Invasive forest pest surveillance: survey development and reliability

John W. Coulston, Frank H. Koch, William D. Smith, and Frank J. Sapio

Abstract: Worldwide, a large number of potential pest species are introduced to locations outside their native ranges; under the best possible prevention scheme, some are likely to establish one or more localized populations. A comprehensive early detection and rapid-response protocol calls for surveillance to determine if a pest has invaded additional locations outside its original area of introduction. In this manuscript, we adapt and spatially extend a two-stage sampling technique to determine the required sample size to substantiate freedom from an invasive pest with a known level of certainty. The technique, derived from methods for sampling livestock herds for disease presence, accounts for the fact that pest activity may be low at a coarse spatial scale (i.e., among forested landscapes) but high at a fine scale (i.e., within a given forested landscape). We illustrate the utility of the approach by generating a national-scale survey based on a risk map for a hypothetical forest pest species threatening the United States. These techniques provide a repeatable, cost-effective, practical framework for developing broad-scale surveys to substantiate freedom from non-native invasive forest pests with known statistical power.

Résumé : À travers le monde, un grand nombre d'espèces de ravageurs potentiels sont introduites dans des endroits situés en dehors de leur aire de répartition naturelle. Malgré la meilleure stratégie possible de prévention, certains de ces ravageurs ont des chances d'établir une ou plusieurs populations localisées. Un protocole complet de détection précoce et de réaction rapide demande une surveillance pour déterminer si un ravageur a envahi d'autres endroits à l'extérieur de sa zone d'introduction originelle. Dans cet article, nous adaptons une méthode d'échantillonnage en deux temps et lui ajoutons une dimension spatiale afin de déterminer la taille de l'échantillon nécessaire pour confirmer l'absence d'un ravageur invasif avec un degré de certitude connu. La méthode est dérivée des méthodes d'échantillonnage des troupeaux de bétail pour détecter la présence de maladies et tient compte du fait que l'activité des ravageurs peut être faible à une échelle spatiale grossière (c.-à-d. parmi des paysages forestiers) mais élevée à une échelle plus fine (c.-à-d. à l'intérieur d'un paysage forestier). Nous illustrons l'utilité de l'approche en générant un inventaire à l'échelle nationale basé sur une carte de risques pour une espèce de ravageur forestier hypothétique qui menacerait les États-Unis. Ces techniques offrent un cadre pratique, reproductible et peu coûteux pour élaborer des inventaires à grande échelle afin de confirmer l'absence de ravageurs forestiers exotiques et invasifs avec une puissance statistique connue.

[Traduit par la Rédaction]

Introduction

Non-native invasive species pose a significant threat to natural resources worldwide. Resource losses, environmental damages, and control costs in the United States due to invasive species have been recently estimated to exceed US$120 billion/year (Pimentel et al. 2005). In particular, non-native insects and diseases affecting US forests have an estimated impact of greater than US$4.3 billion annually in damages and control expenditures (Pimentel et al. 2005). Annual control costs for individual invasive species can exceed several million US dollars, although forgoing control efforts may ultimately cost much more; without active management, invasives that are currently limited in geographic distribution can rapidly become established over large areas and, subsequently, cause extensive environmental degradation (Chornesky et al. 2005; Lodge et al. 2006). Historically, invasives have been responsible for severe impacts to forest foundation species such as jarrah (*Eucalyptus marginata* Donn ex Sm.) in Western Australia or the American chestnut (*Castanea dentata* (Marsh.) Borkh.), which has been nearly eliminated from US forests (Ellison et al. 2005; McDougall et al. 2002). These impacts result in major compositional changes that affect wildlife and overall ecosystem processes (Chornesky et al. 2005; Ellison et al. 2005). Furthermore, invasives place added pressure on many critically imperiled animal and plant species through competition or predation (Wilcove et al. 1998).

In the United States, Executive Order 13112 established a National Invasive Species Council and mandated that federal

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agencies whose activities influence the status of invasive species will "detect and respond rapidly to and control populations of such species in a cost-effective and environmentally sound manner" and "monitor invasive species populations accurately and reliably" (Clinton 1999, p. 6184). Similarly, a 2006 report from the Ecological Society of America (ESA) highlighted potential impacts of invasive species and provided several key policy recommendations, including coordinated efforts to detect invasions while they are still localized, better enabling eradication of species before they become established (Lodge et al. 2006). The report acknowledged the high costs of surveying for rare individuals and, therefore, emphasized the cost-effectiveness of surveillance techniques that focus on locations with high invasion risks. Demonstrating the truly global scope of the invasive pest problem, both Executive Order 13112 and the ESA report echo procedures and policies outlined in the International Standards for Phytosanitary Measures (ISPM) produced under the International Plant Protection Convention. In addition to general surveillance guidelines, ISPM publications address requirements for the determination of an area's pest status as well as conditions for establishing pest-free or low-pest-prevalence areas (FAO 1995, 1997, 1998, 2005).

Pest surveillance is a complex task, particularly at a national or similarly broad spatial scale. This is especially true for forest pests, which can travel, often cryptically, along a wide variety of pathways. Every year, a large number of non-native insects and diseases affecting forest tree species are intercepted at international ports of entry from commercial shipments of live plants, logs and raw wood products, and other commodities, as well as in packing materials and even in airline passenger baggage (see Brockerhoff et al. 2006; Haack 2003; Liebhold et al. 2006; McCullough et al. 2006; Tkacz 2002; Work et al. 2005). Pests that evade the inspection process may be accidentally introduced into forested areas. Potentially serious non-native pests that have recently made inroads into US forests include sudden oak death (caused by *Phytophthora ramorum* Werres et al.), first detected in 1995; the emerald ash borer (*Agrilus planipennis* Fairmaire), first detected in 2002; and the sirex woodwasp (*Sirex noctilio* F.), which was first detected in the United States in 2005 (Hoebeke et al. 2005; Ivors et al. 2006; McCullough and Katovich 2004). Internationally, noteworthy examples include the pine wood nematode (*Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle), native to North America but now established in eastern Asia and also reported in Portugal in 1999, as well as the red turpentine beetle (*Dendroctonus valens* LeConte), a secondary pest of pines in its native North American range that has caused widespread tree mortality in China since it was first detected in 1998 (Brockerhoff et al. 2006; Mota et al. 1999; Schrader and Unger 2003; Yan et al. 2005).

Typically, invasive pests like those just mentioned are introduced to a new country or region at one or no more than a few specific points. However, through both natural and human-mediated pathways (e.g., interstate transportation corridors), they may be subsequently dispersed at multiple spatial scales. In particular, human-mediated pathways may facilitate the rapid spread of a pest species to previously remote locations (NRC 2002; Chornesky et al. 2005). This multiple-scale dispersal pattern results in a clustered geographic distribution of the pest among landscapes (where a landscape is simply an area of some specified size that can serve as a sampling unit for a broad-scale spatial survey). Generally, the proportion of all landscapes that are invaded is low with a newly introduced pest, yet within an invaded landscape, the density within the area actually occupied or affected by the pest can be high. Given that conducting a complete census is cost-prohibitive, a probabilistic approach may instead be used to substantiate freedom from a pest species at an acceptable level of certainty. Such a probabilistic approach is consistent with ISPM guidelines for targeted surveys of recently introduced pests (FAO 1997).

Scientifically sound surveillance techniques are prerequisite to any broad-scale efforts to combat invasive species (Lodge et al. 2006; Rajan 2006). A well-developed surveillance system facilitates early detection, increasing the window of opportunity to initiate management measures (Rajan 2006; Venette et al. 2002). A primary objective of this paper is to document techniques that can be used to estimate the required sample size of surveys to substantiate freedom from non-native forest pests with known reliability. To accomplish this, we extend the one-dimensional methods of Cameron and Baldock (1998) to the spatial domain with vector data; chiefly, we develop a model to calculate the parameters necessary to estimate sample size. We provide an example to illustrate surveillance methodology by (*i*) defining the population of interest and sample frame, (*ii*) setting standards of statistical confidence and certainty, (*iii*) determining the optimal sample size, and (*iv*) using the sample size to create the detection survey scheme.

Methods

Our methodology is intended for cases where a recently introduced forest pest has been found in only a small portion of its estimated potential range. In general, invasions are unpredictable, making it difficult to accurately model their progress or ultimate extents (Kareiva et al. 1996). Nonetheless, analyses that focus on the simpler task of identifying areas with heightened invasion risk may inform the design of cost-effective survey programs for early detection (Andersen et al. 2004, FAO 1997; Kareiva et al. 1996). Forest pest risk maps (e.g., Downing et al. 2005; Kelly et al. 2007; Koch et al. 2006; Poland and McCullough 2006) are analytical combinations of spatial data from three categories that represent where a pest is most likely to be introduced and (or) established: host species distribution, climatic or other environmental constraints, and pathways of pest movement (Bartell and Nair 2004). Because of a lack of quality data, some risk maps omit one or more of these categories, but all serve the same basic purpose: to provide a relative risk rating for all locations within the geographic area of interest, thus indicating where resources for monitoring or other measures should be prioritized.

Risk maps have several applications, but here we use a risk map to define the population of interest (i.e., the at-risk forest area) and to construct the sampling frame based on a global sampling grid (White et al. 1992). However, we must also decide upon the appropriate sampling technique and required level of certainty before determining the required sample size (Cochran 1977). When considering the surveillance of invasive forest pests, two-stage samples are attractive because we expect the species of interest to cluster within the primary sampling units. That is, we expect the likelihood of species occurrence within primary sampling units to be more similar than the likelihood among primary sampling units. Therefore, to take advantage of this clustering, it makes sense economically to use a two-stage sample (Reilly 1996). Our aim is to use the global sampling approach developed by White et al. (1992) to construct our primary sampling units and develop appropriate techniques to determine the stage-two (within primary sampling unit) sample when the stage-two sample unit is a pheromone trap or similar device.

Two-stage sample size formula

Cameron and Baldock (1998) developed a probability formula to estimate the necessary sample size for two-stage sampling where the purpose of the survey is to substantiate freedom from disease at given levels of sensitivity and confidence. They developed their formula under the scenario of sampling animal herds over large areas where diseased animals are considered to cluster within a herd. Such clustering within a herd often occurs because the distribution of disease agents is unbalanced across the population. This is especially true with rare diseases, where the among-herd infection rate may be low, but within a diseased herd, the infection rate can be quite high. Their equation for the probability of observing at least one event (e.g., diseased animal) is

$$[1] \qquad P = 1 - \left[\phi_1\left(P(S_2^-|S_1^+)\right)^{n_2} + (1-\phi_1)\right]^{n_1}$$

where P is the probability of observing at least one event, or the level of certainty; ϕ_1 is the stage-one prevalence, or proportion of stage-one sampling units in which the event occurs, which is also the probability of selecting a stage-one sampling unit with the event; $P(S_2^-|S_1^+)$ is the conditional probability of failing to observe the event based on the second stage sample (S_2^-) given that the event does occur in the selected stage-one sampling unit (S_1^+); n_2 is the number of samples within each primary sampling unit; $(1 - \phi_1)$ is the probability of selecting a stage-one sampling unit where the event does not occur; and n_1 is the number of stage-one sampling units. Equation 1 can be rearranged to solve for the sample size required to stipulate freedom from an event

$$[2] \qquad n_1 = \frac{\ln(1-P)}{\ln\left[\phi_1\left(P(S_2^-|S_1^+)^{n_2}\right) + (1-\phi_1)\right]}$$

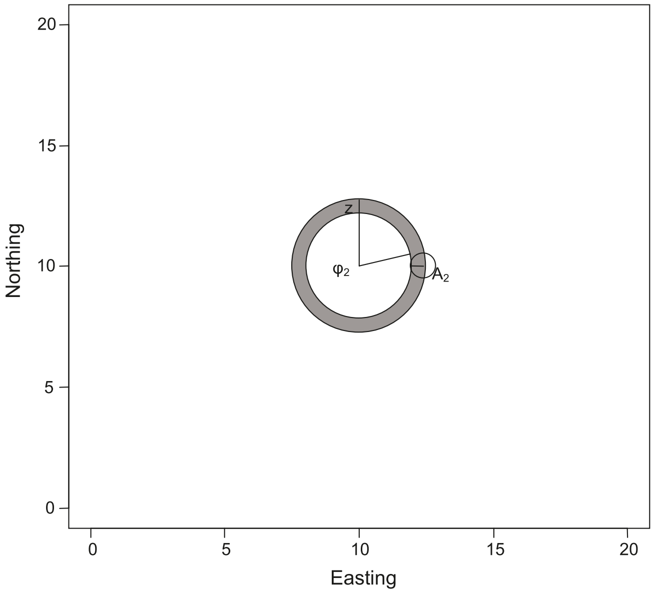
where P is the level of certainty specified a priori and ϕ_1 is specified a priori.

Cameron and Baldock (1998) developed eq. 2 to calculate the required sample size to substantiate freedom from disease in large animal herds. The approximation of $P(S_2^-|S_1^+)$ for large herd sizes was provided by Cannon and Roe (1982):

$$[3] \qquad P(S_2^-|S_1^+) = \left(1 - \frac{\phi_2}{N_a - \frac{n_2-1}{2}}\right)$$

where ϕ_2 is the stage-two prevalence or, in this case, the number of diseased animals in a herd (set a priori); N_a is the mean number of animals in a herd; and n_2 is the number of samples within each primary sampling unit; in this case, the number of animals sampled within the herd.

Fig. 1. The additional area (z) that should be accounted for when estimating $P(S_2^-|S_1^+)$ given the infected area (ϕ_2) and the trap area (A_2).



There are many combinations of n_1 and n_2 that will produce the required level of certainty, and there are often different costs associated with the stage-one sample and the stage-two sample (Clark and Steel 2000; Waters and Chester 1987). Cameron and Baldock (1998) suggested using Cochran's (1977) cost function to select the values of n_1 and n_2 that minimize the overall cost of the survey. The cost function takes the following form:

$$[4] \qquad C_{\text{total}} = C_1 \times n_1 + C_2 \times n_2 \times n_1$$

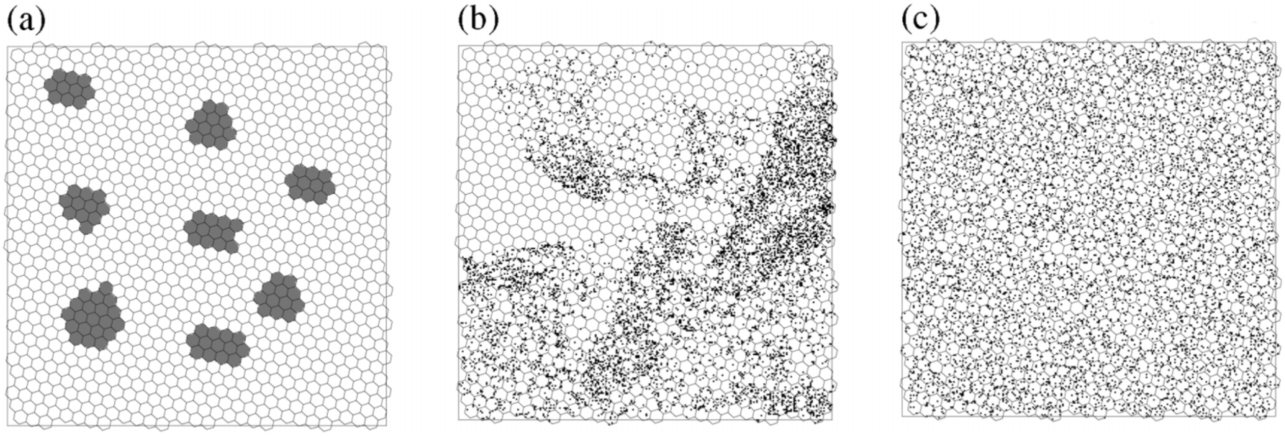
where C_{total} is the total cost of the survey; C_1 the cost of obtaining each stage-one sample; and C_2 is the cost of obtaining each stage-two sample. The combination of n_1 and n_2 that minimizes C_{total} is the optimal solution.

The formulations provided by Cameron and Baldock (1998) and Cannon and Roe (1982) are based on discrete counts and assume samples are drawn from an infinite population. In the case of invasive pest surveillance, changes must be made to adequately estimate the conditional probability $P(S_2^-|S_1^+)$outlined in eqs. 1, 2, and 3; this is due to the spatial nature of the sampling problem. Also, depending on how the stage-one sample is defined, the sampling fraction may be so large that assuming an infinite population is inadequate.

Adjustment to the stage-two probability

Rather than sampling individuals, a detection mechanism (e.g., a pheromone trap or similar device; hereafter, we use the term "trap") is typically used for pest surveillance, with the purpose of determining whether a pest is present in an area of interest. When traps are employed, the sampling problem becomes spatial in character, and subsequently, we

Fig. 2. Examples of the relationship between spatial pattern of risk and sampling fraction when tessellations are used to construct primary sampling units. In each case (Figs 2*a*, 2*b*, and 2*c*), we must draw $n_1 = 100$ primary sampling units with risk from N_1 primary sampling units with risk. The shading denotes risk, and each case has 10% of the total area at risk. In Fig. 2*a*, there are $N_1 = 100$ primary sampling units with risk; therefore, the sampling fraction is $n_1/N_1 = 100/100 = 1$. In Fig. 2*b*, there are $N_1 = 795$ primary sampling units with risk; therefore, the sampling fraction is 100/795 = 0.126. In Fig. 2*c*, there are $N_1 = 1000$ primary sampling units with risk; therefore, the sampling fraction is 100/1000 = 0.10.



must develop a new approach to estimate $P(S_2^-|S_1^+)$). In the two-dimensional case, the value of $P(S_2^-|S_1^+)$ is related to multiple parameters: the size and shape of the stage-one sample unit (A_1), the size and shape of the pest activity (i.e., the infested area) within the stage-one sample unit (ϕ_2), and the effective area of the trap (A_2). When the effective area of the trap is zero (i.e., a point) then $P(S_2^-|S_1^+) = 1 - (\phi_2/A_1)$.

If we consider the simple case, where A_1, A_2, and ϕ_2 are forced to be circles rather than arbitrary shapes, such that A_2 and ϕ_2 are completely contained by a sufficiently larger A_1, then

$$[5] \qquad P(S_2^-|S_1^+) = 1 - \frac{\pi(r_2 + r_{\phi_2})^2}{A_1}$$

where r_2 is the radius of A_2 and r_{ϕ_2} is the radius of ϕ_2. The logic behind eq. 5 is that, because the trap represents an effective area rather than a point, we must account for this additional area when estimating the probability of the trap being negative for presence of the pest when the stage-one sampling unit has actually been infested (Fig. 1). Functionally, eq. 5 extends the radius of the infested area by the radius of the trap's effective area, such that the trap can still be treated as a point for the probability calculation. In eq. 1, the stage-two sample is assumed to be drawn from an infinite population. We consider this assumption to be appropriate for eq. 5 given that A_1 will generally be substantially larger than A_2.

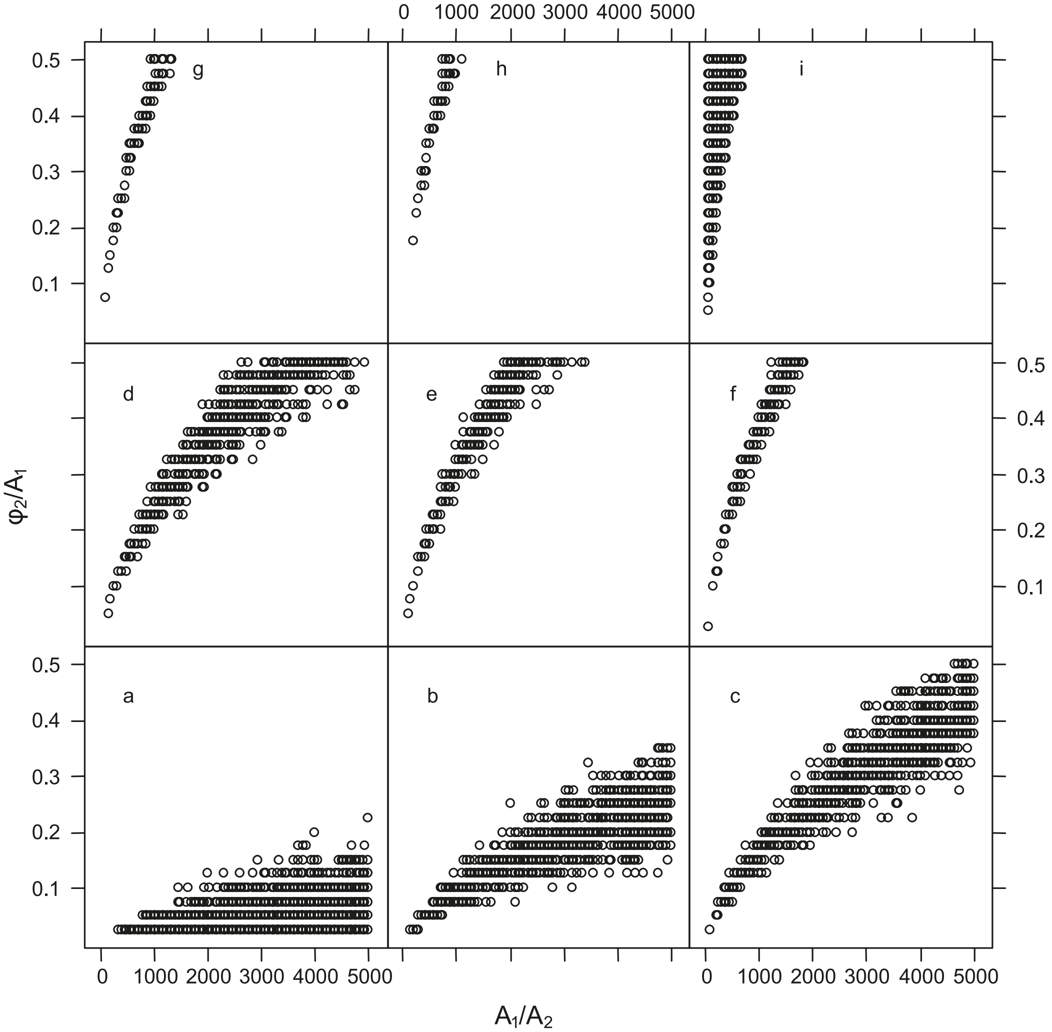
To confirm the validity of eq. 5, we used spatial simulation to estimate the distribution of $P(S_2^-|S_1^+)$. We performed simulations in which we varied the ratio of stage-one sample unit size to effective trap area ($A_1/A_2 = 50, 100, \ldots, 5000$) and the ratio of the infested area to the stage-one sample unit size ($\phi_2/A_1 = 0.025, 0.05, \ldots, 0.5$). Our simulations encompassed 2000 combinations of A_1/A_2, and ϕ_2/A_1. For example, to estimate $P(S_2^-|S_1^+)$ when $A_1 = 250$ km^2 (a circular stage-one sample unit with radius $r_1 = 8.92$ km), $A_2 = 1$ km^2 (a circular trap area, or stage-two sample, with radius $r_2 = 0.564$ km), and $\phi_2/A_1 = 0.05$ ($\phi_2 = 12.5$ km^2, an area with radius $r_{\phi_2} = 1.99$ km), we generated two random points. The first random point served as the centroid of a circle for A_2, and the second random point served as the centroid of a circle for ϕ_2. The circles for both A_2 and ϕ_2 were constrained to remain completely within A_1 and occurred with equal probability in A_1. When the distance between the center points of A_2 and ϕ_2 was less than or equal to the radius of the trap area (r_2) plus the radius of the infested area (r_{ϕ_2}), i.e., the two circles intersected, then the trial was considered a success. When the two circles did not intersect, the trial was considered a failure. In our simulation, there were 1000 trials and 200 replicates for each combination of A_1/A_2 and ϕ_2/A_1. For each replicate, we estimated $P(S_2^-|S_1^+)$ as the number of failures divided by the number of trials. For each combination of A_1/A_2, and ϕ_2/A_1, we adopted the mean of the 200 replicates as the simulated estimate of $P(S_2^-|S_1^+)$. For comparison, we also calculated $P(S_2^-|S_1^+)$ using eq. 5.

Adjustment to the stage-one probability

Cochran (1977) suggested that a finite population correction factor should be used when the sampling fraction (n_1/N_1) is >5%, where in this case N_1 is the total number of possible units from which the stage-one sample is drawn. Our goal is to use a global sampling grid (White et al. 1992) to construct our sample. This global sampling grid is a tessellation; when tessellations are used to define the stage-one sampling unit, the sampling fraction is related to the spatial pattern observed in the underlying risk map that defines the population of interest, i.e., the area at risk for infestation by a pest (Fig. 2). When the risk map displays a random pattern and <5% of the total area is at risk, then a finite correction may not be needed. However, if the pattern of risk has a high degree of clumping, or if >5% of the total area is at risk, then eq. 1 may be adjusted for sampling from a finite population without replacement.

When the sampling fraction is one (i.e., $n_1 = N_1$), eq. 1 simplifies to

Fig. 3. The difference between $P(S_2^-|S_1^+)$ estimated using eq. 5 and the corresponding simulated value (estimated – simulated), by ϕ_2/A_1 and A_1/A_2 in nine categories: (*a*) difference of ≤0.005; (*b*) difference of >0.005 and ≤0.01; (*c*) difference of >0.01 and ≤0.015; (*d*) the difference of >0.015 and ≤0.02; (*e*) difference of >0.02 and ≤0.025; (*f*) difference of >0.025 and ≤0.03; (*g*) difference of >0.03 and ≤0.035; (*h*) difference of >0.035 and ≤0.04; (*i*) difference of >0.04.



$$[6] \qquad P = 1 - \left[\left(P(S_2^-|S_1^+)\right)^{n_2}\right]^{N_1\phi_1}$$

However, in most situations, the sampling fraction will be less than one, and the hypergeometric distribution can be used to extend eq. 6 to a finite population framework. The hypergeometric distribution is a discrete distribution that is used to estimate the probability of observing a set number of successes and failures in a sample from a finite population without replacement. Based on the hypergeometric distribution, the probability of selecting x infested (and $n_1 - x$ uninfested) stage one sampling units is

$$\frac{\binom{N_1\phi_1}{x}\binom{N_1 - N_1\phi_1}{n_1 - x}}{\binom{N_1}{n_1}}$$

The probability of failing to detect the infestation (i.e., all traps are negative) in x infested stage-one sampling units is $(P(S_2^-|S_1^+)^{n_2})^x$. The probability that all traps are negative in the $N_1 - x$ uninfested units is 1^{n_1-x}. Subsequently, the probability of selecting x infested stage one sampling units (and $n_1 - x$ uninfested units) and all traps being negative is

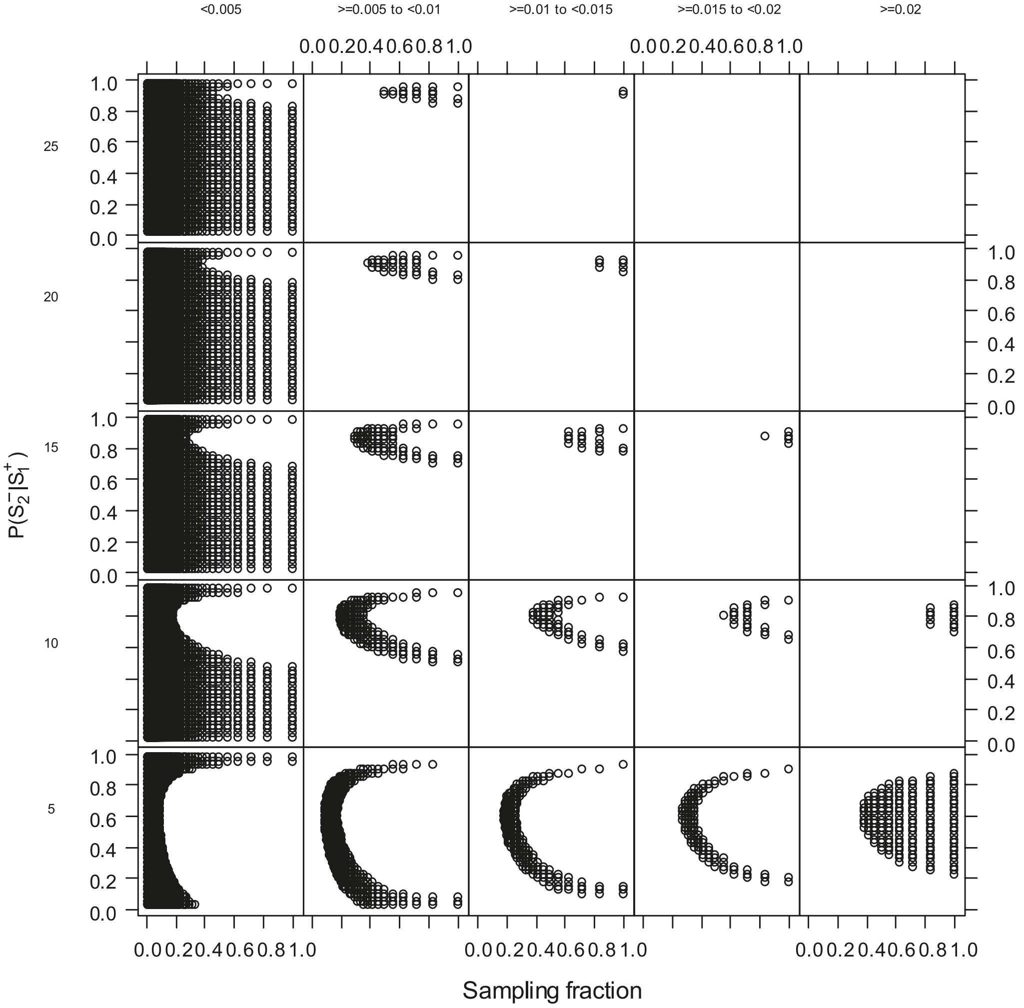
$$\frac{\binom{N_1\phi_1}{x}\binom{N_1 - N_1\phi_1}{n_1 - x}}{\binom{N_1}{n_1}}\left[\left(P(S_2^-|S_1^+)^{n_2}\right)^x \times 1^{n_1-x}\right]$$

The value of x is then restricted to possible values within the population between a lower bound, $l = \max(0, n_1 + N_1\phi_1 - N_1)$, and an upper bound, $u = \min(N_1\phi_1, n_1)$. The probability that all traps are negative for all possible outcomes in the stage-one sample is

$$\sum_{x=l}^{u}\left(\frac{\binom{N_1\phi_1}{x}\binom{N_1 - N_1\phi_1}{n_1 - x}}{\binom{N_1}{n_1}}\left[\left(P(S_2^-|S_1^+)^{n_2}\right)^x \times 1^{n_1-x}\right]\right)$$

and the probability of observing at least one positive trap is

Fig. 4. The difference between eq. 1 and eq. 7 in the probability of observing at least one positive trap, by $P(S_2^-|S_1^+)$ and sampling fraction, for five different values of $\phi_1 n_1$. Each row in the matrix is a different value of $\phi_1 n_1$ and is labeled to the left of each row. Each column represents a separate grouping of the difference, with the range for each group listed at the top of each column.



$$[7] \quad P = 1 - \sum_{x=l}^{u} \left(\frac{\binom{N_1\phi_1}{x}\binom{N_1 - N_1\phi_1}{n_1 - x}}{\binom{N_1}{n_1}} [(P(S_2^-|S_1^+)^{n_2})^x] \right)$$

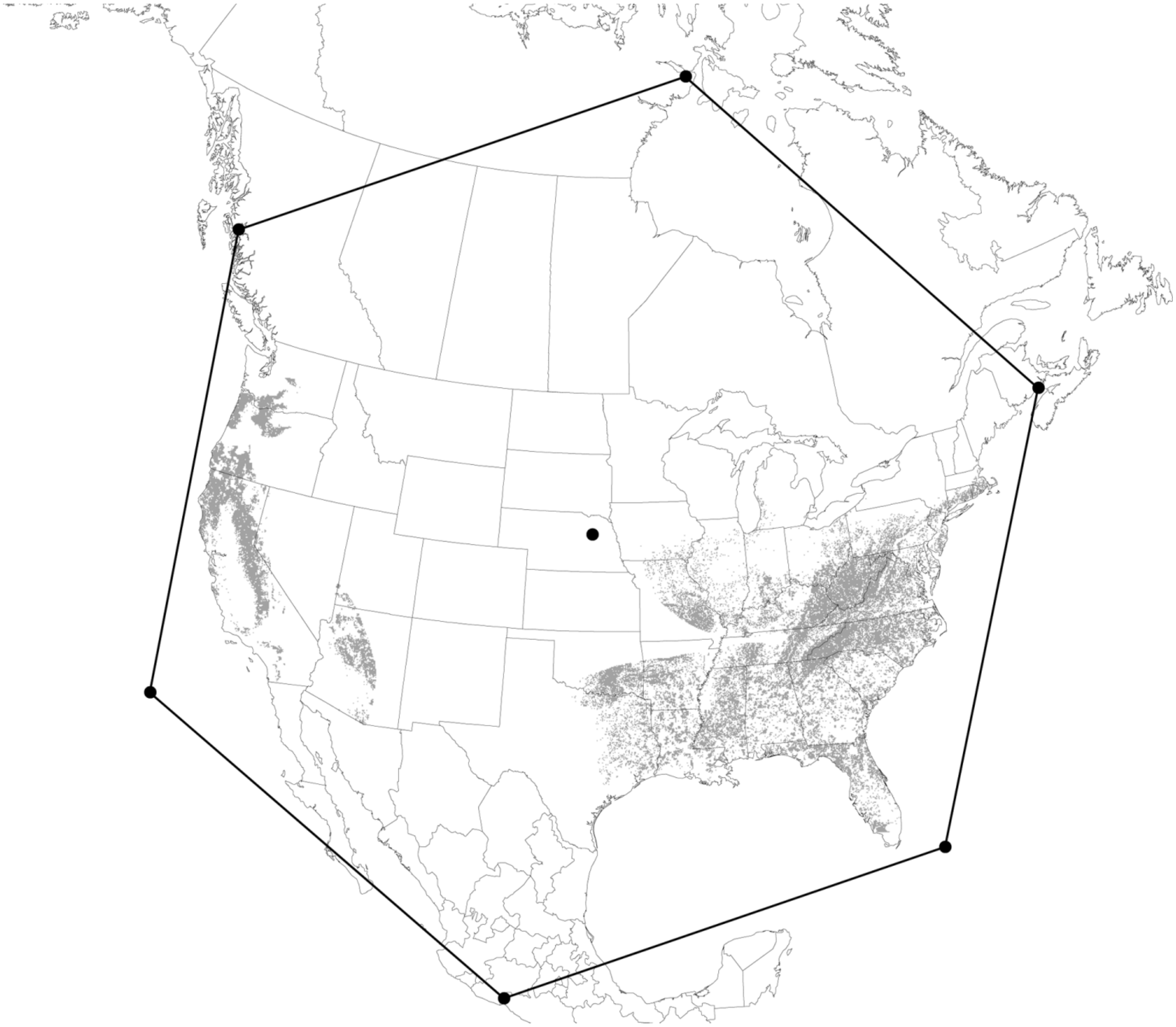
We examined the difference between eq. 1 and eq. 7 when calculating the probability of observing at least one positive trap. To accomplish this, we set n_1 = 1000 and used sequences of values for $P(S_2^-|S_1^+)^{n_2}$ (0.025, 0.05, …, 0.975), $\phi_1 n_1$ (5, 10, …, 25), and $N_1\phi_1$ ($\phi_1 n_1$, $\phi_1 n_1 + 1$, …, 500). We calculated the sampling fraction for each combination as $\phi_1 n_1 / N_1\phi_1$. We computed N_1 as n_1 divided by the sampling fraction and rounded to the nearest integer. The reason for calculating the variables as described was to force rounding to the nearest integer to occur only on N_1.

Results

Overall, estimates of $P(S_2^-|S_1^+)$ computed using eq. 5 were larger than simulated estimates (Fig. 3). The mean difference between the simulated and equation-based estimates was 0.0166 with a root mean square error of 0.025. The largest difference, 0.19, was observed when A_1/A_2 = 50 and the proportion of the stage-one sampling unit with the pest present was 0.5. The smallest difference, 0.000 05, was observed when A_1/A_2 = 4450 and the proportion of the stage-one sampling unit with the pest present was 0.025. Generally, the bias was larger when the trap was large compared with the area of the stage-one sampling unit (A_1/A_2) and when a large proportion of the stage-one sampling unit was infested (ϕ_2/A_1) (Fig. 3). When considering only those situations that are likely to occur when designing surveys at broad spatial scales, the bias is <0.005 (Fig. 3*a*).

We examined differences in estimates of *P* between eq. 1 and eq. 7 for several combinations of sampling fraction, $P(S_2^-|S_1^+)$, and $\phi_1 n_1$. Generally, the difference between the two estimates of *P* was <0.005 (Fig. 4). However, when the sampling fraction was, for instance, >0.3, $P(S_2^-|S_1^+)$ was set to a moderate value (e.g., 0.3–0.8), and $\phi_1 n_1$ was small (e.g., 5), the difference between the estimates of *P* was >0.02 (Fig. 4). Although the difference between the estimates of *P* was often small, these differences in *P* can have a substantial influence on n_1 when the target precision of the survey is high.

Fig. 5. The North American hexagon from the EMAP sampling grid and areas of risk (shaded) for a hypothetical forest pest.



Surveillance methodology example

White et al. (1992) developed a global Environmental Monitoring and Assessment (EMAP) sampling grid that serves as the basis for the US Forest Service Forest Inventory and Analysis Phase 2 (forest mensuration) and Phase 3 (forest health) surveys (Reams et al. 2005). The EMAP sampling grid was developed from a truncated icosahedron made up of 20 hexagons and 12 pentagons covering the planet, with one hexagon advantageously placed to cover North America (Fig. 5). A noteworthy aspect of the EMAP grid's configuration is that this hexagon can be systematically intensified, yielding a wide range of potential sample frames. This provides a straightforward framework for creating a systematic, hexagonal survey lattice. In this case, each hexagon created through intensification represents a stage-one sample unit that may be chosen.

Figure 5 displays a US risk map for a hypothetical nonnative pest that attacks oaks (*Quercus* spp.). Suppose we wanted to design a survey to substantiate freedom from our hypothetical pest outside its currently limited introduction area. More specifically, we want to determine whether the pest exists in >5% of the stage-one sample units (ϕ_1 = 0.05) at a within-unit prevalence (ϕ_2) of >50 km^2, with 90% certainty (P = 0.9). The effective area of our traps is 1 km^2 (A_2); based on the risk map, there are approximately 920 975 km^2 of forest area at risk (R_A). In practice, we use eq. 7 and vary n_1 and n_2 until the desired P is obtained. However, we must also have an estimate of N_1 and A_1 to apply eq. 7. For this example, we estimated these two variables using an iterative approach applied to the risk map in Fig. 5. The risk map was a raster spatial data layer where each cell was coded as either 0 = no risk or 1 = risk (outside of the US boundary = null), and each cell was 2.5 km × 2.5 km. We read the risk map into the R statistical package (R Development Core Team 2006) as a matrix with the same values and dimension as the geospatial data. R_A was the product of the number of cells at risk and the cell size (6.25 km^2). The total area (T_A) was the product of the number of cells (disregarding null values) and the cell size. For any value of n_1, the total number of stage-one

Table 1. Results from hypothetical example.

n_1	n_2	P	C_{total} ($)
859	1	0.900	515 400
588	2	0.908	499 800
484	3	0.900	532 400
421	4	0.906	568 350
381	5	0.911	609 600
346	6	0.908	640 100
317	7	0.900	665 700
303	8	0.900	712 050
290	9	0.915	754 000
271	10	0.900	772 350

Note: Power is based on eq. 7 and using eq. 5 to estimate $P(S_2^-|S_1^+)$ with n_1 primary sampling units and n_2 traps per primary sampling unit. Total cost (C_{total}) was minimized with n_1 = 588 and n_2 = 2.

sample units that would cover the United States (N_T) was calculated as $T_A \times n_1/R_A$. For simplicity, we considered each primary sampling unit to be a square where the length of each side (L), in units of number of cells from the original risk map, was the number of rows in the original risk map divided by $N_T^{0.5}$. A_1, in square kilometres, was then $L^2 \times 6.25$ km^2 and N_1 was the number of stage-one sampling units of size A_1 that contained risk from the original map. We solved eq. 7 for n_1 = 100 to 1000 and n_2 = 1 to 10, estimating N_1 and A_1 for each value of n_1, and selected the first n_1 which achieved our goal of $P = 0.9$ for each value of n_2.

To determine the optimal solution with respect to cost, we used a slightly modified version of eq. 3. The cost of traveling to a single stage-one sample unit to place (and eventually retrieve) a trap was US$400 ($C_a$). The cost of the trap itself and of hiring an entomologist to examine its contents was $200 ($C_T$). The cost of adding a second trap to an already-sampled stage-one unit was $50 ($C_{T2}$). The resulting cost function was

$$C_{\text{total}} = C_a \times n_1 + C_T \times n_2 \times n_1 + C_{T2}(n_2 - 1)n_1$$

The optimal solution was the values of n_1 and n_2 where the total cost was minimized. Based on our hypothetical costs, the optimal solution was n_1 = 588 and n_2 = 2, which had a total cost of approximately US$499 800 (Table 1).

We followed the procedures outlined in Coulston et al. (2008) to develop a survey grid based on sampling n_1 stage-one units. The first step was to estimate the intensification factor for the North American hexagon required to meet our objective of surveying 588 (hexagonal) units. We used the equation given by Coulston et al. (2008):

$$[8] \quad X = 5783883\left(\frac{n_1}{R_A}\right) = 5783883 \times 588 \times 920975^{-1} = 3693$$

where X is the estimated intensification factor for the North American hexagon and 5 783 833 is the coefficient from a nonlinear regression model that relates the estimated sample unit size to intensification factor.

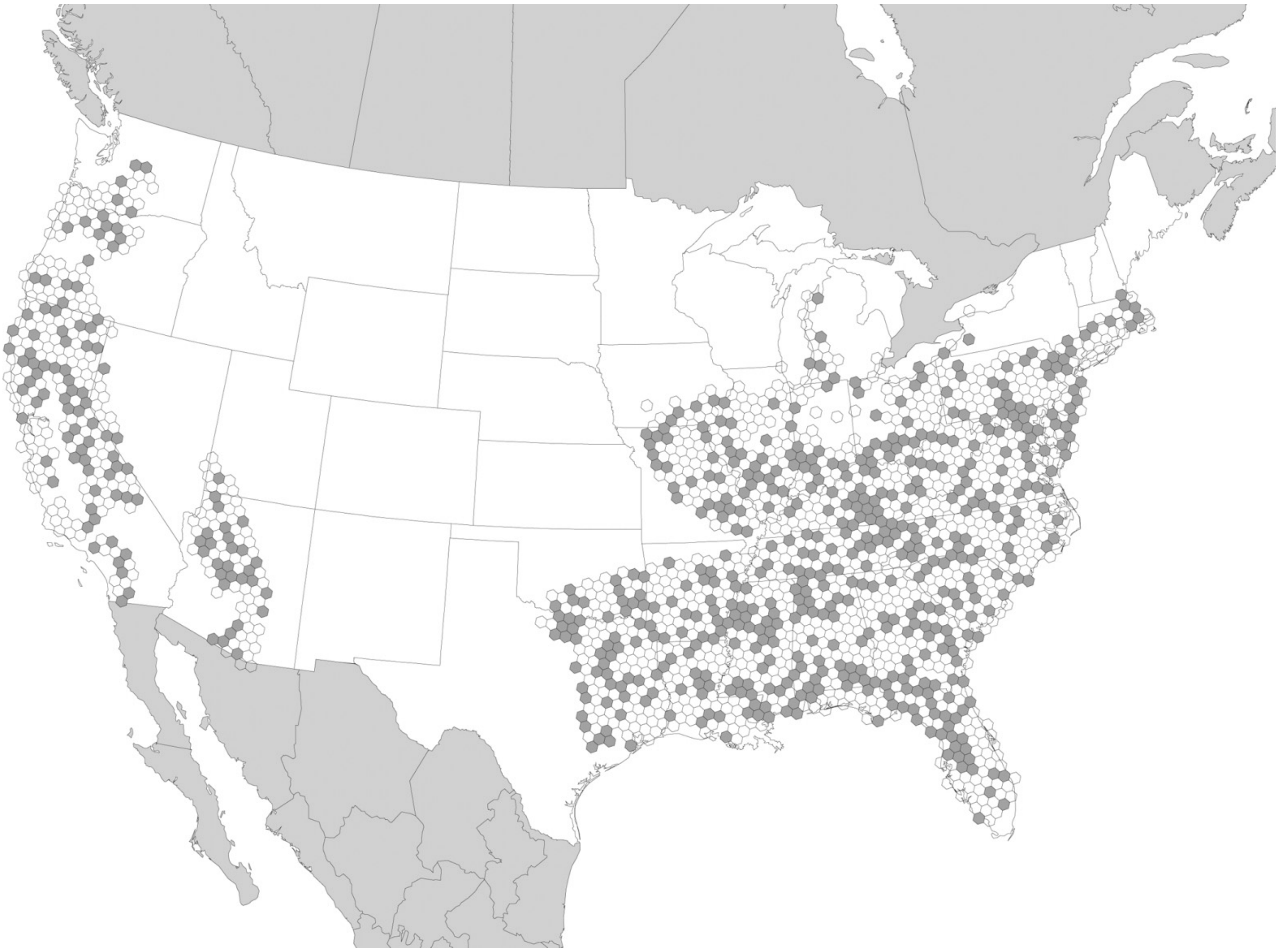
The geometric properties of the North American hexagon and its underlying triangular grid permit it to be systematically intensified by three, four, seven, or any factor sequence combining these numbers (White et al. 1992). Therefore, for computational purposes, the estimated intensification factor X must be rounded to the closest product of an eligible factor sequence. The closest possible product (and sequence) to our target X = 3693 was 3888 (3 × 3 × 3 × 3 × 3 × 4 × 4). Because risk maps generally have moderate to low spatial and thematic accuracy, we considered each hexagon that contains any risk to be completely at risk. Selecting all hexagons at risk yielded N_1 = 1820 total stage one sampling units. From this set, n_1 = 588 sampling units were selected randomly (Fig. 6). The value of A_1 was 1536.8 km^2. We verified the power of our sample design using eq. 7; the probability of detecting at least one positive trap was 0.916.

Discussion

Our chief objective was to develop a practical method for determining an appropriate sample size to substantiate freedom, at a specified level of confidence, from an invasive forest pest using a global sampling grid. Depending on the pattern of risk observed from a risk map, it may be appropriate to treat the stage-one sample as being drawn from an infinite population. However, we suggest that, if the stage-one sampling fraction is high or the desired precision is high, then eq. 7 should be used. Regarding the stage-two portion of the sample size equation, the equations presented by Cameron and Baldock (1998) for sampling animals in livestock herds may be straightforwardly translated to sampling trees in forested landscapes for forest diseases and some insect pests (see also Hall et al. 2005, 2007; Venette et al. 2002). However, in the case of certain mobile pests, a sampling approach based on discrete, count-based variables does not translate directly because the sample is an area (e.g., the effective area of a trap) rather than a number of individuals. As a result, it is necessary to extend the existing approaches to two dimensions. We have shown an analytical method (eq. 5) that applies for most broad spatial scale scenarios in which these techniques would be relevant; however, there are two scenarios where eq. 5 is not appropriate. This occurs when the ratio of the stage-one sample unit area to trap effective area (i.e., the stage-two sample unit area) is very low and when the infested proportion of a stage-one sample unit is high (Fig. 3). Regarding the second scenario, it is unlikely that one would still be trying to establish freedom from a pest if a large proportion of forested land has already been invaded by the pest. Anecdotal evidence of the pest's presence is likely to be substantial at that point. Regarding the first scenario, for most broad-scale surveys, the stage-one sample unit area will be much larger than the trap effective area so this situation is not particularly relevant for national-scale surveys. However, if either of the scenarios described above are applicable, a simulation approach should be used to estimate $P(S_2^-|S_1^+)$.

We have described a two-stage sampling approach that uses the relationship between (i) the number and area of stage-one sample units and (ii) the effective trap area to determine the optimum sample size for a given spatial domain,

Fig. 6. Final set of $n_1 = 588$ stage-one sample units (shaded). The sampling hexagons that were not selected are not shaded. $N_1 = 1820$ and the sampling fraction was 0.323.



provided that the statistical confidence and pest prevalence are specified a priori. As illustrated by our example, a typical population of interest might be the total forest area considered high risk for invasion by a forest pest. It is straightforward to extend this to a multistage sample by applying it to one or more additional risk strata. For instance, an analyst might want to determine appropriate sample sizes for each of three risk levels (high, moderate, or low) in a pest risk map; in such a case, the analyst could adopt different levels of confidence and (or) different levels of pest prevalence to reflect reduced risk in the moderate and low risk strata. As the precision of risk maps increases the use of multistage sampling framework will also likely increase. Increased precision of risk maps will allow for more precise delineation of the population of interest.

Issues of sensitivity and specificity are worth considering for any sampling protocol. As currently formulated, our approach assumes perfect testing; briefly, if the area infested by a pest falls within a trap's effective area, then we assume the pest is always detected, and by extension, that the stage-one sample unit in which the trap falls is positive for presence of the pest. More realistically, any detection process is imperfect, and so, there are possibilities of false positive and false negative results (Hughes 1999; Morrison et al. 2007; Venette et al. 2002). In the case of detecting a forest pest using traps, sensitivity is a more manageable concern, not least because it is likely impossible to calculate the probability of false positives and thus the specificity of a trap-level test. Cameron and Baldock (1998) addressed the issues of sensitivity and specificity by modifying the hypergeometric formula to account for probabilities of false positives and negatives in individual-animal disease screens and showed how these probabilities can be translated to herd-level sensitivity and specificity when calculating sample size. One avenue of further research is exploring an alteration of our analytical formula that would represent effective trap area not as a discrete area but as a circular continuum of confidence, such that confidence is 100% at the center of the circle and decays to zero at a specified distance from the center. Our reasoning is that, regardless of other environmental factors that might affect whether a pest ends up in a trap, the effectiveness of the pheromone or other attractant used in the trap will certainly decline with distance (Byers et al. 1989; Helland et al. 1984). Lure point transect sampling methods, which are intended to estimate the probability of an organism's capture based on distance from a lure, may be informative in this regard (Buckland et al. 2006).

The approach presented here adopts a key simplifying assumption with respect to spatial pattern: that the infested area in a given stage-one unit can be appropriately represented as a single circle. A circle is the most compact of all polygons in terms of perimeter/area ratio, but a pest is likely to infest a potentially complex and irregularly shaped patch of the stage-one unit or, perhaps, multiple disconnected patches. As pattern complexity increases, the perimeter/area ratio also increases. When the perimeter/area ratio increases, the amount of additional area that must be accounted for bcecause of the trap area also increases, and the probability of failing to detect the pest's presence decreases. In other words, the more complex is the pattern (and, thus, the higher the perimeter/area ratio) exhibited by an infestation in a given landscape, the more likely that a trap randomly placed on that landscape will intersect the infested area. Our circle-based approach gives a conservative estimate for the number of samples necessary to demonstrate freedom from a forest pest. However, a second avenue of future research is to extend our analytical formula to incorporate more complex infestation patterns and thus improve both realism and performance.

Our explanation operates largely from the perspective that a user, when calculating sample size, is focused on achieving a desired level of confidence given certain pest prevalence levels within and among forested landscapes. Nonetheless, as Cameron and Baldock (1998) suggest, if prevalence levels are low but the desired confidence is high, a large sample size and potentially expensive survey may result. This emphasizes the utility of a cost function that incorporates expenses such as trap placement as well as analysis of the trap contents through time in determining an optimal solution. However in some situations, the budget will be the limiting factor, i.e., the user will know how many samples or traps he or she can afford to place and analyze. In such cases, it is possible to back-calculate using our formulae to establish the detection power given the affordable sample size.

An overall aim of this research was development of methods, including optimum sample size determination, to generate broad spatial scale (e.g., national-scale) survey schemes for substantiating freedom for invasive forest pests. Our proposed approach is consistent with ISPM guidelines for targeted pest surveys (Food and Agriculture Organization 1995, 1998) and also addresses the recommendation of Lodge et al. (2006) to "use new technology to improve active surveillance of invasive species to increase the success of rapid response and eradication efforts" (p. 2045). Indeed, the techniques we employ provide tools for pest managers to construct surveys rapidly once a risk map has been created. Although other probabilistic methodologies for determining pest-free status have been proposed (e.g., Barclay and Hargrove 2005), our risk map based approach has the further advantage of translating directly into a spatially referenced, cost-effective pest surveillance strategy (Regan et al. 2006). Lodge et al. (2006) also recommended the development of more quantitative approaches to risk analysis. We support their recommendation and expect the cost-efficiency of survey grids to substantiate freedom from an invasive pest to increase with improvement in risk analysis and risk mapping. Our hypothetical pest example demonstrates how, once the sample size is determined, it is possible to use this number to intensify the EMAP hexagon for North America and generate a wall-to-wall tessellation of sampling polygons for the conterminous United States (see also Coulston et al. 2008). With respect to sampling other countries or regions, any of the 19 other hexagonal faces in the EMAP sampling grid may be intensified in a similar manner; some regularity would be lost when intensifying one of the 12 pentagonal faces, but it is relatively straightforward to shift the grid and optimally place a hexagon over any target area of interest (White et al. 1992).

Finally, the USDA Forest Service, in cooperation with other agencies, has recently produced national-scale risk maps for non-native forest pests that have been regularly detected at ports of entry yet never found to be introduced beyond port facilities. This sort of "preemptive" risk analysis has also been completed for pests threatening Europe, New Zealand, and other parts of the globe (e.g., MacLeod et al. 2002; Pitt et al. 2007). Such risk maps may also function as inputs for our sampling method, giving forest health managers a simple way to allocate resources for best determining the current status of these pests within their country or region of interest.

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