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We use mass color-marking to track the local and regional movements of large roosts of blackbirds (Icteridae). Several markings have been done by National Wildlife Research Center scientists, including marking of spring roosts in northeastern Missouri and eastern South Dakota (Knittle et al. 1987, Knittle et al. 1996, Homan et al. 2004), fall roosts in central North Dakota (Linz et al. 1991, Homan et al. 2005), and winter roosts in the southern U.S. (Harsch 1995). Here, we provide a description of the process and methodology of aerial mass color-marking with fluorescent particles.

Marker Formulation

The formulation consists of 50% (v/v) Carboset® 514H (an acrylic adhesive; Noveon™ Incorporated, Cleveland, OH), 25% (v/v) food-grade propylene glycol, 23% water, 2% (w/v) fluorescent pigmented resin (DayGlo® Color Corporation, Cleveland, OH), 0.2% Triton® X-100 (Dow Chemical Company, MI), and 0.2% foam suppressor (e.g., Cleary's Defoamer; Cleary Chemical Corporation, Dayton, NJ). The acute oral LD_{50} for fluorescent resin in rats was >16 g/kg. The acute dermal LD_{50} for rabbits was >23 g/kg (Technical Bulletin 2002, DayGlo® Color Corporation, Cleveland, OH). The marker formulation was nontoxic to 4 species of freshwater fish after 96 hours of exposure at concentrations up to 4,000 µL/L (4,000 ppm) in 12 C° water (Bills and Knittle 1986). Further, no mortalities from exposure to formulation at 12-ppm were detected during a 48-hour acute laboratory bioassay on 2-week old chironomid larvae (*see* Knittle and Johns 1986). At the recommended aerial delivery rate for marker formulation, the expected concentration in a wetland should not exceed 5 ppm.

Mixing and Loading

We use tarps on the mixing apron to keep the area cleaner and facilitate post-operation cleanup. Total time for site preparation, mixing, off-loading, and cleanup is about 2-3 hours. We use a 300-gallon elliptical tank on skids with a 5.5 HP engine and 2 eductors for heavy agitation. A discharge system is also required for back-flushing the tank to keep the talcum-like, fluorescent resin (~4-µm particle diameter) in suspension.

Transfer the desired amount of propylene glycol to the mixing tank, followed by the additions of Triton X-100 and foam suppressor. The Triton X-100, a wetting agent, must be stored above 45° F to avoid solidification. Start the agitation system and slowly mix in the fluorescent resin; to break up clumps of resin, back-flush with the discharge system. After the resin is in suspension, transfer the Carboset 514H to the mixing tank and bring up to final volume with water. Agitate for at least 30 minutes then off-load to the aircraft's holding tank. The aircraft must have an internal agitation system to keep the resin in suspension. It is advisable to keep the waxy-like Carboset 514H warm (e.g., >50° F), thus keeping it mixable, highly fluid and transferable. Use ammonia water to clean the discharge system and mixing tank immediately after off-loading. Save the rinseate in an empty 55-gallon drum or other container for disposal later. Refill the mixing tank with 25-30 gallons of clean ammonia water. Rinse and clean the holding tank after the aircraft returns. Discharge the rinseate through the boom sprayer. If necessary, repeat until holding tank and boom sprayer are thoroughly clean. A pressure washer with a downstream chemical injector (for the ammonia) is very helpful for removing spray blowback from the aircraft. Removal of dried blowback is made much easier by coating the underbelly and sides of the aircraft before takeoff with a non-stick cooking spray.

Aerial Application

The spraying is done during the 20 to 30-minute period of twilight following sunset. Ground-spotters are used to monitor the subsidence of roost activity to help coordinate departure time from the landing strip. The marker formulation is applied through a 24 to 36-nozzle boom sprayer from an altitude 50–100 feet AGL at an air speed of 100-110 MPH. Spray volume should be 3 gallons/acre delivered with 25 lbs/in^2 pressure. A coarse droplet size of approximately 400 microns is recommended. The coarse droplets will leave well-defined splash marks of color on the birds. A 110-gallon load is sufficient to mark about 100,000 birds.

Most pilots make a few preliminary high passes over the wetland to get a lay of their surroundings and to flush low-level flight hazards, such as waterfowl and wading birds. It usually takes about 15 minutes to empty a 110-gallon mix. As twilight fades, the pilot may lose visual contact with the main roost. The ground crew should be ready to direct the pilot's flight path by using strobe lights or other signals. The blackbirds, although disturbed by the aerial passes, will not usually leave the wetland's confines. If there is too much light at the start of the spraying operation, the birds may try to escape the roost. The pilot should be notified to back away from the site for a few minutes to let the birds settle down again. Lastly, if multiple sprays are conducted on the same roost over a period of time, the birds usually become more wary of the overflights and harder to mark. The sprays often have to be pushed back later in the twilight period to overcome the acquired skittishness.

The spray dries in about 3-5 minutes and adheres particularly well to feather surfaces as the birds fly through the descending spray mist (Jaeger et al. 1986, Johns et al. 1989). Studies on marked, free-ranging red-winged blackbirds (*Agelaius phoeniceus*) showed that 30% of the initial marks were lost 4-6 weeks after spraying (Knittle and Johns 1986). Resin particles lodged in the barbules, however, can remain much longer, often several months after the date of application (Knittle et al. 1996). Color-marking should be coordinated with the USGS Bird Banding Laboratory. We always consult with water management agencies and state game agencies prior to conducting color-marking over wetlands.

Identification of Marks and Spray Efficacy

The marks are nearly invisible to the human eye under natural white light; however, the resin particles are highly fluorescent and easily seen in darkroom conditions using a high-intensity, ultraviolet lamp (e.g., Spectroline® SB-100P, Spectronics Corporation, Westbury, New York). UV-contrast goggles and optical magnification further augment viewing of marks. To eliminate false positives, only splash marks and individual droplets firmly attached to the feathers are considered valid evidence of a mark (Knittle et al. 1987). Fluorescent resin particles are ubiquitous in the environment, being used to mark fertilizer mixes, seed coatings, and pesticides. Therefore, dustings of resin particles should not be accepted as a mark (Knittle and Johns 1986).

In large- and medium-sized roosts, the percentage of the roost marked ranges from 50-75% (i.e., spray efficacy). It can be much higher for smaller-sized roosts (Homan et al. 2005). We determine spray efficacy by making total counts of entering birds on the evening the roost is sprayed (Meanley 1965, Arbib 1972). This is followed by collecting a random sample of birds departing the roost the next morning. The total number of newly marked birds is estimated by multiplying the proportion of marked birds in the sample by the number of birds in the roost the evening it was sprayed.

Discussion

The major benefit of aerial mass color-marking is that large numbers of individuals can be marked rapidly because this method does not depend on capture and handling. Knittle et al. (1996) estimated that they marked several hundred thousand birds in a single marking. Additionally, if the roost is repeatedly color-marked with different colors, population dynamics of the roost, including turnover and total number of birds using the roost, can be estimated (Otis et al. 1986, Linz et al. 1991). Although we have only discussed the mass color-marking of blackbirds, any species that becomes highly aggregated at some time in their life cycle may be mass color-marked. Other species of birds that have been mass color-marked are red-billed quelea (*Quelea quelea*) and purple martins (*Progne subis*) (Jaeger et al. 1986, C. E. Knittle, Denver Wildlife Research Center, Denver, CO, unpublished data).

There are a number of factors that can cause variability in results and recovery rates of marked birds. These include (1) failure to accurately estimate the number of birds marked, (2) deterioration and loss of marks, and (3) limits on time and personnel available to adequately follow the sampling design, which can be complex and cover large amounts of geographic area.

Careful consideration of these factors must be taken when planning a marking study. Moreover, despite careful planning, design, and execution of a study, researchers always have to face the conundrum of how to properly interpret null data (e.g., no marks in a sampled quadrat). These can be interpreted in several ways, one of which is that the null results are more a reflection of the vagaries of sampling rather than effects of behavioral phenomena under study. In our experiments, we always try to use either a population index or density estimate to balance or adjust sampling efforts among quadrats. This mitigates the interpretation of null results.

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