Fire Ant Carbohydrate Phagostimulants: Laboratory and Practical Studies

Robert K. Vander Meer *, Emanuel Merdinger **

The fire ant, Solenopsis invicta was accidently imported into the United States in the 1930's from South America. Since then it has infested nine southern states and Puerto Rico (approximately 150 million hectares) (1). It has the potential to spread into Arizona, California, Oregon, and Washington; however, the deserts of Texas and stringent quarantine measures currently hold it in check (2).

Mature fire ant colonies contain up to 250,000 workers and 120 colonies may infest each hectare. The ant is considered a pest for medical and agricultural reasons (3). The highly aggressive workers have a potent sting and the injected venom has a wide variety of physiological effects, the most severe of which is hypersensitivity (4). The stings are painful and may curtail people's outdoor activities.

Fire ant workers attack a wide variety of crops including soybeans, potatoes, corn, citrus, and okra. They feed on germinating seeds in corn and soybeans fields, thus decreasing crop yield (3). This kind of damage previously went unnoticed because all activity was underground. However, in okra the damage done by ants is visibly evident. The worker ants macerate the sepal area of the flower and presumably feed on the plant fluids. Severely attacked flowers may produce deformed fruit or no fruit.

We report here the results of carbohydrate phagostimulant tests, as well as okra and soybean carbohydrate analyses that help explain the observed fire ant attacks on these plants.

MATERIALS AND METHODS

Source of Colonies. Laboratory colonies of S. invicta were reared from newly mated queens collected near Gainesville, Florida using standardized procedures (5).

Carbohydrate Phagostimulant Bioassay. The bioassay was similar to one already described (6), except Whatman Phase Separator (hydrophobic) filter paper squares (2×2 cm) were used to present the aqueous test carbohydrates to the ant.

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All carbohydrates tested (from Sigma Chemical Company, St. Louis, Missouri or Calbiochem, La Jolla, California) were prepared as one percent (W/V) aqueous solutions. Samples (100 µL) were applied to the center of the phase separator filter paper squares and then randomly placed around ten symmetrical locations on the tray floor of the laboratory colony.

The bioassay was evaluated by counting the number of ants feeding at the droplet every 10 minutes for a total of 60 minutes. The results for the six time periods were added and the total used to calculate the ranking. A water control and sucrose standard was included in each test. The test samples were ranked by setting the water response at zero and the sucrose response at 100. The ranking was calculated as follows:

\[
\text{Response (sample)} = \frac{\text{Response (H}_2\text{O)} \times 100}{\text{Response (H}_2\text{O)}}
\]

The mean and standard error for five replicates were calculated for each sample.

**Bioassay for Okra Sepal Fractionation.** The bioassay was similar to one already described (5), except the number of ants within the treated area was recorded every half hour for a total of three hours. The results for each fraction, control, and standard were totaled and the percent response calculated for each. The mean response and standard deviation were calculated for the five replicates. The standard was the precursor to the tested fractions and the solvent was the control.

**Okra Sepal Fractionation.** The sepal area of okra flowers was sliced several places with a razor blade while on the plant and the whole flower collected 10 minutes later. Samples were frozen until used. Just prior to use the flower petals were removed and the sepal and part of the stem homogenized in a Waring blender with 60% aqueous ethanol. The extraction was repeated five times. The residue was extracted three times with chloroform. The combined aqueous alcohol extract was vacuum evaporated until an aqueous solution remained. This solution was extracted three times with chloroform to remove lipids. The aqueous (A) and combined chloroform (B) extracts were bioassayed. The active aqueous extract (A) was further fractionated by passing an aliquot through a column of Dowex 50X-8 strongly acidic resin. Neutral and acidic components (D) were eluted with water, then amino acids and other compounds were eluted with 4N NH₄OH. An additional fraction (E) was obtained by eluting the column with methanol. All fractions were reduced to the volume of the active precursor (in this case aqueous fraction A). All fraction were tested in the bioassay. An aliquot of the active neutral-acidic fraction (D) was passed through a basic Sephadex-25 ion exchange resin, which on elution with water retained acidic compounds (F) and allowed neutral materials to pass through. One neutral fraction was collected (G), and found to be strongly positive for reducing carbohydrates.

**GC Analysis of Carbohydrates.** An aliquot of each sample to be analyzed was evaporated and an equal volume of Tri-Sil Z (trimethylsilylimidazole in dry pyridine, 1.5 mEq, Pierce Chemical Company, Rockford, Illinois) was added. Each sample was heated at 70 C for 45 minutes with a vortex treatment at 30 minutes.

Derivatized carbohydrates were analyzed by GC on a Varian 3700 equipped with a flame ionization detector (Varian Associates, Palo
Alto, California) and a 1.8 m×2-mm i.d. glass column packed with 3% OV-17 on 120/140 mesh Gas-Chrom (Applied Science Laboratories, State College Pennsylvania). The GC oven temperature was programmed from 100 to 250 C at 10 C/min. Qualitative and quantitative data were obtained with a Varian Vista 401 data processor. Sucrose, glucose, and fructose standards for GC analysis were purchased from Calbiochem and Sigma Chemical Company. Quantitative data were obtained using a trehalose internal standard, and peak identification was made by direct GC comparison with silylated carbohydrate standards.

RESULTS AND DISCUSSION

Carbohydrate Phagostimulant Tests. Of nine naturally occurring monosaccharides only glucose, fructose and L-fucose had significant phagostimulant activity (fig. 1). All others had activity either indistinguishable or below that of the water control. The glucose result corresponded to the sucrose standard. The opposite enantiomers (unnatural) of the phagostimulants, glucose and fucose were inactive, as were those of mannose and arabinose (fig. 2). These data emphasize the ant’s discriminatory capabilities and the lock and key concept of phagostimulant substrate/receptor site. Phagostimulant tests conducted with seven modified glucose compounds (fig. 3) further illustrated this concept. In all cases modifying the glucose molecule resulted in decreased activity. Only 2-deoxy-2-fluoro-glucose, 5-thio-glucose and glucose-1, 6, diphosphate had mean activity scores above that of the water control.

![RANKING Diagram](image)

*Figure 1* — Phagostimulant bioassay results for monosaccharides (Ranking based on sucrose = 100; and water = 0).
Figure 2 — Phagostimulant bioassay results for naturally occurring monosaccharides and their diasteriomers (Ranking based on sucrose = 100; and water = 0).

Figure 3 — Phagostimulant bioassay results for glucose and its derivatives (Ranking based on sucrose = 100; and water = 0).
Three of the seven disaccharides tested (including sucrose) had excellent phagostimulant activity (fig. 4). Maltose had activity equal to that of the sucrose standard, while turanose was only slightly below the standard. Only one trisaccharide, raffinose, was tested, and it had a good phagostimulant activity, although less than the active disaccharides.

![Disaccharide Ranking Diagram]

Figure 4 — Phagostimulant bioassay results for disaccharides and one trisaccharide (Ranking based on sucrose = 100; and water = 0).

Nine sugar alcohols were tested (fig. 5). Only myoinositol had greater activity than the water control. It ranked far below the phagostimulant activity of the sucrose standard (13 vs. 100).

These tests reveal that S. invicta can distinguish, by taste, a wide variety of carbohydrates and sugar alcohols. The carbohydrates which act as strong phagostimulants may play important roles in the ant’s choice of food, especially plant sources.

Okra Phagostimulant. Fire ants macerate only the sepal area of okra flowers, but no other plant parts (3). The adult ants ingest only liquids and have a sophisticated filtration mechanism (7). Consequently, they probably feed on fluids exuded from the damaged sepal. Since the ants have an equal opportunity to attack other plant parts, phagostimulants may be associated with the okra sepal. Extraction of okra sepal with 60% aqueous ethanol (A) and chloroform (B) gave polar and lipid fractions, respectively (fig. 6). Lipid fraction (B) was significantly better than the solvent control but much less active than the aqueous fraction. Consequently, fraction (A) was further separated into neutral/acidic...
components (D) and basic/amino acids components (C). Bioassay results clearly showed fraction (D) equivalent to the precursor fraction (A). Fraction (C) was inactive, indicating that amino acids were not phagostimulantes. Fractionation of (D) through basic sephadex 25 resin retained acidic compounds and gave neutral fraction (G). This fraction (G) had activity equivalent to parent fraction (D) and tested positive for reducing carbohydrates. Derivatization of an aliquot and analysis by GC revealed the presence of significant fructose, glucose and sucrose levels. These results coupled with our previous carbohydrate investigations led to a quantitative study of the sugar content of various okra plant parts. The okra sepal contains almost five times more carbohydrate than okra stalks, leaves or fresh seeds. All three sugars identified (fructose, glucose and sucrose) are strong fire ant phagostimulants. Based on the sepal water content the amount of carbohydrate is greater than 3 percent: three times greater than that used in laboratory phagostimulant studies. The other plant parts contain less than 1.0 percent carbohydrate, still adequate to stimulate feeding. Even more enigmatic is the fruit's high carbohydrate content sans seeds. The okra sepal contains almost five times more carbohydrate than okra stalks, leaves or fresh seeds. All three sugars identified (fructose, glucose and sucrose) are

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**Figure 5** — Phagostimulant bioassay results for several sugar alcohols (Ranking based on sucrose = 100; and water = 0).
strong fire ant phagostimulants. Based on the sepal water content the amount of carbohydrate is greater than 3 percent; three times greater than that used in laboratory phagostimulant, studies. The other plant parts contain less than 1.0 percent carbohydrate, still adequate to stimulate feeding. Even more enigmatic is the fruit's high carbohydrate content sans seeds. Although the results appear contradictory, they reflect another important factor, accessibility. The ants cannot get at the carbohydrates in the tough stalk, leaves or fruit, but the soft sepal is easily macerated. The sepal has high amounts of carbohydrates due tot nectaries. Many plant species have extrafloral nectaries, which attract ants and/or other insect (8). The ants benefit from the easily obtained carbohydrates, and the plant benefits by the negative influence ants have on potential herbivores (9). In spite of the damage done to the sepal, the fruit and seeds do develop; however, the deformed fruit no longer has commercial value.

Figure 6 — Flow diagram for the isolation of a fire ant phagostimulant from okra.

**Soybeans.** Fire ants significantly reduce the yield of soybeans, a major crop in the southern United States (10). The ants damaged germinating seeds; once these plants developed a few leaves the ants had no apparent effect on subsequent growth, although radio tracer studies revealed that the ants do feed on the growing plant (10). The net result was a significant decrease in the number of plants per row. Based on our previous work with carbohydrate phagostimulants and okra, we investigated the sugar content of soybeans sprouts and mature plant parts.
The results show that sprouts contain more phagostimulant carbohydrates than roots, stems, or leaves from mature plants. As discovered with okra, all plant parts do have significant amounts of carbohydrates; however, roots, stems and leaves are much more difficult to penetrate than soft tender sprouts. In addition, field preparation for soybean planting eliminates much of the previously existing food material, including insects and other plants. Consequently, the opportunistic fire ant takes advantage of soft tissue sprouts to maintain itself until insect fauna population levels increase.

We have demonstrated that several common plant carbohydrates stimulate fire ants to feed, and that these materials may play a role in host selection. Okra flowers have extrafloral nectaries around the sepal area, which induce feeding and recruitment by fire ant workers. The feeding action damages the sepal and results in deformed fruit which, although not detrimental to the plant ruins their commercial appearance. The ants attack soybean sprouts because of their soft tissue, high carbohydrate content, and scarcity of alternative food materials. Both examples illustrate the opportunistic nature of the fire ant. Undoubtedly we will discover further examples of negative interactions between man and fire ants.

BIBLIOGRAFIE


Indicele de clasificare: 63: 616—022.912.32

REZUMAT

Robert K. Vander Meer, Emanuel Merdinger — HIDRATII DE CARBON, FAGO-STIMULENȚI AI FURNICII DE FOC; STUDII DE LABORATOR ȘI PRACTICE

Furnica de foc era considerată în trecut o mare minătoare de petrol. Astăzi s-a stabilit că ea necesită o hrană diversificată, inclusiv hidrații de carbon. Printr-un screening cuprinzător asupra a numeroși hidrații de carbon s-a constatat că doar un număr limitat au un efect fagostimulant (adică determină furn...
nicile să se hrănească. Intre aceste substanțe se numără și monoazahride (glucoza, fructoza, l-fucoza) și dizahride (sucroza, maltza și furanoza). Prin modificarea glucozei foarte active s-a obținut dispariția efectelor fagostimulante: s-a conchis că activitatea respectivă este foarte sensibilă la structura moleculei. Astfel autorii au putut înțelege mai bine de ce furnica de foc atacă doar anumite tipuri de plante. Anumite părți ale florilor de bane sînt atacate de ele, ceea ce duce la deformarea fructelor. Analiza chimică a hidraților de carbon din diferite părți ale plantei a demonstrat că furnica de foc atacă acele părți ale plantei care conțin o cantitate de hidrați de carbon de cinci ori mai mare decît alte părți.

Р Е З Ю М Е

Роберт Н. Бадер Мерз. Евгений Меркьш — ГИДРАТЫ УГЛЕРОДА ФАГОСТИМУЛЯНТЫ ОГНЕВОГО МУРАВЬЯ, ЛАБОРАТОРНЫЕ И ПРАКТИЧЕСКИЕ ИССЛЕДОВАНИЯ

В прошлом считалось что огневые муравьи нормальны в лучах и сегодня узна-
ваете, что они требуют разнообразных нормация, включая углеводистые гидраты.
Определение окисления на большом числе углеводистых гидратов привело к ут-
верждению, что огневые муравьи имеют фагостимулирующее действие на угле-
водистые муравьи (приспособленные муравьи). Они содержат моносахариды глюкозу,
фруктозу и d-фруктозу, и дисахариды сукрозу, мальтозу и турбозу. Великое изме-
нение в структуре состоящих активных глюкозы привело к отсутствию фагостимуля-рую,
вания, так что деятельность очень чувствительное к форме молекул. Это разрешает
нам понимать почему огневые муравьи нападают на некоторые углеводы.
Вашествинии
цветов бактериолази огневыми муравьями, что приводит к деформации фрукта.
Химический анализ гидратов углерода и различных частей растений показал, что они
содержат пребажественно нь шар ковалентный гидратов углерода фагостимулирую-
щие.

R E F E R A T E

EFECTELE VASODILATATOARE
ALE UNUI NOU DERIVAT DE 1,4 DIHIDROPRIDINĂ, COMPARA-
TIV CU NIFEDIPINA. D. Fruecau,
F. Roy, N. L. Brown, Therapie, 1989,
44, 3, 203-208

Sinteză substanțelor derivate de la
1,4 dihidropiridină a condus la obținerea de medicamente deseori de valoare pentru terapia bolilor aparea-
tului cardiovascular.

Din grupa blocației transportului
ionilor de calciu nifedipina este mult
fotolită în afecțiuni cardiace, ca și
nisoldipina, iar nicardipina sau nimo-
dipina au o mare specificitate ca vasodilatoare cerebrale. Pentru alte sub-
stanțe cum sint: amloidipina, bendidi-
pina și lacidipina s-a stabilit un efect
de întrizire a întrării ionilor de cal-
ciu de lungă durată atât în teșăriile făcute în vivo cât și în vitro.

Autorii testează efectele farmacodi-
namice ale unui nou compus denumit
convențional LP 2025, compus care
blochează intrarea calciului în mu-
șchiul neted vascular, ca urmare a u-

EFECTUL PROTECTOR AL UNEI
FRACȚIUNI FLAVONICE PURIFI-
CATE ASUPRA TULBURĂRI-
LOR DATE DE APARIȚIA RADI-
CALILOR LIBERI DE OXIGEN,
M. Loechampt, B. Guardiola, N. Si-
cot, N. Bertrand, L. Pedrix, J. Du-
hault, Anzeite Mittel forsucning, 1989,
39, II, 8, 802-865

Apariția de radicați libere de oxigen activ este implicată în multe stări patologice cum sint inflamațiile, nترو-
sclerosisă, carcinogeneza, ischemie cere-

Elena Cristea

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brală sau cardiacă, ulcerării ale mu-
coaselor gastrice sau duodenale, opa-
cifieri ale cristalinului, îmbătrânirea
diferitelor organe.

Pleciind de la aceste date, numeroși
autoși se preocupă de găsirea de sub-
stanțe capabile să intervină în forma-
rea și neutralizarea acestor produse
endogene atât de dăunătoare organiz-
mului uman. Știindu-se că o serie de
derivații flavonici au proprietăți anti-
inflamatorii și pentru ei s-a mai pus
în evidență și o influență asupra radia-
calilor liberi, s-a realizat o asceneră de
diosmină 90% și hesperidină 10%
respectiv de două fracțiuni flavonoidice
aduse la un grad avansat de purificare.

Preparatul aflat sub formă de com-
primate (Dafon Franta) cercetat în
vitro și în vivo s-a dovedit activ asup-
tra radicalilor liberi de oxigen apă-
rați în timpul fagocitozei sau în cursul
dezvoltării diabetului experimental
prin injectarea de aloxan.

Efectul protector al asocierii de fla-
vonale explică diversele proprietăți far-
macologice ale preparatului respectiv,
cum și scăderea permeabilității ca-
pilarilor induși prin iradiere, injecta-
tare de antigeni nespecifice sau apli-
care de ciclodorm, dar și acțiunea an-
tinflamatoară și antiedematoasă.

Elena Cristea