BIOCHEMICAL DETERMINANTS OF IRRADIATION IN CODLING MOTH?

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Previous report from Dr. J. Nation at the University of Florida indicated that irradiated Caribbean fruit flies can be differentiated from unirradiated flies by a simple freeze/thaw test. They showed that the levels of phenoloxidase were greatly reduced in treated insects and that this was reflected by a reduced ability to melanize following freezing. They proposed that this method may be applicable for determination of irradiation treatment for all insects since phenoloxidase is a common hemolymph component.

Our laboratory works on quarantine treatments of pome and stone fruits against codling moth. Previous research indicates that radiation treatments of 250 to 300 Gy are sufficient to prevent adult eclosion. It has also been demonstrated that 'Bing' and 'Rainier' cultivars of cherries and 'Red Delicious' apples can tolerate treatments of 300 Gy with little or no loss of quality.

We tested the theory that changes in phenoloxidase could be detected in irradiated codling moth. We treated fifth instar codling moth at 0, 100, 300, 600, and 900 Gy and tested levels of phenoloxidase at 0, 2, 4, and 6 days following treatment. The freeze/thaw experiment along with protein electrophoresis, dot blot, and enzyme activity analyses were performed.

Freeze/thaw: There was a general trend for the irradiated insects to show a decreased rate of melanization. It was more noticeable with increasing days following treatment. However, there was great variation in the rates and were greatly affected by prior damage incurred by the insect during extraction from the diet medium and also by any disease (granulosis virus or nosema) present. We found this method too unreliable to be of any use for the determination of irradiated codling moth.

Protein Electrophoresis: We observed three major isoforms of phenoloxidase in the hemolymph of untreated fifth instars of codling moth. No change in the isoforms were observed on day 0 of the treatments. Slight and inconsistent changes in the banding pattern were observed over subsequent days following treatment. Once again these changes were to inconsistent and unreliable to use as a method for determination of irradiation treatment.

Protein Dot Blot: Diluted samples of fresh hemolymph were transferred to nitrocellulose membrane and reacted against a phenoloxidase reagent. There were again, variable differences in the reactivity of phenoloxidase in relation to irradiation treatment.

Specific Enzyme Activity: This assay was conducted according to the protocol outlined by Nation et al. (1995). We initially tested the phenoloxidase activity and protein content in 100 fifth instar codling moth to obtain a base line and standard deviations. When radiation treated insect phenoloxidase values are compared against the base line and standard deviation of untreated controls, there were no significant differences observed.

The lack of significant differences in phenoloxidase activity in fifth instar codling moth treated with radiation is not surprising. The high titer of phenoloxidase in the hemolymph along with the electrophoretic identification of numerous isoforms most likely contribute to this phenomena. In a recent book chapter, T. M. Koval states that Lepidopteran cell lines contain more DNA repair enzymes than any other insect, invertebrate and vertebrate cell lines. The reason for this is still unclear, however this would explain why Lepidopteran are more resistant to environmental stresses such as high and low temperatures, UV radiation, and combination quarantine treatments than most Dipteran pests.

References:

Koval, Thomas M. 1994. Intrinsic stress resistance of cultured Lepidopteran cells. In *Insect Cell Biotechnology*, (Eds. K. Maramorosch & A. McIntosh), pp.157-185. CRC Press, Boca Raton.

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