Radiol Oncol 2002; 36(3): 231-7.

Effects of 5-Gy irradiation on fertility and mating behaviour of Nezara viridula (Heteroptera: Pentatomidae)

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Background. *The polyphagous and cosmopolitan species Nezara viridula is one of the most important insect pests. The sterile insect technique (SIT) is a pest control strategy that involves sterilising males by exposing them to ionising radiation. Sterile males, released into wild population, mate with females, but eggs are not fertilised and the population gradually declines. Exposing insects to radiation during their growth stage might require lower sterilising dose. The aim of our study was to test whether 5-Gy irradiation of 5th instar nymphs significantly affects: (1) moulting and further development of the irradiated nymphs, (2) the male's and female's reproductive system and (3) the mating competitiveness of treated males, with special focus on vibrational communication.*

Methods: *The 5th instar nymphs were irradiated with 5 Gy using X-ray generator and monitored daily.*

Results: *The observed effects of irradiation were: prolonged moulting, increased mortality during development and during the first day of adult life, decreased males to females ratio, decreased fecundity, egg production, proportion of fertile eggs and progeny survival. The reaction of a male to stimulation with the model female calling song was tested. The irradiated and non-irradiated males responded to stimulation with emission of the courtship song (MCrS). Temporal parameters of MCrS emitted by non-irradiated males differed when compared with those of irradiated ones.*

Conclusions: *The 5-Gy irradiation of 5th instar nymphs did not affect mating behaviour. However since the irradiation during growth stage decreased the fertility and fecundity of emerged adults, this technique, in combination with certain other suppression techniques, could be a successful control strategy for management of Nezara viridula. On the other hand observed effects on moulting and further development of the irradiated nymphs could decrease the efficiency and application of this strategy.*

Key words: gryllidae - radiation effects; insect control; molting; animal communication; vibration

Received 22 August 2002
Accepted 16 September 2002

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Introduction

The southern green stink bug *Nezara viridula* (L.) is one of the most important pentatomid insect pests in the world. As a cosmopolitan and highly polyphagous species it infests many important vegetable crops.[1,2] *Nezara*

viridula and other stink bugs are difficult to control over wide areas, because of the large variety of crops on which they feed and the type of damage they produce.[3] Even a low population density can cause large economic damage to the crops. *Nezara viridula* is a prolific, long-lived bug nourishing on short-season crops and an area wide control strategy may bring success. Autocidal methods and genetic manipulation can be effective against low-density population dispersed across wide ranges and against pest within high-density, but localised population.[4] Knipling proposed that the release of semisterile insects would bring damaging genetic stress on the target population.[5] Partial sterility of adult hemipterans may be achieved after they are exposed to ionising radiation of 30-100 Gy.[6,7] Dyby and Sailer showed that low-level radiation exposure of Nezara viridula during their growth stage (4th instar nymphs) has a greater impact on reproductive fitness.[8] Females laid nonviable eggs in high proportion and had significantly lower fecundity than controls. They reasoned that exposure of 4th instar nymphs to ionising radiation of less than 10 Gy has no serious effect on mating behaviour and survivorship.

The aim of our study was to test whether 5-Gy irradiation of 5th instar nymphs significantly affects: (1) moulting and further development of the irradiated nymphs, (2) the male's and female's reproductive system and (3) the mating competitiveness of treated males, with special focus on vibrational communication.

Materials and methods

The experiment was conducted on southern green stink bugs *Nezara viridula* of the Guadeloupe population. Bugs were reared in the laboratory in plastic cages (38×23×23 cm), at 22 - 26°C, relative humidity 70 - 80 %, 16 L: 8 D daily cycle, and on a diet of green beans (*Phaseolus vulgaris* L.), mung bean (*Vigna mungo* (L.) Hepper), raw peanuts (*Arachis hypoaea* L.) and sunflower seeds (*Helianthus annus* L.). Nymphs and adults were kept in separate cages.

One hour before irradiation, 5th instar nymphs of the same generation (a few days before the final moult) were separated into two groups (20 individuals each) of which one was irradiated and the other (non-irradiated) was used as the control. Experimental animals were placed into 2 plastic petri dishes (2r = 10 cm). The height of the cover was adjusted so that bugs could not move up or down during irradiation. At the Institute of Oncology one group of bugs was irradiated by a dose of 5 Gy (2 Gy/min) using a Darpac 2000-XE (Gulmay Medical, England) X-ray generator filtered with 0,55 mm Cu and 1,8 mm Al filter. The test was repeated six times with different bugs and named irra01 (January 2000), irra02 (February 2000), irra05 (March 2000), irra06 (April 2000), irra07 (November 2000) and irra09 (February 2001). After irradiation the control and irradiated nymphs were placed into plastic cages reared in the way as before treatment. For each group we monitored daily: the number of live individuals, moulted nymphs, male to female ratio, copulating pairs, egg masses, eggs per egg mass, sterile eggs, and the number of hatched eggs. Egg masses were placed into separate petri dishes. Emerging nymphs were then placed into plastic cages. We monitored the time from hatching to the adult stage, so that the overall progeny survival was obtained.

To investigate the effect of irradiation of the 5th instar on vibrational communication during mating behaviour, we used the reaction of a male to female calling song.[9] In this reaction the male responds to the female calling song (FCS) with emission of the male courtship song (MCrS). Responses of the control and treated males were tested in an anechoic and sound insulated chamber (FA Amplaid, Italy) at room temperature

(23±2°C), 65 - 75 % relative humidity and room light. We placed a control or treated male on a membrane of a cone low-middle frequency speaker (WS 13 BF, Visaton GmbH, Haan, Germany, impedance 8 W, 2r = 144 mm, 40 - 6000 Hz). To prevent male's moving from the membrane, the loudspeaker was covered with a 2-mm thick Perspex sheet. The latter was not in contact with the membrane, which acted as a receiver of the male's vibrational signals and as the emitter of a female vibrational signals (FCS). The FCS was synthesised with computer programme (Sound Forge for Windows 95, version 4.0c, Sonic Foundry Inc., Madison, USA). The stimulation programme consisted of 7 stimulation sequences (1 minute each) each followed by 1-minute pause. The 1-minute sequences were composed of 120 Hz pulses repeated every 4 seconds. The duration of pulses varied between different sequences: 200, 500, 800, 1000, 1200, 1600, 2000 ms. The stimulation was played-back from the computer, amplified by laboratory made amplifier and fed into the loudspeaker. The intensity of stimulatory signals was adjusted to the level of male response. Male responses were amplified by a tape recorder (Revox A - 77, Regensdorf, Switzerland) and fed into a PC computer. Digitised signals were stored and analysed later with a computer programme Sound Forge. We tested 5 males from the control group irra05 (group A) and 6 males from the laboratory culture (group B), and 5 males from irradiated group irra06 (group C) of bugs. Each individual was tested several times but only once in a day. Males from group A (N = 5) were tested 20 times (n = 20), from group B (N = 6) 14 times (n = 14) and males from group C 11 times (n = 11). We recorded the overall number of tests during which males responded to the stimulation at least once. We recorded the number of male's responses (MCrS) to the model FCS and we analysed the duration of MCrS pulse trains and the latency (time between the on-set of stimulus and the male's response) (Figure 1). A mean value from the analysed parameters was calculated for each group.

Student's T - test (Microsoft Excel 7.0) was used to determine the significance of difference between the control and irradiated bugs.

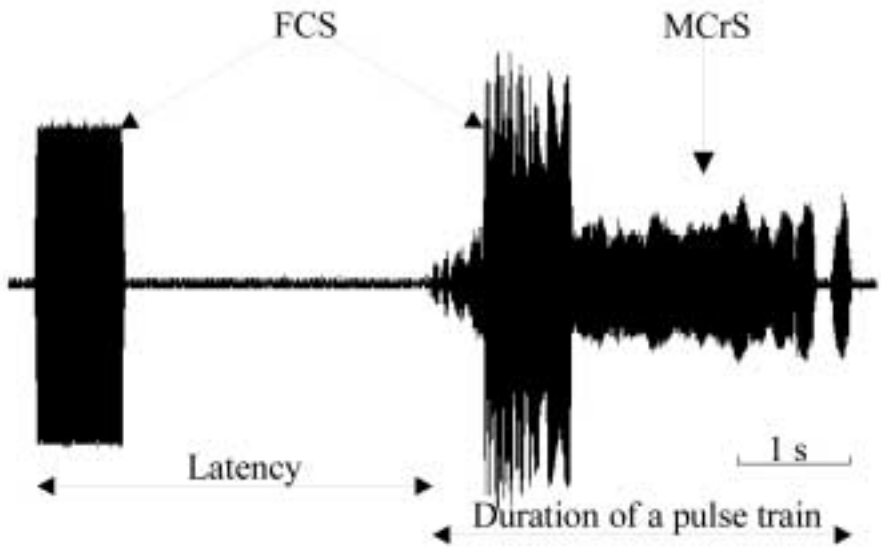

Figure 1. The male response (MCrS) to the model FCS and the analysed parameters: the duration of MCrS pulse train and the latency of the response.

Results

To investigate the effects of radiation we monitored and compared different parameters in control and irradiated group of bugs. Results are shown in the Table 1.

Irradiated groups differed significantly from control groups in the group moult duration (the time between the first and the last observed moult in a group). The time between the first and the last moult within a group was longer in irradiated groups irra01, irra02 and irra05 (20-21 days) than in non-irradiated nymphs (8-11 days). In irra06, irra07 and irra09 groups moult duration was similar for the non-irradiated and irradiated nymphs (7-19 days). All the nymphs survived irradiation but mortality of irradiated nymphs during moult was different in different groups and ranged from 1 (irra07) to 16 (irra02). Mortality was significantly higher when compared with control in three groups: irra01, irra02 and irra05. Nymphal mortality reached highest values 5-7 days after the first observed moult in irradiated and control groups. We found no difference in the time to reach 50% cumulative mortality ($P = 0.47$). In the groups irra01, irra02 and irra05 significantly more non-irradiated (10-15) than irradiated (4-9) nymphs reached adulthood. In groups irra06, irra07 and irra09 similar number of adults emerged from non-irradiated and irradiated nymphs. The number of emerged males was significantly different for the irradiated groups irra01 and irra02 ($P < 0.05$). In the group irra01 only 1 male emerged from irradiated nymphs (in control group 7), in group irra02 no males emerged from irradiated nymphs (in control group 5). On the other hand no significant difference was found in the number of emerged females. In the groups irra05, irra07 and irra09 we observed significantly higher mortality of adults during the first day after the moulting of irradiated nymphs ($P < 0.05$). Adults that successfully emerged from irradiated nymphs lived as long as controls.

No significant difference could be shown

Table 1. Significance difference ($P < 0.05$) between control and irradiated group of bugs, shown separately for each test (irra01, irra02, irra05, irr06, irra07, irra09) for each monitored parameter

Parameters	irra01 (N* = 20)	irra02 (N = 20)	irra05 (N = 20)	irra06 (N = 20)	irra07 (N = 20)	irra09 (N = 20)
Group duration of moult	+#	+	+	-÷	-	-
Mortality of nymphs	+	+	+	-	-	-
50% cumulative mortality	+	+	+	-	-	-
Number of emerged adults	+	+	+	-	-	-
Number of emerged females	-	-	-	-	-	-
Number of emerged males	+	+	-	-	-	-
Number of adults which died a day after the moulting	-	-	+	-	+	+
Longer lifespan	-	-	-	-	-	-
Precopulation period	-	-	-	-	-	-
Copulation period	-	-	-	-	-	-
Duration of copulation	-	-	-	-	-	-
Number of egg masses	-	-	-	-	-	-
Total number of eggs	+	+	+	-	-	-
% of fertilised eggs	/◊	/	/	/	+	/
% of hatched nymphs	+	+	+	-	-	-
Overall progeny	+	+	+	-	-	-

* the number of bugs in the group

The significance of differences ($P < 0.05$).

÷ No significance of differences ($P > 0.05$).

◊ no data avalible

in precopulation period (time between the first emerged adult and the first observed copulation within one group) as well as in copulation period (time between the first and last observed copula). On the contrary we found significant difference between control and irradiated groups in the number of deposited and fertilised eggs ($P < 0.05$), in the percentage of hatched eggs and in overall progeny. Females of control groups (N = 26) laid 927 eggs in 24 egg masses, females of irradiated groups (N = 18) laid in 11 egg masses 297 eggs. In irradiated group irra07 we observed only 23.4% (N = 248) fertilised eggs as compared to 80,4% (N = 386) in control group. Eggs laid by females of control groups (irra01, irra02, irra05) hatched in 87,3% (N = 541) and eggs laid by females of irradiated groups (irra01, irra06) in only 35,7 % (N = 49). In control groups we have obtained 61 adults from 927 eggs, in irradiated only 1 adult from 297 eggs ($P < 0,05$).

In control and irradiated groups typical mating behaviour was observed.[9,10,11] We analysed vibrational communication between males and females of two controls (A, B) and one irradiated group (C). In all three groups we recorded regularly male calling (MCS) and courtship (MCrS) song as a response to the stimulation with the model FCS (Figure 1). We found no difference in the number of responses to the stimulation between the males of control and irradiated groups. The males of control group A (N = 6) responded to stimulation at least once in 30% of tests (n = 20), males of control group B (N = 6) in 35,7% of tests (n = 14). In the irradiated group C males (N = 5) responded to stimulation at least once in 27,3% of the tests (n = 11). The difference between each control group and irradiated one was not significant ($P_{A/C} = 0,46$; $P_{B/C} = 0,11$) (Table 2). In all three groups the number of MCrS pulse trains ranged from 22 to 32 during stimulation, and from 15 to 18 during pauses. We have found no significant difference in the latency of male responses of each control and irradiated group (PA/C = 0,32; PB/C = 0,44). In all three groups most of the MCrS signals were recorded as a response to the stimulation pulse with duration of 1000 ms. The only difference between males of different groups was found in duration of MCrS pulse trains. Males of control group A emitted significantly longer pulses than the males of group B and C ($P < 0,05$) (Table 2).

Discussion

Ionising radiation of 5 Gy had a significant impact on moulting, development of newly emerged adults and on the fecundity of adults that emerged from irradiated 5th instar nymphs.

Moult duration of irradiated nymphs was two times longer than moult duration of non-irradiated nymphs in three groups. In groups showing prolonged moulting and higher mortality of nymphs we also observed lower number of emerged adults and their higher mortality during the first day after moulting. Since Dyby and Sailer [8] reported that low-level radiation exposure of 4th instar nymphs has no serious effect on survivorship, we assumed that 5th instar nymphs are more sensi-

Table 2. Vibrational communication. The significance difference (+), ($P < 0.05$) between control and irradiated groups of bug, shown separately for two control groups (A, B) and for irradiated group (C)

Parameters	A◊ (N* = 5)	B** (N = 6)	C•• (N = 5)
Type of response	-÷	-	-
Number of responses	-	-	-
Duration of pulse trains	+#	+	-
Latency	-	-	-
Number of response regarding of stimulation time	-	-	-

◊ males of control group irra05
** males of control group from laboratory culture
•• males of irradiated group irra06
* The number of tested males
# The significance of differences ($P < 0.05$).
÷ No significance of differences ($P > 0.05$).

tive to low-level radiation then 4th instar nymphs. In two irradiated groups we observed significantly lower proportion of males than females that emerged from nymphs which points to higher sensitivity of males to radiation. On the other hand we observed no significant difference between the control and irradiated groups in the life span, in the time of precopulation period and temporal parameters of copulation. We conclude that the irradiation had greater effect during moulting when the mitotic rate of epidermis cell is very high and the bugs are most vulnerable to external factors. Comparison of the control and irradiated groups also showed that the radiation significantly reduced fecundity and egg production dropped. Semisterility increased after the radiation treatment, the number of fertile eggs and the proportion of hatched eggs decreased. Since the progeny life span of irradiated groups significantly decreased, we could not observe the impact of the radiation on progeny generation. Dyby and Sailer [8] showed that recovery to normal fertility is an all or none event in the progeny generation. Some pairs are sterile and thus bred out of the population, whereas others show complete recovery. Lethal mutations are eliminated within one generation, however some pairs do not recover to normal reproductive fitness, probably because of the environmental stress.

We also investigated whether the 5-Gy irradiation during growth stage changed mating behaviour in *Nezara viridula*. Emission of vibrational signals is an important part of mating behaviour, providing the information needed for mate recognition and location.[10,12] We therefore examined if males that emerged from irradiated nymphs respond differently to the model female calling song than controls. If irradiated males would be unable to recognise the FCS or if their vibrational responses would be significantly altered, their competitiveness would be decreased. Comparison of mating behaviour of control and irradiated males revealed significant difference only in the duration of vibrational signals between the control group A and irradiated group C. Since duration of signals differed also between the two control groups (A, B group), we cannot attribute the difference between groups A and C to irradiation.

The biological effects of radiation on living organisms may be divided into somatic and genetic effects. In our study we observed the somatic effects like prolongation of moulting, the increase of nymphal mortality and increased adult mortality during the first day after the moult. Decreased fecundity and fertility and increased progeny mortality were the genetic effects of 5-Gy radiation on 5th instar nymphs.

In some bugs we observed no effect of irradiation. We assume that overall impact of 5-Gy irradiation is different for different individuals. Some of the bugs could have been parazitised or diseased also, the effects of radiation could be exacerbated by inbreeding depression of laboratory reared bugs.

The 5-Gy irradiation of 5th instar nymphs did not affect mating behaviour. However since the irradiation during growth stage decreased the fertility and fecundity of emerged adults, this technique, in combination with certain other suppression techniques, could be a successful control strategy for management of *Nezara viridula*. On the other hand observed effects on moulting and further development of the irradiated nymphs could decrease the efficiency and application of this strategy.

Acknowledgements

The authors are grateful to Mira Lavrič and Viktor Triler for technical assistance during experimental work. The project was financially supported by the International Atomic Energy Agency (Project No. 10967/RO).

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