CRYOLITE INDUCED MORPHOLOGICAL CHANGE IN THE COMPOUND EYE OF DROSOPHILA MELANOGASTER

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SUMMARY: Treatment of the larvae of the common fruit fly Drosophila melanogaster with 10, 20, and 40 ppm of the F-containing insecticide cryolite (Na₃AlF₆) through its normal food resulted in abnormal morphology of its compound eye. A ridge-like appearance of the mechanosensory bristles of the ommatidia was apparent by scanning electron microscopy (SEM). Interestingly, ommatidial disorganization was greater with 10 ppm cryolite than with 20 and 40 ppm. These results indicate that the use of cryolite as an insecticide in fruit orchards may cause morphological alterations in a non-target organism.

Keywords: Cryolite; Drosophila melanogaster; Fruit fly eyes; Ommatidia; Scanning electron microscopy (SEM).

INTRODUCTION

Among agricultural pesticides, certain inorganic fluorides are noted for having undesirable side effects, e.g., on the reproductive cycle of the silkworm.1 Additionally, sodium fluoride (NaF) and stannous fluoride (SnF₂) have been found to have mutagenic effects on the common fruit fly Drosophila melanogaster.2 Atmospheric HF also increases the frequency of sex-linked recessive lethality and sterility in Drosophila melanogaster.3 Another F compound, cryolite (Na₃AlF₆), is a widely used insecticide used on many fruits, vegetables, and ornamental crops for protection against leaf eating pests. Several studies with different insecticides, fungicides, and pesticides have manifested well-defined effects on the life cycle, hatchability, and emergence of flies in Drosophila melanogaster.4-6 The present work aimed to explore any detectable change in the external morphology of the compound eye of the adult fruit fly as a function of exposure of the eggs and larvae to cryolite. Interestingly, this model insect used in this study is not considered to be an insect pest. Investigation of non-target organisms from this perspective would therefore appear to be beneficial for evaluating the risk under which other organisms including the humans are exposed.7

MATERIALS AND METHODS

For this study of the effect of cryolite on the compound eyes of Drosophila melanogaster, a Scanning Electron Microscope (S-530 HITACHI) was used to examine the external morphology and structural changes in the eyes.

Cryolite exposure: For treatment of the eggs and larvae, three concentrations (10, 20, and 40 ppm) of cryolite (Loba Chemie Pvt. Ltd, India) were prepared in water and mixed with Drosophila food medium containing agar, corn meal, sucrose, and yeast at 22±1°C. Fifty Drosophila eggs were introduced into each food preparation containing the three cryolite solutions, along with a control containing only the normal food constituents. Triplicate sets of each treatment as well as the control...
group were used for the study. The eggs were allowed to hatch and the larvae were allowed to grow in the respective food medium throughout their development.

Adult flies were collected randomly from each treatment group in 2.5% gluteraldehyde for fixation and then dried in graded alcohol. Critical point drying was carried out in the CPD Machine (HCP-2 HITACHI). Finally gold coating of the eyes was done using IB-2 Ion Coater (EIKO ENGINEERING) for study under SEM.

RESULTS

Examination after gold coating of the compound eye of *Drosophila melanogaster*, which consists of a regular, crystalline-like array of some 800 ommatidia (simple eyes), under scanning electron microscopy (SEM) imaging at 300× magnification showed the even distribution of the mechanosensory bristles between ommatidia in the control flies (Figure 1).

![Figure 1](image)

*Figure 1.* SEM imaging at 300× magnification showing the normal, organized alignment of mechanosensory bristles along with properly arranged ommatidia in control adult *Drosophila melanogaster* fed a normal diet from larval hatching.

However, as seen in Figure 2, these bristles became very disrupted and disorganized with disordered ommatidia from the treatment with 10 ppm cryolite. With the 20 ppm cryolite (Figure 3) the bristles were also disoriented and showed a prominent ridge-like appearance, but the effect appeared to be less in comparison to the effect from the 10 ppm treatment.
Figure 2. Complete disorientation of the mechanosensory bristles and disorganized ommatidial structures visible by SEM under 300× magnification in *Drosophila melanogaster* maintained from egg hatching on food containing 10 ppm cryolite.

Figure 3. Disoriented mechanosensory bristles and disorganized ommatidial structures visible by SEM at 300× magnification in adult *Drosophila melanogaster* maintained from egg hatching on food containing 20 ppm cryolite.
With the higher 40 ppm concentration, the ommatidial alignment and bristle orientation appeared to be similarly affected as in the 20 ppm group (Figure 4).

Figure 4. Lesser disorientation of the mechanosensory bristles in the disorganized ommatidial structures visible by SEM at 300× magnification showing ridge-like alignment of ommatidia in adult *Drosophila melanogaster* maintained from egg hatching on food containing 40 ppm cryolite.

**DISCUSSION**

The compound eye of *Drosophila melanogaster* is an effective model for detailed investigation of cell signaling, control of cell proliferation, neuronal connectivity, vesicular transport, etc. Undoubtedly vesicular transport has a very important role in eye development. Rab1 and Rab6 genes have a key role in processing and/or transporting rhodopsins. The *Drosophila* eye is also a model system for analyzing mutations that disrupt trafficking of molecules to lysosomes and lysosome-related organelles. It is also well documented that the Rab11 gene is one of the key players associated with *Drosophila* eye development. Mammalian genomes contain more than 60 known Rab genes.

Pattern formation in the developing *Drosophila* eye begins in the eye disc at the third larval instar and is known to be closely associated with a morphological indentation in the disc called morphogenetic furrow, which moves across the disc epithelium in the direction from posterior to anterior. From there it is apparent that the pattern of classical eye development occurs during the third larval instar. Hence, any change in the developmental programme of third instar larvae would be expected to manifest impairment in adult eye development. As seen in the present study, distinct morphological changes in the eye seen in Figures 2, 3, and 4 indicate that the eggs and larvae subjected to the different concentrations of cryolite during the larval stage caused distinct phenotypic changes in the adult eye.
In *Drosophila melanogaster*, the fat facets (faf) gene encodes a deubiquitinating enzyme essential in differentiating tissues of the eye and ovary of fly.\textsuperscript{15,16} The development of the eye discs in *D. melanogaster* is reported to be impaired due to the facet-inhibitory effect of chemicals like mitomycin-C and nitromine.\textsuperscript{17} Similarly, in the present study, it appears likely that cryolite might have induced aberrant deubiquitinisation resulting in the changes observed here in the morphology of the eye.

Over the years, *D. melanogaster* has been used as a model to study the effect of various pesticides and insecticides. In the present study, the effect of the fluorine-containing insecticide cryolite on the morphology of *Drosophila* has been investigated. To the best of our knowledge, to date there seems to be only few reports on the effect of F on the ommatidia of *Drosophila*. Here, as noted in the results, we have found that treatment of the larvae with cryolite caused distinct changes in the morphology of ommatidia as observed through scanning electron microscopy (SEM). Owing to its precisely organized architecture, the *Drosophila* compound eye can be viewed as a very good model for addressing questions concerning several important biological processes including cell signaling.

Ommatidia are facets that make up the *Drosophila* eye that are connected to the nervous system. The adult compound eye consists of a regular, almost crystalline, array of nearly 800 ommatidia,\textsuperscript{18} each containing 8 photoreceptor neurons. Ommatidia develop in a monolayer epithelium, contained within the eye-antennal imaginal disc in which cells extend from the apical surface to the basal membrane.\textsuperscript{19} During photoreceptor differentiation, ommatidial clusters rotate 90° toward the poles of the eye disc.\textsuperscript{20} Ommatidial rotation can be divided into two phases: the first 45° of quick rotation that is completed by ommatidial row 6 posterior to the morphogenetic furrow, followed by a second 45° of slow rotation.\textsuperscript{21} The nemo (nmo) gene has been identified as the only gene that has been specifically found to affect this rotation process.\textsuperscript{22} Figures 2, 3, and 4 show a distinct disorganization and ridge formation in the compound eye from exposure of the larvae to cryolite that may be due to abnormal functioning of the nemo gene at the time of differentiation. Interestingly, maximum noticeable disorganization occurred with the 10 ppm treatment. At the higher cryolite concentrations similar ommatidial disorganization occurred, but it decreased noticeably with increasing concentration. In agreement with this finding, it should be noted that other studies have shown that F in vivo can often be more genotoxic at lower concentrations than at higher concentrations.\textsuperscript{23,24}

Finally, the rough surface of the compound eyes with unusual ridge and furrow-like changes may be due to subtle defects in cone cells, pigment cells, and/or bristles in the final stages of eye development.\textsuperscript{25} The intensely roughened eye surface seen in Figure 2 may have arisen from these kinds of defects. The comparison of the affected eyes with the normal eye structure (Figure 1) clearly shows that the ordered and well-orchestrated nature of normal eye development seems to be completely lost as a result of exposure of the larvae to the various concentrations of cryolite.
Further study with a wider range of cryolite concentrations (above 40 ppm and below 10 ppm) would no doubt help to determine the instar stage at which these changes occur. In the present study the concentrations of cryolite were selected in accordance with those actually applied in agriculture. Generally, in practice, lower doses are preferable for fruit trees. Since the present study dealt with the effect of cryolite on a single generation of *Drosophila*, it cannot offer a justified theory for the impact of repeated exposure to this insecticide generation after generation. A further investigation with three or more generations of fruit flies can be expected to provide a fuller understanding necessary for proposing a plausible mechanism for the findings reported here.

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**REFERENCES**


