STRESS-INDUCED FAILURE OF OSMOREGULATION IN THE PARASITIC NEMATODE *PSEUDOTERRANOVA DECIPIENS*: INDIRECT EVIDENCE FOR HORMONAL REGULATION

K. G. DAVEY[1], R. I. SOMMERVILLE[2] and M. FUSÉ[1],*

[1]*Department of Biology, York University, North York, Ontario, Canada M3J 1P3* and
[2]*Department of Zoology, University of Adelaide, Adelaide, South Australia 5001*

Summary

When third-stage larvae of *Pseudoterranova decipiens* maintained at 5˚C are placed in either 40% artificial sea water (ASW, iso-osmotic) or 15% ASW (hypo-osmotic) and weighed once at 0h and again at 24h, they neither lose nor gain weight, and the osmotic pressure (OP) of their pseudocoelomic fluid (PCF) remains unchanged. In contrast, when worms are weighed six additional times during the 24h interval, those maintained in iso-osmotic conditions lose weight, while those maintained in hypo-osmotic conditions gain weight. Worms which had been exposed to hypo-osmotic conditions and weighed at various times between 0 and 24h exhibited an increase in weight which was correlated with the number of weighings. Worms exposed to hypo-osmotic conditions and weighed three additional times between 0 and 24h also gained weight, and the OP of the PCF decreased such that worms experiencing the greatest increase in weight suffered the greatest dilution of the PCF. In worms ligatured at the head or tail or at the head and tail, and then exposed to either 15% or 40% ASW, the effect of multiple weighings is exaggerated in a complex way. The presence of a ligature on the tail in worms immersed in an iso-osmotic medium leads to an increase in weight and to a very marked additional increase in weight in worms immersed in a hypo-osmotic medium. The presence of a head ligature in worms in an iso-osmotic medium leads to a decrease in weight and to a smaller weight gain in a hypo-osmotic medium. The addition of a head ligature to worms ligatured at the tail increases the weight gain in both iso-osmotic and hypo-osmotic media. These results demonstrate that stress induced by handling disrupts the normal capacity to osmoregulate in *P. decipiens*; they are consistent with the stress-induced release of postulated diuretic and antidiuretic factors.

Introduction

Recent papers from this laboratory have produced evidence that the nematode *Pseudoterranova (Phocanema, Porrocaecum, Terranova) decipiens* osmoregulates for at least 24h when immersed in media of widely varied osmotic pressures (OP). In brief,

*Present address: Department of Zoology, University of British Columbia, Vancouver, BC, Canada.

when third-stage larvae of *P. decipiens* are immersed for 24h at 5˚C in solutions with OPs as low as 0 and as high as $750 \text{mosmol kg}^{-1}$, the weight of the nematode neither increases nor decreases, and the osmotic pressure of the pseudocoelomic fluid (PCF) remains unchanged, although the worm is freely permeable to water (Fusé *et al.* 1993*a*,*b*). In the course of collecting the data for this work, it became clear that excessive manipulation of the larvae eroded this capacity for osmoregulation. This paper provides the results upon which this conclusion is based, interpreting them as an example of the effects of stress in a nematode. We then propose a working hypothesis that explains all of the observations thus far and forms a basis for further exploration of the mechanisms of osmotic regulation in nematodes.

Materials and methods

Worms were obtained from fisheries on the east coast of Canada, shipped and maintained in 40% artificial sea water (ASW) at 5˚C as described earlier (Fusé *et al.* 1993*a*). All experiments were conducted while the media were maintained at 5˚C in order to avoid the potential complications of development in the worms. The nematodes were weighed using precautions described in earlier papers to ensure uniformity of results. In essence, worms were removed from their medium, blotted gently to remove surface water, and dropped promptly into a tared vial encased in expanded polystyrene insulation and containing the appropriate concentration of ASW at 5˚C. As noted in the earlier papers, worms were out of the medium for no longer than 10s, and the addition of the polystyrene insulation further minimised the effects of any temperature changes and eliminated condensation on the surface of the chilled weighing vials as a possible source of error. These precautions to avoid any alteration in temperature are particularly important because an increase in temperature sets in train the events leading to the development of the worm to the fourth stage (Davey, 1988). Changes in weight from the initial weight were expressed as percentages of the initial weight, and means and standard errors were computed after the data had been subjected to an arcsine transformation. The data appearing in the paper have been transformed to the original percentages after the calculation of means and standard errors. Student's *t*-test was used to test for differences between the means of two groups.

Pseudocoelomic fluid (PCF) was collected and its osmotic pressure (OP) determined in a Clifton nanolitre osmometer (Clifton Technical Physics, Hartford, Conn, USA) as described earlier (Fusé *et al.* 1993*a*).

Results

When intact worms are weighed once at the beginning of a 24h period (0 time), transferred into either 40% or 15% ASW, and weighed again at the end of the period, they neither lose nor gain weight (Fig. 1; Fusé *et al.* 1993*a*). In contrast, when worms are treated identically, but are weighed six additional times between the weighings at 0 and 24h, those exposed to iso-osmotic conditions in 40% ASW lose weight ($3.7 \pm 38\%$; mean $\pm$ s.e.m., $P < 0.001$), while those exposed to hypo-osmotic conditions in 15% ASW make significant gains ($21.8 \pm 0.19\%$; $P < 0.001$). The time course for these changes in worms subjected to repeated weighings is shown in Fig. 2 (40% ASW) and Fig. 3 (15% ASW).

Worms exposed for 24h to 15% ASW and weighed three additional times between the weighings at 0 and 24h also gain weight (mean increase 7.8±2.05%; *P*<0.001). The OP of the PCF is decreased in such worms. The mean OP of the PCF of these worms was
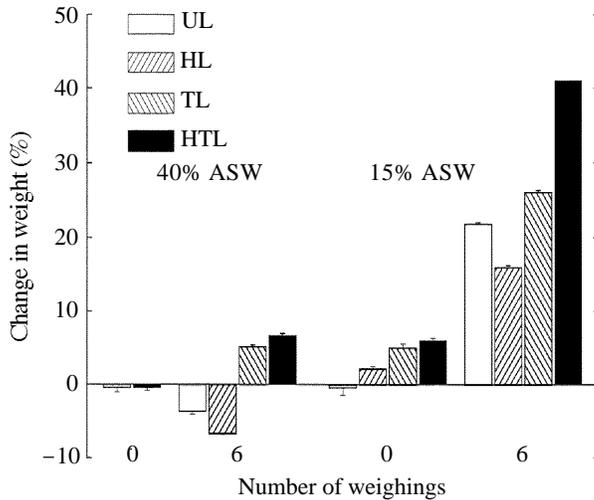

Fig. 1. The effect of weighing ligatured and unligatured worms six times between 0 and 24 h on weight gain over 24h. The data for worms weighed only at 0 and 24h have been taken from Fusé *et al.* (1993*a*). Each bar is the mean of at least 10 determinations, and the vertical lines indicate the standard error of the mean. UL, unligatured; HL, head-ligated; TL, tail-ligated; HTL, head- and tail-ligated.
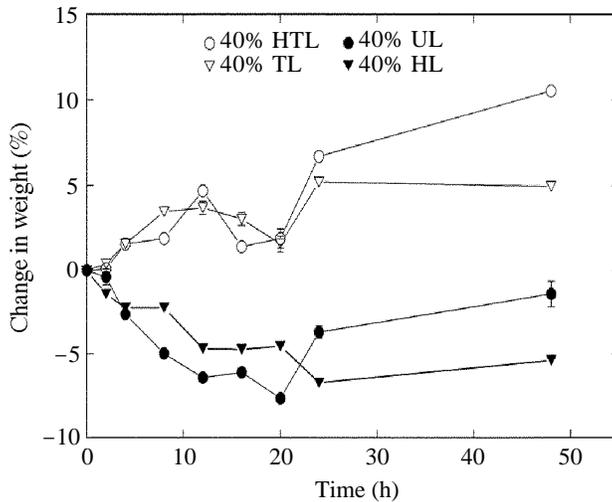

Fig. 2. The time course of weight changes in ligatured and unligatured worms exposed to 40 % ASW and weighed at the indicated times over 48h. Each point is the mean of at least 10 worms, and the vertical bars indicate the standard error of the mean. Where vertical bars are not visible, the S.E.M. is included within the size of the symbol for that time. Abbreviations as in Fig. 1.

Fig. 3. The time course of weight changes in ligatured and unligatured worms exposed to 15 %
ASW and weighed at the indicated times over 48h. Each point is the mean of at least 10
worms, and the vertical bars indicate the standard error of the mean. Where vertical bars are
not visible, the s.e.m. is included within the size of the symbol for that time. Abbreviations as
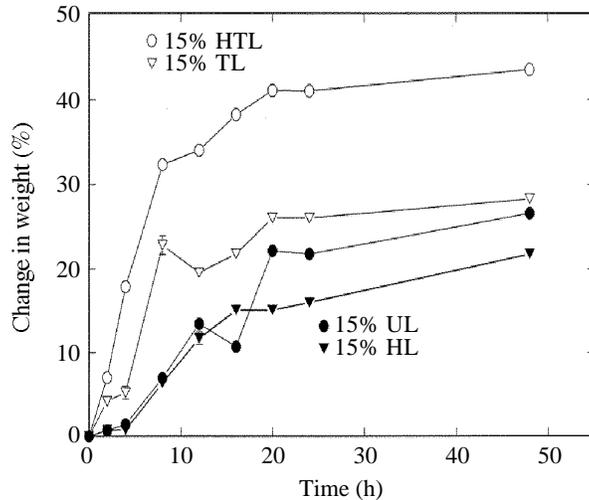in Fig. 1.

337 ± 21.2mosmolkg^{-1}, whereas the mean OP of the PCF of worms which had been
weighed only at 0 and 24h was 435.8 ± 3.2mosmolkg^{-1}. The difference between these
two means is significant ($P<0.001$). There was considerable individual variation in the
weight increase among these worms, but when the OP of the PCF is plotted against the
weight increase, as in Fig. 4, the correlation is strong ($r^2=0.96$), indicating that the larger
the weight gain, the lower the OP of the PCF.

The results from worms that have been ligatured at the head and/or tail, so as to
exclude, for head-ligatured (HL) worms, the mouth and excretory pore, and, for tail-
ligatured (TL) worms, the anus, are more complex. By reference to Figs 1, 2 and 3, the
following observations are particularly important.

In worms exposed to 40% ASW, which is iso-osmotic, HL worms which have been
weighed six times lose significantly more weight ($6.7\pm0.12\%$; $P<0.001$) than unligatured
(UL) worms. However, TL and head- and tail-ligatured (HTL) worms gain weight
compared to unligatured worms ($P<0.001$), the latter a slightly greater, but statistically
significant ($P<0.01$), amount than the former. In contrast, HTL worms weighed at 0 and
24h neither gain nor lose weight ($P>0.05$), confirming observations made in an earlier
paper (Fusé *et al.* 1993*a*).

In worms exposed to hypo-osmotic conditions in 15% ASW and weighed six times,
the application of ligatures to the head alone results in a significantly smaller weight gain
than in worms without ligatures ($P<0.001$). In TL worms, however, there is a marked
increase in the amount of weight gained compared to unligatured and HL worms
($P<0.001$). HTL worms experience a further significant gain in weight ($P<0.001$),
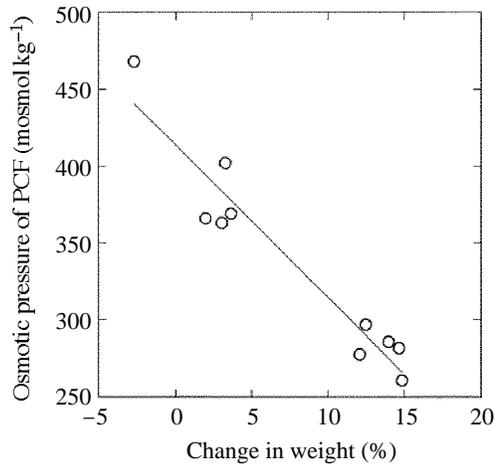
Fig. 4. The regression of weight gained over a 24h period in worms maintained in 40% ASW, and weighed on three additional occasions between the weighings at 0 and 24h, upon the osmotic pressure of the pseudocoelomic fluid (PCF).

resulting in an increase in weight of more than 40% in 24h. As noted in an earlier paper (Fusé *et al.* 1993*a*), TL or HTL worms which have been weighed only at 0 and 24h also increase in weight compared to unligatured worms (*P*<0.001), but this increase is far less (*P*<0.001) than that experienced by worms which have been similarly ligatured and weighed frequently.

Discussion

Earlier papers have demonstrated that *P. decipiens* is capable of osmoregulating over at least a 24h period in either hypo-osmotic or hyperosmotic environments. In media as dilute as distilled water or as concentrated as 75% ASW (750mosmolkg^{-1}), the worms maintain their weight and the OP of their PCF. Worms that are exposed for 10 days to osmotic stress eventually lose the capacity to osmoregulate. The body wall of the worms is freely permeable to water, and the osmoregulatory mechanism is located in the body wall, with the anus providing an additional route for the removal of water in conditions of hypo-osmotic stress (Fusé *et al.* 1993*a,b*).

These conclusions have been reached on worms that were weighed twice, once at the beginning of the 24h period and once at the end of that period. The data presented in this paper, however, demonstrate that more frequent weighings result in a loss of the osmoregulatory capacity, such that worms placed in a hypo-osmotic environment were no longer able to maintain either their weight or the OP of their PCF. Even those worms that had been placed in an iso-osmotic environment, represented by 40% ASW, lost the capacity to osmoregulate although, in this case, there was a decrease in weight. This remarkable loss of osmoregulatory capacity when the worms are weighed at frequent intervals can only be explained as a manifestation of stress, as a result of massive and unaccustomed sensory input occasioned by frequent handling.

The time course of these changes in weight demonstrates that in unligatured worms the departure from the original weight is detectable by the second or third weighing (4 or 8h) after the weighing at 0 time. Even worms that are weighed only three additional times between 0 and 24h exhibit a decrease in osmoregulatory capacity.

If, as we suggest, the loss of osmoregulatory capacity by *P. decipiens* occurs as the result of handling the worms while they are being weighed, great caution needs to be exercised in interpreting physiological data derived from nematodes which are outside their normal environment. In the immediate case, all of the experiments have been conducted at 5°C on worms which are dormant and held in 40% ASW, and all of the evidence suggests that such worms are in a stable and healthy condition, capable of osmoregulation and development for a period of at least 3 months (see Fusé *et al.* 1993*a* for a fuller discussion).

Although it is our principal conclusion that the stressful conditions of the experiments outlined here resulted in a loss of the normal control over osmoregulation in *P. decipiens*, there are other considerations that require examination. The results of experiments on ligatured worms are at first sight puzzling and do not easily fit a simple conceptual model. Both the precise nature of the input that causes the stress-related breakdown in the normal physiology of the worm and the mechanism by which the disturbance in osmoregulation is made manifest remain matters for conjecture.

In seeking to explain the more detailed results obtained from ligatured worms, it is important to understand the mechanisms by which stress is expressed in other invertebrates. Stress is always associated with the inappropriate release of neuroendocrine factors. In the nematode *Ascaris lumbricoides*, removal from the host results in a loss of the capacity to osmoregulate (Harpur and Popkin, 1973) and, indeed, when *Ascaris* is removed from its host, it enters upon a biochemical and physiological decline from which it never recovers (Harpur, 1963, 1964). Among the earliest detectable changes in *Ascaris* is the production and release of neurosecretion from cells in the anterior end of the worm, a process which is in train at least as early as 2h after the death of the host and which results in complete exhaustion of the neurosecretory cells within 24h (Davey, 1964). In insects, hyperactivity resulting from enforced activity or exposure to sublethal doses of insecticides results in the release of a number of hormones and other nerve factors (Davey, 1963; Sternburg, 1963; Raabe, 1982), and even moderate handling of the cockroach *Periplaneta americana* elevates levels of octopamine in the haemolymph (Downer *et al.* 1984).

Given that background, it is reasonable to propose that the disturbances in osmoregulation arising as the result of stress in *P. decipiens* are manifestations of the release of osmoregulatory hormones, and the ensuing discussion explores whether it is possible to explain the results of the experiments on ligatured worms from that perspective. In embarking on that exploration, the authors are aware that osmoregulatory hormones have never been described for any nematode, and the exploration is thus entirely hypothetical. In the discussion which follows, worms that have been weighed six additional times between 0 and 24h are referred to as 'stressed', while those that have been weighed only at 0 and 24h are referred to as 'unstressed'.

Consider first those worms maintained in 40% ASW. It is important to recognise that,

although we refer to 40% ASW as iso-osmotic to the PCF of *P. decipiens*, the worm always maintains the osmotic pressure of the PCF approximately 90mosmolkg^{-1} above the medium in which it is immersed (Fusé *et al.* 1993*a*), a condition which is probably essential to the maintenance of the hydrostatic skeleton. Thus, even in 40% ASW, there will be an osmotic gradient between the inside and the outside of the worm, such that water will tend to enter.

Unstressed worms in 40% ASW do not change weight, indicating that they are managing to eliminate the water which enters down the osmotic gradient. Stressed worms, however, do not gain weight, but lose a small part of their weight (about 3.7%, Fig. 1). Thus, the loss of osmotic control in this case is the result of an inappropriate diuresis.

There are two routes by which water has been identified as leaving the worm: through the body wall and through the anus (Fusé *et al.* 1993*a*). In the unstressed worm in 40 % ASW, it is clear that the water which enters as a result of the normal difference in OP between the PCF and the medium leaves through the body wall, since HTL worms, in which the anus is blocked, do not increase in weight. In the stressed worm, however, blocking the anus, as in a TL or HTL worm, leads to a marked increase in weight, reversing the effect of stress in the unligatured or HL worm. That might suggest that the anus is the route which is affected by stress. However, the magnitude of the changes involved needs to be considered. The difference between the loss experienced by a stressed HL worm and the increase experienced by a stressed HTL worm is about 15%, which amounts to something like 3mg, given the average weight of *P. decipiens*. We have only fragmentary information about the rates of urine production in *P. decipiens* (Fusé *et al.* 1993*a*) but that information suggests that the rate of flow of fluid from the anus under extreme hypo-osmotic conditions is of the order of nanolitres per hour, a rate which is unlikely to account for 3 μl in 24h.

If much of the outward flow of water is taking place across the body wall, then the effects of ligating worms at the tail are best explained by postulating the existence of a diuretic factor which has its origin in the tail, which affects fluid transport across the body wall and which is released in large amounts in stressed worms. Thus, the decrease in weight in unligatured worms is a consequence of the stress-related release of the hypothesised diuretic factor. When that factor is removed, as in TL or HTL worms, the worms gain weight. The tail is the source of at least one other factor in *P. decipiens*, a hormone that influences oxygen consumption (Davey, 1986).

But postulating a diuretic hormone emanating from the tail does not explain all of the observations in this paper. To choose an obvious example, why does the unligatured stressed worm in 15% ASW gain so much weight while the unstressed but otherwise similar worm does not? If there is an increased release of the diuretic factor in stressed animals, then surely the stressed worm would not increase in weight substantially while the weight of the unstressed worm in 15% ASW is unchanged. These and other observations can be accommodated by postulating an antidiuretic factor which is released by stress. Given the general environment in which *P. decipiens* lives, it is more likely to be exposed to hyperosmotic conditions, such as sea water, than to hypo-osmotic conditions, and the existence of an antidiuretic control is thus to be expected.

If an antidiuretic factor is involved in the control of osmoregulation in *P. decipiens*, its source is clearly not the tail, since TL worms gain weight. Equally, it is unlikely to emanate from the head, since HTL animals gain considerably more weight than TL worms, an effect opposite to that to be expected if the head were a source of antidiuretic factor. It is therefore proposed that the source of the antidiuretic factor is in the body of the worm, possibly the ventral nerve cord, which contains some neurosecretory cells (Davey, 1988).

What is the role of the head in this proposed arrangement? Obviously, the head has considerable influence, but that influence is complex. In those worms in 40% ASW in which the tail is present, ligating the head leads to a decrease in weight compared to unligatured worms. In contrast, in worms in which the tail has been ligatured as well as the head, the effect of adding the head ligature is opposite. In other words, the direction of the influence exerted by the head depends on the presence of the tail. If the tail is present, then removal of the head results in a decrease in weight, and the head is thus exerting an antidiuretic effect. In the absence of the tail, the head appears to exert a diuretic effect.

These observations can be explained by assuming that the head, while not the source of either of the proposed factors, is influencing their release, perhaps as the result of sensory information detected by anterior sense organs. That control would take the form of inhibition on release, so that ligation of the head would promote the release of both the diuretic and antidiuretic factors, adding to the release promoted by stress. Does this hypothetical scheme, in which a diuretic hormone emanating from the tail and an antidiuretic hormone released from sources in the body of the worm, both subject to inhibitory controls from the head, explain the results which have been obtained in this study?

For worms in 40% ASW, unligatured and unstressed worms in 40% ASW would be releasing very little in the way of either of the factors, other than that required to maintain the OP of the PCF slightly above that of the external medium. Stress would result in some release of both factors, but the net effect is diuretic, an effect which is enhanced by ligating the head, thus freeing the tail from any inhibition on release. In the absence of the tail, stress results in the release of antidiuretic factor, and this effect is enhanced by the absence of the head and the freeing of the source of antidiuretic factor from its inhibition.

In 15% ASW, there will be a substantial influx of water even in unstressed worms. It is therefore likely that some of the inhibition on the release of diuretic factor is lifted, while the inhibition on the release of antidiuretic factor is maintained or strengthened, in order that the osmoregulatory mechanisms can cope with the influx of water and thus maintain the weight of the nematode. In the absence of the head, the inhibition on the release of antidiuretic factor is lifted and the worms gain some weight. In the absence of the tail, the diuretic factor is removed and the worms gain additional weight. In stressed worms with heads, both the diuretic and the antidiuretic factor are subject to release by stress, and the observation that the worms gain a good deal of weight suggests that under these circumstances the release of antidiuretic factor must be greater. In the absence of the head in stressed worms, the release of diuretic factor is further enhanced and the worms experience some decrease in weight. Removal of the tail, with its diuretic factor, results in an increase in weight, and removal of the head along with the tail permits some further

release of antidiuretic factor, and the osmoregulatory capacity of the worm is so sharply reduced that it gains more than 40% in weight in 24h.

While there is no direct evidence for the existence of either of these factors, the scheme outlined above accounts for all of the observations and provides a conceptual framework for results which would otherwise lack organisation. More importantly, it also provides a working hypothesis within which work on osmoregulation can proceed.

References

DAVEY, K. G. (1963). The release by enforced activity of the cardiac accelerator from the corpus cardiacum of *Periplaneta americana. J. Insect Physiol.* **9**, 375–381.

DAVEY, K. G. (1964). Neurosecretory cells in a nematode, *Ascaris lumbricoides. Can. J. Zool.* **42**, 731–734.

DAVEY, K. G. (1986). A possible hormone controlling O_2 consumption in the nematode *Phocanema decipiens. Gen. comp. Endocr.* **64**, 30–35.

DAVEY, K. G. (1988). Endocrinology of nematodes. In *Endocrinology of Selected Invertebrate Types* (ed. H. Laufer and R. G. H. Downer), pp. 63–86. New York: Alan R. Liss Inc.

DOWNER, R. G. H., BAILEY, B. A., GOLE, J. W. D., MARTIN, R. J. AND ORR, G. L. (1984). Estimation of octopamine and its role in the release of a hypertrehalosemic factor in the American cockroach, *Periplaneta americana.* In *Insect Neurochemistry and Neurophysiology* (ed. A. B. Borkovec and T. J. Kelly), pp. 349–352. New York: Plenum Press.

FUSÉ, M., DAVEY, K. G. AND SOMMERVILLE, R. I. (1993*a*). Osmoregulation in a parasitic nematode *Pseudoterranova decipiens. J. exp. Biol.* **175**, 127–142.

FUSÉ, M., DAVEY, K. G. AND SOMMERVILLE, R. I. (1993*b*). Water compartments and osmoregulation in the parasitic nematode *Pseudoterranova decipiens. J. exp. Biol.* **175**, 143–152.

HARPUR, R. P. (1963). Maintenance of *Ascaris lumbricoides in vitro*. II. Changes in muscle and ovary carbohydrates. *Can. J. Biochem. Physiol.* **41**, 1673–1689.

HARPUR, R. P. (1964). Maintenance of *Ascaris lumbricoides in vitro*. III. Changes in the hydrostatic skeleton. *Comp. Biochem. Physiol.* **13**, 71–85.

HARPUR, R. P. AND POPKIN, J. S. (1973). Intestinal fluid transport: studies with the gut of *Ascaris lumbricoides. Can. J. Physiol. Pharmac.* **51**, 79–90.

RAABE, M. (1982). *Insect Neurohormones*, pp. 173–174. New York: Plenum Press.

STERNBURG, J. (1963). Autointoxication and some stress phenomena. *A. Rev. Ent.* **8**, 19–38.