Relationship between diet and liver carcinomas in roe deer in Kielder Forest and Galloway Forest

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The winter diets of roe deer culled from Kielder Forest, in north-east England, where the incidence of liver carcinomas in roe deer is high, and Galloway Forest, in south-west Scotland, where the incidence of liver carcinomas is low, were compared by microhistological analysis of faeces. Both areas are planted with spruce forests but the diets of the deer from Kielder Forest were less varied and contained more spruce and heather than the diets of the deer from Galloway Forest.

Veterinary Record (2004)
155, 197-200

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IN a survey carried out between January 1992 and March 1994, hepatocellular tumours were found in 40 of 21,894 roe deer (*Capreolus capreolus*) culled in Britain (Munro 1992, Munro and Youngson 1996). The geographical distribution and incidence of the tumours were uneven, and 18 of them were found in the 3184 deer culled in Kielder Forest in north-east England, an area planted with conifers. In contrast, in Galloway Forest, in south-west Scotland and, like Kielder Forest, planted with conifers, only three of 4100 deer culled had a tumour. The difference between the incidence of carcinomas in these apparently similar regions is highly significant (P<0·001). Both areas are similar in terms of elevation above sea level; although afforestation has not generally extended above 400 m, Kielder Forest covers part of the Cheviot Hills, which range up to 820 m, and Merrick in Galloway Forest ranges up to 840 m. Both areas are exposed to airborne industrial pollution from the south-east and south (Battarbee and others 1989, Fowler and others 1989).

Among possible causes, it was considered that naturally occurring oncogens in the diets of the deer might be involved. Spruce (*Picea* species) buds and needles make up an important part of the diet of roe deer in Kielder Forest (De Jong and others 1995), and spruce tissues contain high (although variable) levels of terpenes, which are known to interfere with rumen microbial activity (Oh and others 1967, Duncan and others 1994), and some of which are carcinogenic (Van Genderen and others 1996).

This paper describes an investigation of the relationship between the incidence of liver carcinomas and the proportion of spruce in the diet of the deer in the two forests by determining the botanical composition of their winter diets by microhistological faecal analysis.

MATERIALS AND METHODS

Areas of study

Kielder Forest and Galloway Forest are in areas in the border regions of northern England and south-west Scotland that were planted with non-native conifers during the 19th and early 20th centuries. After afforestation, the populations of roe deer expanded (Ritchie 1920), probably aided by cover and by the food that the young plantations provided (Prior 1968, Rowe 1982, Gill 1994). For food plants, the deer also depend on open spaces, such as roadsides, rides and restocking areas (De Jong and others 1995). The carcase weights and female fertility of the deer in these forests appear to be typical for British populations of roe deer (Ratcliffe and Mayle 1992, McIntosh and others 1995).

Soils and botanical composition

The soils in Kielder Forest above 300 m (half the area) are characterised by deep peat-forming blanket mires and occasional outcrops of shale and sandstone. Below 300 m, boulder clays predominate which produce intractable gleyed soils, often with a peaty surface (Wallace and Good 1995). Small areas of brown earth occur in the valley bottoms. Similar types of soil occur in Galloway Forest (Bown and Heslop 1979).

Kielder and Galloway Forests were planted on soils previously in use as rough pasture. The larger part of both forests was planted around or after 1950, predominantly with Sitka spruce (*Picea sitchensis*) and Norway spruce (*Picea abies*). In open spaces and restocked areas the original plant communities remain or have reappeared, and second-rotation communities contain a larger proportion of dry heath (Wallace and Good 1995). Apart from the planted trees, other plant species characteristic of both areas are heathers (*Calluna vulgaris* and *Erica tetralix*), bilberry (*Vaccinium myrtillus*), heath bedstraw (*Galium saxatile*), purple moor-grass (*Molinia caerulea*), fine bent (*Agrostis tenuis*), hare's-tail (*Eriophorum vaginatum*), sedges (*Carex* species) and rushes (*Juncus* species).

Analysis of diet

Over 70 species of food plants occurring in the two regions were sampled for reference slides. Pieces of relevant parts were cleaned in household bleach overnight, washed in water, and fragments of epidermis were then stripped off and mounted in glycerol. Photomicrographs of these slides were used to identify the fragments of cuticle observed in samples of roe deer faeces.

From October 1993 to March 1994, five faecal pellets were taken from the rectum of each culled roe deer and preserved by freezing at –20°C (Table 1). The Kielder Forest samples were collected from both tumour-affected and apparently healthy deer shot in various parts of the forest where liver carcinomas were known to occur. The Galloway Forest samples were collected from roe deer culled in several locations in the Galloway Forest Park; no liver carcinomas were found in these deer. The faecal samples were generally mixed on a monthly basis but samples from healthy and diseased deer were kept apart. The mixed pellet samples were heated under pressure to 115°C in water for approximately two hours and left to soak overnight. A 5 g subsample was washed in a Waring blender and strained over a plankton sieve, then stored in 70 per cent ethanol. The samples were examined by light microscopy, and at least 100 fragments of cuticle or epidermis in each sample were identified by comparison with photomicrographs of epidermal material and measured by using a grid of 0·01 mm^2 squares in the microscope eyepiece (De Jong and others 1995). The abundance of each species was calculated as a percentage of the total area of the fragments measured (Stewart 1967, Sparks and Malechek 1968, Putman 1984, Cid and Brizuela 1990, Alipayo and others 1992, Homolka and Heroldová 1992).

TABLE 1: Sampling dates and numbers of deer sampled for faecal analysis in Kielder Forest and Galloway Forest

Site and date	Number of deer	Condition of deer
Kielder Forest		
29/11/93	1	Liver damaged
04/01/94	1	Diseased
24/01/94	1	Healthy from 'diseased' area
Winter 93/94	5	Presumed healthy
Galloway Forest		
14/10/93	1	Healthy
November 1993	15	Healthy
December 1993	4	Healthy
January 1994	5	Healthy
February 1994	8	Healthy
March 1994	2	Healthy

To test whether the diets differed significantly from each other, Kulczynski's similarity index (KSI) was used:

$$\text{KSI} = (\Sigma 2C/\Sigma[A+B]) \times 100$$

where C is the smaller percentage of a species or higher category occurring in two diets and A and B are the percentages of this species or category found in each of the diets (Cuartas and Garcia-Gonzalez 1992). The diets were compared first at the species level and after all the species had been grouped into six categories. The KSI of a series of 20 'duplos' (subsamples of the same mixed sample) was assessed and mean values and standard deviations (sd) were calculated. A pair of diets can be considered similar when the KSI is within the sd of the duplo range (H. van der Voet, personal communication). At the species level this occurred when KSI was at least 61; when the species were grouped into six categories it occurred when KSI was at least 77. An analysis of the series of duplos indicated that a diet difference of 1·73 x the sd of the duplo average was significant (P=0·05), 1·73 being the critical *t* value of a one-sided *t* test. At the species level there was a significant difference between two diets when KSI was no more than 55; when the species were grouped into six categories there was a significant difference between two diets when KSI was no more than 72.

TABLE 2: Species composition of fragments of plant epidermis identified in faecal samples from roe deer in Kielder Forest and Galloway Forest, in winter 1993/94. The values are the percentage weighted average of the total epidermis surface area sampled

	Kielder Forest	Galloway Forest
Pteridophyta	1	3
Picea species	18	4
Calluna vulgaris	53	33
Undetermined Compositae	–	<1
Empetrum nigrum	–	1
Erica tetralix	<1	<1
Fraxinus excelsior	–	<1
Galium aparine	–	<1
Galium saxatile	9	10
Undetermined Leguminosae	<1	2
Lonicera periclymenum	–	1
Myrica gale	–	1
Plantago species	<1	–
Quercus species	–	<1
Rhamnus species	–	<1
Rosa species	–	<1
Rubus species	–	2
Rumex acetosella	–	<1
Stellaria species	1	<1
Ulex species	–	<1
Vaccinium species	1	8
Undetermined dicotyledons	1	2
Buds and twigs of dicotyledons	<1	4
Undetermined grasses	<1	6
Agrostis species	1	–
Alopecurus pratensis	–	<1
Anthoxanthum odoratum	–	<1
Dactylis glomerata	–	<1
Deschampsia cespitosa	<1	2
Deschampsia flexuosa	2	5
Elymus repens	–	1
Festuca species	<1	2
Holcus species	2	2
Poa species	–	1
Undetermined monocotyledons	1	2
Carex species	2	1
Eriophorum vaginatum	2	1
Juncus species	3	1
Bryophyta	1	5
Fungus/lichen	–	<1
Mast	–	<1

Kulczynski's similarity index = 59; diet difference not significant

RESULTS

The botanical composition of the mixed samples was assessed at taxon level (Table 2) and summarised at the level of six diet categories (Table 3). Because of the small sample sizes a weighted average was also calculated of all the autumn and winter diets from both sites (Fig 1).

In Galloway Forest the overall winter diet was more varied than at Kielder (Table 2) and contained at least 33 taxa compared with 19 at Kielder. There were also differences in the composition of the diet categories; at Kielder, at least seven dicotyledoneous species were found and at least five grass species, compared with 19 dicotyledenous species and nine grasses at Galloway Forest.

At the category level the weighted averages of the diets were significantly different (Table 3). Spruce constituted a significantly higher proportion of the winter diet at Kielder (18 per cent) than at Galloway Forest, where it contributed only 4 per cent (P=0·015). The diets of the diseased and healthy deer could not be compared statistically because of the small sample size, but the highest proportion of spruce (37 per cent) was found in the diet of a diseased deer.

In all the samples from both areas, dicotyledoneous plants constituted more than half the volume of the botanical content of the faeces. Heather (*C vulgaris*) was the most important food plant during winter; at Kielder it constituted 53 per cent of the average diet and at Galloway Forest 33 per cent, but the difference was not significant. *G saxatile* constituted 9 per cent of the diet at Kielder and 10 per cent at Galloway and was the second most important dicotyledoneous species eaten in both areas, closely followed by *Vaccinium* species, which constituted 8 per cent at Galloway Forest. An autumn sample from one animal in each area contained very little heather; at Kielder *G saxatile* and spruce predominated, at Galloway Forest bramble (*Rubus* species) leaves. At Galloway Forest the overall composition of the diet of a deer culled in October suggested that it had been feeding in a river valley rather than in a spruce forest.

Grasses were a more important component of the deer's diet at Galloway Forest (19 per cent) than at Kielder (5 per cent), and more species of grass were found in the Galloway diets than in the Kielder diets. At Kielder the proportion of grasses in the diet was slightly lower than that of other monocotyledoneous plants.

In both areas ferns and mosses were only minor components of the diet, and mast was virtually absent.

DISCUSSION

The hypothesis that there might be a relationship between the incidence of liver carcinomas and the proportion of spruce in the diets of the deer seems to be borne out by the results. The diets of the deer at Kielder contained much more spruce than

TABLE 3: Major diet categories of roe deer at Kielder Forest and Galloway Forest in autumn/winter 1993/94. The values are the percentages of total epidermis surface measured in faecal samples

Location	Percentage						
Kielder Forest		Nov	Jan	Jan	Winter		Weighted average
Diseased (d)/healthy (h)		d	d	h	h		
Number		1	1	1	5		8
Pteridophyta		0	3	0	0		1
Picea species		37	9	12	17		18
Calluna vulgaris		3	64	53	63		53
Other dicotyledons		48	4	7	7		12
Grasses		1	5	6	6		5
Other monocotyledons		10	14	21	4		9
Rest (not included in KSI)		0	1	1	2		1
Galloway Forest	Oct	Nov	Dec	Jan	Feb	Mar	
Number	1	15	4	5	8	2	35
Pteridophyta	2	4	7	2	0	0	3
Picea species	1	7	1	2	5	2	4
Calluna vulgaris	0	40	17	25	33	56	33
Other dicotyledons	57	24	50	32	27	23	30
Grasses	37	18	19	27	14	19	19
Other monocotyledons	0	4	2	3	13	0	5
Rest (not included in KSI)*	3	3	4	10	9	0	5

* Bryophyta, fungi, lichens
Kulczynski's similarity index (KSI) of weighted averages = 63
Diets differ significantly at category level

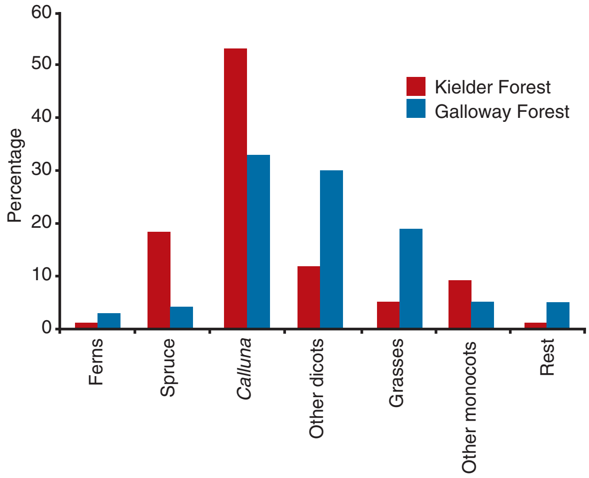

FIG 1: Percentage composition of the winter diets of roe deer in Kielder Forest and Galloway Forest

the diets of those at Galloway Forest. The diets of the deer at Kielder were also much less varied, and contained less grass and fewer dicotyledoneous species. Accepting the idea that spruce is not an optimal food plant for roe deer, it is possible to speculate why this might be so and why the deer in some regions of Kielder Forest eat so much of it.

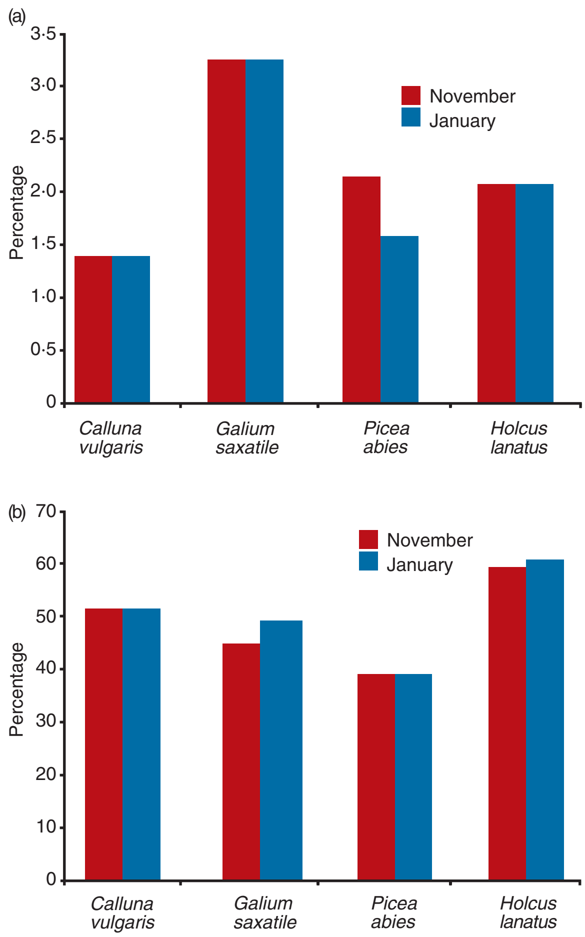

FIG 2: (a) Percentage of nitrogen and (b) percentage of cell walls in some important food plants for roe deer in winter in Kielder Forest and Galloway Forest

As concentrate selectors, roe deer need relatively high-quality food (Van Soest 1994). Indicators of high-quality food for concentrate selectors are the plants' content of nitrogen and cell walls. A low content of cell walls is important because concentrate selectors are less able to digest cell walls than other ungulates such as the intermediate feeders and grazers (Van Wieren 1996a, b), and they are more specialised in making use of the cell contents. Fig 2 shows the content of nitrogen and cell walls of some relevant food plants. Although the nitrogen content of *Picea* species is not very high, it is no lower than that of *C vulgaris*, a much preferred food plant, and their cell wall content is also low, so that from an energetic point of view spruce cannot be considered a low-quality food for roe deer. The reason why spruce may not be an optimal food plant for roe deer may be related to the high levels of other compounds (notably terpenes) in the cell contents (Oh and others 1967, Duncan and others 1994).

Roe deer in Kielder are apparently forced to forage extensively on spruce in winter. The diets at Kielder also contained a high proportion of heather, though not significantly higher than at Galloway Forest. Although heather contains no specific poisonous compounds, it is rich in tannins and other phenolic compounds that inhibit browsing and, to some degree, digestion (McArthur and others 1991, Van Genderen and others 1996). Spruce needles, buds and twig tips are rich in tannins and terpenes (Forrest 1975, 1980a, b, Von Rudloff 1977), and all these parts were present in the deers' faeces. As concentrate selectors, roe deer require a broad range of food plants to maintain a varied but limited consumption of secondary chemicals (Freeland 1991, McArthur and others 1991). It is possible that at Kielder the total load and lack of variety of secondary compounds may play a role in the ill health of the roe deer. In west and central Scotland, where the incidence of liver carcinoma was low (Munro and Youngson 1996), the winter diets of roe deer contained approximately 25 per cent conifers but were much more varied (Latham and others 1999).

The suggestion that spruce may not be a preferred food for roe deer for most of the year is reinforced by the results of a study in which diets from different parts of Kielder were compared (De Jong and others 1995). At Kielder, various habitats can be distinguished as being quite poor or very poor; the winter diets from the very poor area (Highfield, a restock area on deep peat) contained much more spruce (23 per cent) than a less poor area at Pundershaw, a restocked area on surface water gley, where the diets contained only 8 per cent spruce. Only in May, when quality is at its highest, was spruce a preferred food in both areas (39 per cent and 40 per cent). Furthermore, the little that was available of the more preferred species was very heavily browsed, and the spruce in the very poor area was more severely damaged by browsing.

The terpenes present in Sitka leaf oil are known (Von Rudloff 1964, 1977) and none of them is known to provoke cancer. However, another component of leaf oil, α-hexenal, is genotoxic and a possible cause of liver carcinomas (Eder and Schuler 2000); it is a non-specific secondary compound with fungicidal properties that is formed from linoleic acid when living green plant parts are ground in the presence of oxygen (Major and others 1963, Major and Thomas 1972). As this compound is synthesised whenever a herbivore chews fresh green plant parts, any herbivore must be able to cope with it to some degree. However, not all plant species form it in the same concentration, during the same season or at the same pH (Major and others 1972), and there are similar variations between individual trees. Sitka spruce and conifers in general form high concentrations of it, and small amounts have been isolated even from intact Sitka needles collected in winter (Von Rudloff 1975, 1977). Roe deer with home ranges in particularly poor parts of Kielder Forest may be forced to browse from particularly poisonous trees. No specific data on the hexenal content of heathers are available.

The incidence of liver carcinomas in roe deer appears to be associated with high levels of spruce in their diet; these high levels may be induced by the poor quality of the deer's habitat, which forces them to consume a suboptimal diet with high levels of secondary compounds, of which terpenes and α-hexenal probably form a substantial part.

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