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EFFICACY OF DIFFERENT OVITRAPS AND BINOMIAL SAMPLING IN *AEDES ALBOPICTUS* SURVEILLANCE ACTIVITY

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ABSTRACT. The efficacy of plastic, glass, and metal ovitraps has been compared in the Desenzano del Garda (Northern Italy) urban area, infested by *Aedes albopictus* since 1993. Plastic and glass ovitraps gave similar results while metal ovitraps collected a significantly lower number of eggs (−56 and −59%, respectively). The reason may be related to the different internal color of the metal trap with respect to plastic and glass ones. Utilizing the Gerrard and Chiang binomial model we obtained a high r^2 for all 3 ovitrap types (plastic, 0.96; glass, 0.87; metal, 0.79) indicating a good fitting of the observed data to the model. According to the desired level of precision for the estimated population, the number of each type of ovitrap required is calculated. Validation of the model in a different area of northern Italy gave positive results. The simple observation of oviposition strips for presence or absence of *Ae. albopictus* eggs could be adopted in surveillance programs, thus allowing considerable labor saving.

INTRODUCTION

For several years, *Aedes albopictus* (Skuse) has been spreading over a number of countries through the international trade of tires (Reiter and Sprenger 1987). In Italy, where it was reported the first time in 1990 in Genoa (Sabatini et al. 1990), it quickly spread into many central-northern Italian sites. At the end of 1994, 38 municipalities belonging to 7 regions had been infested (Romi 1994).

In the areas of its original distribution, *Ae. albopictus* plays an extremely important part in spreading many arboviruses (Hawley 1988). Moreover, it was noticed that this species has a remarkable bioethological elasticity that permits it to transmit indigenous arboviruses in newly colonized areas (Shroyer 1986, Mitchell 1991).

Laboratory tests have highlighted that some arboviruses (Sindbis, Chikungunya, West Nile, and Rift Valley) and filariasis (*Dirofilaria immitis* Leidy and *D. repens* Railliet and Henry), present in the Mediterranean Basin and spread by indigenous mosquitoes, can also be transmitted by *Ae. albopictus* (Cancrini et al. 1992, Mitchell 1994). Moreover the strong anthropophily causes severe nuisance problems requiring specific control actions to be undertaken in the infested areas (Bellini et al. 1994, 1995).

In the survey programs carried out in Asia and America, *Ae. albopictus* is mainly monitored by means of ovitraps. Ovitraps, specifically set up for monitoring *Aedes aegypti* (Linn.) (Fay and Eliason 1966), are quite sensitive and are characterized by low operating costs.

Programs covering integrated pest management in agriculture often estimate the population of a phytophagous insect by means of binomial sampling (Kuno 1986, 1991; Binns and Bostonian 1990; Binns and Nyrop 1992). This type of sampling allows estimation of the average population density through the frequency of the positive sampling units, consequently reducing required operating time and cost. Mogi et al. (1990) observed that it is possible to apply the binomial sampling model described by Gerrard and Chiang (1970) to monitoring *Ae. aegypti* and *Ae. albopictus* by means of ovitraps.

The purpose of this work was to set up a binomial sampling model to estimate the *Ae. albopictus* population and to check the influence of the type of ovitrap on the model.

MATERIALS AND METHODS

This study was carried out in Desenzano del Garda (10°32′48″E, 45°27′51″N), a tourist resort on the southern coast of Lake Garda in northern Italy with a population of 20,000, where *Ae. albopictus* recently colonized a large part of the urban area of approximately 1,000 hectares (Celli et al. 1994).

Three types of ovitraps were used, chosen because they are commonly manufactured and widely available: 85 plastic ovitraps (550 ml capacity), 32 glass ovitraps (590 ml capacity), and 34 metal ovitraps (440 ml capacity) (Fig. 1). The ovitraps were coated externally with the same shiny black paint. With the use of a clip, a strip of Masonite™ (2 × 12 cm) was fastened to the inner surface and positioned vertically to provide a suitable surface for oviposition. When the ovitraps were placed in the field, approximately 200 cc of dechlorinated tap water was poured inside and replaced every week when the strip was changed. The number of eggs was counted by observing the strips under a dissecting microscope. The ovitraps were distributed

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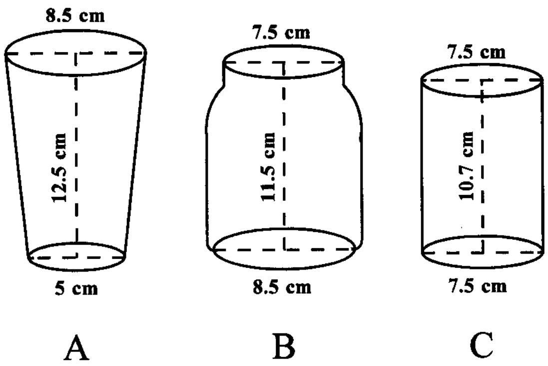

Fig. 1. Dimensions and shapes of the ovitraps tested: A) plastic, B) glass, C) metal.

Table 1. Field comparison of the efficacy of the tested ovitraps.

	No. traps	No. weeks	Total no. eggs/ovitrap ± SE
Block stations			
Plastic	8	18	331.7 ± 72.5a[1]
Glass	8	18	356.2 ± 69.4a
Metal	8	18	149.0 ± 75.4b
Random stations			
Plastic	85	26	310.8 ± 31.6a
Glass	32	26	279.6 ± 45.9ab
Metal	34	26	148.7 ± 33.3b

[1] Values not followed by the same letter are significantly different by Tukey HSD test.

at random, in fixed shaded stations, at a distance of 150–200 m from each other.

The comparison on the capturing efficacy of the ovitraps was performed by 2 tests 1) using data obtained from all the ovitraps from May 19 to November 10, 1994 and 2) using only data collected in 8 selected monitoring stations specifically organized from July 7 to November 3, 1994. In each of these 8 stations 3 traps were placed, one for each type of test, at a distance of 1–5 m from one another, according to the microenvironmental characteristics of the site.

The relation between the sampling frequencies of traps containing eggs and the average number of eggs per trap were calculated by the Gerrard and Chiang (1970) model:

$$\ln(m) = \ln \alpha + \beta \ln[-\ln(1 - p)], \qquad (1)$$

where m = average number of eggs per trap, p = sampling frequency of ovitraps containing eggs, and α and β are the constants. The variance was calculated following the method of Gerrard and Chiang (1970) as reported by Schaalje et al. (1991). As reported by Mogi et al. (1990), the optimum number of traps required to reach different levels of precision was obtained by the equation:

$$n_D = [p/D^2(1 - p)][\beta/-\ln(1 - p)]^2, \qquad (2)$$

where D is the predetermined level of precision. The covariance analysis (ANCOVA) was used to compare the regression lines of the models obtained from the 3 ovitraps. In the comparative test on the efficacy of traps the data relative to the specifically organized stations were analyzed with a 2-way ANOVA followed by a Tukey test; the data relative to the traps arranged at random were analyzed with a 1-way ANOVA followed by the same test.

The model obtained in Desenzano del Garda was validated utilizing data collected with 37 plastic traps placed weekly from July 6 to October 5 in another infested area in northern Italy (Bologna; 10°20′23″E, 44°29′52″N).

RESULTS

When the attracting efficacy of the traps was compared using data obtained from the specifically organized stations, no significant differences were observed between the plastic and glass traps. The metal traps collected significantly fewer eggs than the glass and plastic traps (Table 1). When the comparison was performed using data obtained from all the randomly arranged traps, the efficiency was plastic > glass > metal but with a significant difference only between plastic and metal traps (Table 1).

Figure 2 shows the regression lines calculated by equation (1) for the various traps, which correlate the population mean of eggs per trap with the frequency of the positive traps. As only values other than 0 were considered, the weekly data produced 25 values for plastic traps and 21 values each for glass and metal traps, respectively.

Table 2 shows the parameters relative to the regression analysis. The determination coefficient (r^2) was high for all 3 types of ovitraps, indicating a good data fitting to the model. The ANCOVA showed that there were no significant differences among the 3 regression lines, indicating that they can be considered similar (intercept: F = 0.65 and $P = 0.52$; slope: F = 2.82 and $P = 0.07$). Thus, a model curve can be calculated using the data obtained from the 3 types of traps (Fig. 3). The estimation model results in the following equation:

$$m = 32.62[-\ln(1 - p)]^{1.28}.$$

Should an estimation be carried out on a mixed trap sample, the determination coefficient would be high ($r^2 = 0.87$), indicating that the model fits well to the values observed. The data related to the variance calculation are shown in Table 3. The curves calculated by equation (2) for defining the optimum number of ovitraps required for binomial sampling, for different levels of precision ($D = 0.2 - 0.3 - 0.4$) are shown in Fig. 4. No differences were observed among the 3 ovitraps in the number of eggs collected at different temperature levels recorded

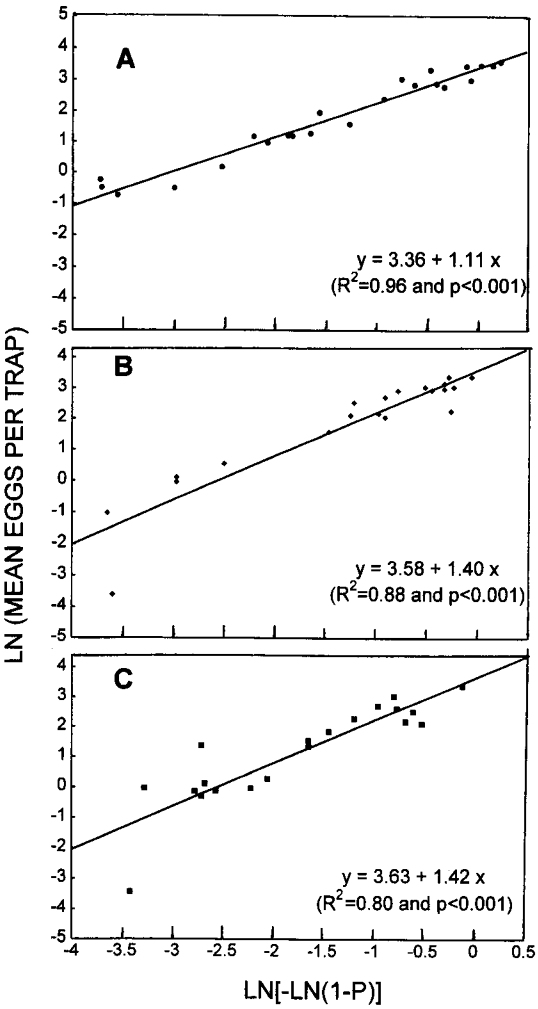

Fig. 2. Population mean density of *Aedes albopictus* as estimated by equation $\ln(m) = \ln \alpha + \beta \ln[-\ln(1 - p)]$ for: A) plastic ovitrap, B) glass ovitrap, C) metal ovitrap.

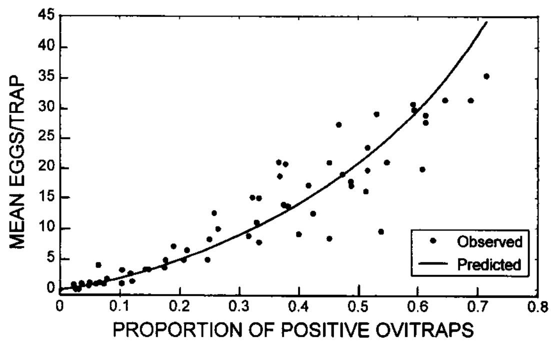

Fig. 3. Binomial estimation model of *Aedes albopictus* population density obtained with all data from the ovitraps tested.

during the season. By pooling the weekly average temperature against the number of eggs collected in the same week we obtained regression lines without any significant differences (slope $p = 0.92$; intercept $p = 0.50$). The r^2 was low (plastic $r^2 = 0.33$, $p < 0.008$; glass $r^2 = 0.34$, $p < 0.006$; metal $r^2 = 0.34$, $p < 0.007$) as expected due to the many factors influencing seasonal egg laying dynamics.

From the data collected in Bologna where the frequency of positive ovitraps was in the range of 6–62%, we obtained the equation $Y = 3.37 + 0.99X$ ($r^2 = 0.75$, $p < 0.0001$) which is similar to the model (intercept: F = 2.27 and $p = 0.14$; slope: F = 0.69 and $p = 0.41$).

DISCUSSION

Female *Ae. albopictus,* when searching for suitable sites for oviposition, use visual and olfactory stimuli (Fay and Perry 1965, Yap et al. 1995). The size, shape, and material that make up the traps widely used in surveillance programs may influence their attracting capacity and the number of eggs the females lay in them (Gubler 1971, Hawley 1988, Holck et al. 1988). Reiter et al. (1991) assume that the addition of attractant substances in the ovitraps can influence the number of eggs laid by the female *Ae. aegypti,* triggering a positive feedback mechanism.

Analogous to what Kloter et al. (1983) had observed with regard to *Ae. aegypti,* in our study no significant differences arose between the number of eggs laid by *Ae. albopictus* in plastic and glass ovitraps. On the contrary, a very minor attraction was observed for the metal traps. A possible explanation might be the brightness of the inner side of the metal traps, whereas the plastic and glass inner sides appeared to be black due to the transparency of the material.

The plastic traps seem to be more sensitive, especially at low population densities. At the end of the season, they continued collecting eggs for 4

Table 2. Parameters by regression of $\ln(m)$ on $\ln[-\ln(1 - p)]$ for tested ovitraps to estimate density of *Aedes albopictus* eggs.

	$\ln \alpha \pm$ SE	$\beta \pm$ SE	r^2	P
Plastic	3.364 ± 0.085	1.112 ± 0.047	0.96	<0.001
Glass	3.583 ± 0.204	1.400 ± 0.121	0.88	<0.001
Metal	3.632 ± 0.322	1.421 ± 0.165	0.80	<0.001
Pooled	3.485 ± 0.114	1.285 ± 0.063	0.87	<0.001

Table 3. Variance for predicting eggs/ovitrap values through the proportion of positive ovitraps (pooled data).

p	m	Var_G[1]	− CI[2]	+ CI[2]
0.05	0.73	0.13	0.64	0.82
0.10	1.83	0.40	1.67	1.99
0.15	3.19	0.81	2.97	3.41
0.20	4.78	1.37	4.50	5.07
0.25	6.62	2.15	6.26	6.98
0.30	8.72	3.27	8.28	9.16
0.35	11.10	4.87	10.56	11.64
0.40	13.81	7.19	13.15	14.46
0.45	16.89	10.60	16.09	17.68
0.50	20.41	15.61	19.44	21.37
0.55	24.46	23.05	23.28	25.63
0.60	29.17	34.23	27.74	30.60
0.65	34.71	51.28	32.96	36.47
0.70	41.37	77.89	39.21	43.53
0.75	49.55	120.87	46.86	52.24
0.80	59.98	194.05	56.58	63.39
0.85	74.04	329.52	69.60	78.47
0.90	94.87	620.80	88.78	100.96
0.95	132.86	1,497.88	123.40	142.33

[1] Var_G is the variance calculated following the method of Gerrard and Chiang (1970).
[2] CI is the confidence interval of estimated population density.

weeks after the glass and metal traps had stopped their collecting activity.

The curves obtained by the Gerrard and Chiang (1970) model show a good fit of the empirical data collected with all 3 types of traps. From our studies we have observed that through binomial sampling it is possible to estimate the population of *Ae. albopictus* rather accurately by using plastic, glass, and metal traps. The curves obtained with the different traps were similar.

The higher r^2 shown by the regression equation relative to the plastic ovitraps compared to those of the glass and metal ones indicates a better correspondence to the model. In general $D = 0.1$ is considered to be the standard level of accuracy in ecological studies. Mogi et al. (1990), studying *Ae. aegypti* in Thailand, observed that D between 0.2 and 0.3 can be sufficient for studying populations with a frequency of $0.11 < p < 0.99$.

Due to the different sensitivity the numbers of ovitraps that have to be used to achieve the same level of accuracy results is: plastic < glass < metal.

The metal trap results are considerably less sensitive than those of the other 2 types of traps and therefore their use is not recommended, especially in low population density conditions. Since the differences between the plastic and glass traps with regard to both sensitivity and conformity with the model are not evident, the choice must be made based on their utility in the field. Evaluation characteristics are unit cost; shock resistance; the possibility of making a hole which, should it rain, will allow water to drain and consequently maintain constant water levels, and the possibility of being stacked during transport. In light of these considerations, the plastic traps seem to be more suitable for *Ae. albopictus* surveillance activities. As an example in the case of $p = 0.30$ the number of ovitraps to be used should be 139 for $D = 0.20$, 62 for $D = 0.3$, and 35 for $D = 0.4$.

As shown for the data collected in the Bologna area, the binomial sampling method should be used not only to compare population densities in time but also to compare densities between areas. Use of the binomial estimation model for monitoring *Ae. albopictus* populations will reduce the time required for observing Masonite strips under a stereomicroscope by 30–70%, depending on population densities.

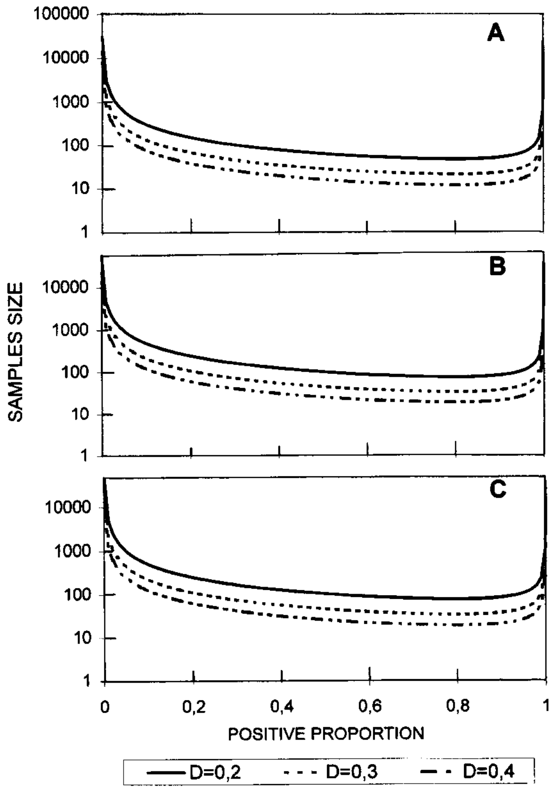

Fig. 4. Numerical sample size curves for *Aedes albopictus* by: A) plastic ovitraps, B) glass ovitraps, and C) metal ovitraps, at three precision levels (D = 0.2, 0.3, and 0.4).

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