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A 4-Alkyl-substituted Analogue Of Guaiacol Shows Greater Repellency To Savannah Tsetse (*Glossina* spp.)

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Abstract The responses of *Glossina morsitans morsitans* Westwood to guaiacol (2-methoxyphenol), a mild repellent constituent of bovid odors, and seven analogues comprising 2-methoxyfuran, 2,4-dimethylphenol, 2-methoxy-4-methylphenol (4-methylguaiacol), 4-ethyl-2-methoxyphenol (4-ethylguaiacol), 4-allyl-2-methoxyphenol (4-allylguaiacol; eugenol), 3,4-methylenedioxytoluene, and 3,4-dimethoxystyrene were compared in a two-choice wind tunnel. The 4-methyl-substituted derivative (2-methoxy-4-methylphenol) was found to elicit stronger repellent responses from the flies compared with guaiacol. None of the other analogues showed significant repellent effects on flies. 4-Methylguaiacol, guaiacol, and eugenol (which was included because of previous reports of its repellency against a number of arthropods) were further evaluated in the field with wild populations of predominantly *Glossina pallidipes* Austen. The presence of guaiacol or eugenol near odor-baited traps caused some nonsignificant reduction in the number of tsetse catches at relatively high release rates (~50 mg/hr). In contrast, the 4-methyl derivative at three different release rates (2.2, 4.5, and 9.0 mg/hr) reduced trap catches of baited traps in a dose-response manner. At 10 mg/hr release rate, it reduced the catches of baited and unbaited traps by ~80 and ~70%, respectively. In addition, the compound not only reduced the number of tsetse attracted to natural ox odor (~ 80%), but also had an effect on their feeding responses, reducing the proportion that fed on an ox by more than 80%. Our study shows that the presence of a methyl substituent at the 4-position of guaiacol enhances the repellency of the molecule to savannah tsetse and suggests that 4-methylguaiacol may represent a promising additional tool in the arsenal of techniques in trypanosomiasis control.

Keywords Tsetse fly · *Glossina pallidipes* · Repellents · Behavior · Trypanosomiasis · Guaiacol · 4-Methylguaiacol

Introduction

Tsetse-transmitted trypanosomiasis continues to cause morbidity and death in human populations and livestock throughout sub-Saharan Africa. Current intervention options

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include parasite control through the use of trypanocidal drugs (Jordan 1986; Holmes, 1997); the promotion of trypanotolerant livestock (FAO 1987a, b; d'Lteren et al. 1999); vector control through the use of visual and odorant baits such as traps (Hassanali et al. 1986; Brightwell et al. 1987, 1991), insecticide-treated targets (Vale et al.1988a), and insecticide-treated animals (Bauer et al. 1995; Hargrove et al. 2000); and sterile insect technique (SIT) (Vreysen et al. 2000).

The potential of repellents to protect cattle and thereby reduce cases of trypanosomiasis has been recognized since the 1970s. Earlier work by Vale (1974a) showed that constituents in human odor reduced the number of tsetse attracted to a host and the number that subsequently fed. The effect was greater on females than on males, and *Glossina pallidipes* was more strongly affected than *Glossina morsitans.* During the process of screening for tsetse attractants, a number of compounds were discovered that had varying levels of repellency against the two savannah tsetse species. These included two synthetic compounds, acetophenone and caproic acid, and two naturally occurring constituents of cattle and human odor, respectively, guaiacol (2-methoxyphenol) and lactic acid (Vale 1977a, 1980; Vale et al. 1988b). Commercially available insect repellents such as N,N-diethyl-meta-toluamide (DEET) and naphthalene were ineffective (Vale 1977a; Torr et al. 1996). Torr et al. (1996) evaluated the effects of controlled release of acetophenone, 2-methoxyphenol, and a series of fatty acids on attractants-baited trap catches of *G. pallidipes* in Zimbabwe. 2-Methoxyphenol reduced the catch by 85%, whereas acetophenone, pentanoic acid, or hexanoic acid halved the respective catches. Further studies on targets baited with a synthetic attractant blend, a source of natural ox odor, and an ox placed at the center of rings of electrified nets showed that whereas 2-methoxyphenol reduced the numbers of tsetse attracted to the attractant sources by ~50% and ~40%, respectively, only pentanoic acid had a small but significant effect on the feeding responses of the flies that arrived closer to the ox. These findings led these authors to conclude that the repellents could not provide any useful degree of protection against trypanosomiasis.

We have explored two approaches to discovering more potent tsetse repellents: (1) by detailed analyses of odors and assays of different blends of candidate semiochemicals of wild animals that are refractory to tsetse (Gikonyo et al. 2000, 2002, 2003); and (2) through structure-activity studies of known natural repellents in the laboratory followed by field evaluations of promising analogues. Herein, we report our discovery of a synthetic analogue of guaiacol (2-methoxyphenol), which has demonstrated greater repellency than the parent compound.

Methods and Materials

Laboratory Bioassays

Wind Tunnel Bioassays Tests were conducted in a two-choice cylindrical plexi-glass tunnel (180 cm long, 24 cm diam) described in detail by Gikonyo et al. (2003). The middle of the tunnel was connected to a PVC pipe in the terminal end of which an air-extracting fan was mounted. The duct in the middle divided the tunnel into two equal arms with a 20-cm wide middle zone where air from both arms mixed. When the fan was switched on, air flowed into the tunnel from both arms, thereby making the middle of the tunnel downwind. The upwind ends of the tunnel were closed with PVC gauze and the down end with a metallic mesh cover. The upwind ends were connected to air filters containing activated charcoal (4-14 mesh, Sigma Chemical Co.) The wind tunnel had three windows, one on each arm (15×10 cm) for introducing the test odors and one in the middle (4.7 cm diameter) for introducing an insect release cage. Light from fluorescent tubes and bulbs controlled with a dimmer switch was diffused through frosted glass sheeting placed 35 cm above the tunnel, giving about 1,000 lx incident light. A white sheet of paper marked with black stripes about 2 cm apart was placed beneath the tunnel floor to provide contact to a fly during anemotaxis. The wind speed inside the tunnel was maintained at 10 cm/sec. The bioassay room was maintained at 25±2°C and 70±5% relative humidity (RH).

Test Compounds Guaiacol (2-methoxyphenol) and seven analogues comprising 2-methoxyfuran, 2,4-dimethylphenol, 2-methoxy-4-methylphenol (4-methylguaiacol), 4-ethyl-2-methoxyphenol (4-ethylguaiacol), 4-allyl-2-methoxylphenol (4-allyllguaiacol; eugenol), 3,4-methylenedioxytoluene, and 3,4-dimethoxystyrene (Fig. 1) (each 98–99% pure, obtained from Sigma-Aldrich, Taufkirchen, Germany) were each tested against a control (blank). Eugenol was specifically included in the tests because of previous reports of its repellency against some arthropods (Chogo and Crank 1981; Hassanali et al. 1990; Mwangi

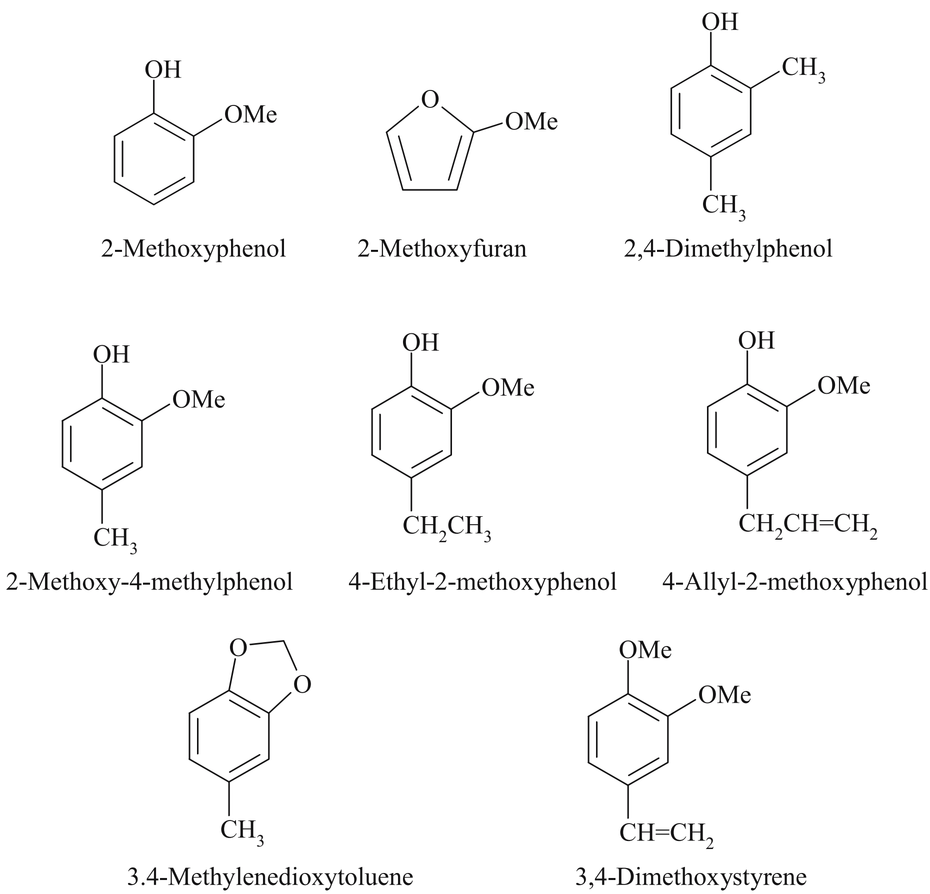

Fig. 1 Structures of 2-methoxyphenol (guaiacol) and analogues screened in the wind tunnel for repellency against *G. m. morsitans*

et al. 1995). A blank test comprising of two blank odor dispensers placed at both upwind ends was also undertaken.

Odor Dispensers The odor dispenser consisted of a black cloth that was secured with Soxhlet-cleaned (dichloromethane) rubber bands placed on one end of an open-ended plexiglass tube (5 cm long and 4.5 cm diam). The dispensers were each placed via the window on the upper side of either arm of the tunnel onto a metallic rack positioned 15 cm from the upwind end such that they were at midheight with the cloth facing downwind. The appropriate dose of the odor was pipetted onto the center of the cloth on one of the dispensers, and air was allowed to flow at 10 cm/sec. A similar dispenser placed in the other arm of the tunnel served as a control. All windows of the tunnel were closed and air allowed to flow for 5 min before the first fly was introduced.

Testing Procedure The procedure detailed in Gikonyo et al. (2003) was followed. One hour before the bioassay, individual flies (3-d-old teneral male tsetse flies) were placed in cylindrical plastic cages (8.0 cm long and 4.5 cm diam) closed on one side with a paper stopper inserted into a semicircular slit. At the start of the bioassay, a release cage containing a quiescent male fly was introduced cautiously through the release hole in the middle of the tunnel (downwind end, middle zone), and the paper plug of the release cage was removed gently so that only air from the charcoal filters carrying odor from the dispensers passed over the release cage. The behavior of each fly was observed and recorded for 3 min, after which the fly was aspirated out with a vacuum pump. The following observations were made: (1) number of flies departing from the midsection; (2) initial direction of flights upwind and any subsequent changes; and (3) return downwind flights and final landing/resting position (middle, control, or treated arms of the tunnel).

In each replicate cycle, involving one dose of a given test odor in 1 day, the above observations on about 10 individual flies were recorded after which the tunnel, the metallic racks, and the release cages were cleaned with water and then with 70% ethanol. With the humidifier switched off, the bioassay room temperature was raised to above 30°C, and air was exhausted from the room for about 6 hr by a powerful extraction fan fixed on the room wall. At the same time, the tunnel was exhausted at maximum wind speed overnight. Blank tests were conducted to confirm no residual effects of previous test materials. Once a week, the tunnel walls were also flushed with hot air from a heat gun. Between three and 10 replicates (corresponding to 30–100 flies observed) were carried out for each treatment. To avoid possible bias, the arms of the tunnel were used alternately for control or test odor in successive replicates. The percent responses were rank-transformed before being subjected to analysis of variance (ANOVA) by using PROC GLM (SAS institute, 2002) with significance level set at 5%.

Insects Newly emerged male *G. morsitans morsitans*, available from the ICIPE colony maintained in a rearing room at 25±2°C, 65±5% RH on a 12:12 (L/D) photocycle, were used as a representative of savannah morsitans species to initially screen the relative repellency of the test compounds in the laboratory.

Field Experiments

Field Site All experiments were carried out at ICIPE's Nguruman field station, southwest of Kenya where *G. pallidipes* Austen are predominantly present.

Traps All catches were made by NG2G traps, baited with acetone at ~500 mg/hr and cow urine aged 1–4 wk at overall (water plus volatiles) release rate of ~1,000 mg/hr, which provide relatively uniform catches (Brightwell et al. 1991). Traps were placed 100 m apart along the forest edge. The odors were placed on the ground 30 cm downwind of the trap.

Effects of Various Candidate Repellents on Trap Catches The effect of 2-methoxyphenol (guaiacol), 4-allyl-2-methoxyphenol (eugenol) and 2-methoxy-4-methylphenol (4-methylguaiacol) on trap catches were compared in a series of Latin square design experiments comprising of the following treatments:

(a) Baited trap, baited trap with 1.0 or 10.5 mg/hr eugenol, and baited trap with 1.0 or 9.0 mg/hr guaiacol, respectively. In the second experiment, higher doses (25.0 or 50.0 mg/hr) of the two compounds were evaluated. The repellents were dispensed undiluted from glass vials (1.7 cm wide and 4.3 cm deep) with lids containing 1- or 2-mm-diam holes to give the desired release rates.
(b) Unbaited trap, baited trap, baited trap with 2-methoxy-4-methylphenol at a dose of 10.0 mg/hr. The repellent was dispensed from glass vials, as above.
(c) Baited traps with 2.2, 4.5, or 9.0 mg/hr, 2-methoxy-4-methylphenol. In this set of experiments, the repellent was dispensed from sealed polythene sachets (25–50 cm^2 surface area and 50 μm thickness). The surface area of the sachets was varied to obtain the different release rates.

In all the above experiments, the repellents were incorporated into a series of replicated Latin squares consisting of days × sites × treatments. The catches (n) were normalized by using a $\log_{10}(n + 1)$ transformation and subjected to analysis of variance (ANOVA with PROC GLM, SAS Institute, 2002). The detransformed means are reported accompanied by their transformed means and standard errors.

4-Methylguaiacol Effects on Glossina pallidipes Responses to Natural Attractants This set of experiments was conducted to determine the number of tsetse attracted to a source of ox odor with or without the synthetic repellent. The odor was generated by placing an ox (~250 kg) in a pit (5 m long, 2 m wide, and 2 m deep with iron sheet roofing and entrance door) and exhausting the air from the pit at 2,000 l/min via a ventilation shaft fitted with a 12-V co-axial fan at the end (Vale 1974b). Two such pits were constructed. To estimate the number of tsetse attracted to the odor, an electric net (1.0×1.0 m) was placed 1 m downwind of the odor source (Vale 1974b). Sheets of corrugated fiberglass coated with a thin film of polybutene were placed underneath the screens to collect the electrocuted flies. As tsetse orientate imprecisely to an odor source in the absence of a visual stimulus (Vale 1974b), a visual model (metal drum 50 cm long and 37 cm diam covered with black cloth) was placed between the screen and odor source. A balanced incomplete Latin square design was used for these experiments with the following treatments: (1) ox odor, (2) ox odor + model, (3) ox odor + repellent, (4) ox odor + model + repellent. The repellent (10 mg/hr) was released from a polythene sachet placed near the odor outlet. The experiments were conducted for 3 hr in the morning (0800–1100 hours) and 3 hr in the afternoon (1400–1700 hours).

Effect of 4-Methylguaiacol on Feeding Efficiency of Glossina pallidipes The feeding efficiency of *G. pallidipes* on an ox with or without the repellent was determined by using the method developed by Vale (1977b). In this experiment, an ox was tethered in the middle of an incomplete ring of five electric screens (1.0×1.0 m). The screens covered 20% of the circumference of the circle. These rings were constructed at two different locations. The

Table 1 Behavioral responses of male teneral *Glossina mositans morsitans* to guaiacol and analogues in a wind tunnel

Test Odor	Dose (µl)	*N*	Percent files departing from midsection	Final resting position			
				Control	Center	Treated	*P*
2-methoxyphenol (guaiacol)	100	100	45.8	60.90±5.99a	3.33±3.33^{c}	35.80±8.52b	0.01
	200	119	57.1	40.63±11.78	27.50±8.28	31.90±11.68	Ns
	400	88	58.1	46.25±8.69	20.72±5.23	33.05±5.80	Ns
2-methoxyfuran	100	40	62.5	46.65±9.71	21.48±6.25	31.87±5.24	Ns
	200	30	60	36.47±5.87	40.37±14.91	23.13±12.85	Ns
	400	30	76.67	20.83±6.35	40.27±5.01	38.87±5.57	Ns
2,4-dimethylphenol	100	30	53.33	49.03±4.96	18.10±11.7	32.87±8.92	Ns
	200	30	50	38.43±14.35	20.93±12.36	40.63±9.78	Ns
	400	30	66.67	39.53±6.18	35.93±5.53	24.53±2.49	Ns
2-methoxy-4-methylphenol	25	80	65	30.13±5.71^{a}	63.53±5.70^{b}	6.35±3.22^{c}	0.001
	50	71	64	30.83±7.68^{ab}	56.78±11.29^{a}	12.38±4.59^{b}	0.05
	100	80	65	56.24±11.93^{a}	25.23±7.03^{b}	18.54±5.21^{b}	0.05
	200	80	76.5	53.56±6.03^{a}	21.83±6.23^{b}	24.61±2.69^{b}	0.001
	400	70	75	63.53±5.70^{a}	15.99±4.83^{b}	20.59±3.45^{b}	<0.001
4-ethyl-1,2-dimethylphenol	100	35	62.9	26.20±14.48	28.7±14.85	45.2±4.77	Ns
	200	24	83.3	75.00±1.00	12.5±5.5	12.5±12.5	Ns
	400	30	70.3	27.70±1.82	41.7±7.20	30.6±5.60	Ns
4-allyl-2-methoxyphenol	100	30	50	38.90±20.04	19.43±10.01	41.67±30.05	Ns
	200	30	56.7	6.67±6.67	49.00±24.79	44.43±29.39	Ns
	400	30	62.5	24.47±2.68	43.43±7.05	32.10±8.60	Ns
3,4-methylenedioxytoulene	100	30	76.67	40.73±15.44	12.17±6.49	47.10±9.26	Ns
	200	30	73.33	48.53±4.56^{a}	14.53±2.76^{b}	36.93±1.95^{a}	0.05
	400	30	36.67	24.50±15.28^{a}	11.50±7.58^{ab}	64.00±15.28^{c}	0.01
3,4-dimethoxystyrene	100	35	56.7	52.8±13.90	11.1±11.1	36.1±21.69	Ns
	200	34	46.7	53.9±17.12	32.8±4.34	13.3±13.33	Ns
	400	30	65	29.0±5.51	19.4±1.00	51.7±10.27	Ns

experiment was a randomized complete block design and treatments were: (1) ox alone and (2) ox + repellent, which were randomly assigned to the two sites. At the site that received the repellent treatment, sachets containing the repellent (10 mg/hr) were placed on the post to which the animal was tied. The animals were kept stationary, whereas the treatments were rotated after each cycle of the experiment. Because in a preliminary experiment a relatively higher number of flies were caught in the afternoon, all the experiments were run for 3 hr from 1500–1800 hours. At the end of the experiment, the numbers of flies caught inside and outside the ring were recorded. Flies were also classified as fed or unfed based on the presence of fresh blood visible through the abdominal wall. The number of flies approaching the target (animals) with/without a repellent were estimated and statistically analyzed. Following Vale (1977b), feeding efficiency was estimated as a percentage of the fed tsetse relative to the total catch (fed + unfed) inside the ring of nets.

Results

Responses in the Wind Tunnel Equal number of flies flew upwind to the control (44%) or treated (47%) arms of the wind tunnel (N=145) when no odor was dispensed (blanks). Table 1 shows the responses of *G. m. morsitans* to the eight methoxyphenol analogues tested in the two-choice wind tunnel. Only the final resting position was statistically affected by the presence of a candidate repellent (Table 1) as previously shown with waterbuck repellents, a refractory host of savanna tsetse (Gikonyo et al. 2003). Only one 4-substituted methoxyphenol derivative, 2-methoxy-4-methylphenol, elicited consistent and significant repellent responses. The final resting position was dependent on the dose of this phenol: at lower doses (25 and 50 μl), more of the flies flying downwind settled in the midsection of the tunnel; however, at higher doses (100–400 μl) the flies tended to fly further away from the repellent-treated side and landed on the control arm of the wind tunnel (no odor present). Guaiacol (2-methoxyphenol) itself elicited milder responses, and

Table 2 Mean catches (detransformed and transformed ± SE) of *Glossina pallidipes* from NG2G-baited (cow urine + acetone) traps with various doses of eugenol or guaiacol (N=10)

Treatment	Trap catch	
	Detransformed	Transformed (±SE)
Expt 1		
Baited trap (BT)	524.807	2.720±0.138
BT + 1.0±0.18 mg/hr eugenol	644.169	2.809±0.137
BT + 10.5±0.03 mg/hr eugenol	516.416	2.173±0.140
BT + 0.9±0.27 mg/hr guaiacol	347.536	2.541±0.125
BT + 9.18±0.19 mg/hr guaiacol	218.273	2.339±0.104
Expt 2		
Baited trap (BT)	368.978	2.567±0.095
BT + 25.7±0.16 mg/hr eugenol	246.604	2.392±0.119
BT + 50.2±0.03 mg/hr eugenol	157.036	2.196±0.091
BT + 24.36±0.28 mg/hr guaiacol	285.759	2.456±0.137
BT + 49.81±0.17 mg/hr guaiacol	220.800	2.344±0.102

Table 3 The detransformed mean catch (transformed ± SE in brackets) of *Glossina pallidipes* from unbaited or baited (cow urine + acetone) NG2G traps plus 4-methylguaiacol

Treatment	Mean catch	Index	Percent reduction
Unbaited trap	214.2 (2.333±0.070)*	0.29×	71
Baited trap	740.7 (2.870±0.048)	1.0×	
Baited trap + repellent	138.8 (2.146±0.050)*	0.19×	81

The catch index is the detransformed mean catch of tsetse expressed as a proportion of that from a baited trap (N=24)

The asterisk indicates that means are significantly different from baited trap catch (P<0.05, F test)

in one dose (100 µl), the number of flies that flew downwind and settled on the control arm was statistically significant. Contrary to our expectation, eugenol did not elicit any significant repellent responses from *G. m. morsitans*.

Effect of Candidate Repellents on Baited Trap Catches In field experiments, neither 2-methoxyphenol (guaiacol) nor 4-allyl-2-methoxyphenol (eugenol) reduced the trap catches of *G. pallidipes* ($F_{8,41}$=0.13 and $F_{8,41}$=0.43, respectively, Table 2).

The inclusion of 4-methylguaiacol with unbaited and baited (acetone + cow urine) traps reduced trap catches (>70 and 80%, respectively) ($F_{4,67}$ =31.38; P<0.001, Table 3). There was no significant difference in the responses of males and females, and hence the data for the sexes were pooled.

Effect of 4-Methylguaiacol on Attraction to Natural Attractants In both experiments, *G. pallidipes* catches to ox odor (ox placed in a ventilated pit) with 4-methylguaiacol were reduced by ~80% ($F_{3,20}$=18.54, P<0.001 and $F_{3,20}$= 100.77, P<0.001, respectively, Table 4). In the presence of the visual stimulus (metal drum covered with black cloth placed between the screen and odor source), the repellent caused reductions of ~70 and ~80% in the two experiments, respectively.

Table 4 The detransformed mean catch (transformed ± SE in brackets) of *Glossina pallidipes* from a target at a source of natural ox odor with and without 4-methylguaiacol

Treatment	Mean catch	Index	Percent reduction
Expt 1			
Ox odor	25.6 (1.424±0.093)	1.0×	
Ox odor + model	31.8 (1.516±0.092)ns	1.24×	
Ox odor + repellent	5.3 (0.799±0.082)*	0.20×	80
Ox odor + model + repellent	7.9 (0.949±0.085)*	0.31×	69
Expt 2			
Ox odor	358.1 (2.555±0.354)	1.0×	
Ox odor + model	379.3 (2.580±0.036)ns	1.06×	
Ox odor + repellent	66.8 (1.831±0.031)*	0.19×	81
Ox odor + model + repellent	64.7 (1.818±0.062)*	0.1×	82

The catch index is the detransformed mean catch of tsetse expressed as a proportion of that from an ox odor source (N=6)

The asterisk indicates that means are significantly different from ox odor (P<0.05, F test)

Table 5 The detransformed mean catch (transformed ± SE in brackets) of *Glossina pallidipes* from NG2G-baited (cow urine and acetone) traps with various doses of 4-methylguaiacol

Treatment (baited trap + repellent)	Mean catch	Index	Percent reduction
A Trap alone (control)	117.8 (2.075±0.084)[a]	1×	
B Trap + 2.2 mg/hr	41.6 (1.629±0.071)[b]	0.35×	65
C Trap + 4.5 mg/hr	38.6 (1.598±0.068)[b]	0.33×	67
D Trap + 9.0 mg/hr	29.8 (1.489±0.071)[b]	0.25×	75

The catch index is the detransformed mean catch of tsetse expressed as a proportion of that from a baited trap (control) (N=16). Means followed by different letters are significantly different (P<0.05, F test)

Effect of 4-Methylguaiacol on Trap Catches All release rates of 4-methylguaiacol significantly reduced trap catches, with 9.0 mg/hr showing maximum reduction ($F_{3,60}$=12.61, P<0.001, Table 5).

Effect of 4-Methylguaiacol on Feeding Efficiency of Glossina pallidipes on Host The studies on feeding levels of *G. pallidipes* in the field (Table 6) showed that in addition to reducing the proportion of the tsetse attracted to an ox by >80% (Table 4), the candidate repellent also reduced host feeding from 27 to 4.8%, a >80% reduction.

Discussion

In a previous study undertaken in Zimbabwe (Torr et al. 1996), guaiacol at 5–10 mg/hr reduced the numbers of *G. pallidipes* attracted to baited and unbaited Epsilon (Hargrove and Langley 1990) traps (~85 and ~60%, respectively), baited targets (~50%), and a source of natural ox odor (~40%). In the present study, the compound elicited relatively mild repellent responses from *G. m. morsitans* in a two-choice wind tunnel, but no significant reductions in baited trap catches of *G. pallidipes* even at relatively high doses. The difference in the two studies may be due to different efficiency of the two trap designs used and/or some differentiation in the sensory responses of the two *G. pallidipes* populations. Detailed comparison of the electrophysiological and behavioral responses of the two *G. pallidipes* populations and their close-range behaviors to the two traps may shed some light on the question.

Despite the relatively high repellency demonstrated in different experiments in Zimbabwe, guaiacol had no significant effect on the numbers of flies that fed on an ox, which makes it an ineffective tool for protecting cattle against trypanosomosis (Torr et al.

Table 6 Mean catches and percent feeding efficiency of *Glossina pallidipes* on cattle with and without 4-methylguaiacol

Treatment	Total catch (inside the screen)	Total catch (inside and outside the screen)	Total fed (inside the screen)	Feeding efficiency (%)
Ox alone	445±8.5[a]	818±23.5[a]	120±10.3[a]	27
Ox + repellent	83±4.5[b]	154±9.6[b]	4±0.2[b]	4.8

Synthetic repellent reduces the feeding efficiency on treated cattle by 82%. Feeding efficiency is the catch of fed flies from inside the ring of nets expressed as proportion of the total catch (fed + unfed). Number of replicates = 6. Means followed by different letters are significantly different at (P<0.05, F test)

1996). In the present study, the presence of a methyl substituent at the 4-position of guaiacol significantly enhanced the repellency of the molecule to savannah tsetse *G. m. morsitans* in the wind tunnel, and reduced numbers of *G. pallidipes* attracted to baited and unbaited traps and to ox odor. In addition, the compound reduced the number of flies that landed on the ox and those that fed on the host (>80%).

These results suggest that 4-methylguaiacol represents a promising tool for protecting individual cattle against trypanosomiasis (Saini and Hassanali 2004). We are currently exploring two strategies for integrating the repellent with existing tools and tactics. First, we are studying its efficacy in controlled release devices carried by individual cattle owned by nomadic communities who need a mobile technology to protect their cattle and reduce their dependence on trypanocidal drugs. Such devices may also provide an effective means of reducing challenge to trypanotolerant cattle, which succumb to the disease at high infection rates (Jordan 1986). Second, use of the repellent as a "push" component with bait technologies (traps, targets, or live baits with insecticides as "pull") in "push–pull" tactics in areas where cattle are the dominant source of blood meal may provide a more effective way of suppressing tsetse among settled pastoralist and agro-pastoralist communities.

The enhanced repellency of 4-methylguaiacol (and lack of activity of the other analogues tested) against savannah tsetse discovered in the present study raises some interesting questions about the sensory mechanism that underlies its action. This phenol reduced the catch of *G. pallidipes* in unbaited traps that were only visually attractive and thus, according to Dethier et al. (1960) definition, qualifies as a true repellent. This suggests that these phenols probably interact with a specific group of receptors different from those of the two tsetse kairomonal phenols, 4-methylphenol and 3-*n*-propylphenol (Owaga et al. 1988; Saini and Hassanali 1992). They not only inhibit or counter the response of sensory signals from the receptors of these attractants, but also those associated with close-range visual attraction (Davis 1985). However, the close structural relationship between 4-cresol and 4-methylguaiacol and lack or mild activity of the respective parent phenols and those of ethyl analogues (Owaga et al. 1988) suggests a close similarity of the binding sites of their respective receptors. Our current studies on the odor binding proteins of savannah tsetse are expected to provide some insights into the relationship between the two sets of receptors and their relative specificity.

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