

Looking for the gold standard: assessment of the effectiveness of four traps for monitoring mosquitoes in Italy

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ABSTRACT: Several kinds of traps are available for the collection of Culicidae species creating nuisance problems and/or a potential risk of pathogen transmission. The choice of the most appropriate sampling device should take into consideration the objective of the monitoring activity (e.g., faunistic research, vector control evaluation, arbovirus surveillance, etc.), the ecological and behavioral characteristics of the target mosquito species, and the ecology of the sampling areas. However, there are few factual criteria technical personnel can rely on to choose the most suitable sampling method, particularly when the targets are represented by mosquito species in temperate areas. We carried out a Latin square experiment in three ecologically different settings in Mantua municipality (northern Italy) to compare the performance of four different traps targeting host-seeking mosquitoes: two traps specifically designed for mosquito monitoring purposes (Centers for Disease Control and Prevention CO₂ trap and Biogents BG Eisenhans de Luxe trap) and two designed to reduce mosquito densities in outdoor domestic settings (Activa Acti Power Trap PV 440 and Activa Acti Power Trap MT 250 Plus). Overall, 1,930 specimens belonging to nine species were collected and differences in the performance of the four traps with reference to their ability to detect overall species diversity, as well as to collect single species, were highlighted. These observations, coupled with an analysis of the costs associated with the trap's purchase, operation, and servicing, provide useful indications for the implementation of mosquito monitoring in temperate areas. *Journal of Vector Ecology* 37 (1): xxx-xxx. 2012.

Keyword Index: Culicidae, mosquito traps, vector surveillance, *Aedes albopictus*.

INTRODUCTION

Monitoring mosquito populations represents a key aspect for identifying risks of pathogen transmission to humans and animals, necessary for an efficient targeting of control measures against pest and vector species. Several approaches for mosquito monitoring are available, but their specificity and sensitivity vary in relation to the ecological context and the target mosquito species. Human landing catch is a sampling method traditionally used in several contexts and can be highly effective to evaluate biting rates, but it requires a large effort, is not ethically feasible in the case of disease vectors, and could provide biased results in the case of species with limited windows of feeding activity. Other widely used sampling procedures are “resting catches,” realized with different methods (e.g., aspirators, insecticide spraying), which have the disadvantage of being time consuming, strongly dependent on mosquito resting habits, and although largely used in tropical rural villages, difficult to be applied in urbanized areas. Alternatively, trapping devices are widely used to estimate species abundance and composition. Light-traps have been the primary tool for monitoring mosquito populations (Sudia and Chamberlain 1988, Odetoynbo 1969, Service 1970), and their sensitivity has been improved by the release of CO₂ as an attractant (Cooperband and Carde 2006). Other

traps actively capturing host-seeking females attracted by artificial baits, simulating the complex odor blend produced by a host, have been recently developed (Mathenge et al. 2002, Hoel et al. 2007, Brown et al. 2008, Kaufman et al. 2008, Ritchie et al. 2008, Kweka and Mahande 2009). Each of these traps has peculiar characteristics that influence estimates of species abundance and diversity (Huffaker and Back 1943, Acuff 1976).

Ideally, it could be possible to employ a multiplicity of trap designs and attractants to ensure that all possible species are adequately detected (Van Essen et al. 1994, Russell 2004, Muturi et al. 2007), but time and resource constraints often favor simpler, and less precise, monitoring systems. In this case, in order to minimize sampling biases, the choice of the most appropriate sampling device should take into consideration the objective of the trapping activity (e.g., monitoring for faunistic purposes, vector control evaluation, arbovirus surveillance, etc.), the ecological and behavioral characteristics of the target species, and the ecology of the sampling areas. However, indications of objective criteria for technical personnel to base their choice on are scarce, particularly when the targets are represented by mosquito species in temperate areas.

This paper aims to compare the performance of four different traps targeting host-seeking mosquitoes, two traps specifically designed to monitor mosquito species

(Centers for Disease Control and Prevention CO₂ trap and BG Eisenhans de Luxe) and two designed to reduce mosquito densities in outdoor domestic settings (Acti Power Trap PV 440 and Acti Power Trap MT 250 Plus), in different ecological settings in Mantua Province (northern Italy). Results are discussed not only with reference to their relative performance (i.e., sensitivity and specificity) in the study sites but also taking into consideration economic aspects and management features in order to propose objective criteria for choosing the most appropriate tool for monitoring and surveillance protocols in European mosquito-infested areas.

MATERIALS AND METHODS

Traps

Four traps specifically developed to collect host-seeking mosquitoes have been tested. All these traps collect specimens by an aspirating system, but each of them is differentiated by several characteristics:

The CDC CO₂ trap is a widely used trap for mosquito sampling and is generally considered to be the gold standard for monitoring of host-seeking mosquitoes. The mosquitoes are aspirated by a fan in a collection bag. In this experiment, it has been equipped with dry ice as a source of CO₂ with no chemical lures added in order to maintain the “classical” set-up of the trap. In our experimental setting, the CDC was placed suspended from a support at about 1.5 m high, whereas the other three traps were placed on the ground.

The Biogents BG Eisenhans de Luxe (hereafter called BG-Eisenhans) represents an advanced model of the better known BG-Sentinel trap (http://www.biogents.com/cms/website.php?id=/en/traps/mosquito_traps.htm). Both traps are conceived to collect mosquitoes with an aspirating system based on a recirculation of the air-flow mimicking convection currents created by a host; one single fan draws the attracted mosquito into a catch bag below the central dark intake of the trap. The airstream then exits the trap through a white surface around the central intake. The Eisenhans model is different from the Sentinel one because it is equipped with a CO₂ cylinder as a source of carbon dioxide whose release is regulated by a computer. The trap is also equipped with the odor attractant of one bag of Sweetscent attractive and UV light. During the experiments, the latter was deactivated to reduce collection of insects other than mosquitoes (such as Lepidoptera).

The Acti Power Trap PV 440 (hereafter called PV) is a residential trap produced by ACTIVA S.r.l. (www.noflyzone.net), aspirating insects by a fan through a circular slot and storing them in a collection drawer. It is equipped with three Sweetscent attractive bags (as recommended by the producer), a UV light (deactivated during these experiments), and some flashing colored LEDs (which are conceived to make the trap also attractive for flies, but that were impossible to remove or deactivate).

The Acti Power Trap MT 250 Plus (hereafter called as MT) is also produced by ACTIVA S.r.l. and is very similar to the PV trap. This residential trap utilizes propane as a

source of CO₂, water vapor, and heat by computer-regulated platinum catalysis. A Sweetscent bag was added as additional odor attractant.

Study sites

The study was carried out in the municipality of Mantua (northern Italy), an urban area surrounded by highly variable ecological settings (i.e., woods, gardens, rivers, lakes, channels, and swamps) and characterized by a very abundant and diverse mosquito fauna. Based on preliminary larval sampling carried out in 2008 (data not shown), three ecologically different sites were chosen to carry out the experiments: Bosco Virgiliano (45°08'N, 10°48'E), a wild forested area near a lake (Lago Inferiore), mostly characterized by the presence of several *Aedes vexans* breeding sites along its shores; Cittadella (45°10'N, 10°47'E), a typical urban environment showing high densities of *Aedes albopictus* and *Culex pipiens*; and Belfiore (45°09'N, 10°46'E), a green urban area localized on the bank of a lake (Lago Superiore) with a large island of lotus plants (*Nelumbium lucifera*) where *Anopheles maculipennis*, *Culex modestus*, *Cx. pipiens*, and *Coquillettia richiardii* were found to be abundant.

Experimental design

Two replicates of a Latin square experiment were performed in each site in August-September, 2009, for a total of six experiments. In each replicate, the four traps were placed at about 5 m from each other and their position was rotated daily so that, at the end of the four-day replicate, each trap had occupied each corner of the Latin square. Dry ice was recharged and batteries changed each day in the CDC, while the other traps did not require any maintenance during a single experiment. Sample collections were carried out each 24 h since trap activation. At the end of the fourth day, the traps were removed.

Statistical analysis

The data obtained were analyzed by non-parametric methods, as not normally distributed. Kruskal-Wallis test were used to compare differences in trap collections, whereas the two-by-two trap comparisons were carried out by the Mann-Whitney test. Multiple tests were corrected with stepwise Holm's method for multiple comparisons (Holm 1979). All analyses were carried out using Statsoft STATISTICA software, version 8.

Simpson's Diversity Index (Simpson 1949) was applied to evaluate species diversity, using the formula:

$$1 - D = \frac{1 - \sum n_i (n_i - 1)}{N(N - 1)}$$

The 95% confidence limits of this index were calculated as:

$$\pm 2 \sqrt{\frac{\sum \left(\frac{n_i}{N}\right)^3 - \left(\sum \left(\frac{n_i}{N}\right)^2\right)^2}{N(N - 1) \cdot 4}}$$

where n_i is the abundance of the species i and N is the total

number of individuals per sample.

The Simpson's index of evenness (E) was calculated to obtain a measure of the relative abundance of the different species in the sample, using the formula $E=1-D/S$, where S is the number of species sampled.

RESULTS

Overall, 1,930 specimens, 96% female, belonging to nine species, were collected during the six Latin square experiments: *Culex pipiens* (38%), *Culex modestus* (35.1%), *Anopheles maculipennis* sensu lato (10.8%), *Aedes albopictus* (7.9%), *Aedes caspius* (4.6%), *Aedes vexans* (3.1%), and *Coquillettidia richiardii* (0.4%), *Anopheles claviger* (0.1%) (Table 1). Sixty-five percent of the mosquitoes, mostly *Cx. modestus* and *Cx. pipiens* (Table 2), were collected in Belfiore, 27% in Bosco Virgiliano (mostly *Cx. modestus* and *Cx. pipiens*), and 8% in Cittadella (mostly *Ae. albopictus* and *Cx. pipiens*). A significantly higher diversity of species was observed in Bosco Virgiliano (Simpson's $1-D=0.72$) with respect to the other two sites (Table 3).

The relative capture rates of the four traps were very different: CDC collected 53.5% of the overall specimens, BG-Eisenhans 35.6%, MT 7.3%, and PV 3.6%. Also, the relative proportion of mosquito species collected by each trap was significantly different (Table 4): CDC trap was most effective in collecting *Cx. modestus* and *Cx. pipiens*, whereas BG-Eisenhans trap was shown to be highly effective in collecting *Ae. albopictus*, *Ae. vexans*, *Ae. caspius*, and *An. maculipennis* s.l.

Table 5 summarizes the results of the comparison among trap performances for each species. Significant differences were observed for *Ae. albopictus* (Kruskal-Wallis test, $H=33.51$; $p<0.001$), *Ae. caspius* ($H=12.09$; $p=0.007$), and *Cx. pipiens* ($H=9.36$; $p=0.02$). In the case of *Ae. albopictus*, these differences were due to the higher number of specimens collected with the BG-Eisenhans trap with respect to the other traps (Mann-Whitney test, $p<0.001$ in each comparison, Table 6) and were consistent among sampling sites and periods of the sampling (Kruskal-Wallis test, $0.001<p<0.019$). In the case of *Cx. pipiens* and *Ae. caspius*, the difference was due to a higher performance of BG-Eisenhans trap with respect to PV trap only (Mann-Whitney test, $p<0.008$ and $p<0.001$, respectively, Table 6).

The Simpson's index, based on the total numbers of mosquitoes collected by each trap, is significantly higher for BG-Eisenhans and PV than for CDC and MT (Table 3). Moreover, BG-Eisenhans was shown to collect the different species more homogeneously than the other traps, as indicated by the highest Evenness index (4.58) obtained with this trap, with respect to those obtained with PV (3.31), CDC (1.05), and MT (0.80).

DISCUSSION

The development of commercial devices aimed to reduce pest nuisance in domestic environments recently increased the panorama of trapping instruments to be

used to collect mosquitoes for monitoring and surveillance programs (Kline 2006, Brown et al. 2008, Hoel et al. 2009). The results of our comparative field evaluation of the reference CDC trap and of three commercially available traps targeting host-seeking female mosquitoes provide relevant indications for people involved in the monitoring and surveillance of mosquito species in European infested areas.

Representativeness of adult collections and effectiveness in collections of species diversity

The comparison was carried out in three sites of the Municipality of Mantua characterized by high diversity and high abundance of Culicidae species, as shown by preliminary larval surveys carried out in 2008 highlighting a differential presence of different typologies of breeding sites (e.g., regularly flooded river banks, natural ponds, swamps, irrigated cultivation, domestic water containers) in each site. *Cx. pipiens* and *Cx. modestus* were found to be highly abundant in all sites. Bosco Virgiliano was identified as the site with the significantly highest diversity of species (Table 3). On the other hand, as expected, the highest densities of *Ae. albopictus* were found in the most urbanized site, i.e., Cittadella, confirming the strong association of this species with domestic environments in Italy.

The analysis of trap effectiveness in collections of species diversity shows that the ability of BG-Eisenhans and PV traps to collect the highest number of mosquito species was significantly higher than that of CDC and MT (Table 3). BG-Eisenhans trap showed the highest Evenness index, indicating that this trap collected the different species more evenly than the other traps and could represent the best choice in case of entomological surveys carried out in areas where the mosquito fauna is not known in advance.

Trap effectiveness in collections of single species

The four traps tested in the study showed strong differences in collecting single mosquito species. In particular, the BG-Eisenhans trap showed the greatest effectiveness in collecting *Ae. albopictus*, even in ecological situations where this wasn't the predominant species, and a significantly higher effectiveness than PV-trap in collecting *Ae. caspius* and *Cx. pipiens*. It should be emphasized that these results represent the first comparative field evaluation of the BG-Eisenhans trap, showing that the performance of this trap is similar to those of the BG-Sentinel trap, which has been shown to be particularly efficient in collecting *Aedes* mosquitoes in different areas (i.e., *Ae. aegypti*: Brazil, Krockel et al. 2006, Maciel de Freitas et al. 2006; Australia, Williams et al. 2006, 2007. *Aedes albopictus*: Australia, Ritchie et al. 2006; Virginia, U.S.A., Meeraus et al. 2008; New Jersey, U.S.A., Farajollahi et al. 2009; Virginia, U.S.A., Bhalala and Arias, 2009. *Aedes japonicus*, *Ae. triseriatus*, *Ae. vexans*, and *Anopheles punctipennis*: New Jersey, U.S.A., Farajollahi et al. 2009). Moreover, our collections of high numbers of *Cx. pipiens* and *Cx. modestus* with the BG-Eisenhans trap are in agreement with evidence of the BG-Sentinel trap being effective in collecting *Cx. pipiens* and

Table 1. Culicidae collected during Latin square experiments carried out in three sampling sites in Mantua municipality, subdivided by species and gender. Abbreviations: BG = Biogents Eisenhans de Luxe; CDC = Centers for Disease Control and Prevention CO2 trap; PV = Acti Power Trap PV 440; MT = Acti Power Trap MT 250 Plus; f = female; m = male.

Site	Trap	<i>Aedes albopictus</i>		<i>Aedes vexans</i>		<i>Anopheles claviger</i>		<i>Anopheles maculipennis s.l.</i>		<i>Coquillettidia richiardii</i>		<i>Culiseta annulata</i>		<i>Culex modestus</i>		<i>Culex pipiens</i>		<i>Aedes caspius</i>		Total
		f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	
Belfiore	BG	9	1	2		85	1					34		166	1			22		321
	CDC	3		8		29				6		495		239				19		799
	MT			1		22	14					4	3	29	10			13		96
	PV		1	2		4	15					1		11	2					38
	total	12	2	13		140	30			6		534	3	445	13			54		1,254
Bosco Virgiliano	BG	52	2	33		16	1			1		88	8	49	2			20		273
	CDC	6		8		6						27		160				4		211
	MT	3		1		1						13		5	1			2		26
	PV	1		2		1	1					1		6	2					14
	total	62	2	44		24	2			1		129	8	220	5			26		524
Cittadella	BG	51	8	1		6						1		24				3		94
	CDC	1				5								12				4		22
	MT	7			1	1								7	2					18
	PV	7		1		1						2	1	5				1		18
	total	66	8	2	1	13					3	1	48	2				8		152
Grand Total		140	12	59	1	177	32			7	2	666	12	713	20			88		1,930

Table 2. Relative proportions of species collected during Latin square experiments carried out in Mantua municipality grouped per sampling site.

Site	<i>Aedes albopictus</i>	<i>Aedes vexans</i>	<i>Aedes caspius</i>	<i>Anopheles maculipennis s.l.</i>	<i>Culex modestus</i>	<i>Culex pipiens</i>	other species*
Belfiore	1.1%	1.0%	4.3%	13.6%	42.8%	36.5%	0.6%
Bosco Virgiliano	12.2%	8.4%	5.0%	5.0%	26.1%	42.9%	0.4%
Cittadella	48.7%	2.0%	5.3%	8.6%	2.6%	32.9%	0.0%

Table 3. Simpson's Diversity Index and Evenness Index calculated for each sampling site in Mantua municipality and for each trap tested. Abbreviations: BG = Biogents Eisenhans de Luxe; CDC = Centers for Disease Control and Prevention CO₂ trap; PV = Acti Power Trap PV 440; MT = Acti Power Trap MT 250 Plus; c.f. = confidence limits.

	Site			Trap			
	Belfiore	Bosco Virgiliano	Cittadella	BG	CDC	MT	PV
Simpson's 1-D	0.66	0.72	0.65	0.97	0.86	0.79	0.96
upper 95% c. l.	0.65	0.70	0.60	0.97	0.85	0.75	0.94
lower 95% c. l.	0.68	0.75	0.70	0.98	0.88	0.83	0.98
Evenness	0.37	0.45	0.47	4.58	1.05	0.80	3.31

Table 4. Relative proportions of trap captures per each species collected during Latin square experiments carried out in three sampling sites in Mantua municipality. Abbreviations: BG = Biogents Eisenhans de Luxe; CDC = Centers for Disease Control and Prevention CO₂ trap; PV = Acti Power Trap PV 440; MT = Acti Power Trap MT 250 Plus.

Trap	<i>Aedes albopictus</i>	<i>Aedes vexans</i>	<i>Aedes caspius</i>	<i>Anopheles maculipennis s.l.</i>	<i>Culex modestus</i>	<i>Culex pipiens</i>	other species*
BG	80.9%	60.0%	51.1%	52.2%	19.3%	33.0%	20.0%
CDC	6.6%	26.7%	30.7%	19.1%	77.0%	56.1%	60.0%
MT	6.6%	5.0%	17.0%	18.2%	2.9%	7.4%	0.0%
PV	5.9%	8.3%	1.1%	10.5%	0.7%	3.5%	20.0%

Cx. restuans in Germany (Rose et al. 2006).

The planning of mosquito monitoring campaigns requires, besides a deep knowledge of efficacy of the different traps in collecting the target species, an analysis of the campaign costs, which include the purchase of the traps and of additional instruments, such as pressure regulators for carbon dioxide or propane cylinders, their operational costs, and their servicing costs. The impact of these costs on the monitoring campaigns depends on its dimension, its duration, and its recurrence in different years. In our case, the purchase cost was lowest for the CDC trap and highest for the MT (if cost for CDC=1, then costs were 1.4, 2 and 4 for PV, BG-Eisenhans and MT, respectively). In the attempt to define a relationship between trap costs and the collection effectiveness, we associated the numbers of mosquitoes collected with the actual purchase and operational costs related to our experiments, thus highlighting that the cost of a single collected specimen was very different for the four traps. If a value of 1 is assigned to the cost of a single mosquito captured by CDC trap, the relative cost was 2.7, 22.1, and 30.7 for BG-Eisenhans, PV, and MT, respectively. However,

it should be considered that these figures significantly overestimate the relevance of purchase costs, which are very high for the latter two traps, while underestimating that of the operational and servicing costs, due to the fact that our experiments involved only eight monitoring events per trap per site. This scenario could not be taken as representative of the costs of a regular monitoring campaign, which implies a much more extensive exploitation of the traps utilized, thus decreasing the impact of the initial purchase costs while increasing those of the operational and management costs. Thus, we used the actual costs of our experiment to estimate the costs of hypothetical monitoring campaigns, in which each single trap is supposed to be utilized 23 times/year (i.e., weekly between May and September, corresponding to a typical mosquito reproductive season in Italy) for either one, two, or three years. Interestingly, although the overall costs strongly decrease with the increase of trap use (between 1/2 and 1/3 of the overall costs associated with our experiment when the three-year scenario was considered), the ranking among the four traps utilized (i.e., costs for CDC<PV< BG-Eisenhans<MT) did not vary, whatever

Table 5. Results of statistical analysis (Kruskal-Wallis test) for differences in mosquito captures among the four traps per each species collected during Latin square experiments in Mantua municipality.

Species	H(3,N=192)	p-value
<i>Aedes albopictus</i>	33.51	< 0.001*
<i>Aedes vexans</i>	4.85	0.18
<i>Aedes caspius</i>	12.09	0.007*
<i>Anopheles maculipennis s.l.</i>	1.46	0.69
<i>Culex modestus</i>	3.72	0.29
<i>Culex pipiens</i>	9.36	0.02*

scenario was considered.

Finally, it is crucial to note that the four traps also require a very different servicing effort, which has not been considered in the above calculations, but has a very relevant impact on the cost of monitoring campaigns. First, the weight of the trap, which is related to the effort required for their transportation, was about 4 kg for CDC, 9 kg for PV, 16 kg for MT (which requires a propane cylinder), and 27 kg for BG-Eisenhans (which requires a carbon dioxide cylinder). Second, the time required for trap positioning, activation, and deactivation is considerably shorter for the CDC trap, intermediate for BG-Eisenhans and PV traps and longer for the MT trap, due to the need of a warming up to activate the catalytic process, which should consist of a few minutes but often needs consecutive resetting. Third, the time necessary to collect the samples is short for CDC and BG-Eisenhans traps where the mosquitoes are collected in a net-bag and the procedure for changing the bag is fast and easy. On the other hand, in MT and PV traps, which are designed for long-lasting mass-captures in permanent locations, the insects are stored in a collection drawer and show a tendency to slip between it and the net, forcing the operator to use a brush and a pair of forceps to remove them, thus making this step very time-consuming.

Our results show that, among the four traps tested, the CDC is probably the best choice for routine monitoring of mosquito species with nocturnal/crepuscular activity, while the BG-Eisenhans trap, which represents an evolution of the better known BG-Sentinel, is the best choice for *Ae. albopictus* monitoring schemes as well as in faunistic surveys. On the other hand, the two Activa traps, which have been originally designed to reduce the nuisance of mosquitoes in peridomestic environments, are not recommended for monitoring activities.

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Table 6. Two-by-two trap comparison of mosquito captures for the species with significant results of Kruskal-Wallis test reported in Table 5 (Mann-Whitney test with Holm's stepwise correction). Abbreviations: BG = Biogents Eisenhans de Luxe; CDC = Centers for Disease Control and Prevention CO2 trap; PV = Acti Power Trap PV 440; MT = Acti Power Trap MT 250 Plus; Z-adj.= Z-adjusted value; * = 95% significant p-value.

Trap comparison	<i>Aedes albopictus</i>		<i>Aedes caspius</i>		<i>Culex pipiens</i>	
	Z-adj.	p-value	Z-adj.	p-value	Z-adj.	p-value
PV vs BG	-4.52	< 0.001*	-3.48	< 0.001*	-2.66	0.008*
BG vs CDC	3.87	< 0.001*	1.38	0.17	0	1
PV vs CDC	-1.05	0.29	-2.25	0.025	-2.38	0.017
MT vs PV	0.57	0.57	2.22	0.026	2.36	0.018
MT vs CDC	-0.42	0.67	-0.08	0.94	-0.65	0.52
MT vs BG	-4.12	< 0.001*	-1.54	0.12	-1.07	0.29

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