TECHNIQUES TO IDENTIFY IRRADIATED FRUIT FLY LARVAE

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There has been considerable interest in developing tests to identify irradiated insects that may be found in commodities treated for quarantine purposes. These insects, in many instances, will be immature forms such as fruit fly larvae. Rahman et al. (1990) reported a decrease in the size of the supraoesophageal ganglion (brain) of Mediterranean fruit fly larvae following irradiation. Irradiation had no effect on the size of the proventriculus so a correlation could be made to serve as an indicator of irradiation. Other fruit fly larvae have been reported to exhibit similar reductions.

We (Nation et al. 1995a and b) recently completed a study of the Caribbean fruit fly response to irradiation using three techniques - brain development, melanization, and phenoloxidase - to evaluate response of larvae to irradiation. Larvae were irradiated at doses up to 150 Gy using a Cesium-137 source or a 10 MeV linear accelerator.

To evaluate brain development as an indicator of irradiation, larvae were irradiated at hatching and dissected for measurements of the brain and proventriculus as mature third instars. Cross-sectional area of a plane through the brain and proventriculus, and simple dorsal width measurements of the two organs were evaluated as indicators of radiation exposure. Mature third instars irradiated at hatching with $\geq$ 20 Gy had significantly reduced brain area, brain width, and brain/proventriculus ratios from controls. The area of the brain of day - 3 third instars that had been irradiated on the first day of the first, second, and third instar was reduced by 62, 45, and 28%, respectively, in comparison to control third instars. Detailed dissections of hatching larvae exposed to 50 Gy revealed reductions in brain growth, small and misshapen compound eye and leg imaginal disks, and a ventral nerve cord that was elongated and sinuous.

To evaluate melanization as an indicator of irradiation, hatching larvae were irradiated, later removed from diet as first, second, third instars, and frozen for at least 15 minutes. Within 15 minutes after removal from the freezer, third instar control larvae extensively melanized and became black. Larvae irradiated with 5 or 10 Gy showed similar melanization, but the black color developed more slowly. Larvae irradiated as first instar at $\geq$ 20 Gy became only slightly grey, never brown or black, even after 17 hours at room temperature. Neither first or second instar controls, nor first or second instars irradiated as first instars melanized after death.

To evaluate phenoloxidase levels as an indicator of irradiation, larvae were irradiated, prepared, and frozen as in melanization tests. Five third instar larvae (at least 10 first or second instar) were ground in buffer solutions, centrifuged, filtered, and placed in an enzyme reaction tube with 2-methyl-DOPA. The intensity of the red color produced is measured in a Spectronic 20D and phenoloxidase units determined by the change in light absorbance. There was $>85\%$ reduction in phenoloxidase activity of larvae irradiated at hatching with $\geq$20 Gy and assayed as third instar. Larvae irradiated on the first day of the first instar and on the first day of the second instar had $\geq 90\%$ reduction in phenoloxidase activity as late third instars. Larvae irradiated on the first day of the third instar had 50% reduction in phenoloxidase activity as they were leaving the food to pupate.

A simple spot test for phenoloxidase, utilizing 2-methyl-DOPA on transparency film, was developed that produced a red color with a crushed control larvae and no color with a larvae irradiated at $\geq$25 Gy. First and second instars, whether control or irradiated, do not have sufficient phenoloxidase activity to cause color development.

All of these methods have potential as a method to identify larvae that have been irradiated. All work best if larvae are irradiated as eggs or first instar larvae and evaluated as third instar larvae. The evaluation of brain size would be difficult for quarantine inspectors to use. The melanization test has potential use since it requires only a freezer and evaluation of color change or no color change. The use of the phenoloxidase simple spot test, in our opinion, has the greatest potential to identify irradiated fruit fly larvae.

References

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