

**GUIDELINES FOR AUTHORS — CAMERA-READY**  
**MANUSCRIPTS**  
**(Instructions, layout and examples)**

TITLE OF ARTICLE

AUTHOR NAME(S)

*Author Affiliation(s)*

SUMMARY

The font size should be 8 pts.

1. INTRODUCTION

These instructions are intended to provide guidance to contributors of an edited volume when preparing a camera-ready manuscript on a word processor. Please read these general instructions carefully before beginning the final preparation of your camera-ready manuscript.

2. FORMATTING INSTRUCTIONS

*2.1. Format and Style*

The text should be in clear, concise English. Please be consistent in punctuation, abbreviations, spelling (British), headings, and the style of referencing. Please make sure your text has been proofread with care.

We recommend using the formatting styles function in your word processing application for the text, subheads, etc. rather than changing layout settings in every place. This way you will obtain maximum consistency in layout. Changes in the layout can be made by changing the relevant styles.

When formatting an article, please work with either WORD or WordPerfect. Do not mix applications within one article.

This document is an example of formatting and layout.

*2.2. Fonts and Setup*

The font for your manuscript should be Times/Times New Roman. The text should be justified, and the text area is 12 x 18.5 cm (excluding running head) or 12 x 19.5 cm (including running head). Body text should be 10 pts, and Quotations, Notes,

References and Summaries 8 pts. Apart from exceptions that will be mentioned below, all spacing should be single.

In the Page Setup in WORD, the following settings are appropriate:

Margins: Top 4.9 cm, Bottom 5.5 cm, Inside 4.6 cm, Outside 4.4 cm, Header 4.3 cm, Footer 5.0 cm. Mirror margins.

Paper size: A4, portrait orientation.

Layout: Section start – Odd Page; Headers and Footers – Different odd and even, Different first page; Vertical Alignment – Top.

Use only one space, not two, between sentences.

### 2.3. *Layout of the Opening Page*

The opening page of a contribution should always be a right-hand page, and should show the contributor's name, the title, and possibly a subtitle. The contributor's name should be in 12 pts, capital letters. The chapter title should be in 14 pts, capital letters. The subtitle, if used, should be in 11 pts, upper and lower case, italicized. All the above should be centred. This should be followed by the summary, and then the first heading and the opening text. Leave 10 pts between title and subtitle if there is a subtitle. Leave 40 pts after the subtitle (if used) or the title (if no subtitle is used).

*Table 1. Formatting instructions*

Text item	Font size	Case	Alignment	Spacing
Author name	12 pts	capitals	centred	30 pts below name
Article title	14 pts	capitals	centred	No subtitle: 40 pts below title Subtitle: 10 pts below title
Subtitle (if used)	11 pts	upper and lower case	centred	40 pts below subtitle

## 3. SUBHEADS

If numbering is used in headings, it should be Arabic, not Roman. Please distinguish between the following four levels of headings:

### 4. SUBHEADS, FIRST ORDER

10 pts, capitals, centred. Leave 16 pts space above and 8 pts space below.

#### 4.1. *Subheads, Second Order*

10 pts, upper and lower case, italics, left aligned. Leave 16 pts space above, 8 pts space below.

#### 4.1.1. Subheads, Third Order

10 pts, upper and lower case, italics, left aligned. Leave 16 pts space above, 0 pts space below.

*Subheads, Fourth Order.* 10 pts, upper and lower case, italics, left aligned. No numbering is used. Text continues directly after subhead. Leave 16 pts space above.

#### 4.2. Remarks

Where a subhead appears directly after another subhead (such as happens with paragraph 2. and 2.1. in this document, see above) less space should be left in between them. In this case, the space above the first subhead remains the same. 8 pts space below the first subhead becomes 4 pts space below. If the second subhead has 16 pts above, this is reduced to 8 pts space above (combined = 12 pts). The space below the second subhead remains the same.

### 5. RUNNING HEADS AND NEW PARAGRAPHS

The running heads of a contribution to an edited volume should be as follows:

- left-hand pages: page number flush left  
author's name centred, in (small) capitals
- right-hand pages: page number flush right  
title of contribution centred, in (small) capitals

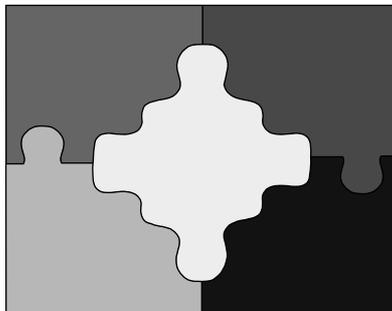
If the title is very long, please make an abbreviated title of not more than 40 characters (including spaces) to be used as a running head. The font size is 10, leave 12 pts space below. Exception: the opening page of an article does not have a running head.

New paragraphs should be indented by 0.5 cm, except after titles and subheadings.

### 6. FIGURES AND PHOTOGRAPHS

Normal figures should preferably be embedded in the text (rather than supplied separately). Legends for figures and illustrations should not be incorporated into the figure itself, and they should be listed in numerical order (headed as *Figure x. Title*). Legends should be italicized, centred, 9 pts, below the figure. Leave 12 pts above and below the legend. See Figure 1 below for an example. It is best to put everything (figure plus legend) into a box (but showing no border), and then the box can be positioned on the page (using WORD) with Format Text Box, Layout, and Advanced Layout. Take care to position the anchor so that the box stays where you want it to be in the layout. Centre the figure on the page.

If you use a figure (or table) that is taken directly from an existing publication, you must receive official permission in writing from the copyright holder of that publication to reproduce the figure/table in your paper.



*Figure 1. Sample figure.*

## 7. EQUATIONS

Equations should be italicized and centred on the page, with the equation number in parentheses, flush right. Please put 12 pts space above and below the equation.

$$E=mc^2 \quad (1)$$

Wherever possible, try to avoid breaking equations between parentheses, brackets, or braces.

## 8. QUOTATIONS

Quotations are 8 pts, and should be indented 1 cm on the left and on the right, with 6 pts space above and below the quotation.

After long quotations, follow indentation or lack of indentation depending on whether a new paragraph is required.

## 9. TABLES

Please centre tables on the page, unless it is necessary to use the full-page width. Exceptionally large tables may be placed landscape (90° rotated) on the page, with the top of the table at the left-hand margin. Legends should be italicized, centred, 9 pts, above the table. (Type the legend in the top row of the table.) Leave 12 pts above and below the legend. Use 8 pts characters in the table. As with a figure, it is helpful to put the table and its legend into a text box.

The decimal place of numbers (even if not typed in) should be vertically aligned in columns of numbers.

An example of a table is given below.

*Table 2. Vegetable colours*

Vegetable	Colour
Carrot	Orange
Leek	Green/White
Red pepper	Red
Parsnip	Off-white

## 10. BOXES

Put information, not directly a part of the article's content in the text, into boxes (with border lines). For example, theoretical background material, case study, list of definitions, reference equations, etc. Use 8 pts characters, but use 9 pts characters in headings (italicised).

## 11. NOTES

If necessary, use Notes, positioned before the list of References. Consider Notes title as a Subhead first order. All the Notes should be in 8 pts. Use superscript for numbering the Notes, and indent slightly after the number.

## 12. KEY WORDS

Provide up to 10 key words, for help in preparing the subject index.

## 13. REFERENCES

Consider References title as a Subhead first order. The font size should be 8 pts. Second and subsequent lines of each reference are to be indented slightly (0.5 cm). Author names and publication year should be in **bold** letters.

In the text, use the Name-Date system of citing references. If there is more than one citation at one place in the text, show the earliest one first.

The list of references (generally in alphabetical order), at the end of the article, should follow the examples shown below. Write out the full name of journals.

Journal article:

**Calkins, C. O., Ru Nguyen, K. Corwin, and J. R. Brazzel. 1988.** Evaluations of quality of irradiated Mediterranean fruit fly, *Ceratitidis capitata* (Weidemann) (Diptera: Tephritidae), at the release site in Miami, Florida during an eradication program in 1985. *Florida Entomologist* 71: 346–351. [http://fulltext10.fcla.edu/DLData/SN/SN00154040/0071\\_003/98p0531w.pdf](http://fulltext10.fcla.edu/DLData/SN/SN00154040/0071_003/98p0531w.pdf)

**Delafosse, A., Z. Bengaly, and G. Duvallet. 1996.** Use of *Trypanosoma* antigen detection ELISA during an epidemiological survey in the Sideradougou area, Burkina Faso. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux* 49: 32–37.

**Flint, S. 1985.** A comparison of various traps for *Glossina* spp. (Glossinidae) and other Diptera. Bulletin of Entomological Research 75: 529–534.

Book, and article/chapter in a book:

**Bloem, K., S. Bloem, N. Rizzo, and D. Chambers. 1993.** Female medfly refractory period: effect of male reproductive status, pp. 189–190. In M. Aluja and P. Liedo (eds.), *Fruit flies. Biology and management*. Springer-Verlag, New York, NY, USA.

**Parzen, E. 1960.** *Modern probability theory and its applications*. John Wiley, New York, NY, USA.

Proceedings:

**Caceres, C., J. P. Cayol, W. R. Enkerlin, G. Franz, J. Hendrichs, and A. S. Robinson. 2004.** Comparison of Mediterranean fruit fly (*Ceratitidis capitata*) (Tephritidae) bisexual and genetic sexing strains: development, evaluation and economics, pp. 367–384. In B. N. Barnes (ed.), *Proceedings, Symposium: 6<sup>th</sup> International Symposium on Fruit Flies of Economic Importance, 6–10 May 2002, Stellenbosch, South Africa*. Isteg Scientific Publications, Irene, South Africa.

**Dyck, V. A., S. H. Graham, and K. A. Bloem. 1993.** Implementation of the sterile insect release programme to eradicate the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Olethreutidae), in British Columbia, Canada, pp. 285–297. In *Proceedings: Management of Insect Pests: Nuclear and Related Molecular and Genetic Techniques*. FAO/IAEA International Symposium, 19–23 October 1992, Vienna, Austria. STI/PUB/909. IAEA, Vienna, Austria.

**Hancock, D. L., R. Osborne, S. Broughton, and P. Gleeson. 2000.** Eradication of *Bactrocera papayae* (Diptera: Tephritidae) by male annihilation and protein baiting in Queensland, Australia, pp. 381–388. In K. H. Tan (ed.), *Proceedings: Area-Wide Control of Fruit Flies and Other Insect Pests. International Conference on Area-Wide Control of Insect Pests, and the 5<sup>th</sup> International Symposium on Fruit Flies of Economic Importance, 28 May–5 June 1998, Penang, Malaysia*. Penerbit Universiti Sains Malaysia, Pulau Pinang, Malaysia.

**Zapfen, G., J. Hendrichs, P. Liedo, and A. Cisneros. 1983.** Comparative mating behaviour of wild and mass-reared sterile medfly *Ceratitidis capitata* (Weid.) on a field cage host tree — II. Female mate choice, pp. 397–409. In R. Cavalloro (ed.), *Proceedings, Symposium: Fruit Flies of Economic Importance*. CEC/IOBC International Symposium, 16–19 November 1982, Athens, Greece. A. A. Balkema, Rotterdam, The Netherlands.

No author given:

**(FAO/IAEA/USDA) Food and Agriculture Organization of the United Nations/International Atomic Energy Agency/United States Department of Agriculture. 2003.** Manual for product quality control and shipping procedures for sterile mass-reared tephritid fruit flies. Version 5.0. IAEA, Vienna, Austria. <http://www.iaea.org/programmes/nafa/d4/index.html>

**(IAEA) International Atomic Energy Agency. 2003.** Automation for tsetse mass rearing for use in sterile insect technique programmes. Final report of a co-ordinated research project 1995–2001. IAEA-TECDOC-1353. IAEA, Vienna, Austria.

## **EXAMPLE OF FIRST THREE PAGES OF AN ARTICLE:**

# GENETIC BASIS OF THE STERILE INSECT TECHNIQUE

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## SUMMARY

The use of the Sterile Insect Technique (SIT) for insect control relies on the introduction of sterility in the females of the wild population. This sterility is produced following the mating of these females with released males carrying, in their sperm, dominant lethal mutations that have been induced by ionizing radiation. The reasons why the SIT can only be effective when the induced sterility in the released males is in the form of dominant lethal mutations, and not some form of sperm inactivation, are discussed, together with the relationship of dominant lethal mutations to dose, sex, developmental stage and the particular species. The combination of genetic sterility with that induced by radiation is also discussed in relation to the use of genetic sexing strains of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) in SIT programmes. A case is made to lower the radiation dose used in SIT programmes so as to produce a more competitive sterile insect. Increased competitiveness can also be achieved by using different radiation environments. As well as radiation-induced sterility, natural mechanisms can be recruited, especially the use of hybrid sterility exemplified by a successful field trial with tsetse flies *Glossina* spp. in the 1940s. Genetic transformation will make some impact on the SIT, especially regarding the introduction of markers for released flies, and the construction of genetic sexing strains. It is concluded that using a physical process, such as radiation, will always have significant advantages over genetic and other methods of sterilization for the large-scale application of the SIT.

## 1. INTRODUCTION

E. F. Knipling realized in the 1930s (Lindquist 1955) that, if male insects could be sterilized genetically without affecting their ability to mate, then they could be used to introduce a genetic load into a wild population in the field that would lead to its suppression or even eradication. For some time geneticists were aware that X-rays could induce mutations in insects (Runner 1916, Muller 1927), but it was not until A. W. Lindquist showed a publication by Muller (1950) to Knipling that applied entomologists realized the great potential it offered (Baumhover 2001, 2002). The results from the first experiments to sterilize the New World screwworm *Cochliomyia hominivorax* (Coquerel) were published in 1951 (Bushland and

Hopkins 1951). This demonstration, that X-rays could indeed induce sterility, was the first small step on the way to the eradication of this serious livestock pest in the southern states of the USA, and then in Mexico and all the countries of Central America as well as Panama (Wyss 2000). A permanent barrier of sterile insects has been established in eastern Panama to prevent the reinvasion of the pest from South America. Baumhover (2001, 2002) provided a historical account of the early days of the screwworm eradication programme, and Klassen and Curtis (this volume) describe the Sterile Insect Technique (SIT), in general, from an historical perspective.

During the first field trials of sterile screwworms in Curaçao, the genetic basis of sterility was poorly understood, but it was realized that sterility resulted from the induction of dominant lethal mutations in the irradiated sperm (Bushland and Hopkins 1951, LaChance et al. 1967). At that time the level of understanding of the genetics of the screwworm led Bushland to comment that (quoted by LaChance 1979):

. . . we eradicated screwworms from Curaçao and the south-eastern United States without knowing how many chromosomes it had.

Prior to the adoption of radiation to sterilize insects, chemical mutagens were evaluated (Borkovec 1966), but difficulties relating to toxicity, handling and residues were considerable, and so radiation has usually been the method of choice. Even though field trials with chemosterilized *Anopheles albimanus* Wiedemann mosquitoes in El Salvador were successful (Breland et al. 1974), it is unlikely that today such releases could be carried out.

## 2. STERILITY REQUIREMENTS FOR THE SIT

It is very important that the word “sterility” be precisely understood in terms of its use in the SIT. The word “sterility” describes one of many possible end points of the reproductive process, but it can cover a multitude of causal factors. The following definitions of sterility were taken at random from three biological dictionaries:

- Structural or functional inability to reproduce
- Involuntary total inability to reproduce
- Any complete or partial failure to produce functional gametes or viable zygotes

These definitions cover genetic, physiological, morphological or even “psychological” factors, which can lead to a final end point of sterility, and clearly many of these manifestations would not be useful for sterility in SIT programmes. For the SIT to be effective, females of the wild population in the field have to be permanently prevented from reproducing, and any factor(s) transferred by the released male that accomplishes this would, in fact, be sufficient. True genetic sterility in released male insects requires: (1) production of viable sperm, (2) their transfer to the wild female during mating, (3) their use in fertilization of eggs, and (4) the inability of the fertilized zygote to complete development to a fertile adult. In other words, an irradiated male insect must be able to carry out all the functions of a normal fertile insect — it must produce fully functional sperm that succeed in

fertilizing eggs and initiating the development of fertilized eggs. In the SIT, the radiation-induced sterility is actually produced in the generation following the release of the males, i.e. with the death of the embryo, larva, pupa or adult, or the production of F<sub>1</sub> adults that themselves produce gametes that result in zygotes that do not develop. A male insect that cannot mate, is aspermic, or that transfers non-functional sperm, could be classed as sterile, but males with any of these defects would probably not be effective for the SIT.

Irradiated males must also be able to transfer the appropriate accessory gland fluid during mating, ensuring that female behaviour corresponds to that following mating with a fertile male. In some insects, this female post-mating response involves temporary or permanent refractoriness to further mating, and a change in female behaviour. In *Drosophila* sp. the peptides transferred in the accessory fluid, that are involved in the female post-mating behavioural changes, have been well studied (Chen 1996), and it has even been possible to sterilize females by the ectopic expression of a transgene which codes for the sex peptide (Aigaki et al. 1991). In fact, a male that only transferred accessory gland fluid, and which could elicit the correct post-mating female response, could theoretically “sterilize” the female. In the Mediterranean fruit fly *Ceratitidis capitata* (Wiedemann), it has been shown that irradiated sterile males produce the same kind of change in wild female behaviour, from mating to oviposition, as do fertile males (Jang et al. 1998, Jang 2002).

In species where females remate, sterility for use in the SIT must be efficiently induced in sperm without affecting sperm function and its capacity to compete with other sperm, and exert its effect only after fertilization of the female egg; dominant lethal mutations are such sterility factors. They are readily induced in all chromosomes by irradiation, and they have little effect on the phenotype of the sperm, at least at the doses usually used for the SIT (Bakri et al., this volume). Lethality occurs when the haploid nucleus, carrying such a mutation or mutations, is combined with a normal haploid nucleus, resulting in the death of the early embryo at the moment when the genetic information required for normal development is absent or incorrect (Muller 1927). In addition, cell division can become asynchronous and lead to the death of the zygote.

### 3. DOMINANT LETHAL MUTATIONS

The mechanisms by which these mutations cause lethality in Diptera in the developing zygote are now well documented (Smith and von Borstel 1972, LaChance 1967); they are illustrated in Figure 1.

