

The Effect of Single and Combined Heat and CO₂ Stimuli at Different Ambient Temperatures on the Behavior of Two Plant-Parasitic Nematodes

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Abstract: *Pratylenchus penetrans* and *Ditylenchus dipsaci* were reared at 15-16 C, and their behavior towards single and combined heat and CO₂ stimuli was studied at ambient temperatures of 8.6 and 27.3 C. At the lower temperature, attractivity of the heat source was prevalent in both species, but CO₂ was also attractive. At the higher ambient temperature (27.3 C), the reaction to CO₂ was more positive and more rapid than to heat. In fact, at this temperature only *D. dipsaci* was attracted to the heat source, whereas *P. penetrans* did not react positively. The combined stimulation of heat and CO₂ caused *D. dipsaci* to aggregate more strongly than did a single stimulus; this applied to both ambient temperatures. For *P. penetrans* exposed to the low temperature (8.6 C), the combined stimuli were about as attractive as was the better of the single stimuli; i.e., heat. At the high temperature (27.3 C), the combined stimulation was less effective than the better of the single stimuli; i.e., CO₂. At this ambient temperature, the thermonegative reaction seems to dominate over the CO₂-positive one. The reaction of *D. dipsaci* was generally stronger in all experimental variants than that of *P. penetrans*. Insofar as temperature gradients play a role in locating host plant roots, their efficacy would seem to be restricted to a favorable temperature range. Within this range, combined heat and CO₂ stimuli might improve attractivity. **Key words:** thermal response, response to CO₂, *Pratylenchus penetrans*, *Ditylenchus dipsaci*.

Zusammenfassung: Die Wirkung einfacher und kombinierter Wärme- und CO₂-Reize bei verschiedenen Umwelttemperaturen auf das Verhalten zweier pflanzenparasitischer Nematoden. *Pratylenchus penetrans* und *Ditylenchus dipsaci* wurden bei 15-16 C gezüchtet und ihr Verhalten gegenüber einfachen und kombinierten CO₂- und Wärmereizen bei Umwelttemperaturen von 8.6 und 27.3 C untersucht. Bei der niederen Temperatur (8.6 C) dominierte die attraktive Wirkung der Wärmequelle in beiden Arten, doch wirkte auch CO₂ anziehend. Bei der höheren Temperatur (27.3 C) war es umgekehrt: die Reaktion auf CO₂ war rascher und positiver als jene auf Wärme. Tatsächlich wurde bei dieser Temperatur nur *D. dipsaci* zur Wärmequelle angezogen, *P. penetrans* nicht. Die kombinierte Reizung mit Wärme und CO₂ führte mit *D. dipsaci* bei beiden Umwelttemperaturen zu stärkeren Ansammlungen als die einzelnen Reize. Für *P. penetrans* war die kombinierte Reizung bei 8.6 gleich wirksam wie die Wärmequelle allein. Bei 27.3 C hingegen war die kombinierte Reizquelle—ähnlich wie die einfache Wärmequelle—kaum oder nicht attraktiv, im Gegensatz zur CO₂-Quelle. Bei dieser Umgebungstemperatur scheint also die thermonegative Reaktion über die CO₂-positive zu dominieren. Von den beiden Arten reagierte *D. dipsaci* in allen Versuchsvarianten allgemein besser als *P. penetrans*. Insofern Temperaturgradienten bei der Lokalisierung der Wirtspflanzenwurzeln eine Rolle spielen, ist ihre Wirksamkeit wahrscheinlich auf einen gewissen, je nach Art und Adaptation verschiedenen Temperaturbereich begrenzt. In diesem Bereich könnten kombinierte CO₂- und Wärmereize wirksamer sein als Einzelreize.

El Sherif and Mai (7) expressed the opinion that the positive thermotactic behavior of some plant-parasitic nematodes might play a vital part in locating plant roots. On the other hand, it has been shown that chemical stimuli (such as CO₂ which is liberated by growing roots and germinating seeds) are attractive for many nematode species (literature reviewed under DISCUSSION).

This paper deals with the effect of single CO₂ and/or heat stimuli on the behavior of *Pratylenchus penetrans* and *Ditylenchus dipsaci* at two different temperatures.

MATERIALS AND METHODS

P. penetrans and *D. dipsaci* were reared at

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15-16 C on alfalfa callus tissue and on onions, respectively. *P. penetrans* was obtained from W. F. Mai, Cornell University, Ithaca, N.Y., U.S.A. in 1968 and maintained since on alfalfa callus tissue. *D. dipsaci* was extracted from onion collected locally and maintained on onion.

The heat source was constructed according to the details given by El Sherif and Mai (7). However, we did not form a spiral with the heating wire but a "zig-zag" of similar dimension (~ 3 × 3 mm) in order to avoid short circuits. This zig-zag was fixed on the outside of the bottom of a 9-cm glass petri dish. An agar-nematode suspension, 2-3 mm thick and containing about 500 to 1000 nematodes, was poured into this dish, which was covered during the experiments. The voltage applied to the heating wire was 1,000 mv of direct current in all cases. This created a temperature gradient as represented in Fig. 1-A. This gradient built up finally to average 1.04 C above the ambient

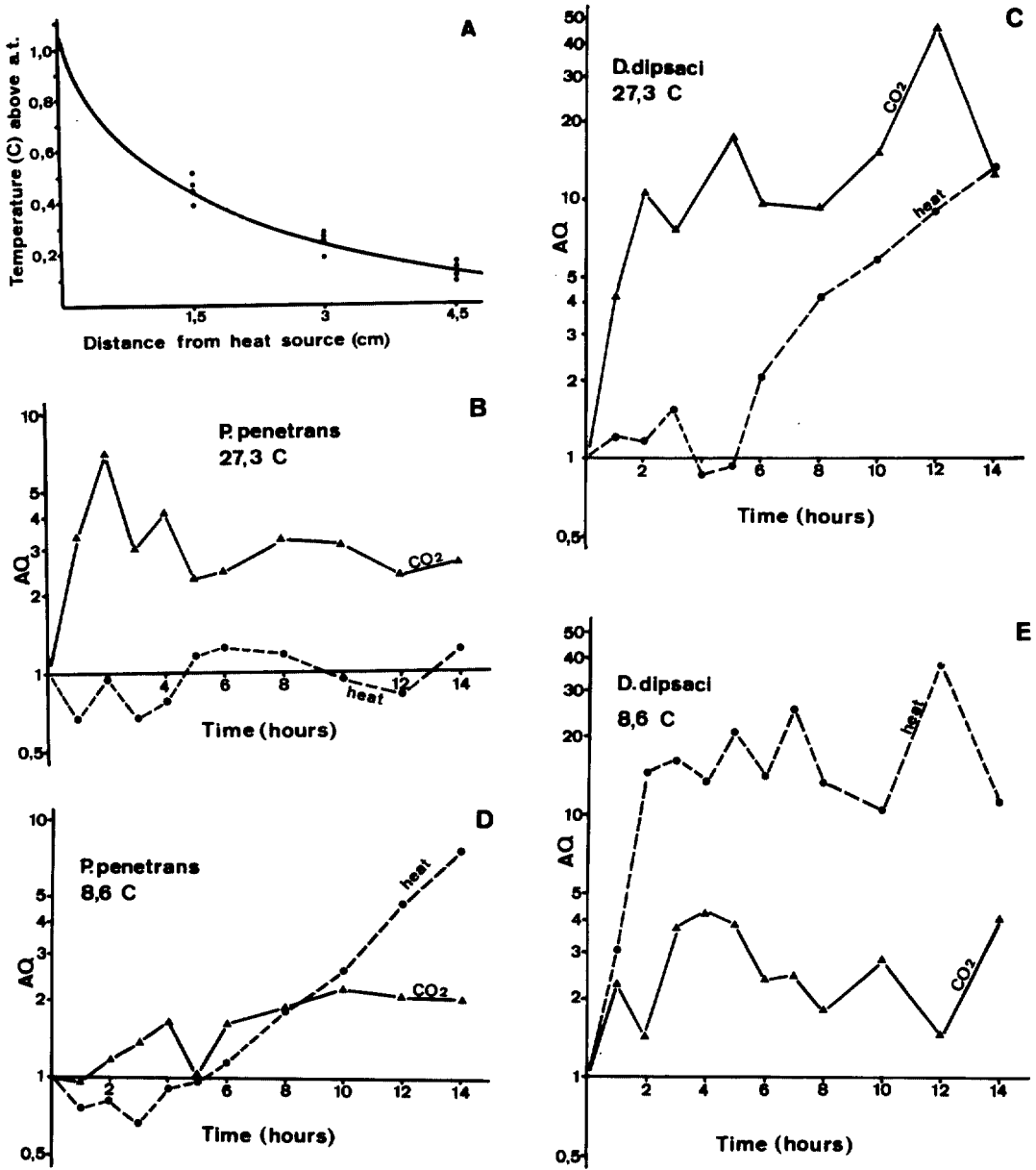


FIG. 1. A. Experimental temperature gradient used. The temperature differences between the heat source (0 cm) and 1.5, 3 and 4.5 cm away were measured in four experiments. The temperature curve declines and is approaching asymptotically the ambient temperature (a.t.); i.e., 8.6 and 27.3 C, respectively. B, C, D, E. Course and intensity of aggregation at the single CO₂ and heat sources exposed to the two different ambient temperatures (8.6 and 27.3 C). Ordinate: Aggregation Quotient (AQ), in logarithmic scale.

temperature within about 15 min. The temperature differences between the heat source and the surroundings were measured with precision thermistors (Yellow Springs Instruments Co., Inc.) and recorded by a thermograph.

CO₂ was introduced across the cover of the

petri dish by means of a glass capillary tube, which ended 1 mm above the agar surface. Gas flow was between 1.3 and 1.8 ml/hr. The effect of the given CO₂ and heat source was studied at ambient temperatures of 8.6 C and 27.3 C. All experiments were run in the dark.

Two series of experiments were conducted:

In one series, we investigated the course of the aggregation at the CO₂ and heat sources. The two sources were offered in the same dish at a distance of 3 cm from each other, and the experiments were run for 1, 2, 3, 4, 5, 6, 8, 10, 12, and 14 hr. Four repetitions were made for each time. In the other series, we compared the effect of single and combined CO₂ and heat sources in 14-hr experiments. The single sources were offered at a distance of 3 cm from each other, the combined ones at a common point. Eight repetitions were made for each variant.

The degree of aggregation was determined as follows: an agar disc of 12.5 mm diam with the stimulus source in its center was taken out, and the nematodes were counted. In the surroundings, six discs of the same size were sampled at random. The number of nematodes in the "source disc" divided by the average of the number of nematodes in the six "random discs" was chosen as a measure of aggregation. This we designate as "Aggregation-Quotient" (AQ). Values of AQ > 1 point to aggregation, values < 1 to repulsion, and values = 1 to indifference. In order to figure AQ values > 1 and < 1 in comparable relations, the logarithmic scale was used in the figures.

RESULTS

The course of the aggregations of the two species is given in Fig. 1-B, C, D, E. At 27.3 C ambient temperature, both species aggregated rapidly and distinctly at the CO₂ sources, *D. dipsaci* more strongly than *P. penetrans*. To the heat source, the reaction of *D. dipsaci* became positive only after 6 hr. However, after 14 hr an AQ similar to that for CO₂ was reached. The heat source was not attractive for *P. penetrans* (Fig. 1-B).

At 8.6 C ambient temperature, the heat source was the dominant attractive factor for both species. The reaction to CO₂ was also positive but weaker than to heat, and also weaker than at 27.3 C. A delay in the response to the heat stimulus occurred for *P. penetrans* (aggregating after 6-8 hr) but not for *D. dipsaci*. At both ambient temperatures, *D. dipsaci* reacted more strongly to both stimuli than did *P. penetrans*.

The effect of combined CO₂ and heat stimuli as compared with the single stimuli is given in Fig. 2-A, B. For *D. dipsaci*, the combined stimuli were more attractive than the single ones at both low and high ambient temperatures. For *P. penetrans* at 8.6 C, the

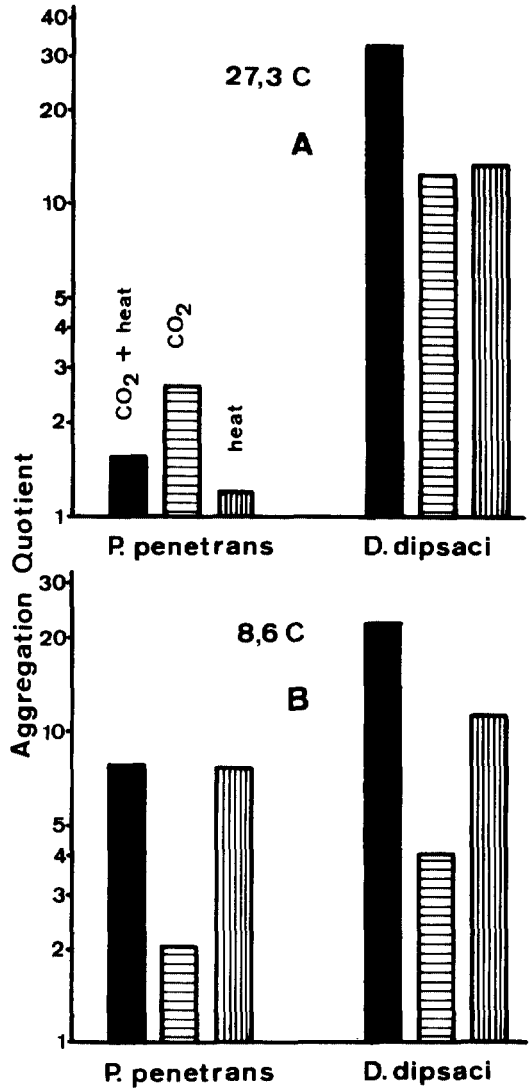


FIG. 2. A, B. Comparison of the effect of single and combined stimulation with CO₂ and heat at the two ambient temperatures of 8.6 and 27.3 C. Aggregation Quotient in logarithmic scale. 14-hr experiments.

combined source was about equivalent to the single heat source, with CO₂ apparently having no distinct additional effect. At 27.3 C, however, aggregation at the combined source was weaker than for CO₂ alone, indicating a negative effect of the heat source.

The results in Fig. 2 agree with those in Fig. 1 in that both species tend to react more positively to heat at the low temperature and more positively to CO₂ at the high temperature.

DISCUSSION

Behavior in temperature gradients. Rode (18, 19) observed that larvae of *Heterodera rostochiensis* orient towards a thermal preferendum. When the preliminary storage temperatures were 4, 18, and 24 C, this preferendum lay between 9 and 18 C. It changed in the same sense as the storage temperature, but to a lesser degree. According to Rode's observation, the orientation is strongly negative thermotactic when the ambient temperature is higher than the preferendum. This behavior is the more pronounced the greater the difference between the former and the latter. When the ambient temperature was below the thermal preferendum, no distinct positive thermotactic response was observed. This could mean that a thermonegative reaction, which leads the animal out of too warm zones, has a greater significance for survival than has a thermopositive one. Such a tendency is apparent also in the behavior of *H. rostochiensis* larvae in a combined chemo-thermic gradient. In larvae with a thermal preferendum of about 11 C which were studied at 19.5 and 17.5 C ambient temperature, the thermonegative reaction had priority; i.e., they migrated down a temperature gradient even when they came into otherwise repellent or inactivating concentrations of chemicals. Only at ambient temperatures of 16 C did the chemical stimuli mainly dominate (20).

Croll (3) found that *D. dipsaci* aggregated at that temperature at which it had been stored before the experiments. He calls this phenomenon acclimatization [see Schwertfeger (22)]. Animals which were stored for 30 days at 10, 20, and 30 C aggregated at 10-15, 20-25, and 30-35 C, respectively. However, an interpretation of Croll's graphs shows that there is also a tendency to aggregation on the cooler side of the gradient (10-40 C). Wallace's experiments (25) with the same species in a gradient of 2-30 C led to aggregations at 10 C. The pre-experimental storage temperature was possibly 10 C (Wallace, 1970, in lit.). Herter (9) designated the pre-experimental storage temperature as adaptation temperature, a term we shall accept.

El Sherif and Mai (7) investigated the behavior of nematodes in temperature gradients and towards infrared sources with reference to their possible roles in locating a plant host.

Their experiments were carried out at 22 C; the adaptation temperature (in this case rearing temperature) was 20-22 C, sporadically less (Mai, 1971, in lit.). *P. penetrans*, *D. dipsaci*, and *Tylenchorhynchus claytoni* reacted positively to heat sources, increasing the temperature by 0.14; 0.28, and 0.56 C; *Trichodorus christiei* and *Xiphinema americanum* did not. The possible significance of temperature gradients in host finding was also referred to by Staar (23).

Our own experiments confirm the attractivity of heat sources in a cool environment. At elevated temperatures (27.3 C), only *D. dipsaci* was attracted but not *P. penetrans*. As the two species were exposed to the same adaptation temperature (15-16 C), their different reactions might be based on inherent, genetically fixed differences in the species-specific optimum temperatures. As regards the development of *D. dipsaci*, this temperature seems to be near 27 C (17); for *P. penetrans* it is said to be 21 C (10).

These results and those of Rode (18, 19, 20) suggest that the localization of biological heat sources (roots, etc.) by means of temperature gradients apparently is limited to a certain temperature range.

Our results and those of El Sherif and Mai (7) with *D. dipsaci* are in contrast to those of Croll (3) and Wallace (25), whose nematodes aggregated at the adaptation temperature and at 10 C, respectively. Our nematodes migrated to a heat source with a temperature many degrees above the adaptation temperature. However, the experimental technique was different: ours corresponded to that of El Sherif and Mai; Croll's to that of Wallace.

A strange phenomenon is the delay in the reaction to the temperature gradients (Fig. 1-C, D). This was also observed by El Sherif and Mai (7). The question arises whether the thermopositive response is, under certain conditions, preceded by a phase of "sensitization".

As a whole, the behavior of nematodes in temperature gradients seems to be rather complex and requires more investigations.

Behavior in CO₂ gradients. In 1957, we referred to CO₂ as a possible attractant for nematodes. Later, aggregations of *D. dipsaci* at CO₂ sources and directed orientation to them were observed (12, 13, 14). The positive reaction to CO₂ was confirmed by several authors and for the following species. Bird (2): *Meloidogyne javanica*, *H. schachtii*, *P. minyus*,

Pratylenchus sp., various rhabditids; Johnson and Viglierchio (11): *D. dipsaci*, *M. hapla*, *M. javanica*, *H. schachtii*; Edmunds and Mai (6): *P. penetrans*; Croll and Viglierchio (4): *D. dipsaci*; Klingler (15): *Aphelenchoides fragariae*.

Bird (1) reported negative results with CO₂ dissolved in agar, but when he used gaseous CO₂ liberated from a glass capillary tube, aggregations did occur (2). El Sherif and Mai (7) did not obtain accumulation of *P. penetrans* at a source supplied with CO₂ from germinating seeds. However, the same species was attracted to CO₂ if it was introduced from a cylinder via a hypodermic needle (6). The following considerations might possibly explain the negative results. CO₂ of the germinating seeds diffused through the stem of an otherwise closed funnel onto an agar surface, diffusion probably being very weak, assuming that the Respiration Quotient (RQ) was ≤ 1 , which seems to be the rule for germinating seeds (24). The small quantity of CO₂ finally reaching the agar surface might be dissolved in water and dissociated. There are, however, indications which suggest that perception at the receptors is based on the CO₂ molecule itself, the bicarbonate ion probably being ineffective. Rogers (21) postulated a receptor model for CO₂—the latter releasing exsheathment in zooparasitic nematodes—which is based on dissolved CO₂ and/or undissociated carbonic acid (H₂CO₃). For phytoparasitic nematodes, Croll and Viglierchio (4) proposed a detector which depends on the spatial configuration of the CO₂ molecule as such. In insects, specific CO₂ receptors were demonstrated electro-physiologically and shown to respond to gaseous CO₂ (16). On the basis of observations made in orientation experiments with *D. dipsaci* on agar surfaces, we supposed that gaseous CO₂ was necessary for a reaction (14).

As CO₂ is dissolved and may dissociate in agar rich in water at relatively great quantities before a CO₂ gradient is built up, orientation experiments in agar apparently require not too weak a gas source or a previous partial CO₂ saturation of the agar. Moreover, point-like sources give better results than diffuse ones. In soil, a partial CO₂ saturation of soil water may be assumed because of the generally rather high carbon dioxide content of soil air.

The results of our own experiments confirm the positive response of *D. dipsaci* and *P. penetrans* to CO₂ and at the same time

demonstrate a species-specific difference in the intensity of this response. Moreover, it is shown that the response is temperature-dependent, being more positive at a higher than at a lower one. Probably the locomotory activity plays a role in this respect.

Behavior in combined gradients. Intensity of growth, increase of temperature, and enhancement of respiration of plant organs are correlated to some degree (5, 8). A nematode migrating to a root generally will encounter an increasing temperature and CO₂ concentration. Up to now, these stimuli have been investigated independently. The effect of the combined stimuli (Fig. 2) was different from that of the single stimuli. These differences were influenced by the species as well as by the ambient temperature. Whereas for *D. dipsaci* the combined stimulation was more attractive at the low and high ambient temperature, the effect was different in *P. penetrans*. At the low ambient temperature, the combined stimuli were only as good as the better of the single stimuli; i.e., heat. At the high ambient temperature, however, the combined stimulation was clearly less effective than the better of the single sources; i.e., CO₂. So whereas CO₂ apparently did not increase the attractivity of the heat source at the low temperature, the heat source reduced the attractivity of CO₂ at the high temperature. The thermonegative reaction seems to dominate under this condition over the CO₂-positive one. This is reminiscent of the results of Rode (20) where at the higher ambient temperatures (17.5, 19.5 C) the thermonegative orientation of *H. rostochiensis* also dominated over the reaction to chemicals.

The localization of the host plant by means of ascending temperature gradients would seem to be limited, therefore, to a certain temperature range varying according to species and possibly to adaptation temperature. Within this range, however, combined chemical (CO₂) and thermal stimuli might be more effective for certain species than the single stimuli.

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