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Comparative Evaluation of Pyrethroid Insecticide Formulations against *Triatoma infestans* (Klug): Residual Efficacy on Four Substrates

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*We investigated the residual efficacy of four insecticide formulations used in Chagas disease vector control campaigns: cyfluthrin 12.5% suspension concentrace (SC), lambda-cyhalothrin 10% wettable powder (WP), deltamethrin 2.5% SC, and 2.5% WP on four types of circular blocks of wood, straw with mud, straw with mud painted with lime, and mud containing 5% of cement. Three concentrations of these insecticides were tested: the LC₉₀ (previously determined on filter paper), the double of the LC₉₀, and the recommended operational dose. For each bioassay test, 15 third-stage nymphs of *Triatoma infestans* (Klug) (Hemiptera: Reduviidae) were exposed for 120 h to each treatment at 24 h, 30, 60, 90, and 180 days post-spraying. Mortality rates, moulting history and behaviour were recorded at 24, 48, 72, and 120 h of exposure. Mortality rates were highest during the first 30 days post-spraying. Highest mortality rates (above 50%) were observed for deltamethrin 2.5% SC and lambda-cyhalothrin 10% WP on wood blocks up to three months post-spraying. Mud was the substrate on which treatments showed lowest persistence, with the other two substrates showing intermediate residual efficacy of all treatments. During the first 30 days WP formulations were not as effective as SC flowable formulations but, overall in the longer term, WP gave grater mortality rates of *T. infestans* nymphs exposed at up to six months post-spraying. Porous surfaces, especially mud, showed most variability presumably due to absorption of the insecticide. In contrast the less porous surfaces (i.e. wood and lime-coated mud) kept mortality rates high for longer post-treatment, irrespective of the insecticide concentration used.*

Key words: bioassays - efficacy - insecticides - pyrethroid - residuality - substrates - *Triatoma infestans*

Compounds from every class of insecticide have been tested against triatomine bugs (Hemiptera, Reduviidae), the vectors of Chagas disease in Latin America (Schofield 1985, Sequeda et al. 1986, Zerba 1999). Among the organochlorines, DDT showed relatively poor effect but gamma-HCH-BK was found to be very effective in early trials against *Panstrongylus megistus* Burmeister and *Triatoma infestans* (Klug) in Brazil and Argentina (Dias & Pellegrino 1948, Romana & Abalos 1948). Dieldrin was used with good results against *Rhodnius prolixus* (Stal) in Venezuela but mild resistance was observed in some areas (Sequeda et al. 1986). Various organophosphate and carbamate insecticides were also tested but they were more expensive and had no operational advantages over BHC (Schofield 1985). During the late 1970's and early 1980's, trials of synthetic pyrethroids showed them to be more effective than BHC at lower doses (Pinchin et al.

1980, 1981). Since the mid 1980's, several of the most common pyrethroids (deltamethrin, lambda-cyhalothrin, cyfluthrin, and cypermethrin) have become the main insecticides used operationally for control of Triatominae, because of their efficacy and persistence, as well as the minimal environmental pollution associated with their use (Pinchin et al. 1980, 1981, Schofield 1985).

Limiting factors for activity include the initial insecticide dose rate, nature of the sprayed surface as well as the age of insecticide deposits and the environmental conditions such as humidity and temperature (Nocerino 1981, Penna et al. 1984, Giga & Canhao 1992, Fletcher & Axtell 1993). The type of substrate, in terms of its porosity, is of particular importance. On porous surfaces such as mud, the insecticide deposit seems to lose activity much faster than on surfaces such as wooden boards, ceramics and tiles (Barlow & Hadaway 1949, 1952, Penna et al. 1985). In the case of pyrethroids, chemical breakdown may also be expected on highly alkaline surfaces and on surfaces exposed to sunlight (Leahey 1985). In order to get a better knowledge of insecticide/substrate interaction, we evaluated the residual efficacy of four commercial pyrethroid formulations currently used in bug control operations on four substrate materials commonly used for house construction in Paraguay. The experiments were performed in the Tropical Medicine Laboratory of the Instituto de Investigaciones en Ciencias de la Salud (IICS), Asunción, Paraguay.

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MATERIALS AND METHODS

Insects - *T. infestans* susceptible Paraguayan strain was maintained at the IICS. Groups of 30 bugs were kept in 18 x 38 cm jars at 28°C and 60-70% relative humidity and fed weekly on pigeons for 20 min. Nymphs which had moulted to third stage < 1 week previously were used for tests. After the period of exposure to insecticide, the insects were blood fed on a pigeon and observed for 15 days.

Substrate block - Four types of circular blocks (9 cm diameter, 0.5 cm thick) were used as substrates to be treated with insecticide. The blocks were made of either wood, straw with mud, straw with mud painted with lime, or mud containing 5% cement, all prepared at the Centro de Tecnología Apropiada (CTA), Universidad Católica Nuestra Señora de la Asunción, Paraguay. A total of 220 blocks were sprayed and held in cardboard boxes away from light and wind at laboratory conditions of temperature and humidity until used.

Wood blocks were made of "Petereby" *Cordia trichotoma* (Vell.), a typical wood used in rural areas of Paraguay to build windows, doors, and roof supports.

Soil-cement blocks were made of a hardened mixture of 5% cement and soil components (sand, lime, and clay, proportions 50:20:30). The soil was pulverised mechanically and then sieved (sieve # 4-4.8 mm). Portland cement was then added uniformly and mixed by hand to homogeneity with rainwater until a dense paste was obtained. Compacting was carried out in layers until a final thickness of 0.5 cm in moulds of 9 cm diameter was obtained. Resulting blocks were cured by coverage with plastic foil and occasional spraying with water for one week to avoid rapid drying.

Mud blocks were made from soil composed of 60:20:20 sand, lime, and clay. Straw or grass was added as a fibre to reduce cracks during the drying process. Compacting and curing were performed as with the soil cement blocks. To simulate the type of whitewash commonly used on walls, a mixture of lime (calcium oxide) and water (500 mg/500 ml) was prepared and continuously stirred before application by brushing onto dried mud blocks. Two coats were applied with a 15 min-drying interval.

Insecticides - Four commercial products: cyfluthrin 12.5% SC, deltamethrin 2.5% suspension concentrate (SC), and 2.5% wettable powder (WP), and lambda-cyhalothrin 10% WP were evaluated at three concentrations (Table I).

Concentration-response curves were first established by exposing 3rd stage nymphs of *T. infestans* (n = 15/test x 2 replicates) for 24 h to insecticide residues on disks of filter paper (Whatman nr 1). Six dilutions of each insecticide were prepared: concentrations of active ingredient (a.i. expressed in mg/m²) were 6, 12.5, 25, 50, 100, and 200 mg ai/m². Each insecticide was first mixed in water following the manufacturer instructions, with vigorous shaking to ensure an even suspension and serial dilutions were prepared quickly to prevent sedimentation. Each filter paper disk placed on an inverted 9 cm diameter Petri dish was treated with 1.2 ml of freshly shaken insecticide suspension of sufficient volume to wet the filter paper with no run-off. Duplicate experiments to

determine concentration-response curves were run two weeks apart. Log probit analysis used the Toxicologie TM Programme (G Febvay & Y Rahbe, Insa-Inra 406, F-69621 Villeurbanne cedex, France) to calculate the LC₅₀ and LC₉₀.

Bioassays of treated substrates - The four types of blocks were then sprayed with each pyrethroid formulation at three concentrations: the recommended commercial dose, the LC₉₀, and 2 x the LC₉₀. To do this, the blocks were put on an horizontal paper surface forming an area of 0.22 m² to be sprayed with a 10-15 cm swath from a 500 ml plastic sprayer with the nozzle held 45 cm from the block.

The amount of insecticide required to cover the area of 0.22 m² at the target rate was diluted in 50 ml of distilled water for application. Pre-weighed filter papers were also treated by the same procedure, dried (with a hair dryer) and re-weighed immediately to give a further estimate of the amount of insecticide applied to the surface. Applied concentrations were confirmed by HPLC residue analysis by Roussel Uclaf (later Aventis, now Bayer) Berkhamsted, UK.

Efficacy of insecticide treated substrates was bioassayed with unfed 3rd stage nymphs of *T. infestans* exposed to three concentrations of treatment on each of the four substrates. The insects were held in place using WHO (1995) bioassay cones (9 cm diameter designed for mosquito bioassays on walls) nailed to the treated blocks. The nymphs (n = 15 per test x 2 replicates) were held on untreated (control) and treated surfaces for 120 h.

Mortality rates, moulting history and behaviour were recorded after 24, 48, 72, and 120 h exposure at 24 h, 30, 60, 90, and 180 days post-spraying. Surviving insects were transferred onto clean filter papers and held at 28°C and 60-70% relative humidity for 15 days to check further mortality.

An insect was considered dead if no locomotory activity could be seen when it was prodded. Moribund insects were considered dead at the time of reading if they did not show any subsequent recovery of activity during the following 15 days.

Mortality data (mean of two independent tests) were analyzed using the statistical package MINITAB. A chi square contingency analysis was performed to investigate the relationship between insect mortality and persistence of deposits at different post-spray times for each substrate. Chi square analysis of the contingency tables was also performed to establish significant differences in mortality rates between different insecticide concentrations on the various substrate types. The most effective concentration per block was determined by subdividing contingency tables and pooling the different concentrations in order to analyse variations in chi square with changes in degrees of freedom.

RESULTS

The initial LC₅₀ value (determined from treated filter paper test) closely approximates the commercially recommended operational application rate of each insecticide tested (Table I). Therefore, further tests evaluated these operational application rates as well as the LC₉₀ and the double of the LC₉₀ (for 24 h exposure on

TABLE I
Technical information on insecticides applied

Compound	Trade name	Formulation	Recommended application rate	Concentration-response-curve(mg a. i/m ²)		Manufacturer
				LC ₅₀	LC ₉₀	
Lambda-cyhalothrin	ICON	10% WP	30 mg/m ²	32.7(24.6-43.4)	62.5(59.5-114.5)	Zeneca (now Singenta), UK
Deltamethrin	K-Othrine	2.5% SC	25 mg/m ²	31.1/20.5-47.2)	187.8 (106.6-330.7)	Aventis (now Bayer) Germany
Deltamethrin	K-Othrine	2.5% WP	25 mg/m ²	38.4-4-54.4)	164.4 (105.4-246.6)	Aventis (now Bayer)Germany
Cyfluthrin	Responsar	12.5% SC	25 mg/m ²	32.2 (23.1-36.4)	182.4 (110.2-241.1)	Bayer, AG, Germany

WP: wettable powder; SC: flowable suspension concentrate

filter paper) to evaluate the residual efficacy of insecticides on different treated substrates blocks.

High mortality rates (50-100%) were observed on three of the four substrates (i.e. wood blocks, lime-coated mud, and soil cement blocks) during the first three months of post-treatment with cyfluthrin 12.5% SC at 25 mg ai/m², deltamethrin 2.5% SC at 25 mg ai/m² or lambda-cyhalothrin 10% WP at 30 mg ai/m². The activity of deltamethrin 2.5% WP at 25 mg ai/m² decayed drastically after one month and, as post-spraying time increased, longer exposure periods were required for all four products to achieve peak mortality rates. Also, for all four insecticides at each dosage, longer exposure was required on treated mud than on the other three substrates to achieve the same mortality (data not shown). Nevertheless, 100% mortality was observed when bugs were exposed on mud blocks freshly treated with deltamethrin SC or lambda-cyhalothrin WP for 72 h (data not shown). Control mortality on all types of untreated substrate was usually zero and never surpassed 7% (2/30). Therefore no correction was made for control mortality in data analysis.

One month post-spraying the wood, lime-coated mud and soil-cement blocks still showed good efficacy against nymphs exposed for 24-48 h but at 3-6 months post-spraying, 72-120 h of exposure were required to achieve maximum mortality. Insecticide activity was still present six months post-spraying of all four products, at least on wood and soil-cement substrates, provided the exposure time was sufficient (data not shown). Nine months post-spraying, a low mortality rate (20% or less) was only observed in substrates sprayed with wettable powders except on wood blocks sprayed with deltamethrin WP where mortality was zero.

Table II compares mortality of bugs exposed by 24 h on blocks with 30, 60, 90, and 180 days post-spraying. The decline of insecticide activity with time on each substrate was clearly demonstrated for all four products. On wood and soil-cement blocks with each treatment, mortality rates of *T. infestans* nymphs were significantly higher ($p < 0.001$) at 1-3 months than at 6 months post-spraying. On lime-coated mud blocks, the two WP formulations were initially less effective than the two flowable formulations but performance of lambda-cyhalothrin WP was similar to that of the SCs over 3-6

months. Deltamethrin SC gave higher initial mortalities on all surfaces, with persistence similar to that of lambda-cyhalothrin WP. Cyfluthrin SC performed better on wood and lime-coated mud blocks but very poorly on uncoated mud blocks and on soil blocks. For all products, wood and lime-coated mud blocks were better surfaces than uncoated mud or soil-cement blocks.

Table III shows chi-square contingency table analysis applied to determine the most effective insecticide at 6 months after spraying (excluding cyfluthrin SC which had entirely lost residual efficacy). The highest mortalities were pooled and compared over all concentrations; if no statistical significance was observed between different degrees of freedom, new pools were performed until significant differences were found. No simple pattern emerges from these data except that the persistence of each treatment clearly depends upon the substrate. It is interesting to note, though difficult to explain, that the highest dose of the products did not produce the longest persistence. Deltamethrin 2.5% WP was the most residual insecticide on all substrates when applied at the highest dose tested (i.e. LC₉₀ x 2). At doses more likely to be used in field (i.e. recommended rate and LC₉₀), the flowable formulation of deltamethrin SC showed best effects overall, especially on lime-coated mud blocks.

DISCUSSION

Many previous studies (Burkholder & Dicke 1966, Jain & Yavav 1989, Fletcher & Axtell 1993) indicated that the insecticide formulation, the insect stage, as well as the insecticide exposure method are critical in insecticide evaluation. This study was designed to compare residual efficacy of two formulations of four pyrethroids against nymphs of triatomine bugs on substrates representing those commonly used in domestic habits of rural South America. Products chosen were considered to be those most likely to be of benefit in campaigns of house spraying to control vectors of Chagas disease. Insecticide trials against triatomines, including treatments on different types of surfaces, have been previously reported by Penna et al. (1985), Schofield and Williams (1985), Oliveira Filho (1992, 1999), Diotaiuti and Texeira Pinto (1991), Guillén et al. (1997), Rojas de Arias et al. (1999), and Rojas de Arias and Fournet (2002).

TABLE II

Triatoma infestans mortality rates following 24 h exposure to four substrates treated with four products tested one, three, six months post-treatment

Insecticide	Dead triatomines/total(percent)			Chi-square values (DF:1) ^a ; p values	
	30	Post-treatment days		30 vs (90 + 180) days	(30 + 90) vs 180 days
		90	180		
	Wood blocks				
Deltamethrin 2.5% SC	30/30 (100)	30/30 (100)	6/30 (20)	14.38; < 0.001	61.43; < 0.001
Deltamethrin 2.5% WP	23/30 (76.7)	2/30 (7)	2/30 (7)	43.39; < 0.001	10.06; < 0.001
Lambda-cyhalothrin 10% WP	30/30 (100)	18/30 (60)	8/30 (27)	24.96; < 0.001	21.99; < 0.001
Cyfluthrin 12.5% SC	23/30 (76.7)	18/30 (60)	30/30 (100)	0.01; 0.93	10.28; < 0.01
Control	2/30 (7)	0/30 (0)	0/30 (0)		
	Mud blocks				
Deltamethrin 2.5% SC	17/30 (56.7)	0/30 (0)	0/30 (0)	38.30; < 0.001	8.71; < 0.01
Deltamethrin 2.5% WP	13/30 (43.3)	0/30 (0)	2/30 (7)	20.25; < 0.001	2.91; < 0.05
Lambda-cyhalothrin 10% WP	17/30 (56.7)	6/30 (20)	4/30 (13)	13.39; < 0.001	4.82; < 0.01
Cyfluthrin 12.5% SC	0/30 (0)	6/30 (20)	0/30 (0)	-	-
Control	2/30 (7)	0/30 (0)	0/30 (0)		
	Lime-coated mud blocks				
Deltamethrin 2.5% SC	30/30 (100)	10/30 (33)	16/30 (53)	24.96; < 0.001	1.0; 0.31
Deltamethrin 2.5% WP	15/30 (50)	8/30 (27)	2/30 (7)	9.48; < 0.01	8.48; < 0.01
Lambda-cyhalothrin 10% WP	16/30 (53.3)	10/30 (33)	14/30 (46)	0.95; 0.33	0.01; 0.94
Cyfluthrin 12.5% SC	21/30 (70)	4/30 (25)	0/30 (0)	39.85; < 0.001	15.25; < 0.001
Control	2/30 (7)	7/30 (7)	0/30 (0)		
	Soil-cement blocks				
Deltamethrin 2.5% SC	30/30 (100)	2/30 (7)	0/30 (0)	81.56; < 0.001	24.55; < 0.001
Deltamethrin 2.5% WP	25/30 (83.3)	8/30 (27)	4/30 (13)	30.57; < 0.001	12.69; < 0.001
Lambda-cyhalothrin 10% WP	10/30 (33.3)	0/30 (0)	0/30 (0)	19.25; < 0.001	4.06; < 0.01
Cyfluthrin 12.5% SC	6/30 (20)	4/30 (13)	0/30 (0)	2.38; 0.06	4.06; < 0.01
Control	2/30 (7)	0/30 (0)	0/30 (0)		

^a: Chi-square. Fifteen nymphs (2 x 15/test) were exposed for 24 h on each substrate sprayed with insecticide at commercial dose and mortality was scored after 15 days recovery period.

We defined similar values to those found by Schofield and Williams (1985) when deltamethrin concentration-response was evaluated using a residual exposure method to test different insecticides against triatomines. For instance, LC_{50} at 24 h in second stage nymphs of *T. infestans* nymphs reached at 32 mg/m² while 136 mg/m² is mentioned as LC_{90} . Using technical grade deltamethrin and following 24 h exposure, Zerba et al. (1988), reported a LC_{50} of 0.03 µg/cm² for fifth instar-nymphs. Therefore, concentration-response data using filter paper as standard procedure provided important baseline information under laboratory conditions and could be a useful tool in evaluating differences in insecticide susceptibility in further studies.

The interaction between insecticide and substrate surface resulted in a rapid decrease of its activity (data not shown). The nature of the substrate plays an important role in the rate at which this decay occurs. In these trials, insecticide was most persistent on wood and lime-coated mud surfaces. These surfaces are less porous in nature avoiding the quick penetration of the insecticide occurring on mud blocks (Williams et al. 1982, 1983, Giga & Canhao 1992).

The most rapid loss in insecticide toxicity occurs on mud blocks indicating strong absorption or breakdown of the insecticide by alkaline substances (White 1982,

Diotaiuti & Texeira Pinto 1991). The lack of insecticide persistence on porous surfaces has been noted before (Barlow & Hadaway 1949, 1952, Williams et al. 1982, 1983, Jain & Yarav 1989, Giga & Canhao 1992). Moreover, the notable variability in mortality on porous substrates, such as those reported here, have also been noted by other authors (Diotaiuti & Texeira Pinto 1991).

Concrete surfaces are also porous substrates and, for example, the application of corn dust to them causes a decline in the pH and a reduction in its porosity, improving the residual effect of insecticides (White 1982). While testing commercial sealants, Arthur (1994) found that applying sealants to concrete prior to insecticide application formed a waterproof barrier and improved residual efficacy of cyfluthrin WP. As reported here, lime-coated mud blocks showed improved insecticide efficiency. This may be due to various reasons but most probably because of improved particle adhesion and decreased porosity of the mud. Lime is obtained by the application of heat to calcium carbonate that produces carbonic anhydride (gas) and calcium oxide (quick lime). Slow hardening in normal conditions is one of the main characteristics of lime reacting with atmospheric carbon dioxide which begins at 24 h of kneading the paste and is completed at 6 months forming neutral calcium carbonate which is finally insoluble (CTA 1992). This property and

TABLE III
Insecticide bioassays results for persistence analysis at six months post-spraying according to the applied concentration

Insect status	Deltamethrin 2.5% SC			Deltamethrin 2.5% WP			Lambdacyhalothrin 10% WP		
	Concentration (mg a.i./m ²)			Concentration (mg a.i./m ²)			Concentration (mg a.i./m ²)		
	OD25	LC ₉₀ 188	2 x LC ₉₀ 376	OD25	LC ₉₀ 164	2 x LC ₉₀ 329	OD30	LC ₉₀ 62	2 x LC ₉₀ 125
Wood blocks									
Dead	3	14 ^{a,b}	14 ^{a,b}	1	2	14 ^{a,b}	4	2	10 ^b
Alive	12	1	1	14	13	1	11	13	5
Mud blocks									
Dead	0	1	2	1	0	4 ^a	2	2	11 ^a
Alive	15	14	13	14	15	11	13	13	4
Lime-coated mud blocks									
Dead	8 ^{b,c}	11 ^{a,b,c}	7 ^c	1	5	14 ^{a,b,c}	7 ^c	1	10 ^{a,b,c}
Alive	7	4	8	14	10	1	8	14	5
Soil cement blocks									
Dead	0	9 ^a	1	2	0	14 ^a	0	1	4
Alive	15	6	14	13	15	1	15	14	11

OD: operational dose; Wood blocks: Chi-square = 71.362 df= 8 p < 0.001; a: first pooling = 71.362 df= 6 p < 0.001; difference = 71.362 - 71.362 = 0; b: second pooling = 68.154 df= 5 p < 0.001; pooling effect = 71.312 - 68.154 = 3.208 0.10 > p > 0.05; Mud blocks: Chi-square = 43.498 df= 8 p < 0.001; a: first pooling = 31.942 df= 6 p < 0.001; pooling effect = 43.498 - 31.942 = 11.556 df= 7 p < 0.001; Lime-coated mud blocks: Chi-square = 40.346 df= 8 p < 0.001; a: first pooling = 38.028 df= 6 p < 0.001; difference: 2.318 df= 2 0.50 > p > 0.25 (not significant), b: second pooling = 35.332 df= 5 p < 0.001; difference = 2.696 df= 1 0.25 > p > 0.10 (not significant), c: third pooling = 35.332 df= 5 p < 0.001; pooling effect = 35.332 - 30.319 = 5.013 df= 2 0.10 > p > 0.05; Soil cement blocks: Chi-square = 72.441 df= 8 p < 0.001; a: pooling = 12.178 df= 7 0.10 > p > 0.05; pooling effect = 72.441 - 12.178 = 60.263 df= 1 p < 0.001

This table shows the best concentration per block sub-dividing contingency tables and pooling the different concentrations. Variations in chi-square with changes in degrees of freedom were calculated per each block. The three concentrations of each product correspond to commercial doses, LC₉₀ and 2 x LC₉₀.

the decline in the pH value on the surface would avoid the breakdown of the applied insecticide. Further studies should be done to determine the period of time required to neutralize the effect of lime on insecticides and to maximize their sealant properties.

Our data have shown that the flowable formulation (deltamethrin SC 25) killed a high percentage of insects at an early age of insecticide deposits (up to 30 days). Nevertheless, wettable powder on lime-coated blocks such as lambda-cyhalothrin WP10 killed less insects in the short term but the effect was expanded up to 9 months, showing a similar performance for both formulations (Table II).

Independence of triatomine mortality and insecticide concentration was observed for flowable formulations but not in all wettable powders (Table III). Wettable formulations are often more effective at controlling insects than emulsions on porous surfaces (Schofield & Pinchin 1979, Williams et al. 1982). In WP, active ingredients tend to filter out the liquid and remain on the surface of the porous materials rather than penetrating deeply into the substrate being readily available to contact insects (Williams et al. 1982). Overall, the dependence of mortality and concentration begins to disappear with the age of the spray deposit.

In field studies, initial sprayings with pyrethroids have eliminated domestic bug populations for several years at low dose and cost (Gurtler et al. 1994, Rojas de Arias et al.

1999). Studies carried out in laboratory conditions have confirmed these results (Diotaiutti & Teixeira Pinto 1991, Ferro et al. 1995) indicating that pyrethroids persistence depends on the product and type of substrate. However, it should be remarked that the good performance of pyrethroids does not entirely depend on the residual effect of the insecticide as residual effect relies on the amount of insecticide remaining on the treated surface while reinfestation is a consequence of many different factors mainly related to the initial impact on the insect colony and the surface type. Effectiveness of pyrethroids in their initial impact is more relevant than the residual effect that promptly decreases on all types of surfaces. The results of this laboratory study showed a very low residual effect of the pyrethroids on all substrate types used beyond 90 days post-spraying with the only exception of deltamethrin SC 25 on wood blocks but it is important to note that lime-coated surfaces sprayed with flowable or wettable formulations have shown a residual activity beyond 90 days, time enough to eliminate any nymph that hatches from eggs after the initial clean-out.

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