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

C. B. de Jong, S. E. van Wieren, R. M. A. Gill, R. Munro

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.

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 3,184 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 4,100 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 communities that 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 communities characteristic of both areas are heathers (Calluna vulgaris and Erica tetralix), bilberry (Vaccinium myrtillus), heath bedstraw (Galium saxatile), 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 mm² 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 Malekech 1968, Putman 1984, Cid and Brizuela 1990, Alipayo and others 1992, Homolka and Heroldová 1992).

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), fine bent (Agrostis tenuis), hare’s-tail (Eriophorum vaginatum), sedges (Carex species) and rushes (Juncus species).
Species level this occurred when KSI was at least 61; when the range (H. van der Voet, personal communication). At the standard deviations (sd) were calculated. A pair of diets can be significant (P=0.05), 1.73 being the critical value of a one-sided 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.

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

$$KSI = \left( \frac{\sum C}{2(A+B)} \right) \times 100$$

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 García-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 duo 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 duo average was significant (P=0.05), 1.73 being the critical 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.

### 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 dicotyledoneous 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 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 Forest 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

<table>
<thead>
<tr>
<th>Location</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kielder Forest</td>
<td>Nov 15 Jan 4 Dec 5 Jan 8 Feb 2 Mar 35</td>
</tr>
<tr>
<td>Galloway Forest</td>
<td>Oct 1 Nov 4 Dec 5 Jan 8 Feb 2 Mar 35</td>
</tr>
<tr>
<td>Pteridophyta</td>
<td>2 4 7 2 0 0 0 3</td>
</tr>
<tr>
<td>Picea species</td>
<td>1 7 1 2 5 2 4 3</td>
</tr>
<tr>
<td>Calluna vulgaris</td>
<td>0 40 17 25 33 56 33</td>
</tr>
<tr>
<td>Other dicotyledons</td>
<td>57 24 50 32 27 23 30</td>
</tr>
<tr>
<td>Grasses</td>
<td>37 18 19 27 14 19 19</td>
</tr>
<tr>
<td>Other monocotyledons</td>
<td>0 4 2 3 13 0 5</td>
</tr>
<tr>
<td>Rest (not included in KSI)*</td>
<td>3 5 4 10 9 0 5</td>
</tr>
</tbody>
</table>

* 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

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 Calluna 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 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 1991), 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

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
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 promote 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.

References


