133. THE RELATIONSHIP BETWEEN NITROGEN METABOLISM AND THE DURATION OF THE LARVAL STAGE OF THE DEATH-WATCH BEETLE (*XESTOBIUM RUFOVILLOSUM* DE G.) REARED IN WOOD DECAYED BY FUNGI

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FISHER [1941] has reviewed existing knowledge on food relations of the death-watch beetle. The conclusion of Campbell & Bryant [1940], that fungus benefits the insect largely because of the depreciation which it induces in the mechanical strength of the substrate, is accepted, but the suggestion is made that the insect may nevertheless benefit in some more direct manner from the presence of fungus or fungal remains in its substrate. For example, fungal mycelium may be the means of concentrating certain ferments in the larval gut or, alternatively, it may provide a rich source of nitrogen. It is known from the work of Findlay [1934] that fungi growing in wood are capable of accumulating N to the extent that their content of that element may be as much as forty times as great as that of their woody substrate. Fisher [1941] therefore draws attention to the work of Becker [1938], in which conclusive evidence is given that the addition of an organic source of N (peptone) to wood results in a marked increase in the growth rate of the larvae of *Hylotrupes bajulus* boring in it. Becker [1938] made the further observation that fungal attack in wood can also favour the development of *Hylotrupes* under certain conditions, and suggested the possibility that the controlling factor here is the enrichment of the N compounds in the wood. The mechanism of the enrichment process is not discussed, but the biological observations of Becker [1938] form an important contribution to the N metabolism of wood-boring insects, and they have prompted the present re-examination of the relationship between the N metabolism of *Xestobium* and the duration of its larval stage in wood which has been decayed in known amounts by fungi.

In contrast to carbohydrates, N compounds usually form a very small proportion of wood. The total N content of the oak wood used in this and previous work was of the order of 0.2 %, and since the N content of the *Xestobium* larva has been found to be 3.91 % [Campbell & Bryant, 1940], it is obvious that a considerable amount of the substrate must be comminuted by each individual before its N requirements for body-building purposes can be satisfied. Pupation presumably cannot normally take place until these requirements are met, and there would thus appear to be good grounds for the belief that any agency which is effective in increasing the rate of supply of N for conservation purposes

to the growing larva will tend to shorten the larval stage. It is important to note that when Becker [1938] added peptone to wood infested by *Hylotrupes* he found that the more rapid larval growth which ensued was caused by a greater intake of protein and not by a greater intake of wood. Treated wood was not disintegrated to a greater extent than wood to which no peptone was added. On the other hand, in wood previously decayed by fungi the larvae grew at a greater rate, but the extent of damage caused in the substrate by boring on the part of the larvae was greater than in sound, control samples of the same wood. The full significance of these findings will be discussed later.

From an investigation of the N contents of sound and decayed woods, Huntgate [1940] has concluded that there is little change in the total N content during fungal decay, and it is obvious that if this conclusion holds for the decayed oak wood used in the previous work on *Xestobium* carried out in this laboratory [Fisher, 1935; 1937; 1940; 1941; Campbell & Bryant, 1940] the progressive shortening of the life cycle which has been found to accompany increases in the extent of fungal decay in the substrate cannot be based on any simple enrichment process of the nitrogenous components of the wood, although the direct bearing of N metabolism on the duration of the larval stage cannot be denied.

EXPERIMENTAL

The present study has fallen naturally into two parts, (1) the effect of fungal decay on the N content of oak sapwood, and (2) the N economy of *Xestobium* larvae reared in decayed oak sapwood under controlled conditions. All N determinations were carried out according to the modified Kjeldahl procedure of Stubblefield & De Turk [1940]. 2 g. (air-dry) samples of wood were digested with 10 g. K_2HPO_4 , 6 g. $Fe_2(SO_4)_3$, 0.6 g. Hg and 25 ml. H_2SO_4 : digestion was usually complete within 45 min. The method was preferred to that used previously [Campbell & Bryant, 1940].

The effect of decay by Phellinus cryptarum Karst. on the N content of oak sapwood

The material consisted of remnants of samples of sound wood, and of wood decayed to known losses in weight by the fungus, used in previous studies [Fisher, 1941; Campbell & Bryant, 1940]. The greater part of five of the samples of decayed wood had been used by Fisher [1941] for beetle infestation. The samples which had lost 22.65, 30.8 and 51.20 % of their respective dry weights owing to decay were not so used, but N values have been found for them for present purposes (Table 1).

With one exception, the results in Table 1 show that each sample of decayed wood has a higher N content than sound wood of the same origin. At the same time, in terms of original sound wood, N is progressively removed by the fungus as the extent of decay increases. This latter observation is explained by the fact that a considerable weight of fungal mycelium containing a relatively high proportion (0.68 %) of N derived from the wood [Campbell & Bryant, 1940] grew on the exterior of the test pieces and was removed before beetle infestation and analysis. Despite this net loss of N from the system, the percentage amount of N present in the decayed wood is greater than in sound wood because, during decay, major wood components are transformed into water and CO_2 in the vital processes of the fungus at a greater rate than N is removed via the external mycelium. Because of possible variations in N content between sample and sample which cannot entirely be eradicated by any system of matching, the

Table 1. *N contents of oak sapwood before and after decay by Phellinus cryptarum*

(All results expressed as % by weight of oven-dry material.)

Loss in weight due to fungal decay as % of sound wood	N content	
	Found	Calc. as % of sound wood
0-00 (Sound wood)	0-203 } 0-197 } 0-200	0-200
17-70	0-230 } 0-226 } 0-227 } 0-228	0-187
22-65	0-209*	0-162
30-80	0-258 } 0-247 } 0-246 } 0-250	0-173
36-48	0-225 } 0-225 } 0-225	0-143
40-00	0-208 } 0-210 } 0-209	0-125
51-20	0-198 } 0-193 } 0-196 } 0-196	0-096
59-90	0-234 } 0-243 } 0-239	0-096
73-30	0-281 } 0-284 } 0-283	0-076

* Only one sample available.

results (Table 1) have been examined statistically. The determined average N values, calculated as percentages by weight of sound wood (oven-dry), have been plotted against corresponding values for loss in weight due to decay (Fig. 1) and a regression line determined to express the general relationship between the two quantities. Included in this figure is a probability limits band, within which 95 % of the N content values for all samples of decayed oak sapwood of similar origin would be expected to fall when plotted against the appropriate percentage losses in weight due to decay. Percentage N contents corresponding to each sample of decayed wood actually exposed to beetle attack have been read from the mean line (Fig. 1) to provide the data in Table 2.

The data in Table 2 show that the rate of increase in the N content of decaying oak sapwood is not great and that, until the original wood has lost not less than *ca.* 20 % of its weight by decay, the significance of any increase in the N content of the residue is difficult to establish experimentally when due allowance is made for error in the N determination itself. In view of the nature of the material at his disposal, it is not surprising therefore that Huntgate [1940] concluded that the N content of wood shows little change during decay. It is none the less striking, however, that, in the extreme case, wood which has been so severely decayed as to lose 73.3 % of its original weight (Table 2) may, in the process, have some two-thirds of its N removed via external fungal mycelium and yet leave a residue of N content over 25 % greater than that of the wood itself at the outset of decay. Wood decayed to this extent no doubt contains a relatively high proportion of fungal mycelium.

Fig. 1. Relationship between percentage N content and percentage loss in weight due to fungal decay (based on original oven-dry wood).

Table 2. *Calculated mean N content of oak sapwood at various stages of decay by Phellinus cryptarum*

(All results expressed as % by weight of oven-dry material.)

Loss in weight due to fungal decay as % of sound wood	N content	
	As % of sound wood read from Fig. 1	As % of sample read and calc. from Fig. 1
0.00 (Sound wood)	0.210	0.210
17.70	0.176	0.214
25.62	0.162	0.218
32.80	0.148	0.222
36.48	0.142	0.224
40.00	0.135	0.225
50.60	0.114	0.231
59.90	0.097	0.241
73.30	0.071	0.266

The N economy of the Xestobium larva reared in decayed wood

Fisher [1941] concludes from the biological investigation that the effect on larval development of the presence of fungus in the substrate is most pronounced when decay itself is relatively not far advanced, since the rate of decrease in the length of the life cycle was found to diminish considerably when the loss in weight due to decay in the substrate exceeded 45%. When considered in conjunction with this conclusion the data in Tables 1 and 2 strongly suggest that change in the N content of the substrate is not by itself a determinant of the duration of the larval stage of *Xestobium*. Apart from the fact that up to a loss in weight due to decay of 50% the N content of the substrate (Table 2) is only about 10% greater than that of sound wood, the substantial increase in N content of the substrate brought about by advanced decay is not accompanied by a proportionate decrease in the length of the life cycle of the insect. It is therefore of importance to determine as far as possible how the *Xestobium* larva deals with the N which is available to it in substrates of common origin but which differ in the extent of their fungal decomposition. Three sets of material were available for this purpose, and the results of N determinations upon them are given in Table 3.

Table 3. *N* contents of three samples of oak sapwood decayed by *Phellinus cryptarum* before attack by *Xestobium rufovillosum* and of the frass produced in them by the larvae

Loss in weight in sample due to fungal decay as % of sound wood	Average N content (% of oven-dry weight)	
	Decayed wood before insect attack (from Table 1)	Frass
17.9	0.230	0.235 } 0.235
	0.226	
	0.227	
36.48	0.225	0.228 } 0.225
	0.225	
	0.225	
40	0.208	0.221 } 0.216
	0.210	
	0.209	

When due allowance is made for error in the analytical method used and for possible error due to variations in the distribution of nitrogenous components in the starting material, it must be concluded that for losses in weight due to decay in the substrate up to approximately 40%, the N content of the frass of the *Xestobium* larva is not significantly different from that of the decayed wood in which it bores. The implications of this conclusion are of far-reaching importance, as will now be shown.

DISCUSSION

Throughout these researches data have been obtained for loss in weight due to insect attack and for the weight of frass produced per unit weight of wood disintegrated by boring, so that it is now possible to assess the average N intake of the *Xestobium* larva in the three separate instances dealt with in Table 3 and to relate this intake quantitatively to the total amount of N available in the wood disintegrated (Table 4).

Table 4. *Relationship between N intake and N available to the Xestobium larva reared in oak sapwood decayed by Phellinus cryptarum*

(Results, except those in columns (1) and (7), expressed as % by weight (oven-dry) of wood disintegrated by larval boring)

Loss in weight due to fungal decay in substrate as % of sound wood	Loss in weight due to insect attack = abstraction coefficient	Frass produced by larva	N available to larva in wood disintegrated (from Table 1)	N recovered in frass (calc. from Table 3)	N abstracted and retained by larva (by difference)	N abstracted as % of N available per unit of wood disintegrated = N-abstraction coefficient
17.9	47.56	52.44	0.228	0.123	0.105	46.05
36.48	23.93*	76.07*	0.225	0.171	0.054	24.00
40.00	21.15*	78.85*	0.209	0.170	0.039	18.60

* Determined previously by Campbell & Bryant [1940].

The data in Table 4 permit the remarkable conclusion that, irrespective of the extent of decay in the substrate over the range examined, the N-abstraction coefficient of the *Xestobium* larva is not significantly different numerically from the abstraction coefficient for the total food. This means not only that N is removed from the wood digested in the proportion in which it occurs in the substrate, but also that all of the N digested is retained in the larval body

The data in Table 4 actually show that, on the average, 95 % of the N in the wood digested is thus retained, but for reasons already given this figure is probably an underestimate. Since, as has already been shown [Campbell & Bryant, 1940], the dietetic value of oak sapwood is not appreciably altered even after extensive fungal decay, it is reasonable to assume that, so far as its N metabolism is concerned, the *Xestobium* larva, to all intents and purposes, always retains all of that element which it absorbs, for if any considerable proportion of metabolized N had been excreted in the frass in the form, for example, of uric acid, amino-acids, or even NH_3 , it could not have escaped detection by the methods used. The frass of *Xestobium* must therefore be regarded as consisting essentially of wood which has been comminuted, compressed in part or in whole into pellets in the larval gut, and excreted without having been modified chemically by digestive processes. Wood which is digested, on the other hand, is transformed into soluble products and absorbed virtually *in toto*. Previous claims to have established the composition of the food of *Xestobium* [Campbell, 1929; Ripper, 1930; Norman, 1936] by comparing the composition of frass and sound wood or wood adjacent to larval tunnels are therefore without foundation. In the light of present knowledge, the analytical data recorded in these earlier publications merely give an indication of the changes brought about in the wood by the fungal decay which normally precedes or accompanies beetle attack, and the most accurate estimate of the composition of the larval diet is that afforded by the data of Campbell & Bryant [1940] supplemented by those of the present study. It is realized that this conclusion has an important bearing on the chemical study of the feeding habits of other wood-boring insects.

This demonstration that at least 95 %, and probably all, of the N absorbed by the growing *Xestobium* larva is retained in the body, makes it clear that the rate of N intake is one of the principal determinants of the duration of the larval stage in wood.

The experimental data of Fisher [1941], showing the relationship between the length of life cycle and the extent of fungal decay in the substrate, as well as the data of Campbell & Bryant [1940], showing the relationship between the larval abstraction coefficient and the same variable, have been treated statistically to obtain the curves shown in Fig. 2. These two curves are essentially of the same shape, and the common variable may be eliminated by plotting values for length of life cycle (curve A) against corresponding values for abstraction coefficient (curve B). This leads to the curve in Fig. 3 which, because of previous findings (Table 4), illustrates the relationship between length of life cycle on the one hand, and either the total amount of food digested or the amount of N digested per 100 g. of wood disintegrated on the other. It is conceivable that, but for unavoidable errors in the various biological, manipulative and chemical determinations upon which all the foregoing curves are based, the curve in Fig. 3 might have been a straight line. Even as it is, when considered from the standpoint of the N metabolism of *Xestobium*, this curve presents a similar paradox to that encountered in the previous study [Campbell & Bryant, 1940], that as the length of life cycle decreases, so does the amount of N abstracted by the larva from every 100 g. of wood which it comminutes by boring. As has already been shown, this can only be accounted for by an increase in the rate of boring on the part of the larva. The conclusion is thus reached that, in common with the major components [Campbell & Bryant, 1940], the N in decayed as opposed to less decayed or sound wood is made more readily available to the *Xestobium* larva principally on account of the softening effect of fungal decay on the substrate. The larva thereby encounters less resistance to boring and,

Fig. 2. Curve A. Relationship between duration of life cycle and loss in weight due to fungal decay in the substrate. Curve B. Relationship between larval abstraction coefficient (total food) and loss in weight due to fungal decay in the substrate.

Fig. 3. Relationship between duration of life cycle and (a) larval abstraction coefficient (total food), (b) larval N-abstraction coefficient.

because it retains substantially all of the N which it digests, it is enabled to complete its development in a shorter time. It follows, of course, that, as the rate of conservation of N increases, so must the rate of conservation of the other elements which are essential to larval growth. For instance, N only represents 1/6.25 of the protein and probably 1/14.5 of the chitin [Armstrong & Armstrong, 1934] synthesized during metabolism. It is calculated from the foregoing data that the larva reared in wood which has lost by decay 73.3 % of its weight must comminute unit weight of wood in approximately 1/12 of the time taken by its counterpart reared in wood decayed to a loss in weight of 17.9 %. Unfortunately an independent method of determining the rate of boring is not as yet available but, for *Xestobium* living either in sound or decayed wood, this is the prime factor which determines the duration of the larval stage and, as shown in previous work [Campbell & Bryant, 1940], when the extent of decay in a suitable substrate is the only variable in the environment of the beetle, rate of boring is determined by the degree of softening thereby induced in the substrate. It is only on a basis of this close integration of effects, which resolve into a reduction in the length of life cycle but emanate from a common cause, that all of the observations, biological and chemical, can be correlated. For instance, there can be little doubt that the shape of curve (Fig. 2), which is characteristic of the relationship between loss in weight due to decay in the substrate on the one hand and each of 'length of life cycle', 'abstraction coefficient (total food)' and 'N-abstraction coefficient' on the other, would also be characteristic of the relationship between loss in weight due to decay (or density) and mechanical strength (or resistance to larval boring) of the substrate. If this be granted, it is readily seen from the slope of the type curve (Fig. 2) why it was observed [Fisher, 1941] that after a loss in weight due to decay in the substrate of about 45 % the rate of decrease in length of life cycle began to decline.

In view of the similarity in the effect of fungal decay on larval growth in both *Hylotrupes* [Becker, 1938] and *Xestobium* [Fisher, 1941], it is reasonable to assume that both insects deal with the N available in the diet in the same way. The results reported by Becker [1938] can best be explained by assuming that when peptone is added to the substrate the *Hylotrupes* larva increases its rate of N intake without having to increase its rate of boring to the extent which it must in decayed wood, where an increase in N intake is only achieved by virtue of an increase in rate of boring.

SUMMARY

1. N estimations have been carried out on a series of samples of oak sapwood decayed to various stages by *Phellinus cryptarum* Karst. The fungus is shown to remove a considerable proportion of the wood N via the mycelium which grows on the exterior of test pieces.

2. After removal of the external mycelium the decayed test pieces were found to be of significantly higher N content than sound wood of similar origin, but the rate of increase in N content of the wood residue during progressive decay was not great. Specimens which had lost approximately 50 % of their original weight by decay might contain, on the average, some 10 % more N than sound wood.

3. The N content of the frass of *Xestobium rufovillosum* De G. reared in oak sapwood decayed by *Phellinus cryptarum* has been compared in three separate instances with that of the original decayed wood, and no significant differences were found between the two quantities on a percentage basis.

4. It is concluded that the growing *Xestobium* larva retains in its body at least 95 %, and probably all, of the N available to it in the wood which it digests and that, because this is so, the rate of intake of N is a determinant of the duration of the larval stage.

5. The N in decayed as opposed to less decayed or sound wood, is made more available to the *Xestobium* larva by the softening effect produced by decay on the substrate. Less resistance is thereby encountered to boring and the duration of the larval stage decreases accordingly.

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REFERENCES

- Armstrong, E. F. & Armstrong, K. F. (1934). *The Carbohydrates*, p. 102.
London: Longmans, Green and Co.
- Becker (1938). *Naturwissenschaften*, **28**, 462.
- Campbell (1929). *Biochem. J.* **23**, 1290.
- & Bryant (1940). *Biochem. J.* **34**, 1404.
- Findlay (1934). *Ann. Bot., Lond.*, **48**, 109.
- Fisher (1935). *Nature, Lond.*, **135**, 102.
- (1937). *J. roy. Soc. Arts*, **85**, 407.
- (1940). *Ann. appl. Biol.* **27**, 545.
- (1941). *Ann. appl. Biol.* **28**, 244.
- Huntgate (1940). *Bot. Gaz.* **102**, 382.
- Norman (1936). *Biochem. J.* **30**, 1135.
- Ripper (1930). *Z. vergl. Physiol.* **13**, 315.
- Stubblefield & De Turk (1940). *Industr. Engng Chem., Anal. ed.*, **12**, 396.