



Ground application of mating disruption against the gypsy moth (Lepidoptera: Erebidae)

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Abstract

The gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Erebidae), is a non-native defoliating insect that continues to expand its range in North America and undergo periodic outbreaks. In management efforts to suppress outbreaks, slow its spread and eradicate populations that arrive outside of the invaded range, aerial deployments of mating disruption tactics and pesticides are generally used. However, in some cases, such as in heavily urbanized areas or other landscapes where aerial deployments are not feasible or permitted, ground applications are required. Ground applications tend to be labour-intensive to ensure adequate coverage. To better inform optimal deployment of ground applications of mating disruption, we measured the effectiveness of a pheromone formulation designed for ground application, SPLAT® GM, in forested areas of Virginia from 2011 to 2014 using different dosages and number of point applications. We observed that SPLAT® GM applied to the tree trunks at the dosages of 49.4 and 123.6 g AI/ha in 11 × 11 systematic grids (i.e., every 11 m) reduced male trap catch by >90% relative to untreated control plots, which based on previous studies corresponds to >95% reduction in gypsy moth mating success. Our observations suggest that ground applications of gypsy moth mating disruption can be a successful management tool when circumstances require it.

KEYWORDS

insect pest management, invasive species, *Lymantria dispar*, pheromone treatment, SPLAT

1 | INTRODUCTION

The gypsy moth, *Lymantria dispar* (L.), was introduced into North America in 1869 where it undergoes periodic outbreaks, continues to expand its range along a leading population front and is a target of eradication efforts when introduced outside of its current range (Tobin, Bai, Eggen, & Leonard, 2012). The larvae feed on the leaves of over 300 different species of trees, such as aspen, larch, oak and willow, as well as a number of fruit and nut crops such as apple, apricot, blueberry, filbert, pear, pistachio and plum (Liebhold et al., 1995; Miller, Hanson, & Dowell, 1987). In addition to the direct costs of management, gypsy moth outbreaks in urbanized and suburbanized areas can lead to reductions in residential property values as a result

of defoliation, increased home cooling costs, damage to public green spaces, general nuisance and allergic reaction in humans (Bigsby, Ambrose, Tobin, & Sills, 2014; Dreistadt & Dahlsten, 1989; Wirtz, 1984). These landscapes can also pose additional management challenges, relative to contiguous forested areas, due to increased human interactions (Sawyer & Casagrande, 1983).

Under current gypsy moth management programmes, available treatment tactics include chemical pesticides (e.g., insect growth regulator tebufenozide), biological pesticides (e.g., *Bacillus thuringiensis kurstaki* and the gypsy moth nucleopolyhedrosis virus), mass-trapping and mating disruption (U.S. Department of Agriculture, 2012). Of these tactics, mating disruption tends to be the least expensive option, the most effective at low-to-medium population densities

and has the least amount of non-target effects as even the target insect (gypsy moth) is not killed (Onufrieva, Hickman, Leonard, & Tobin, 2019; Sharov, Leonard, Liebhold, & Clemens, 2002; Thorpe, Reardon, Tcheslavskaja, Leonard, & Mastro, 2006; Thorpe et al., 2007; Tobin, 2008). Mating disruption is a technique in which synthetic pheromone is deployed to disrupt mating communication in insect populations (Cardé & Minks, 1995). It is the dominant tactic used in efforts to slow the spread of the gypsy moth under the Slow the Spread Program (STS) (Tobin, Sharov, & Thorpe, 2007), which is a U.S. state and federal management programme (Sharov, Leonard, Liebhold, Roberts, & Dickerson, 2002).

In the overwhelming majority of mating disruption treatments, synthetic pheromone is applied aerially to treatment blocks in non-overlapping swaths, similar to conventional pesticides (Tcheslavskaja et al., 2005; U.S. Department of Agriculture, 2012). This method of application works well for operational treatments in large treatment areas such as contiguous forests or natural areas (Thorpe et al., 2006). However, aerial applications have several limitations. For example, aerial applications are often prohibited near ecologically sensitive areas, sensitive military installations and bodies of water and, thus, may require buffer zones around the target area to prevent drift and contamination (U.S. Environmental Protection Agency, 1999). For many target areas, these requirements can make it challenging or infeasible to aerially apply mating disruption. Moreover, aerial treatments are not always effective in small or fragmented areas, perhaps due to difficulty in ensuring adequate pheromone coverage in small treatment blocks (Onufrieva et al., 2019). Lastly, in areas populated by humans, there can be intense public resistance to aerial deployments of any insect pest management strategy (Czerwinski & Isman, 1986; Philp, 2012).

In lieu of aerial applications, ground-based treatments are often the only feasible method of deploying mating disruption tactics. One formulation available for ground application against gypsy moth is Hercon Luretape® (Kolodny-Hirsch, Webb, Olsen, & Venables, 1990), which, though effective, is not biodegradable and needs to be

removed at the end of the flight season. Another tactic used in gypsy moth management programmes is SPLAT® GM (ISCA Technologies), which is biodegradable and thus does not need to be removed after application, and has been shown to be effective against gypsy moth (Onufrieva et al., 2010). SPLAT® is a controlled-release technology currently used to formulate a wide range of semiochemicals for insect pest control (Mafra-Neto et al., 2013). Previous studies demonstrated its efficacy when used in ground-based applications against other pests (Soopaya et al., 2015). Although this tactic has already been used in the past to manage gypsy moth (Phillips et al., 2011), no prior research has addressed the optimal deployment of mating disruptant (i.e., spacing between applications) and the dose at which treatments would successfully reduce gypsy moth mating. To optimize the deployment of ground applications of mating disruption against gypsy moth, we measured the effectiveness of SPLAT® GM at different spatial deployments and dosages over four years and quantified its effectiveness to disrupt gypsy moth mating when applied manually to individual trees.

2 | MATERIALS AND METHODS

2.1 | Study area

The study was conducted in 2011–2014 in the Goshen Wildlife Management Area (GWMA) located in Rockbridge and Augusta Counties, Virginia, USA. This area has been infested by gypsy moth for >20 years; however, our study area had never experienced detectable levels of defoliation. The study area consisted of generally closed canopies that were dominated by *Quercus* spp. (oak), with overstorey trees reaching heights ~25 m.

In all years, each study plot had a male moth release point established at its centre and surrounded by four pheromone-baited traps placed 25 m to the N, S, W and E of the release point to prevent trap interference (Elkinton & Cardé, 1988; Figure 1a,b). Treatment efficacy was assessed based on capture rates of male moths. The same

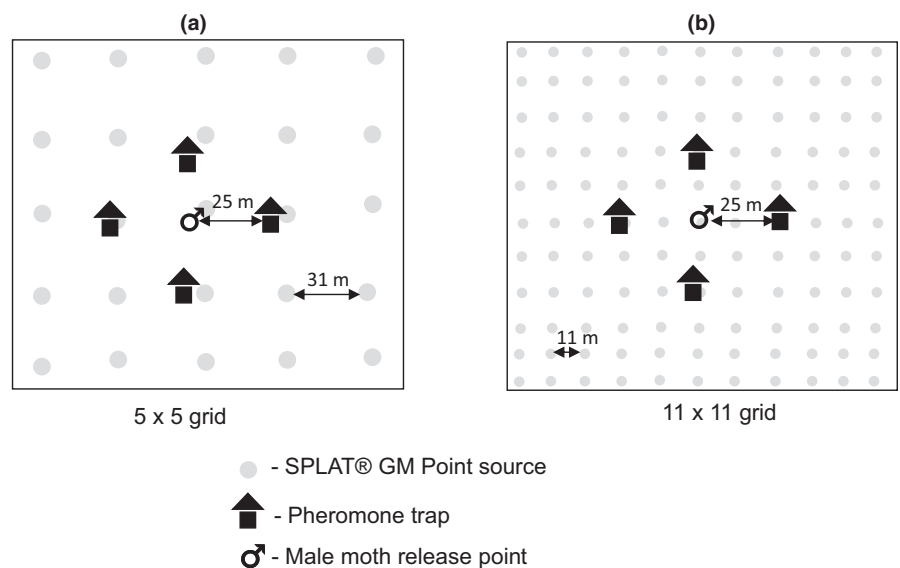


FIGURE 1 Treated experimental plot layouts in 2012 and 2013

number of laboratory-reared and marked male moths (30–200) was released at each release point each week. The numbers of released males used for each release were similar to the ones used to evaluate efficacies of aerial applications of SPLAT® GM (Onufrieva et al., 2010). These artificially created populations were shown to appropriately model background populations managed by the STS program (Onufrieva et al., 2019; Onufrieva & Onufriev, 2018), in which male gypsy moth catches in pheromone-baited traps could be used as a single measure of the effectiveness of pheromone treatments against gypsy moth populations (Tchesslavskaja et al., 2005).

Male gypsy moths were obtained as pupae from the USDA Animal and Plant Health Inspection Service, Pest Survey Detection and Exclusion Laboratory, OTIS Air National Guard Base, MA, USA. A red dye was added to the larval diet, which remains visible in the bodies of adults, to help distinguish laboratory-reared males from feral individuals. Pupae were kept in paper cups with polyethylene food wrap lids until emergence. Traps were checked at the time of release, and all captured male moths were removed and checked for the red dye present in the body to separate laboratory and feral males. Only catches of laboratory-reared males were used for the analysis to ensure equal population densities among experimental plots.

2.2 | Pilot study, 2011

We conducted a preliminary study in 2011 to inform an experimental design in subsequent years. We randomly assigned four plots 63 m × 63 m (3,969 m² in area) one of the following four treatments: (a) untreated control; (b) an overall dosage of 6.4 g AI/ha when applied at 22 point locations in the plot (5 × 5 grid, ~31 m between points); (c) an overall dosage of 6.4 g AI/ha when applied at 62 point locations in the plot (8 × 8 grid, ~16 m between points); and (d) an overall dosage of 6.4 g AI/ha when applied at 121 point locations in the plot (11 × 11 grid, ~11 m between points). Five releases were made between 4 August and 26 August 2011. The results indicated that male trap catch was not significantly reduced by any of the treatments compared with untreated control plots; however, trap catch was lowest when SPLAT® was applied in an 11 × 11 grid. Based on these results, we conducted subsequent studies using either a 5 × 5 grid or an 11 × 11 grid.

2.3 | Subsequent studies, 2012–2014

Based upon this preliminary study, we modified our study to include different doses when using a 5 × 5 or an 11 × 11 grid deployment pattern of SPLAT®. In 2012, a randomized block design was used with two blocks each consisting of 3 plots grouped according to proximity. Within each block, one plot was left untreated and used as a control; the rest of the plots were treated with 16 g AI/ha SPLAT® GM applied in a 5 × 5 or an 11 × 11 grid (Figure 1). Therefore, each treatment was replicated twice. Releases were made on weeks 1, 2, 4, 8, 9, 10 and 11 after the treatment application, 1–2 releases/week, totalling 11 releases in each plot.

In 2013, the same experimental design was used. Experimental plots were located over 1 km away from the plots used in 2012 to prevent possible treatment interference (Sharov, Thorpe, & Tchesslavskaja, 2002). In each of the 2 blocks, 1 plot was left untreated and used as control, 1 plot was treated with SPLAT® GM at 49.4 g AI/ha applied to an 11 × 11 grid, and 1 plot was treated with SPLAT® GM at 123.6 g AI/ha applied to an 11 × 11 grid (Figure 1b). We released male moths for once a week for 15 weeks and twice on week 6, for a total of 16 releases in each plot.

In 2014, the experimental plots treated in 2013 were monitored to evaluate gypsy moth mating disruption one year after the treatment application given that past research demonstrated persistence of mating disruption tactics one year after treatment (Onufrieva et al., 2013; Thorpe et al., 2007). Male moths were released once a week for 10 weeks.

2.4 | Treatment applications

SPLAT® GM designed for ground application comes in plastic tubes that fit a caulking gun. We used a calibrated caulking gun for SPLAT® products provided by ISCA Technologies, Inc.

In 2011, the tested pheromone dosage was small; thus, we weighed the amount of SPLAT® GM for each row using a laboratory scale and applied it using plastic knives to achieve an overall dosage of 16 g AI/ha in each of the treated plots.

In 2012 and 2013, we tested a higher dosage of pheromone, which required larger volume of SPLAT® GM. We calculated the amount of SPLAT® GM needed to achieve the overall dosage and weighed it. We also weighed the amount of SPLAT® GM needed to be applied to an individual tree and used the amount as a visual reference to apply SPLAT® GM to tree trunks using a caulking gun to achieve a required overall dosage. Therefore, there was some variability in dollop sizes; however, the plots were treated with correct overall dosages.

We applied SPLAT® GM at chest height to trees of various heights and sizes in regular grids. Several plots were treated in each day, so time of day and temperature was not consistent. We

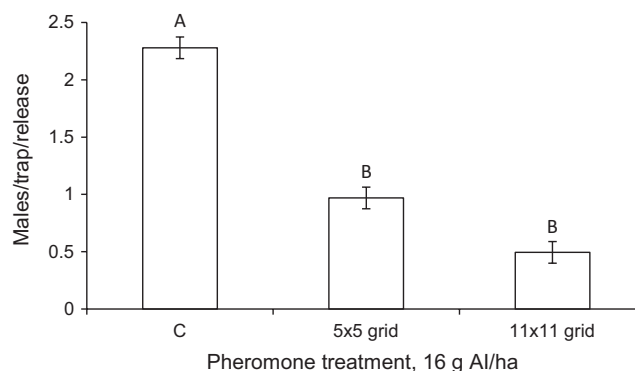
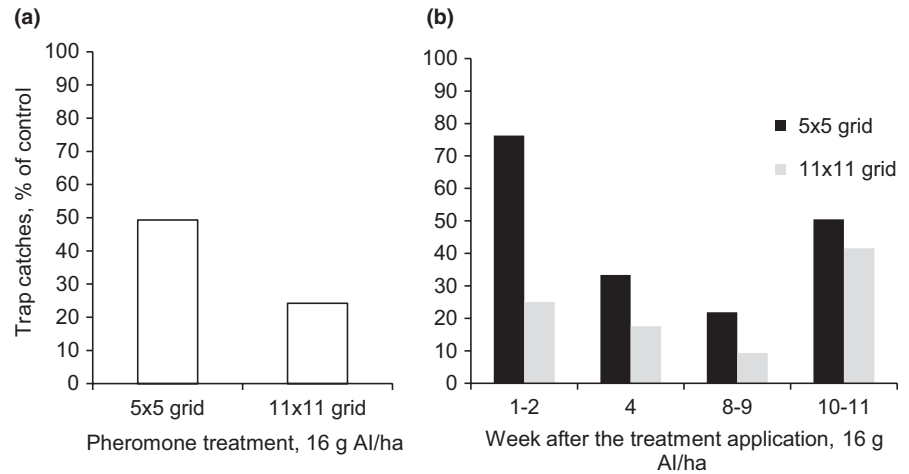


FIGURE 2 Male moth catches (\pm SE) in pheromone-baited traps in plots treated with 16 g AI/ha applied every 31 m (5 × 5 grid), 11 m (11 × 11 grid) and untreated controls (C), 2012. Bars with the same letters are not significantly different, Tukey's HSD ($\alpha < .05$)

FIGURE 3 Overall (a) and weekly (b) male moth catches in pheromone-baited traps in treated plots compared with untreated control plots, 2012



followed the manufacturer's recommendation to apply SPLAT in ambient temperatures of 55°F to 95°F and >3 hr before any expected rainfall.

2.5 | Statistical analysis

Prior to statistical analyses, we transformed moth counts using $\ln(y + 1)$ to satisfy the assumption of normality. All results were back-transformed for easier interpretation (McDonald, 2014). We tested the main effects of treatment (number of point sources in 2012, and dosage in 2013–2014) and week, and their interaction, using analysis of variance (ANOVA). A block effect was also added as a random effect. We used Tukey's adjustment for multiple comparisons of mean values in post hoc tests (JMP® Pro2016, 2016). In treated plots, we also calculated trap catches as a percentage of untreated control plots at several time intervals after the treatment application to determine the persistence of trap catch reduction.

3 | RESULTS

In 2012, trap catches in pheromone-baited traps were significantly reduced in all treated plots compared with untreated control plots ($F = 14.18, df = 2, 62, p < .01$, Figure 2). The dosage of 16 g AI/ha reduced trap catches by 50% and 70% compared with untreated control plots when applied to a 5 × 5 and an 11 × 11 grids, respectively (Figure 3a). The trap catches in all treated plots were higher during the first 2 weeks following the treatment applications compared with weeks 3–9, but the differences were not statistically significant (Figure 3b).

In 2013, the overall trap catches were significantly reduced by both treatments compared with untreated control plots ($F = 55.38, df = 2, 56, p < .01$, Figure 4) and both treatments reduced overall trap catches by >90%. Although significantly reduced by both treatments during all of the time intervals, trap catches were highest during the first week after the pheromone application (Figure 5).

In 2014, one year after the application, both dosages of ground applied SPLAT® GM continued to significantly reduce trap catches compared with untreated control plots ($F = 16.62, df = 2, 61, p < .01$). In plots treated with SPLAT® GM at 49.4 g AI/ha, trap catches were reduced by 61%, while in plots treated with 123.6 g of AI/ha trap catches were reduced by 80% compared with untreated control plots. However, the differences between the two treatments were not significant (Figure 6).

4 | DISCUSSION

In this study, we evaluated various dosages and methods of application of SPLAT® GM designed for ground application. To achieve successful mating disruption in management programmes, the synthetic pheromone must be present in the air in sufficient quantities for the entire period of sexual activity of moths (Cardé, Doane, Granett, & Roelofs, 1975; Howse, Stevens, & Jones, 1998). In the STS program, applications of pheromone for mating disruption are required to reduce trap catches by at least 90% and to be effective for a period of at least 8 weeks to ensure covering the entire period of gypsy moth flight (up to 6 weeks, Tobin, Klein, & Leonard, 2009) and to provide a

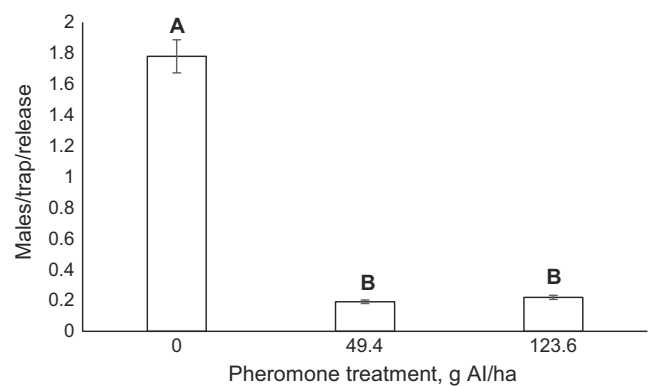


FIGURE 4 Male moth catches (±SE) in pheromone-baited traps, 2013. Bars with the same letters are not significantly different, Tukey's HSD ($\alpha < .05$)

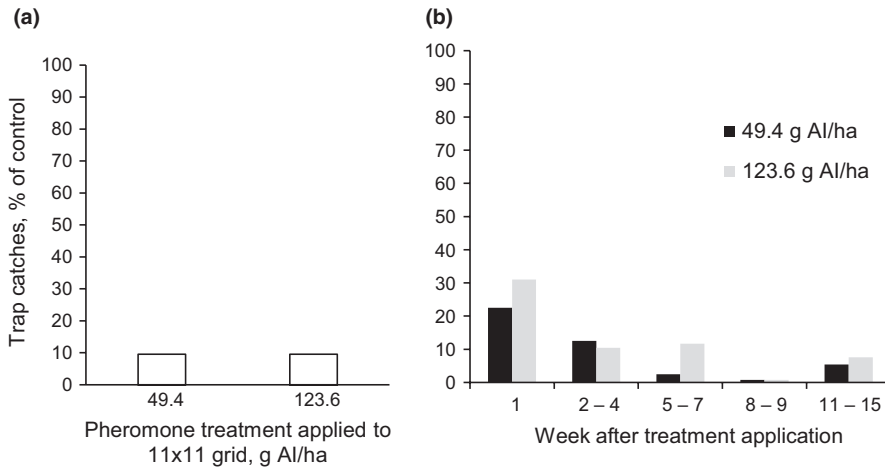


FIGURE 5 Overall (a) and weekly (b) male moth catches in pheromone-baited traps in treated plots compared with untreated control plots, 2013

safety margin for uncertainties associated with the logistics of treatment planning and with gypsy moth phenology (Thorpe et al., 2006).

In the plots treated with 16 g AI/acre dosage, trap catches were not reduced enough for the treatment to be considered effective in the STS program. Even though there were no significant differences between trap catches in plots with a 5 × 5 and an 11 × 11 grids, based on these results and results of preliminary study, an 11 × 11 grid is recommended for ground applications of SPLAT® GM. Using a finer grid provides a more uniform coverage of the area with smaller dollops of SPLAT® GM, which increases the rate of pheromone release and improves mating disruption in treated plots.

The dosages of 49.4 and 123.6 g AI/ha applied to an 11 × 11 grid reduced overall trap catches by >90%; however, the efficacy during the first week after the pheromone application did not meet the 90% trap catch reduction requirement of the STS program. It appears that in both years, trap catches in treated plots were higher during the first week after the application, which means that it could require one week for a sufficient amount of pheromone to be emitted from ground-based treatments. Consequently, SPLAT® GM designed for ground application should be applied at least 1 week prior to estimated beginning of gypsy moth flight, which can be predicted using a phenology model (Régnière & Sharov, 1998). Applications that are

a week prior to flight should not present a problem as SPLAT® GM applied at 49.4 and 123.6 g AI/ha sufficiently reduced trap catch for 9 weeks (Figure 5), which exceeds the approximate 6-week flight period of adult males. Trap catches in plots treated with 49.4 and 123.6 g AI/ha were still significantly reduced 1 year after the application, which means that SPLAT® GM could be applied once every two years. However, a partial application during the second year may increase efficacy of the mating disruption treatment. Overall, for ground-based mating disruption treatments of SPLAT® GM, a dosage of at least 49.4 g AI/ha in an 11 × 11 grid (every 11 m, 121 release points/ha) should be applied at least one week prior to the beginning of gypsy moth flight. This application method is similar to the one recommended for Hercon Luretape®, which is applied at 50 g AI/ha to 100 release points/ha (Kolodny-Hirsch et al., 1990). However, because SPLAT® GM does not require removal after the flight season it is likely less expensive and time-consuming to apply relative to Hercon Luretape®.

These results are also in agreement with the results of a similar study conducted to evaluate efficacy of ground applied SPLAT® in mating disruption of light brown apple moth *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) (Sooaya et al., 2015): SPLAT® was shown to be effective when applied to 500 release points/ha at a rate of 40 g AI/ha. In the case of light brown apple moth, ground and aerial application rates are the same (Brockhoff et al., 2012), while in gypsy moth, full efficacy of aerial application of SPLAT® GM is reached at a much lower dosage (15 g AI/ha) compared with the ground treatment. This research highlights the effectiveness of ground-based treatments of a mating disruption tactic against the gypsy moth when applied at the appropriate dosage and spacing. Although this work was specific to gypsy moth, mating disruption is a tactic used against several species, especially moth species (Casado, Cave, & Welter, 2014; Louis & Schirra, 2001; Pfeiffer, Kaakeh, Killian, Lachance, & Kirsch, 1993; Stelinski, Gut, & Miller, 2013; Stelinski, Lapointe, & Meyer, 2010; Stelinski, Miller, Ledebuhr, Siegert, & Gut, 2007; Witzgall, Kirsch, & Cork, 2010). This study also contributes to a framework for development of ground-based mating disruption treatments. Generally, ground-based treatment application is a useful tool in

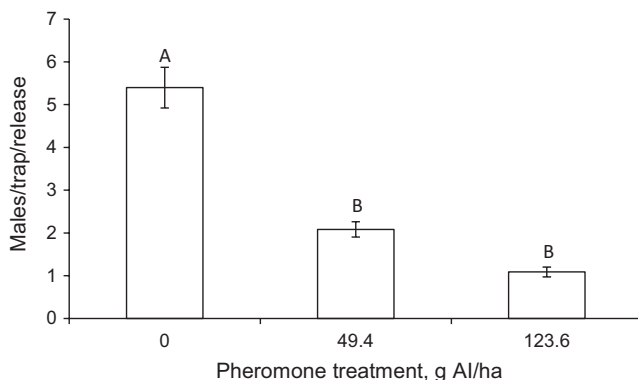


FIGURE 6 Male moth catches (\pm SE) in pheromone-baited traps in plots treated with ground applied SPLAT® GM at 49.4 and 123.6 g AI/ha one year prior to monitoring, 2014. Bars with the same letters are not significantly different, Tukey's HSD ($\alpha < .05$)

pest management as it may be the only option in environmentally and socially sensitive areas.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

KSO, ADH, DSL, and PCT conceived research. KSO and ADH conducted experiments. KSO analysed data and conducted statistical analyses. KSO and PCT wrote the manuscript. KSO and DSL secured funding. All authors read and approved the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in VTechData at <https://doi.org/10.7294/nmjg-pq40>.

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