



Fig. 1. General schematic of the third floor of the pilot-scale flour mill used for all experiments. The black boxes, white circles, and rectangles represent large structural features on the third floor such as support columns, milling equipment, sifters, and large storage bins. The bioassay area positions are numbered 1–10. The location of each aerosol application position is denoted as A1, A2, and A3 inside the gray squares. Aerosol application 4 was released from all three locations A1, A2, and A3 (1/3 dose at each position). Source: Scheff et al. (2018), used by permission.

of the mill at an approximate 45° angle and applied using a sideways sweeping motion toward the left and right sides of the floorspace. The total aerosol application time was approximately 2–5 min, followed by a 1-h exposure period. After the 1-h exposure, the air ventilation system was turned on for approximately 10 min to remove any residual aerosol particles that may be present in the ambient air. The next step was to pick up all bioassay arenas that were exposed to the aerosol insecticide treatment and replace with new bioassay arenas for the next aerosol treatment, which took approximately 5–15 min. The aerosol application position was changed after each treatment and each aerosol application location was repeated twice for each aerosol formulation. Due to logistical constraints of the milling facility (time available for testing) and time required for each aerosol application the pyrethrin + pyriproxyfen aerosol treatments were conducted first, followed by the pyrethrin + methoprene aerosol treatments. Day 1 of testing had five trials, day 2 had six trials, and day 3 had five trials, for a total of 16 aerosol applications.

Bioassays

The *T. confusum* larvae used in the study were from a pesticide-susceptible strain maintained at the United States Department of Agriculture–Agricultural Research Service–Center for Grain and Animal Health Research (USDA-ARS-CGAHR, Manhattan, KS). *Tribolium confusum* colonies are reared on 95% organic whole-wheat flour and 5% brewer's yeast at 27°C, 60% RH, and 0:24 (L:D) h in an environmental chamber. These colonies have been maintained at the USDA-ARS-CGAHR for more than 30 yr.

Testing arenas used in this study were individual 60 × 15 mm (~22 cm²) Petri dishes partially filled with concrete and placed inside larger 150 × 20 mm (~137 cm²) plastic Petri dishes. Concrete dishes were prepared based on Arthur (2015). Briefly, a dry powder driveway patching material (Rockite, Hartline Product Co., Inc., Cleveland, OH) was mixed with water to create a thin slurry, poured in the bottom of Petri dishes to a depth of ~0.5 cm, and held at ambient conditions for approximately 7 d. Inside each Petri dish, four concrete arenas were placed. Three of the arenas were used in the current study for residual bioassays at 2, 4, and 6 wk post-aerosol treatment and contained no diet at time of exposure to aerosol. The other arena was used as described by Scheff et al. (2018).

These groups of testing arenas were placed at ten different positions on the third floor (Fig. 1). Nine positions also had aerodynamic particle sizer units (APS 3321, TSI Inc., Shoreview, MN) placed to collect data on aerosol particle concentration and size distribution.

Two additional testing arenas were placed on the first floor of the mill during each aerosol treatment and served as untreated controls. Arenas were placed in each of the four major corners of the mill floor (arenas 4, 5, 8, 10). Bioassay arenas along walls were placed approximately 0.5 m away from the wall (arenas 4, 5, 6, 8, 9, 10). Bioassay arena 2 was placed in between two large pieces of milling equipment and pneumatic conveying ducts. Bioassay arena 7 was placed underneath a piece of milling equipment. Relative to the application locations, bioassay arena 1 was placed directly in front of aerosol application location 1 and bioassay arenas 9 and 4 were placed next to aerosol application locations 2 and 3, respectively.

After aerosol applications, each bioassay arena was covered and transported back to the USDA-ARS-CGAHR and held at 27°C, 60% RH, and 0:24 (L:D) in an environmental chamber. At 2, 4, and 6 wk post-aerosol application, five (3–4 wk old) *T. confusum* larvae along with ~400 mg of diet were added to one concrete arena from each bioassay arena position. Number of larvae available for bioassays was limited, but five larvae per dish does provide sufficient resolution to evaluate IGR effects on development. Larvae were examined twice weekly, up to 4 wk, for adult emergence. Emerged adults were removed from dishes to prevent cannibalism.

At the end of 4 wk, all five individuals were classified as larvae, pupae, or adults. Means and SEs were calculated for adult emergence and data analyzed using statistical analysis software (version 9.4, SAS Institute, Cary, NC). Adult emergence data were transformed to angular values prior to analysis (Zar 2010) before using a three-way analysis of variance based on bioassay position, application position, and residual week as the main effects.

Aerosol Dispersal Patterns

To assess the strength of the effect of the aerosol application, an efficacy value index was created to convert the three morphological states (larvae, pupae, adult) to a single value for comparison (Campbell et al. 2014). The efficacy index ranged from 1, the weakest efficacy response with five adults, to 21, the strongest response with five larvae. All of the index values with the corresponding numbers of adults, pupae, and larvae indicated in brackets were the following: 1 [5,0,0], 2 [4,1,0], 3 [4,0,1], 4 [3,2,0], 5 [3,1,1], 6 [3,0,2], 7 [2,3,0], 8 [2,2,1], 9 [2,1,2], 10 [2,0,3], 11 [1,4,0], 12 [1,3,1], 13 [1,2,2], 14 [1,1,3], 15 [1,0,4], 16 [0,5,0], 17 [0,4,1], 18 [0,3,2], 19 [0,2,3], 20 [0,1,4], 21 [0,0,5].