THE EFFECT OF FUMIGANTS ON THE PHYSIOLOGY OF RESPIRATION IN PERIPLANETA AMERICANA (L.)

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Received August 26, 1970

(Communicated by Dr. T. S. Mahabale, F.A.Sc.)

ABSTRACT

(1) Toxicity of three fumigants is in the order trichloro-ethylene > EDC T > 1, 2, dibromo-3-chloropropane.
(2) Toxicity to the insects investigated was directly related to the amount of carbon dioxide produced during fumigation.
(3) A high percentage of spiracular openings per minute is necessary for high toxicity.
(4) Penetration of three fumigants through integument was in the order trichloroethylene > 1, 2, dibromo-3-chloropropane > EDCT.
(5) Spiracular action during fumigation plays an important role for the entry of gases.

The effect of toxic agents on the mechanism of respiration in insects has been the subject of interest since over a century (Davy, 1851; see Packard, 1898). Even so, available information on the effects of gaseous toxicants on insect respiration is meagre and very limited. Moreover, the precise physiological responses to the action of fumigants have not received the close attention that the subject deserves. It was Hazelhoff (1928) who first showed that carbon dioxide facilitates the entry of fumigants by its action on tracheal valves. Wigglesworth (1935, 1941) has provided some information on the effect of pyrethrum on the spiracular regulation of respiration in Xenopsylla cheopis Roths. With the exception of this work most other studies (Brinley and Baker, 1927; Munro 1959; p. 378) are pure speculations without any support of observed facts. Direct observations on the spiracular responses to fumigants have never been made before except by Bond (1961) whose observations on Phormia regina contradict his own views on Schistocerca gregaria. In addition, there are several factors that influence the supply of oxygen to insect tissues. Among these factors are...
percentage of ambient oxygen (Bond, 1965), percentage of carbon dioxide, spiracular control, the rate and percentage of integumental penetration, diffusion of gases through the tracheal cuticle and the effect of gases on the cells of the tissues. All these factors affect the oxygen requirements and the oxygen consumed. Assessment of respiratory responses on the basis of changes in O₂, N₂, CO₂ or other atmospheric gases will not pinpoint the level at which any adjustment may have occurred. However, if one measures other variable parameters such as ventilation, characteristics of spiracular movements, control and mode of gas penetration at the same time, one can better locate the levels of respiratory responses.

MATERIALS AND METHODS

The common cockroach *Periplaneta americana* (L.) was used primarily because the structure and physiology of their spiracles are relatively similar to those of *Schistocerca* which has been exhaustively investigated (see Miller, 1964).

The fumigants tested were

(a) Ethylene dichloride 75%, carbon tetrachloride 25%, (EDCT), (3:1);
(b) 1, 2 Dibromo-3-chloropropane 60% known as Nemagon®
(c) Trichloroethylene 95%. The concentration of the fumigants was 0.2 cc in 250 cc in air and 0.5% in Ringer's solution.

The methods used to investigate the effect of the above fumigants on ventilation, spiracular movements and mode of penetration are given elsewhere (Bhatia and Tonapi, 1968).

OBSERVATIONS AND RESULTS

Ventilation in the normal insect shows that the cockroach ventilates 6-8 times per minute. With each contraction the first spiracle opens while the second spiracle closes. This spiracular action is reversed during the relaxation of the abdomen (Figs. 1A, 2A and 5A). As the spiracular activity is correlated with ventilation movements of the abdomen, the spiracles open and close 6-8 times per minute. The antagonistic action of the first and second spiracles is shown in Figs. 2A and 5A.

Ventilation.—The phases and duration of contraction are appreciably reduced during the first five to ten minutes when treated with ethylene dichloride and carbon tetrachloride (3:1). After this period of fumigation the abdomen was relaxed, there were no contractions at all (Fig. 1 B and 1 C),
Respiration in Periplaneta americana (L.) 211

However, frequency of contraction and relaxation is suddenly increased when treated with 1, 2, dibromo-3-chloropropane. In general the activity recorded by kymograph showed relatively more relaxed condition than contraction (Fig. 1 D). On the other hand there is a greater degree of contraction than relaxation when the insects are treated with trichloroethylene. The frequency of at least partial contraction is then increased to almost three times that of normal (Fig. 1 E).

Fig. 1. Kymographic traces of ventilatory movements of Periplaneta americana (L.). A, Normal; B, with EDCT (after five minutes of fumigation); C, with EDCT (after fifteen minutes of fumigation); D, with 1, 2, dibromo-3-chloropropane; E, with trichloroethylene. CT, Ventilatory movements during contraction.

It is most important to note here that with 1, 2 dibromo-3-chloropropane the insect tends to spread its wings as if to take to flight. This act is significant and helps to explain the behaviour of the spiracles in relation to fumigants. This characteristic posture is not taken by the insect when treated with the other two chemicals.

First thoracic spiracle.—With EDCT the spiracle tended to remain open and the duration of the open condition was lengthened during the first five
to ten minutes (Figs. 2 B and 3 B). There was a corresponding decrease in the duration of its closing. After this initial reaction the spiracles retained a completely open condition (Figs. 2 C and 3 C). The frequency of closing and opening was doubled when treated with 1-2-dibromo-3-chloropropane. However, the overall tendency was to achieve greater duration of the open condition rather than the closed one (Figs. 2 D and 3 D). The frequency of opening and closing is increased almost to ten times per minute, while the duration of the open condition was increased as compared to that of closing (Figs. 2 E and 3 E) with trichloroethylene.

![Diagram](image)

**FIG. 2.** Valvular movements of the first thoracic spiracle with different fumigants. A. Normal; B, with EDCT (after five minutes of fumigation); C, with EDCT (after fifteen minute-D—with 1, 2 dibromo-3-chloropropane; E with trichloroethylene; CL, closed; O, open.

Second thoracic spiracle.—The spiracle remains completely open when treated with EDCT (Figs. 5 B and 6 B). But it remains closed with 1, 2 dibromo-3-chloropropane (Figs. 5 C and 6 C). The duration of the open condition is greater, while the number of quick closing movements remain same, viz., 6-8 per minute when treated with trichloroethylene (Figs. 5 D and 6 D).

Action of fumigants when mediated through Ringer’s solution*.—The abdominal ventilation movements and the synchronized spiracular activity were obtained even when the insect was partially flooded with the Ringer's

Fig. 3. Valve movements of the first spiracle, showing the degree of opening and closing with different fumigants. A, Normal; B, with EDCT (after five minutes); C, with EDCT (after fifteen minutes). CL, closed; O, open.

Fig. 3. Valve movements of the first spiracle, showing the degree of opening and closing with different fumigants. D, with 1, 2, dibromo-3-chloropropone; E, with trichloroethylene. CL, Closed; O, Open.
Fig. 4. Valve movements of the first spiracles when the fumigants were allowed to penetrate the body through Ringer's solution. A, Normal (with Ringer's solution only); B, Ringer's solution with EDCT; C, Ringer's solution with 1, 2, dibromo-3-chloropropane; D, Ringer's solution with trichloroethylene; CL, closed; O, open.

Fig. 5. Opening and closing valvular movements of the second spiracle with different fumigants. A, Normal; B, with EDCT; C, with 1, 2, dibromo-3-chloropropane; D, with Trichloroethylene. O, open; CL, closed. (Compare difference in sequence of spiracular action with that of the first spiracle (Fig. 4).

solution (Figs. 4 A and 7 A). The fumigant was allowed to pass through this fluid, which acted as a carrier. There was no penetration of these chemicals through the spiracles or tracheae. The spiracular behaviour
under these conditions was altered when so treated with different chemicals. The first spiracle opened completely when EDCT was allowed to pass through the fluid (Fig. 4 B). There was considerable increase in the frequency of spiracular opening, with corresponding decrease in the duration of the closed condition, when the cockroach was similarly treated with 1, 2, dibromo-3-chloropropane (Fig. 4 C). However, with trichloroethylene the frequency of closing and opening were both increased, but the period of open condition during each minute was increased to nearly seven times the normal (Fig. 4 D).

The second thoracic spiracle when treated similarly or as above with EDCT showed frequency of closing decreased from 6 times per minute to 4 times per minute (Fig. 7 B) and the duration of open condition was increased. The effect of 1, 2, dibromo-3-chloropropane was to increase the frequency of the closed condition and the duration of the open condition was decreased. The increase in the frequency of closing is almost twice the normal (Fig. 7 C). There was a considerable change in the behaviour of the second thoracic spiracle when trichloroethylene was allowed to pass through Ringer's solution. The spiracle remained open almost all the time (Fig. 7 D).

Carbon dioxide produced during fumigation with these fumigants was also measured and the results are shown in Table I. Time for integumental penetration was also noted as shown in Table II.

**TABLE I**

*Percentage of CO₂ produced during fumigation with different fumigants*

<table>
<thead>
<tr>
<th>Fumigant</th>
<th>Difference in levels in manometer cm</th>
<th>Mean cm.</th>
<th>Vol. of CO₂ cc per insect</th>
<th>% of CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>EDCT</td>
<td>..</td>
<td>2.0</td>
<td>2.5</td>
<td>2.8</td>
</tr>
<tr>
<td>1, 2, dibromo-3-chloropropane</td>
<td>2.7</td>
<td>2.1</td>
<td>3.2</td>
<td>3.0</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td>..</td>
<td>3.7</td>
<td>3.2</td>
<td>3.5</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The primary effect of EDCT (3 : 1) is to relax the abdominal ventilatory muscles. Many changes take place due to relaxation of the muscular system,
TABLE II

Integumental penetration of different fumigants. 0·1 cc in 250 cc

<table>
<thead>
<tr>
<th>Fumigant</th>
<th>Condition of spiracles</th>
<th>Time in which the insect was killed (minutes)</th>
<th>Mean time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 2 3 4</td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>Unsealed</td>
<td>∞ ∞ ∞ ∞</td>
<td>357</td>
</tr>
<tr>
<td></td>
<td>Sealed</td>
<td>1 2 3 4</td>
<td></td>
</tr>
<tr>
<td>EDCT</td>
<td>Unsealed</td>
<td>30 33 35 34</td>
<td>33 (1·8 times faster)</td>
</tr>
<tr>
<td></td>
<td>Sealed</td>
<td>18 13 18 25</td>
<td></td>
</tr>
<tr>
<td>1, 2, dibromo-3-chloropropane</td>
<td>Unsealed</td>
<td>125 120 115 120</td>
<td>120 (4·8 times faster)</td>
</tr>
<tr>
<td></td>
<td>Sealed</td>
<td>27 20 27 25</td>
<td></td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td>Unsealed</td>
<td>15 12 16 18</td>
<td>15 (5·0 times faster)</td>
</tr>
<tr>
<td></td>
<td>Sealed</td>
<td>1 3 6 2</td>
<td></td>
</tr>
</tbody>
</table>

It is well known that the first thoracic spiracle has two muscles operating on its closing mechanism (Miall and Denny, 1886). The contraction of the dilator muscle and the simultaneous relaxation of the occlusor opens the spiracle.

![Fig. 6. Valvular movements of second spiracle showing degree of opening and closing of the spiracle with different fumigants. A, Normal; B, with EDCT; C, with 1, 2, dibromo-3-chloropropane; D, with trichloroethylene; O, open; CL, closed.](image-url)
Respiration in Periplaneta americana (L.)

In short they are antagonistic in action. However since both these muscles are relaxed (EDCT causes relaxation of the muscles), the spiracle remains open. 1, 2, dibromo-3-chloropropane also produces parallel effects, the duration of relaxation of the abdominal muscles is relatively less than that of EDCT. And also the duration of the open condition of the first spiracle is not as pronounced. Although trichloroethylene does not induce such widespread muscular relaxation, even the slight relaxation of the two muscles is responsible for the partial open condition. In any case this gas is the least relaxant in its action on muscular system.

![Graph showing opening and closing movements of the valve of the second spiracle with different fumigants when used in Ringer's solution. A, Normal; B, with EDCT; C, with 1, 2, dibromo-3-chloro-propane; D, with trichloroethylene. O, open; CL, closed.]

The behavior of the second thoracic spiracle can also be explained similarly. It is of interest to note that the second spiracle is provided with only a single muscle, the occlusor muscle. The second spiracle remains open due to relaxation of the occlusor when the insect is treated with EDCT. The open condition of the spiracle is consistent with that of the relaxed abdomen as in the normal cockroach.

The effect of Nemagon (1, 2, dibromo-3-chloro-propane) on the second spiracle can be understood only in terms of the explanation offered earlier (Miller, 1960). As pointed out previously this fumigant acts on the musculature in a manner very similar to that of an insect in preparation for flight, which often tries to spread its wings but fails to do so. After a period of ten minutes in this condition, the second spiracle assumes full closure, as in an insect immediately after flight (see Miller, 1960-III, p, 266). It can be readily
appreciated that 1, 2, dibromo-3-chloropropane affects more the frequency of closing of the second spiracle when it is carried directly into the insect by means of Ringer's solution.

Trichloroethylene, which causes the contraction of the muscles, should cause the second thoracic spiracle to close more often due to the contraction of its occlusor muscle. But this is not so; the carbon dioxide which is produced by trichloroethylene is more increased by about 1.7 per cent (see Table I). This carbon dioxide causes the open condition of the second thoracic spiracle by its action on the peripheral mechanism of the spiracle (Miller, 1960).

Effect on the spiracle of the fumigants when introduced via the body fluid is similar to the effect from fumigation.

It can be appreciated that the percentage of carbon dioxide produced (Table I) has an effect on the time taken to kill the insect. Time taken by trichloroethylene to kill the insect with sealed spiracles is less than with the other two chemicals. This is probably because trichloroethylene produces the highest percentage of carbon dioxide. 1, 2, dibromo-3-chloropropane comes next in production of carbon dioxide and the time taken to kill the insect with sealed spiracles is little more than with trichloroethylene. EDCT takes much more time to kill the insect with sealed spiracles and this is due to less production of carbon dioxide.

Table II also shows the degree of integumental penetration. It can be readily seen from the table that integumental penetration is highest by trichloroethylene, as it kills the insect with sealed spiracles 5.0 times faster than with unsealed spiracles. Integumental penetration with 1, 2, dibromo-3-chloro-propane is less than with trichloroethylene; it causes the death of the insects with sealed spiracles 4.8 times faster than the insects with unsealed spiracles. EDCT is the least penetrating of the three fumigants, as it causes the death of the insect with sealed spiracles only 1.8 times faster than insect with unsealed spiracles.

Figure. 8 shows histograms of per cent of opening and closing per minute of both first and second thoracic spiracles with three different fumigants. From Figs. 2 and 5, histograms were plotted to show that for highest toxicity, some degree of closing of the spiracles is required, as is shown in the case of trichloroethylene, which is the most toxic of the fumigants studied. EDCT is second in toxicity, and with this fumigant both first and second
Respiration in *Periplaneta americana* (L.) 219

Spiracles are continuously open. In the case of 1, 2, dibromo-3 chloropropene the spiracles are open for a lesser period per minute resulting in less entry of gas through the spiracles.

![Diagram showing percent of time thoracic spiracles of adult *Periplaneta americana* (L.) are open and closed per minute.](image)

Fig. 8. Per cent of time the two thoracic spiracles of adult *Periplaneta americana* (L.) are open and closed per minute (data from Figs. 2 + 5).

Apparently the accumulation of CO₂ in the body of the insect caused by the sealing of the spiracles in some way increases the toxic action of the fumigant.

Acknowledgements

Acknowledgements are due to authorities of Poona University for facilities and to Prof. (Dr.) L. Mulherkar, Head of the Department of Zoology, for interest and encouragement. I am grateful to Prof. (Dr.) Walter Ebeling University of California, Los Angeles for many stimulating discussions and help in the preparation of this manuscript.

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