

## Protocol for a small-scale In2Care<sup>®</sup> Mosquito Trap field test by Pest Management Professionals

### Product information

The In2Care Mosquito Trap is an EPA-registered outdoor contamination station against container-breeding and day-biting *Aedes* mosquitoes, with the goal to reduce the mosquito population to a level that greatly reduces nuisance and the spread of diseases such as Dengue and Zika virus. This auto-dissemination station lures and contaminates *Aedes* mosquitoes with a special slow-killing larvicide and lets them spread this to other breeding sites so that mosquito larvae are not only killed inside the trap but also in other breeding sites in its vicinity. This is combined with a biological adulticide that kills the contaminated mosquito after a few days to prevent her from transmitting arboviruses. See <http://www.in2care.org/videos>.



Trap bioactives are provided in separate sealed aluminium refill sachets that contain In2Care's special (patented) statically charged gauze strips, the bioactive powder mixture and 2 odour tablets. For optimal efficacy, In2Care recommends area-wide coverage at densities of approx. 1 trap per 400 m<sup>2</sup> (10 traps per acre) and that each Trap is serviced every 4-6 weeks; replacing the powder-treated gauze strip with a new one and refreshing the water. As explained in the [User Manual](#), this is done using fresh refill sachets that contain 2 other tablets and the following bioactive ingredients (EPA registered):

- Pyriproxyfen (PPF). This slow-killing larvicide consists of a juvenile hormone analogue that is safe for mammals and humans and targets mosquito larvae at the pupal development stage. PPF is effective against mosquito larvae when applied in very low concentrations in the water (<10 ppb). Inside the trap, PPF slowly kills all mosquito larvae that develop from eggs laid by mosquitoes; it prevents the last stage of development into adult. Therefore, live larvae will remain present inside the trap, but no adult mosquitoes will emerge. Additionally, contaminated mosquitoes that obtain PPF particles attached to their legs can transfer PPF to other breeding sites surrounding the trap and kill larvae there. Field studies have shown that skip-ovipositing *Aedes* mosquitoes can spread PPF in the environment and achieve effective larval control in breeding sites where PPF accumulates. Pyriproxyfen is not lethal to the adult mosquito, which is why an additional adulticide is added.
- *Beauveria bassiana*. The In2Mix contains spores of this insect-specific fungus, which are the non-growing stages that can persist for long time periods. Spores attach to the mosquito upon contact and need an insect-specific cue to penetrate the skin and cause infection inside the insect (fungus spores will not infect mammals or humans). The fungus takes several days to kill, depending on mosquito species and temperature. Prior to death, fungal infection can also reduce the mosquito's ability to feed and transmit arboviruses. The slow kill of the fungus allows infected mosquitoes to spread PPF for a few days.

### Field test Objectives

This protocol provides suggestions for a small-scale field test by PMPs at client sites to test the In2Care Mosquito Trap and validate its mosquito-killing impacts. Trap efficacy can be assessed in terms of its attraction to gravid *Aedes* mosquitoes, the larvicidal impact achieved inside the trap and in surrounding mosquito breeding sites, and reductions in nuisance reported by the clients.

Our recommendation is to test the intended use of In2Care Mosquito Traps; *i.e.* in an integrated mosquito control approach and confirm that it can achieve mosquito reductions and protection for the client.

For optimal impacts, we recommend a combination yard treatment using:

1. A barrier treatment with a fast-acting insecticide spray to achieve rapid knockdown of all nuisance mosquitoes and reduce the influx of *Aedes* mosquitoes from outside the treatment area. For example, treating border vegetation & high risk areas with a residual adulticide like Demand or Polyzone spray.
2. In2Care Mosquito Traps at a density of 10 Traps per acre.

### Field test recommendations

This protocol described the steps for a 2-month evaluation in at least 10 selected yards, each with a 0.2-acre treatment area (= approx. 8500 ft<sup>2</sup>) using 2 In2Care Traps per yard. Larger yards can also be used, but these will require more In2Care Traps; a density of 10 Traps per acre (1 Trap per 4000 ft<sup>2</sup>) is recommended. Suitable yards will have much vegetation, water and shade, and clients reporting high mosquito numbers; many day-time biting nuisance and lots of larval breeding sites. We recommend doing the test in a cluster of 10 adjacent residential yards when possible, or at least have the yards being similar in size, layout and vegetation and make sure they are not near/adjacent to untreated sites with a lot of mosquito breeding.

The actives inside the In2Care Trap need time to get spread into the environment and will mainly impact the next generation of mosquitoes by reducing their breeding success. Therefore, Trap impacts will be most pronounced and easy to observe at 4, 6 and 8 weeks post-deployment.

When possible, we recommend prolonging the study to a 2-month evaluation by servicing the Traps with fresh water and a new refill after 1 month and repeating the monitoring steps at week 6 and 8. In this way there will be more data points to confirm the long-term activity & continuous protection by the product.

We advise to monitor in each yard the following parameters **2 days prior to In2Care Trap deployment**:

- Mosquito nuisance reported by the clients.  
Using a standard customer survey; noting the reports of day-time mosquito bites. See Appendix 2 for an example survey we recommend using.

This will serve as a baseline measurement with which In2Care Trap impact results can be compared.

*Note: Considering that customer complaints will be the only measurement of adult mosquito populations in this study (and not quantitative measurements with monitoring traps), we advise this before-and-after comparison in the same yards. Different clients can have varying attitudes or nuisance thresholds, so we do not recommend comparing the 10 treatment yards with 10 completely different yards (that get a different treatment) from different clients.*

We advise to monitor in each yard the following parameters **up to 8 weeks post-deployment**:

- Mosquito nuisance reported by the clients (using a customer survey as provided in Appendix 2).
- Quality of the placed In2Care Traps.
- Trap attraction to gravid *Aedes* females (larvae observed inside the In2Care Traps).
- Larvicidal efficacy of pyriproxyfen (PPF) inside the In2Care Trap container.
- Auto-dissemination impact: larvicidal efficacy of PPF in breeding sites near In2Care Traps.

## Materials

- 20 In2Care Mosquito Traps: containers, lids, floaters, time indicator caps & securing tools
- 40 In2Mix refill sachets
- Water (1.3 gallon per In2Care Trap)
- 5 black glass ovipots (to assess larvicide spread)
- Netting & fish food
- Barrier treatment materials

## Methods

### Site inspection

- Select 10 suitable 0.2-acre yards. Preferably sites with a known *Aedes* infestation (complaints from day-time biting mosquitoes) and not near/adjacent to untreated areas with a lot of mosquito breeding.
- At the start, check the premises for potential mosquito breeding sources. Check & remove flower pots, vases, fountains, bins, buckets, rain gutters, containers, waste material etc. Note the location of *Aedes* mosquito breeding sites (water with larvae) on the site monitoring form.

- Determine potential In2Care Trap placement locations (see Appendix 1) using this site map; place Traps where mosquito breeding was observed or can be expected.

### Barrier Treatment

- At the start, spray all vegetation at the borders of the treatment site using a residual insecticide spray.
- Depending on the desired combi approach and persistence of the spray product as stated on the product label, this may be repeated every ..x.. weeks.

### Trap deployment

- As described in Appendix 1, place the In2Care Traps in suitable spots. Criteria: presence of *Aedes* mosquitoes, vegetation, shade, close to the house, low risk of disturbance. When possible place at the sites where mosquito breeding sources were found. Per yard, we recommend placing 1 Trap in the front and 1 Trap in the backyard.
- Number & Label each In2Care Mosquito Trap. Use stickers with contact information and warning not to touch or remove the product.
- Assemble the In2Care Traps as described in Appendix 1. When needed, use the securing tools and/or ground pins. Note on the site map where the In2Care Traps were placed exactly.
- After 1 month, service each Trap as advised in our User Manual. Clean each Trap, add fresh water and use a new refill sachet to re-activate.

### Placement of monitoring tools

- Randomly select 5 yards, and place in each 1 ovipot (small black glass jars with water) at a shaded site approx. 2 - 5 meters from an In2Care Mosquito Trap (= 5 ovipots in total).
- Collect the ovipot contents at week 2, 4, 6 & 8 for monitoring the larvicide spread as explained below.

### Impact Monitoring

#### *1. In2Care Trap product quality (week 2, 4, 6 & 8)*

- Monitor the water levels in all Traps biweekly (when possible, or else monthly). If some Traps show relatively more water evaporation, relocate these to a more shaded spot. When < 5 cm of water is observed, top up to the 1.3 gallon max capacity.
- Note if the netting has become wet. The actives work best when kept dry!
- Note any product damages, relocations or thefts. Replace missing Traps during this test.

#### *2. In2Care Trap Mosquito Attraction (week 2, 4, 6 & 8)*

- Count/estimate the numbers of *Aedes* larvae observed in all In2Care Trap containers biweekly. For instance, score if there are 0, <10, 10-50, or >50 mosquito larvae in the water of the Trap.
- If some Traps show relatively much fewer larvae than the other In2Care Traps, relocate them to other (more suitable) shaded/vegetated spots and note this on the site form.

*Presence of mosquito eggs & larvae will indicate good Trap attraction. PPF is an insect growth regulator and kills the pupal stage, not the young larvae. The young larvae will attract more mosquitoes to come and lay their eggs.*

*3. Larvicidal efficacy (PPF action inside the In2Care Trap) – week 4*

- At week 4 (when the Traps are serviced), select and collect In2Care Traps that contain live *Aedes* larvae and transport them to a monitoring room.
- For each collected Trap, remove the lid, floater & netting, add larval fish food in the water, and cover the container with netting (using a rubber band).
- Monitor for each collected Trap the adult mosquito emergence rates: count how many larvae die in the pupal stage & how many become a mosquito adult. Wait until all larvae have died or emerged before scoring the final numbers.

*0% adult emergence will confirm that PPF has remained fully effective in killing all larvae inside the Trap container.*

*4. Auto-dissemination efficacy (PPF action outside the In2Care Trap) - week 2, 4, 6 & 8*

- Biweekly, collect the ovipots that have *Aedes* larvae in them. Relocate the ovipots that had not been visited (no eggs) to another shaded spot close to the In2Care Trap.
- Pour the water (with eggs/larvae) in a collection container (label these carefully) and place the ovipots back with fresh water.
- Transport the collected contents to a monitoring room and cover the containers with netting.
- Add fish food and monitor larval development until all have emerged into adults or all have died.
- Note any dead black-coloured pupae with de-curved tails (= distinctive for PPF mode of action).
- Calculate for each container the adult emergence rate ( $\frac{\# \text{ died pupae}}{\# \text{ all pupae}} \times 100$ ).

*Reduced adult emergence rates (<40% adults = >60% dead pupae) in ovipots will confirm that the PPF has been successfully spread by the mosquitoes from the In2Care Trap to the ovipots.*

*5. Impact on *Aedes* mosquito population (before, during & after treatment)*

- Collect nuisance reports from each yard using customer surveys before, during & after treatment. See appendix 2. We advise to ask the clients to fill out the questionnaire 2 days before the treatment, between week 4 & 6, and after week 8.
- Compare the numbers of daytime mosquito bites/nuisance complaints before and after treatment.

*Reductions in mosquito nuisance reports are expected somewhat at the start due to the misting barrier treatment but will be most noticeable after 2 weeks of Trap deployment since PPF & Beauveria are slow-killing actives and will reduce the next generation of mosquitoes.*

For more information, please contact:

In2Care BV  
Marijkeweg 22  
6709 PG Wageningen  
The Netherlands  
[www.in2care.org](http://www.in2care.org)  
Email: [marit@in2care.org](mailto:marit@in2care.org)

## Appendix 1 - In2Care Trap deployment instructions

### Trap Placement

In2Care Mosquito Traps need to be placed outdoors in shaded, secluded areas preferably away from kids and pets. When possible, use a mapping tool/device to record & track placement. The ideal placement location is a site where *Aedes* mosquito breeding is observed. Make sure that In2Care Traps are placed on level surfaces at spots with continuous shade at all sun angles. In residential areas, this would be near the house; next to garden sinks, patios, sheltered dark/moist corners. Make sure that Traps are only placed in spots that are suitable; not on roads, open grassland, or other large open (non-shaded) areas. Full Traps (>1 gallon of water) are quite stable and heavy enough to not get knocked over easily. In2Care Traps need to stand firmly and horizontally at ground level, if they are on an uneven underground this can cause issues with Trap placement and gauze getting wet. In most areas, placement on sturdy, level floors or soil should suffice. When possible, place Traps in secluded spots or corner areas to minimize chances of disturbance. In case of domestic animals being present, try to place Traps in spots that are not easily accessed by the animals as they may try to get to the water inside. Adding official markings/stickers is recommended to minimize removal or theft.



### Trap Assembly

Trap assembly instructions are detailed in the User Manual, which can be downloaded from: <http://www.in2care.org/how-does-it-work>. For more visual assembly detail, please see In2Care's User Instruction video on: <http://www.in2care.org/products/videos>. It is important that the water should be placed in the Trap prior to putting the floater with the gauze in the Trap.

1. First decide where to place the Trap before proceeding with assembly.
2. Secure the Trap with fixation tools when needed.
3. Add approximately 1.3 gallon of clean tap water to the container by using a watering/jerry can or garden hose. This maximum water level is reached when water starts coming out at the bottom via the overflow openings (Note this does not indicate leakage).



4. Prepare the floater component. Shake the refill sachet before opening. Use gloves to place the powdered netting strip from the sachet onto the floater, as described in the User Manual. It is normal for some parts of the netting will be whiter than others. Pour remaining powder and 2 yeast tablets contained in the packing into the water. Picture of steps & refill/sachet contents below:



5. Place the floater with the powdered gauze strip carefully on top of the water and empty the sachet contents (leftover powder and odor tablets) in the water, see picture.  
*Be careful with handling the Trap after this point; the actives work best when kept dry.*
6. Next, attach the lid which can be secured with a twisting movement.
7. Finally, turn the green calendar indicator to either approximate date to show when the Trap was put in place or to show a future date to show when the sachet/refill will need to be replaced.



Use stickers to number the Traps and alert people not to disturb, damage, replace or remove them. Keep track of the final placement of each Trap on a map and/or via a mapping tool.

## Appendix 2 - Customer Survey

*Client Survey for Mosquito Control programs with In2Care Mosquito Traps & residual barrier treatment:*

### Questionnaire before treatment

1. How would you classify the level of nuisance and burden you are experiencing from mosquitoes at your property?
  - Extremely high
  - High
  - Moderate
  - Low
  
2. How often are you or your family members bitten by mosquitoes in your yard?
  - All the time: multiple times a day
  - Often: every day
  - Sometimes: some days
  - Only a few times per week
  
3. At what time during the day are you experiencing the most nuisance and mosquito bites at your property?
  - At night
  - In the evening (after dark)
  - Around sunset
  - During the day
  - In the early morning
  
4. Are there specific sites in your yard where you experience the most issues with mosquitoes?
  - No
  - Yes, please specify where: \_\_\_\_\_
  
5. Do you have a lot of mosquito breeding sites at your property?
  - Yes, we have a lot of flower pots and places with still-standing water
  - I do not know
  - No, we keep our yard clean and have no or few water containers
  
6. Do your neighbors have a lot of problems with mosquitoes?
  - Yes
  - I do not know / not relevant
  - No
  
7. Are you satisfied that our staff clearly explained the mosquito control services we will be performing?
  - Very satisfied, I know exactly what to expect
  - Satisfied, I understand the key points of your services
  - No opinion, I do not need to know
  - Dissatisfied, please provide me with more details about the treatment



**Questionnaire during treatment (4 - 6 weeks after start)**

1. How would you classify the level of nuisance and burden you are experiencing from mosquitoes at your property now?
  - Extremely high
  - High
  - Moderate
  - Low
  - Very low
  - No nuisance at all anymore
  
2. How often are you or your family members getting bitten by mosquitoes in your yard?
  - All the time: multiple times a day
  - Often: every day
  - Sometimes: some days
  - Only a few times per week
  - No more at all
  
3. Have you observed an improvement? Do you have less problems with mosquitoes now than before the treatment??
  - Yes, we see a big improvement
  - Yes, we see a slight improvement
  - It is not clear yet
  - No, we still experience the same burden from mosquitoes
  - No, we have even more problems with mosquitoes
  
4. If any, at what time during the day are you experiencing the most nuisance and mosquito bites at your property?
  - At night
  - In the evening (after dark)
  - Around sunset
  - During the day
  - In the early morning
  
5. Have there been any major changes in your yard's landscape?
  - Yes, we added a pond / pool / other places with still-standing water
  - Yes, we added new plants and vegetation
  - Yes, we altered other things: \_\_\_\_\_
  - No, our yard is the same as before
  
6. Are you satisfied that our technician spent the appropriate amount of time servicing your property?
  - Very satisfied
  - Satisfied
  - Unknown or no opinion
  - Dissatisfied

**Questionnaire after treatment (8 weeks after start)**

1. How would you classify the level of nuisance and burden you are experiencing from mosquitoes at your property now?
  - Extremely high
  - High
  - Moderate
  - Low
  - Very low
  - No nuisance at all anymore
  
2. How often are you or your family members getting bitten by mosquitoes in your yard?
  - All the time: multiple times a day
  - Often: every day
  - Sometimes: some days
  - Only a few times per week
  - No more at all
  
3. Have you observed an improvement? Do you have less problems with mosquitoes now than before the treatment?
  - Yes, we have seen a big improvement
  - Yes, we have seen a slight improvement
  - It is not clear
  - No, we still experience the same burden from mosquitoes
  - No, we have even more problems with mosquitoes
  
4. If any, at what time during the day are you experiencing the most nuisance and mosquito bites at your property?
  - At night
  - In the evening (after dark)
  - Around sunset
  - During the day
  - In the early morning
  
5. Have there been any major changes in your yard's landscape?
  - Yes, we added a pond / pool / other places with still-standing water
  - Yes, we added new plants and vegetation
  - Yes, we altered other things: \_\_\_\_\_
  - No, our yard is the same as before
  
6. Are you satisfied with our mosquito control program?
  - Very satisfied
  - Satisfied
  - No opinion
  - Dissatisfied
  - Very dissatisfied
  
7. Are the results as you expected?

- Yes, exceeding our expectations
- Yes, same as expected
- No, less than expected
- Not sure / no expectations

8. Are you satisfied with your overall experience you had with our company and our staff?

- Very satisfied
- Satisfied
- No opinion
- Dissatisfied

9. Do you have any suggestions to help us improve our company and services?

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