CHAPTER 6.3.

PROCEDURES FOR DECLARING PEST FREE STATUS

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SUMMARY

Procedures are presented for declaring an area to be "pest free" following an area-wide eradication programme against a population of an insect pest. These involve two probability models to deal with null trapping results, and a growth model to help verify that pests were no longer present when control actions were stopped. The two probability models are presented for a situation in which trapping for adult insects of the pest (and/or sampling for its immature stages) is ongoing, and for which the results are all negative. The models calculate the probability of such negative results if in fact insects were present. If this probability is sufficiently low, then the hypothesis that insects are present is rejected. The models depend on knowledge of the efficiency of the traps, and also the area of attractiveness of the traps. The possibility of a rebound of an incipient but non-detectable population that remains after control measures are discontinued is considered. Using a growth model, the rate of increase of an insect population that starts from one or two insects is examined. An example is given for tsetse flies — both means and confidence limits are calculated for a period of 24 reproductive periods after control has been terminated. If insects are disease vectors, it is also suggested that the progress of the disease be monitored to detect continuing transmission. This should be done in conjunction with a disease transmission model.

1. INTRODUCTION

Following an area-wide programme that has attempted to eradicate an insect population from a specific area, it is important to confirm the "pest free status". The criterion of programme success used is that the pest population has been eradicated from the target area (FAO 1996), a very different concept from that of the World Health Organization (WHO) regarding species eradication at a global level (WHO 2001).

There have been several successful insect pest eradication programmes, e.g. the New World screwworm *Cochliomyia hominivorax* (Coquerel) has been eradicated from North and Central America (Galvin and Wyss 1996; Wyss 2000a, b; Klassen et al., this volume; Vargas-Terán et al., this volume), and from Libya, using the sterile insect technique (SIT) together with wound treatments and quarantine measures (FAO 1992; Lindquist et al. 1992; Krafur 1998; Wyss 2000b; Vargas-Terán et al., this volume). Many tephritid fruit fly populations around the world have been similarly eradicated by integrating the SIT with other methods (Sproule et al. 1992; MAG/SAG 1995; Kuba et al. 1996; Reyes F. et al. 2000; Villaseñor et al. 2000; Koyama et al. 2004; Enkerlin, this volume) or the male annihilation technique (MAT) for *Bactrocera* species that respond to the strong attractant methyl eugenol (Steiner et al. 1970; Koyama et al. 1984; Hancock et al. 2000; Seewooruthun et al. 2000; Suckling et al. 2016).

If continued attempts to trap adult insects or detect immature stages after the termination of control actions are unsuccessful, this could be taken as evidence that the population has been eliminated from the target area, but with the realization that null results do not necessarily imply non-existence (Mitchell 1980; Richards and Tarry 1992; Clift and Meats 1997; Barclay and Hargrove 2005; Hargrove 2005;

Meats 2014). Low-density populations are often very difficult to detect. For example, even when trapping the mountain pine beetle *Dendroctonus ponderosae* Hopkins using pheromones that are known to be effective, if the species is endemic and at a very low density (e.g. one infested tree per 10 hectares), it may be almost impossible to trap beetles (Bartos and Schmitz 1998; L. Safranyik, personal communication).

Perkins (1989) listed seven scientific questions pertinent to eradication, including:

What accommodations need to be made for the limitations in the detection methods of ultra-low populations?

In interpreting null trapping (or other sampling) results, some knowledge of the detectability of the species (when using specified traps), as well as a measure of the area of attraction surrounding traps, must be available (Shelly et al. 2010). Once these measures are known, probability estimates of non-existence can be formulated from null results. An added complication arises if detectability itself is density-dependent, or if the area of attraction depends on weather or other factors.

The Food and Agriculture Organization of the United Nations (FAO) recognizes three categories of pest status: presence, absence, and transience (FAO 1999). Absence is determined by one or more of the following factors: (1) the lack of any records of its presence, (2) the action of eradication, (3) a shift in a pest's range, or (4) the interpretation of positive records as being only temporary. The three stages in the establishment of a pest free area are: (1) systems to establish freedom from pests, (2) measures to maintain freedom, and (3) checks to verify that freedom has been maintained (FAO 1996). However, since these guidelines are very general (applying to all pests — plant, animal, and microbial), they do not include methods specifically applicable to insects.

At present, insect population eradication is usually declared after the pest has not been detected for a reasonable period of time, which includes a considerable safety margin. The usual procedure is to continue control measures for several generations after the last insect was captured, then monitor the area intensively for additional generations (section 2.2.), and finally, if no more insects are detected, declare success. The New World screwworm in Costa Rica was declared eradicated about 1 year after the last fly was found (Galvin and Wyss 1996; Wyss 2000b, 2001). In Libya, sterile releases were discontinued 6 months after the last wild fly was trapped, and eradication was formally declared 8 months later (FAO 1992; Lindquist et al. 1992; Wyss 2000b). The Mediterranean fruit fly *Ceratitidis capitata* (Wiedemann) was considered eradicated in Chile after trapping (with a parapheromone and food attractants) for three generations without catching any wild flies (C. Flores and J. Gonzalez, personal communication). On the island of Kume, Japan, the melon fly *Zeugodacus cucurbitae* (Coquillett) was considered eradicated 6 months after the last capture, and following examination of 70 000 fruits (Iwahashi 1977). When the population of *Glossina austeni* Newstead was eradicated using an integrated approach on the island of Unguja, Zanzibar, sterile releases were maintained for six generations (after the last wild fly was captured) before eradication was deemed complete (Vreysen et al. 2000). The oriental fruit fly *Bactrocera (papayae) dorsalis* (Hendel) was introduced into Mauritius in 1996, and,

immediately after detection, eradication activities were begun. The last fly was trapped in May 1997, and trapping continued for 2 years, after which time eradication was declared (Seewooruthun et al. 2000). Following detection of the last wild fly, and a 4-month effort using the MAT and SIT, the oriental fruit fly was declared eradicated from the Mariana Islands (Steiner et al. 1970). One year (about six generations) after the detection of the last wild fly, the oriental fruit fly was considered eradicated from the Okinawa Islands using the MAT (Koyama et al. 1984). Subsequent to seeing the last individual, and trapping for 190 days and examining over 300 000 fruits, this same pest was judged eradicated on Amami Island (Ushio et al. 1982). In Perth, Australia, a large infestation of the Queensland fruit fly *Bactrocera tryoni* (Froggatt), after integrating the SIT, was declared eradicated 12 months after the last wild fly was trapped (Sproule et al. 1992). Using the MAT in north Queensland, Australia, against the oriental fruit fly *B. dorsalis*, area-freedom was claimed 12 months after the capture of the last fly, and after another 12 months eradication was claimed (Hancock et al. 2000).

Under the auspices of the FAO and the International Atomic Energy Agency (IAEA), a meeting was held in Vienna, Austria, in August 2003, to review the procedures for declaring areas free of tsetse flies and the trypanosomosis problem. The results of that meeting are described in section 2.1.3.

2. ASSESSMENT METHODOLOGIES

Three methodologies, suitable for addressing the question of the non-existence of insects in a given area, are presented. These include probability models to assess the results of trapping, a model of a rebounding population following suppression, and the use of disease transmission information in the case of pest species that are disease vectors. These methodologies are illustrated for tsetse flies *Glossina* spp. (Barclay and Hargrove 2005; Hargrove 2005).

2.1. Probability Models

2.1.1. Developments in Japan

Itô (1977) apparently made the first quantitative attempt to solve the problem of declaring a species to be absent from the area of interest or at minimal density, but since it was published in Japanese, it received little attention outside of Japan. Itô (1977) recommended the use of Wald sequential sampling to determine the minimum number of samples required (such as the number of fruits) in SIT projects for fruit flies. However, Kuno (1978) pointed out that the lower limit in sequential sampling need not be considered in eradication projects, so he recommended that the sample size (n) be based on either the binomial distribution or the Poisson distribution, giving:

$$n = \log(\beta) / \log(1-p) \quad (1)$$

for the binomial distribution, or

$$n = \log(\beta) / p \quad (2)$$

for the Poisson distribution, where n is the sample size (the number of examined sampling units), β is the error rate that is permissible, and p is the permissible proportion of infested sampling units. The symbol “ β ” is usually used for this probability instead of α , since it is related to Type II error rate rather than Type I error rate. The above sample size regulates the risk in the sense that the actual error rate is always smaller than β if the actual proportion of infested units is larger than p . Equations (1) and (2) are not effective if the total number of existing sampling units for survey is limited. Hence, in a later paper, Kuno (1991) recommended another equation:

$$n = N (1 - \beta^{1/Np}) \quad (3)$$

where N is the total number of sampling units. Kuno noted that the length of the sequence of zero captures increases nearly inversely with the size of the assumed p_0 ; p_0 is assigned to be some very small probability of pest occurrence that is deemed acceptable. Thus, for very small values of p_0 , the required length of the sequence becomes impossibly long. For example, if $N = 50\,000$, and the required value of $p_0 = 0.0001$, then the length of the sampling sequence yielding zero captures would need to be 30 095, even though this may be achievable if routine trapping is being done.

Yamamura and Sugimoto (1995) summarized the relation between equations (1), (2), and (3) as follows: let Y be the number of infested units in the sample, P be the actual proportion of infested sampling units, N be the total number of sampling units, and n be the sample size. Then one of the criteria used in the Japanese quarantine procedure is that

$$Pr(Y = 0 | P = p_t) < \beta \text{ for all } p_t \text{ satisfying } p < p_t \leq 1.$$

The probability $Pr(Y = 0 | P = p_t)$ is most precisely expressed by a hypergeometric distribution:

$$Pr(Y = 0 | P = p_t) = (N - N p_t)! (N - n)! / N! (N - n - N p_t)! \quad (4)$$

The minimum number of samples, n , which satisfy the above condition for a given set of p and β , can be given approximately by a simple formula (Japanese Industrial Standards Committee 1956):

$$n = (N - (Np - 1)/2) (1 - \beta^{1/Np}) \quad (5)$$

The n calculated by the above formula is slightly larger than the exact n for given β , N and p . In that sense, the above approximation is a slightly conservative approximation. We can use the more conservative approximation given by equation (3) if p is sufficiently small. If the sampling proportion n/N is sufficiently small, we can use another conservative approximation given by equation (1) based on the binomial distribution. If both p and n/N are sufficiently small, we can use a much

more conservative approximation given by equation (2) based on the Poisson distribution.

2.1.2. *Developments in Australia*

Clift and Meats (1997), using risk management software and trapping data up to May 1997, established criteria for 99% probability of localized eradication. Except for this last report, no probabilities were presented in any of the previous examples of eradication in Australia. Most authors simply did not provide the criteria on which eradication was judged, e.g. Schwarz et al. (1989).

An operational protocol, previously established in Australia (Clift and Meats 2004), specified that eradication could be claimed three generations (based on degree-day models) plus 28 days after the last fly was trapped. If one gets negative results, normally after three generations in some programmes (Tassan et al. 1983), one rejects the null hypothesis that flies are present. However, trapping records from a *B. dorsalis* campaign indicated that when trapping at only one trap per 1.5 km², 12 weeks of successive zeros could occur when flies were still present (Clift and Meats 1997; Clift et al. 1999). In addition, if there is a residual population and it is too small to be easily trapped or detected in fruit, then it will naturally increase when control stops, and so it becomes more detectable over time (Barclay and Hargrove 2005). In insects, such as tsetse, that have a slow rate of reproduction, this requires a long period, whereas for fruit flies, that have a much higher reproductive rate, any remnant population should soon become detectable (McInnis et al. 2017). The analytical methods of Meats and Clift (2005) represent a quantitative refinement of the ad hoc code of practice previously in use.

In countries such as Australia, Chile, Mexico, and the USA, cases of the eradication of spot infestations of pests (such as the Mediterranean fruit fly) in areas normally claiming area-freedom are much more common. Such eradications are virtually routine, and are not reported in the scientific literature (Suckling et al. 2016). Codes of practice for such procedures are also unpublished, and details differ according to both the country and pest involved. Meats et al. (2003) reviewed the codes of practice current in Australia for the Mediterranean and Queensland fruit flies, examined 25 years of data from spot eradications of each species, and calculated the radii of the areas of infestation where the probability of exceeding such radii was equal to probit 9 (containing 99.9968% of probability). If zones of area-freedom are to be established for other species, similar codes will be required. Obviously, it would be desirable if there were methods, with a pre-determined degree of probability, to calculate the radius of an affected area (where area-freedom would be suspended) and the length of the no-detection period needed to reinstate area-freedom within the previously calculated radius.

Clift and Meats (2004) and Meats and Clift (2005) assessed the probability of eradication of fruit flies in Australia by computing the probability that the fly population is below a certain density; this density can be adjusted to represent the critical density for population persistence, below which eradication would occur via the Allee effect (Allee 1931; Yamanaka and Liebhold 2009), whereby the rate of population growth declines or even becomes negative when the population declines below a given threshold (the Allee threshold). The number of traps and the length of

trapping time can then be computed knowing the maximum acceptable density, somewhat similar to the approach of Kuno (1991). The method of Meats and Clift (2005) involved a probability model using the concept of catch per trap per week, called c_{tw} , where t is the number of traps and w is the number of weeks. They defined

$$m = c_{tw} tw. \quad (6)$$

The zero term of the Poisson distribution was then used to calculate the probability, P_0 , of a zero catch with time as

$$P_0 = \exp(-m) \quad (7)$$

Then, to calculate the number of weeks with zero catches to achieve a critical value of P_0 (e.g. 0.01, 0.001, etc.), one forms

$$w = (-\ln P_{0,crit})(c_{tw} t) \quad (8)$$

This allows the specification of a lower catch limit, c_{tw} , that signifies a pest density that is below the Allee threshold; then the population can be left to decline to zero by itself without any additional control being required.

In a comprehensive review of the potentials and limitations of trap arrays for fruit fly detection, Meats (2014) examined the efficiency of detection systems, required spacing of traps, surveillance for resurgence following eradication attempts, effects of weather and insect age on trappability, and expectation of success in eradication and the ability to declare eradication. The efficiency of existing programmes in Australia and California is compared with recommendations for trap spacing. In addition, considerations of generation time, as predicted by heat accumulation and its use in predicting phenological events, are reviewed.

2.1.3. Results of 2003 FAO/IAEA Meeting on Tsetse Flies

The following probability models are based on trapping (or other method of sampling) with zero results, while assuming that insects are present. The models then give the probability of a zero catch for this assumption; if the probability is sufficiently low, one can reject the hypothesis that insects are present. For simplicity, all sampling systems are referred to below as “traps”. Techniques for declaring that eradication has occurred are nearly the same as those for a declaration that an infestation has not yet occurred (Yamamura and Katsumata 1999).

Two models are presented: (1) local sampling involving one trap or one group of traps (suitable for spot infestations), and (2) area-wide sampling (suitable for an area-wide eradication programme involving either an established pest or a large outbreak). Both models involve sampling a population that is close to elimination. Either approach can be used, and the results should be rather similar; when residual population sizes are very low, the models converge (Barclay and Hargrove 2005; Feldmann et al. 2018).

For an insect to be caught in a trap on a given day, the following conditions must be met:

- A trap must be operative in the vicinity of the insect.
- The insect must be active.
- Given the above, the insect must succeed in finding the trap and be captured by it.

Local Sampling with One Trap. Regarding the probability of a zero catch in each of a number of traps, consider a single trap and the “circle” (area) of attraction around it, within which the probability of catching a given insect with a given trap during one day is σ , called the detectability; the probability of not catching a given insect is $1 - \sigma$. This assumes that detectability is constant within the circle; Meats (2014) has pointed out that attractiveness of baits falls off with distance, so the detectability might better be treated as a mean over the circle of attractiveness. If there are k insects in the “circle”, the mean number caught per activity period is $k\sigma$. If there are k insects present, and if the insects are caught independent of each other, then the conditional probability of catching no insects during an activity period (or sampling period) is:

$$p(0|k) = (1 - \sigma)^k \quad (9)$$

The probability of both zero catch and a given number of insects being in the circle is $p(0 \cap k) = p(0|k) f(k)$, where the symbol $\cap$ refers to “and” or conjunction (Parzen 1960), and $f(k)$ is the probability of k insects being present. The conditional probability of no catch, given that there is an undetermined positive number of insects present, is:

$$p(0|k > 0) = \sum p(0|k) f(k) = \sum (1 - \sigma)^k f(k), \text{ where the sum is for } k \geq 1 \quad (10)$$

This equation could be used to construct probabilities of a zero catch, assuming that there are insects present near the trap. The problem arises in stipulating a distribution for $f(k)$, called the prior distribution on k .

Since the choice of a particular prior distribution is arbitrary unless there is some information about it, and since the probability given the presence of one insect has an easy closed form solution (i.e. $p(0|1) = 1 - \sigma$) and is more conservative, the latter probability is used in the development below (Barclay and Hargrove 2005).

The probability of a zero catch, given that there is one insect present, is $p(0|1) = 1 - \sigma$, so the probability of a succession of n zero catches on n independent sampling occasions is

$$P(0) = (1 - \sigma)^n \quad (11)$$

and this is true for each trap. For example, if the hypothesis (that there are pests present) is to be rejected at the $\beta = 0.01$ level, and if σ was 0.1, then the number of trapping days, n , needs to be such that $(1 - 0.1)^n \leq 0.01$. This can be found using the

equation: $n = \log(0.01) / \log(0.9) = -2.0 / -0.0458 = 43.7 \approx 44$. More generally, the equation is:

$$n = \log(\beta) / \log(1 - \sigma) \quad (12)$$

where β is the chosen rejection level. The base of the logarithms is immaterial, as long as both logs are of the same base. Once the rejection level has been chosen, and the value of detectability, σ , is known, the required value of n can be computed easily (however, the number of trapping days should be linked to the generation time under defined climatic conditions).

SAMPLING FRACTION OF POPULATION. Each trap has an area of attraction such that, within that small area, the probability of catching a given insect approximates the average detectability. If the number of traps is not sufficient to cover the whole area, and therefore the sum of the areas of attraction is less than the area to be evaluated for “pest free status” (called the “assessment area”), then one of two scenarios may occur. If the pests are sufficiently mobile so that they move in and out of areas of attraction in their normal daily or weekly movements, then the detectability is simply reduced, compared with the situation in which they are in the area of attraction all of the time. Alternatively, the traps could be moved from day to day or week to week, so as to cover the assessment area; then the detectability would similarly be reduced. Assuming that every insect spends roughly the same amount of time in areas of attraction to traps, then the detectability will be reduced by the sampling fraction. If the sum of the areas of attraction to traps is a fraction f of the assessment area, then the average detectability will be σf . In that case, the criterion becomes:

$$P(0) = (1 - \sigma f)^n < \beta \quad (13)$$

and solving for n :

$$n = \log(\beta) / \log(1 - \sigma f) \quad (14)$$

The size of the area of attraction will be crucial to the calculation of the sampling fraction, and this area may depend on weather, since odour plumes from lures in traps will vary in size with wind speed, and may also vary with topography, season, competing natural stimuli, and surrounding vegetation.

Area-Wide Sampling. Another way of looking at the problem, which leads to the same kind of result, is to consider area-wide or total population sampling, covering the whole assessment area. Having selected one or more sampling systems, the next problem is to decide on the required intensity of sampling, and for what period of time, before a series of zero catches can be interpreted as indicating eradication at some specified level of probability.

The parameters are defined as follows:

- A Area sampled (km^2), assumed to be closed to immigration and emigration (either naturally or through the maintenance of a wide-enough barrier)
- k Total number of surviving pest insects, assumed to be randomly distributed in A
- σ Trap efficiency (detectability), i.e. the conditional probability that an insect is caught by a given trap, given that there is only one trap present in a 1 km^2 area containing the insect, and given that the insect is active
- s Number of traps present in all of A
- n Number of days that each trap is operated

If there are s traps in the assessment area deployed for n days, and if s is sufficiently small that the traps act independently of each other, then the probability ($C'(k, s, \sigma, n)$) that none of the traps catches any of the k surviving insects is:

$$C'(k, s, \sigma, n) = \exp(-s n \sigma k / A) \quad (15)$$

assuming that the capture probability is identical for all insects, and is independent of time t (Hargrove 2003). The result of interest is the function relating to the probability, $p(0)$, of observing a sequence of zero results, if in fact insects are in the assessment area:

$$p(0) = \exp(-s n \sigma \rho) \quad (16)$$

where $\rho = k/A$ is the population density, and other symbols are as defined above. The objective is to know when a series of zero catches is sufficiently long that the null hypothesis of the existence of pests at the assumed level can be rejected. For example, for the probability of a sequence of zero catches in the presence of insects to be below 0.01, then it is required that:

$$\exp(-s n \sigma \rho) < 0.01 \quad (17)$$

from which

$$-s n \sigma \rho < \ln(0.01) = -4.605 \quad (18)$$

One can solve for one of the variables in terms of the others that are known. For example, if t is determined, σ is known, and ρ can be surmised, then

$$s > 4.605 / n \sigma \rho \quad (19)$$

When this condition is met, the required probability has been achieved. If this calculation leads to an unreasonably large number of traps being required, then a different criterion may be considered. The value of s obtained from the inequality above assumes a risk level of 0.01 in concluding that no insects are present, and this is shown in Fig. 1 for various values of the number of traps used (s) and number of insects present (k).

Figure 1. Probability of catching a single tsetse fly in a small remnant population (numbers in body of figure) in an area of 8000 km² as a function of number of traps used and duration of trapping (when using a trap with efficiency of $\sigma = 1\%$). (Figure from Barclay and Hargrove 2005, reproduced with permission.)

It must be emphasized that the calculated number of traps (s) is independent of the size of the assessment area to be controlled (unless the area is very small). However, with a very large area, there is a greater probability that “pockets” of insects still exist (where the control treatments and/or released sterile males are locally overwhelmed), and these pockets must be identified and treated accordingly (Shiga 1991).

As a simple example of the application of the inequality (19) above, consider the insecticide aerial-spraying operation (sequential aerosol technique (SAT)) against tsetse flies in Botswana (Okavango Delta, about 16 000 km²) (Allsopp and Phillemon-Motsu 2002; Tsetse News 2002), where apparently the last fly was caught on 30 August 2002, although very few traps had been installed in the Delta. Possibly the population of *Glossina morsitans centralis* Machado was actually eradicated in the SAT-treated area. However, suppose that 100 insects survived the sprays, what chance would there be of catching one of them? If the insects were distributed at random, 10 traps were deployed for about 3 months (90 days), $\sigma = 0.01$, and using inequality (19), the probability of catching at least one insect is $C(k, s, n) \approx 0.06$. Thus, with this low level of trapping, there is a 94% chance that detection of the presence of insects will fail. To be 90% certain that at least one insect is detected (given that 100 insects were present), the application of inequality 19 suggests that more than 400 traps must be deployed for 3 months. To be 99% certain, this figure rises to more than 800 traps. Note that these figures assume a

rather even distribution of traps. Deploying traps for a longer period, such as 1 year to include all seasons, may permit a reduction in the number of traps. Spreadsheets, that incorporate a variable number of trapping days, are available to calculate the minimum number of traps required. (It is important to be aware of the possible existence of insects in places that were avoided or ignored by field personnel, substantially reducing the chances of detecting a residual population.)

In inequality 19, ρ must be surmised; the size of any residual population is not known. To some extent, this uncertainty is balanced by the fact that, if there is a residual population and it is too small to be easily trapped, naturally over time it will increase since it is no longer under control, and it will become more detectable; this is the subject of the next section.

2.2. Incipient Non-Detectable Populations

If control actions have reduced the pest population until very few insects are present, so few that they cannot be detected by trapping, then when control is terminated natural reproduction will cause the population to increase if the population is not below the Allee threshold. How long will it take for this small population to increase to a detectable level? (It is assumed that surveillance trapping will continue or be increased (detection trapping/sampling) until there is a decision regarding the “pest free status” of the area.) The population can be modelled by a simple equation:

$$N_{t+1} = \lambda N_t \quad (20)$$

where λ is the rate of increase each generation, and N_t is the population size at generation t . Starting with a very small initial population of size N_0 (following termination of the eradication activity), the size of the population t generations later would be $N_t = N_0 \lambda^t$. When t is large enough for the population to have become easily detectable, but continued trapping still yields no insects, then a declaration of “pest free status” can be made. In calculating this critical value of t , allowance must be made for dormant or non-growing periods when equation (20) above does not apply. In addition, the time covered by t generations must be adjusted based on available pest-specific degree-day models to make allowance for slow-growing periods during unfavourable climatic conditions. Furthermore, before such a declaration could be made, the allowance of an adequate time-buffer is needed to permit sufficient time to elapse for the population to increase to perhaps 10 times the minimal detectable level. Since equation (20) is deterministic, and events in nature usually involve random elements, reasonable lower limits of λ should be used, not mean values (often used in calculations of ordinary population growth). This is illustrated below by examples from fruit flies and tsetse flies (which have much lower rates of increase than fruit flies or most other insect pests).

2.2.1. Observed Populations of *Bactrocera dorsalis* in an Eradication Context

Clift and Meats (1997) described the trap catches obtained during a population resurgence of *B. dorsalis* part-way through an eradication programme in tropical northern Queensland, Australia. In discussing localized extinction and reinfestation

of various areas within the pest-quarantine area, they noted intervals of up to 12 weeks between catches, and with the data available were unable to define localized extinction. After a declaration of eradication in 1999, subsequent data indicated that at least 16 weeks (favourable for the development of a fruit fly population) were needed to attain confidence that there were no flies in the area. Simulation data for 16 weeks were consistent with this result (Clift and Meats 2004). In practice, after 12 months of no catches under continued control, area-freedom was claimed, and after another 12 months with no catches and no control procedures applied, eradication was claimed. Throughout this interval, a 1 km grid of traps, with efficiency of at least 10%, was maintained over an 8000 km² area, and traps were checked at least weekly.

2.2.2. Monte-Carlo Simulation of Population Growth of Tsetse Flies

Females give live birth to a larva about every 9 days (Hargrove 1994), which then pupates, and about 30 days later an adult emerges (Phelps and Burrows 1969a, b). Assuming this rate of reproduction, and the least detectable population (one gravid female), a Monte-Carlo simulation was performed to compute the mean population numbers after each of 24 reproductive periods (i.e. a simulation length of about 1 year, with the first period including the pupal maturation and the rest following at about 2-week intervals), assuming that gender determination of larvae is a random phenomenon, and that at meiosis males and females are equally likely. At each reproductive period in this simulation, a decision was made (on the basis of a random number) for each adult gravid female (whether her offspring was to be male or female), and then the new numbers were tallied. This was repeated for 10 000 24-reproductive growth periods.

Mean values for the 10 000 simulation runs were calculated for each of the 24 reproductive periods. A cumulative frequency distribution of population sizes was computed for each reproductive period, and the lowest 1% of the 10 000 runs was noted. Population values bounding these proportions for the 24th reproductive period are shown in Table 1 (the bound for the proportion 0.01 is the upper bound of the first percentile). Four mortality values for adult flies were used (daily mortality of 0, 0.5, 1, and 2 %). If these are accumulated over a 2-week period, they translate into 14-day survivorships of 1.00, 0.93, 0.87, and 0.75, respectively, and these were used in the Monte-Carlo simulation. Since tsetse has a reproductive rate of half a female every 2 weeks (or 10 days, etc.), the fertility rate for 2 weeks is 0.5. Hargrove (1988) suggested that a tsetse population could not sustain 4%-added daily mortality regardless of the amount of density-dependence in its natural mortality.

Table 1 shows that, with 1% daily mortality, the mean number of adult females (after 24 reproductive periods) resulting from one gravid female reached almost 711, and one would expect that most of them would still be within some local common area, if it were suitable. Since these population numbers could vary widely, effective lower limits of the population were established (for the numbers below which it is expected that the total female population would be in 1 out of 100 cases) (Table 1). At this point, the questions become, "What is the minimum detectable density of flies?" and "What is the maximal tolerable error level?" Once these questions are answered, an appropriate waiting period can be determined.

The proportions of the 10 000 simulation runs that by chance ended in population extinction are shown in Table 2 (for the conditions used). Most extinctions occurred during the first five reproductive periods.

Table 1. Number of tsetse flies (means and 1% confidence lower values) resulting from 10 000 simulated growing populations after 24 reproductive periods starting with one gravid female or with one male and one female

Initial population		Survivorship per reproductive period			
		1.00	0.93	0.87	0.75
One gravid female	Mean	7480	2176	711	65
	Below 1%	830	1	1	1
One male and one female	Mean	11 211	3397	1157	112
	Below 1%	907	217	51	2

Table 2. Proportions of 10 000 simulated growing tsetse populations that went extinct during 24 reproductive periods (table from Barclay and Hargrove 2005, reproduced with permission)

Initial population	Survivorship per reproductive period			
	1.00	0.93	0.87	0.75
One gravid female	0.00	0.14	0.26	0.52
One female, one male	0.00	0.08	0.16	0.36
Two gravid females	0.00	0.01	0.04	0.18

2.3. Monitoring Vectored Infections

If pests are difficult or impossible to detect at very low density, but nevertheless act as vectors of a disease, then, in addition to surveillance trapping, one supplementary method of detection is to use antigen tests and assess if the prevalence of vectored infections is decreasing (Delafosse et al. 1996). An epidemiological model using a zero-biting frequency could be applied to test the hypothesis that insects are absent (but bites from non-infected insects would not be monitored by this procedure (Dyck et al. 2000)). The models of Rogers (1988) or Baker (1992) could be used for tsetse, assuming that the insect density is zero. Infection rates in hosts should decrease with time. Also, if insects are truly absent, new host recruits should not be infected. A similar approach was used in the UK to decide if a warble fly (*Hypoderma* sp.) had been eradicated. In addition to trapping warble flies, blood sera from cattle were examined for antigens to the warble fly (Richards and Tarry 1992), and actually

these blood antigens were detectable for several years after the last capture of a warble fly in a trap.

However, as this method has problems associated with it, it should usually be used only to provide additional information. The vector population may be too low for effective transmission, in which case the disease would decline even in the presence of a small vector population. On the other hand, diseases that do not kill the host may linger for a long period in the population after the vector is no longer present. Also, in antigen tests, false positives and false negatives are possible, and disease symptoms may be present without the real presence of the disease (Tazé and Gruvel 1978), giving the impression that eradication of the vector is not yet complete. Nevertheless, if the vector species is difficult or impossible to trap, this method may be the only one available.

2.4. Summary of Requirements for Models

- Select sampling methods that will sample all individuals and stages of the population.
- Determine the detectability of each sampling method; to obtain good estimates, this is best done before the eradication programme begins.
- Determine the range of attraction for each trap and other sampling method used.
- Determine the fraction of the assessment area that is actually being sampled (product of the number of traps and the area of attraction of each trap, divided by the total assessment area).

3. ACTIVITIES BEFORE, DURING, AND AFTER ERADICATION PROCEDURES

3.1. Pre-Eradication Activities

Before embarking on an eradication programme for any insect pest, a variety of actions must be taken and information collected. These include the acquisition of scientific background information, and also the creation of a plan for these activities. This information will be of value for monitoring and surveillance activities during, and subsequent to, the eradication programme. At least one, and preferably several, sampling techniques suitable for each of the pest species must be available (Itô et al., this volume; Vreysen, this volume). Costs and logistic difficulties associated with each trap and other sampling method should be known.

3.1.1. Data Requirements and Complicating Factors

Choice of Risk Level. It is impossible to conclude with complete certainty that a population has been eradicated. The best that can be achieved is to state that the probability, that observed zero or null trapping/sampling results are consistent with the presence of insects, is sufficiently low to reject the hypothesis of insects being

present. For this hypothesis-testing approach, a level of probability (the β or type II error level) must be specified; thus, when the probability of null results from trapping falls below this level, the hypothesis that insects are present will be rejected. The choice of β is arbitrary, as it is in ordinary science where the rejection level, α , is usually 0.05. However, if human and animal lives, or a large investment in an eradication programme, are at stake, this level is probably too liberal. On the other hand, a lower level of β means that more trapping is needed to satisfy the probability requirement. Thus, it is recommended that β be 0.01, and this value is used below; Kuno (1991) also used this level. Kuno (1978) pointed out that null hypotheses are usually framed in terms of absence, rather than presence. Thus, he used a type II error with level β , the convention used by Kuno (1991) and Yamamura and Sugimoto (1995).

Sampling Methods. Different sampling methods, that sample different portions of a population, are often applied in area-wide integrated pest management (AW-IPM) programmes (Vreysen, this volume). Insects with free-living immature stages will usually require sampling methods different from those applicable to mature individuals, and hungry insects may be attracted to lures that are different from those that attract sexually active or ovipositing individuals (Jang et al. 1999). Traps that minimize the capture of sterile males, and instead capture mainly females, are a special advantage in the case of male-only sterile releases (Katsoyannos et al. 1999). In attempting to sample a sparse (or perhaps non-existent) population, careful planning is needed to ensure that all portions of the population receive attention. If there are portions of a population that cannot be trapped or sampled otherwise, then, before applying the probability models to them, sufficient time must be given for them to become responsive to a trap. Given the propensity of insects to congregate in certain areas, detection trapping needs to be done throughout the assessment area that will eventually be designated “pest free”. Any information about the location of aggregated insects, times of greatest activity, and relative efficiency of various trap types and other sampling methods should be utilized to improve the chances of detecting insects that are at very low densities. In addition, to cover all life stages and physiological states, all effective trap types and other sampling methods should be utilized.

Detectability. Insect detectability (σ) can be estimated using a variety of methods (Thompson 2002); one method uses mark-recapture techniques (Shelly et al. 2010; Itô et al., this volume). Before an eradication programme commences, this should be done for each trap type used. Detectability can be estimated by the ratio of the number of marked individuals in the second sample (r) divided by the total number of marked individuals (m) released in the first sample:

$$\sigma = r / m \quad (21)$$

and σ has an approximate variance given by

$$\text{Var}(\sigma) = (n / mP) (1 - m/P) (P - n) / (P - 1) \quad (22)$$

in which P is either the known or estimated total population size (Thompson 2002). The standard error can be calculated from the variance. A further complication is that detectability may not be constant, but density-dependent. In the probabilistic approach outlined above, accurate measurement of detectability is of crucial importance; the way that detectability varies with density must be determined. If detectability declines at low density, then even estimating it presents a sampling problem since sparse captures will give a poor estimate of detectability. As an example of detectabilities that may be encountered in some pests, Table 3 shows the approximate estimated mean efficiencies for two tsetse species. The results in Table 3 underline the importance of selecting, for each pest species, the appropriate sampling systems, and of providing at least approximate estimates of the efficiency of each system that is used. If trap efficiency is unknown, it is necessary to conduct studies that estimate this parameter; only then can one use the technique to make defensible statements about the probability of an area being free of a pest species.

Table 3. Estimates of probabilities (converted to percentages) of catching Glossina morsitans morsitans Westwood and G. pallidipes Austen using a variety of stationary and mobile baits (capture probabilities in this table for stationary baits apply to the probability for 1 day for flies in a 1 km² neighbourhood of the sampling device; for mobile baits the system may sample more than 1 km², but the time frame is the same (Vale (1974a, b) and Flint (1985) described the various sampling systems); table from Barclay and Hargrove 2005, reproduced with permission)

Baits	<i>Glossina m. morsitans</i>		<i>Glossina pallidipes</i>	
	Males	Females	Males	Females
Mobile baits				
Standard fly-round	4.0	0.3	0.2	c. 0.1
Ox fly-round	4.0	0.5	0.8	0.2
Land Rover (electric net)	12.3	2.4	1.5	0.7
Decoy (electric net)	5.6	c. 0.1	< 0.1	< 0.1
Trolley (electric net)	9.5	2.1	3.9	1.4
Stationary baits				
Stationary ox	c. 0.1	< 0.1	0.2	0.2
Odour-baited F3 trap	c. 0.1	c. 0.1	1.0	1.0
Odour-baited electric net	c. 0.1	c. 0.1	1.9	2.0

Range of Trap Attraction. To apply the concept of detectability, it is important to define the area in which a trap will attract insects. This area will differ for each trap type, and therefore each type must be estimated independently, and perhaps also in different seasons and weather conditions. If there are areas not covered by a trap,

then the traps should be moved around so that the total area is susceptible to trapping.

Problem of Immigration. Unless formidable natural barriers to movement exist, most insects will eventually move into a favourable habitat. Therefore, eradication is possible only if such barriers exist or eradication programmes have long-term commitments to address whole populations or treat large eradication areas sequentially (Wyss 2000a; Hendrichs, Vreysen et al., this volume). Even with barriers, such as large lakes, oceans, mountain ranges, forests, large tracts of grassland, etc., invasions may still occur occasionally as a result of phoresy, strong winds, persistent flying, etc. If trapping is not carried out systematically and at sufficient intensity, it may be difficult to distinguish between a reinvasion and an incipient population that went undetected, unless population genetics methods are used (Krafsur and Ouma, this volume). Indeed, to verify the eradication of the oriental fruit fly and melon fly in the Mariana Islands, trapping continued for several years; several reintroductions were detected (Mitchell 1980). (In this case, another fly species that was also attracted to the traps verified the traps' attractiveness.)

Unequal Propensity to be Trapped. There is genetic variability in virtually all biological characteristics. Many insects exhibit variability in their attraction to pheromones and other natural attractants (Yaninek and Geraud 1989; Clearwater et al. 1991; Whittle et al. 1991). If eradication is attempted using only traps with odour attractants, there is a possibility that insects with a lower propensity to be trapped will eventually predominate (Barclay 1990, 1996; Shelly 1997). As the population declines, ultimately it could form a residual population that would not be detected by further trapping, and from which a rebound could occur. To avoid this problem, eradication should follow an integrated approach combining various control tactics, and assessment of the status of the population should deploy simultaneously several methods of sampling.

3.1.2. *Characteristics of Targeted Pest Species*

- *Population density.* Population density is important in assessing both the control method and the progress towards eradication, and in determining the detectability of the trap types and possibly the effects of density on detectability.
- *Reproductive capacity.* In population growth and ease of control, the following parameters are important: fertility rate, generation length, number of reproductive periods per individual, number of generations per year, probability of finding a mate at low density (the Allee effect), and density-dependence of fertility.
- *Mortality.* Age-specific and stage-specific mortality, and density-dependence of mortality, are important in assessing both the method and difficulty of eradication, and also the speed of rebound from a small population after an attempted eradication or a pest reintroduction. Mortality is also important in assessing the probability of a small population becoming extinct by chance.

- *Population movement.* Dispersal rate and distance, probability of exotic introductions, home range or territoriality, and density-dependence of movement are important in determining the size of sampling areas and the area to be covered with monitoring traps. Individuals may move from a suboptimal habitat into areas of known concentration that have been eradicated.
- *Periods of inactivity.* Certain portions of the population may be inactive and therefore cannot be trapped or otherwise sampled. Periods of inactivity are important in determining how long each trap type has effectively been trapping flies, and thus how many sampling periods may be counted in determining the probability that no insects are present.
- *Spatial distribution patterns.* Knowledge of spatial distribution is important in placing the control effort and simultaneous monitoring in the most effective positions. The distribution of the pest population within the targeted area, with particular regard to terrain, vegetation, and other features likely to affect distribution and density, should be ascertained. This information is important in case it is unnecessary or undesirable to uniformly apply control actions over the whole target area.

3.1.3. *Characteristics of Control Area*

- *Nature of terrain.* The topography, and availability and quality of roads, will determine the ease and cost of control and monitoring, and the logistics of trap location and visitation.
- *Boundaries, size of eradication areas, and barriers to reinvasion.* To prevent reinvasion, these must be known before eradication activities proceed.
- *Vegetation patterns.* These will affect the monitoring of insect density and distribution in suitable habitats.
- *Host availability.* This applies to host plant density for plant pests, or host animals for livestock or human pests.
- *Temperature and precipitation regimes.* Temperature will affect the speed of pupal development, influencing both the reproductive rate and the time required to ensure that no relict population exists after an eradication attempt, and precipitation regimes will affect the seasonal changes in vegetation patterns and also the availability of breeding habitats of insects such as mosquitoes.

3.2. *Pest Control Phase: Suppression*

If the target insect is detected after eradication measures have stopped, delimitation trapping and more traps must be deployed immediately in the area where the insect was caught, and further control measures initiated. If, on continued monitoring, more insects are caught, control treatments should be continued (Mangan and Bouyer, this volume).

By collecting field data before and during control operations, the success of the control treatment can be assessed (Itô et al., this volume; Vreysen, this volume). As the density of the target population declines, the probability of a positive catch decreases. Simulation studies for tephritid fruit flies indicated that at very low

population levels (capturing 0.001 flies per trap per week), consecutive zero catches for 16 weeks occurred in at least 5% of simulation runs (Clift et al. 1999).

However, given that the trap grid includes all the areas where the insects could be present, any relict population will increase and hence be detected eventually (McInnis et al. 2017). In the case of tephritid fruit flies, this occurs after three generations (Lance and Gates 1994). Trapping records from *B. dorsalis* showed that 12 weeks of successive zero catches occurred, even though wild insects were still present (Clift and Meats 1997; Clift et al. 1999). Often this represents a trade-off; it is not practical to have an optimal trapping grid of, for example, 400 m throughout the assessment area, but if the existing 1 km grid is maintained, some insects may go undetected until numbers build up to a detectable level.

3.3. *Post-Eradication: Detection Trapping*

The intensity of surveillance or detection trapping should be highest after control ceases (Hendrichs, Vreysen et al., this volume; Vreysen, this volume). Immediately following an eradication attempt, surveillance traps must be increased throughout the target area. These surveillance traps should be chosen for their efficiency in detecting the target species at low populations (Lance and Gates 1994; Katsoyannos et al. 1999; Papadopoulos et al. 2001). The trapping time needs to be sufficient to conform to the probability model, and allow the eventual probability of occurrence of any insect in the trapping area to be lower than the chosen risk level, e.g. a probability of 0.01 of being wrong.

Vreysen and Van der Vloedt (1992) described a sensitive biological method to determine whether or not eradication has been achieved. This consists of the release of virgin sterile female flies (female sentinels), and their recapture and dissection, to determine if, while in the field, they became inseminated by wild males.

It should be recognized that remnant insects might still exist in areas where control actions were less effective, e.g. sterile insects that did not reach narrow canyons or insecticidal sprays that did not penetrate dense vegetation. If there is a way to identify such potential problem areas before eradication activities start, then particular attention must be given to these areas.

3.4. *Evaluation Phases*

The first evaluation phase would normally be the calculation of the probability of insects being present given a sequence of null trapping results while control actions continue. The calculation can be done using any of the probability models, either on a trap-by-trap basis (most useful for spot infestations), in which all traps must satisfy the risk criterion, or on an area-wide basis (more useful for most programmes dealing with established pests), which gives one probability. When this probability becomes small enough, then the conclusion is reached that no insects are present at that trap site.

If, after terminating control actions, the number of insects remaining is extremely low, such as one insect in 10 or 100 km², the small number of traps indicated by the second probability model might not catch any of the remaining insects, simply

because the insects never come into contact with the traps (they are too widely separated). In this case, either more traps must be deployed, or they should be moved around to ensure that for some time they are located in the vicinity of the insects. Alternatively, the SIT may be continued for some time as insurance, as in the eradication of *G. austeni* in Zanzibar (Vreysen et al. 2000).

The second phase involves stopping control actions but continuing detection trapping and waiting for an appropriate period (its length dictated by the population growth model (equation (20)) to assess the possibility of resurgence of a small remnant population. Generally, before declaring “pest free status”, it would be advisable to wait long enough to allow the potential small population to grow to at least 10 times the minimum detectable level. If continued trapping proceeds without catching any insects, confidence in the eradication procedure will increase, either because elimination has succeeded, or an increase in the small remnant population would make detection easier.

In addition, there may be species for which, at present, no efficient trap exists. In this case, the trapping time would be unrealistically long or may even be incalculable, and there is little alternative except to wait (while still trapping) and see if a population rebound occurs during the minimal period. In such cases, a supportive technique proposed by Vreysen and Van der Vloedt (1992) is to release only virgin sterile females and, after recapture, assess if any of them had mated with wild males.

In both phases, it is advisable to monitor the progress of any diseases vectored by the insect pest, possibly using sentinel animals, trap trees, or other highly attractive features in highly suitable pest habitats. This is especially important in cases where detection trapping is very inefficient.

3.5. Criteria for Declaration of “Freedom from Pests”

The criteria for making a declaration of eradication involve the above two phases of monitoring, and both should be satisfied before making a declaration. In the first phase, a series of zero results will have been obtained from surveillance traps (while still continuing control actions). From these results, the probability of such a sequence is calculated in accordance with one of the two probability models. If this probability is sufficiently low (below 0.01), then control activity can be stopped but surveillance trapping must continue. If the calculation results in an unreasonably large number of traps, then only the second phase of the criterion might be required.

The second phase involves calculating the minimal expected size of a population resulting from the rebound of a remnant population after a period of time. Calculating the population growth rate for the species is done using an appropriate population growth equation, such as the one in equation (20), and waiting until the expected population is at least 10 times the size of the minimally detectable population, assuming that a rebound is occurring. When both phases pass the numerical test, then eradication may be declared.

Throughout both phases, any diseases vectored by the pest should be monitored. By observing the presence or absence of new infections, especially in young hosts, the existence of continuing disease transmission can be ascertained (section 2.3.).

As an illustration, the practical implementation of both phases has occurred in Australia, where experience in eradicating incursions of both endemic and exotic tephritid fruit flies has accumulated for over 50 years (Madge et al. 1997; Hancock et al. 2000). If a fruit fly species is introduced (usually in infested fruit) into an area that was free of that species, the young adults will disperse from the point of introduction, and could take from 1 or 2 days to several weeks to mature, mate, and infest more fruit. Dispersal can happen during and after the pre-maturation period. In a fly free area, an invading group of flies will disperse into a mate free void, so that only the few that stay around the origin will be at a sufficiently high density to encounter each other and breed (Meats 1998a, b). However, once mated, a female can disperse any distance that her lifetime permits, and spread the infestation as a new generation. Thus, we can expect that the occurrence of adults in a usually fly free zone would be clustered around the origin, and that the occurrence of larvae would be even more clustered. Transport of infested fruit is often the main problem.

In the case of the Mediterranean fruit fly, catching one or more male insects in a male-targeted trap indicates that supplementary traps (preferably baited with food lures attractive to females (Katsoyannos et al. 1999)) should be set up around it (CDFA 1999). In addition, a search for larvae is made within the array of supplementary traps. A catch of three male insects in the same or adjacent traps within 14 days, a catch of one female, or the detection of larvae in fruit, indicate the beginning of an outbreak and the need for the release of sterile insects within a suitable radius of probable movement within the time elapsed since detection. Also, the localized movement of soil or fruit leaving that area has to be prohibited, and a wider "suspension zone" imposed. A formula is then applied to establish criteria for the reinstatement of "area-freedom" status, involving a period free of both control measures and fly detection. This period is equivalent effectively to 12 weeks, or the length of one generation plus 28 days, whichever is longer (Tassan et al. 1983; Madge et al. 1997; Hancock et al. 2000).

3.6. *Quarantine Issues*

For agricultural pests that are transported in produce, it is feasible to impose quarantine on fruits, vegetables, etc. entering an area where a pest has been eradicated. Such quarantine would not cause financial hardship to local growers who benefit from the absence of the pest, although the quarantine process itself might be expensive. For pests of veterinary importance, it is advisable to establish a programme of quarantine and testing of hosts that are imported from infested areas. For pests of medical importance, humans are often the host and quarantine is not feasible. Therefore, a medical history should be required of people travelling to areas in which a disease has been eradicated; luggage should also be inspected to prevent the unintentional introduction of exotic pests.

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