11 Gamma-H2AX
A Promising Biomarker for Fruit Fly Phytosanitary Irradiation Exposure

Mohammad Sabbir Siddiqui[*]*, Phillip Taylor, and Peter Crisp*

CONTENTS

Abstract DNA double-strand breaks (DSBs) are one of the most biologically significant DNA damage lesions. Exposure to ionizing radiation (IR) causes DSBs in living organisms, which trigger intrinsic DNA repair mechanisms. Phosphorylation of the C-terminal of the core histone protein H2AX (termed γH2AX when phosphorylated) is an early known response to DNA DSBs. Quantification of the γH2AX response offers a highly sensitive and specific assay for detecting DSB formation and repair. Postharvest exposure to IR of 150–400 Gy is an increasingly prominent phytosanitary measure in a variety of Australian (and imported) fruit. The radiation-induced γH2AX response has been shown to be highly persistent in the Queensland fruit fly ("Q-fly"; *Bactrocera tryoni*), Australia's most economically damaging insect pest of horticultural crops, lasting at least 17 days after exposure to IR. The presence of persistent γH2AX, indicating ongoing repair of impaired DNA, can be used to assess irradiation exposure in fruit flies. A direct and reliable assay using γH2AX as a marker of prior IR exposure in fruit flies has the potential to facilitate domestic and international trade in commodities that have been irradiated for disinfestation.

11.1 INTRODUCTION

Fruit flies are the most economically damaging insect pest of Australian horticulture. Between 2006 and 2009, the average value of fruit fly susceptible production in Australia was approximately AU$5.3 billion and exports of fruit fly susceptible horticulture products were around AU$406.9 million (Abdalla et al. 2012; Hyam 2007; Plant Health Australia 2018). The risk of exotic fruit flies—in the form of eggs, larvae, pupae, or adult—entering and establishing in Australia is increasing (Abdalla et al. 2012; Hallman 2011; Hallman et al. 2011). Phytosanitary treatments, such as fumigation and other chemical and physical (e.g., heat, cold) treatments, are commonly used to disinfest imported and exported commodities of quarantine pests (Hallman 2011; Hallman et al. 2011, 2018). Over the past 40 years, the standard postharvest insect disinfestation chemicals dimethoate and fenthion have provided phytosanitary assurance, but the use of these insecticides has been

[*] Corresponding author.

greatly restricted (Richard et al. 2003). Finding alternatives to chemical treatments is necessary to prevent introduction and establishment of exotic pests in new areas (Hallman 2011).

Ionizing radiation (IR) is a safer alternative than fumigation and other chemical and physical (heat/cold) disinfestation methods (Follett 2009, Follett et al. 2011; Hallman 2011; IAEA-TECDOC-1427 2004). Numerous countries use IR to disinfest fruit and vegetables from a multitude of quarantine pests (Richard et al. 2003; Hallman 2011; Hallman et al. 2011), including approximately 30,000 metric tons (and increasing by ~10% each year) of sweet potatoes. Increasing quantities of irradiated tropical fruit, such as mangoes, papayas, litchis, capsicums, and tomatoes, are now successfully being exported from Australia to New Zealand consumer markets (Lynch 2010; Lynch and Nalder 2015).

For biosecurity treatments, fresh produce in finished pallet loads is exposed to a minimum generic dose of 150–400 Gy of IR (e.g., electron beam, X-ray, or gamma ray from cobalt-60) (Follett 2009; Hallman et al. 2011). When IR comes into contact with a cell of a pest insect, it breaks chemical bonds in DNA and other molecules, rendering the insect unable to complete development and to reproduce, and thus preventing the establishment of viable pest populations. Verifying irradiation treatment is difficult because quarantine pests are often found alive during inspection in exported and imported commodities. Currently, the only means of assessing quality of imported and exported fruit is through quarantine audits and treatment facility certification. For commercial disinfestation, a regulatory framework exists with the use of generic irradiation doses for a wide range of pest groups. However, the lack of a reliable test to retrospectively confirm radiation exposure can reduce market confidence in a situation where live pests are detected in exported and imported fruit and costs must be incurred to destroy or export the infested consignment.

11.1.1 Can Gamma-H2AX Be Used as a Biomarker of Phytosanitary Radiation?

On exposure to IR, DNA double-strand breaks (DSBs) are induced in the nuclei of all living cells, inducing a DNA repair mechanism characterized by the phosphorylation of the histone protein H2AX (producing the active form gamma-H2AX [γH2AX]) (Rogakou et al. 1998, 1999). Gamma-H2AX is highly conserved across a wide taxonomic range of organisms and is a well-characterized histone protein known to be responsive to IR-induced DNA DSBs (Downs et al. 2000; Foster and Downs 2005; Redon et al. 2002). Gamma-H2AX assay is a standard and well-established method for biological dosimetry of IR exposure. Quantification of the γH2AX response has been used widely as a highly sensitive and specific assay in radiation biodosimetry and cellular radiosensitivity responses during chemotherapy and radiotherapy and to identify regions of the genome where DSBs fail to repair (Bhogal et al. 2010; Ivashkevich et al. 2012; Redon et al. 2012). However, the γH2AX test has not yet been exploited as a retrospective test for identifying the irradiation status of live insects found in exported or imported consignments of fruit and vegetables.

In the γH2AX assay, the DSB level and corresponding IR dose exposure in the nuclei of cells are measured either by measuring the overall γH2AX protein level or by counting discrete "foci" in individual nuclei, which can be visualized and quantified using numerous methods, including fluorescence microscopy and flow cytometry (Figure 11.1) (Hamasaki et al. 2007; Nakamura et al. 2006; Pilch et al. 2004). Two types of γH2AX foci have been found in cells: the first is transient γH2AX foci that are associated with rapid DSB repair and dephosphorylation of γH2AX to H2AX, usually in minutes to hours (Markova et al. 2007, 2011). The second type of γH2AX foci is residual and tends to persist for days to months (Figure 11.1). The measurement of persistent γH2AX signals has been widely used in many applications in recent years, such as for monitoring cancer patients' response to chemotherapy and radiotherapy, radiation biodosimetry, drug biodosimeters, environmental genotoxicity, and in disease (Siddiqui et al. 2015). A study on mini-pig skin cells showed that γH2AX was significantly elevated in irradiated cells after 70 days post-IR exposure (Ahmed et al. 2012). Another study on mouse skin found γH2AX signals up to 7 days post exposure

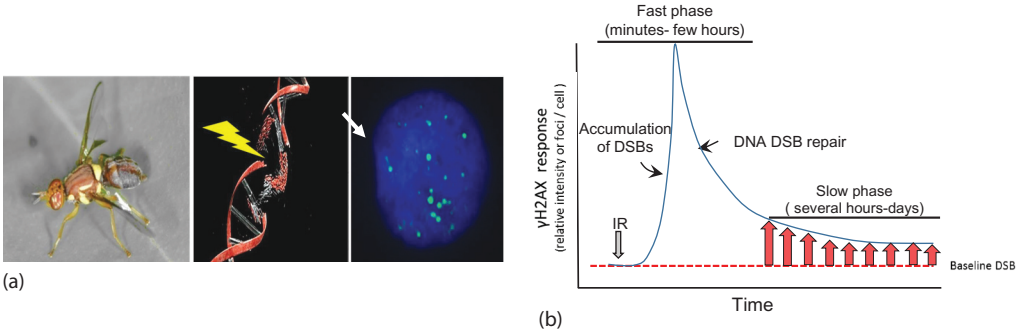

FIGURE 11.1 (a) Ionizing radiation (IR) causes DNA double-strand breaks (DSBs) in Q-fly (arrow indicates γH2AX foci). The number of γH2AX foci represents the number of DSBs. Representative fluorescence image of human buccal cell nuclei containing discrete or diffuse γH2AX foci. Human buccal cell nuclei were visualized (stained with DAPI) with a fluorescence microscope. (b) Schematic representation of the short-term kinetics and persistent γH2AX response in relation to DSB repair. The kinetics of DNA DSB repair follows two phases, a fast phase lasting up to a few hours, which is followed by a slower phase that may persist for several hours to days. On exposure to DNA damaging agents, such as IR, the γH2AX foci appear in the fast phase within minutes after the DSBs are formed and reach a maximum level after about 30 min. This level then declines rapidly and corresponds to repair of DNA DSBs. A small portion of γH2AX (above baseline, as indicated by the dashed line) may persist for up to several months (slower phase) after the initial DSB-induction event and is known as the persistent γH2AX response (as indicated by the bold red arrows). Persistent γH2AX may represent unrepaired DSBs, which are either in the process of slow ongoing repair or too complex to repair. (Adapted from Siddiqui, M.S. et al., *Mutat. Res-Rev. Mutat. Res.*, 766, 1–19, 2015.)

and proposed that they may be used as a biodosimeter in accident scenarios (Bhogal et al. 2010). Linking radiation-induced DNA damage and persistent γH2AX signals is of fundamental importance in establishing a molecular tests capable of detecting and quantifying a prior radiation dose and the resulting DNA damage. The objective of this short communication is to propose the use of the γH2AX test for confirming whether fruit fly species of quarantine concern found in irradiated exported and imported consignment have actually been irradiated and to quantify the dose absorbed.

The proof-of-concept study uses the commercially important pest, Queensland fruit fly (Q-fly; *Bactrocera tryoni* [Froggatt]), as a model to test whether irradiation exposure can be measured retrospectively. In Q-fly, IR exposure leads to a persistent γH2AvB response (a fruit fly variant of γH2AX) for up to 17 days after exposure and can be detected using the γH2AvB test (Siddiqui et al. 2013). Because H2AvB is conserved for all fruit flies of major quarantine concern in which the histone has been sequenced (including *Ceratitis capitata* [Wiedemann], *Bactrocera dorsalis* [Hendel], *Rhagoletis zephyria* Snow, *Bactrocera latifrons* [Hendel], *Bactrocera oleae* [Rossi], *Zeugodacus cucurbitae* [Coquillet]), the γH2AvB test may offer promise in providing rapid, sensitive, and specific detection of prior irradiation exposure to a wide range a fruit flies of market access and biosecurity concern.

11.1.2 Potential Limitations of Gamma-H2AvB as a DNA Damage Biomarker in Fruit Flies

Specimen processing challenges: Once fruit fly specimens are acquired, they must be handled during transport and in the laboratory according to rigorously defined and controlled processes to avoid γH2AvB protein degradation (Valdiglesias et al. 2013).

γH2AvB kinetics differs among species: Persistent γH2AX levels vary in different tissues and cell types and may be affected by genomic status as well as by the type of DNA-damaging agent.

The kinetics (e.g., persistent response) of γH2AvB response in different fruit flies is still unknown. It would be interesting to test whether γH2AvB can be used to assess the kinetics of persistent γH2AvB responses in diverse fruit flies of quarantine concern (Siddiqui et al. 2015).

11.2 CONCLUSION

Currently, for recipients of shipments, certification by the treatment facility is the only available assurance of prior irradiation treatment of live pests discovered in imported and exported fruits and vegetables. A test that directly assesses the dose received by insects in irradiated produce would improve confidence in commercial irradiation treatments, thus offering potential production and market access advantages. Because persistent γH2AX responses have been reported in different cell and tissue types, an assay based on measuring these responses should be investigated for its potential as a method to detect and quantify prior phytosanitary irradiation exposure. This γH2AX test may provide producers an advantage by increasing market acceptance of irradiation as a phytosanitation treatment. A key advantage of the test focusing on measuring the persistence of γH2AvB is that the biomarker has been identified in many insect species and could form the basis of a similar test in diverse pest insects of quarantine concern. The next steps involve broadening the range of insects in which γH2AvB can be detected and validating or modifying the γH2AvB test for operational conditions so that it can be incorporated in commercial and quarantine facilities.

ACKNOWLEDGMENTS

The project Raising Q-fly Sterile Insect Technique to World Standards (HG14033) is funded by the Hort Frontiers Fruit Fly Fund, part of the Hort Frontiers strategic partnership initiative developed by Hort Innovation, with co-investment from Macquarie University and contributions from the Australian Government.

REFERENCES

Abdalla, A., N. Millist, B. Buetre, and B. Bowen. 2012. Benefit–cost Analysis of the National Fruit Fly Strategy Action Plan. ABARES report to client prepared for Plant Health Australia, Canberra, (December): 1–41. http://www.planthealthaustralia.com.au/wp-content/uploads/2012/12/BCA-Fruit-Fly-Strategy-Action-Plan.pdf.

Ahmed, E. A., D. Agay, G. Schrock, M. Drouet, V. Meineke, and H. Scherthan. 2012. Persistent DNA damage after high dose *in vivo* gamma exposure of minipig skin. *PLoS One* 7(6) e39521.

Bhogal, N., P. Kaspler, F. Jalali, O. Hyrien, R. Chen, R. P. Hill et al. 2010. Late residual Gamma-H2AX foci in murine skin are dose responsive and predict radiosensitivity in vivo. *Radiation Research* 173(1): 1–9.

Downs, J. A., N. F. Lowndes and S. P. Jackson. 2000. A role for *Saccharomyces cerevisiae* histone H2A in DNA repair. *Nature* 408(6815): 1001–1004.

Follett, P. A. 2009. Generic radiation quarantine treatments: The next steps. *Journal of Economic Entomology* 102(4): 1399–1406.

Follett, P. A., T. W. Phillips, J. W. Armstrong, and J. H. Moy. 2011. Generic phytosanitary radiation treatment for Tephritid fruit flies provides quarantine security for *Bactrocera latifrons* (Diptera: Tephritidae). *Journal of Economic Entomology* 104(5): 1509–1513.

Foster, E. R., and J. A. Downs. 2005. Histone H2A phosphorylation in DNA double-strand break repair. *The FEBS Journal* 272(13): 3231–3240.

Hallman, G. J. 2011. Phytosanitary applications of irradiation. *Comprehensive Review in Food Science and Food Safety* 2: 143–151.

Hallman, G. J., K. Guo, and Liu T.X. 2011. Phytosanitary irradiation of *Liriomyza Trifolii* (Diptera: Agromyzidae). *Journal of Economic Entomology* 104(6): 1851–1855.

Hallman, G. J., L. Wang, G. Demirbas Uzel, E. Cancio-Martinez, C. E. Caceres-Barrios, S. W. Myers, and M. J. B. Vreysen. 2018. Comparison of populations of *Ceratitis capitata* (Diptera: Tephritidae) from three continents for susceptibility to cold phytosanitary treatment and implications for generic cold treatments. *Journal of Economic Entomology* 112(1): 127–133.

Hamasaki, K., K. Imai, K. Nakachi, N. Takahashi, Y. Kodama, and Y. Kusunoki. 2007. Short-term culture and gammaH2AX flow cytometry determine differences in individual radiosensitivity in human peripheral T lymphocytes. *Environmental and Molecular Mutagenesis* 48(1): 38–47.

Hyam, L. 2007. National Program to Coordinate Fruit Fly Fight, Plant Health Australia, Canberra. http://www.planthealthaustralia.com.au/wp-content/uploads/2012/12/Draft-National-Fruit-Fly-Strategy-Mar-2008.pdf

IAEA-TECDOC-1427. 2004. Irradiation as a Phytosanitary Treatment of Food and Agricultural Commodities. Proceedings of a Final Research Coordination Meeting Organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture 2002. *International Atomic Energy Agency* (*IAEA*): 181 pp.

Ivashkevich, A., C. E. Redon, A. J. Nakamura, R. F. Martin, and O. A. Martin. 2012. Use of the Gamma-H2AX assay to monitor DNA damage and repair in translational cancer research. *Cancer Letters* 327(1–2): 123–133.

Lynch, M. 2010. Irradiated Tropical Fruit Exports to New Zealand. https://steritech.com.au/wpcontent/uploads/downloads/Mango%20Trade%20Fact%20Sheet.pdf.

Lynch, M., and K. Nalder. 2015. Australia export programmes for irradiated fresh produce to New Zealand. *Stewart Postharvest Review* 3: 8.

Markova, E., N. Schultz, and I. Y. Belyaev. 2007. Kinetics and dose-response of residual 53BP1/gamma-H2AX Foci: Co-Localization, relationship with DSB repair and clonogenic survival. *International Journal of Radiation Biology* 83(5): 319–329.

Markova, E., J. Torudd, and I. Belyaev. 2011. Long time persistence of residual 53BP1/gamma-H2AX Foci in human lymphocytes in relationship to apoptosis, chromatin condensation and biological dosimetry. *International Journal of Radiation Biology* 87(7): 736–745.

Nakamura, A., O. A. Sedelnikova, C. Redon, D. R. Pilch, N. I. Sinogeeva, R. Shroff et al. 2006. Techniques for Gamma-H2AX detection. *Methods in Enzymology* 409: 236–250.

Pilch, D. R., C. Redon, O. A. Sedelnikova, and W. M. Bonner. 2004. Two-dimensional gel analysis of histones and other H2AX-related methods. *Methods in Enzymology* 375: 76–88.

Plant Health Australia. 2018 Http://www. Planthealthaustralia. Com.au/national-programs/fruit-Fly/

Redon, C., D. Pilch, E. Rogakou, O. Sedelnikova, K. Newrock, and W. Bonner. 2002. Histone H2A variants H2AX and H2AZ. *Current Opinion in Genetics & Development* 12(2): 162–169.

Redon, C., U. Weyemi, P. R. Parekh, D. Huang, A. S. Burrell, and W. M. Bonner. 2012. Gamma-H2AX and other histone post-translational modifications in the clinic. *Biochimica Et Biophysica Acta* 1819(7): 743–756.

Richard, D., S. Winter, D. Michael, and J. Oakeshott. 2003. Irradiation in Horticulture-An Australian Perspective. Australian horticulture industry prepared for Horticulture Australia Limited. http://www.hortaccess.com.au/files/irradiation_in_horticulture_-_april_2003.pdf. Accessed April 2003.

Rogakou, E. P., C. Boon, C. Redon, and W. M. Bonner. 1999. Megabase chromatin domains involved in DNA double-strand breaks in vivo. *The Journal of Cell Biology* 146(5): 905–916.

Rogakou, E. P., D. R. Pilch, A. H. Orr, V. S. Ivanova, and W. M. Bonner. 1998. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *The Journal of Biological Chemistry* 273(10): 5858–5868.

Siddiqui, M. S., E. Filomeni, M. Francois, S. R. Collins, T. Cooper, R. V. Glatz et al. 2013. Exposure of insect cells to ionising radiation in vivo induces persistent phosphorylation of a H2AX homologue (H2AvB). *Mutagenesis* 28(5): 531–541.

Siddiqui, M. S., M. François, M. F. Fenech, and W. R. Leifert. 2015. Persistent γH2AX: A promising molecular marker of DNA damage and aging. *Mutation Research/Reviews in Mutation Research* 766: 1–19.

Valdiglesias, V., S. Giunta, M. Fenech, M. Neri, and S. Bonassi. 2013. γH2AX as a marker of DNA double strand breaks and genomic instability in human population studies. *Mutation Research* 753(1): 24–40.