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EFFECTS OF TWO (*DIPTERA:CULICIDAE*) MOSQUITO CONTROL AGENTS ON GROWTH AND REPRODUCTION OF RED-WINGED BLACKBIRDS (*AGELAIUS PHOENICEUS*)[†]

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ABSTRACT

We compared red-winged blackbird (*Agelaius phoeniceus*) reproductive success and nestling growth in 1988 in 10 wetland sites treated with methoprene and 10 sites treated with *Bacillus thuringiensis israelensis* (Bti) to 30 sites that had never received any mosquito (*Diptera:culicidae*) control treatment. No differences were detected between reference and treatment sites for clutch size, egg volume, growth rates, or fledge weight. The probability that an egg survived to the nestling stage was greater in methoprene-treated than reference sites. No other differences were detected among sites for reproductive success estimated using the Mayfield method. We conclude that treatment of the wetlands with methoprene or Bti did not directly or indirectly affect red-winged blackbird growth or reproduction within the first two years after treatment.

INTRODUCTION

Methoprene (tradename Altosid) and *Bacillus thuringiensis israelensis* (Bti) are two larvicides commonly used to control emergence of adult mosquitoes (*Diptera:culicidae*). Methoprene is a juvenile growth inhibitor that delays or prevents metamorphosis from larval to pupal stages. Bti is a soil bacterium that, when ingested, acts as a stomach poison killing insects that have a larval gut pH in the range of 7 to 10. These larvicides are reported to be among the safest in terms of toxicity to non-target species, but some negative effects on short-lived aquatic insects and zooplankton have been reported (Miura and Takahasi, 1974; Mulla et al., 1979; Mulla et al., 1982; Noriand and Mulla, 1975). These organisms are the major prey species for many aquatic predators (for example, dragonflies and damselflies), so their selective removal could reduce the number of prey available. Because many wetland bird species consume aquatic predators during the breeding season, lesser abundance or availability of aquatic predators could then affect reproduction of birds that use wetlands for feeding and nesting.

Our objective was to determine if treatments of wetlands with methoprene or Bti indirectly affected bird reproduction or growth. The red-winged blackbird (*Agelaius phoeniceus*) was chosen because it: (1) depends on an aquatic-based food supply during the breeding season (Orians 1961, 1980), (2) nests in high densities in many wetlands, and (3) can be used to assess possible effects of mosquito control

treatment on other insectivorous altricial songbirds with similar life histories (for example, marsh wren [*Cistothorus palustris*]), but for which adequate samples are difficult to obtain.

We did not expect methoprene or Bti to be directly toxic to red-winged blackbirds. Adult birds were not exposed to great concentrations (either by direct contact or by ingestion) and the rat oral LD₅₀ for methoprene is >34.6 g kg⁻¹. Altricial nestling birds however, may be sensitive to indirect effects of pesticides/insecticides because they depend on adults to provide food and maintain their body temperature. For example, potential decreases in insect abundance or availability after treatment might alter foraging behavior and ultimately affect nestling growth and survival (Whitmore et al., 1993.)

MATERIALS AND METHODS

The study area was located in Wright County in south-central Minnesota. Many wetlands in this region are treated by the Metropolitan Mosquito Control District (MMCD). The MMCD has been applying mosquito control agents in this region since the 1950's. We chose study sites (50 total) that had no history of treatment with persistent organochlorine compounds (for example, DDT).

Wetlands (30 reference; 10 Bti; 10 methoprene) were chosen randomly from about 1400 wetlands that the MMCD had treated or had designated for future treatment. The treated sites were not randomly assigned to treatment, but represent the types of

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wetlands that would receive the specific treatments under the MMCD's standard operating procedures. Sample sizes were unequal among groups because we used the 30 reference sites in a before-and-after experiment that began in 1991 (Hanowski et al. in press). Sites chosen were >1.0 ha in area, had suitable nesting habitat for red-winged blackbirds, and were not adjacent to roadsides that were treated with herbicides.

Methoprene sites were treated in the early spring of 1988 with a 60 day release briquet to achieve a 55 ug L⁻¹ concentration in the wetlands Bti was applied. by helicopter at a rate of 56 kg ha⁻¹ in April of 1988 and again after each rainfall >2.5 cm. Sites with a history of mosquito control treatment had been treated from 1 to 3 years before 1988, but total treatment applied to each site varied depending on water levels in previous years.

Red-winged blackbird nests were located and marked with flags placed from 1 to 3 m from the nest in early May 1988, before egg-laying. We measured water depth under the nest, nest height above water, and noted plant species used for support of the nest. Egg breadth and length were measured with needle point dial calipers to the nearest 50 µm. Egg volume was calculated using the equation from McGuire (1986):

$$V = 0.524 \times \text{Length} \times \text{Breadth}^2$$

We visited nests every other day so we could accurately determine hatching date. Nails of young birds were clipped to identify individuals. Birds were weighed (to the nearest 0.5 g) in plastic bags suspended from Pesola spring scales; birds were allowed to defecate before being weighed. Tarsus length was also measured, but, because it was highly correlated with weight, we did not analyze this variable.

We used Mayfield's formula (Mayfield 1961, 1975) to compute daily estimates of nest success at each site. The following were computed:

Hatch rate = number of eggs that hatch per number of eggs present at hatch time

Nest survival rate during incubation = 1 - (number of nests lost during incubation per incubation exposure days)

Egg survival rate during incubation = 1 - (number of eggs lost from clutches [excluding eggs lost from total clutch loss] per egg exposure days)

Nest survival rate with young = 1 - (number of nests lost with young per total nestling days on site)

Individual nestling survival rate = 1 - (number of young lost from nest [excluding young lost from total nest loss] per number of nestlings days on site)

Probability that an egg will produce a fledgling = the product of the five probability values calculated above.

Mayfield's correction is necessary when nests are found at different stages of reproduction. This method reduces data to units of exposure (egg or nest days), reflecting length of time each nest was under observation. Calculations of nest success that do not take into account the time span of observations for each nest usually underestimate losses. By using this method, data between sites are comparable regardless when nests were first observed on each site (Johnson and Shaffer, 1990).

Although several nests, eggs, and nestlings were measured on each site (for example, samples), we used the mean value of all measurements on a site in statistical analyses. This was done to ensure that treatment replicates (for example site) were statistically independent (see Hurlbert, 1984). Therefore, in this experiment we had 10 methoprene, 10 Bti, and 30 reference replicates for statistical analyses.

All data were first examined for normality and homogeneity of variances (Sokal and Rohlf, 1981) and transformations (for example, log, rank, or arcsine) were used for variables that failed assumptions of parametric statistical tests. We used one-way analysis of variance (ANOVA) with contrasts to compare reference and methoprene-treated, and reference and Bti-treated sites for several measures (for example, clutch size, egg volume, and all Mayfield variables). The power for all statistical tests which compared treatments to reference was determined using the standard error of the difference between the mean of the reference and treatment group. The minimum percent difference detectable for a power of 0.80 and alpha of 0.05 for a two-sided test was calculated.

Two measures of bird growth were used. The first was a measure of rate of growth calculated by the ratio of weight on day eight: weight on day four. The second measure of growth was weight of the young on day eight. We used this measure instead of "fledge weight" in the analysis because it was more accurate mainly because we were unable to get an accurate fledge weight for all birds. For those birds that were not measured on day eight, a day-eight weight was interpolated using regression of weights from surrounding days. A bird was considered a fledgling when it was > eight days of age.

Because nest success for red-winged blackbirds is affected by nest height and water depth (Goddard and Board 1967; Holcomb and Twiest 1968; Holm 1973; Brown and Goertz 1978; Shipley 1979), we examined our data to determine if nests that were placed higher in vegetation or in areas with deeper water were more successful. No differences in water depth or nest height were detected between successful and unsuccessful nests within each group so this factor was ignored in our data analyses and interpretation of results.

RESULTS

Clutch size averaged 3.6 eggs per nest and did not differ between reference and either treatment group (Table 1). Egg volume was unaffected by Bti or methoprene treatment.

The probability of an egg surviving to the nestling stage was greater (1.0 versus 0.98) in methoprene-treated than in reference sites (Table 1). There were no other differences between reference and treatment groups for Mayfield measures of survival. All nests survived during the egg-laying stage and the overall egg hatch rate was 86 %. The probability of a nest surviving the incubation state was 93 % and the survival of a single nest to the fledgling stage was 90 % (Table 1).

We found no differences between reference and treatment groups for any growth parameter (Table 1). Mean weight at day eight was 27.0 to 29.3 g and fledge age was 9.6 to 10.3 days. The ratio of day eight:day four weight ranged from 2.2 to 2.3 (Table 1).

We were able to detect a 5 % to 100 % difference between control and treatment means at a power of 0.80 and alpha of 0.05. In general, all variables physically measured (for example, clutch size, weight) had lesser percent differences detectable than the percentage values (for example, Mayfields). The actual differences observed between group means ranged from 0 to 8 % (Table 1). Therefore, the actual measured difference for most variables was less than the detection limit.

DISCUSSION

Have mosquito control activities in these wetlands decreased the amounts or availability of food for the red-winged blackbird? Benthic aquatic insect data

collected in wetlands during May and June indicated no significant decreases in numbers of insects and biomass in either Bti-treated and methoprene-treated sites relative to reference sites (Niemi et al. 1990). Niemi et al. (1995), found that both methoprene and Bti depressed insect numbers after three years of treatment, however, no differences were found during the first sampling period (for example, early-May). This result has important implications to our assessment of mosquito control treatment effects on red-winged blackbirds and the entire avian community. If reduction in numbers of benthic insects occurred after mid-May, or at all as indicated in this study, this decrease may not be apparent in emerging aquatic insects for another month or more (depending on the life cycle period for each insect species (A. Hershey personal comm.)). This suggests that red-winged blackbird food resources were not depressed during the period that food was required for reproductive activities (at least during the first two to three years of treatment of a particular site). Therefore, we would not expect to see a negative effect of either Bti or methoprene treatment on the red-winged blackbird parameters that we measured.

Due to the difficulty in working with other species in these wetlands, we were only able to assess potential effects of mosquito control agents on one representative species, the red-winged blackbird. We were able to collect adequate samples for this species which would have been impossible for other species within these sites, and the power for detecting treatment effects for this species was quite high. No other species was abundant enough (Niemi et al. 1995) within these sites to collect adequate samples or would lend itself to study due to difficulty in finding nests.

Table 1. Mean, standard deviation (SD), and probability values (P) from ANOVAs that compared reproductive parameters of red-winged blackbirds between reference and methoprene treated and between reference and Bti-treated wetlands in south-central Minnesota, 1988.

Parameter	Reference			Methoprene			P	Bti			P
	Mean	Std Dev	n	Mean	Std Dev	n		Mean	Std Dev	n	
Clutch size, no of eggs	3.7	0.3	30	3.6	0.3	10	0.74	3.6	0.2	10	0.46
Egg volume, cm ³ †	3.8	0.1	30	3.8	0.3	10	0.54	3.8	0.1	10	0.99
Probability nest survival egg-laying‡	1.00	0.00	30	1.00	0.0	10	0.59	1.00	0.01	10	0.42
Probability survival of nest incubation‡	0.94	0.06	30	0.92	0.08	10	0.35	0.92	0.05	10	0.19
Probability egg survival to nestling‡	0.98	0.02	30	1.00	0.01	10	0.00	0.99	0.01	10	0.37
Probability survival nest to fledging‡	0.94	0.07	30	0.89	0.12	10	0.42	0.90	0.05	10	0.06
Hatch rate of eggs‡	0.83	0.15	30	0.86	0.16	10	0.36	0.88	0.10	10	0.28
Weight at day 8, gm†	27.0	3.7	29	29.3	2.5	8	0.10	28.8	4.0	10	0.17
Fledge age, days	10.3	1.1	29	9.6	1.2	8	0.14	10.1	1.2	10	0.58
Wt day 8:day 4†	2.2	0.3	29	2.2	0.2	8	0.83	2.3	0.3	10	0.50

†Ln transformation

‡Arcsine transformation

SUMMARY

Based on results from the red-winged blackbird and aquatic insect data, it is unlikely that the treatments affected avian species that breed in these wetlands two to three years after treatment. This result agrees with our before-and-after treatment study (Hanowski et al. In press). Most individuals completed their reproductive cycles before the period when the aquatic food resource was depressed by mosquito control treatment (most by late-June). Although red-winged blackbirds are apparently unaffected by treatment of wetlands with Bti and methoprene during the first three years of treatment, several questions remain to be answered. First, will continued treatment of these sites (especially the smaller sites) depress aquatic insects beyond some critical density required for recovery? Second, if aquatic insects communities are depressed during July and August, will there be longer-term implications on dispersion patterns of young birds to these sites from other areas? And finally, will birds that use wetlands as migration stopover sites be affected by the lesser numbers of aquatic insects that are likely present on these sites during late summer and early fall?

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