ON THE DIGESTION OF WOOD BY INSECTS

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(With One Text-figure.)

I. INTRODUCTION.

The digestion of wood in insects, and consequently the fate of cellulose, has proved to be a prolific field for speculation. Wood-feeding insects are generally of a fairly small size, which does not permit a thorough study of their digestive processes. For this reason perhaps the literature dealing with the physiology of their nutrition is very scanty. Moreover, the problem is made more difficult to settle owing to the presence of micro-organisms, with intimate relations to the alimentary canal, in a number of these insects. An important rôle in the digestion of wood has been assigned to these micro-organisms, and the symbiotic relations between wood-feeding insects and bacteria or yeasts have often been quoted. Uvarov (1929), in his interesting summary of the literature on insect nutrition and metabolism, writes: "the admission of the rôle of micro-organisms in the nutrition of wood-eating insects clears the way to a better understanding of their physiology" (p. 266). The same author admits clearly that all the evidence in favour of this view is indirect, but nevertheless he stresses the importance of the "so-called symbiotic micro-organisms" in more than one aspect of the nutrition of insects, especially of those with wood-eating habits.

In the cases where extracellular intestinal micro-organisms occur, such as the Protozoa-harbouring termites (Cleveland, 1924, 1925, 1928, 1930; Beckwith and Rose, 1929), the wood-eating roach, Cryptocercus punctulatus (Trager, 1932), and insects feeding on rotting wood, such as Potoria cuprea (Werner, 1928 a, b) and other Lamellicorn larvae (Wiedemann, 1930), it is comparatively easy to aim at estimating the precise rôle which such organisms might play in the nutrition of their host. Both Cleveland and Werner infer that the insects they have investigated depend directly on the products of cellulose splitting by the intestinal micro-organisms. Wiedemann, on the other hand, concludes from a very thorough physiological study that the intestinal micro-organisms of some Lamellicorn larvae are utilised as a direct food supply.
The case of the intracellular micro-organisms on the other hand is more
difficult to investigate. Though so far there is not a single piece of direct evidence
for the help the host is supposed to derive from the micro-organisms, yet they
have been accepted by numerous authors (e.g. Buchner, 1928, 1930) as playing a
vital rôle in the nutrition of wood-eating insects, especially in connection with the
digestion of cellulose.

This hypothesis is based on the assumption that insects are unable to digest
wood through the activity of their own enzymes. Falck (1930), however, has shown
clearly that the larvae of Hylotropes bajulus, which do not harbour any micro-
organism, have enzymes capable of breaking down cellulose from the wood. More-
over, the rôle assigned to the intracellular micro-organisms has been doubted both
for bionomical reasons (Mansour, 1930) and on physiological grounds (Ripper,
1930).

In view of this confusion with regard to a group of insects of great economic
importance, the present work has been undertaken.

II. MATERIAL AND METHODS.

(a) Wood-eating insects.

The larvae of Macrotoma palmata and Xystrocera globosa, two wood-eating
Cerambicids, were used in this research.

The first species lives in the wood of Morus alba and bores in both inner and
outer regions. The second species lives in the wood of Albizzia lebbek (Mimosa-
cae) and confines its ravages to the sap wood. The larvae of both species were
procured from felled trees; the second species was more easy to extricate owing to
its position next to the bark. The collected larvae, if not made use of immediately,
were put separately in Petri dishes full of moist frass and chips of the wood on which
the particular larvae fed. The Petri dishes were then kept till wanted in a 30° C.
incubator.

The stomach juice for the enzyme experiments was procured by cutting a
longitudinal slit in the middle region of each larva. The mid-gut clear from the
surrounding tissues immediately bulges out from this slit. It was then pricked and
the oozing juice was collected in a suitable receptacle.

The gastric juice of a few animals was mixed together and, after being centri-
fuged, it appeared as a clear fluid with a reddish brown colour.

The pH of the fresh fluid was estimated colorimetrically for every lot. It was
found to be about 6·4 for both species. The measurements (five in number for each
species) varied in both cases from 6·3 to 6·5.

Each Xystrocera larva gave about 0·13 c.c. of gastric juice, while those of
Macrotoma gave about 0·25 c.c.

1 We are indebted to Mr Alfieri (Secretary of the Royal Entomological Society of Egypt) for the
identification of this species whose larvae, according to Willcock (1922), are very difficult to dis-
tinguish from those of the giant Longicorn, Rhesus serricollis Motsch.
(b) Other material.

The phytophagous caterpillars of Prodenia littoralis and Agrotis ypsilon were used for comparative purposes.

The first species is the cotton-leaf worm of Egypt. It is also common on many Malvaceous plants and Trifolium Alexandrinum. The second species is a surface cutworm which attacks cotton, Trifolium and a number of other plants too. The two species are very common in Egypt, especially during the warmer part of the year, and consequently they are very easy to procure.

The collecting of the gastric juice of these two caterpillars was carried out by inducing vomiting. This was done by exposing the caterpillars to chloroform fumes for a few minutes (Shinoda, 1930). The larvae generally recovered after a few days and, if necessary, the operation could be repeated.

(c) Qualitative experimental methods.

For demonstrating the digestion of cellulose qualitatively a few drops of the gastric juice were put on sections of the rib of a lettuce leaf or sections of a date stone placed in hollowed out slides. A few drops of toluol were also added to prevent bacterial action. The section was then covered and the whole slide was put in a moist chamber in a 30°C incubator. After a day or two, the sections were rinsed with distilled water, soaked in chlor-zinc-iodine and examined microscopically. If a cellulase were present, most of the cell walls would have disappeared.

(d) Quantitative experimental methods.

For quantitative work on the digestion of cellulose, a number of methods have been described by several authors.

Karrer (1925), in his work on the cellulose-splitting enzyme of Helix pomatia, followed the method by which the arising glucose is titrated with the help of Fehling's solution. He found that the cellulase could hydrolyse cellulose, treated with Schweizer's reagent, etc., totally to glucose (Karrer, 1924, and Karrer and others, 1925), while untreated cellulose, like pure filter-paper, was more resistant and could only be hydrolysed completely after the addition of fresh enzyme (Karrer and Schubert, 1926); here also the product of hydrolysis was always glucose only. Woodman (1927), studying the mechanism of cellulose digestion in the ruminants, also arrived at a similar conclusion.

The method of titrating the arising glucose was also followed by Boynton and Miller (1927) in their work on the presence of cellulase in the shipworm Bankia setacea.

Earlier, Dore and Miller (1923) in their study of the digestion of wood by Teredo navalis (the shipworm) demonstrated the presence of cellulase by showing that the ejected matter contained a much lower percentage of cellulose (in percentage of dry weight) than the wood from which it was derived. This method has been followed by Falck (1930) and Ripper (1930) for proving the presence of cellulase in some wood-eating larvae of insects.
Another method used by Ripper (1930) is based upon the determination of the decrease in weight of cellulose after the action of the digestive juice of some insect larvae.

In the present research the first method, i.e. of measuring the glucose arising from the breaking down of cellulose, has been followed.

The estimation of the glucose was carried out with the micro-method of Hagedorn-Jensen (1922a,b). By this method it is possible to determine quantitatively amounts of glucose as small as 0.06 mg. with considerable accuracy. This made it possible to work accurately with the small quantities of digestive juice available. The proteins present in the juice, which might make the method less accurate, were precipitated with ZnSO₄ and NaOH, as prescribed by the original authors.

In most cases 1/10 N glycocol buffer of pH = 6.1 was added to the digestive mixture. The resulting pH was then 6.3.

Starch-free filter-paper cut up in very small pieces was used as cellulose. According to Karrer and Schubert (1926), natural cellulose (e.g. filter-paper) is more resistant to the action of enzymes than cellulose which has been treated with zinc chloride, ammoniacal copper solution, etc. For this reason in the case of Xystrocera, where no cellulase could be detected in the experiments with ordinary filter-paper, cellulose treated previously with Schweizer's reagent (modified cellulose) has been used. For this purpose starch-free filter-paper was dissolved in Schweizer's reagent and then precipitated with acetic acid.

The quantitative experiments were started soon after the gastric juice had been procured. Immediately after adding the enzyme solution to the mixture of buffer and filter-paper, a small portion of this digestive mixture was taken out for control titration, and the rest put in the thermostat at 32° C. A few drops of toluol were added to each mixture to prevent bacterial action, of which, even after a period of 10 days, there was no trace. After the time allowed for the digestion, a given amount of the mixture was titrated and the pH measured. The titration figures obtained in c.c. 1/200 N thiosulphate were calculated into mg. glucose with the help of the table given by Hagedorn-Jensen (1922a), on the assumption that only glucose is responsible for the reduction. The results of the splitting of cellulose, expressed in mg. glucose, were then calculated in percentage of the total quantity of glucose, which might originate through total hydrolysis of the quantity of cellulose used.

The increases in reducing power, due to the autolysis of the gastric juice and to the hydrolysis of the cellulose, were determined as controls simultaneously with each digestion experiment. Autolysis controls were made by preparing digestive mixtures, similar to the ordinary digestive mixtures described above, without adding cellulose. The controls for the hydrolysis of cellulose were made by adding "boiled" gastric juice (5 min. at 100° C.) to the mixture of buffer and substrate.

The actual digestion figure is then the difference between that of the digestion experiment and the total of the autolysis and hydrolysis figures.
Partial wood analyses, for the estimation of soluble sugars and starch in the different kinds of wood attacked by the insects studied in this research, have also been carried out. The wood was filed, powdered in a mortar and then separated by a sieve having 120 meshes per inch. A definite weight of this fine powder was extracted first with cold and then with hot water, according to the methods described by Waksman and Tenney (1927). Cold water extracts the sugars (glucose, saccharose, mannose and pentoses), whereas hot water extracts the starch and other hexosans, as well as other substances.

The cold-water extract was made by treating the powdered wood with a definite quantity of distilled water (30 c.c. per 1 gm.) and allowing the mixture to stand in the frigidaire for 24 hours, with a few drops of ether added to prevent bacterial action. It was then filtered and the residual matter thoroughly washed several times with distilled water. The filtered aqueous extract was then made up to volume.

The hot-water extract was made by mixing the residual matter of the cold extraction with a definite quantity of water (30 c.c. per 1 gm.) and boiling in water for 30 min. After filtration and washing the residue thoroughly with hot water, the filtrate was made up also to a definite volume.

For the estimation of the water-soluble sugars (in the cold-water extract) and the starch (in the hot-water extract) respectively, definite volumes of the two extracts were hydrolysed with HCl. This breaks down the carbohydrates in both extracts to monoses. Titration of these reducing sugars was then carried out for a small volume of both extracts according to the method of Hagedorn-Jensen (1922a, 1922b) previously referred to, and the total amount of glucose in the whole quantity of the hydrolysed extracts then calculated.

The quantity of carbohydrates present in the original cold-water extract is expressed in mg glucose, whereas for the hot-water extract the quantity of starch (in mg) was calculated from the glucose found after hydrolysis. Both were then calculated per hundred parts of the dry weight of the quantity of wood originally used for the extractions.

The kinds of wood analysed in this fashion are: sapwood of Albizzia lebbek, which harbours Xystrocera larvae; heartwood of Albizzia lebbek, which occasionally harbours Macrotoma larvae; wood of Morus alba, in which Macrotoma larvae were found; wood of Poinciana regia and Tamarix sp., which harbour the larvae and the adults of some Bostrychid beetles (Sinoxylon ceratoniae and Bostrychoplites Zickeli).

III. THE ALIMENTARY CANAL.

Externally, the larvae of Macrotoma resemble very closely those of Xystrocera. Internally, however, the Macrotoma larva is immediately recognised by the presence of a well-developed gizzard (Fig. 1a). Such an organ is totally absent in the larva of Xystrocera. The oesophagus in this latter species leads directly to the mesenteric region.
The mesenteric region in the two species is dilated anteriorly into a stomach which passes insensibly into a long intestinal region with a well-marked loop. The hind-gut is also looped in a fashion similar to that of the mesenteric part. The Malpighian tubules are four in number. In the advanced stages (at least in *Xystrocera*) two tubules are found to be greatly dilated and chalky in colour owing to the presence of huge quantities of calcium carbonate. This substance is passed to the outside during the pupal stage and is utilised in building the pupation cell.

Fig. 1. *a*, anterior region of alimentary canal of *Macrotoma palmata*, × 8. *b*, anterior region of alimentary canal of *Xystrocera globosa*, × 8. *giz* gizzard; *mes* mesenteron; *oes* oesophagus.

The gizzard of *Macrotoma* has a thick chitinous lining which is studded with sharp uniform teeth. Apparently the ingested food material is milled in this region into finer particles. This perhaps increases the efficiency of the gastric juice in the process of digestion. The finer condition of the gut content in the case of *Macrotoma* compared with that of *Xystrocera* is also evident from a microscopic study.

Thorough examination of the two larvae by means of sections has proved the absence of mycetocytes from all parts of the body. A search of the different regions
IV. PHYSIOLOGICAL INVESTIGATION.

(a) Qualitative enzyme tests.

The method followed here has been described in detail on p. 245.

The results of these tests are given in Table I below. The experiments with distilled water have been put as controls.

Table I. Qualitative experiments on the digestion of cellulose by the gastric juices of some insect larvae.

<table>
<thead>
<tr>
<th>Sections</th>
<th>Gastric juice of</th>
<th>After 1 day</th>
<th>After 2 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rib of lettuce</td>
<td>Distilled water</td>
<td>Intact</td>
<td>Intact</td>
</tr>
<tr>
<td>Macrotoma</td>
<td>Xystrocera</td>
<td>Intact</td>
<td>Intact</td>
</tr>
<tr>
<td>Prodema</td>
<td>Macrotoma</td>
<td>Partial dissolution</td>
<td>Intact</td>
</tr>
<tr>
<td>Date stone</td>
<td>Distilled water</td>
<td>Intact</td>
<td>Total dissolution</td>
</tr>
<tr>
<td>Macrotoma</td>
<td>Xystrocera</td>
<td>Partial dissolution</td>
<td>Intact</td>
</tr>
</tbody>
</table>

(b) Quantitative enzyme determinations.

(1) The digestion of cellulose by gastric juice of Xystrocera.

The method followed has been described on p. 245.

The digestive mixture was the same in all cases, i.e. 0.5 c.c. digestive mixture, containing:

- 6 mg. cellulose (starch-free filter-paper in experiments Nos. 1-4, in experiment No. 5 starch-free filter-paper treated with Schweizer's reagent).
- 0.2 c.c. glycol buffer 0.1 N.
- 0.3 c.c. gastric juice.
- Temp. = 32° C.

Per titration was used 0.1 c.c. in experiments Nos. 1-4, 0.05 c.c. in experiment No. 5.

The results are given in Table II.

(2) The digestion of cellulose by gastric juice of Macrotoma.

The digestive mixture was the same in all cases, i.e. 0.5 c.c. digestive mixture, containing:

- 6 mg. cellulose (starch-free filter-paper).
- 0.25 c.c. glycol buffer 0.1 N.
- 0.25 c.c. gastric juice.
- Temp. = 32° C.

Per titration was used 0.05 c.c. in experiment No. 1, 0.025 c.c. in experiments Nos. 2 and 3.

The results are given in Table III.
### Table II. Quantitative experiments on the digestion of cellulose by the gastric juice of Xystrocera.

<table>
<thead>
<tr>
<th>No. of exp.</th>
<th>Gastric juice from</th>
<th>pH of mixture</th>
<th>Time of digestion in hours</th>
<th>Increase in mg. glucose in</th>
<th>Actual digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Digestion exp.</td>
<td>Autolysis exp.</td>
<td>Hydrolysis exp.</td>
</tr>
<tr>
<td>1</td>
<td>6 animals = 1 c.c. juice</td>
<td>6·3</td>
<td>2 × 24</td>
<td>0·124</td>
<td>0·126</td>
</tr>
<tr>
<td>2</td>
<td>8 animals = 1 c.c. juice</td>
<td>6·3</td>
<td>2 × 24</td>
<td>0·120</td>
<td>0·123</td>
</tr>
<tr>
<td>3</td>
<td>5 animals = 0·8 c.c. juice</td>
<td>8·0</td>
<td>3 × 24</td>
<td>0·124</td>
<td>0·129</td>
</tr>
<tr>
<td>4</td>
<td>8 animals = 0·7 c.c. juice</td>
<td>4·3</td>
<td>24</td>
<td>0·172</td>
<td>0·168</td>
</tr>
<tr>
<td>5</td>
<td>6 animals = 0·6 c.c. juice</td>
<td>6·3</td>
<td>24</td>
<td>0·002</td>
<td>0·009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6·3</td>
<td>2 × 24</td>
<td>0·002</td>
<td>0·005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6·3</td>
<td>3 × 24</td>
<td>0·005</td>
<td>0·001</td>
</tr>
</tbody>
</table>

### Table III. Quantitative experiments on the digestion of cellulose by the gastric juice of Macrotoma.

<table>
<thead>
<tr>
<th>No. of exp.</th>
<th>Gastric juice from</th>
<th>pH of mixture</th>
<th>Time of digestion in hours</th>
<th>Increase in mg. glucose in</th>
<th>Actual digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Digestion exp.</td>
<td>Autolysis exp.</td>
<td>Hydrolysis exp.</td>
</tr>
<tr>
<td>1</td>
<td>2 animals = 0·5 c.c. juice</td>
<td>6·3</td>
<td>24</td>
<td>0·112</td>
<td>0·019</td>
</tr>
<tr>
<td>2</td>
<td>4 animals = 1 c.c. juice</td>
<td>6·3</td>
<td>2 × 24</td>
<td>0·220</td>
<td>0·39</td>
</tr>
<tr>
<td>3</td>
<td>4 animals = 1 c.c. juice</td>
<td>6·3</td>
<td>24</td>
<td>0·113</td>
<td>0·20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6·3</td>
<td>2 × 24</td>
<td>0·225</td>
<td>0·40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6·3</td>
<td>24</td>
<td>0·057</td>
<td>0·009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6·3</td>
<td>3 × 24</td>
<td>0·151</td>
<td>0·025</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6·3</td>
<td>4 × 24</td>
<td>0·187</td>
<td>0·032</td>
</tr>
</tbody>
</table>

### Table IV. Quantitative experiments on the digestion of starch by the gastric juice of a few larvae.

<table>
<thead>
<tr>
<th>Species used</th>
<th>Gastric juice from</th>
<th>pH of mixture</th>
<th>Time of digestion in hours</th>
<th>Increase in mg. glucose in</th>
<th>Actual digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xystrocera</td>
<td>6 animals = 0·6 c.c. juice</td>
<td>6·3</td>
<td>2 × 24</td>
<td>0·061</td>
<td>0·003</td>
</tr>
<tr>
<td></td>
<td>8 animals = 1·1 c.c. juice</td>
<td>6·3</td>
<td>3 × 24</td>
<td>0·091</td>
<td>0·003</td>
</tr>
<tr>
<td></td>
<td>10 animals = 0·8 c.c. juice</td>
<td>6·3</td>
<td>2 × 24</td>
<td>0·102</td>
<td>0·032</td>
</tr>
<tr>
<td>Prodenia</td>
<td>15 animals = 1·1 c.c. juice</td>
<td>6·3</td>
<td>24</td>
<td>0·092</td>
<td>0·051</td>
</tr>
<tr>
<td>Aphidis</td>
<td>2 animals = 0·6 c.c. juice</td>
<td>6·3</td>
<td>24</td>
<td>0·036</td>
<td>0·007</td>
</tr>
<tr>
<td>Macrotoma</td>
<td>4 animals = 1 c.c. juice</td>
<td>6·3</td>
<td>2 × 24</td>
<td>0·045</td>
<td>0·011</td>
</tr>
</tbody>
</table>
On the Digestion of Wood by Insects

(3) The digestion of starch by gastric juice of some larvae.

The digestive mixture was the same in all cases, i.e. 0.5 c.c. digestive mixture, containing:

- 3 mg. starch.
- 0.25 c.c. glycol buffer o-i N.
- 0.25 c.c. gastric juice.

Temp. = 32° C.

Per titration was used 0.025 c.c.

The results are given in Table IV.

(c) Wood analyses.

The method followed has been described in detail on p. 247.

The results are given in Table V.

### Table V. Quantity of soluble sugars and starch in some kinds of wood, expressed as percentage of dry weight.

<table>
<thead>
<tr>
<th>Wood</th>
<th>Sugars %</th>
<th>Starch %</th>
<th>Total (sugar and starch) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poinciana (coarse filings)</td>
<td>1.0</td>
<td>3.9</td>
<td>4.9</td>
</tr>
<tr>
<td>Poinciana (60 mesh)</td>
<td>1.0</td>
<td>4.1</td>
<td>5.1</td>
</tr>
<tr>
<td>Poinciana (120 mesh)</td>
<td>1.1</td>
<td>4.5</td>
<td>5.6</td>
</tr>
<tr>
<td>Tamartx (120 mesh)</td>
<td>2.8</td>
<td>4.4</td>
<td>7.2</td>
</tr>
<tr>
<td>Albizia sap wood (120 mesh)</td>
<td>6.2</td>
<td>3.9</td>
<td>10.1</td>
</tr>
<tr>
<td>Albizia heart wood (120 mesh)</td>
<td>2.0</td>
<td>0.5</td>
<td>2.7</td>
</tr>
<tr>
<td>Mora (120 mesh)</td>
<td>0.27</td>
<td>0.2</td>
<td>0.47</td>
</tr>
</tbody>
</table>

(d) Conclusions.

*Macrotoma palmata*. The gastric juice of this larva was found to dissolve the cell walls of date stone and lettuce ribs. Filter-paper was also seen to be dissolved; the presence of reducing sugars in this last solution could be demonstrated with Fehling's fluid and by the glucosazon test; the second test is only specific for the glucose.

Quantitative determinations showed that cellulose in the form of filter-paper was broken down by the enzyme present in the gastric juice; in 4 x 24 hours 47 per cent. of the cellulose present had been hydrolysed. This figure is of the same order as that found by Karrer and Schubert (1926) for the breaking down of cellulose by the enzymes of *Helix pomatia*, and thus indicates a strong cellulase in the gastric juice of *Macrotoma palmata*.

The soluble sugars and starch in the food of the larvae amount only to 0.47 per cent. of the dry weight of the wood. This figure compared with that of the food of the larvae of the *Xystrocera* (10.4 per cent.) is certainly exceedingly low. It is obvious therefore that *Macrotoma* larvae derive their necessary carbohydrates from

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1 The question whether the action of the cellulase of *Macrotoma* on wood cellulose (bound to lignin) is of the same order as that on filter-paper cellulose, is the subject of a research in progress.
the products of cellulose splitting through the action of their suitable gastric secretion.

*Xystrocera globosa.* The qualitative experiments with the gastric juice of the larvae of this species indicated the total absence of cellulase. The quantitative experiments with natural and modified cellulose also gave similar results.

Further enzyme investigations revealed the presence of a strong amylase. This enzyme is of about the same strength as that found in the phytophagous larvae of *Prodenia littoralis* and *Agrotis ypsilon* (Table IV).

Maltase and saccharase were also found to be present.

Analysis of the sap wood of *Albizzia lebbek*, in which the larvae of *Xystrocera globosa* live, showed a high content in soluble sugars and starch (Table V). This indicates the suitability of this kind of wood for the enzyme complex of these larvae. Since no cellulase is present at all, it is not unreasonable to conclude that the larvae of *Xystrocera* derive their necessary carbohydrates from the content of soluble sugars and starch of the wood they eat, without attacking the cellulose. Estimation of the soluble sugars and starch in the heart wood of *Albizzia lebbek* (Table V) showed that this region of the wood is very poor in these substances; only 0.7 per cent. is present, compared with about 10 per cent. in the sap wood. This probably explains the fact that the larvae of *Xystrocera* are never found in this region of the wood.

The smaller branches of *Albizzia lebbek* are also attacked by the larvae and adults of *Sinoxylon ceratoniae* and *Bostrychoplites Zickeli*, two Bostrychid beetles which also flourish in the woods of *Poinciana* and *Tamarix*. These two last-mentioned kinds of wood have been found to be rich in sugars and starch, just as in the case of *Albizzia lebbek* (Table V). Probably the choice of these sugar- and starch-rich woods indicates that the beetles in question have feeding habits similar to those of *Xystrocera* larvae.

V. DISCUSSION.

The two species of wood-eating insects which have been the subject of this investigation have proved to be different in their feeding habits as far as the source of carbohydrates is concerned. *Xystrocera globosa* can only utilise the soluble sugars and the starch in the wood it lives on, while *Macrotoma palmata* is able to break down cellulose through the activity of its enzymes. The two species have been searched thoroughly for micro-organisms, but with negative results.

In their mode of feeding, the larvae of *Xystrocera globosa* resemble those of *Cossus cossus* (living in the wood of poplar), where Ripper (1930) has proved that no cellulase is present. In these two species, the larvae confine their ravages to the sap wood. Our analysis of the inner wood (heart wood) of *Albizzia lebbek* (Table V) shows that this region is comparatively poor in starch and sugars, and this explains the immunity of this region against the attacks of the larvae of *Xystrocera*. Probably a similar analysis of the wood of poplar might indicate a higher content of the sap wood in starch and sugars.
On the Digestion of Wood by Insects

The cases of the larvae of *Xystrocera* and *Cossus*, where the carbohydrate supply comes from a relatively poor source (sugar and starch content of wood), are analogous to the well-known cases of earthworms and Holothurians, where the percentage of available nutriment in the ingested material is exceedingly low (Jordan, 1913 and Oomen, 1926). As in these latter cases, the nutritive value of wood, as far as the simpler carbohydrates are concerned, is comparatively low. Consequently *Xystrocera* and *Cossus* must ingest huge quantities of their food material in order to extract from it the carbohydrates sufficient for their growth. This is quite evident also from the big accumulations of excreta which fill up the galleries of such insects. For this reason perhaps insects with such feeding habits very quickly cause serious damage to the trees they attack.

Insects of this type could for their feeding habits be put quite close to those living on hard substances which are rich in starch and sugars, such as grains, some roots, etc. Interesting in this connection is the observation of Ripper (1930) that the larvae of *Cossus cossus*, when fed on beetroot, which thus forms a concentrated food supply, reach the imaginal stage in one year, whereas in their natural habitat this stage is reached after 2½ years.

*Macrotoma palmata*, the second species, represents a type with a totally different feeding habit. The larvae possess a strong cellulase and an amylase, less in strength than that of *Xystrocera*. A weak saccharase has been also detected.

Contrary to those of *Xystrocera*, the *Macrotoma* larvae therefore can live on wood very poor in sugars and starch, and can derive the necessary carbohydrates direct from cellulose. An analysis of the wood of *Morus alba*, from which the larvae were extricated, showed a total content of 0.47 per cent. only of sugars and starch.

*Macrotoma palmata* is recorded also from the wood of *Albizia lebbeck*, infested with *Xystrocera globosa*, and it is significant to note that whereas the second species confines itself entirely to the sap wood, the first species lives mainly in the heart wood.

The type of cellulose digestion exhibited by the larvae of *Macrotoma palmata* is also known to exist in the larvae of *Cerambyx cerdo* (Ripper, 1930) and of *Hylotrupus bajulus* (Falck, 1930). These two species, like *Macrotoma*, are free from micro-organisms. Ripper has demonstrated the presence of cellulase in the former species, whereas Falck has shown a decrease in cellulose when comparing food and excreta of the latter. In other members of the same family, *Leptura rubra* and *Rhagium* spp., where fungus- and yeast-like micro-organisms occur respectively in the epithelium of the gut, Ripper (1930) has found also cellulase. Owing to the near relation between these two species and the previous ones, this author concludes that the cellulase of *Rhagium* and *Leptura* originates similarly from the digestive epithelium and not from the intracellular micro-organisms.

*Xestobium rufovillosum* also has been the subject of a similar investigation by Ripper (1930), who has found a cellulose decrease when comparing food and excreta. In this species the same author has succeeded in demonstrating quantitatively the presence of cellulase. Campbell (1929) also found a similar cellulose decrease in an unidentified species of *Xestobium*. 
The larvae and adults of *Xestobium rufovillosum*, as well as those of many other Anobiid species, harbour intracellular yeast-like micro-organisms in the anterior region of the mesenteron (Buchner, 1921, and Breitsprecher, 1928). These yeast-like micro-organisms have often been cited as helping in the digestion of cellulose, but as Ripper (1930) has pointed out, yeasts are not known to attack cellulose. Moreover, Heitz (1927) has cultivated similar intracellular yeast-like micro-organisms from wood-eating Anobiids (*Ernobius abietis*), and has failed to get any sign of cellulose attack. We therefore agree with Ripper in concluding that the intracellular micro-organisms present in *Xestobium* play no part at all in the digestion of cellulose. The cellulase in this species originates from the digestive epithelium just like that of *Macrotoma* and *Cerambyx*.

Wood-eating insects of this cellulose-digesting category probably ingest less wood material than the starch and sugar-digesting type, and consequently are probably less conspicuous in their ravages.

The finding of two categories of wood-eating insects, *i.e.* with cellulase (*Macrotoma*) and without cellulase (*Xystrocera*), helps to clear up the confusion about the nutrition of wood-eating insects. Species of wood having a comparatively high content of starch and sugar are open to the ravages of the *Xystrocera* type, while those with a much lower content in these substances are only subject to the attacks of the *Macrotoma* type. The two types of wood-eating insects live on sound wood, and in their digestive processes they are quite independent of the action of micro-organisms, whether intracellular or extracellular.

The presence of a large well-developed proventriculus in *Macrotoma palmata* (Fig. 1a) and its absence in *Xystrocera* (Fig. 1b) can now also be explained. Probably in the first species, owing to its digestive habits, the food material must be in a very fine condition for a higher efficiency of the cellulase. It is not unlikely therefore that the food material in this region is milled up to very fine particles. In the second species apparently such a process is not necessary. This is confirmed by a microscopical comparison of the sizes of food particles in the alimentary canal of the two species.

Wood-eating insects which are supposed to derive some digestive help from the micro-organisms they harbour, *e.g.* Protozoa-harbouring termites and Lamellicorn larvae with intestinal micro-organisms, have been referred to above (p. 243).

In view of the fact that termites can live for some time after the removal of their intestinal Protozoa (Cleveland, 1928, p. 232) and also in view of the fact that these Protozoa ingest solid wood (Cleveland, 1925a) and that they are digested in the alimentary canal of their host (Cleveland, 1925d, p. 315), we do not find it unreasonable to conclude that the termites utilise their intestinal Protozoa as a direct and only supplementary food source. It is highly probable also that a physiological study of the nutrition of Protozoa-harbouring termites might show that some possess cellulase and are similar in their feeding habits to *Macrotoma palmata*, while others lack such an enzyme and are similar to *Xystrocera globosa*. In this manner the findings of Cleveland could be explained. The termites with cellulase could live indefinitely on wood poor in starch and sugar content or even on pure
On the Digestion of Wood by Insects

cellulose after being defaunated, while the ones which lack this enzyme could only live on the soluble sugars and starch content of the wood they attack and supplement their diet directly from the culture of Protozoa they harbour.

The Lamellicorn larvae with intestinal micro-organisms on the other hand have been the subject of a thorough physiological study by Wiedemann (1930). According to this author, the micro-organisms are utilised as a direct food source (loc. cit. p. 254).

In the light of Wiedemann’s results and in view of what has been mentioned above concerning the Protozoa-harbouring termites, we are led to conclude that the wood-eating insects under consideration (i.e. termites with Protozoa and Lamellicorn larvae with micro-organisms) are not true wood feeders. They utilise the micro-organisms taken in with the wood, or growing in special chambers of the proctodaeum, as a main or supplementary direct food source.

The view that such insects live directly on the products of cellulose splitting by the micro-organisms they harbour falls to the ground.

As to the view that the intracellular micro-organisms, present in a number of wood-eating insects, play an important rôle in the nutrition of their hosts, especially in helping to break down cellulose, there is not a single case to our knowledge which could be quoted in its favour. On the contrary, all the cases with intracellular micro-organisms, better known to us, indicate very clearly that such micro-organisms are of no significance in the nutrition of their hosts1.

VI. SUMMARY.

1. The true wood-feeding insects do not depend on micro-organisms for the digestion of wood. Such insects are of two types:

   (a) Without cellulose-breaking enzymes. Insects of this type derive the necessary carbohydrates from the soluble sugars and starch in the wood they live on, and consequently such insects can only live in kinds of wood comparatively rich in these substances.

   (b) With cellulose-breaking enzymes. Insects of this type can utilise the cellulose of the wood through the activity of their own secretions. They therefore can live on woods very poor in the simpler carbohydrates.

2. Wood-eating insects with free-living intestinal micro-organisms (such as the termites and the Lamellicorn larvae) use the micro-organisms as direct food and derive no digestive help from them. Insects of this type are better referred to as micro-organism feeding.

1 The biological meaning of the intracellular micro-organisms, especially of some coleopterous insects, is dealt with separately by one of us (see K. Mansour, "On the so-called symbiotic relationship between some coleopterous insects and intracellular micro-organisms," Quart. Journ. Micr. Sci. (in press)).
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