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Source: Florida Entomologist, 88(2) : 208-210

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/0015-4040(2005)088[0208:HRRSOP]2.0.CO;2

HYPOXIA REDUCES REPRODUCTIVE SUSCEPTIBILITY OF PLUM CURCULIO (COLEOPTERA: CURCULIONIDAE) TO IONIZING RADIATION

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Ionizing irradiation is used in Florida and Hawaii to disinfest several fruits and sweetpotatoes of fruit flies (Diptera: Tephritidae) or other insects (Hallman 2004a). Importation of fruit irradiated against 11 fruit fly species and the mango seed weevil, *Cryptorhynchus mangiferae* (F.) (Coleoptera: Curculionidae), has been approved (APHIS 2002). The treatment shows promise for widespread implementation, as it is safe, broadly efficacious, accepted by consumers, cost-effective, may be applied after packing, and widely tolerated by fresh agricultural commodities (Hallman 2002).

A large body of research has determined minimum absorbed doses of ionizing radiation to prevent development or reproduction for many different species of arthropods <http://www-infocris.iaea.org/ididas/start.htm>. Some of this research has shown that hypoxia can reduce some of the detrimental effects of irradiation to insects used in sterile release programs. The effect of hypoxia on irradiation disinfestation treatment efficacy has not been studied until recently, although some agricultural commodities are stored under hypoxic conditions, a strategy that is increasing in application. Hallman (2004a) found that while no oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), fifth instars developed to the adult stage when irradiated at 200 Gy in ambient atmosphere, 5.3% of those irradiated in hypoxic atmospheres developed to the adult. Hypoxia caused a small increase in the ability of apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae), third instars to emerge as adults after irradiation (Hallman 2004b).

Plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), is native to the eastern Neartic and is a quarantine pest of stone and pome fruits exported from the United States and Canada. Hallman (2003) determined that a minimum absorbed dose of 92 Gy (the maximum recorded dose when 80 Gy was sought) prevented reproduction of adults, the most radiotolerant stage of the insect. The objective of this research was to determine the effect of hypoxia on reproduction of the plum curculio.

Plum curculios originally collected near Gainesville, Florida, were obtained from a colony at the United States Department of Agriculture, Agricultural Research Service facility in Byron, Georgia. The insects were reared at about 25EC, 70% RH, 12: 12h (L: D), on immature apples that were picked when about 3 cm in diameter. Larvae emerging from the apples were placed on sterilized potting soil until adult emergence.

A radiation source of ^{137}Cs (Husman Model 521A, Isomedix, Inc., Whippany, NJ) that delivered a gamma ray dose rate of about 40 Gy·min^{-1} was used in this research. Routine dosimetry was done with radiochromic film (Gafchromic MD-55, ISP Technologies, Inc., Wayne, NJ) and read with a spectrophotometer at 510 nm (Milton Roy Spectronic 401, Ivyland, PA).

Adult plum curculios were irradiated in ambient atmospheres and in atmospheres of mostly nitrogen. Cylinders (polyvinyl chloride, 37.5 cm inside length, 10 cm inside diameter) fitted on one end with a screw cap sealed with vacuum grease and on the other end with 2 brass, barbed-nipple compression hose fittings (25 mm long, 4-mm inside diameter) were constructed. Two-week-old plum curculio adults were placed inside the cylinder with a few immature apples, and the atmosphere was purged through the hose fittings with nitrogen at a pressure of about 3 kPa for 2 minutes 20, 16, and 2 h before irradiation with an absorbed dose of 40 Gy. After purging, the hose fittings were sealed with rubber septa and the cylinders held at about 24°C. About 1.5 h after irradiation, the cylinders were opened to return the insects to ambient atmosphere. There were 6 replicates of 300-600 each for adults irradiated in ambient or hypoxic atmospheres. Controls consisted of 6 replicates of 30-100 insects each in a cylinder under ambient atmosphere that were not irradiated.

After irradiation, adults were maintained on immature apples at about 25°C, and mortality was determined every week. The apples were replaced every week and maintained at 25°C for development of any immatures inside. Larvae emerging from apples were collected every 1-3 days and placed on potting soil for pupation and adult emergence. After larvae were no longer emerging, the apples were opened and any remaining insects collected. Analyses of variance were done with Prism 4 (www.graphpad.com).

The mortality rate for the irradiated plum curculios was greater than that for the control until 15 weeks after irradiation when the rate for the control accelerated (Fig. 1). There was no significant difference among the treatments for time to reach 95% mortality (overall mean = 27.4 ± 2.6 weeks, $F = 1.56$, $P = 0.26$, df: 2,15).

Reproduction under irradiation and hypoxia was increased by over 20-fold compared with irradiation in ambient atmosphere (Table 1). Reproduction was greater than the 34.4 Gy for 4th instars per female reported by Hallman (2003) for

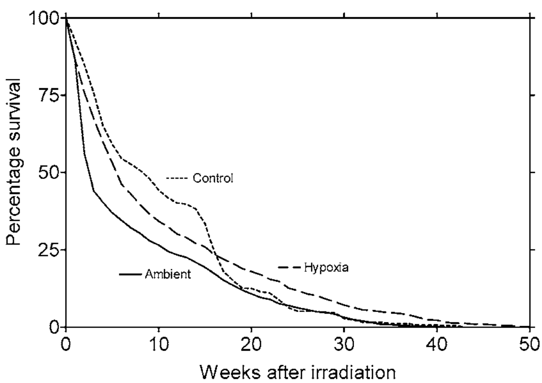

Fig. 1. Mortality rate for control and irradiated (40 Gy) plum curculios.

the control, but similar to that reported for 40 Gy under ambient atmosphere (0.31). Although few 4th instars were produced with irradiation under ambient conditions, significantly more of these became adults compared with the control and irradiation under hypoxia. Adults irradiated under ambient conditions did not live significantly less time than control or adults irradiated under hypoxia, but their reproductive period was shorter and peaked earlier.

Previous studies on the effect of hypoxia on irradiation phytosanitary treatment efficacy concluded that, although an effect could be observed, it did not necessarily threaten the ability of the treatment to prevent an infestation. Although 5.3% of irradiated (200 Gy) oriental fruit moth 5th instars developed to adults, they all died within 10 days of emergence without ovipositing, while non-irradiated controls lived up to 28 days and laid abundant eggs that hatched (Hallman 2004a). Even though apple maggots irradiated as 3rd instars in apples subject to hypoxia had an estimated increase in the dose required to prevent adult emergence of 17% compared with those irradiated in ambient atmosphere, no adults emerged in large-scale testing (Hallman 2004b). In both of these cases the measure of efficacy was prevention of adult emergence, which allows for a greater margin of error than a treatment against adults where prevention of successful reproduction is the only viable efficacy standard. In a treatment designed to prevent adult emergence, even if some adults emerge, as long as they will not successfully reproduce, establishment of the pest in a new area is prevented. But in a pest where adults may be present, such as plum curculio, any failure in prevention of reproduction means that some individuals would be capable of a new infestation.

The difference in reproductive success between plum curculios irradiated under hypoxia and ambient atmosphere is approximately of the same order of magnitude as the difference between 20 and 40 Gy under ambient conditions (Hallman 2003). This may give a rough indication that hosts of plum curculio irradiated under low oxygen storage might require twice the dose as under ambient conditions, or about 180 Gy to prevent reproduction. This dose would need to be confirmed by large-scale testing before it could be used as a phytosanitary treatment. Until that is done, irradiation against plum curculio should not be used for fruits under low oxygen storage.

Miguel Diaz and Sandra Ramos, USDA-ARS, Weslaco, Texas, are acknowledged for technical help. Lisa Neven, USDA-ARS, Wapato, Washington, is thanked for providing immature apples. Walter P. Gould, USDA-APHIS-PPQ, Riverdale, MD, and Lisa Neven, USDA-ARS, Wapato, WA, are thanked for reviewing the manuscript. This research was supported by funding from USDA-APHIS-PPQ.

Summary

Adult plum curculios irradiated in a hypoxic atmosphere accomplished by flushing a cylinder with nitrogen gas were more tolerant of ionizing radiation than plum curculios irradiated in ambient atmosphere. Some hosts of plum curculio,

Table 1. Reproduction of plum curculio irradiated with 40 Gy in hypoxic and ambient atmospheres.[a]

Treatment	Mean 4th instars/female	4th instars developing to adult (%)	Week of peak reproduction post irradiation	Mean 4th instars/ female during week of peak reproduction	Final week of reproduction	Final contiguous week of reproduction
Control	89.8 ± 10.1 a	42.5 ± 1.9 b	4.8 ± 0.79 a	18.4 ± 3.6 a	24.3 ± 3.1 a	17.3 ± 0.67 a
Hypoxia	5.9 ± 0.91 b	40.0 ± 3.7 b	5.8 ± 1.1 a	1.9 ± 0.30 b	28.8 ± 2.5 a	17.5 ± 1.4 a
Ambient	0.27 ± 0.12 c	64.7 ± 2.9 a	1.5 ± 0.34 b	0.18 ± 0.08 c	8.8 ± 1.5 b	2.8 ± 0.48 b
—Results of statistical analyses (degrees of freedom for all are 2, 15):						
F value	72.4	25.9	6.8	25.1	17.6	102
P value	0.0001	0.0001	0.014	0.0001	0.0005	0.0001

[a]Means followed by the same letter are not significantly different, Tukey's, 95% confidence.

such as apples, are stored under hypoxia. An irradiation quarantine treatment against plum curculio for apples stored in hypoxia would probably need to be greater than the 92 Gy determined to be efficacious in ambient atmosphere.

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