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Integrated control *Bactrocera dorsalis* using sterile insect technique and complementary methods in Trok Nong subdistrict, Thailand

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Abstract

As part of an area-wide integrated approach to fruit fly control, the sterile insect technique (SIT) was implemented in Trok Nong Subdistrict, Chanthaburi Province, Thailand. The project aimed to establish an area of low pest prevalence of fruit flies (FF-ALPP) under certification by the National Plant Protection Organization (NPPO), and to produce fruits that meet safety standards for consumption and export in accordance with the International Standard for Phytosanitary Measures (ISPM). The project includes a site suitability study, delimitation of core and buffer areas, and training programs to raise awareness among growers. SIT, using a genetic sexing strain of *Bactrocera dorsalis* (Hendel), was conducted alongside male annihilation technique (MAT) and host sanitation. The average number of flies captured per trap per day (FTD) in the core area was significantly lower than in the neighboring area in both 2022 and 2023. Moreover, no economic fruits were damaged in the core area during 2023.

Keywords: Sterile insect technique, Area of low pest prevalence, *Bactrocera dorsalis* (Hendel), Fruit fly management, Irradiation, Genetic sexing strain, Area-wide Integrated Pest Management

1. Introduction

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) originated in Asia and has since spread globally [1]. This species is a significant economic pest, attacking a broad range of fruits and vegetables in tropical and subtropical regions. It is ecologically adapted to these climates [2]. In Thailand, a tropical country, fruit flies are a major economic concern. The larvae feed inside fruits, causing yield losses and quality degradation, which lead to quarantine restrictions from importing countries. For example, nations such as the United States and Australia, only import fruits and vegetables from certified pest-free or low-pest-prevalence areas or they require the products to undergo post-harvest treatment. These treatments are costly and reduce farmers' income [3]. Managing *B. dorsalis* requires an integrated approach, as no single method is sufficient. A combination of mechanical, cultural, behavioral, chemical, and biological control methods, along with host plant resistance, is necessary [4].

The sterile insect technique (SIT) is an eco-friendly biological control method involving the area-wide release of sterile insects to suppress pest populations over time [5]. SIT operation requires mass rearing, sterilization, transport, and release of insects to induce sterility within wild pest populations. The first SIT program established in the 1970 stopped the invasion of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) into southern Mexico [6]. However, the simultaneous release of sterile males and females can incur high costs and may result in crop damage from oviposition by sterile females. Research has shown that efficiency and cost-effectiveness are significantly improved when only sterile males are released [7,8]. Genetic sexing strains (GSS) have been developed for several species, with success shown in *Ceratitis capitata* and *Anastrepha ludens*, where the sexing system has been improved for large-scale and prolonged application [9]. The construction of GSS requires the

availability of recessive morphological or conditional lethal mutations and the linkage of wild-type alleles of the marker genes to the male determining region. This is typically achieved using radiation-induced reciprocal translocation between the Y chromosome and the autosome carrying the wild-type alleles of the selectable markers, T (Y-A), enabling early-stage physical or mechanical sex separation [10]. The first GSS was developed for *Ceratitidis capitata* (Mediterranean fruit fly or medfly) using the pupal color as a selectable marker. This marker is determined by the white pupae (*wp*) gene. Heterozygous males emerge from brown pupae, while homozygous females emerge from white pupae [11].

Trok Nong Subdistrict, located in Khlung District, Chanthaburi Province, eastern Thailand, is a key region for economic fruit production, particularly mangosteen for export. The region produces an annual average of 5,000-6,000 tons, valued at approximately US\$5.53 million [12]. To address the challenge of fruit fly infestation, the Thailand Institute of Nuclear Technology (TINT) has collaborated with the Department of Agricultural Extension (DOAE) and the Trok Nong Subdistrict Administrative Organization (SAO). Together, they implemented an integrated pest management strategy that combines the sterile insect technique (SIT) with the male annihilation technique (MAT), orchard sanitation, destruction of infested fruits and mass trapping methods. These integrated methods have been implemented in the target area since 2009 [13]. In Thailand, GSS of *B. dorsalis* was developed [14]. In 2022, the SIT program began with the release of sterile GSS fruit flies to establish an area of low pest prevalence (FF-ALPP). This initiative has significantly improved fruit yield and quality, enhancing the region's export potential.

2. Materials and methods

2.1 Ecological Study

Trok Nong Subdistrict, located at 12°27.1' N and 102°13.17' E, encompasses a 2,300-hectare area dedicated to plant production. Key fruits cultivated include mangosteen (*Garcinia mangostana* Linn.), durian (*Durio zibethinus* Murray), rambutan (*Nephelium lappaceum* Linn.), longkong (*Lansium domesticum* Corr.), and sala or snake fruit (*Salacca zalacca* (Gaertn.)). Additional crops include rubber (*Hevea brasiliensis* Muell. Arg), palm trees (*Elaeis guineensis* L.), and rice (*Oryza sativa* L.). Without effective control measures, fruits such as rose apple (*Eugenia javanica* Lamk.), guava (*Psidium guajava* Linn.), dragon fruit (*Hylocereus undatus* (Haw.)), banana (*Musa* spp.), and sapodilla (*Manikara zapota* L.) face nearly 100% infestation. Moderately affected fruits include mango (*Mangifera indica* L.), santol (*Sandoricum koetjape* Burn. f. Merr.), rose apple (*Syzygium jambos* (L.) Alston), kumquat (*Citrus japonica* Thunb.), papaya (*Carica papaya* L.), star fruit (*Averrhoa carambola* L.), jujube (*Ziziphus jujuba* Mill.), and marian plum (*Bouea macrophylla* Griff). Fruits with lower infestation rates, where larvae can still survive, include betel nut (*Areca catechu* L.), longkong (*L. domesticum* Corr.), patana oak (*Careya arborea* Roxb.), mangosteen (*G. mangostana* Linn.) [15,16,17], fig (*Ficus carica* L.), cherry (*Malpighia glabra* Linn.), satol (*S. koetjape* Burm.f. Merr.), and jambul (*Syzygium cumini* (L.)).

Chanthaburi Province experiences a tropical monsoon climate with high temperatures and substantial rainfall throughout most of the year. During the rainy season from May to October the temperatures typically vary between 25°C to 32°C. In 2022, the total precipitation was 2,873 mm. During the hot season (March to May), temperatures range from an average high of 35.3°C to a low of 17.1°C, with average humidity reaching 97% [18].

The predominant fruit fly species in the study area include *B. dorsalis*, *B. correcta* (Bezzi), *B. (Zeugodacus) tau* (Walker), *B. umbrosa* (Fabricius), and *B. (Bactrocera) tuberculata* (Bezzi). Population dynamics studies were conducted from May to June 2009 using the marking and recapture method. It was found that the highest fruit fly population in the area was approximately 665,408 flies, averaging 41,558 flies per km². The flies were predominantly clustered in specific habitats and border zones [19].

The target area was delineated into core and buffer zones using geographic information system (GIS) and global positioning system (GPS) via a mobile application (Handy GPS). A fruit fly protection barrier was established with a width of 1 km, covering a total area of 26.2 km² (calculated by the mobile application). This included a core area of 8.62 km² and a buffer zone of 17.58 km² (Figure 1).

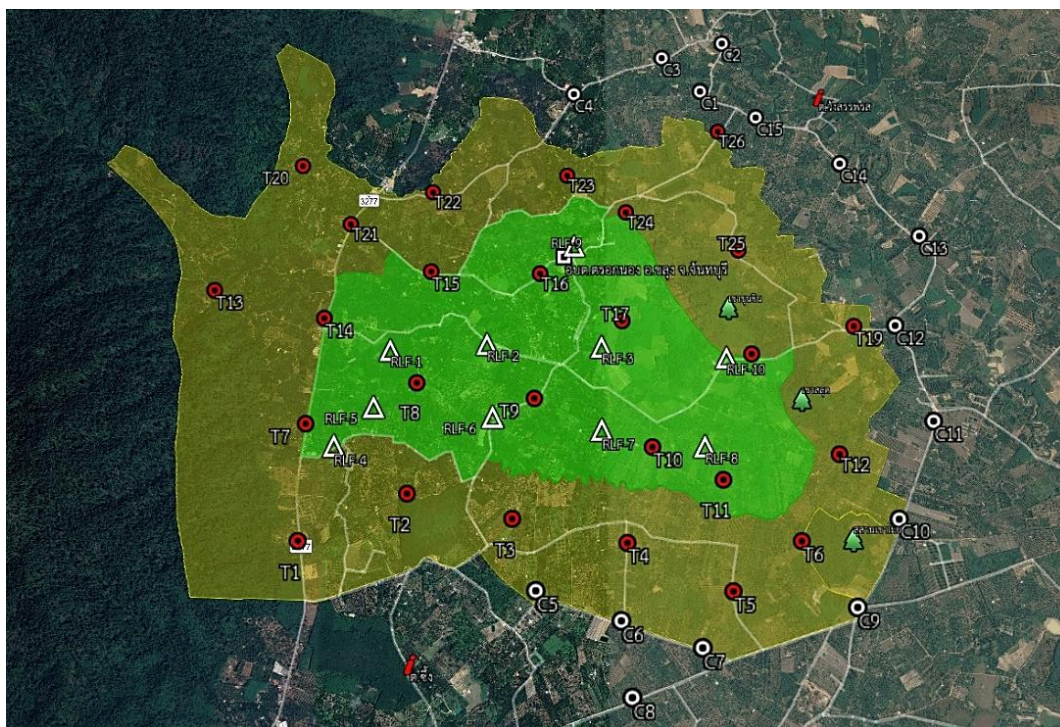


Figure 1 The boundaries of the core (green shade) and the buffer (yellow shade) zones are shown in Google Earth. Monitor traps (marked as circles) were placed to evaluate fruit fly populations at the target and neighboring areas. Sterile flies were released at the locations marked as triangles.

2.2 Public Relation

At knowledge dissemination workshop was conducted to train community leaders and growers, raising awareness about the project and objectives. Additionally, the research team held a meeting with the working group from Trok Nong Subdistrict Administrative Organization to discuss the operation plan.

2.3 Sterile Insect Technique

The GSS of *B. dorsalis* adults were maintained in rectangular mesh cages measuring 75 cm (L) × 75 cm (W) × 95 cm (H), constructed of stainless steel. Each cage contained seven egg collection tubes with oviposition holes, supporting a population of approximately 68,000 flies per cage with a 1:1 female to male ratio. The flies were fed an artificial diet that consisted of yeast hydrolysate and sugar in a 1:3 weight-to-weight (w/w) ratio. Two PVC pipes contained a moist absorbent material provided water for the flies, while guava juice was introduced into the tubes to stimulate oviposition and prevent egg desiccation. Eggs were seeded onto a larval diet (7 mL eggs / 7 kg diet) in plastic trays measuring 59 cm (L) × 43 cm (W) × 10 cm (H). The diet consisted of wheat bran, dried yeast, sugar, sodium benzoate, methyl-p-hydroxybenzoate, citric acid, and water, as modified from Tanaka et al. [20].

Third-instar larvae, upon maturing, ceased feeding and self-popped by jumping out of the diet into a stainless-steel tray containing water. Both adults and larvae were reared under room-controlled conditions maintained at $25 \pm 2^\circ\text{C}$, $80 \pm 10\%$ relative humidity (RH). The larvae pupated on a thin layer of wood sawdust, and six-day-old pupae were separated from the sawdust using a mechanical sifter (JCS Technic line Co., Ltd.) operating at 6 rpm.

Male pupae (brown) were separated from female pupae (white) using a color sorting machine (Gladiator GD1, Belt, and Bearings Co., Ltd.). Male pupae were then packed in plastic bags (500 cc per bag) and irradiated with a dose of 90 gray (Gy) using an X-ray irradiator (RS 2400V, Rad Source Technologies, Inc.) at a dose rate of 18 Gy/min or cobalt-60 source installed in a gamma ray irradiator (Gamma chamber 5000, BRIT, India) at a dose rate of 25 Gy/min (Figure 2). Following irradiation, the pupae were transported to Trok Nong Subdistrict Administrative Organization. Pupae were kept in release containers (10 × 43 × 59 cm) at a controlled temperature of $25 \pm 2^\circ\text{C}$. Adult flies were provided with a diet and water upon emergence. Weekly releases of 2.5-5 million sterile flies, five days old, were conducted at designated release points in the core area from January to September each year [21,22].



Figure 2 The mass rearing facility of genetic sexing strain (GSS) sterile fruit flies at TINT, Ongkharak, Nakhon Nayok, Thailand. (A) Rearing adults and eggs collection; (B) The larval diet preparation with artificial food; (C) Larvae rearing; (D) Pupae collection; (E) Pupae color sorting; (F) Genetic sexing strain (GSS) Male (brown pupae) and Female (white pupae); (G) Pupae irradiation at 90 Gy.

2.4 Monitoring, Control, and Evaluation

Monitoring systems were established to survey the fruit fly population in the targeted area, following the standard for maintaining a low-level fruit fly population [23]. Steiner traps were deployed in the core area (13 traps) and the buffer area (16 traps) at a density of one trap per square kilometer. To evaluate the effectiveness of the sterile fly release, the traps were also deployed in neighboring areas: Wang Sapparot Subdistrict (8 traps) and Sue Subdistrict (7 traps) [24-27]. The number of flies caught was recorded weekly throughout the year, and the species of fruit flies were identified.

In addition to the sterile insect technique (SIT), fruit fly control in the targeted area was implemented through integrated methods, including sanitation and male annihilation technique (MAT). Sanitation involved removing and destroying unwanted host plants, as well as collecting ripe and fallen fruits for bio-fertilizer production during the fruiting season. For MAT, approximately 11,376 plywood blocks (5 cm × 5 cm × 1.6 cm) each containing a mixture of 75% methyl eugenol and 25% malathion were deployed in the buffer area throughout the year. These blocks aimed to suppress or eliminate the male population of wild fruit flies and intercept the flies that might migrate from neighboring areas, with a deployment density of 6 traps per hectare [28,29]. However, as the sterile flies were not released after the harvest season (October to December), farmers installed MAT traps in the core area to reduce the number of wild male fruit flies. These traps were replaced monthly to maintain boundaries and minimize the risk of wild fruit flies entering the targeted area.

Fruit fly damage on the economic important fruits was assessed during the harvest season. Approximately 200 kg of mangosteen, longkong, and rambutan were collected and transported to the laboratory. The evaluation included analyzing the fruit fly population in the area and determining the percentage of fruit damage caused by fruit flies (Figure 3).



Figure 3 Area-wide management of fruit flies using sterile insect technique (SIT) combined with other methods in Trok Nong Subdistrict. (A) Public Relation; (B) Male Annihilation Technique (MAT); (C) Mass Trapping; (D) Orchard sanitation; (E) Sterile Insect Technique; (F) Fruits sampling; (G) Monitoring and Evaluation.

3. Results and discussion

The number of fruit flies (*Bactrocera dorsalis*) captured in monitoring traps within the targeted area was observed weekly and used to calculate the average number of fruit flies per trap per day (FTD) captured. From January to December 2022, the average FTD values in the core, buffer, and neighboring areas were 0.62 ± 0.43 , 0.81 ± 0.74 , and 49.1 ± 73.7 FTD, respectively. For the same period in 2023, the corresponding FTD values were 0.69 ± 0.37 , 0.99 ± 1.41 , and 33.1 ± 30.3 . The monthly FTD data for the three areas is shown in Figure 4.

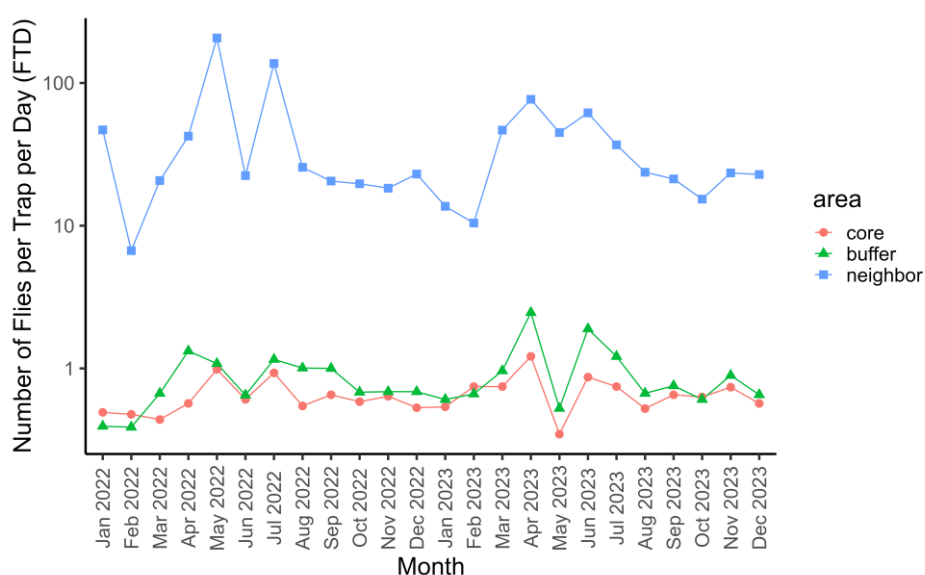


Figure 4 The average number of fruit flies per trap per day (FTD) captured in different areas, by month. Y-axis is shown on a logarithmic scale for clarity.

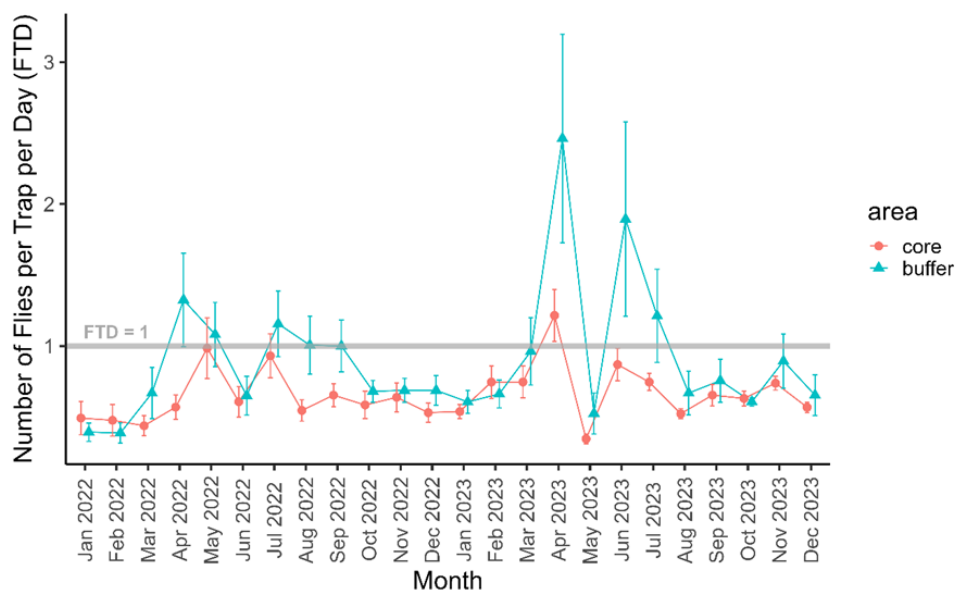


Figure 5 The average number of fruit flies per trap per day (FTD) captured in the core and buffer areas, by month. Error bars represent the standard errors of the mean by month. The data points were slightly offset horizontally for clarity.

In April 2023, the FTD in the core area reached 1.22, exceeding the specified threshold for an area of low pest prevalence (ALPP), which is 1 FTD (1.22) [30]. An emergency action plan was implemented to address this outbreak. MAT traps were deployed at a density of six traps per hectare within a 100-meter radius of the outbreak point to reduce the fruit fly population. An evaluation of fruit damage caused by fruit flies revealed that mangosteen and longkong were unaffected in both 2022 and 2023. In 2022, only 1% of rambutans were damaged and no damage to economic fruits was observed in 2023 within the target area. The yearly FTD distributions in each area are illustrated in Figure 6 and summarized in Table 1. The integrated methods implemented in this project achieved a significant reduction in fruit fly populations in the target area compared to the neighboring area, where no interventions were applied.

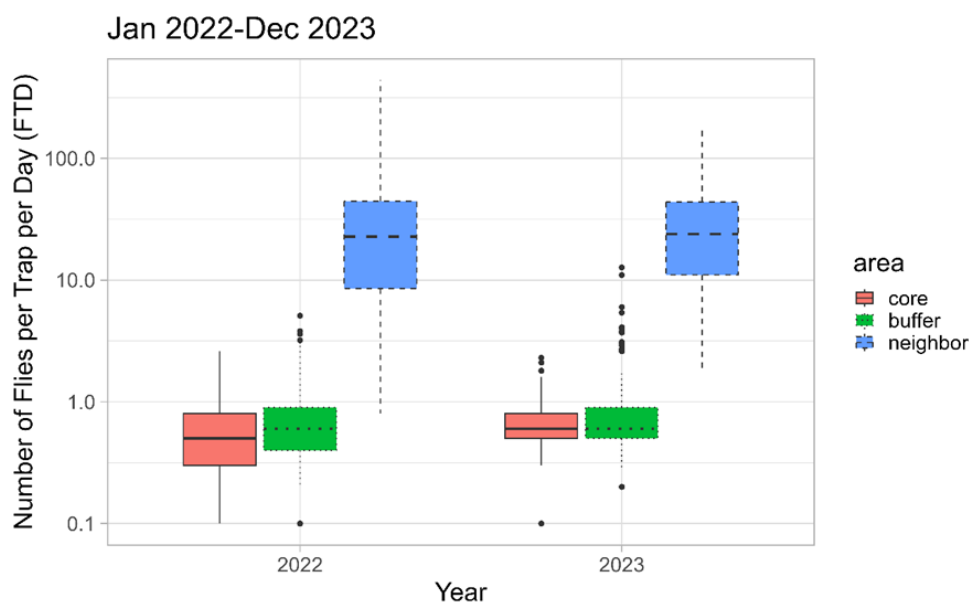


Figure 6 Distribution of fruit flies per trap per day captured in different areas, grouped by year. Y-axis is shown on a logarithmic scale for clarity.

Table 1 Summary of fruit flies per trap per day for each area during 2022-2023 (SD = standard deviation).

Year	Areas	mean FTD \pm SD
2022	core	0.62 \pm 0.43
	buffer	0.81 \pm 0.74
	neighbor	49.14 \pm 73.66
2023	core	0.69 \pm 0.37
	buffer	0.99 \pm 1.41
	neighbor	33.10 \pm 30.30

For an area to be certified by the National Plant Protection Organization (NPPO) as a low prevalence area, the average number of captured fruit flies must be lower than 1 FTD throughout the period of two years. This pilot project in Trok Nong demonstrates a successful model for fruit fly suppression or eradication. Consequently, fruits grown in this area are now eligible for export to various countries. Similar area-wide integrated pest management approaches, which sometimes include the sterile insect technique (SIT), have been used to establish low fruit fly prevalence and fly-free zones in Central America and Panama. These initiatives have enabled the export of fresh peppers and red tomatoes from Costa Rica, El Salvador, Guatemala, and Nicaragua without the need for quarantine treatments [31]. Under new phytosanitary measures, an area of low pest prevalence can serve as an intermediate step between a pest-infested area and a pest-free zone. This approach permits the implementation of control measures to prevent the establishment of breeding populations while maintaining market access [23].

4. Conclusion

The integration of mass rearing and the continuous release of sterile *Bactrocera dorsalis*, effectively controlled the fruit fly populations in Trok Nong Subdistrict, Thailand. The results demonstrated a significant reduction in fruit fly densities. In 2022, the fly per trap per day (FTD) in the core area was 0.62 ± 0.43 , compared to 49.14 ± 73.66 FTD in the neighboring area. Similarly, in 2023, the core area maintained low fruit fly densities at 0.69 ± 0.37 FTD, while in the neighboring area recorded 33.10 ± 30.30 FTD. Fruit damage assessments further confirmed the efficacy of the intervention, with only 1% rambutans damaged by fruit flies in 2022, and no economic fruit damage observed in the target area in 2023. These findings indicate that the integrated pest management methods, incorporating SIT, can effectively suppress fruit fly populations and protect economically important fruits. Furthermore, the approach holds potential for achieving certification as a Fruit Fly Area of Low Pest Prevalence (FF-ALPP) by the National Plant Protection Organization (NPPO) in the future.

5. Ethical approval

All procedures involving animals were approved by the Institutional Committee for the Care Use of Laboratory Animals of Thailand Institute of Nuclear Technology (Public Organization) (approval code AAP62-01).

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7. Conflicts of Interest

The authors declare no conflict of interest.

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