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R. K. Vander Meer
USDA/ARS

C. S. Lofgren
USDA/ARS

J. A. Seawright
USDA/ARS

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SPECIFICITY OF THE RED IMPORTED FIRE ANT (HYMENOPTERA: FORMICIDAE) PHAGOSTIMULANT RESPONSE TO CARBOHYDRATES

R. K. VANDER MEER, C. S. LOFGREN AND J. A. SEAWRIGHT
USDA/ARS
Medical and Veterinary Entomology Research Laboratory
P.O. Box 14565
Gainesville, FL 32604 USA

ABSTRACT

The red imported fire ant, *Solenopsis invicta* Buren, is considered an oil-loving feeder, however, carbohydrates are essential ingredients in the diet of the fire ant also. Comprehensive screening of mono-, di-, and tri-saccharides demonstrated that glucose, fructose, fucose, sucrose, maltose, turanose and raffinose were significant phagostimulants for fire ant workers. It was also found that while D-glucose and L-fucose, the naturally occurring isomers, were active, the opposite diastereomers were not. Any structural modification of the glucose molecule resulted in loss of activity. None of the sugar alcohols evaluated were active. The fire ant is an agricultural pest and the many reports of fire ant damage to food crops may be linked to their need for dietary carbohydrates. A knowledge of carbohydrate phagostimulants may help to understand specificity of fire ant /plant interactions.

Key Words: Feeding, sugars, *Solenopsis invicta*, diet, agriculture

RESUMEN

La hormiga de fuego, *Solenopsis invicta*, era considerada como una de las que gusta de alimentarse de aceite. Sin embargo, a partir de estudios de laboratorio se hizo evidente que los carbohidratos eran ingredientes esenciales en la dieta de estas hormigas. Estudios de tamizaje de mono- di-, y tri-sacáridos demostraron que la glucosa, fructosa, fucosa, maltosa, turanosa y rafinosa fueron fagoestimulantes de las obreras de las hormigas de fuego. Se encontró además que mientras que la D-glucosa y la L-fucosa, los isómeros de aparecen en la naturaleza, eran activos, los diastereómeros opuestos no lo eran. Cualquier modificación estructural de la molécula tuvo como resultado pérdida de actividad. Ninguno de los alcoholes de los azúcares evaluados fué activo. La hormiga de fuego es una plaga agrícola y muchos reportes de daños por las hormigas a los cultivos deben relacionarse con su necesidad de carbohidratos. El conocimiento de los fagoestimulantes carbohidratados puede ayudar a entender la especificidad de las interacciones de las hormigas de fuego con las plantas.

The red imported fire ant, *Solenopsis invicta* Buren, was accidently imported into the United States in the 1930's from South America. Since then it has spread to infest over 150 million hectares in nine southern states and Puerto Rico (Lofgren 1986a). Its potential spread includes Arizona, California, Oregon and Washington; however, the deserts of Texas and stringent quarantine measures currently restrict its spread (Lofgren 1986b). Mature fire ant colonies contain up to 250,000 workers and 120 colonies may infest each hectare. The ant behaves like a "weed species", thriving in disturbed habitats (farmland, pastures, around homes, and playgrounds) where contact with man is frequent (Tschinkel 1987). It is the high population density and sympatry with man's activities that have made the fire ant one of the most important medical and agricultural pest ant species (Adams 1986).

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. As with honey bees, about one percent of the population may develop allergic reactions and each year about one-third of the people in infested areas are stung. Consequently, the number of hyperallergic cases is much higher than for bee stings (Adams & Lofgren 1981). Even without an allergic reaction, the stings are painful and curtail people's outdoor activities.

The fire ant is agriculturally important because it is an opportunistic omnivore that has an excellent food recruitment system (Vander Meer 1986). The workers attack a wide variety of crops including soybeans, potatoes, corn, citrus, and okra. They feed on germinating seeds in corn and soybean fields, thus decreasing the crop yield (Adams 1986). This kind of damage was noted soon after research began on the imported fire ants (Wilson & Eads 1949); however, the extensive use of persistent chlorinated hydrocarbon insecticides in the 1950's and 1960's subdued the agricultural effects of fire ants until these residual compounds dissipated in the 1980's to the present (Adams 1986).

Fire ants are considered a "grease or oil loving" ant and toxic baits were developed for fire ant control based on a vegetable oil phagostimulant/active ingredient solvent (Banks et al. 1985). However, feeding studies have also indicated the importance of carbohydrates in the fire ant diet (Lofgren et al. 1961; Ricks & Vinson 1970), and it has been shown that honey-water added to the standard laboratory diet enhanced both colony weights and queen survival (Williams et al. 1980). In addition, several food preference studies have also indicated the importance of dietary carbohydrates (Vinson 1968; Howard & Tschinkel 1981; Sorensen & Vinson 1981). In many other insects, the larval stages do not require dietary carbohydrates, instead relying on amino acid and fatty acid oxidation for their energy needs. In contrast, adults usually consume large amounts of carbohydrates (Chippendale 1978).

We report here results of carbohydrate phagostimulant tests that define the scope of effective sugar feeding stimulants and the specificity of the taste receptors of the ant for the structural integrity of naturally occurring carbohydrates.

MATERIALS AND METHODS

Source of Colonies

Laboratory colonies of *S.invicta* were reared from newly mated queens collected near Gainesville, Florida using standardized procedures (Banks et al. 1981). Each colony attained an estimated population of at least 50,000 workers prior to use in phagostimulation bioassays.

Carbohydrate Phagostimulant Bioassay

The bioassay was similar to one already described for assessing fire ant recruitment/aggregation (Vander Meer et al. 1988). Colonies with two or more nest cells (10,000 to 20,000 workers each) were used for the bioassays. A colony nest cell and foraging workers were transferred to the center of a clean bioassay tray. Worker ants were allowed to acclimate at least one h before testing. Each of the five replicates consisted of a different colony. No attempt was made to manipulate the ratio of larvae to workers nor to assess the condition of the queen, except to periodically determine if she was still producing eggs and worker brood. The bioassay trays had numbered positions from 1 to 10 marked equidistant from each other and in a 15 cm radius from the tray center. The tray sides were painted with Fluon^{R} to prevent ant escape. Whatman Phase Separator filter paper squares (2x2 cm), were placed on slightly larger aluminum foil squares that protected the tray from sample contamination. A water control and 1% (W/V) sucrose standard were included in each test, thus a total of 8 treatments could be evaluated for each replicate. All samples, standard, control and treatments were applied (100uL) to the center of the phase separator filter paper squares and then randomly placed around the ten symmetrical locations on the tray floor. Bioassays were carried out at about 30°C with a variable light/dark cycle.

All sugars, sugar alcohols, sugar derivatives and artificial sweeteners (Sigma Chemical Company, St. Louis, Missouri or Calbiochem, La Jolla, California) tested were prepared as one percent (W/V) aqueous solutions. The chemical names for all compounds tested appear in the Figures.

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. The test samples were ranked by setting the water response at zero and the sucrose response at 100. This nullified much of the natural colony to colony variation and allowed comparison of results from one test to another. The ranking was calculated as follows:

$$\frac{(\text{No. ants, sample}) - (\text{No. ants, } H_2O \times 100)}{(\text{No. ants sucrose}) - (\text{No. ants } H_2O)}$$

The mean and standard error for five replicates was calculated for each treatment. After each bioassay the colony cells were returned to their rearing trays. The same sets of colonies were used multiple times for the bioassays.

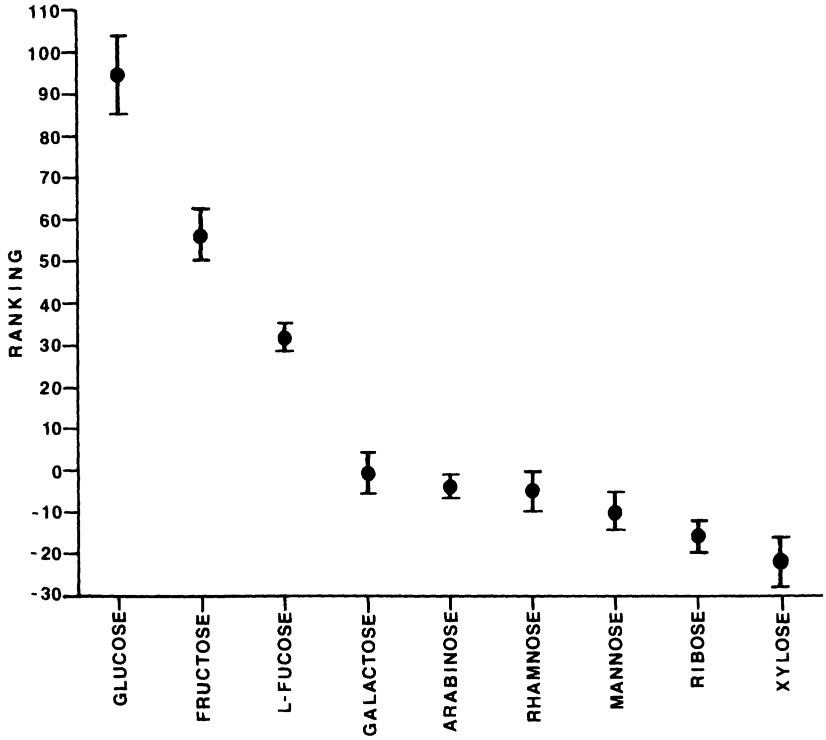


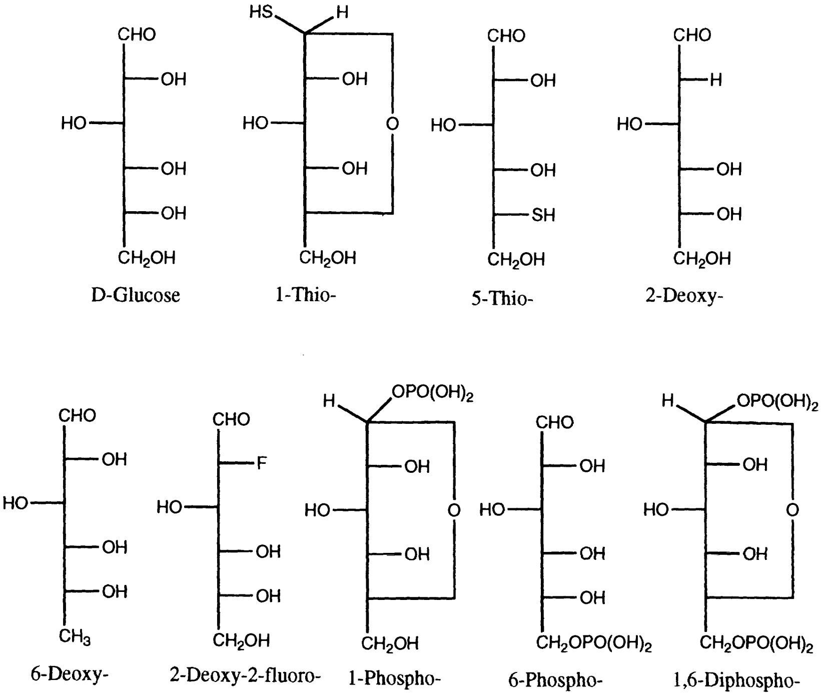
Fig. 1. Phagostimulant bioassay results for monosaccharides (Ranking based on sucrose = 100; and water = 0). The mean and standard error of five replicates are presented.

RESULTS

Carbohydrate Phagostimulant Tests

Nine naturally occurring monosaccharides were tested for phagostimulant activity. Of these, only D-glucose, D-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 was indistinguishable from that of the sucrose standard (Ranking = 100%). All three active monosaccharide phagostimulants were significantly different from each other.

D-Glucose was clearly the most effective of the monosaccharides tested, we then conducted phagostimulant tests with eight chemically modified glucose compounds to determine what affect structural changes would have on fire ant phagostimulation. The structures are shown in Fig. 2 and the bioassay results are shown in Fig. 3. Substituting sulfur for oxygen, 1-thio- and 5-thio- resulted in no significant phagostimulation activity. Similarly, removal of a hydroxyl group (2- and 6-deoxy-) reduced the activity to insignificant levels. Mono or di-phosphorylation gave no phagostimulation 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.



D-Glucose 1-Thio- 5-Thio- 2-Deoxy-

6-Deoxy- 2-Deoxy-2-fluoro- 1-Phospho- 6-Phospho- 1,6-Diphospho-

Fig. 2. Chemical structures of glucose and the glucose derivatives tested for phagostimulant activity.

The opposite enantiomers of several of the naturally occurring monosaccharides shown in Fig.1 were tested in the phagostimulation bioassay. The two enantiomers of each compound have identical physical and chemical properties. They differ only in that they rotate plane polarized light in opposite directions. The results are shown in Fig. 4. Naturally occurring D-glucose and L-fucose were active phagostimulants; however, the opposite enantiomers were inactive. Both unnatural enantiomers of the monosaccharides, mannose and arabinose were inactive in the phagostimulation bioassay as were their natural counterparts.

Results for seven disaccharides and one trisaccharide tested for phagostimulation activity are shown in Fig. 5. The three disaccharides, sucrose, maltose, and turanose had excellent phagostimulant activity. Maltose had activity equal to that of the sucrose standard, while turanose was only slightly below the standard. The one trisaccharide, raffinose, tested had good phagostimulant activity, although less then the active disaccharides.

Nine sugar alcohols were tested (Fig. 6). Only myo-inositol had greater activity than the water control, but it still ranked far below the phagostimulant activity of the sucrose standard (13 vs. 100). The two enantiomers of arabinitol were tested, but each showed equally poor phagostimulation results.

DISCUSSION

Relatively soon after their accidental importation into the United States, fire ants were reported to feed on the seeds of corn, peanuts, and beans, as well as crop seed-

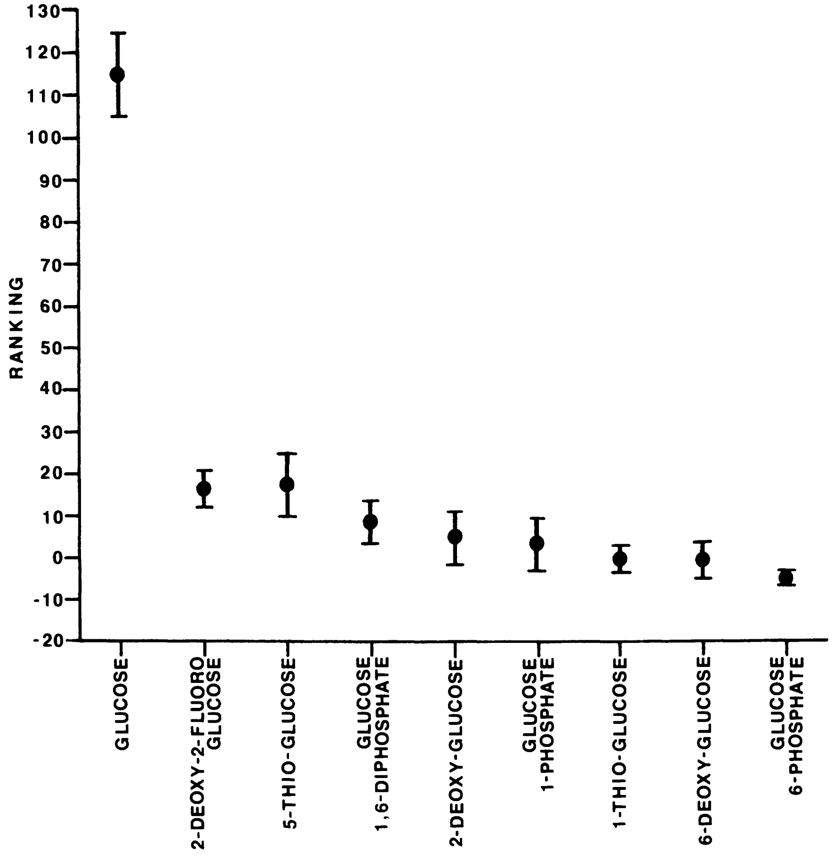


Fig. 3. Phagostimulant bioassay results for glucose and its derivatives (Ranking based on sucrose = 100; and water = 0). The mean and standard error of five replicates are presented.

lings (Wilson & Eads 1949). More recent studies have described significant loss of soybean yields due to fire ant infestations (Adams et al. 1983). In addition, studies using the radioisotope phosphorous-32 (^{32}P) demonstrated that the fire ant workers feed on corn, okra and soybeans (Smittle et al. 1983). The nature of the ^{32}P feeding experiment dictated that the radiolabel was obtained by the workers via ingestion of aqueous solutions. The authors observed that although fire ants were not observed feeding on okra seedlings or soybeans, the ants in the immediate vicinity had high levels of radioactivity. They concluded that the ants were feeding extensively on the plant roots. Similar experiments demonstrated that the ants feed on citrus (Smittle et al. 1988). Tennant & Porter (1991) examined the crop contents of returning foraging workers and concluded that carbohydrates represented a large proportion of what was being brought back to the colony. These authors suggest that the fire ant must be feeding on plant roots and/or the exudate from root associated coccids. All of the above emphasize that the fire ant is much more than an oil loving ant and that phagostimulant effects of water soluble substances may play a dominant role in directing their interactions

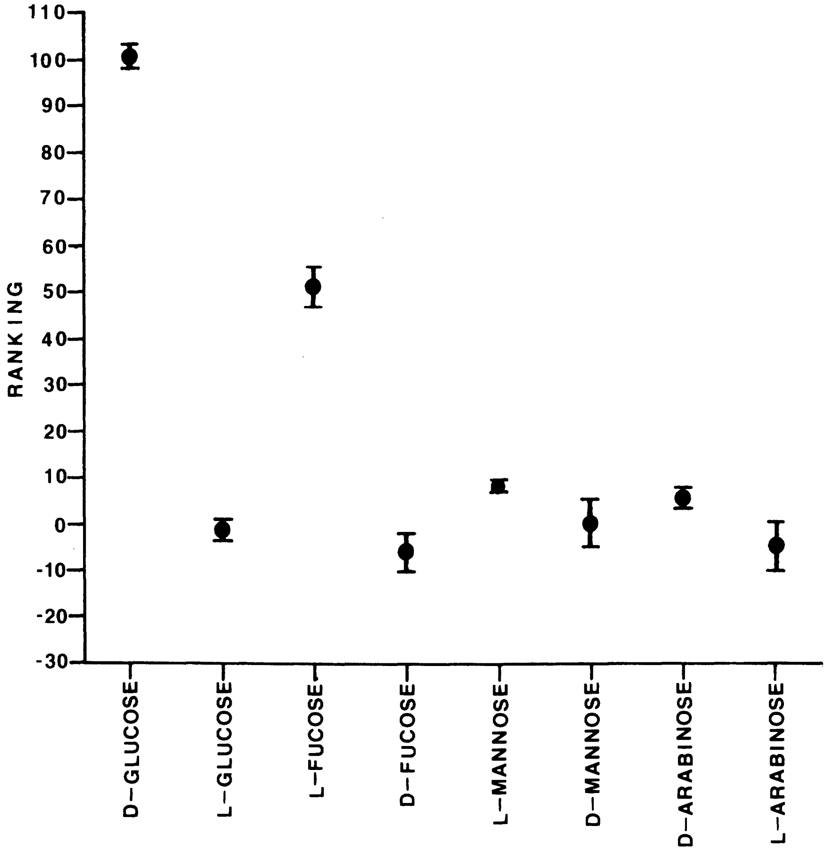


Fig. 4. Phagostimulant bioassay results for naturally occurring monosaccharides and their diasteriomers (Ranking based on sucrose = 100; and water = 0). The mean and standard error of five replicates are presented.

with plants. No comparison of phagostimulant activity was made between an aqueous sucrose solution (carbohydrate) and soybean oil (an oil), however, it is clear that both elicit strong recruitment (R. K. V. M., personal observation). When the interaction is with crop plants, then the fire ant becomes an agricultural pest. An understanding of phagostimulation and its specificity can provide insight into feeding preferences and may help us to develop better bait formulations.

A previous report investigated the phagostimulant effects of aqueous extracts of arthropods, amino acids, vitamins, and sugars (Ricks & Vinson 1970). The studies were carried out with the dark and light varieties of imported fire ant, *Solenopsis saevissima richteri*. These two forms probably corresponded to what is currently known as *S. richteri* and *S. invicta*, respectively (Buren 1972). In spite of different experimental conditions and evaluation procedures, our results for the same sugars were mostly congruent. The two exceptions (out of 11) were that we did not find phagostimulant activity for trehalose, and we found that fructose was an active phagostimulant. However, fructose was active for the dark phase of imported fire ant (Ricks & Vinson

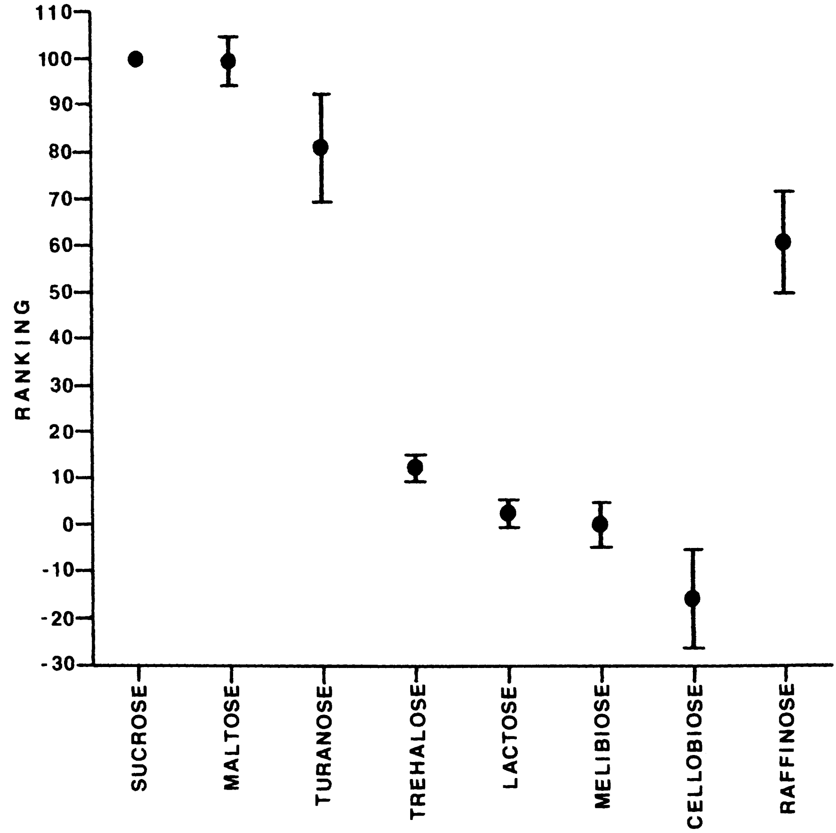


Fig. 5. Phagostimulant bioassay results for disaccharides and one trisaccharide (Ranking based on sucrose = 100; and water = 0). The mean and standard error of five replicates are presented.

1970). Of the additional sugars and sugar alcohols evaluated in our bioassay only turanose and L-fucose were found to be phagostimulants.

The fire ant has an excellent recruitment system (Vander Meer 1986), thus worker numbers can accumulate at a food source by either additive independent discoveries or by recruitment of workers. Initial discovery could be the result of random foraging or of attraction to volatiles emitted by the food source. In our bioassay the carbohydrates are non-volatile; however, the workers could be attracted to water vapor. Initial contact with treatments and water control are expected to be identical. After discovery, the quality of the food is evaluated, most likely through chemo-and mechanoreceptors at the tips of the labial and maxillary palps. Feeding is initiated if the sensory input is favorably interpreted by the central nervous system (CNS). Water was the common carrier of the phagostimulants in our bioassay, therefore, the response of workers coming in contact with the treatments was dictated by chemoreceptors and the translation of the interpretation of the CNS into feeding behavior. We do not know if the test compounds were all detected by the ant's chemoreceptors, but interpreted

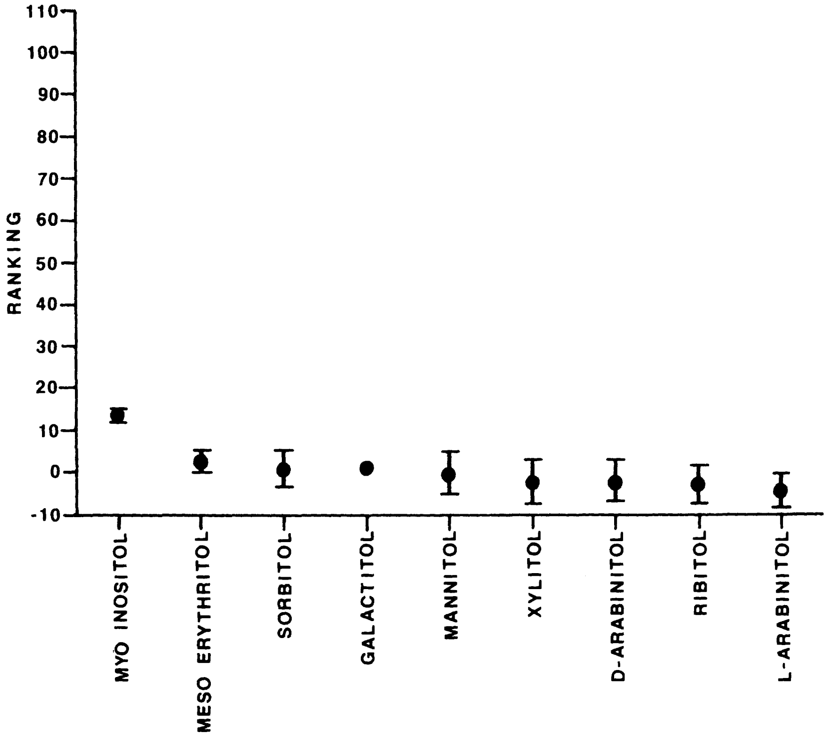


Fig. 6. Phagostimulant bioassay results for several sugar alcohols (Ranking based on sucrose = 100; and water = 0). The mean and standard error of five replicates are presented.

differently by the CNS, or if the compounds were differentially detected (see Bernays and Simpson 1982 for general review).

The sensitivity of the fire ant worker to changes in phagostimulant structure is clear from our results. L-fucose (6-deoxy-L-galactose) and L-rhamnose (6-deoxy-L-mannose) are naturally occurring deoxyaldoses. L-fucose was one of three monosaccharides that showed phagostimulant activity. Rhamnose was inactive. Neither monosaccharide from which these were derived, galactose and mannose, showed phagostimulant activity. In sharp contrast to the excellent phagostimulant activity of glucose, 2-deoxy- and 6-deoxy-D-glucose were also inactive. It is evident from Fig. 3 that any chemical modification to the glucose structure eliminates phagostimulant activity. The structural changes reflected in these compounds are obvious and lead to differences in physical properties, e.g. spectra (IR, NMR), melting point, and solubility. It is logical that the variation of molecular fit of these compounds with taste receptors and/or the CNS interpretation is reflected in the phagostimulation scores. This is most dramatically illustrated by the lack of phagostimulant response of worker ants to the unnatural diasteriomers of glucose and fucose. In this case the structural changes do not result in differences in physical properties. However, all chiral centers have the opposite configuration.

These data reveal that *S. invicta* can distinguish, by taste, a wide variety of carbohydrates. These carbohydrate phagostimulants may play important roles in the fire ant's choice of food, especially from plant sources. Glucose, fructose, and sucrose are known components of plant nectars (Lanza 1991, Lanza et al. 1993) and have been shown to act as phagostimulants in nectars and other plant fluids fed on by ants. Investigation into the stage of plant development susceptible to fire ant damage and the carbohydrate content of that stage may reveal a rationale for the selective agricultural impact of the fire ant.

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