The Influence of Environmental Factors on the Respiration of Plant-parasitic Nematodes[1]

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Abstract: Respiration of selected nematode species was measured relative to CO_2 level, temperature, osmotic pressure, humidity, glucose utilization and high ionic concentrations of sodium and potassium. In general, respiration was stimulated most by the dominant environmental factors at levels near those expected in the nematode's "natural" habitat. Soil-inhabiting nematodes utilized O_2 most rapidly with high (1–2%) CO_2 whereas a foliar nematode (*Aphelenchoides ritzemabosi*) did so with 0.03% CO_2, the concentration typically found in air. Temperature optima for respiration corresponded closely to those for other activities. *Ditylenchus dipsaci* and *Pratylenchus penetrans* adults and *Anguina tritici* and *A. agrostis* second-stage larvae respired within the range of osmotic pressures from 0 to 44.8 atm and respiration of their drought-resistant stages was stimulated by increasing osmotic pressure which accompanies the onset of drought. Rehydration of *A. tritici* and *A. agrostis* larvae with RH as low as 5% stimulated measurable respiration. Glucose utilization from liquid medium by *A. tritici* larvae or *A. ritzembosi* was not detectable. Supplemental Na^+ stimulated respiration of *Anguina tritici*, K^+ did not.
Key Words: Respiration, Temperature, CO_2 concentration, Osmotic concentration, Relative humidity, Ionic composition, *Anguina tritici, Anguina agrostis, Pratylenchus penetrans, Ditylenchus dipsaci, Aphelenchoides ritzemabosi.*

Plant-parasitic nematodes appear to be well adapted for surviving extremes of temperature, moisture stress and carbon dioxide concentration. The present study attempts to define the limits of these adaptations in terms of respiratory activity. Respiratory physiology of nematodes parasitic on animals has been extensively investigated (5, 18, 25), whereas only a few studies have been made on the respiration of freeliving nematodes. Overgaard-Nielsen (13) was first to use the Cartesian diver technique to measure the rate of respiration of several kinds of soil-borne nematodes. Santmeyer (20) showed candidate nematicides can be screened in the laboratory by measuring their effect on the respiration rate of *Panagrellus redivivus*.

Tolerance to desiccation varies greatly in different plant-parasitic nematode species (23, 27). Second stage larvae of *Anguina tritici* and *A. agrostis*, fourth stage larvae of *Ditylenchus dipsaci* and the adults of *Aphelenchoides ritzemabosi* are known to survive desiccation for many years (27). Little is known, however, regarding the exact degree of desiccation and the corresponding levels of metabolic activity of the desiccated stages of these nematodes. Wallace and Greet (28) reported the resistance of *Tylenchorhynchus icarus* to desiccation is accompanied by increased respiratory activity. Respiration rates of several species of plant-parasitic nematodes have been shown to be higher in air (0.03% CO_2) than in either a CO_2-free atmosphere or in higher concentrations of CO_2 (19). Carbon dioxide has been proposed as one of the factors which attract plant-parasitic nematodes to roots in soil (7).

The respiratory rates of some free-living nematodes have been recorded at different temperatures by Overgaard-Nielsen (13) and Santmeyer (20).

MATERIALS AND METHODS

Second stage (L_2) larvae of *Anguina tritici* (Steinbuch) Chitwood, were obtained from ten-year-old wheat galls and from one-year-old galls harvested in 1965. L_2 larvae of *Anguina agrostis* (Steinbuch) Filipjev, were

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TABLE 1. Oxygen consumption of dry larvae of *Anguina tritici* from one-year-old wheat galls during the fifth hour of exposure to different relative humidities at 25 C.

RH (%)	Respiration nl $O_2/\mu g/hr$
5	0.009
50	0.028
75	0.033
95	1.600
95 (24 hr) + 5 (5 hr)	0.021

obtained from bent grass seed galls harvested in 1965. Adults of *Pratylenchus penetrans* (Cobb) Chitwood and Oteifa, *Ditylenchus dipsaci* (Kühn) Filipjev, and *Aphelenchoides ritzemabosi* (Schwartz) Steiner, were obtained from monoxenic cultures maintained on alfalfa callus tissue growing in nutrient agar (9).

Wheat galls were surface sterilized in 2.5% sodium hypochlorite for 5 min and bent grass galls for 1–2 min and then rinsed several times with sterile distilled water. Galls were opened in sterile distilled water and the larvae obtained treated with 0.05% (w/v) dihydrostreptomycin sulfate and 0.05% (w/v) neomycin sulfate for 5 min with a final rinse in sterile distilled water. Nematodes were aseptically extracted from callus tissue during the 20 hr immediately preceding respiration measurements.

Respiration was measured by three methods: the Cartesian diver technique, the Warburg direct method, and Gilson's differential method.

For most experiments, a Cartesian diver microrespirometer was used similar in design to that of Holter and Linderstrom-Lang (6). Divers having a total volume ranging 8–15 μl were constructed from pyrex glass capillaries. Five to ten nematodes were placed in 0.5 to 1.00 μl of medium in the bulb of each diver and the neck seals were added.

With the Warburg and Gilson respirometers, 7-ml flasks were used. Each flask received 8,000 to 180,000 nematodes and

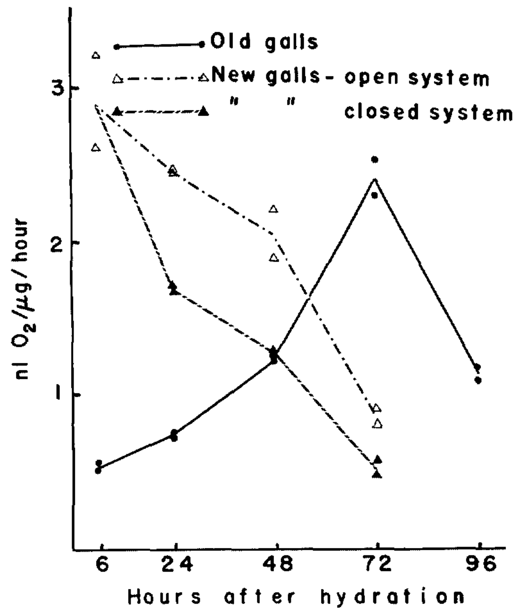

FIG. 1. Respiratory rates of second stage larvae of *Anguina tritici* after removal from one-year-old galls and placement in water. In a closed system, the same respirometer vessel was used for 72 hours, in an open system, a new vessel was prepared each day. Each point is the mean of three determinations. Rates for new galls are significantly different at 24 and 48 hours.

was shaken at 95 strokes per min with a stroke length of 4.5 cm. Except where otherwise stated, all measurements were made at 22 ± 0.01 C.

Nematodes, glassware and media were aseptic at the beginning of each experiment. A blank containing the same medium from which nematodes had been removed, was run with each experiment. Gas consumption in these occurred only very rarely, and when it did the experiment was discarded. Contamination was also detected by directly plating the nematode suspension liquid on potato dextrose agar at 35 C. If more than 50 colonies per ml of medium were counted after 48 hr incubation, the experiment was discarded.

TABLE 2. Respiration rates of L_2 larvae of *A. tritici* from wheat galls from May 1965 harvest six hours after hydration.

Measured (mo/yr)	Respiration nl $O_2/\mu g/hr$
July, 1965	2.929
August, 1965	1.484
February, 1966	1.221
March, 1966	1.031
July, 1966	0.882

Rate of respiration has been expressed as nl oxygen/μg dry weight/hr. Dry weight was determined for each species both as suggested by Roberts (16) and by Myers and Krusberg (12). For each dry weight determination, 1,000 to 15,000 nematodes were used and each was repeated five times.

RESULTS

REHYDRATION: To study the influence of humidity alone on respiration, *A. tritici* larvae taken directly from galls were placed inside the divers and the concentration of sodium hydroxide in the neck seal of the diver was changed to produce the desired relative humidity (10).

Respiration of L_2 larvae of *A. tritici* was measurable in relative humidities as low as 5% but below 95% RH, O_2 consumption was very low (Table 1). During the first six hr after larvae were exposed to 95% RH, the rate of oxygen consumption gradually increased. The initial rate of respiration of *A. tritici* larvae from ten-year-old wheat galls was lower than that from one-year-old galls (Fig. 1).

When *A. tritici* larvae from old galls were placed in water, their rate of respiration rose for 72 hr and then decreased (Fig. 1). The rate of respiration of larvae from one-year-old wheat galls was highest 6 hr after hydration after which it dropped rapidly (Fig. 1). With the methods used, respiration measurements could not be made earlier than 6 hr

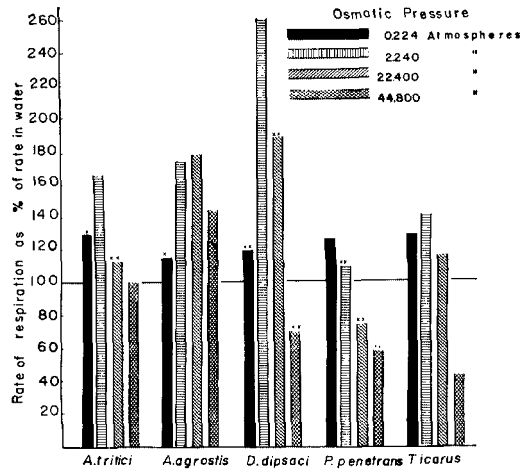

FIG. 2. Respiratory rates of plant-parasitic nematodes in urea solutions. Each value is the mean of 10 determinations. Crosses over bars indicate significant differences between adjacent bars (x = .05 level, xx = .01 level). Values for *Tylenchorhynchus icarus* calculated from Wallace and Greet, 1964.

after removal of larvae from galls. The most rapid decrease was noted when larvae were placed in Cartesian divers and respiratory measurements were made for 72 hr without changing the neck seals during this period. Such an arrangement may be called a "closed system." On the other hand, if divers were emptied daily and reloaded with new larvae from the original population stored in water (to provide an "open system"), a different response was obtained (Fig. 1) and the respiratory rate did not fall as rapidly.

Larvae from old galls became motile about 60 hr after being placed in water, whereas larvae from one-year-old wheat galls were active in 4–6 hr. The rate of respiration of L_2 larvae of *A. tritici* recovered from one-year-old galls decreased sharply during the first few months after harvest and then declined more gradually (Table 2). The time required for these larvae to resume activity after hydration gradually increased during the year following harvest.

TABLE 3. Influence of osmotic pressure on oxygen consumption of nematodes (nl/μg/hr).

Solution	Respiration (oxygen consumption) 2.24 atm	22.4 atm
Anguina tritici (from one-year-old galls, hydrated 6 hr)		
Urea	1.710 a[1]	1.168 b
D-Mannitol	1.684 a	
Sodium chloride	1.810 a	1.429 a
Potassium chloride	1.020 b	0.390 c
A. agrostis (from one-year-old galls, hydrated 6 hr)		
Urea	2.112 a	2.140 a
Sodium chloride	2.740 b	2.840 b
Ditylenchus dipsaci		
Urea	6.309 a	4.500 b
Pratylenchus penetrans		
Urea	5.690 a	3.809 c
D-Mannitol	5.121 b	

[1] For each species, values followed by the same letter do not differ significantly at the 5% level.

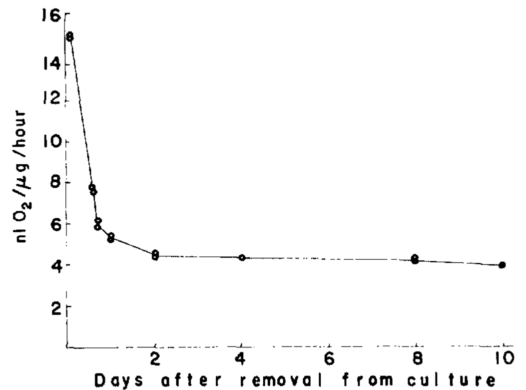

FIG. 3. The effect of storage in aerated distilled water on respiration of *Pratylenchus penetrans*. Each point is the mean of three determinations.

OSMOTIC PRESSURE AND IONIC COMPOSITION OF MEDIUM: To study the effect of osmotic pressure on respiration, 0.01M, 0.1M, 1.0M and 2.0M urea and D-mannitol solutions were used to produce osmotic pressures of approximately 0.224, 2.24, 22.4 and 44.8 atm, respectively. NaCl and KCl solutions were used to study the osmotic effect and also the influence of sodium and potassium ions on respiration.

The rate of respiration of *D. dipsaci* adults and *A. tritici* and *A. agrostis* larvae increased as the osmotic pressure of the medium was raised from zero to 2.24 atm. The highest rate of respiration for *P. penetrans* was at an osmotic pressure 0.224 atm (Fig. 2). Osmotic regulation at 2.24 atm with D-mannitol in place of urea yielded the same respiration response data with *P. penetrans* and L_2 larvae of *A. tritici* (Table 3).

Sodium chloride osmo-regulation (2.24 atm and 22.4 atm) increased. The rates of respiration of L_2 larvae of *A. tritici* and *A. agrostis* were the same in isotonic (2.24 or 22.4 atm) sodium chloride or urea solutions; but those of *A. tritici* in potassium chloride solutions were significantly less than in isotonically comparable urea or sodium chloride solutions. In 0.5M KCl, the rate of respiration was only 27% of the rate attained in an isotonic NaCl solution (Table 3).

NUTRIENTS AND STARVATION: Three attempts were made to determine whether the addition of glucose to the medium from the side arm of the Warburg flask would increase the rate of respiration of L_2 larvae of *A. tritici* from one-year-old wheat galls. A single similar attempt was made with adults of *A. ritzemabosi*. No respiration increase was detected for either of these two species after the addition of 0.1 mg/ml glucose.

The respiratory rate of *P. penetrans* adults dropped sharply for 20 hr after their removal from culture, reaching 50% of the original rate after 15 hr. Forty-eight hr after removal of nematodes from culture, respiration was nearly constant at ⅓ the original rate (Fig. 3). The respiratory rate of *Tylenchorhynchus claytoni* (Steiner) decreased similarly over a period of 5 days after removal from culture.

CARBON DIOXIDE: To study the effect of carbon dioxide concentration on respiration, Krebs' carbon dioxide buffer (8), bubbled overnight with the desired CO_2 concentration,

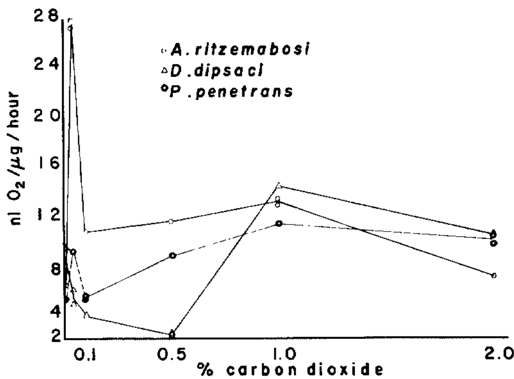

FIG. 4. The influence of CO_2 concentration on respiration of nematodes. Each point is the mean of four determinations.

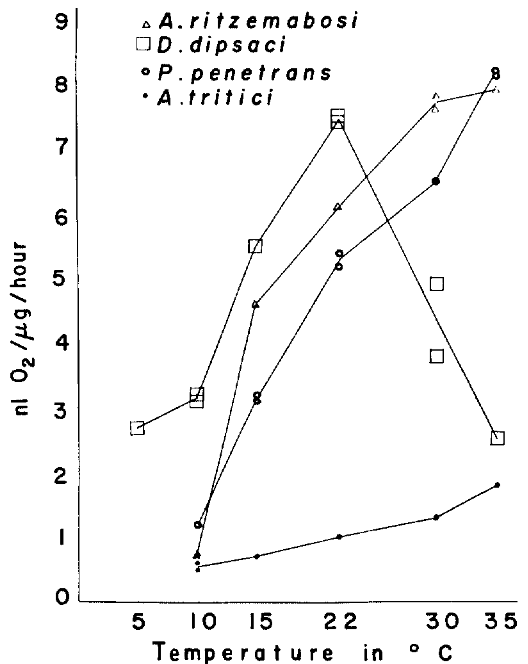

FIG. 5. The influence of temperature on respiration of nematodes. Each point is the mean of four determinations.

was used in place of the sodium hydroxide seal in the neck of the Cartesian diver.

The rate of respiration of *A. ritzemabosi* and *P. penetrans* was higher in air (0.03% CO_2) than in the total absence of CO_2 in the gaseous phase. The Q_{O_2} of *D. dipsaci* decreased as the CO_2 concentration was increased from 0.03 to 0.1% and the respiratory rates of *P. penetrans* and *A. ritzemabosi* dropped significantly. The respiratory rate of *D. dipsaci* rose more than 6-fold when the CO_2 concentration was increased from 0.5 to 1.0%. An increase of CO_2 concentration from 1 to 2% decreased the respiratory rate of *D. dipsaci* and *A. ritzemabosi*, but not of *P. penetrans* (Fig. 4).

All three species were actively motile during eight hr exposure to CO_2 concentration from 0.03 to 2.0%.

EFFECT OF TEMPERATURE ON THE RATE OF RESPIRATION: The rate of respiration of adults of *P. penetrans* and *A. ritzemabosi* and L_2 larvae of *A. tritici* and *A. agrostis* increased as the temperature was raised from 10 to 35 C. The rate of respiration of *D. dipsaci* remained high even at 5 C, but decreased sharply as temperature was raised above 22 C. The respiratory rates of other species were not detectable at 5 C (Fig. 5).

DISCUSSION

Respiratory rates of the selected plant-parasitic nematodes studied fell within the range of Q_{O_2} values known for nematodes parasitic in animals; however, it must be kept in mind that respiratory rates for animal-parasitic nematodes are usually measured at 37 C and those of plant-parasitic nematodes in the present studies were determined at 22 C.

Plant-parasitic nematodes may often be exposed to greater ranges of temperature, carbon dioxide concentration, osmotic pressure, and moisture than are most animal-parasitic nematodes. Osmotic pressure of a tomato root may vary from 5 to 16 atm (22). Osmotic pressure and CO_2 concentration in soil may change sharply after a rain or as a result of drought. It is probable that each nematode species has developed tolerances

to different degrees of environmental stress. Plant-parasitic nematodes pass at least part of their life cycle in soil, where they live in water films surrounding soil particles (27). Carbon dioxide produced as a result of respipiration diffuses out of water films slowly. Moreover, CO_2 is readily soluble in water, resulting in a high concentration of CO_2 in the soil environment to which soil-borne nematodes appear to be adapted.

Respiration of plant-parasitic nematodes is reduced in the absence of carbon dioxide. The rate of oxygen consumption of plant-parasitic nematodes tends to decrease after about 5 hr storage inside Cartesian divers with a potassium hydroxide neck seal (2). A relatively low rate of respiration of *A. tritici* larvae, when measured by using the same divers without changing the neck seals during a 72-hr period (closed system, Fig. 1) and may be due to total absence of CO_2 inside the divers for long periods. A similar inhibition of respiration in *P. penetrans* in the absence of CO_2 was reported by Rohde (19). Such a decline in the respiratory rate cannot be explained on the basis of a drop in O_2 tension within the respiration vessel. In the present studies the oxygen tension inside the Cartesian divers did not fall more than 10% in 6 hr. The oxygen tension in divers with CO_2 buffer was even lower, and yet nematodes in these divers respired at a higher rate.

P. penetrans passes its life cycle in soil or in root lesions only a few cells deep. The lesions are invaded by soil microorganisms so that CO_2 concentration in the environment of these nematodes is likely to be high, and the nematode appears to be stimulated by these high concentrations. Fleshy tissues invaded by *D. dipsaci* are the site of hydrolytic enzyme activity and are high in CO_2. On the other hand, respiration of *A. ritzemabosi* is highest in air, suggesting an adaptation conditioned by its life in leaves and other above-ground parts of plants, where CO_2 concentrations are closer to that of the atmosphere.

Adults of *P. penetrans* and *D. dipsaci* and second stage larvae of *A. tritici* and *A. agrostis* respire within a range of osmotic pressures from 0 to 44.8 atm. *P. penetrans*, a typical plant-parasitic nematode, respires maximally at 0.224 atm osmotic pressure, which is close to the range of optimum osmotic pressure for some of the animal-parasitic nematodes (22). The tolerance range of *P. penetrans* is wide on either side of 0.224 atm. Even at 2.24 atm its rate of respiration is higher than in distilled water. The high respiratory rate of plant-parasitic nematodes over a wide range of osmotic stress suggests there may be an osmoregulatory mechanism (15). The presence of such a mechanism in *Heterodera* and *Meloidogyne* has been suggested by Dropkin (4).

Second stage larvae of *A. tritici* and *A. agrostis* and pre-adults of *D. dipsaci* are well adapted to resist drying and the increased respiration of these nematodes at 2.24 to 44.8 atm osmotic pressure may reflect extra work necessary to retain or replace water.

At the stage when the surrounding medium as well as the tissues of the nematodes are dried up, osmotic stress is probably no longer a problem for drought-resistant nematodes in the absence of free water. Nematodes in a state of anhydrobiosis, such as *A. tritici* and *A. agrostis* in galls have a measurable respiration, but is not possible to say how much oxygen intake was due to the plant tissue also present.

The increase in rate of respiration of *A. tritici* larvae during increase in relative humidity from five to 95 percent is not logarithmic, as has been reported for the drought-resistant tardigrade, *Macrobiotus hufelandi* (14). The respiration rate of *A. tritici* larvae remains very low in humidities below 95% RH. When desiccated larvae of *A. tritici* are exposed to a given humidity, there is a linear

rise in the rate of respiration (Table 1). This might arise because, as the moisture penetrated the mass of larvae, more and more larvae began to respire, or because the metabolic rate of individual larvae increased as their tissues absorbed more water.

A. tritici larvae from old galls take longer (72 hr) to reach the level of highest metabolic activity than do larvae from one-year galls (approximately 6 hr). This might be because the former are more dehydrated during storage inside the wheat galls. The initial high rate of respiration after hydration of larvae might indicate an oxygen debt, which could be expected in close-packed, desiccated storage conditions.

A fall in the rate of respiration of *A. tritici* larvae, after an initial rise, may be due to either starvation or aging, whose effects on Q_{O_2} are difficult to separate (23). Similarly, aging and starvation may be responsible for the fall in the respiration rate of *P. penetrans* and *T. claytoni* stored in distilled water. Fall in the rate of respiration of *P. penetrans* stored in distilled water after removal from culture follows the pattern obtained by Rohde (19) for this nematode after removal from soil. The rates of respiration of some free-living soil nematodes (*Dorylaimus obtusicaudatus, Mononchus papillatus, Plectus granulosus, Pontonema vulgare*) have been reported, however, to remain stationary over a seven day period following extraction of the nematodes from the soil (13).

No stimulation of respiration of adults of *A. ritzemabosi* or second stage larvae of *A. tritici* resulted from addition of glucose to the medium. Plant-parasitic nematodes normally feed by piercing cell walls with the stylet. Possibly these nematodes are unable to ingest exogenous glucose without tactile stimulation as is required by some animal-parasitic nematodes (17).

Potassium ions do not stimulate respiration of L_2 larvae of *A. tritici* as do sodium ions. A 0.5 M potassium chloride solution inhibits respiration. In this respect *A. tritici* larvae resemble *Litomosoides carinii* (3) and differ from the larvae of *Eustrongyloides ignotus* (24).

The rate of most biological processes, including respiration, increases with an increase in temperature, reaching a maximum, beyond which the rate of activity tends to decline. The temperature vs. respiratory rate curves (Fig. 7) for several plant-parasitic nematodes show that the optimum temperature for respiration varies with the species. The temperature optima for respiration are very near those for other activities such as motility, infectivity, and reproduction of a given nematode species. The optimum temperature range for motility and reproduction of *D. dipsaci* is 15–20 C (1, 21, 26) which coincides with the temperature at which the rate of respiration is maximum. Adaptation of this nematode to low temperature is shown in its high rate of respiration at 5 C. *A. ritzemabosi* is found in temperate climates (29) and its highest rate of respiration was in the range of 30 to 35 C. The highest rate of respiration of *P. penetrans* occurred at 35 C, which coincides with the optimum soil temperature for activity of a related species, *P. minyus* (11).

Temperature not only has a direct bearing on the rate of biological processes, but influences other environmental factors as well. Solubility of CO_2 in water decreases with increase in temperature. On the other hand, CO_2 concentration in soil may increase at higher temperatures because of higher microbial activity. In soil, higher temperatures may result in a long term rise in osmotic pressure, due to increased evaporation of water. Osmotic pressure inside plants tends to drop slightly with a rise in temperature.

Aphelenchoides ritzemabosi inside aboveground parts of plants exposed to sunlight, lives in an environment of high temperature

and low CO_2 concentration due to low solubility of this gas at high temperature and its removal by photosynthesis.

The species is able to tolerate high osmotic pressure due to photosynthetic products inside the foliar parts of plants. As measured by respiration, it is well adapted to its environment.

Pratylenchus penetrans, on the other hand, appears to be well adjusted to life in soil, since it can respire effectively at the high concentration of CO_2 associated with decay and microbial activity and the high osmotic pressure accompanying high temperature.

Ditylenchus dipsaci is well adapted to survive inside storage organs of plants, where carbon dioxide concentration and osmotic pressure are high, particularly when these organs begin to rot. Storage organs of plants can survive well at lower temperatures than growing parts and *D. dipsaci* inside the overwintering storage organs are able to tolerate low temperatures.

Second-stage larvae of *Anguina tritici* inside developing wheat galls should have high metabolic activity, as the high temperatures accompanied by high osmotic pressures would stimulate its respiration. After gall formation, however, these larvae go into a state of anhydrobiosis.

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