Fly Paraquat Treatment Protocol

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Protocol reference: Willoughby et al. 2006.

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.

Paraquat treatment:

From David Rand (pers. comm.): “For paraquat, dissolve in 2% sucrose and wick on to squares of Watman filter paper and expose to flies in a vial with parafilm. Works pretty well."

From Hreve Tricoir (pers. comm.): "According to the paraquat treatment, we have devised a way to administer paraquat to adult flies based on incorporation of paraquat in a simple nutrition medium including sucrose, that is described below. An important point is that we used low melting agarose instead of standard agar to make the medium to be able to incorporate paraquat at a low temperature (typically 37°C). I guess that this could be used also for other compounds that may be temperature sensitive. The medium that we used is quite simple and for your experiments it may be more relevant to use a yeast-containing medium made also with LMA. We think that the use of a medium is better for reproducibility to paraquat soaked paper used by some labs, which could be sensitive to dryness. Another issue is the duration of paraquat treatment. We choose to make 24h treatment instead of acute treatment with 6h-starved flies to do not confound stress treatments. Obviously one drawback is to have only access to long-term response. We

did not test shorter treatments but, in this case, I advice you to test for the reproductibility of the volume of ingested food in different flies.
With 24h paraquat treatment viability of flies were 100% for 5mM paraquat and around 20mM for 20mM treatment. Transcriptome changes may be observed with bth concentrations."
Willoughby, L., H. Chung, C. Lumb, C. Robin, P. Batterham & P. J. Daborn (2006) A comparison of Drosophila melanogaster detoxification gene induction responses for six insecticides, caffeine and phenobarbital. Insect Biochem Mol Biol, 36, 934-42.

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

Treatment	Stage	% Lethality	Notes
5mM Paraquat	Adults	1.7%	48 HR Feeding
10mM Paraquat	Adults	1.7%	24 HR Feeding

Adults were fed 5mM paraquat for 48 hours and 10 mM paraquat for 24 hours. The notes have no information on larval treatments, but I will do an initial treatment of 10 mM paraquat for 6 hours feeding time.