determination. Simple comparison of the solubilities of dusts using fixed conditions may give misleading results, and the correlation of solubility with pathological action by *in vitro* means will require a much more exhaustive study than has so far been attempted. In experiments designed to show the dissolution of dusts in the body, which will be reported elsewhere, cement has shown a relatively high solubility as compared with flint, sand (harmful dusts) and carborundum (harmless).

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FIXATION OF FREE NITROGEN BY BARK BEETLES

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IN 1912 and 1916 one of us isolated *Azotobacter* (*A. Sulci*) from Aphides^{1,2}. More recently, L. Tóth and others³ proved by micro-Kjeldahl determinations that species of *Rhynchota* (Aphides, etc.) can also rapidly and considerably increase their nitrogen content by utilizing atmospheric nitrogen, though Smith⁴ has not been able to confirm these results. This year, we made more progress in studying nitrogen fixation by the bark beetle, *Ips*, which has done much damage lately in Czech spruce forests.

The larvæ were mostly collected in mountain forests. Over successive intervals they were crushed with oxalacetic acid solution or similar reagent, oven-dried at 100° C., and subjected to Kjeldahl estimations. Using large amounts (0.5–0.8 gm.), as described by Csaky and Tóth⁵, did not give good results. Samples often showed a decrease in nitrogen compared with the controls. Hence, only small numbers of larvæ (30–50) were used. It was also found that results depended on the age of the larvæ. Half-grown (approximately three weeks old) larvæ fixed atmospheric nitrogen, whereas fully fed and fat ones often showed a decrease. This agrees with our view⁶ that the microbial symbionts of *Ips*, which occur in the peripheral (so-called adipose) tissue, soon find their way into the alimentary canal, where they are digested. Pupæ and young beetles do not fix nitrogen.

Though they were often found under the bark in great quantities, it was found inadvisable to collect larvæ directly in the forest. It was best to collect large samples (10–15 kgm.) of the infested bark and to isolate the larvæ in the laboratory, grouping them according to size. Even so, it was sometimes difficult to ensure that only one species of *Ips* was under examination, since several can occur together.

In the first series, using *Ips amitinus* L., from the Jizerské Mountains, in July 1948, the reagent used was either: (A) 0.088 per cent oxalacetic acid, 0.5 per cent dextrose, 0.7 per cent sodium chloride, 5.4 per cent dipotassium hydrogen phosphate; or (B), which contained 0.2 per cent succinic acid in

place of the oxalacetic acid and 9 per cent of phosphate. Both had pH = 7.2, and 3 c.c. were used with each sample of larvæ.

Sample	Dry weight (gm.)	Per cent N in dry material	Duration of fixation	Increase of N
1	0.2466	1.7	control	—
2	0.2480	1.92	13 hr.	13 per cent
3	0.2473	1.81	24 "	6 "
4	0.2552	2.08	48 "	21 "
5	0.3590	1.52	control	—
6	0.3727	1.46	13 hr.	decrease
7	0.3634	1.61	24 "	6 per cent
8	0.3820	1.57	48 "	—

Reagent A was used for the first four samples, and reagent B for the others. Oxalacetic acid is considered preferable to succinic acid for this work.

Further results were obtained using samples of the same species from the same locality. Small larvæ and small amounts were used, but with slightly different reagents. Reagent C contained 0.1 per cent of aspartic acid and had a pH = 7.2; reagent D contained 0.0669 per cent of oxalacetic acid, 1 per cent dextrose and 12 per cent phosphate besides the sodium chloride; its pH = 7.7. Reagent E was the same as A except for dextrose (1.5 per cent) and 5.4 per cent K₂HPO₄, pH = 7.0. Reagent F contained 0.132 per cent of oxalacetic acid and the higher amounts of dextrose and phosphate, pH = 7.0.

In the following samples, reagent C was used for 1 and 2; reagent D for 3 and 4; reagent E for 5 and 6; and F for 7 and 8.

Sample	Dry weight (gm.)	Per cent N in dry material	Duration of fixation	Increase of N
1	0.0922	2.89	control	—
2	0.0923	3.19	12 hr.	10 per cent
3	0.0950	3.46	control	—
4	0.0885	3.64	12 hr.	5 "
5	0.1804	1.78	control	—
6	0.1798	1.84	12 hr.	3 "
7	0.3816	0.84	control	—
8	0.2572	1.33	12 hr.	60 "

A third series of tests was made with *Ips typographus* L. and *Ips (Pityogenes) chalcographus* L., from Broumov Rocks (August 1948), using an oxalacetic acid reagent.

Sample	Dry weight (gm.)	Per cent N in dry material	Duration of fixation	Increase of N
1	0.1919	1.46	control	—
2	0.1872	1.39	14 hr.	—
3	0.1819	1.12	24 "	—
4	0.2016	1.59	control	—
5	0.2086	1.67	14 hr.	increase
6	0.2142	1.57	24 "	—
7	0.1944	1.63	48 "	increase
8	0.2024	1.28	control	—
9	0.1887	1.11	14 hr.	—
10	0.1929	0.98	control	—
11	0.1863	1.09	14 hr.	increase 11 per cent

In the first three samples, the *I. typographus* were very small; small to medium specimens were used in samples 4–7 and medium ones in 8 and 9. The last two samples were young larvæ of *I. chalcographus*. In the first three samples the adipose tissue with symbionts had probably not developed, hence no nitrogen fixation. In the other samples of *I. typographus* the larvæ were probably too old, hence only a small increase or decrease of nitrogen was observed.

In the last two, *I. chalcographus* definitely fixed free nitrogen.

Another series of samples, also collected at Broumov in August 1948, were similarly treated with the same reagent, samples 1-6 with 2 c.c. and 7-11 with 3 c.c. *I. typographus* larvæ longer than 4 mm. were used in samples 1 and 2; *I. chalcographus* (small, young larvæ up to 2 mm.) was used in samples 3-6; samples 7 and 8 were medium-sized larvæ of the same species (up to 3 mm. in length), and samples 9-11 consisted of larvæ up to 5 mm. long.

Sample	Dry weight (gm.)	Per cent N in dry material	Duration of fixation	Increase of N
1	0.1597	1.36	control	—
2	0.1604	1.44	13 hr.	about 6 per cent
3	0.1457	lost	—	—
4	0.1414	1.09	13 hr.*	—
5	0.1492	1.27	26 "	6 "
6	0.1423	1.33†	72 "	21 "
7	0.2211	1.39	control	—
8	0.2314	1.59	13 hr.	14 "
9	0.2348	1.43	control	—
10	0.2388	1.41	13 hr.	—
11	0.2356	1.39‡	26 "	—

* Control. † In contrast with 4. ‡ Larvæ too old.

Thus, on the whole, in all four series there is an increase in the nitrogen content of the larvæ, agreeing with our cytological considerations. This also coincides with the rapid breeding of these insects, though it is not so remarkable as with Aphides. It affords an explanation of the damage caused by *Ips*, and the question arises whether this behaviour can be controlled and even put to some use.

More than a hundred eggs as well as many larvæ were used in order to isolate the symbiotic organisms. In the adipose tissues it is mainly *Torulopsis* (*Cryptococcus*), an extremely variable organism, in contrast with previous experience. In addition, many strains of *Candida* were isolated as well as various species of bacteria, some resembling *Azotobacter*.

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MUSHROOM RESEARCH STATION AT YAXLEY

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RESEARCH on some of the problems of mushroom growing has been carried on for many years, particularly in France and in the United States. Two aspects have received special attention—the chemical and microbiological changes in horse manure during composting, and the fungus diseases of mushrooms and mushroom beds. The principal British work on mushroom cultivation is that of Ware on diseases and of Pizer on horse-manure composts. Recently the mechanization of transport has caused an increasing shortage and rise in price of horse manure, and this has become a major problem. Research on synthetic composts was in progress in the United States twenty years ago, and several formulæ have been published; but many of

the materials used are not available in Great Britain. Since the end of the War, American growers and research workers have devoted more attention to methods of using the available supplies of horse manure more economically, and to mechanization of their cultural operations. Synthetic composts are not widely used. Some work on synthetic composts was done in France during the 'thirties, but horse manure is still the only medium used commercially.

In 1945 a group of British mushroom growers decided to set up their own research station at Yaxley, with the development of a commercially satisfactory synthetic compost as the main problem. A laboratory, two test-houses, and a covered turning shed were built, and work started in 1946. Six experimental crops have been grown during the past three years, and the main requirements for making a productive synthetic compost from wheat straw have been established. Small-scale tests on a commercial farm have given a yield comparable with the best obtained from horse manure.

As a result of consultations between the Mushroom Research Association, Ltd., which has financed the research station until now, the Mushroom Growers' Association (a specialist branch of the National Farmers' Union) and the Ministry of Agriculture, arrangements are being made for the Research Association to receive a grant from the Ministry and to publish all its results.

Each main cropping-experiment now includes twenty-four composts of about half a ton each, made up side by side in two rows, and separated by iron sheets, so that aeration and losses of heat and moisture are similar to those occurring in one long stack of compost. In this way the replicated comparisons and factorial experiments familiar in agricultural research can be carried out. Analyses and microbiological examinations of the composts are made at suitable stages. Some of the results obtained in this way are likely to interest workers on agricultural composts.

The main nutrients studied are nitrogen, phosphorus, potassium and calcium. The source of nitrogen is very important, organic nitrogen giving higher yields than the inorganic forms so far tested. An empirical mixture of twelve trace-elements has quite a pronounced effect; one problem for the future is to determine which of these are essential and in what proportions. Preliminary experiments with growth substances suggest that further work in this field may give results of practical value, in addition to its scientific interest. The number of mushrooms produced has been increased in several experiments, and earlier fruiting has been obtained.

Variations in the depth of beds and in the intervals at which spawn is sown have quite marked effects on the time of fruiting and on yield. In the course of these experiments the number and size of mushrooms produced are recorded daily, and some measurements of their rate of growth and the relation between dimensions and weight are made. In several instances factors affecting the number of mushrooms produced have been distinguished from those affecting their size, and this work will add much to our knowledge of the appearance of mushrooms in flushes, the opening of the veil, which is subject to considerable variations, and the exhaustion of mushroom beds. These fundamental aspects of mushroom 'macrobiology' have been almost entirely ignored in the past, and their elucidation is of scientific and practical interest.