10609

to procedural modifications of irradiation. It was interesting to note that in mice of the *X/Gf* strain, in which it was difficult to detect virus in thymic tissues, the incidence of leukaemia induced by X-rays was as high as that observed in the *NIH* strain. In the latter strain, virus detection was relatively easy (Table 1) although we cannot disregard the sampling problem in electron microscopy.

The results recorded here and previously[1–4] support the hypothesis that all strains of laboratory mice carry leukaemia virus[14,15] and that induction of leukaemia varies with an as yet undefined threshold of susceptibility. Study of the viral aetiology of human leukaemia would necessitate the use of a virus-free susceptible experimental animal. So far, germ-free rats seem to be virus-free[2,16]; and they are susceptible to the leukaemogenic effects of murine leukaemia viruses[17] and of chemical agents[18]. Further characterization of germ-free rats for viral flora is in progress.

We thank Dr A. Goldfeder, Dr L. W. Law, Dr W. P. Rowe, Dr J. C. Solomon and Mr J. G. Gilbey for mice, and Miss B. J. Minczewski and Mrs P. Luckert for technical assistance. This work was supported by funds from the Leukemia Society, Inc., and the US Public Health Service.

Masahiro Kajima
Morris Pollard

Lobund Laboratory,
Department of Microbiology,
University of Notre Dame,
Indiana.

Received March 1, 1968.

1 de Harven, E., *J. Exp. Med.*, 120, 857 (1964).
2 Kajima, M., and Pollard, M., *J. Bacteriol.*, 40, 1448 (1965).
3 Kajima, M., and Pollard, M., in *Electron Microscopy 1966* (edit. by Uyeda, R.), 2, 215 (Maruzen Co., Tokyo, 1966).
4 Pollard, M., and Kajima, M., *Proc. Intern. Conf. Radiat. Biol. and Cancer*, 175 (Publ. Radiation Soc. of Japan, 1967).
5 McEndy, D. P., Boon, M. C., and Furth, J., *Cancer Res.*, 4, 377 (1944).
6 Kaplan, H. S., *J. Nat. Cancer Inst.*, 8, 191 (1948).
7 Rappaport, H., and Baroni, C., *Cancer Res.*, 22, 1067 (1962).
8 Dmochowski, L., Grey, C. E., Dadgett, F., and Sykes, J. A., in *Virus, Nucleic Acids and Cancer*, 85 (The Williams and Wilkins Co., Baltimore, 1963).
9 Feldman, D. G., and Gross, L., *Cancer Res.*, 26, 412 (1966).
10 Dalton, A. J., and Moloney, J. B., in *Interpretation of Ultrastructure* (edit. by Harris, R. J. C.), 385 (Academic Press, Inc., New York and London, 1962).
11 Goldfeder, A., *Radiat. Res.*, 16, 601 (1962).
12 Rinere, M. R., Chouroulinkov, I., Marty, C., and Guerin, M., *C.R. Soc. Biol.*, CIVI, 1035 (1962).
13 Kaplan, H. S., and Brown, M. B., *J. Nat. Cancer Inst.*, 13, 185 (1952).
14 Miller, J. F. A. P., in *Advances in Cancer Research* (edit. by Haddow, A., and Weinhouse, S.), 6, 291 (Academic Press Inc., New York and London, 1961).
15 Gross, L., in *Advances in Cancer Research* (edit. by Haddow, A., and Weinhouse, S.), 6, 150 (Academic Press Inc., New York and London, 1961).
16 Kajima, M., and Pollard, M., *Cancer Res.*, 27, 980 (1967).
17 Pollard, M., and Kajima, M., *Proc. Soc. Exp. Biol. and Med.*, 121, 585 (1966).
18 Pollard, M., and Kajima, M., *J. Nat. Cancer Inst.*, 39, 135 (1967).

BIOLOGY

Behavioural Responses of Parasites to the Symbiotic Fungus associated with *Sirex noctilio* F.

Several hymenopterous parasites have been released in Australia and New Zealand as possible biological control agents for the woodwasp *Sirex noctilio* F. These include *Ibalia leucospoides* (Hochenw.), *Rhyssa persuasoria* (L.) and *Megarhyssa nortoni nortoni* (Cresson). *I. leucospoides* attacks the siricid egg or newly hatched larva, whereas the other two species attack late stage larvae, pupae and (rarely) emerging adults.

There is a symbiotic association between *Sirex noctilio* and the fungus, *Amylostereum* sp.[1]. The fungus is inoculated into the coniferous tree during oviposition by *Sirex* females and, on successful establishment, exerts a phytotoxic effect on the tree and by killing it creates a suitable milieu for the development of *Sirex* larvae. Some aspects of the behaviour of the parasites have been studied in the laboratory and it has been shown that responses to material produced by the presence and action of *Amylostereum* within the host tree *Pindus radiata* D. Don are important in the location of hosts by their parasites.

Logs were exposed to *Sirex* females so that oviposition drills of different ages were available in a series of logs or in the same log. When these were exposed to *I. leucospoides* females it was found that maximum preference was shown for drills 2–3 weeks old, which coincides approximately with the time of egg eclosion.

Dissection of drills selected by the female parasites for antennal search and probing with the ovipositor revealed that 35 per cent (of a total of 123) did not contain *Sirex* eggs. That these were investigated by the parasites during the same period as those which contained eggs provides evidence that the stimuli involved were not associated with the presence of the eggs themselves but rather with the fungus and other material introduced by *Sirex* at the time of oviposition.

When presented with flasks containing cultures of *Amylostereum* of different ages (in a sawdust-base medium) female parasites investigated the cotton wool plugs of certain culture flasks with their antennae in a way identical to that observed in response to suitably aged drills in infested logs. The females showed a preference for cultures 2–3 weeks old. When air was withdrawn from "attractive" flasks with a syringe and then directed at their antennae, females adopted a searching attitude, the antennae being lowered to seek out and explore the tip of the hypodermic needle. There was no response in control tests to air collected from above sawdust based culture medium not inoculated with the fungus.

Acetaldehyde is known to be present in the fungal cultures during the period for which they are attractive to *I. leucospoides* (Titze and Madden, unpublished). Antennal response to the tip of a syringe containing low concentrations (in air) of vapour collected over acetaldehyde was obtained from up to 60 per cent of the females in a series of tests each involving thirty individuals.

Attraction to the youngest drills occurred on logs in which the moisture content was about 60 per cent (of oven dry weight), and as the moisture content increased so did the age of the preferred drills. It is known[2] that the fungus grows more rapidly in wood of moderate moisture content, and the earlier attraction of *I. leucospoides* to drills in wood of lower moisture content may be related to this.

R. persuasoria and *M. nortoni* responded to filter papers immediately after their removal from between transverse cuts in infested logs. This response took the form of antennal investigation and insertion of the ovipositor through the filter paper at the point of maximum antennal interest. Insertion was chiefly confined to areas of the paper which had been in contact with frass recently produced by *Sirex* larvae.

Identical positive responses were shown by these species in the subsequent bioassay of suitably concentrated water, methanol, ethanol and acetone extracts of fungal cultures, of frass and of the filter papers that had been in contact with infested wood. The threshold concentration for the two species was different.

The extracts also promoted a male response in *M. nortoni* which was expressed in aggregation and precopulatory activity. This behaviour is observed on infested billets and trees from which female insects are emerging. Preliminary experiments with acetaldehyde failed to elicit a positive response in either sex to any of a range of test concentrations, although antennal palpation was obtained in response to the polymer paraldehyde in bioassay.

The evidence presented here suggests that the fungus *Amylostereum* is involved in the host location behaviour of three parasite species. Attraction is associated with

active fungal growth, but it is not clear at this stage if the same component is responsible for the observed behavioural responses of each of the species studied. The fact that the timing of oviposition by *I. leucospoides* relative to that by *Sirex* is very different from that by the rhyssine species suggests that parasites respond to different chemical stimuli produced by the fungal infections at the different times, but the situation is made complex by the obvious difference in the chemical and physical properties of the trees. When *I. leucospoides* oviposition is taking place the tree as a whole and the oviposition drills of *Sirex* are characterized by high moisture and volatile oil contents. The rhyssines show a positive response to 2–3 week old *Amylostereum* cultured on basal media, but little response to infested billets until some 5 months after inoculation. At the time of their oviposition in trees, both wood moisture and volatile oil contents are usually much lower than at the time of oviposition by *I. leucospoides*, although pockets of high moisture content are associated with frass, recently deposited by well developed *Sirex* larvae. Furthermore, this frass is characterized by a relatively high nitrogen content. Gradients of moisture along larval galleries and within the wood permit different degrees of fungal growth and modify the concentration of the attractant released. Differences of these kinds could well modify the responses to the same fungal-produced material so as to produce the observed differences in the timing of oviposition.

Positive responses, similar to those obtained with *R. persuasoria* and *M. nortoni*, have been shown by females of *Rhyssa crevieri* (Prov.), *Megarhyssa greenei greenei* Vier. and *M. macrurus* (L.) which were received from eastern Canada during 1967. All other ichneumonid parasites attacking *Sirex* in the insectary have similar oviposition behaviour to that of the rhyssines tested and it is reasonable to assume that the fungus is also involved in their attraction. Similarly, the behaviour of *Ibalia ensiger* Nort. is identical with that of *I. leucospoides*.

J. L. MADDEN

CSIRO Division of Entomology,
Stowell Avenue,
Hobart, Tasmania.

Received February 9, 1968.

[1] Talbot, P. H. B., *Austral. J. Bot.*, 12 (1), 46 (1964).
[2] Coutts, M. P., and Dolezal, J. E., *Austral. Forest Res.*, 1 (4), 3 (1965).

Sensitivity of Three Stored Product Insect Species exposed to Different Low Pressures

THE effect of low pressures on the mortality of stored product insects has been investigated by Back and Cotton[1] and by us[2]. The relative sensitivity of six stored product insects was determined and the possible use of low pressures for stored product insect control was considered. In a preliminary study on the effect of different low pressures on *Sitophilus oryzae* (L.)[3], the highest mortality was obtained when the insects were exposed to 5 cm of mercury for 8 h, while lower pressures resulted in lower mortality. We have recorded the sensitivity of two more species to different low pressures, and compared it with that of *S. oryzae*.

Low pressure measurements were taken in metal containers filled with grain, using a method described earlier[3]. *Sitophilus oryzae* (L.) (adults 2–3 days after emergence), *Callosobruchus maculatus* (F.) (adults 2–3 days after emergence) and *Trogoderma granarium* Everts (larvae 2·0–2·5 mm length) were exposed to the low pressures. The insects were taken from the stock of the controlled temperature culture room at 26° ± 1° C and 70 per cent ± 2 relative humidity. Different low pressures were obtained in each of the metal containers of 20 l. capacity containing 12 kg of imported 'Hard Red' winter wheat of 12 per cent M C. Fifty insects in 40 c.c. glass jars containing 1 g of wheat or chick peas (*Cicer arietinum*) were inserted at a depth of 5 cm below the surface layer of grain.

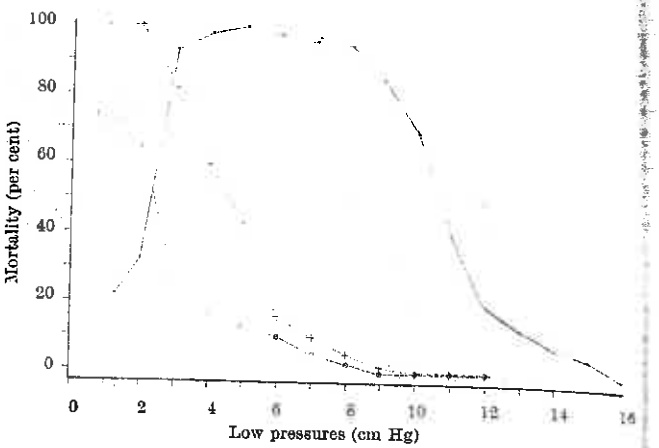

Fig. 1. Effect of low pressures on the mortality of three stored product insect species. ●—●, *Sitophilus oryzae* (L.) adults; + – · – +, *Callosobruchus maculatus* (F.) adults; ○ – – – ○, *Trogoderma granarium* Everts larvae.

The low pressures tested varied from 1 to 16 cm of mercury and the time of exposure was always 24 h. Treatment was carried out in the controlled temperature room and the mortality was checked 24 h after exposure.

The results are the average of four measurements and the percentages were modified according to Abbott's[4] formulae. The sensitivity of three insects to different low pressures is shown in Fig. 1. The curve for *S. oryzae* (L.) is clearly different from the other two. Very low pressures of 1 to 3 cm of mercury resulted in a lower percentage mortality of *S. oryzae* (L.), while the maximum effect was obtained with a range of 4 to 6 cm of mercury. With increased pressure, the percentage of mortality decreased, as expected. The mortality curves of the two other insects were very similar, showing the highest percentage of mortality at the lowest pressures and the decrease in mortality with the increase of the pressure. In the case of *T. granarium* Everts, the lowest pressure tested (1 cm of mercury for 24 h) did not result in total mortality. This finding confirms data already recorded[2].

The sensitivity of various insect species to different low pressures has not yet been investigated sufficiently. Exposure to the lowest pressures was thought to cause maximum mortality of the insects, but our results show that different insect species can react differently. Bhambhani[5], in his work on responses of pests to fumigation, noted that *S. oryzae* (L.) and *S. granarius* (L.) were less susceptible at 3–4 mm of mercury than at 2–4 cm of mercury. Sharplin and Bhambhani[6] found that when treating *S. granarius* (L.) the lowest pressures caused closure of the spiracular structure. We cannot offer any other explanation for our results. Further research is needed to investigate the effect of low pressures on insects with different morphological features.

These results may be important in considerations of low pressure treatment for insect control.

M. CALDERON
S. NAVARRO

Ministry of Agriculture,
Department of Plant Protection,
Stored Products Research Laboratory, Jaffa, Israel.

Received February 27, 1968.

[1] Back, E. A., and Cotton, R. T., *Agric. Res.*, 31, 1035 (1925).
[2] Calderon, M., Navarro, S., and Donahaye, E., *J. Stored Prod. Res.*, 2, 135 (1965).
[3] Calderon, M., and Navarro, S., *Progress Report for the Year 1966/67*, Department of Plant Protection, Jaffa, Israel.
[4] Abbott, W. S., *J. Econ. Entomol.*, 18, 265 (1925).
[5] Bhambhani, H. J., *Bull. Entomol. Res.*, 47, 749 (1955).
[6] Sharplin, T., and Bhambhani, H. J., *Canad. Entomol.*, 95, 352 (1963).