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EXTRAS

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Recent Biochemical Studies of the Fire Ant, Solenopsis invicta

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A B S T R A C T *Laboratory and field colonies of* Solenopsis invicta *showed no significant difference in carbohydrate composition. Fourth-instar larvae were found to have an unusually high percentage of trehalose compared to adults and first-instar larvae. Amino acid analysis of sexuals and minor workers showed an absence of threonine, and amounts of amino acids in larval forms were significantly larger than in adults. The fire ant queen poison sac was found to contain piperidine alkaloids and is the storage site for a pheromone that elicites orientation and attraction in workers. This pheromone may be useful in providing a species-specific bait toxicant formulation.*

About 40 years ago the red imported fire ant, *Solenopsis invicta Buren*, was accidently imported into the southern United States from South America. The ant spread rapidly from its initial port of introduction, Mobile, Alabama, and now infests all or parts of nine states (Fig. 1). In recent years the northward migration of this aggressive ant has been slowed by cold winters. Movement to the west will probably be restricted by the arid conditions of west Texas.

Mature ant colonies may consist of 100 to 200 thousand worker ants that build large mounds or nests for the protection of their queen

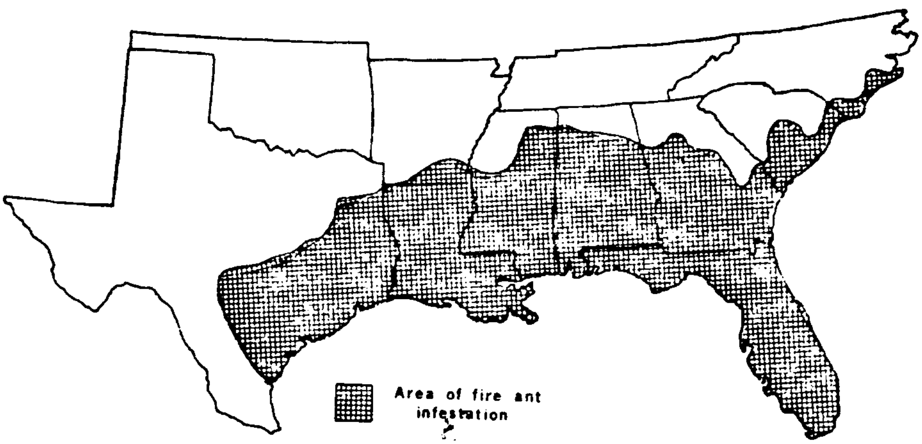


Fig. 1

and rearing of brood. Infestations of 40 to 100 colonies per hectare is common in pastures, hayland, along roadsides and in some cropland. The ants potent sting is painful and kills the cells near the point of injection causing a postule to form. Some people develop hypersensitivity to the venom, a condition which has resulted in several deaths (11). Also, the ants are a problem on farmland because they sting when disturbed and interfere with the harvest of crops.

The density of the ants in infested areas became severe enough in the early 1950's that the U.S. Congress initiated a cooperative federal-state control program in 1957 to assist farmers in eliminating the pest (7). Initially, heptachlor (1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene) and dieldrin (1,2,3,4,10,10-hexachlore-6,7-epoxy-1,4,4a,5,6,7.8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene) were used chemicals to nontarget organisms they were banned and replaced by a bait formulation containing a toxicant mirex (dodecachlorooctahydro-1,3,4 mehtano-1M-cyclobuta cd pentalene). This new bait was very effective and was less environmentally hazardous than heptachlor or dieldrin, although it too was a chlorinated hydrocarbon. However, in 1978 its use was also prohibited by the Environmental Protection Agency because residues were found in animals (17) and humans (6), and it was shown to be a possible carcinogen (12). The U.S. Department of Agriculture has been conducting an intensive research program for many years to find new chemical, biological and ecological approaches to control of the red imported fire ant. Since 1958, over 5,000 chemicals have been bioassayed for toxicity in baits. More recently, insect growth regulators, chitin inhibitors and sterilants have been tested, and surveys have been initiated in South America for pathogens and parasites that might be released in the United States to help control the pest.

As part of our overall research program, we have begun studies on the biochemistry of the fire ant. This research should aid in developing control methods compatible with the environment. In this paper we present some basic data on the carbohydrates and amino acids of larvae and workers, as well as comments on the function and contents of the poison sac.

METHODS AND MATERIALS

Adults and larvae used in our studies were obtained from well-established laboratory colonies originating from newly-mated field collected queens. We isolated carbohydrates by extracting ant samples with 95% ethanol (Figure 2). Lipids were removed from the concentrated ethanol extract with hexane. Carbohydrates were further purified by treatment with zinc sulfate and barium hydroxide. Free amino acids remaining in the aqueous extract did not interfere with subsequent sugar analysis. The purified carbohydrates were silylated with trimethylsilylimidazole in pyridine (10), and the derivatized sugars were analyzed on a Varian 37000 gas chromatograph equipped with a flame ionization detector and coupled to a Mewlett Packard 3385A data processor. The columns used were 3% OV101. 3% OV17 and 3% OV225 all on 100/120 Gas Chrom Q and packed in 1.8 m $\times$ 2 mm glass columns. Oven temperature programming varied.

Samples for free amino acid analysis were immediately frozen with dry ice and ground in 10% trichloroacetic acid. Lipids were removed by extraction with hexane or ether, and the samples were dried under a stream of nitrogen. Amino acids were analyzed with a Beckmen 120 C Amino Acid Analyzer.

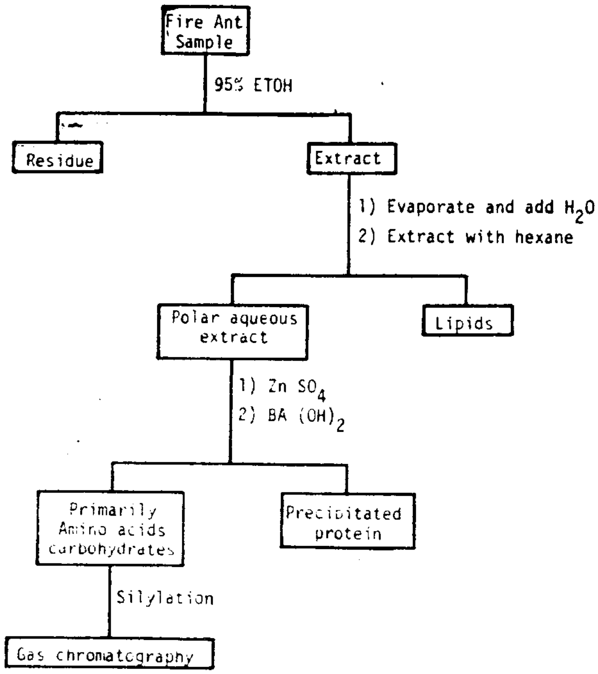


Fig. 2

RESULTS AND DISCUSSION

Carbohydrates. Research on other insects has clearly shown that the role of carbohydrates is associated not only with energy production but also with growth factors, detoxification, metamorphosis, reproduction and feeding behaviour (4). The red imported fire ant has long been thought of as an oil feeder. However, the observation that addition of honeywater (1 : 1 ratio) to their normal diet enhanced both laboratory colony weights (making them comparable to field colonies) and the survival of newly mated queens emphasizes the importance of dietary carbohydrates and carbohydrate metabolism (15).

As a first step in understanding carbohydrate utilization in fire ants, we have identified the major simple carbohydrates found in the ant (Figure 3). Identification was made by comparison of Gas Chromatograph retent on times and coinjection with standards on two columns of different polarity.

The primary carbohydrates present are fructose, glucose and trehalose, with smaller amounts of sucrose and some unidentified compounds. Both anomeric forms (alpha and beta) of reducing sugars appear on the chromatogram. Percentage composition for several laboratory and field colonies is shown in Table I. Differences between the two are not significant in spite of the fact that laboratory colonies had a continous supply of honey-water.

Trehalose, an important reserve disaccharide in insects, is readily hydrolyzed to glucose, which is then oxidized to provide energy. Although the relative percentage of trehalose is very similar in field

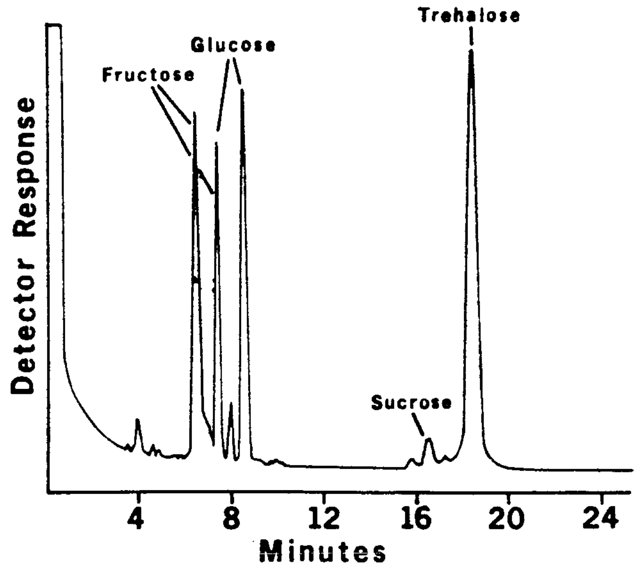


Fig. 3

Table I

Carbohydrates found in Solenopsis invicta laboratory and field colonies

Lab. colony	Percentage carbohydrate			
	Fructose	Glucose	Sucrose	Trehalose
1	17	66	3.3	14
2	22	55	1.4	22
3	25	41	1.0	32
4	21	46	6.3	27
Mean (SD)	21.3 (3.3)	52.0 (11.0)	3.0 (2.4)	23.8 (7.7)
Field colony				
1	17	65	0.6	18
2	30	51	4.2	15
3	13	64	0.6	23
Mean (SD)	20.0 (8.9)	60.0 (7.8)	1.8 (2.1)	18.7 (4 0)

and honey-fed laboratory colonies the percentage of trehalose dramatically decreases when honey-water is removed from the diet of laboratory colonies.

These data suggest that fire ants, although generally thought of as being oil feeders (7), have very definite carbohydrate requirements. In addition, the relative amount of trehalose found in the ants may be indicative of a colony's general well being. We are currently trying to determine the source of imported fire ant carbohydrates in the field.

Table II shows the carbohydrate composition in adults and larval stages of fire and castes and sexes. Of particular note are the large amounts of trehalose found in fourth-instar larvae. Larvae in certain other hymenoptera play an integral role in colony nutrition. Wasp larvae are fed various food materials by workers, and in return the larvae regurgitate a secretion rich in carbohydrate, which is solicited by the workers (16). We are currently testing the hypothesis that fire and larvae may play a similar role. The large amount of trehalose found in fourthinstar larvae lends some support to this idea.

Table II

Simple carbodydrates found in adults and larval stages of *Solenopsis invicta* castes

Sample	Percentage carbohydrate			
	Fructose	Glucose	Sucrose	Trehalose
Adults				
Males	3.7	38.0	1.5	56.0
Females, alate	0.6	28.0	1.5	70.0
Major workers	10.0	27.0	4.5	58.0
Minor workers	20.0	40.0	9.0	31.0
First Instars				
Males	2.3	62.0	13.0	23.0
Females, alates	14.0	66.0	2.8	17.0
Major workers	2.4	40.0	4.2	54.0
Minor workers	6.3	56.0	3.3	35.0
Fourth Instars				
Males	3.0	28.0	7.2	61.0
Females, alates	5.2	11.0	5.0	79.0
Major workers	2.5	6.0	3.0	88.0
Minor workers	3.3	9.4	4.4	83.0

Amino acids. Insects are characterized by having unusually high concentration of free amino acids in their tissues and hemolymph. Besides serving as substrates for protein synthesis, they may regulate osmotic pressure, detoxify waste products, participate in a number of morphogenetic and reproductive processes, and act as energy sources under certain conditions (3). With such multiple role possibilities, it is important in our understanding of fire and biochemistry to have a knowledge of their amino acid requirements and functions.

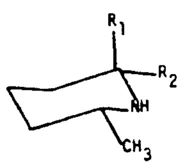
The amount of free amino acids per gram of worker, female alate, male alate, larvae and adult are shown in Table III. The most obvious feature of the data is the absence of threonine in all samples examined. Also conspicuous are the considerably higher amounts of the aromatic amino acids tryptophan, phenyl alanine, and to a less extent tyrosine in fourth-instar larvae as oposed to adults. Similarly, the total u moles of free amino acids per gram of sample is significantly higher in the larval forms than in adults. Sexual adults differ from workers primarily in increased amounts of taurine and decreased amounts of valine. Quantitative and qualitative differences may simply reflect a difference in larvae-adult physiology, or they may be indicative of possible larval

Free amino acids found in *Solenopsis invicta* adults and larvae
u Moles amino acid per g sample

Amino acid	Minor workers		Female alates		Male alates	
	Larvae	Adults	Larvae	Adults	Larvae	Adults
Tryptophan	1.24	—	0.60	—	0.50	—
Lysine	10.53	1.47	6.17	0.70	7.68	0.42
Histadine	6.83	2.26	4.31	1.19	4.30	1.55
Ammonia	11.57	—	6.63	3.27	4.71	5.08
Arginine	3.18	5.28	2.47	1.45	0.64	1.64
Taurine	4.97	3.59	1.90	5.79	3.24	10.29
Aspartic acid	0.15	0.57	0.80	0.10	0.15	0.10
Threonine	—	—	—	—	—	—
Serine	8.46	4.63	8.72	3.51	7.48	4.22
Glutamic acid	6.02	7.25	3.04	3.33	4.06	3.28
Proline	12.75	21.94	11.38	6.58	13.64	10.58
Glycine	5.50	2.38	4.52	1.59	4.00	1.79
Alanine	8.35	5.26	5.18	1.63	10.25	4.78
Half cysteine	—	—	—	0.53	—	—
Valine	4.73	13.50	4.79	0.89	5.15	1.51
Methionine	0.97	0.51	1.57	0.08	2.02	0.22
Isoleucine	1.73	0.47	2.84	0.17	3.02	0.17
Leucine	0.91	0.53	2.18	0.24	1.40	0.28
Tyrosine la	2.19	0.67	8.35	2.10	6.88	0.58
Phenylamine	4.85	0.13	3.50	0.31	4.70	0.12
Total	94.93	70.44	79.23	33.45	83.94	46.61

participation in overall colony and queen nutrition (as previously suggested). Workers are known to solicit material secreted or regurgitated from fourth-instar larvae (9), and that the larval stomach is very rich in substances which give positive ninhydrin tests, indicative of amino acids and or proteins. The implications of the amino acid data presented here will become clearer after samples of earlier larval stages, eggs, and larval parts are analyzed.

Poison sac chemistry. The major components of the poison sac are the piperidine alkaloids identified as alkyl piperidines (see Figure 4)



Female Sexual Alkaloids

R_1 = H R_2 = $C_{11:0}$
R_1 = $C_{11:0}$ R_2 = H
R_1 = H R_2 = $C_{13:1}$
R_1 = H R_2 = $C_{13:0}$

Worker Alkaloids

R_1 = $C_{11:0}$ R_2 = H
R_1 = $C_{13:1}$ R_2 - H
R_1 = $C_{13:0}$ R_2 = H
R_1 = $C_{15:1}$ R_2 = H
R_1 = $C_{15:0}$ R_2 = H

Fig. 4

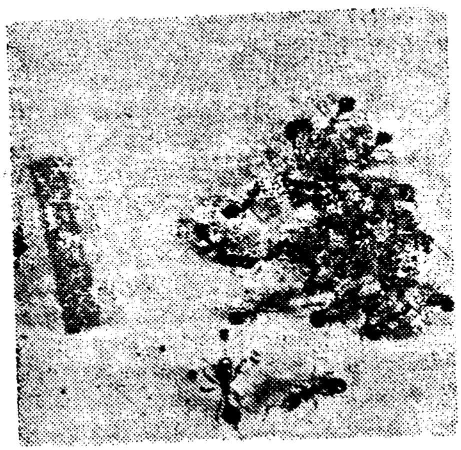


Fig. 5

(2, 8 and 13). These compounds have antibacterial, fungicidal and necrotic activity and are used by the workers in defense and in securing prey (1, 5). The queen does not sting yet she does have a well developed poison gland and sac. We have recently discovered that the queen's poison gland is the probable site of synthesis of a queen pheromone and that the poison sac is the site of pheromone storage (14). The pheromone elicits orientation and attraction in workers and promotes the deposition of brood near her. Figure 5 illustrates the effects on workers of 0.2 queen equivalents of queen poison sac extract applied to a piece of a rubber septum. We hope that use of the pheromone in bait toxicant formulations will provide a species — specific toxicant formulation. In addition to the piperidine alkaloids, about 10% of the content's residue is proteinaceous. Several laboratories in the USA are investigating the composition and physiological activity of these peptides and proteins.

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REZUMAT

Robert K. Vander Meer, Emanuel Merdinger, Clifford S. Lofgren — STUDII BIOCHIMICE RECENTE PRIVIND FURNICA *SOLENOPSIS INVICTA*

Date fiind cunoştinţele tot mai numeroase despre structura complexă socio-biochimică a furnicilor *Solenopsis invicta* importate, sperăm să putem prezenta cîteva metode de control noi şi sigure. Datele prezentate în acest referat sînt preliminare, dar ele reflectă unele diferenţe interesante între adulţi şi lucrători, care fac să înţelegem mai uşor modul complex de hrană în colonii. Metodele de dezmembrare a funcţiilor fiziologice şi de comportare, pot conduce la metode de control mai sigure şi mai eficace.

РЕЗЮМЕ

Роберт К. Вандер Меер, Эмануел Мердингер, Клиффорд С. Лофгрен — НЕДАВНО ПРОВЕДЕННЫЕ БИОХИМИЧЕСКИЕ ИССЛЕДОВАНИЯ В СВЯЗИ С МУРАВЬЕМ *Solenopsis invicta*

Имея в виду все более обильные сведения в связи с комплексной социальной и биохимической структурой импортных муравьев *Solenopsis invicta*, авторы полагают, что им удается изложить несколько новых и надежных контрольных методов. Сообщаемые ими в настоящей статье данные являются предварительными, но они указывают на существование ряда интересных различий между взрослыми и рабочими муравьями, что облегчает понимание сложного образа кормления колонии. Методы расчленения физиологических функций и образа их жизни могут привести к разработке некоторых более надежных и более действенных контрольных методов.