Fly Rotenone Treatment Protocol

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Protocol reference: Brown JB et al. 2014 (PMID: 24670639)

Treatment feeding schedule for Larvae:

For each treatment, approximately 50 (mixed sex) young mated adults were transferred to each fresh food vials and maintained for 12 hours. Vials were cleared and allowed to age 3.5 to 4 days. Vials were then rinsed into a series of sieves using tepid water; feeding third instar larvae were collected form the #40 sieve and transferred to a hard agar plate with a pot of yeast to induce crawling. Prior to reaching the yeast, larvae were captured and 50 larvae were transferred to new food vials containing the treatment of interest (details below), and larvae were allowed to feed for 4 hours. Treated larvae were captured and transferred to 2 ml vials, flash frozen in liquid nitrogen and stored at -80° C prior to RNA preparations. The number of survivors was recorded and the mean lethality calculated for each treatment.

Treatment feeding schedule for Adults:

For each treatment, 40 newly eclosed males and females (1:1) were transferred to fresh food (BDSC corn meal agar) vials and maintained at 25° C for two days. To treat flies, two Kimwipes were folded into a square and put in the bottom of a one-pint glass bottle. Kimwipes were saturated with 4 ml of the treatment solution, (10% sucrose solution and one drop of green vegetable coloring per 50 ml solution, plus the treatment of interest). Harvesting time for adults varied by treatment. Upon harvesting, flies were placed in 2 ml tubes, flash frozen in liquid nitrogen and stored at -80° C prior to RNA preparations.

Rotenone treatment:

From Kristi Montooth (pers. comm.): "My experience with AntimycinA and Rotenone is mainly from their use in in vitro biochemical assays of the OXPHOS enzyme complexes. I have protocols on what concentrations can be added to an in vitro assay to inhibit the complexes, but don't know how best to expose individuals. For inhibiting OXPHOS in an in vitro assay here are some suggested amounts from the Drosophila literature: Antimycin A: 2-5 ug/mL

Rotenone: 2 ug/mL or 5-10 ug per 50 ug of mitochondrial protein"

From David Rand (pers. comm.): "Rotenone was a pain - hard to dissolve and we had a hard time doing anything to flies. It may help to dissolve in DMSO, but we have not done this. We read several papers where rotenone affects fly phenotypes, but their descriptions were very vague and multiple concentrations could not kill or harm flies when dissolved in fly food. Some pre-dissolving must be needed, since it is suppose to be an "insecticide" but flies loved it."

Take note of the following table concerning % lethality of rotenone:

Treatment	Stage	% Lethality	Notes
2 ug/mL Rotenone	Larvae	7.8%	Melanization
8 ug/mL Rotenone	Larvae	13.6%	Melanization

Adults will not eat rotenone so this test will not be done. This is not due to the DMSO solvent, as they readily take up 5% DMSO in 10% sucrose and display no obvious negative effects. Larvae were fed 2ug/ml and 8ug/ml for 6 hours. Rotenone caused melanization of trachea and cuticle in both samples.