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Uptake, transport and seasonal recycling of ^{134}Cs applied experimentally to bracken (*Pteridium aquilinum* L. Kuhn)

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Abstract

Solutions containing ^{134}Cs were applied to cultivated clones of bracken. The radionuclide was taken up by roots, rhizome tips and leaf tips, and its subsequent retention and transport was monitored over a year. ^{134}Cs was transported through growing plants, recycled down to rhizomes at the end of the growing season and concentrated in meristems and second generation leaves. Accidentally released ^{137}Cs may therefore be taken up and recycled to succeeding generations of leaves in this important component of extensive upland and heathland ecosystems. The long-lived nature of bracken means that ^{137}Cs could remain environmentally available for many years longer than its physical half-life (30.2 yrs) and concentration in meristems could potentially cause genetic and phenotypic change in clones.

Keywords: Radiocaesium; Uptake; Bracken; Recycling

1. Introduction

$^{137\&134}$Caesium (Cs) (released in the activity ratio 2:1) were the most potentially dangerous radionuclides released during the 1986 Chernobyl accident. In Britain ^{137}Cs (radioactive half-life 30.2 yr) and ^{134}Cs (radioactive half-life 2.1 yr) were found

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to be the main contaminants of vegetation one week after the accident (Howard & Livens, 1987). Predictions based on earlier studies suggested that radioactive caesium would rapidly become unavailable to plants (Russell, 1965) because caesium would bind to clay minerals, particularly illite (Cremers, Elsen, Depreter & Maes, 1988; Russell, 1963), in the soil. However, these predictions were based on mathematical models formulated from investigations pertaining to lowland agricultural systems where soils are predominantly mineral soils (Howard & Livens, 1987). The British areas most affected by the Chernobyl accident were permanent pastures on organic soils. Sandalls, Gaudern & Nason (1989) found that, for upland pastures, transfer factors (quantitative expressions of the amount of element transferred from soil to plant parts) for radiocaesium were two orders of magnitude higher on organic soils than mineral soils. The high organic to low clay content of these soils ensures that the $^{134\&137}$Cs remained mobile (Barber, 1964; Howard & Livens, 1987). This mobility maintains radiocaesium availability for transfer into plants.

Most models of radionuclide behaviour following the Chernobyl accident also neglected potential internal recycling of radionuclides within long-lived clonal plants which, in contrast to lowland annual agricultural crops, characterise the upland areas of Great Britain which received the most fallout. As the safety of some reactors still gives cause for concern, it is necessary to have a better understanding of the impacts of accidentally released radionuclides. The main aim of this investigation was to determine whether (1) Cs is taken up by long-lived clonal plants from substrates where it is freely available for uptake, (2) if so, by what route it enters the plants and (3) to what extent is it recycled internally or lost to the rhizosphere during subsequent growth.

Bracken was chosen as the experimental species because it is the dominant component of many British upland ecosystems. It occupies 2900–4200 km^2 of Great Britain (Barr et al., 1993) and is a clonal plant constructed of ramets, the production of which could theoretically perpetuate the clone indefinitely (Oinonen, 1967). Ramets, rather than clones, may live indefinitely. Calculations derived from extensive plants in Britian indicate that clones do live for several hundred years (Sheffield, Wolf & Haufler, 1989), while larger individuals perhaps over 1000 yr old have been detected elsewhere in the world (Parks & Werth, 1993). This plant therefore offered a relevant long-lived and extensive system in which to study the long-term cycling and effects of an important radionuclide which has been accidentally released in the past.

2. Materials and methods

Dormant rhizome fragments of bracken (*Pteridium aquilinum* L. Kuhn) bearing leaf (frond) primordia, rhizome meristems, senescent fronds and roots measuring 20 to 25 cm in length were dug up from areas less than 10 m^2 in February and March. They were taken from 24 sites at least 500 m apart to ensure genetic diversity (Sheffield et al., 1989). The fragments were planted in large round pots 30 cm in diameter containing perlite (an inert derivative of volcanic lava that reduces the possibility of substrate–ion binding and standardises the rooting medium).

The pots were placed in trays so that the plants could be fed from below by capillary action. Pilot experiments indicated that 10% Hoagland's solution (Hoagland, 1920) was satisfactory for long-term cultivation and did not interfere with radionuclide uptake (Tyson, 1993). This solution is relatively low in nutrients, which prevents any interference in the uptake of caesium by potassium.

The plants were initially cultivated in a greenhouse at 18°C in 12 h of artificial light per day. When the plants began to produce fronds they were hardened-off in a cold house, before being moved outside after late frosts in late May. The plants were grown outside under a polythene tunnel to subject the bracken to seasonality and also prevent the interception of rain (that would otherwise have led to an unwanted input of ions and flooding). The young bracken was transplanted into containers measuring 74 cm × 33 cm × 20 cm (0.24 m^2) which were sunk in troughs in the ground lined with polythene to prevent contamination from the experiment to the soil and vice versa, leakage of nutrients, and frost damage in cold weather. The plants were left to establish until the middle of the growing season (July) of the next year before the first experiments were performed. The plants developed very similarly to a pioneer stand of an immature clone in the field (Tyson, 1993) in that by the end of the experiments all plants had mature fronds, some plants also had immature or sporulating fronds, and all bore extended rhizome tips and good root growth.

2.1. Experimental procedure

Three replicates of each of the following treatments were set up randomly within a plot of 45 plants: radionuclide application to either root, rhizome tip or frond tip. Sufficient plants were treated to allow for five harvests. The experiment was arranged using a random block design. Each plant used for root or rhizome application of radionuclides was carefully excavated to expose either roots or rhizome tips. A plastic centrifuge tube was cut down to fit over the organ of application. Fronds from the centre of the plant were chosen (to allow for the possibility of both proximal and distal translocation following uptake). 10% Hoagland's solution was then added to the tube and plasticine was used to seal the tube. The perlite was then replaced and the plants were left overnight.

The next day a cannula and syringe were used to remove the nutrient solution from the tubes. 37,000Bq ^{134}Cs in 7 ml distilled water was then added to cover a single root, rhizome tip or frond tip.

The radionuclide solution was left in contact with each plant organ for 24 h, then removed, and distilled water was pipetted into the tubes and left for 15 min to remove radionuclide from the apoplast. This was then repeated to remove any residues, and excavated plant organs reburied in perlite.

2.2. Sampling times

After radionuclide application there were five sampling times: (1) one month later (August) when fronds were still expanding; (2) three months later (October) when fronds were senescing; (3) six months later (January) during frond senescence; (4) nine

months later (April) during the emergence of second generation fronds; (5) one year later (July) during expansion of the second generation fronds.

The plants were stored at 4°C until ready for processing which began immediately and carried on for a week after sampling. Component parts were separated as follows: (1) "old roots" — roots growing from the original rhizome; (2) "old rhizome" — the original rhizome; (3) "roots" — roots growing out of newly-grown rhizome; (4) "rhizome" — new rhizome generated from the original rhizome; (5) "meristem" — buds on rhizomes that were not turning upwards; (6) "frond primordium" — buds on rhizomes that were turning upwards; (7) "crozier" — fronds before they unfurled; (8) "young frond" — fronds between the stage 7 and a fully unfurled frond; (9) "frond" — green fully-unfurled fronds; (10) "senescent frond" — yellow to brown fronds standing and intact; (11) "dead frond" — fronds as in 10, but limp, broken or not intact.

Each component was cut up into approximately 0.5 cm sized pieces. The geometry of the plant parts used for each sample was similar for each category of plant part used. The plant parts were dried in an oven at 105°C for one week, then weighed (recorded as biomass) and placed in low-density glass vials for activity measurement on a sodium iodide gamma spectrometer connected to a 2-channel CMTE Electronik GmbH data processor (MCA) which recorded the 605 and 796 keV gamma energy peaks for ^{134}Cs. As perlite proved difficult to separate completely from the roots, a correction factor was calculated for each plant by removing the perlite carefully from one sample of root. The perlite and root tissue were then weighed separately and the percentage of the weight made up by perlite for the samples used was then calculated (typically this was around 30% of the total weight). This factor was then used to work out the approximate weight of roots alone. An empty vial was used to monitor the background for three days. Cleaned plant parts from unlabelled material were used to determine any difference in the background readings of the empty vessel and of the different plant parts.

2.3. Statistical analysis

The data were recorded as counts per second of radioactivity emitted by the material after background subtraction. Readings were recorded as significantly greater than background at the 0.05 level using a one-tailed t-test. Standard statistical methods for ascertaining errors in radioactivity measurement were used (N.C.R.P., 1978). Calculations were made to adjust the data for radioactive decay.

The patterns of biomass allocation to different plant parts and overall dry weights were broadly similar between experiments; total biomass of plants approximately trebled during the experimental period. The results for ^{134}Cs are therefore expressed as mean amounts per plant part sampled, rather than as concentrations and each set of ^{134}Cs data gives the % dry weight of the plant part concerned (see Table 1a).

A non-parametric method, the Mann–Whitney U test, was used to compare differences between data sets. With this test a fixed value of $p = 0.081$ ($n = 3$) indicated non-overlapping data with large differences between their median values (Minitab, 1989).

Table 1
(a) Mean total dry weight (g) of plant taken from bracken sampled several times after application of radionuclide to roots

Plant part	Month and year									
	Aug 1990	s.e.	Oct 1990	s.e	Jan 1991	s.e.	April 1991	s.e	July 1991	s.e.
Dead fronds	—		—		0.59 (0.66)	1.13	0.54	0.12	3.03 (1.47)	0.88
Senescent fronds	3.92 (6.65)	0.67	5.61 (8.16)	2.25	21.53 (22.51)	5.71	18.34 (25.35)	2.99	15.74 (7.28)	4.51
Fronds	13.64 (23.37)	0.34	10.19 (16.07)	1.07	—		—		57.25 (30.11)	7.80
Young fronds and croziers	0.51 (0.85)	0.31	0.37 (0.56)	0.11	0.86 (1.07)	0.06	0.4 (0.53)	0.10	0.47 (0.25)	0.14
Frond primordia	0.39 (0.68)	0.06	0.37 (0.56)	0.11	0.88 (0.99)	0.15	0.77 (1.05)	0.07	1.95 (0.95)	0.53
Meristems	0.83 (1.41)	0.16	0.97 (1.48)	0.27	0.88 (0.99)	0.15	0.77 (1.05)	0.07	1.95 (0.95)	0.53
Rhizome	32.51	1.04	38.69 (60.22)	2.63	51.8 (58.17)	7.25	36.56 (50.00)	1.60	90.04 (42.15)	29.97
Roots	6.57 (11.25)	0.18	8.83 (13.35)	2.13	15.28 (16.61)	3.51	13.27 (17.66)	3.98	33.37 (17.79)	7.21
Total	58.37	1.67	64.79	5.41	900.94	16.28	73.45	4.83	201.20	46.21

(b) Mean amounts of ^{134}Cs (c.p.s.) found in plant parts of bracken sampled several times after application of radionuclide to roots

Plant part	Months after sampling									
	One (Aug)	s.e	Three (Oct)	s.e.	Five (Jan)	s.e.	Nine (April)	s.e.	Twelve (July)	s.e.
Dead fronds	—		—		0.07	0.03	0.79	0.44	2.16	0.91
Senescent fronds	6.09	3.13	14.55	9.87	135.80	61.21	124.60	19.39	16.79	5.34
Fronds	116.10	36.69	117.20	18.69	—		—		74.39	9.06
Young fronds and croziers	11.53	7.02	0.68	0.56	—		17.35	0.65	—	
Frond primordia	10.53	4.64	2.63	1.26	4.51	1.64	2.47	0.70	0.41	0.14
Meristems	12.43	3.27	9.47	3.78	4.54	1.70	6.26	2.54	2.56	0.60
Rhizome	122.20	42.54	124.70	40.46	112.10	44.62	122.00	29.11	25.97	8.64
Roots	22.42	9.08	18.93	0.96	32.93	8.64	18.43	2.76	12.39	1.18
Total	301.30	75.50	288.60	56.45	289.90	115.60	291.90	47.11	134.70	21.01

("—" denotes organ absent; s.e. = standard error; $n = 3$; parentheses show the % d.wt. of each plant part at that time).

3. Results

3.1. Root application

Over the first month more radiocaesium had been transported to rhizome and fronds ($p = 0.081$) than to other organs (Table 1b, Fig. 1). As there was more than twice as much biomass of rhizome as there was of fronds ($p = 0.081$) (Table 1a) but the amount of ^{134}Cs in each was similar, the concentration of ^{134}Cs must have been lower in the rhizomes. Amounts of ^{134}Cs in young fronds and croziers, frond primordia and meristems were similar, at about one-tenth of those found in the rhizome (Fig. 1). The lowest amount of ^{134}Cs measured was in the senescent fronds. These fronds had much greater biomass ($p = 0.081$) than the young fronds, frond primordia and meristems so transport into them must have been minimal.

After a further 2 months there had been very little change in ^{134}Cs allocation or percentage concentration in most organs (Table 1b, Fig. 2) but when the fronds grown in summer had finally senesced 3 months later, there was far more ^{134}Cs in senescent fronds than in any other organ (Table 1a). Increases in number of frond primordia and roots at this time were matched by increases in radionuclide allocation to these organs.

After 9 months, relatively large amounts of radionuclide remained in senescent fronds and rhizomes (Table 1a). However, whereas the biomass of the senescent fronds

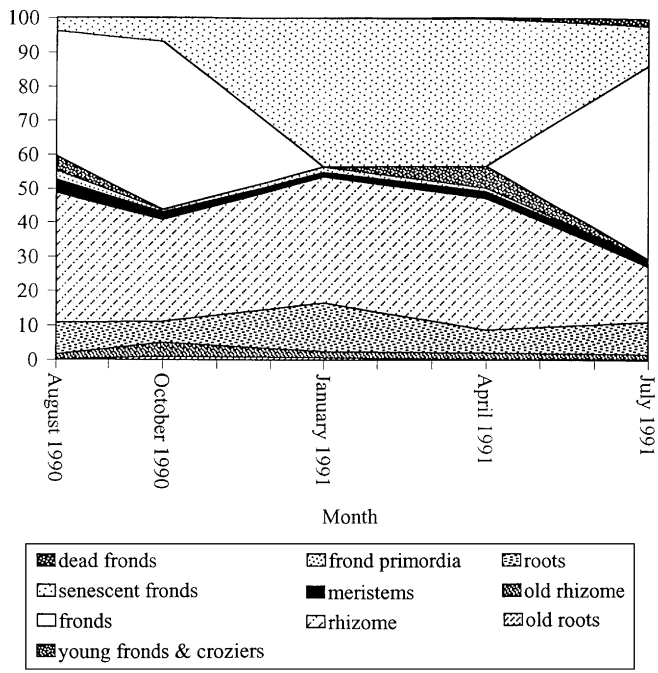

Fig. 1. Relationship between relative content (%) of ^{134}Cs in different organs and after different times in cultivated bracken plants following application of radionuclide to roots.

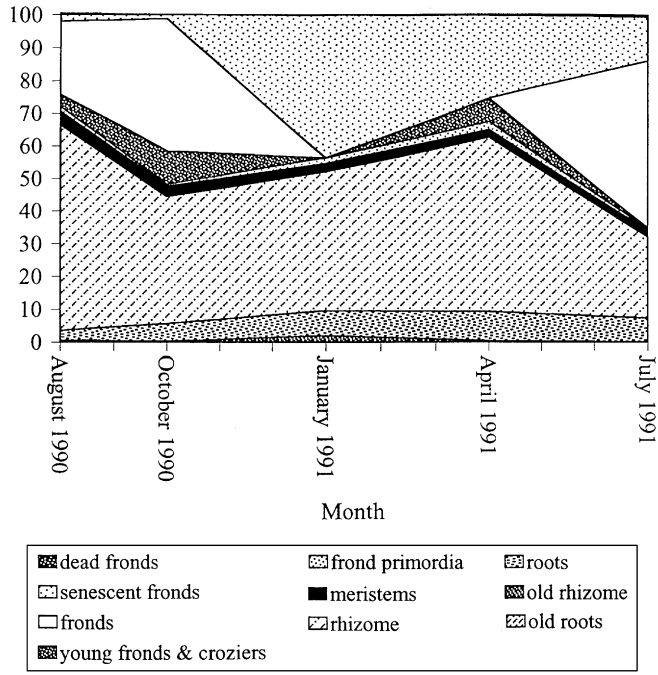

Fig. 2. Relationship between relative content (%) of ^{134}Cs in different organs and and after different times in cultivated bracken plants following application of radionuclide to rhizomes.

had remained steady, there had been a drop in the dry weight of rhizome tissue over the winter period (Table 1a). As young fronds and croziers developed, they contained more radiocaesium than would be expected from their dry weight, giving a relatively high radiocaesium concentration (Fig. 1). Biomass and radionuclide in frond primordia declined as they became young fronds and croziers, but biomass and radionuclide allocation did not change to any extent in meristematic tissue at this time. The amount of root tissue remained the same over the winter period, but contained half as much ^{134}Cs at the end than it had at the beginning of winter.

The patterns of allocation in the second summer were very different from those recorded a month after the experiment started. There was far more ^{134}Cs in newly developed fronds ($p = 0.081$) than any other organ. This coincided with changes in the senescent fronds which had remained since the spring but in the summer contained much lower radionuclide levels ($p = 0.081$). There was more radionuclide in the fronds and senescent fronds than in the first summer (Fig. 1) and less in the rhizome at this point than at any other time sampled.

3.2. Rhizome application

The overall amounts of ^{134}Cs in these plants were higher than those of the root-applied plants (Table 2). Relative radiocaesium allocation was broadly similar

Table 2
Mean amounts of ^{134}Cs (c.p.s.) found in organs of bracken sampled several times after application of radionuclide to rhizome

Plant part	Month and year									
	Aug 1990	s.e.	Oct 1990	s.e.	Jan 1991	s.e.	April 1991	s.e.	July 1991	s.e.
Dead fronds	—		—		0.84	0.45	1.81	0.54	5.96	3.26
Senescent Fronds	27.71	19.77	16.18	6.79	614.50	97.95	216.20	4.61	209.80	25.38
Fronds	295.20	30.76	1,008	288.00	—		—		839.10	159.30
Young fronds and croziers	46.46	13.71	23.18	18.84	—		71.87	21.71	—	
Frond primordia	18.30	7.66	9.68	3.93	22.57	7.57	28.19	12.25	10.08	5.49
Meristems	51.42	3.05	62.17	11.19	39.19	10.64	26.62	9.19	32.60	2.55
Rhizome	849.60	110.50	744.10	146.20	643.80	102.00	558.90	149.50	396.70	63.65
Roots	37.96	17.89	90.35	4.27	106.60	18.05	92.36	27.90	114.50	2.97
Total	1.33	106.90	1.95	426.20	1.43	192.50	996.00	205.50	1.61	122.80

("—" denotes organ absent; s.e. = standard error; $n = 3$).

in the first month to that of the root-applied plants except that the rhizome had even greater amounts ($p = 0.081$) (about 60% concentration, Fig. 2), than the rest of the plant organs. Fronds and frond primordia contained relatively less ^{134}Cs than in the root-applied plants.

3.3. Frond application

Overall levels of ^{134}Cs in the plants receiving the radionuclide via fronds approached those of the rhizome-applied plants, and were higher than those in the root-applied plants (Table 3). ^{134}Cs allocation in the first month was very similar to that of the root-applied plants, except there was very little senescent frond material (Table 3, Fig. 3). The high levels of ^{134}Cs in the rhizome of the rhizome-applied plants were not found in these plants.

There had been a large allocation of ^{134}Cs to the fronds by the third month, similar in relative terms to the other two treatments, but again with little allocated to or concentrated in senescent fronds (Table 3, Fig. 3).

After 6 months (January), less ^{134}Cs had been translocated to senescent fronds and more to frond primordia and meristems than in the other treatments (Table 3) but patterns of radiocaesium for both spring (April) and summer (August) were broadly similar to the other two treatments (Table 3, Fig. 3).

The experimental period showed a peak of radionuclide allocation to the rhizome in winter. This did not occur in the other treatments, in which there was either a gradual decline (rhizome application) or a sudden decline (root application; Table 3).

4. Discussion

Three particularly important conclusions can be drawn from this investigation. Firstly, bracken takes up, retains and recycles radiocaesium. Secondly, not only the fronds and roots but also the rhizome tip of bracken can act as a site of uptake of solutes. Thirdly, ^{134}Cs is transported to and concentrated in developing organs. No attempt was made to quantify net uptake of radionuclide as the investigation was conducted under conditions very unlike those of natural ecosystems, and other authors have published data relating to transfer factors into bracken. Lux et al. (1995), found low transfer factors of ^{137}Cs to bracken from soil in a forest near Chernobyl. The authors suggested that bracken was deriving nutrients primarily from the deep mineral horizon, where ^{137}Cs would have been locked up in the clay lattice. Transfer factors for the fern *Athyrium filix femina* rooted in the organic horizon of neighbouring sites were far higher. It therefore seems likely that net uptake into bracken would be higher in the British soils most affected by the Chernobyl accident, which have organic horizons far deeper than those studied by Lux, Kammerer, Rühm and Writh (1995) and in which caesium remains mobile and available for uptake (as in our experimental design).

Table 3
Mean amounts of ^{134}Cs (c.p.s.) found in organs of bracken sampled several times after application of radionuclide to fronds

Plant part	Month and year									
	Aug 1990	s.e.	Oct 1990	s.e.	Jan 1991	s.e.	April 1991	s.e.	July 1991	s.e.
Dead fronds	—		—		—		5.07	2.38	1.77	1.44
Senescent fronds	2.57	1.31	4.15	2.84	485.10	59.21	562.90	239.60	237.30	41.47
Fronds	281.50	65.78	1,184	398.60	—		—		445.20	156.30
Young fronds and croziers	47.34	30.69	12.93	8.43	—		66.70	22.46	—	
Frond primordia	9.25	2.88	17.47	5.28	74.40	23.36	15.89	9.53	6.10	2.97
Meristems	37.84	12.86	52.78	14.98	65.24	10.90	11.90	4.02	17.31	5.25
Rhizome	306.90	116.20	635.50	229.80	904.50	184.90	456.60	191.60	184.50	28.20
Roots	27.54	11.50	70.02	24.25	149.40	18.64	71.45	24.93	74.34	21.03
Total	712.9	197.5	1.98	652.6	1.68	275.1	1.19	41.65	966.5	246.1

("—" denotes organ absent; s.e. = standard error; $n = 3$).

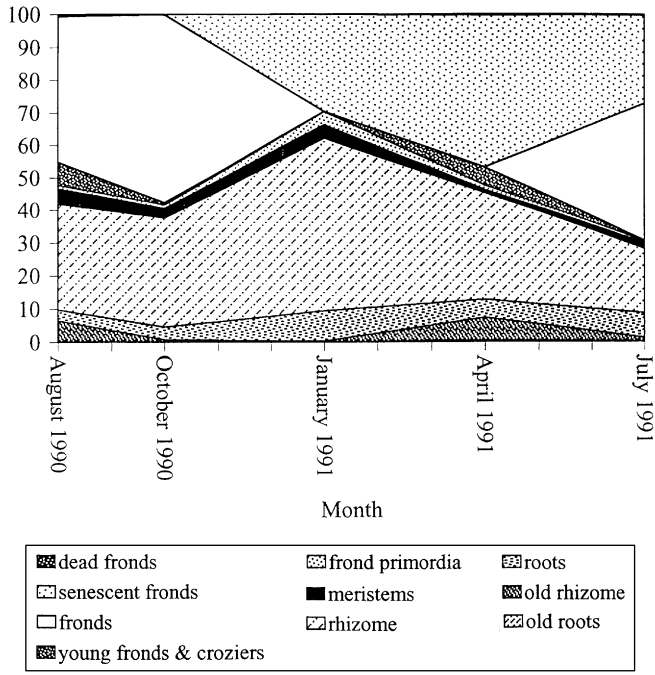

Fig. 3. Relationship between relative content (%) of ^{134}Cs in different organs and after different times in cultivated bracken plants following application of radionuclide to fronds.

Despite differences in detail of the allocation of the radionuclide between application organs, the three treatment methods yielded plants with almost equal concentrations of ^{134}Cs in the rhizomes and fronds in summer, which re-translocated radionuclide into second generation fronds. The initial accumulation in organs of the same type as those which received application probably related to the initial retention of radionuclide by the organs exposed to radionuclide as found, for example, in the retention of ^{32}P by roots of other clonal pteridophytes (Headley, Callaghan & Lee, 1988).

A similar distribution in dry weight allocation for the senescent fronds of all treatments was not matched by their amounts of ^{134}Cs. The three modes of application differed in the details of ^{134}Cs distribution, but the presence of ^{134}Cs in the senescent fronds of the root application and rhizome application plants provides strong evidence that these fronds have functional vascular tissue. An indication of functional vascular bundles in senescent fronds of bracken was also found by Callaghan, Scott and Whittaker (1981). Ferguson and Armitage (1944) measured higher amounts of potassium in the rachis of bracken in November than in the lamina. This may have been because of the translocation of potassium down the rachis at this time. This supports the theory that translocation and/or transport of ions can take place

from senescent fronds to the rhizome in autumn for storage and recycling the next year (Hunter, 1953). The overall decrease in ^{134}Cs concentrations in the rhizome in the final summer probably reflects translocation to the fronds.

The peak in the concentration of ^{134}Cs in the senescent fronds in spring in the frond-applied plants may have resulted from contact of some senescent fronds with the fronds to which the radionuclide was applied (which had also become senescent) before harvesting. Rain in the natural environment could lead to external cycling, where radiocaesium is leached from the fronds and senescent fronds to the ground and then taken up after some time by the roots or by other plants. Examination of nutrient cycling by the measurement of throughfall, stemflow, etc. in bracken ecosystems has indicated external as well as internal cycling of nutrients (e.g. Sponder, 1979; Williams Kent & Ternan, 1987).

An important aspect of the recycling of radiocaesium to meristematic tissues is the damage potentially suffered by these tissues due to β-particle and γ emissions (Miller, 1968; Whicker & Fraley, 1974). As the half-life of ^{137}Cs is approximately 30 yr, the recycling of ^{137}Cs could lead to chronic irradiation of the meristems and possible DNA damage. Boyle and Boyle (1968) reported that heterozygosity confers radioresistance and bracken is an outcrossing species, so has high levels of heterozygosity (Wolf, Sheffield & Haufler, 1991). Jubrael (1987) had difficulty in isolating bracken mutants from spores treated with mutagens, which perhaps indicates the existence of efficient repair systems, again possibly conferring radioresistance.

However, Zavitkovski (1977) selected bracken as being one of the most radiosensitive species in the ground flora of an irradiated aspen, maple, birch forest. As bracken growth originates from a single meristematic cell, whereas that of the other species studied originates from multicellular meristems, bracken may be relatively sensitive to radiation. The long-lived nature of bracken means that ^{137}Cs could remain environmentally available for many years longer than its physical half-life (30.2 yrs) and concentration in meristems could potentially cause genetic and phenotypic change in clones. Also, as bracken is productive and widely distributed, such damage could be extensive in space as well as time.

The results of this study emphasise the importance of bracken in the long-term bioavailability of radiocaesium accidentally released in the environment in the past and perhaps, potentially, in the future also. The recycling of radiocaesium would maintain its presence in the plant, and therefore within surface layers of the soil, for far longer than if it had been taken up by an annual plant, which would transfer radiocaesium to the soil relatively quickly. The constant recycling and slow release from the dead fronds of bracken would ensure a constant danger in the bracken ecosystem for many years.

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