Trail pheromone of the red imported fire ant *Solenopsis invicta* (Buren)[1]

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Summary. Z,Z,Z-Allofarnesene (2Z,4Z,6Z)-2,6,10-trimethyl-2,4,6,10-dodecatetraene) was identified as the trail pheromone of the red imported fire ant by comparing chromatographic and spectral properties of the pheromone obtained from ant Dufour's glands with those of synthetic compounds.

Since its introduction from Brazil in 1940[2], the spread of the red imported fire ant *Solenopsis invicta* (Buren) across the southeastern United States has posed one of our most serious pest control problems. The large size of the colonies, the aggressiveness of individual ants, and the tendency of nest members to attack and sting en masse combine to make this species potentially dangerous to humans and livestock. In addition, the large, concrete-like mounds housing mature colonies often damage expensive farm equipment.

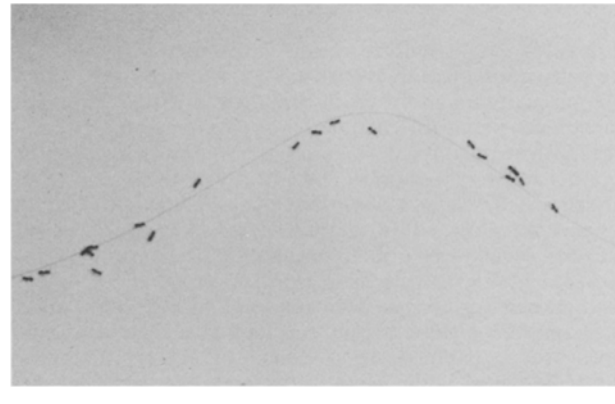
Non species-specific ant baits using chlorinated hydrocarbon insecticides have proven ineffective in controlling the spread of *S. invicta,* and according to some evidence, actually hastened its advance by eliminating competing species[3]. Use of a species-specific attractant increases the effectiveness of a bait in controlling a target species. For example, the leaf cutting ants *Atta cephalotes* (L.), *A. sexdens* (L.) and *Acromyrmex octaspinosis* (Reich) will readily pick up orange pulp baits impregnated with their trail pheromone methyl 4-methylpyrrole-2-carboxylate[4]. Other ant pheromones such as faranal (6*E*,10*Z*)-3,4,7,11-tetramethyl-6,10-tridecadienal-1), the trail pheromone of the pharaoh's ant *Monomorium pharaonis,* and triolein, the sexual brood pheromone of *Solenopsis invicta,* are possible components of baits[5,6]. A similar effect may be possible with the trail pheromone of *S. invicta.*

The source of the trail pheromone for *S. invicta* is the Dufour's (venom accesory) gland[7]. Early studies showed that the 2 imported *Solenopsis* species: *S. invicta* and *S. richteri,* readily follow trails of either species, but do not follow those of *S. geminata* and *S. xyloni,* 2 native species[8,9]. GC analysis shows the *S. invicta* pheromone has a shorter retention time than the *S. richteri* pheromone, which has a mol.wt of 218[9].

For this study, imported fire ants collected in the vicinity of College Station, Texas were maintained in dirt in plastic containers by the method of Vinson[10]. A paper bridge provided access to a paper covered foraging table. Bioassays of trail-following activity consisted of replacing a section of natural trail with a trail prepared by streaking the material to be tested along a pencil line. Controls of solvent and pencil line with no additive showed no activity. Ants followed a trail showing strong activity as they would their own, following a brief period of confusion. A 2nd, more definitive assay consisted of drawing a U-trail connecting 2 sections of a natural trail. A positive test in this case was indicated when the ants left their natural trail to follow the applied material. For each test of natural material, 10–30 Dufour's glands were excised and crushed in pentane to obtain a solution of trail pheromone.

Material was fractionated by preparative GC on 2 m × 4 mm inner diameter 1% OV101 (column A), and 1% carbowax 20 M (column B), both on 80–100 mesh chromosorb G-HP, programmed 80–225 °C at 5°/min. On column A, the active material co-eluted with pentadecane, on column B, with heptadecane. The peak showed tailing on both columns, indicating thermal instability. Conversions to an all-glass system did not lessen this instability.

Analysis of a pentane extract of 100 *S. invicta* Dufour's glands by GC-MS on column B showed that the active compound had a molecular ion at 204 and a fragmentation pattern almost identical to that of farnesene (2,6,10-trimethyl-2,6,9,11-dodecatetraene) which is the major component found in the Dufour's gland of the related myrmecine ant *Aphaenogaster longiceps*[11]. However, on testing these compounds were found to be 100 or more times less active than the natural material. GC-MS analysis of a sample containing these isomers under the same conditions showed that they had slightly different retention times and mass spectra with reduced peaks at 135, indicating that the active compound could produce a more stable fragment with a possible formula $C_{10}H_{15}$. The conjugated portion of allofarnesene would produce such a fragment; but, although allofarnesenes were previously reported as constituents of several plant oils[12,13], no mass spectral data was available. A sample of *E,E,E*-allofarnesene was prepared by reaction of the triphenylphosphine ylide prepared from geranyl bromide with tiglic aldehyde. Mass spectra of this compound matched that of the pheromone almost exactly, but GC retention time was somewhat longer. A 2nd isomer prepared by reaction of the ylide of neryl bromide with tiglic aldehyde gave similar results. Since separation of isomers was possible by GC, a mixture of isomers could be prepared and tested. Reaction of commercial pseudoionone ((3*E*,5*E* and 3*E*,5*Z*)-3,5,9-undecatriene-2-one) with triphenylphosphonium ethylide gave the 4 *E*-4 allofarnesene isomers, all of which were less active and had longer retention times than the pheromone. The *Z*-4 allofarnesene isomers could be prepared by reaction of the *E*-3 pseudoionone isomers (prepared using the procedure of Zakharova et al.[14a,b]) with the same ylide. The mixture produced by this reaction was biologically active but the isomers were heat sensitive and could not be separated by GC for further analysis and testing. HPLC using silica gel and ODS columns also proved ineffective in separating the isomers, but a 20% silver nitrate on Rsil (10u, 25 cm × 4.7 mm inner diameter) column, with 5% $CHCl_3$ in pentane gave near baseline separation of the isomers with no decomposition. Comparison of retention times showed that the 2nd peak eluting corresponded to material from ant Dufour's glands.



Imported fire ants following a trail of synthetic Z,Z,Z-allofarnesene at 500 pg/cm concentration.

GC analysis on columns A and B and mass spectral data of this peak were also identical. The pseudoionone isomers used as starting materials were inseparable by GC, even with capillary columns, but could be separated using silica gel HPLC with 50% $CHCl_3$ in pentane as solvent. Since Zakharova et al. has previously determined the configuration of the pseudoionone isomers[14b], the possible products of the Wittig reactions were also determined. The active material was found to have the *Z,Z,Z*-configuration.

The pheromone was found to be identical spectrally and chromatographically with *Z,Z,Z*-allofarnesene, but all isomers with *Z*-4 configuration were biologically active. Testing using serial dilutions showed the syntetic *Z,Z,Z* isomer to have noticeable activity at the 5 fg/cm level, but optimum activity was found at the 100–500 pg/cm level (fig.). GC analysis of the pheromone deposited in a 10-cm glass tube by a colony of actively moving ants was also within this range. Ants reaching a section of trail of higher or lower concentration than the one they were moving along hesitated before proceeding, regardless of whether the trail they had been following was natural or artifical. This indicated that the ants could detect differences in pheromone concentration, a useful ability since trails must cross in the field.

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2 C.S. Lofgren, W.A. Banks and B.M. Glancy, A. Rev. Ent. *20*, 1 (1975).
3 J.W. Summerlin, A.C.F. Hung and S.B. Vinson, Environ. Ent. *6*, 193 (1977).
4 S.W. Robinson and J.M. Cherrett, Bull. ent. Res. *68*, 159 (1978).
5 F.J. Ritter, I.E.M. Bruggemann-Rotgens, P.E.J. Verweil, E. Talman, F. Stein, J. La Brijn and C.J. Persoons, Proc 8th int. Congr. IUSSI, Wageningen (Netherlands) 1977, p.41.
6 W.S. Bigley and S.B. Vinson, Ann. ent. Soc. Am. *68*, 30 (1975).
7 E.O. Wilson, Anim. Behav. *10*, 134 (1962).
8 D.P. Jouvenaz, C.S. Lofgren, D.A. Carlson and W.A. Banks, Fla Ent. *61*, 224 (1978).
9 M.R. Barlin, M.S. Blum and J.M. Brand, J. Insect Physiol. 839 (1976).
10 S.B. Vinson, J. econ. Ent. *61*, 712 (1968).
11 G.W.K. Cavill, P.J. Williams and F.B. Whitfield, Tetrahedron Lett. *1967*, 2201.
12 Y.R. Naves, Helv. chim. Acta *49*, 1029 (1966).
13 T.S. Sakai and Y. Hirose, Bull. chem. Soc. Jap. *42*, 3615 (1969).
14 *a* N.I. Zakharova, M.A. Miropol'skaya, T.M. Filippova, I.M. Kustanovich and G.I. Samokhvalov, Zhurn. org. Khim. *9*, 512 (1973); *b* N.I. Zakharova, T.M. Filippova, A.R. Bekker, M.A. Miropol'skaya and G.I. Samokhvalov, Zhurn. org. Khim. *14*, 1413 (1978).

Effect of exogenous iron on the viability of pathogenic *Naegleria fowleri* in serum

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Summary. When *Naegleria fowleri* (Lee) was incubated in newborn calf and human serum an amebicidal effect was observed. Heat inactivation of both sera resulted in the recovery of viable amebae after incubation in these sera. Exogenous iron added to non-heat inactivated calf serum improved viability slightly but was without effect when added to human serum not heat inactivated. Exogenous iron greatly enhanced growth and/or viability in heat inactivated calf serum. Viability of amebae also was considerably enhanced in human serum which was heat inactivated when pH was lowered in conjunction with iron supplements.

Naegleria fowleri is one of several species of free-living amebae which can cause primary amebic meningoencephalitis (PAME). It is usually a fatal disease of the central nervous system, and nearly all cases have been related to recent association with water sports or other aquatic activity[2].

Numerous axenic culture media have been developed for the cultivation of *Naegleria* species[3–8]. Most of these media contain mammalian serum in conjunction with a variety of other components for enhanced *Naegleria* growth.

The growth of amebae in serum-enriched media suggests serum itself might support the growth or maintenance of amebae. It has been shown that mammalian serum could support the growth of some procaryotic parasites provided sufficient iron was initially available or the serum was supplemented with exogenous iron[9, 10].

Mammalian serum has been reported to have an amebicidal or amebostatic effect on amebae[11–15]. These results have been attributed to a variety of factors including antibodies, heat labile factors, and complement. The purpose of this investigation was to quantify the viability and/or growth of pathogenic *N. fowleri* in newborn calf and human serum and to determine if addition of iron could enhance viability and growth.

Materials and methods. Newborn calf serum (Grand Island Biological Co., Grand Island, N.Y.) and pooled human serum (from a local hospital) were heat inactivated at 56 °C for 30 min unless designated otherwise. Penicillin and streptomycin (200 µg/ml each) were added, and the serum was sterilized by filtration (0.45 µm). 2-ml aliquots were pipetted into 15 × 165 mm screw-capped tubes. Iron was added to serum in the form of $FeCl_3 \cdot 6\ H_2O$ or $FeSO_4 \cdot 7\ H_2O$. Stock iron solutions were prepared in distilled water, filter sterilized, and diluted in distilled water so that 0.5 ml delivered the appropriate amount of iron. In addition, the pH of some tubes was lowered with 1 N HCl. Iron and HCl were added to sera 24 h before inoculation with amebae. Control tubes received Page ameba saline (PAS)[16]. *Naegleria fowleri* (Lee) was grown at 37 °C in tubes containing 3 ml of Chang's SCGYEM medium[6]. For inoculum, amebae were harvested by centrifugation (1000 × g for 10 min) and resuspended in PAS. The number of amebae/ml were determined by hemocytometer counts and dilutions were prepared in PAS so that 0.5 ml delivered the