

Fly Caffeine Treatment Protocol

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Protocol reference: Willoughby et al., (WILLOUGHBY et al. 2006, Phillip Daborn and Philip Batterham pers. com.)

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 from 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.

Caffeine treatment:

Take note of the following table concerning % lethality of caffeine before doing treatment:

Treatment	Stage	% Lethality	Notes
1.5 mg/ml Caffeine	Larvae	10.4%	Feed for 4 HR
2.5 mg/ml Caffeine	Adults	41.9%	48 HR Feeding
25 mg/ml Caffeine	Adults	12%	100% Lethal in 24 HR, Feed flies for 8 HR