

The indoor use of plastic sheeting pre-impregnated with insecticide for control of malaria vectors

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Summary

OBJECTIVE To evaluate the efficacy of permethrin-treated plastic sheeting (ITPS) applied as a lining to the ceiling or walls of rooms against pyrethroid-susceptible and pyrethroid-resistant *Anopheles gambiae*. **METHOD** Overnight tests were carried out in veranda-trap experimental huts in Vallée du Kou, where two molecular forms of *A. gambiae*, S and M, occur. The S form is mostly pyrethroid resistant due to the *kdr* mechanism, and the M form is mostly *kdr* susceptible. A variety of ITPS covered surfaces were tested, ranging from ceiling only to all walls plus ceiling covered.

RESULTS ITPS had a major effect on the mortality of mosquitoes, the proportion killed being dependent upon the surface area covered. Homozygotes for *kdr* resistance showed lower rates of mortality than did heterozygotes or homozygotes for susceptibility. Deterred entry of mosquitoes and inhibition of blood feeding were also correlated with surface area covered. The mode of action and efficacy of ITPS seems to bear closer resemblance to that induced by indoor residual spraying (IRS) than to that induced by insecticide-treated nets.

CONCLUSIONS ITPS might be conceived as being equivalent to long-lasting or permanent IRS but without some of the operational constraints normally associated with spraying. High coverage of ITPS could potentially have a mass population effect on mosquitoes and give rise to long-term community protection against malaria. A phase III trial is justified to assess the acceptability of ITPS and its efficacy against malaria.

keywords experimental huts, *Anopheles gambiae*, insecticide treated plastic sheeting, pyrethroid

Introduction

The two main methods of malaria vector control are indoor residual spraying (IRS) and insecticide-treated nets (ITN). In Africa and South Asia, ITNs and IRS are both very effective (Curtis *et al.* 1999; Rowland 1999). There is mixed evidence concerning the relative cost-effectiveness of these two approaches: in some settings IRS appears to be more cost-effective than ITNs, while in other settings the reverse is true (Curtis *et al.* 1999; Rowland 1999; Curtis & Mnzava 2000). As it is impossible to make a generalized statement favouring one approach over the other, the choice depends not only on the question of epidemiological effectiveness but also on the considerations of feasibility and existence of appropriate delivery systems. Where populations are exposed to unstable or epidemic malaria, for example in Asia, African highlands or southern Africa, IRS has the advantage of being readily targeted to places of highest risk (Najera & Zaim 2001). But IRS requires good

infrastructure and logistics, campaign planning and timing to have any chance of success (Rowland 1999), ruling out most countries of sub-Saharan Africa where rural populations are exposed to stable malaria and systems for large-scale IRS do not exist. In these places ITNs have the advantage of being less demanding to implement than IRS and being able to be targeted on individuals most at risk, such as pregnant women and children. This advantage is further enhanced by the recent development of long-lasting insecticidal nets (LLIN) which resist washing and greatly extend the effective life of the insecticide (N'Guessan *et al.* 2001; Graham *et al.* 2005).

In situations of conflict and disaster, the affected populations may be severely exposed to vector mosquitoes, and physical structures in displacement camps for applying IRS or hanging ITN may not exist (Rowland & Nosten 2001). To address this problem, attempts have been made in the last decade to render temporary shelters insecticidal, either by spraying tents with pyrethroid (Hewitt *et al.*

1995) or by impregnating polyethylene tarpaulins with insecticide during manufacture (Graham *et al.* 2002). Insecticide-treated plastic sheeting (ITPS), the common name given to the factory-impregnated polyethylene tarpaulins are being manufactured commercially for refugee camps and displaced populations affected by natural disasters (Allan & Guillet 2002).

The recent entomological evidence from ITPS trials (Graham *et al.* 2002, 2004) has raised hopes for a new solution to malaria in displaced populations. This development, coupled with the widely recognized operational constraints associated with IRS campaigns in conventional rural settings, has given rise to the concept of using ITPS indoors, fixed to ceilings or walls, as a long-lasting alternative to IRS, in the same way as LLIN has overcome the constraints associated with having to retreat ITN in places where systems for retreatment are rudimentary or absent. This approach might have the additional benefit of increasing not only the residual efficacy of the insecticide but of reducing the hazard associated with insecticide spraying. ITPS put to such application might achieve effective vector control with limited logistic or technical resources.

An entomological evaluation of ITPS was therefore conducted in Burkina Faso in experimental huts that simulate normal mosquito–host contact in domestic environments. The trial was intended as a proof of principle study, the ITPS not having been tested indoors before, to assess the efficacy of ITPS in an area where pyrethroid-susceptible and resistant populations *Anopheles gambiae* occur sympatrically.

Materials and methods

Study area

The efficacy of ITPS was evaluated in experimental huts at the Vallée du Kou field station, Bobo Dioulasso (4°25' W, 11°24' N). Kou is a rice-growing, irrigated valley comprising seven villages, surrounded by wooded savannah. Few insecticides are used on the rice crop, but insecticides are used extensively for cotton protection. The rainy season extends from May to October and the dry season from November to April. The Kou River is a permanent source of irrigation water and there are two rice crops per year from July–November and January–May. The irrigation system and rice fields provide almost year-round mosquito breeding. High densities of *A. gambiae* (up to 200 bites/person/night) are recorded during the rainy season. Two molecular forms of *A. gambiae*, M and S (Gentile *et al.* 2001), occur in the area. The *kdr*-based mechanism conferring resistance to pyrethroids and DDT predominates in the S form.

Experimental procedures

Insecticide-treated plastic sheeting is an experimental, high-density polyethylene, multi-layer film containing 2% w/w of permethrin, manufactured by Sumitomo Chemical Co. The experimental huts used in this study were similar to those described from Côte d'Ivoire (Darriet *et al.* 1998; Guillet *et al.* 2001; N'Guessan *et al.* 2001). Each hut consists of a single room with entry windows on three sides and a screened verandah on the fourth side. Six treatments were randomly allocated to huts: (i) ceiling ITPS (36.5% of available surface), (ii) two walls ITPS (40.5%), (iii) four walls ITPS (62.8%), (iv) four walls and ceiling ITPS (full coverage), (v) untreated plastic sheeting (full coverage) and (vi) control with no plastic sheeting. The huts were occupied by sleepers from 20:00 to 6:00 hours, six nights a week, from September to November 2004. The sleepers were rotated between huts on consecutive nights. Host-seeking mosquitoes entered the huts via the window slits and could exit into the verandah traps. Mosquito collections were started 1 week after introduction of the plastic sheeting. Each morning all female mosquitoes were collected from each hut and scored as alive or dead and unfed or blood-fed. Surviving mosquitoes were provided with 10% honey solution and held for 24 h before scoring delayed mortality.

Before plastic sheeting was installed, we collected mosquitos for several days to check that there was no difference between huts in attractiveness to mosquitoes. To assess the residual activity of insecticide on the plastic sheeting, we did cone bioassays *in situ* each month for 6 months using laboratory-reared, susceptible *A. gambiae* (Kisumu strain). In accordance with the WHO protocol for assessing IRS treatments (WHO 1996), three batches of 15 unfed females, 3–5 days old were exposed to ITPS surfaces for 30 min in each hut. Knocked down mosquitoes were counted at the end of the exposure period and all mosquitoes held for 24 h before scoring mortality.

Genomic DNA was extracted from field-collected mosquitoes and PCR amplified to determine the presence of the molecular forms M or S using the method of Favia *et al.* (2001). Samples of live and dead mosquitoes were taken from selected huts (the control, two walls ITPS, four walls plus ceiling ITPS) for detection of *kdr* alleles in individual mosquitoes using the method of Martinez-Torres *et al.* (1998).

Ethical considerations

Volunteers from the study village were recruited after obtaining informed consent. A medical doctor was on hand during the trial to respond to any side effects of the ITPS or

to treat any cases of fever. Confirmed falciparum parasitaemia was treated with Coartem (artemether 20 mg/lumefantrine 120 mg). The protocol received approval from the ethics committees of Centre Muraz (a national research centre), IRD, LSHTM and WHO.

Data analysis

The effect of each treatment was assessed relative to the control arm in terms of detergency (the number of mosquitoes caught in each hut), excito-repellency (the proportion of mosquitoes in the verandah traps), the proportion blood feeding and the proportion killed; the proportional data were analysed using logistic regression (STATA 6 software). Because of non-normality in the number of mosquitoes collected from each hut, this data was analysed using the Wilcoxon rank sum non-parametric test. Genotype frequencies were tested using chi-squared or Fisher's exact test.

Ethical approval

The present study obtained approval from the ethics committees of Centre Muraz, a national research centre close to the study area, and the London School of Hygiene & Tropical Medicine.

Results

In situ bioassays

Eighty-three cone bioassay tests and 1354 mosquitoes were tested on ITPS in two treatment huts and one control over the 6 months of the trial. There was a consistent 100% knockdown and 100% mortality on ITPS throughout this period. Mosquito mortality in the control huts was 1.4% (576 mosquitoes tested) over this period.

Experimental huts trial

Before the ITPS were installed, we collected 859 *A. gambiae* females over 54 hut-nights (9 days \times 6 huts). There were no significant differences between huts in the number of mosquitoes collected ($F = 6.9$, d.f. = 5, $P = 0.23$), and hence no evidence of differential attractiveness. This was important to establish in view of the fact that the treatments could not be rotated between huts.

There were 426 hut-nights (71 days \times 6 huts) and a total of 5321 *A. gambiae* females collected during the trial. Genotyping of 166 specimens randomly selected from the six huts showed the *A. gambiae* population was composed of M (49.4%) and S (50.6%) molecular forms. The frequency of *kdr* was 0.45 overall. The *kdr* allele was predominantly found in the S molecular form (frequency of *kdr* allele = 0.95) and was present only at low frequency in the M form (0.04).

The main effects of the intervention are shown in Table 1. The hut with untreated plastic sheeting and the unlined control hut showed very consistent results for each of the biological parameters studied. Lining only 36% (ceiling) or 40% (two walls) of available surface was insufficient to deter mosquitoes from entering the huts but lining 63% (four walls) or 100% (four walls and ceiling) had a significant effect, with both treatments deterring over 40% of *A. gambiae* from entering. The permethrin treated sheeting was markedly repellent: the average rate of insecticide-induced exophily was 60%, and there was no significant difference in exit rates between treatments.

Blood-feeding rates were universally high (>70%). Feeding was partially inhibited by the ITPS treatments. The level of inhibition seemed to be correlated with surface area covered, with four walls or four walls plus ceiling covered giving the best protection (Table 1). Mortality rates ranged from 12% to 46% between ITPS treatments. The proportion killed was correlated with the percentage area covered

Table 1 Numbers of *Anopheles gambiae* s.s. collected from experimental huts over 71 nights at Vallée du Kou, Burkina Faso. Numbers in the same column sharing a letter superscript do not differ significantly ($P > 0.05$)

Treatment	Total number in hut	% Deterred	% Blood-fed	95% CI	% Feeding inhibition	% Dead after 24 h	95% CI	% In exit trap	95% CI
Untreated hut (control)	1041 ^a	–	96.9 ^a	(95.7–97.8)	–	1.7 ^a	(1.1–2.7)	27.8 ^a	(25.1–30.6)
Non-insecticidal plastic sheeting	1097 ^a	–	96.2 ^a	(94.9–97.2)	–	1.5 ^a	(0.9–2.4)	29.7 ^a	(27.1–32.5)
Ceiling ITPS	1174 ^a	–7.1	93.7 ^b	(92.2–95.0)	3.3	11.9 ^b	(10.2–13.9)	73.9 ^b	(71.3–76.4)
Two walls ITPS	787 ^{ba}	28.3	87.7 ^d	(85.2–89.8)	9.5	19.8 ^d	(17.2–22.8)	67.7 ^d	(64.4–70.9)
Four walls ITPS	631 ^b	42.5	78.3 ^c	(74.9–81.3)	19.2	44.5 ^c	(40.7–48.4)	78.3 ^c	(74.9–81.3)
Ceiling plus four walls ITPS (full coverage)	591 ^b	46.1	70.6 ^c	(66.8–74.1)	27.1	46.4 ^c	(42.4–50.4)	77.0 ^{bc}	(73.4–80.2)

ITPS, insecticide-treated plastic sheeting.

with ITPS, with four walls plus ceiling generating the highest mortality.

Modifying effect of *kdr* resistance on mortality and blood-feeding

The results of *kdr* genotyping of 135 *A. gambiae* from the experimental huts (control, two walls treated, four walls plus ceiling treated) are shown in Table 2. Because *kdr* is incompletely recessive (Chandre *et al.* 2000), the results for *kdr^s/kdr^s* and *kdr^r/kdr^s* genotypes were pooled for analysis. There was evidence for a significantly higher frequency of *kdr^r/kdr^r* in those that survived the ITPS treatments than in those that died on them. Mortality in the control hut was low, and there was no evidence for or against selection of *kdr^r/kdr^r* in this hut. Blood-feeding rates were very high for all genotypes in the control hut and for *kdr^r/kdr^r* in the ITPS huts. There was evidence of blood-feeding inhibition in *kdr^s/kdr^s* and/or *kdr^r/kdr^s* genotypes in the hut fully covered with ITPS.

Discussion

Insecticide-treated plastic sheeting was initially developed as a combined shelter and malaria control tool for newly established refugee camps (Allan & Guillet 2002). The present study indicates the material has considerable potential as a wall lining in conventional habitations in rural Africa against indoor resting mosquitoes. The main effect of ITPS was on the mortality of field mosquitoes. In bioassay ITPS was fully effective against susceptible *A. gambiae* throughout the 6-month period of the study and showed no signs of decay. ITPS is derived from Olyset long-lasting net technology in which permethrin is incorporated into polyethylene before extruding into fibres during net manufacture (WHO 2001). Olyset nets are known to remain effective for several years under field use (Tami *et al.* 2004), and from our initial results with ITPS it might be reasonable to expect the residual effect of insecticide to persist for an equivalent period. To verify this assumption, one of the ITPS samples has been kept aside as a wall lining in a nearby house and will be subjected to regular bioassay testing until a significant loss of efficacy is detected. Used outdoors ITPS deteriorates within a year under the hot tropical sun, even with UV filters (Graham *et al.* 2002, 2004). Indoors, the material would be protected from the adverse effect of UV light on polyethylene.

For most biological parameters measured, except exophily, the impact of ITPS was correlated with surface area covered. Maximum efficacy was attained with the hut fully lined with ITPS. The ingress of mosquitoes and blood

Table 2 Mortality and blood-feeding inhibition among *kdr* genotypes. *Kdr* is incompletely-recessive and hence heterozygotes are pooled with homozygotes for susceptibility

	Number tested	Mortality		Bloodfeeding		Feeding inhibition		
		<i>kdr^s/kdr^s</i> + <i>kdr^r/kdr^s</i> (n)	<i>kdr^s/kdr^s</i> + <i>kdr^r/kdr^s</i> % dead (n)	<i>kdr^r/kdr^r</i> 0 (0) 10.5 (2) 19.2 (5)	<i>P</i> (χ^2 or Fisher's exact test)	<i>kdr^s/kdr^s</i> + <i>kdr^r/kdr^s</i> (%)	<i>kdr^s/kdr^s</i> + <i>kdr^r/kdr^s</i> (%)	<i>kdr^r/kdr^r</i> (%)
Control	24	17	8.3 (2)		0.502	98.8 (23)	100 (17)	–
Two walls	27	19	44.4 (12)		0.033	85.0 (23)	100 (19)	0
Four walls + ceiling	22	26	63.6 (14)		0.005	36.4 (8)	88.5 (23)	11.5

P, probability of H0 resistant homozygotes frequencies were not different between dead/alive mosquitoes or fed/unfed.

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feeding rates were only partially inhibited by ITPS. This indicates that ITPS would confer limited personal protection inside the home but should result in satisfactory transmission control when applied at community level through an effect on mosquito longevity. An effect on mortality was the primary outcome when ITPS was erected as makeshift refugee shelter in 'outdoor platform' trials in Pakistan (Graham *et al.* 2002, 2004). Blood-feeding inhibition was greater in the present study because of the stronger anthropophily of African vectors. The protective effect of ITPS was apparent against susceptible phenotypes but little protection was observed against homozygotes for *kdr* resistance. It is worth noting that in early 2000 South Africa failed to control malaria through deltamethrin residual spraying because of the development of pyrethroid resistance. The S. African programme was obliged to revert from deltamethrin to DDT to achieve control, as *A. funestus* was susceptible to DDT still (Hargreaves *et al.* 2000; Najera & Zaim 2001). An increased level of mixed function oxidase activity rather than *kdr* resistance was associated with the failure of pyrethroids in southern Africa.

Insecticide-treated plastic sheeting may favour the selection of pyrethroid resistance, as a higher proportion of mosquitoes homozygous for resistance survived in the ITPS huts. By contrast in a previous study in experimental huts permethrin treated nets seem unlikely to select for pyrethroid resistance, at least in areas where the *kdr* mutation is rare and present mainly in heterozygous form (Corbel *et al.* 2004). ITN and ITPS act at different stages of the gonotrophic cycle, and the difference in selection rates probably reflects a difference in mosquito behaviour during these two reproductive stages. Blood is a limited resource, and host-seeking mosquitoes on encountering an ITN en route to a host may persist in their attempt to penetrate the toxic barrier particularly if they have a degree of resistance and are less irritated by the toxin. Resistant females would be killed as readily as susceptible females if they stay longer on the toxic surface while persisting in their attempt to probe for a blood meal; consequently they too would pick up a lethal dose of insecticide (Chandre *et al.* 2000). By contrast, once they are fed, females become relatively quiescent and seek a refuge where they can digest their meals without risk of predation. Resistant blood-fed females, on first signs of irritation in a pyrethroid treated 'refuge', stand to gain nothing by persisting on that surface so presumably move away to the verandah more readily than they would when seeking a blood meal at the earlier stage in the gonotrophic cycle. Being resistant they are more likely to survive the encounter with the toxic surface than blood-fed susceptible phenotypes.

The rapid spread of pyrethroid resistance in the major malaria vectors reinforces the need to identify alternative insecticides and to develop and implement effective and sustainable resistance management strategies (Chandre *et al.* 1999; Hargreaves *et al.* 2000; Kolaczinski *et al.* 2000; Diabaté *et al.* 2002, 2004; Etang *et al.* 2003; Asidi *et al.* 2005). The use of ITPS as a long-lasting alternative to IRS has the potential to be widely implemented at community level with relatively limited technical or logistical infrastructure in place. The persistence of the insecticide on the plastic sheeting is a crucial point, and it is reasonable to expect a residual effect for several years. A trial at community level would be necessary to estimate the acceptability and efficacy of ITPS against malaria and the operational factors involved in implementation. A square metre of thin ITPS costs about 10 cents according to the manufacturer (T. Itoh, personal communication), i.e. about \$4 required to cover a 40 m² bedroom. In that situation, lining a wall and ceiling with ITPS could be cheaper than buying a family size LLIN (estimated to \$5) and if it were to last for more than a year or two ITPS might be more cost-effective than IRS which requires vertical organization and repeated six-monthly campaigns. There remains the speculation of how ITPS could be implemented in practice. To have a major impact on malaria a majority of sleeping rooms should be furnished with ITPS, necessitating good popularity or subsidized distribution or erection of ITPS by malaria control programmes. But once erected the team may not have to return for several years, with considerable cost savings over IRS. Looking beyond the pyrethroids, it would be also interesting to assess the efficacy of ITPS treated with a different class of insecticide to which there is no irritability and no resistance, particularly in areas where pyrethroid resistant mosquitoes predominate. A dual intervention with LLIN and ITPS should also be investigated, as it could have a strong protective effect in areas of pyrethroid resistance by reducing both the blood-feeding rate and the population density of mosquitoes.

Acknowledgements

The study was funded by the WHO Pesticide Evaluation Scheme, the Institut de Recherche pour le Développement and the Gates Malaria Partnership of the London School of Hygiene & Tropical Medicine. We are grateful to the heads of IRSS and Centre Muraz for their support during the study. Our thanks also go to A Ouattara, D Traoré, T Kabero and to the sleepers in the huts for their participation in the present work. Finally we wish to thank Professor Martin Akogbéto of the Centre Entomologique de Cotonou for his support and to Luc Djogbénou, Joseph Chabi and Sébastien Koudénoukpo for their help with the

genotyping. The ITPS was kindly provided by Dr Takaaki Itoh of Sumitomo Chemical Co., Japan.

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Utilisation de recouvrements d'intérieur en plastique pré-imprégnés d'insecticide pour le control des vecteurs de la malaria

OBJECTIF Evaluer l'efficacité de recouvrements en plastique traité à la perméthrine et appliqués comme doublures aux plafonds et aux murs des chambres pour lutter contre *Anopheles gambiae* sensible ou résistant à la pyréthroïde.

MÉTHODE Des tests ont été effectués durant la nuit en utilisant des pièges de véranda sous forme de huttes expérimentales, dans la Vallée du Kou, où sont présentes deux formes moléculaires de *An. gambiae*, les formes S et M. La forme S est surtout résistante à la pyréthroïde par le mécanisme *kdr* et la forme M plutôt *kdr* susceptible. Une variété de surfaces recouvertes de plastique traité à la perméthrine a été testée et ce, allant du traitement du plafond seul au traitement du plafond plus tous les murs.

RÉSULTATS Les recouvrements en plastique traité à la perméthrine avaient un effet majeur sur la mortalité des moustiques; la proportion tuée dépendant de la surface recouverte. Les homozygotes pour la résistance *kdr* avaient un taux de mortalité plus faible que les hétérozygotes ou les homozygotes pour la sensibilité. La dissuasion de l'entrée des moustiques dans les pièces et l'inhibition de la succion de sang étaient en corrélation avec la surface de la zone recouverte. Le mode d'action et l'efficacité des recouvrements en plastique traité à la perméthrine semble avoir une similitude plus proche de celle induite par la pulvérisation résiduelle des intérieurs que de celle induite par les moustiquaires imprégnés d'insecticide.

CONCLUSION Les recouvrements en plastique traité à la perméthrine pourraient être conçus comme étant équivalents à une pulvérisation résiduelle de longue durée ou permanente des intérieurs, mais sans les contraintes opérationnelles normalement liées à la pulvérisation. L'application étendue des recouvrements en plastique traité à la perméthrine pourrait avoir un effet de masse potentiel sur la population de moustiques et donner lieu à une protection de longue durée aux communautés contre la malaria. Un essai de phase III se justifie alors, afin d'évaluer l'acceptabilité des recouvrements en plastique traité à la perméthrine et leur efficacité contre la malaria.

mots clés huttes expérimentales, *Anopheles gambiae*, recouvrements en plastique traité aux insecticides, pyréthroïde

Uso intradomiciliario de laminado plástico pre-impregnado con insecticida para el control de vectores de malaria

OBJETIVO Evaluar la eficacia del laminado plástico tratado con permetrina (LPTP) aplicado como cobertor del cielorraso o las paredes de habitaciones, contra *Anopheles gambiae* tanto sensible como resistente a piretroides.

MÉTODO Se llevaron a cabo pruebas durante la noche con trampas en barracas experimentales en el Vallée du Kou, en el cual co-existen dos formas moleculares, S y M, de *An. gambiae*. La forma S es en su mayoría resistente a piretroides, debido al mecanismo *kdr*, y la forma M es en su mayoría *kdr* sensible. Se probó el cubrir una variedad de superficies con LPTP, desde solo el cielorraso hasta paredes y cielorraso conjuntamente.

RESULTADOS El LPTP tuvo un efecto importante sobre la mortalidad de los mosquitos, siendo la proporción de muertes dependiente de la superficie cubierta. Los homocigotos para resistencia por *kdr* mostraron una menor tasa de mortalidad que los heterocigotos o los homocigotos sensibles. También se encontró una correlación con el área cubierta y la disminución en la entrada de los mosquitos o la inhibición de la alimentación por sangre. El modo de acción y la eficacia del LPTP se parece más al inducido por el rociamiento residual intradomiciliario que al de las mosquiteras impregnadas.

CONCLUSIONES Se puede concebir el LPTP como equivalente al rociamiento intra-domiciliario de larga duración o permanente, pero sin algunas de las restricciones operacionales asociadas al mismo. Una alta cobertura de LPTP podría, potencialmente, tener un efecto de masa sobre los mosquitos y proteger, a largo plazo, a la comunidad frente a la malaria. Está justificado el realizar ensayos de fase III, para evaluar la aceptabilidad del LPTP y su eficacia frente a la malaria.

palabras clave barracas experimentales, *Anopheles gambiae*, laminado plástico tratado con insecticida, piretroïde