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Carbon Dioxide and Temperature Gradients in Baermann Funnel Extraction of *Rotylenchulus reniformis*[1]

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Abstract: Vermiform *Rotylenchulus reniformis* were anesthetized in water by 10–40% CO_2 but were fully motile for 24 hours in water below 5% CO_2. When air containing 2.5% CO_2 was blown onto agar, nematodes accumulated at the point of highest CO_2 concentration. Nematodes also accumulated when chilling (0.2–1 C) of agar by the gas flow at the accumulation point was offset with heat from a fiber optic. In Baermann funnels containing *R. reniformis* in silt loam and sandy clay loam soils, CO_2 in funnel water increased during 24 hours from 0 to ca. 1%; more CO_2 accumulated below the soil layer than above. Bubbling air with 2.5% CO_2 into water below soil in covered funnels increased the CO_2 gradient and increased nematode extraction, whereas bubbling air without CO_2 below soil purged CO_2 from the water and decreased nematode extraction. Manipulation of CO_2 within funnels usually increased extraction by only 30% and never by more than 3-fold. Controlling temperature gradients consistently increased extraction by 2–30-fold.

Key words: Baermann funnel, carbon dioxide, nematode behavior, nematode extraction, *Rotylenchulus reniformis,* temperature gradient.

Use of behavioral stimuli to extract nematodes from soil and plant tissue has been explored little since Overgaard (10) heated Baermann funnels with an electric light bulb. We controlled temperature gradients in Baermann funnels and found that extraction efficiencies for *Rotylenchulus reniformis* Linford & Oliveira, *Meloidogyne incognita* (Kofoid & White) Chitwood, and *Tylenchulus semipenetrans* Cobb after 24 hours were increased 2–100-fold whenever funnel water was not chilled by evaporation (15). Covering funnels and heating uncovered funnels from above with light bulbs gave similar results. Changes in extraction were associated with the magnitude and direction, vertically, of temperature change but not with changes in median soil temperature. Thus, a behavioral response to temperature, or to something associated with temperature, appeared responsible. *Rotylenchulus reniformis* and several other nematodes move toward or away from heat on artificial gels; the direction of movement in most cases depends on ambient and adaptation temperatures (2,4,5,7,14, 16).

Carbon dioxide also attracts a diversity of nematodes on artificial gels (1,6,8, 9,11,13). Thresholds for attraction by *M. incognita* (11) and *Ditylenchus dipsaci* (Kuhn) Filipjev (9) to CO_2 are ca. 0.01%/cm and 0.1%/cm, respectively, low enough to indicate that CO_2 gradients in Baermann funnels may influence the directional movement of nematodes. Behavioral responses of *R. reniformis* to CO_2 have not been examined. Our objective was to compare the anaesthetic and behavioral effects of CO_2 on *R. reniformis* in vitro with the effects of gradients of CO_2 and temperature on nematode movement from soil in Baermann funnels.

MATERIALS AND METHODS

Direct observations of CO_2 effects

Anesthesia threshold: Mixed developmental stages of *R. reniformis* were extracted from soil by Baermann funnel and suspended in tap water purified by reverse osmosis (< 20 μmho/cm). The nematode suspension (ca. 500 nematodes/ml) was split among loosely capped vials into which was bubbled breathing-grade air (78% N_2, 21% O_2, less than 0.1% CO_2) charged with 0, 2.5, 5, 10, 20 or 40% CO_2 or with 50% N_2 (by volume). After 30 minutes, 90 minutes, or 24 hours, nematodes from each vial were poured into a petri dish. Within 5 minutes of pouring from each vial, the

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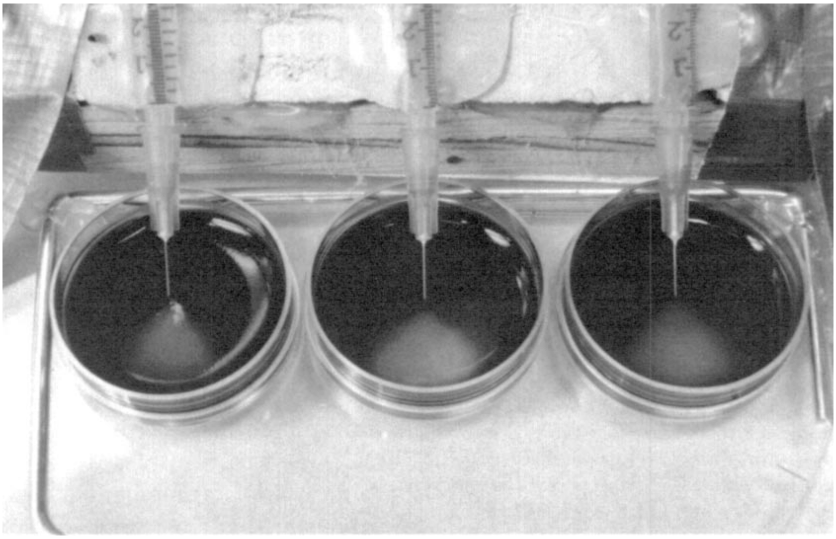

FIG. 1. Apparatus used to generate and verify v-shaped flow pattern of air containing 2.5% CO_2 across agar surface. Note color change of bromthymol blue caused by pH drop under the airflow.

percentage of 100 nematodes that moved spontaneously during 2 seconds of individual observation was determined. The 24-hour count included suspensions only at 0, 2.5, and 5% CO_2. The experiment was repeated once.

Attraction to CO_2 in agar: Suspensions of *R. reniformis* were mixed 1:1 with 1.5% water agar at 41 C and dispensed into 35-mm-d petri dishes (1.5 ml/dish, 1,500–4,000 nematodes/ml). Air, or air charged with 2.5% CO_2, was passed over water to increase humidity and blown (30 ml/minute) across the agar surface through a 28-gauge needle fastened at a 45-degree angle 2 mm above the center of the dish. Flow pattern from each needle was verified beforehand to be v-shaped by observing color changes induced by blowing air with 2.5% CO_2 onto agar containing bromthymol blue in 0.6 N NaOH (3), adjusted to pH 8 with HCl (Fig. 1). Nematode distribution within each dish was observed periodically and quantified after 55 minutes by removing a 5-mm-d agar disk from six predetermined positions relative to the flow pattern (Fig. 2). Nematodes in disks were dispersed into water and counted. The number of nematodes at position A (directly in front of the needle) and the average numbers of nematodes at positions B (two points downwind from A) and positions C (5 mm to either side of the flow pattern) were standardized by dividing by the number of nematodes at position D (1 cm behind the needle tip). Two experiments were done. In the first, three replications of dishes containing nematodes stored overnight at 17 or 30 ± 0.5 C were exposed for 55 minutes to air or to air charged with 2.5% CO_2. The experiment was done at ambient temperatures of 25, 28, and 31 ± 0.5 C. In the second experiment, six dishes containing freshly extracted nematodes were exposed to 2.5% CO_2 blown from the needle as described. In three of the dishes, gentle heat was applied to a 6-mm-d spot directly in front of the needle by a fiber optic positioned 2 mm above the agar. Temperature and pH changes in the dish were measured with a tissue implantation thermistor and pH microelectrode. The experiment was done twice qualitatively (no agar cylinders removed) and once quantitatively. All experiments were analyzed as completely randomized designs.

Baermann funnel extractions

The Baermann apparatus (Fig. 3, diagram A) was a 15-cm-d polyethylene funnel full of water, a latex drain tube with clamp, and a cylindrical ring cut from polyvinyl

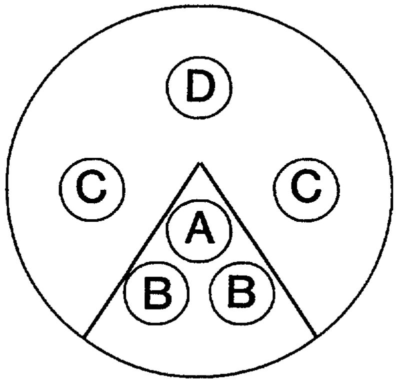

FIG. 2. Diagram of positions within 35-mm-d petri dish where 5-cm-d agar disks were removed to measure nematode response. Diagonal lines indicate edge of v-shaped region in which CO_2 concentration was increased by blowing air containing 2.5% CO_2 onto agar surface.

chloride pipe (4.5 cm high, 10 cm d) with two layers of two-ply facial tissue stretched across its bottom and secured with a rubber band (15). A 100-g soil sample in a layer ca. 1 cm deep was loaded into each ring and the ring was lowered into the funnel so that the soil became covered by water 2 cm deep. Soil for each experiment was a silt loam (6% sand, 70% silt, 24% clay) or a sandy clay loam (67% sand, 12% silt, 21% clay) in which *R. reniformis* had been propagated for several generations on tomato. Soil population density varied substantially during the course of the study; however, the soil for each experiment was thoroughly mixed before use. Unless otherwise indicated, water used in the funnels was purified by reverse osmosis and supplemented with 3.4 mM $CaCl_2$ to reduce clay dispersion (15). Temperature was measured with a surface thermistor (Yellow Springs Instruments Co., Yellow Springs, Ohio), the lead wire of which was bent to permit contact with the upper surface of the soil layer or with the under surface of the soil-retaining tissue. Carbon dioxide was measured in 200-μl aliquots from funnels with a CO_2 microelectrode (Microelectrodes, Londonderry, New Hampshire). The electrode was calibrated against water in equilibrium with breathing-grade air and with air containing 2.5% CO_2 by volume. For CO_2, temperature, and pH, the definition of gradient was the measurement below the soil minus the measurement above. Each experiment was a completely randomized design, and differences between treatments were compared with LSD, unless otherwise indicated. Nematode counts were log transformed prior to analysis.

Naturally occurring CO_2 gradients in Baermann funnels: The first experiment (Fig. 3, diagram B) included three replications of uncovered and loosely covered funnels containing silt loam with 0, 3.4, or 34 mM $CaCl_2$ added to funnel water. After 5 and 24 hours, CO_2 concentrations were measured in water at the upper soil surface and at the under surface of the retaining tissue in each funnel. Nematodes were counted in a 1:10 dilution of a 15-ml sample drained from each funnel. Repeated drainings of 15-ml aliquots at 24 hours indicated that the first aliquot drained at 24 hours usually contained more than 90% of the nematodes extracted. The experiment was repeated, but with measurements only at 24 hours.

The second experiment included three replications of four kinds of funnels with silt loam soil, each immersed in a water bath (Fig. 3, diagram C): uncovered funnel, loosely covered funnel, uncovered funnel heated above with a light bulb, and covered funnel chilled above with a copper cold water coil. Water bath temperatures were adjusted to maintain median soil temperatures of 23.6 ± 0.5 C. After 5 and 24 hours, temperature and CO_2 gradients were measured and nematode samples were drained.

Independent control and reversal of vertical gradients of CO_2 and temperature: Three preliminary tests were done with silt loam to develop a method to control CO_2 gradients. Each included three replications of uncovered and loosely covered funnels with and without a vinyl capillary tube positioned in the water near the bottom of the funnel or in the air pocket under the cover (Fig. 3, diagram D). The tissue supporting the soil was reinforced with a layer of

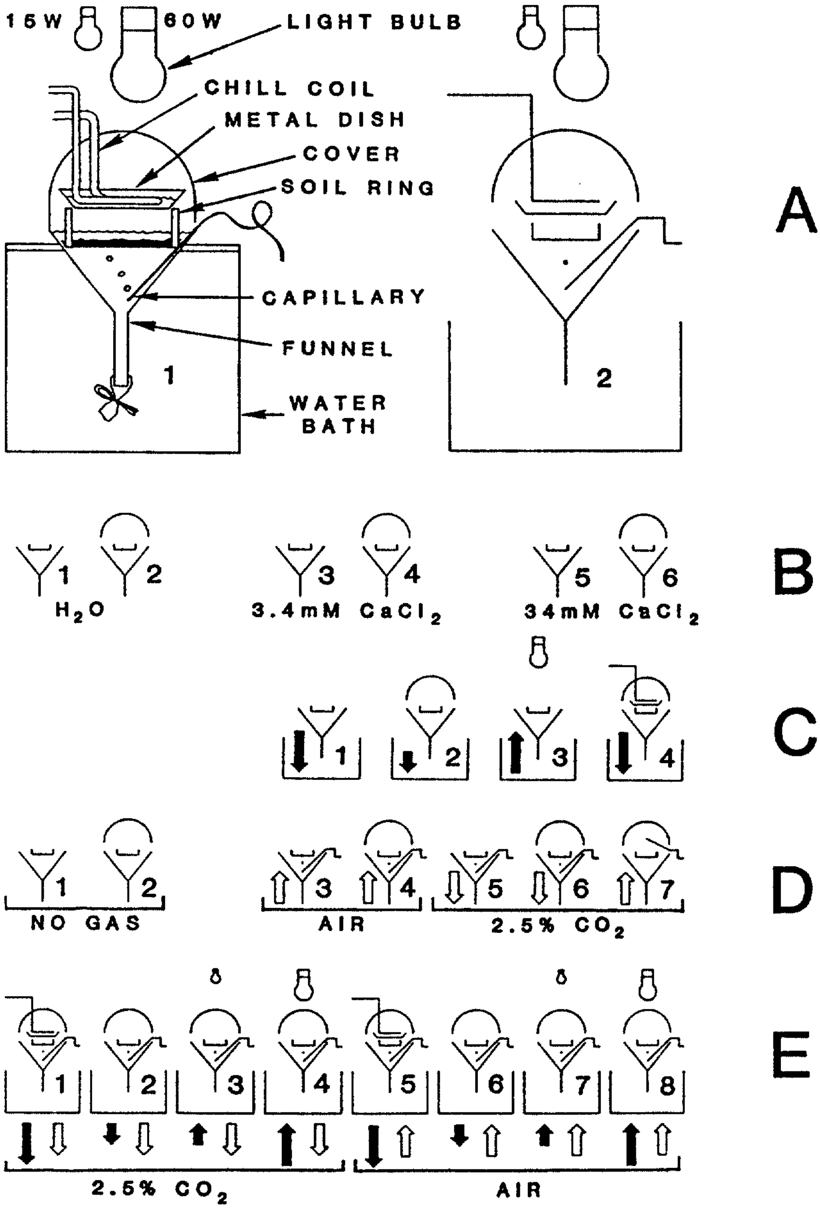

FIG. 3. Baermann funnel modifications compared for effects on extraction of *Rotylenchulus reniformis.* A) Detail of components and corresponding diagram of symbols used to depict components in diagrams B–E. B) Effects of covering and of $CaCl_2$ in funnel water. C) Effects of temperature gradient control. D) Control of CO_2 gradients without temperature control. E) Simultaneous control of temperature and CO_2 gradients. Where temperature and (or) CO_2 gradients were controlled, arrows indicate the direction in which temperature (solid arrow) or CO_2 (open arrow) increased. Relative magnitude of temperature increase is indicated by arrow length.

cheesecloth. Air or air charged with 2.5% CO_2 was gently blown through each capillary at 30 ml/minute; mechanical agitation of the soil was not noticeable. After 5 and 24 hours, temperature, CO_2, and pH gradients were measured in every funnel. After 24 hours, capillaries and soil rings were removed, nematodes were allowed to settle for 4 hours, and nematode samples were drained. Each test was done on a different day with a new collection of soil. Daily nematode counts were divided by the

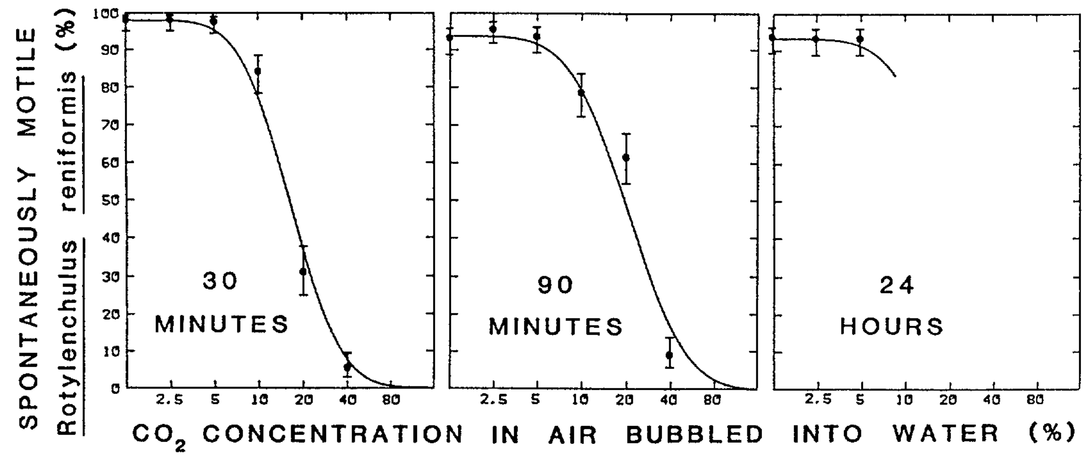

FIG. 4. Anesthetic effect of CO_2 on *Rotylenchulus reniformis* after three exposure periods. Each percentage is the mean of two experiments and is based on 100 randomly selected nematodes per experiment. Nematode suspensions included juveniles, males and preparasitic adult females, which were counted collectively. Brackets indicate binomial confidence limits ($P = 0.05$).

average number extracted from control funnels, which were covered and had no capillaries.

To independently control and reverse vertical gradients of CO_2 and temperature within Baermann funnels, a funnel was devised with a loose cover, a capillary opening near the bottom of the funnel for gas delivery, reinforcement of the tissue with cheesecloth, and a water bath. Funnels were optionally equipped with a 60 W or 15 W light bulb above for heat or a copper chill coil suspended in water within an aluminum dish crimped on top of the soil retaining ring (Fig. 3, diagram E). These modifications maintained temperature differences through the soil layer of ca. + 2 C, + 0.2 C, − 0.2 C, or − 2 C. Water bath temperatures were adjusted to compensate for heating from above or chilling to achieve median soil temperatures of 24 ± 0.5 C in most cases. Each funnel received 30 ml/minute air or air charged with 2.5% CO_2 through the capillary.

The first two of four experiments included three replications of funnels containing silt loam soil with positive and negative CO_2 gradients combined with positive or negative temperature gradients of ca. 2 C through the soil layer. The third experiment substituted sandy clay loam soil for silt loam soil. These experiments were of a completely randomized design. The fourth experiment included seven replications through time (one each day) with silt loam soil collected on that day and treated as in experiments 1 and 2, plus a duplicate set of funnels that had temperature gradients of + or − 0.2 C instead of + or − 2 C. This experiment was analyzed statistically as a randomized complete block with data from each soil collection as a separate block. In each experiment, temperature and CO_2 gradients were measured in every funnel after 24 hours. Then capillaries and soil retaining rings were removed, nematodes allowed to settle for 4 hours, and nematode samples drained.

RESULTS

Direct observations of CO_2 effects

Anesthesia threshold: Five percent CO_2 (20% O_2, 74% N_2) did not measurably decrease spontaneous motility of *R. reniformis,* but 40% CO_2 (13% O_2, 47% N_2) stopped most movement (Fig. 4). Loss of motility was apparent within 30 minutes. Exposure to a 1:1 mixture of air and N_2 (10.5% O_2) did not decrease motility; thus, the anesthetic effects of CO_2 were not the result of decreased O_2 concentration.

Attraction to CO_2 in agar: In most cases, nematodes accumulated in front of gas de-

TABLE 1. Effects of ambient and overnight storage temperature on accumulation of *Rotylenchulus reniformis* in agar when air with or without 2.5% CO_2 was blown onto the agar surface in a v-shaped pattern.

		Relative nematode density†			
		Air		Air + 2.5% CO_2	
Ambient temperature	Position‡	17 C	30 C	17 C	30 C
25 C	A	3.0***	0.9	7.6***	2.1
	B	0.8	1.2	1.5	1.4
	C	0.8	1.2	0.9	0.9
28 C	A	1.7*	1.4	6.4***	8.4***
	B	1.0	1.2	1.3	1.8
	C	1.0	1.0	1.0	0.9
31 C	A	4.8***	3.3***	10.0***	9.6***
	B	1.4	1.2	1.2	1.0
	C	0.9	1.1	0.8	0.7

Each number is the mean of three replications. *, *** indicate 1 LSD greater than 1.0 at $P = 0.05$ and 0.001, respectively.
† Relative nematode density is the number of nematodes within a 5-mm-d agar cylinder at one of the three positions indicated divided by the number of nematodes at a fourth position, D.
‡ A = directly under the airflow. B = within the spray pattern downwind from A. C = out of airflow 5 mm to either side of A.

livery needles within 55 minutes, whether air with 2.5% CO_2 or without CO_2 was emitted, suggesting that a cooling effect by the airflow contributed to accumulation (Table 1). In all cases, however, accumulation was greater, usually 2–4 times greater, when air contained 2.5% CO_2. Accumulated nematodes were highly motile, distributed within as well as on top of the agar, and did not appear trapped by water films. The agar dried noticeably after 1 hour. Juveniles, males, and preparasitic adult females appeared similarly attracted. Nematodes stored overnight at 30 C were attracted to the air source without CO_2 only at 31 C, the temperature at which the response to CO_2 was the strongest. They appeared repelled by evaporative chilling at 25 C ambient temperature. Nematodes stored overnight at 17 C accumulated in a chilled region relative to 25, 28, and 31 C, and thus moved toward their storage temperature. When agar directly in front of the delivery needle was heated with a fiber optic, agar temperature near the needle tip was elevated ca. 1 C (Table 2). Nematodes freshly extracted from soil accumulated whether agar near the source of 2.5% CO_2 was heated or chilled.

Baermann funnel extractions

Naturally occurring CO_2 gradients in Baermann funnels: When funnels with and without loose covers at three $CaCl_2$ concentrations in funnel water were compared, covering funnels in all cases increased

TABLE 2. Accumulation of *Rotylenchulus reniformis* and changes in temperature and pH in agar when air containing 2.5% CO_2 was blown through a capillary (30 ml/minute) across the agar surface in a v-shaped pattern, with or without application of gentle heat directly under the airflow with a fiber optic bundle.

	Relative nematode density†		Temperature (C)		pH	
Position‡	Agar heated	Agar not heated	Agar heated	Agar not heated	Agar heated	Agar not heated
A	6.2***	4.8***	22.4	18.9	6.6	6.9
B	1.4	1.4	22.2	19.9	6.6	6.9
C	0.9	1.0	21.8	19.9	6.9	7.1
D	1.0	1.0	21.3	19.8	6.9	7.2

Each number is the mean of three replications. *** indicates 1 LSD greater than 1.0 at $P = 0.001$.
† Number of nematodes within a 5-mm-d agar cylinder at position indicated divided by number at position D.
‡ A = directly under the airflow. B = within the spray pattern downwind from A. C = out of airflow 6 mm to either side of A. D = out of airflow 1 cm behind A. (See Fig. 1.)

TABLE 3. Changes in CO_2 gradients and numbers of *Rotylenchulus reniformis* extracted from a silt loam soil as a result of loosely covering† Baermann funnels or adding $CaCl_2$ to funnel water.

Extraction time	$CaCl_2$ (mM)	Carbon dioxide (%) Conc. under soil Unc	Conc. under soil Cov	Net gradient‡ Unc	Net gradient‡ Cov	Nematodes extracted per 100 g soil Log$_e$ Unc	Log$_e$ Cov	Arithmetic mean Unc	Arithmetic mean Cov
Experiment 1 at 5 hours	0	0.1	0.2	0.1	0.1	3.3	4.8	27	133
	3.4	0.2	0.3	0.2	0.1	3.8	5.8	53	427
	34	0.2	0.2	0.2	0.1	4.0	6.3	53	567
LSD 0.05		0.06		0.07		1.0			
Experiment 1 at 24 hours	0	0.1	0.5	0.0	0.3	5.5	8.8	250	7,500
	3.4	0.2	0.4	0.2	0.4	6.9	9.0	1,010	8,350
	34	0.1	0.3	0.1	0.2	6.6	9.1	730	9,280
LSD 0.05		0.11		0.17		0.5			
Experiment 2 at 24 hours	0	0.3	0.4	0.1	0.2	5.8	9.3	370	11,030
	3.4	0.3	0.5	0.2	0.5	6.2	9.1	650	9,410
	34	0.2	0.3	0.0	0.3	7.5	9.6	2,090	14,460
LSD 0.05		0.14		0.15		1.1			

Numbers are averages of three replications. Nematode counts were analyzed after log transformation.
† Cov = covered. Unc = uncovered.
‡ Measurement at bottom of soil layer minus measurement at top of soil layer.

nematode extraction after 5 and 24 hours by about one order of magnitude (5–30-fold) (Table 3). Calcium chloride addition also increased extraction in uncovered funnels, but to a much smaller extent. Measurable differences in CO_2 were not related to $CaCl_2$ addition. In all funnels, more CO_2 accumulated in the water under the soil layer than above it. The net CO_2 gradient that resulted after 24 hours was on the order of 0.2%/cm and about two times stronger in covered than in uncovered funnels.

When temperature but not CO_2 gradients were controlled, the pronounced effect that covering status had on extraction efficiency was reversed by changing the temperature gradient (Table 4). At the same median soil temperature, large numbers of nematodes were extracted from uncovered funnels heated above and small numbers were extracted from covered funnels chilled above. At 24 hours, the unchilled covered funnels and the heated uncovered funnels had the greatest extraction efficiency, the least chilling above the soil layer, and the strongest positive CO_2 gradients.

Independent control and reversal of vertical gradients of CO_2 and temperature: Bubbling 2.5% CO_2 or air into the water below the soil controlled the direction of the net CO_2 gradient through the soil layer (Tables 5, 6). Air purged CO_2 from the water so that CO_2 accumulation in the unpurged water above the soil caused a negative gradient. Bubbling air with 2.5% CO_2 increased the CO_2 concentration below the soil resulting in a positive gradient. The pH of funnel water (Table 5) was higher below the soil layer in all funnels and was unrelated to nematode extraction or to the direction of CO_2 change. In uncovered funnels (Table 5), temperature gradients were appreciably altered by evaporative chilling during bubbling, making the effects of CO_2 on nematode extraction difficult to distinguish from the effects of temperature. In covered funnels, however, temperature gradients were changed by no more than 0.2 C/cm. Covered funnels with CO_2 bubbled below the soil layer yielded 2.8 times as many nematodes as did covered controls, and covered funnels with air bubbled below the soil layer yielded less than half as many nematodes as covered controls.

When gradients of CO_2 and temperature were independently controlled and reversed in covered funnels, funnels heated above usually yielded 2–4 times as many

TABLE 4. Changes in CO_2 gradients and numbers of *Rotylenchulus reniformis* extracted from a silt loam soil when temperature gradients in covered and uncovered Baermann funnels were controlled by immersing funnels in water baths and applying heat or cooling to the tops of funnels.

		Temperature (C) for run #1		CO_2 (%) for run #1		Nematodes extracted per 100 g soil			
						Run #1		Run #2	
Funnel†	Diagram (Fig. 3)	Net gradient‡	Median	Above soil layer	Under soil layer	Log_e	Arithmetic mean	Log_e	Arithmetic mean
		Collection after 5 hours							
Unc	C1	2.8	24.0	0.3	0.5			3.9	47
Cov	C2	0.1	23.8	0.7	0.5			5.8	323
Unc + light bulb	C3	−1.9	23.1	0.3	0.4			5.7	287
Cov + chill coil	C4	2.4	23.7	0.2	0.4			3.1	23
	LSD 0.05	0.6	0.7	0.2				0.6	
		Collection after 24 hours							
Unc	C1	2.4	24.1	0.3	0.5	7.5	1,880	5.0	157
Cov	C2	0.1	23.9	0.4	0.8	9.0	8,160	8.5	5,007
Unc + light bulb	C3	−1.7	23.8	0.2	0.6	9.0	8,253	8.6	5,603
Cov + chill coil	C4	2.1	23.7	0.2	0.3	7.1	1,280	4.8	123
	LSD 0.05	0.4	0.8	0.3		0.6		0.4	

Numbers are averages of three replications. Nematode counts were analyzed after log transformation.
† Cov = covered; Unc = uncovered.
‡ Temperature at under surface of soil-retaining tissue minus temperature at upper surface of soil layer.

TABLE 5. Changes in temperature, CO_2, pH, and numbers of *Rotylenchulus reniformis* extracted from a silt loam soil when air or air charged with 2.5% CO_2 was bubbled into Baermann funnel water below the soil layer or blown into the funnel covers at a rate of 30 ml/minute.

	Net gradients†						Nematodes extracted after 24 hours	
	After 5 hours			After 24 hours				
Treatment	Temp (C)	CO_2 (%)	pH	Temp (C)	CO_2 (%)	pH	Log_e	% of covered
			Experiment 1					
Covered	0.2	−0.2	0.7	0.2	0.2	0.4	7.7	100
+ CO_2 under	0.1	0.8	1.1	0.2	1.1	0.6	8.7	280
Uncovered	1.2	−0.2	0.7	1.1	−0.1	0.4	5.9	16
+ air under	0.6	−0.3	0.8	0.5	−0.2	0.4	6.7	35
LSD 0.05	0.3	0.5	0.7	0.3	0.4	0.4	0.5	
			Experiment 2					
Covered	0.3	0.0	0.7	0.2	0.6	0.2	7.9	100
+ air under	0.1	−0.6	0.7	0.1	−0.1	0.4	7.0	43
Uncovered	1.3	0.0	0.6	0.9	0.0	0.2	6.4	22
+ CO_2 under	0.7	1.9	0.6	0.4	1.4	0.3	7.2	50
LSD 0.05	0.5	0.3	0.2	0.3	0.9	0.2	1.1	
			Experiment 3					
Covered	0.3	0.1	0.3	0.2	0.3	0.1	8.6	100
+ CO_2 over	0.5	−0.3	0.3	0.2	0.0	0.1	8.2	68
Uncovered	1.2	0.0	0.3	1.4	0.1	0.1	6.9	19
LSD 0.05	0.5	0.2	0.4	0.6	0.3	0.4	0.6	

Numbers are means of three replications.
† Measurement just under soil layer minus measurement just above soil layer.

TABLE 6. Changes in numbers of *Rotylenchulus reniformis* extracted from a silt loam soil when combinations of positive and negative temperature and CO_2 gradients were imposed through the soil layer in Baermann funnels.

	Experi-ment	CO₂ gradient† and funnel‡								LSD 0.05
		+ E 1	− E 5	+ E 2	− E 6	+ E 3	− E 7	+ E 4	− E 8	
Median soil temp. (C)	1	23.7	23.7					24.6	24.2	0.8
	2	23.6	24.0					25.4	24.7	0.7
	3	23.6	23.7					25.4	25.0	0.6
	4	24.4	24.5	24.4	24.4	24.3	24.3	24.1	23.9	0.2
Net temp. gradient (C)	1	2.2	2.2					−1.6	−1.6	0.9
	2	2.2	1.7					−2.5	−1.8	0.6
	3	2.2	2.1					−3.0	−2.2	0.5
	4	1.7	1.6	0.2	0.2	−0.1	−0.2	−1.6	−1.5	0.4
Net CO_2 gradient (%)	1	1.4	−0.4					1.1	−0.4	0.4
	2	1.1	−0.2					0.6	−0.4	0.6
	3	0.8	−0.1					1.1	−0.4	0.2
	4	1.4	−0.6	1.3	−0.4	0.8	−0.6	1.6	−0.6	0.4
Nematodes extracted (Log_e Y)	1	6.9	7.0					8.5	7.8	0.6
	2	8.4	8.4					9.3	9.2	0.3
	3	6.1	6.7					7.5	7.2	0.6
	4	8.5	8.6	9.2	8.8	9.3	9.1	9.3	9.2	0.6
Nematodes extracted (mean)	1	1,000	1,140					4,850	2,490	
	2	4,680	4,640					11,040	9,760	
	3	480	860					1,820	1,480	
	4	6,560	5,850	10,690	7,640	11,370	9,910	12,270	11,270	

Numbers given for experiments 1–3 are the means of three replications. Numbers given for experiment 4 are the means of seven replications.
† Measurement just under soil layer minus measurement just above soil layer.
‡ Funnel modifications E 1–8 depicted in Fig. 3.

nematodes as did funnels chilled above (Table 6). These differences were appreciably smaller than the 5–30-fold differences associated with temperature in previous experiments, suggesting that mechanical agitation from bubbling increased extraction in funnels that had behaviorally unfavorable gradients. However, comparing unagitated covered and uncovered control funnels in one experiment revealed only a twofold increase due to covering. Thus, smaller extraction differences in this series of experiments appeared inherent to the soil collections used. In every experiment, all funnels with negative or with weakly positive (+ 0.2 C/cm) temperature gradients (i.e., gradients comparable to those in standard covered funnels) yielded more nematodes when 2.5% CO_2 was bubbled than when air was bubbled. These differences averaged less than 30% and were significant only for paired comparisons of the log-transformed nematode counts among all replications across experiments (critical $t = 3.58$, significant at $P = 0.01$).

Discussion

In agar, vermiform developmental stages of *R. reniformis* strongly accumulated around a CO_2 source, provided temperature gradients and thermal adaptation were appropriate. Accumulation occurred at 2.5% CO_2, which is not anesthetic, and thus appears to result from attraction. Several other plant-parasitic and insect-parasitic nematodes are attracted to CO_2 in agar (1,6,8,9,11,13). Movement toward storage or adaptation temperatures in agar was reported previously for *R. reniformis* (14) and other species (2,4,5,7,16).

In Baermann funnels, manipulation of CO_2 gradients at subanesthetic concentrations had a much smaller effect on extraction efficiency than did the manipulation of temperature gradients. Favorable CO_2 gradients usually increased extraction by about 30% and never by more than threefold. Favorable temperature gradients increased extraction by 2–30-fold. This result suggests that, within funnels, behavioral responses to temperature are much stronger than responses to CO_2. Interactive effects of temperature and CO_2 on nematode behavior were examined previously for *D. dipsaci* and *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans Stekhoven in agar (9), but they have not been examined in soil, to our knowledge. Net gradients in funnels were well above the 0.01%/cm threshold measured for *M. incognita* (11) and the 0.1%/cm that attracts *D. dipsaci* in agar (9) and still failed to reverse temperature gradient effects. Appreciably stronger CO_2 gradients could have been generated only by introducing toxic concentrations.

Carbon dioxide gradients that occur naturally within Baermann funnels do appear to have some influence on the movement of *R. reniformis* and, most likely, other nematodes. The net vertical gradient is fortuitously in a favorable direction. The saturated soil layer undoubtedly has greater diffusive resistance than water, and CO_2 that evolves from the lower surface of the soil layer is trapped and accumulates. This effect is surely offset to some extent by the direct effect of evaporative chilling on CO_2 solubility in water above the soil. However, the relative solubility of CO_2 in water increases as temperature decreases by only about 1%/C; this is less than one-tenth the relative difference that was measured in most undisturbed funnels.

The pH of water in funnels was always higher below the soil than above it; this insensitivity of pH to CO_2 may result from buffering by clay in the water. Increased extraction due to addition of $CaCl_2$ to funnel water in these and previous (15) experiments remains unexplained. Calcium carbonate precipitation could cause behaviorally significant microscopic gradients of CO_2, pH, or Ca^{++}. *Meloidogyne javanica* (Treub) Chitwood, for example, is strongly repelled in sand by 10 mM $Ca(NO_3)_2$ (12).

Covering or otherwise modifying Baermann funnels to minimize or offset evaporative chilling may be of practical importance in some diagnostic and research laboratories. In two laboratories in Texas, we have consistently obtained markedly increased extraction efficiencies year-round for three root-parasitic nematode species (15) by covering funnels. In both laboratories, however, relative humidity is consistently low enough to cause appreciable temperature gradients in the funnels; a behaviorally significant degree of evaporative chilling may not occur in all laboratories or in all kinds of Baermann extractors. We did not obtain increased extraction of *T. semipenetrans* from soil by covering Baermann saucers and we have not tested for the covering effect on funnels of the various sizes that are used. Of greater interest, perhaps, is the reproducibility of the effect in the funnels we use, which indicates the response to temperature may be stronger than to any other stimulus present in numerous soil samples examined. The ecological roles of behavioral responses of nematodes to temperature in natural soil profiles may merit serious investigation.

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