The effect of gamma radiation on sterility and mating ability of brown planthopper, *Nilaparvata lugens* (Stål) in field cage

W Limohpasmanee¹, T Kongratarpon and T Tannarin
Thailand Institute of Nuclear Technology, Ongkharak, Nakhon Nayok, 26120 Thailand

¹ Corresponding author e-mail: wanitch1@yahoo.co.th

Abstract. The brown planthopper, *Nilaparvata lugens* (Stål) is the major rice pest in Thailand. Adults and nymphs suck the sap from the rice plant causing it to wilt and transmitting the grassy stunt and the ragged stunt diseases. The population suppression by the sterile insect technique is overwhelmingly a function of mating between sterile males and wild females. The objectives of these experiments were to determine the suitable dose which induces partially sterile in *N. lugens* and their effect on wild population in the field cages. One-day-old 4th and 5th instar nymphs and adults were irradiated in a ⁶⁰Co irradiator at the doses of 30, 60, 90 and 120 Gy. It was found that irradiation at the dose of 90 Gy induced complete sterility in females and 78.47 % sterility in males. The inherited sterility were transferred to their progenies and induced 51.46 and 77.00 % sterility in F-1 males and females. The irradiation as the mention dose did not affect mating ability. The competitiveness index was increased when the ratio of irradiated males per normal male was increased. The releasing irradiated males at 10 fold of normal males in field cages could suppress F-1 population 80.11 % and suppress F-2 population 80.32 % when compare with the control. This technique may be applied to delay and/or reduce seasonal increase of brown planthopper.

1. Introduction
Brown planthopper (BPH, *Nilaparvata lugens* (Stål)) is the major rice pest. Adults and nymphs suck the sap from the rice plant causing it to wilt and transmitting the grassy stunt and the ragged stunt diseases. In Thailand, *N. lugens* was found in the central, the lower north and some parts of north eastern regions. In 2012, the outbreak areas were covered 970,620 rai, which was approximated to 10.07 % of the total cultivated area. The usual chemical method of control has not always prevented crop damage but to some extent may have even magnified the problem, through the development of insect resistance to the chemicals and the elimination of the hopper's natural enemies. The sterile insect technique (SIT) was first developed in 1927 and could be applied as a component of an integrated program to control several major insect pests. The advantages of this technology are species-targeted, environmental-friendly, highly efficient when the population density of the target pest is partly reduced, and replacement application of the harmful insecticide. The technique was first applied to screwworms (*Cochliomyia hominivorax*) in south-eastern USA and Mexico. SIT is also being used to suppress codling moths, (*Cydia pomonella* (L.)) in Canada, fruit flies (*Bactrocera* spp.) in Argentina, Chile, USA, Mexico, Japan, Israel and Jordan, and tsetse flies (*Glossina* spp.) in many African countries. In Thailand, Thailand Institute of Nuclear Technology (Public Organization) has conducted an integrated control of fruit flies by using the sterile insect technique together with other
control methods in several areas including Doi Ang Khang in Chiang Mai province, Troklong Subdistrict in Chantaburi province and Long district in Phrae province. Successful application of SIT requires the ability to rear, sterilize and distribute sufficient number of insects to achieve a sufficiently high “overflooding” (sterile: wild insect) ratio in the field for the sterile males to successfully compete and mate with their wild counterparts. However, because Lepidoptera and Hemiptera are radioresistant, irradiation at a full-sterility dose will cause adverse effects such as morphological and behavioral abnormality as well as a decrease in mating ability (Lance and McInnis 2005). But irradiation at sub-sterility dose could induce partially sterile in parent and transfer inherited sterility to F1 offspring. The lower dose of radiation used to induce F1 sterility increases the quality and competitiveness of the released insects (North 1975).

In general, males irradiated at the completely sterilizing dose often cannot compete with untreated males in mating although irradiation did not significant reduce longevity of the males (Economopoulos, 1972). In addition, mass rearing of insect at constant temperature and using artificial diets for many generations affects physiological and behavioral characteristics. Kakinohana (1980) reported mass rearing with artificial diet in laboratory caused the qualitative changes in oviposition, sexual maturation, diurnal rhythm of mating, mating competitiveness and dispersal ability in the field of the melon fly. Hibino and Iwahashi (1992) showed differences in mating behavior between mass-reared melon flies and wild flies. Iwahashi (1993) showed a non-synchronized daily activity pattern with wild melon flies of the released flies. Sexual compatibility is the degree to which two sympatric groups of insect tend to mate randomly without regard to their group of origin rather than mating selectively with members of their own groups. Due to sterile insects did not reproduce, and therefore their fitness was largely a matter of survival, dispersion, adequate behavioral response, habitat finding, and successful mating, i.e. fitness ends with mating or insemination (LaChance 1979). Therefore the general sexual competitiveness of sterile insect was largely defined and influenced by components such as survival, mating propensity, mating compatibility, post-mating factors, etc. (FAO/IAEA/USDA 2003; Lance and McInnis, 2005).

Field releases of partially sterile insects have demonstrated the usefulness of inherited sterility to control many lepidopteran pests, including the cabbage looper Trichoplusia ni (Hubner) (North and Holt 1969), corn earworm Helicoverpa zea (Boddie) (Carpenter and Gross 1993), gypsy moth Lymantria dispar (L.) (Mastro 1993), codling moth (Bloem et al. 2001). Season-long releases of irradiated H. zea in mountain valleys of North Carolina, USA, delayed and/or reduced seasonal increases of wild H. zea males.

The objectives of these experiments were to determine the suitable dose of gamma radiation which induces partially sterile in N. lugens and their effect on wild population in the field cages.

2. Methods

The brown planthoppers used in these experiments were obtained from Pathumthani Rice Research Center and mass-reared at Thailand Institute of Nuclear Technology (Public Organization) Ongkarak, Nakhon Nayok, Thailand. One-day-old 4th and 5th-instar nymphs and adults were irradiated in a 60Co irradiator (GC-5000, BRIT, India) at the doses of 30, 60, 90 and 120 Gy; the dose rate was 4 kGy/h. At the 5th nymph stage, the insects were sexed and reared separately. To assess the sterility of parental males and females, irradiated males were mated with normal females and normal males were mated with irradiated females. While normal males were mated with normal females in the control cage, the number of the offspring was counted every 3 days and the progenies obtained from each cages were reared on the rice seedlings until they emerge to adult. To assess the sterility of F-1 males and females, F-1 males were mated with normal females and F-1 females were mated with normal males. The number of the offspring was counted every 3 days. For the remaining calculations, data were angle (arcsine) transformed and replications were polled.

To assess sexual competitiveness, one-day-old adults were irradiated at the doses of 90 and 120 Gy and placed as ratio 1:1 and 3:1 of normal males in mating cages which 10 virgin females were provided. While normal males were mated with normal females and irradiated males were mated with
normal females in the control cages. The number of F-1 progenies was counted every 3 days and reared with rice seedling until all released insects died. The number of offspring of normal and irradiated males which mated with normal females in each treatment was given, as well as the resulting competitiveness values, estimated by modified the determination of sterile insect competitiveness (Fried, 1971).

\[ CI = \frac{(Ha - Hb)}{(Hb - Hc)} \times \frac{N}{S} \]

Where  
CI = Competitiveness Index;  
\( H_a \) = number of offspring from a mating with normal males;  
\( H_b \) = number of offspring from a mating with irradiated and normal males for an N/S ratio;  
\( H_c \) = number of offspring a mating with irradiated males;  
N = Number of normal males  
S = Number of irradiated males

Value of 1 indicates the equal competitiveness between the sterile and normal males, and < 1 indicates normal females mated with sterile males less than normal males.

To assess effect of partially-sterile insect releasing on population of brown planthopper in the 100x100x150 cm. field cages. The 14 days rice seedlings were provided in the cages as their food. The one-day-old adults were irradiated at the doses of 90 Gy and released 0, 1, 5 and 10 fold of normal males in the cages, which 100 normal males and females were released before. The number of F-1 and F-2 progenies were counted every 7 days and reared with rice seedling for 60 days. The data were analyzed with ANOVA.

3. Results

When the 4th instar nymphs of \( N. \) lugens were irradiated at the doses of 30 and 60 Gy, the resulting females only laid a few eggs after mating with normal males, and all eggs did not hatch. On the other hand, males irradiated at the same doses exhibited only 66.99 and 90.96 % sterility, respectively. At the doses of 90 and 120 Gy, all irradiated nymphs died before adult emergence.

The results involving the irradiation of \( N. \) Lugens at the 5th instar nymph stage are shown in figure 1. The females irradiated at the doses of 30, 60 and 90 Gy laid only a few eggs, and all of the eggs did not hatch. Females irradiated at the dose of 120 Gy did not laid any eggs. The average sterility of
males irradiated at the doses of 30, 60, 90 and 120 Gy was 20.93, 40.75, 79.84 and 97.13 % respectively. Radiation-induced sterility increased with increasing doses; the relationship between the radiation dose and sterility could be expressed as \( y = 0.0181x + 3.244 \) with \( R^2 = 0.9527 \). The sterility of the corresponding F1 males whose male parents had been irradiated at the doses of 30, 60, 90 and 120 Gy were 39.70, 43.88, 57.83 and 43.82 %, respectively. On the other hand, the sterility of F1 females whose male parents had been irradiated at the doses of 30, 60 90 and 120 Gy were 62.03, 44.56, 24.40 and 35.89 %, respectively.

![Graph showing the sterility of males irradiated at different doses](image)

Means in the pattern color columns followed by the same letter are not significantly different \((p<0.05)\).

**Figure 2.** Effects of gamma radiation on the sterility of *N. lugens* when irradiated the adults.

The result of adult irradiation was shown in the figure 2. The females irradiated at the doses of 30, 60, 90 and 120 Gy laid a few eggs and all eggs did not hatch. The average sterility of males irradiated at the doses of 30, 60, 90 and 120 Gy was 24.54, 52.99, 78.47 and 86.00 % respectively. The sterility increased with increasing doses, the relationship between doses and sterility were \( y = 46.008\ln(x) - 132.54 \), \( R^2 = 0.9883 \). The sterility of the corresponding F1 males whose male parents had been irradiated at the doses of 30, 60, 90 and 120 Gy were 21.87, 69.70, 51.46 and 61.01 %, respectively. On the other hand, the sterility of F1 females whose male parents had been irradiated at the doses of 30, 60 90 and 120 Gy were 56.92, 87.92, 77.00 and 86.36 %, respectively.

The results of the sexual competitiveness assay were shown in table 1. The competitiveness indices of the 90 Gy irradiated males were 1.03 and 3.43 when released at the ratios 1:1 and 3:1 overnormal males. The competitiveness index increased as the ratio of irradiated males per normal male increased. These values were truncated to 1 and equal competitiveness between sterile and normal males was assumed. The competitiveness indices of the 120 Gy irradiated males were 0.99 and 0.95 when released at the ratios 1:1 and 3:1 over normal males. The competitiveness index decreased slightly when the ratio of irradiated males per normal males increased. The results also indicated that gamma irradiation at the dose of 90 Gy had no effects on mating ability of *N. lugens* males, while irradiation at the dose of 120 Gy slightly decreased the competitiveness.

The results of partially-sterile insect releasing on population of brown plant hopper in the field cages were shown in table 2. The average number of F-1 nymph at the peak were decreased every treatments but only the treatment which released irradiated males 10 fold of normal males was highly significant \((F= 7.19, \ P<0.01)\). The average number of F-1 females and F-2 nymph at the peak of all treatments which released irradiated males were decreased high significantly \((F= 11.52, \ P<0.01 ; F=40.58, \ P<0.01)\) when compare with control, but were non-significant among the treatments. The 90 Gy irradiated males releasing at 1, 5 and 10 fold of normal males suppressed F-1 population 3.77, 33.86 and 80.11 % respectively and suppressed F-2 population 75.44, 76.58, 80.32 % respectively.
Table 1. The average number of offspring and the competitiveness index of irradiated males at different ratios of normal and irradiated males.

| Treatments          | Average number of offspring (nymphs) | Sterility (%) | Competitiveness index |
|---------------------|--------------------------------------|---------------|-----------------------|
| 10 NM x 10 NF       | 58.40                                | -             | -                     |
| 10 IR_{90}M x 10 NF | 10.60                                | 81.88 ± 3.37b | -                     |
| 10 NM x 10 NF x 30 IR_{90}M | 15.00 | 74.37 ± 2.82b | 3.43 ± 0.84b       |
| 10 IR_{120}M x 10 NF | 5.80                                 | 89.98 ± 4.32b | -                     |
| 10 NM x 10 NF x 10 IR_{120}M | 34.00 | 41.35 ± 16.79a | 0.99 ± 0.61a     |
| 10 NM x 10 NF x 30 IR_{120}M | 21.60 | 62.75 ± 12.26b | 0.95 ± 0.60a     |

Means in the same column followed by the same letter are not significantly different (p< 0.05)
NM : normal male   NF : normal female   IR_{90}M : 90 Gy irradiated male  IR_{120}M : 120 Gy irradiated male

Table 2. Effect of sterile insects releasing on brown planthopper population in the field cages.

| Treatment            | Maximum number of the brown planthopper |
|----------------------|-----------------------------------------|
|                      | Nymphs (F-1)   | Females (F-1)  | Nymphs (F-2)   |
|                      | Mean   | SD  | Mean    | SD   | Mean   | SD   |
| 100 NMx100 NF        | 3509.75a | 517.45 | 1104.75a | 130.08 | 6035.50a | 1304.76 |
| 100 NMx100 NFx100 IRM| 3377.50a | 1138.90 | 355.50b  | 118.21 | 1482.50b | 306.00  |
| 100 NMx100 NFx500 IRM| 2321.25ab | 1299.26 | 398.75b  | 387.28 | 1413.25b | 591.26  |
| 100 NMx100 NFx1000 IRM| 698.00b  | 89.35  | 355.50b  | 85.25  | 1188.00b | 501.18  |

Means in the same column followed by the same letter are not significantly different (p< 0.05)
NM : normal male   NF : normal female   IRM : 90 Gy irradiated male

4. Discussion and conclusion
Adult of *N. lugens* was more resistant to radiation than 4th and 5th nymph, due to somatic division is at a minimum (Robinson 2005). Furthermore, radiosensitivity appears to be influenced by cell repopulation, tissue and organ regeneration ability, and biological repair. And males were more resistant to radiation sterilization than females, like the most species. Due to the basic difference between male and female germ cells (sperm are haploid post-meiotic, whereas mature eggs are pre-meiotic). Females were completely sterile when irradiated at the dose of 30 Gy. While males were sterile only 24.54 %, when irradiated at the same dose. Mochida (1973b) found that egg formation was interrupted when fifth-instar female nymphs were irradiated at 15 to 20 krad.

The Lepidoptera and Homoptera which have the diffuse centromeres, some dominant lethal mutation may be expressed just prior to hatch, but most of the broken chromosome pass on to the F-1
generation. It is mainly in the F1 generation of Lepidoptera that induced chromosome abnormalities express themselves as dominant lethal mutations (Carpenter 2005). Insect in this group was radiation resistant, the irradiation at the fully lethal dose caused adverse effects on insect such as abnormality, mating ability decrease. To maximize genetic damage to mature germ cells and minimize somatic damage, insect should be irradiated at 1-day adult as the dose of 90 Gy although irradiation at the dose of 120 Gy gave higher sterility both in parent and F-1. Irradiation adult at the dose of 90 Gy, females were completely sterile while male were sterile 78.47 %. and F-1 males and females were sterile 51.46 and 77.00 % respectively.

The sexual competitiveness assay indicated that gamma irradiation at the dose of 90 Gy had no effects on mating ability of N. lugens males, while irradiation at the dose of 120 Gy slightly decreased the competitiveness. And the competitiveness index increased as the ratio of irradiated males per normal male increased when released the 90 Gy irradiated males. When 90 Gy irradiated males were released at the ratio of 3:1 over normal males, 74.37% suppression of F1 fertility was achieved. When the dose of 120 Gy was used, the suppression level was reduced to 62.75%, indicating that 90 Gy irradiation showed a higher efficiency of controlling N. Lugens populations. At lower doses, irradiated insects were partially sterile and would carry minimal somatic and morphological damage. The partially sterile males appeared more active, dispersed over a greater distance, and generally were more competitive than males with a higher level of sterility (Bloem et al. 2001). The releasing 90 Gy males at 1, 5 and 10 fold of normal males in field cages showed 3.77, 33.86 and 80.11 % suppression in F-1 population and 75.44, 76.58, 80.32 % suppression in F-1 population. Their efficiency was decreased when compare results with mating competitiveness assay, because of the difference of space and population density. However, this technique could reduce the increasing rate of brown planthopper only. But the number of insects in filed cages still increases because the fraction of males that remained fertile was higher than the fraction of increasing rate. This technique was applied to delay and/or reduce seasonal increases of wild corn earworm Helicoverpa zea (Boddie) in mountain valleys of North Carolina, USA. Release ratios average less than 5:1 overall, but reduce the wild population of H. zae by more than 70 %. In another case, season-long field studies of the codling moth were conducted in apple orchards in Washington State, USA, both release of partially sterile codling moth and combination of mating disruption plus the release of partially sterile codling moth gave the significantly lower fruit damage when compared with control plots located outside the treatment areas.

The suitable dose of gamma radiation which induces partially sterile in N. lugens adults was 90 Gy, irradiated females were completely sterile and irradiated males were sterile 78.47 %. The abnormal chromosome were transfer to their progeny and induced 51.46 and 77.00 % sterility in F-1 males and females. The irradiation as the mentioned dose did not affect mating ability and the competitiveness index was increased when the ratio of irradiated males per normal male was increased. The releasing irradiated males at 10 fold of normal males in field cages could suppress F-1 population 80.11 % and suppress F-2 population 80.32 %, when compare with the control.

References
[1] FAO 2005 Glossary of phytosanitary terms Secretariat of the International Plant Protection Convention (IPPC), FAO
[2] LaChance LE 1967 Genetics of insect vectors of disease (Elsevier, Amsterdam, The Netherlands) pp 617-50
[3] LaChance LE, CH Schmidt, and RC Bushland Pest control Biological, physical, and selected chemical methods (Academic Press, New York, USA) pp 147-196
[4] LanceDR and DOMcINNIS 2005 Sterile Insect Technique (Springer, The Netherlands) pp 70-85
[5] FAO/IAEA/USDA 2003 Manual for Product Quality Control and Shipping Procedures for Sterile Mass-Rearing Tephritid Fruit Flies, Version 5.0, IAEA. 85 pp
[6] North DT 1975 Annual Review of Entomology 20 167-82
[7] BloemS, K ABloem, JE Carpenter and CO Calkins 2001Environmental Entomology 30
763-69
[8] Carpenter JE, ANSaprks and HLCromroy 1987aJ. of Econ. Ent. 801233-37
[9] Carpenter JE., and HR Gross 1993Environmental Entomology22 1084-91
[10] Economopoulus AP 1972Environmental Entomology1:490-97
[11] KakinohanaH 1980 Qualitative change in the mass-reared melon fly, Dacus cucurbitae Coq.
Proc. of Symp.on Fruit Fly Problems27-36
[12] Hibino Y and Olwahashi 1992Appl. Ent. Zool.24152-54
[13] Iwahashi O 1993Follow-up Mission Report for THA/5/038/02.IAEA. 33p
[14] La ChanceLE1979 Genetics in relation to insect management (The Rockefeller Foundation, New York, N.Y., USA) pp 8-19
[15] FAO/IAEA/USDA 2003 Product quality control and shipping procedures for sterile mass-reared tephritd fruit flies. Version 5.0 IAEA, Vienna, Austria
Http://www.iaea.org/programmes/nafa/d4/index.html
[16] Fried M 1971 J. of Econ. Ent.64869–72
[17] Bauer H 1967Chromosoma22102-25
[18] Klassen W and J F Creech 1971Miscellaneous Publication USDA/ARS Number 1182
[19] North DT and G G Holt 1969 Canada Entomologist101513-20
[20] Mastro VC1993 Final Research Co-ordination Meeting IAEA STI/PUB/929 pp 125-129
[21] Muller HJ 1954 Radiation Biology1351-473
[22] LaChance L E and J G Riemann 1964Mutation Research1 318-33
[23] Robinson AS 2005Sterile Insect Technique (Springer, The Netherlands) pp 70-85
[24] Mochida O 1973 Effect of gamma radiation on the development and reproduction of the brown planthopper, Nilaparvata lugens (Stal) (Homoptera: Delphacidae) 42A4DC1A93E823038C
[25] Carpenter JE, S Bloem and F Marec 2005Sterile Insect Technique (Springer, The Netherlands) pp 115-46
[26] Carpenter JE, Hidryani N Nelly, and BG Mullinix 1997 J. of Econ. Ent.90: 444