VALIDATION OF CO_2 TRAP DATA IN THREE EUROPEAN REGIONS

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Abstract - CDC traps are employed as an efficient tool in monitoring mosquito population changes, evaluation of climatic and human made changes on species abundance and composition as well as for estimation of success of control programs. Despite the great advantage in labor saving and sampling the species which are slightly or not attracted to man, compared to classical human bite sampling, usage of traps is constrained to the districts which can provide technical support, gaseous CO_2 or dry ice. The sampling efficacy and accuracy depend on many factors such as: the mosquito species, the weather conditions, the terrain features, the trap construction, the trap site selection, the height of trap position, the amount of CO_2 release and the sucking power, although several other elements could play an important role as well. Several attempts have been made to produce results of trap catches less variable on the site, more uniform at different sites and more reliable in practical application. In Germany females of *Ae. vexans* dominated between at ground level whereas at the height of 10 m *Cx. pipiens* was by far the most abundant species. In urban conditions, *Cx. pipiens* fly up to the level of 45 m. *Ae. vexans* actively migrates 1-2 km per night while, snow-melt mosquitoes migrate less than 2 kilometers during adult life. In Italy the mosquito nuisance would be considered intolerable with over 30 females of *Aedes*/trap/night or 200 females of *Culex*/trap/night. Our investigation demonstrated that *Ae. geniculatus*, *Ae. sticticus* and *An. plumbeus* were attracted more by human bait than by carbon dioxide. There was no significant differences between both numbers and species of mosquitoes caught by CO_2 + light and by CO_2 alone.
Key words - Mosquitoes, CO_2, dispersal, distribution, tolerance

INTRODUCTION

Most European countries with developed mosquito control programs, include in their monitoring systems CO_2 baited traps which give more confidence in making qualitative and quantitative investigations of the mosquito fauna. The precise knowledge of the biology of the mosquito species in question, as well as their relative abundance and phenology is inalienable to fulfill the requirements of an economical and ecological successful treatment. Thus a monitoring program for adult mosquitoes serves to achieve important data about the species composition, abundance and phenology (population dynamics), the spatial and temporal distribution related to migration, the tolerance threshold as a basis for the needs of mosquito control activity, the reduction of the mosquito population after control operations and the effect of various visual and olfactory stimuli on mosquitoes. The monitoring of the adult mosquito population can be efficiently conducted by CO_2 baited light traps which could be modified to fulfil the specific needs of each study. CO_2 traps were engaged in the investigations of mosquito ecology and biting behavior in Yugoslavia in 1983, and for both scientific and regular monitoring purposes in Italy and Germany since the begining of the 1990‘s. In the recent years they were also accepted as a regular monitoring tool by the mosquito abatement districts in Spain and in some other countries.

Control programs aimed at reduction of primarily nuisance mosquitoes ought to be conducted on the basis of appropriate cost/benefit evaluation considering that mosquito density reduction is basically subject to the principle of decreasing productivity per unit of economic investment. In the Upper Rhine Valley dispersion of snow melt and floodwater mosquitoes, the effect of temperature changes on sampling size and vertical distribution of different species have been determined. According to the trapping data in Italy, threshold number of mosquitoes requiring control has been established too. Depending on the results obtained by CO_2 traps in Yugoslavia, some aspects of blood searching behavior have been verified, control strategies developed and justified.

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MATERIALS AND METHODS

Suction traps are used which are baited with CO_2 (dry ice, approx. 1 kg per catch) to catch adult female mosquitoes. The mosquitoes are lured close to the trap by the bait where the air stream of the ventilator sucks them into the net. Samples are taken from late afternoon until the next morning, during the main period of flight activity. After the collection of the traps, the mosquitoes are counted and identified after Mohrig (1969**),** Gutsevich *et al.* (1974) and Cranston *et al.* (1978).

The routine monitoring program in Germany is based on a biweekly-sampling interval carried out from late April through end of September. More than 40 trapping sites are chosen along the Upper Rhine River from Freiburg in the south, to the city of Mainz in the north (300 km stretch of the river). For vertical distribution monitoring traps were set on 6 different poplar trees each at different height of 0.5, 2, 4, 6, 8 and 10 m from ground level to investigate the vertical distribution. The distance between the different trapping sites (trees) did not exceed 20 m. Beginning of trapping was introduced soon after the first seasonal emergence of adult floodwater mosquitoes and continued throughout four consecutive nights.

To study the dispersal behavior of the floodwater mosquito *Aedes vexans* (Meigen), an isolated mass breeding site was choose where no control activity took place and masses of mosquitoes developed after a flood of the Rhine River. Before the emergence of the adults and up to two weeks after mass occurrence of the mosquitoes the trapping was conducted on a daily (1st week) or bi-daily (2nd week) basis. The traps were located in the uncontrolled area (2 traps) and in concentric circles of 2.5 and 10 km (8 traps on each circle). For the snowmelt mosquitoes monitoring program the traps were placed in an area of swampy forest where all breeding sites of *Aedes communis* (De G. Ellis and Brust), *Ae. rusticus* (Rossi), *Ae. punctor* (Kirby) and *Ae. cantans* (Meigen) were treated with *B.t.i.*-preparations to kill mosquito larvae within a radius of 1.5 kilometer around a settlement. Traps were operating at 500 m distance along a west-east and a north-south transects from the center of the village. Trapping was conducted on weekly bases from April (before emergence) until August.

In Italy the study was carried out from 1993 to 1996 in three urban areas and two agricultural areas in the Provinces of Bologna (Crevalcore and Amola) and Modena (Albareto), where mosquito control is regularly performed with B.t.i. application only. In this study, only the capture of *Culex pipiens* L. (99 % of the culicid population) was analyzed. Data with more than 5 "non *Cx. pipiens*" specimens were not considered. The CO_2 traps were in operation on a weekly base from the middle of June to the middle of October. The day after activating the traps, the 9–12 families involved in the study for each monitoring station were contacted by telephone. Families were selected in a radius of 0-300 meters from the traps and for each station the same families were contacted for the entire period of study. The people were asked about the nuisance level experienced using four subjective indices: absent S_1, tolerable S_2, severe S_3, intolerable S_4. For every interview and each nuisance level, the average number of adult mosquitoes captured in the trap was calculated per S_1, S_2, S_3 and S_4. The threshold value S was determined as the mean S_2 and S_3 value. The threshold of tolerance is higher than S_2 where the nuisance is tolerable and definitely lower than S_3 where it is severe. The data were analyzed using the 2- and 3-way ANOVA methods while the linear regression data were analyzed with the ANCOVA method.

To investigate the relative performance of human bait and CO_2 baited CDC traps, samples were collected with these two techniques on four days during the first week of every month from April to October in two consecutive years. Human bait sampling was done five times a day for one hour each time (five hours total) whereas the traps were running 24 hours continuously, being sampled every fifth or fourth hour (24 hours total). Catching was done in several localities of Vojvodina, the northern-most province of Yugoslavia.

Separate studies to investigate the relative importance of light and CO_2 was conducted in reed-beds. Four traps (light only, CO_2 only, light + CO_2, and fan only) were spaced 300-500 m apart and run continuously from 21.00 to 02.00. The same procedure was conducted with three groups of aforementioned

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combination of the traps but at the distances of 3-5 m between them. Vertical distribution was monitored in urban areas up to the level of the 15th floor of a building.

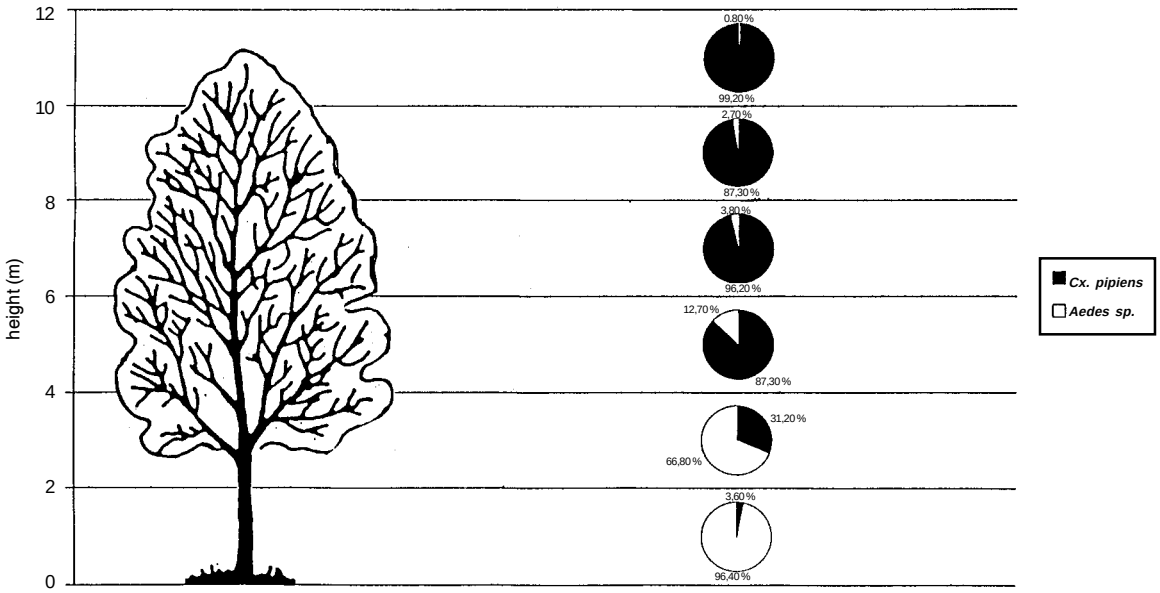

Figure 1. Relative dominance of *Culex* and *Aedes* species registered at different heights

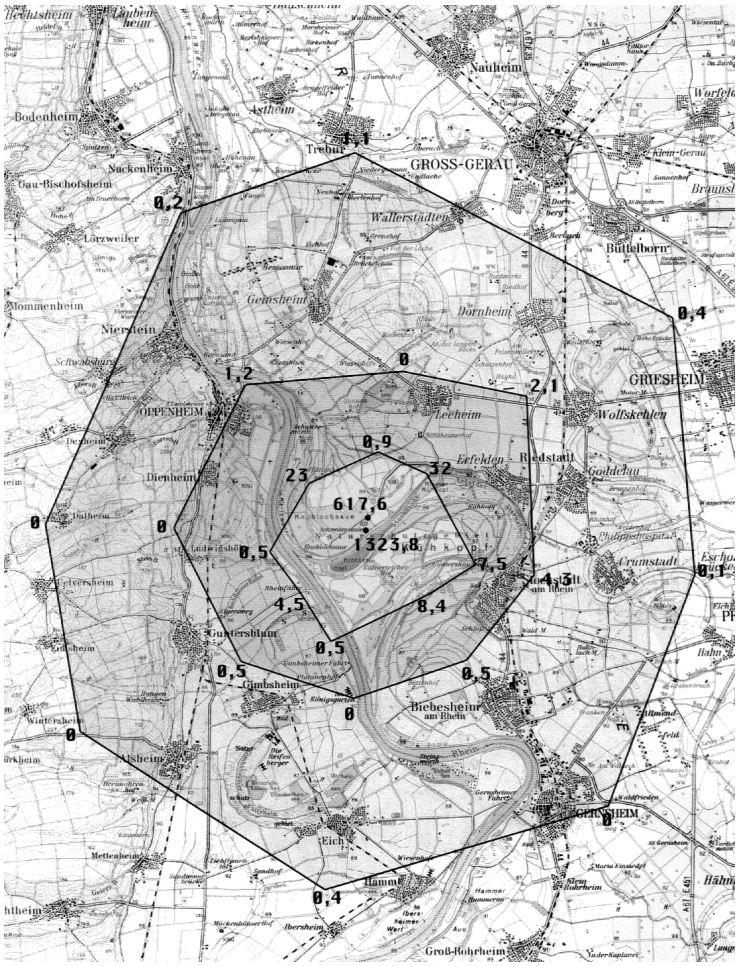

Figure 2. Ten day trapping average of *Aedes vexans* females at breeding site and distances of 2.5 km, 5 km and 10 km (dispersal monitored during fortnight).

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RESULTS

In the Upper Rhine Valley, between 1994 and 1997, 47,000 to 77,000 mosquitoes representing 22 species and 5 genera, were caught during each season. *Ae. vexans* was by far the most abundant species, with a proportion of about 80% or more, depending on the water level. Females of *Ae. vexans*, *Ae. rossicus* Peus, *Ae. cinereus* Meigen and *Ae. sticticus* (Meigen) dominated between ground level and 4 meters (species composition in 2 m: 98.5% of *Aedes* spp. and 1.5% of *Cx. pipiens*), whereas at the height of 10 m *Cx. pipiens* was with 99.2% by far the most abundant species (Fig. 1). In urban conditions *Cx. pipiens* hungry females were caught in traps positioned both outside and inside the buildings up to the level of the 15th floor, app. 45 m high. Females of *An. maculipennis* complex were sampled on the ground and the first floor only.

The floodwater-mosquito *Ae. vexans* was actively migrating 1-2 km per night. Within one week after emergence females could be caught already 10 km far away from the breeding site. On the Fig. 2 average number of *Ae. vexans* females/24 hours caught at breeding site, and 2.5, 5 and 10 km apart from it during 7 daily (1st week) and 3 bi-daily (2nd week) 24- hour trapping periods are shown. The snow-melt mosquitoes (e.g. *Ae. punctor*, *Ae. rusticus* and *Ae. cantans*) migrated in total less than 2 kilometers. During the whole season they never appeared in large numbers more than 2 km from the breeding sites.

The 3-way ANOVA method (year – area – nuisance level) showed that the average density of mosquitoes captured in the traps varies depending on the area, year and nuisance levels (Carrieri *et al.*, 1998). There are significant differences between the average number of adult mosquitoes trapped at the different nuisance levels (Fig.3).

The threshold value (defined as the mean value between S_2 and S_3) also varies depending on the year ($F=244.7$ and $P<0.00001$) and area ($F=11.9$ and $P<0.00004$). It is not possible, therefore, to calculate a single threshold value of S. In the agricultural areas, the 3-way ANOVA method (year, area and nuisance level) showed that the average density of mosquitoes captured is significantly different only in relation to the place and nuisance levels. The threshold of tolerance also differs in relation to the place ($F=154.77$ and $P<0.00001$) but not in relation to the year ($F=1.51$ and $P=0,23$). It is important to note,

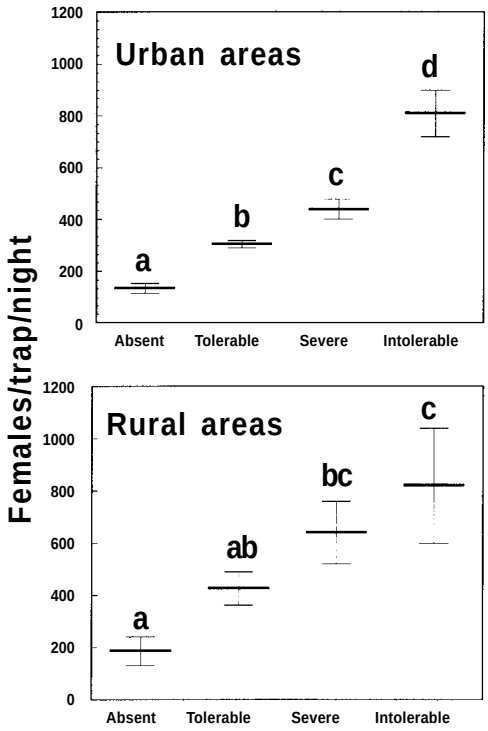

Figure 3. Average captures of *Cx. pipiens* in relation to the different muisance levels reported.

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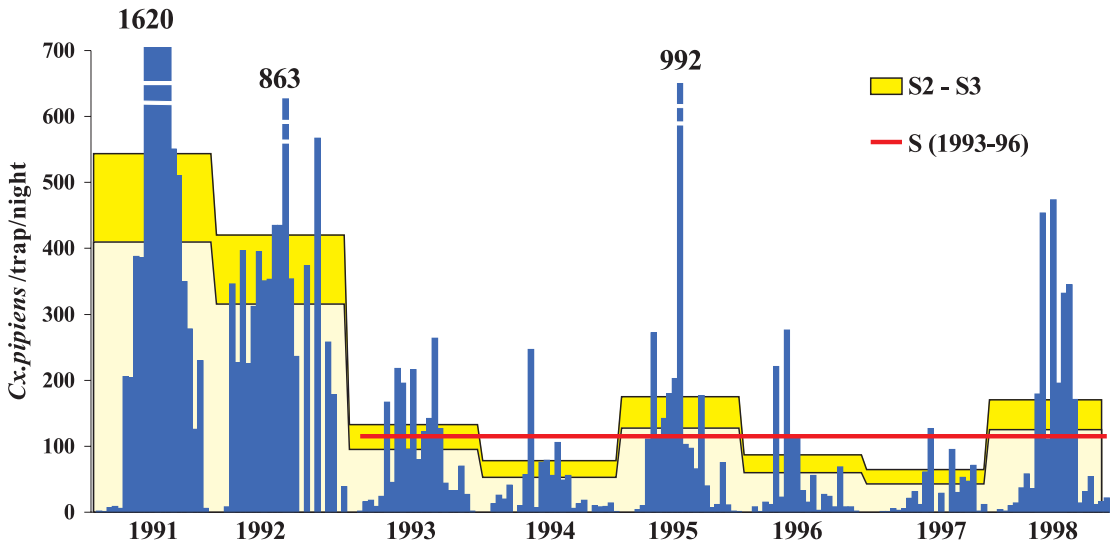

Figure 4. Captures of *Culex pipiens* females in an urban area and estimated tolerance thresholds (Bentivoglio - Bologna).

however, that the changes in the culicid population were similar over the two year of the study period. In the agricultural area it was also observed that the number of adults captured differed in accordance to the nuisance levels reported by the interviewed persons (Fig.3).

The tolerance threshold varied linearly as a function of the average annual density according to the following equations. In the urban areas: $S_{Ua} = 49{,}71 + 0{,}91\ D_{Ua}$ ($F = 250.54$ e $R^2 = 0.80$) and in the rural areas: $S_{Ra} = 29.35 + 1{,}05\ D_{Ra}$ ($F = 146.47$ e $R^2 = 0.81$), where S is the tolerance threshold and D is the average annual density of *Cx. pipiens*.

The test of parallelism shows that the lines of regression calculated for the urban and agricultural areas are parallel and coincident. It is therefore possible to calculate a single equation to determine the tolerance threshold of *Cx. pipiens*: $S = 18.65 + 1.03\ D_{Cx}$ ($F = 440.86$ e $R^2 = 0.81$).

Empirically and on the basis of direct observations and telephone complaints to the operational base at a monitoring station, it was established that the nuisance would be considered intolerable with over 30 females of *Aedes*/trap/night and 200 females of *Culex*/trap/night. The round natural logarithm of 30 is 1.5. The same value is obtained by rising to 0.7, the number 200. On the whole, this arithmetical artifice fits, since the aggressiveness of *Cx. pipiens* and *Cx. modestus* Ficalbi is lower than that of *Ae. caspius* (Pallas) and *Ae. detritus* (Hal.). Finally, to take into account the cumulative effect of the main culicid species in causing discomfort, the number of females captured, belonging to the two genera mentioned above, was integrated in the final formula:

INM= log (n *Aedes* + $n^{0,7}$ *Culex* + 1). Where: "INM" is the Nuisance Numerical Index; "n Aedes" is the number of females/trap belonging to the genus *Aedes*, and "$n^{0,7}$ Culex" is the number of females/trap belonging to the genus *Culex*. The capture values obtained from the traps positioned the previous night are entered into a computer daily in a spread sheet which automatically applies the formula and transforms the number of females captured into the corresponding Nuisance Numerical Index (INM). Finally, the INM is graphically represented for each location, a method which is easily understood and represent particular situation at the monitoring site. The graph, together with the current information on the ongoing control activities, is circulated by a specific bulletin, which is sent daily by Fax to the associations and tourist-hotel facilities involved. If the pre-set tolerance threshold is exceeded, adulticide activities in Bologna and Modena Provinces are implemented by ULV spraying, using pyretroid insecticides (Bellini and Veronesi, 1994).

Figure 4 shows the data relative to captures in one urban area (Bentivoglio, Bologna) in the period 1991-98. Control activities with *B.t.i.* conducted in 1991 and fully applied by 1993 accomplished

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a major reduction in culicid density. The threshold S was calculated using the average density of the adult population captured in the period 1993-96 when control activities were already consolidated, while S2 – S3 represents the tolerance threshold calculated on the basis of the average annual density. It could be observed that in 1997 the number of *Cx. pipiens* captured was always below the established threshold while in 1998 from the end of June to the beginning of August there were levels of density sufficient to justify extraordinary control activities in order to keep the mosquitoes within the tolerance threshold.

With CO_2 baited trapping all species of practical importance for Vojvodina were collected. During three years of investigations 24 out of 34 species and subspecies known to occur in the region (Srdic *et al.*, 1986) have been recorded. In Table 1 data from 280 hours of human bait catching and 56 days of 24-hour trapping during the period April-October are presented. In the third column the relationship between mosquito caught on human bait and by trapping is shown; no allowance being made here for the difference in the duration of trapping by the two techniques. Clear differences in the number of mosquitoes collected, especially for some species, are evident. *Anopheles maculipennis* complex mosquitoes were caught only by the trap. This method of catching also showed high efficiency in sampling the *Cx. pipiens, Culiseta annulata* (Schrank), *Cx. modestus* Ficalbi, *An. claviger* (Meigen), *Ae. caspius, Coquillettidia richiardii* (Ficalbi), *Ae. vexans, Ae. cinereus* and *Ae. dorsalis* (Meigen). The efficiency of the two sampling methods can be considered equal if the ratio in the third column is 1 : 4.80 because the duration of sampling by bait and the trap is in this proportion (5 : 24 hours). In this way it is seen that human bait catching is more efficient in the case *of Ae. geniculatus* (Olivier), *Ae. sticticus* and *An. plumbeus* Stephens. The above mentioned coefficient reflects the host preference of the individual species: the lower the coefficient, the more anthropophilic the species.

Considering the relative significance of light and CO_2 in Vojvodina, it was found that the efficiency of the trap in terms of the total number of specimens caught (sexes combined) was significantly lower in the absence of CO_2. The control trap collected only *Cx. pipiens*, which were predominant at the sampling site. Further information was obtained by considering the sexes separately (Table 2). In both experiments, the CO_2 tarp yielded a very large proportion of females (99-96%), whereas the light trap gave only 4-

Table 1. Number and species relation between two mosquito catching methods.

No.	Species	Human bite catches (HBC)	CO_2 baited trap catches (TC)	HBC : TC
1.	*Ae. geniculatus*	129	272	1 : 2
2.	*An. plumbeus*	17	53	1 : 3
3.	*Ae. sticticus*	197	616	1 : 3
4.	*Ae. dorsalis*	17	118	1 : 7
5.	*Ae. cinereus*	14	136	1 : 10
6.	*Ae. vexans*	1.438	16.142	1 : 11
7.	*Cq. richiardii*	26	573	1 : 22
8.	*Ae. caspius*	27	665	1 : 24
9.	*An. claviger*	1	47	1 : 47
10.	*Cx. modestus*	11	1.504	1 : 137
11.	*Cs. annulata*	2	582	1 : 291
12.	*Cx. pipiens*	21	9.817	1 : 467
13.	*An. maculipennis* complex	0	526	-

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Table 2. Sex ratio in samples from traps containing two attractants used either alone or in combination, compared with a control in which no attractant was provided.

Sex	CO_2		Light		CO_2 + light		Control	
	No.	%	No.	%	No.	%	No.	%
5 m separation								
G	9	1	216	96	662	71	47	92
E	759	99	9	4	269	29	4	8
Total	768	100	225	100	931	100	51	100
300 - 500 m separation								
G	13	4	57	63	52	17	15	56
E	339	96	34	37	248	83	12	44
Total	352	100	91	100	300	100	27	100

37% females i. e. attracted mainly males. In experiments with well separated and closely placed traps the light + CO_2 trap gave 29-83% females, and the control trap gave 8-44% females, respectively.

DISCUSSION

In our experience the efficiency of CO_2 baited trap is very high because it enables a larger number of adults of most species of the designated region to be sampled, in both low and high population density conditions than would be obtained by other sampling methods. If compared to human bait catches, the traps save a great deal of time and manual labour and have the added advantage of also catching zoophilic species. Thus they have clear advantage over human bait catches even when sampling *Ae. geniculatus, Ae. sticticus* and *An. plumbeus.* Necessity to use CO_2 traps for monitoring *Cx. pipiens* and *An. maculipennis* complex mosquitoes is proven but the fact that only some or none of these mosquitoes are caught by human bait catches.

The dispersal behavior of mosquitoes is influenced by physiological needs (carbohydrates, blood, oviposition sites), habitat needs (resting and searching), terrain features (mass and vegetative coverage pattern) and meteorological factors (illumination level, temperature, humidity and wind velocity). Dispersal serves mostly to bring the blood-sucking insects in to the contact with a suitable signal from a potential host. Most temperate zone *Aedes* species have the peak of flight activity during the twilight when the temperature is dropping and the humidity is increasing. They are usually more active on nights when the moon is shining. Species that tend to fly extensively over long distances usually show two different dispersal behaviors (Provost, 1953), a drift with the wind (so-called migration flight or passive migration) and an active dispersal (so-called appetitive flight).

During the migration flight the mosquitoes are ascending in swarms and drifting passively with the wind over long distances to occur suddenly in large numbers far away from their breeding places. This is a non-oriented flight activity which is especially influenced by the speed and direction of the wind. Passive migration (although phases of take off and lending are well under the control of individuals engaged in flight) occurs in swarms only a short time after emergence (Bidlingmayer, 1985). During the appetitive flight female mosquitoes, usually older than 24 hours after emergence, are dispersing actively. They are flying upwind when the wind velocities are below mosquito flight speed which is approximately 1 m/s (Bidlingmayer and Hem, 1981). The flight against the wind increases the likelihood of encountering stimuli deriving from a host. However, strong wind prevents the appetitive flights. Usually females are flying close to the ground or slightly above the top of the vegetation (Becker *et al.*, 1996). Experiments

documented that *Ae. vexans* migrates approximately 1 km per night during warm and humid weather periods with moderate wind speeds (Becker *et al.*, 1998). Increasing numbers of *Ae. vexans* females could be caught in CDC-traps in a distance of about 5 km eight days after emergence, and within two weeks in a distance of 10 km and more. Clarke (1943) recorded migration distances of marked *Ae. vexans* females of 22 km and Gjullin *et al.* (1950) and Mohrig (1965) up to 48 km.

Snow melt mosquito species stay near their breeding sites and do not regulary migrate long distances. Joslyn and Fish (1986) in mark-recapture experiments collected *Ae. communis* females at distances up to 1600 m from their breeding sites. Nielsen (1957) reported a maximum flight range of about 1600 m for *Ae. communis* and *Ae. cinereus* with an average dispersal range of less than half this distance.

In Germany, *Ae. rusticus* females were found resting in the forest during daytime and migrating to the forest edge and the adjoining fields with increasing dusk. Females prefered to migrate along rows of trees in open areas. The mosquitoes obviously follow their hosts, mostly red deer, when these animals browse on the meadows next to the forest. The migration distance of this species was only a few hundreds of meters. To overcome a discretionary judgement of the discomfort level and base adulticide activity on objective proof, the technique of adult monitoring is systematically used by means of carbon dioxide baited traps. The number of females captured/night/trap is transformed into a Nuisance Numerical Index (INM) and the adulticide treatment is applied once a pre-set limit of tolerance (tolerance threshold) has been reached. In this case, monitoring is directly connected to the operational control activities. Supply of important elements is in function of evaluation of the infestations/re-infestations trend and the effectiveness of larvicide and adulticide treatments. Ever since the CO_2 traps were implemented in Italy, the crucial question has been how to make use of the value of daily captures more practical, and meet the twofold requirement of assessing the effectiveness of the operational activities, and demonstrate the objective drops in infestation levels year by year. The experience in the agricultural field of IPM against phytophagous insects with the aid of pheromone traps, and the concept of economic threshold of damage, have provided a decisive contribution in setting an innovative direction for the usage of CO_2 trapping data.

On the basis of the data collected, it was observed that the nuisance level of *Cx. pipiens* appears to be related to their density with respect to the average trend. The reduction of yearly average mosquito densities, caused by mosquito control activities, tends to have a decrease in the tolerance threshold density, thus creating an increasing demand for control activities. This means that it is not possible to modulate control activities on the basis of expectations because this would result in a continuous intensification of control measures. It would probably be more correct, to apply an analysis of cost/benefits and the historical records of adult monitoring for setting a tolerance threshold a priori and use this to modulate control activities. It was found that *Ae. geniculatus*, *Ae. sticticus* and *An. plumbeus* were attracted more by human bait than by carbon dioxide. This was probably because mosquitoes orientate to hosts on the basis of olfactory and termo cues in addition to just CO_2 (Jaenson, 1985). Hence, it can be presumed that *Ae. geniculatus*, *Ae. sticticus* and *An. plumbeus* are anthropophilic, and other mosquito species caught are zoophilic to varying degrees. There was no significant differences between both numbers and species of mosquitoes caught by CO_2 + light and by CO_2 alone. An advantage of not using light in the traps was that few other insects (Neuroptera, Lepidoptera, Diptera and Hymenoptera) were caught, thus simplifying sorting and identification. Similar was reported by Ceresia and Savage (referred to in Service, 1976). In the results we obtained the total number of mosquitoes taken by CO_2 + light was in both experiments less than sum of the individual catches from CO_2 alone or light alone. Therefore we are of the opinion that there is some negative interaction between the attractive qualities of CO_2 and the light. Considering the sexes separately, fewer females were caught by CO_2 + light than by CO_2 alone, even when traps were placed side by side. This suggests that light may be actually repellent to females.

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