*European Mosquito Bulletin, **27** (2009), 33-46.*
Journal of the European Mosquito Control Association
ISSN 1460-6127; w.w.w.e-m-b.org
First published online 1 October 2009

Efficacy and lasting activity of four IGRs formulations against mosquitoes in catch basins of northern Italy

Bellini Romeo[1], Albieri Alessandro[1], Carrieri Marco[1], Colonna Roberta[1], Donati Luciano[1], Magnani Maurizio[1], Pilani Roberto[1], Veronesi Rodolfo[1], Chiot Giancarlo[2], Lanza Nazario[2].

[1]Centro Agricoltura Ambiente "G.Nicoli", Via Argini Nord 3351, 40014 Crevalcore, Italy
[2] Chemtura Italy – Technical Department

Abstract

IGRs (Insect Growth Regulators) are chemical insecticide compounds that cause physiological alteration during the development of the insects. Within the IGRs, pyriproxyfen and diflubenzuron are characterized by a low acute toxicity towards mammals and a good safety level for non-target organisms. Both active ingredients are in use as larvicides in mosquito control programmes and in Italy they are registered as liquid, granular and tablet formulations.

These products are good candidates for larval control in catch basins where conditions are suitable for the development of *Culex pipiens* and *Aedes albopictus.*

The trials conducted in four localities of the Emilia-Romagna Region during 2006-2007 were planned to estimate the efficacy and the lasting action of different formulations under conditions in Northern Italy. Three diflubenzuron formulations (DEVICE® GR-2, DEVICE® TB-2 and DEVICE® SC-15) and one pyriproxyfen formulation (SUMILARV® 0.5G) were tested and compared.

Key words: diflubenzuron, pyriproxyfen, *Aedes albopictus*, *Culex pipiens,* mosquito control, catch basin.

Introduction

Following the introduction of new European regulations requiring higher safety standards, and for market reasons, the number of insecticide products available in Europe has suffered a strong reduction over recent years.

Therefore the need for maximum exploitation of available products with the ability to ensure high efficacy and long lasting activity is required.

These characteristics are certainly verifiable in the IGR products, which act by determining physiological modifications during the development of the target species. Among the IGRs, *diflubenzuron* and *pyriproxyfen* are characterised by a low acute toxicity against mammals and a good safety margin with respect to non-target organisms including fish and birds (WHO, 2001; WHO, 2005; WHO, 2006). Both have been used for some time as larvicides in mosquito control (Chamberlain, 1975; Mulla *et al*., 1975; Mulla *et al*., 1986; Mulligan III &

Schaefer, 1990; Mulla, 1995) and they are currently registered in liquid, granular, and tablet formulations.

Diflubenzuron mainly acts through ingestion which leads to inhibition of the synthesis and deposition of the chitin in the immature stages of the insects. Pyriproxyfen, analogue of the juvenile hormone (JH), acts on the larva by eliminating the typical biochemical changes required for metamorphosis leading to abnormally shaped individuals that cannot regularly complete their development, and consequently die.

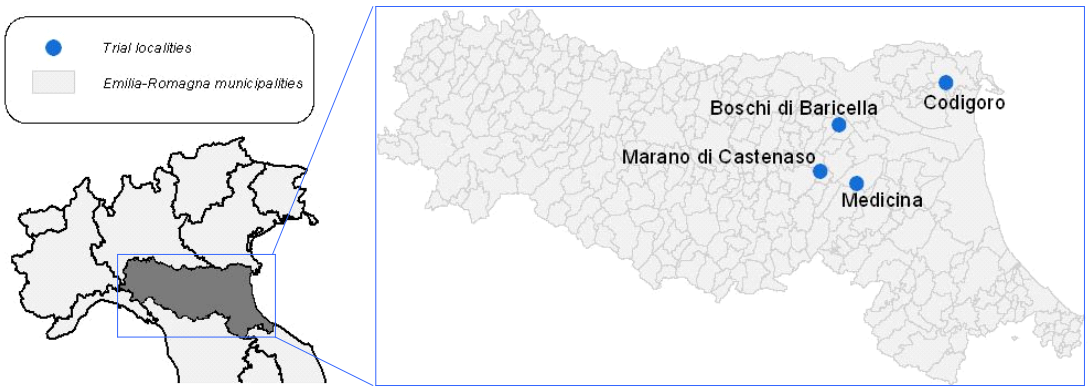
Owing to the favourable eco-toxicological profile of these IGRs, diflubenzuron and pyriproxyfen are considered as excellent candidates in the use in road drains in public and private environment (Rutz *et al*., 1980; Gabinaud *et al*., 1985; Ishii & Rak Sohn, 1987; Rey *et al*., 2006). Two key mosquito species which favour the ideal development conditions found in Italian catch basins are: *Culex pipiens* biotype *molestus* Forskål and *Aedes albopictus* (Skuse) (Celli *et al*., 1994). The study described here, performed during 2006-2007, aims to assess the efficacy and lasting activity of four commercial formulations, three based on diflubenzuron (DEVICE® TB-2, DEVICE® GR-2, DEVICE® SC-15) and one on pyriproxyfen (SUMILARV® 0.5G).

Materials and Methods

The trials were conducted in 4 urban areas of the Emilia-Romagna Region, situated in the Po Plain (Northern Italy), during the summers of 2006 and 2007.

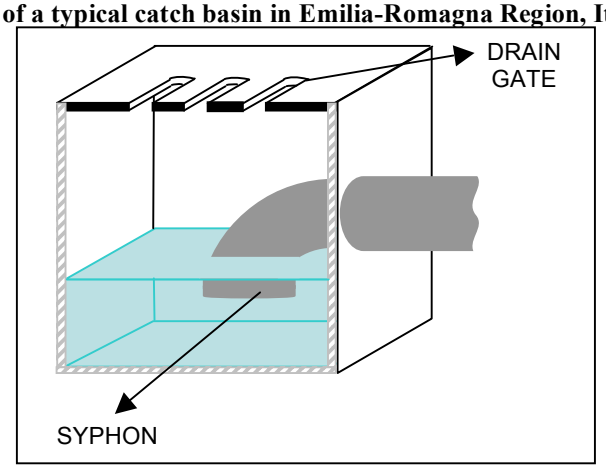
In 2006 the urban areas of Codigoro (FE) (44° 49’ N - 44° 49’ E) and Medicina (BO) (44° 28’ N – 11° 38’ E) were involved, while the 2007 trials were carried out in Marano di Castenaso (BO) (44°30'35" N, 11°28'14" E) and Boschi di Baricella (BO) (44°41’31”N, 11°33’23”E) (Figure 1).

Figure 1. Localities where trials were conducted during 2006-2007



In the situation study the catch basins could contain up to 40 litres of water which is typically characterized by a high amount of floating organic material and mud at the bottom (Figure 2).

Figure 2. 3-D section of a typical catch basin in Emilia-Romagna Region, Italy



The drains were chosen upon preliminary control of the larval presence and the treatment was attributed randomly.

The dose of the granular formulations was prepared with a calibrated measurer in the laboratory using a precision balance.

The granules and the tablets were introduced manually ensuring that they all entered the water. In 2007 trials included a liquid diflubenzuron formulation, DEVICE® SC-15, which was sprayed into the drains after suitable dilution using a knapsack pump. A volume equal to 25 ml/drain sprayed over 3 seconds was calculated to keep conditions similar to operational.

Weekly, from every drain two water samples were collected using a 0.5 l dipper, after having waited 2 minutes from the opening of the grid and between one collection to the next. The presence of pre-imaginal stages was recorded according to three categories: L_1-L_2; L_3-L_4 and pupae. Samplings continued up to week 7-8 post-treatment in Codigoro and up to week 5 in Medicina, whereas in Marano and Boschi they continued up to week 6 post-treatment in both the tests.

In 2006 during the second test carried out in Medicina from August 30 to September 28, the same insecticides doses were initially used but 7 days after the treatment, the amount of SUMILARV® 0.5G increased to 4 g/catch basin (Tables 1 and 2).

Therefore in the case of DEVICE® GR-2 and DEVICE® TB-2 the maximum dose indicated on the label for very polluted and organic waters was used, while in the case of SUMILARV 0.5G® the dose was about 1.6 and, only in the 2nd trial of 2006, 3.2 times higher than indicated on the label.

Table 1. Scheme of the tests conducted in 2006

1st TRIAL 23 June - 17 August , Codigoro (FE)		
DEVICE® GR-2 (2% diflubenzuron)	DEVICE® TB-2 (2% diflubenzuron)	SUMILARV® 0,5G (0.5% pyriproxyfen)
Dose		
g/catch basin	n. tablets/catch basin	g/catch basin
2	1	2
Treated catch basins (n.)		
30	30	30

Control catch basins (n.)		
20		
2nd TRIAL 30 August - 28 September, Medicina (BO)		
DEVICE® GR-2 (2% diflubenzuron)	DEVICE® TB-2 (2% diflubenzuron)	SUMILARV® 0.5G (0.5% pyriproxyfen)
Dose		
g/catch basin	n. tablets/catch basin	g/catch basin
2	1	4
Treated catch basins (n.)		
14	14	15
Control catch basins (n.)		
21		

Table 2. Scheme of the tests conducted in 2007

1st TRIAL: 17 May - 28 June // 2nd TRIAL: 13 August – 25 September - Marano and Boschi (BO)			
DEVICE® GR-2 (2% diflubenzuron)	Device® TB-2 (2% diflubenzuron)	DEVICE® SC-15 (15% diflubenzuron)	SUMILARV® 0.5G (0,5% pyriproxyfen)
Dose			
g/catch basin	n. tablets/catch basin	ml/catch basin	g/catch basin
2	1	0.28	2
Treated catch basins (n.)			
25	25	25	25
Control catch basins (n.)			
20			

Evaluation of mosquito adults' production inhibition by IGR products

When available a sample of 10-35 L_3-L_4-pupae was collected from a single catch basin, placed in plastic glasses with screw caps and transported in a cool box to the laboratory.

The dead individuals and the emerged adults were counted every 24-48 hours up to exhaustion of the samples (Figure 3).

This method was necessary to know the natural mortality in the control catch basins and to evaluate pyriproxyfen delayed activity. The adults unable to fly after the emergence were considered as an effect induced by the treatment and therefore considered in the calculation of the mortality.

In the diflubenzuron treated catch basins, due to its mechanism of action, L_1 and rarely L_2 were present, and therefore the samples were not collected for several weeks after treatment.

The efficacy of the tested formulations has been expressed as the ability to inhibit adult production from the catch basin and calculated according to the formula:

also $\mathbf{\%AIE = 100 - \{[(L_{3\text{-}4} + P)_{Tr} \times (\%Sf)_{Tr}] / [(L_{3\text{-}4} + P)_{Ts} \times (\%Sf)_{Ts}] \times 100\}}$

where:

%AIE is the percentage of adult emergence inhibition;
$\mathbf{(L_{3\text{-}4} + P)_{Tr}}$ is the mean density of L_3-L_4 and pupae in the treated catch basins;
$\mathbf{(\%Sf)_{Tr}}$ is the percentage of adults obtained from the samples collected from the treated catch basins;
$\mathbf{(L_{3\text{-}4} + P)_{Ts}}$ is the mean density of L_3-L_4 and pupae in the control untreated catch basins;

$\mathbf{(\%Sf)_{Ts}}$ is the percentage of adults obtained from the samples collected from the untreated catch basins;

Figure 3. "Emergence container" used to evaluate the adults' emergence rate from the larvae-pupae samples collected from each road drain.

The data have been submitted to block ANOVA, previous angular transformation of the percentages values. The Newman-Keuls test has been used to evaluate the meaningful effects of the differences among the averages.

In each area a pluviometer was placed, while the temperature data have been obtained from the nearest meteorological station ARPA Emilia-Romagna (Ostellato for Codigoro, Sasso Morelli for Medicina and Mezzolara for Marano and Boschi).

Results

Culicidae population dynamic and climatic conditions during the trials

In all trial localities the species of Culicidae recovered were *Culex pipiens* and *Aedes albopictus.*

In 2006, with respect to the L_3-L_4-pupae sampled in all the road drains in the first trial (Codigoro), *Cx. pipiens* represented 72.1%; *Ae. albopictus* the remaining 27.9%. In the second trial (Medicina) the proportions between the two species were 80.0% *Cx. pipiens* and 20.0% *Ae. albopictus*.

During 2007 the larval population was composed of 95.1% *Cx. pipiens* and 4.9% *Ae. albopictus* and 80.7% and 19.3% in the first and second trial respectively.

Figures 4 and 5 report the total pre-imaginal population trend as sampled in each road drain chosen as control. Figures 6-7 and 8-9 show weather data during the trials of both the years. The control population density resulted in a range similar in Medicina and Codigoro.

In 2006, in Codigoro, a clear decline in larval population density was registered from the first sampling date (June 23) to week 6 (August 3), followed by a slight increase in the two last sampling dates (Figure 4). During the trial precipitation was limited (47 mm in total) with a single notable stormy event on August 12 (25 mm). Air temperatures were high, exceeding 37 °C for several days especially during weeks 4-5 post-treatment (Figure 6). The minimum average temperature resulted 17.6 °C; the maximum average was 31.7°C.

Figure 4. Dynamics of total pre-imaginal population in control catch basins (*Culex pipiens* + *Aedes albopictus*) in Codigoro and Medicina in 2006

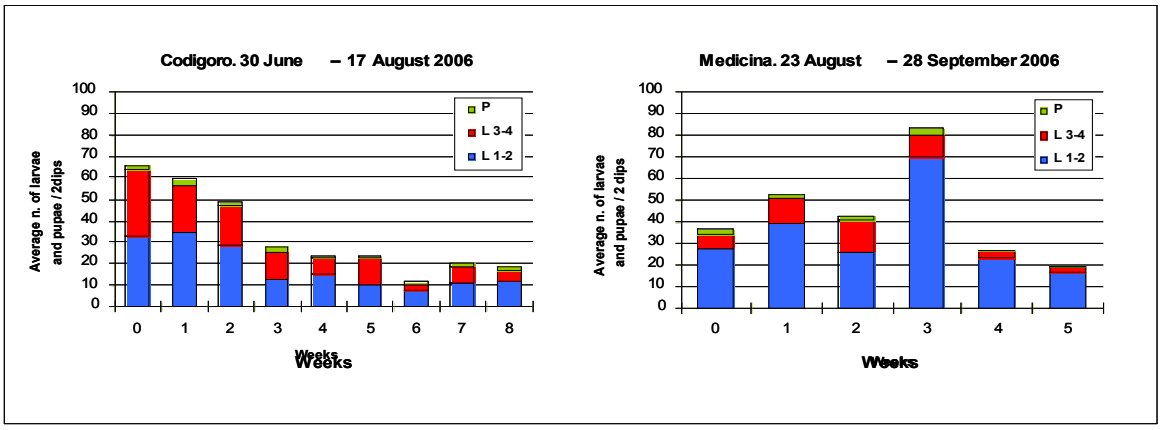


Figure 5. Dynamics of total pre-imaginal population in control catch basins (*Culex pipiens* + *Aedes albopictus*) in Marano and Boschi in 2007

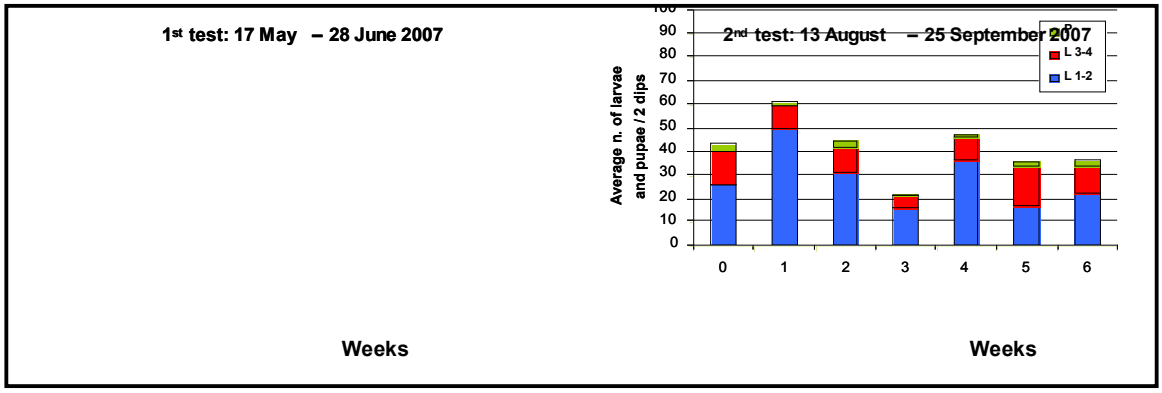
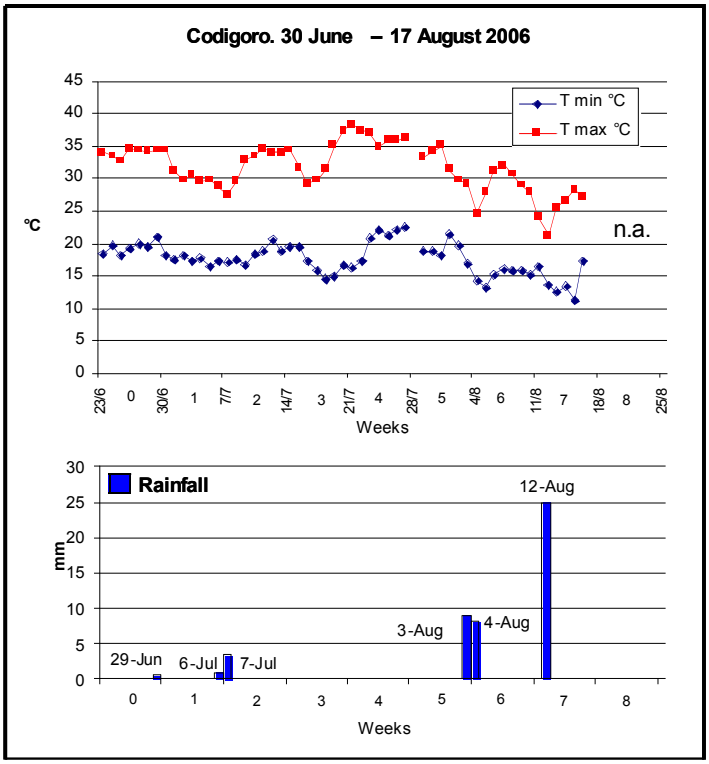
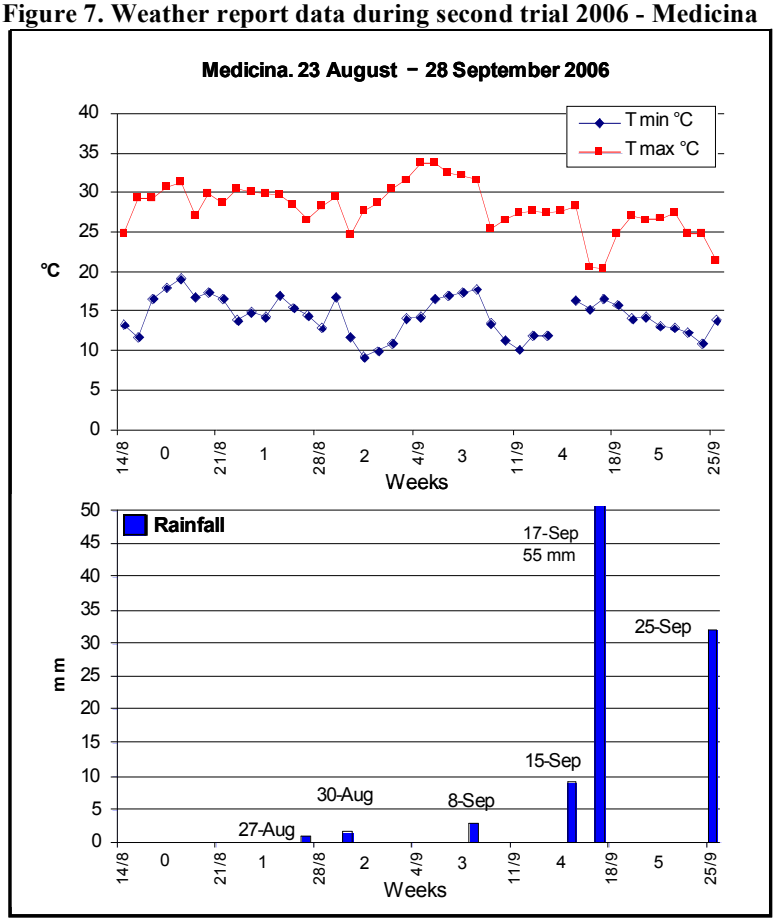


Figure 6. Weather report data during first trial 2006 - Codigoro



n.a.: data not available

Figure 7. Weather report data during second trial 2006 - Medicina



In Medicina a collection of more than 80 larvae + pupae/2dips was registered on September 14 (week 3). Rainfall was higher during the Medicina trial (101.5 mm in total) than during the Codigoro trial (47 mm in total). The minimum average temperature has been 14.0°C; the maximum average was 27.9°C (Figure 7).

Relative proportions of L_{1-2}, L_{3-4}, and pupae were 35%, 49%, 16% in Codigoro and 63%, 31% 6% in Medicina.

In 2007 the observed larval population densities resulted in a range similar to 2006.

In both trials the mean larval densities in control drains fluctuated between 20 and 60 individuals per two dips without a clear seasonal trend (Figure 5). The climatic conditions during the first trial (Marano, 17 May – 28 June 2007) was characterised by 6 days of rain (total rainfall of 79 mm) but without relevant storm events, except the 29 mm of rainfall on June 6. The year 2007 was characterised by abnormally hot winter and spring. Moreover for a good part of the second half of June the maximum temperatures remained above 30°C and the minimums above 15°C, values higher than the average of the period (Figure 8).

In the second trial (Boschi, 13 August – 25 September 2007) the precipitations was similar with 6 days of rain and a total rainfall of 54 mm. The main rainfall was on 31 August with 22 mm. Temperatures can be considered as normal for the season (Figure 9). The minimum average temperature was 14.0°C; the maximum average 28.2°C.

Figure 8. Weather report data during the first trial 2007 – Marano and Boschi

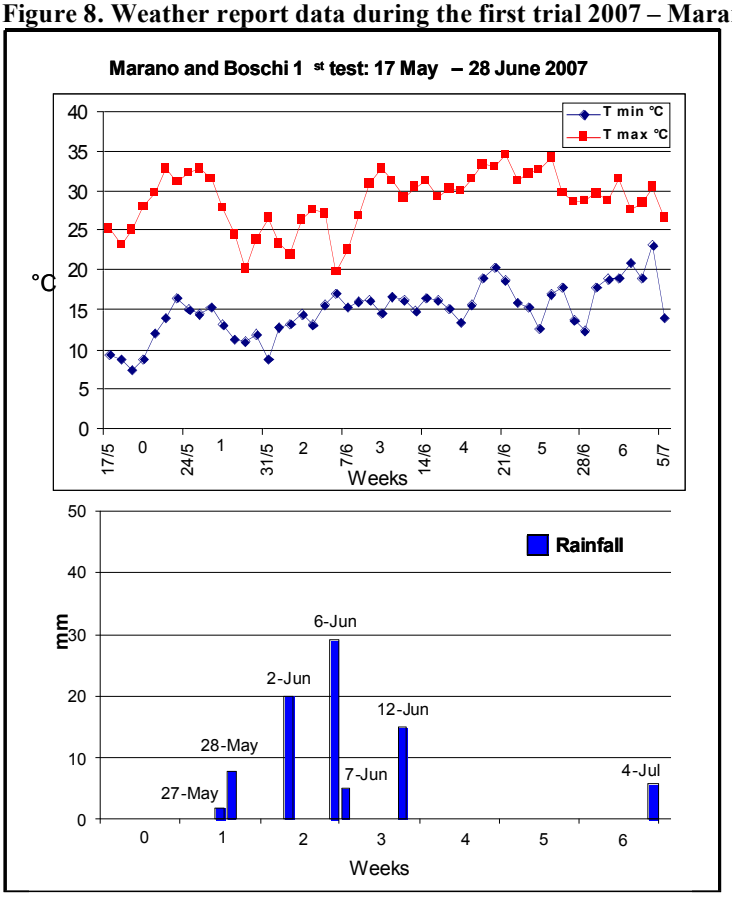
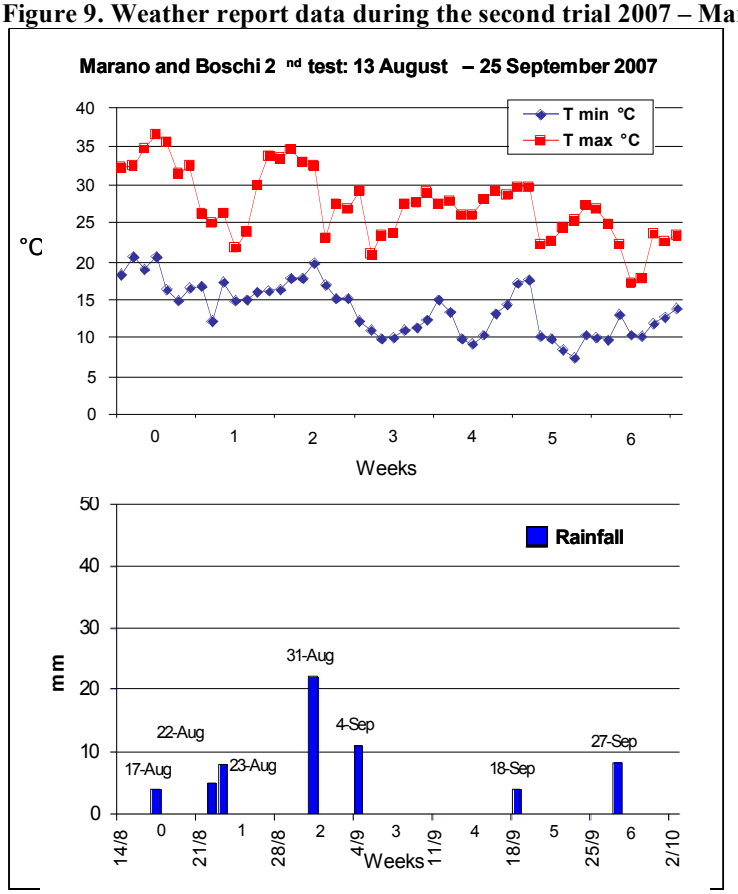


Figure 9. Weather report data during the second trial 2007 – Marano and Boschi



Performances of the tested formulations

Figures 10 and 11 report the dynamics of adult production reduction (corrected with Abbot) in the treated road drains for each formulation in the two years survey.

Figure 10. AIE (corrected with Abbot) in the treated road drains of Codigoro and Medicina - 2006

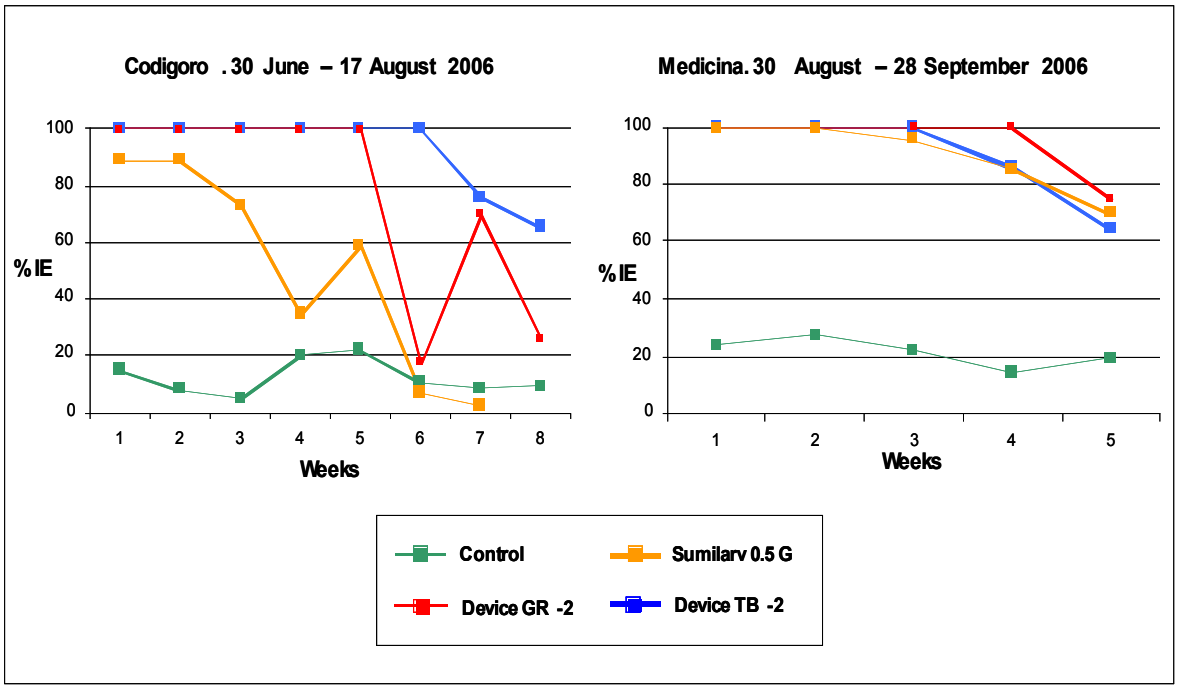
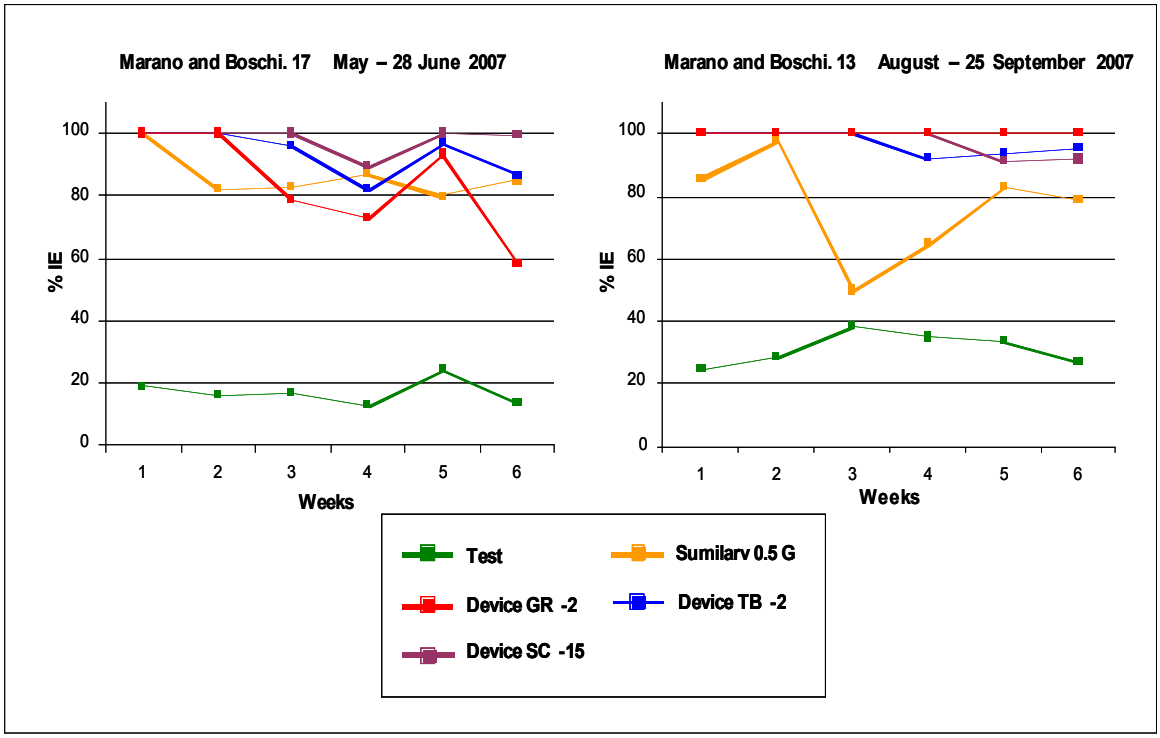


Figure 11. AIE (corrected with Abbot) in the treated road drains of Marano and Boschi - 2007



DEVICE® TB-2

2006. In the trial of Codigoro Adult Inhibition Emergence was 100% until week 6 post-treatment, while in the trial carried out in Medicina this value has been observed until week 3 only, then efficacy declined to 86 % at week 4 and 65% at week 5 (Fig. 10).

The discrepancy between results in the two trials cannot be assigned to precipitations characterized by a strong stormy event on September 17 (55 mm), 4 days before the week 4 data sampling, with not enough time for the larvae to grow up to L_{3-4}.

2007. The AIE was 100% up to week 2 post-treatment in the first and up to week 3 in the second trial. During the first trial, at week 4, the adult reduction percentage lowered to 81.6% and then increased the following week. Maybe it is possible to put in relation this phenomenon with the rain occurred on June 2 and June 6-7 with partial re-distribution of the a.i. eventually blocked in the mud.

The trend was more linear in the second trial (Figure 11) where, after three weeks of complete inhibition the product activity remained very high for three more weeks.

DEVICE® GR-2

2006. In Codigoro the AIE has turned out 100% up to week 5 with a sharp loss at week 6 (Figure 10). In Medicina the inhibition has been 100% until week 4 post-treatment with a decrease to 75% at week 5 (Figure 10).

2007. In the first trial AIE was 100% up to week 2 post-treatment, then a trend similar to Device TB-2 was observed, thus confirming the hypothesis regarding the possible influence of rain in re-distributing the product from the mud to the water column.

In the second trial the product showed 100% adult inhibition up to week 6, when the sampling was stopped (Figure 11).

DEVICE® SC-15

In 2006 no trials were carried out using this formulation.

2007. In the first trial the inhibition was complete up to week 3. At week 4 a drop to 89.4% was observed with a recover to very high values at week 5 (99.7 %) and week 6 (99.5%). The trend was similar on higher level to what observed for other products (Figure 11), with a probable re-distribution effect due to rainfalls.

In the second trial results were also excellent (Figure 11) with close to 100% inhibition in the first 3 weeks, followed with very positive values up to week 6 post-treatment.

SUMILARV® 0.5G

2006. In Codigoro the AIE was around 80% during the first three weeks, than declined sharply (Figure 10). In Medicina reduction resulted close to 100% for two weeks, than gradually lowering down to 70% at week 5 (Figure.10).

The doubling of the dose in Medicina trial evidently improved efficacy of the product in comparison to the previous Codigoro trial.

2007. In the first trial Sumilarv 0.5G® achieved 100% AIE in the first week only. For the remaining part of the test the inhibition was mostly regular but not completely satisfying

(percentages in the range 79.9 - 87.2%). AIE reduction during week 2 post-treatment cannot be ascribed to rainfalls and to consequent product removal from catch basins because the two most important events took place later (June 2 and 6). These rainfalls, instead, probably caused a partial larvicidal a.i. re-distribution inside basins with a slight AIE increment during week 3 and 4.

Different was the result of the second test, with a more irregular trend and with scarce results at week 3 and 4 (Figure 11). It is difficult to say if the rainy event of August 31 (between week 2 and 3 sampling) could be responsible for the increasing AIE observed at week 4 and 5.

Conclusions

From data obtained during the two years' trials it is possible to compare the products' efficacies and lasting activities. Block ANOVA calculated on all the trials conducted showed differences between formulations performance starting at week 3 post-treatment (Table 3).

The three diflubenzuron-based formulations (Device®) assured good efficacy and persistence standards until week 5 post-treatment, whereas Sumilarv® 0.5G tested at the dose of 2 g/catch basin didn't achieve the same efficacy and persistence. This difference was partially compensated when the Sumilarv 0.5G® dose was doubled.

No significant differences were observed between AIE measured in diflubenzuron-based formulations (F=0.18; P=0.71) in relation to the seasonal period (1st part - 2nd part of summer).

Results of the tests confirmed the high efficacy and the good insecticide persistence of diflubenzuron based formulations at the dosages given on the labels in the Italian road drain system.

Best results for efficacy, persistence, and constancy of results were given by DEVICE® TB-2 AND DEVICE® SC-15. This latter formulation is also suitable for the high practicality of use in the treatment of the road hopper windows, a road drain typology very common in most Italian towns.

The performances of SUMILARV® 0.5G confirmed themselves as problematic in the test environment in both years where it results that, at label doses, efficacy and persistence were not satisfying.

Table. 3. Comparison among IGRs formulations efficacy during the two years' trials

Week	Product	N	Mean	SD	Duncan test
W1	Device GR2	4	98,77	1,44	NS
	Device TB2	4	100,00	0,00	
	Sumilarv 0,5G (2g)	3	91,84	7,16	
	Device SC15	2	100,00	0,00	
	Sumilarv 0,5G (4g)	1	99,29	0,00	
W2	Device GR2	4	99,64	0,56	NS
	Device TB2	4	99,57	0,85	
	Sumilarv 0,5G (2g)	3	89,60	7,87	
	Device SC15	2	100,00	0,00	
	Sumilarv 0,5G (4g)	1	99,42	0,00	
W3	Device GR2	4	94,13	10,45	A
	Device TB2	4	96,64	4,28	A
	Sumilarv 0,5G (2g)	3	68,67	17,28	B
	Device SC15	2	98,45	0,52	A
	Sumilarv 0,5G (4g)	1	95,85	0,00	A
W4	Device GR2	4	92,77	14,46	A
	Device TB2	4	89,69	8,29	A
	Sumilarv 0,5G (2g)	3	61,93	25,99	B
	Device SC15	2	91,46	3,70	A
	Sumilarv 0,5G (4g)	1	85,55	0,00	A
W5	Device GR2	4	91,33	11,21	A
	Device TB2	4	88,49	16,35	A
	Sumilarv 0,5G (2g)	3	73,72	13,01	AB
	Device SC15	2	95,21	6,38	A
	Sumilarv 0,5 G (4g)	1	69,71	0,00	B

When comparing the efficacy of the four products on the two mosquito target species we observed that:

- no significant differences resulted when performances of each product were analysed against singular species;
- Sumilarv 0.5G® showed a slightly less (non significant) pronounced efficacy on *Ae. albopictus* than on *Cx. pipiens* during the period week 2-6 post-treatment (Figure 12). This evidence must be investigated in a larger trial in order to be confirmed;

- the three diflubenzuron formulations showed no evidences of different efficacy on the two mosquito species (Figure13).

Figure 12. AIE trend on *Cx. pipiens* and *Ae. albopictus* in catch basins treated with Sumilarv 0.5G (2 g/catch basin)

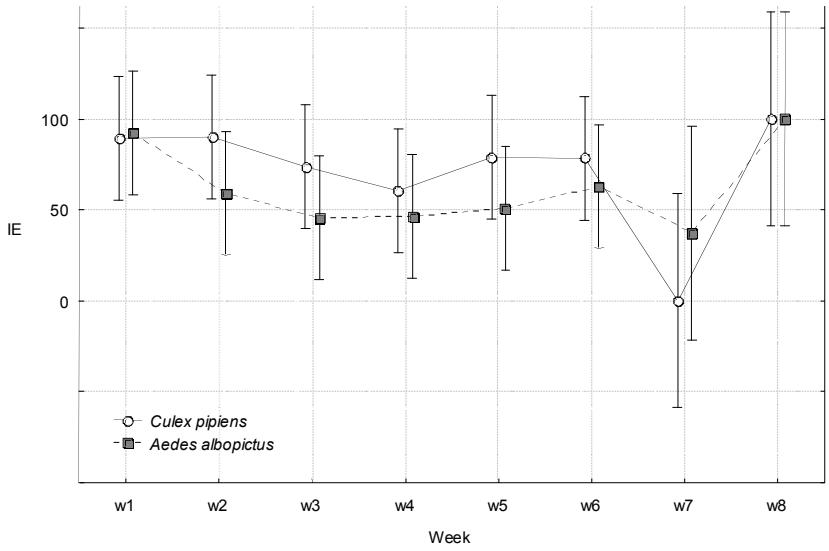
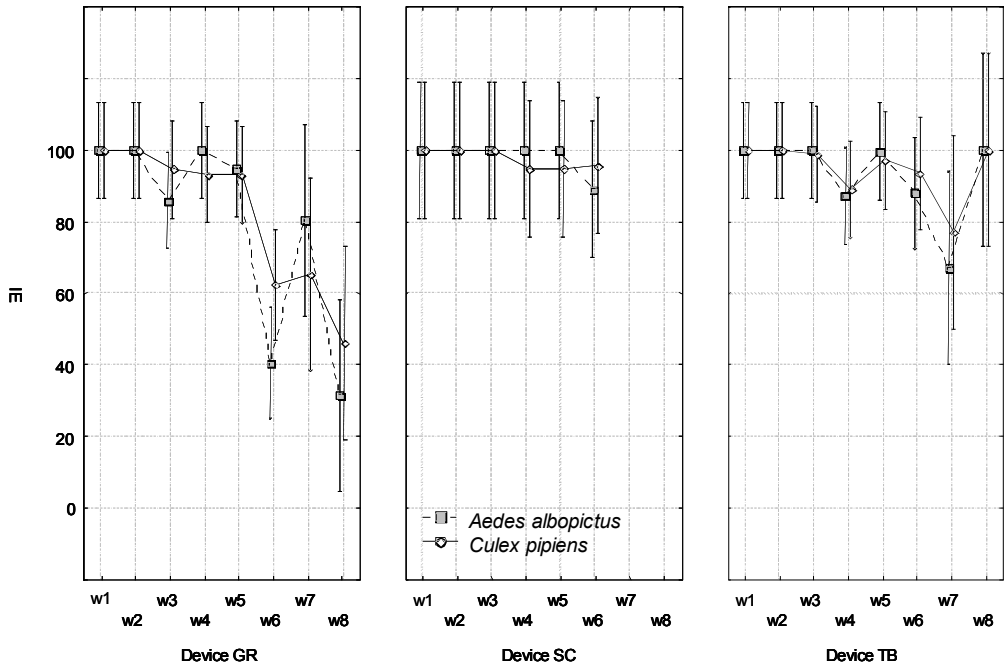


Figure 13. AIE trend on *Cx. pipiens* and *Ae. albopictus* in catch basins treated with diflubenzuron-based formulations



Acknowledgements

We thank CHEMTURA for financing the study, the municipalities of Baricella, Castenaso, Codigoro, and Medicina for kindly hosting the study.

References

Celli, G., Bellini, R. & Carrieri, M. (1994) Survey on *Aedes albopictus* (Skuse) (*Diptera*: *Culicidae*) infestation in Desenzano del Garda (Brescia province-Italy). *Bollettino dell' Istituto di Entomologia "G. Grandi", Università di Bologna* **48**, 211-217.

Chamberlain, W.F. (1975) Insect growth regulating agents for control of arthropods of medical and veterinary importance. *Journal of Medical Entomology* **12**, 395-400.

Gabinaud, A., Vigo, G., Cousserans, J., Roux, M., Pasteur, N. & Croset, H. (1985). La mammophilie des populations de *Culex pipiens* L., 1758 dans le Sud de la France; variations de ce caractere en fonction de la nature des biotopes de developpement larvaire, des caracteristiques physico-chimiques de leurs eaux et des saisons. Consequences pratiques et theoriques. *Cahiers O.R.S.T.O.M. Service Entomologie Medicale et Parasitologique* **23**, 123-132.

Ishii, T. & Rak Sohn, S. (1987) Highly polluted larval habitats of the *Culex pipiens* complex in central Sweden. *Journal of the American Mosquito Control Association*, **3**, pp. 276-281.

Mulla, M.S., Majori, G. & Darwazeh, H.A. (1975) Effect of the insect growth regulator Dimilin or TH 6040 on mosquitoes and some nontarget organism. *Mosquito News* **35**, 211-216.

Mulla, M.S., Darwazeh, H.A., Kennedy, B. & Dawson, D.M. (1986) Evaluation of new insect growth regulators against mosquitoes with notes on nontarget organisms. *Journal of the American Mosquito Control Association* **2**, 314-320.

Mulla, M.S. (1995) The future of insect growth regulators in vectors control. *Journal of the American Mosquito Control Association* **11**, 269-273.

Mulligan III, F.S. & Schaefer, C.H. (1990) Efficacy of a juvenile hormone mimic, pyriproxyfen (S-31183), for mosquito control in dairy wastewater lagoons. *Journal of the American Mosquito Control Association* **6**, 89-92.

Rey, J.R., O'Meara, G.F., O'Connell, S.M. & Cutwa-Francis, M.M. (2006) Factors affecting mosquito production from stormwater drains and catch basins in two Florida cities. *Journal of Vector Ecology* **31**, 334-343.

Rutz, D.A., Axtell, R.C. & Edwards, T.D. (1980) Effect of organic pollution levels on aquatic insect abundance in field pilot-scale anaerobic animal waste lagoons. *Mosquito News* **40**, 403-409.

World Health Organization (2001) Review of: IR3535; KBR3023; (RS)-Methoprene 20% EC, Pyriproxyfen 0.5% GR; and Lambda-cyhalothrin 2.5% CS. Report of the fourth WHOPES working group meeting. WHO/HQ, Geneva 4-5 December 2000, pp.103.

World Health Organization (2005) Specifications and evaluations for public health pesticides. Diflubenzuron.; Geneva, Switzerland, pp.27.

World Health Organization (2006) Report of the ninth WHOPES working group meeting WHO/HQ review of: Dimilin GR and DT, Vectobac DT, Aqua K-Othrine, Aqua Reslin Super. Geneva, Switzerland; pp. 96.