MAJOR AND TRACE ELEMENT DETECTION
IN INSECTS BY ION INDUCED
X-RAY FLUORESCENCE

II. A. VAN RINSVELT, R. DURKES, JR., R. LEVY,
AND H. L. CROMROY

ABSTRACT

The application of ion induced X-ray fluorescence to study the relative
concentration of several major and trace elements present in the tissues of 9
species of adult and immature insects was investigated. Results of the qualita-
tive analyses indicated that K, Ca, Ti, V, Mn, Fe, Ni, Cu, Zn, Pb, Rb, and Sr
could easily be identified from a single spectrum. In addition, between and
within species differences and similarities were observed by comparing the
pulse heights of specific elements.

A high concentration of Rb, with respect to K, was found to be present in
the ash of all adult and immature insects analyzed. Therefore, a species
comparison was made between the relative concentration of Rb and K (Rb/K
ratio).

Knowledge of the importance of major and trace elements present in insect
tissues has been hampered by the lack of good analytical techniques having a
sufficient sensitivity, selectivity, and practical applicability to allow precise
study of the tiny absolute quantities and concentrations present. Sensitive
techniques such as neutron activation analysis, atomic absorption spec-
troscopy, and X-ray fluorescence spectrometry have been widely used to deter-
mine the concentration of various elements in biological tissues. However,
their application for studying insect tissues has been limited. Levy and
Cromroy (1973) made a comprehensive study using atomic absorption spec-
troscopy to determine the concentration of several major and trace elements
in 41 species of adult and immature insects.

In the classical X-ray fluorescence technique, in particular, the emitted
characteristic X-rays are analyzed by low efficiency crystal spectrometers or
by low resolution proportional counters and scintillation detectors. Order of
magnitude or better improvement in sensitivity now is possible through (a)
the excitation of the characteristic X-rays of the elements present in the
sample by heavy ion bombardment and, (b) the use of more sensitive semi-
conductor X-ray detectors.

The purpose of this study was to determine the feasibility of using ion
induced X-ray fluorescences for the observation and the quantification of
major and trace elements present in adult and immature insects.

METHOD AND MATERIALS

ION INDUCED X-RAY FLUORESCENCE TECHNIQUE

Characteristic X-rays excited by other X-rays or by electrons have long
been used for classical elemental analyses. Recently Johansson et al. (1970)
showed the great potential for the use of heavy ions for excitation of characteristic X-rays. In this technique, charged particles are accelerated to several MeV and then collimated and focused on the sample to be analyzed. The X-rays, characteristic of the elements present in the samples, are produced during the bombardment and detected by a lithium drifted silicon X-ray detector, [Si(Li)]. The large efficiency of the Si(Li) detector and the intense yield of X-rays emitted during the bombardment permit trace element analysis at the $10^{-12}$ gram level and the possibility of extrapolating the sensitivity to the $10^{-15}$ gram level. The resolution of the Si(Li) detector is such that it will allow the identification through their characteristic X-rays of consecutive elements in the periodic table as long as $Z > 10$. A further advantage stems from the fact that the system is non-dispersive, permitting the simultaneous determination of all elements in a short single run.

**Sample Preparation and Irradiation**

Approximately 1 g of whole adult or immature insects were freeze-dried by lyophilization and then ashed in a low temperature radio frequency asher. In most instances, the insects were not fed during the 24 hrs preceding the lyophilization to assure that their guts would be voided and that the elemental analyses would not be affected by undigested food in the gastrointestinal tract. Finally a few milligrams of the residual material were glued to 20 $\mu$g/cm$^2$ thick self-supported pure carbon films with a small drop of diluted polystyrene glue. The glue was previously analyzed and found to be relatively free of impurities in the region of interest.

The dried samples were then bombarded with 4 MeV helium ions produced by the University of Florida Van de Graaff accelerator and the emitted X-rays were detected by a 80 mm$^2$ active area Si(Li) detector. The pulses from the Si(Li) detector were energy sorted and stored in a 512-channel pulse-height analyzer which finally delivered a complete spectrum of the emitted X-rays, permitting the identification through their characteristic X-rays of all elements present in the sample. A 0.25 mm thick teflon absorber was inserted between the detector and the radiating sample in order to keep the counting rate in the detector at a level such that the detector would not lose its large efficiency and its good resolution. The intensity of the ion beam was kept below 150 nA so that highly volatile elements would not escape from the overheated sample during bombardment.

**Results and Discussion**

Nine insect samples were investigated in this research and typical X-ray spectra obtained for 3 species are shown in Fig. 1 and 2. In these figures, most observed elements are characterized by 2 X-rays, the $K_\alpha$ and $K_\beta$ lines. For the lead component, however, only the L series (L$\alpha$, L$\beta$, and L$\gamma$) is observed. The decrease in the counting rate for low energy X-rays is due to the larger absorption from the teflon absorber as $Z$ becomes smaller.

It is clear from these 4 examples that the measured resolution is in line with the predicted results mentioned earlier and that a qualitative analysis can be made just by identification of the various peaks present in the spectrum.
Some quantitative information can also be drawn from the results presented here, merely by the determination of the relative heights of the X-ray peaks. It can be seen, for example, that the manganese (Mn) peak is high in *Photuris B* compared with the 3 other samples and that only *Plecia nearctica* contains a significant amount of titanium (Ti) and vanadium (V). *Plecia nearctica* also shows the largest amount of lead (Pb) and strontium (Sr) while *Photuris B* shows the largest rubidium (Rb) peak. The high Pb concentration in adult "love bugs" may be due to the accumulation of this environmental contaminant from automobile exhaust fumes, since the "love bugs" were collected along a highway (Callahan and Denmark 1973). Since rubidium has not been reported in insect tissues, a rubidium survey was made of the nine samples by comparing the rubidium peak to the potassium peak. This comparison was made since a general relationship has been shown between rubidium (Rb) and potassium (K) for a variety of biochemical and

![Graph showing X-ray spectra of adult and nymphal Periplaneta americana.](image)

Fig. 1. X-ray spectra obtained from the bombardment of samples of adult and nymphal *Periplaneta americana* by 4 MeV helium ions. The charge accumulated on the target was 90 μC (beam intensity: 100 nA) and 80 μC (beam intensity: 50 nA) for the adult and immature insects, respectively. The excellent resolution of the system and the favorable peak to background ratio are clearly demonstrated in this semi-logarithmic plot.
Fig. 2. X-ray spectra obtained from the bombardment of samples of *Photuris B* and *Plecia nearctica* by 4 MeV helium ions. The charge accumulated on the target was 200 μC (beam intensity: 130 nA) and 300 μC (beam intensity: 125 nA) for the *Photuris B* and the *Plecia nearctica*, respectively.

physiological processes (Christian and Feldman 1970). The results are shown in Table 1. It should be noted that the Rb/K ratio represents the area under the rubidium peak divided by the area under the potassium peak, after background subtraction. The large variations in this ratio demonstrate the sensitivity of the method. For the most part it is not within the scope of this research to explain the presence or the absence of particular elements in the insects and to present reasons for the wide changes in the Rb/K ratio from the various samples. The results presented here, however, show that a careful investigation, using this new technique, could certainly answer some of the questions related to major and trace elements present in insects.

Exact quantitative studies could finally be achieved by some standard technique. It seems that the best method in this case would be the use of internal standards. The limit of the sensitivity will depend on many factors (element observed, ion and energy available, cross section for X-ray production) and preliminary results obtained by Duggan et al. (unpublished, 1971) showed that the 10^{-12} gram level can easily be reached.

In conclusion, the ion induced X-ray fluorescence technique is well suited
TABLE 1. COMPARISON OF RUBIDIUM TO POTASSIUM RELATIVE RATIOS IN 9 SAMPLES OF INSECTS. *

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common Name</th>
<th>Rb/K Ratio (x10⁻⁵)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Periplaneta americana</em> (L.) (Adult) American cockroach</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td><em>Periplaneta americana</em> (Nymph)</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td><em>Photuris D</em> (Adult)</td>
<td>Firefly</td>
<td>37</td>
</tr>
<tr>
<td><em>Photuris B</em> (Adult)</td>
<td>167</td>
<td></td>
</tr>
<tr>
<td><em>Photuris A</em> (Adult)</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td><em>Plecia nearctica</em> Hardy (Adult)</td>
<td>&quot;Lovebug&quot;</td>
<td>25</td>
</tr>
<tr>
<td><em>Sitophilus oryzae</em> (L.) (Adult)</td>
<td>Rice weevil</td>
<td>10</td>
</tr>
<tr>
<td><em>Trichogramma castaneum</em> (Herbst) (Adult)</td>
<td>Red flour beetle</td>
<td>9</td>
</tr>
<tr>
<td><em>Pseudoplasia includens</em> (Walker) (Larva)</td>
<td>Soybean looper</td>
<td>4</td>
</tr>
<tr>
<td><em>Plodia interpunctella</em> (Hubner) (Larva)</td>
<td>Indian meal moth</td>
<td>23</td>
</tr>
</tbody>
</table>

*Photuris complex—species identified by Dr. J. E. Lloyd, Department of Entomology and Nematology, University of Florida.

for the analyses of insect samples; the resolution of the system is very good within its range of application and its sensitivity is probably more than adequate.

ACKNOWLEDGEMENT

The authors wish to acknowledge the Insects Affecting Man and Animals Research Laboratory and the Insect Attractants, Behavior, and Basic Biology Research Laboratory, USDA, for supplying many of the insects used in the analyses.

LITERATURE CITED


