STUDIES IN WOOD DECAY.

II. Enzyme Action in Polyporus Volvatus Peck and Fomes Igniarius (L.) Gillet.

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The present paper is the third of a series,[1,2] of papers dealing with the enzyme action in the wood-destroying fungi. It is the second of a series[3] of papers to be issued by this Laboratory dealing with the decay of wood in the broadest sense.

From the standpoint of parasitism, *Polyporus volvatus* is one of the most interesting of the wood-destroying fungi. Although no inoculation experiments have been made, numerous observations tend to confirm the opinion of the writer that *Polyporus volvatus* is truly parasitic. Throughout Washington, Oregon, and Idaho it is not at all unusual to find fruiting bodies of *Polyporus volvatus* appearing in great numbers over practically the entire surface of the trunk of Douglas fir, white fir, and western hemlock. This condition may be observed on trees still having a green, healthy foliage as well as on trees which to all appearances have been killed by the fungus.

[1] Schmitz, H., and Zeller, S. M., Studies in the physiology of the fungi IX. Enzyme action in *Armillaria mellea* Vahl., *Daedalea confragosa* (Bolt.) Fr., and *Polyporus lucidus* (Leys.) Fr., *Ann. Missouri Bot. Garden*, 1919, vi, 193–200.

[2] Schmitz, H., Enzyme action in *Echinodontium tinctorium* Ellis and Everhardt, *J. Gen. Physiol.*, 1919–20, ii, 613.

[3] Schmitz, H., and Daniels, A. S., Studies in wood decay I. Laboratory tests on the relative durability of some western coniferous woods with particular reference to those growing in Idaho, School of Forestry, Univ. Idaho, Bull. 1, 1921, 1–12.

The fact that *Polyporus volvatus* may be parasitic seems to have been first suggested by Zeller[4] in an unpublished paper. Dr. Zeller writes in part, "The fact that there were still needles on the last year's growth and that the mycelium had spread from the base of the tree up the trunk for 45 to 50 feet indicates that the fungus is parasitic. The writer has no absolute proof of this statement."

In this region *Fomes igniarius* is the cause of a serious white heart rot in the common aspen (*Populus tremuloides*) and also causes a a similar heart rot in mountain birch (*Betula fontinalis*). Quite recently Weir[5] has reduced *Fomes igniarius* and *Fomes nigricans* Fr. to synonomy.

Thus, the two fungi considered here are not without considerable interest both from the general and economic point of view.

Methods.

The cultures of the fungi used in the present study were obtained from young sporophores by the tissue method. As before, the cultures were grown on sterile carrots and while still in an active growing condition, removed from the flasks, dried, and ground. All of the enzyme cultures were set up in duplicate and 0.25 gm. of fungous meal was invariably used. Otherwise, the methods followed are those previously described.

Esterases.

The esterase activity of *Polyporus volvatus* and *Fomes igniarius* was studied by the use of 1 per cent solutions of methyl acetate, ethyl acetate, ethyl butyrate, triacetin, and olive oil emulsion. After twenty-one days incubation, hydrogen ion concentration determinations were made of the various filtrates. Positive esterase activity was obtained in the case of both fungi when methyl acetate was used as the substrate. The action on all the other substrates was negative.

[4] Zeller, S. M., Wood destroying fungi of Washington. Unpublished paper.

[5] Weir, J. R., Some observations on abortive sporophores of wood destroying fungi. *Phytopathology*, 1915, v, 48–50.

Carbohydrases.

The action of carbohydrases was determined on 1 per cent solutions of maltose, lactose, sucrose, raffinose, potato starch, inulin, white fir cellulose, and hemicellulose from date seed endosperms. After varying periods of incubation, the cultures were filtered and 5 cc. samples of the filtrates treated with 20 cc. of Fehling's solution. The results in the following table are the average of two titrations and are expressed as the number of cubic centimeters of 0.05 N potassium permanganate solution required to oxidize the dissolved copper.

TABLE I.

Carbohydrase Action of Polyporus volvatus and Fomes igniarius.

Incubation period.	Substrate.	*Polyporus volvatus.*			*Fomes igniarius.*	
		With fungous meal.	With fungous meal autoclaved.	Without fungous meal.	With fungous meal.	With fungous meal autoclaved.
		0.05 N $KMnO_4$				
days		*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
14	Maltose	21.85	14.70	13.25	30.40	17.15
20	Lactose	26.65	21.40	18.30	29.60	22.00
3	Sucrose	4.35	3.65	0.75	13.10	6.00
4	Raffinose	8.80	3.55	0.10	11.50	4.35
4	Potato starch	19.00	2.75	0.10	8.10	3.55
10	Inulin	5.00	3.10	0.15	7.50	5.00
30	White fir cellulose	6.00	2.85	0.20	8.00	4.95
30	Hemicellulose	7.80	3.45	1.80	8.70	5.25

Glucosidase.

The presence of a glucosidase in the fungous meal of the two fungi here discussed was determined by the action of the fungous meal upon a 1 per cent solution of salicin. After a 5 day incubation period, the enzyme cultures were filtered and sugar determinations made of 5 cubic centimeter samples of the filtrates. The results, expressed in the number of cubic centimeters of 0.05 N potassium permanganate solution required to oxidize the dissolved copper are shown in Table II.

Tannase.

Under the action of tannase, digallic acid is probably converted into gallic acid in accordance with the general formula,

$$C_{14}H_{10}O_9 - H_2O = 2C_6H_2(OH)_3COOH$$

Whether or not this reaction took place was determined by titrating the filtrates against 0.05 N iodine after the digallic acid had been precipitated out by the addition of albumin and the albumin salted out with NaCl. As indicated in Table III, the results are negative.

TABLE II.

Glucosidase Action of Polyporus volvatus and Fomes igniarius.

	Polyporus volvatus.	*Fomes igniarius.*
	0.05 N $KMnO_4$	
	cc.	*cc.*
1 per cent salicin + fungous meal	11.10	15.15
1 " " " + " " autoclaved	3.50	4.50
1 " " "	0.40	0.40

TABLE III.

Tannase Action of Polyporus vovatus and Fomes igniarius.

Enzyme Culture.	*Polyporus volvatus.*	*Fomes igniarius.*
	0.05 N iodine.	
	cc.	*cc.*
1 per cent digallic acid + fungous meal	4.7	3.9
1 " " " " + " " autoclaved	4.7	3.9
1 " " " "	3.5	3.5

The absence of tannase in *Polyporus volvatus* is difficult to account for since the fungus inhabits the barks of trees having a high tannin content. Its absence may, however, be due to the fact that the culture medium upon which it had been grown contained little or no tannin. The influence of the culture medium on the production of tannase in fungi has been fully investigated by Knudson.[6]

[6] Knudson, L., Tannic acid fermentation. I, *J. Biol. Chem.*, 1913, xiv, 159, Figs. 1 and 2; Tannic acid fermentation. II, 185.

Urease and Amidase.

To determine the presence or absence of enzymes which split amino-acids and urea into ammonia and hydroxy acids, the indicator method suggested by Schmitz and Zeller[1] was employed. The results are shown in Table IV.

TABLE IV.

Urease and Amidase Activity of Polyporus volvatus and Fomes igniarius.

	Enzyme culture.		Approximate change in hydrogen ion concentration.	
			Urea.	Acetamid.
			pH	*pH*
Polyporus volvatus.	Substrate + fungous meal.....	1	5.6–6.0 3 min.	5.6–5.6 3 min.
		2	5.6–6.0 3 min.	5.6–5.6 3 min.
	Substrate + fungous meal autoclaved...	3	5.6–6.0 3 min.	5.6–5.6 3 min.
		4	5.6–6.0 3 min.	5.6–5.6 3 min.
Fomes igniarius.	Substrate + fungous meal.....	1	5.6–7.8 45 sec.	5.6–5.6 3 min.
		2	5.6–7.8 45 sec.	5.6–5.6 3 min.
	Substrate + fungous meal autoclaved...	3	5.6–6.0 3 min.	5.6–5.6 3 min.
		4	5.6–6.0 3 min.	5.6–5.6 3 min.
Control.	Substrate alone.....	1	5.6–6.0 3 min.	5.6–5.6 3 min.
		2	5.6–6.0 3 min.	5.6–5.6 3 min.

Positive results were obtained only in the case of *Fomes igniarius* when urea was used as a substrate.

Rennet.

When 0.25 gm. of fungous meal was added to 10 cc. of fresh milk, coagulation occured in 3 hours in the case of *Polyporus volvatus*, and in $4\frac{1}{2}$ hours in the case of *Fomes igniarius*.

Catalase.

The presence of catalase was demonstrated by the action of the fungous meal upon a 3 per cent solution of hydrogen peroxide. When 0.25 gm. of the meal was added to 100 cc. of solution, 9.5 cc. of oxygen were liberated in the case of *Polyporus volvatus*, and 12 cc. in the case of *Fomes igniarius* in a period of 5 minutes.

Protease.

Tryptic and ereptic fermentation was studied by the use of albumin, peptone, casein, and fibrin in enzyme cultures having a neutral acid and alkaline reaction. The biuret test was employed in testing for the action of erepsin. The enzyme cultures were filtered, crystalline ammonium sulphate was added to the filtrate to precipitate the native proteins, and the solutions were again filtered through bone-black to remove the precipitate. In no case was a pink color produced when sodium hydroxide and dilute copper sulphate were added to the second filtrate.

A tryptophane test was also made of the filtrate by adding a few drops of glacial acetic acid and a few drops of strong bromine water. In no case was a pink color produced.

SUMMARY.

Circumstantial evidence is presented which indicates that *Polyporus volvatus* is parasitic.

Cultures of *Polyporus volvatus* and *Fomes igniarius* may be obtained from the young sporophores by the tissue method.

In *Polyporus volvatus* the presence of the following enzymes was demonstrated: esterase, maltase, lactase, sucrase, raffinase, diastase, inulase, cellulase, hemicellulase, glucosidase, rennet, and catalase.

In *Fomes igniarius* the presence of the following enzymes was demonstrated: esterase, maltase, lactase, sucrase, raffinase, diastase, inulase, cellulase, hemicellulase, glucosidase, urease, rennet, and catalase.