Merlewood Research and Development Paper
Number 36

EFFECTS OF CERTAIN FULL AND PARTIAL STERILIZATION TREATMENTS ON LEAF LITTER AND SOIL

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R & D 72/36

Introduction

For some years we have been carrying out litter decomposition studies, mostly in connection with the International Biological Programme. In many cases, we have found it necessary to treat the plant litter in some way to achieve full sterilization, in others, the litter was treated with X-rays as a partial sterilant, i.e. to kill only soil animals and their resting stages. We therefore carried out experiments described below to test the effect of different treatments on the organisms and on the chemical composition of the litter. We also include a short review of the literature on this subject.

Several treatments are available for full or partial sterilization of biological materials, notably gamma-irradiation, autoclaving, dry heat, X-rays, and chemical fumigants. There seems to be little information on the detailed effects of such treatments on plant litter, particularly in terms of chemical changes induced by the treatment.

Gamma-radiation is used widely for sterilizing a range of biological materials. McArdle and Hehemias (1956) recorded changes in pectic constituents of fruit and vegetables after gamma-irradiation. Bowen and Cawse (1964a) found no changes in dry peat and plant tissue after irradiation at 2 Mrad, but, when these materials were very wet, there was an increase in ammonium. Coleman and MacFadyen (1966) recorded increases in ammonium, nitrate, and respiration of litter-soil cores irradiated at 2.5 or 5.0 Mrads. Hering (1967) compared oak leaf litter irradiated at 2.5 Mrad with autoclaved litter. Decomposition by Trichoderma viride was rather more rapid in the autoclaved litter, although differences in total carbon, total nitrogen, hemicelluloses or alpha-cellulose were not found in the treated litters. On the other hand, Frankland (1969) found that, after irradiation at 2.5 Mrad, bracken (Pteridium aquilinum) rachides showed an increase in soluble carbohydrates.

More is known about the effects of gamma-radiation on soil, and Cawse (1969) gives an up-to-date, authoritative review of gamma-irradiation as a tool in soil research. Several advantages have been quoted in favour of this method for sterilizing soil; it is highly efficient, and a dose of about 2.5 Mrad usually gives complete sterility of soil (Cawse, 1969); penetration is relatively good, and appreciable thicknesses of material can be treated; using a ^{60}Co source, there is no induction of radioactivity in the irradiated samples, which are safe to handle; there is no time lag between treatment and readiness of samples for use; there are no phytotoxic residues (McLaren, 1969); the rise in temperature on treatment is small; there is only minor physical damage and the total chemical changes are relatively slight (Cawse, 1969). Indeed, McLaren (1969) states that, for soil sterilization, gamma-irradiation is perhaps the most nearly ideal treatment. However, some chemical effects of radiation treatment of soil have been reported. In particular, gamma-irradiation of soils appears to bring about marked changes in the proportions of different forms of nitrogen. There is often an increase in available nitrogen, usually in the ammonium form (Bowen and Cawse, 1964a, 1964b; Singh and Kanehiro, 1970), but sometimes as nitrate (Cawse, 1968, 1969). Cawse and Crawford (1967) and Cawse and White (1969) observed that in some soils after gamma-irradiation, nitrite accumulated and nitrate decreased (cf Singh and Kanehiro, 1970). The factors affecting the formation of nitrite are discussed by Cawse and

Cornfield (1971). Increases in available phosphorus have also been reported (Eno and Popenoe, 1963; Bowen and Cawse, 1964b; McLaren, 1969). Bowen and Cawse (1964b) also found substantial increases in carbon and organic nitrogen in soil solutions after gamma-irradiation. There were smaller increases in K, Ca, Mn, and organically-bound Mg, and the amount of nitrate was lower. Only some 4% of the increase in exchangeable nitrogen came from humus decomposition, the remainder came from lysis of micro-organisms. McLaren (1969) noted the release of small amounts of Mn, NH_4^+, soluble C, organic nitrogen and phosphorus from soil micro-organisms, and to a lesser extent from humus, after irradiation. Changes were smaller if the soils were dry when irradiated. Bowen and Cawse (1964a) observed that irradiated soil dried out more slowly than did untreated soil, and the rate of water percolation was reduced.

The dosage of gamma-radiation lethal to soil micro-organisms depends on the organism and the conditions in which irradiation is carried out. Kashkina and Abaturov (1967) treated soils with different levels of gamma-radiation. Bacteria were the most resistant soil micro-organisms, while fungi were the most radio-sensitive. In general, micro-organisms were more susceptible in wet than in dry soil. Low radiation doses brought about changes in the qualitative composition of the microflora. Jackson et al (1967) found that microbial counts in soils at 30% water content (by weight) were generally reduced to zero at lower radiation doses than those in air dry soils. One Mrad killed all fungi, but 2 to 3 Mrads were required to kill all bacteria in a 30 g soil sample. At a given water content, samples with higher initial microbial populations required greater radiation doses for sterilization than did samples with lower populations. They concluded that gamma-irradiation is a rapid and efficient method for sterilizing small quantities of soil.

Cawse (1967) studied the effects on soil of sub-sterilizing doses of gamma-radiation ($\leq$ 200 kr). He found an immediate increase in carbon in the soil solution after irradiation, and the increase was significantly correlated with both subsequent nitrogen mineralisation and soil respiration. Nitrification was rapid in all soils after 200 kr, probably because of the absence of effects on the oxidation process in non-proliferating cells of the nitrifying bacteria rather than increased proliferation of survivors.

Clark and Coleman (1970) found that, one week after treatment, respiration values (as CO_2 evolution) of litter-soil cores treated with 800 kr of gamma-radiation had a significantly lower variance than had the controls or samples treated with 2500 kr. The cores which received 800 kr showed an initial slight stimulation of CO_2 production. Bacteria were eliminated by 2500 kr and numbers were low after 800 kr.

Autoclaving and dry heating have been used by microbiologists for a number of years as sterilizing treatments. Little is known about the effects of such treatments on plant litter, although it is known that they produce pronounced changes in soils. Thus, Johnson (1919) observed the presence of phytotoxic substances in some heat-sterilized soils, and Bowen and Rovira (1961) found that growth of clover was retarded in heat sterilized soil. Hervey and Williams (1963) showed that autoclaved soil, unlike gamma-irradiated soil, was toxic to cotton root rot fungus. Autoclaving of soils, unlike irradiation, affects cation exchange capacity, particle size distribution, and moisture desorption (McLaren, 1969). Salonius et

al (1967) found that, compared with soil irradiated at 3 Mrads, autoclaved soil contained more soluble organic matter, soluble carbohydrate, and water-extractable ions. Furthermore, autoclaved soil had greater aggregate stability and was toxic to certain micro-organisms.

Various chemicals have been used to sterilize soils, but again little seems to be known about the effects of such treatments on plant litter. Uemura and Yambe (1962) obtained effective sterilization of soil, seeds and other materials, using propylene and ethylene oxides. Glathe et al (1967) found ethylene oxide to be an efficient soil fumigant at concentrations of 1 kg/m^3 or more. Adams (1966) observed large increases in extractable phosphorus when soil was treated with ethylene oxide. Increases were also found in pH, organic matter, and aggregate water stability, but there was no reduction in available water. Wolcott et al (1967) examined some effects on soil of two weeks' exposure to dichloropropene or two days' exposure to chloropicrin. After treatment, the soils were leached with distilled water until no nitrate could be detected in the leachates. Both treatments produced an increase in ammonium and a smaller increase in nitrate.

Propylene oxide (1, 2-epoxy propane) vapour has been used extensively for the sterilization of heat-sensitive organic materials in laboratory experimentation since it was recommended by Hansen and Snyder (1947), and has been accepted for standard tests, e.g. by the British Standards Institute (1961). It is said to be highly selective and normally appears to have no adverse effects on the material, although Smith (1965) reported an instance of residual toxicity to Lentinus lepideus Fr. after sterilizing wood specimens, and Roff (1963) mentioned the possibility of residual effects on brown rot fungi. Da Costa and Osborne (1970) observed that in some cases it is possible to demonstrate a residual effect of propylene oxide treatment. Bartlett and Zelazny (1967) described a simple technique for preparing sterile soils. They exposed soils in polythene bags to propylene oxide in a warmed pressure cooker; propylene oxide diffuses rapidly through polythene film in these conditions. The residual gas is removed under vacuum at 45°C and the soils can be kept in the polythene bags until required for use.

Information on the effects of X-rays is also sparse. Peterson (1962) found that soil exposed to X-rays respired at a rate approaching that of untreated soil.

Methods

Freshly-collected leaf litter of oak and hazel from Moathop Wood IBP site (Nat. Grid Ref. SD436795) was air dried (AD) at laboratory temperature and weighed into glass specimen tubes, approximately 0.4 g in each tube. Sub-samples were oven dried at 105°C (OD) and weighed to get an OD/AD weight conversion factor. The tubes of litter were plugged with cotton wool and were divided into batches of 16 tubes for each treatment. Treatments were: (1) control, no treatment, (2) a total of 20 kr of X-rays given in three separate doses, (3) autoclaved for one hour at 20 lb/in^2 and 126°C, (4) gamma-irradiation of 2.5 Mrads from a ^{60}Co source, (5) and (6) were propylene oxide treatments in which tubes of litter with the cotton wool plugs removed were placed in a large desiccator containing a

dish of propylene oxide and left for 24 hours, the cotton wool plugs being left in the desiccator. Dogassing was carried out in a filtered inoculating room, with the usual precautions, in two different ways. In treatment (5), the lid of the desiccator was partly opened and allowed to stand for seven hours, after which time the cotton wool plugs were replaced. In treatment (6), air was drawn via a sterile cotton wool filter through the desiccator overnight, after which the cotton wool plugs were replaced.

After treatment some of the tubes were examined immediately, the remainder were examined after intervals of up to 8 weeks. Examination consisted of (a) tests for sterility, (b) measurement of respiration, and (c) chemical analysis.

The criterion for sterility was absence of visible growth of micro-organisms in some standard culture media. Czapek-Dox medium with addition of 0.05% yeast extract (CDY) was used as a relatively non-selective medium to isolate fungi, and Oxoid tryptone soya supplemented with soil extract (TS) for bacteria. These media were inoculated with litter and incubated at 25°C and 50°C. The higher temperature was used to isolate thermophiles which it was thought might survive sterilization by heat more readily than would mesophiles.

Three litter samples of each tree species were tested for sterility one week after each sterilization treatment as follows:-

From each sample, forty fragments of litter 1-2 mm^2 were cut out under a sterile hood and plated. Five fragments were arranged equidistantly in each Petri dish and covered with 12 ml molten CDY or TS agar cooled to 45°C. Two plates per medium were prepared for each incubation temperature, together with control plates which contained particles of sterile filter paper in place of litter. The plates were incubated for a month; those at 50°C were wrapped in polythene to prevent rapid drying out of the agar. A total of 24 plates per sterilization treatment were examined for each tree species.

To detect very low densities of viable micro-organisms, a whole leaf from each sample was incubated for one month in 25 ml CDY or TS broth in 100 ml plugged Erlenmeyer flasks at 25°C and 50°C. One flask was prepared from each litter sample per medium and temperature, i.e. for each tree species, a total of 12 flasks per sterilization treatment were examined for growth. Sterile filter paper replaced litter in control flasks.

Approximately eight weeks after sterilization, a further sample from each treatment was examined for changes in the microbial population by plating on agar media as above.

Litter irradiated by X-rays to remove animals was examined for any major changes in the natural population of fungi and bacteria by plating leaf fragments in nutrient agar. The same procedures, including the same media, incubation temperatures and replication of plates, were used as in the sterility tests. Fungal and bacterial colonies which grew out from the litter fragments were compared with those on similar plates of air-dry non-irradiated litter. Comparisons were made after 10 days and again after one month.

Table 1. Frequency of occurrence of bacterium T(1-3) in fragments (1-2 mm^2) of oak litter on tryptone soya agar. Thirty fragments per treatment

Incubation temp. °C	Controls	Gamma radiation	Auto-claving	Propylene oxide
25	0	11	24	28
50	0	7	21	27

Table 2. Total number of colonies of micro-organisms on oak (O) and hazel (H) before and after X-ray treatment

Incubation temp. °C	25				50				
Medium	CDY		TS		CDY		TS		Total
Litter	O	H	O	H	O	H	O	H	O H
Controls	14	31	32	24	2	5	14	5	62 65
After X-ray treatment	21	32	26	28	0	1	16	4	62 65

Respiration was measured immediately after treatment and after 2, 4 and 8 weeks. To measure respiration, the unplugged tubes were placed in a Gilson respirometer (Howard, 1968) after addition of 2 ml of sterile distilled water to each tube with shaking to moisten the litter. After equilibration overnight, the oxygen uptake was measured over seven hours. The results are expressed on a per hour basis corrected to Normal Temperature and Pressure (NTP, 0°C and 760 mm).

Chemical analyses were for soluble tannins by a colorimetric method using the Folin-Denis reagent (A.O.A.C., 1965), soluble carbohydrates by the method of Deriaz (1961), organic carbon by dry combustion, total nitrogen by a modified micro-Kjeldahl method, and ash after ignition at 550°C.

Results

a) Tests for sterility

i) Agar cultures

Fungal growth did not develop from any of the 720 fragments of litter plated in nutrient agar after autoclaving, gamma-irradiation, and propylene oxide treatments. A bacterium appeared to be the only organism which had survived these total sterilization treatments. Minute brown submerged colonies were observed on treated oak litter 10 days or more after plating in TS agar. Three distinct colonial variants, possibly mutants of one organism, were present in sub-cultures obtained from these colonies. One variant T(1) was a Gram negative rod and the other two, T(2) and T(3), were a species of Bacillus (V. G. Collins, personal communication). Morphological and biochemical details of this component culture T(1-3) are given in the Appendix. Growth of T(1-3) on litter at 50°C was rather more rapid than at 25°C; it occurred only on TS agar and was not recorded in hazel litter. The frequency of its occurrence in oak is given in Table 1. The competition of fast-growing species apparently prevented its growth on control plates, but it appeared in controls of later experiments after storage had reduced the number of competitors (see Appendix).

Not only was T(1-3) less frequent on irradiated litter, but also the time lag between plating and visible growth into the agar was greater, and mature colonies were more restricted than on litter sterilized by other methods. Radiation, in particular, appeared to have interfered with normal growth in some way. Further evidence of this was the usual failure of sub-cultures from irradiated litter whereas those from autoclaved and gassed litter grew well. After the treated litters had been stored for eight weeks and replated, hazel remained sterile and the bacterium T(1-3) was again the only organism detected in oak.

ii) Liquid cultures

The bacterium described above was not detected in liquid cultures of litter and there was no evidence from this test that any micro-organisms within the litter had survived the total sterilization treatments. However, in half the flasks of litter gassed by method (5) and in three-quarters of the flasks of method (6) 1-3 colonies of bacteria and fungi developed. They were chiefly Penicillia and were known to occur as laboratory contaminants.

As they were not present in the control flasks it was concluded that a low level of contamination by aerial micro-organisms had occurred, probably during degassing.

iii) Biological effects of treatment with 20 kr X-rays

No major changes were found in the composition of the microbial population after removal of animals by X-ray treatment.

The total number of colonies of fungi, bacteria and actinomycetes on control litter equalled that on treated litter (Table 2) and Aureobasidium sp. was dominant in all samples as is usual on freshly fallen oak and hazel leaf litter in Meathop Wood (Frankland, unpublished data). The only sporulating fungus not previously recorded on Meathop litter was a single colony of the thermophile Thermomyces lanuginosus Tsiklinsky, which occurred on X-rayed hazel incubated at 50°C in TS agar.

b) Respiration

Comparison of the respiration results is complicated by differences in moisture contents of the litter samples. However, this was allowed for by analysis of covariance (Steel and Torrie, 1960). The adjusted respiration values were then compared using Snedecor's Q method (Snedecor, 1957). Comparisons were made in two ways:

i) within weeks between treatments and control

ii) within treatments between weeks.

The results for the comparison within weeks between treatments and control are shown in Table 3. In most cases, respiration in the treated samples is significantly less than in the control, but only autoclaving reduced the respiration to zero. The structure of the experiment will not stand comparison between treatments, nor was this the intention. It is noteworthy, however, that X-rays reduce the respiration less than do the other treatments. Comparison within treatments between weeks did not show a clear pattern. In some treatments (oak control and X-rays, hazel control, X-rays, irradiated, and propylene oxide 2) there were significant differences between weeks, the respiration tending to decrease with time.

c) Chemical analyses

The chemical analyses were submitted to analysis of variance and the results are given in Tables 4 and 5. Analyses in which the F ratios from analysis of variance suggested a significant difference between treatments were examined in detail using Snedecor's Q test (Snedecor, 1957), to find which treatment was significant. In two cases with hazel litter, the Q test did not reveal a significant difference although the F test indicated that the results were significant at the 5% level. With the oak litter, no significant chemical differences were found immediately after treatment, but eight weeks after treatment the value for soluble tannins was significantly lower in the autoclaved litter and the value for soluble carbohydrates was significantly greater in gamma-irradiated litter. The hazel litter also showed a significantly lower content of soluble tannins in the autoclaved

litter after eight weeks, but also showed the same effect immediately after treatment.

Discussion and Conclusions

The results show that autoclaving, gamma-irradiation, and propylene oxide treatments are effective as total sterilizing treatments. A bacterium was the only survivor of those treatments detected. Organisms rarely survive treatments of 2 to 3 Mrads, but Robinson et al (1970) found that a naturally-occurring bacteriophage for Bacillus megaterium strain KM could survive in soil after 5 Mrad of gamma-irradiation, although the number of survivors was small. There are also various reports of bacteria resisting extreme exposures of irradiation. For example, Anderson et al (1956) found a Micrococcus following gamma-irradiation of meat. In pure culture, this organism survived a dose of approximately 6 Mrads.

Low values for oxygen uptake were observed after gamma-irradiation and propylene oxide treatments. Because of the total sterilizing effects of these treatments it seems likely that the respiration in the gamma-irradiated samples is due to respiratory enzymes. This conclusion was also reached by Roberge (1971) who found that Black spruce (Picea mariana) humus treated with 1.1 to 3.3 Mrads of gamma-irradiation continued to show oxygen uptake. Cawse and Cornfield (1971) concluded that radio-resistant enzyme systems, in their case from denitrifying organisms which could no longer proliferate, could continue to function in soils treated with 0.75 Mrad. The oxygen uptake which we observed after propylene oxide treatment may be due to a similar effect, but as propylene oxide is a powerful oxidizing agent, the oxygen uptake could be due to the residual chemical effect, or possibly to contaminating organisms introduced during degassing.

Our results confirm that autoclaving is the most drastic of the treatments used and it appears to reduce the content of soluble tannins in the litter. Propylene oxide treatment is effective in killing organisms, although its activity is only about half that of ethylene oxide. Propylene oxide is less flammable than ethylene oxide, but the latter is more readily removed because it has a lower boiling point. Both of these substances are unpleasant to use and can be dangerous (Sykes, 1958). Because both propylene and ethylene oxides are powerful oxidising agents, it is essential to ensure that the experimental material is free of the gas before use. Degassing can be difficult to achieve without introducing contaminant spores; it is more efficiently done at temperatures greater than normal laboratory temperature, but this could be dangerous.

Gamma-irradiation is at least as effective as autoclaving in killing organisms, and may be more so as the Bacillus species appeared to be damaged more by irradiation than by the other treatments. This treatment appears to leave at least some enzyme systems functioning, but storage of the treated material for some weeks before use allows free enzyme activity to decline. The effect of storage time on free enzymes, depending also on such factors as temperature and moisture, requires further investigation before more exact recommendations can be made for specific materials. Practical experience also suggests that physical damage to plant litter is less after irradiation $\leq$ 2.5 Mrads than after autoclaving.

Because the covariance analyses were performed on relatively few samples, too much emphasis should not be placed on the actual adjusted respiration values in Table 3. However, they do serve to show that the respiration of litter treated with X-rays was generally greater than in the other treatments and it was generally significantly less than the controls. This was to be expected if the treatment had succeeded in killing the fauna while leaving the microbial population intact. As the X-ray treatment produced no major changes in the composition of the microbial population, nor significant changes in the chemical composition of the litter, but killed soil animals and their resting stages, it is clearly a useful treatment for litter if micro-organisms are to be studied in the absence of animals, or if a different population of animals is to be introduced.

Acknowledgements

We are grateful to: Mrs. D. M. Howard for setting up and maintaining the experiment, for making respiration measurements, and for preparing the samples for analysis; Miss S. Dawson for microbiological assistance; the Chemical Service at Merlewood for chemical analysis of the litters; Miss Barbara D'Sylva and Dr. Vera Collins for examing some bacterial cultures; Mr. J. N. R. Jeffers and Mr. D. K. Lindley for statistical advice; Mr. D. C. Pickering of the Liverpool Regional College of Technology who very kindly treated the litter with X rays; and the staff of the United Kingdom Atomic Energy Authority, Wantage, for helpful advice on gamma-irradiation.

Table 3

Comparison of respiration means ($\mu l O_2/g \cdot D/h$) adjusted for moisture content, within weeks between treatments and control

Oak

Treatment	Week 1	Week 2	Week 4	Week 8
Control	51.16	29.62	44.79	17.78
X-rays	26.34*	22.09	10.91*	13.48
Autoclaved	4.54*	X	X	X
gamma-irradiated	6.61*	7.76*	9.13*	1.81*
prop. oxide 1	12.12*	3.64*	3.70*	-
prop. oxide 2	4.73*	5.63*	3.72*	8.44*

Hazel

Control	248.56	137.45	97.85	30.43
X-rays	74.39*	46.76*	25.02*	29.49
Autoclaved	X	1.66*	X	X
gamma-irradiated	13.22*	10.37*	2.04*	5.63*
prop. oxide 1	6.80*	-2.48*	4.35*	-
prop. oxide 2	2.44*	20.74*	16.00*	11.46*

* Difference from control significant at 5% level

X Respiration value of zero in original measurements.

Table 4

Oak litter, chemical analysis Results from analysis of variance

	Treatment	Mean values of duplicate analyses percent OD basis: Soluble tannins	Soluble carbohydrates	Total hydrogen	Total nitrogen	Total carbon	Total ash
Week 0	control	9.65	3.75	4.80	1.00	53.20	4.48
	X-rays	9.70	4.80	4.40	1.13	53.20	4.62
	autoclaved	7.55	4.40	5.00	1.06	53.30	5.27
	gamma-irradiated	9.00	3.90	5.30	1.06	51.85	4.77
	prop. oxide 1	9.30	3.65	4.55	1.02	52.35	5.40
	prop. oxide 2	9.60	3.30	5.45	1.12	54.75	5.12
	SE of group mean	0.57	0.32	0.47	0.04	1.10	0.44
	F ratio	2.06	2.93	0.84	1.55	0.88	0.71
Week 8	control	9.75	3.85	5.15	1.09	52.05	4.52
	X-rays	9.10	3.70	4.70	0.98	52.00	5.45
	autoclaved	6.60*	3.85	4.70	1.12	53.30	5.29
	gamma-irradiated	9.60	4.45*	4.40	1.11	51.60	5.20
	prop. oxide 1	9.20	3.65	4.80	1.08	53.05	5.57
	prop. oxide 2	8.70	3.70	4.90	1.08	53.60	4.96
	SE of group mean	0.41	0.09	0.30	0.05	0.54	0.27
	F ratio	7.79*	10.65**	0.70	0.86	2.31	1.96

* significant at 5% level

** significant at 1% level

Table 5

Hazel litter, chemical analysis Results from analysis of variance

Mean values of duplicate analyses percent OD Basis

	Treatment	Soluble Tannins	Soluble carbohydrates	Total hydrogen	Total nitrogen	Total carbon	ash
Week 0	control	4.10	3.10	3.80	1.09	51.00	7.87
	X-rays	3.90	2.80	4.45	1.24	48.90	9.06
	autoclaved	2.20*	2.80	5.30	1.22	51.45	9.17
	gamma-irradiated	4.00	3.85	5.10	1.24	50.55	8.51
	prop. oxide 1	3.45	2.70	4.60	1.21	50.65	8.62
	prop. oxide 2	3.25	2.25	4.80	1.30	50.50	8.93
	SE of group mean	0.17	0.36	0.82	0.06	1.05	0.32
	F ratio	17.30**	2.18	0.46	1.43	0.74	2.19
Week 8	control	3.10	2.85	5.15	1.20	50.05	8.77
	X-rays	3.75	3.40	5.00	1.17	50.75	9.78
	autoclaved	1.60*	3.25	4.90	1.32	51.85	8.93
	gamma-irradiated	3.75	3.50	3.60	1.27	49.70	8.46
	prop. oxide 1	3.90	2.75	4.95	1.25	51.05	8.83
	prop. oxide 2	2.95	2.15	4.20	1.27	50.15	8.82
	SE of group mean	0.15	0.30	0.38	0.06	0.50	0.20
	F ratio	32.50**	6.67*	1.90	0.71	2.33	5.17*

* significant at 5% level

** significant at 1% level

Appendix

Further investigations of the effects of gamma-radiation on the bacterium, T(1-3), isolated from oak litter

Well-grown sub-cultures of the bacterial complex T(1-3) on slopes of TS agar failed to grow when streaked on TS agar plates after autoclaving (15 mins. at 15 lb/in^2) or gamma-irradiation (2.5 Mrad). In the original samples, some protection may have been given by the leaf tissue. On close examination of the leaf fragments it could be seen that the organisms had grown more frequently from the cut ends of the leaf veins than from tissue of the lamina. Since, biologically, gamma-irradiation appeared to be the most effective of the sterilization methods used, the resistance of T(1-3) in litter to irradiation was investigated further with a view to finding a lethal dose.

Oak leaf litter from the original air-dried collection was gamma-irradiated in glass flasks with doses of 2.5, 3.0, 4.0, 4.5, 5.0, 5.5 and 6.0 Mrad. The litter was cut into fragments (1-2 mm^2) under sterile conditions and plated on TS agar (5 fragments per plate) as before, but fragments of veins and lamina were plated separately. Five plates of veins and five plates of lamina were prepared for each radiation dose. Control plates of chopped sterile filter paper and of non-irradiated litter which had travelled to the irradiation plant with the other samples were also prepared. The plates were randomized and incubated at 50°C. Growth was recorded after 2-3 weeks.

Table 6 Frequency of occurrence of bacterium T(1-3) on fragments (1-2mm^2) of oak litter on TS agar after various doses of gamma-radiation

	No. of fragments on which the bacterium T(1-3) was observed		
No. of fragments of leaf tissue examined	Veins 25	Lamina 25	Total 50
Mrad			
0.0	7	9	16
2.5	21	9	30
3.0	15	14	29
3.5	16	3	19
4.0	11	2	13
4.5	13	0	13
5.0	7	0	7
5.5	5	1	6
6.0	2	2	4
Filter paper controls			0
Total for irradiated material	90	31	

A decrease in numbers of colonies occurred over the dose range 2.5-6.0 Mrad with an indication of greater survival in veins than in the lamina, but it

was difficult to exclude all small veins from fragments of lamina. A smaller number of colonies on non-irradiated litter than on some irradiated material was expected, because of the presence of microbial competitors, which were killed by irradiation.

Sub-cultures of bacterium T(1-3) failed to grow from the irradiated material used above, but the bacterium was found to be alive and motile in scrapings of the plated litter, even in those from leaves exposed to 6.0 Mrad, although the number of bacteria per microscope field decreased with increasing dose of radiation (B. T. D'Sylva, personal communication). A full lethal dose had not therefore been reached, but the evidence suggests very low biological activity in the most heavily irradiated samples, where severe chemical and physical damage to leaf tissue can be expected. The leaves were noticeably more powdery when cut with scissors after a dose of 3.5 Mrad or above than after lower doses. For most practical purposes, the use of a dose lethal to this highly-resistant bacterium would obviously be unsuitable.

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