

# Effects of Organophosphate Insecticide Application to the Conditioned Taste Aversion of Red-winged Blackbirds, *Agelaius phoeniceus*, Icteridae

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An experiment was conducted among free-ranging red-winged blackbirds (*Agelaius phoeniceus*) that acquired illness-induced conditioned taste aversion (CTA) by consuming insect prey tainted with a dose of parathion up to 2.0 mg/kg consumer body weight. Birds quickly acquired CTA and avoided all four insect prey during a lengthy posttest without parathion. This experiment proved that organophosphate insecticide application in the field might decrease the food consumption of wild birds and may also affect the reproductive success of breeding birds. Thus, CTA acquired accidentally after eating insecticide contaminated insect prey appears to be one of the reasons for the decreasing number of breeding songbirds in North America.

Organophosphates have become the most widely used insecticides in the U.S. as well as in many other industrialized nations. This is because their great toxicity enhances cost effectiveness and since they are thought to be relatively less persistent, they have been considered to be environmentally safer than some earlier pesticides (Pimentel and Greiner, 1997). These substances are widely applied in developing nations as well, but the regulation of their use and monitor of their effects is usually much less organized (Bull, 1982; Pimentel and Levitan, 1991).

Over-wintering neotropical migratory birds that breed in North America may be affected by insecticides in Central America, but their use and effects cannot be clearly quantified because of insufficient monitoring (Terborgh, 1989). It is known, however, that non-target, neotropical migrant birds have been killed through secondary poisoning by organophosphate insecticides applied in Central America (Gard et al., 1993). In developed nations where monitoring and reporting is most regulated, organophosphates have been directly implicated in the mass death of birds (Grue et al., 1983). Bird death have occurred when recommended guidelines have been violated (White et al., 1979; Graham, 1982), but the most frequent incidences have occurred when insecticides were applied according to recommended guidelines (Grue et al., 1983; Mineau, 1991).

Although mortality is the most easily quantified effect of organophosphate poisoning, studies with sublethally dosed, captive and free-living birds have documented

many physiological and behavioral effects with the potential to directly or indirectly impair productivity (Grue et al., 1983; White and Seginak, 1990). However, only inconsistent reproduction has been observed in wild populations exposed to single or multiple applications (DeWeese et al., 1979; Powell, 1984).

Analysis of brain acetylcholinesterase (AChE) activity confirms that many birds receive sublethal doses of insecticide and recover from varying levels of illness rather than actually die (Grue et al., 1991; Nicolaus and Lee, in press). Sublethal acute doses of organophosphates might have important long-term effects on bird feeding behavior. In particular, illness alters food preference and thus the feeding behaviors of virtually all of the vertebrates and at least some invertebrates (Garcia et al., 1974; Riley and Tuck, 1985). This process is known as the Garcia Effect or conditioned taste aversion (CTA). When consumption of a palatable favored diet for a subject animal is followed by gastrointestinal illness, preference for that food taste is reduced in a single trial (Garcia et al., 1955, Garcia and Koelling, 1967). Animals avoid the referent taste in any context and display signs of disgust in its presence (Garcia et al., 1974). The tendency to associate taste and illness is apparently ancestral: virtually all vertebrates and many invertebrates share this trait (Gustavson, 1977). The key variables important in the induction of CTAs are (1) the salience and novelty of taste (Garcia et al., 1974), (2) illness intensity (Nachman and Ashe, 1973) and (3) the interval of time between food consumption and illness (Revusky, 1968).

In real agricultural application of organophosphate insecticide, birds may form CTA to the illness producing insect prey and avoid them. Thus, the non-lethal doses of parathion may disturb the feeding behavior of

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birds and reduce food availability. Thus, this experiment demonstrated that free-ranging red-winged blackbirds (*Agelaius phoeniceus*) readily consumed insects tainted with a hidden, illness-causing dose of parathion and then formed a very powerful conditioned aversion to referent prey.

### Methods and Materials

The feeding behavior of breeding red-winged blackbirds was evaluated because these birds establish relatively small, clearly defined territories. This species should serve, in some sense, as a representative neotropical migrant passeriform whose abundance should make it convenient for others to replicate this work. Five territorial males out of 10 experimental sites that attack mounted male red-winged blackbirds in spring traps can be captured and individually marked with minimal disturbance.

Parathion was used (99.5%, Chem. Service) to breed red-winged blackbirds because its action as an acetylcholine (AChE) inhibitor is both well-known and similar to other AChE-inhibiting insecticides (White et al., 1983). It is very widely used and, although it is among the most toxic of the organophosphates, it is by no means the most toxic member of its class (Schafer and Brunton, 1979; Hudson et al., 1984). The LD<sub>50</sub> acute oral dose in red-winged blackbirds is 2.3 mg/kg BW (Schafer et al., 1983). This substance is either relatively tasteless, or if detectable, the taste is not very noxious (White et al., 1979; DeWeese et al., 1983).

In 1996, the region selected for experiment was a long strip of marshland along Illinois State Highway 30 approximately 21 km south of DeKalb, Illinois, USA (41° 80' N, 88° 80' W). A single railroad track runs along Highway 30 for a distance of some 18 km between the towns of Shabonna and Hinkley, Illinois. For 11.2 km of this distance, the railroad right-of-way provided an 18 m strip of marshy prairie habitat to the south of the highway which was suitable for breeding red-winged blackbirds and appeared strikingly uniform the entire distance along the road.

The insect prey in this experiment were obtained from Grubco Inc., Hamilton, Ohio. The four insect prey were larvae of mealworms (Coleoptera, *Tenebrio molitor*; mean weight 0.088 g, mean length 1.7 cm), waxworm's larvae (Lepidoptera, *Galleria mellonella*; mean weight 0.210 g, mean length 1.5 cm), crickets (Orthoptera, *Acheta domestica*; mean weight 0.151 g, mean length 1.5 cm), and frozen fly larvae (Diptera, *Musca domestica*; mean weight 0.079 g, mean length 0.8 cm). Dead fly larvae were used in this experiment because live fly larvae could climb and escape from the plastic bowl. The hind legs of crickets were removed to prevent their escape from bowls. The mealworms and waxworms were stored in a 5°C refrigerator, fly larvae in a -5°C refrigerator, and crickets at 22°C.

As much as 2.0 mg/kg BW of parathion, used in this

experiment, is at a slightly lower dose than the LD<sub>50</sub> of red-winged blackbirds. The injection procedure was designed to minimize the loss of the parathion and assured that each bird would receive a precise dose. It improved the chance that this dose could pass beyond the stomach and so not be lost through emesis and reduced the likelihood that parathion could be tasted by the birds. A 27-gauge syringe needle, 20 mm long, ran from the extreme posterior to the extreme anterior of an intact insect prey where a 0.01 ml volume of corn oil carrier was injected.

### Preliminary phase

The preliminary phase (maximum 54 d) was from April 7 to May 31, 1996. Beginning on April 7 and extending through April 15, 1996, a single clear plastic feeding bowl 11 cm wide × 5 cm deep was placed within 5 m of the primary display site in each of the 10 prospective red-winged blackbird territories. For this part of the experiment, each bowl was on a 15 cm × 15 cm white painted platform atop a 1.3 m high stake filled with a daily ration of 30 shelled sunflower seeds. On April 16, 1996, an additional clear plastic bowl was added separately from the first by 4 m at each site. The daily rations of 30 shelled sunflower seeds in one bowl and 8 mealworms in the other bowl were given in the morning. Every day the food locations were switched randomly and remained for the entire day. Food distribution began within 30 min before sunrise each morning.

The food consumption and the movement of territorial male and females were recorded daily at each site. When the actual feeding from the bowls by primary eaters, secondary eaters, or intruders was observed, the bowls were examined immediately after the eaters left the bowls. The kind and number of food items eaten, time, and bird identity were all recorded. Territorial males in 5 of the ten sites were successfully trapped live, weighed, and individually marked with three colored leg bands and an USFWS aluminum band. The experimental design is summarized in Fig. 1.

### Pretest phase

During the pretest (10 d) from June 1 to 10, 1996, the arrangement of bowls in each of the 10 randomly selected territories were altered. Each of these had four clear, plastic bowls in a square within 5 m of the primary display site. Every morning, four different kinds of insect prey were randomly distributed so that only one kind of prey was present in each of the bowls. The prey were 2 mealworm larvae, 2 waxworm larvae, 2 crickets, and 4 fly larvae. Fly larvae were substantially smaller than other prey. For the purpose of statistical analysis, the consumption of one fly larva equivalent to 0.5 of other prey were considered. Food was placed 30 min before sunrise each morning and observations lasted 3-5 min at each site before going on to

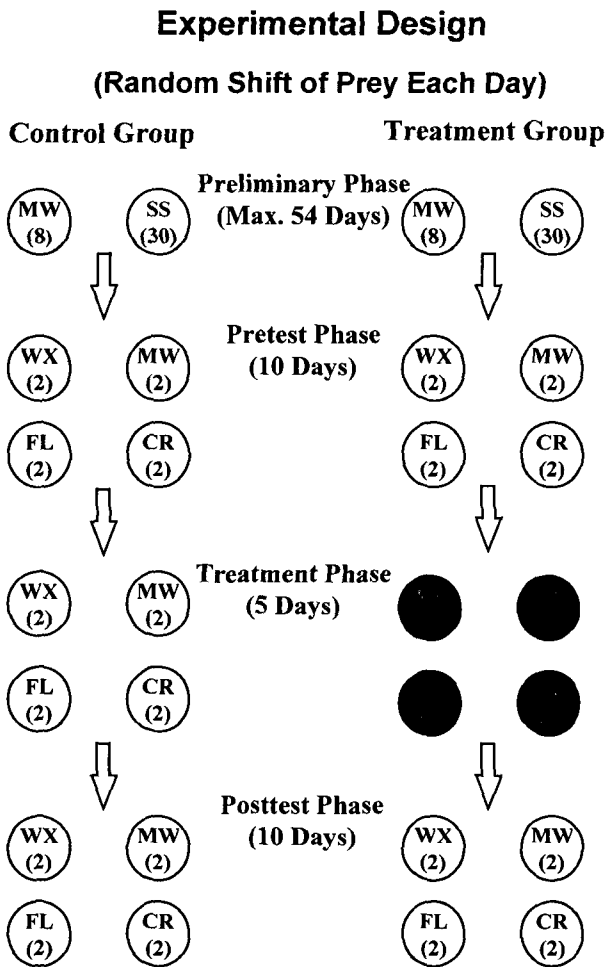


Fig. 1. Experimental design of the distribution of insect prey in each phase. Each circle represents a feeding bowl, food, and the number of prey (parenthesis). Only one of each prey of the treatment group (dark circle) was injected with 0.5 mg/kg BW parathion during the treatment phase. Control group was identical, except no parathion was included during the treatment phase. SS; sunflower seeds, MW; mealworms, WX; waxworms, FL; fly larvae, CR; crickets.

the next site on the route. After 3 h, each territory was revisited to record food consumption and territorial behavior and to remove unconsumed food.

#### Treatment phase

During the treatment phase (5 d) from June 11 to 15, 1996, the experimental design included offering birds four very different, but presumably palatable insect prey with which they had largely become familiar. Of the 10 territories, 5 were randomly assigned to the control group (sites 'A' to 'E') and the remaining 5 sites were the treatment group (sites 'F' to 'J'). Both in the treatment and control group, each of the four prey were presented in separate bowls throughout the pretest, treatment, and posttest phases of the experiment.

For the treatment group during the treatment phase, each one of the four kinds of insect prey was injected with 0.01 ml of corn oil containing 0.5 mg/kg BW para-

thion. All of the other insect prey remained uninjected. In the control group, each one of the four kinds of insect prey was injected with 0.01 ml of plain corn oil. Thus, the maximum dose would be 2.0 mg/kg BW. That dose of parathion is slightly below LD<sub>50</sub> for red-winged blackbirds (Schafer and Brunton, 1979). Treatment was synchronized so that 5 territories would undergo treatment at the same time. It was assured that one remained long enough at the treatment sites to record that insect prey were actually consumed by residents who then survived the treatment.

#### Posttest phase

During the posttest (10 d) from June 16 to 25, 1996, all territories reverted back to pretest conditions so that each of the four kinds of insect prey without parathion were present separately in each of the four bowls. The schedule of visits and the other details of the study remained the same as that of the pretest.

#### Data analysis

The average daily numbers of insect prey consumed by primary eaters during the pretest and posttest phases were used for statistical analysis to prove the bird's alteration in food consumption before and after the treatment phase. A paired sample *t*-test between the pretest and posttest was calculated for each insect prey (Zar, 1996). Test was calculated by using the SPSS statistical program. All statistical tests used a significance level of  $P < 0.05$ .

### Results

#### Preliminary phase

During the preliminary phase, although resident birds were plainly present nearby, the resident birds did not eat sunflower seeds and mealworms when these were first provided early in the morning. After 2 to 26 days at the 10 sites (average 12.4 days), the birds became increasingly habituated and they sampled at least one type of food within 24 h. As days progressed, the birds lost their fear, and in some cases the birds stayed near the bowls before both types of foods were distributed. Observations on the movements of birds, indicating each site included in this experiment was an independent replicate territory as confirmed by the consistency in which individual males bearing bands remained visible in their territories day after day.

#### Pretest phase

The birds were familiar with sunflower seeds and mealworms during the preliminary phase, but waxworms, fly larvae and crickets were novel. Birds at all sites came and fed quickly enough for one to witness until the end of the pretest. The birds continued to eat at least mealworms and waxworms within 3 h test interval

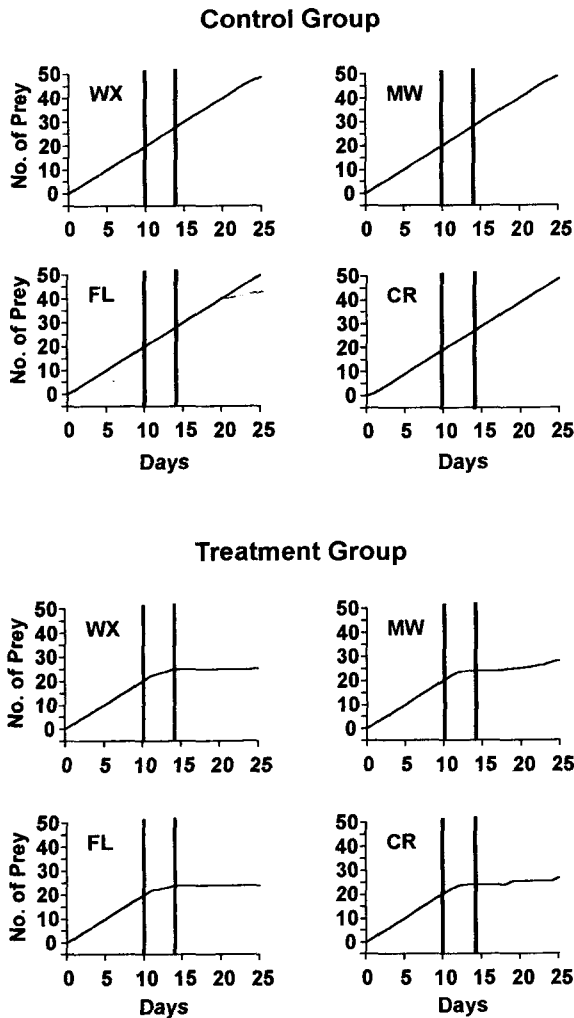


Fig. 2. Cumulative daily mean consumption of waxworms (WX), mealworms (MW), fly larvae (FL) and crickets (CR) consumed by primary eaters at each group which excluded non-consumption sites. Pretest phase (days 1-10), treatment phase (days 11-15), and posttest phase (days 16-25).

during the pretest (Fig. 2). The 10 territories had 1-3 resident females in addition to the territorial male, and the foods were consumed by males at 5 territories and by females at 5 territories. No other avian species consumed the food in this experiment.

Pooled pretest data for the full pretest phase indicated that not all foods were equally preferred. The birds at three sites, did not eat fly larvae and at 6 sites did not eat crickets. Thus, prey preference were ranked on the basis of mean consumption data (waxworms, mealworms > fly larvae > crickets; Table 1).

**Treatment phase**

For the control group, food consumption by primary eaters did not change during the treatment phase, so birds continued to consume the preferred insect prey (Fig. 2). For the treatment group, the birds were very familiar with the four insect prey during the pretest

Table 1. Average number of consumed insect prey per day during the pretest and posttest phases by primary eaters, and the results of a paired-sample t test

Insect Prey	Pretest	Posttest	t value	df	P
<b>Control Group</b>					
Waxworm	2.0	1.9	1.00	4	0.374
Mealworm	2.0	1.9	1.50	4	0.208
Fly Larvae	1.6	1.6	1.00	4	0.374
Cricket	0.8	0.8	1.00	4	0.374
<b>Treatment Group</b>					
Waxworm	2.0	0.1	39.19	4	0.001
Mealworm	2.0	0.2	10.41	4	0.001
Fly Larvae	1.2	0.0	2.45	4	0.070
Cricket	0.8	0.1	1.62	4	0.181

phase, so they did not hesitate to eat the four insect prey on the first day of the treatment phase.

The primary eater at site 'F' consumed all waxworms and mealworms including treated prey (a total of 1.0 mg/kg BW parathion) on the first day of treatment. The bird ate a treated waxworm, a treated cricket and all fly larvae (a total of 1.5 mg/kg BW parathion) on the second day of treatment. On the next day, all insect prey were eaten by unknown birds within 3 h. After that, no birds ate the insect prey throughout this phase. The primary eater at site 'G' consumed all insect prey (a total of 2.0 mg/kg BW parathion) while observed near the territory on the first day of the treatment phase, and then only one treated cricket on the second day and one treated fly larva on the third day were consumed by unknown birds until the end of this phase. The primary eater at site 'H' consumed all waxworms and fly larvae on the first day of treatment (a total of 1.5 mg/kg BW parathion). The bird ate all mealworms and crickets (a total of 1.0 mg/kg BW parathion) on the second day, and then all mealworms and fly larvae (a total of 1.0 mg/kg BW parathion) on the third day. The bird ate all waxworms (a total of 0.5 mg/kg BW parathion) on the fourth day. After wards the bird stopped eating any insect prey. A female at site 'I' ate all waxworms and mealworms on the first and the second days (a total of 1.0 mg/kg BW parathion on each day). Then, only one treated mealworm was disappeared on the fourth day. The primary eater female at site 'J' ate all four insect prey (a total of 2.0 mg/kg BW parathion) on the first treatment day. After wards only all waxworms and mealworms were consumed by unknown birds on the third day.

**Posttest phase**

Primary eaters of the control group continued to eat their preferred foods during the posttest (Fig. 2), and each food consumption by the primary eaters was not significantly different between the pretest and posttest (Table 1). The territorial birds in the treatment group clearly remained in their territory during the posttest phase. Waxworm and mealworm consumption, however, by primary eaters in the treatment group, significantly decreased during the posttest when compared to the

consumption rate of the pretest phase (Table 1). Also, the primary eaters stopped eating fly larvae and crickets during the posttest even though it was not supported statistically (Fig. 2) because of the non-consumption of birds during the whole period. Only the primary eater at site 'J' resumed eating fly larvae continuously from the third day of posttest phase.

## Discussion

The pretest record of the number of birds present at each site and the record of food consumption assured that sites were equivalent. Random assignment did not include bias and at least the primary consumers were familiar with prey of all four kinds.

For the treatment group, some birds might have consumed as many as 4 consecutive doses of parathion at the rate from 0.5 to 2.0 mg/kg BW parathion in a day. When the birds had illness after eating insect prey that were located in four separate bowls on the first day of the treatment phase, they delayed their food consumption each morning for the rest of the treatment phase. When they did come in to feed, they took more time to move among the bowls. These delays might have made it possible for birds to consume a slow sequence of food. However, none of the food was safe in this experimental setting, so the birds could not avoid intoxication.

As long as taste is paired with illness, nongustatory senses also play a role in the avoidance behavior (Nicolaus et al., 1983). Avian species usually search for prey visually, so at least some birds, such as bobwhite quails (*Colinus virginianus*), buteo hawks (*Buteo* sp.) and crows (*Corvus brachyrhynchos*), avoid prey by visual cues associated with gustatory illness (Wilcoxon and Dragoin, 1971; Brett et al., 1976; Nicolaus et al., 1983). Therefore, it was possible that when the birds consumed four chemically treated insect prey and got sick, illness was associated with all four insect prey because of their toxicity. After the birds in the treatment group acquired CTA, they avoided all four foods visually.

When treated birds neglected insect prey, other resident birds at sites 'F' and 'J' consumed some prey. However, many other residents still failed to eat the food even when treated birds neglected it because resident birds usually had several days of neophobia before eating novel foods. The food consumption of red-winged blackbirds had been increased by observations of conspecifics consuming food (Mason and Reidinger, 1981). In this experiment, however, the food avoidance behavior of primary eaters did not affect the food preference of the secondary eater in a territory, and also the food consumption by the secondary eater did not change the feeding behavior of birds which acquired CTA and then avoided the foods.

In real agricultural application of organophosphate insecticide, red-winged blackbirds would form CTA to

the illness producing insect prey and avoid them. Thus, the non-lethal dose of parathion can disturb the feeding behavior of birds and reduce food availability. Moreover, the birds may tend to avoid most of their prey when the situation is similar to this experiment. This means that in the specific situation of insecticide application on agricultural land, the insecticide may severely affect the food consumption of wild birds. In the real world, insecticide application is applied as many as 50 times in a crop growing season and leaves the contaminated insects in the field for more than two days and is available all day long (WHO, 1986; Pimentel and Levitan, 1991). Thus, it is possible that the insecticide application would contaminate all the insect species in the field. The birds would then be repeatedly treated in a day with a high dose of insecticide and with different insect species. In the worst situation, the birds may avoid all the insect prey which they consumed in the insecticide-applied field, and it would affect their survival in nature.

The decreasing number of breeding songbird populations in North America is known mainly as a result of habitat destruction or cowbird brood parasitism (Terborgh, 1989). However, inadvertent development of CTA among birds made ill by consuming insect prey contaminated with pesticides may be another reason. Many species of birds require a reliable supply of protein-rich insects to maintain breeding territories and feed their demanding nestlings. Choice and fidelity to breeding territory may be powerfully affected by the quality of the food supply nearby. If some or several insect prey contain sufficient pesticide levels to induce illness, breeding birds that have acquired CTA might either abandon territories or fail to exploit available insect prey sufficient to successfully fledge the full number of young needed to sustain a stable regional population.

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