Field Evaluation of Traditionally Used Plant-Based Insect Repellents and Fumigants Against the Malaria Vector *Anopheles darlingi* in Riberalta, Bolivian Amazon

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ABSTRACT Inexpensive insect repellents may be needed to supplement the use of impregnated bed-nets in the Amazon region, where the primary malaria vector, *Anopheles darlingi* (Root), is exophilic and feeds in the early evening. Three plants that are traditionally used to repel mosquitoes in Riberalta, Bolivian Amazon, were identified by focus group, and then they were tested against *An. darlingi* as well as *Mansonia indubitans* (Dyar & Shannon)/*Mansonia titillans* (Walker). *Cymbopogon citratus* (Staph), Guatemalan lemongrass, essential oil at 25% was used as a skin repellent, and it provided 74% protection for 2.5 h against predominantly *An. darlingi* and 95% protection for 2.5 h against *Mansonia* spp. *Attalea princeps* (name not verified) husks, burned on charcoal in the traditional way provided 35 and 51% protection against *An. darlingi* and *Mansonia* spp., respectively. Kerosene lamps, often used to light rural homes, were used as a heat source to volatilize 100% *Mentha arvensis* (Malinv ex. Bailey) essential oil, and they reduced biting by 41% inside traditional homes against *Mansonia* spp., although they were ineffective outdoors against *An. darlingi*. All three plant-based repellents provided significant protection compared with controls. Plant-based repellents, although less effective than synthetic alternatives, were shown by focus groups to be more culturally acceptable in this setting, in particular para-menthane-3, 8, idol derived from lemon eucalyptus, *Corymbia citriodora* (Hook). Plant-based repellents have the potential to be produced locally and therefore sold more cheaply than synthetic commercial repellents. Importantly, their low cost may encourage user compliance among indigenous and marginalized populations.

KEY WORDS plant-based, repellent, *Anopheles darlingi*, *Cymbopogon citratus*, *Mansonia* spp.

In the Amazon basin 804,632 malaria cases were reported in 2004 (PAHO 2005), and recent modeling work using population and endemnicity data has estimated that the malaria burden may be 10 times that figure (Hay et al. 2004).

The major malaria vector in the Amazon basin is *Anopheles darlingi* (Root) (Tadei and Dutary Thatcher 2000). It is a highly anthropophilic vector (Oliveira-Ferreira et al. 1992) that bites throughout the night, with peaks in biting activity around dusk and dawn (Roberts et al. 1987, Lourenco-de-Oliveira et al. 1989, Klein and Lima 1990, Tadei et al. 1998, Voorham 2002). *An. darlingi* is generally exophagic, although some indoor feeding does occur (Roberts et al. 1987, Rozendaal 1989, Tadei et al. 1998, Tadei and Dutary Thatcher 2000). Because of the early evening biting peak, personal protection may be necessary to supplement bed-net use in areas where remoteness of small communities and outdoor resting vectors preclude control through indoor residual spraying.

In this region, the Roll Back Malaria initiative is focused upon indigenous and forest-dwelling peoples, for whom malaria is a major disease burden (PAHO 2000). Living as they do in small, isolated, dispersed communities, these people are difficult to reach with health care, and interventions have to be tailored to suit their life styles, cultural beliefs, and occupational attitudes (WHO 2000). Many nonimmune people move into the Amazon to work in mining and logging. Migrants tend to have low socioeconomic status, many lack the knowledge or money to protect them from vector-borne disease, and they tend to live in transient settlements of substandard housing without adequate healthcare. This movement of nonimmune people spreads malaria, and it has contributed to the rise in drug-resistant strains of *Plasmodium falciparum* throughout the region (Bloland 2001). Therefore, malaria prevention methods that are culturally acceptable, cheap, and portable must be investigated.

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Riberalta is a small frontier town on the border between Bolivia and Brazil. Malaria incidence remains high, despite >90% bed-net coverage (Lenglet 2001). In 2004, the reported annual parasite incidence (API) was 42.77 per 1,000 slides (PAHO 2005), although ≈80% of cases are unreported (Mollinedo 2000). Malaria incidence peaks around April, when workers (beneficiadores) return from the forest where they have been harvesting Brazil nuts, *Betholletia excelsa* (Humb. &. Bonpl.). Approximately 21,000 people from Riberalta were employed in the forest extraction industry in 1998 (PSI 1999), and this reliance on the forest for employment is an important factor in malaria epidemiology in the region. Forty-five percent of cases are imported, although 40% of cases involve peridomestic transmission (Districto de Salud Riberalta 2003).

The introduction of repellents to this region may be successful because knowledge of transmission and prevention is good with 87% of respondents recognizing that malaria is transmitted by the bite of an infected mosquito (Lenglet 2001). A recent clinical controlled trial was conducted into the effect of paramenthane-3, 8, idol (PMD), on malaria incidence. PMD is an insect repellent derived through the acid modification of *Corymbia citriodora* (Hook), lemon eucalyptus, essential oil. The cohort that used PMD in addition to insecticide-treated nets (ITNs) resulted in 80% fewer episodes of *Plasmodium vivax* malaria compared with a matched ITN-only group (N.H., unpublished data). Therefore, the potential for use of local plants as repellents was investigated, because they are familiar and may provide a cheaper alternative to imported synthetic repellents.

Materials and Methods

Focus Groups. Five focus groups were carried out in rural and periurban villages around Riberalta in March 2001 to establish which plants are used by the indigenous population against mosquitoes. Carmen Ruiz, a moderator working for Population Services International (PSI) conducted the discussions. As well as responding to questions, volunteers from the groups were given several repellents in unmarked cups to evaluate 1) 50% diethyl toulamide (Deet) in ethanol (Sigma Chemie, Deisenhofen, Germany); 2) 30% PMD (Masta, Leeds, United Kingdom); 3) Treo (Primavera Labs, Inc., NY), made up of 0.05% citronella, 0.06% geraniol, 0.08% rhodinol extra, 0.06% terpineol, and <0.5% *p*-menthane-diol in a moisturizing cream with a floral odor; 4) 2% neem, *Azadirachta indica* (A. Juss), oil with the azadirachtin fraction removed (Bioforce, Irvine, United Kingdom), in ethanol; and 5) 15% citronella, *Cymbopogon nardus* (L.), cream (Boots Plc., Nottingham, United Kingdom). Each group also was given several bottles to choose between: 1) bottle with cap, 2) roll on applicator, 3) stick applicator, 4) tube, and 5) spray. Volunteers smelled the repellents and applied a small amount to their skin (<0.2 ml) to evaluate the "feel" of the repellents. Volunteers rated the products from 1 (favorite) to 5 (least favorite), and these scores were used to calculate a weighted average, where the overall score (sum of each score) was divided by the number of people attending each focus group. For example, if product A was rated first choice by five people, and one person each rated it their second, third, fourth, and fifth favorite, the weighted average would be $((1 \times 5) + (2 \times 1) + (3 \times 1) + (4 \times 1) + (5 \times 1))/9 = 2.11$.

Study Sites. The study was performed between 30 April and 27 May 2003 in the transition period between the wet and dry seasons. Two field sites were selected, both of which are located <30 km from Riberalta (11° 01′ S, 066° 06′ W), Vaca Diez, near the northern border of Bolivia with Brazil.

Site 1, "El Prado," a military base, consists of a large open field within which are 34 dormitory blocks that provide quarters for 300 soldiers (Distrito de Salud Riberalta 2003). The base is surrounded by disturbed forest with a lake and river located at one end that supports the larvae of *An. darlingi* and lower numbers of *Mansonia titillans* (Walker)/*Mansonia indubitans* (Dyar & Shannon) (J. Carvajal, personal communication).

Site 2, "Warnes," is a rural village that comprises 86 traditional wooden houses and 362 inhabitants (Distrito de Salud Riberalta 2003). It is located 2 km from the River Beni, and it is surrounded by disturbed forest and marshland that supports high numbers of *Ma. indubitans/titillans* as well as maize, *Zea mays* L., fields. Tests were conducted in a large grassy area within the center of the village.

Ethical Clearance and Volunteer Safety. Because the following trials required human-landing catches, local volunteers experienced in conducting human-landing captures were recruited. These individuals all had good knowledge of malaria transmission, because they worked for the government malaria control project, and they had access to free malaria diagnosis. Additionally, free Primaquine prophylaxis was available. Tests were carried out at sites where there was little disease transmission. Ethical clearance was obtained from the London School of Hygiene and Tropical Medicine Ethics Board and the Bolivian Ministry of Health.

Repellent Testing. Three of the plants that are traditionally used in the region, identified by focus group, and positively identified by botanists at Instituto para el Hombre, Agricultura y Ecologia, were tested. Each evaluation was a Latin square design using three volunteers over nine nights to test the plant product in comparison with a known repellent and a control. Volunteers were allocated one of three locations within the open field sites that were a minimum of 10 m from each other and a minimum of 20 m from alternate sources of kairomones such as houses, people, and livestock. Because insect repellents act over a distance of <1 m and the maximum distance of host attraction to a single human is 10 m (Gillies and Wilkes 1970), the design eliminates any "relativity effect" where insects choose between two hosts simultaneously. Treatments were rotated each night and volunteers changed locations every three nights to ensure that each treatment was tested by each volunteer in each location during the evaluation. Each plant was evaluated in two tests, one test conducted at El Prado and one test conducted at Warnes. Each test was performed between 1830 and 2030 hours, because this is the time of peak mosquito activity in the area (Harris et al. 2006). During the human-landing catches, the volunteers sat on low stools and wore shorts to the knee and work boots to standardize the area of the lower legs exposed to the mosquitoes. In addition, the volunteers did not wash using soap after midday, and smoking before testing was prohibited to minimize variation in headspace kairomones (Magnon et al. 1991, de Jong and Knols 1995).

Volunteers collected mosquitoes from their lower legs once they had settled, without the need to wait for biting, by using a mouth aspirator, flashlight, and a collection vessel designed for this purpose. Mosquitoes were maintained overnight with glucose-soaked cotton wool and identified the following day by using a key (Faran and Linthicum 1981).

Cymbopogon citratus (Staph). Twenty-five percent Guatemalan lemongrass essential oil (The Essential oil Co., Hampshire, United Kingdom), was compared with a 15% Deet (Sigma Chemie), and 15% baby oil (Boots Plc.) was used as control. All three treatments were diluted in locally bought rubbing alcohol (96% ethanol). Three milliliters of each treatment was applied. This volume was chosen as the volunteers had an average leg surface area of 600 cm^2, and most people apply 2 mg/cm^2 ad libitum (W. G. Reifenrath, personal communication) in Rutledge (1988). Repellents were measured using a pipette and applied evenly to the lower legs by the volunteer wearing a latex glove to minimize absorption of material onto their hand. They were applied at 1800 hours, 30 min before testing.

Attalea princeps (name not verified). Focus groups revealed that *A. princeps* ("motaçu") seeds are commonly burned on the hot embers of a fire to create a thick smoke. To test its repellency, 250 g of plant material (roughly six kernels, the amount normally used as identified by focus group) was placed onto 250 g of charcoal that had been alight for 30 min, so that it was glowing. Smoldering charcoal (250 g) was used as control and a locally bought mosquito coil (10 mg of δ-allethrin) also was compared. The volunteers sat <1 m from the repellent source and were placed 40 m apart at sufficient distance from each other to ensure that drifting smoke from one treatment did not affect mosquito landings on other volunteers.

Mentha arvensis (Malinv ex Bailey). The potential for kerosene lamps, modified to vaporize *M. arvensis* was investigated, because this plant has been shown to exhibit high vapor toxicity to stored grain pests, affecting their acetyl cholinesterase activity (Lee et al. 2001).

The lamps were based on the design by Pates et al. (2002), although they were much larger with a distance of 5 cm between the vaporizing tin and the wick. In El Prado, the lamps were tested in the open air, and 25% *M. arvensis* (The Essential Oil Co.) was compared with 0.2% δ-allethrin. At Warnes, the lamps were tested inside houses, comparing 100% *M. arvensis* against 0.5% δ-allethrin. For both evaluations, the *M. arvensis* and δ-allethrin were diluted in local Soya oil (Bunge Alimentos, Brazil), and the control was pure soya oil. Soya oil (10 ml), plus essential oil or insecticide, was placed inside the vaporizing tin, and fresh oil was used each night.

Table 1. Weighted average score for repellent based on smell and texture (scores closest to 1 indicate the preferred products)

	Citronella	Deet	Neem	PMD	Treo
Rural women (n = 23)	3.30	3.65	3.74	1.03	0.93
Periurban women (n = 29)	0.76	0.55	0.83	1.03	0.93
Women's preference	2.03	2.10	2.29	1.03	0.93
Rural men (n = 24)	3.25	6.58	3.21	2.79	1.67
Beneficiadores (n = 9)	4.22	3.22	2.89	2.67	2.00
Men's preference	3.74	4.90	3.05	2.73	1.84
Overall avg	2.88	3.50	2.67	1.88	1.38

Data are given as percentages.

Statistical Analysis. All data were transformed with natural log + 1 to account for a left-skew in the data that is a consequence of low numbers collected on repellent-testing volunteers and analyzed by analysis of variance (ANOVA) with a General Linear model (GLM), on MINITAB 11 for Windows (Minitab, Inc., State College, PA). Residuals of GLM were plotted to test for correct distribution. The effect of volunteer, position, and treatment along with the interactions between each parameter was analyzed. Average percentage protection for the 2-h duration of each test was calculated using the formula: percentage protection = 100 − (treatment/control) × 100. Data for each of the six tests were analyzed separately and cannot, therefore, be directly compared.

Results

Focus Groups. Eighty-five individuals attended the focus groups. All of the communities interviewed thought that mosquitoes were a nuisance, especially inside their houses. Most respondents used smoke from burning plants year-round inside their homes to reduce this nuisance. All of the focus group members, whether rural or periurban, showed interest in using repellents, and three of the men said that they used diesel or kerosene on their skin to prevent bites when working outdoors. However, a major consideration was cost: no one was prepared to pay >15 Bolivianos (US$1.98) per 250 ml, although the commercial repellent Autan (Bayer) was for sale in Riberalta for 35 Bolivianos. Other important factors mentioned were smell and ease of application (Tables 1 and 2). The preferred repellents were Treo and PMD, by both men and women—overall weighted average 1.38 and 1.88, respectively (Table 1). Treo was liked for its cream formulation, and PMD for its pleasant odor. Methods that allowed repellent application without the need to wash hands afterward were preferred, i.e., roll on and spray—weighted average 1.19 and 1.41,

Table 2. Weighted average preferred method of application (scores closest to 1 indicate the preferred products)

	Roll on	Bottle	Tube	Stick	Spray
Rural women (n = 23)	2.13	3.65	3.26	2.83	2.48
Periurban women (n = 29)	0.41	2.89	2.78	0.07	0.62
Women's preference	1.27	3.27	3.02	1.45	1.55
Rural men (n = 24)	1.54	4.21	4.63	2.92	1.54
Beneficiadores (n = 9)	0.67	2.89	2.78	1.56	1.00
Men's preference	1.11	3.55	3.71	2.24	1.27
Overall avg	1.19	3.41	3.36	1.85	1.41

Data are given as percentages.

respectively (Table 2), because water is not always available.

Plants Mentioned during Focus Groups. The addition of *A. princeps* kernels (motaçu) to fires to create a repellent smoke was mentioned by all but one of the communities. *A. princeps* is abundant throughout Beni, associated with secondary forests. Two members of the Lamiaceae were mentioned: *Mentha arvensis* (locally known as "Hortelă-del-Campo") and *Cymbopogon citratus* ("Paha Cedron"). These plants were both found growing in peoples' gardens and wild around Riberalta, and both were selected for follow-up evaluation as repellents. In addition, *Carapa guianensis* (Aubl.) (locally known as "Andiroba") was rubbed upon the skin by the rural community, but it was not field-tested based upon poor performance during preliminary laboratory testing. This finding of low repellence has been confirmed (Miot et al. 2004). *Cedrella odorata* (L) ("Cedro") leaves also were burned to repel insects, although this plant was not tested due to the unpleasant odor of the leaves.

C. citratus. During the first evaluation conducted in El Prado, 753 mosquitoes were captured in nine nights: 80.1% *An. darlingi* and 11.9% *Ma. indubitans/titillans.* In the second study at Warnes, 1,195 mosquitoes were captured in nine nights of which 99.3% were *Ma. indubitans/titillans.* At the field site where *An. darlingi* was the predominant species, the skin repellents were extremely effective: *C. citratus* provided 73.68% protection and 15% Deet provided 94.65% protection for 2.5 h with an average of 33 landings per human-hour (F = 83.90, df = 2, P < 0.0001) (Table 3). Against *Mansonia* spp. at Warnes, *C. citratus* provided 95.15% and Deet provided 99.56% protection with 55 landings per human-hour (F = 737.53, df = 2, P < 0.0001). Although the treatments and positions were rotated to reduce the effect of position and variation in individual attractiveness to mosquitoes, these factors still affected the data when analyzed statistically. There was positional bias at El Prado (F = 15.87, df = 2, P = 0.002), perhaps because one collection site was located closer to the lake than the others. However, there was no significant interaction between position and treatment or tester and treatment, indicating that

Table 3. Mean and percentage protection provided by repellents at two field sites: 1) El Prado, where mosquitoes captured were predominantly *An. darlingi* (80%) and *Mansonia* spp. (11%); and 2) Warnes, where mosquitoes captured where predominantly *Mansonia* spp. (>95%)

Treatment		Exp. no. 1 El Prado *An. darlingi*	95% CI	Exp no. 2 Warnes *Mansonia* spp.	95% CI
15% Deet	AM	2.56		0.11	
	WM	0.65a	−0.24–2.60	0.08a	0.01–0.28
	% P[a]	94.65	83.55–105.75	99.56	99.80–100.08
30% *C. citratus*	AM	14.67		3.56	
	WM	8.97b	3.10–23.53	2.03b	0.48–5.23
	% P	73.68	51.11–96.24	95.15	89.75–100.56
Oil control	AM	66.44		129.11	
	WM	57.58c	37.09–88.12	111.17c	71.24–173.16
		3 El Prado *An. darlingi*		4 Warnes *Mansonia* spp.	
δ-Allethrin mosquito coil	AM	127.78		99.00	
	WM	115.75a	80.45–166.34	91.76a	65.69–126.74
	% P	−51.83	−97.95 to −5.72	−75.12	−176.88–26.65
A. princeps	AM	44.78		31.67	
	WM	35.23b	18.30–66.36	28.08b	36.80–65.53
	% P	34.68	−12.95–82.32	51.17	
Glowing carbon control	AM	93.67		64.89	
	WM	82.10ac	54.15–124.21	63.07c	51.46–77.26
		5 El Prado *An. darlingi*		6 Warnes *Mansonia* spp.	
0.5% δ-Allethrin	AM	89.00		48.33	
	WM	70.41a	39.48–128.74	45.06ab	32.78–61.18
	% P	−4.82	−62.44–52.81	−15.66	−119.46–88.15
M. arvensis	AM	104.56		33.11	
	WM	75.44a	37.97–150.91	30.19b	21.42–42.82
	% P	−1.14	−70.69–68.41	40.99	12.88–69.10
Oil only control	AM	112.00		68.89	
	WM	98.51a	63.18–153.93	58.74a	34.87–98.48

Note that each of the six experiments was performed separately; therefore, the results of each cannot be compared directly. Values followed by the same letter are not significantly different from each other, P > 0.05.

AM, arithmetic mean; WM, Williams' mean.

[a] % P, percentage protection = 100 − (treatment/control) × 100.

data were not significantly biased by this factor and can still be used to evaluate the effectiveness of the repellents. In Warnes, there was no positional bias. There was, however, a significant difference between the numbers of mosquitoes collected by different individuals ($F = 52.79$, df = 2, $P < 0.0001$), and there was a significant interaction between individual and repellent ($F = 16.60$, df = 4, $P = 0.004$), indicating that the repellents did not protect each individual equally. However, this result may be caused by one individual in this group that had low attraction to mosquitoes, and highlights the need for studies to enroll large numbers of subjects (Rutledge and Gupta 1999).

A. princeps. In El Prado, 2396 mosquitoes were captured over nine nights, of which 83.3% were *An. darlingi* and 11% were *Mansonia* spp. In Warnes, 1,760 mosquitoes were captured comprising 94.5% *Mansonia* spp. and 5.2% *An. darlingi.* In both sites, the mosquito coil that was intended as a repellent had, in fact, more landings than the control (glowing charcoal) with a total of 48 versus 35.2%, and 50.6 versus 33.2% of mosquitoes collected from the coil and charcoal users in El Prado and Warnes, respectively (Table 3).

Compared with the charcoal-only control, *A. princeps* provided significant protection, giving 34.68% protection at site 1 (El Prado) ($F = 8.98$, df = 2, $P = 0.009$) and 51.17% protection at site 2 (Warnes) ($F = 19.61$, df = 2, $P < 0.0001$) (Table 3). Comparison with the mosquito coil showed that motaçu provided 60.09% protection against *An. darlingi* ($F = 16.32$, df = 2, $P = 0.001$) and 59.03% against *Mansonia* spp. ($F = 25.78$, df = 2, $P < 0.0001$). This indicates that the heat from the glowing charcoal provides around 20% protection against mosquitoes, which was statistically significant in the Warnes study with *Mansonia* spp. ($F = 5.03$, df = 2, $P = 0.039$) but not against *An. darlingi* at El Prado. There was no positional bias in either location, neither was there a significant difference between collectors in the first evaluation in El Prado ($F = 1.71$, df = 2, $P = 0.240$). Again, there was a significant difference between the attractiveness of collectors in study two at Warnes ($F = 18.52$, df = 2, $P = 0.005$), and again the interaction was significant between tester and treatment ($F = 12.22$, df = 8, $P = 0.0009$).

M. arvensis. The lamps were tested outdoors at El Prado and 2,750 mosquitoes were captured, 80% *An. darlingi.* However, the lamps did not have any significant effect on the numbers of mosquitoes landing on the volunteers ($F = 0.24$, df = 2, $P = 0.792$).

When the lamps were tested indoors at Warnes, 1,353 mosquitoes were captured over nine nights, of which 98% were *Mansonia* spp. The lamps were marginally effective when used indoors, with no protection (−15.66%) from 0.5% δ-allethrin and 40.99% protection from 100% *M. arvensis* ($F = 4.34$, df = 2, $P = 0.053$) (Table 3). There was no significant difference between collectors ($F = 0.18$, df = 2, $P = 0.841$) or positions ($F = 0.18$, df = 2, $P = 0.841$). Further analysis by one-way ANOVA revealed that there was no significant difference between δ-allethrin and the control ($F = 1.66$, df = 1, $P = 0.216$), but 100% *M. arvensis* significantly reduced the number of mosquitoes landing on volunteers ($F = 7.07$, df = 1, $P = 0.017$).

Discussion

From this Bolivian field study, it may be suggested that plant-based repellents could contribute to malaria prevention in this region and possibly the whole of the Amazon Basin. The plant-based repellents have potential to be used by those located in remote areas, and their traditional use and low cost may increase their accessibility to marginalized, indigenous, or migrant populations.

In focus groups conducted by PSI, a repellent was the preferred method for preventing bites when offered alongside coils, permethrin soap, vaporizers, and insecticidal spray, because it is quick and easy to use, and it can be used in the forest (Ruiz 2000). In the focus groups, conducted in Bolivia for the current study, plant-based repellents were more popular than Deet. Ease of use was also an important factor identified in these focus groups, because rural men and beneficiadores liked packaging that allowed the bottle to be carried comfortably in the pocket and applied without having to wash the hands afterward.

C. citratus at 25% proved to be surprisingly effective against *An. darlingi* with 74% protection under biting pressure of 31 mosquitoes per person-hour. This is superior to citronella oil that has a duration of 2 h at 100% concentration in laboratory tests (USDA 1954) and similar to 66.7% repellency recorded with the related *C. excavatus*, after 3 h in a field trial with *Anopheles arabiensis* Patton (Govere et al. 2000). Ideally, the longevity of *C. citratus* repellents could be improved by combination with low concentrations of PMD that has known efficacy and longevity (Trigg 1996, Moore et al. 2002) or in combination with a simple slow-release formulation, to reduce its volatility, e.g., mineral oil or vanillin (Tawatsin et al. 2001). This is especially important as under realistic user conditions, such as working in the forest, duration of protection will be lower than under test conditions due to increased sweating and abrasion when people are active (Wood 1968, Khan et al. 1972, Gabel et al. 1976, Rueda et al. 1998).

Of the repellents tested, *A. princeps* smoke was perceived as unpleasant, but it was commonly used because it is freely available and was considered effective. *A. princeps* provided 35 and 51% reduction in biting, probably due to the thick smoke it generates and the volatilization of several insecticidal actives including capric acid, palmitic acid, and oleic acid (Clay and Clement 1993). The protection provided is lower than that of burning *Daniella oliveri* (Rolfe) bark (66%) (Lindsay and Janneh 1989) and coconut husks (77%) (Vernede et al. 1994). However, the use of *A. princeps* outdoors, especially during overnight forest visits could provide significant protection from disease. That *A. princeps* husks are a freely available waste product is of particular importance for the beneficiadores who are one of the poorest social groups in Bolivia with an average annual income of 4,700

Bolivianos, barely sufficient to feed a family (Enever 2003). Encouraging use of this plant against mosquitoes alongside the social marketing of bed-nets, hammock-nets, and repellents could help prevent malaria. It is inadvisable to encourage the use of this plant indoors, because although the burning of traditional material does have a protective effect against malaria in regions of intermediate API (van der Hoek et al. 1998), the respiratory effects of burning biomass fuels indoors causes the loss of 38,539,000 disability-adjusted life years globally each year (WHO 2002). It is interesting to note that smoldering charcoal alone provided some protection from mosquitoes, most likely due to reduction in humidity near the fire, because insect odor receptors are very responsive in the presence of moisture (Davis and Bowen 1994).

Volatilization of *M. arvensis* essential oil by using a kerosene lamp is a safer means of volatilization than direct combustion. However, the lamps provided a disappointing level of personal protection when used with δ-allethrin, in contrast to the high reductions in mosquito numbers measured by Pates et al. (2002). It is possible that higher concentrations of δ-allethrin may be needed for personal protection by using vaporizing lamps in traditional Bolivian buildings, with their open design, in contrast to compared with the breeze-block constructions in which Pates et al. (2002) tested their lamps. However, it was interesting to note that such lamps may provide a practicable way of volatizing plant-based repellents, particularly those that have short protection times due to their rapid evaporation. Many plants from the family Lamiaceae are repellent and have similar ED_{90} values to Deet (Curtis et al. 1987, Barnard 1999), *Nepata cataria* has a greater spatial repellency than Deet (Bernier et al. 2005), and many terpene-rich plants have high vapor toxicity (Osmani et al. 1974). However, their repellency declines rapidly as they evaporate from the skin. The lamp would provide a cheap means for continuous volatilization of the oils to maintain repellency. A second advantage is that the lamps are commonly used for lighting homes in rural Bolivia. Therefore, they are only used between sunset and the time when the users retire to the protection of their bed net, precisely the time when mosquito protection is required.

Finally, although the plant-based repellents are not as efficacious as synthetic alternatives, they may actually be more effective as a disease prevention tool, because they are actually used by the population, and they were proved in this trial to be more efficacious than a commercially available mosquito coil.

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