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Radiation-induced chromosomal aberrations in grape phylloxera

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Key words: chromosome aberrations, grape phylloxera, irradiation, reproduction.

Abstract: Chromosomal aberrations in phylloxera females induced by different doses of gamma irradiation were detected. The results showed that the chromosomes of all tested embryos of irradiated phylloxera had aberrations, regardless of dose. When phylloxera nymphs were irradiated, the chromosomal number on the metaphase plate of some embryos' cells was increased. The result indicated that the chromosomal aberrations influenced the mortality, longevity and reproduction of phylloxera. Eight autosomal chromosomes were identified according to their length. Additionally, the karyotype of irradiated and unirradiated populations of local phylloxera strain was defined.

1. Introduction

Grape phylloxera, *Daktulosphaira vitifoliae* Fitch (Homoptera: Phylloxeridae), an aphid-like gall-forming parasite, is one of the most destructive insect pests of cultivated grape *Vitis vinifera* L. world wide. Grape phylloxera causes direct damage to grapevine by forming damaging root galls. These galls are metabolically active organs suited to match the nutritional requirements of phylloxera and can support populations with high reproductive rates. Granett *et al*. (1985) reported that there was frequent decline of commercial vineyards as result of this pest, and consequently losses of quality and yield of grapes.

The use of resistant rootstocks is the most common and effective means of managing phylloxera. Our previous studies showed that some rootstocks were more resistance than others to grape phylloxera (Makee *et al*., 2003). However, for unknown reasons, the resistance of some rootstocks may break down and farmers must replant vineyards (Granett *et al*., 1983; Song and Granett, 1990; De Benedictis and Granett, 1993). Therefore, additional ways to control this pest should be considered.

Sanitation and quarantine can be considered as required procedures to prevent the movement of this serious pest. Insecticides and hot water dip treatments are used as quarantine treatments (Granett *et al*., 2001). Ionizing radiation has been recognized as an alternative method for treating agricultural products to overcome quarantine barriers in trade (FAO, 2003). Irradiation treatment does not influence the quality of many commodities; it is reasonably safe to the consumers and environment. Previously, several authors have presented reviews of this subject (Hallman, 1998; Johnson and Marcotte, 1999; Hallman, 2000, 2001).

Irradiation has been successfully used for the control of many insect pests such as codling moth *Cydia pomonella* (L.), beetle *Prionolplus reticularis* White (Lester *et al*., 2000), apple maggot, *Rhagoletis pomonella* (Walsh), the borer *Eucosma notanthes* Meyrick (Lin *et al*., 2003), coconut scale *Aspidiotus destructor* Signoret (Follett, 2006), the weevil *Sternochetus mangiferae* (F.) (Follett, 2001), cigarette beetle, *Lasioderma serricorne* (F.) (Hu *et al*., 2002), and the rice weevil *Sitophilus zeamais* Motschulsky (Hu *et al*., 2003).

Makee *et al*. (2008) proposed that gamma irradiation could be economically very useful in quarantine treatments against phylloxera. The results showed that the percentage of matured phylloxera females and fecundity were markedly reduced when higher doses of gamma irradiation were used (Makee *et al*., 2008). However, to our knowledge efforts of associate performance parameters of irradiated phylloxera with chromosomal rearrangements have not yet been studied. Therefore, the purpose of the present research was to detect chromosomal aberrations in phylloxera females induced by different doses of gamma irradiation. The influence of such chromosomal aberrations on longevity and reproduction of phylloxera was examined. Moreover, determination of the number and length of autosomal and sex chromosomes was undertaken. Such

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study will allow definition of the karyotypes of irradiated and unirradiated populations of the local phylloxera strain.

2. Materials and Methods

Establishment of phylloxera colony

Grape phylloxera was originally collected from field-infested roots of the local grape varieties in southern parts of Syria. All insects were reared on fresh and healthy pieces of local grape variety Balady roots, 4-7 mm in diameter and 5-7 cm long as outlined in Makee *et al*. (2003). The experiments were conducted at 25±1°C with 70±5% RH, and 24 hr darkness. Egg sterilization was carried out as described by Makee *et al*. (2003). A Co^{60} source (Issledov Gamma Irradiator, Techsnabexport Co. Ltd., Moscow, Russia) delivering a dose rate of 60 Gy/min was used to treat the insects.

Effect of irradiation on matured females, fecundity, and oviposition period

New phylloxera eggs were placed on fresh root pieces and left until hatching. A group of three-week-old feeding phylloxera nymphs was taken. Nymphs were irradiated at different doses: 0-10-20 and 30 Gy (n= 25 nymphs at each dose). Irradiated and unirradiated nymphs were kept at 25±1°C with 70±5% RH, and 24 hr darkness.

A daily microscopic inspection of all phylloxera stages at each applied dose was carried out. The number of feeding nymphs, which were able to develop to adult stage, was observed to determine the percentage mortality at each dose. Female fecundity and longevity was determined at each tested dose.

Chromosome preparations

Mitotic metaphase chromosomes were obtained from 24 to 36-hr-old embryos. At each dose, 10 eggs were taken and placed in a 1.5 ml tube. The eggs in each tube were fixed in Carnoy's fixative (ethanol:chloroform:acetic acid 6:3:1) and shaken for 10 min. Then a drop was taken and transferred onto a clean slide. Shortly before drying, a drop of 60% acetic acid was added and macerated for 2-3 min with fine tungsten needles. Then the specimen was spread on the slide using a heating plate at 45°C to allow evaporation of the acetic acid. The preparation was then stained and mounted in lactic acetic orcein for 5 min; redundant stain was removed with a piece of filter paper. The cover glass was sealed with nail polish. Chromosome preparations were examined in phase contrast micrographs.

Chromosome measurement

The lengths of the chromosomes from 12 well spread orcein-stained metaphase chromosomes were measured in digital images using Digitizier software, version 1 (developed by the Mathematics Department, Atomic Energy Commission of Syria). Chromosome lengths were ranked for each cell nucleus and means and standard deviations (SD) were calculated. Relative chromosome lengths were calculated as percentages of the total length of all chromosomes in the diploid set.

3. Results

Effect of irradiation on mortality, longevity and fecundity

Our results show that the percentage mortality of irradiated phylloxera nymphs was significantly higher than that of unirradiated ones. A regression line was fitted to present the relationship between gamma irradiation and percentage mortality of phylloxera (Fig. 1), showing that the percentage mortality was positively correlated with dose (t= 7; P=0.05). The lowest percentages of mortality were recorded at 10 Gy, after which the percentages started to increase. Only about 16% of phylloxera nymphs were able to reach matured female stage at 30 Gy.

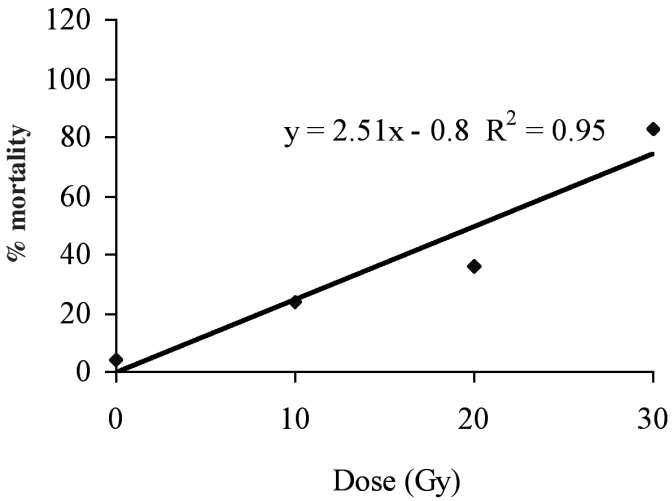

Fig. 1 - Effect of gamma irradiation on percentage mortality of grape phylloxera.

Table 1 illustrates that phylloxera longevity and number of eggs were considerably influenced by the applied dose (Table 1). The mean value for longevity and the mean number of eggs were significantly reduced by increasing the dose (F=75.67; df=3, 96; P=0.05 and F=106.78; df=3, 96; P=0.05, respectively).

Table 1 - Effect of different doses of gamma irradiation on mean number of eggs and longevity of phylloxera

Dose (Gy)	Mean no. eggs (±SE)	Mean longevity (d) (±SE)
0	60.0±4.0 a	14.6±0.75 a
10	24.5±2.7 b	10.0±0.88 b
20	10.7±1.0 c	7.0±0.69 c
30	0.7±0.2 d	0.6±0.01 d

Means followed by different letters (columns) are significantly different at $P< 0.05$ (Tukey HSD test).

Chromosomal analysis under a light microscope

From matured phylloxera females, 24 to 36-hr-old embryos, which contain a higher proportion of dividing cells, were taken to analyze the metaphase chromosomes. It was revealed that the wild-type metaphase karyotype of phylloxera consists of 10 chromosomes. There were eight autosomal chromosomes and sex chromosomes (XX) (Fig. 2 A). A karyotype of 2n=9 was also found (Fig. 2 B). Based on our observations, phylloxera metaphase chromosomes appeared like thick condensed rods. In some cells, an additional very small chromosome was detected (Fig. 3).

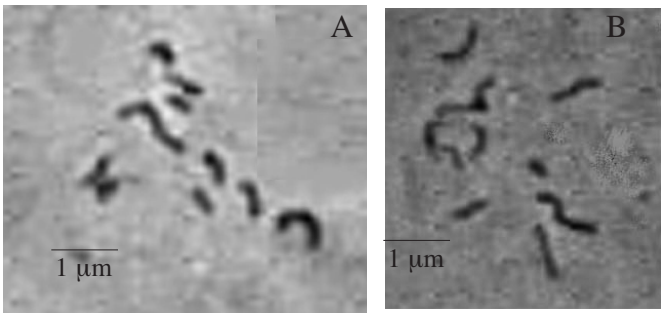

Fig. 2 - Normal metaphase kryotype from embryonic cells of Grape phylloxera, *Daktulosphaira vitifoliae* Fitch: A) 2n= 10; B) 2n=9 + small chromosome (the arrow).

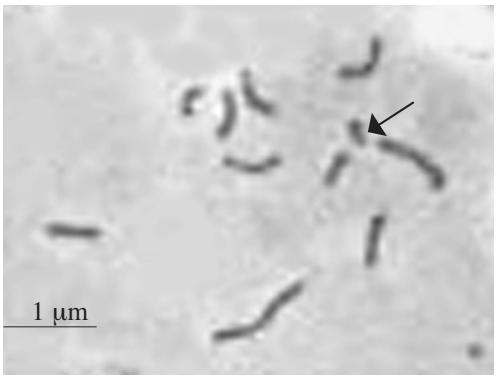

Fig. 3 - Normal metaphase kryotype from embryonic cells of Grape phylloxera: 2n= 10 + small chromosome (the arrow).

To study the effect of different doses of gamma irradiation on phylloxera chromosomes, 24 to 36-hr-old embryos were examined at each tested dose, and it was found that the chromosomes of all tested embryos of irradiated phylloxera had aberrations, regardless of dose. At all different doses, sticky chromosomes were observed in the embryo cells (Fig. 4). When 20 Gy was applied, inter-chromosome translocations were clearly visible (Fig. 5). However, increasing in the chromosomal number on the metaphase plate in some cells was noticed in embryos when phylloxera nymphs were irradiated (Fig. 6).

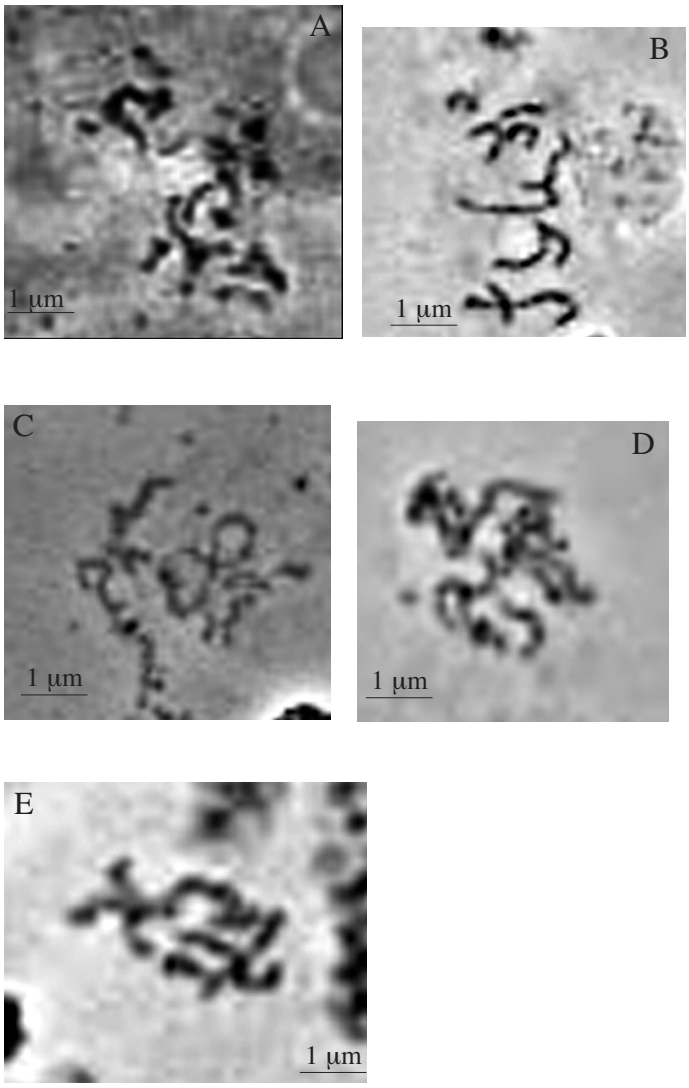

Fig. 4 - Mitotic metaphase chromosomes from embryos of treated Grape phylloxera with gamma ray, showing sticky chromosomes at deferent doses: A-B at dose 10 Gy; C) at dose 20 Gy, D-E) at dose 30 Gy.

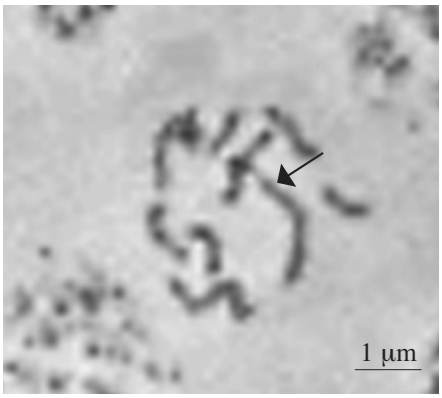

Fig. 5 - Orcein-stained preparations from embryos of irradiated phylloxera at dose 20 Gy showing mitotic metaphase chromosomes with a translocation on the large chromosome (the arrow).

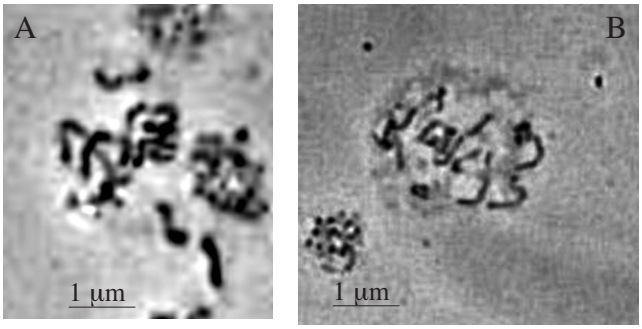

Fig 6 - Orcein-stained preparations from embryos of irradiated phylloxera showing: A) at dose 20 Gy: a cell with about 15 chromosomes which some of them are stuck together; B) at dose 30 Gy: a cell with 14 chromosomes.

Chromosomes measurement

It was possible to identify eight autosomal chromosomes according to their length when different metaphase chromosomes were investigated. Table 2 illustrates the mean length and relative length for each identified chromosome. The total mean length of complete metaphase chromosomes was 12.17±1.97 µm.

Chromosome pair no. 1 is an extra large chromosome with an average total length of 2.31±0.3 µm and relative length of 18.18%; chromosome pair no. 2 is a large chromosome with an average total length of 1.33±0.3 µm and relative length of 10.5%; chromosome pair no. 3 is a medium chromosome (1.12±0.65 µm); and chromosome pair no. 4 is a short chromosome (0.90±0.160 µm) (Table 2).

The sex chromosome (XX) was clearly recognized. The sex chromosome is the shortest chromosome in the metaphase complements with an average total length of 0.69±0.1 µm and relative length of 5.51%.

The mean length of the additional chromosome, which was observed in several cells, was only 0.5±0.1 µm.

Table 2 - The mean length and relative length (%) of phylloxera chromosomes

No. chromosome	Mean length (µm±SD)	Relative length (%)
1	2.31±0.3	18.18
1	2.31±0.3	18.18
2	1.33±0.3	10.5
2	1.33±0.3	10.5
3	1.12±0.65	8.81
3	1.12±0.66	8.81
4	0.90±0.16	7.1
4	0.90±0.16	7.1
X	0.69±0.1	5.41
X	0.69±0.1	5.41
Total mean length	12.17±1.97	100

4. Discussion and Conclusions

Several studies demonstrated that Dipteran, Coleopteran and Hemipteran species tend to be more radiosensitive than Lepidopteran species. However, considerable variation was noted among the species tested within these orders (Makee and Saour, 1999; Bakri *et al*., 2005; Follett *et al*., 2007). In fact, a few studies were carried out to determine the radioresistance of Hemiptera (scales, mealy bugs, aphids, and whiteflies).

A previous study showed that the egg hatch of phylloxera decreased when eggs were subjected to high doses of gamma irradiation and the percentage of matured phylloxera females significantly increased as older nymphs and lower doses were used (Makee *et al*., 2008). On the contrary, fecundity was markedly reduced when older nymphs and higher doses were employed. However, a relationship between chromosomal aberrations induced by irradiation and phylloxera biology was not determined in the study.

Phylloxera can be considered a cytogenetically exciting insect species because of abnormal features related to its cyclical parthenogenesis, and because it has holocentric chromosomes (chromosomes that lack a localized centromere). Phylloxera populations can consist totally of parthenogenetic (thelytokous) females. Several studies showed that grape phylloxera mainly reproduces asexually (*parthenogenesis*): an egg cell can develop into offspring without fertilization by a sperm. Thus, the offspring and its siblings are assumed to be genetically identical to the mother (Vorwerk and Forneck, 2006). Parthenogenetic reproduction of phylloxera has been observed in the field and can be easily maintained under constant conditions in the greenhouse or *in vitro*. This type of reproduction allows phylloxera populations to be replicated several fold, thus several asexual generations can be analyzed within a short period. Once a year, XX parthenogenetic phylloxera females, like aphids, produce one egg that develops as an XO male, having lost half its X chromatin during the single maturation division (Blackman and Hales, 1986; Blackman, 1987).

The current study has shown that phylloxera nymph mortality increased with irradiation (Fig. 1), confirming the results reported by Makee *et al*. (2008). Correspondingly, Dohino *et al*. (1998) found that the survival of aphids was significantly decreased when they were treated with doses of 400-600 Gy. The high death rate, especially when phylloxera nymphs were exposed to higher doses of gamma irradiation, can be attributed to the effects of the dominant lethal mutations induced in phylloxera nymphs' chromosomes by irradiation (LaChance, 1967). When low doses were applied, a small portion of irradiated nymphs successfully completed development and produced matured females, survival which was due to the holokinetic nature of phylloxera chromosomes. Irradiation can cause fragmentation but the resulting fragments are still able to move on the mitotic spindle so that chromosome breakage does not lead automatically to the loss of genetic material (Hughes-Schrad and Ris, 1941).

Our results reveal that the fecundity and longevity

of surviving matured females, irradiated as nymphs, were greatly impacted by irradiation (Table 1). Comparable results were reported when crawls and nymphs of mealybug, *Maconellicoccus hirsutus* (Green), were irradiated (Jacobsen and Hara, 2003). Therefore, at low doses some matured phylloxera females were recorded, but they laid only few eggs and lived for a short period of time. It could be that the induced chromosomal aberrations in irradiated nymphs prevent the normal process of mitotic division, which leads to egg production. Therefore, the matured females were unable to produce a normal number of eggs.

To study the effect of different doses of gamma irradiation on phylloxera chromosomes, 24 to 36-hr-old embryos were examined at each tested dose. It was noted that the chromosomes of all tested embryos of irradiated phylloxera had aberrations, regardless of dose. We noticed sticky chromosomes, inter-chromosome translocations, and increases in the chromosomal number on the metaphase plate in some cells. All these chromosomal aberrations in the embryos were expected as the phylloxera, like Lepidptera species, has holocentric chromosomes. It is reported that irradiation causes fragmentations and translocations in many species of Lepidptera (Traut *et al*., 1986; Makee and Tafesh, 2006, 2007). And because the chromosomes are holocentric when a break occurs, the fragments are usually not lost and can still be attached on the spindle. In the present study on phylloxera, a lot of chromosomal breaks occurred during the formation of eggs in the ova of the nymphs which gave sticky chromosomes and inter-chromosome translocations in the cells of the embryos. It can also be said that spindles were affected by the irradiation which caused an increase in the chromosomal number on the metaphase plate in some embryo cells.

In this work, the embryos of laid eggs varied greatly in their chromosomal rearrangements (Figs. 2, 3, 4 and 5). However, such rearrangements allow the formation of embryos but it is unknown if they will permit the development of embryos until egg hatch. In mealybug only embryos with an approximately normal amount of paternal chromosomal material were able to survive (Nelson-Rees,1962).

The current study has shown that the metaphase complement of the Syrian strain of the phylloxera female consists of 10 chromosomes, representing eight autosomal and two sex chromosomes, confirming the results presented by Forneck *et al*. (1999) and Maillet (1957). We found also normal karyotype with 2n= 9 in the embryonic cells coincidently. Forneck *et al*. (1999) found one karyotype containing 2n = 9 in the somatic cells of phylloxera and they interpreted it as a male sexual phylloxera, although they did not find any spermatides during their study, which leads us to think that maybe this deficiency in chromosome number is a kind of variety of the karyotype.

When defining the karyotype of six phylloxera populations from Germany, Forneck *et al*. (1999) noticed extra chromosomes. Similarly, in our study an additional very small chromosome was observed in some examined cells of the Syrian phylloxera strain. The detection of supernumerary chromosomes was reported in aphids as well (Blackman, 1976; Wilson *et al*., 2003). Such supernumerary or accessory chromosomes are not essential for the life of a species and are lacking in most of the individuals; they do not carry genes necessary for basic growth, but may have some functional significance such as to increase asymmetry chiasma distribution or increase variation by increasing crossing over and recombination frequencies.

In aphid, Blackman (1980, 1981) suggested that the differences in chromosome numbers might be due to dissociations or fusions involving elements of the normal diploid set, or to the presence of supernumerary B chromosomes. The centromeric activity of holocentric chromosomes, dispersed along its full length, allows the broken chromosomal fragments to segregate during mitosis (Ris, 1942). Moreover, Blackman (1980) proposed that thelytokous reproduction of aphids is a factor that permits karyotype variation within populations of the same species.

The phylloxera karyotype consists of 10 chromosomes. The total complement length is about 12Ìm and the chromosomes range in length from 0.7 to 2 Ìm.

When mitosis of *Agallia constricta* (leafhopper) was examined, the metaphase chromatin appeared to be a 2-3 Ìm wide (Rieder *et al*., 1990). Based on embryo metaphase, the chromosomes of phylloxera females could be sorted into five different size-dependent groups: extra long, long, medium, short and extra short (Table 2). However, in some cells a dot-like chromosome, that represents the additional chromosome, was observed. Forneck *et al*. (1999) classified phylloxera chromosomes into two classes: one pair of large chromosomes and four pairs of shorter chromosomes. Nevertheless, in their study they did not mention the exact length of each pair.

The X chromosome is the shortest one in phylloxera karyotype Forneck *et al*. (1999). However, Blackman *et al*. (2003) reported that in most aphids species the X chromosomes could be identified as the longest or second longest pair. On the contrary, in some aphids the X chromosome was the shortest pair (Blackman, 1986; Blackman *et al*., 2003).

The present study confirms the efficiency of cytogenetic techniques in analyzing the karyotype and chromosomal length of phylloxera, as well as tracing chromosome aberrations in irradiated phylloxera populations. This investigation is a contribution to the search for genetic variation of phylloxera behaviour and development from different populations and provides useful information that can be taken into account in pest management and quarantine measurements against phylloxera. However to apply irradiation technology more comprehensive studies are still needed.

References

BAKRI A., HEATHER N., HENDRICHS J., FERRIS I., 2005 - *50 Years of radiation biology in entomology: lessons learned from IDIDAS*. - Ann. Entomol. Soc. Am., 98: 1-12.

BLACKMAN R.L., 1976 - *Cytogenetics of two species of* Euraphis *(Homoptera, Aphididae)*. Chromosoma, 56: 393-408.

BLACKMAN R.L., 1980 - *Chromosome numbers in the Aphididae and their taxonomic significance*. - Syst. Entomol. 5: 7-25.

BLACKMAN R.L., 1981- *Species, sex and parthenogenesis in aphids*, pp. 75-85. - In: FOREY P.L. (ed.) *The evolving biosphere*. Cambridge University Press, London, UK.

BLACKMAN R.L., 1986 - *The chromosomes of Japanese Aphididae (Homoptera), with notes on the cytological work of* Orihay Shinji. - Cytologia, 51: 59-83.

BLACKMAN R.L., 1987 - *Reproduction, cytogenetics and development*, pp. 163-195. - In: MINKS A.K., and P. HARREWIJN (eds.) *Aphids, their biology, natural enemies and control*, Vol. 2A. Elsevier Science Publishers, Amsterdam, The Netherlands.

BLACKMAN R.L., BROWN P.A., RAMIREZ C.C., NIEMEYER H.M., 2003 - *Karyotype variation in the South American aphid genus Neuquenaphis (Hemiptera, Aphididae, Neuquenaphidinae)*. - Heredity, 138: 6-10.

BLACKMAN R.L., HALES D.H., 1986 - *Behaviour of the X chromosomes during growth and maturation of parthenogenetic eggs of* Amphorophora tuberculata (*Homoptera*, *Aphididae*), *in relation to sex determination*. - Chromosoma, 94: 59-64.

DE BENEDICTIS J., GRANETT J., 1993 - *Laboratory evaluation of grape roots as host of California grape phylloxera biotypes*. - Am. J. Enol. Vitic., 44(3): 285-291.

DOHINO T., MATSUOKA I., TAKANO T., HAYASHI T., 1998 - *Effects of electron beam irradiation on* Myzus persicae *(SULZER) (Homoptera: Aphididae)*. - Research Bulletin of the Plant Protection Service, 34: 15-22.

FAO, 2003 - *Guidelines for the use of irradiation as a phytosanitary measure. International Plant Protection Convention, ISPM no. 18*. - Food and Agricultural Organization (FAO), Rome, Italy.

FOLLETT P.A., 2001 - *Irradiation as a quarantine treatment for mango seed weevil (Coleoptera: Curculionidae)*. - Proc. Hawaiian Entomol. Soc., 35: 95-100.

FOLLETT P.A., 2006 - *Irradiation as a phytosanitary treatment for* Aspidiotus destructor *(Homoptera: Diaspididae)*. - J. Econ. Entomol., 99: 1138-1142.

FOLLETT P.A., YANG M.M., LU K.H., CHEN T.W., 2007 - *Irradiation for postharvest control of quarantine insects*. - Formosan. Entomol., 27: 1-15.

FORNECK A., JIN Y., WALKER A., BLAICH R., 1999 - *Karyotype studies on grape phylloxera (*Daktulosphaira vitifoliae *Fitch)*. - Vitis, 38(3): 123-125.

GRANETT J., BISABRI-ERSHADI B., CAREY J., 1983 - *Life tables of phylloxera on resistant and susceptible grape rootstocks*. - Ent. Exp. & Appl., 34: 13-19.

GRANETT J., TIMPER P., LIDER L.A., 1985 - *Grape phylloxera (*Daktulosphaira vitifoliae*) (Homoptera: Phylloxeridae) biotypes in California*. - Journal of Economic Entomology, 78: 1463-1467.

GRANETT J., WALKER M.A., KOCSIS L., OMER D.A., 2001- *Biology and management of grape phylloxera*. - Annu. Rev. Entomol., 46: 387-412.

HALLMAN G., 1998 - Ionizing radiation quarantine treatments. - An. Soc. Entomol. Brasil, 27: 313-323.

HALLMAN G., 2000 - *Expanding radiation quarantine treatments beyond fruit flies*. - Agric. for Entomol., 2: 85-95.

HALLMAN G., 2001 - *Irradiation as a quarantine treatment*. - In: MOLINS R.A. (ed.) *Food irradiation: principles and applications*. Wiley, New York, USA.

HU T., CHEN C., PENG W.K., 2002 - *The lethal effect of gamma radiation on* Lasioderma serricorne *(Fabricius) (Coleoptera: Anobiidae)*. - Formos. Entomol., 22: 157-162.

HU T., CHEN C., PENG W.K., 2003 - *Lethal effect of gamma radiation on* Sitophilus zeamais *(L.) (Coleoptera: Curculionidae)*. - Formos. Entomol., 23: 145-150.

HUGHES-SCHRADER S., RIS H., 1941 - *The diffuse spindle attachment of coccids, verified by the mitotic behavior of induced chromosome fragments*. - J. Exptl. Zool., 87: 429-456.

JACOBSEN C.M., HARA A.H., 2003 - *Irradiation of* Maconellicoccus hirsutus *(homoptera: Pseudococcidae) for phylosanitation of agricultural commodities*. - J. Econ. Entomol., 96(4): 1334-1339.

JOHNSON J., MARCOTTE M., 1999 - *Irradiation control of insect pests of dried fruits and walnuts*. - Food Technol., 53: 46-48.

LACHANCE L.E., 1967 - *The induction of dominant lethal mutations in insects by ionizing radiation and chemicals as related to the sterile male techniques of insect control*. - In: WRIGHT J., and R. PAL (eds.) *Genetics of insects vectors of disease*. Elsevier Science Publisher, Amsterdam, The Netherlands, pp. 813.

LESTER P.B., ROGERS D.J., PETRY R.J., CONOLLY P.G., ROBERTS P.B., 2000 - *The lethal effects of gamma irradiation on larvae of the huhu beetle,* Prionoplus reticularis: *a potential quarantine treatment for New Zealand export pine trees*. - Entomol. Exp. Appl., 94: 237-242.

LIN J.Y., HORNG S.B., HUNG C.C., 2003 - *Effects of gamma radiation on survival and reproduction of the carambola fruit borer,* Eucosma notanthes *Meyrick (Lepidoptera: Tortricidae)*. - Formos. Entomol., 23: 189-197.

MAILLET P., 1957 - *Sur les chromosomes de quelques phylloxerides de France*. - Vitis, 1: 153-155.

MAKEE H., CHARBAJI T., AYYOUBI Z., IDRIS I., 2003 - *Evaluating resistance of some rootstocks to grape* phylloxera *with an* in vitro *and excised root testing systems*. - *In vitro* Cell. Dev. Biol. - Plant, 40(2): 225-229.

MAKEE H., SAOUR G., 1999 - *Non-Recovery of fertility in partially sterile male* Phthorimaea operculella *(Lepidoptera: Gelechiidae)*. - J. Econ. Entomol., 92(3): 516-520.

MAKEE H., TAFESH N., 2006 - *Sex chromatin body as a marker of radiation-induced sex chromosome aberrations in the potato tuber moth,* Phthorimaea operculella *(Lepidoptera: Gelechiidae)*. - J. Pest Sci., 79: 75-82.

MAKEE H., TAFESH N., 2007 - *Sex chromatin body as a cytogenetic marker of W chromosome aberrations in* Cydia pomonella *females*. - In: VREYSEN M.J.B., A.S. ROBINSON, and J. HENDRICHS (eds.) *Area-wide control of insect pests. From research to field implementation*. Springer, Dordrecht, The Netherlands, pp. 792.

MAKEE H., TAFESH N., MAREC F., 2008 - *Analysis of radiation-induced W chromosome aberrations in codling moth* Cydia pomonella (*L.) by fluorescence in situ hybridization techniques*. - J. of Pest Science, 81: 143-151.

NELSON-REES W.A., 1962 - *The effects of radiation damaged heterochromatic chromosomes on male fertility in the Mealy Bug*, Planococcus Citri *(Risso)*. - Genetics, 47(6): 661-683.

RIEDER C.L., BOWSER S.S., COLE R., RUPP G., PETERSON A., ALEXANDER S.P., 1990 - *Diffuse kinetochores and holokinetic anaphase chromatin movement during mitosis in the hemipteran Agallia constricta (leafhopper) cell line AC-20*. - Cell Motility and the Cytoskeleton, 15: 245-259.

RIS H., 1942 - *A cytological and experimental analysis of the meiotic behavior of the univalent X chromosome in the bearberry aphid Tamalia (= Phyllaphis) coweni (Ckll.)*. - J. Exp. Zool., 90: 267-330.

SONG G.C., GRANETT J., 1990 - *Grape phylloxera (Homoptera: Phylloxeridae) biotypes in France*. - J. Econ. Entomol., 83: 489-493.

TRAUT W., WEITH A., TRAUT G., 1986 - *Structural mutants of the W chromosome in* Ephestia *(Insecta, Lepidoptera)*. - Genet-

ica, 70: 69-79.
VORWERK S., FORNECK A., 2006 - *Reproduction mode of grape phylloxera* (Daktulosphaira vitifoliae, *Homoptera: Phylloxeridae) in Europe: molecular evidence for predominantly asexual populations and a lack of gene flow between them*. - Genome, 49: 678-687.
VORWERK S., SONNTAG K., BLAICH R., FORNECK A., 2008 - *Application of current* in situ *hybridization techniques for grape phylloxera (*Daktulosphaira vitifoliae, Fitch) and grapevine *(Vitis spp. L.)*. - Vitis, 47(2): 113-116.
WILSON A.C.C., SUNNUCKS P., HALES D.F., 2003 - Heritable genetic variation and potential for adaptive evolution in asexual aphids (Aphidoidea). - Biological Journal of the Linnean Society, 79: 115-135.