5. Insecticide-impregnated screens used under 'multi-target method' for haematophagous fly control in cattle: a proof of concept

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Abstract

Livestock are seasonally subjected to the nuisance of haematophagous flies, such as tabanids and stomoxyine flies. Topical application of insecticides has short term efficacy (a week or so), is expensive, and generates pesticide residues in animal products and environment. Attractive insecticide-impregnated blue fabrics are used for tsetse fly control in Africa; however, they are expensive and were never evaluated for other haematophagous flies. In previous works, we defined specifications of a white and blue screen specifically attracting haematophagous flies, particularly *Stomoxys* spp. In the present study, an assay was carried out in Kantchanaburi Province, Thailand, with around 30 of such screen prototypes, made of a multilayer polyethylene film incorporated with deltamethrin. Screens (also called 'targets') were deployed in 12 test farms, to evaluate the efficacy of a so-called 'multi-target method' (MTM); four control farms were also enrolled. A Vavoua trap was deployed one day/week in each farm to follow-up the density of insects. In the test-farms, during the 4 months post treatment, the mean density of haematophagous flies was significantly and consistently reduced by 63-73% compared to the control group. Laboratory tests indicated that insecticidal activity of these screen prototypes lasted around 3-4 months. However, in the field, significant reduction of fly densities was observed in all test farms up to 7 months after screen deployment, possibly as a consequence of the early impact of the screens on fly population dynamics. The significant effects obtained in test farms provided evidence for the proof of concept that MTM is effective for on-farm control of haematophagous and common flies. Durability of the screens will be increased in the next prototype generation. This innovative control method will be evaluated more extensively and in other livestock and poultry farms.

Keywords: polyethylene film, toxic target, livestock, screens, tabanids, *Stomoxys* spp.

Constantianus J.M. Koenraadt, Jeroen Spitzen and Willem Takken (eds.)
***Innovative strategies for vector control** – Ecology and control of vector-borne diseases Volume 6*
DOI 10.3920/978-90-8686-895-7_5, © Wageningen Academic Publishers 2021

Introduction

Livestock are seasonally subjected to nuisance, bites and blood loss caused by obligatory haematophagous flies, such as tabanids and stomoxyine flies, including *Musca crassirostris* which is highly abundant in cattle (Desquesnes *et al.* 2018). The economic impact of haematophagous flies on livestock is huge, with estimates indicating a loss of 130 kg of milk and 25-60 kg of meat per year, respectively in dairy and feeder cattle (Taylor *et al.* 2012). However, these flies are not only direct pests but also mechanical vectors of a number of pathogens, such as parasites (*Trypanosoma* spp., *Besnoitia besnoiti*), bacteria (*Bacillus anthracis*, *Anaplasma marginale*, *Francisella tularensis*, etc.) and viruses (Equine infectious anemia virus, Bovine leukosis virus, etc.) (Baldacchino *et al.* 2013, 2014) which economic impact must be considered, even if it would hardly be quantified. Very few and poorly efficient methods are available for the control and/or prevention of these haematophagous flies. Keeping animals under permanent protection of buildings or mosquito nets is an option, but it is not convenient for groups of large animals, such as cattle. Chemical or physical repellents, such as smoke, may only ensure limited and temporary prevention. The most employed methods are those that use synthetic insecticides. Insecticide paints or sprays indoor or on farm buildings are used for mosquito control (Mosqueira *et al.* 2010, Schurrer *et al.* 2006). However, for fly control, so far, the most employed method is direct spraying of the insecticides on the animals under contact sprays or fog. These methods have been used as early as in the 1950s, for example in the control of horseflies in cattle using a combination of pyrethrins and piperonyl butoxide (Bruce and Decker 1951). Later, organophosphate preparations were also used (Matthysse 1974). Not only are such sprays costly and of very short-term efficacy (lasting a week or so), they also generate high pesticide residues in animal products and/or byproducts (milk, meat, faeces). Contamination occurs by direct dispersion of insecticide droplets into surface water and by drainage systems or when rain wash-off pesticides from the animals and the run-off find their way into water systems, ending in large environmental contaminations.

An alternative method, developed for the control of tsetse flies in Africa is the use of attractive screens made of insecticide impregnated blue fabrics that are either blue squares (1×1 m) or alternate black net and blue fabric measuring 75×50 cm. A more recent development is the 'tiny target' (25×50 cm) made of small blue fabric panel flanked by a small black net (Lindh *et al.* 2009, Rayaisse *et al.* 2011). Very specific fabrics, mostly made of cotton (making them quite expensive), and dyed with phthalogen blue (a toxic dye now forbidden in Europe (Choudhury 2018)) are considered to be the most efficient in terms of attractivity. Tsetse fly attractivity toward colour is highly selective and only very specific blue fabrics that exhibit a wavelength reflectance around 460nm perform properly (Lindh *et al.* 2012). This method would be very costly, especially if high numbers of screens were needed to control tsetse flies in their natural habitats. However, thanks to the low reproductive capacity of tsetse flies, a very limited number of screens is sufficient to impact their population dynamics (Bouyer *et al.* 2015). Indeed, being larviparous, female tsetse flies produce only one progeny at a time, depositing a third instar larva every 8-12 days (depending on temperature and humidity), thus generating a maximum of 8-10 offspring in a lifetime (Bursell 1963, Wang *et al.* 2013). Blue-black fabric screens are currently used in Africa, especially for the control of riverine tsetse flies. The screens are deployed at intervals of 50-100 m or so along river borders (Tirados *et al.* 2015).

Although the blue-black screens have been used extensively against tsetse flies, they have never been evaluated for the control of tabanids and *Stomoxys* spp., because it is presumable that a very high number of screens would be required to impact their populations. Indeed, stomoxyine flies and tabanids may lay 60-130 and 200-800 eggs at a time, respectively, 8-10 times in a lifetime, for

a total of 480-8,000 eggs, respectively (Baldacchino *et al.* 2014, Foil and Hogsette 1994). These flies are, then, highly prolific; it is considered that, for example in tabanids, if only 2% of the female flies oviposit at least once, this would be sufficient to maintain the fly population (Foil and Hogsette 1994). As expected, this population would increase rapidly if more flies and ovipositions occur. Being so prolific, the control of these flies would require the use of more potent tools. It is on the basis of this need that we considered developing toxic screens that can attract and kill tabanids and *Stomoxys* spp. To the best of our knowledge, such efforts have not heretofore been attempted.

In a series of previous developments, we designed, assayed and defined blue and white fabric screens specifically attracting tabanids, *Stomoxys* spp., *M. crassirostris* and other *Musca* spp. These screens did not attract non-target insects, such as butterflies, bees or other pollinators. These so-called 'fly-screens' are made of a white screen 60×60 cm interspersed with a horizontal blue rectangle on the upper part, which wave-length reflectance that peaks at 450-460 nm (Figure 1). However, the use of fabrics presents a number of disadvantages such as high cost, toxic dying procedure, difficult color monitoring and maintenance, high soaking capacities of insecticides (high cost and loss of insecticide), easy wash-off of the insecticide with rains, etc. These challenges experienced with fabrics led us to develop a new type of screen, thanks to a collaborative project implemented by a consortium called *FlyScreen*, that brings together a number of public institutions and a private/industrial partner. These new screens are made of multilayer and multi-functionalised polyethylene plastic film (a patented protected technology), in which a pyrethroid insecticide is incorporated during the polymer extrusion. The attractivity of haematophagous flies by such polyethylene white and blue screens was demonstrated, using sticky films, in previous studies (unpublished). In the present study, these fly-attracting insecticide impregnated screens (also called 'targets') were evaluated in cattle farms, for their efficacy to control haematophagous flies, under a so-called 'multi-target method' (MTM) (a method using multiple targets (20-30) per farm).

Material and methods

Dairy cattle farms

For the purpose of this study, in order to assess the efficacy of MTM in on-farm situation, small to medium size dairy farms were selected from the dairy production area of Nong Pho, Rachaburi Province, central Thailand. Thanks to a local veterinary worker, pre-selected dairy farms were visited, and the farmers issued with a questionnaire concerning the nuisance of flies and the

Figure 1. The 'multi-target method' (MTM): 18-38 screens were set-up around walking areas (A; left) or in the dung drying area (B; right) in dairy farms, Nong Pho, Kantchanaburri, Thailand.

arthropod control practices regularly implemented in the farm. Farms with too low fly activity, or farms systematically / routinely using smoke or insecticide / acaricide sprays were excluded from the study. For the included farms, the historical frequency of insecticides and smoke usage were recorded, but the use of insecticides was 'proscribed' throughout the experiment. In non-rejected farms, an entomological survey was initiated by using Vavoua traps for one day, once in a week for 2-3 weeks before setting up the screens. Farms exhibiting the lowest fly densities were excluded from the study. Selected farms were then randomly assigned into Test and Control groups, taking into account the fly densities, in order to obtain very close densities on average, in the two groups at the beginning of the experiment.

Mean comparisons of farm size (m^2) and number of cows per farm were made amongst Control group and Test group, using T-student test.

Insect trapping, counting and identification

Insect trapping was performed using Vavoua traps, made according to the available recommendations (Laveissiere and Grebaut 1990), using a 100% polyester blue fabric (CR Solon No 41., Chai Rung Textiles, Thailand). This fabric had previously been characterised as the best polyester blue fabric in terms of attractivity to haematophagous flies in Thailand (Onju *et al.*, unpublished results). For stratified flies sampling, one trap was set-up in the best location of each farm, generally in the centre of the farm, for 24 hours each week of the follow-up, from 18th May 2017 to 22nd February 2018. Grease was placed at the lower part of the iron rods used to set up the traps, to avoid interference of ants in the insect catches.

Stomoxyine flies were identified using a reference key (Zumpt 1973) and previous descriptions made in Thailand (Masmeatathip *et al.* 2006). Tabanids were identified using reference keys (Burton 1978, Philip 1960, Schuurmans Stekhoven 1926), and *Musca crassirostris* were identified using a key for *Musca* spp. from Thailand (Tumrasvin and Shinonaga 1978). However, statistics were carried out at the family level for tabanids and genus for *Stomoxys* and *Musca*, with the exception at species level, of *M. crassirostris* (an abundant obligatory haematophagous *Musca* species). Insect counts were reported in table-data files for statistical analyses.

Fly-screens

Multi-layer multi-functionalised polyethylen plastic films 120 µm thick, including deltamethrin (incorporated during the polyethylen extrusion), were produced by AtoZ Textile Mills Ltd. (Arusha, Tanzania) according to a process protected by a patent (Patent pending no. 1856676, deposited on 18/07/18). Screens are made of a white square plastic sheet, 60×60 cm, with a horizontal blue rectangular section (30×50 cm) located in the center and at 5 cm from the top of the screen (Figure 1). Upper and lower parts of the screens are equipped with grooves that allow fixation using a 10 mm diameter plastic pipe (electric sheath). Screens were set-up at 30 cm above the ground or grass level, on bamboo sticks (hammered 80 cm apart) using hay strings; this optimal highness of the screens had been previously studied and validated (Lescure 2014).

Multi-target method

Due to the generally high density of flies inside dairy cattle farms, and to ensure a high probability that flies land on a screen during their flight in the farm, high number of screens, from 20 to 40 screens, were set up per farm. The number of screens to set up in each farm was defined as:

(number of cows × 0.8) ± 20%. More, or less screens might be necessary, according to topographical conditions in each farm. It is the act of deploying multiple targets per farm that we refer to as MTM.

The distance between the screens varied depending on the situation and size of the farms, ranging from 3-5 m in the smallest farms, to 30-50 meters in the largest ones. Screens were deployed at the most visible and easily accessible locations inside the farms, preferably around stables and walking areas (Figure 1A), or, inside the area used to dry cattle dungs (Figure 1B). This was to enhance visibility by insects emerging inside the farm or coming from outside and including special areas that could be considered as 'ways of passage' or 'channels' for the insects. However, the best spots could not always be used since the screens need to stay out of reach of the animals. Indeed, in some preliminary observations, when some screens were reachable by the cattle, the animals tended to smell, lick and chew the screens, thus reducing the efficacy of the screens and compromising the study protocol. When necessary, the grass was cut prior to setting up the screens, and regularly thereafter to keep the screens as visible as possible.

Statistical analyses

To compare farms size, cattle numbers, density of cattle in tests and control groups, and to compare total screen numbers and the mean numbers of screens set-up per head of cattle, we used mean comparisons according to Student t test; a difference was significant when the calculated t value was below the critical 'Tc' value, at the appropriate degree of freedom, with an assumed *P*-value of 0.05.

Insects density analyses were carried out on *Stomoxys* spp., tabanids, *M. crassirostris*, 'haematophagous flies' (*Stomoxys* spp. + tabanids + *M. crassirostris*), 'common flies' (*Musca* spp. at the exception of *M. crassirostris* which was included in 'haematophagous flies'), and 'total flies' (haematophagous flies + common flies). The mean total number of insects trapped in control farms versus test farms were compared before setting up the screens (2-3 weeks of trapping from 18th May until 7th June 2017) and after setting up the screens; numbers of insects from control versus test farms were compared for every 4 weeks periods (i.e. monthly trappings) for up to 9 months. For the comparison of insect densities in test and control farms, the two-way repeated measures ANOVA was used. Before running the ANOVA, and since our data do not have equal sample sizes (number of farms in control and test groups are different) we used the Welch test to check the homogeneity of variance assumptions (hypothesis of homogeneity of variances was accepted if calculated *P*-value was >0.05). In the next step, the mean numbers of insect trapped in serial measures (per periods of 4 weeks) were compared under the different conditions, in control farms and test farms, using 'R program' (R-Development-Core-Team 2005). The mean insect densities were significantly different when the *P*-value was below 0.05 (Schober and Vetter 2018).

Results

Selection of the dairy cattle farms and farm grouping

Thirty-five pre-selected dairy farms were visited with a local veterinarian to select suitable farms for the purpose of the study. Questionnaires revealed that 6 farms had no problem with flies; these farms were generally very clean, and using automatic or systematic manual spraying of water, twice a day, generally linked with milking time. Four farms were treated regularly using insecticides sprays on cattle, 3 farms were treated using slow fires to produce a repellent smoke on a daily basis; these 7 farms were also excluded from the study. Other farms met the selection

criteria and fly trapping was initiated in a total of 22 farms. In six of these farms, the insect densities recorded during the first two weeks of the survey were too low to be suitable for this study. Thus, these six were excluded. A total of 16 farms were included in the study. Their mean size was 2,888 m^2 (ranging from 700 to 8,800 m^2) and their mean cattle number was 43.5, ranging from 20 to 60 heads.

These 16 farms were randomly split into 2 groups of 4 farms (Control group) and 12 farms (Test group), taking into account the mean numbers of flies trapped during the first 2-3 weeks of the survey. Fly numbers mean comparisons were made to ensure that the two groups exhibit similar flies' densities before setting up the screens. Only mean densities of *Stomoxys* spp, *M. crassirostris* and common flies were compared. Tabanid' densities were too low to be considered independently. Results from the baseline studies (before setting up the screens) indicated that there were no significant differences in mean densities of flies in the control vs test farms for different types of flies and for all flies considered together (Table 1). Overall, mean fly densities were more or less similar even though slightly higher in test farms than control farms.

Multi-target method screen-setting and maintenance

The screens were set up in the 12 test farms, between 15th and 18th June 2017. In the five smallest farms (<1,200 m^2; mean size 818 m^2) with an average of 32.6 cattle, 18-21 screens (average 20 screens) were deployed at a mean interval distance of 3-8 meters, mainly in the open area (where farmers expose cattle dung under the sun for drying). Some other screens were set up around the stables at a distance of 1-2 meters from the animal shelter, depending on the available space around the stable. Screens were never set up inside the stables and were always kept out of the reach of cattle. The number of screens to set up in each farm (number of cows × 0.8 ± 20%) was respected in all small farms.

Table 1. Means, standard deviations and P*-values of two-way repeated measures ANOVA for comparison of flies trapped in Control (n=4) and Test (n=12) farms before screen-setting (18th May-7th June 2017).*

Flies	Group	Mean	Standard deviation	*P*-value
Stomoxys spp.	Test	60.9	8.7	0.54
	Control	59.6	16.8	
Musca crassirostris	Test	43.5	4.4	0.98
	Control	32.2	11.1	
Hematophagous flies[1]	Test	104.6	8.6	0.68
	Control	91.9	21.7	
Common flies	Test	271.4	30.4	0.98
	Control	205.1	31.3	
Total flies	Test	375.9	30.0	0.88
	Control	328.4	58.3	

[1] 'Hematophagous flies' is the total of *Stomoxys* spp. + tabanids + *M. crassirostris*; 'Common flies' includes all *Musca* spp., at the exception of *M. crassirostris* which is included in 'haematophagous flies' (as an obligatory haematophagous fly (Desquesnes *et al.* 2018)); 'total flies' includes haematophagous flies and common flies.

In the 7 medium and large size farms (1,800-8,800 m^2; mean size 4,054 m^2), with a mean number of 49 cows, between 25 and 38 screens (average 30 screens/farm) were set up, at a mean interval distance of 5-10 meters. For the largest farms (6,320 and 8,800 m^2, with 38 and 60 cattle, respectively), a mean interval distance of 20-30 meters was maintained between the screens. Typical screens-deployments under this MTM are presented on Figure 1. The number of screens to set up (number of cows × 0.8 ± 20%) was respected in all farms, except Farm 6, having 60 cows, which received only 30 screens instead of 38-58, thanks to easy and correct coverage of the space, and Farms 13 and 16, which received respectively 2 and 4 more screens to ensure a better coverage of their land space.

Size of the farms, cattle numbers and numbers of screens set up in each farm are presented in Table 2 with some meaningful meta-data. In total, 311 screens were set up in the 12 test farms, with 406 cows. The MTM was implemented with a little less than one screen per cow (average 0.79±0.10 screen per cow). In other words, a mean of 4 screens for every 5 cows. There were no significant differences in mean farms size (in m^2), number of cows and cow densities between control and test farms.

Table 2. Characteristics of the farms and screen-settings in the test and control farms.

Farm no.	Farm size (m^2)	Number of cows	Cow density (m^2/cow)	Number of screens set up	Screen density m^2/screen	Number of screen/ cattle	Smoke used as repellent	Insecticide sprays frequency
Test farms								
F1	700	27	26	20	35	0.74	+++	+++
F2	700	29	24	21	33	0.72	+++	+++
F3	780	27	29	18	43	O.67	++	0
F4	760	35	22	21	36	0.60	0	0
F5	1,845	36	51	30	62	0.83	+	+
F6	8,800	60	147	30	293	0.50	++	0
F7	6,320	38	166	30	211	0.79	+	0
F13	2,706	24	113	25	108	1.04	++	0
F14	3,710	36	103	25	148	0.69	+	0
F15	1,150	25	46	20	58	0.80	+	++
F16	2,720	35	78	38	72	1.09	0	0
F17	2,275	34	67	33	69	0.97	+	0
Totals	32,466	406		311				
Means (±95% CI)	2,706±1,428	33.8±5.4	73±28	25.9±3.5	97±46	0.79±0.10		
Control farms								
F12	3,300	30	110				0	0
F20	700	20	35				0	0
F21	465	31	15				+	++
F22	680	23	30				0	0
Totals	5,145	104						
Means (±95% CI)	1,286±1,320	26.0±5.2	47±42					

Screens were maintained during the weekly insect-trapping. Servicing of screens consisted in checking and reinforcing the firmness of the strings and sticks and repairing screens that had been torn off either by strong wind or animals. The grass beneath and around the screens was also cut twice a month, depending on the season, to insure full visibility of the screens by the flies, on at least 180°, and, when possible 360° around. All screens lasted 6 months in the farms and were then removed mid-December 2017. However, trappings of insects were implemented for another 3 months, until February 2018.

Dynamics in insect densities in the two groups

Welch tests carried out prior to run the ANOVA, demonstrated that the homogeneity of variance assumptions was acceptable; indeed, before the screens were set up, all *P*-values were above 0.05, ranging from 0.58 for *Stomoxys* spp., up to 0.98 for common flies. The weekly insect catches performed using Vavoua traps are presented on the figures; after screen settings, data were averaged by periods of 4 weeks for representation in the figures. The density of flies at the beginning of the study are the means of weekly trappings made at the end of May-early June 2017, just before the screens were set up. Further on, monthly average of four consecutive weekly trappings were made to represent the monthly trends of fly densities from June 2017 to February 2018. Figure 2 represents the mean apparent densities per trap (ADT) of haematophagous flies in Control (n=4; black interrupted line) and Test group (n=12; grey line). Although the Test group

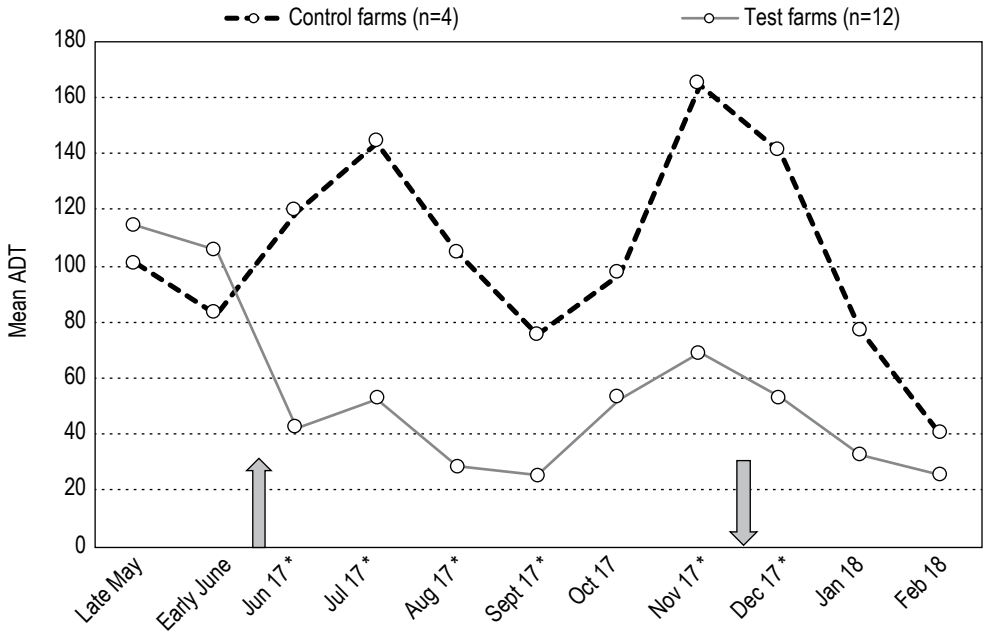

Figure 2. Variations of the average monthly apparent density per trap (ADT) of haematophagous flies in Control farms (black interrupted line) and Test farms (grey line) along the study. Asterisks placed after month-labels are indicating significant difference between Test and Control groups apparent densities; Pointing-up arrow indicates the date of screen deployment (15th-18th June 2017); Pointing-down arrow indicates the date screens were removed (14-15th December 2017).

exhibited slightly higher insect densities than Control group before screens were deployed, the two-way rerepeated ANOVA did not show a significant difference (*P*-values 0.54 for *Stomoxys* spp, 0.98 for 'common flies' and 0.88 for 'total flies'). The two groups were therefore considered as exhibiting similar fly densities before the screens were set up (Table 1, columns 'Before screens setting'). Mean comparisons made before screens-setting and, monthly after screens-setting are summarised in Table 3 for both *Stomoxys* spp., *M. crassirostris*, total haematophagous flies (including tabanids), common flies (*Musca* spp. with the exception of *M. crassirostris*) and total flies. Densities of all flies observingly decreased (Figure 2 and Figure 3) and significantly so (Table 3; all *P*-values <0.05 in June, August and September 2017) in the test farms compared to the control farms just after the screens were set up. Except for *Stomoxys* spp in July, all flies were significantly decreased during the 4 months post treatment (June to September). As shown on Table 4 and Figure 2, in October, the natural decrease of fly populations seems to cancel out the difference between Test and Control groups (all *P*-values >0.05), but significantly lower densities appear again in Test farms in November-December, due to a huge natural increase in fly densities observed in the Control group. From January 2018, the differences between Test and Control groups disappeared, 8 months after screen-setting.

The natural seasonal trend of fly density is shown by the apparent density of the flies in the Control farms, but the trend is different in test farms, although it tends to follow the same pattern especially for *Stomoxys* spp. and as a consequence in haematophagous flies.

Figure 3 is representing the percentage of flies in control farms versus test farms ('mean ADT in test farms' divided by 'mean ADT in control farms'); from 100% flies and more, before screen setting, the percentage of flies trapped in control farms fell down by 63-73% during the first 4 months, and was slowly recovering, reaching only 63% of control farms, 9 months after screen-setting (Feb 2019).

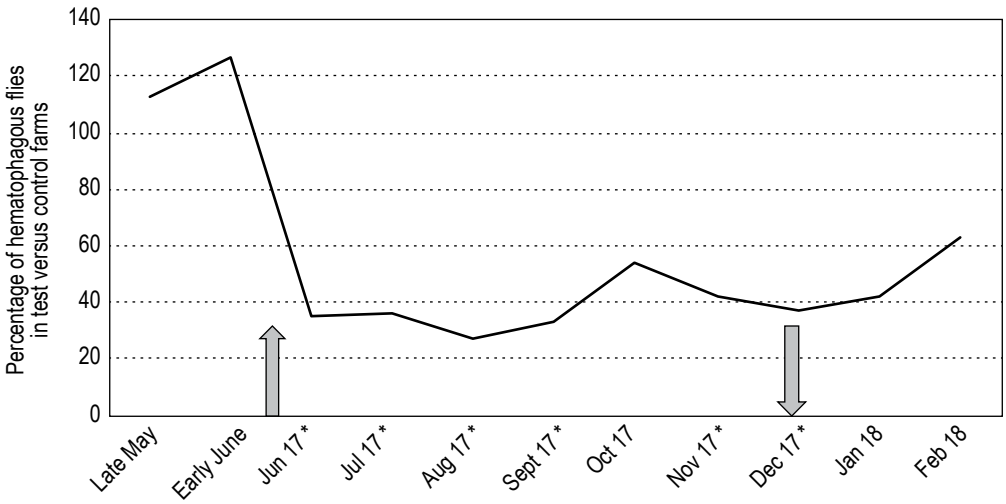

Figure 3. Percentages of flies trapped in test farms (n=12) versus control farms (n=4) along the study (mean apparent density per trap (ADT) in test farms / mean ADT in control farms).

Table 3. Means and P*-values of two-way repeated measures ANOVA for comparison of flies trapped in Control (n=4) and Test (n=12) farms before screen-setting (18th May-7th June 2017) and from June to September 2017.*[1]

	Farms	Before screens setting		Jun 17		Jul 17		Aug 17		Sept 17	
Flies	groups	mean	*P*-value	mean	*P*-value	mean	*P*-value	mean	*P*-value	mean	*P*-value
Stomoxys spp.	Test	61	0.545	22	**0.028**	32	0.140	19	**0.020**	16	**0.017**
	Control	60		68		54		52		44	
Musca crassirostris	Test	44	0.979	20	**0.003**	20	**0.000**	10	**0.000**	9	**0.000**
	Control	32		51		89		52		31	
Hematophagous flies	Test	105	0.676	42	**0.006**	53	**0.002**	29	**0.000**	25	**0.000**
	Control	92		119		144		105		75	
Common flies	Test	271	0.984	67	**0.000**	77	**0.036**	47	**0.000**	55	**0.002**
	Control	205		186		207		148		142	
Total flies	Test	376	0.881	109	**0.000**	130	**0.014**	76	**0.000**	80	**0.001**
	Control	297		306		351		252		217	

[1] Differences between Test and Control farms are significant when *P*-value is <0.05; which is indicated in bold.

Table 4. Means and P*-values of two-way repeated measures ANOVA for comparison of flies trapped in Control (n=4) and Test (n=12) farms from October 2017 to February 2018.*[1]

	Farms	Oct 17		Nov 17		Dec 17		Jan 18		Feb 18	
Flies	groups	mean	*P*-value	mean	*P*-value	mean	*P*-value	mean	*P*-value	mean	P-value
Stomoxys spp.	Test	39	0.082	45	0.071	30	**0.025**	14	**0.045**	9	**0.012**
	Control	77		86		72		36		19	
Musca crassirostris	Test	13	0.384	23	**0.003**	22	**0.004**	18	0.107	16	0.394
	Control	20		77		68		41		21	
Hematophagous flies	Test	53	0.059	69	**0.003**	53	**0.002**	32	0.058	25	0.119
	Control	97		165		141		77		40	
Common flies	Test	67	0.104	125	**0.000**	146	**0.019**	94	0.064	117	0.579
	Control	142		550		394		265		147	
Total flies	Test	120	0.076	194	**0.000**	199	**0.011**	126	0.060	142	0.467
	Control	239		715		535		342		187	

[1] Differences between Test and Control farms are significant when *P*-value is <0.05; which is indicated in bold.

Indeed, in control farms, *Stomoxys* spp. (Figure 4A), and consequently 'haematophagous flies' (Figure 2) exhibit the first peak of activity in end of June-early July, followed by a gradual decrease during the heavy rainy season, reaching a minimum in September and peaking again in October-December. Thereafter, the density declines to a minimum in February, likely due to cool and dry season. In the test farms, the peak of June is completely prevented, either reduced to a very

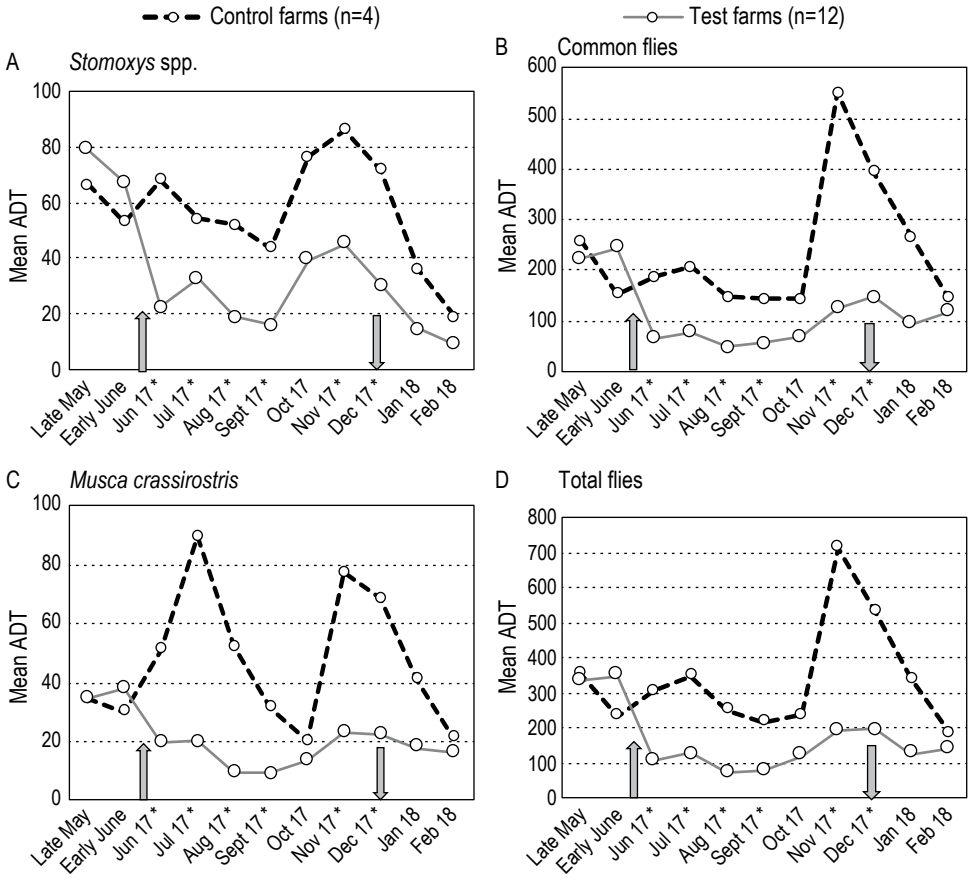

Figure 4. Variations of the average monthly apparent density per trap (ADT) of (A) Stomoxys *spp., (B) common flies, (C)* Musca crassirostris *and (D) total flies, in Control farms (black interrupted line) and Test farms (grey line) along the study. Asterisks placed after month-labels are indicating significant difference between Test and Control groups apparent densities; pointing-up vertical arrows indicate the date of screen deployment (15th-18th June 2017); pointing-down arrows indicate the date screens were removed (14-15th December 2017).*

low level in some farms, or to medium level in others (data not shown), but the general profile of fly dynamics remains the same in all farms, with two conspicuous peaks roughly in July and November (Figure 2 and 4).

In the Control group, *M. crassirostris* density peaks a little later than *Stomoxys* spp., in July to August, and then follows the same trend as *Stomoxys* spp., while common flies have a minor peak in July and a major one in November-December. In the test farms, both peaks of July and November of *M. crassirostris* and common flies in November are almost completely prevented (Figure 4B and 4C).

Overall, fly densities exhibited two peaks, i.e. in July and November. The peaks were clearly brought under control in all test farms, although the second peak of *Stomoxys* spp. was not completely under control. The effect of the treatment seems to disappear completely by January-February 2018, 8-9 months after screen-setting (2-3 months after the screens were removed), when insect populations are naturally decreasing due to the dry season.

Discussion

Although fly density in test farms was slightly higher than in control farms at the beginning of the study, they were very close before the screens were deployed, thus, validating the comparison between the groups. As shown by fly catches (and confirmed by farmer testimonies), there was a reduction of 63-73% in fly densities during the 4 months after the screens were deployed. Despite a slight increase in October (month 5 after screen setting), the fly reduction was still around 60%, up to the seventh month after screens were deployed. At month nine post screen-deployment, the fly density recorded in test farms was still only 37% of the control farms. These values show a medium-term effect of the treatment, quite longer than the toxic activity of the screens which was estimated though a tarsal contact test (Makoundou *et al.* 1995) in laboratory reared *Stomoxys calcitrans*, to be around 4-5 months (data not shown).

The natural trend in fly density observed in the Control group showed two peaks in July and November-December. In the Test group, the first peak is almost completely prevented and the second one is lowered. As a consequence, the effect of the screens can be split into four phases, (1) a first phase for 3-4 months, just after the screen deployment, during which the fly densities in Test group clearly decreases while that of the Control group increases, (2) a natural decrease in fly populations observed in the Control group in October (5 months after screen-setting) during which the difference between Test and Control groups is no more significant, although the all flies density in the Test group is still below that of the Control group (Figure 4D), (3) a huge peak of fly density observed at months 6-7 (November-December 2017) in Control group, and a much lower one in the Test group, thus, making the differences between Test and Control groups significant again during this window of time (likely as a consequence of the early impact of the treatment on fly population dynamic), and (4) a final phase of natural decline in fly density in both groups (January-February 2018), during which the effect of the treatment seems to disappear completely.

Overall, the toxic effect of the screens in the field, as was evaluated in weathering studies carried out on laboratory reared *Stomoxys calcitrans* (data not shown), was estimated to last around 3-4 months. Although the residual effect might extend a little longer, the effect of the screens was expected to last around 4 months in the field. Indeed, the effect of the treatment seems to disappear at month 5 (October), when no significant difference is recorded between Test and Control groups. However, this event coincides with the natural decrease of the fly population shown by the decrease of flies in Control group. Further effects observed in the field might therefore not be due to the killing effect of the screens during the period November-December, but rather because of the early impact of the screens, affecting fly population dynamic for up to 7 months after screen deployment. Considering that the toxic effect of the screens is expected to only the first 3-4 months, a population control of up to 7 months is quite satisfying, in our view. Of course, an extended durability of the toxicity of the screens, lasting for example, up to 12 months, would have a bigger and more sustainable impact on fly densities in the field.

Five farms of the test group (F1, 2, 3, 4, 15) individually showed lower effects than the others, especially for *Stomoxys* spp. (details not provided). In these farms, the effect of the screens was

visible for all flies during the 4 months following screens-setting, but not statistically significant for *Stomoxys* spp., and inconsistently significant for common flies, suggesting possible development of insecticide resistance. Supporting a chemoresistance suspicion, the regular use of insecticides in these farms was higher than the average of other farms, including Control farms (Table 2A and 2B), with one farm using insecticide sprays regularly (F15: ++), and two farms using them systematically (F1 & F2: +++); the other two farms (Farm 3 & 4) were not using them, but they are very close neighbours of Farms 1 & 2.

Beside insecticide resistance, other parameters could be considered, possibly contributing to the lower response to the treatment observed in these 5 test farms, in comparison with the others. These farms were smaller in size (818 m^2/4,054 m^2), their mean number of cows was also lower (29/38), but they exhibited a higher density (1 cow/41 m^2) than the other group (1 cow/104 m^2); they received lower mean numbers of screens (20.0/30.1) and thus the number of screens set-up per cow was lower in these farms (0.72) compared to the others (0.84). Considering these observations, it can be hypothesised that a balance between the density of screens and cows needs to be considered in order to optimise the effects of the treatment.

The cost of production of screens produced under this new technology being quite low (around 1 €/screen), the MTM has a real potential to be easily and early adopted by the farmers.

Conclusions

The effect of the insecticide-impregnated screens was obvious as soon as the screens were set up, as was repeatedly mentioned by the farmers, and confirmed by the mean apparent density of flies per trap (ADT) observed in test farms versus control farms; indeed, ADT in test farms fell between 63-73% of the control farms, during the first 4 months after the multiple targets were set up.

The proof of concept of the MTM was demonstrated by this study. Results obtained suggest that, on average, setting-up of around 1 screen per cow could be the optimal rate appropriate for the control of haematophagous flies. Thanks to a reasonable cost of these screens, the MTM has a fair potential for early and large-scale adoption.

Durability of the toxic effects of the screens should be improved in the next generation of screens, with the aim of achieving one-year (12 months) efficacy for practical timeline reasons.

Other farming systems might also benefit from this control method which should be evaluated, for example, in horse, pig and poultry farms.

Acknowledgements

The authors are grateful to AtoZ Textile Mill Ltd. (Arusha, Tanzania) for providing the insecticide impregnated screen prototypes used in this study. This work was funded by the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), The Thailand International Cooperation Agency (TICA 'One Health') and the French National Research Agency [Agence Nationale de la Recherche (ANR)], under the 'FlyScreen' project' (ANR-15-CE35-0003). The authors declare no conflict of interest.

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