and temporal spread of West Nile virus. Their goals were to (a) identify or rule out potential mosquito vectors and wild bird reservoirs, and (b) detect field transmission using sentinel chicken flocks. In both cases positive individuals were expected to be rare or absent.

In the veterinary sciences a variety of culturing protocols, immunodiagnostic tests, and DNA- or RNA-based identification methods have been developed to test for current or past presence of bacteria, viruses, and some helminths and arthropods. Direct inspections are more commonly used to detect other helminths and most ectoparasitic arthropods. The latter are often sought in subsamples of feces, skin snips, or partings of fur or feathers on chosen animals.

A conceptually simple goal may be to certify that a population is free of a chosen trait, i.e., that $f = 0$. A key question is, "How many individuals need to be examined to be $100(1 - P)\%$ certain that a trait is absent in a population?" In

this case the null hypothesis is that $f \geq f_c$, where f_c is a critical frequency level that the sampler sets by the circumstances of the problem (72, 74, 89). A similar problem exists in public health programs in which a critical frequency of disease (or vaccination) is used to target intervention (see 80). Inference about a population would be made from observing $x \leq X$ positives in a probability sample of $n \leq N$ individuals.

In situations where individuals with a trait are known to be present, the sampler's goals may be to estimate f with a desired margin of error, or to set an upper bound on f given that n individuals have been examined and found to be negative (28, 33). As in sampling to declare absence, the lower the true frequency, the greater the effort required to achieve desired precision and confidence. Unfortunately, sample sizes that would be necessary to achieve even modest goals may exceed the resources (labor and materials) available to obtain the individuals and test them individually.

Statistics and Strategies

BINOMIAL-, BETA-BINOMIAL-, AND HYPERGEOMETRIC-BASED SAMPLING Cannon & Roe (28) and subsequent authors (25, 26, 32, 73, 74, 89, 106, 109) derived sample size calculators from the binomial and hypergeometric distributions, with and without adjustment for test sensitivity (Se) and specificity (Sp). The binomial distribution is appropriate where sampling is with replacement or without replacement from an effectively infinite population, whereas the hypergeometric distribution is correct when sampling is without replacement, especially where N is relatively small (≤ 100). Both distributions assume individuals with the trait would be independently distributed throughout a source population. Software [HERDACC (73), FREECALC (25, 26)] is available to estimate required sample size and to solve probabilistic models appropriate for a variety of field and laboratory situations (25, 26, 43, 73, 74). The software and a comprehensive manual that illustrates a wide variety of applications (24) are available through the EpiVetNet web site (<http://epiweb.massey.ac.nz>). In situations where positive individuals are aggregated, that is, their presence is correlated in clusters, alternative approaches are to use the beta distribution (43, 74) or the beta-binomial distribution (16).

To illustrate the effect of population size using the hypergeometric distribution, if $f_c = 0.01$ in a population of $N = 100$, then an n of 91 must be examined with a perfect test to have a 90% chance of containing one or more positive individuals. Furthermore, necessary n rapidly approaches N if f_c is lower, greater confidence is desired, or both. In some situations goals may be unachievable even with $n = N$ because individual-level sensitivity (Se) is less than 1 (89). Where N s are much larger, sample sizes increase asymptotically to levels determined by f_c and desired confidence (Figure 1). As examples, with N of 1,000 or 10,000, respective n s would have to be 205 and 227 if $f_c = 0.01$, or 900 and 2056 if $f_c = 0.001$. Thus, for a given f_c , the larger the population, the smaller the proportion (or percentage) of individuals that will need to be examined to achieve a desired level of confidence (28, 83).

GROUP TESTING An alternative procedure, called group testing, is also known as “pool,” “composite,” or “batch” testing, and as “weighing designs.” The procedure was developed to reduce costs of processing large samples. Group testing is particularly useful for estimating frequency when frequency is expected to be low (<0.2) and obtaining individuals (or test material from them) is cheap, but testing itself is expensive (71 cited in 79). Group testing was originally developed to reduce the costs of screening large numbers of blood samples to exclude syphilitic military inductees (44) and was later adapted for studies of aphid vectors of plant viruses (52, 129) and mosquito vectors of arboviruses (31). More recent needs to reduce costs of screening large numbers of blood samples for antibodies to human immunodeficiency virus (HIV) and processing environmental samples for a variety of pollutants have prompted renewed interest and theoretical development (see 79 for a review). Examples of recent applications of group testing exist in the literature in medical entomology (1, 41, 42, 48, 75, 107, 136) and plant pathology (15, 63).

The basic idea is to choose n independently and identically distributed individuals from the focal population's N using a probability method, and then to combine them into m groups of $k = n/m$ units per group. The individuals themselves or material from them, such as leaf tissue, serum, feces, or skin scrapings, can be processed, provided the amount per individual is held constant to maintain equal statistical weightings. Each group is then tested with the expensive procedure, yielding a count of x ($0 \leq x \leq m$) positive groups. If f is low, then the number of tests can be reduced, because most of the groups will contain no positives and all k individuals in each negative group will have been judged negative with one test. Put another way, if cost limits the number of tests, group testing can allow the sampler to greatly increase the sample size (number of individuals).

If positive individuals need to be identified, then individuals in each positive pool can be retested individually as done by Dorfman (44), or subgrouped and retested with the same or a more expensive confirming test (81). An ingenious approach to reducing the costs of retesting is to arrange material from individuals in rectangular arrays (as in a microtiter plate) and then combine aliquots from cells along each row and again separately down each column (121). Each positive individual would be at the intersection of any corresponding positive row and column. Regrouping schemes and rectangular arrangements will always be more efficient than Dorfman's individual retesting scheme (81, 121).

Group testing provides a particularly efficient approach to estimating frequency where frequency is low. Assuming there were just one positive individual in each positive group, the minimum frequency will be $f_{MIR} = x/(km)$ with variance of $(f[1-f])/(mk)$. This estimate is known in the arbovirus literature as “minimum infection rate” (MIR), and although this estimator provides an underestimate of true f , its bias diminishes as f approaches zero. The better maximum likelihood estimator (MLE) of f is $f_{MLE} = 1 - (1 - x/m)^{1/k}$ with asymptotic (large sample) variance of $(f[1-f]^{2/(k-1)})/(mk^2)$. This MLE estimator of f gives an overestimate of true f , but the bias is small when $f \leq 0.1$ and $k = 15$ or less per group, and

trivial when $f \leq 0.05$ and $k = 25$ or less (121). Cowling et al. (35) evaluated f_{MIR} and f_{MLE} and five other frequentist and Bayesian approaches, including ones that incorporate varying knowledge about a test's Se and Sp . They found that if f_{MLE} is not five times greater than its variance, then its lower confidence interval can be negative, as a consequence of being based on large sample theory. A derived upper bound or confidence interval is unreliable. Furthermore, both f_{MIR} and f_{MLE} are only appropriate if the test is perfect (Se and $Sp = 1$). See (35) for more details and the alternative estimators.

CHOICE OF GROUP SIZE A key operational problem in group testing is to choose group size, k , that minimizes costs and bias in estimates of f for arbitrary sample size, n . With perfect Se and Sp and no dilution effect, the most efficient k is the next integer greater than $1/\sqrt{f}$ and the expected number of tests per individual is approximately $2/\sqrt{f}$ (44, 102). For example, if $f = 0.1$, the choice of $k = 4$ would be best, and would require 0.63 tests per individual (or 37% fewer than if each of the n individuals were tested individually). With $f = 0.01$, the best $k = 11$ and expected tests would be 0.2. Swallow (121, 122) notes that when choosing k , knowledge of f will be sketchy at best, and a k that is too great (for true f) may cause unacceptable bias in f_{MLE} . He advises investigators to calculate k as above, but to use a guess for f that is slightly greater than the best prior estimate (122). Doing so will retain most of the benefits of group testing.

More recent work (23, 81, 131, 132) demonstrates that most efficient k also depends on the test's Se and Sp and the extent to which each is affected by grouping. Grouping can lower the concentration of the target analyte, the substance being identified or measured during an analysis, below the test's detection threshold (reduce Se) and introduce masking analytes that increase the frequency of falsely positive tests (reduce Sp). Performance of a test being considered must be evaluated experimentally to define its limits of detection, Se and Sp under operational conditions (for examples, see 23, 57, 104). Provided Se and Sp are both greater than 0.5, effects of dilution can be compensated by reducing k , usually to within a range of 4–25, depending on f (68).

AUTOCORRELATION One situation arises when sampling is systematic and positive individuals are aggregated in the sampling universe, as occurs when fecal samples are collected from herd mates in a herd infected with *Escherichia coli* (e.g., 74). Spatial aggregation leads to serial correlation among neighboring individuals, which violates the assumption that positives are identically and independently distributed, as required by binomial or hypergeometric theory. Such aggregation leads to underestimates of f when low and renders confidence limits unreliably small. If the objective is solely to estimate f , then autocorrelation can be overcome by randomly assigning individuals or specimens to groups prior to testing. On the other hand, if the objective is also to identify (and perhaps remove or treat) positive individuals, then biasing effects of positive autocorrelation can be overcome by avoiding small group sizes (68).

SAMPLING FOR RARE ARTHROPODS WITH INSECTICIDE RESISTANCE

Goals

This area of application addresses a common question: What is the frequency of rare individuals in a population?

Historically, one of the most critical challenges in arthropod pest management has been the need for early detection of rare individuals, or alleles, that confer resistance to one or more insecticides. Over 500 species of arthropods have developed resistance to one or more pesticides (51), with some pests having developed resistance within 3 years after the introduction of a new insecticide (103, 128). More recently, at least 12 species of insects have developed resistance to various toxins from the bacterium, *Bacillus thuringiensis* (Bt) (62, 124), including the first case of resistance to Bt in field populations of the diamondback moth, *Plutella xylostella* (L.) (125). Resistance to Bt toxins has heightened the need for proactive resistance management plans, including the use of more sensitive methods for early detection of resistance in major insect pests of Bt transgenic crops (2, 3, 54, 95, 101, 134).

The proportion of resistant insects or resistance alleles in a pest population is typically assumed to be low. This assumption is based in large part on population genetics theory of expected frequency of mutation rates (36, 111). Although many models assume an initial resistance-allele frequency of 10^{-3} to 10^{-5} (2, 123), resistance management models have arbitrarily been set with allele frequencies as high as 10^{-2} or as low as 10^{-13} (111). Because of the practical constraints to implementing extensive sampling methods that are sensitive enough for early detection of rare individuals or rare resistant alleles, empirical estimates for the initial frequency of resistant alleles have also been the exception, rather than the rule (6, 55).

Despite the increasing need for effective methods to detect rare resistant genotypes or phenotypes, the often-used laboratory bioassay, with typical sample sizes of 150–300 individuals, lacks the sensitivity needed for early detection (112) or suffers from a weak correlation with resistance development in field populations (47). In addition, novel biochemical or immunological methods hold considerable promise but are often presented with no recommendations for minimum sample size; the resistance mechanism (marker gene) must already be known, which may preclude early detection in field populations (e.g., 39, 47).

Once resistance occurs, the focus really becomes resistant-pest management (6), in which monitoring methods can be used that are less dependent on large sample size (18, 39, 47, 103). One caveat to this worst-case scenario is that if a new resistance event is fortuitously limited to one geographic area, statistically sensitive and logistically feasible resistance monitoring can then be developed and implemented in other at-risk locations (agriculture, forests, urban habitats). For the purposes of this review, we use a general and well-accepted definition of resistance by Sawicki (113): “Resistance is a genetic change in response to

selection by toxicants that may impair control in the field.” The degree to which genetic change is measured is dependent upon the method and is discussed in the context of each method.

Statistics and Strategies

We review seven strategies to detect resistant arthropods and the underlying statistical foundations of each method. Because the sampling unit in each strategy may differ slightly, our ability to make direct inferences about the phenotypic frequency of resistant individuals or the genotypic frequency of resistance-conferring alleles may also differ. With several assumptions about the genetic structure of a population and the level of dominance of a resistance allele, calculation of phenotype frequencies from genotype frequencies (or vice versa) may be possible; however, these estimates are sensitive to initial assumptions (134).

LETHAL DOSE BIOASSAYS Methods developed for detection and monitoring of pesticide resistance have relied heavily on laboratory bioassays, typically conducted on the progeny of field-collected arthropods (47, 112). For example, eggs and larvae of many Lepidoptera are usually field-collected, then laboratory-reared for one or more generations before bioassays can be conducted on neonate progeny (e.g., 17, 103, 115). The traditional lethal dose bioassay consisted of comparing shifts in the amount of a pesticide necessary to kill 50% of a test population, measured as the lethal dose (LD_{50}) or lethal concentration (LC_{50}) (47). The LC_{50} of the test (field) population divided by the LC_{50} in an unselected laboratory population provides the resistance ratio; the ratio can be compared with other populations, or among numerous toxicants for the same population.

LC_{50} assays have often suffered from at least four drawbacks: (a) each test population must be exposed to a range of pesticide concentrations (5 minimum), with a minimum of ~ 270 individuals (108); (b) a given method may be used for a number of years that is not indicative of variable toxicant exposure in the field (47, 90); (c) variable responses in unselected (check) populations can have a large effect on the resistance ratio, with no connection to selection response by the field population; and (d) given the typical sample sizes used, the assay can be insensitive to early detection of genetic resistance in the field (112). Roush & Miller (112) showed that the frequency of resistant individuals must be fairly high (at least 20%) before the LC_{50} is appreciably changed. Thus, regardless of sample size, this method is limited to the confirmation of high levels of resistance in field populations; consequently, no increase in sample size above 300 individuals will appreciably improve sensitivity.

DIAGNOSTIC DOSE ASSAYS Because of the cost and insensitivity of 50% mortality assays, several researchers have developed diagnostic dose assays, based on the use of a discriminatory dose at the LC_{99} level (47, 88, 103, 112, 116). Minimizing the assay to a single dose allows for higher numbers of individuals to be screened,

which results in greater sensitivity and considerably reduces expense. However, a discriminatory dose may also be greatly limited by sample size. As shown by Roush & Miller (112), binomial probability theory indicates that to detect resistant phenotypes that represent 1% of the population, a minimum of 300 individuals must be sampled, with a 95% probability of detection. However, when using a diagnostic LC_{99} , one cannot always assume that we are using a perfect diagnostic concentration; e.g., there is still a risk that the LC_{99} will kill a few resistant individuals. Thus, to account for the potential errors of misclassification, a statistical (Z) test can be used to show with 95% confidence the probability of using the correct diagnostic concentration. With this test, and a 1% phenotypic rate, an average of 1400 randomly sampled individuals must be screened. If the initial frequency of a recessive resistance allele is 10^{-3} , the expected frequency of homozygous-resistant individuals is 10^{-6} . Thus, over 1,000,000 individuals would need to be screened. A diagnostic dose is therefore most likely to detect dominant resistance alleles (6) and would be inefficient at detecting recessive alleles.

For completely recessive alleles, a sample of 1400 random individuals in a diagnostic dose assay provides an allele frequency estimate of only plus or minus 27 in 1000 (6). With Bt crops, the refuge/high dose strategy could begin to fail when the allele frequency becomes greater than one in 1000 (2). Thus, a diagnostic assay is not likely to be effective for early detection and a timely, adaptive response to resistance.

BIOCHEMICAL METHODS During the past decade several biochemical methods have been proposed for detecting insecticide-resistant individuals, including enzyme electrophoresis, enzyme assays, and immunoassays (39, 47). Esterases have been most suitable for monitoring, particularly when resistance involves amplification of the gene (61). DNA techniques can be used to detect and monitor target-site mechanisms. Biochemical methods typically require more time and expense to develop, but the sensitivity of the resulting assay is also much greater. Owing to high specificity, these methods should require lower sample sizes than the conventional diagnostic dose, i.e., there should be no uncertainty regarding the insect's response to the marker of interest. Thus, based on similar binomial probabilities, a sample size of 300 individuals should be adequate to detect at least one resistant individual with a phenotypic frequency of 1% (112).

FERAL SCREENS With the benefit of Bt-resistant, laboratory populations, Gould et al. (55) and Tabashnik et al. (126) devised innovative techniques for directly estimating the initial frequency of resistance alleles for tobacco budworm, *Heliothis virescens* (F.) and diamondback moth, respectively. This approach involves the use of single-pair matings of feral insects with individuals from one or more resistant laboratory strains. For the tobacco budworm, >2000 feral males were collected from 4 southeastern states. The Bt-resistant laboratory strain was estimated to be >2000-fold more resistant than a susceptible line (based on LC_{50} resistance ratio) and able to survive on Bt cotton. Subsequent bioassays provided an initial allele

frequency estimate of 1.5×10^{-3} . As noted by the authors, this could be considered an overestimate because of the high resistance level used for paired matings. For diamondback moth, the frequency estimate was high at 0.12; this strain also had resistance to at least four Bt toxins (126). Given these examples, it is difficult to recommend a single, robust, sample size requirement. However, when resistance is assumed to be rare (10^{-3}) and not all feral/laboratory matings result in sufficient progeny (e.g., 1025 of the original 2289 paired matings with tobacco budworm), it appears that the method should continue with a minimum of 2000 feral insects. As with biochemical assays, the primary limitation is that one or more resistance alleles must already be identified, and preferably fixed, in a homozygous resistant strain. As more resistant colonies become available, the method will likely gain broader application.

F₂ SCREEN Based on a concept similar to the feral/resistant screen used by Gould et al. (55), Andow & Alstad (3) devised the F₂ screen as an innovative approach to estimate initial resistance allele frequencies. Parental mated females are caught in the field (via light traps). The F₁ progeny are sib-mated to produce the F₂ generation, which is exposed to a discriminatory dose of toxin. In the F₂ generation one sixteenth of the larvae are expected to be homozygous for the rare resistance allele. A Bayesian approach (20) is used to estimate an expected allele frequency and 95% credibility intervals for the population sampled (4, 114). The method was successfully used to estimate the expected frequency of resistant alleles in populations of European corn borer, *Ostrinia nubilalis* (Hübner) at $<5.3 \times 10^{-3}$ (5, 7). Bentur et al. (11) used the F₂ screen to estimate the frequency of Bt resistance alleles at $<3.6 \times 10^{-3}$ for *Scirpophaga incertulas* (Walker). To be 95% confident that the frequency of a resistance allele is $<10^{-3}$, approximately 750 isofemale lines must be screened (114). To be equally confident that the allele frequency is $<10^{-4}$, nearly 7500 lines must be assayed (4).

IN-FIELD SCREEN With the advent of Bt transgenic crops, the possibility of using the crop itself as an in-field discriminatory dose has recently been examined for lepidopteran pests of Bt corn (134) exposed to the Cry1Ab toxin and Bt cotton (127) using the Cry1Ac toxin. The concept with an in-field screen is to allow the Bt plant to provide the field-level diagnostic concentration to wild populations of insects as they encounter the toxin under field conditions. With this approach, either sentinel plots can be planted at multiple locations, including both a Bt and non-Bt isoline plot for comparison (134), or commercial Bt fields can be used (127).

Two common Bt corn events, BT-11 (Syngenta) and MON-810 (Monsanto) (both Cry1Ab) are considered to provide a high dose for the European corn borer (8, 21, 101), as well as a significant level of control for the corn earworm, *Helioverpa zea* Boddie (21, 82). Likewise, Bt cotton (Monsanto) is considered to provide a high-dose Bt exposure to the tobacco budworm and pink bollworm, two primary pests targeted by the technology; some control is provided for corn earworm,

but under severe infestations up to 50% of cotton fruit can be infested with corn earworm.

Necessary sample size for the in-field screen is dependent upon insect population density exposed to the Bt crop and the frequency of resistant individuals in the population (Figure 3). Final sensitivity of the method is dependent upon these two factors and the final sample size taken. For example, with an assumed resistance frequency of 10^{-3} , and a background density (on non-Bt corn) of 2 larvae per ear, $\sim 1 \times 10^3$ Bt-plants must be examined to achieve 95% sensitivity.

The expected phenotypic frequency of resistance is based on the ratio of late instar larvae found on Bt plants to larvae observed on non-Bt plants (both expressed on a per plant basis). For example, if two late instar larvae are found in 5000 Bt plants (and the infested plants also test positive for Bt toxin), and an average density of 1.125 larvae per plant occurs on nearby non-Bt plants, the expected frequency of resistant phenotypes is $f = 2/(1.125 \cdot 5000) = 3.55 \times 10^{-4}$. These larvae are considered putatively resistant, pending follow-up laboratory bioassays (134). As

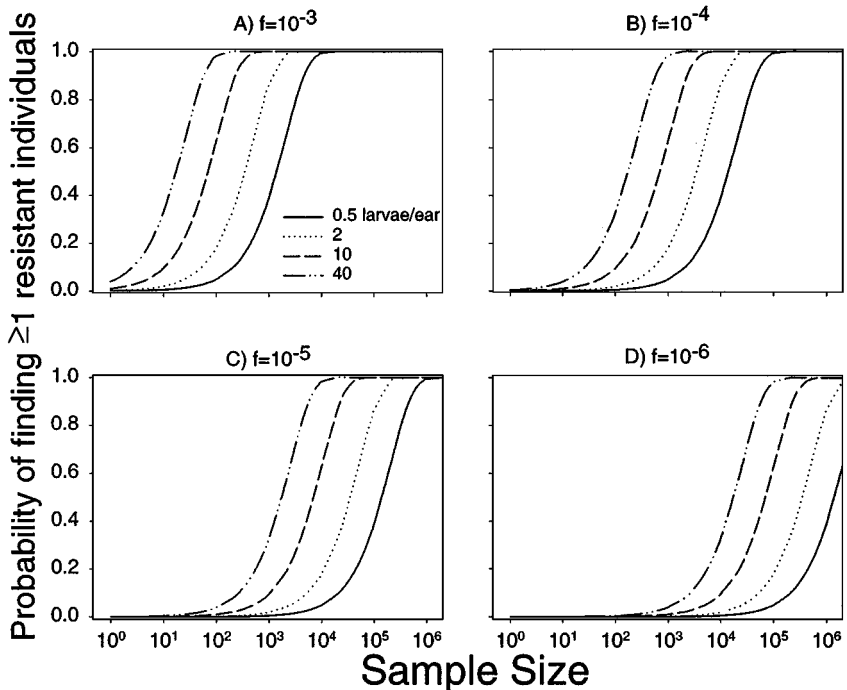


Figure 3 Probability of detecting at least one Bt-resistant individual [$P(X \geq 1)$] as affected by number of sample units taken from Bt plants, average insect density on isogenic non-Bt variety, and a presumed phenotypic frequency of resistance. (Redrawn with permission of the Entomological Society of America.)

shown in Appendix I (see the Supplemental Material link at www.annualreviews.org), the 95% confidence interval for this estimate is 3.08×10^{-5} to 1.305×10^{-3} .

Tabashnik et al. (127) used commercial Bt cotton fields to monitor for rare surviving pink bollworm larvae. They used field data, collected over 5 years (sampling of 140,950 cotton bolls), to estimate a phenotypic frequency per year. As with the in-field screen (134), and assuming recessive gene action, they used the square root of the phenotypic frequency to estimate resistance allele frequency. This “indirect” estimate of resistance allele frequency was also compared with a “direct” estimate of Bt resistance, based on traditional bioassays of laboratory-reared progeny of parental pink bollworm larvae collected in nearby non-Bt fields. Notably, the in-field (or indirect) screen approach compared well with their direct approach of conducting laboratory bioassays with pink bollworm larvae collected from non-Bt fields. However, for both the in-field and laboratory assay results, the initial allele frequencies for resistance were high, at 0.13 and 0.16, respectively.

These resistance allele estimates are much higher than those of <0.013 for European corn borer (7) and 1.5×10^{-3} for tobacco budworm (55). However, despite the high estimates for the frequency of resistant alleles, the frequency did not increase over a 4-year period, and there was no significant shift in phenotype (larval) frequency ($\sim 10^{-4}$). Each of these examples, using more sensitive methods than previously available with diagnostic assays, underscores the importance of empirical evidence for critical reviews of our understanding of the evolution of resistance.

FEEDING DISRUPTION ASSAYS Most recently, a novel feeding disruption assay was developed for both rapid species identification and resistance monitoring for the tobacco budworm and corn earworm in Bt cotton (9). The method combines the concept of a repeatable diagnostic dose of Cry1Ac Bt, with fecal pellet production as an indicator for Bt resistance; a lack of fecal production from larvae exposed to a diagnostic concentration of Cry1Ac in a blue indicator diet suggests a lack of resistance in the individual insect. Eggs and early instars of each species cannot be accurately identified by visual observation in the field. Although immunoassays are commercially available for these species (138), the assays are expensive, do not work for larvae, and require that the eggs be destroyed in the process. These obstacles preclude use for resistance monitoring. The feeding disruption assay uses the inherent differential Bt susceptibility of each species to distinguish species, while simultaneously allowing for an estimate of the proportion of resistant individuals. This method has many advantages, including rapid processing (24 h), and could thereby provide a rapid method for monitoring phenotypes over large geographic areas.

Binomial statistics were used to assess the precision of this method, with 95% confidence intervals generated from assay results with sample sizes (n) of 10, 50, and 100 insects. The authors show that a sample size of 100 is clearly preferred. For example, for a population of corn earworm, a sample of 100 larvae with 80%

of the larvae classified as feeders, provides a confidence interval of 67.2–87.2%. Sample sizes exceeding 100 insects may be necessary where resistance is likely to be low, or when tight confidence intervals are warranted for comparisons among populations. The added precision must be compared with any tradeoffs in additional cost. This method has considerable potential for wide-scale implementation and may also be applicable to other targeted insects that must ingest toxins for biological activity.

CONCLUSIONS

Although the early detection of rare individuals offers significant advantages from a management perspective, implementing appropriate sampling strategies can be problematic. When individuals are particularly rare, hundreds if not thousands of sample units must be examined to have a significant chance of finding at least one individual. Fiscal and time constraints may preclude the execution of a theoretically optimal strategy. Nevertheless, poorly planned surveys are unlikely to detect rare individuals, and even when ample resources are available, inferences about a population must be drawn with care. A common error is to conclude that a pest is absent from a habitat after collecting a limited number of samples. In fact, rare individuals may be present but at a frequency below the level of detection. Final characterization of the sampling universe should account for this possibility.

The arenas of quarantine inspection, veterinary/medical entomology, and resistance monitoring share many common aspects (in addition to the constraints imposed by time and money), yet currently differ in important ways when sampling for rare individuals (Table 3). Whereas all three arenas share the goal of detecting rare individuals, veterinary/medical entomology and resistance monitoring have the equally important goal of estimating, or placing an upper bounds on, the frequency of rare individuals within the sampling universe. Currently, the detection of a single pest during quarantine sampling is sufficient evidence to justify further regulatory action. In terms of the statistical foundation of detection strategies, resistance monitoring relies heavily on binomial statistics, whereas the other two arenas also (could) consider hypergeometric and beta-binomial statistics.

The three arenas probably differ most in sensitivity and specificity of the methods used to achieve their goals (Table 3). Sensitivity of a sampling program depends on sample size and on the techniques/technologies used to collect and process a sample. During quarantine inspections inspectors may collect a minimal number of sample units that are examined visually, and visual inspections may not detect small organisms. Thus, routine inspections have a weak potential to correctly classify all shipments with exotic pests as positive. The sensitivity of methods used for veterinary/medical entomology and resistance monitoring is variable, depending on

TABLE 3 Comparison of sampling programs from three arenas within entomology to detect rare individuals

Aspect	Quarantine inspection	Veterinary/medical entomology	Resistance monitoring
Primary goals	Detection	Detection, estimation	Detection, estimation
Primary statistical foundation	Binomial, beta-binomial, hypergeometric	Binomial, beta-binomial, hypergeometric	Binomial
Sensitivity of method	Weak	Variable	Variable
Specificity of method	Strong	Variable	Strong

the specific techniques employed. Strategies such as group testing and the in-field screen improve sensitivity by effectively increasing the number of sample units that can be processed. In general, both quarantine sampling and resistance monitoring use methods with a high degree of specificity and have a strong potential to correctly classify truly noninfested shipments or susceptible individuals, for example, as negative. In veterinary/medical entomology, particular technologies have a higher rate of false positives. Thus, specificity of methods used within the discipline is variable.

Technological advances may or may not improve the specificity or sensitivity of strategies designed to detect rare individuals. Technology has the potential to increase the number of sample units that can be processed, improve the accuracy of test results, or both. Technologies that reduce the likelihood of obtaining false positive results will, by definition, provide detection methods with a greater degree of specificity. However, gains in specificity do not necessarily translate into improved sensitivity. Technological advances that effectively increase the number of sample units that can be processed are more likely to improve our ability to detect rare individuals. Thus, a need exists to characterize gains in specificity and sensitivity from new technologies in an operational setting. Before a new technology is widely adopted, one should understand precisely where gains are being made and evaluate whether or not the technology is truly likely to improve detection capabilities. For the detection of rare individuals, gains in sensitivity are often more valuable than gains in specificity.

In this review we provide several statistically based approaches to estimate the amount of effort necessary to encounter rare individuals. The relevance of a particular method depends more on the size and organization of the sampling universe than on the type of organism we are searching for. Statistical methods from specialized fields within entomology (and from disciplines outside of entomology) can be applied across disciplines to solve the common challenge of locating scarce individuals.

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