DUPLEX™-G TESTING PROTOCOL

INTRODUCTION:

Duplex™-G is a unique dual-active larvicide that consists of a *Bti* coated (S)-methoprene sand-based granule. At application, the *Bti*-coating is released and will begin controlling larvae immediately. Within hours of application after the *Bti* release, (S)-methoprene will begin releasing and controlling adult emergence. During the initial application, you will get both *Bti* and IGR effects. After the first week post-application, there will be IGR effects only.

To evaluate the effectiveness of an application, there are a few simple steps to take that are outlined in the following protocol. Since Duplex™-G is a dual-action larvicide, the first indicators of effectiveness will be direct mortality to larvae caused by *Bti*. After an application, observations should be made in the first 72 hours to determine mortality. Dip counts taken at various locations at the treated site are a good way to observe larval mortality. The *Bti* effectiveness will decline after the first 72 hours following the initial application.

Once the *Bti* effectiveness has declined, the IGR effect from methoprene will control subsequent broods of mosquito larvae for up to 28 days post-treatment. Dip counts are still a good way to evaluate control but the evaluation procedures are different. Pupae must be collected from the field and held in the lab to determine the inhibition of adult emergence. Collecting larvae is not an effective method for determining inhibition of adult emergence. Mosquito larvae must be in a treated environment upon molting to pupae. Water brought back from the treatment site will lose methoprene and there may not be enough left in the water to cause an effect.

HELPFUL EVALUATION TIPS:

- Choose a site that is readily accessible containing abundant larval populations
- Ensure proper calibration of equipment and uniform application
- Existing pupae present at the site of treatment will not be affected by *Bti* or the IGR, wait 48 hours following the application to evaluate pupae for IGR effect
- Untreated sites may be used as a comparison to treatment sites
- Try to collect 25 larvae per dip for *Bti* evaluation and 25 pupae for IGR evaluation
- Record data and observations in a lab book or in prepared data sheets
- Take dip counts at random locations within the treatment site, dip count numbers will vary based on site size and is up to the investigator to determine the appropriate sampling size and location
- Duplex™-G has a range of application rates, it is suggested that on first evaluation 7.5 pounds per acre be used

DUPLEX™-G FIELD TESTING GUIDELINES:

Pre-treatment larval abundance (first and second instar larvae, third and fourth instar larvae, and pupae) should be recorded in both experimental and control sites. The sampling method should be appropriate to the type of breeding habitat, and the appropriate number of samples should be taken from each habitat based on the type and size of the habitat. Larval instars and pupae from each sample are counted and recorded.

Post-treatment larval abundance (all stages) should be monitored 24, 48 and 72 hours post-application and then weekly. Data should be recorded in a lab book or prepared data sheets. 10 random dip counts at each location will be recorded on the provided form for the first 72 hours minimum. *Bti* effects from Duplex™-G can persist for a week in some cases and should be monitored accordingly. After the first 48 hours, pupae may be collected if available and reared out for inhibition of emergence calculations. Inhibition of emergence begins at application, but waiting 48 hours after the initial treatment ensures that all pupae collected have developed in the presence of (S)-methoprene.

Collection of pupae starts at 48 hours, 1 week, 2 week, 3 week, 4 week and 5 week post-treatment, if possible.

Characterization of the habitats in terms of abiotic and biotic factors aids the interpretation of results. Rainfall and any change in water level or other parameters, such as algal bloom, water quality, outflow, temperature or predators in the habitats, should be recorded.

DATA COLLECTION:

Use the attached data sheets to record dip counts and pupae collected as follows:

1. Record conditions at treatment site and any factors that may affect application.
2. Record pre-treatment larval populations on the data sheet with 10 random dip counts for the treatment and control areas.
3. Make 10 random dip counts in treatment and control (if used) area every 24 hours until control drops below, usually after 96 hours for *Bti* evaluation.
4. Begin collecting pupae (if present) after the first 48 hours and rearing them for emergence calculations.
5. Make pupal collections weekly or when present and check for emergence inhibition until control percentages drop below 70%.
6. Pupae may be collected after the first 48 hours for IGR affects.

CALCULATION METHODS FOR IN-FIELD EFFICACY EVALUATIONS

The efficacy and residual activity of the larvicide is determined from the post-treatment counts of live larvae and pupae in treated and control sites compared with the pre-treatment counts or the control.

The assessment of an IGR's efficacy is based on the level of inhibition of emergence of adults and the percentage reduction in larval and pupal densities. Larvae and pupae are sampled as described above.

Adult emergence can be monitored directly in the field by using floating sentinel emergence traps in treated and untreated habitats, by pupal isolation, or by sampling and counting pupal skins. Adult emergence may also be assessed by collecting pupae (20–40 per replicate) and bringing them to the laboratory in appropriate containers with the water from the respective habitats, then transferring them to small cups inside the holding cages. Dead larvae and pupae found in the cups should be removed and any morphological abnormalities recorded.

When adult emergence is monitored in the laboratory using pupae collected from treated and untreated habitats, IE% is calculated using the following formula, on the basis of determining adult emergence from the number of pupae collected:

$$IE(\%) = \left(\frac{IC}{C\text{-}T}\right) \times 100$$

Where C = percentage emerging or living in control habitats and T = percentage emerging or living in treated habitats.

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