STERILE INSECT TECHNIQUE: A MODEL FOR DOSE OPTIMIZATION FOR IMPROVED STERILE INSECT QUALITY

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ABSTRACT

The sterile insect technique (SIT) is an environment-friendly pest control technique with application in the area-wide integrated control of key pests, including the suppression or elimination of introduced populations and the exclusion of new introductions. Reproductive sterility is normally induced by ionizing radiation, a convenient and consistent method that maintains a reasonable degree of competitiveness in the released insects. The cost and effectiveness of a control program integrating the SIT depend on the balance between sterility and competitiveness, but it appears that current operational programs with an SIT component are not achieving an appropriate balance. In this paper we discuss optimization of the sterilization process and present a simple model and procedure for determining the optimum dose.

Key Words: SIT, model, competitiveness, sterility, radiation dose

RESUMEN

La técnica de insecto estéril (TIE) es una tecnología de control de plagas favorable para el medio ambiente con una aplicación de un control integrado de plagas claves para toda la área, incluyendo la supresión o eliminación de poblaciones introducidas y la exclusión de nuevas introducciones. La esterilidad reproductiva es normalmente inducida por radiación ionizada, un método conveniente y consistente que mantiene un grado razonable para la capacidad de competencia en insectos liberados. El costo y la eficacia de un programa de control que incluye TIE dependen en tener un balance entre la esterilidad y la capacidad para competir, pero parece que los programas operacionales corrientes con TIS como un componente no están logrando el tener un balance apropiado. En esta publicación, nosotros discutimos la optimización del proceso de esterilización y presentamos un modelo y procedimiento sencillos para determinar la dosis óptima.

The sterile insect technique (SIT) was conceived in the 1930s (Knipling 1955), and first applied on a significant scale in the 1950s against the New World screwworm *Cochliomyia hominivorax* (Coquerel) (Baumhover et al. 1955; Knipling 1960) and subsequently to a number of other pest species (Dyck et al. 2005). The principle of the technique is to introduce sterility by rearing large numbers of the target pest, reproductively sterilize them, and release them into the wild. When the sterile males mate with wild females, the females produce no viable offspring. With a constant rate of release of sterile insects this results in an increasingly rapid decline in the overall population over several generations. This technique has been used successfully against a number of pest species such as Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), melon fly *Bactrocera cucurbitae* (Coquilett), pink bollworm *Pectinophora gossypiella* (Saunders), codling moth *Cydia pomonella* (L.) and tsetse fly *Glossina austeni* Newstead (Tan 2000; Wyss 2000; Hendrichs et al. 2005; Klassen & Curtis 2005).

The attractive features of the SIT are that it is absolutely specific to the targeted pest, integrates well with other controls, reduces the use of toxic insecticides, and its action is inverse-density dependent. This latter characteristic implies that as the field population declines, the pressure increases on the population from a constant rate of sterile insect release; this characteristic makes it desirable for eradication, suppression, containment, or the exclusion of sporadic introductions in a preventive release program (Hendrichs et al. 2005). The inverse-density dependence of the technique makes it possible, as part of a systems approach, to eliminate or reduce pests to such low levels as to allow export of important commodity crops to areas with quarantine restrictions against the pest.

Sterility can be induced by chemicals or ionizing radiation. Chemical sterilization was used in early work (Bořkovec 1966; LaChance 1967; Labrecque & Smith 1968), but because of the hazard of handling these substances, problems with controlling the dose, and the risks of environmental

contamination, chemical sterilization has been replaced by irradiation (Hayes 1968; Bakri et al. 2005a; Bakri et al. 2005b).

When biological material is irradiated, free radicals are formed, and breaks are created in the chromosomes. If breakage occurs in chromosomes of the germ line, this leads to the formation of dominant lethal mutations in eggs and sperm (LaChance 1967; Curtis 1971). Radiation sterilization is a simple process with easy and reliable quality control procedures. The action of the radiation is immediate so there is no requirement to hold the sterile insects after treatment, and radiation can pass through packaging material allowing the insects to be treated after sealing in secure packaging enhancing biosecurity and reducing handling.

Dose Optimization

The radiation absorbed dose (referred to hereafter as dose) that is used to induce sterility is of prime importance to programs that include the release of sterile insects. Insects that receive too low a dose are not sufficiently sterile and those that receive too high a dose may be uncompetitive, reducing the effectiveness of the program by requiring that a greater number of sterile insects must be released (Robinson 2002).

While competitiveness has often been investigated (Hooper 1970; Hooper & Katiyar 1971; Hooper 1972; Katiyar 1973a, b; Hooper 1975; Zumreoglu et al. 1979; Winstead et al. 1990; Haynes & Smith 1992; Boshra 1994; Saour & Makee 1997; Bloem et al. 1998; Bloem et al. 1999; Bloem et al. 2004; Toledo et al. 2004), the critical balance between sterility and competitiveness has rarely been investigated or discussed in sufficient detail, and few data have been presented in the literature in a form that permits a proper analysis of this balance (Bakri et al. 2005a). In order to perform the analysis, data are required simultaneously for the variation of both sterility and competitiveness with dose. Where competitiveness has been studied, frequently only one or two doses have been investigated. Further, for the competitiveness data to be realistic, the tests should be performed in field cages or open plots.

The relationship between residual fertility and log(dose) is well known and is sigmoid in form. Not enough data are available to be certain of the relationship between competitiveness and log(dose), but for simplicity we assume it to be similar to most response-to-dose relationships, which are sigmoid (Finney 1971); however any monotonic decreasing function will lead to similar conclusions to those presented below. Fig. 1 illustrates the relationships of fertility and competitiveness to log(dose) following this assumption, where the scale on the x-axis is such that one unit represents the change in log(dose) needed to produce one σ change in the response (competitiveness or fertility). The displacement, δx (in units of σ) of the competitiveness curve to the right (or left) of the fertility curve is generally unknown, but must vary with species and other factors such as the oxygen content of the atmosphere and temperature during irradiation, free radical scavengers provided in the diet, quality of rearing, and possibly other factors. Considerable research related to the SIT is to improve the competitiveness of the insects for a given sterility level, that is to move the competitiveness line as far to the right as possible, and thus to increase the value of δx.

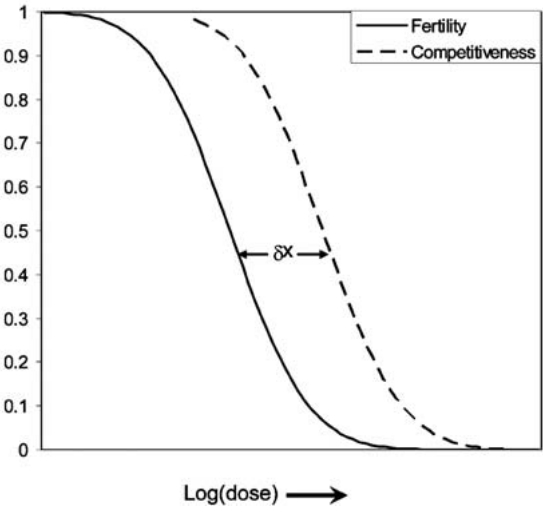

Fig. 1. Schematic relationship of residual fertility and competitiveness to log(dose). δx is the separation of the two response curves (based on data of Hooper, 1972).

Knipling (1955) presented a simple relationship for the effect of released sterile insects on a wild population. This may be written as:

$$[1] \qquad F_1 = P \times (1 - S) \times R$$

where F_1 is the population size in the filial generation, P is the parental generation size, R is the net population growth rate per generation, and S is the sterility induced by the released sterilized insects (IAEA 1992, pp. 108-109). In practice R is likely to be density dependent, but for this simple model it is assumed to be density independent, and S is dependent on the number of sterile insects released (N) if it is assumed that the released insects are both fully sterile and fully competitive:

$$[2] \qquad S = \frac{N}{(N + P)}$$

This, however, does not take into account either of incomplete sterility induced by the irradiation (S_l), or of the reduced competitiveness (Q). To sim-

plify the equations, the reduced competitiveness of the N released insects can be represented as NQ fully competitive insects (and $N(1\text{-}Q)$ non-competitive insects that have no effect on the target population), and the reduced sterility as NQS_l sterile and $NQ(1\text{-}S_l)$ fertile insects. This simplification does not affect the final form of the relationship. These $NQ(1\text{-}S_l)$ fertile individuals add to the pool of breeding individuals, so that:

$$[3] \qquad P' = P + NQ(1 - S_l)$$

and equation [2] becomes:

$$[4] \qquad S' = \frac{NQS_l}{NQS_l + P + NQ(1 - S_l)} = \frac{NQS_l}{NQ + P}$$

The original equation [1] now becomes:

$$[5] \qquad F_1 = P' \times (1 - S') \times R$$

or:

$$[6] \qquad \frac{F_1}{R} = (P + NQ(1 - S_l)) \times \left(1 - \frac{NQS_l}{NQ + P}\right)$$

Regression analysis of both Probit transformed competitiveness (Q) and residual fertility ($1\text{-}S_l$) against log radiation dose will yield a relationship that may be used to predict both parameters for any radiation dose. Equation [6] can then be solved numerically by iteration to find the minimum value of F_1/R for given values of δx and N/P. Using values of $R = 1, P = 1$ and $N = 9$, Fig. 2 shows the effect on the subsequent generation (F_1) of log(dose) at 3 values of δx for a fixed release rate. This clearly shows that as the value of δx increases the value of $F_{1\text{Minimum}}$ decreases and this minimum point occurs at a higher sterility. At the same time the slope of the F_1 curve each side of the optimum point gets shallower, implying that a larger dose variation may be tolerated This has the potential to increase the throughput of the irradiation process as less strict limits need to be applied.

This indicates that research is essential to establish the relationship of dose to the level of sterility and competitiveness in the treated insects, and that a standardized dosimetry system and recognized dosimetry procedures are used (ISO/ASTM 2005a). The dose to be used for any given SIT program is then based on the results of such studies. The program manager should specify the optimum dose to achieve the best combination of competitiveness and sterility (Table 1), and this dose should be reviewed when changes in any procedure alter the value of δx.

Ideally, all the insects should be irradiated to receive this optimum dose, but as the dose rate varies spatially within a container, it is inevitable that insects within will receive a range of doses. Because of this dose variability the program manager should also specify the minimum and maximum acceptable dose that insects may receive. If the dose variability within the container is too high, it may be necessary to modify the radiation field (e.g., with a field flattener, a shaped lead shield that improves the dose uniformity ratio) or limit the volume used for irradiation by blocking off areas with unacceptably high and/or low dose rates. The range of acceptable doses should be approximately symmetric about the optimum dose (in log(dose)), as shown by the symmetry of the F_1 curves (Fig. 2). We suggest that the maximum and minimum dose should be set to yield F_1 values not more than 110% of $F_{1\text{Minimum}}$. For many insects, the dose required in the late pupal stage to stop egg production or egg hatch in females is lower than the dose required to induce sterility in males (Bakri et al. 2005a). For most purposes, therefore, the minimum dose will be set higher than the dose at which egg production or hatch stops. For legal or other justifiable program requirements a higher minimum dose may be specified, but it must be recognized that this may affect the program efficiency (Toledo et al. 2004).

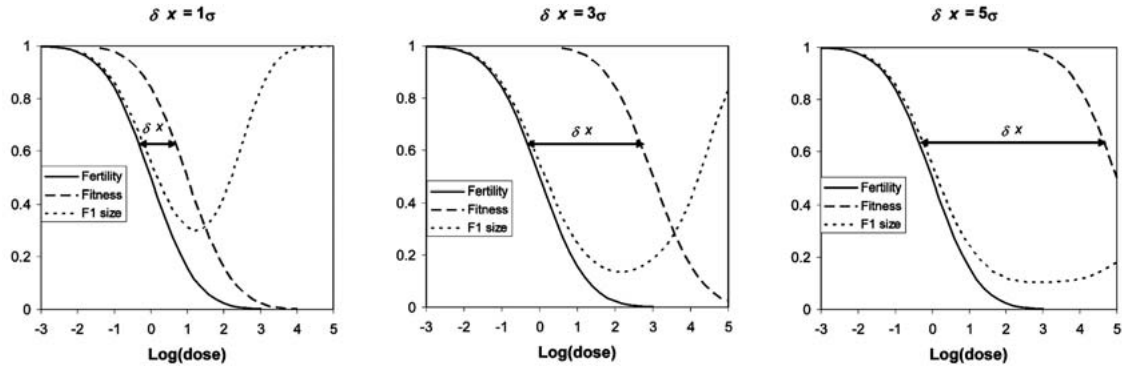

Fig. 2. Size of next generation (F_1) as a function of log(dose) for $\delta x = 1\sigma$, 3σ and 5σ.

TABLE 1. VALUES FOR OPTIMUM DOSE (IN σ ABOVE THE LOG(DOSE) THAT YIELDS 0.5 RESIDUAL FERTILITY), $F_{1\,Minimum}$, THE FERTILITY AND COMPETITIVENESS CORRESPONDING TO THE OPTIMUM DOSE, AND THE RANGE OF LOG(DOSE) FOR $F_{1\,Minimum}$ + 10% FOR SELECTED VALUES OF δx (DISPLACEMENT OF THE COMPETITIVENESS CURVE RIGHT OF THE FERTILITY CURVE). THIS ANALYSES IS FOR N/P = 9.

δx/σ	Optimum log(dose)	$F_{1\,\text{Minimum}}$	Fertility	Competitiveness	Maximum log(dose) range for $F_1 \le 1.1 \times F_{1\,\text{Minimum}}$	
0	0.70	0.480	0.240	0.24	0.25	1.10
0.5	1.00	0.380	0.160	0.31	0.60	1.35
1	1.25	0.300	0.110	0.40	0.85	1.60
1.5	1.50	0.240	0.067	0.50	1.15	1.85
2	1.75	0.190	0.042	0.61	1.35	2.10
2.5	1.90	0.160	0.027	0.71	1.55	2.35
3	2.15	0.140	0.016	0.80	1.70	2.60
3.5	2.30	0.120	0.010	0.88	1.85	2.85
4	2.55	0.110	0.005	0.93	2.00	3.15
4.5	2.75	0.106	0.003	0.96	2.10	3.50
5	2.95	0.103	0.002	0.98	2.20	3.85

The actual dose applied in different programs and research projects has varied widely, by a factor of almost 3 for some species (i.e., *Sitophilus granarius* L. which varies between 50 and 135 Gy) as shown by the International Database on Insect Disinfestation and Sterilization website (IAEA 2003; Bakri et al. 2005a). From Table 1 it can be seen that the optimum dose only yields 95% sterility (5% residual fertility) when δx is about 1.8 and 99.9% when δx is greater than 5. It is unlikely that δx will ever be as large as 5, but because of the lack of appreciation for the insect competitiveness issues involved, many programs continue to use 99.9% sterility when lower doses would yield better control.

The optimum dose depends furthermore on the ratio N/P (Table 2). In the early stages of a suppression or eradication campaign, while the wild population is still relatively large and the ratio N/P is small, the optimum dose is lower than later in the program when the value of N/P is larger. It would thus appear that current operational programs releasing sterile insects are not achieving the appropriate balance between sterility and competitiveness at each stage in the program. Table 2 may be used to estimate the optimum dose in Gy for any given value of δx at various ratios of N/P. If the regression equation for the dose-fertility relationship, with dose in Gy transformed to log(*dose*) and fertility to normal equivalent deviates (*NED*) is:

$$[7] \qquad NED(fertility) = a + b \times \log(dose)$$

then the actual dose in Gy can be calculated from the values of D in Table 2 as:

$$[8] \qquad dose(Gy) = 10^{\frac{-(D+a)}{b}}$$

The value of δx can be estimated from a simple field cage experiment, but an adequate set of field cage data to determine the dose-response relationship has not been published. In order to illustrate the concept, the extensive set of laboratory data for the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) presented by Hooper (1972) for fertility and competitiveness (Haisch 1970; Fried 1971) at various treatment doses is used. The main purpose of this illustration is to demonstrate the procedure for determining the optimum dose from relevant data. Using Hooper's data, we show the relationships of fertility and competitiveness to the radiation dose in Fig. 3, with the linear regression lines and 95% confidence intervals for the regressions. The regression fit for the fertility is very good, but there is a larger scatter in the competitiveness values. This is inherent in the method of measuring and calculating competitiveness (Haisch 1970; Fried 1971; Hooper & Horton 1981; Iwahashi et al. 1983). The regression coefficients for competitiveness and fertility do not differ significantly from each other (competitiveness: regression coefficient = -3.4032, SE = 0.3326; fertility: regression coefficient = -3.8866, SE = 0.5403) (Sokal & Rohlf 1981). The value of δx from these data is 1.44, and from the fertility relationship the increase in log(dose) that results in a 1σ change in fertility is 0.26, the reciprocal of the slope of the linear regression line.

From these values the optimum dose can be estimated from Table 2 and equation [8]. In the present example, where δx = 1.44, for N/P = 8, from the table the value of D is 1.45. Based on equation [8]:

$$[9] \qquad dose(Gy) = 10^{\frac{-(1.45+5.4955)}{-3.8866}} \cong 61$$

TABLE 2. OPTIMUM RADIATION DOSE (D, IN UNITS OF σ ABOVE THE LOG(DOSE) THAT YIELDS 0.5 RESIDUAL FERTILITY) AND CORRESPONDING STERILITY LEVEL (IN ITALICS) FOR SELECTED VALUES OF δ*x* AND *N/P* (THE RATIO OF STERILE TO WILD MALES).

	N/P							
δ*x*	1	2	4	8	16	32	64	128
0	0.22	0.34	0.50	0.67	0.86	1.04	1.22	1.40
	58.7%	*63.3%*	*69.1%*	*74.9%*	*80.5%*	*85.1%*	*88.9%*	*91.9%*
0.5	0.48	0.61	0.77	0.95	1.14	1.32	1.50	1.68
	68.4%	*72.9%*	*77.9%*	*82.9%*	*87.3%*	*90.7%*	*93.3%*	*95.4%*
1	0.73	0.87	1.03	1.21	1.40	1.58	1.76	1.94
	76.7%	*80.8%*	*84.8%*	*88.7%*	*91.9%*	*94.3%*	*96.1%*	*97.4%*
1.5	0.98	1.11	1.28	1.45	1.64	1.82	2.00	2.17
	83.6%	*86.7%*	*90.0%*	*92.6%*	*94.9%*	*96.6%*	*97.7%*	*98.5%*
2	1.22	1.35	1.51	1.68	1.86	2.04	2.22	2.39
	88.9%	*91.1%*	*93.4%*	*95.4%*	*96.9%*	*97.9%*	*98.7%*	*99.2%*
2.5	1.46	1.58	1.73	1.90	2.08	2.25	2.42	2.59
	92.8%	*94.3%*	*95.8%*	*97.1%*	*98.1%*	*98.8%*	*99.2%*	*99.5%*
3	1.70	1.81	1.95	2.11	2.28	2.44	2.61	2.77
	95.5%	*96.5%*	*97.4%*	*98.3%*	*98.9%*	*99.3%*	*99.5%*	*99.7%*
3.5	1.93	2.03	2.17	2.31	2.47	2.63	2.79	2.94
	97.3%	*97.9%*	*98.5%*	*99.0%*	*99.3%*	*99.6%*	*99.7%*	*99.8%*
4	2.16	2.26	2.38	2.52	2.66	2.81	2.96	3.11
	98.5%	*98.8%*	*99.1%*	*99.4%*	*99.6%*	*99.8%*	*99.8%*	*99.9%*
4.5	2.40	2.49	2.60	2.72	2.86	3.00	3.14	3.28
	99.2%	*99.4%*	*99.5%*	*99.7%*	*99.8%*	*99.9%*	*99.9%*	*99.9%*
5	2.64	2.72	2.82	2.93	3.06	3.19	3.32	3.45
	99.6%	*99.7%*	*99.8%*	*99.8%*	*99.9%*	*99.9%*	*100.0%*	*100.0%*

Table 3 presents the calculated optimum dose, $F_{1\,\text{Minimum}}$, the corresponding values for sterility and competitiveness and the minimum and maximum doses to remain within 110% of $F_{1\,\text{Minimum}}$ for δ*x* = 1.44 and various values of N/P. Also shown are the values corresponding to 99% and 99.9% sterility, 103 and 162 Gy. For this value of δ*x* the optimum dose lies below 90 Gy, with a range from 52-89 Gy, which corresponds well with Hooper's own conclusion that the optimum dose is about 70 Gy. Both 99% and 99.9% sterility doses fall outside this dose range for all values of N/P shown.

The value of δ*x* is overestimated by the data in Hooper (1972), as the competition was between irradiated and unirradiated colony flies under laboratory conditions, not between irradiated colony flies and wild flies in the field. This colony has been maintained under artificial conditions for many generations and can be expected to have competitiveness less than 1 before irradiation. Irradiated colony flies could therefore be expected to perform worse against wild flies than against colony flies. Wong et al. (1983) compared mating success between irradiated and wild males of *Ceratitis capitata*, and found no difference over a range

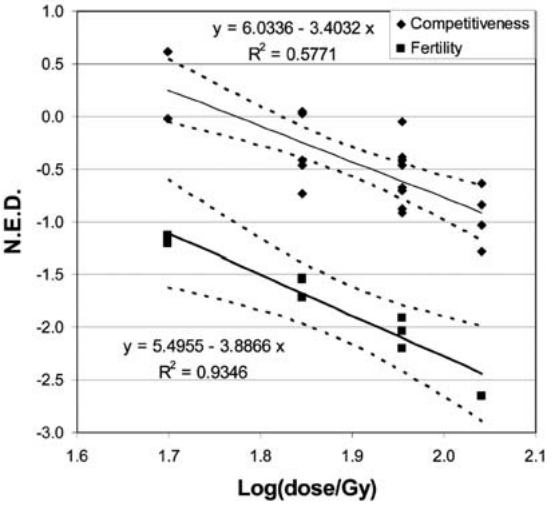

Fig. 3. Relationships of fertility (squares) and competitiveness (diamonds) with dose for the Mediterranean fruit fly (data from Hooper 1972). NED = normal equivalent deviates.

TABLE 3. OPTIMUM DOSE, MINIMUM AND MAXIMUM DOSES, AND THE CORRESPONDING STERILITY, COMPETITIVENESS AND F_1 FOR MEDITERRANEAN FRUIT FLY, FOR $\delta x = 1.44$ AND VARIOUS VALUES OF N/P (DERIVED FROM DATA IN HOOPER 1972).

Dose	Gy	Sterility	Competitiveness	F_1	$F_1/F_{1Minimum}$
$\delta x = 1.44$	N/P = 3				
Minimum	39	74.8%	78.0%	0.476	1.10
Optimum	52	88.0%	60.4%	0.433	1.00
Maximum	70	95.3%	40.7%	0.476	1.10
103 Gy	103	99.0%	18.8%	0.643	1.49
162 Gy	162	99.9%	4.9%	0.871	2.01
$\delta x = 1.44$	N/P = 9				
Minimum	49	85.9%	64.1%	0.267	1.10
Optimum	61	92.7%	49.3%	0.243	1.00
Maximum	77	96.6%	34.8%	0.267	1.10
103 Gy	103	99.0%	18.8%	0.378	1.56
162 Gy	162	99.9%	4.9%	0.692	2.85
$\delta x = 1.44$	N/P = 100				
Minimum	77	96.7%	34.7%	0.060	1.10
Optimum	89	98.1%	26.0%	0.055	1.00
Maximum	103	99.0%	18.5%	0.060	1.10
103 Gy	103	99.0%	18.8%	0.060	1.09
162 Gy	162	99.9%	4.9%	0.169	3.08

of doses, but there is an urgent need for additional field cage or field data on competitiveness over a range of radiation doses to determine the dose relationship and thereby the magnitude of δx.

DOSIMETRY AND THE IRRADIATION PROCESS

Dosimetry plays a crucial role throughout the radiation sterilization process of insects. At the research phase, where the effect of radiation on sterility as well as on competitiveness of the insects is investigated, radiation dose is the key quantity. At the production facility, dosimetry also has several essential roles. First, it assists in the characterization of the irradiator, and in the regular monitoring of its consistent operation. It also helps in determining the correct size and shape of the canister and other key process parameters for irradiation of the insects. And later during the sterilization process, it provides an important element of process control.

Considering the importance of dosimetry in programs applying the SIT, the selection of an appropriate dosimetry system is critical. Such a system should provide a systematic and repeatable means of estimating the dose and its associated confidence interval (ISO/ASTM 2005b). The system should be verifiable and traceable (referenced) to national or international standards. Considering various factors, the Gafchromic® dosimetry system (Gafchromic HD-810 film; International Specialty Products, Wayne, NJ 07470, U.S.A.) has been selected by the IAEA based on the specific requirements of SIT programs, especially the useful dose range of 50-600 Gy and a low cost (IAEA 2004). This reference also describes relevant dosimetry procedures as well as various components of this dosimetry system.

Accidental release of insects that are significantly under-dosed will require rapid correction by release of additional sterile insects and other measures, especially for programs like those in California and Florida, USA., where SIT is used for eradication of extremely small introductions and/or as a prophylactic measure to prevent establishment of newly introduced flies (Dowell et al. 2000). Besides administrative control, there are 3 main process control elements that are in place that would minimize the chances of such accidents (FAO/IAEA/USDA 2003). These different elements control various steps in the process and thus complement each other as follows: (1) Sterility Testing—In any SIT program, sterility testing through bioassays should be carried out on a regular basis to confirm that all the procedures are being followed correctly, including the rearing, the pre-irradiation preparation (such as age-based selection of insects, packaging for hypoxia or nitrogen, if used), temperature control, irradiation dose control, and post irradiation handling, (2) Routine Dosimetry—The purpose of dosimetry in process control is to monitor that all the canisters (and hence all the insects) are receiving the dose within the specified range, and (3) Radiation-Sensitive Indicators—This control element provides an immediate visual check at irradiation facilities and at pupal reception/fly emergence

centers that a given container has gone through the irradiation process.

CONCLUSIONS

For SIT, ionizing radiation is the method of choice for inducing reproductive sterility. The sterilization process is important in determining the quality of the released insects and their ability to compete with the wild population. Thus, optimization of the sterilization process is critical for the efficacy of SIT programs and should be given due consideration. We believe that doses lower than currently applied will result in a more effective SIT program, with any increase in residual fertility more than compensated for by the increased competitiveness of the released insects.

We have developed a quantitative procedure for determining the optimum dose based on fertility and competitiveness data. In order to estimate the optimum dose, it will be necessary to calculate correlations between dose and both fertility and competitiveness. The fertility relationship is already known for many insects, so attention should be concentrated on collecting data on competitiveness over a suitable range of doses. As the optimum also depends on the ratio of sterile to fertile males, the treatment dose should be reviewed constantly during the progress of a program. Optimization can lead to significant reduction in program cost and increase in programme efficiency.

The dose of radiation can be readily measured with a standardized dosimetry system, such as the Gafchromic® system (IAEA 2004; ISO/ASTM 2005c). A dosimetry system that is traceable to national or international standards can be reliably used both for setting the dose for the radiation sterilization process and for routine process control.

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