

Carbon dioxide sensing by *Panagrellus silusiae* and *Ditylenchus dipsaci*

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SUMMARY

Chemosensing constitutes a key mechanism by which a nematode perceives the external environment. Reports of the last half century testify to an assortment of substances that attract or repel nematodes. In this report, the accumulation of *Panagrellus silusiae* about a carbon dioxide source was used as an assay to evaluate many substances at various concentrations for their ability to modify nematode chemosensing. Accumulation varied with compound and dilution and often exhibited one or more peaks of activity. With many substances, accumulation of treated and untreated nematodes was similar; however, a modest number of substances promoted great accumulation while a few reduced accumulation substantially. Sequential treatments demonstrated that inhibited or enhanced accumulation was reversible and effected by oxidation-reduction or solubility product type considerations. The modification of carbon dioxide sensitivity was generally consistent with the notion of altered conformation of the receptive site on the nematode sensor structures.

RÉSUMÉ

Détection du dioxyde de carbone par Panagrellus silusiae et Ditylenchus dipsaci

La chémodétection constitue l'un des mécanismes clés permettant aux nématodes la perception de leur environnement. Durant la première moitié de notre siècle, ont été signalées diverses substances qui attirent ou repoussent les nématodes. Lors de la présente étude, l'accumulation des individus de *Panagrellus silusiae* autour d'une source de dioxyde de carbone a été utilisée pour tenter d'évaluer un certain nombre de substances, à diverses concentrations, au regard de leur aptitude à modifier la chémodétection du nématode. Cette accumulation varie avec le composé utilisé, et sa dilution, et montre souvent un ou plusieurs pics d'activité. Pour beaucoup de substances, l'accumulation des nématodes traités et non traités était identique; cependant un certain nombre de substances provoquent une accumulation importante tandis que quelques-unes réduisent notablement celle-ci. Des traitements successifs ont montré que l'inhibition ou l'accroissement de l'accumulation est réversible et sensible aux caractéristiques de réduction/oxydation et de solubilité du produit. La modification de la sensibilité au dioxyde de carbone correspond généralement à la notion d'une modification dans la conformation des sites récepteurs appartenant aux structures sensibles du nématode.

Chemosensing has been long considered one of the principal mechanisms by which nematodes perceive their external environment. For a half century, it has been known that roots and excised shoot tissues attract certain nematodes (Linford, 1939). Subsequently, a number of workers (Wieser, 1955; Wallace, 1958; Lownsbery & Viglierchio, 1958, 1960; Bird, 1959) demonstrated that a number of different phytoparasitic nematodes were attracted by roots of various plants. For a recent review see Zuckerman and Jansson (1984). Other research (Klingler, 1959; Bird, 1959; Johnson & Viglierchio, 1961) established that carbon dioxide and sometimes oxygen could attract a number of plant parasitic nematodes. Klingler (1963) showed that the accumulation was not a consequence of random movement and retention at the site of CO₂ injection by observing that *Ditylenchus dipsaci* was active and could move away but return to the CO₂ site. Only when the nematodes moved into the capillary insertion tube carry-

ing the carbon dioxide gas stream did the nematodes suffer anoxia and become quiescent. A later study (Croll & Viglierchio, 1969b) indicated that the carbon dioxide receptor of *D. dipsaci* exhibited reversible inhibition, i.e., a KI₃ treatment preventing the sensing of CO₂ could be reversed by a subsequent H₂S treatment to restore sensitivity. It was of interest, therefore, to study receptor function by exploring carbon dioxide sensitivity modification of *Panagrellus silusiae*, an easily reared free-living nematode and to compare some of their responses to those of *D. dipsaci*.

Materials and methods

NEMATODE PREPARATION

Stock supplies of *Panagrellus silusiae* were maintained on culture plates with bacteria as a food source and *Ditylenchus dipsaci* were obtained from garlic scales

using Baermann funnel extraction. As needed, the nematodes were washed off a plate (mostly adults) or extracted with a Baermann funnel and collected on a 38- μm /aperture sieve, backwashed into a beaker and stored under aeration at 15 °C for not more than a week. For use, nematodes were aliquanted from the storage suspension into a centrifuge tube and centrifuged, after which the supernatant was decanted. Ten millilitre of test solution at the desired concentration, or water for untreated controls of each batch, was added to the nematode pellet and incubated at room temperature for 10 min while being shaken vigorously. At the end of the treatment, the suspension was centrifuged, the supernatant was decanted and the nematode pellet was washed twice with deionized water before use in a bioassay. As a normal practice, 6 000-10 000 nematodes were used in each bioassay.

PREPARATION OF AGAR BIOASSAY PLATES

Agar buffer (0.9 %) was prepared by dissolving 2 g of Difco agar in 225 ml of boiling phosphate buffer (0.02 M; pH 6.8) to reduce pH variation and to eliminate dissolved carbon dioxide. The hot solution was cooled under running tap water to about 40 °C then stored in a 40 °C bath.

The treated or untreated nematodes prepared as previously described were added to the agar buffer held at 40 °C, stirred vigorously for 2 min at this temperature and then poured onto the prewarmed glass plate (30 cm \times 50 cm edged with disposable tape to create a shallow receptacle) on a level counter to yield an agar stratum approximately 1.5 mm in depth containing a random dispersion of nematodes. Once the agar had gelled (about 1 min), the edge tape was removed and discarded and the gel slab edges were trimmed. A plastic film (Goodyear Prime Wrap [®] permeable to CO₂, O₂, N₂) was cut slightly larger than the gel slab and laid directly on the gel surface, avoiding the entrapment of gas bubbles between the film and the gel surface. The film edges were folded under the glass plate so as to provide a taut, bubble-free, smooth surface. The gel slab assembly was then placed directly under the gas delivery assembly.

THE BIOASSAY

The gas delivery assembly consisted of a fixed template (30 cm \times 50 cm plastic foam slab) holding fifteen delivery pipettes each 10 cm from its closest neighbor. Each gas from its own pressurized cylinder was fed through a flow meter then humidified by bubbling through an 0.1 N sulfuric acid solution before passage through a glass wool filter and delivery to a dispersion manifold. Gas from the manifold was delivered through latex rubber tubing to each pipette at the rate of approximately 500 ml/min. The types of gas to the delivery pipettes were arranged randomly and at the delivery sites

the pipette tip to gel surface gap was 2 mm. Gas delivery site loci were permanently etched onto the glass plate for ease of orientation under the gas delivery assembly and for subsequent counting. Each bioassay (run for 3 h) included five replicates of each gas, CO₂ for attraction, N₂ as an inert gas and O₂ for possible changes in response to oxygen as a consequence of nematode treatment.

The accumulation counts were made immediately, directly on the gel with the aid of a dissecting microscope and a plastic template placed directly on the gel surface and inscribed with the center of each delivery site and a concentric 1 cm radius circle to designate the area within which nematodes were to be counted.

Each solute candidate for sensor modification was pre-tested by immersing nematodes for 20 min in a range of concentrations to establish the highest concentration at which no motility inhibition could be detected. All modification treatments reported were conducted at concentrations below this threshold to ensure the use of motile nematodes. Each test substance dilution series was conducted within a week with the same stock source of nematodes.

In view of the extensive accumulation of data from assays of many agents, the results have been condensed and presented in summary form. In these semi-log plots ordinate is the accumulation index (A.I.), where :

$$A.I. = \frac{\frac{\text{The number of treated nematodes accumulating about CO}_2}{\text{The number of treated nematodes accumulating about N}_2}}{\frac{\text{The number of untreated nematodes accumulating about CO}_2}{\text{The number of untreated nematodes accumulating about N}_2}}$$

while the abscissa indicates the concentration of the agents. A.I. constitutes a dimensionless measure of change in accumulation about CO₂ as a function of concentration of treating agent; among other factors A.I. reduces variation due to numbers of nematodes in the agar, ambient temperature effects on motility and storage effects on the nematodes. An A.I. of 1.0 signified a response equal to untreated nematodes (normal). An A.I. in the region of 0.06-0.10 indicated that the number of nematodes accumulating about the CO₂ source was equal to the number accumulating about the N₂ source.

Results

Pre-treatments of nematodes with magnesium salts (Fig. 1) serve to illustrate typical modulation effects on sensing in the form of a transformation of the number of nematodes accumulating about a point carbon dioxide source. Multiple peaks were commonly observed with pre-treatments by various agents; one to four peaks were common but for one exception, potassium permanganate, which initiated with an accumulation index of less than 0.1 at 10⁻³ M and rose linearly to normal at a concentration of 10⁻⁸ to 10⁻⁹ M.

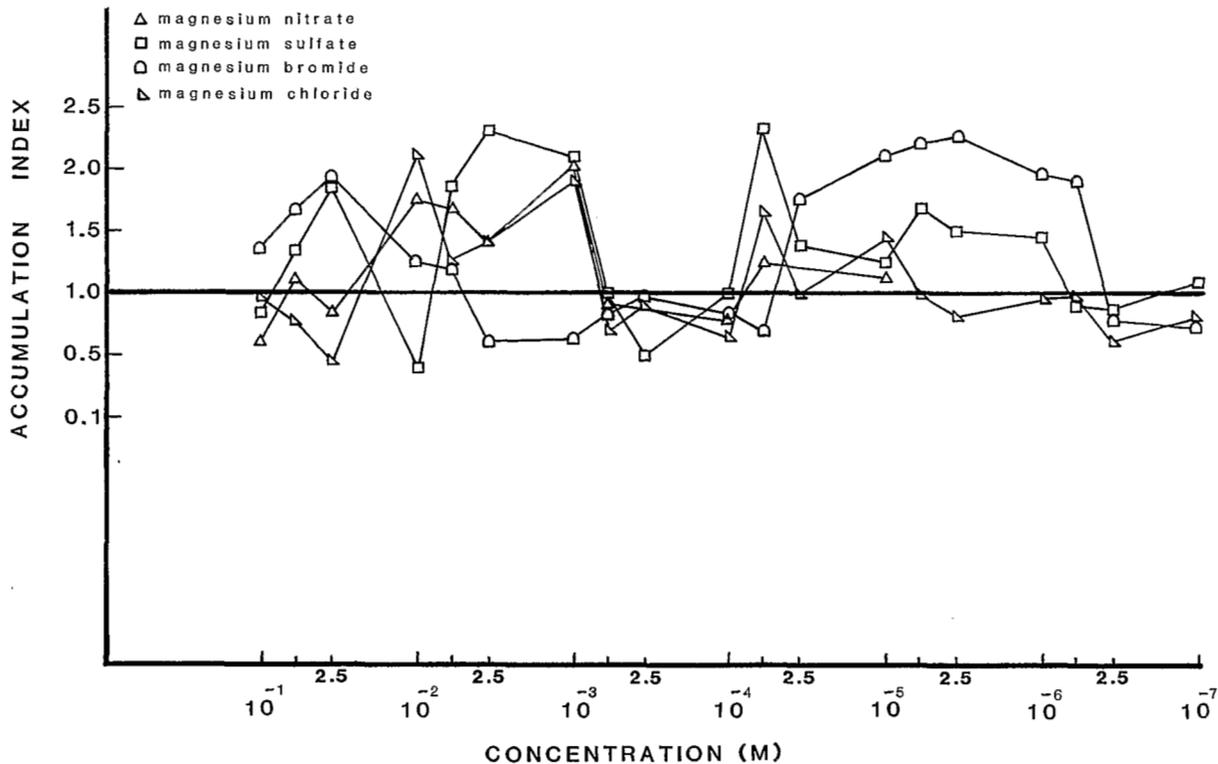


Fig. 1. The accumulation index of *Panagrellus silusiae* at various concentrations of magnesium salts. Accumulation index value is the ratio of the subratio of the number of treated nematodes accumulating about CO₂ to the number accumulating about N₂ to the subratio of untreated nematodes accumulating about CO₂ to the number of untreated nematodes accumulating about N₂ (see text equation).

PANAGRELLUS SILUSIAE

For *P. silusiae* untreated controls, the ratio of accumulation about CO₂ to accumulation about N₂ ranged from 10-25 depending upon batch with a year-long batch mean of about 17. Accumulation about oxygen did not occur with *P. silusiae*; the A.I. ratio O₂/N₂ for untreated controls and all treatments was normally about 1.

The influence of pre-exposure to inorganic salt solutions on the accumulation of *P. silusiae* about CO₂ was strongly controlled by the cation in terms of the number of peaks and their general height and location over the concentration range tested. However, the anion did modify the cation effect either by increasing or decreasing the peak or shifting its location somewhat (Fig. 1). When the A.I. for each dilution was averaged for a cation series, averages well above 1 were obtained for the monovalent cations NH₄⁺, Rb⁺, K⁺, Na⁺, Cs⁺, Li⁺ and divalent cations Mg⁺⁺ and Ca⁺⁺ as nitrates. Sulfates, chlorides and bromides were usually less effective than nitrates in maintaining high averages.

Most of the dilution responses ranged within $\pm 50\%$ of normal (A.I. between 0.5 and 1.5). Many responses

were found to be $> 50\%$ above normal (A.I. > 1.5), while very few responses were observed to be below 50% of normal (A.I. < 0.5). The events considered of primary interest involved the lowest concentration at which a pre-treatment agent produced a response 50% or greater, above or below normal (Fig. 2); other agents that affected responses between A.I. 5-1.5 throughout the concentration range were not deemed noteworthy. With all active agents, a positive response of A.I. of 1.5 or greater occurred with dilute solutions; whereas, an inhibitory response of A.I. 0.5 or less occurred at higher concentrations (Fig. 2). With three agents, AgNO₃, KMnO₄ and I₂ A.I.'s of < 0.1 were obtained. Other positive peaks, A.I. of 1.5 or greater or negative peaks, A.I. 0.5 or less, when they occurred, were at higher concentrations than those noted (Fig. 2). The concentration range between the lowest negative peak and the highest positive peak of individual agents ranged from a factor of approximately 0.5-6 orders of magnitude. The accumulation response of *P. silusiae* to CO₂ appeared to be very highly sensitive to barium nitrate in terms of solute dilution.

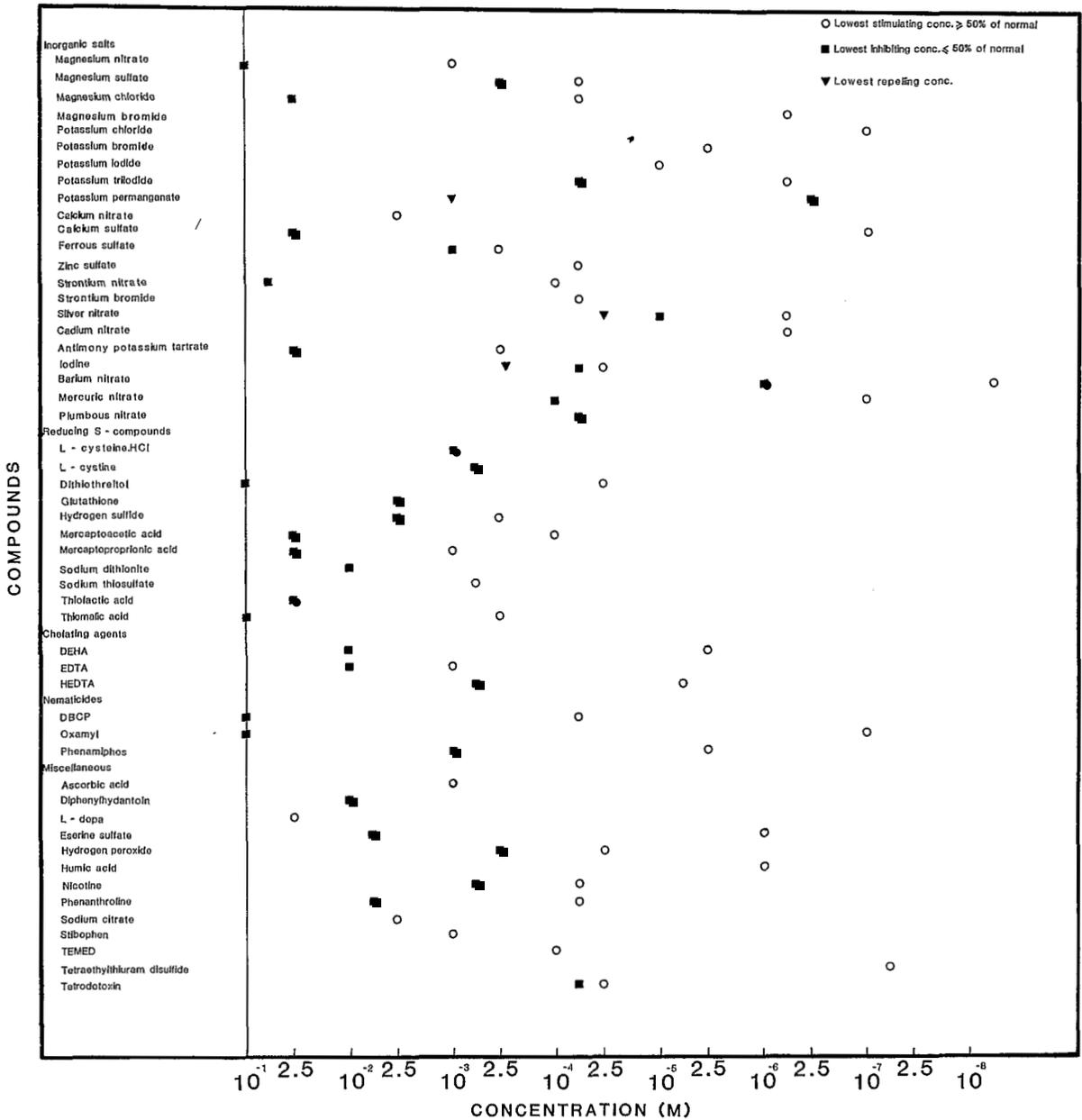


Fig. 2. Compounds used in treatment of *Panagrellus silusiae* at the lowest concentration resulting in a > or < 50% in accumulation index, i.e., the lowest concentration low and the lowest concentration high; to avoid confusion, other peaks were ignored.

The pre-exposure compounds tested included oxidizing and reducing agents as well as cations and anions that could be involved in solubility product considerations. Various combinations at concentrations that strongly affected sensing to exhibit positive or negative peaks of activity were tested as sequential treatments for influence on carbon dioxide sensing.

After the first treatment, conducted as previously

described, the nematodes were washed twice then treated with a second agent for a like time before washing and placing on a migration plate for regular bioassay. The results of various treatment combinations are illustrated (Fig. 3) as accumulation indices. The effect of the silver treatment remained essentially unchanged when followed by EDTA, ascorbic acid, ferrous sulfate, and potassium triiodide but moved modestly

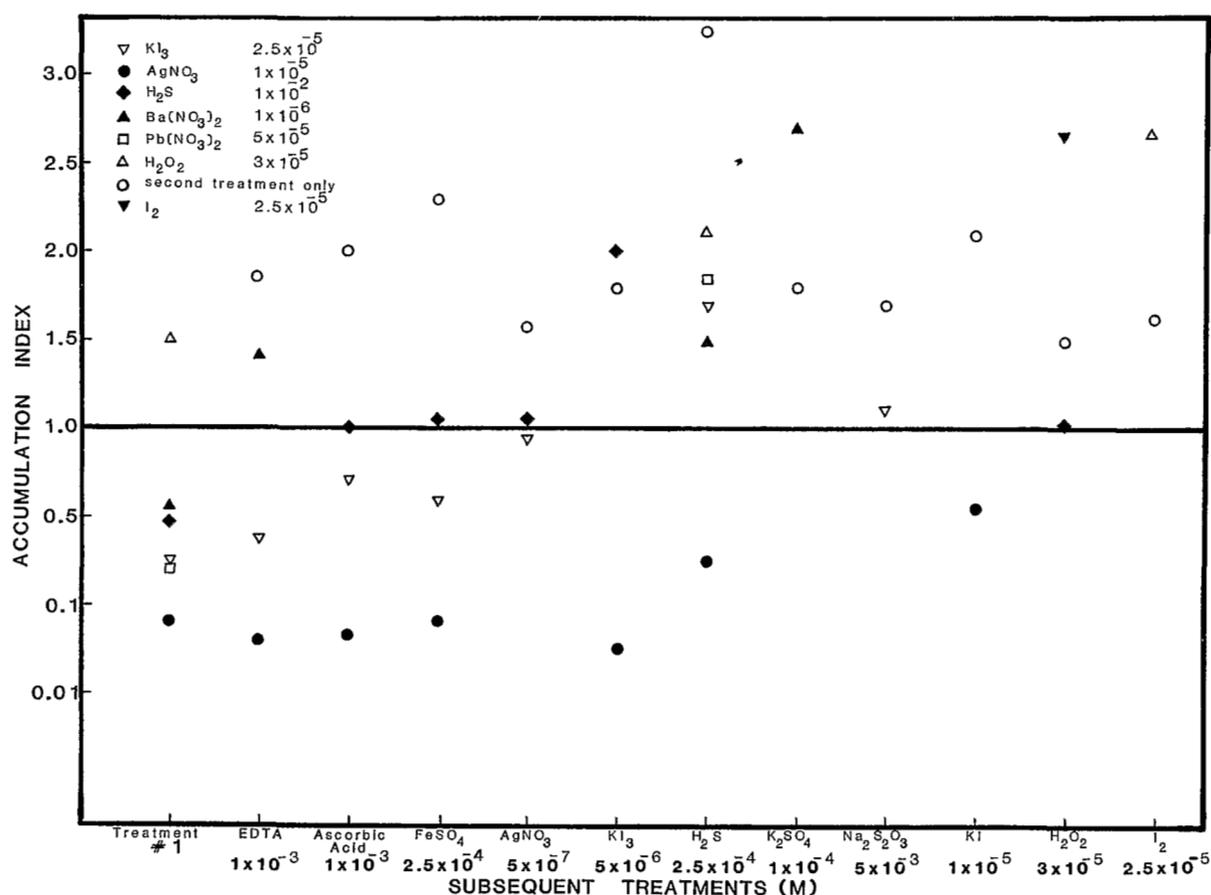


Fig. 3. The accumulation index of *Panagrellus silusiae* receiving sequential treatments involving oxidation-reduction and solubility product considerations. The A.I. of the first treatment alone is indicated in the Treatment 1 column and of a second treatment without a first, by the open circles.

towards normal when followed by hydrogen sulfide or potassium iodide, though remaining strongly inhibitive. The effect of barium nitrate followed by potassium sulfate increased from an A.I. of approximately 0.5 to 2.7, substantially above potassium sulfate by itself. The negative effect of hydrogen sulfide was strongly reversed when followed by potassium triiodide (A.I. < 0.5 to > 2.0). All other combinations except for the double oxidation increased the A.I. but remained intermediate between the first treatment or second treatment alone. The effect of the double oxidation combination of hydrogen peroxide and molecular iodine, like barium nitrate followed by potassium sulfate, was synergistic to produce an A.I. in excess of 2.5 and greater than the sum of individual treatments; moreover, the results were the same whichever treatment came first. In other sequence tests in which either of two cations at some concentration gave an A.I. of 1.5 or greater, the results were the same whether each cation was tested separately or sequentially in either order.

DITYLENCHUS DIPSACI

Some of the same compounds were used to test the modification of sensing by *D. dipsaci*. The general response was similar to *P. silusiae* but not identical. The dilution curves were similar, generally, with one or more peaks; however, they did not necessarily occur at the same concentration for both nematodes. A direct comparison of some 24 compounds demonstrated that six were active positively at higher concentrations for *D. dipsaci* than for *P. silusiae*, nine were active at lower concentrations for *D. dipsaci* than *P. silusiae* and that nine were active at about the same concentration for both nematodes.

The results of sequence treatments, confirmed the previous observations between the two nematodes, of similar but not identical CO₂ sensing. For example, if the concentrations of each component giving optimum responses for *D. dipsaci* were tested on *P. silusiae*, the A.I. was low; however, if the optimum concentrations

for *P. silusiae* were tested on *D. dipsaci*, the A.I. was much higher but still below the A.I. observed with *P. silusiae*. Moreover, using the lowest concentration optimum for *P. silusiae* of barium nitrate at 5×10^{-9} M followed by potassium sulfate at 10^{-4} M or by the reverse sequence (individual treatments showed no effect on *D. dipsaci*), the *D. dipsaci* A.I. was more than two times greater than the sequence barium nitrate 5×10^{-7} M followed by potassium sulfate at 10^{-6} M (individual treatment optima for *D. dipsaci*). Similarly, strontium nitrate 2.5×10^{-7} M followed by zinc sulfate at 10^{-6} M (individual treatment optima for *P. silusiae*) gave an A.I. two times greater than a sequence using strontium nitrate at 10^{-3} M followed by zinc sulfate at 10^{-2} M (individual treatment optima for *D. dipsaci*).

ATTRACTION TO OXYGEN

P. silusiae was essentially indifferent to oxygen; the number of nematodes about an oxygen source was the same as that about the nitrogen source. Pretreatments with salt solutions made no difference with one exception; this was the treatment with thiosulfate solution at 5×10^{-3} M which effected a strong attraction to oxygen. Treatment with reducing agents e.g., dithionite, hydrogen sulfide, ascorbic acid, ferrous sulfate, mercaptoacetic acid, L-cysteine and dithiothreitol caused a mild repulsion to oxygen. Although the attraction to CO_2 varied greatly with treatment agent and concentration, sources of oxygen and nitrogen were the same with the exceptions noted.

In *D. dipsaci*, the long-term accumulation ratio of CO_2/N_2 varied from 3-8, and the accumulation ratio O_2/N_2 averaged about 1.2. Treatments with the agents used in the CO_2 studies which included salts, reducing agents and miscellaneous organic compounds effected no changes in the O_2/N_2 ratios from normal.

Discussion

The ability of nematodes to respond to a gradient indicates a capacity to detect the agent effecting a gradient (Ward, 1978), which in these experiments was carbon and oxygen are coplanar with bond angles of as generated by momentary binding of carbon dioxide to the sensing receptor site followed by its release. With an increasing concentration gradient more catch and release episodes result and therefore, more pulses and a stronger signal for the nematode to interpret and transform into motor activity. The carbon dioxide molecule is linear, $\text{O} = \text{C} = \text{O}$; however, upon dissolution in an aqueous medium, it combines with one molecule of water to form carbonic acid. In the carbonate form, the carbon and oxygen are coplanar with bond angles of 120° , carbon to oxygen distances of 1.31 \AA and the resultant oxygen to oxygen distance of 2.27 \AA (Pauling,

1948). The complementary sensor site receptive to the carbonate is likely to conform to these dimensions. Under the buffered assay conditions, the maximum carbonate concentration possible in solution can be calculated (using equilibrium constants) to have been approximately 5.5×10^{-3} M with the conformation resonating among three principal forms (Fig. 4).

The resonant energy among the three forms is approximately 42 kcal per mole (Pauling, 1948) and CO_2

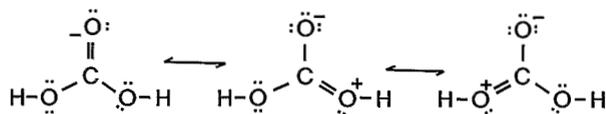


Fig. 4. Resonant forms of carbonate in solution.

sensing can be postulated as a momentary binding of a resonant form to the complementary sensor site to create a signal in the sensor with a concomitant decrease in the resonant energy of the carbonate to effect detachment and readiness for the next cycle. This hypothesis is similar in substance to the transition-state theory currently advocated for enzyme catalysis (Kraut, 1988). To improve the reality of perception and thereby avoid spurious signals, it is likely the sensor would possess multiple sites receptive to CO_2 . Moreover, to ascertain gradient direction in a three-dimensional environment, the nematode would require at least three sensors arranged symmetrically about the anterior end. An alternate system that could accomplish gradient perception with less than three receptors would require the inclusion of a short-term memory capability. Signals generated at the receptor sites would move down the sensor nerve to a small ganglion for integration and forwarding of the resultant impulse to a central ganglion for initiation and coordination of the appropriate motor activity. Either operational hypothesis is consistent with sensor nervous system structure in the region (Maggenti, 1981).

The bioassay used herein indicates the number of nematodes found in a prescribed area about the gas source. Upwards of 90 % of normal untreated nematodes found in the carbon dioxide counting area migrated from regions adjacent to the zone. Therefore, for an A.I. > 1 , more nematodes migrated from the outer regions; correspondingly for an A.I. < 1 , fewer nematodes than normal migrated from the surrounding region. It appears that sensor modifying agent may effect an unmasking of potential sites to render them receptive in normally insensitive nematodes or to effect a masking of normally receptive sites in sensitive nematodes.

In an early report (Croll & Viglierchio, 1969b), it was suggested that "concentration sensing, necessary in orientation, may be favored by a spatial configuration type of detector for CO_2 ". In that case, the attraction of

D. dipsaci by CO₂ was inhibited by treatment of the nematodes with triiodide ion and reversed by a subsequent treatment with hydrogen sulfide. The explanation involved sulfhydryl groups oxidized by triiodide ion to the disulfide form which rendered the detector inactive followed by reduction to the sulfhydryl form by hydrogen sulfide to restore detector activity. In essence receptivity was dependent upon the conformation of the receptor surface in the binding zone and the immediate vicinity. Conformational change can also be brought about by hydrogen bonding (5 kcal/M) and van der Waals forces of molecular attraction. The modified spatial configuration would consist of physical surface change and/or a partial charge redistribution on the sensor surface. The results reported herein were in large measure consistent with this notion. The treatments utilized low concentrations for short periods to accentuate surface effects and reduce internal ones, notwithstanding earlier reports (Croll & Viglierchio, 1969a; Viglierchio, 1974) of low transcuticular solute transport at much higher osmotic concentrations. With few exceptions, the peaks observed with A.I.-concentration curves could be explained in part by the varying "activity" of solutes in solution, as a function of concentration in terms of hydrogen bonding and van der Waals forces for salts and organic compounds. This effect also appears to occur in nature by virtue of the positive stimulus, on *P. silusiae* accumulation to CO₂, effected by treatments with natural soil solutions. The variation in activity observed with salts and organic solutes was also consistent with that from extracts of different soil types: three soil extracts were active at higher dilutions than a fourth which came from a very sandy soil. Certain organic compounds, by virtue of their solubility properties, could be expected to penetrate the nematode cuticle, particularly at the higher concentrations, such that their resultant effect on accumulation would be a consequence of internal as well as surface activity. Nematicides are such compounds whose utility depends upon the internal disruption of membrane or nerve conduction functions. At high concentrations, the reduction in accumulation about CO₂ could be explained in part by these disruptive functions but the stimulations occurring at very low concentrations cannot.

Mediating effects, other than the mild van der Waals forces already discussed, affected conformation. For example, if in the region of the receptor site the surface manifested a potential disulfide bond then reducing agents, in particular hydrogen sulfide, would favor the reduced sulfhydryl form allowing for more conformational flexibility and enhanced receptivity. This characteristic was observed with ascorbic acid, ferrous sulfate and several thiol compounds. Whereas, 2.5×10^{-4} M hydrogen sulfide produced the greatest increase in A.I., moderate oxidizing agents as I₂ and H₂O₂ produced a smaller increase in A.I. Perhaps differentially modified states of S-S bonding effected by

oxidants or reductants of varying strengths can explain, in part, the levels of CO₂ sensing observed.

The sequence treatments offer striking insight into the complexities of chemosensing. The reversal of inhibition of chemosensitivity previously observed (Croll & Viglierchio, 1969b) with *D. dipsaci* treatments with triiodide followed by hydrogen sulfide was not an isolated case but reflected a general phenomenon (Fig. 3). The outcome from a pair combination may result in an intermediate stimulation of A.I. between those of each compound by itself or a synergistic stimulation of A.I. greater than the sum of the components. For *P. silusiae*, these reversals appeared to involve oxidation-reduction, oxidation-oxidation or solubility product considerations. The oxidation-reduction mechanism has been discussed; however, the double oxidation involving hydrogen peroxide and iodine was of special interest. The resultant stimulation of A.I. was synergistic and greater than the sum of the component activities; moreover, it was irrelevant in which order the treatments occurred; the final stimulation was the same. The results suggested the presence of at least two oxidizable sites, possibly disulfide bonds, that greatly facilitated conformational accommodation of the sensor surface for carbon dioxide receptivity.

Sequence treatments involving certain heavy metals revealed some striking responses. Barium ion that 10^{-6} M provided an A.I. of approximately 0.4 but when followed by sulfate ion at 10^{-4} M, the A.I. was stimulated synergistically to a very high value, 2.7 (Fig. 3). On the other hand, a silver treatment at 10^{-5} M was repellent (less nematodes about CO₂ than N₂) but followed by sulfide at 10^{-4} M or potassium iodide at 10^{-5} M, the A.I. was raised modestly above the repellent level. Using solubility product considerations, the maximum allowable barium ion concentration at the end of the sulfate treatment was 1×10^{-6} M, the same concentration of the initial treatment which was inhibitory. The concentration of barium in the bathing solution before and at the end of the sulfate treatment was the same, though accumulation was greatly different; moreover, similar results prevailed if sulfate was followed by barium. It appeared that barium sulfate as a molecule bound to the reception surface to increase availability of CO₂ receptive sites. More dilute solutions of barium were much less stimulatory offering a more modest 50% increase in A.I. at 10^{-9} M. The maximum allowable silver ion concentration at the end of the iodide treatment was 1.5×10^{-11} M and at the end of a sulfide treatment, 5×10^{-44} M. While both of these treatments resulted in a A.I. of < 0.7, a single treatment of silver ion at 10^{-7} M resulted in a A.I. of 1.5.

However, either second treatment enhanced chemoattractive behavior compared to silver alone at higher concentrations. Silver, therefore, was bound tightly to inhibit sensing at high concentration with greater affinity than could be provided by free iodide and sulfide

ions, but facilitated sensing at lower concentrations. In the unusual combinations outlined above, strong mechanisms were involved, apparently other than the conformational changes effected by van der Waals forces applicable to most of the situations examined as illustrated by a strontium nitrate treatment that effected a modest stimulation of accumulation which followed by zinc sulfate or vice versa remained unchanged.

For attraction responses, CO₂ sensing could be explained to a large degree, in terms of an increase or decrease in the intensity of the "like" signals generated by the sensor. However, repulsion, in which the nematode number about CO₂ would be less than that about N₂, as effected by Pb(NO₃)₂, KMnO₄, I₂ and AgNO₃ at high concentrations, would also be a function of CO₂ sensing but one which generates an escape rather than an attraction response. Apparently, the CO₂ sensed initiated a different signal perceived by motor ganglia as a "dislike" signal that generated the escape motor activity. Although the carbonate conformational constrictions remained the same, the strong activity of the agents, (cationic attachment, oxidation and substitution) may have distorted the sensor surface to create a new site receptive to CO₂, but which generated a different signal from the sensor that would be perceived by the motor function ganglia as a "dislike" signal. This possibility would be consistent with and facilitated by the view that the limited number of sensory structures available to the nematode would be multipurpose sensing organs able to detect a range of compounds found in the environment. Moreover, a secondary set of sensors, posterior to the primary, generating a like signal, in the absence of a similar signal from the first, could, in part, explain the "reversals" in locomotion reported by Croll (1975).

Chemosensing remains an intriguing and complex process that merits further study. Although evolutionary development of chemosensitivity may have resulted in a basic similarities in nematode sensing processes, different nematodes are likely to manifest individual characteristic mechanisms. It would be useful to look at other chemosensing systems amenable to a reliable bioassay for an assessment of any similar modifying effects. A better understanding of the events taking place in the chemosensor and the nature of the signal generated may be provided by future electrophysiological measurements of the functioning cell in the manner used for odor-induced membrane currents in vertebrate-olfactory receptor neurons (Firestein & Werblin, 1989).

REFERENCES

- BIRD, A. F. (1959). The attractiveness of roots to the plant-parasitic nematodes *Meloidogyne javanica* and *M. hapla*. *Nematologica*, 4 : 322-335.
- CROLL, N. A. (1975). Behavioral analysis of nematode movement. In : Dawes, B. (Ed.) *Advances in Parasitology*, 13 : 71-122.
- CROLL, N. A. & VIGLIERCHIO, D. R. (1969a). Osmoregulation and the uptake of ions in a marine nematode. *Proc. helminth. Soc. Wash.*, 36 : 1-9.
- CROLL, N. A. & VIGLIERCHIO, D. R. (1969b). Reversible inhibition of chemosensitivity in a phytoparasitic nematode. *J. Parasitology*, 558 : 895-896.
- FIRESTEIN, S. & WERBLIN, F. (1989). Odor-induced membrane currents in vertebrate-olfactory receptor neurons. *Science*, 244 : 79-82.
- JOHNSON, R. N. & VIGLIERCHIO, D. R. (1961). The accumulation of plant-parasitic nematode larvae around carbon dioxide and oxygen. *Proc. helminth. Soc. Wash.*, 28 : 171-174.
- KLINGLER, J. (1959). Anziehung von Collembolen und Nematoden durch Kohlendioxid-Quellen. *Mitt. schweiz. ent. Ges.* 32 : 311-316.
- KLINGLER, J. (1963). Die Orientierung von *Ditylenchus dipsaci* in gemessenen künstlichen und biologischen CO₂-Gradienten. *Nematologica*, 9 : 185-199.
- KRAUT, J. (1988). How do enzymes work? *Science*, 242 : 533-540.
- LINFORD, M. B. (1939). Attractiveness of roots and excised shoot tissues to certain nematodes. *Proc. helminth. Soc. Wash.*, 6 : 11-18.
- LOWNSBERY, B. F. & VIGLIERCHIO, D. R. (1958). Mechanisms of accumulation of *Meloidogyne hapla* around roots of tomato seedlings. *Phytopathology*, 48 : 395.
- LOWNSBERY, B. F. & VIGLIERCHIO, D. R. (1960). Mechanism of accumulation of *Meloidogyne incognita acrita* around tomato seedlings. *Phytopathology*, 50 : 178-179.
- MAGGENTI, A. R. (1981). *General Nematology*. New York, Springer-Verlag, 372 p.
- PAULING, L. (1948). *The nature of the chemical bond*. Ithaca, N.Y., Cornell University Press, 450 p.
- VIGLIERCHIO (1974). Osmoregulation electrolyte uptake in antarctic nematodes. *Trans. am. microsc. Soc.*, 93 : 325-338.
- WALLACE, H. R. (1958). Observations on the emergence of cysts and the orientation of larvae of three species of the genus *Heterodera* in the presence of host plant roots. *Nematologica*, 3 : 235-243.
- WARD, S. (1978). Nematode chemotaxis and chemoreceptors. In : Hazelbauer, G. L. (Ed.). *Taxis and Behavior: Elementary sensory systems in Biology, Series B. Receptors and Recognition*, London, Chapman & Hall, 5 : 143-167.
- WIESER, W. (1955). The attractiveness of plants to larvae of rootknot nematodes. 1. The effect of tomato seedlings and excised roots on *Meloidogyne hapla* Chitwood. *Proc. helminth. Soc. Wash.*, 22 : 106-112.
- ZUCKERMAN, B. M. & JANSSON, H. B. (1984). Nematode chemotaxis and possible mechanisms of host/prey recognition. *Ann. Rev. Phytopathol.*, 22 : 95-113.

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