Onderstepoort J. Vet. Res. (1964), 31 (1), 59–68
Printed in the Republic of S. Afr. by the Government Printer, Pretoria

THE ROLE OF CARBON DIOXIDE AS STIMULANT AND ATTRACTANT TO THE SAND TAMPAN, *ORNITHODOROS SAVIGNYI* (AUDOUIN)

E. M. NEVILL, Veterinary Research Institute, Onderstepoort

INTRODUCTION

Sand tampans, although not important as disease transmitters (Heisch & Harvey, 1960) are responsible for considerable irritation and stock losses in the sandveld ranching areas of the North West Cape (Kalahari) and South West Africa.

All classes of stock may be attacked and if unable to protect themselves, e.g. newly-born calves or sick animals, are drained of blood and killed within a few hours. The economic losses among healthy stock from restlessness, loss of blood, and because the shade of trees is often denied them due to the abundance of tampans in these situations, must be substantial.

Farmers are concerned about the rate at which this problem is spreading, for while tampans were initially found in and around the kraals near homesteads, they are now spread over most parts of the farms.

Sand tampans live below the sand surface especially in the shade of trees, shrubs and fences. It is impossible to seek shade in an infested area around a tree especially on a hot summer day, for within minutes, tampans of all sizes emerge from the sand in hordes and converge on their prey.

How they detect their food has been the subject of surmise by farmer and scientist and has been attributed to various factors, e.g. vibration and smell (Theiler, 1962).

Observations in the laboratory and in the field suggested that the stimulus necessary for tampan emergence and attraction is of a chemical nature and would be found in the breath of animals or contained in a body odour. It was noted that on breathing into a jar containing tampans in sand the tampans soon appeared on the surface. In the field when a steady breeze was blowing only tampans on the down-wind side of a host would surface and be able to locate the host animal. Sound, vibration, or pressure on the sand exerted by the host did not seem to be responsible for tampan stimulation or attraction. Sound and vibration also had no effect on tampans in sand in a stoppered container. Only by tilting such a container so that the tampans' position in the sand was altered could they be stimulated to surface, but they soon returned under the sand.

It was thought advisable to investigate the nature of the stimulus involved. This could then perhaps lead to control measures other than the use of insecticides which require extremely high dosage rates to be of any effect.

Received for publication on 9 January, 1964.—Editor

Dethier (1957) has reviewed the findings of workers who have investigated the process by which a blood-sucking arthropod locates its food-source. He shows that ticks in general are more or less stimulated by or sensitive to light, gravity, temperature, humidity, tactile stimuli, and certain odours especially those from hair and skin of various animals.

El-Ziady (1958) showed *Ornithodoros erraticus* to respond in various ways to temperature, humidity, gravity, light and smell. However, *O. erraticus* only detected a host after coming into actual contact with it.

The effect of carbon-dioxide (CO_2) on blood-sucking arthropods has been studied mainly in mosquitoes, but also in two species of *Trombicula* (Sasa, Taraka, Ueno & Muira, 1957), in the tropical rat mite (Sasa & Wakasugi, 1957), the dog flea (Sasa, 1957), and in bed-bugs (Dethier, 1957).

The attractiveness of CO_2 to mosquitoes has been studied for at least 40 years. Many workers agree that CO_2 is attractive to, or at least stimulates certain species, although it is not the only factor involved. Brown (1952) working mainly with *Aëdes aegypti*, showed warmth to be attractive in the absence of moisture and that this was due to heat of convection as opposed to radiation. He also showed CO_2, sweat at low concentration, diethyl ether and gasoline to be attractive.

Kellogg & Wright (1962) blame the conflicting results obtained with mosquitoes on methods used in evaluating the effect of an attractant. They state that it is unnecessary to look for additional specific chemical attractants in that their experiments show that the process of seeking a host relies on " a simple type of response to CO_2, heat, moisture, and visual appearance, when combined and presented in the proper way ".

Garcia (1962) is the first worker to have shown that CO_2 is an attractant for some ticks. He used CO_2 to sample areas for *Ornithodoros coriaceus*, when he also attracted *Dermacentor occidentalis* and a few *Ixodes pacificus;* these two together formed 5 per cent of the ticks collected.

METHODS

To investigate the suggestion that tampan stimulation is of a chemical nature, laboratory tests were conducted using first stage tampans in sand in 750 cc Erlenmeyer flasks. These flasks were connected to each other in series by means of glass and rubber tubing. Air was drawn from outside the room and passed over flasks each containing 50 first stage nymphs to act as control and then over a rat or guinea-pig in a desiccator. From here the air was passed over an equal number of test flasks similar in all respects to the control flasks.

The number of flasks depended upon the number of tampans to be used, as it was almost impossible to count more than 50 active first stage tampans in one flask.

In the first test a total of 10 flasks was used. The number of tampans surfacing in the flasks after drawing air through the system for 10 minutes is shown in Table 1.

These results indicate that tampans will respond to some chemical emanation from an animal. This could be a body odour or could be contained in the breath.

TABLE 1.—*Number of tampans surfacing in flasks after drawing air through a system of 10 flasks*

Flasks		Number of First Stage Nymphs on sand surface					
		A	B	C	D	E	Total
Air from over rat	At Start	5	5	5	9	9	33
	After 10 minutes	15	31	23	32	26	127
	Number surfacing	10	26	18	23	17	94
Air alone	At start	14	1	6	7	4	32
	After 10 minutes	8	1	6	7	3	25
	Number returning under sand	6	0	0	0	1	7

This test was repeated a number of times using both rats and guinea-pigs with similar results. It was noted, however, that the same tampans could not be used indefinitely as they ceased to be activated when used too often. This non-activation could be due to exhaustion or depletion of reserves similar to that noted by Lees (1952) in the sheep tick, *I. ricinus*. In this instance the life expectation was reduced from 62 to 38 weeks by " disturbance caused by removing the ticks from their tubes three times weekly . . . ".

THE IMPORTANCE OF CARBON DIOXIDE IN TAMPAN STIMULATION

The tests described show tampans to be stimulated by air passed over rats or guinea-pigs. Also in the field many different animals stimulate and attract tampans. It must thus be assumed that tampan stimulation and attraction relies on a chemical substance common to all these animals. Carbon dioxide is present in varying quantities in the exhaled breath of all the higher animals and its role in tampan stimulation was therefore investigated.

Initially CO_2 was removed from the system already described by inserting a U-tube containing soda-lime to absorb CO_2, between the test animal and the test flasks. On passing air through the system no tampans surfaced in any of the flasks but soon did so in the test flasks when the CO_2 absorber was removed. This served as a good indication that CO_2 is responsible for tampan emergence.

A more complicated system was then arranged in which six flasks each containing 50 first stage tampans in sand, two desiccators each holding a guinea-pig, and one soda-lime U-tube were used. This system allowed for a direct comparison to be made between flasks receiving air from a guinea-pig, and those receiving this air minus CO_2. Two guinea-pigs were necessary since tampan stimulation decreased rapidly with distance from the guinea-pig. This meant that the number of tampans brought to the surface by air from over a guinea-pig was far greater in the first three than in the last three flasks, due possibly to CO_2 absorption and dilution.

Air from outside the room was drawn over the first guinea-pig, then through the U-tube, three flasks of tampans, over another guinea-pig and over another three flasks of tampans. The number of tampans surfacing in the two groups of flasks were then compared as shown in Table 2.

TABLE 2.—*Number of tampans surfacing after exposure to air over guinea-pig minus CO_2 and air over guinea-pig*

Attractant source	Number of First Stage Nymphs on sand surface		
	At start	After ten minutes	After forty minutes
Air over guinea-pig minus CO_2	7	7	5
Air from over guinea-pig	0	12	22

On removing the soda-lime from the system a further 27 tampans surfaced in the first group of flasks, whereas the number in the second group remained constant.

These results point to CO_2 being a factor responsible for the activation of tampans.

TESTS WITH CARBON DIOXIDE IN THE LABORATORY

5% CO_2

In testing the effect of CO_2 on tampans, 5 per cent CO_2 was used to approximate the percentage contained in bovine breath.

Air containing 5 per cent CO_2 was passed over six flasks each containing 50 first stage tampans, with the soda-lime absorber interposed after the first three flasks. In all cases 14 litres of air plus CO_2 were used.

Within a few minutes after passing the 5 per cent CO_2 over, the number of tampans on the sand surface in the first three flasks had risen from 12 to 49; from 5 to 6 in the flasks receiving air alone.

Two further tests with eight flasks each showed similar activation. The number of tampans on the surface in the first four flasks which received CO_2, increased within a few minutes from 18 to 57, and from 6 to 62 respectively. Where CO_2 was eliminated the numbers remained almost constant.

10% CO_2

Using the same system with only six flasks, 10 per cent CO_2 was tested twice. Results were similar, numbers increasing from 32 to 96, and 32 to 95 in the flasks receiving 14 litres of 10 per cent CO_2. Tampan numbers in the control flasks remained constant or a few tampans even returned under the sand surface.

All these laboratory tests were conducted in a room kept at 70° F and 40 to 50 per cent relative humidity.

THE ATTRACTIVENESS OF CARBON DIOXIDE IN FIELD EXPERIMENTS

To test the laboratory findings under field conditions, CO_2 in cylinders, dry-ice (solidified CO_2) and guinea-pigs were taken to a heavily infested sandveld area of the Kalahari, approximately 60 miles north-west of Kuruman.

100% CO_2 *in Cylinder*

CO_2 liberated from the cylinder was led to the experimental site through rubber tubing 50 feet long and of $\frac{1}{4}$ inch diameter. The end of the tubing was placed in an enamel tray (18 × 12 × 2 inches) buried so that its rim was level with the sand surface. This tray acted as a trap for any tampans approaching the CO_2 outlet, in that the sides of the tray were too smooth to allow the tampans to climb out.

Carbon-dioxide was liberated at approximately 18 litres per minute into this tray placed successively in a number of different locations. Usually a site was chosen under a tree or group of trees, mostly *Acacia giraffae*, where tampans were known to be present. On each occasion a fairly strong breeze was blowing. Atmospheric temperatures were in the neighbourhood of 75° F.

Within a few minutes of liberating the gas, tampans would start emerging from the sand and head towards the CO_2 outlet and eventually fall into the enamel tray. It was very noticeable that tampans only surfaced and approached the tray from the downwind side. On the windward side of the tray no tampans were ever observed to surface.

The distance over which the tampans were attracted varied with the strength of the wind; the furthest distance noted being 24 feet away from the gas outlet.

Since tampans move fairly slowly, especially over rough terrain, the gas had to be liberated for some time, usually 30 to 80 minutes, so that the furthest tampans could reach the tray before the gas was turned off.

On stopping the flow of CO_2 the tampans ceased moving towards the outlet and tested the air with their fore-legs. After searching around for a few minutes they returned underground.

Tampans of all stages were caught, a total of 1693 being collected in 190 minutes. These numbers are only a fraction of those which could have been caught had conditions been more favourable, for at the time of the experiments rain fell almost nightly and temperatures were nearly 20° lower than mid-summer temperatures. Wet sand is known to restrict the movement of tampans, especially the earlier nymphal stages.

Dry-ice

Dry-ice was also found to be effective in attracting tampans and proved easier to handle than a cylinder containing CO_2. On two occasions pieces of dry-ice were placed in enamel bowls buried in the sand to the level of their rims.

On the first occasion eight bowls 9 in. in diameter were buried 6 yd apart in a tampan infested area and dry-ice was placed in each alternate bowl. Small boxes of foam-plastic insulating material were used to hold the dry-ice and prevent too rapid CO_2 liberation. This experiment was conducted at night under very unfavourable conditions as, apart from low temperatures ($\pm 65°$ F), the sand was wet and rain fell an hour after setting up the bowls. Nevertheless when the bowls were visited the next morning a total of 112 tampans was found in the dry-ice bowls as compared with nil in the controls.

On the second occasion the attractiveness of guinea-pigs was compared with that of dry-ice. The test was conducted overnight and replicated three times. The guinea-pigs were kept separately in wire cages suspended about 1 in. above trap bowls. Only three tampans were found in the guinea-pig bowls the following morning as compared with 173 in the bowls containing dry-ice.

The apparent unattractiveness of the guinea-pigs was possibly due to the cool conditions coupled with the relatively small amount of CO_2 exhaled by these small animals.

It was also found that a fowl left overnight in a drum sunk into the sand, attracted no tampans. The same set-up in the middle of the summer, however, had collected more than 1,000 tampans which confirms the suggestion that tampan activity is materially reduced by low temperatures.

Later in the same month (April, 1963), in another area, dry-ice was again used. The same enamel bowls were used to hold pieces of dry-ice wrapped in newspaper to slow down vaporization. Conditions were fairly dry and night temperatures very low. However, in the morning once the sand started to warm up there was considerable tampan activity.

Bowls were buried along a fence bordering a cattle kraal, and dry-ice was placed in each bowl when the sun was already well up. After two hours the bowls were revisited and all contained tampans; one of the bowls placed in an area which had been disturbed a good deal, collected 4,553 tampans.

Thereafter dry-ice was used to sample plots treated with insecticides; it showed the presence of tampans where none could be found by sifting the sand.

5% CO_2 in field tests

The above tests in the field clearly showed tampans to be stimulated by and attracted to 100 per cent CO_2. Bovines and humans, however, exhale from 4 to 5 per cent CO_2 which will be rapidly diluted in the open so as to perhaps be undetectable to tampans beneath the sand-surface.

To test its effectiveness in the open, 5 per cent CO_2 was liberated into an enamel bowl sunk in sand in an enclosure at Onderstepoort artificially infested with approximately 1500 tampans of all stages.

When liberated at 6 litres per minute tampans appeared within 5 minutes from up to 9 ft from the bowl and after 40 minutes 141 had fallen into the bowl. This was repeated at 1 litre per minute with similar results. However, when liberated at 6 litres per minute at heights 5 ft and 3 ft above ground level no tampan activity was noticed even after 10 minutes. All these tests were conducted while a slight variable breeze was blowing and at about 80° F.

These tests provide strong evidence that the amount of CO_2 exhaled by higher animals is sufficient to account for the stimulation and attraction of tampans in the immediate vicinity of such animals.

THE EFFECT OF LOW TEMPERATURES ON TAMPAN ACTIVITY

This was investigated using 100 per cent CO_2 from a cylinder just before sunrise when the temperature was 52° F. After 15 minutes of CO_2 liberation no tampans had emerged from the sand.

Previous tests at the same spot during the day at 75° F resulted in marked tampan activity within a few minutes, 190 being trapped in 30 minutes. Consequently the inactivity of the tampans at 52° F could only be attributed to the low temperature.

Emergence was then encouraged by loosening the sand around the gas outlet so as to disturb the tampans present in this sand. After another 15 minutes five tampans were trapped and more could be seen approaching from the downhill-side of the bowl. As there was no movement of air CO_2, being heavier than air, presumably flowed downhill, which explains why the tampans only emerged on the down-hill side. Had the sand not been disturbed it is fairly certain that no tampans would have emerged at this temperature.

THE ATTRACTIVENESS OF WARMTH AND MOISTURE TO THE SAND TAMPAN

In laboratory tests use was made of an air-tight box with a glass front. The bottom of the box was covered with sand to a depth of 3 in. and about 500 tampans of all stages were introduced. These soon disappeared under the sand.

Three test tubes were covered with calico; two were filled with boiling water, the third with cold water. All the tubes were well stoppered. The calico on one warm tube and the cold tube was moistened and the three tubes were pushed into the sand in the box so as to stand upright.

No tampans surfaced in response to these warm and moist tubes, but did so only when 5 per cent CO_2 was introduced into the box. Upon introducing CO_2, a number of tampans was attracted to the warm tubes but never to the cold tube. The warm tubes were at first too hot for the tampans to climb up onto. After five minutes the tubes had cooled sufficiently to permit of the tampans moving freely on them.

It was obvious that a greater number of tampans was present on the warm-moist, than on the warm-dry tube. On the warm-dry tube they moved around, did not settle and soon left. On the warm-moist tube they moved around less, and only started leaving after 20 minutes when the tube had cooled.

The cold-moist tube attracted no tampans.

Heat does therefore seem to play a part in attracting and orientating CO_2-activated tampans to the host. A warm-moist object will not necessarily attract more tampans than a warm-dry one, but these attracted tampans remain longer to investigate.

DISCUSSION

These experiments and observations have shown that tampans are capable of detecting extremely low concentrations of CO_2 while below the sand surface. The fact that they are stimulated to the surface and then attracted to a source liberating only 1 litre of 5 per cent CO_2 per minute is very strong evidence that the CO_2 present in the exhaled breath of higher animals is *the* factor responsible for primary tampan stimulation and attraction.

Once stimulated by CO_2 they are then responsive to heat radiating from objects warmer than the surroundings. They are not attracted to moist objects alone but moisture does serve to make warm objects more attractive.

These findings seem to substantiate those of Kellogg & Wright (1962) who concluded from their experiments with mosquitoes, that host-seeking relies on " a simple type of response to CO_2, heat, moisture, and visual appearance, when combined and presented in the proper way ", and suggested that it is unnecessary to look for additional specific chemical attractants.

Although 100 per cent CO_2 is effective in attracting tampans over distances up to 24. ft from the source, there does not at present seem to be an inexpensive and practical way of using it in trapping devices to eradicate tampans over a large area. Not all tampans react simultaneously to CO_2, which further complicates the problem of eradication by trapping. Trapping with CO_2 over a limited area may, however, be effective in reducing or eradicating localized tampan populations.

Carbon-dioxide will provide a very useful means of sampling tampan populations in distribution surveys and in insecticide trials, which hitherto relied on the laborious and time-consuming sifting of sand samples.

SUMMARY

Sand tampans cause irritation and losses among cattle in the sandveld areas of the Kalahari and South West Africa.

They appear above the ground in response to a stimulus. The nature of this stimulus was investigated in the laboratory and in the field, in the hope that the knowledge so gained would lead to a means of control.

These studies showed carbon-dioxide to be the main factor which caused tampans to surface. In the laboratory concentrations of 5 per cent CO_2 and higher proved to be effective stimulants. In the field 100 per cent CO_2 was used, liberated from a cylinder and from dry-ice. It caused marked stimulation and attraction of tampans, so that large numbers could be collected at the source of liberation. In one instance 4553 tampans were collected in 2 hours when using a small piece of dry-ice as attractant.

At Onderstepoort 5 per cent CO_2 was liberated in a tampan-infested open air enclosure covered with an 8 in. depth of sand to resemble Kalahari conditions. As little as 1 litre per minute of 5 per cent CO_2 caused tampans to surface and move towards the CO_2 source. Even a slight breeze did not prevent them from surfacing and eventually locating the CO_2 source.

At low temperatures, e.g. 52° F, tampans were not attracted by CO_2 unless activated by disturbing the sand in which they occurred.

Laboratory investigations into the role of heat and moisture as tampan attractants showed that CO_2 activation was necessary before tampans would react to heat and moisture. Moist objects alone were unattractive, warm objects attractive, but warm-moist objects were the most attractive.

In all experiments in the laboratory only a proportion of the tampans present reacted to the stimuli tested.

It seems at present impractical and uneconomical to attempt to control tampans over large areas with traps containing CO_2, but it will be of great use in sampling tampan populations.

ACKNOWLEDGEMENTS

The author wishes to convey his thanks to Professor R. M. du Toit for his helpful suggestions during the course of the work; and to Dr. G. Theiler and Professor du Toit for their constructive criticism of the manuscript.

To Miss S. Voorendyk and Mr. D. W. du Plooy for their valuable assistance in the field experiments, and to Mr. J. Boshoff, manager of Flora Dora ranches for the facilities placed at our disposal, gratitude is expressed.

REFERENCES

BROWN, A. W. A., 1952. Factors in the attractiveness of bodies for mosquitoes. *Trans. Ninth Int. Congr. Ent.*, Vol. 1, 1952, p. 895–900.

DETHIER, V. G., 1957. The sensory physiology of blood-sucking arthropods. *Exp. Parasitol.*, Vol. 6, No. 1, p. 68–122.

EL-ZIADY, S., 1958. The behaviour of *Ornithodoros erraticus* (Lucas, 1849), small form (*Ixodoidea, Argasidae*), towards certain environmental factors. *Ann. Ent. Soc. Amer.*, Vol. 51, No. 4, p. 317–336.

GARCIA, R., 1962. Carbon-dioxide as an attractant for certain ticks (Acarina: *Argasidae* and *Ixodidae*). *Ann. Ent. Soc. Amer.*, Vol. 55, No. 5, p. 605.

HEISCH, R. B. & HARVEY, A. E. C., 1960. Is *Ornithodoros savignyi* (Audouin) a vector of relapsing fever in Africa? *Ann. Trop. Med. Parasitol.*, Vol. 54, p. 205–207.

KELLOGG, F. E. & WRIGHT, R. H., 1962. The guidance of flying insects. V. Mosquito attraction. *Can. Ent.*, Vol. 94, No. 10, p. 1009–1016.

LEES, A. D., 1952. Aspects of the physiology of the sheep tick. *Ann. Appl. Biol.*, Vol. 39, No. 1, p. 146–148.

SASA, M., TARAKA, H., UENO, Y. & MUIRA, A., 1957. Notes on the bionomics of unengorged larvae of *Trombicula scutellaris* and *Trombicula akamushi*, with special reference to the mechanisms of cluster formation and reaction to carbon-dioxide expired by the hosts. *Japan J. Exp. Med.*, Vol. 27, p. 31–43.

SASA, M. & WAKASUGI, M., 1957. Studies on the effect of carbon-dioxide as the stimulant on the tropical rat mite, *Bdellonyssus bacoti* (Hirst, 1913). *Japan J. Exp. Med.*, Vol. 27, p. 207–215.

SASA, M., 1957. Notes on the effect of carbon-dioxide in the breath as the stimulant on the flea *Ctenocephalides canis* (Curtis, 1826). *Japan J. Exp. Med.*, Vol. 27, p. 401–410.

THEILER, G., 1962. The sand tampan *Ornithodoros savignyi:* an ecological puzzle and an economic problem. *Ann. Cape Prov. Mus.*, Vol. 2, p. 212–222.