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Zoospore Chemotaxis in the Rumen Phycomycete *Neocallimastix frontalis*

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Neocallimastix frontalis zoospores showed chemotaxis to a range of carbohydrates, but not to the common amino acids, purines, pyrimidines or vitamins. Four chemoreceptors were identified: the glucose receptor, sensitive to D-glucose, D-galactose, D-xylose, L-sorbose, D-fucose and 2-deoxy-D-glucose; the sucrose receptor, sensitive to sucrose, D-fructose and raffinose; the mannose receptor, sensitive to D-mannose and D-glucose; and the sorbitol receptor, sensitive to D-sorbitol and D-mannitol. Growth of the vegetative stage of *N. frontalis* did not occur with D-xylose, L-sorbose, D-fucose, 2-deoxy-D-glucose, raffinose, D-mannose or D-sorbitol. The zoospores were attracted to carbohydrate mixtures representing the soluble carbohydrates found in different barley tissues; the highest response was with those mixtures representing carbohydrates of awn and inflorescence tissue. Chemotaxis also occurred preferentially to the awn and inflorescence tissue carbohydrate combinations rather than to carbohydrate combinations representing other tissues. Germination of the zoospores occurred in medium containing glucose in excess of 10^{-4} M.

INTRODUCTION

The flagellated zoospores of the rumen phycomycete fungus *Neocallimastix frontalis* are released from sporangia borne on the vegetative stage of the organism soon after the host animal eats (Orpin, 1975). The zoospores may invade, germinate upon and grow at the expense of plant tissue present in the rumen (Orpin, 1977). Not all plant tissues are invaded by the zoospores to the same extent; a marked preference is shown for inflorescence tissue where the zoospores germinate principally at the stomata and on damaged areas. Growth of the single rhizoid results in penetration of the plant tissue through the stoma or damaged epidermis. Because of the marked substratum selection shown by the zoospores and their germination at the stomata, chemotaxis was thought likely to be operative. Some phycomycete zoospores show positive chemotaxis to L-leucine and L-lysine [*Allomyces macrocygnus* and *A. arbuscula* (Machlis, 1969)] and to carbohydrates [*Pythium aphanidermatum* (Royle & Hickman, 1964)].

The chemotactic response of *N. frontalis* zoospores with a range of carbohydrates, amino acids, purines, pyrimidines and vitamins has now been examined. On the basis of these results, a mechanism is suggested for the preferential attraction of the zoospores to inflorescence tissues of grasses in the rumen.

METHODS

Animals. Sheep, Clun Forest wethers, each fitted with a permanent rumen cannula, were fed 1 kg hay chaff and 100 g rolled oats daily.

Preparation of rumen fluid and zoospore suspensions in fresh rumen fluid. Centrifuged rumen fluid and sterile reduced rumen fluid supernatant were prepared according to Orpin (1977). Rumen fluid, used as a

source of neocallimastix zoospores, was aspirated 17 to 23 h after the sheep was fed, filtered through a layer of muslin and 40 ml filtrate was centrifuged for 10 min at room temperature at 1500 *g*. The pellet was resuspended in 15 ml supernatant fluid and incubated at 39 °C for 0·5 h with 'inducer' (Orpin, 1975) extracted from 5·0 g dry wt oats. This resulted in the release of *N. frontalis* zoospores from the sporangia. The suspension was used for some chemotaxis experiments.

Culture in vitro. Stock cultures of *N. frontalis* were grown using the methods of Orpin (1975). The organism was also grown in liquid medium consisting of (g l^{-1}, except where stated): yeast extract, 10·0; tryptone, 20·0; L-cysteine hydrochloride, 1·0; glucose, 1·0; α-linolenic acid, 0·05; streptomycin, 0·1; sodium benzyl penicillin, 10^5 i.u. %; and centrifuged rumen fluid, 10 % (v/v). The medium constituents, except the antibiotics, were autoclaved in 99 % of the final volume, allowed to cool, and the antibiotics (in sterile distilled water, 1 % of final medium volume) were added aseptically. The medium was dispensed in 8 ml quantities into 125 × 13 mm rimless test-tubes inoculated with organisms from a thriving stock culture (0·5 ml) and incubated at 39 °C under CO_2; the cultures were passaged every 2 days. Suspensions of zoospores were prepared by filtering the cultures (100 ml) through a 10 mm pad of cotton-wool placed in the stem of a 50 mm diam. glass funnel, and centrifuging the filtrate containing the zoospores (but no vegetative growth) at 1000 *g* for 5 min at room temperature. The pellet was washed by centrifuging in reduced centrifuged rumen fluid (10 ml) and finally suspended in reduced centrifuged rumen fluid to a zoospore density of approximately 10^4 ml^{-1}. These suspensions were used throughout except when chemotaxis was determined in fresh filtered rumen fluid.

Chemotaxis experiments. Chemotaxis experiments using suspensions of zoospores grown *in vitro* were performed in chambers similar to that of Palleroni (1976), using 0·9 ml of spore suspension in the chambers. The compound being assayed was dissolved in CO_2-saturated sterile reduced centrifuged rumen fluid, and the solution was taken up in 50 μl Drummond microcap capillaries (Drummond Scientific Co., U.S.A.) which were then placed in the chemotaxis chambers. After 30 min incubation at 39 °C each capillary was removed, washed externally (Palleroni, 1976) with sterile reduced rumen fluid, and the contents were expressed on to a microscope slide. The number of neocallimastix zoospores was determined microscopically.

Experiments with zoospore suspensions in fresh rumen fluid were performed in chambers similar to that designed by Adler (1973) using 20 μl Drummond microcap capillaries. One end of the capillary was sealed with latex, the other was inserted into the rumen fluid preparation (0·2 ml). Incubation and counting were performed as described for the Palleroni (1976) chamber.

For experiments with organisms cultured *in vitro*, the same reduced centrifuged rumen fluid was used both for suspending the zoospores and for the attractant solution. For experiments with fresh rumen fluid, attractants were dissolved in reduced centrifuged rumen fluid taken from the same sheep 24 h previously.

The pH of reduced centrifuged rumen fluid used in the pH experiments was adjusted as required with 10 % (w/v) $NaHCO_3 . 10H_2O$ or acetic acid with CO_2 flowing through the medium (acetate did not affect chemotaxis).

Optical configuration of carbohydrates and amino acids. Unless otherwise specified, carbohydrates (where appropriate) were of the D-configuration, and amino acids the L-configuration.

Determination of specificity and number of chemoreceptors. One attractant at its optimum concentration was put in the capillary tube, and the other, at 0·01 M, was put in both the capillary tube and the suspension of zoospores. The reciprocal experiments were also performed. In each case the attractant was dissolved in reduced centrifuged rumen fluid, and equilibrated with CO_2.

Determinations. Sucrose was determined enzymically using the method of Bergmeyer & Klotzsch (1963), fructose by the method of Klotzsch & Bergmeyer (1963) and glucose by that of Dahlqvist (1961).

Plants. Barley (*Hordeum distichon*) plants were kindly supplied by Dr A. A. Jenkins, Agricultural Research Council, Plant Breeding Institute, Trumpington, Cambridge. Seven days after anthesis the plants were harvested and dissected into roots, stem, leaves (including ligules), awns and the remaining inflorescence tissue. Yellowing leaves were discarded. Soil was removed from the roots by washing them gently with 0·9 % (w/v) NaCl. The separate tissues were homogenized in distilled water [100 ml (g dry wt tissue)$^{-1}$] using an Ultra-turrax homogenizer, filtered, and the filtrates were heated at 100 °C for 5 min to destroy enzymic activity. The filtrates were centrifuged at 38000 *g* for 1 h, and the supernatants were assayed for glucose, sucrose and fructose.

Removal of soluble materials from barley awns. No method could be devised that would specifically remove soluble carbohydrates from plant tissues without also removing other soluble compounds. Two compromise methods were used, each starting with 0·1 g dry wt awns: (i) the awns were submerged in distilled water (1 l), stirred for 48 h at 4 °C, removed and washed with distilled water; (ii) the awns were incubated in a nylon bag in the rumen of a sheep devoid of phycomycete fungi (Orpin, 1977) for 24 h, removed and washed with distilled water. Awns prepared by these methods were then incubated in nylon bags in a rumen containing *N. frontalis* as the sole phycomycete (Orpin, 1977); the bags were removed after 19 h and the invasion of the

tissue was assessed using the methods of Orpin (1977). Sucrose, glucose and fructose present in the tissues after treatment to remove soluble compounds were determined as described above.

Germination experiments. Zoospore suspensions (10^4 spores ml^{-1}) in chemotaxis medium containing glucose at between 10^{-7} and 1 M were incubated under CO_2 at 39 °C. Samples were taken every 5 min for the first 30 min, at 15 min intervals until 2·5 h, and then hourly up to 4·5 h. The proportion of zoospores which had germinated in each sample was determined by microscopy, and plotted against time. The graph was extrapolated to determine the time for 100 % germination if this had not been reached during the experiment.

RESULTS

If *N. frontalis* were attracted to plant tissue by materials diffusing into the rumen fluid, no invasion of tissues devoid of soluble materials should occur. The degree of invasion of barley awns, treated to remove all soluble materials, was assessed. Untreated awns, after incubation in the rumen for 19 h followed by exposure to the inducer of zoosporogenesis, released $1{\cdot}1 \times 10^7$ zoospores (g dry wt tissue)$^{-1}$; awns treated by prior incubation in a rumen devoid of phycomycetes released $3{\cdot}1 \times 10^4$ zoospores (g dry wt tissue)$^{-1}$ and those leached with distilled water released $14{\cdot}4 \times 10^4$ zoospores (g dry wt tissue)$^{-1}$. This suggested that soluble diffusible materials in the plant tissue were acting as chemotactic agents. The capacity of carbohydrates, amino acids, purines, pyrimidines and vitamins (all likely to be present in plant tissues, though many only at low concentrations) to elicit chemotaxis by the zoospores was then examined.

As preliminary experiments showed that *N. frontalis* zoospores were attracted to glucose; glucose at 10^{-3} M was used to determine the optimum conditions (temperature, pH, cell density, reproducibility) for chemotaxis, using cells grown *in vitro*.

Rate of accumulation of zoospores in capillaries. The number of zoospores which entered capillaries not containing attractant was small in comparison with the number present and with the number entering the capillary containing 10^{-3} M-glucose. This background accumulation never exceeded 0·3 % of the total zoospores and was presumably due to random swimming of the zoospores. The rate of accumulation of cells into capillaries containing 10^{-3} M-glucose was initially slow but increased after 5 min to reach a constant maximum rate between 5 and 30 min (Fig. 1). The rate then decreased markedly. The time at which this point was reached varied with the glucose concentration but it was always within 1 h.

At low zoospore densities, the rate of accumulation in capillaries was proportional to density; at densities in excess of 5×10^3 ml^{-1}, increasing the zoospore density had no significant effect on the number of spores entering the capillary (Fig. 2). All subsequent experiments were performed using a zoospore density of 10^4 ml^{-1} to minimize the effect of density on the rate of accumulation in the capillary.

Reproducibility. When 20 capillary tubes, containing 10^{-3} M-glucose, were incubated with zoospore suspensions for 1 h at 39 °C, $6{\cdot}2 \pm 0{\cdot}8 \times 10^3$ spores accumulated.

Effect of temperature and pH. Chemotaxis to 10^{-3} M-glucose was markedly affected by temperature, the optimum being 40 ± 2 °C. Outside these limits chemotaxis decreased rapidly; no chemotaxis was recorded at 50 or 36 °C (Fig. 3). Chemotaxis to 10^{-3} M-glucose was independent of pH in the range 5·5 to 7·0 but outside this range taxis decreased (Fig. 3). All subsequent experiments were performed at pH 6·5 to 7·0 at 39 °C.

Effect of soluble protein. Plant fraction 1 protein (ribulose-1,5-bisphosphate carboxylyase) is the major protein released from plant tissue by mastication of food by the host animal (Reid, Lyttleton & Mangan, 1962) and the concentration of the soluble protein in the bolus may attain 100 μg ml^{-1}. Because of this, and because *N. frontalis* zoospores are most abundant in the rumen in the first hour after the host animal has commenced feeding (Orpin, 1975), i.e. when soluble protein will be at its highest concentration, the effect of fraction 1 protein (100 μg ml^{-1}) on chemotaxis was investigated. No taxis to fraction 1 protein was recorded and it neither stimulated nor inhibited taxis to 10^{-3} M-glucose.

Chemotaxis to different carbohydrates. A range of carbohydrates at 10^{-3} M or 0·01 %

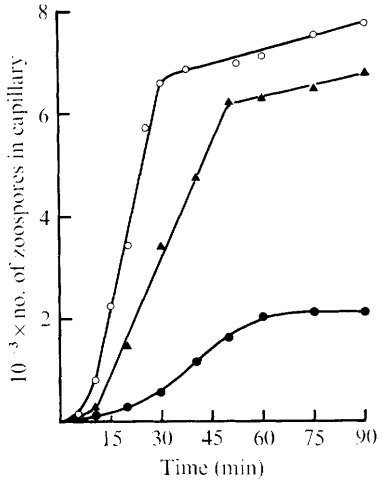

Fig. 1

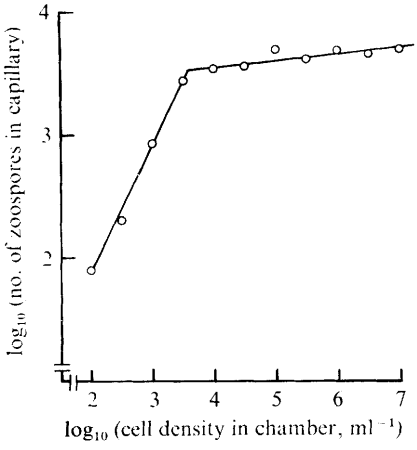

Fig. 2

Fig. 1. Accumulation of *N. frontalis* zoospores in capillaries containing different concentrations of glucose: ○, 10^{-3} M; ▲, 10^{-4} M; ●, 10^{-5} M. The chemotaxis chambers each contained 0·9 ml of zoospore suspension (10^4 spores ml^{-1}) and were incubated at 39 °C. At intervals, the zoospores which had accumulated in the capillaries were counted. Results are corrected for random entry by the zoospores. Average of three experiments.

Fig. 2. Effect of zoospore density on accumulation in capillaries. The chemotaxis chambers each contained 0·9 ml of zoospore suspension containing between 10^2 and 10^7 spores ml^{-1}. The chambers were incubated at 39 °C for 30 min and the zoospores which had accumulated in capillaries containing 10^{-3} M-glucose were counted. Results are corrected for random entry by the zoospores. Average of three experiments.

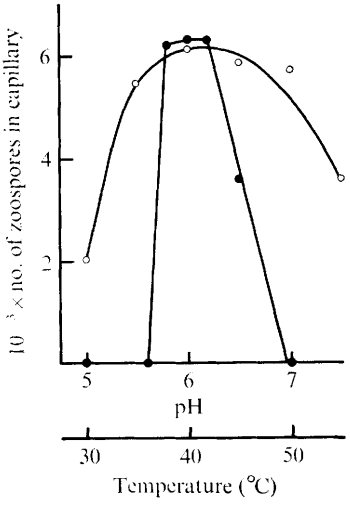

Fig. 3

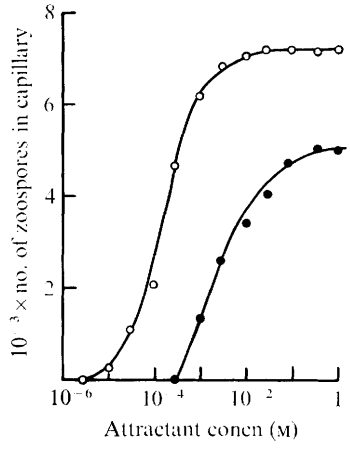

Fig. 4

Fig. 3. The effects of temperature (●) at pH 6·5 and pH (○) at 39 °C on chemotaxis by *N. frontalis* zoospores. The chambers each contained 0·9 ml of zoospore suspension (10^4 spores ml^{-1}) containing 10^{-3} M-glucose as attractant. In each experiment incubation was for 30 min. Results are corrected for random entry by zoospores. Average of two experiments.

Fig. 4. Concentration–response curves for chemotaxis by *N. frontalis* zoospores to glucose (○) and mannose (●). The chambers each contained 0·9 ml of zoospore suspension (10^4 spores ml^{-1}) and were incubated at 39 °C for 30 min. Results are corrected for random entry by zoospores. Average of two experiments.

Table 1. *Threshold and optimum concentrations for taxis to different carbohydrates and use of these carbohydrates for growth by N. frontalis*

The threshold concentration is the minimum concentration tested which elicited chemotaxis. The optimum concentration is that which attracted the most zoospores per unit time. Columns (1) refer to washed suspensions of *N. frontalis* zoospores cultured *in vitro*; columns (2) refer to suspensions of zoospores in fresh filtered rumen fluid. Growth is recorded as: −, no growth; (+), poor growth; +, good growth.

	Threshold concn (M)		Optimum concn (M)		
Compound	(1)	(2)	(1)	(2)	Growth
Xylose	10^{-5}	5×10^{-4}	$>5\times10^{-1}$	10^{-1}	−
Fructose	5×10^{-6}	10^{-5}	10^{-1}	10^{-1}	+
Glucose	5×10^{-6}	10^{-5}	$>10^{-2}$	5×10^{-2}	+
Galactose	10^{-4}	5×10^{-3}	5×10^{-2}	5×10^{-1}	+
Mannose	5×10^{-4}	5×10^{-4}	$>5\times10^{-1}$	10^{-1}	−
Sorbose	5×10^{-4}	10^{-3}	10^{-3}	5×10^{-2}	−
Sucrose	10^{-7}	5×10^{-4}	5×10^{-2}	10^{-1}	+
Raffinose	5×10^{-3}	10^{-2}	10^{-2}	5×10^{-1}	−
Sorbitol	5×10^{-4}	5×10^{-3}	5×10^{-1}	10^{-1}	−
Mannitol	5×10^{-3}	10^{-3}	10^{-1}	10^{-1}	(+)
Fucose	10^{-4}	ND	10^{-2}	ND	−
2-Deoxy-D-glucose	10^{-5}	ND	5×10^{-2}	10^{-1}	−

ND, Not determined.

(polysaccharides) were tested for their ability to elicit chemotaxis. Zoospores, produced from vegetative growth grown on glucose as the major energy and carbon source, moved towards glucose, galactose, xylose, fructose, mannose, L-sorbose, sucrose, raffinose, mannitol, sorbitol, fucose and 2-deoxy-D-glucose (Table 1). A concentration–response curve was plotted for each attractant (see Fig. 4 for response to glucose and mannose) and the optimum concentration for chemotaxis and the threshold concentration (the lowest concentration of attractant at which a response was first detected) were derived (Table 1). The following compounds at 10^{-3} M did not elicit chemotaxis: erythrose, L-arabinose, ribose, L-rhamnose, cellobiose, maltose, trehalose, melibiose, melezitose, aesculin, salicin, adonitol and dulcitol; nor did the following at 0·01 % (w/v): rice starch, soluble starch, cellulose (Sigmacel MN30), glycogen, dextrin and pectin.

Experiments with fresh rumen fluid. Since conditions in the rumen are different from those we had used *in vitro*, we repeated the experiments using *N. frontalis* zoospores suspended in fresh filtered rumen fluid after induction of zoosporogenesis *in vitro*. We found that zoospores responded to the same range of carbohydrates but the threshold values and optimum concentrations of the attractants were both higher (Table 1). To ascertain if the zoospores responded to the products of fermentation of carbohydrates produced by other rumen micro-organisms, we tested acetate, propionate, *n*-butyrate, 2-methylpropionate, lactate and succinate separately at 10^{-3} M; none attracted the zoospores.

Amino acids, purines, pyrimidines and vitamins. Since *N. frontalis* zoospores were attracted by soluble compound(s) diffusing from the plant tissue, taxis to the common amino acids, purines, pyrimidines and vitamins, which may occur free in plant tissues at low concentrations, was investigated. No taxis to any of these compounds was detected. The amino acids tested (at 10^{-4} M) were: glycine, alanine, serine, threonine, valine, leucine, isoleucine, phenylalanine, tyrosine, methionine, tryptophan, proline, histidine, aspartate, glutamate, arginine, lysine and asparagine. The nucleotide bases tested (at 10^{-4} M) were: adenine, guanine, cytosine, and thymine. The vitamins tested (at 10^{-5} M) were: α-tocopherol, ascorbate, thiamin, riboflavin, nicotinamide, pyridoxal phosphate, pantothenate, biotin, folic acid, inositol and cyanocobalamin.

Chemotaxis to carbohydrates in the presence of a second attractant. To ascertain how many chemoreceptor sites were present on zoospores and to determine their specificity, an attractant, at its optimum concentration, was put in the capillary and a second attractant, at 0·01 M, was put in both the zoospore suspension and the capillary to saturate the receptor sites for that compound. If the two attractants use the same receptor no taxis should occur (Adler, 1969). If not, taxis should not be inhibited. The results (Table 2) suggest that there are separate receptors for glucose, sucrose, mannose and sorbitol. The glucose receptor was sensitive to glucose, galactose, xylose, L-sorbose, fucose and 2-deoxy-D-glucose; the sucrose receptor to sucrose, fructose and raffinose; the mannose receptor to mannose and glucose; and the sorbitol receptor to sorbitol and mannitol.

Metabolism of carbohydrates. Neocallimastix frontalis failed to grow in liquid medium on any of the carbohydrates (at 1 g l^{-1}) which did not elicit chemotaxis (i.e. those not shown in Table 1). Growth did occur on many, but not all, of the carbohydrates eliciting chemotaxis (Table 1); the exceptions were xylose, mannose, L-sorbose, raffinose, sorbitol, fucose and 2-deoxy-D-glucose. It is not surprising that fucose and 2-deoxy-D-glucose, which are analogues of galactose and D-glucose respectively and can inhibit galactose and glucose metabolism, were not utilized as carbon sources. Mannose and sorbitol both elicited taxis and may be utilized by other rumen micro-organisms (Hungate, 1966) but neither was utilized by *N. frontalis.*

Distribution of sucrose, fructose and glucose in tissues of barley. Sucrose, glucose and fructose are the major soluble carbohydrates present in grass tissues (Bailey, 1964), so their levels in the different barley tissues were determined (Table 3). The highest concentrations of sucrose fructose and glucose were in the awns and inflorescence tissue. All three sugars were detected in all the tissues except the roots which were devoid of sucrose.

Taxis to multiple attractants. Sucrose, glucose and fructose, the abundant carbohydrates of barley tissues, were examined in various combinations (Table 4). Their effects were synergistic for chemotaxis at low concentrations, but at high concentrations this effect decreased (Table 4). When sucrose, glucose and fructose were added together in the proportions found in the different barley tissues (cf. Table 3), their capacity to attract the zoospores was strongest with mixtures representing awns and inflorescence tissue (Table 5). In addition, if the zoospores were exposed to two capillaries containing the carbohydrate mixtures representing two different types of tissue, taxis was shown principally to that containing the inflorescence or awn tissue carbohydrates (Table 6).

Germination of zoospores in the presence of glucose. Glucose, at 0·08 M, stimulated the encystment and germination within 30 min of all the zoospores attracted to capillaries. As smaller numbers of germinated cells occurred in capillaries containing less glucose, the time required for zoospores to germinate in chemotaxis medium containing different glucose concentrations was investigated. The results (Fig. 5) show that complete germination occurs at glucose concentrations of 0·001 to 0·5 M. Germination at the lowest glucose concentration took 6 h.

DISCUSSION

Little information on chemotaxis of phycomycete zoospores to specific attractants is available. Royle & Hickman (1964) showed that the zoospores of *Pythium aphanidermatum* were attracted to a mixture of $1{\cdot}83 \times 10^{-2}$ M-glucose and -fructose and $0{\cdot}97 \times 10^{-2}$ M-sucrose in 1 % casein hydrolysate. These levels of carbohydrates were much higher than the concentrations of the same carbohydrates eliciting chemotaxis in *N. frontalis* zoospores. Chet & Mitchell (1976) concluded that phycomycete zoospores were much less sensitive to low concentrations of attractants than were bacteria, but we have found *N. frontalis* zoospores to be nearly as sensitive to glucose (threshold 5×10^{-6} M) and more sensitive to fructose (threshold 5×10^{-6} M) than *Escherichia coli* which has thresholds of 3×10^{-6} M and 1×10^{-5} M respectively for these sugars (Adler, 1975). The threshold concentrations for *N. frontalis*

Table 2. *Chemotaxis in the presence of a second attractant*

Results are expressed as $10^{-2}\times$ the number of zoospores accumulated in the capillary after incubation for 1 h at 39 °C. The compounds tested for chemotaxis were at their optimum concentration; the compounds saturating the system were at 0·01 M.

	System saturated with:												
Chemotaxis to:	No other carbohydrates	Xylose	Fructose	Glucose	Galactose	Mannose	L-Sorbose	Sucrose	Raffinose	Sorbitol	Mannitol	Fucose	2-Deoxy-D-glucose
Xylose	54	—	47	0·22	0·30	42	0·38	36	42	44	41	0·35	0·66
Fructose	80	72	—	74	70	68	72	1·2	0·70	75	69	74	81
Glucose	72	0·6	70	—	0·8	65	0·8	60	61	73	66	0·82	1·2
Galactose	71	0·22	58	0·40	—	61	0·70	60	65	72	64	1·1	0·75
Mannose	48	47	38	0·75	42	—	45	41	43	44	49	32	8·3
L-Sorbose	53	1·2	48	0·8	1·1	51	—	46	47	39	42	0·81	1·0
Sucrose	76	65	0·5	68	69	71	70	—	0·2	69	71	66	72
Raffinose	44	40	0·20	36	32	37	37	0·17	—	38	42	45	36
Sorbitol	52	54	47	49	46	50	51	32	38	—	0·21	48	50
Mannitol	38	38	30	33	36	49	38	38	32	0·5	—	37	33
Fucose	53	0·50	47	0·75	1·1	45	0·82	55	47	45	48	—	1·4
2-Deoxy-D-glucose	47	1·0	49	0·92	1·2	45	0·35	42	49	45	47	0·8	—

Table 3. *Sucrose, glucose and fructose concentrations (% dry wt) in different tissues of barley*

Tissue	Sucrose	Glucose	Fructose
Awns	10·8	4·7	1·8
Inflorescence	9·5	4·7	1·9
Leaves	4·3	1·4	0·8
Stem	1·7	0·4	0·5
Roots	0	0·6	0·4

Table 4. *Effect of combinations of glucose, sucrose and fructose on chemotaxis by N. frontalis zoospores*

Results show an average of two experiments, corrected for random entry of zoospores. In the carbohydrate mixtures, each component was at the concentration shown in the first column.

Concn of each component (M)	Rate of attraction of zoospores (spores min^{-1}) to carbohydrate mixtures containing:						
	G	S	F	G+S	F+S	G+F	G+F+S
10^{-5}	105	210	117	235	212	242	240
2×10^{-5}	120	238	110	226	227	244	NT
10^{-6}	15·2	108	0	216	207	233	246
10^{-7}	0	5·6	0	16·0	11·0	14·6	18·0

G, Glucose; F, fructose; S, sucrose. NT, Not tested.

Table 5. *Taxis of N. frontalis zoospores to soluble carbohydrate mixtures representing those found in different tissues of barley*

Compositions of soluble carbohydrate mixtures were calculated from the known soluble carbohydrate composition of each tissue, converted to a wet wt basis. The rate of attraction of zoospores was calculated from the number of spores present in the capillaries after 10 min incubation at 39 °C.

Composition of carbohydrate mixture (mM)			Tissue represented	Rate of zoospore attraction (spores min^{-1})
Sucrose	Glucose	Fructose		
40·0	33·0	12·5	Awn	870
35·0	33·0	13·0	Inflorescence	820
16·0	10·0	5·6	Leaf	750
6·0	2·7	3·4	Stem	620
0	4·2	2·7	Root	430

Table 6. *Attraction of N. frontalis zoospores to soluble carbohydrate mixtures representing two different types of tissues*

Compositions of soluble carbohydrate mixtures were calculated from the known soluble carbohydrate composition of each tissue, converted to a wet wt basis. Two capillaries, each containing one of the mixtures under examination, were inserted into the same zoospore suspension (0·9 ml) containing 10^4 spores ml^{-1}, and incubated for 30 min at 39 °C. The total numbers of zoospores entering each tube were determined by microscopy. Results are corrected for random entry of zoospores, and show the average of three experiments.

Other tissue carbohydrate mixture offered:	Percentage of total zoospores attracted to carbohydrate mixture representing:				
	Inflorescence	Awn	Leaf	Stem	Roots
Inflorescence	ND	53	18	8	3
Awn	47	ND	22	10	5
Leaf	82	78	ND	ND	ND
Stem	92	90	ND	ND	ND
Roots	97	95	ND	ND	ND

ND, Not determined.

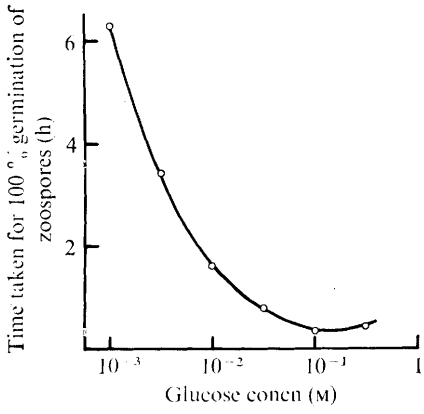

Fig. 5. Germination of zoospores of *N. frontalis* in the presence of glucose. Zoospores (10^4 ml^{-1}) were incubated under CO_2 in the chemotaxis medium containing different concentrations of glucose. The time for 100 % germination was determined by plotting percentage germination against time, and extrapolating to 100 % germination. Average of two experiments.

chemotaxis in the presence of fresh rumen fluid containing large numbers of bacteria were considerably higher than those in the absence of other organisms. Probably metabolism of the attractants occurs near to the orifice of the capillary, effectively decreasing the attractant concentration and reducing the zoospore chemotaxis. No doubt this situation also occurs near fresh plant particles in the rumen.

There are four different chemoreceptors in *N. frontalis* zoospores: for glucose, sucrose, sorbitol and mannose. Glucose and sucrose occur commonly in the diet of the host animal, and were readily metabolized by *N. frontalis* vegetative growth. The significance of the sorbitol receptor is difficult to understand since neither sorbitol nor mannitol, which used the same chemoreceptor, occur commonly in the diet of the host animal. The sorbitol receptor may be sensitive to other, as yet unidentified, dietary constituents. The mannose receptor was also sensitive to glucose. Since mannose occurs in the diet chiefly as mannan (Hungate, 1966) very little free mannose enters the rumen, and as mannose does not support the vegetative growth of *N. frontalis*, taxis to mannose probably does not occur *in vivo*. The mannose receptor may therefore be used for glucose taxis rather than mannose taxis. Other carbohydrates to which *N. frontalis* showed taxis, but which could not support the growth of the organism, are either not normally free in the diet (xylose, L-sorbose) or are usually absent (fucose, 2-deoxy-D-glucose).

The synergistic effect of glucose, fructose and sucrose at low concentrations, and preferential taxis to mixtures of soluble carbohydrates representing those found in awn and inflorescence tissue, is strong evidence that the soluble carbohydrates alone are responsible for the substratum selection by the zoospores. In addition, the glucose concentration in the awns ($3{\cdot}3 \times 10^{-2}$ M) was sufficient to trigger the germination of the zoospores. In the leaves of barley, the glucose present ($1{\cdot}0 \times 10^{-2}$ M) would also be sufficient to trigger germination but taxis to the carbohydrates diffusing from the awn and inflorescence tissue would take precedence over those diffusing from the leaf tissue. In the absence of inflorescence tissue, invasion of and growth upon leaf tissue will probably occur, as *N. frontalis* grows vegetatively on several tissues when in the rumen (Orpin, 1975, 1977) and has been found in the rumen of a sheep on an inflorescence-free diet. The glucose content of leaves of perennial rye grass (*Lolium perenne*), a major dietary constituent in Great Britain, is approximately $0{\cdot}8 \times 10^{-2}$ M (Waite & Boyd, 1953); this would also be sufficient to elicit chemotaxis and to trigger germination of the zoospores.

Taxis to, and growth in regions high in, soluble carbohydrates will enable the organism to

grow in a microenvironment rich in other compounds such as proteins, amino acids, purines, pyrimidines and vitamins, which may also be required for growth. It may also allow the organism access to these compounds prior to, or at the same time as, their consumption by other rumen organisms.

Glucose, sucrose and fructose are depleted from the diet of sheep within 2 to 3 h of feeding (Phillipson & McAnally, 1942). After this period *N. frontalis* zoospores would not be able to identify the inflorescence tissue by its soluble carbohydrate content. Since the zoospores are present in small numbers at any time of day in sheep fed once daily (Orpin, 1975), their subsequent germination may be delayed or may occur free in the rumen fluid. Germination under these conditions may be a function of time rather than substrate or substratum availability.

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