# The transformation of *Saperda calcarata* (Coleoptera: Cerambycidae) into a cellulose digester through the inclusion of fungal enzymes in its diet

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**Summary.** The larvae of the aspen borer, *Saperda calcarata*, which feed on the inner bark and sapwood of living aspen stems, are unable to digest cellulose. However, they can be transformed into cellulose digesters by adding the active cellulase complex of the fungus, *Penicillium funiculosum*, to their diet. *S. calcarata* larvae are preadapted to exploit the digestive potential of ingested microbial enzymes. We argue that ingested fungal enzymes may be responsible for cellulose digestion in many, perhaps most or even all, cellulose digesting cerambycid beetles.

**Key words:** *Saperda calcarata* – Cerambycidae – Cellulose digestion – Cellulase – Acquired digestive enzymes

The ability to digest cellulose is common among the wood-boring larvae of anobiid and cerambycid beetles (Martin 1983). At one time it was believed that intracellular symbionts might play a role in cellulose digestion in these insects (Buchner 1928; Uvarov 1929). However, that possibility was conclusively ruled out when it was demonstrated that cellulose is digested as efficiently by symbiont-free species as by symbiont-containing species (Ripper 1930; Mansour and Mansour-Bek 1934a, b; Müller 1934) and that the symbionts, which are yeasts, are not cellulolytic (Müller 1934). After it was recognized that cellulose digestion was not dependent upon endosymbionts, it was concluded that anobiid and cerambycid beetles must be able to produce their own cellulolytic enzymes. It is important to recognize, however, that this conclusion was arrived at by default, not because of compelling evidence in its favor.

Recently we have shown that the ability of the balsam fir sawyer, *Monochamus marmorator* (Cerambycidae), to digest cellulose is not due to enzymes produced by the beetles, but rather to fungal enzymes ingested by the larvae during the consumption of fungus-infected wood (Kukor and Martin 1986a). We also proposed that this mechanism for cellulose digestion might be widespread among xylophagous beetle larvae, and that there was actually no evidence that any anobiid or cerambycid species produced its own cellulase complex.

In this study we have investigated polysaccharide digestion in the larvae of another cerambycid, the aspen borer, *Saperda calcarata*. Species of the genus *Saperda* do not attack the decayed wood of dead trees. The females oviposit in slits chewed in the bark of live trees, and the larvae move into the inner bark and sapwood to feed. Thus, *Saperda* larvae rarely encounter or consume fungus-infected wood, precluding a reliance upon ingested microbial enzymes for the digestion of polysaccharides. If our interpretation of the origins of cellulolytic capacity in the cerambycids is correct, we would not expect *S. calcarata* to be able to digest cellulose.

Our research on *S. calcarata* is part of an ongoing series of investigations of the role of acquired fungal enzymes in the digestive processes and nutritional ecology of wood- and detritus-feeding arthropods (Martin and Martin 1978, 1979; Sharma et al. 1984; Kukor and Martin 1983, 1986a, b).

## Methods and materials

### Collection and maintenance of animals

*S. calcarata* larvae were collected from stems and large branches of trembling aspen, *Populus tremuloides,* at the University of Michigan Biological Station, Emmet County, Michigan. Since *S. calcarata* cannot be maintained for extended periods on artificial laboratory diets, larvae were kept in 50-cm long aspen branch sections with paraffin-sealed ends. Larvae could be kept in this way for 2–3 weeks under laboratory conditions.

### Extract preparation and enzyme assays

Midguts were dissected from *S. calcarata* larvae as described by Martin et al. (1980). Since the pH of the midgut fluid ranges between 7.05 and 7.20, extracts were prepared in 100 mM Tris-HCl buffer (tris[hydroxymethyl]aminomethane), pH 7.1. For enzyme assays, the pooled midguts from 8–10 larvae were prepared as described in Martin et al. (1981).

Activities toward potato amylose (Calbiochem), carboxymethylcellulose (CMC) (Sigma), laminarin (United States Biochemical), citrus pectin (Sigma), larchwood xylan (United States Biochemical), microcrystalline cellulose (Polyscience), and chitin (Sigma) were assayed by measuring

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**Table 2.** Utilization of [U-$^{14}$C]-cellulose by *S. calcarata* larvae. The duration of the experiment was 24 hours. Each value is the mean ± the standard error of the mean for three replicates

| Treatment prior to transfer to $^{14}$C-cellulose-containing diet | Percent of label from $^{14}$C-cellulose in diet released as $^{14}$CO$_2$ |
| --- | --- |
| Collected from natural galleries in aspen | 0.0 ± 0.0 |
| Cultured for 3 days on aspen wood chips plus *P. funiculosum* cellulase | 1.3 ± 0.2 |
| Cultured for 3 days on aspen wood chips plus heat-inactivated *P. funiculosum* cellulase | 0.03 ± 0.02 |

in the gut fluids of *S. populnea,* a European species with feeding habits very similar to those of *S. calcarata* (Schlottke 1945). Schlottke also reported that cellulase activity in the gut fluid was lower, and amylase activity higher, in this species than in other species of cerambycids with diets of dead or decayed wood. In assaying for cellulase, Schlottke used a ten-day incubation time, and detected cellulase activity in the Malpighian tubules and the hemolymph, neither of which are plausible sources for the digestive enzymes of insects. Schlottke, aware of the enigma posed by cellulases from these two sources, hypothesized that the observed activity might not be due to digestive cellulases, but rather to chitinases that normally function during molting, but which also possess some activity toward other $\beta$-1,4-glucans, such as cellulose. Whether or not Schlottke's explanation is correct, we believe that there is sufficient ambiguity in his result that it cannot be accepted as a convincing demonstration of cellulolytic capacity in *S. populnea* larvae.

*The acquisition of cellulolytic capacity by S. calcarata larvae on a diet containing fungal cellulase*

Although *S. calcarata* larvae do not normally ingest fungal enzymes, there seemed to be no reason a priori to expect that such enzymes would be any less stable or active in the gut fluid of this species than in *M. marmorator*. We, therefore, undertook experiments to determine whether *S. calcarata* larvae could acquire cellulolytic capacity by ingesting a food that contained fungal cellulase.

Larvae were cultured on aspen wood shavings that had been impregnated with the cellulase complex of the fungus, *Penicillium funiculosum.* Although the larvae did not consume large amounts of wood when it was presented to them in this unnatural form, they did feed at a reduced rate, and after three days their gut fluids exhibited significant activity toward microcrystalline cellulose (Table 1). No cellulase activity was detectable in the gut fluids of larvae that had fed on aspen wood shavings to which heat-inactivated fungal cellulase had been added.

Larvae with ingested fungal cellulase in their gut fluids are able to digest [U-$^{14}$C]-cellulose (Table 2). Labeled cellulose was incorporated into the diet of the larvae by feeding them aspen wood shavings amended with $^{14}$C-cellulose fiber, and cellulose digestion was monitored by counting the $^{14}$C in the respiratory carbon dioxide produced by the larvae. In these experiments it was not possible to achieve a clean separation of frass and uneaten food. As a consequence, it was not possible to obtain an accurate measure of the amount of food or labelled cellulose actually ingested, and we had to settle for a determination of the fraction of $^{14}$C presented to the larvae in their food that was released in the form of carbon dioxide. Since the larvae did not consume very much of the $^{14}$C-cellulose-amended aspen shavings during this experiment, the value we obtained, 1.3%, grossly underestimates the efficiency with which the larvae assimilated the cellulose actually ingested.

**Conclusions**

Ingested fungal enzymes have been shown to be responsible for cellulose digestion in the termite, *Macrotermes natalensis* (Martin and Martin 1978, 1979), the siricid woodwasp, *Sirex cyaneus* (Kukor and Martin 1983), and the cerambycid beetle, *Monochamus marmorator* (Kukor and Martin 1986a). Although *S. calcarata* larvae do not normally digest cellulose, they are transformed into cellulose digesters when their diet contains active fungal cellulases. The larvae are preadapted for the exploitation of ingested enzymes.

The conditions which prevail in the gut fluids of cerambycid larvae are quite compatible with a role for ingested fungal enzymes. The pH of the gut fluid is close to neutrality, generally ranging between 6.8 and 7.2 (Swingle 1931; Schlottke 1945; Grayson 1958; Mishra and Singh 1978). Although this range is slightly above the pH at which most fungal cellulases manifest their maximum activity, it is nonetheless a range in which such enzymes are stable and exhibit significant activity. The proteolytic enzymes of cerambycid gut fluid have not been thoroughly studied. Schlottke (1945) detected proteolytic activity in the gut fluids of several species, and noted that activity was not evenly distributed in the midgut. Activity was quite low in the anterior portion of the midgut, which is the region where cellulase activity is generally highest, and was higher in the posterior portion where cellulase activity is lower. He also noted that the anterior portion of the midgut is slightly acidic while the posterior portion is slightly alkaline, and that the proteases are more active under alkaline than acidic conditions. These results all point to the conclusion that ingested enzymes would probably not be subject to rapid digestion in the midgut fluids. The anterior portion of the midgut in particular would seem to be a region of the alimentary tract in which ingested fungal cellulases would be stable and active.

We predict that ingested fungal enzymes will prove to be responsible for cellulose digestion in many, perhaps most or even all, cellulose-digesting cerambycid beetles. We also maintain that there is no longer any justification for believing that the larvae of any cerambycid species are able to secrete all of the enzymes required for cellulose digestion. To be sure, many additional species must be examined before these sweeping generalizations can be accepted as anything more than provocative challenges. We hope the challenges will be accepted, and the generality of the importance of ingested enzymes in the nutritional ecology of wood-feeding beetles can be established.

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