The Journal of Infectious Diseases

MAJOR ARTICLE

Novel Sterile Insect Technology Program Results in Suppression of a Field Mosquito Population and Subsequently to Reduced Incidence of Dengue

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Background. There is a steady rise in the global incidence of *Aedes*-borne arbovirus disease. It has become urgent to develop alternative solutions for mosquito vector control. We developed a new method of sterilization of male mosquitoes with the goal to suppress a local *Aedes aegypti* population and to prevent the spread of dengue.

Methods. Sterile male mosquitoes were produced from a locally acquired *Ae. aegypti* colony by using a treatment that includes double-stranded RNA and thiotepa. A field study was conducted with sterile mosquito releases being performed on a weekly basis in predefined areas. There were 2 intervention periods (INT1 and INT2), with treatment and control areas reversed between INT1 and INT2.

Results. During INT1, releases in the treated area resulted in up to 91.4% reduction of live progeny of field *Ae. aegypti* mosquitoes recorded over time, while the control neighborhoods (no releases of sterile male mosquitoes) remained highly infested. The successful implementations of the program during INT1 and INT2 were associated with 15.9-fold and 13.7-fold lower incidences of dengue in the treated area compared to the control areas, respectively.

Conclusions. Our data show the success of this new sterile insect technology-based program in preventing the spread of dengue.

Keywords. SIT; suppression; dengue; *Aedes aegypti*; dsRNA; thiotepa.

Mosquito-transmitted arboviruses are the cause of substantial human mortality and morbidity. Dengue is endemic in more than 100 countries in several continents, and around 500 000 people with severe disease require hospitalization every year [1, 2]. Despite the fact that dengue itself is rarely fatal, severe dengue is a potentially fatal complication [2].

Although the World Health Organization announced in 2017 its intention to reduce the global incidence of dengue by 75% within a decade [2], current data show that this goal is far from being achieved [3]. Hyperurbanization and climate change are driving expansion of the range of the primary mosquito vector of dengue and other arboviruses [4–6]. Consequently, the high morbidity and economic and resource burden on health services in endemic settings are substantial and increasing [1].

Because specific vaccines to arboviruses have been presenting limited efficacy and no treatments are available other than management [7], the main strategy to prevent the outbreak of dengue epidemics still remains the control of the main mosquito vector (Aedes *aegypti*) [8]. However, traditional methods of vector control (mechanical removal of potential breeding sites for mosquitoes and the use of insecticides [9]) have been insufficient to prevent disease outbreaks [3]. This has created an urgent need for alternative solutions.

One of the alternative solutions is the sterile insect technique (SIT), which is based on the massive and continuous release of sterile male mosquitoes that mate with the wild females. If releases are sustained, these females generate fewer and fewer viable offspring, resulting in the gradual reduction of the local mosquito population [10]. As a method of insect control, SIT has several fundamental advantages, such as species specificity [11] and the absence of evidence for the development of resistant mosquito populations, which is one of the main criticisms related to insecticides [9].

Several SIT-type vector control programs have already been shown to suppress mosquito populations in field studies [12–15]. These include the sterilization of mosquitoes by irradiation [10], the use of genetically modified mosquitoes carrying a dominant lethal gene [13], and incompatible insect technology, which utilizes mosquitoes infected with *Wolbachia* bacteria [12, 15–17]. None of these pilot programs have, to our knowledge,

Received 13 October 2020; editorial decision 20 January 2021; accepted 22 January 2021; published online January 28, 2021.

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The Journal of Infectious Diseases® 2021;224:1005–14

DOI: 10.1093/infdis/jiab049

attempted to directly assess the effect of mosquito population suppression on the incidence of dengue or other arbovirus [12–14].

Herein, we present a novel SIT method, which we call natural vector control (NVC). The sterilization method includes a first step where larvae are treated with double-stranded RNA (dsRNA) and subsequently pupae are exposed to thiotepa. DsRNA was successfully used in previous studies to sterilize various insect species, including *Ae. aegypti* [18–20], but none of them were done on a large scale. Herein, the gene *AAEL013723* is the target for gene-silencing of the dsRNA. This gene encodes a polypyrimidine tract binding protein (PTB), an RNA binding protein implicated in various aspects of cellular mRNA metabolism [21]. In *Drosophila*, the PTB gene encodes an abundant male germline-specific mRNA isoform whose expression correlates with male fertility [22]. Thiotepa is an alkylating agent that induces dominant lethal mutations in the sperm and ova of many species of insects [23]. Thiotepa treatment alone was successfully used in past SIT-based intervention programs in the 1960s and 1970s [24, 25]. However, these programs were discontinued because high concentrations and/or long exposure times of pupae that are needed to achieve full sterilization resulted in thiotepa residues in the released mosquitoes. This was deemed environmentally unacceptable [26–29]. Moreover, long exposure times hamper large-scale production. We developed a new protocol that enables the large-scale use of thiotepa to fully sterilize these insects without leaving residues of this chemical in the adults. Herein, we demonstrated the efficacy of this new approach in a field study and showed not only the successful suppression of an *Ae. aegypti* field population, but also provided direct demonstration that a SIT vector-control program by itself can substantially reduce the spread of dengue.

METHODS

NVC Sterile Male Mosquito Production

The *Ae. aegypti* colony was established from eggs collected in Jacarezinho (2017), using ovitraps [30]. All mosquitoes used in this study were reared based on the protocol of Rutledge et al [31], with adaptations (Supplementary Material).

To evaluate the PTB gene silencing, first instar larvae were soaked in a PTB-1 dsRNA (12 µg/mL) solution for 1 hour then reared until adulthood. The PTB gene expression was analyzed by real-time polymerase chain reaction (PCR). To optimize the production of sterile male mosquitoes, male pupae were treated with different concentrations of thiotepa solution (ranging from 0.1% to 0.6%) for different incubation times (from 30 minutes to 2 hours). The final production protocol used in the small-scale tests involved treating the larvae with a formulation containing 1 mg of PTB-1 dsRNA (from first to third instars), followed by treatment with a 10 µg/mL solution of PTB-2 dsRNA from third instar to the pupal stage. The 2 dsRNA sequences used are shown in the Supplementary Material. Each one targets different regions of the PTB gene and were selected for inducing greater gene silencing compared with other sequences tested. Finally, male pupae were mechanically separated from females (Larval-Pupal Separator, Model 5412, John W. Hock Company) and treated with 0.1% thiotepa solution overnight.

For the field trial, NVC male mosquitoes were produced essentially as described above, with modifications necessary to adapt the protocol for large-scale production, which included (1) a higher number of larvae treated per batch (250 000 larvae instead of 100); (2) reduction of PTB-2 dsRNA concentration (20 ng instead 10 µg per larvae); and (3) mode of dsRNA delivery during the first phase of NVC male mosquito production (soaking for 1 hour instead of feeding). Batches of 120 000 male pupae were treated in 0.6% thiotepa solution for 2.5 hours, then washed in 0.0025 N H_2SO_4 (pH 2–3) to inactivate any external thiotepa remnants (Supplementary Material).

A fertility bioassay was performed to assess the fertility status of NVC mosquitoes as described [32], with adaptations (Supplementary Material). The competitive capacity of NVC mosquitoes was tested in a semifield trial using a cage system exposed to environmental conditions (Supplementary Material).

Field Trial

Jacarezinho is a city in the northern region of Paraná state, southern Brazil. The study areas were chosen based on *Ae. aegypti* infestation rates and historical dengue epidemics. Control and treated areas were similar in size (around 80 hectares), as were the number of inhabitants and socioeconomic characteristics, based on demographic data provided by the local municipality. Two intervention periods (ie, massive releases of NVC sterile male mosquitoes) were conducted during the study period, the first (INT1) occurred from September 2018 to April 2019 and the second (INT2) occurred from September 2019 to January 2020 (Supplementary Material).

The releases of NVC mosquitoes were made on a weekly basis during INT1 and INT2. NVC male mosquitoes were packed in plastic containers (Supplementary Material) and manually released from a car driving along streets of the designated area. The number of NVC male mosquitoes released in each microregion was defined based on monitoring of eggs collected in the areas in the previous week.

Monitoring of *Ae. aegypti* abundance was performed in INT1 by the weekly installation of 100 ovitraps [33, 34] in houses or in the peridomiciliary area of the residences of both treated and control area [35]. Eggs from each ovitrap were counted and hatched for a 48-hour period. The larvae that hatched in this period were considered viable progeny.

Estimation of field population suppression for INT1 was performed as previously described in Gorman et al [36], with modifications. Weekly moving averages relative to the same period at each control area were calculated according to the equation $M = (T_a/C_a)/(T_b/C_b) - 1$, where M is the population change, T_a

is mean larvae per point in the treated area after release, C_a is mean larvae per point in the control area after release, T_b is mean larvae per point in the treated area before release, and C_b is mean larvae per point in the control area before release. This was done by comparing weekly data against baseline data obtained across the 3 weeks prior to the beginning of releases. The corresponding 95% confidence intervals (CIs) were calculated by a 10 000-loop bootstrap [36] for the entire period of releases and for each period of 7 weeks, to follow the effect along the project and considering the similar effect in coming weeks. The CIs were calculated using R version 3.5.2.

In addition to our own *Ae. aegypti* monitoring, official mosquito infestation data were provided by the Sanitary Surveillance of the Health Department of Jacarezinho according to the *Ae. aegypti* Infestation Index Rapid Survey (LIR*Aa*) [35], when indicated.

Epidemiological Data

Epidemiological data regarding dengue cases were provided by the Epidemiological Surveillance of the Health Department of Jacarezinho. All the patients presenting dengue symptoms were reported to the local authority and tested by enzyme-linked immunosorbent assay (ELISA, detection of NS1 using both IgM and IgG), RT-qPCR MULTIPLEX, and dengue serological identification.

Dengue incidence in indicated periods was calculated as (number of dengue cases/exposed population of the area) × 100 000 for control (I_{DC}) and treated (I_{DT}) areas. The number of exposed people in each area was based on demographic data provided by Jacarezinho authorities. The rate ratio (RR) is the ratio between the 2 incidences ($RR = I_{DT}/I_{DC}$). Values of $RR < 1$ indicate that the NVC treatment is protective against dengue and values of $RR > 1$ indicate that intervention is a worsening factor for dengue; 95% CIs were calculated using R software.

Ethics

Animal care and use protocol were performed in accordance with the Brazilian guidelines (law 11 794), the standards issued by the National Council for Animal Control and Experimentation, and were approved by the Animal Use Ethics Committee of the Paraná Institute of Technology (protocol number 013/17).

An Environmental License from the Environmental Institute of Paraná was obtained (operation license 36127) to perform the releases of NVC mosquitoes in Jacarezinho. Agreements from Sanitary and Epidemiological Surveillance, Health Secretariat, City Hall, and Public Ministry were also obtained.

Anonymized information regarding occurrence of dengue cases was provided by the Epidemiological Surveillance of the Health Department of Paraná and is available to the general public as epidemiological bulletins.

RESULTS

Generation of Fully Sterile and Competitive *Ae. aegypti* Male Mosquitoes

Reduced PTB protein in the male *Drosophila* results in sterility [22]. The gene encoding this protein is also found in *Ae. aegypti* [37], and so we evaluated whether treatment of *Ae. aegypti* larvae with specific dsRNA would induce silencing of AaPTB. Treatment with PTB dsRNA induced gene silencing 24 hours after treatment compared to control (nontreated) samples. Interestingly, the mosquitoes treated with PTB dsRNA during the larval stage showed strong upregulation of PTB mRNA compared with untreated or nonspecific dsRNA controls during the pupa and adult mosquito phases (Figure 1A). Although PTB dsRNA interferes with expression of the PTB gene, this effect did not cause the sterility of the male mosquitoes per se (data not shown).

We also evaluated different conditions of treatment using thiotepa as a chemosterilant alone. As can be seen in Figure 1B, none of the initial conditions tested provided 100% sterility of the adult *Ae. aegypti* mosquitoes.

Several tests were carried out to optimize the sterilization process to ensure a process that would provide 100% sterile and thiotepa residue-free mosquitoes. Treatment of the larvae with 1 mg PTB dsRNA followed by incubating the pupae with 0.1% thiotepa for an overnight period ensured that it was effective in inducing 100% sterility (Figure 1C) and no detectable thiotepa residues in adult male mosquitoes (Supplementary Material).

Although these results suggested that NVC-generated male mosquitoes were 100% sterile, it is possible that this outcome was due to an inability of male mosquitoes to mate with females. Therefore, a semifield trial was conducted and showed that treated mosquitoes were competitive with nonsterile mosquitoes under environmental (noncontrolled) conditions and suppressed the viable progeny of the next generation (Figure 1D).

Large-scale Releases of NVC Male Mosquitoes Successfully Suppressed a Field *Ae. aegypti* Mosquito Population

To evaluate whether the massive release of NVC sterile male mosquitoes could suppress a wild mosquito population, we conducted a field trial in Jacarezinho, a Brazilian city with a long history of dengue epidemics. Several adaptations of the NVC production process were made to adapt it to large-scale production ("Methods" and Supplementary Material). The first intervention (INT1) of NVC mosquito releases started in September 2018 and continued weekly until mid-April 2019. The number of NVC male mosquitoes used is shown in Figure 2A. Female contamination was below <0.01% (data not shown).

The suppression effect of NVC male mosquito releases on the *Ae. aegypti* field population was quantified by measurement of weekly moving averages of viable larval sampling that hatched from eggs collected during field surveillances in both treated and control areas [36]. The mean number of eggs per ovitrap

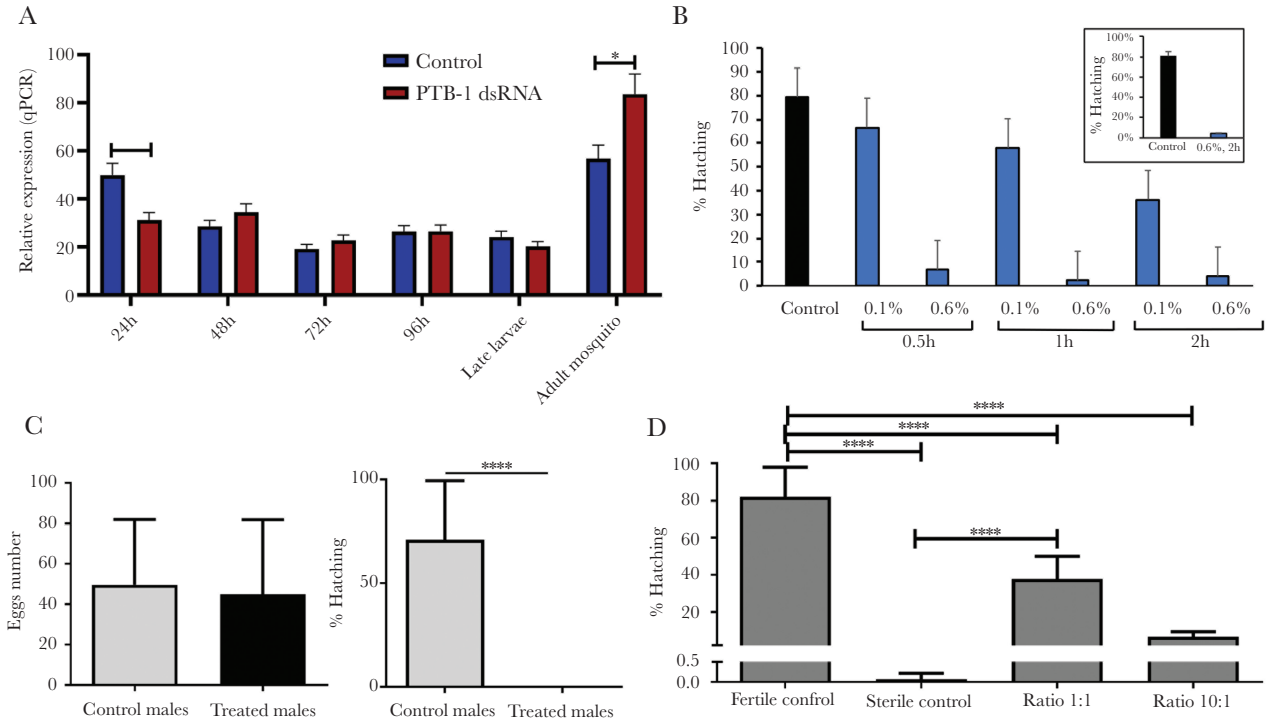

Figure 1. Optimizations of sterilization treatment and semifield testing. *A*, First instar larvae were soaked in water (control) or PTB-1 dsRNA (12 µg/mL) for 1 hour, then reared until adults. Samples from larvae collected after indicated time points were analyzed for PTB expression using real-time PCR. Data are mean and SEM of 6 replicates. Ten experiments were performed and provided similar results. Statistics: JUMP software. Asterisks indicate statistically significant differences. *B*, Groups of 50 male pupae were treated with 0.1% or 0.6% thiotepa, or water (control) for the indicated times, then washed to remove any external residues of thiotepa and reared until adulthood. These adults were mated with virgin females and the viable progeny was determined as the percentage of larvae hatched from their eggs. Data show the mean and SEM of 10 replicates and 3 experiments were performed with similar results. Inset: Evaluation of thiotepa solution (0.6%, for 2 hours) treatment in large-scale production of NVC males (120 000 male pupae were treated in each batch). Data show the mean and SEM from 3 indepedent experiments. *C*, *Aedes aegypti* larvae were treated with a formulation containing 1 mg PTB-1 dsRNA (from first to third instars stages) then with 10 µg/mL solution of PTB-2 dsRNA (from third instar to the pupal stage). Subsequently, male pupae were treated with 0.1% thiotepa solution overnight (15 hours), and then reared until adulthood. Seven-day-old NVC (treated males) or normal fertile males (control males) were allowed to mate with untreated virgin females. The eggs from these females were counted and hatched to determine the percentage of viable larvae: left, average number of eggs per female with SEM and, right, percentage mean of viable larvae hatched from the eggs with SEM. Data represent 2 independent experiments. Statistical analysis: unpaired *t* test, **** $P < .0001$. *D*, Competitive competence of NVC in the semifield trial. Different proportions of NVC and fertile (untreated) male mosquitoes were placed in cages installed in a pre-defined area in the city of Jacarezinho and allowed to mate with untreated (fertile) virgin females, according to the protocol described in "Methods" and Supplementary Material. After the mating period, females from all groups were fed with blood and allowed to oviposit in appropriate containers placed inside the semifield cages. Data are the mean percentage of hatching with SEM (viable larvae derived from eggs) for each group (3 experiments). Statistical analysis: 1-way analysis of variance, Tukey multiple comparison test, **** $P < .0001$. Abbreviations: dsRNA, double-stranded RNA; NVC, natural vector control; PTB, polypyrimidine tract binding protein; qPCR, quantitative polymerase chain reaction.

collected is shown in the Supplementary Material. In the first intervention period (INT1), data showed that treatment with NVC male mosquitoes in the treated (Aeroporto) area reduced the field population compared to the mosquito population of the control (São Pedro) area, reaching up to 91.4% (95% CI, 82.8% to 91.4%) reduction in the number of viable larvae between week 0 and weeks 17-21 (Figure 2B).

Evidence for population suppression was further supported by data provided by the Brazilian authorities based on the LIR*Aa* index [35], which represents the percentage of houses that test positive for the presence *Ae. aegypti* larvae. High *Ae. aegypti* infestation rates were found in both the control and treated areas before NVC male mosquito releases (week −32) (Figure 2C). Six months after the beginning of the NVC releases (week 26), the treated area presented a large decrease in the rates of infestation of *Ae. aegypti*.

Suppression of *Ae. aegypti* Mosquito Population by NVC Males Was Associated With Lower Incidence of Dengue in the Treated Areas

In early March 2019 (week 24), an outbreak of dengue began in Jacarezinho (Figure 3A). During the period of NVC releases (weeks 1–29), 72 dengue cases originated in neighborhoods of the control area, while only 8 cases of dengue were reported in the treated area (Figure 3A). It is worth noting that the lowest number of dengue cases in the treated area coincides with the periods of lower mosquito infestation in this area (Figure 3B). In week 35, the total number of dengue cases originating in the treated area remained much lower (24 cases) compared to the control area (287 cases).

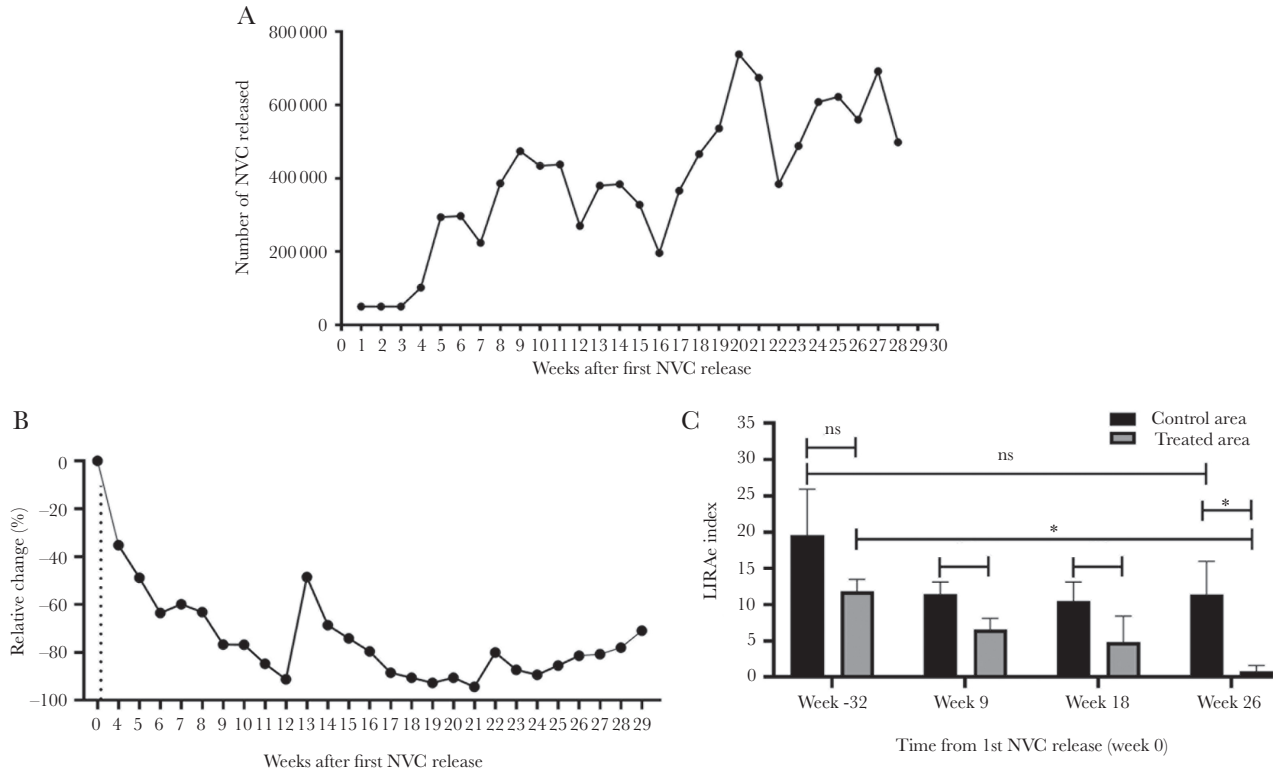

Figure 2. Large-scale releases of NVC male mosquitoes can suppress local *Aedes aegypti* populations (INT1). *A*, Number of NVC mosquitoes released at the treated area, Aeroporto, Jacarezinho, per week. *B*, Suppression of *Ae. aegypti* wild population in Jacarezinho after treatment with NVC mosquitoes. Weekly moving averages showing percentage change in *Ae. aegypti* abundance at the treated area, measured by mean number of larvae per trap relative to control area. In weeks 17–21 there was a 91.4% (95% CI, 82.8% to 91.4%) reduction in numbers of mosquito compared to week 0. *C*, Mean LIR*Aa* index in the control (São Pedro) and treated (Aeroporto) areas. *Ae. agypti* infestation before the implementation of first NVC program is indicated by week –32. Weeks 9, 18, and 26 LIR*Aa* indexes after the beginning of NVC release program. **P* < .05. Abbreviations: CI, confidence interval; INT1, intervention period 1; LIR*Aa, Ae. aegypti* infestation index rapid survey; ns, nonsignificant; NVC, natural vector control.

The incidence of dengue in the control area during the entire period analyzed (weeks 1–35) was 6320 cases per 100 000 inhabitants, while the incidence of dengue in the treated area, in the same period, was only 396 cases per 100 000 inhabitants, which is nearly 16 times lower. The RR was 0.0627 (95% CI, .0414–.0949), indicating that the treatment with NVC had a protective effect for residents of the treated area in terms of dengue.

Approximately 5 months after completion of INT1, we reversed the treatment and control areas. In this second intervention (INT2), the releases of NVC sterile male mosquitoes were carried out in Vila São Pedro (treated area) while Aeroporto was now designated the control area. Except for this change, the other procedures used in INT2 were made essentially as for as INT1. The number of mosquitoes released per week are indicated in Figure 4A. Another dengue outbreak started around week 55 (Figure 4B). This time, the number of dengue cases in the treated area (São Pedro) remained very low over the entire period of INT2 (weeks 51 to 70), while in the control area (Aeroporto) the number of accumulated cases of dengue rapidly increased throughout the same period. The incidence of dengue cases during the INT2 period in the control and treated areas were 8070 and 590 per 100 000 inhabitants, respectively (RR = 0.0737; 95% CI, .0501–.1083), representing an almost 14-times lower incidence of dengue in the treated area relative to the control area.

DISCUSSION

One of the key factors for successful SIT implementation is identifying a technology that induces sterility while at the same time retaining the competitive fitness of the sterilized treated males relative to the endemic male population in the release area. Furthermore, to be implemented widely, such a method needs to be robust, cost-effective, easily scalable, and easy to implement anywhere [38].

In this study, the effectiveness of male sterile mosquitoes was evaluated in Jacarezinho. During INT1, the suppression of the mosquito population resulting from the SIT program in the treated region was more than 90% relative to the control region, which correlated with the lower incidence of dengue cases in this same region during the outbreak that started in the city. Subsequent reversal of the control and treated areas for INT2 was performed 5 months after the termination of INT1. Strikingly similar results were obtained during this second

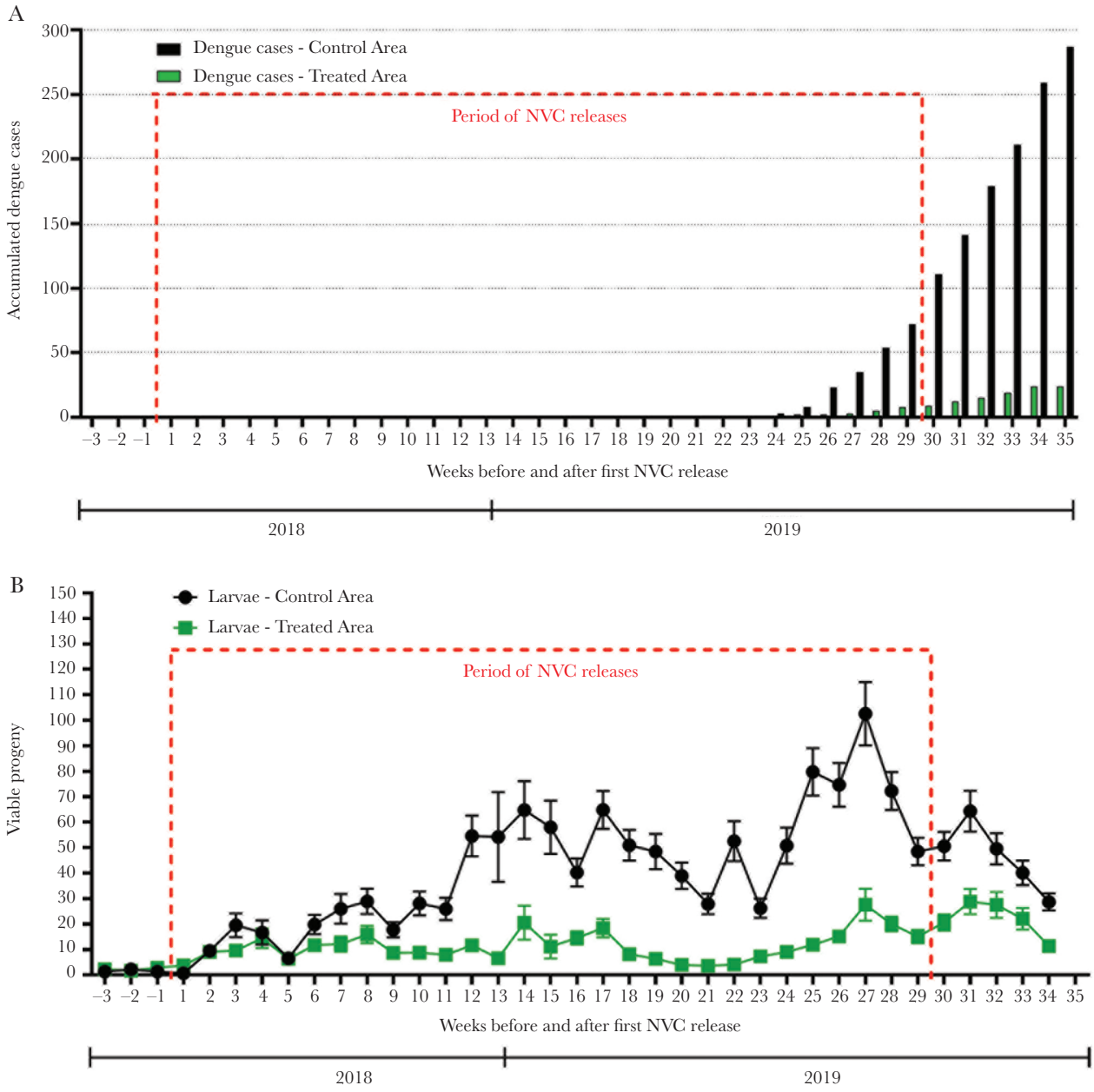

Figure 3. Viable *Aedes aegypti* progeny and dengue cases in the treated and control areas of Jacarezinho during INT1. *A*, Releases of NVC male mosquitoes in the treated area occurred weekly between 28 September 2018 and 22 April 2019 (weeks 1 to 29). Week 35 corresponds to 28 May 2019. The cumulative number of confirmed dengue cases in the control (black) and treated (green) areas were provided by the Parana Health Department. The incidence of dengue in the control area (I_{DC}) was 6320 cases per 100 000 inhabitants (95% CI, 5650–7070) and in the treated area (I_{DT}) was 396 cases per 100 000 inhabitants (95% CI, 270–590). The rate ratio (I_{DT}/ I_{DC}) was 0.0627 (95% CI, .0414–.0949). Multivariate analysis of variance (correlation between viable progeny in the treated and control areas and dengue cases) provided *P* values < .05. *B,* Control and treated areas were monitored with egg collection for 37 weeks (week –3 to 34). Eggs collected from the control and treated areas were transferred to the laboratory, where they were hatched. The mean number of larvae hatched from eggs of each ovitrap (100 per area) over the 37 weeks was defined as viable progeny. Statistical analysis: analysis of covariance provided a *P* value < .0001 for the difference between slopes for mean larvae per trap from control and treated areas. Abbreviations: INT1, intervention period 1; NVC, natural vector control.

intervention (INT2): the incidence of dengue in the area where sterile male mosquitoes were released was found to be 14-times less than the control area.

The generation of sterile male mosquitoes (NVC) used in this study involved 2 different treatments, namely larval exposure to PTB dsRNA and pupal exposure to thiotepa. It was previously demonstrated that *Drosophila* PTB encodes an abundant male germline-specific mRNA isoform (dmPTB) whose expression correlates with male fertility [22]. Also, *Drosophila* PTB expression is necessary for proper spermatid individualization, the terminal step necessary for production of motile sperm [39]. Loss of dmPTB results in severe disruption of the actin

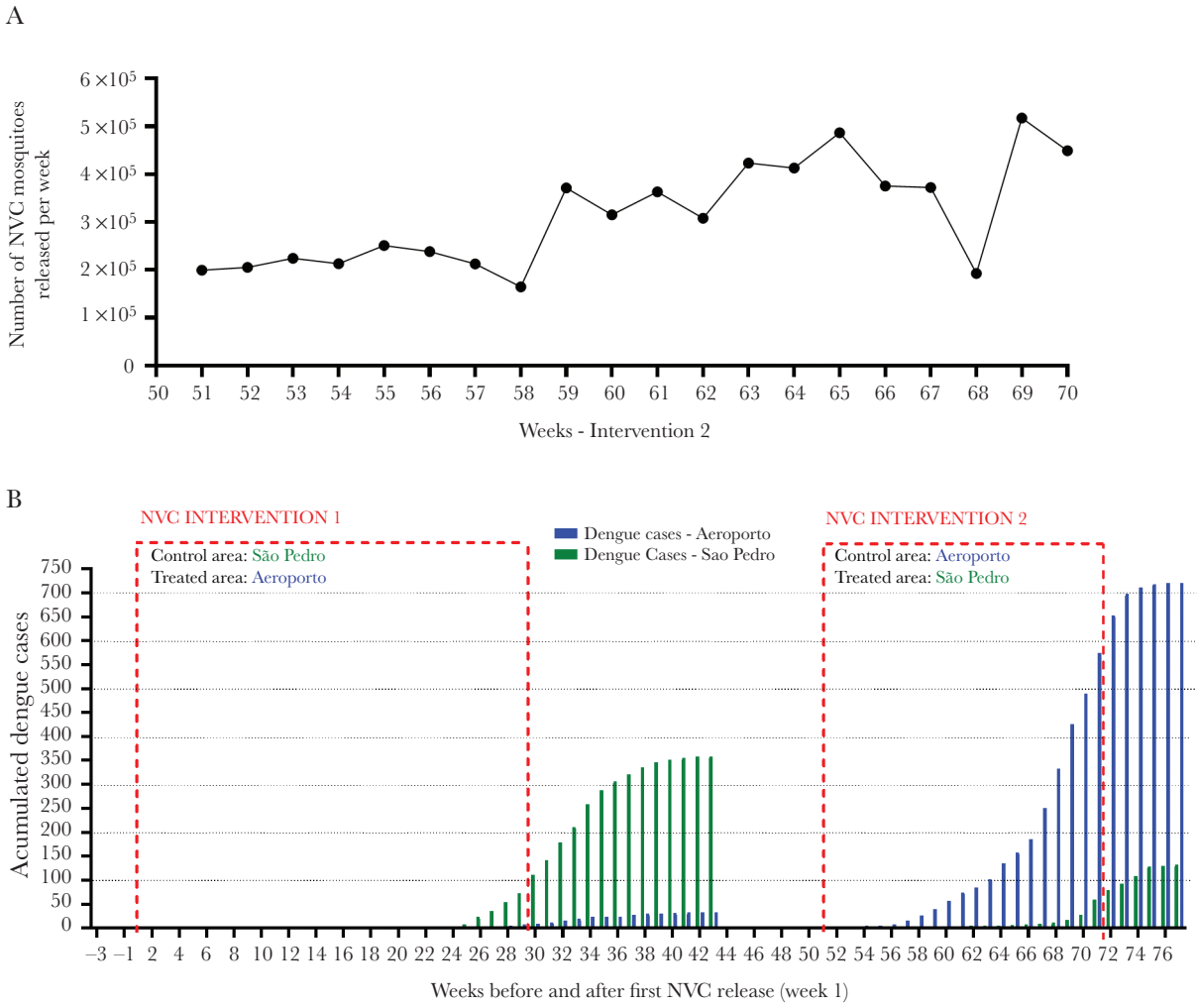

Figure 4. Dengue cases in Aeroporto and São Pedro areas of Jacarezinho during INT1 and INT2. *A*, Number of NVC mosquitoes released at the treated area (Sao Pedro) per week during INT2 (weeks 51 to 70, 16 September 2019 to 31 January 2020). The y-axis shows the number of NVC mosquitoes released each week. *B*, Cumulative number of confirmed cases of dengue in Aeroporto (green bars) and São Pedro (blue bars) study areas during and immediately after INT1 and INT2. For INT1, São Pedro neighborhood was the control area and Aeroporto was the treated area, while for INT2 these areas were switched (Aeroporto was the control area and São Pedro the treated area, as indicated). The periods of NVC sterile male mosquito releases are shown by red dotted lines. The incidence of dengue cases in the control area (Aeroporto) in INT2 was 8070 per 100 000 inhabitants and 590 per 100 000 inhabitants in the treated area (São Pedro). The rate ratio was 0.0737 (95% CI, .0501–.1083). Data on dengue cases were provided by the Parana Health Department after confirmation using ELISA and RT-qPCR multiplex methods. Abbreviations: CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; INT, intervention period; NVC, natural vector control.

cones of the spermatid individualization complex, resulting in lack of motile sperm and accumulation of cysts of elongated spermatidis [39]. The treatment of mosquitoes during the larval stage using specific AaPTB dsRNA caused silencing and subsequent upregulation of the PTB mRNA at the pupal stage (Figure 1A). The second stage of treatment involves thiotepa, a known insect chemosterilant that has been used in the past [24, 28, 40]. Thiotepa chemosterilization was discontinued because it either promoted incomplete sterility (when used in low doses or for short periods of incubation) or resulted in residues of thiotepa in the released mosquitoes when used in doses that induced 100% sterility [41]. Here, we show that thiotepa treatment for NVC was effective at a lower concentration (semifield trial) or shorter exposure times (field trial). This facilitated scaled-up production and, most importantly, no detectable residues of the thiotepa in the released mosquitoes in the current NVC process (Supplementary Material). Although we have not yet demonstrated the mechanism of action of the apparent interaction between the dsRNA and thiotepa, it is possible that the first treatment with dsRNA, which silences the PTB in the male germ line, potentiates the effects of thiotepa that acts later in the same tissue. Subsequent adaptations in the production process made for the field releases have enabled us to decrease further the quantities of PTB dsRNA used (which considerably reduces the production costs), contributing to the viability of the production necessary for larger intervention programs.

Gradual mosquito population suppression, as was demonstrated in this study, shows the importance of sustaining

a continuous release program that is dependent on real-time monitoring of the mosquito population. Indeed, the slight increases in viable progeny (weeks 14, 17, and 27; Figure 3B) reflect the smaller numbers of sterile male mosquitoes released in the previous weeks (Figure 2A). In addition, the follow-up INT2 underscores the need for an effective SIT suppression program to be continued over at least 2 seasons and over a large area (area wide) to avoid a rebound of the mosquito population and subsequent outbreaks of dengue.

Several other methods of SIT-based vector control have been tested in field studies and have shown good efficacy in suppressing mosquito populations during intervention periods [12, 13, 42, 43]. This is very important as it shows that biological control of the mosquito vectors may become the most viable and effective solution in the fight against arboviruses. However, considerations regarding the impact of these programs on preventing outbreaks of arboviruses were essentially based on mathematical models [13, 44] and not on actual measurements of the incidence of dengue during the intervention. To our knowledge, our data presented in this study are the first real demonstration that a program based on SIT can, in fact, dramatically reduce the incidence of dengue in the treated regions (Figure 3 and Figure 4), corroborating the mathematical models proposed in those previous studies. In addition, the nature of NVC mosquitoes (nongenetically modified, free of residues, and adapted to local climatic conditions), the possibility of scaling production as needed, and the possibility of enabling local communities to conduct the intervention programs themselves in the future, are other advantages of the method presented here.

Subsequent to the work described herein we adapted the system to produce millions of mosquitoes per week. This successful demonstration of the INT1 and INT2 has led the state of Parana to request the expansion of the use of NVC sterile male mosquitoes (Supplementary Material) in additional areas of Parana state that are currently experiencing an ongoing dengue epidemic. This underscores the practicality of SIT programs for arbovirus disease prevention.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Financial support. This work was supported by Forrest Innovations Ltd.

Potential conflicts of interest. N. P. is employee of Forrest Innovations and L. C. P., F. A. A, D. A. O, D. R., R. N. O., and M. L. F. are employees of Forrest Innovations and Forrest Brasil Tecnologia Ltda. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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