EFFECTS OF GAMMA RADIATION ON
LICHENS AND LICHEN-FORMING FUNGI
AND ALGAE

Final Report

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ABSTRACT:

Initial literature reports indicated that lichens were highly resistant to gamma radiation. A series of experiments were undertaken to determine why lichens were so resistant. In experiments with Cladonia mitis, C. sylvatica, C. verticillata, Parmelia sulcata and P. subaurifera, using both acute and chronic radiation, it was found that these lichens were not highly resistant to gamma radiation. The lichens appeared to be resistant as long as they were in a dormant condition. But, when allowed to pass through a period which was suitable for growth, radiation damage was quickly manifested.

Radiation damage was seen in various ways, including the change of thallus color, disintegration of phycobiant chloroplastids, coalescing of fungal hyphae, reduced CO₂ uptake, and reduction in thallus tensile strength.

INTRODUCTION:

When this project, effects of gamma radiation on lichens, was initiated there were only 3 published investigations (Brodo, 1964; Jones and Platt, 1969; Woodwell and Cannutz, 1967). All of these studies indicated that lichens were highly resistant to gamma radiation. My initial work with crustose lichens from Nevada and gamma radiation tended to support the idea of lichens having great resistance. Based on the work with the Nevada lichens, support was requested to determine why lichens are so resistant to gamma radiation. This study was to be confined to one lichen species, Cladonia mitis, so a data base could be compiled and used for comparison in other lichen radiation studies.

CLADONIA STUDY:

All acute gamma radiation studies were done at Michigan Technological University using a gamma cell ⁶⁰Co source. Cladonia mitis was the primary lichen used in these studies.

The initial work was to determine how high a gamma radiation dose was required to cause the lichen to manifest physical damage. The first macroscopic change noted was a browning of podetia at 2.000 krad. No physical damage could be seen with lower radiation doses.

Using a Warburg apparatus the rate of oxygen uptake was determined for lichens which had received varying doses of gamma radiation, 0.1 krad to 1.000 krad. It was found that when compared to the control, over a six week period following irradiation, there was no significant change in oxygen uptake (Figure 1). It was felt that the respiration data tended to corroborate the hypothesis of the lichens resistance to gamma radiation.

The algal component of the lichen was examined microscopically in conjunction with the oxygen uptake study. Algal cell damage was found

-Figure 1. Oxygen uptake of acutely irradiated Cladonia mitis podetia.

A and B uptake by podetia which had been in the growth chamber 1-1/2 weeks prior to irradiation. C and D uptake by podetia which were irradiated immediately after collection.

5 weeks after irradiation in those lichens receiving 100 krad, 1 week after receiving 1000 krad and immediately after 2000 krad (Figure 2). Damage was seen in the clumping and disintegration of the chloroplastid. However, less than 10% of the algal cell population appeared to be damaged. Again, this study indicated the resistance of the lichen to gamma radiation.

In an effort to determine cell membrane damage irradiated lichens were placed in solutions containing ^{32}P . The retention of ^{32}P was determined for non-irradiated lichens and lichens which had been irradiated 6 weeks earlier. Only those lichens which received 1000 krad differed from the controls (Figure 3). The 1000 krad lichens had a retention approximately 1/2 of the control. Lichens receiving doses of less than 1000 krad did not vary significantly from the growth chamber control.

All the results of the first study with Cladonia mitis indicated that lichens showed great resistance to gamma radiation; at least for 6 weeks after acute irradiation. The question arises: What about longer times following acute radiation or chronic gamma radiation?

A gamma field, chronic gamma radiation, was established by the North Central Forest Experiment Station, Genetics Institute, Rhinelander, Wisconsin. Permission was obtained to set up an experiment in the gamma field. Clumps of Cladonia mitis were transplanted from Keweenaw County, Michigan to the gamma field in Wisconsin. The lichens were placed so they received doses ranging from approximately 10 to 900 rad. per day. Comparisons of oxygen uptake, ^{32}P retention, and algal cell structure during the first year of radiation indicated that the radiation had little or no effect on the transplanted lichens. So, physically, cytologically, and physiologically the chronically irradiated lichens paralleled the control lichens. This information strengthened the idea of lichen resistance to gamma radiation.

Figure 2. Algal cells from the control lichen and from irradiated *Cladonia mitis podetia* immediately after irradiation, one, two, and five weeks thereafter. Small vacuoles, seen in non-irradiated algae after two weeks were attributed to growth chamber conditions. Radiation damage is shown by shrunken cytoplasm with irregular margins, condensed and stained cytoplasm, and cytoplasm broken into many stained globules.

Figure 3. Comparison of ^{32}P uptake of irradiated and non-irradiated lichens six weeks after irradiation.

Early in the spring, following the summer of chronic irradiation, and before the season's irradiation period began (early April), all of the irradiated lichens appeared normal. However, by late May, still before the new irradiation period, those lichens which had received 900^+ rads per day exhibited severe damage. These damaged lichens were brown, rather than yellow-green, were flattened, rather than upright, and had many broken podetia. It was felt that the damage could be attributed to the gamma radiation, but there was no conclusive proof. New lichens were transplanted into the field and received a summer of gamma radiation. In the fall, after the irradiation season, more lichens were brought into the field and placed adjacent to those which had been irradiated. The following spring, only those lichens which had been irradiated, 900^+ rad per day, manifested damage, the non-irradiated ones did not. The experiment was repeated the following year and the results were the same. This was the first series of experiments which indicated that lichens are affected by gamma radiation.

The obviously damaged lichens had a greatly depressed ^{32}P retention (Figure 4), had few viable algal cells (Figure 5), chromatographically had the same pigment systems, but with reduced quantities (Figure 6) and reduced O_2 uptake.

This experiment indicated that a prolonged period suitable for growth in combination with high doses of gamma radiation was needed before gamma radiation damage was manifested.

An acute radiation study using Cladonia verticillata tended to substantiate the findings of the gamma field study. (Pullum and Erbsich, 1972). The lichen showed no adverse effects of the gamma radiation until after 6 weeks in a growth chamber where conditions were suitable for lichen growth. The physical appearance and ^{32}P retention of the 1000 krad lichen was much different than the control or less severely irradiated thallus (Figure 7).

Fig.4 . ^{32}P uptake by podetia of *Cladonia sylvatica*

Figure 5. Algal cell configurations and ratios from podetia which had been in the gamma field for one year. The upper figures (a - e) represent clumps of algal symbionts. Cells with only an outer wall were devoid of chloroplasts; while living cells have the cell wall surrounding the chloroplastid. a - c: Algae from control podetia. The lower figure indicates the % green algal cells, versus those less a chloroplastid, found in gamma field irradiated podetia. The dashed line indicates the number of green cells found in non-irradiated podetia.

Figure 6. Results of thin layer chromatography of pigments from irradiated and non-irradiated Cladonia podetia. Control = podetia brought in from the field, non-irradiated; site 1 = podetia 8' from the gamma source in the radiation field, irradiated one summer (900 rad/hr.); site 8 = podetia 75' from the gamma source in the radiation field, irradiated one summer (2 rad/hr.); 1000 Kr = podetia which had been irradiated 6 weeks earlier and kept in the growth chamber.

Figure 7. Uptake of ^{32}P by Cladonia verticillata 24 hr. and 6 weeks after gamma irradiation. Controls received no irradiation. Those which received 10 krad are shown in the graph on the left; those receiving 1000 krad on the right.

Some of the algal cells of the 1000 krad thallus were physically damaged (20 - 30%) and none that were isolated could be cultured; the latter indicating that all algal cells of the 1000 krad material were adversely affected.

The effect of gamma radiation on $^{14}\text{CO}_2$ uptake by C. mitis was determined (Barstow and Erbisch, 1977). The rate of CO_2 uptake was greatly reduced within 24 hours following a radiation dose of 1000 krad. Over a longer time period the effect on CO_2 uptake was increased (Figure 8). It had been felt that photosynthesis may continue under the deep snow cover and that the lack of this winter photosynthesis of the irradiated lichens may have been the cause of the damage manifested. The combination of low temperature ($1-2^\circ\text{C}$) and low light intensities (3-5 ftc.) reduced CO_2 uptake (Figure 8) so much in non-irradiated lichens that winter photosynthesis is not considered as an important factor in causing radiation damage to be manifested.

Since lichen acids or substances are unique to lichens it was felt that these may offer protection to the lichen during irradiation. In a qualitative study there were indications that the concentration of some acids decreased after irradiation (Sartorelli, 1972). However, no significant qualitative differences were noted in irradiated and non-irradiated C. mitis podetia. A quantitative study of the effect of gamma radiation on one of the acids, usnic acid, which appeared to decrease was undertaken. In this work it was shown that gamma radiation had no effect, in vivo or in vitro, on usnic acid (Funky, 1974) (Figure 9). A change in usnic acid concentration was noted if the irradiated lichen were allowed to pass through a suitable, prolonged growth period (Figure 10). From the results of these two studies it can be concluded that lichen acids have little or no protective properties in relation to gamma radiation.

Podetial Treatment	Light and temperature conditions			
	1500 ft. c.	1500 ft. c.	5 ft. c.	5 ft. c.
	<u>20° C</u>	<u>3° C</u>	<u>20° C</u>	<u>3° C</u>
Control	116.12	18.95	3.66	2.99
Irradiated 48 hours prior to ¹⁴ C uptake	57.22	10.47	2.59	2.30
Irradiated 10 months prior to ¹⁴ C uptake	5.93	3.10	2.98	3.13
Autoclaved (Dead)	2.33	2.47	1.02	2.09

Figure 8. Average ¹⁴C uptake in nmoles/gram dry lichen ($\times 10^{-5}$) Cladonia mitis.

Figure 9. Results of the in vivo analysis of usnic acid, given as mean % per gram of dry thallus. All samples were tested immediately after irradiation. The hair-line indicates one standard deviation within each group, A = control group; B = irradiated to 10 krad; C = irradiated to 100 krad; D = irradiated to 1000 krad.

Figure 10. Results of the *in vivo* analysis of usnic acid, given as mean % per gram of dry thallus. Samples A - D were irradiated in November, 1973 and sample E was irradiated June 1973. All samples were tested in April, 1974. The hairline indicates one standard deviation within each group; A = control; B = irradiated to 10 krad; C = irradiated to 100 krad; D and E = irradiated to 1000 krad.

PARMELIA STUDY:

The development of the Enterprise Radiation Forest by the North Central Forest Experiment Station in Rhinelander, Wisconsin, provided an opportunity to study the effects of gamma radiation on lichens in their natural habitats. So, in addition to the Cladonia project, a study was initiated in the Enterprise Forest to determine the effects of gamma radiation on two species of Parmelia, P. sulcata and P. subaurifera.

In the Parmelia study environmental changes as well as radiation effects were monitored and correlated (See Figure 11 for dosages, host tree, etc.). For both species it was found that there were few effects noted during the radiation period and it was not until late the following spring when damage was evidenced (Erbisch, 1977a, 1977b, 1974). Damage in P. sulcata was seen in the lifting of thallus lobes and their subsequent breaking, coalescing of hyphae, misshapen algal cells, and brown pigment formation in the thallus. P. subaurifera damage was noted in the breaking up of the thallus, misshapen algal cells and a bleaching of the thallus. For both species the growth rate of highly irradiated thalli slowed (Figure 12). Those thalli which survived the high dose of gamma radiation and were able to resume growth formed numerous small circular outgrowths (Figure 12).

The damage found on the Parmelia thalli was considered to be primarily caused by radiation. Changes caused by environmental variations were minimal. Measurements of light, temperature, and humidity were essentially the same throughout the radiation forest and were not significantly different in the highly irradiated area (Crow and Buech, 1977, Erbisch, 1977a) during the radiation year and the following spring. It was during the late spring, when the trees were beginning to leaf out, when radiation damage was noted.

As with Cladonia radiation damage was manifested only after the irradiated lichens went through a prolonged period which was suitable for growth.

Location and radiation data for the Parmelia subaurifera thalli studied in the Enterprise Radiation Forest.

Tree No.	Tree Species	Distance from source, m	Exposure rates, r	
			Daily r/20 hr day	Cumulative total r
2001	<u>Acer rubrum</u>	5.1	900	122,272
2002-3 *	<u>Acer rubrum</u>	7.8	580	78,797
3009	<u>Acer rubrum</u>	8.8	490	66,570
3064	<u>Acer rubrum</u>	22.4	84	11,412
3194	<u>Acer rubrum</u>	44.1	14.4	1,956

Location and radiation data for the Parmelia sulcata thalli studied at Site 1 of the Enterprise Radiation Forest. The lichens studied were within 30 cm of the BBI mark and in direct line with the 137Cs source.

Tree Number	Tree Species	Distance from Source (meters)	Exposure rates (Roentgens) Daily R/20 hr. day	Cumulative Total R	Number of <u>P. sulcata</u> Thalli Studied
2001	<u>Acer rubrum</u>	5.1	900	122,272	5
2002	<u>Acer rubrum</u>	7.8	580	78,797	10
2003	<u>Acer rubrum</u>	7.7	590	80,156	7
3008-9	<u>Acer rubrum</u>	8.8	490	66,570	13
3040-41	<u>Acer saccharum</u>	11.8	310	42,116	11
3064	<u>Acer rubrum</u>	22.4	84	11,412	3
3164	<u>Fraxinus niger</u>	33.3	30.6	4,157	4
3176	<u>Acer rubrum</u>	39.6	19.0	2,581	4
3194	<u>Acer rubrum</u>	44.1	14.4	1,956	4

Figure 11. Parmelia sulcata and P. subaurifera site data for the Enterprise Radiation Forest.

Control Data for *Parmelia sulcata*.

<u>Tree Number</u>	<u>Average Radial Growth Increase, in Millimeters</u>		
	April-April 1972 1973	April-Nov. 1973 1973	Nov.-Nov. 1973 1974
2001	0.76	-1.72	-0.98
2002	1.22	0.34	0.53
2003	1.10	0.33	0.38
3008-9	1.37	0.70	0.94
3040-41	1.32	0.97	1.28
3064	2.20	1.00	1.40
3164	1.15	0.45	0.85
3176	1.04	0.94	0.92
3194	1.20	1.75	1.10
Controls	1.23	0.73	1.23

Increase in average radial growth during the radiation period (April, 1972 - April, 1973), the period following radiation in which damage was manifested (April, 1973 - November, 1973), and the following year (November, 1973 - November, 1974).

Figure 12. Growth data for *Parmelia sulcata* and *P. subaurifera* prior to, during and following gamma radiation in the Enterprise Radiation Forest. Because many *P. sulcata* thalli were studied data for this species is averaged, while for *P. subaurifera*, only a few thalli were followed, so individual thallus data is given.

tree No.	Radial growth increase, mm		
	Apr. 1972- Apr. 1973	Apr. 1973- Nov. 1973	Nov. 1973- Nov. 1974
2001	0.6	-1.0	-0.1
	0.5	-0.5	-0.3
	1.2	-3.4	-1.1
	0.2	-0.5	-1.2
	-0.1*	1.1*	-1.1
	0.5	-3.1	-4.2
2002-3	0.5	-0.2	0.1**
	-1.7	2.6	-9.5
	0.0	-0.5	-0.6**
	1.5	0.6	-0.8**
3009	0.6	0.1	0.2
	0.2	0.2	0.2
3054	-0.3	2.3	2.0
	1.1	0.5	1.5
	0.8	0.3	1.0
	0.1	0.3	-0.1
3194	0.8	0.4	-1.5
	0.7	1.0	0.1
	0.4	1.0	1.5
	0.7	0.9	0.5
control	2.3	0.6	-2.7
	1.2	-	1.4
	1.9	-	2.0
	1.0	0.7	-0.1
	1.1	-0.7	-1.5
	0.8	-	0.8
	0.9	0.5	-2.5
	0.2	0.2	0.2

Figure 12. Cont. Growth data for Parmelia subaurifera.

* This thallus was partially overgrown by Parmelia sulcata, which subsequently died.

Figure 13. Outline drawing of a tree 2002 P. subaurifera thallus with circular thalline outgrowths (arrows). The scale line equals 1 cm.

LONG-TERM CLADONIA STUDY:

The Cladonia and Parmelia studies both indicated that lichens would physically manifest radiation damage only after being in a suitable, prolonged growth period. However, it may not be the growth period which induced the physical damage manifestation, but simply the time period. It may be that lichens must have a minimal time before the damage becomes evident and its (the damage) appearance was just coincidental with the growth period.

To determine if time or growth period was critical a long term experiment was designed. For this experiment large mats of Cladonia mitis were collected. One half of each mat was irradiated to 1000 krad. Then the irradiated and non-irradiated material was divided in half with one bunch of non-irradiated and one irradiated placed in a growth chamber and the remainder placed back into its natural habitat. The growth chamber was set to simulate summer conditions with 16 hours of light at 72° F and 8 hours of dark at 65° F. The growth chamber lichens were watered periodically with distilled water. At monthly intervals lichens from the growth chamber and the field were brought into the laboratory and examined for physical changes, CO₂ uptake, O₂ evolution, tensile strength, usnic acid content, and the condition of the algal component.

From this experiment I hoped to determine if time or the growth period was most important. If the lichens only needed to have a minimal time period before physical damage could be seen then both the irradiated lichens in the growth chamber and the field would exhibit damage. But if a prolonged growth period were necessary, then only the irradiated lichens in the field would manifest damage. Growth chamber lichens, not having the opportunity to have the growth period (spring), should be much the same as the non-irradiated lichens or at least the same as the non-irradiated ones in the growth chamber.

Lichens were collected and irradiated in the fall, placed in the growth chamber or field and examined monthly through June. The experiment was repeated twice. The collection and analysis of data has not yet been completed, but enough data is available to reach a conclusion.

The field irradiated lichens first showed physical effects of the radiation during May (late spring). These lichens lost their yellow-green color, becoming brownish-gray, and appeared to be crushed. Neither the field control nor the growth chamber lichens became flattened or brownish-gray. The growth chamber lichens, controls and irradiated ones, became lighter green, but did not evidence any other physical difference when compared to the field control.

An internal examination of these same lichens did show some differences. In the field irradiated material there was some browning and coalescing of hyphae and many of the algal cells had died. There was no coalescing or browning of hyphae in the growth chamber irradiated material, but many of the algal cells were devoid of chloroplasts. The non-irradiated growth chamber lichen also exhibited the decrease in algal cells. Apparently the loss of algae was due to the growth chamber conditions. Chloroplasts in the field irradiated material seems to be in various stages of disintegration, while those in the growth chamber simply seemed to be bleached.

The usnic acid analysis was inconclusive because the amount of usnic acid in the variously treated lichens was about the same. There were no significant differences in usnic acid levels.

The CO₂ uptake study did show differences (Figure 14). The irradiated lichens, field and growth chamber, had the most depressed CO₂ uptake. The control growth chamber lichens also had a decreasing CO₂ uptake rate; this decrease was probably due to the reduced number of algal cells.

Figure 14. Labeled CO₂ uptake by irradiated (1000 krad) and non-irradiated Cladonia sylvatica podetia. Growth chamber lichens were kept at summer conditions - 75° F day, 60° F night, 16 hr. light, 8 hr. dark.

□ = non-irradiated, field grown

■ = irradiated, field grown

○ = non-irradiated, growth chamber grown

● = irradiated, growth chamber grown

The O_2 uptake varied considerably over the experimental period, but by June all of the lichens, irradiated and control, were at the same level (Figure 15). Apparently, gamma radiation had very little effect on the O_2 uptake mechanism of the lichens.

Tensile strength measurements were done with a specially designed apparatus (Figure 16) (Erbisch, et al., 1977). Variations occurred in tensile strength, but field grown irradiated lichens consistently had lower tensile strength than did any of the other lichens (Figure 17). The data is somewhat misleading in that field irradiated lichens had a much lower tensile strength than recorded. The most fragile of the podetia were not measured because they broke while being prepared for measurement. Refinements in the preparation techniques should alleviate this problem.

The information, while not yet complete, indicates that the extended growth period hypothesis is applicable for lichen radiation damage manifestation. The physical appearance, algal cell structure, and tensile strength measurements supports the hypothesis.

CONCLUSION:

Lichens are not as resistant to gamma radiation as was earlier believed. Lichens as long as they are in dormant, summer, state appear to be highly resistant to gamma radiation. However, damage is quickly manifested as the irradiated lichens pass through an extended suitable, growth period. Evidence for the need of an extended growth period for damage manifestation was obtained from:

1. Chronically irradiated Cladonia podetia,
2. chronically irradiated Parmelia thalli,
3. acutely irradiated Cladonia podetia which had been monitored for a long period of time - 1 year.

Figure 15. Oxygen uptake of irradiated (1000 krad) and non-irradiated Cladonia sylvatica podetia. Growth chamber lichens were kept at summer conditions - 75° F day, 60° F night, 16 hr. light, 8 hr. dark.

- = non-irradiated, field grown
- = irradiated, field grown
- = non-irradiated, growth chamber grown
- = irradiated, growth chamber grown

STRAIN INDICATOR CIRCUIT

Figure 16. Schematics of the tensile testing apparatus and indicator circuit.

Figure 17. Comparison of tensile strength of irradiated and non-irradiated field and growth chamber grown lichens. Tensile strengths (grams/mm²) of the irradiated and growth chamber controls were compared to the field grown control and results are given as % of control tensile strength.

- = control field grown lichens
- = irradiated field grown lichens
- = control growth chamber lichens
- = irradiated growth chamber lichens

Although the project is no longer supported by ERDA, work is continuing. In particular, cytological and tensile strength analyses are being carried out for another year with the 1000 krad irradiated lichens and with lichens which received doses of less than 1000 krad.

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