Effects of asulam and glyphosate on the leaves and rhizomes of *Pteridium esculentum*

N. D. Hallam
Botany Department, Monash University, Clayton, Victoria 3168
C. Herrmann
Botany Department, Monash University, Clayton, Victoria 3168
R. G. Richardson
Keith Turnbull Research Institute, Frankston, Victoria 3199

Summary

The first effect of the application of asulam and glyphosate to immature fronds of *Pteridium esculentum* is cytological damage observable after 3 days. The upper epidermis and hypodermis were the first tissues affected. Glyphosate had a more severe effect than asulam as collapsed leaf cells and necrotic tissue were observed 1 week earlier than in asulam-treated material. Asulam caused initial extensive chlorosis in immature fronds that regained their normal colour after 6 weeks. No cytological damage was evident in mature frond tissue treated with asulam. In the case of glyphosate-treated mature fronds, degeneration of chloroplasts occurred 6 weeks after treatment with the herbicide. The initial site of action of both herbicides at a subcellular level appears to be the chloroplast.

Even though ^{14}C-labelled herbicides could be located in the rhizome apex 2 weeks after foliar application, no cytological damage occurred until 6 weeks after glyphosate, and 8 weeks after asulam application. In the case of glyphosate-treated plants, the rhizome apex was heavily infected with microorganisms, suggesting that this herbicide damaged the tissue to the extent that it becomes vulnerable to microbial invasion. No dead tissue or microbial invasion appears to occur in the rhizomes of asulam-treated plants. Surface examination of fronds by scanning electron microscopy showed that the upper surface of the mature fronds was covered by a smooth cuticle, whereas the under surface was covered with hairs and farinaceous outgrowths. Only twisted hairs were evident on both the upper and lower surface of immature fronds.

Introduction

Pteridium esculentum (Forst. f.) Cockayne (F. Dennstaedtiaceae), commonly known as Austral Bracken, is a fern indigenous to south-eastern Australia. Two other species occur in Australia, *P. revolutum* (Bl.) Nakai and *P. semihastatum* (Wall. ex Agardh) Andrews, and are found in north-eastern Queensland (Clifford and Constantine 1980). The genus *Pteridium* has a world-wide distribution and many of its species are regarded as serious weeds of pasture land. For discussion on the controversial taxonomy of this plant see Tryon (1941), Copeland (1947), Page (1976) and Clifford and Constantine (1980).

The success of bracken as a weed, and its ability to resist control measures, are related to its morphology. The extensive rhizome system allows bracken to spread and infest areas independent of sexual reproduction. Prothalli and sporelings of *P. esculentum* are rarely found in the field in Australia.

The extent, longevity and large reserves of starch within the rhizomes enable continual regeneration of fronds even when bracken is persistently slashed or burnt. Additionally, the fronds, which can vary in height from 15–300 cm (O'Brien 1963), suppress the growth of pasture species and other plants through shading and litter production, and possess toxic compounds which make them relatively unpalatable to insects and stock. Methods of control that have been used successfully against bracken include husbandry techniques such as ploughing and stocking, and a variety of herbicide treatments. Recently, Holroyd *et al.* (1970) found asulam to be very effective against bracken, and it subsequently became the most widely used herbicide treatment for the control of bracken. More recently, glyphosate has been found to be effective against bracken (Scragg *et al.* 1974; Williams and Foley 1975).

Trials carried out on *Pteridium aquilinum* in the United Kingdom have shown that asulam, when applied as a foliar spray, is absorbed into the fronds and translocated to phloem sinks such as frond buds and rhizome apices. Translocation of the herbicide to the rhizome system occurs mainly from the mature fronds which are unaffected by asulam (Veerasekaran *et al.* 1977*b*). Immature fronds, which act as phloem sinks, exhibit chlorosis and necrosis as a result of the application and accumulation of the herbicide (Veerasekaran *et al.* 1977*b*). Asulam has been found to kill frond buds and rhizome apices and reduce the frond density of field bracken 1 year after treatment, in both Scotland (Veerasekaran *et al.* 1978) and Australia (Martin 1977), although the degree of damage was considerably less in the Australian trials. Veerasekaran *et al.* (1977*a*) found that asulam inhibited protein and RNA synthesis in frond buds, and it is believed that asulam, like other carbamate herbicides, is a mitotic poison. In support of this, Watts and Collin (1979) found that asulam inhibits cell division in cultures of celery tissue.

Glyphosate, also a phloem-mobile herbicide, was found to be effective against *P. aquilinum* (Williams and Foley 1975) in field trials, causing damage to the rhizomes and reducing their carbohydrate content. In an ultrastructural study on the effects of glyphosate on *Sinapis alba* seedlings by Uotila *et al.* (1980) it was found that starch grains disappeared from chloroplasts and membrane structures became disrupted. Work by Jaworski (1972) suggests that glyphosate inhibits the aromatic amino acid biosynthetic pathway by repressing chorismate mutase and/or prephenate dehydratase, thus preventing the formation of phenylalanine and tyrosine.

The objectives of this study were to (i) investigate the morphological effects of asulam and glyphosate on field-grown *Pteridium esculentum*, at both the macroscopic and microscopic level; (ii) investigate the surface structure of fronds by means of the scanning electron microscope in order to correlate the surface appearance with the retention, spread and penetration of herbicide spray drops (Cook *et al.* 1979); and (iii) locate the site of action of the herbicides by using carbon-14 labelled asulam and glyphosate.

Materials and methods

Field spraying

The bracken (*P. esculentum*) used in the study was field grown in the grounds of the Keith Turnbull Research Institute at Frankston, Victoria. The site had been slashed some months prior to the study, so that the bracken was an open stand (100 m^2) of both mature and immature fronds, with an average height of 40 cm.

Asulam, or 4-aminophenylsulphonylcarbamate, is available commercially as a solution of the sodium salt

(400 g asulam/l) under the trade name Asulox, and is manufactured by May and Baker Limited. Asulox was applied at a rate of 12 l ha^{-1} (equivalent to 4.8 kg ha^{-1} asulam, i.e. within the manufacturer's recommended range of 4–6 kg ha^{-1}) with diesel oil (20% v/v); Agral 60 was used to emulsify the final solution. An adjacent area (100 m^2) was sprayed with diesel oil (20% v/v) plus Agral 60 as a control plot.

Glyphosate, or N-(phosphonomethyl) glycine, is commercially available as the mono(isopropylamine) salt in a water-based solution (as 360 g glyphosate l^{-1}) under the trade name Round-Up (Monsanto). Round-Up was applied at the recommended rate of 9 l ha^{-1}, equivalent to 3.24 kg ha^{-1} of glyphosate. A spraying of water alone was used as a control spray for a similar sized plot as for the asulam spraying (above).

The herbicide solutions were applied in early autumn by a C.D.A. ultralow-volume sprayer at a rate of 200 ml per 100 m^2 (20 l ha^{-1}). A low volume method of application was chosen, as the retention of spray on bracken fronds increases with a reduction in spray volume (Catchpole and Hibbitt 1972).

Light microscopy

Samples of fronds and rhizome apices from the herbicide-treated plots and controls were collected at 3 days, 1, 2, 3, 4, 6, 8, 10 and 12 weeks after treatment and prepared for light microscopy.

Tissue was sampled randomly from control fronds and fronds with no necrosis, but selectively sampled from those with dead tissue. Samples were taken from the boundary area between dead and non-dead tissue in these fronds.

Rhizomes were excavated and apices of both long and short shoots were sampled. Only actively growing apices (white and covered with hairs) were selected, while small brown apices were regarded as dormant (Conway and Forrest 1961) and not sampled, since dormant apices have been found to be unaffected by asulam treatment (Veerasekaran *et al.* 1978; Martin 1977).

Pieces of frond pinnules (approx. 2 cm in length) were placed in fixative (5% glutaraldehyde in 0.025 M phosphate buffer) in the field, and in the laboratory ultimate segments were cut into 1-mm lengths (mature fronds) or 3 mm lengths (immature fronds). Excavated apices of rhizomes were taken back to the laboratory, where the upper 0.5 cm of the apices were cut longitudinally at right angles to the lateral line of the rhizome into 1-mm slices under fixative.

All tissues were fixed in 5% glutaraldehyde in buffer for 1 h, and after fixation the samples were rinsed four times in cold 0.05 M phosphate buffer over 1 h, post fixed in 1% OsO_4 in 0.025 M phosphate buffer for 1 h and rinsed in distilled water for 10 min before dehydration through an ethanol series. They were transferred to 100% acetone for 1 h, then placed in a 50–50 100% acetone–Spurr's resin mix for 24 h. After being infiltrated in pure Spurr's resin (Spurr 1969) for 7 days (the resin being changed daily) blocks were polymerized at 70° for 24 h. Embedded specimens were sectioned on a Sorvall JB-4 microtome. Transverse sections of frond tissue (1–1.5 μm thick) and longitudinal sections of rhizome apices (1.5 μm thick) were cut with glass knives and sections transferred to 10% acetone droplets on microscope slides, dried at 75°C and then stained with Toluidine Blue (pH9) before being observed.

Scanning electron microscopy

Pinnules from immature and mature fronds were cut into 0.75–1 cm pieces and plunged into liquid Freon 22 at −100°C and freeze dried at −30°C for 72 h. The dry specimens were mounted on aluminium stubs with silver Electrodag, gold coated and examined in an ISI 60 SEM.

Investigations with ^{14}C herbicides

Preparation and application of labelled herbicides. The carbon-14 asulam (Na salt, ring labelled, specific activity 2.564 mCi/mM) was prepared to obtain a solution of the same strength as was used in the field spraying, i.e. 240 mg asulam ml^{-1} and applied to four mature fronds. The upper surface of each frond was treated with 50 μl of solution (with an activity of 9.95 microcuries) in 5 μl aliquots, such that all pinnae received herbicide.

The carbon-14 glyphosate (ring labelled, specific activity of 1.95 mCi/mM) was converted to the isopropylamine salt by the addition of isopropylamine (Sprankle *et al.* 1975) (4 μl to 2.17 mg glyphosate) and then made up to the previously used field strength (162 mg glyphosate ml^{-1}) by the addition of distilled water and unlabelled glyphosate. The upper surfaces of two mature fronds were treated with 40 μl of labelled herbicide.

Preparation of cellular extracts. Fronds and associated rhizome apices were sampled 1 and 2 weeks after application of herbicide. Cellular extracts were prepared by two methods – anhydrous extraction and aqueous extraction – and the fractions radioassayed. Both methods have been described by Bretherton and Hallam (1979).

Determination of radioactivity. The pieces of filter paper containing the samples were pelleted and then oxidized in a Packard tricarb Sample Oxidizer 306 and radioassayed in a Packard tricarb Liquid Scintillation Spectrometer using Perma fluor V as a scintillant.

Results

Field observations of asulam-treated and diesel-oil control bracken. The immature fronds in the diesel-oil control plot showed damage 14 days after spraying. The majority of circinate pinnules were killed by the treatment. The extent of necrosis did not increase over the remaining 8 weeks, while the undamaged areas continued to develop and mature.

Asulam-treated fronds appeared chlorotic after 2 weeks but also showed apparent diesel-oil damage. By 4 weeks the asulam-treated fronds were completely chlorotic and many fronds had dead circinate pinnules, apparently due to diesel-oil damage. Fronds sprayed with diesel oil alone showed only necrotic areas of the unfurling pinnae. By 6 weeks the fronds, which had now matured, had returned to the same green colour as the diesel-sprayed controls. The areas of dead tissue still present on the control fronds had not changed in extent from those observed at 4 weeks.

Mature fronds treated with asulam appeared the same as the controls (green) throughout the 12 weeks. Diesel-oil controls of mature fronds showed no damage.

Excavated apices of the rhizomes from both asulam-treated and diesel-oil control plots appeared unaffected throughout the study period.

Glyphosate-treated bracken. The immature fronds were the first parts of the bracken affected by the glyphosate treatment. Two weeks after spraying, the fronds were partially chlorotic with necrotic circinate and unfurled pinnules. After 3 weeks, the fronds with circinate pinnules were completely dead, while those with unfurled or unfurling pinnules still had some areas of unaffected tissue. This necrosis was further advanced after 4 weeks and only some ultimate segments were unaffected. Within 6 weeks, all the immature fronds were completely dead.

The mature fronds appeared to be the same as the water-sprayed controls until 6 weeks after spraying, when approximately 50% had turned a distinct brown colour. These fronds died over the following 6 weeks, while the green fronds remained unchanged.

The rhizome apices excavated 6 weeks after spraying were all partially or totally necrotic (i.e. brown and very soft), as were all apices excavated over the remainder of the study period.

Light microscopy of asulam-treated bracken. Even though no macroscopic effects were noticed in immature fronds until 2 weeks after application of the asulam herbicide, cytological changes could be observed after 3 days, many of the upper surfaces of epidermal cells and hypodermal cells having disrupted cytoplasm (Figure 1). Palisade, spongy mesophyll, lower epidermis and vascular bundles appeared normal.

Two weeks after treatment, the mesophyll also showed cytoplasmic disruption with almost all the mesophyll tissue consisting of collapsed cells (Figure 2). By 3–4 weeks, necrotic tissue was evident with some areas of the tissue shrunken and dead, except for that near the midnerve. Parenchyma cells associated with the midnerve were usually last to collapse, often being the only recognizable cells in the specimen apart from those in the vascular bundles.

Damage was observed in LM sections of immature fronds that had been treated with diesel oil. Fourteen days after the application, areas of the upper epidermis of some samples were found to be dead, i.e. the cells had collapsed.

None of the asulam-treated, nor the diesel-oil treated mature frond tissue samples showed any cytological damage.

Sections of rhizome apices cut from asulam-treated bracken during the first 6 weeks after spraying showed no damage either. Meristematic cells within the apical mound could be observed in division (Figure 3); large multicellular hairs surrounded the apical meristem and each cortical parenchyma cell contained 5–10 starch grains. The 8, 10 and 12-week samples of apices had dead or collapsed hairs infected with fungi and bacteria. (Microorganisms were never seen in association with rhizome apices in the control plants.)

Light microscopy of glyphosate-treated bracken. Initial damage was evident in immature frond tissue 3 days after treatment, and consisted of patches of cytoplasmically disrupted upper epidermal cells, and slightly plasmolyzed hypodermal cells (Figure 4). The mesophyll tissue and lower epidermis of the leaves appeared unaffected. Damage was more extensive in the immature fronds 1 week after spraying; in addition to the plasmolyzed hypodermal and cytoplasmically disrupted epidermal cells, totally collapsed palisade mesophyll cells were evident (Figure 5). The chloroplasts of palisade and mesophyll cells appeared to be swollen.

Many of the samples, 2 weeks after treatment, were dead, i.e. the cells of

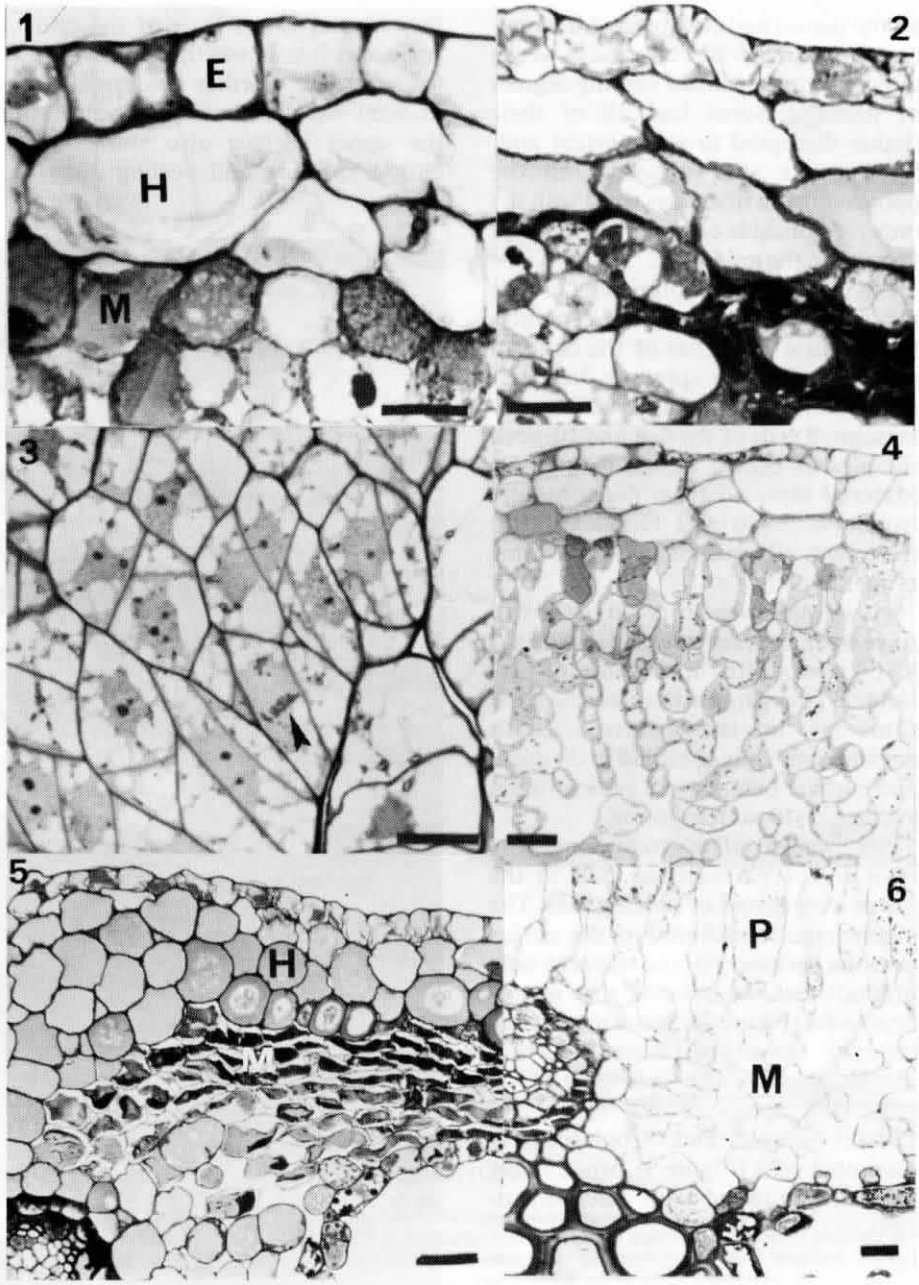

Figure 1 Immature frond, 3 days after asulam treatment. The cytoplasm of the epidermis (E) and hypodermis (H) shows disruption, whereas the mesophyll cells (M) appear normal. (Scale bar, 100 μm.)

Figure 2 Immature frond 2 weeks after asulam treatment showing cytoplasmic disruption throughout the leaf. (Scale bar, 100 μm.)

Figure 3 Meristematic cells of rhizome apex 6 weeks after asulam treatment. Note cell in division (arrow). (Scale bar, 100 μm.)

Figure 4 Immature frond 3 days after glyphosate treatment. Little damage is evident apart from minor shrinkage of the cytoplasm from the walls of some hypodermal cells. (Scale bar, 200 μm.)

Figure 5 Immature frond 1 week after glyphosate treatment showing epidermal (E) and hypodermal (H) cells with disrupted cytoplasm and dead mesophyll cells (M) containing phenolic deposits. (Scale bar, 200 μm.)

Figure 6 Mature frond 6 weeks after glyphosate treatment showing palisade cells (P) virtually devoid of chloroplasts and mesophyll tissue (M) with degenerate chloroplasts. Note the heavily lignified vascular tissue and the lignified lower epidermis. (Scale bar, 300 μm.)

all the tissues had collapsed. All immature frond material collected 3 weeks after spraying exhibited varying degrees of damage. Some had all of their tissues disrupted to some extent and, by 4 weeks after spraying, all the photosynthetic tissue was dead with the only recognizable cells being the xylem vessels of the midnerve.

No change was observed in the appearance of the treated, mature-frond tissue from that of the control, until 6 weeks after spraying. Very few chloroplasts were observed in the mesophyll cells of the 6, 8 and 10-week samples of brown fronds (Figure 6). Material sampled from green mature fronds, 6, 8, 10 and 12 weeks after treatment, all appeared to be the same as in the controls.

No damage was observed in the rhizome apex samples collected during the first 4 weeks after application of herbicide. Tissues appeared to be much the same as in the controls, with meristematic cells observed in division, and cortical parenchyma cells with, on average, 5–10 starch grains.

Six weeks after treatment, the majority of parenchyma cells in the cortex were devoid of starch grains. The parenchyma and fibres of the cortex near the periphery of the rhizome were plasmolyzed and infected with microorganisms (Figure 7). Some meristemic cells of these specimens had very darkly stained nucleoli (Figure 8).

All the apices of the 8, 10 and 12-week samples had cytoplasmically disrupted cells (Figure 9), while many were also infected with microorganisms.

Scanning electron microscopy of the frond surface. Observations showed the upper surface of mature fronds to be covered with a smooth cuticle (Figure 10). The lower surface, however, was covered with hairs and farinaceous outgrowths. The small lateral veins, emanating from the midnerve on the under surface of ultimate segments, were covered with short straight hairs which also occurred along the sides of the midnerve. The areas between veins were covered by farinaceous outgrowths (Figure 11).

On immature fronds, long, twisted hairs were present on both the upper and lower surfaces (Figures 12, 13). The short hairs found on the lower surface of mature fronds were also present along the sides of the midnerves and on the lateral nerves of immature fronds (Figure 12). The outer indusium of immature segments terminates near the midnerve, unlike that of mature fronds, and leaves little of the under surface visible. It was therefore difficult to determine whether epidermal outgrowths were present. Observation of the upper surface also showed the cuticle splitting and peeling away to reveal new cuticle underneath (Figure 13).

Aqueous extraction of cell organelles. Most of the radioactivity in the frond extracts occurred in the soluble fraction

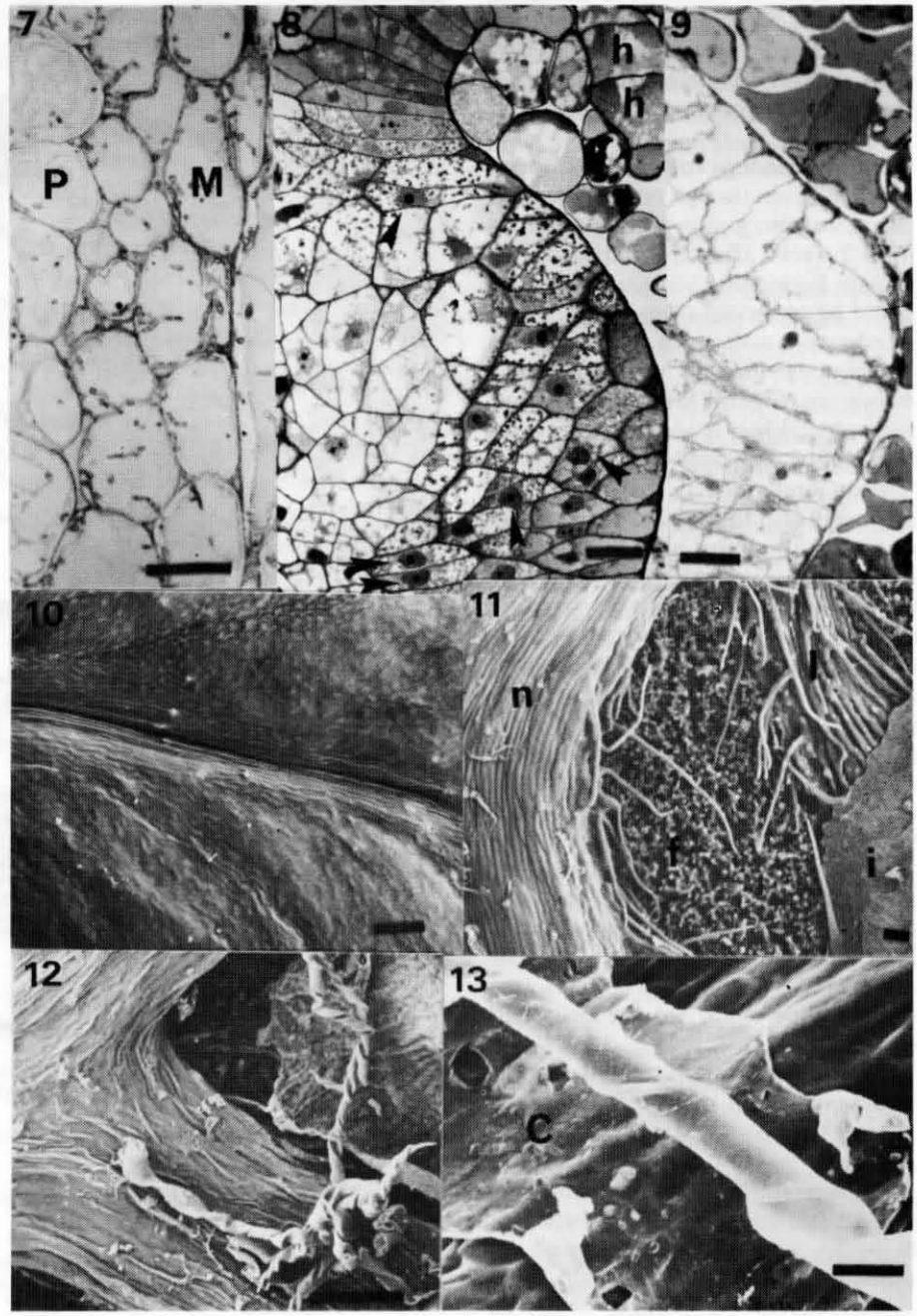

Figure 7 Cortex of rhizome apex 6 weeks after glyphosate treatment. Note the disrupted parenchyma cells (P) and microorganisms (M). (Scale bar, 300 μm.)

Figure 8 Rhizome apex 6 weeks after glyphosate treatment. The tissue appears normal except that many nuclei contain densely staining nucleoli (arrowed). Note the multicellular hairs (h) surrounding the apex. (Scale bar, 200 μm.)

Figure 9 Rhizome apex 10 weeks after glyphosate treatment. The meristematic cells have disrupted cytoplasm (compare with Figure 8). (Scale bar, 200 μm.)

Figure 10 Smooth cuticle over the upper surface of a mature frond ultimate pinna. The central indentation indicates the position of the mid-nerve. (Scale bar, 100 μm.)

Figure 11 Under surface of a mature frond showing the midnerve (n), lateral veins with hairs (l), farinaceous outgrowths (f) and the indusium (i). (Scale bar, 200 μm.)

Figure 12 Lower surface of an immature frond showing long, twisted hairs. (Scale bar, 13 μm.)

Figure 13 Upper surface of an immature frond showing a twisted hair above the peeling cuticle (c). (Scale bar, 100 μm.)

of the cells following centrifugation (Figure 14). In both the ^{14}C-glyphosate and ^{14}C-asulam treated frond extracts there was a decrease in activity from the 1-week sample to the 2-week sample, suggesting that some carbon-14 had been transported away from the fronds in that time.

Anhydrous extraction of cell organelles. After centrifugation, all the centrifuge tubes containing frond extracts were a translucent golden-green colour in the specific gravity mixtures of 1.36 and 1.34, (fractions 3–9) (Figure 15). The specific gravity mixture of 1.052 remained clear (fractions 1 and 2). Chloroplasts formed an opaque band between fractions 3 and 4 and, in some tubes, there was also a diffuse band of chloroplast fragments between fractions 4 and 6 where there are peaks of radioactivity. A green cell-pellet in the base of all the tubes (fraction 10) contained radioactive whole cells and cell-wall debris.

No significant radioactivity was obtained from any of the extracts of rhizome apices. Significant activity was obtained from the frond preparations, particularly for the 1-week samples. As in the aqueously extracted samples, there was reduced activity in the frond preparations after 2 weeks.

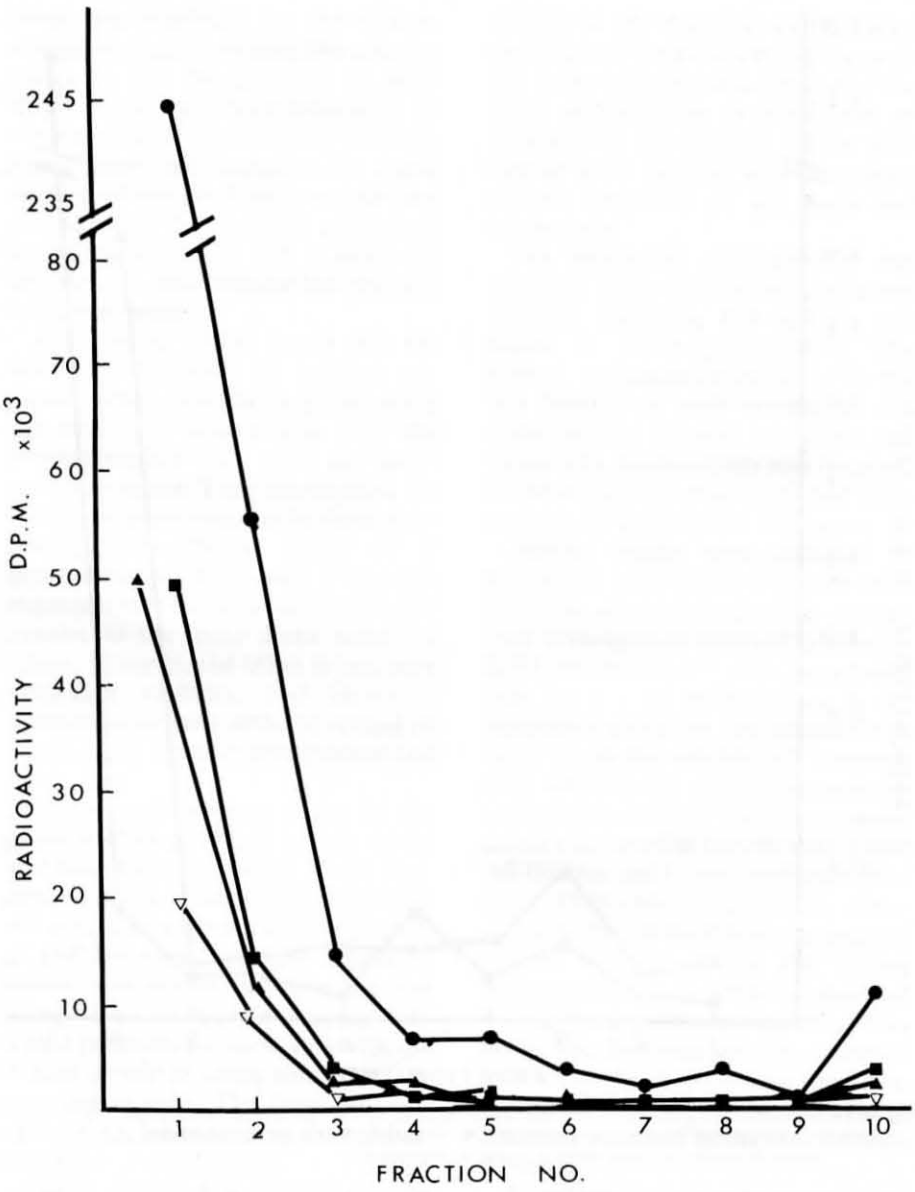

Figure 14 Localization of radioactivity in aqueously extracted cellular fractions of ^{14}C-asulam and ^{14}C-glyphosate-treated frond tissue. ●, 1 week after treatment (asulam); ■, 2 weeks after treatment (asulam); ▲, 1 week after treatment (glyphosate); and ▽ 2 weeks after treatment (glyphosate). Fractions 1 and 2, high speed supernatant; 4 and 5, mitochondria; 7 and 8, nuclei, chloroplasts; and 10, wall and cell debris.

Discussion

The initial effect of both herbicides and the diesel-oil spray was to destroy the meristematic regions of the immature fronds. Due to the diesel-oil component of the asulam spray, it is possible that necrosis observed was caused by the diesel oil and not the herbicide. Other workers have found necrosis on immature fronds caused by asulam alone. Veerasekaran *et al.* (1977*b*), working with glasshouse grown *P. aquilinum* plants, found that asulam treatment caused the 'scorch and collapse' of young fronds after 2 weeks, and Catchpole and Hibbitt (1972), working with bracken and other perennial weeds, found that the meristems of fronds and leaves were killed within 1 or 2 weeks after asulam treatment. It may be possible that asulam in some way 'cushions' the effect of diesel oil on immature frond tissue, such that any effects are delayed in time and overshadowed by those caused by the herbicide.

Less herbicide damage was noted in the rhizome apices of asulam-treated bracken than that found by Veerasekaran *et al.* (1976). The only deviations in anatomical appearance from the controls occurred 8, 10 and 12 weeks after treatment. Meristematic cells, many nucleoli of which appeared darkly stained, had apparently ceased division and microorganisms were observed among the hairs of the apices but separated from the apical tissue proper by a barrier of lignified cell walls. Veerasekaran *et al.* (1976) found that the epidermis and adjacent cell layers of rhizome apices became progressively more distorted and lignified from 1 week after asulam treatment. They also observed fissures in the apices 8 and 12 weeks after treatment, and that the exposed internal tissues were broken down by microbial action. The changes described by Veerasekaran *et al.* (1976) are similar in nature, but much more severe, than those observed in this investigation. This may be explained by the different plant materials used, as Veerasekaran *et al.* worked with pot-grown rhizome cuttings of *P. aquilinum*, not field-grown *P. esculentum*. Another feature of asulam-treated apices noted by Veerasekaran *et al.* (1976), was the accumulation of starch grains in the cortical tissues, which was also found in 4-CPA (4-chlorophenoxyacetic acid) treated *P. aquilinum* apices, by Conway and Forrest (1961). This was not observed in any of the asulam-treated apices examined for this study. Veerasekaran and Kirkwood (1972) noted that the rhizome apices of bracken plants were hard, swollen, fissured and black in colour (as opposed to the normal white colour) 30 days after treatment.

The meristematic cells of apices 6 weeks after treatment had darkly staining nucleoli, while all the cells of the

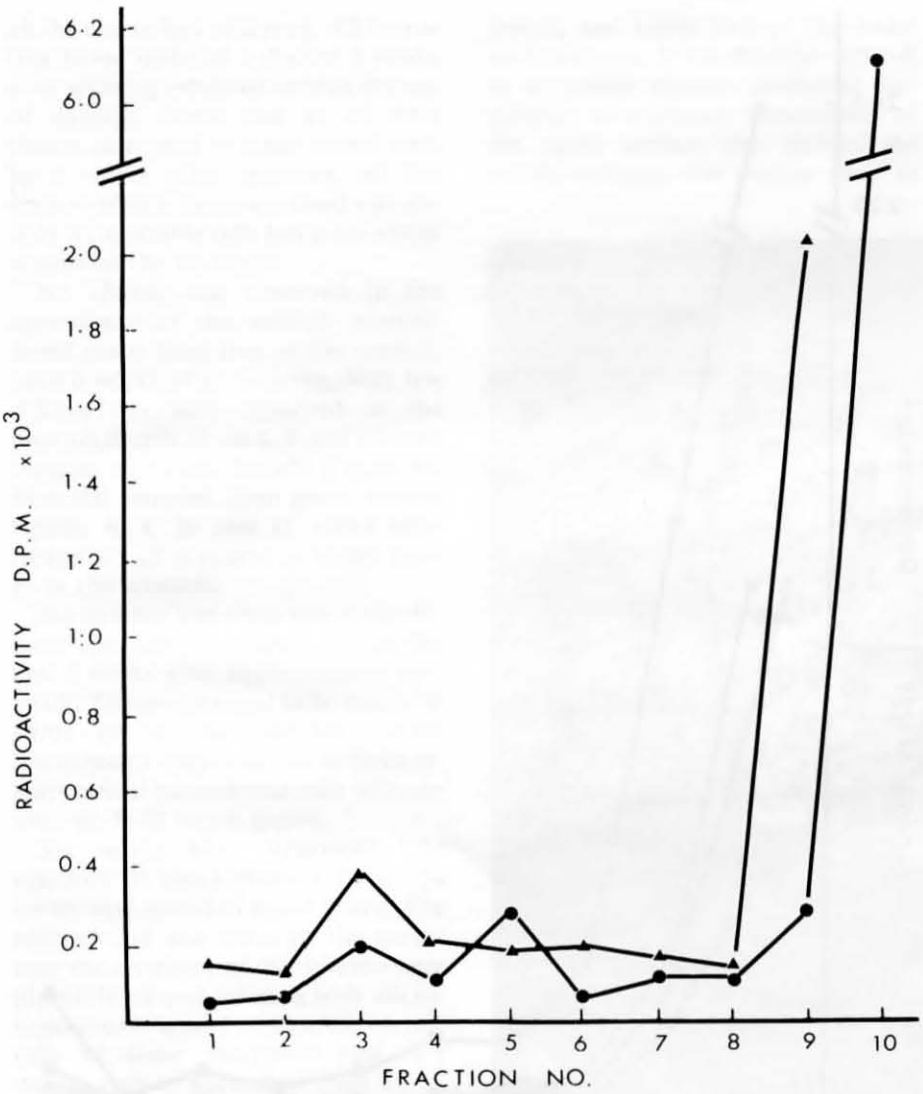

Figure 15 Localization of radioactivity in anhydrously extracted cellular fractions of frond tissue. 1 week after ^{14}C asulam and ^{14}C glyphosate treatment. ●, ^{14}C asulam; ▼, ^{14}C glyphosate. Fraction 3, wall and cell debris; 5, nuclei and mitochondria; 8 and 9 chloroplasts.

8, 10 and 12-week samples had disrupted cytoplasm. Areas of the cortex of 6-week samples were plasmolyzed and infected with microorganisms, while the majority of tissue of 8, 10 and 12-week samples contained microorganisms. The symptoms observed in this study (soft and necrotic tissue) were different from those described for other herbicide-affected apices. Both asulam and 4-CPA have been found to cause apices to become lignified and fissured (Conway and Forrest 1961; Veerasekaran *et al.* 1976). Williams and Foley (1975) mention in their brief article that glyphosate applied to field *P. aquilinum* causes some 'destruction of rhizome' but they do not elaborate further.

The rhizome apices of the glyphosate-treated bracken were found to be affected 6 weeks after application of herbicide. This coincided with the appearance of necrotic tissue in mature fronds. The cortical cells of affected rhizome apices were found to be nearly devoid of starch grains. Williams and Foley (1975) found that glyphosate caused a reduction in the carbohydrate content of *P. aquilinum* rhizomes; it seems probable therefore that glyphosate in some way either interferes with the production of starch, or causes its breakdown.

Both herbicides caused cellular damage in immature fronds within 3 days of application. The first tissues to be affected were the upper epidermis and the hypodermis. At first, only patches of these cells were affected. (Such patches, no doubt, correspond to the spray drops of the applied herbicide.) The mesophyll tissues were the next to become affected. These were found to be damaged in the majority of samples 1 week after glyphosate treatment and 2 weeks after asulam treatment. It seems that the palisade mesophyll becomes affected before the spongy mesophyll, as in some samples only the palisade cells were damaged, while in others the palisade cells showed a greater degree of damage than the spongy mesophyll cells. The lower epidermis and parenchyma cells of the midnerve were the last tissues to become affected.

The gradation of damage from the upper epidermis to the lower, with time, indicates that the penetration of herbicide occurs from the upper, or adaxial, surface. The findings therefore of Veerasekaran *et al.* (1977*b*) and Cook *et al.* (1979) that herbicide uptake is greater through the abaxial surface of *Pteridium aquilinum* pinnules, are somewhat irrelevant if the majority of field-applied spray droplets reach only the upper surface of the fronds.

The majority of young fronds survived the asulam treatment, being no longer chlorotic 6 weeks later, and had matured. Immature fronds act as phloem sinks and have only acropetal translocation, whereas mature fronds exhibit the basipetal translocation of ^{14}C-asulam (Veerasekaran *et al.* 1977*b*). Therefore the accumulated asulam in the immature fronds (resulting in chlorosis and necrosis) may be translocated out of the fronds as the undamaged areas develop and the frond matures. However, Veerasekaran *et al.* (1976) say that 'it is unlikely that at frond maturity the herbicide is subsequently translocated out of these (originally immature) tissues'.

Only the very immature fronds (those still with circinate pinnae) were killed by the asulam treatment, whereas after the glyphosate treatment all the young fronds were killed within 6 weeks. The symptoms of chlorosis and necrosis on the glyphosate-treated fronds, prior to death, were also noted by Uotila *et al.* (1980) on the leaves of *Sinapis alba* 3 days after glyphosate application.

Necrotic tissue was observed on immature fronds 2 weeks after glyphosate treatment and 2 weeks after asulam treatment. (Samples observed under the light microscope showed that the tissue had been totally destroyed.)

The chlorosis observed in the immature fronds from 2 to 4 weeks after asulam treatment has also been observed by other workers. Veerasekaran *et al.* (1977*b*) found glasshouse grown *P. aquilinum* plants with chlorotic and necrotic young fronds 2 weeks after treatment, and Veerasekaran and Kirkwood (1972) found the young fronds of greenhouse grown *P. aquilinum* plants to be chlorotic 30 days after treatment.

The damage observed in the diesel-oil treated immature frond tissue, corresponds in appearance to that

observed in the asulam-treated tissue, but it does not correspond in time. That is, damage (collapsed upper epidermal cells), was not found until 14 days after diesel-oil treatment, whereas plasmolyzed upper epidermal cells were first observed in asulam-treated tissue after 3 days. The diesel oil therefore probably has little effect on immature frond tissue when applied in conjunction with asulam, as the herbicide damages the tissue before the diesel oil has time to act.

The mature fronds were unaffected by the asulam treatment. This was also found in studies by Veerasekaran and Kirkwood (1972) and Catchpole and Hibbitt (1972). Mature fronds were, however, affected by the glyphosate treatment. Many of them developed a brown coloration 6 weeks after spraying. This was not observed in any of the other bracken areas. The brown colour may have resulted from the oxidation of phenolic materials normally present and a reduction in or loss of chlorophyll (Catchpole and Hibbitt 1972). The diesel-oil treatment also had no effect on mature fronds, which would be expected, since no damage was caused by the asulam spray which included diesel oil.

Glyphosate-treated mature fronds showed the effects of the herbicide 6 weeks after spraying. Few chloroplasts were visible in the mesophyll cells which appeared 'empty' except for the globular material normally present in the palisade cells. These samples were from the brown-coloured fronds observed in the glyphosate-treated plot. It seems therefore that the herbicide affects the chloroplasts of the mature frond, initially resulting in a change of frond colour. The brown coloration is probably due to the globular materials, which may be phenolics, observed in the mesophyll cells, the brown colour of which (as observed under the light microscope) becomes unmasked with the loss of chlorophyll.

The farinaceous outgrowths on the lower surface of fronds seem to be only a mature feature, as they were not observed either under the SEM or light microscope on immature fronds. They may develop as a form of protection for the frond since the indusium, which covers all but the midnerve in young fronds, only covers a small area of the under surface of mature fronds.

Boize *et al.* (1976) investigated the influence of leaf surface roughness on the spreading of oil-spray droplets and found that drops larger than 20 μ in diameter spread according to the pattern of epidermal grooves and other micro and macroscopic features. The drop size produced by the C.D.A. sprayer was approximately 280 μ in diameter, so that the spreading of spray drops would have been influenced by the topography of the frond surfaces. Spray drops that landed on the upper surface of mature fronds would have spread along the midnerve grooves of ultimate segments as well as along the depressions that indicate the position of lateral veins.

Cook *et al.* (1979) found that the uptake of herbicide by bracken was greater when spray drops spread along the midnerve groove and over the general surface than when the drops remained intact. They investigated the uptake of aminotriazole by three morphologically different forms of *P. aquilinum*. One form, with a very corrugated upper surface, exhibited a low uptake, as the spray drops remained intact. However, the other types, with smoother surfaces, had increased uptakes associated with the spread of drops along the midnerve grooves and other areas.

The spread of spray drops on the upper surface of young fronds would also have been influenced by the midnerve grooves as well as the hairs and old cuticle fragments. Veerasekaran *et al.* (1977*b*) found that the uptake of asulam was greater in young bracken fronds than in mature fronds. This would probably be associated with the thinner cuticle of young fronds and the presence of hairs. The basal portions of hairs are believed to be sites of herbicide entry (Hull 1970).

Spray drops that landed on the under surface of fronds would have been influenced in their manner of spread by the presence of nerves, hairs, indusium and, in the case of mature fronds, epidermal outgrowths. Cook *et al.* (1979) and Veerasekaran *et al.* (1977*b*) both found that herbicide uptake was greater from the lower surface of bracken fronds. Cook *et al.* (1979) found that one form of *P. aquilinum*, with very few hairs on the lower surface, had a much lower uptake of aminotriazole than two other forms with many hairs over the veins and midnerves. These two forms also had thicker cuticles than the other, and Cook *et al.* (1979) suggests that uptake is not dependent on cuticle thickness. On the other hand, Veerasekaran *et al.* (1977*b*) attributed the increased abaxial uptake of asulam to the thinner cuticle (compared to that on the upper surface) and the presence of stomata.

In all the aqueously extracted cell preparations, much of the radioactivity was found in the soluble cell components. There was also substantial radioactivity in the fractions of the frond and rhizome preparations, and especially in the mitochondria fraction of the frond preparations. A small peak of radioactivity was found in the last fraction of most samples and corresponded to fragments of cell walls and whole cells.

No significant radioactivity was obtained from anhydrously extracted rhizome fractions, but activity was found in frond preparations. The highest radioactivity occurred in the last fraction of each sample, as this contained the cell-wall fragments and whole cells. Radioactivity also occurred in those fractions that contained chloroplasts or chloroplast fragments.

Similar results were obtained by Bretherton and Hallam (1979) for both methods of extraction, even though their investigation involved labelled 2, 4, 5-T and *Rubus procerus*. They found that the highest radioactivity in the aqueously extracted cell preparations occurred in the soluble cell components, while in the anhydrously extracted cell preparations, the highest radioactivity occurred in the cell wall/whole cell fraction and in the chloroplast fractions. They concluded that the radioactive herbicide must leak out into the soluble fraction of the cell during aqueous extraction, while it remains at its site(s) of action during anhydrous extraction. This may also have occurred in this investigation because the distribution of radioactivity in the cellular fractions obtained by the two methods differed.

Frond tissues treated by either herbicide showed significant radioactivity in the chloroplasts when prepared by the anhydrous extraction method 1 week after treatment. This suggests that glyphosate and asulam became in some way associated with the chloroplasts.

The amount of radioactivity present in the 2-week samples of frond preparations, both anhydrously extracted and aqueously extracted, was considerably less than that present 1 week after application of herbicide. This indicates that there was movement of radioactivity, and hence herbicide, out of the frond after 1 week. Both asulam and glyphosate are known to be phloem mobile and were therefore most likely translocated out of the fronds. Sandberg *et al.* (1980) found ^{14}C label in the phloem of field bindweed roots and stems after application of ^{14}C-glyphosate. They also found that basipetal translocation of ^{14}C-glyphosate occurred in Canada thistle and wild buckwheat between 3 and 14 days after treatment. The appearance of radioactivity in the aqueously extracted

rhizome apex preparations 2 weeks after treatment, suggests that the ^{14}C-glyphosate is translocated out of the fronds to phloem sinks such as rhizome apices. Because of the anomalous data obtained for the aqueously extracted frond preparations 1 week after ^{14}C-asulam treatment, one cannot say whether there was reduced activity in the fronds after 2 weeks. However, the appearance of substantial amounts of radioactivity in the rhizome apices after 2 weeks suggests that translocation had occurred from the fronds. Veerasekaran *et al.* (1977*b*) found that ^{14}C-asulam when applied to mature fronds of *P. aquilinum*, was translocated in a basipetal direction to phloem sinks such as rhizome apices and frond buds.

A difficulty associated with this sort of investigation, is that one does not know whether the assayed radioactivity can be attributed to the labelled compound originally applied to the plant. Metabolites of the compound, or synthates, may become labelled in the plant. Sandberg *et al.* (1980) found that only a very small percentage of potential glyphosate metabolites (such as aminomethylphosphonic acid, glycine and sarcosine) occurred in tissues of Canada thistle, field bindweed and tall morning glory 30 days after the application of ^{14}C-glyphosate and may have been present in the glyphosate initially applied. It is believed that asulam, in bracken, becomes slowly hydrolyzed to sulfanilamide (Veerasekaran *et al.* 1976), and it was found that 10 weeks after ^{14}C-asulam application to bracken, 65% of the extracted ^{14}C was in ^{14}C-asulam (Veerasekaran *et al.* 1977*b*). Therefore, the radioactivity assayed 1 and 2 weeks after ^{14}C-asulam and ^{14}C-glyphosate application in this investigation can be assumed to be ^{14}C-asulam and ^{14}C-glyphosate.

Asulam and glyphosate have both been found to affect field-grown *Pteridium esculentum*. In this field study, however, glyphosate had a greater effect over a shorter period of time than the more widely used herbicide asulam.

References

Boize, L., Gudin, C., and Purdue, G. (1976). The influence of leaf surface roughness on the spreading of oil spray drops. *Annals of Applied Biology* **84**, 205–11.

Bretherton, G., and Hallam, N. D. (1979). The movement of 2, 4, 5-trichlorophenoxyacetic acid into the leaves of *Rubus procerus* P. J. Muell. and its effects of chloroplast ultrastructure. *Weed Research* **19**, 307–13.

Catchpole, A. H., and Hibbitt, C. J. (1972). Studies on the retention, penetration and translocation of asulam in some perennial weed species. Proceedings of the 11th British Weed Control Conference, 77–83.

Clifford, H. T., and Constantine, J. (1980). 'Ferns and Fern Allies and Conifers of Australia'. (University of Queensland Press: St Lucia.)

Conway, E., and Forrest, J. D. (1961). The effects of 4-chlorophenoxyacetic acid on the rhizome of *Pteridium aquilinum* (L.) Kuhn. *Weed Research* **1**, 114–30.

Cook, G. T., Carr, K. E., and Duncan, H. J. (1979). The influence of morphological differences in bracken pinnules on the foliar uptake of aminotriazole. *Annals of Applied Biology* **93**, 311–17.

Copeland, E. B. (1947). 'Genera Filicum, the Genera of Ferns'. (Ronald Press: New York.)

Holroyd, J., Parker, C., and Rowlands, A. (1970). Asulam for the control of bracken (*Pteridium aquilinum*). Proceedings of the 10th British Weed Control Conference, 371–6.

Hull, H. M. (1970). Leaf structure as related to absorption of pesticides and other compounds. *Residue Reviews* **31**, 1–150.

Jaworski, E. G. (1972). Mode of action of N-phosphonomethylglycine: inhibition of aromatic amino acid biosynthesis. *Journal of Agricultural Food Chemistry* **20**, 1195–8.

Martin, R. J. (1977). Control of bracken (*Pteridium esculentum* G. Forst. (Cockayne)) in pasture with asulam. *Weed Research* **17**, 49–54.

O'Brien, T. P. (1963). The morphology and growth of *Pteridium aquilinum* var. *esculentum* (Forst.) Kuhn. *Annals of Botany N.S.* **27**, 253–67.

Page, C. N. (1976). The taxonomy and phytogeography of bracken — a review. *Journal of the Linnean Society* (Botany) **73**, 1–34.

Sandberg, C. L., Meggitt, W. F., and Penner, D. (1980). Absorption, translocation and metabolism of ^{14}C-glyphosate in several weed species. *Weed Research* **20**, 195–200.

Scragg, E. B., McKelvie, A. D., and Kilgour, D. W. (1974). Further work on the control of bracken in the N. of Scotland. Proceedings of the 12th British Weed Control Conference, 761–9.

Sprankle, P., Meggitt, W. F., and Penner, D. (1975). Absorption, action and translocation of glyphosate. *Weed Science* **23**, 235–40.

Spurr, A. R. (1969). A low viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructural Research* **26**, 31–42.

Tryon, R. M. (1941). Revision of the Genus *Pteridium*. *Rhodora* **43**, 1.

Uotila, M., Evjen, K., and Iversen, T. H. (1980). The effects of glyphosate on the development and cell-ultrastructure of white mustard (*Sinapis alba* L.) seedlings. *Weed Research* **20**, 153–8.

Veerasekaran, P., and Kirkwood, R. C. (1972). The effect of stage of frond development on the absorption and translocation of asulam in bracken. Proceedings of the 11th British Weed Control Conference 17–23.

Veerasekaran, P., Kirkwood, R. C., and Fletcher, W. W. (1976). The mode of action of asulam [methyl (4-aminobenzenesulphonyl) carbamate] in bracken. *Journal of the Linnean Society* (Botany) **73**, 247–68.

Veerasekaran, P., Kirkwood, R. C., and Fletcher, W. W. (1977*a*). Studies on the mode of action of asulam in bracken (*Pteridium aquilinum* (L.) Kuhn) II. Biochemical activity in the rhizome buds. *Weed Research* **17**, 85–92.

Veerasekaran, P., Kirkwood, R. C., and Fletcher, W. W. (1977*b*). Studies on the mode of action of asulam in bracken (*Pteridium aquilinum* (L.) Kuhn) I. Absorption and translocation of (^{14}C) asulam. *Weed Research* **17**, 33–9.

Veerasekaran, P., Kirkwood, R. C., and Fletcher, W. W. (1978). Studies on the mode of action of asulam in bracken (*Pteridium aquilinum* (L.) Kuhn) III. Longterm control of field bracken. *Weed Research* **18**, 315–9.

Watts, M. J., and Collin, H. A. (1979). The effect of asulam on the growth of tissue cultures of celery. *Weed Research* **19**, 33–7.

Williams, G. H., and Foley, A. (1975). Effects of herbicides on bracken rhizome survival. *Annals of Applied Biology* **79**, 109–11.