Scientific Review 2000-2003

The Scottish Agricultural Science Agency

An Agency of the Scottish Executive Environment and Rural Affairs Department (SEERAD)

Scientific Review 2000-2003

Scottish Agricultural Science Agency

Edited by RKM Hay
AJ Milne

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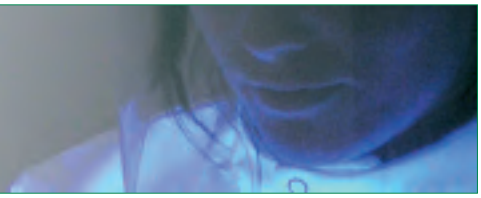
Chapter 1 The Scientific Role of SASA (RKM Hay)



The Scottish Agricultural Science Agency exists to provide government, and the Scottish Executive in particular, with the scientific expertise that is necessary to secure many aspects of the quality, safety and security of food supplies, and protection of the environment. Much of this work is relatively routine provision of advice, monitoring and diagnoses, based on sound scientific foundations, including the long experience of seasoned scientists. SASA scientists are, however, called upon to deal with unexpected tasks, as well as new areas developing from its established disciplines.

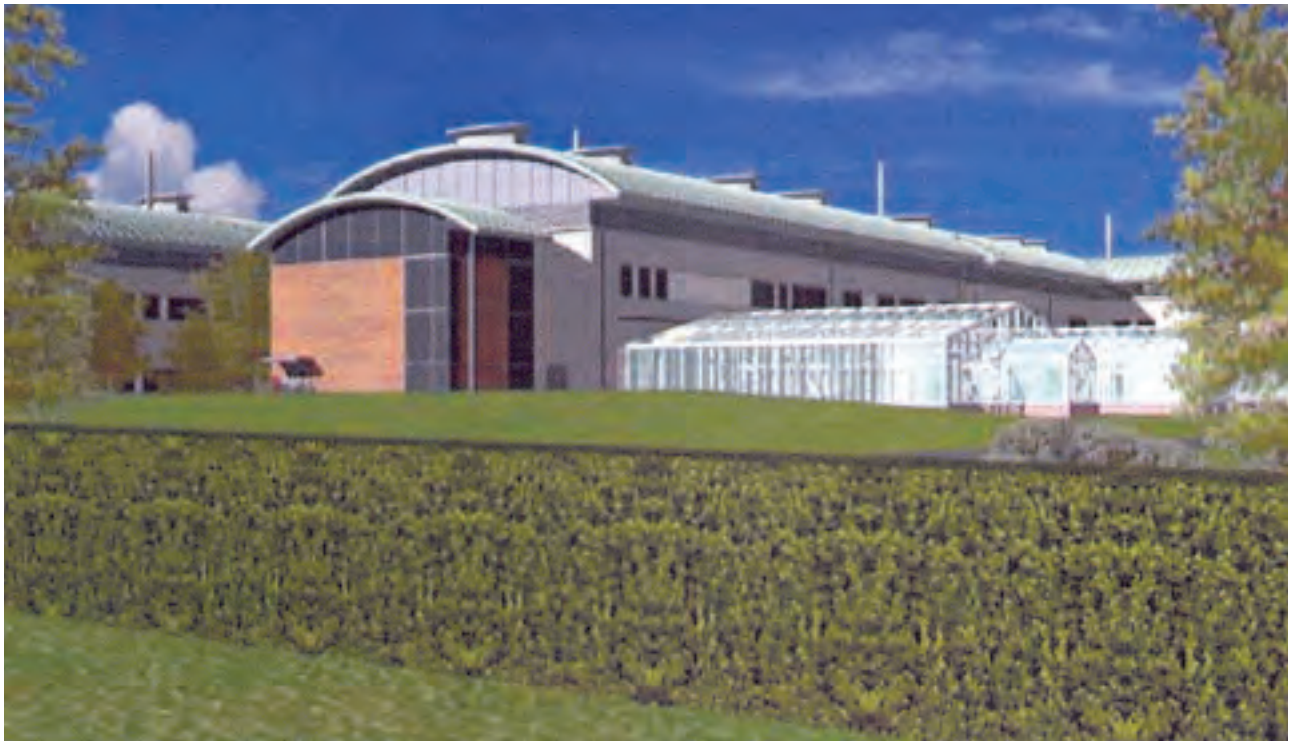
This, the third, Scientific Review, covering the years 2000-2003, provides a balanced view of these duties, featuring:

- the steady evolution of long-standing areas of work, for example in seed testing, variety testing, disease diagnosis, plant health and pesticide science
- areas where molecular biologists are introducing powerful new techniques that improve the accuracy and timeliness of scientific diagnoses (Chapter 6)
- the undertaking of new work, building on existing competence and reputation (e.g. variety testing of additional species – 3.3; the work of the GM Inspectorate – Chapter 7; lamb predation by eagles – 9.2)
- responses to emergencies (e.g. sudden oak disease – 5.2; exotic pests – 5.5; ring rot of potatoes – 6.2)
- the wider significance and usefulness of data collected as part of statutory and regulatory work (e.g. aphid monitoring records as a model for studies of climate change – 9.3)
- examples of national and international collaboration that enrich the scientific work of the Agency, and lead to the development of improved methods and tools (e.g. in the diagnosis of virus and viroid diseases – 4.5, 5.3; in studies of insecticide resistance of insects – 4.6).

Although the review stands alone as a scientific publication, to avoid repetition, it builds on work already published in the two preceding reviews. Interested readers are, therefore, recommended to consult earlier reviews (1992-97; 1997-2000) in which complex areas of work such as crop certification, the PCR reaction and modern approaches to mass spectroscopy are described in more detail. In line with this policy, this review includes authoritative articles on pea variety testing (3.4); the incidence of PCN in Scotland (4.3); trends in herbicide usage (8.3); and pesticide poisoning of wildlife (8.4).

The most novel development in this review is the inclusion of five more informal and personal articles from SASA scientists, explaining how they have met particular challenges in their work. These challenges were very varied: working abroad for an international organisation; responding at very short notice to the foot and mouth outbreak; establishing the GM Inspectorate for Scotland; applying specialist expertise to a new plant disease, which has the potential to cause major plant health problems; deploying molecular biological techniques, common to a wide range of species including humans, to improve and speed up diagnostic tests. These articles demonstrate the high calibre of scientists in the Agency, but they also give the reader some idea of what it is like to work there, and how a career in science can develop in many different ways.

As this is the third and last scientific review that I will edit, I take the opportunity to express my admiration for the high quality of scientific work done by the Agency staff, and wish them well in their new purpose-built laboratories under their new director, Gordon Machray.



Visualisations of the south east corner of SASA's new headquarters from Roddinglaw Road

Visualisations of the aerial view of SASA's new headquarters

2 Seed Testing

2.1 Introduction (R Don)

2.2 The Effect of Glyphosate on the Quality and Performance of Barley Seed in Scotland (G McLaren and R Don)

2.3 The Incidence of Loose Smut (*Ustilago nuda*) In Scottish Barley Seed Lots (V Cockerell, M Anderson and M Jacks)

Chapter 2 Seed Testing

2.1 Introduction (R Don)

The Official Seed Testing Station for Scotland (OSTS) is the principal centre for seed testing and seed quality information in Scotland. For ninety years it has played a central role in ensuring the quality of seed traded in Scotland by testing for analytical purity, the presence of seed of other species, germination and seed health. The continuity of seed testing by the OSTS gives SASA a unique perspective of historical trends in Scottish agriculture, particularly the arable sector, and overall seed quality. This chapter considers some recent trends in Scottish barley seed and how the work of the OSTS can assist growers in their quest to produce high quality seed.

Since 1998 there has been a dramatic increase in the use of glyphosate as a pre-harvest desiccant of barley crops to assist in harvesting and this has been accompanied by an increase in the number of germination tests that have shown glyphosate-induced abnormal seedlings. The occurrence and levels of these abnormal seedlings vary between samples of seed from different crops receiving the same pre-harvest glyphosate treatment. Responding to these developments, to provide customers with information and advice on glyphosate application and seed quality, the OSTS has conducted laboratory and field experiments over the last 4 years. The first article (2.2) reports on the findings of these experiments. The second article (2.3) reports on increasing levels of loose smut (*Ustilago nuda*) in barley seed in the last 12 years. Reasons for this increase are examined and advice is given on how the disease should be managed to ensure low levels of smut in growing crops.

2.2 Effect of Glyphosate on the Quality and Performance of Barley Seed in Scotland (G McLaren and R Don)

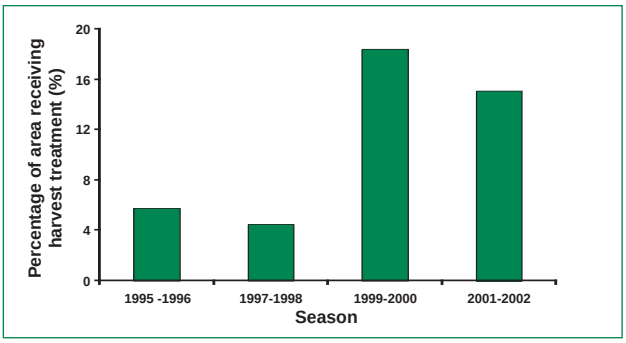
Glyphosate

Glyphosate is a non-selective, broad-spectrum, systematic, phosphoglycine herbicide used to control annual and perennial grasses, sedges, broad-leaved weeds and woody plants. It is applied to the foliage where it is readily absorbed. Once absorbed, glyphosate is extensively translocated symplastically and *via* the phloem, being partitioned within the plant according to the same pattern as photosynthate, and accumulating in areas of active growth, where it inhibits protein synthesis. Glyphosate inhibits the shikimic acid pathway, which is responsible for, among other things, the biosynthesis of aromatic amino acids, phenylalanine, tyrosine and tryptophane. This pathway is also responsible for the biosynthesis of such diverse plant compounds as phytoalexins, plastoquinone, alkaloids, cinnamate, coumarin and flavonoids. Cessation of growth occurs almost immediately after the herbicide reaches the apical meristems, and the plant tissues slowly degrade due to lack of proteins. Symptoms include gradually increasing chlorosis/necrosis, and death ultimately results from dehydration and desiccation (Anon., 1996).

Glyphosate Use in Scottish Cereal Crops

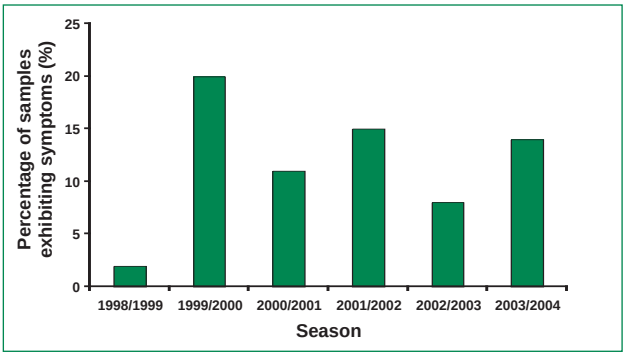
In Scottish cereal crops it is used for sward destruction in direct drilling/minimum cultivation systems, and pre-harvest spraying of cereal grain crops is recommended for the control of perennial weeds such as *Elytrigia repens* L. (couch grass). It is also recommended for use as a pre-harvest desiccant. However, glyphosate should not be used as a desiccant where the crop grain moisture is greater than 30%, and the manufacturer does not recommend it for use in crops grown for the production of seed (Don *et al.*, 1990; Anon., 1998). Despite this, and although its use is contrary to recommendations of the UK certifying authorities, there is increasing evidence of its application to seed crops.

Figure 2.1 The proportion of the Scottish barley area receiving pre-harvest glyphosate treatment



Between 1997/98 and 1999/2000 there was a 260% increase in the area receiving pre-harvest glyphosate treatment (Snowden and McCreath, 1997; Snowden and Thomas, 1999; Kerr and Snowden, 2001; Snowden and Thomas, 2003) (Fig. 2.1). Over the same period, there was up to a ten-fold increase in the number of barley samples with glyphosate-induced abnormalities, in germination tests conducted at the OSTS (Fig. 2.2).

Figure 2.2 The proportion of barley samples exhibiting glyphosate symptoms in germination tests conducted at the OSTS

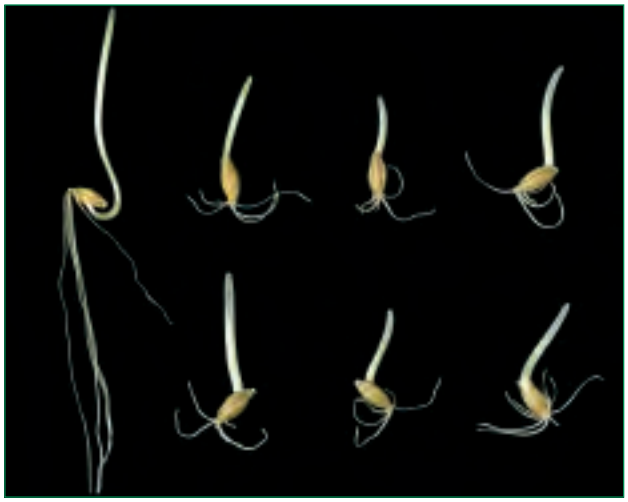


The Effect of Glyphosate on Germination

The main symptoms of glyphosate damage expressed in the germination test are: negative geotropism, where roots grow upwards and are commonly splayed; stunting; and the absence of root hairs (McLaren and Don, 2002). Coleoptiles are also affected and can be empty, twisted, stunted or split (Fig. 2.3).

Paper towelling (PT) is the recommended germination substrate (Anon., 2004), as it provides conditions that are conducive to the expression of damage caused by glyphosate. Samples with 5% or more seedlings showing glyphosate symptoms in PT, are retested using a proprietary potting compost. Compost reduces glyphosate symptoms in some samples and thereby gives a more accurate assessment of germination potential.

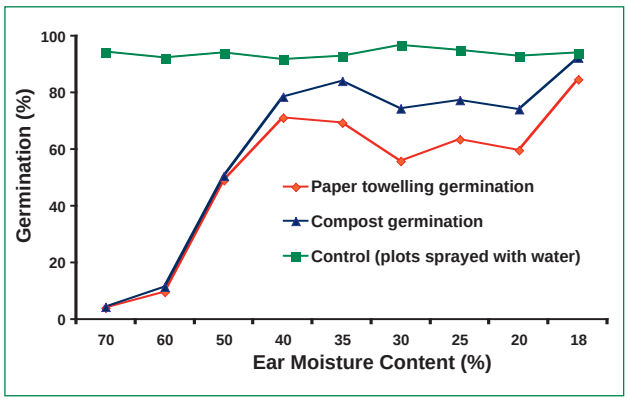
Figure 2.3 Seedlings showing glyphosate-induced abnormalities in paper germination tests (normal seedling for comparison is on the left of the plate)



The Effect of Pre-Harvest Glyphosate Treatment at Different Crop Maturities on the Germination of the Resultant Seed

In experiments where crops were sprayed with glyphosate at different crop maturities, as indicated by ear moisture content, germination of the harvested seed was significantly lower when tested in paper towelling compared with compost substrate (p = 0.05) (McLaren and Don, 2002). This was particularly marked at ear moisture contents of less than 35% (Fig. 2.4).

Figure 2.4 The effect of glyphosate application at different ear moisture contents on germination in compost and paper towelling



The levels of glyphosate-induced abnormalities were not significantly related to ear moisture content in the range 18% to 70% (Fig. 2.5). However, they were significantly related (p<0.01) to the amount of rainfall experienced after treatment with glyphosate (Fig. 2.6).

Figure 2.5 The relationship between ear moisture content at time of glyphosate treatment and the levels of glyphosate-induced abnormalities in harvested seed

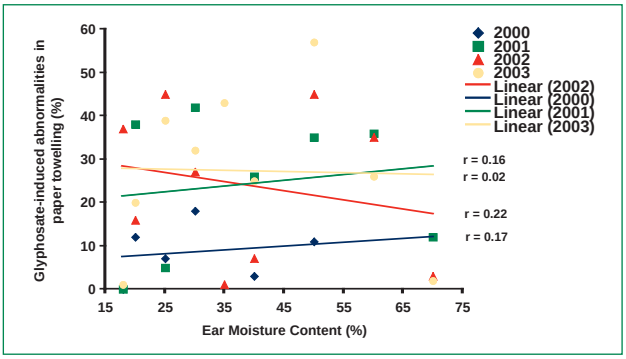
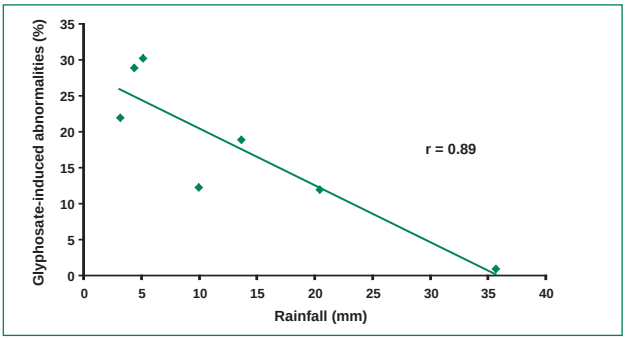


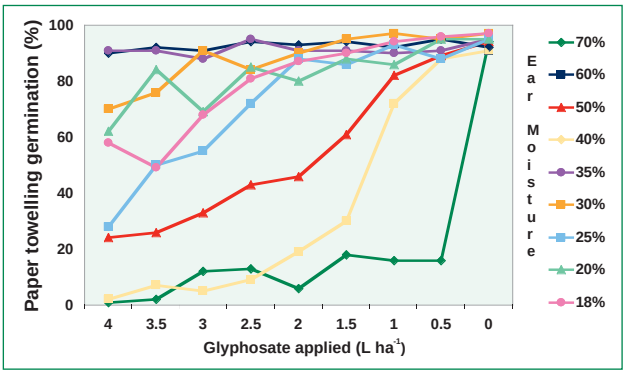
Figure 2.6 The relationship between rainfall after glyphosate application and the level of glyphosate-induced abnormalities in the resultant seed in paper towelling tests



The Effect of Glyphosate Application Rate

Sensitivity to glyphosate decreased as the crop matured and ripened (McLaren and Don, 2004). At an ear moisture content of 70%, glyphosate sprayed at 0.5 L ha^{-1} and above had a detrimental effect on germination but, as ear moisture content decreased, the effect of glyphosate, at concentrations as high as 4 L ha^{-1}, was minimal (Fig. 2.7).

Figure 2.7 Interactions between crop maturity and rate of glyphosate application in determining the level of glyphosate-induced abnormalities in the resultant seed



The Field Performance of Glyphosate-Affected Barley Seed

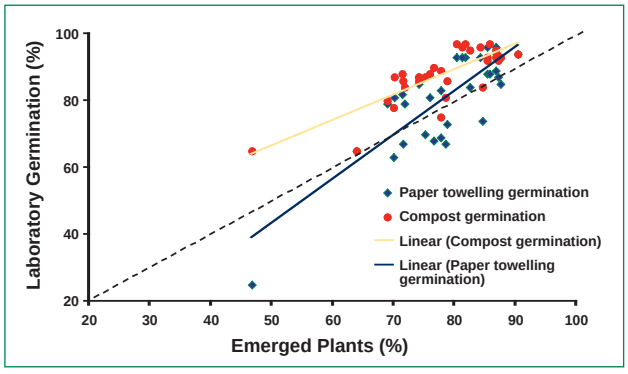
The performance of glyphosate-affected seed was investigated in emergence and establishment experiments, at Gogarbank Farm (McLaren, Don and Roberts, 2004), using samples of seed submitted to the OSTS for germination testing. These samples, of 4 varieties, were intended for sowing, and control samples, which showed no glyphosate symptoms, were also included (Table 2.1).

Table 2.1 Samples of barley used in the field performance experiment

Variety	Number of Samples	Mean Paper Towelling Germination (%)	Mean Compost Germination (%)
Chalice – Glyphosate Symptoms	3	81	91
Chalice Control	2	93	96
Decanter – Glyphosate Symptoms	3	83	88
Decanter Control	2	94	94
Optic – Glyphosate Symptoms	13	74	85
Optic Control	2	93	97
Riviera – Glyphosate Symptoms	5	74	82
Riviera Control	2	96	93

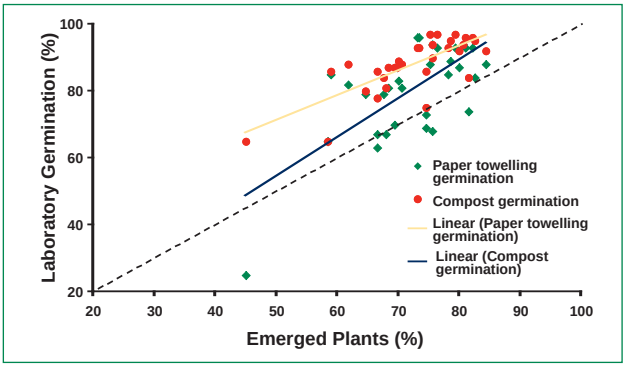
There was a clear relationship between field emergence and both paper towelling (r = 0.80; p<0.001) and compost (r = 0.81; p<0.001) germination tests. However, when laboratory germination test results were plotted against field emergence, it was clear that the compost test over-predicted the field emergence, with all but two samples having a field emergence that was lower than the compost germination (Fig. 2.8). For the paper towelling germination results, there was a more even distribution of the results, with 34% of samples under-performing and 66% of samples achieving a higher emergence than predicted. For both paper towelling and compost results there were considerable differences between samples with similar levels of germination. For example, samples with germinations of between 65% and 93% achieved similar levels of field emergence.

Figure 2.8 Relationships between laboratory germination test results and emerged plants in the field



As with field emergence, there was a clear relationship between field establishment, number of plants reaching maturity in the crop, and both paper towelling (r = 0.72; p<0.001) and compost (r = 0.79; p<0.001) germination test results. When plotted against field establishment, it was clear that the compost test over-predicted, with all samples having a field establishment that was lower than the compost germination (Fig. 2.9). For the paper towelling germination results there was a more even distribution of the results with 25% of samples over-performing and 75% of samples achieving a lower establishment than predicted by the paper towelling result. For both paper towelling and compost results there were considerable differences between samples with similar levels of germination. For example, samples with germinations of between 68% and 94% achieved similar levels of field establishment

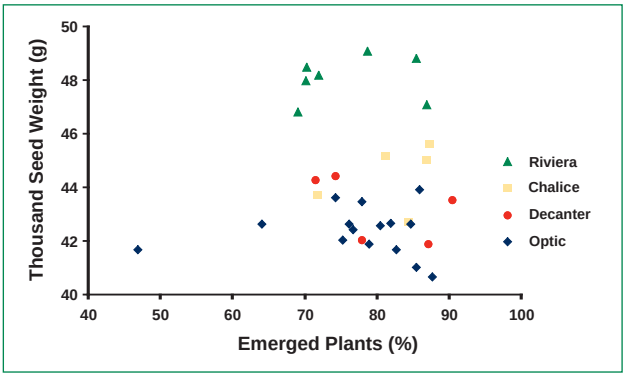
Figure 2.9 Relationships between laboratory germination test results and established plants in the field



The Quality of Seed Harvested from Crops Sown with Glyphosate- Affected Seed

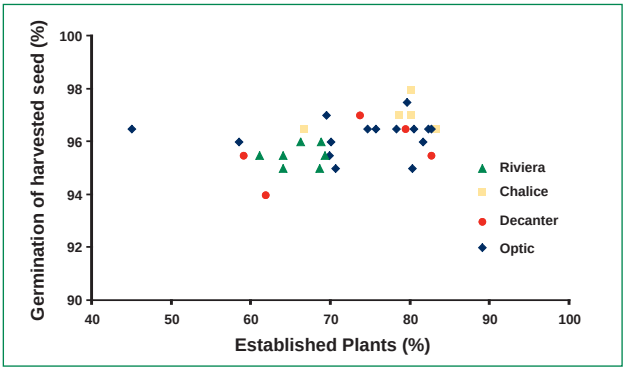
There was no evidence that the thousand seed weights of harvested seed were related to either field emergence (p=0.96) or field establishment (p=0.65). For example, similar thousand seed weights of 41.7g were obtained where seedling emergence ranged from 46.8% to 82.6% (Fig. 2.10). However, there were clear differences in thousand seed weight between the varieties, irrespective of the field emergence or establishment. Between samples of individual varieties there were no significant differences in thousand seed weight.

Figure 2.10 Relationships between emerged plants in the field and thousand seed weight of harvested seed



The overall mean germination of the harvested seed was 96.1% and, using tolerance tables (Miles, 1963), there was no significant difference between the highest (98%) and lowest (94%) germinations. Similar germinations of 96.5% were obtained where seedling establishment ranged from 45% to 83.2% (Fig. 2.11).

Figure 2.11 Relationships between established plants in the field and the germination of the harvested seed



Conclusions

Pre-harvest application of glyphosate can have a detrimental effect on the quality and performance of barley seed. There would appear to be two separate effects of glyphosate treatment. When applied at ear moisture contents greater than 35% there was a direct biochemical effect on the seed that was irreversible and resulted in seed death. At these high moisture contents there was no significant difference between the results of germination tests conducted in paper towelling and compost media. At moisture contents less than 35% the effect was physiological, resulting in the occurrence of glyphosate-induced abnormalities. Testing in compost lowered the occurrence of these abnormalities.

The frequency of glyphosate-induced abnormal seedlings was related to ear moisture content and the level of rainfall experienced in the period immediately after glyphosate application. There was also an interaction between rate of application and crop maturity. When applied at an ear moisture content of 70% or more, glyphosate concentrations as low as 0.5 L ha^{-1} reduced the germination of the resultant seed to less than 20%. On the other hand, this concentration has a minimal effect on crops of ear moisture contents of 60% or less. At concentrations above 2 L ha^{-1}, and ear moisture contents less than 50%, rainfall experienced after spraying was the most significant factor in determining seed viability.

The reduction in germination of seed from crops receiving pre-harvest glyphosate treatment was minimised when seed was tested in compost rather than paper towelling media. However, compost germination tests over-estimated the field emergence and establishment of glyphosate- affected seed and this was most pronounced in seed lots with the lowest germinations. The paper towelling germination test result may be more relevant when used with precision sowing systems where accurate predictions of emergence and establishment are required. Seed identified as having glyphosate-induced abnormalities in paper towelling tests are of lower vigour. When subjected to the stresses of field conditions, these low vigour seed are less likely to emerge and those that emerge are less likely to establish to maturity (McLaren, Don and Roberts, 2004).

The effect of glyphosate on the performance of barley seed was limited to the seed produced from crops receiving pre-harvest treatment. The germination and thousand seed weight of harvested seed was unaffected by the quality of the mother crop and whether its performance, in terms of field emergence and establishment, was affected by pre-harvest glyphosate treatment.

References

Anon. 1996. *Glyphosate Fact Sheet. Pesticide News,* **33**.

Anon. 1998. *Roundup Biactive Product Guide,* Monsanto plc.

Anon. 2004. *International Rules for Seed Testing,* Edition 2004. The International Seed Testing Association (ISTA), P.O. BOX 308, 8303 Bassersdorf, CH-Switzerland.

Don R, Bartz J, Bryant GFM, Geffen Av, Lunn G, Overaa P, Steiner AM. 1990. Germination and tetrazolium testing of treated barley seed samples from glyphosate treated crops in seven ISTA stations. *Seed Science and Technology* **18**: 641-651.

Kerr J, Snowden JP. 2001. *Arable crops 2000.* Pesticide Usage in Scotland Report. Edinburgh: Scottish Agricultural Science Agency.

Miles S R. 1963. Handbook of tolerances and measures of precision for seed testing. *Proceedings of the International Seed Testing Association* **28**: 520 – 686.

McLaren G, Don R. 2002. The effect of glyphosate treatment on the germination potential of resultant crops. *Proceedings of Crop Protection in Northern Britain 2002,* 103 – 108.

McLaren G, Don R. 2004. The effect of glyphosate treatment on the germination potential of barley seed. *Proceedings of Crop Protection in Northern Britain 2004,* 153 – 158.

McLaren G, Don R, Roberts AMI. 2004. The performance of glyphosate affected barley seed. *Proceedings of Crop Protection in Northern Britain 2004,* 223 – 228.

Snowden JP, McCreath M. 1997. *Arable crops 1996.* Pesticide Usage in Scotland Report. Edinburgh: Scottish Agricultural Science Agency.

Snowden JP, Thomas, LA. 1999. *Arable crops 1998.* Pesticide Usage in Scotland Report. Edinburgh: Scottish Agricultural Science Agency.

Snowden JP, Thomas, LA. 2003. *Arable crops 2002.* Pesticide Usage in Scotland Report. Edinburgh: Scottish Agricultural Science Agency.

2.3 The Incidence of Loose Smut (*Ustilago nuda*) in Scottish Barley Seed Lots (Valerie Cockerell, Moray Anderson and Margaret Jacks)

The fungus *Ustilago nuda* causes loose smut of barley. The fungus is present as mycelium within seed embryos, lying dormant until the seed is sown. As the seed germinates, the fungus is carried systemically within the plant and infects the ear at an early stage of development. Instead of grain, masses of black fungal spores are produced. The spores are released as the ear emerges and are spread by the wind on to open flowers, infecting developing grain on healthy plants.

Loose smut has been controlled in certified seed since the introduction of compulsory certification for marketed seed in 1975. The maximum permitted infection in a seed lot is 0.5% for seed sold at the EC standard but most Scottish spring barley seed is certified at the UK Higher Voluntary Standard (HVS), where the maximum permitted infection is 0.2% for certified seed of the first and second generation and 0.1% for basic seed. Where growers choose to use farm-saved seed, a threshold of 0.5% (based on EC standard seed) is advised. Seed with greater than 0.5% should either be discarded or treated with a fungicide that controls loose smut.

In recent years, levels of loose smut have been noticeably higher in samples of spring barley seed tested at SASA for advisory and statutory purposes and in spring barley certification plots. To determine the extent of the problem, loose smut data from seed samples submitted to the OSTS for testing by growers and producers, together with data from plots sown from seed of certified seed lots as part of the UK Seed Certification Scheme, have been examined.

Seed Samples

Data from testing from 1992-2003 support the observation that loose smut levels have increased in recent years (Table 2.2 and Table 2.3). The mean percentage of farm-saved winter barley samples with more than 0.2% loose smut, in the period 2001-2003, was 14% compared with 4% during 1993-2000. This is, however, significantly less than in 1992 when 35% of samples had greater than the 0.2% HVS standard. The results for certified winter barley seed suggest a similar increase but few samples have been received in recent years. Overall, incidence of loose smut infection was significantly higher (P=0.006) in farm-saved winter barley samples than in certified samples.

Table 2.2 Incidence of loose smut in farm-saved and certified winter barley samples tested at the Official Seed Testing Station 1992-2003

	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003
Farm-saved												
Percentage samples infected	58	13	15	25	14	9	15	19	8	21	28	39
Percentage samples >0.2%	35	4	8	3	6	2	4	4	2	12	13	16
Percentage samples >0.5%	27	2	8	0	6	1	0	1	2	7	9	8
Number of samples tested	57	45	26	32	36	76	55	100	94	78	78	88
***Certified seed**												
Percentage samples infected	8	5	7	13	10	3	6	2	5	13	16	17
Percentage samples >0.2%	3	0	0	3	0	0	0	2	0	7	4	0
Percentage samples >0.5%	0	0	0	3	0	0	0	2	0	0	4	0
Number of samples tested	37	41	41	38	51	64	50	41	19	15	25	18

*The category 'certified seed' includes samples intended for sale as certified seed submitted by merchants and certified samples taken as part of SEERAD's enforcement programme.

Table 2.3 Incidence of loose smut in farm-saved and certified spring barley samples tested at the Official Seed Testing Station 1992-2003

	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003
Farm-saved												
Percentage samples infected	21	14	10	17	16	14	19	20	26	29	40	35
Percentage samples >0.2%	9	0	7	13	6	2	5	6	8	13	17	14
Percentage samples >0.5%	4	0	2	13	2	0	5	3	6	8	11	8
Number of samples tested	73	78	61	24	51	95	109	86	117	168	176	159
Certified												
Percentage samples infected	7	9	6	4	9	6	18	30	13	14	25	30
Percentage samples >0.2%	1	1	0	1	1	0	3	6	3	5	6	11
Percentage samples >0.5%	1	0	0	1	1	0	2	3	0	4	3	7
Number of samples tested	104	100	109	137	132	50	89	90	103	162	128	130

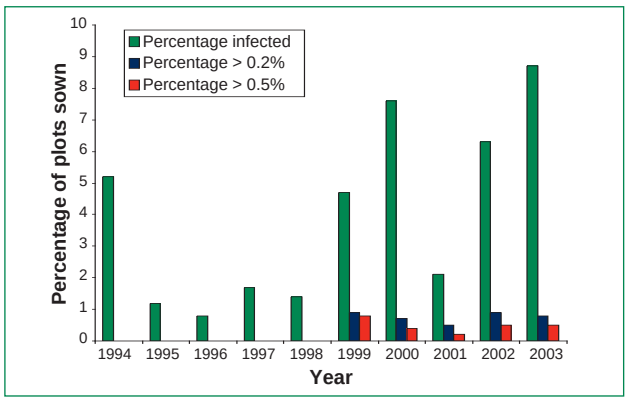
*The category 'certified seed' includes samples intended for sale as certified seed submitted by merchants and certified samples taken as part of SEERAD's enforcement programme.

As with winter barley, the incidence of infection has increased in both farm-saved and certified spring barley samples. Farm-saved seed had significantly higher (P<0.001) levels of loose smut than certified seed in the years 1992-1997, but there was no significant difference (P=0.175) between levels in farm-saved seed and certified seed between 1998-2003. Infection levels for farm-saved seed were highest in 2002, with 11% of samples having levels greater than 0.5%. For certified seed the highest levels were found in 2003, with 7% of samples having levels greater than the 0.5% standard. The highest infection level recorded for an individual sample of farm-saved seed in the years 2001-2003 was 19.3% and, for a certified seed sample, 6.6%.

UKSCS Certification Plots 1994-2003

Of the 1,710 plots of winter barley sown in the ten years, 1994-2003, no plots failed to meet the 0.2% standard and there was no increase in the percentage of plots with infection from 1994 to 2003. The percentage of spring barley plots with loose smut infection increased significantly from 1999 onwards (Fig. 2.12), with the level of smut found in some plots greater than the 0.5% limit for EC standard seed. The highest level of infection found over the ten-year period was 10.8% in a plot of Chalice spring barley in 2003. It should be noted that most barley seed lots are certified after fungicide treatment and that a high proportion of plots are sown with a fungicide seed treatment that controls loose smut.

Figure 2.12 The incidence of loose smut found in SASA Certification plots and the proportion failing to meet the EC (0.5%) and HVS (0.2%) standards



Previous Surveys

Previous surveys of loose smut in Scotland (Rennie, 1987; Rennie and Cockerell, 1990) have shown that, in general, farm-saved barley has a higher incidence of loose smut than certified seed. However, infection levels were particularly high in certified seed during the period 1980-1985 when over 36% of winter barley samples and 20% of spring barley samples did not meet the 0.2% HVS standard. High levels were attributed to varietal susceptibility (many stocks of certified seed of the variety Panda had over 5% seed infection) and the incomplete control of loose smut by the systemic fungicide carboxin. By 1990, Cockerell and Rennie reported a reduction in the incidence of loose smut in Scottish barley seed produced for certification. This was most likely to be due to the use of more effective fungicides during seed multiplication than during the period 1980-85 when carboxin was shown to provide incomplete control. During the 1980s and early 1990s, growers were less likely than merchants to apply a seed treatment that controlled loose smut infection because of the relative costs of fungicide products. Those effective against loose smut were at least 10 times the price of those that were not effective. It was, therefore, more likely that higher levels of seed infection would be found in farm-saved seed than in certified seed.

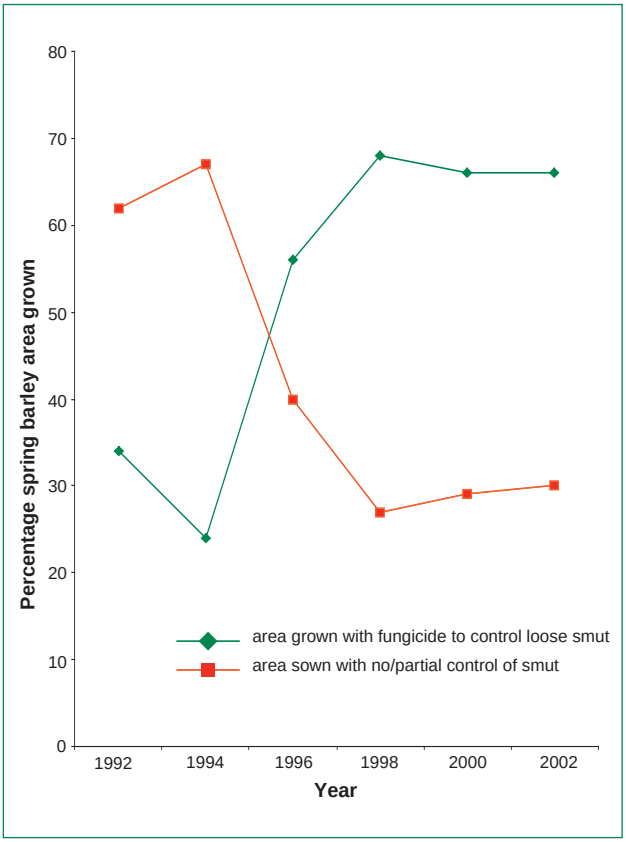
Evidence from these earlier surveys suggests that it is possible that the increase in loose smut observed in spring barley in the 1992-2003 survey was a result of either changes in the use of/or effectiveness of fungicides to control loose smut, or a shift in the market to more susceptible varieties.

Fungicide Use

Data from SASA's pesticide usage survey reports (*Section 8.1*) show an increase since 1994 in the area of spring barley sown with fungicides that control loose smut effectively, and a decline in the area sown without effective control (Fig. 2.13). The introduction of low-cost fungicides that give control of the major diseases of barley, including loose smut, from the mid-1990s accounts for the increase in effective fungicides. Although the area without effective control of loose smut has declined, the area of spring barley sown untreated increased from 2% in 1996 to 7% in 2002 (Snowden and McCreath, 1997; Snowden and Thomas, 2003). The rise in untreated area can be accounted for by an increase in organic grain production and an increase in the number of growers choosing to sow seed untreated where their seed meets recommended thresholds for the major seed-borne diseases.

Where growers sow untreated seed for more than one generation without managing loose smut levels, through testing and treatment, there is a risk of increased inoculum levels. The spread of spores from infected crops to healthy crops will lead, potentially, to an increase in overall levels of loose smut. Similarly, if spring barley crops are sown with fungicides that give no control of smut, or do not control the disease effectively, increases in smut levels may be seen.

Figure 2.13 Trends in seed treatments used on Scottish spring barley (SASA Pesticide Usage Reports)



Varieties

It is difficult to compare the annual incidence of loose smut in individual varieties as the number of samples of each variety submitted varies among years, reflecting variety popularity, but by considering only the varieties, for which 10 or more samples were submitted each year, it is possible to examine changes over time (Fig. 2.14). Optic has increased in popularity since 1995 becoming by far the most popular from 2000 onwards. A few varieties have remained popular (e.g. Tyne, Delibes and Maresi), whereas others have been popular and then declined, (e.g. Derkado in the early nineties followed by Prisma and Chariot in the mid nineties). Chalice, Decanter and Riviera have been more widely grown since 1998. Comparison of levels of loose smut infection in each variety against the overall levels in the spring barley samples submitted (Fig. 2.15) shows that the increase in overall level of smut is not associated with the most poplar variety Optic. There does, however, appear to be a relationship between increasing infection levels and the levels of infection found in Chalice and, to a lesser extent Decanter and Riviera. The variety Chalice accounted for 82% of certification plots where infection was greater than 0.2%.

Figure 2.14 Comparison of the overall percentage of spring barley samples with more than 0.2% loose smut and those of individual varieties

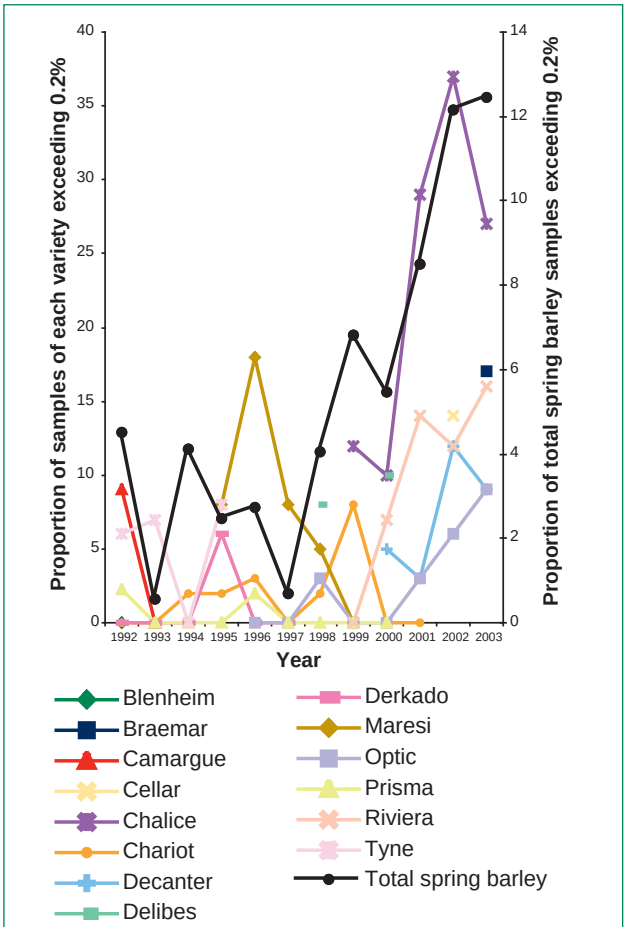
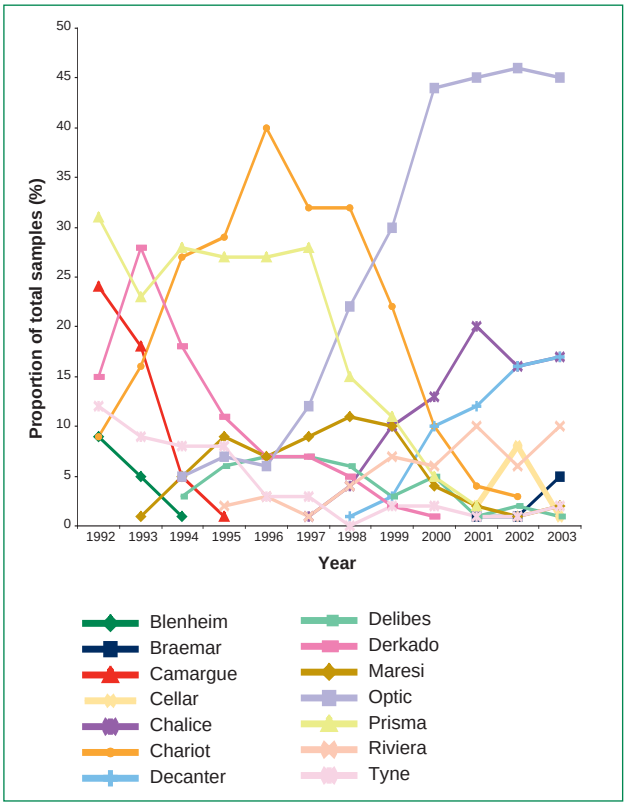


Figure 2.15 Changes in the proportions of spring barley varieties submitted to the OSTS for testing 1992-1993



Conclusions

The incidence of loose smut in Scottish barley varies according to season and variety. From the data presented it can be concluded that the introduction and popularity of more susceptible varieties since 1997 has contributed to the high levels of smut recorded in the survey. Unfortunately disease resistance scores for loose smut are not available in the HGCA Recommended List for cereals since, if data had been available, growers might have dealt with the multiplication of susceptible varieties in a more appropriate way. The increased use of seed treatments that are effective against loose smut since 1994 has not prevented an increase in the number of samples infected above the HVS standard in either certified or farm-saved seed. However, where untreated seed or seed with a seed treatment not effective against loose smut is multiplied for more than one year, these crops may contribute to the increasing levels of smut found in the seed. In addition a number of growers in Scotland have revealed that they use reduced levels of fungicides, which do not give the same level of control of loose smut as full rate application.

Over the 12-year survey, most spring (83-98%) and winter (65-98%) barley seed lots met the HVS loose smut standard of 0.2% permitted infection. However, in some years, a significant proportion did not, with some samples tested having more than 10% infection in both certified and farm-saved seed. Although it is unlikely that seed with less than 1% infection will result in a yield loss, the disease can spread not only within the crop but also to crops on neighbouring farms, potentially reducing the quality of seed produced.

To maintain low levels of smut in their growing crops, growers and merchants must manage loose smut. The following options are available to ensure a healthy crop.

- Treat all seed with a fungicide effective against loose smut.
- Test seed.
  - If test result meets seed standard,

a) a treatment that does not control loose smut is adequate.

b) consider sowing seed untreated if leaf stripe meets voluntary standard (2%) and the germination is above 85%.
  - If test result above standard,

a) do not use as seed

b) use an effective seed treatment.

References

Rennie WJ. 1987. Incidence and control of loose smut in Scottish barley 1980-1985. *Proceedings of Crop Protection in Northern Britain 1987,* Dundee 15-17 March, 85-90.

Rennie WJ, Cockerell V. 1990. Incidence of loose smut in Scottish barley 1986-1988. *Proceedings of Crop Protection in Northern Britain 1990,* Dundee 20-22 March, 135-138.

Snowden JP, McCreath. 1997. *Arable Crops 1996,* Pesticide Usage in Scotland Report, Edinburgh: The Scottish Office Agriculture, Environment and Fisheries Department.

Snowden JP, Thomas LA. 2003. *Arable Crops 2002,* Pesticide Usage in Scotland Report, Edinburgh: Scottish Executive Environment and Rural Affairs Department.

3 Varieties

3.1 Introduction (S Cooper)

3.2 The Role and Storage of Genetic Resources in Cereal Section (R Tulloch)

3.3 Distinctness, Uniformity and Stability Tests for Establishing Plant Breeders' Rights in Parsnip (*Pastinaca sativa* L.) (FN Green, M Thomson, GD Campbell, L McCarthy, G Hull, AMI Roberts and GW Horgan)

3.4 Testing Pea Cultivars (*Pisum sativum* L. *sensu lato*) for Distinctness, Uniformity and Stability in the UK since 1968. (FN Green and T Christie)

Chapter 3 Varieties

3.1 Introduction (S Cooper)

The testing of varieties for Distinctness, Uniformity and Stability (DUS), either to enter varieties on to a National List, or for the granting of Plant Breeders' Rights, began in the UK in 1965 with cereals.

Other crops were gradually phased in, with peas, the subject of the third paper in this section, being tested since 1968. Paper 3.4 describes the different types of pea variety in cultivation and how the use of the harvested product is determined by different varietal characteristics. It also charts how fresh peas were replaced by vining peas in the vegetable sector in the 1960s and how combinable peas became an increasingly important source of protein in the late 1970s.

SASA ceased to be a DUS test centre for cereals after a UK-wide review in 1988. Nevertheless, since then, it has had access to cereal variety candidates in their second year of test so that they can be characterised botanically under Scottish conditions for seed certification purposes. This material, together with the official reference collections held by SASA before 1988, forms the basis of a large well-characterised collection of cultivated cereal varieties including heritage varieties. The first paper (3.2) describes how this material is characterised and stored, including a glimpse into future work on genetic fingerprinting.

SASA recently became the EU DUS test centre for parsnips. The second paper (3.3) describes how a DUS testing regime for this crop was set up from scratch – a broadly similar approach would be needed by any organisation undertaking DUS tests for any new crops/species. It illustrates how the different components of the process – trial design, character set, recording of characters and statistical analysis, together with a comprehensive reference collection – all interact to produce the final result.

3.2 The Role and Storage of Genetic Resources in Cereal Section (R Tulloch)

The genetic resources held by the Cereal Section of SASA have been collected over the last 40 years and are held in three collections: herbarium cards, herbarium ear storage and a genebank.

From the 1960s until 1989, SASA was one of three test centres for DUS (Distinctness, Uniformity and Stability) for cereal crops in the United Kingdom. Once a variety was entered for testing, a sample of seed was received from the breeder for testing using the protocols of the time. Some of the test material was harvested and herbarium cards were produced to allow further study of the variety. Any harvested material remaining, after the production of a herbarium card, was placed in herbarium ear storage. If the variety passed DUS and VCU (value for cultivation and use, which was tested elsewhere) testing successfully it was entered into the National List.

In the 1960/70s, Cereal Section did not have a genebank of frozen material. All varieties on the National List, those used regularly in commerce, or landraces (traditional varieties) were grown each year in a living collection (together the varieties were termed as "common knowledge"). The living collection grew in size each year as more varieties were added to the National List. This made the living collection difficult to maintain, in terms of size and staff hours. In 1979 common knowledge was altered, and Cereal Section was no longer required to grow each variety every year. The cereal genebank at SASA was set up at that time to allow all the varieties that were grown in the living collection, but that were no longer on the National List, to remain in the collection.

In the late 1980s, an interdepartmental review led to a reduction in the number of DUS test centres in the United Kingdom to one – in Cambridge, resulting in a reduction in the workload in Edinburgh and Belfast.

The Cereal Section at SASA continued to build its genetic resources until the changes in the 1980/90s, when SASA became an executive agency of SEERAD. The Service Level Agreement (SLA) between SASA and SEERAD allows for the continuation of a variety collection of cereal crops, now in support of cereal certification. The agreement stipulates that the genetic resources must be based in Scotland and cover traditional Scottish varieties and all new varieties that are grown in Scotland.

SASA is relocating to a purpose built facility in 2006 and this will affect the storage of genetic resources.

Herbarium Cards

Once varieties reach the end of testing, herbarium cards are produced using material grown at Gogarbank Farm in 7 row plots (6.73m, or 22ft in length). Each variety is harvested by hand with 125 ears (barley and oats) or 105 ears (wheat) cut from different plants, chosen randomly throughout the plot, and then air-dried. Each variety has two herbarium cards made from black mounting board measuring 17.5cm by 12.5cm: a grain card and a rachis card for barley and wheat and two grain cards for oats. The variety name/breeders designation, AFP number (each variety is assigned an accession number as a variety may have several names through its life, but the AFP number remains unchanged), card number, the plot number and year of harvest are recorded in the top right of the card using a white pen. Ears from the selected bundle are then dissected to give the required parts, which are then glued to the card using "Copydex" glue. This glue was chosen as it is visible when wet, allowing ease of use, but dries clear, so that it does not obscure the material mounted on the card. The glue is also flexible, allowing the mounted items to be moved slightly during characterisation without damaging the card integrity. Once the card is completed it is studied using a microscope and the states of the characters shown on the card are recorded on a Varietal Description Card.

Once the completed herbarium card has been studied it is given a unique, sequential number, which is recorded on the Variety Maintenance Card. The herbarium cards are then stored in drawer units for future reference. Examples of completed herbarium cards can be seen in Figs. 3.1, 3.2 and 3.3.

Figure 3.1 Example of a Completed Wheat Herbarium Card

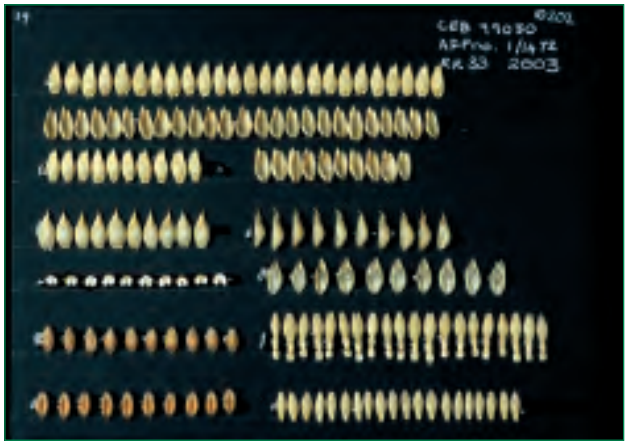


Figure 3.2 Example of a Completed Barley Herbarium Card

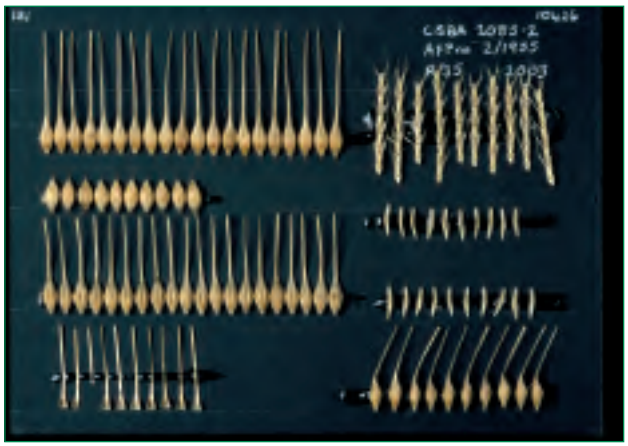
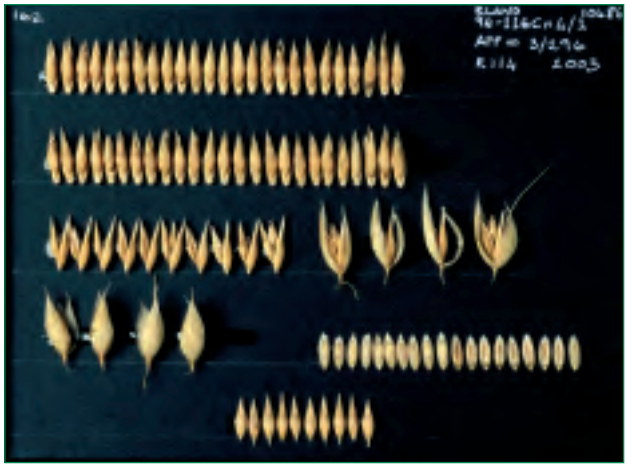


Figure 3.3 Example of a Completed Oat Herbarium Card



Herbarium Ear Storage

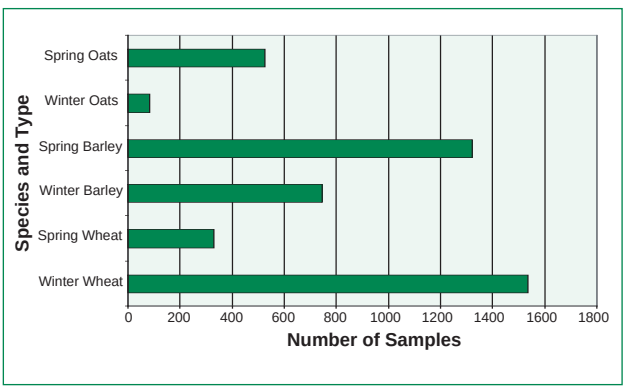
Once the herbarium card for a variety has been completed, the remainder of the harvested bundle and any surplus bundles are placed in the herbarium ear storage, which consists of cardboard boxes measuring 13 x 15 x 50cm, on fixed wooden and metal shelving units. Each variety entered for DUS and VCU testing is allocated a box, the boxes are numbered sequentially so that the varieties can be placed in AFP number order, and the number of each variety is recorded on its Variety Maintenance Card.

The room in which the boxes are stored is heated by wall mounted fan heaters, to protect the room from freezing in winter. The humidity is not controlled or monitored in the room and, although the room is water tight, humidity levels can vary according to weather conditions. As such changes may lead to fungal infections, such as *Fusarium* spp., Cereal Section staff monitor the condition of the collection regularly (HGCA, 2003). The room is also monitored by Cereal Section staff and outside contractors for signs of insects, such as grain weevils, and vermin infestation, such as mice. (HGCA, 2003) The herbarium store planned for the new building is designed to overcome these problems: the room will be air-conditioned, controlling the temperature and humidity of the air, which limits insect activity and fungal infections. Should the area become infected it will be easy to fumigate as it has been designed for complete air sealing and extraction.

Genebank

All varieties sent to SASA as final year DUS candidates are selected for addition to the genebank (Fig. 3.4). The seed arrives at SASA from breeders *via* the National Institute of Agricultural Botany (NIAB) or the Scottish Agricultural College (SAC) in 400g bags. Upon arrival, the samples are sorted into AFP order and a Microsoft Excel spreadsheet produced; this is used to create sowing and freezer plans. This spreadsheet is also used to produce labels for the storage containers used in the freezer. Varieties are also added if they are maintained on the Common Catalogue (which contains all European Community countries' national lists together) and grown in Scotland using seed obtained from the variety breeder or maintainer.

Figure 3.4 Number of accessions held in the cereal genebank, SASA



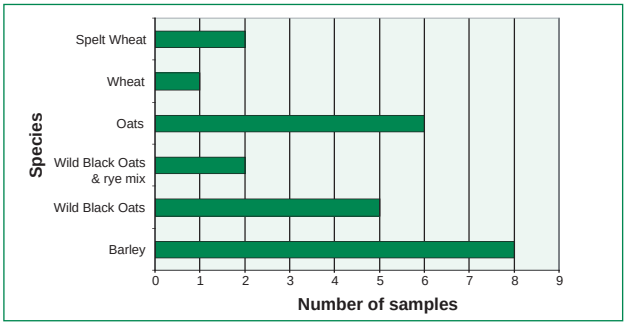
Once the seed has been sorted it is tested for moisture level: one sample each from the DUS and the VCU seed is selected at random and tested using a Prometer moisture meter. If the seed has a water content above 5% it is air-dried and, once the level is 5% (FAO/IPGRI, 1994) or below, packaging can commence.

The seed is used to grow plots (for herbarium harvesting and green characterisation) and 3.35 metre rows (the living collection). The remaining seed (approximately 200g) is used for genebank storage. A 60g Nalgene container (High-density polyethylene container with a leak-proof, screw lid) is filled and packed with seed and sealed tightly. The remaining seed (approximately 140g) is placed in a 250g Nalgene container (same specifications as before) with any air gap filled with cotton wool. The 60g container is placed in the main genebank and the 250g container in the safety duplicate genebank. Each container has two labels; the main label attached to the outside of the container is a laser printable polyester label, which is water resistant for durability in the freezer. Should the outer label become damaged each container also has an inner label, which are inserted in the clear plastic container with the writing facing out.

The main genebank is located in freezers (-20°C) within the Cereal Shed at East Craigs and the safety duplicate genebank is located in freezers (-20°C) in Overgogar House at Gogarbank Farm. The duplicate is kept at a separate location to reduce the risk of total loss of the genebank owing to fire or electrical failure. The duplicate genebank has been operating for three years and is therefore much smaller than the main genebank, but it is increasing as new varieties are added and the process of regenerating varieties from the main bank continues.

The genebank also houses the Scottish Heritage Collection of traditional and landrace varieties from Scotland, which have been grown over the last fifty years (Fig. 3.5). There was a risk of these varieties disappearing completely from agricultural use, but there has been an increase in interest in recent years. SASA is actively searching for samples of traditional varieties such as bere barley and strigosa (black) oats. The samples that are currently in the collection are being stored for three main purposes. They are available for study, both as seed and as green material, and they act as a depository for growers: if their crops fail they can restock with seed that is adapted to their region. Finally the Diagnostic and Molecular Biology department at SASA generates a genetic fingerprint of each sample, which may show differences in the crops among geographical areas, and any population differences within each sample.

Figure 3.5 Number of traditional varieties held in the Scottish Heritage Collection



Cereal Section stores its data on a purpose built computer system known as the Cereal Certification Management System (CCMS). As varieties are placed in the genebank their details are added to CCMS, including variety name (approved and breeders designation), AFP, first frozen year, seasonalilty, species and freezer number.

Each year a random sample is taken from the oldest stock and tested for germination, by the Official Seed Testing Station (OSTS) at SASA using the tetrazolium method. This gives a quick (within 24 hours) and reliable estimate of germination levels (the percentage of grains that would germinate) The results from these tests are used to decide if the year and species tested should be regenerated. If the result is less than 80% viability then all the entries of the species for the year tested are regenerated. Wheat is currently being regenerated after a period of 20 years in the freezer.

The Future

SASA is due to relocate to a purpose built facility by 2006 and this should allow the genetic resources of the Cereal Section be managed more effectively. The Plant Varieties and Seeds wing of the new building is to feature drying and freezer rooms.

In the future, herbarium cards could be digitally photographed, or scanned, and the image stored electronically, reducing the amount of storage required by the section. A photograph does have its limitations, as it is difficult to measure depth. An alternative, for certain herbarium card measurements, is an automated measurement system: a photograph is used to input data for each variety into the computer and a program is used to alter the image into a measurable image. There are problems with this type of technology, such as the need to distinguish the small changes in length and depth of a grain, which distinguish one variety from another. NIAB designed a system to overcome these problems that worked in the short term, but was not practical for long term use. This form of herbarium card may be used in the future, but at the present time it is not particularly effective for use on cereal varieties.

Some of the visual characters studied on herbarium cards are dependent on the environmental conditions and are therefore not stable from year to year, and others vary between geographical locations. (Such as Scotland and Southern England). Work is in progress at SASA and elsewhere to record the genetic fingerprints of cereal varieties, which could reduce the reliance on visible characteristics. The main problem with this technology is variation and cross-pollination. Each character studied has a range of possible states. For example, the possible states could be Small, Medium or Large. For a specific species, a character may be described as “medium to large”, because most of the plants exhibit the character in the medium state, but some as large. At the present time it is not known if these differences are due entirely to environmental factors, or if some are due to a genetic mixture within the population. If the differences are purely due to environmental factors, then genetic fingerprinting will distinguish varieties easily, but if the differences are due to genetic differences within a variety then genetic fingerprinting would not separate varieties as easily.

References

FAO/IPGRI. 1994. *Genebank Standards.* Rome; Food and Agricultural Organisation of the United Nations/International Plant Genetic Resources Institute.

HGCA. 2003. *The Grain Storage Guide.* Home Grown Cereals Authority.

3.3 Distinctness, Uniformity and Stability Tests for Establishing Plant Breeders' Rights in Parsnip (*Pastinaca sativa* L.). (FN Green, M Thomson, GD Campbell, L McCarthy, G Hull, AMI Roberts and GW Horgan)

Background

Plant Breeders' Rights are a form of intellectual property designed to protect new cultivars (varieties) of plants, and may be granted by a national authority, e.g. UK, or by an international authority, e.g. European Union. Since 1994, European Plant Breeders' Rights legislation (EC, 1994) has increasingly been used instead of national legislation, as a cultivar can be protected throughout the European Union. A cultivar is eligible for an award of Plant Breeders' Rights if it is Distinct, Uniform and Stable (DUS), has an approved name and is novel (new). A DUS test involves the submitted candidates being grown and compared with other similar cultivars in field trials. At least two independent growing periods are required.

Plant Breeders' Rights entitle the holder to prevent anyone from producing or multiplying, offering for sale, selling or marketing, exporting, or importing the protected cultivars without authority. The basis for cultivar protection internationally, is the International Convention for the Protection of New Varieties of Plants (UPOV, 1991), which was first established in 1961 with subsequent revisions in 1972, 1978 and 1991. The first parsnip applications for European Plant Breeders' Rights (EUPBR) were submitted to the Community Plant Variety Office (CPVO) in 2000. SASA was appointed the sole centre for testing DUS for EUPBR parsnip applications, and started testing in 2001.

As parsnip is not subject to seed marketing regulations in the European Union, there is no requirement for a National List. As a result, there is no register of known cultivars, and there are no official descriptions or seed reference samples. When EUPBR tests started, because only two cultivars were known to have been submitted for national registration or protection, the available information was very limited. Before DUS testing could start, there was a need to create a reference collection of known cultivars, and to define a set of characteristics to describe and discriminate among them. The testing of the candidates submitted for EUPBR was undertaken simultaneously with the characterisation of all available cultivars in commerce.

Creating a Seed Reference Collection

A search of seed catalogues and seed company websites established that there were 26 cultivars used or marketed for processing or for sale in supermarkets. National DUS test and certification authorities were asked for names of cultivars for which seed was sold in their countries, but this did not produce any new names. Requests for seed were sent to all known maintainers and suppliers to clarify whether cultivars were being actively maintained, and to inform them that samples received would be treated as 'definitive' for Plant Breeders' Rights purposes. Seed of 24 cultivars and four candidates was received in 2001 and a further 14 cultivars were obtained in 2002 from the UK Vegetable Genebank at Horticulture Research International and from suppliers of 'heritage' seed for the amateur market. Currently the collection comprises 46 different accessions.

As parsnip germination can deteriorate from 100% to 0% in one year if seed is not kept under optimal conditions, all accessions were tested by the Official Seed Testing Station at SASA to determine the germination percentage. 100 seed weights were also measured for each accession, so that the quantity of seed to be sown to establish an even plant density in field trials could be calculated. Most seed accessions received were in good condition; 69% of accessions had over 80% germination, and 85% had over 70% germination. All seed was stored in a deep freeze (-22°C) to minimise the rate of deterioration and retain long term viability.

Establishing an Appropriate Trial Design for DUS Testing

Plot spacing, replication, plant number, plant density, fertiliser regime and cultivation methods were varied in the first two years of testing, before an optimal four replicate, randomised block trial design was established. A bed shaper is used to create a 4 metre long raised bed, into which 3 rows, 35 cm apart, are sown for each plot (Fig. 3.6). Seed is sown using a Wintersteiger drill and seedlings are singled to a density of 10 plants per metre. Approximately 120 plants are grown per plot, and 640kg ha^{-1} of a 14-14-21 NPK fertiliser is applied.

Figure 3.6 Parsnip DUS Trial at Gogarbank Farm in 2003

An experiment, undertaken to investigate the effects of plant population density, confirmed that the higher densities used by commercial growers (target 5 cm plant spacing in the row), did not give as good discrimination between cultivars as that used in DUS tests (target 10 cm plant spacing in the row). Lower densities are not suitable for DUS testing as plant-to-plant competition is weak, and foliage is subject to damage by wind.

Defining a Set of Characteristics for DUS Testing

Parsnips are biennial plants grown for their edible fleshy roots, which develop from tissues of the taproot and hypocotyl. As there is little extension of the hypocotyl beyond the cotyledon stage, it is a true root, which is almost entirely underground. Parsnip leaves are pinnately compound, with petiolate lower leaflets. (Rubatzky *et al.,* 1999).

Initially, a draft set of characteristics was based on two other Umbelliferous crops: carrot root and celery leaf characteristics. This list was modified and refined and, in 2003, resulted in the presentation of a draft UPOV guideline for discussion at a meeting of the Technical Working Party for Vegetables. The draft, which was approved early in 2004 and will be published later this year, lists the internationally agreed characteristics that can be used in DUS tests for Plant Breeders' Rights.

Sampling and Recording Procedure

All plants in each plot were observed visually and, where there were clear differences between cultivars, these were recorded. A sample of 15 single plants from each plot was then scored and measured for continuous leaf and root variables. The sample was taken from the central row of each plot, avoiding plants at each end of the row. Before root measurement, all plants in each plot were topped (removal of leaves), lifted and washed. Undeveloped, damaged or aberrant roots were discarded. Fifteen roots were randomly sampled from the remaining plants, taking care to ensure that no atypical roots were selected.

Statistical Analysis/Methodology

Internationally-agreed statistical methods, Combined Over Year Distinctness analysis (COYD) and Combined Over Year Uniformity analysis (COYU), were used to analyse plot score values and single plant data recorded in 2001, 2002 and 2003 for Distinctness and Uniformity respectively.

Results

After two years of DUS testing, the candidates Panache, MS2, MS5 and P14 were awarded EU Plant Breeders' Rights. Palace will be awarded EU PBR in the near future, and there is one candidate, Sport, currently under test. As other cultivars in the reference collection have been commercialised, they are not novel, and ineligible for Plant Breeders' Rights.

Distinctness

After three years of trials, 29 accessions (cultivars and breeding lines) have been characterised; 25 of these were found to be Distinct following COYD analysis: Alba, Andover, Arrow, Avonresister, Countess, Guernsey, Gladiator, Javelin, Lancer, MS2, MS5, New White Skin, P14, Palace, Panache, Paragon, Patriot, Polar, Tender and True, Tenor, White Gem, White King, White Spear and two different accessions of Yatesnip.

Four accessions were not clearly Distinct (Archer, Cobham Improved, Dagger and Imperial Crown), whereas three new accessions, Merlin, Excalibur and Sport, are still being characterised.

Uniformity

The variation within cultivar populations was satisfactory when assessed using the Combined Over-Year Uniformity (COYU) analysis. No cultivars had 'off-type' plants.

Stability

Accessions received into the UK Vegetable Genebank at HRI in the early 1990s were compared with similarly named accessions received from seed companies. No significant changes in identity were noted, though there is evidence of improvement, through continual selection, of some older open-pollinated cultivars such as Tender and True and Avonresister. Such changes in cultivars cannot be controlled, without enforcement through seeds regulations.

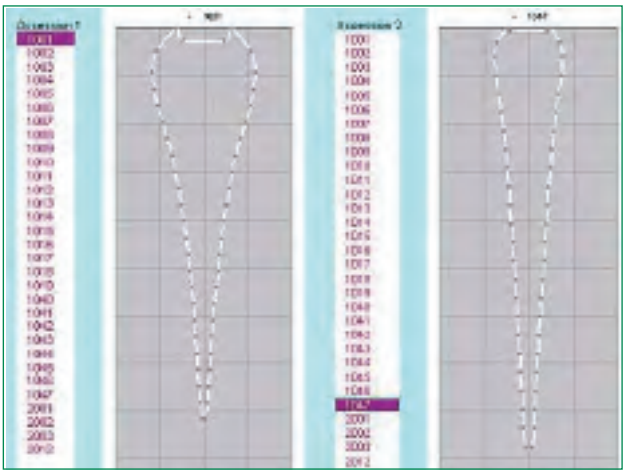
Heritage Cultivars

Small quantities of seed of fourteen accessions, not thought to be in commercial production, were obtained from genebanks and heritage seed collections: Bayonet, Bayonetta, Bedford Monarch, Cambridge Improved Marrow, Exhibition Long, Harris Model, Hollow Crown, Hollow Crown Improved, Improved Marrow, Lisbonnais, Offenham, The Student, Viceroy and White Diamond. Characterisation of these accessions will be completed in 2005 or 2006 and their descriptions will be compared with those cultivars known to be in commercial production.

Classification

There is little published information on cultivated parsnip, but classification used by commercial growers is based on three main root shapes: Bayonet (narrow obtriangular), Wedge (broad obtriangular/obovate) and Bulbous (ADAS, 1984; NIAB, 2000). This classification has, with slight modification, been adopted for use in a new UPOV parsnip guideline, which is awaiting publication. Visual separation of these types is not easy as there is a continuum of shape (NIAB, 2000), but a new mathematical tool developed by Biomathematics and Statistics Scotland (BioSS) allows the comparison of average shape from a sample of roots, which makes classification, and comparison between cultivars, easier (Fig. 3.7).

Figure 3.7 Average root shapes for any two varieties can be compared using landmark measurements derived from of images of 30 roots. 'Avonresister' (accession 1001) is obovate and 'P14' (accession 1047) is narrow obtriangular



Automatic Measurement

Automated measurement systems have recently been developed for DUS testing in the UK (Horgan *et al.,* 2004; Smith *et al.,* 2004). A joint BioSS/SASA project to develop a system to measure parsnip roots automatically using a computer, has been running in parallel with manual recording methods. This system may be the standard recording method in future, as it is suited to measuring simple dimensions, area and complex shapes, and leads to improved discrimination between cultivars over manual measurements (Fig. 3.8).

Figure 3.8 Characteristics measured automatically on a longitudinal root section:

A-B Depth of crown depression, A-D Length, C-I Width at widest point, E-F Width of crown depression, G-H Core width at widest point, L-M Width 2 cm below widest point.

Roots are cut at J-K – the point where the root is 2 mm wide.

References

ADAS. 1984. *Parsnips.* Booklet **2395.** Alnwick. Ministry of Agriculture, Fisheries and Food.

EC. 1994. Council Regulation (EC) No 2100/94 of 27 July 1994 on Community plant variety rights. Luxembourg. Office for Official Publications of the European Communities.

Horgan GW, Roberts AMI, Mann A, Green FN. 2004. Automatic measurement of Pea characteristics. International Union for the Protection of New Varieties of Plants (UPOV). Geneva, Switzerland. *Technical Working Party on Automation and Computer Programs.* Paper **TWC/22/7.**

NIAB. 2000. Descriptive Lists of Vegetables. *Vegetable Variety Handbook.* Cambridge. National Institute of Agricultural Botany.

Rubatzky VE, Quiros CF, Simon PW. 1999. *Carrots and Related Vegetable Umbelliferae.* Wallingford. CABI. ISBN 0 85199 129 7.

Smith D, Farrell R, Norris C, Jarman R, Law JR, Cooke RJ. 2004. Image analysis in DUS testing at NIAB. International Union for the Protection of New Varieties of Plants (UPOV). Geneva, Switzerland. *Technical Working Party on Automation and Computer Programs.* Paper **TWC/22/9** and *Technical Working Party for Agricultural Crops.* Paper **TWA/33/7.**

UPOV 1991. International Convention for the Protection of New Varieties of Plants. Geneva, Switzerland. *International Union for the Protection of New Varieties of Plants (UPOV).*

3.4 Testing Pea cultivars (*Pisum sativum* L. *sensu lato*) for Distinctness, Uniformity and Stability in the UK since 1968. (FN Green and T Christie)

Seed of cultivars of certain agricultural or vegetable crops can be legally sold only if registered on a National List (HMSO, 1972) and, for peas, there are separate National Lists for vegetable (Pea) and agricultural (Field Pea) cultivars. As explained in section 3.3, one of the requirements for registering a cultivar on a National List, or for awarding a Plant Breeders' Right, is that all cultivars must be Distinct, Uniform and Stable (DUS) (HMSO, 1964). Since 1968, over 900 Vegetable and Agricultural Pea cultivars have been submitted for DUS testing in the United Kingdom, the majority of which have been tested at SASA.

The pea is a self-pollinating crop with many simply inherited, discontinuous characteristics, which enable easy classification of cultivars into groups. In the UK, six Cultivar Groups and approximately 120 subgroups have been defined in order to facilitate DUS testing, which involves the comparison of new candidates with existing cultivars in the same subgroup. Although the industry selects cultivars on yield and quality characteristics, these are not used in DUS testing, as both are considered to vary with harvest time, site and season. DUS tests, therefore, have relied on morphological characteristics to discriminate new candidates. Where a large number of very similar cultivars occur in a subgroup, measurement of plants and statistical analyses of the results can be necessary to establish Distinctness.

In order to support DUS tests, the UK seed reference collection of pea cultivars is stored at SASA; it contains nearly 2,800 cultivars, which have been registered in Europe since 1968. Approximately 780 of these cultivars are currently registered or protected in the European Union. All seed samples in the collection are stored at 2°C and duplicated for safety in deep freeze storage (-22°C).

Commercial Use

Vegetable peas are usually harvested when the seed is green and immature, either as Fresh Harvest peas, where pods are picked by hand and sold without processing, or as Vining peas which are combine-harvested and mechanically shelled for canning or freezing. Agricultural peas are either grown for forage or combine-harvested when the seed is dry. Dry seed is mainly used for compounding animal feed, but a small amount of high quality seed is used for direct human consumption, being marketed in packets for re-hydration and cooking for the catering industry, or in the home.

Modern Cultivar Development

When UK National List legislation was introduced in 1973, commercial pea breeding supplied cultivars for the freezing and canning industry, although a few cultivars were used for the production of fresh harvest peas, dry seed for human consumption and for forage production. Initially the processing industry focused on canning cultivars but, with the increase in freezer ownership, freezing cultivars predominated. The processing industry demanded very high uniformity of seed colour at the fresh harvest stage, as this was an important factor determining the appearance of the pea in the freezer pack or in the can.

Although the production of Fresh Harvest peas has been declining in favour of Vining Peas since the 1960s, the recent popularity of Snap and Mangetout Sugar peas has reversed some of this decline. The growing of Vining peas increased rapidly in the 1970s, when canned and frozen products became more popular, and freezer ownership increased. Vining Peas then accounted for most of the total UK pea area, but now for only about 37% (35,000ha) (Gent, personal communication). Semi-leafless (*Afila*) combining peas were introduced in the late 1970s to produce dry seed for protein production. Within five years, the area of combining peas increased rapidly, eventually overtaking Vining peas in 1984 (Biddle *et al.*, 1988) and they now account for 63% (60,000ha) of the total pea area (Gent, personal communication).

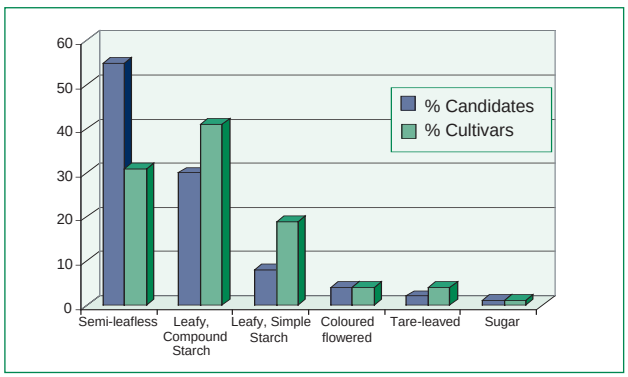
Classification of Pea Cultivars

Cultivars can be classified into six mutually exclusive Cultivar Groups (Table 3.1) using morphological characteristics, and these Groups relate broadly to commercial use. The number of cultivars in each Group varies considerably (Fig. 3.9), with *Afila* and Vining peas accounting for 70% of the total, whereas the Leafy, Simple Starch Grain Group has 19%. The number of cultivars in the remaining Groups is small, each with less than 5% of the total number of cultivars.

Table 3.1 Cultivar Groups in cultivated Pea

Cultivar Group	Defined by:
Sugar	Pod parchment absent or partially present. Plants with, or without, anthocyanin pigment.
Coloured flowered	Pod parchment entirely present. Plants with anthocyanin pigment.
Semi-leafless/Leafless (*Afila* Peas)	Pod parchment entirely present. Plants without anthocyanin pigment, leaflets absent and/or stipules reduced.
Leafy, Simple Starch Grain	Pod parchment entirely present. Plants without anthocyanin pigment, leaflets present, seeds not wrinkled. Foliage not tare-leaved.
Leafy, Compound Starch Grain (Vining Peas)	Pod parchment entirely present. Plants without anthocyanin pigment, leaflets present, seeds wrinkled. Foliage not Tare-leaved.
Tare-leaved	Pod parchment entirely present. Plants without anthocyanin pigment. Tare-leaved, foliage reduced in width and with pointed apex, stipule habit parallel, flowers with raised standard base shape.

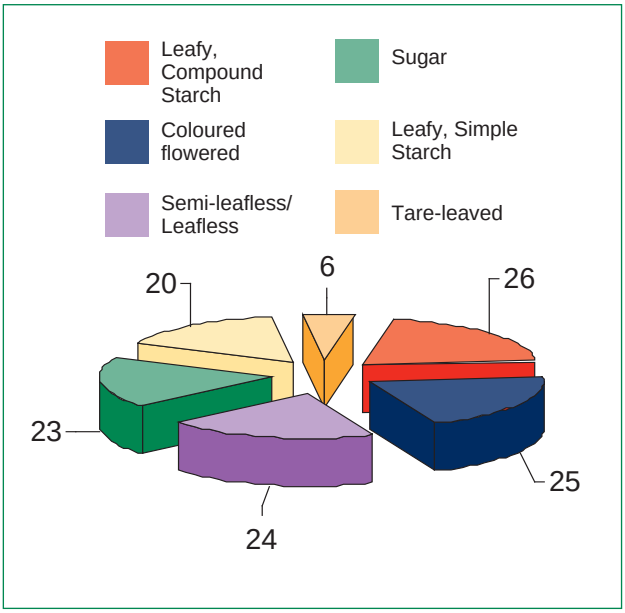
Figure 3.9 Distribution of cultivars and DUS candidates in Cultivar Groups



The number of candidates submitted for UK DUS tests is not correlated with the number of cultivars. *Afila* peas account for over half of the total candidates, almost double that of Vining peas, the next largest group.

Within these 6 Groups, cultivars can be classified into several Subgroups, defined by combinations of discontinuous morphological characteristics (Green, 1995). With the exception of the Tare-leaved Group, the number of subgroups in each Group is similar (Fig. 3.10). Subgroups vary in size, containing between 1 and 265 cultivars.

Figure 3.10 Number of subgroups in each Cultivar Group

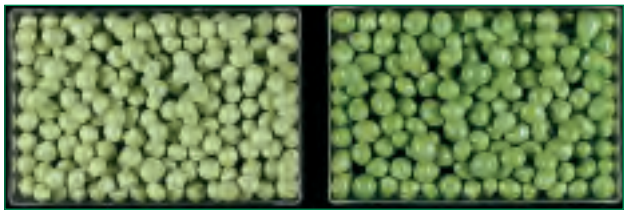


Although the Coloured Flowered and Sugar Groups make up a relatively small proportion of the total number of cultivars and relatively few candidates, there is a wide range of variation, which is illustrated by the number of subgroups. Fewer cultivar comparisons need to be made, as the subgroups are small, and a wide range of variation in flowering date, plant height and seed weight make DUS testing relatively simple. The characteristics of each group are considered in the following sections.

Leafy, Compound Starch Grain Group

This Group contains wrinkled-seeded Vining pea cultivars bred for use by the freezing and canning industry. The main breeding aims have been to improve yield, yield reliability, disease resistance and seed quality, although earlier crops and smaller seed size have also been important niche markets. Good yield is essential for the commercial success of Vining peas, but the best prices are paid for the right combination of earliness, smaller size and sweetness, and good even colour, as required by the market. Pea cultivars used for freezing have a dark green immature seed colour, whereas those for canning are pale green (Fig. 3.11). This Group also contains a large number of traditional cultivars used by amateur growers, which were well known before the UK National List was created in 1973.

Figure 3.11 Immature seed colour: pale (used for canning) and dark (used for freezing)



Freezing Peas

'Sprite' and 'Scout' reached the UK market from the USA in the mid-1960s and complemented the use of 'Dark Skinned Perfection'. Shortly after, 'Puget', followed as the first triple-podded maincrop cultivar registered in 1969. Earlier cultivars extended the maturity range, and continued breeding has raised yields and improved quality. 'Scout' and 'Puget', the two long serving successful cultivars, have only recently been replaced by cultivars with smaller seeds and better colour.

Although Petits Pois have been marketed since the 1960s, their share of the Vining market has now grown to about 8%. The growth of this premium product followed the introduction of 'Waverex', which was registered in Germany.

Canning Peas

Breeding pale seeded cultivars for canning peas diminished in the 1970s, but a few dual purpose freezing and canning cultivars have been bred, with a medium green fresh seed colour, which are not too pale for freezing and not too dark for canning.

A total of 266 UK candidates have been submitted for test, of which, 236 were dark green cultivars for the freezing market, and the remaining 30 were pale seeded for canning.

Semi-leafless and Leafless Group

The semi-leafless (*af*) and leafless (*st*) genes were introduced by the John Innes Institute to develop a new plant model for the pea plant. In semi-leafless and leafless peas, leaflets are converted to tendrils (Fig. 3.12), which grow together to help the crop stand until the seed is dry. The plants are then combine-harvested.

Figure 3.12 Foliage types: Leafy and Afila (Semi-Leafless/ Leafless)

The market for peas in this group is primarily for tannin-free dry peas for compounding for animal feed, but also for dry seed products such as protein and starch. However, the semi-leafless gene has also been bred into cultivars used for human consumption (fresh harvest, dried, freezing and canning) and for forage.

In the late 1970s, there was a growing market for UK and European produced plant protein, from which *Afila* Peas benefited. In the mid 1980s, the USA stopped exports of soya due to a shortage in their home market, and this seriously affected the supply of protein for livestock feed in Europe, giving a further impetus to the production of UK-grown plant protein. By 1984 the area of semi-leafless peas exceeded that of Vining peas for the first time (Biddle *et al.,* 1988). More recently, both BSE, which peaked in 1992/3, and the Foot and Mouth disease epidemic of 2001, have caused increased demand for the replacement of animal feed made from fish and animal products, and raised the profile of peas as a source of non-animal protein.

However, apart from the demand for protein, peas were also popular as a break crop, as their nitrogen fixing ability contributed to improved yields of following cereal crops, such as wheat and to a lesser extent barley. The development of new products and technologies, has also continued to extend the market for peas in this group, e.g. micronisation, which involves the production of flakes or flour from dry seed.

The first cultivars, registered in 1978, established the new plant model for dry seed harvest. Two, 'Filby' and 'Barton', were leafless and three, 'Eaton' 'Melton', and 'Wensum', were semi-leafless. With the exception of 'Filby' all had wrinkled seeds. These early cultivars were crossed with leafy types to produce the first smooth seeded agricultural cultivars, 'Sentinel', 'Consort' and 'Folio', between 1982 and 1984. Further breeding targeted stem strength and standing ability to reduce losses from lodging. These factors, and improved downy mildew resistance and ease of harvest, all contributed to the raising of yield by approximately 30% between 1980 and 2004. The first vegetable semi-leafless cultivars registered were the Vining pea 'Bikini' and the Marrowfat pea 'Princess'. Both are still in commercial production.

This is one of the most difficult Groups to test for DUS, as the absence of leaflets reduces the number of available characteristics, and many varieties are very similar, as the source of the semi-leafless gene originates from one of four lines. Measurement of plants is necessary for establishing Distinctness. A total of 494 UK candidates in the Semi-leafless/Leafless Group have been submitted for DUS tests; 394 of these had simple starch grains (282 yellow seeded, 113 green seeded) and 100 had compound starch grains (35 yellow seeded and 65 green seeded).

Leafy, Simple Starch Grain Group

This group contains green and yellow seeded cultivars that have the same market as semi-leafless/leafless Group. The main use is for the production of tannin-free dry seed for pig and poultry feed. However, green seeded Marrowfat cultivars are also grown for human consumption and smooth seeded cultivars are grown for canning.

In 1970, 'Vedette', the first green seeded combining pea was registered, to compete with the import of dry seed of 'Alaska' from the USA, and it was succeeded by both green and yellow seeded cultivars from the UK and Europe. The Group has diminished in importance since the introduction of semi-leafless peas, as there is less foliar disease due to the absence of leaflets, better standing ability due to increased tendrilation, and easier harvesting as the plants are less bulky.

A total of 76 candidates have been submitted for UK DUS tests, 36 of which were yellow seeded and 40 were green seeded, six of which were of the Marrowfat seed type.

Marrowfats

Although not one of the six Cultivar Groups, Marrowfat cultivars have distinctive rhomboid or wedge shaped seeds with an irregular surface, and occur in four different Cultivar Groups: Leafy, Simple Starch Grain, Semi-leafless/Leafless, Tare-leaved and Coloured Flowered. This seed is quite different from smooth and wrinkled types (Fig. 3.13), but has simple starch grains and is therefore well suited to dry seed harvest.

Figure 3.13 Different types of dry seed: smooth (simple starch grains), Marrowfat (simple starch grains) and wrinkled (compound starch grains)

A few yellow seeded Marrowfat cultivars are grown for compounding, but green seeded Marrowfats grown for human consumption is the most important market. The best price is paid for dry seed that retains the natural green colour. This seed is sold in packets for rehydration and is used in the catering industry, in the home and for the fish and chip market. Poorer quality seed is canned and sold as 'Processed Peas'. Marrowfat cultivars can also be grown as Vining peas where seed is immature at harvest and is marketed as processed peas (seed soaked and canned whole) or as mushy peas (seed coat is removed before canning or freezing).

'Maro', registered in the 1960s, has been one of the longest-grown cultivars for canning and for dry seed sold in packets. It is still in commercial production today, as is 'Progreta', the first tare-leaved Marrowfat registered in 1978, and 'Princess', the first semi-leafless Marrowfat registered in 1986. Other Marrowfat cultivars have been bred to increase seed weight, but until recently none has had better seed quality than 'Maro'. A total of 40 UK candidates have been submitted for DUS test, of which 6 were leafy, 22 semi-leafless (*Afila*) and 12 tare-leaved.

Tare-leaved Group

Tare-leaved plants occur as spontaneous mutants in a number of cultivars. The foliage is reduced and leaflets and stipules are narrow with a pointed apex and the stipule habit is parallel, giving rise to their description as 'rabbit-eared'. The base of the flower standard is also distinctive. The inheritance of the tare-leaf phenotype is unknown and crosses have revealed that it does not behave in a Mendelian manner, with selections sometimes reverting to the original plant type. Despite this, the tare-leaf characteristic has been successfully incorporated into several cultivars.

'Progreta', which arose from 'Maro', was the first tare-leaved cultivar registered in 1978. The plants were recognised as having good potential, as they stood well and were easier to combine than 'Maro', but seed quality was not as good. The popularity of tare-leaved cultivars has declined and few are now submitted for DUS test. A total of 22 candidates have been submitted for DUS test, of which 14 were Marrowfats, 6 were smooth seeded and 2 had wrinkled seed.

Sugar Peas

Although both Snap and Mangetout Sugar peas (Fig. 3.14) had been in existence for over 100 years, their popularity in the UK was limited to the amateur market. New breeding in the USA resulted in several new cultivars of Snap peas, developed for the American market, but the early cultivars were too tall for commercial production and were mainly used by amateur growers. As, due to gene interaction, only a small reduction in stem length could be achieved with each crossing, the development of shorter cultivars was a slow process. These new Snap pea cultivars ('Sugar Snap' was the first, registered in 1978) were popular with gardeners but, over time, cultivars with shorter habit have led to commercial fresh market production of pods for supermarket sale. 'Sugar Gem' and 'Sugar Luv' registered in 1984, further improved Snap pea quality by reducing the development of strings in the pod sutures.

Figure 3.14 Sugar Pea pod types: Snap and Mangetout

A total of 13 cultivars have been tested in the UK comprising 2 Mangetout and 11 Snap Peas, 2 of which are 'stringless'.

Coloured Flowered Group

When the National List was set up, only two coloured flowered pea varieties, 'Marathon' and 'Minerva', were registered. As Early European forage cultivars probably originated from landraces with coloured flowers, and were maintained by continuous selection, not all characteristics are uniform as they were not positively selected. However, cultivars bred by crossing and purified by single seed descent, are much more uniform. Coloured flowered cultivars are grown primarily for silage, though some cultivars are dual-purpose, with dry seed being compounded for animal feed or used as pigeon feed. They have seeds with tannin, some of which are marbled and/or have anthocyanin spotting on the testa. Some attempts were made to reduce seed tannins with the aim of reducing bitterness in compounded animal feed. This was achieved by introducing genes that modify anthocyanin, resulting in cultivars with pink and white flowers ('Rosakrone' and 'Magnus') or white flowers with a pink blush ('Golf') (Fig. 3.15). 'Magnus', registered in 1986, was one of the earliest dual-purpose cultivars with semi-leafless habit and it is still in commercial use.

Figure 3.15 Flower colour: modified anthocyanin, normal anthocyanin and white

A total of 32 cultivars have been tested in the UK, 20 being leafy and 12 semi-leafless.

Testing DUS for new cultivars in the future

The number of Semi-leafless/Leafless cultivars continues to grow as *Afila* peas replace the leafy types. Unfortunately, from a DUS testing perspective, the range of variation in *Afila* peas is narrow, making it difficult to find clear differences between cultivars. For many agricultural cultivars, establishing distinctness is currently dependent on measurement of plant parts and statistical analyses, and there is an urgent need to find improved methods of discrimination

Although molecular techniques can now be used to identify cultivars, their current use in DUS testing is very limited. Maintainers can not maintain their cultivar, which is effectively a population, with only one molecular banding pattern, as selection and regeneration of plants in the field could not achieve such uniformity. The high rate of mutation in peas, much of it unseen, could also pose a problem in maintaining a high level of molecular uniformity.

The recent use of imaging for the automatic measurement of plant parts in peas offers some hope for achieving better discrimination among cultivars (Green *et al.,* 2001). It could improve accuracy, reduce cost and allow measurement of some more complex characteristics, such as shape, which would not be possible to record using manual methods. Another advantage of imaging is that new characteristics could be accommodated at relatively little extra cost.

As, in practice, several methods are used in DUS testing, any developing technologies which have the potential to improve discrimination among cultivars need to be evaluated, to ensure that the tests used are the most efficient.

References

Biddle AJ, Knott CM, Gent GP. 1988. *The PGRO Pea Growing Handbook 1988.* Peterborough: Processors and Growers Research Organisation.

Green FN. 1995. Registration of Pea (*Pisum sativum* L.) cultivars in the United Kingdom: Documentation, Classification and Description. *Acta Horticulturae* **413**: 99-106.

Green FN, McCarthy L, Horgan GW, Mann AD, Roberts AMI. 2001. The development of an automated image measurement system to discriminate among varieties of Peas (*Pisum sativum* L.) submitted for Distinctness, Uniformity and Stability Tests, in Hay, RKM. ed. *Scientific Review 1997-2000*. Scottish Agricultural Science Agency, Edinburgh. **3.3**: 25-29.

HMSO, 1964. *Plant varieties and Seeds Act.* London: Her Majesty's Stationary Office.

HMSO, 1972. *European Communities Act.* London: Her Majesty's Stationary Office.

4 Seed Potatoes

4.1 Introduction (KJ O'Donnell)

4.2 Evaluation of Late Blight Resistance in Sarpo Varieties (SF Carnegie and AM Cameron)

4.3 The Incidence of Potato Cyst Nematodes in Scottish Seed Potato Land 1998-2003 (J Pickup and Y Cole)

4.4 Development of Symptoms of Potato Mop-Top Virus (PMTV) during Seed Multiplication (SF Carnegie and M McCreath)

4.5 Potato Tuber Necrotic Ringspot Disease (I Browning)

4.6 Aphicide Resistance in the Peach-Potato Aphid, *Myzus persicae* in Scotland (L King and J Pickup)

4.7 Aphid Transmission of the Potato Viruses PVY^O and PVY^N (L King, A Fox, I Browning and J Pickup)

Chapter 4 Seed Potatoes

4.1 Introduction (KJ O'Donnell)

Potato is the fifth largest crop in Scotland, in terms of area grown, yet the potato crop assumes an importance out of proportion to this crude ranking; there are no equivalent scientific resources devoted to, for example, oilseed rape. Scotland produces 75% of the UK's seed potatoes – potatoes used for planting. It is therefore a key part of the agriculture sector, bringing up to £80 million into the Scottish economy.

The work of the Potato and Plant Health Division at SASA is, in large part, directed at servicing the needs of the Scottish seed potato industry. SASA is the Certifying Authority for seed potatoes in Scotland, and responsible for the management of the Scottish Seed Potato Classification Scheme (SPCS). This is a crop certification scheme, which sets tolerances for disease, trueness to type etc., providing an assurance of the quality of the Scottish seed potato crop.

SASA is responsible for the provision of scientific services relating to seed potato production, including:

- The maintenance and production of nuclear stock – the *in vitro* starting material for seed potato production
- Testing services for potato cyst nematode (PCN), viral diseases and other potato pests
- Training of SEERAD Agricultural Inspectors
- Monitoring of levels of aphids that can spread disease
- Monitoring the Scottish potato crop to verify the absence of quarantine diseases such as ring rot and brown rot (which have never been found in Scottish potatoes).
- National List trialling of new potato varieties for Distinctness, Uniformity and Stability (DUS), and Value for Cultivation and Use (VCU). (SASA is the main UK centre for official potato variety testing).

SASA also carries out R&D in support of these functions. No biological system stands still, and continual scientific input is needed to ensure that SASA has the skills and knowledge needed to underpin the continued success of the Scottish seed potato industry. Some of that work is described in this chapter.

Sustainable agriculture has become increasingly important over the last few years, and a key part of any reduction in chemical inputs is the development of disease resistant varieties. In their paper *Evaluation of Late Blight Resistance in Sarpo Varieties,* SF Carnegie and AM Cameron describe SASA's assessment of promising varieties, using the techniques used for VCU testing.

Another pest of concern to potato growers is potato cyst nematode (PCN). Land can be used for seed potato production only if it is tested, and the two PCN species, *Globodera rostochiensis* and *G pallida,* not detected. J Pickup and Y Cole's paper *The Incidence of Potato Cyst Nematodes in Scottish Seed Potato Land 1998-2003,* describes SASA's PCN testing work over a 6 year period. While levels of PCN in potential seed potato land remain low, there does appear to be a relative increase in *G pallida.* This is important because, although seed potato crops cannot be grown on land where PCN is present, it is permitted to plant ware crops using resistant varieties. There are, at present, no varieties fully resistant to *G pallida* and therefore land where it is found is taken out of potato production.

The SPCS sets strict tolerances for the levels of virus found in seed potato crops. One question that SASA has had to consider is whether potato mop-top virus (PMTV) should be added to the list of diseases covered by the regulations. This issue is addressed by SF Carnegie and M McCreath in *Development of Symptoms of Potato Mop-Top Virus (PMTV) during Seed Multiplication.*

Another viral disease of recent concern is the subject of *Potato Tuber Necrotic Ringspot Disease* by I Browning. This disease is not found in Scotland but is present in other European potato-growing countries. The work described in the paper is an example of how SASA must be proactive in looking for such problems before they occur.

Many types of virus are spread by aphids. A fortunate side effect of Scotland's climate is that aphid numbers – and therefore disease – are relatively low, compared with other European countries. SASA monitors aphid populations closely and *Aphicide Resistance in the Peach-Potato Aphid,* Myzus persicae (*in Scotland*) by L King and J Pickup describes a change in aphid populations in 2001, and explores the reasons for it.

Finally, L King *et al.*, bring entomology and virology together, in *Aphid Transmission of the Potato Viruses PVY^{O} and PVY^{N}.* This also illustrates one of SASA's strengths: the cross-pollination that results from members of the same scientific community working together and sharing ideas and results.

The papers in this chapter, although on a diverse range of subjects, have a unifying theme – helping to ensure that the Scottish seed potato industry continues to thrive.

4.2 Evaluation of Late Blight Resistance in Sarpo Varieties (SF Carnegie and AM Cameron)

Varietal resistance to late blight can be provided by a single major gene acting against the fungus, *Phytophthora infestans;* in most cases the effect of such genes is to confer plant immunity to the pathogen. In the 1950s and 60s breeding for late blight resistance in potato used 'R' genes derived from *Solanum demissum* but the strategy failed because isolates of *P. infestans* that were able to overcome these genes developed fairly rapidly by mutation. For example, var. Pentland Dell contained R genes 1, 2 and 3, and the variety was initially resistant in commercial production. However, after a few years, new isolates that were able to infect Pentland Dell developed and multiplied, resulting in an apparent breakdown of resistance, even though the variety was still resistant to the original isolates (Malcolmson, 1969).

This type of resistance, which reacts differentially to isolates, is commonly classified as race specific resistance. Subsequently, potato breeding has concentrated on developing race non-specific (durable) resistance which is conferred by the combined action of several genes. In order to obtain a true estimate of the degree of race non-specific resistance of a variety, new varieties still need to be tested using isolates that can overcome as many of the 11 known R genes as possible.

Following the introduction of the A2 mating type into Europe in the late 1970s, the occurrence of new populations of *P. infestans* has resulted in greater late blight pressure on European potato production, and renewed awareness of the benefits of breeding varieties for resistance to late blight. In addition, the development of markets for organic potatoes has also increased interest in new potato varieties with improved resistance to late blight. Varieties, originally bred in Hungary by Sarpo KFT, are being developed in the UK by Sárvári Research Trust and are claimed to have high, durable resistance to late blight based on a unique source of resistance. Initial tests by SASA, using an isolate from var. Pentland Dell from Northern Ireland, confirmed that the Sarpo varieties first imported into Scotland showed very high resistance. However, over the past 3-4 years, late blight has developed on a number of Sarpo varieties being grown in Scotland and Wales, and isolates of *P. infestans* have been collected from some crops. In view of this apparent failure of resistance, the reaction of a range of Sarpo varieties was assessed over 2 years using 7 of these isolates.

Methodology

The virulence (ability to infect) of isolates was determined by inoculating detached leaflets from glasshouse-grown plants of a range of varieties, each containing a single R gene (R1 to 11) and a variety containing no R genes. One drop of a zoospore suspension derived from 3,000 sporangia ml^{-1} was placed on the adaxial surface. After incubation for 6-7 days, the leaflets were examined for growing, sporulating lesions. The reaction was classified as virulent if there was sporulation and the lesion had expanded more than a few mm outside the inoculation drop. The origin and virulence of the test isolates are shown in Table 4.1.

Tests for varietal resistance were conducted on whole plants grown in a glasshouse or polytunnnel for *c.* 45 days, by which time the plants were either flowering or had flower buds. The experiments were laid out in a fully randomised block design with 3 replications. Sporangia were washed from detached leaflets that had been incubated for 6-7 days after inoculation and the concentration adjusted to 3,000 sporangia ml^{-1} before chilling to release zoospores. Plants were sprayed to run-off, with separate sprayers and inoculation areas for each isolate to minimise the risk of cross contamination, and then incubated in a growth chamber at 15-18°C for 7 days with high humidity for 48 hours after inoculation. The % leaf area affected by late blight was recorded on a 1 (susceptible) to 9 (resistant) scale using the key of Cruickshank *et al.,* (1982).

Table 4.1 Isolates of *Phytophthora infestans* used in the tests in 2001 and 2002

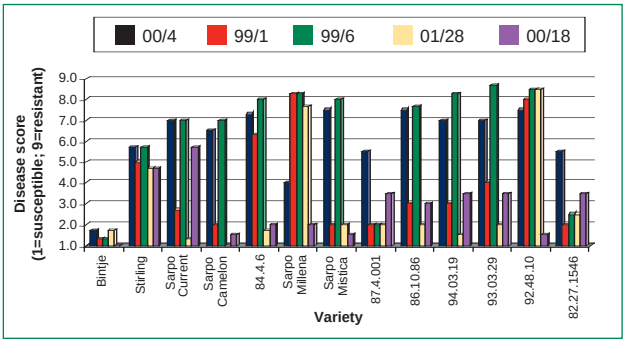
Isolate	Variety from which the isolate was derived	Site	Year of isolation	Virulence*
		2001		
00/4	Pentland Dell	Midlothian, Scotland	2000	1.2.3.4.6.7.11
99/1	Sarpo Camelon	Nairn, Scotland	1999	3.4.7.8.11
99/6	Sarpo Current	Nairn, Scotland	1999	1.3.4.7.8.11
00/18	84.4.6	Bangor, Wales	2000	1.3.4.7.8.10.11
01/28	84.4.6	Bangor, Wales	2000	1.2.3.4.6.7.11
		2002		
00/4	Pentland Dell	Midlothian, Scotland	2000	1.2.3.4.6.7.11
99/1	Sarpo Camelon	Nairn, Scotland	1999	3.4.7.8.11
01/29	Sarpo Mira	Bangor, Wales	2001	1.2.3.4.6.7.8.10.11
01/30	84.4.6	Bangor, Wales	2001	1.2.3.4.6.7.11
02/31	Sarpo Axona	Ross-shire, Scotland	2002	3.4.10.11

* ability of an isolate to infect potato varieties containing various R genes

Results

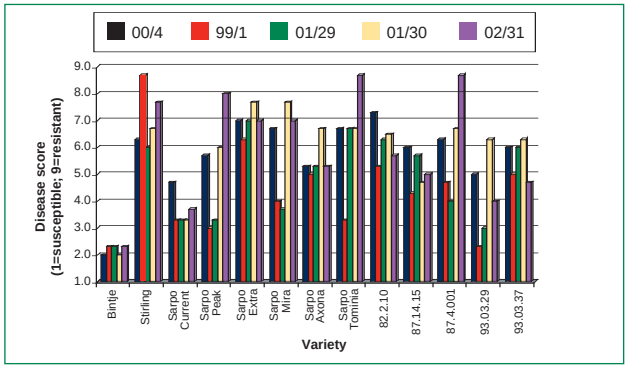
2001 Experiment. The isolates differed considerably in their degree of virulence, with 99/1 being the least complex, and 00/18 and 01/28 being the most complex. The reaction of the standard varieties, Bintje and Stirling, was similar with all isolates (Fig. 4.1) but the 11 Sarpo varieties reacted differentially to the isolates. Two varieties, 87.4.001 and 82.27.1546, were susceptible to all Sarpo-derived isolates and were moderately susceptible to isolate 00/4. The remaining 9 varieties were largely resistant to 00/4 but were susceptible to at least one of the four Sarpo-derived isolates. The virulence of the isolates differed with variety. Isolate 99/6 infected only 2 Sarpo varieties, 87.4.001 and 82.27.1546, whereas inoculation with 99/1, collected from the same field as 99/6 in 1999, produced infection on all but 3 varieties. Of the Welsh isolates, isolate 01/28 produced avirulent reactions on 2 varieties (Sarpo Millena and 92.48.10) and isolate 00/18 produced only one, on var. Sarpo Current, on which large, hypersensitive lesions developed resulting in a moderately resistant score of 5.7.

Figure 4.1 Reaction of Sarpo varieties in 2001 to inoculation with 4 isolates (99/1, 99/6, 01/28, 00/18) of *Phytophthora infestans* collected from Sarpo varieties infected in the field and an isolate (00/4) from var. Pentland Dell



2002 Experiment. There was greater variability among some of the replicate results in 2002 than in 2001, particularly with the Sarpo varieties, and this was reflected in a higher LSD value in 2002. Bintje was susceptible to all isolates (Fig. 4.2). Stirling was relatively resistant to all isolates although there was more variability in its reaction to the isolates than in 2001. All Sarpo varieties reacted with moderate or high resistance to inoculation with isolate 00/4 except for Sarpo Current, which appeared to be more susceptible than in 2001. Five out of the 11 Sarpo varieties reacted similarly to inoculation with each of the Sarpo-derived isolates. These were Sarpo Extra, Sarpo Axona, 82.2.10, 87.14.15 and 93.03.37. The remaining 6 Sarpo varieties appeared to be moderately susceptible to at least one of the isolates. Isolate 02/31 produced 4 avirulent reactions on the six varieties, isolate 01/30 produced 5 and isolate 99/1 produced none.

Figure 4.2 Reaction of Sarpo varieties in 2002 to inoculation with 4 isolates (99/1, 01/29, 01/30, 02/31) of *Phytophthora infestans* collected from Sarpo varieties infected in the field and an isolate (00/4) from var. Pentland Dell



Summary

The virulence of isolates collected from Sarpo varieties, infected naturally by *P. infestans,* varied from relatively simple races with four virulence genes, to those with 8 or more. Isolates collected from the same field or the same cultivar also differed in their virulence. Isolates containing virulence to R8 have not been detected in previous screening by SASA of isolates for variety testing, and the presence of this virulence in some of the Sarpo-derived isolates suggests that R8 may be present in the breeding material. Isolate 99/1 was more virulent to the range of Sarpo varieties tested than isolate 99/6, although both were taken from the same field in 1999. This confirms the results of previous tests of the virulence of these 2 isolates by SASA and by Leonard and Shaw (2001). Isolate 99/1 was generally as virulent as other more complex isolates (e.g. 00/18, 01/29) with a few notable exceptions (e.g. no infection on Sarpo Millena and 92.48.10). Isolates 01/28, 00/18 and 01/30 collected from var. 84.4.6 differed slightly in their virulence and in which Sarpo varieties they were able to infect. Isolates 00/28 and 01/30 were identical in their virulence to R genes; however isolate 00/28 produced a susceptible reaction with var. 93.03.29 and var. 87.4.001 whereas isolate 01/30 produced avirulent reactions. This suggests that there may be major resistance genes, other than the known R genes, operating in Sarpo cultivars.

Overall, the results demonstrate that part of the late blight resistance shown by some Sarpo cultivars is race specific but also suggest that some varieties may also contain an underlying, useful degree of race non-specific resistance. Van der Plank (1984) predicted that the degree of race non-specific resistance in varieties containing R genes would generally be low. For example, Pentland Dell is immune to races of *P. infestans* which are not virulent to R genes 1, 2 and 3 but, when challenged by race 1.2.3, its resistance score is 4. Such reactions were recorded in the present tests (e.g. Sarpo Millena), and were most evident in the varieties tested in 2001. Other varieties (e.g. Sarpo Mira) did not appear to be as susceptible as Sarpo Millena when they were challenged by Sarpo-derived isolates. Van der Plank (1984) also demonstrated that race specific resistance delayed the start of an epidemic but did not subsequently slow it. This pattern of late blight development has been observed with some Sarpo varieties in the field, again suggesting that, in these varieties, resistance may be partly race specific. In such cases, lesions have been large and sporulating freely. Care will, therefore, need to be taken in interpreting the results of late blight field trials of Sarpo varieties exposed to current UK races because the absence of some virulence genes in the population may give a misleading impression of the level and durabilty of the resistance, as occurred with var. Pentland Dell (Malcolmson, 1969). Testing Sarpo selections at as early a stage as possible with isolates collected from Sarpo varieties may be an effective strategy for selecting varieties with true durable resistance.

References

Cruickshank G, Stewart HE, Wastie RL. 1982. An illustrated assessment key for foliage blight of potatoes. *Potato Research* **25**: 213-214.

Leonard B, Shaw D. 2001. Resistance to late-blight disease in Sarpo accessions from Hungary. Internal report, University of Wales, Bangor.

Malcolmson JF. 1969. Races of *Phytophthora infestans* occurring in Great Britain. *Transactions of the British Mycological Society* **53**: 417-423.

Van der Plank JE. 1984. *Disease Resistance in Plants.* New York and London Academic Press, 2nd edition.

4.3 The Incidence of Potato Cyst Nematodes in Scottish Seed Potato Land 1998-2003 (J Pickup and Y Cole)

Potato cyst nematodes (PCN) *Globodera rostochiensis* and *G. pallida* are the two most important pests of potatoes in the UK. They are specialist parasites, feeding on potato roots, causing losses of yield that vary according to the level of infestation present, the soil type and the tolerance to PCN attack of the variety of potato under cultivation. PCN are considered as quarantine pests by plant protection organizations throughout the world, including the European Plant Protection Organization (EPPO), which categorizes both species as A2 pests (i.e. quarantine pests present in the EPPO region but not widely distributed and under official control). Within the European Community, statutory control measures are laid down by the EC PCN Directive of 1969 (69/465 EEC), the revision of which has been discussed on several occasions within the EC Plant Health Standing Committee, although a final revised text has yet to be agreed.

The SASA Scientific Review of 1992-1997 included an analysis of the distribution of PCN in Scotland between 1992 and 1997, based on the results of SASA's statutory pre-crop soil tests carried out before seed potato production (Pickup, 1997). The analysis presented here examines the results of the pre-crop testing for the following six-year period, 1998-2003. A six-year period is chosen to correspond to the minimum rotation period for seed potato production in Scotland so that compiling data over this period should not involve any significant double-counting.

Table 4.2 Diagnosis of PCN at SASA, 1998-2003. Clean land contains no cysts of PCN, viable or non-viable. Failed land contains viable PCN. Where percentages are presented they represent the portion of the total area tested, unless otherwise stated.

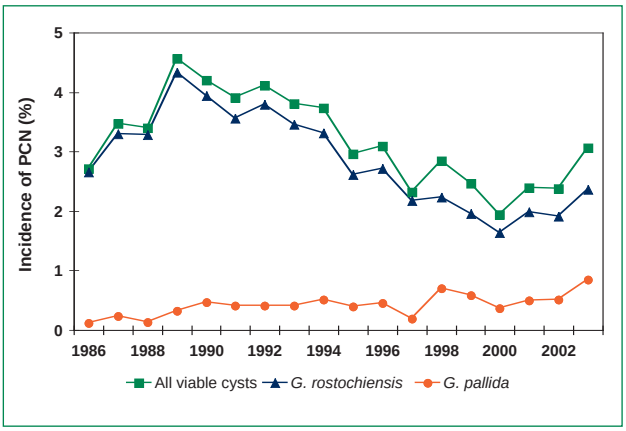
Region	Area sampled (ha)	Clean land (ha)	Viable & non-viable PCN (%)	Failed land (%)	*G. rostoch.* (%)	*G. pallida* (%)	*G. pallida* as % of area failed
North	5,155	5,100	1.1	0.1	0.1	0.0	0
Grampian	44,129	42,419	3.9	1.2	1.0	0.2	18
Angus & NE Fife	47,582	41,809	12.1	3.8	2.8	1.1	29
Perth & Kinross	22,207	19,402	12.6	2.9	2.6	0.3	12
South	9,100	8,820	3.1	1.4	1.1	0.3	19
Scotland	128,172	117,550	8.3	2.4	1.9	0.6	23

For the growing seasons 1998-2003, over 40,000 statutory pre-crop soil samples from an area of over 128,000ha were examined for the presence of PCN (Table 4.2). Of the total area tested, 8.3% (10,622ha) was found to contain PCN (both viable and non-viable cysts). Viable cysts were found on 2.4% of the land tested (3,094ha). Land in which viable cysts were found was placed under quarantine ('scheduling'), restricting the cultivation of potatoes and the movement of soil from the contaminated land, e.g. in association with plants for transplanting. Crops of ware potatoes may be grown on scheduled land, but such cultivation can be permitted only under licence from SASA. The licence stipulates that the crop grown has to be of a variety that is resistant to the species of PCN found in the pre-crop test. As SASA does not recognise any varieties on the UK National List as having full resistance to *G. pallida*, no potato production can be permitted on land contaminated by this species. Where non-viable PCN cysts were found in a pre-crop sample, restrictions were placed on seed potato production to permit the cultivation of resistant varieties only. The incidence of *G. pallida* was 0.6% of the total samples, or 23% of the total samples with viable cysts. In comparison, *G. rostochiensis* was found in 1.9% of the total samples, or 80% of the samples with viable cysts. A very small proportion of samples contained both species of PCN.

It is clear from Table 4.2 that there are distinct regional differences in the incidence of PCN in land submitted for testing. A very low density of PCN was present in the North (Highland and Orkney), and *G. pallida* was not found in any samples examined between 1998 and 2003. Both Grampian and the South (mostly Borders, Dumfriesshire, Lothians and Lanark) had a relatively low incidence of PCN, with viable cysts present in only 1.2% of samples. The highest incidence of PCN was found in the traditional potato-growing areas of central eastern Scotland, where the incidence of viable PCN in Angus & North-East Fife was 3.8%. In Perth & Kinross the incidence of *G. rostochiensis* was similar to that in Angus & North-East Fife, whereas the incidence of *G. pallida* was lower, giving an overall incidence of viable PCN of 2.9%.

Fig. 4.3 shows the change in incidence of viable PCN over the period 1986-2003. Although the overall incidence of both species shows a peak at 4.5% in 1989, the level in 2003 is similar to that recorded in 1986. The most significant change illustrated by this figure is the increase in the proportion of samples containing *G. pallida.*

Figure 4.3 Proportions of pre-crop samples containing viable PCN cysts, 1986-2003



Several observations can be drawn from a comparison with the review of pre-crop sampling results for 1992-1997 (Pickup, 1997). There has been little change in the incidence of viable and non-viable cysts in samples, declining slightly from 8.5% to 8.3% since 1997. However, the proportion of samples containing viable cysts has fallen from 3.3% to 2.4%, largely as a result of a decline from 3.0% to 1.9% of samples containing *G. rostochiensis.* The incidence of *G. pallida* has increased from 0.4% to 0.6%, and the proportion of samples with viable cysts that contain *G. pallida* has increased markedly from 12% to 23%. The emerging pattern shows populations of *G. rostochiensis* in decline, indicating that effective control of this species has been achieved, whereas *G. pallida* populations are increasing. This contrast may be explained by the different responses of the two species of PCN to the two main measures for control: host resistance and long rotation. Commercial varieties with resistance to *G. rostochiensis* are grown widely (over 40% of the seed production area of Scotland is planted with such varieties, e.g. Maris Piper), whereas no commercial varieties have full resistance to *G. pallida.* Furthermore, few varieties that have been bred to exhibit good partial resistance to *G. pallida* have gained commercial success. Although the natural decline of *G. rostochiensis* within the soil over a six-year rotation is usually sufficient to counteract the increase following cultivation of a susceptible crop, the decline of *G. pallida* is slower and a longer rotation is required to achieve an equivalent decline.

The amount of land taken out of seed potato production due to scheduling between 1998 and 2003 was 3,094ha. Over the same period a similar amount of previously scheduled land was allowed back into unrestricted potato production, following a clear soil test. As over 117,000ha was cleared for seed production over this period, and since the area of seed potatoes entered for classification over the same period was less than 81,000ha, scheduling of *c.* 3000ha of land is unlikely to have a very significant reduction on the availability of land for seed production.

Reference

Pickup J. 1997. The incidence of potato cyst nematodes in Scottish seed potato land. *Scottish Agricultural Science Agency Scientific Review 1992-1997,* 28-29

4.4 Development of Symptoms of Potato Mop-Top Virus (PMTV) during Seed Multiplication (SF Carnegie and M McCreath)

Potato Mop-Top Virus (PMTV) is transmitted by the soil borne fungus *Spongospora subterranea,* the cause of powdery scab (Jones and Harrison, 1969; Arif *et al.,* 1995). Primary infection by *S. subterranea* is favoured by cool wet conditions during the growing season (Cooper and Harrison, 1973). Generally, there are no foliar symptoms of PMTV in the year of infection but spraing symptoms may develop in the tubers (brown necrotic flecks, lines or arcs in the flesh, or brown rings or lines on the surface), depending on variety and environmental conditions (Harrison and Reavy, 2002). The development of spraing is particularly favoured by sudden changes in temperature (Harrison and Jones, 1971a) and it renders tubers unusable. The presence of spraing in tubers will result in discoloured patches in cooked potatoes, crisps or French fries and means that crops will be rejected for processing or sale for table consumption. Foliar symptoms occur the year following primary infection and their development is favoured by cool temperatures (Harrison and Jones, 1971b). The virus produces two types of symptom: yellow blotches and chevrons on the leaves and/or a shortening of the internodes, resulting in a bunching effect or 'mop-top'. Secondarily infected plants may produce misshapen or cracked tubers, often with 'elephant-hide' blemishes on the skin. PMTV infection in the growing crop may, therefore, reduce yield and increase the incidence of unattractive, unsaleable tubers.

In the last few years, but particularly in 2002, plants affected by PMTV have been seen more frequently in seed potato crops by inspectors and growers (Table 4.3). A tolerance for PMTV has not been included in the Scottish Seed Potato Classification Scheme because the virus is considered to be self eliminating (Cooper *et al.,* 1976). Whereas most viruses, e.g. PVX or PVY, are transmitted to all daughter tubers derived from an infected seed tuber, PMTV is transmitted to only a proportion of the daughter tubers and, in turn, to only a proportion of daughter plants. In addition, in many cases, PMTV infection affects only 1 or 2 stems on a plant compared with all stems on plants infected with other viruses. Since 2000, a series of experiments has examined the relationship between the incidence of PMTV-affected plants in successive generations and the occurrence of spraing to assess the need for a tolerance for the disease in seed potato classification.

Table 4.3 Percentage seed area containing plants affected by potato mop-top virus (PMTV) between 2001 and 2003

	Year		
Incidence of PMTV in crop	2001	2002	2003
Nil	99.5	96.3	98.8
>1%	0.06	0.24	0.06
>2%	0.02	0.13	0.02

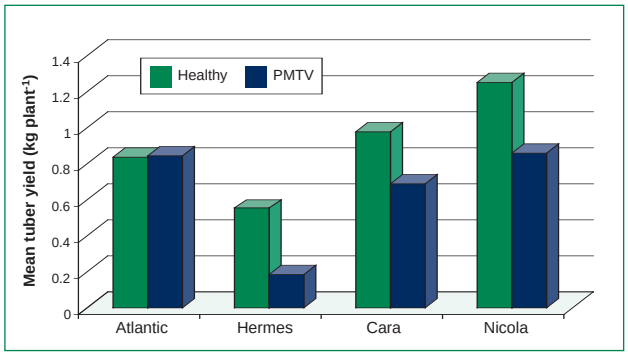
Four seed potato crops from Aberdeenshire, Kincardine, Angus and Perthshire containing *c.* 10% plants affected by PMTV were selected over 2 years (Table 4.4). The type of symptom varied with variety. Paired replicates of healthy and affected plants were marked by stakes along a number of beds in each crop towards the end of July or early August. A sample of leaflets, symptomatic in the case of affected plants, was collected from each plant and tested for PMTV by ELISA. The crop was then desiccated according to farm practice. The tubers produced by each plant were harvested by hand, just before commercial harvesting of the crop. The number and weight of tubers in 6 size fractions were recorded soon after harvest. A proportion of the tubers was assessed for external and internal symptoms of spraing after *c.* 3 months of storage. The remaining tubers were planted in replicate plots at Gogarbank Farm and the daughter plants were assessed for foliar symptoms of PMTV.

Table 4.4 Percentage of plants in which PMTV was detected by ELISA in relation to symptom expression in 4 seed potato crops (values in brackets indicate the numbers of plants sampled)

Year	Variety	Symptom on affected plants	Healthy	PMTV affected
2000	Atlantic	Yellow chevrons on leaves of some stems; size of leaflets and plant unaffected	0 (30)	47 (30)
2000	Hermes	Stunted, mopped plants	0 (40)	40 (20)
2002	Nicola	Stunting of some stems; faint mottling on leaves on affected stems	0 (32)	41 (64)
2002	Cara	Stunting of some stems; faint mottling on leaves on affected stems	0 (30)	13 (30)

Symptoms in four seed crops varied from very mild on var. Atlantic to very severe on var. Hermes (Table 4.4). The percentage of symptomatic plants in which PMTV was detected in leaves was less than 50% and detection did not appear to be related to the severity of symptoms (Table 4.4). The lowest rate of detection was 13% with var. Cara. Virus was not detected in leaf samples from unaffected plants. As might be expected, tuber yield was affected in proportion to the severity of symptoms: affected plants of var. Atlantic with mild symptoms had a similar yield to unaffected plants and the size distribution of the tubers was also similar for both sources (Fig. 4.4). The yield of PMTV-affected plants of vars Hermes, Nicola and Cara was considerably less than that of healthy plants, with the greatest reduction for var. Hermes, which showed the most severe distortion. In addition, the tubers from affected plants were virtually unmarketable, being misshapen.

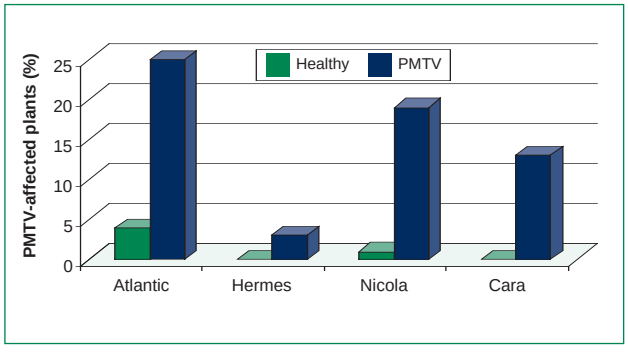
Figure 4.4 Tuber yield of healthy and PMTV-affected plants in seed crops of 4 varieties



Although leaf samples from affected plants were taken from parts of the plants clearly showing PMTV symptoms, PMTV was not detected by ELISA in a relatively high proportion of symptomatic plants. This may have occurred because the distribution or concentration of virus was variable within the plant, resulting in a very low virus titre within some parts. Consequently, testing for suspect PMTV infection in seed crops may require at least 5 samples to be taken. One positive test result would be interpreted as confirming that the symptoms on all plants were caused by PMTV.

Spraing was found in 1.3%, 1.8% and 15% of tubers from PMTV affected plants of vars Atlantic, Hermes and Nicola respectively. While spraing did not occur in tubers from unaffected plants of either var. Atlantic or Hermes, 4% of tubers from unaffected plants of var. Nicola had spraing symptoms. Development of PMTV symptoms in plants derived from the seed of affected plants was generally low, occurring in 25%, 19%, 13% and 3% of daughter plants of vars Atlantic, Nicola, Cara and Hermes respectively (Fig. 4.5). Although PMTV symptoms did not develop on daughter plants derived from unaffected plants of vars Hermes and Cara, symptoms did develop on plants of vars Atlantic and Nicola, occurring in 4% and 1% of daughter plants respectively.

Figure 4.5 Incidence of daughter plants affected by PMTV in relation to the health of mother plants



Spraing developed, primarily, in the tubers of plants showing symptoms of PMTV infection. The highest incidence (15%) was found in var. Nicola. Spraing symptoms in tubers from plants without foliar symptoms were uncommon and may have resulted from primary PMTV infection or from symptomless plant infection. Based on the results of these experiments, the estimated incidence of spraing developing on daughter tubers from crops with 10% PMTV affected plants would have been 0.2% for vars Atlantic and Hermes and 1.5% for var. Nicola. However, as the incidence of infection by PMTV in tubers was not assessed, potentially, the incidence of spraing might have been higher, if symptomless infection had been present and environmental conditions had been optimal for disease development.

The transmission of PMTV from one generation to another, as measured by symptomatic plants in the crop, was generally low, ranging from 25% in var. Atlantic to only 3% in var. Hermes. This confirms that, unlike most other virus diseases (e.g. leafroll in which all tubers from an infected plant are infected), PMTV is translocated only to a proportion of the tubers and daughter plants. This means that, in the absence of further primary infection, the incidence of PMTV would be negligible within 1-2 years. For example, in var. Hermes, the incidence of PMTV affected plants would have decreased from 10% in 2000 to 0.3% in 2001 and then to 0.009% in 2002. Calvert (1968) estimated that self elimination would take 3 years. Furthermore, healthy plants in an affected crop rarely produced affected plants. Roguing of diseased plants could, therefore, help to increase the natural rate of elimination of PMTV in crops, if the initial incidence of disease was low enough for this to be practicable. This limited data set confirms that a tolerance for PMTV in seed potato classification would have minimal impact on the incidence of spraing in the following crop because of the relatively low rate of transmission from plant to tuber to plant. More detailed studies on PMTV transmission are being undertaken with support from the British Potato Council.

References

Arif M, Torrance L, Reavy B. 1995. Acquisition and transmission of potato mop-top furovirus by a culture of *Spongospora subterranea* f.sp. *subterranea* derived from a single cystosorus. *Annals of Applied Biology* **126:** 493-503.

Calvert EL. 1968. The reaction of potato varieties to potato mop-top virus. *Record of Agricultural Research, Ministry of Agriculture for Northern Ireland* **17:** 31-40.

Cooper JI, Harrison BD. 1973. Distribution of potato mop-top virus in Scotland in relation to soil and climate. *Plant Pathology* **22:** 73-78.

Cooper JI, Jones RAC, Harrison BD. 1976. Field and glasshouse experiments on the control of potato mop-top virus. *Annals of Applied Biology* **83:** 215-230.

Harrison BD, Jones RAC. 1971a. Factors affecting the development of spraing in potato tubers infected with potato mop-top virus. *Annals of Applied Biology* **68:** 281-289.

Harrison BD, Jones RAC. 1971b. Effects of light and temperature on symptom development and virus content of tobacco leaves inoculated with potato mop-top virus. *Annals of Applied Biology* **67:** 377-387.

Harrison BD, Reavy B. 2002. Descriptions of plant viruses. *Potato mop-top virus. In CD-ROM. Warwick: Association of Applied Biologists*

Jones RAC, Harrison BD. 1969. The behaviour of potato mop-top virus in soil, and evidence for its transmission by *Spongospora subterranea* (Wallr.) Lagerh. *Annals of Applied Biology* **63:** 1-17.

4.5 Potato Tuber Necrotic Ringspot Disease (I Browning)

Introduction

Symptoms of Potato Tuber Necrotic Ringspot Disease (PTNRD) consist of dark markings or rings on the tuber surface which subsequently become necrotic and sunken (Fig. 4.6). Following its emergence in Hungary in 1979 the disease was found to be caused by Potato Virus Y^N (Beczner *et al.,* 1984), a strain of Potato Virus Y that induces veinal necrosis in *Nicotiana tabacum.* At a meeting of the European Association for Potato Research (EAPR) in Paris in 1993, it was decided that tuber-necrosing isolates of PVY^N should be referred to as PVY^{NTN}. PVY^N generally causes a mild mottle in potato, whereas symptoms of PVY^{NTN} have been reported to vary in severity depending on cultivar, and may include strong mottling, necrotic spots, veinal and stem necrosis (Kerlan and Ramage-Charlet, 1998).

Figure 4.6 Symptoms of PTNRD in Yukon Gold

In the 1980s the disease spread to the Czech Republic, Austria, Slovenia, Belgium and the former Yugoslavia. Subsequently it was reported in Denmark, Lebanon, France, Switzerland, Japan, Serbia, England, The Netherlands, Romania, Tunisia, Spain, Portugal, Greece, Peru, Israel, Italy, Germany and Brazil. PTNRD has had serious consequences for potato production, particularly in Eastern Europe. In Slovenia, for example, seed potato production was almost destroyed (Kus, 1995).

Control of the disease

Cultivars react to PVY^{NTN} in a number of ways, being immune, sensitive to tuber necrosis to varying degrees, or tolerant (susceptible, but not showing symptoms of PTNRD). PVY^{NTN} does not break the extreme resistance gene Ry_{sto} derived from *Solanum stoloniferum,* which confers immunity to PVY (Schiessendoppler, 1996) and production of resistant varieties is likely to be the most economical way of reducing losses due to PVY (Wolf *et al.,* 1998). In Hungary a breeding programme was started in the 1960s to incorporate resistance into new varieties (Lonhard *et al.,* 1995) and this work has resulted in several immune cultivars. In addition, some countries have tested existing cultivars for resistance to PTNRD but few have been found to be immune (Dedic and Ptacek, 1996; Kus, 1996). In recent years only resistant and high quality susceptible cultivars have been accepted on the Slovenian National List (Dolnicar, 2001) with the aim of restoring seed potato cultivation. In France, control measures have been based on breeding for resistance, implementation of ELISA tests for PVY^N, and the setting of a maximum limit for the percentage of tubers carrying superficial necrotic symptoms caused by PVY^{NTN} and other viruses (Charlet and Kerlan, 2001).

Development of test methods

In the last ten years many institutes throughout the world have been working towards development of a test method for detection of PVY^{NTN}. Such a test could be used to detect PVY^{NTN} isolates in certification schemes, providing another mechanism for control of this potentially damaging virus. Molecular tests have been developed which separate tuber-necrosing isolates from those not previously associated with tuber necrosis. However there is some doubt about their reliability in view of the fact that the part of the virus genome responsible for inducing tuber necrosis has not yet been identified. Another problem is that separation of necrosing from non-tuber necrosing isolates is not clearly defined. Indeed, Kerlan and Tribodet (1996) reported that several PVY^N isolates, never associated with PTNRD under natural conditions, could induce tuber necrosis following manual inoculation in the glasshouse.

Do PVY^{NTN} isolates exist in Scotland?

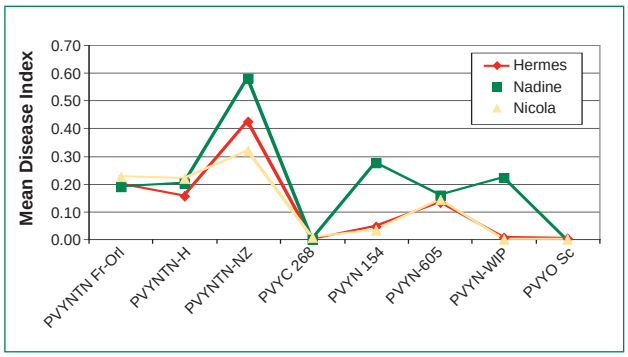
To date no symptoms of PTNRD have been observed during official tuber inspections in Scotland. This could be due to one or a combination of reasons: low levels of PVY^N in Scottish seed; environmental conditions not favourable for expression of tuber symptoms; lack of sensitivity to tuber necrosis of cultivars grown; or a lack or low level of isolates that can readily induce PTNRD. SASA has been interested in the last question for some time in relation to the marketing of Scottish seed.

EAPR PVY^{NTN} Working Group

In 2001, a working group, coordinated by the author, was set up under the umbrella of the European Association for Potato Research (Virology Section) with the aim of developing a bioassay for detection of PVY^{NTN}. A prerequisite for development of any test method for detection of PVY^{NTN} is the identification of a range of PVY isolates whose ability to induce tuber necrosis has been clearly established. Thus the first stage of this work involved testing eight PVY isolates in three sensitive potato cultivars. Plants were manually inoculated in the glasshouse in seven countries, and the severity of tuber necrosis was calculated by means of a disease index based on the proportion of tubers with PTNRD and the surface area affected. In some countries tests were also carried out in the field using varying numbers of isolates and cultivars.

The results of the glasshouse tests are presented in Fig. 4.7 The PVY^O and PVY^C isolates tested induced little or no tuber necrosis in the cultivars examined. All PVY^N isolates, including PVY^N-605 and PVY^NWi-P, which have never been reported to be tuber-necrosing, were capable of inducing tuber necrosis to varying degrees depending on cultivar and country of testing. Most severe tuber necrosis was obtained with a PVY^{NTN} isolate from New Zealand and Nadine proved to be particularly sensitive. Results in the Czech Republic, Hungary and France indicated that there was no greater propensity for tubers to develop PTNRD in glasshouse than field conditions.

Figure 4.7 Mean disease indices for isolates by country and cultivar (guide s.e.d.=0.06)



It was unfortunate that no PVY^{N} isolate was identified that failed to induce tuber necrosis in all cultivars. However, identification of isolates that are likely to cause significant tuber necrosis may be possible by setting a DI threshold. Further tests are required on other PVY^{N} and PVY^{N}-W isolates with a view to identifying isolates that fail to induce PTNRD under any conditions. The results of this study will provide useful data in support of sequence analyses of the viral genome, and a bioassay, using test plants that provide rapid results, would be a valuable alternative to molecular tests.

The issue of PVY^{NTN} is complex: there is variability among PVY^{NTN} isolates and development of PTNRD depends on environmental conditions and on the cultivar/isolate combination. Though there is variation in severity of tuber necrosis produced by different PVY^{N} isolates, it remains questionable whether any isolates exist, which fail to induce tuber necrosis in all cultivars.

References

Beczner L, Horvath J, Romhanyi I, Forster H. 1984. Studies on the etiology of tuber necrotic ringspot disease in potato. *Potato Research* **27:** 339-352

Charlet K, Kerlan C. 2001. Control methods against potato tuber necrotic ringspot disease: strategies implemented in France. *Proceedings 11th EAPR Virology Section Meeting, Trest, Czech Republic, October 2001,* 68-69

Dedic P, Ptacek J. 1996. The reaction of Czech potato cultivars to PVY^{NTN}. *Abstracts of 13th conference of EAPR, Veldhoven, The Netherlands, July 1996,* 298-299

Dolnicar P. 2001. Monitoring of PVY strains in Slovenia. *Proceedings 11th EAPR Virology Section Meeting, Trest, Czech Republic, October 2001,* 15-17

Kus M. 1995. The epidemic of the Potato Tuber Necrotic Ringspot Strain of Potato Virus Y (PVY^{NTN}) and its effect on Potato Crops in Slovenia. *Proceedings 9th EAPR Virology Section Meeting, Bled, Slovenia, June 1995,* 159-160

Kus M. 1996. Sensitivity Assessment of European and North American Cultivars to the Slovenian PVY^{NTN} isolate. *Abstracts of 13th conference of EAPR, Veldhoven, The Netherlands, July 1996,* 63

Kerlan, C, Ramage-Charlet, K. 1998. *Expression of Potato Tuber Necrosis Ringspot Disease (PTNRD) in field conditions in France.* Proceedings 10th EAPR Virology Section Meeting, Baden, Austria, July 1998, 50-55

Kerlan C, Tribodet M. 1996. Are all PVY^{N} isolates able to induce potato tuber necrosis ringspot disease? *Abstracts 13th EAPR Conference, Veldhoven, The Netherlands, July 1996,* 65-66

Lonhard M, Horvath S, Polgar Zs, Wolf I. 1995. Results, possibilities and problems in potato breeding for resistance to PVY in Hungary. *Proceedings 9th EAPR Virology Section Meeting, Bled, Slovenia, June 1995,* 31-33

Schiessendoppler E, 1996. Change of susceptibility of potato cultivars to PVY-strain group as response of PVY^{NTN} introduction. *Abstracts 13th EAPR Conference, Veldhoven, The Netherlands, July 1996,* 310-311

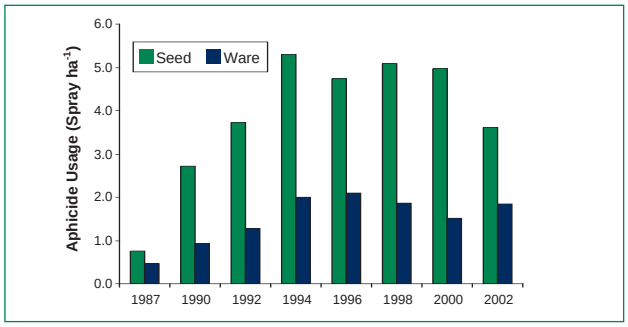
Wolf I, Horvath S, Polgar ZS. 1998. Resistance of some Hungarian potato varieties against Potato LeafRoll Luteovirus and the NTN strain of Potato Y Potyvirus. *Abstracts 10th EAPR Virology Section Meeting, Baden, Austria, July 1998,* 5-6

4.6 Aphicide Resistance in the Peach-Potato Aphid, *Myzus persicae* in Scotland (L King and J Pickup)

The colder and wetter Scottish climate usually ensures that the Peach-Potato aphid *Myzus persicae,* which is the major vector of aphid-borne potato viruses, flies later and in fewer numbers than in southern England and most other seed potato producing areas within Europe. This is one of the major reasons behind the exceptionally high standards of virus health attributed to Scottish seed potatoes.

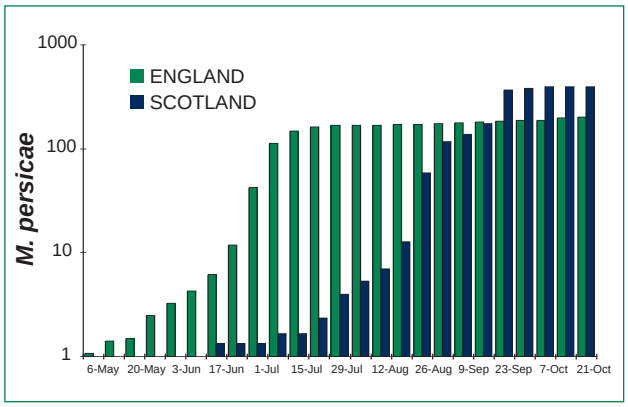
To ensure that uncontrolled aphid activity does not lead to unacceptable increases in virus incidence within the Scottish potato crop, as well as ensuring that statutory tolerances for virus incidence are met in progeny crops, a programme of monitoring populations of aphids in potato crops was introduced into the SPCS in 1992. This programme operates at two levels. At level one, early potato crops are checked for aphid infestations in each of seven geographical areas. If the aphid populations exceed set thresholds, aphid monitoring in that geographical area progresses to the second level, where all seed crops are checked regularly for aphids. If the number of colonies exceeds a further threshold, classification is withheld until a post-harvest virus test has been completed on the progeny tubers from that crop. The introduction of the aphid monitoring programme into the SPCS coincided with a marked increase in the application of insecticides to both seed and ware crops. Between 1994 and 2000, each seed crop received an average of five applications of insecticide (Fig. 4.8).

Figure 4.8. Aphicide usage on seed and ware potatoes in Scotland, 1987 – 2002



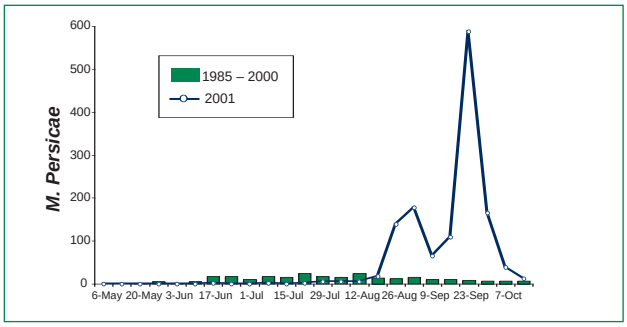
During 2001, the monitoring of aerial populations of aphids carried out by SASA's network of aphid suction traps showed that *M. persicae,* after being very scarce in the early part of the season, was exceptionally abundant in late August and September. By the end of the season the totals of aphids caught in Scottish traps were considerably higher than catches from traps in England (Fig. 4.9). Furthermore, in central eastern Scotland during August 2001, very high infestations of *M. persicae* were observed on many potato crops that had yet to be burned down. These aphids were able to survive treatment with a wide variety of insecticides.

Figure 4.9 Cumulative mean catch of Peach-potato aphids (*Myzus persicae*) in English and Scottish suction traps in 2001



Comparing the seasonal pattern of flight (phenology) of *M. persicae* in 2001 with the historical data for this species in other years confirmed that the data collected in 2001 were exceptional (Fig. 4.10). In fact, the total of 550 aphids caught in the Dundee suction trap during the week ending 23 September 2001 was greater than the total annual catch for this trap for the previous 34 years of operation of this trap, with the sole exception of 1989. As well as the Dundee trap, the Edinburgh trap also showed a similar, albeit less extreme, pattern of aphid activity. Unfortunately, since the Elgin trap was not operating in 2001, no data from northern Scotland are available for comparison. The field aphid problem in late summer 2001 became so severe that SAC obtained an emergency off-label approval for the use of Nico Soap (nicotine) for the control of insecticide-resistant *M. persicae* on oil-seed rape.

Figure 4.10 Peach-Potato aphid *Myzus persicae* – weekly catches



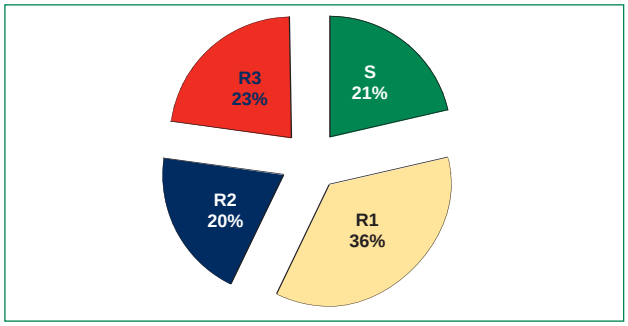
During 2000, field-collected *M. persicae* exhibiting a variety of types of insecticide resistance were discovered in north-east Scotland. Consequently, in 2001, SEERAD funded a survey to establish the incidence of insecticide resistant *M. persicae* in central eastern Scotland. This work has now been extended and forms part of a larger SEERAD flexible fund project, involving collaboration between SCRI, SASA and SAC, with insecticide resistance testing carried out by Rothamsted Research.

In late August and early September the survey work was augmented by field-collected samples of *M. persicae* from sites in Angus, Fife and the Lothians. Many of these were submitted by agronomists who had witnessed very high *M. persicae* infestations on potato crops yet to be burned down. These aphids were able to survive treatment with a wide variety of insecticides. All field-collected aphids were cultured, and samples from the resulting clones were sent to Rothamsted Research for evaluation of resistance mechanisms and to SCRI for DNA fingerprinting. Insecticide resistance assays were also carried out on aphids from the suction trap located at East Craigs, Edinburgh.

The analyses carried out by Rothamsted Research revealed that many aphids exhibited at least two of the mechanisms of insecticide resistance that *M. persicae* is known to possess: R3 (carboxylesterase) and MACE (**m**odified **a**cetyl**c**holin**e**sterase resistance). The presence of a third mechanism, kdr (**k**nock **d**own **r**esistance to pyrethroid insecticides) was not investigated, although it is likely to have been present.

The incidence of carboxylesterase resistance in the aphids from the Edinburgh suction trap was high, with only 21% of the 70 aphids analysed susceptible (Fig. 4.11). Slightly more of these aphids (23%) showed extreme (R3) resistance. Although organophosphate insecticides are effective in controlling susceptible aphids, no control is achieved against aphids with R3. Ten aphids (22%) from a sample of 45 aphids were found to exhibit MACE resistance, which confers full resistance against dimethyl carbamate insecticides (pirimicarb and triazamate). Of the aphicides approved for use on potatoes, only pymetrozine can control aphids that combine MACE and R3 resistance. The first MACE aphid was caught on 15 August and the last on 13 September. Although aphids were tested from five suction traps in England in 2001, only one other MACE aphid was found.

Figure 4.11 The frequency of esterase resistance in Peach-potato aphids (*Myzus persicae*) caught at Edinburgh in 2001. (S: normal (susceptible). R1: 4x normal (moderate resistance). R2: 16x normal (severe resistance). R3: 64x normal (extreme resistance). n = 70)



Molecular characterisation conducted at SCRI, by analysis of four microsatellite loci and fingerprinting of the IGS region of the ribosomal DNA, has demonstrated that field populations collected in Scotland comprise relatively few clones of *M. persicae.* Only seven clones were identified from the samples collected in 2001. Two of these clones were subsequently demonstrated to exhibit MACE resistance. In one of these clones, MACE was associated with R3 esterase resistance and kdr, in the other with R1/R2 esterase resistance and kdr.

Aphid life cycles are complex and there are a great number of variations between species. Many aphids overwinter as cold-hardy eggs produced by sexually reproducing parents on woody host plants. In spring, populations build up on the winter (primary) host plants, and asexual (parthenogenetic) females are produced, which move on to herbaceous summer hosts. For the Peach-Potato aphid, the primary host on which sexual reproduction can take place is the peach (*Prunus persicae*) and the summer host is typically the potato, although many other plants are suitable summer hosts. Therefore, in northern Europe the distribution of the primary host, the peach, present only in very sheltered and protected environments in northern Britain, limits the opportunities for sexual reproduction. As a result, peach-potato aphid populations in Scotland are maintained almost exclusively by asexual reproduction.

The production of a new genotype or clone is most likely to occur following meiosis during sexual reproduction, rather than through mitotic asexual reproduction. However, for meiosis to occur in *M. persicae*, the sexual stages of the aphid life cycle have to be produced and that only occurs in areas with significant populations of peach trees, the primary host. Hence, far greater genetic diversity has been observed in studies carried out in more southerly areas of Europe (e.g. France, Spain) than is observed in Scotland. Any new genotypes found here are more likely to originate from warmer environments to the south. Their success in Scotland will depend upon their ability to survive both the biotic and abiotic environment. Clearly, any selective advantages, such as enhanced insecticide resistance, will be important factors with regards to their likelihood of becoming established alongside, or in place of, existing Scottish clones.

The clonal composition of populations of *M. persicae* may have important implications for aphid control on potatoes beyond just the immediate issue of aphicide resistance. For example, the flight phenology observed in 2001 was significantly different from the pattern typical of previous years. Studies carried out by the University of York demonstrated that populations of *M. persicae* deteriorated rapidly on potatoes at the end of July, as the plants became unpalatable. If these results were equally applicable to the aphids present in Scotland in 2001, then the failures in aphid control observed as late as the end of August could not have occurred. Preliminary evidence from SCRI indicates that MACE resistant *M. persicae* are more efficient transmitters of virus than are some more established clones.

Since the outbreak on potatoes in 2001 and subsequent control failures on oilseed rape in the autumn of that year, there has been no significant recurrence of high populations of insecticide resistant *M. persicae*. Although the 2001 – 2002 winter was very mild, only one of the two MACE resistant clones has been recorded since 2001. SASA introduced modifications into the aphid-monitoring programme within the SPCS, encouraging seed potato growers to refrain from routine prophylactic insecticide applications. Fig. 4.8 shows a slight reduction in insecticide use compared with recent years.

Conclusion

The development of insecticide resistance in *M. persicae* presents a major challenge for the future control of this aphid and is therefore of great significance for the management of viruses within the seed potato crop. Recent developments indicate that existing understanding of the biology of *M. persicae* and the potato viruses it transmits may be based on the behaviour of relatively few genotypes. There is, therefore, the potential for new aphid genotypes to have a major impact on the ability to manage viruses within the Seed Potato Classification Scheme. In addition to monitoring aphid populations, SASA is now in a position, through collaborative work, to monitor changes in the population genetics of this important pest species.

4.7 Aphid Transmission of the Potato Viruses PVY^O and PVY^N (L King, A Fox, I Browning and J Pickup)

Virus transmission and the SPCS

During SPCS inspections of growing crops of seed potatoes, three diseases symptoms caused by aphid-transmitted viruses are recorded: leaf roll, severe mosaic, and mild mosaic. Leaf roll is caused by *Potato leafroll virus* (PLRV), which is transmitted in a persistent manner by only a few aphid species. Persistent viruses are transmitted by aphids that remain viruliferous throughout their lives. Three PLRV vector species (*Myzus persicae, Macrosiphum euphorbiae* and *Aulacorthum solani*) colonise Scottish potato crops in most years, of which *M. persicae* is considered to be by far the most efficient vector. Although leaf roll symptoms were recorded from 27% of seed crops as recently as 1991, improvements in virus management practices have limited the incidence of leaf roll to presence in fewer than 2% of crops since 1997.

Non-persistently transmitted potyviruses: *Potato virus Y* (strains PVY^O and PVY^N), *Potato virus A* (PVA) and occasionally *Potato virus V* (PVV), cause both mild and severe mosaic symptoms. The contact-transmitted virus *Potato virus X* (PVX) is also a common cause of mosaic symptoms in some years. As mosaics can be caused by several viruses, laboratory tests are needed for reliable determination of the causal virus. The ordinary strain of *Potato virus Y* (PVY^O) is more frequently associated with severe mosaic symptoms in some cultivars. Symptoms of mild mosaic are now the most frequently recorded virus disease within the SPCS, with symptoms recorded in 8% of seed crops in 2001. Severe mosaic is less frequently recorded, rarely being present in more than 2% of the crop in recent years.

Non-persistent viruses are usually rapidly acquired by aphids, but the virus remains on the feeding stylets and is rarely retained for longer than a few hours. *M. persicae* is again the most efficient vector of PVY, but there are approximately 40 other vector species, which may land on potatoes, but do not settle for long and do not colonise. Although the relative vector efficiency of these non-colonising species is low in comparison with that of *M. persicae*, large numbers of some species, including the cereal aphids, *Rhopalosiphum padi, Metopolophium dirhodum* and *Sitobion avenae,* land in Scottish potato crops in many years.

Virus risk assessments – a decision-support system

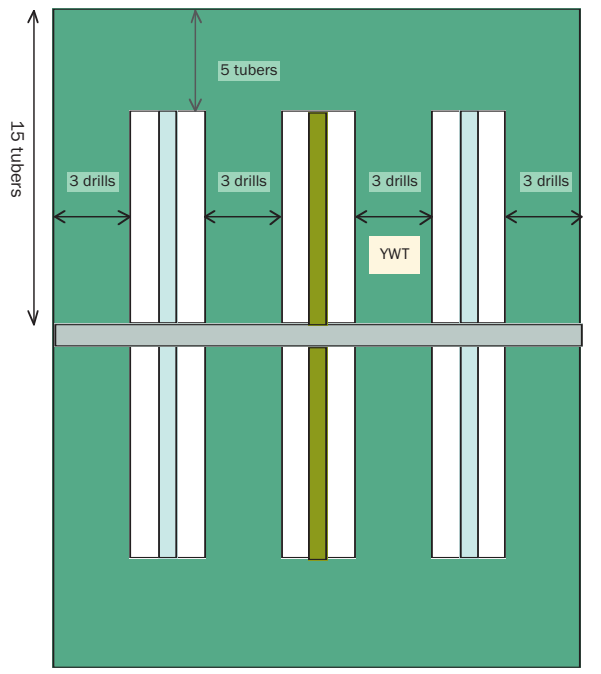
In many countries in which seed potato crops are grown, aphid populations are monitored to provide information on the likelihood of virus transmission. For example, in the UK, the British Potato Council (BPC) has funded the Central Science Laboratory (CSL) of Defra to provide potato growers with a rapid crop-specific assessment of the risk of virus transmission. In 2003, SASA participated in this project, providing rapid assessment of field-collected aphids, for dissemination to growers *via* the BPC website.

In addition, the SASA website provides regional information on virus risk based on the catches from aphid suction traps at Elgin, Dundee and Edinburgh. The methods used on both websites assess risk as combined 'aphid vector pressure indices', calculated by multiplying the frequency of each species by its relative vector efficiency, and summing the results for all species within the catch. To evaluate the value of such assessments of virus risk, and their potential for use in decision making within the SPCS, a field study of the extent and timing of virus transmission has been carried out at SASA's farm at Gogarbank near Edinburgh. By monitoring aphids throughout the study, both locally within the field (using yellow water traps) and on a regional basis (using 12.2m suction traps), published values of relative vector efficiencies can be evaluated under Scottish conditions for the two trapping methodologies. This knowledge should provide SASA with greater ability to identify seed crops at high risk of virus transmission within the growing season, and hence to make appropriate refinements to the aphid monitoring arrangements within the SPCS. The results of work carried out during the 2003 season are reported here.

Field study

Two experimental plots of healthy tubers of cv. Maris Piper were planted on 22 April 2003 at Gogarbank Farm according to the plan detailed in Fig. 4.12. In each plot, one row of 21 Maris Piper tubers infected with PVY was planted across the 21 drills. Tubers infected with PVY^O were used in one plot and tubers with PVY^N in the other. The timing and extent of virus transmission were determined by exposing four-week old *Nicotiana debneyi* indicator plants for successive periods of one week, starting on 3 June (two weeks after emergence of the Maris Piper plants) and ending on 27 August. Aphid populations were monitored each week by yellow water traps sited within the plots and by the suction trap at East Craigs, approximately 3km north-east of the trial site.

Figure 4.12 Trial design, 2003



Healthy Maris Piper

Maris Piper infected with PVY

Potted N. debneyi

Potted Maris Piper

Unplanted drill

Aphid species	PVY Factor
Acyrthosiphon pisum	0.7
Aphis fabae	0.1
Aulacorthum solani	0.2
Brachycaudus helichrysi	0.21
Brevicoryne brassicae	0.01
Hyperomyzus lactucae	0.16
Macrosiphum euphorbiae	0.2
Metopolophium dirhodum	0.3
Myzus ascalonicus	0.2
Myzus ornatus	0.2
Myzus persicae	1
Rhopalosiphoninus latysiphon	0.2
Rhopalosiphum padi	0.4
Sitobion avenae	0.01

Results

Only subtle differences were found in the extent and timing of transmission of PVY^O and PVY^N to indicator plants. Overall, the transmission of PVY^N was slightly higher each week. Transmission of virus reached a maximum in both plots during the week beginning 8 July when 64% and 71% of indicator plants were infected with PVY^O and PVY^N respectively (Fig. 4.13).

Fig. 4.13 also presents the weekly aphid vector pressure indices calculated from the aphid data from yellow water traps and the East Craigs suction trap. These indices also reached their maximum values during the week beginning 8 July. The same relative vector efficiencies were used to calculate the indices for each trapping method. These efficiencies were derived from published data from studies carried out in Sweden and the Netherlands (see column 3 of Table 4.6). As the aphid catches are typically larger in suction traps than in yellow water traps the respective indices are relatively higher. Both indices show a good correlation with the virus transmission data. Using logistic regression models, significant relationships ($p<0.001$) were found between the extent of virus transmission and the aphid vector pressure indices calculated using data from both aphid trapping methods. However, the vector pressure indices derived from the yellow water trap data provided the better fit to the transmission rates of both viruses, as indicated by the higher deviance ratios (Table 4.5).

Figure 4.13 Weekly transmission of PVY^O and PVY^N and changes in aphid vector pressure indices in 2003. Aphid vector pressure indices are calculated using data from suction traps (STD) and yellow water traps (YWT)

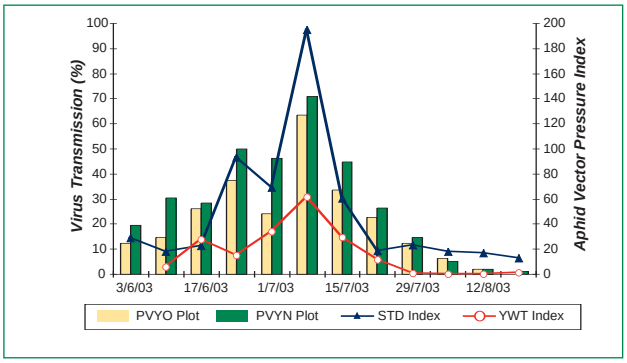


Table 4.5 Regression analysis of weekly transmission of PVY^O and PVY^N and aphid vector pressure indices in 2003. As the PVY infection levels are in the form of binomial data, a logistic regression model following square root transformation (sqrt) was used.

Logistic Regression Model	Deviance ratio	Intercept (standard error)	Slope (standard error)
PVY^O on sqrt(STD)	23.2	-3.29 (0.50)	0.285 (0.062)
PVY^O on sqrt(YWT)	30.5	-2.84 (0.41)	0.404 (0.081)
PVY^N on sqrt(STD)	21.2	-2.98 (0.54)	0.304 (0.071)
PVY^N on sqrt(YWT)	31.3	-2.52 (0.43)	0.414 (0.087)

The relative contributions of individual aphid species to the overall vector pressure indices differed markedly between the two methods of aphid monitoring (Table 4.6): not surprisingly, aphids that colonise potatoes (*Myzus persicae* and *Macrosiphum euphorbiae*) are better represented amongst the field-collected aphids (yellow water trap data). The suction trap catches contain a higher proportion of cereal aphids (particularly *Rhopalosiphum padi* and *Metopolophium dirhodum*). Only *M. dirhodum* made a major contribution to the indices calculated from both monitoring methods.

Table 4.6. Known aphid vectors of *potato virus Y:* their vernacular names, relative vector efficiencies and contribution to the aphid vector pressure indices over the duration of the 2003 trial

Aphid Species	Vernacular name	Efficiency	YWT(%)	STD (%)
Myzus persicae	Peach-potato aphid	1.0	32.6	1.7
Acyrthosiphon pisum	Pea aphid	0.7	1.5	4.2
Rhopalosiphum padi	Bird cherry-oat aphid	0.4	1.7	21.4
Metopolophium dirhodum	Rose-grain aphid	0.3	31.1	48.7
Brachycaudus helichrysi	Leaf-curling plum aphid	0.21	3.6	12.4
Aulacorthum solani	Glasshouse-potato aphid	0.2	0.5	0.5
Macrosiphum euphorbiae	Potato aphid	0.2	23.1	5.5
Myzus ornatus	Violet aphid	0.2	0.5	0.0
Hyperomyzus lactucae	Blackcurrant-sowthistle aphid	0.16	4.5	0.7
Aphis fabae	Black bean aphid	0.1	0.5	1.0
Sitobion avenae	Grain aphid	0.01	0.2	3.8

Conclusions

This work will be repeated in future years, providing further data on the transmission of virus to aphid activity. Regression analysis of the data should enable the relative vector efficiencies to be refined. The significant relationships found in 2003, linking virus transmission to aphid vector pressure indices, demonstrate that it should be possible to derive revised relative vector efficiencies for the aphid vectors collected by the two methods of monitoring. The predictive value of these vector efficiencies can then be tested through further experimental work. However, the different contributions of each species to the two indices suggest that the relative vector efficiencies assigned to any species will each have to be weighted by a factor accounting for the bias associated with the method of monitoring used. Early results suggest that field monitoring using yellow water traps may have more value than suction traps as a basis for a decision support system for managing virus transmission within a particular crop. However, this assessment is based on the vector efficiencies derived from work carried out in other areas of Europe. It will be possible to establish the relative merits of both methods of monitoring only after relative vector efficiencies have been revised according to the levels of virus transmission observed during this series of trials.

5 The Identification and Control of Harmful Organisms

5.1 Review of Plant Health 2000-2003 (JM Chard)

5.2 *Phytophthora ramorum* (Sudden Oak Death) (A Schlenzig and JM Chard)

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Chapter 5 The Identification and Control of Harmful Organisms

5.1 Review of Plant Health 2000-2003 (J Chard)

SASA provides scientific advice and technical support to SEERAD in support of a range of EC, GB and Scottish plant health legislation, mainly associated with potato, soft fruit and other horticultural crops. This work involves bacterial, fungal, insect, nematode and viral pest species. In addition, SASA's UK Potato Quarantine Unit is responsible for testing potato material from outside of the EC (third countries) to meet EC plant health requirements. This chapter reviews the challenges posed by changes in plant health legislation and in pest and disease incidence over the past three years.

Legislative Changes Affecting Plant Health Work

The legislation covering this area of work is the EC Plant Health Directive (2000/29/EC). This consolidated version of the Directive includes all the amendments to the previous Directive (77/93/EEC), particularly provisions to implement measures for the European Single Market in 1993. The major impact of the single market for plant health had been the introduction of plant passports for movement of certain plants and plant products within the Community and the creation of "Protected Zones", where certain organisms were absent, or not widely distributed and being controlled.

The phytosanitary requirements for imports of plants and plant materials from third countries have been reviewed, and harmonised import arrangements agreed (Directive 2002/89/EC, which comes into force on 1 January 2005). The main requirements are for imports to be made through specified points of entry, which have certain minimum facilities, and for 100% inspection of regulated material (at least checks for documentation and identity) before Customs clearance. There is a provision for harmonised rules to be drawn up to allow plant health inspections to be done at points of destination (such as nurseries) and for reduced numbers of inspections on certain commodities on the basis of low risk.

The EC phytosanitary requirements for certain regulated pests were reviewed during the reporting period as a result of occurrences of new pests, or interceptions of regulated pests on traded material. Protected Zone status of parts of the Community are also regularly reviewed. The major changes affecting SASA's work include changes in the requirements for certain *Liriomyza* spp (leaf miners) and *Bemisia tabaci* (tobacco whitefly) particularly the requirement for freedom from these pests on cut flowers, celery and herbaceous plants, rather than on all plant material (2002/36/EC). New measures, including testing for latent infections for *Erwinia amylovora* (fireblight) were introduced for plants moving to fireblight Protected Zones (2003/116/EC). The UK ceased to have protected zone status for *Beet necrotic yellow vein virus* (rhizomania).

Emergency measures were introduced for the damaging pests *Phytophthora ramorum* (2002/57/EC) and *Diabrotica virgifera virgifera* (2003/766/EC), including a requirement for surveys to determine the extent of their spread within the Community. Measures for Pepino mosaic virus were renewed (2004/200/EC). (See section 5.2 for a description of SASA's involvement with *P. ramorum*, and the Scientific Challenge article for a personal view of this work from A. Schlenzig).

Surveillance and Diagnosis

Plant health surveillance is undertaken in order to determine the presence or absence of pests in host plants in Scotland. Pests are also diagnosed on imported plant material; new or unusual organisms are identified in domestic or imported material; and diagnosis is done in support of SEERAD's certification schemes.

Horticultural Crops

Pest diagnoses were done for a wide range of pests and diseases on horticultural crops (Table 5.1).

Table 5.1 Number of diagnoses associated with horticultural crops 2001-2003 samples examined (number positive samples)

		Number of samples examined (number of positive samples)		
Pest	**Common name**	**2001**	**2002**	**2003**
Insects				
Leptinotarsa decemlineata	Colorado beetle	3 (3)	4 (4)	1 (1)
Bemisia tabaci	Tobacco whitefly	12 (12)	2 (2)	9 (9)
Thrips sp		3 (3)	2 (2)	2 (2)
Helicoverpa sp		1 (1)	6 (6)	0
Other entomological diagnoses		48 (2 x *Liriomyza* spp)	26	35 (1 x *Popillia japonica*)
Nematodes				
Nursery stock samples for PCN diagnosis		91	85	149
Other nematological analyses		6	2	2
Fungi				
Colletotrichum acutatum	Strawberry black spot	29	19	8
Erysiphe euphorbiicola	American Poinsettia Powdery Mildew	4 (3 cases on imported cuttings)		3 (3 cases on imported cuttings)
Phytophthora fragariae var. *rubi*	Raspberry root rot	16 (2)	78 (12)	125 (6)
Phytophthora ramorum	Sudden oak death	32	121 (32)	202 (15)
Bacteria				
Erwinia amylovora	Fireblight	12	16	26 (2)
Ralstonia solanacearum	Bacterial wilt	1	7	11
Viruses				
Beet necrotic yellow vein virus	Rhizomania	115	160	130
Pepino mosaic virus		48 (4 EU fruit)	42	7
Plum pox virus			308	
Raspberry bushy dwarf virus		552	600	650
Tomato bushy stunt virus		199		
Other pathogen diagnoses		25	20 (2[1])	18 (3[2])

[1] Rhododendron powdery mildew, *Pelargonium* rust

[2] *Plasmopara obducens* (Impatiens downy mildew) on imported cuttings and 2 cases of *Fusarium sp.* on *Narcissus* bulbs

See section 5.4 for results of the survey for raspberry root rot (*Phytophthora fragariae* var. *rubi*) in spawn beds in 2002-03.

Potatoes: Germplasm And New Varieties – Potato Quarantine Testing

The entry of potato material for planting into the EU from third countries is prohibited unless it is imported with a plant health licence and is subjected to post entry quarantine testing in one of the EU's potato quarantine facilities, such as the UK Potato Quarantine Unit at SASA, which are responsible for testing in accordance with EC requirements as specified in Directive 95/44/EC (as amended by 97/46/EC). European material that does not meet the EC plant health requirements is also tested. The testing done by the Unit during the reporting period is given in Table 5.2.

Table 5.2 Numbers of potato lines subject to post entry quarantine testing

Lines were *in vitro* unless indicated and were tested in accordance with Directive 95/44/EC (as amended by 97/46/EC).

	2000/2001[1]	2001/2002	2002/2003
Bolivia	1		
Canada	2		
England	2 (1 line PVS +PVX 1 line PVM+PVY)	1 (infected with PVM, PVS, PVX, PVY)	
Germany	2 (1 line PVS)		
Hungary		18 tuber lines (3 lines PVM; 2 lines PVY; 1 line PVM, PVS and PVY)	8
Israel			10 tuber lines
Japan	1 + 2 tuber lines (1 tuber line infected with PVS)		
New Zealand	1		12 + 1 tuber line
Peru			3
South Africa			1
USA	12 + 14 Tps lines	15	16
Total no. lines	**37**	**34**	**51**

[1] Data from 1 July to 30 June annually

Abbreviations: PVM, *Potato virus M*; PVS, *Potato virus S;* PVX, *Potato virus X;* PVY, *Potato virus Y;* Tps, true potato seed

Plants regenerated from the Commonwealth Potato Collection held at SCRI (http://bitrws400.scri.sari.ac.uk/cpc/default.htm) were also tested by the Unit for seed-borne viruses and *Potato spindle tuber viroid* (PSTVd) in accordance with EC requirements (Table 5.3). No pathogens were detected in this material. Seedlings that had been germinated, but not grown to maturity, were also tested for freedom from PSTVd (data not shown).

Table 5.3 Numbers of plants tested from the Commonwealth Potato Collection

	2001	2002	2003
No. plants tested:	797	1,772	551

As part of a continuing programme to improve virus detection for potato quarantine testing and for nuclear stock production, the reliability of detection of *Potato mop-top virus* (PMTV) was investigated (Nisbet *et al*., 2004) in microplants, glasshouse-grown plants and tubers. Naturally infected microplants (cvs Cara, Nicola and Slaney) were tested using ELISA and real-time PCR (TaqMan). The results of experiments over three years showed that although testing of microplants, small glasshouse plants or tubers was more reliable than testing leaves from large glasshouse plants, none of them was fully reliable. A major disadvantage of tuber testing is that all progeny tubers must be tested, because as few as 5% of tubers from each plant may be positive for PMTV. To reduce the risk of PMTV escaping detection it is recommended that several microplants derived from each candidate plant should be tested either as microplants or as 10 cm tall glasshouse plants (Nisbet *et al*., 2004) (see also section 4.4).

The programme to improve virus detection for potato quarantine testing also involved characterisation of potato virus P (PVP) and potato rough dwarf virus (PRDV). Differential symptoms were produced in indicator plants and potatoes after mechanical inoculation of the two viruses. A monoclonal antibody was produced to PVP (see section 6.5) and partial sequence data was obtained for both viruses. This work is continuing in order to characterise the viruses fully.

Surveillance Of Seed And Ware Potatoes

The ring rot and brown rot Directives (93/85/EEC and 98/57/EC respectively) require that Member States undertake surveys of their seed and ware potato production, and watercourses, for *Ralstonia solanacearum* (brown rot). Surveillance in Scotland included testing for *Clavibacter michiganensis* subsp. *sepedonicus* (ring rot) and *R. solanacearum* on all material entered for quarantine (Table 5.2), testing of all material for nuclear stock production and annual surveys of seed and ware tubers (Table 5.4). In addition, watercourses in Scotland associated with irrigation of potato crops and potato processing were sampled and tested for *R. solanacearum* (Table 5.4).

In order to ensure that potatoes in Scotland are free from PSTVd, and to meet some exporting countries' requirements, material entered for quarantine, nuclear stock production and export were tested for PSTVd. In addition certain imported potato material was tested (Table 5.4). Annual surveys were also done for *M. chitwoodi* and *M. fallax.* None of these organisms was found (Table 5.4).

Table 5.4 Numbers of samples tested for potato quarantine organisms

(see also potato quarantine testing, Tables 5.2 & 5.3)

	2001	2002	2003
Clavibacter michiganensis* subsp. *sepedonicus*			
Nuclear stock testing	118	160	169
Scottish seed potatoes	840	981	1,070
Scottish ware potatoes	66	77	63
Other potatoes	4	13	12
Ralstonia solanacearum*			
Nuclear stock testing	118	160	169
Scottish seed potatoes	840	981	1,070
Scottish ware potatoes	66	77	63
Other potatoes	4	13	12
Solanum dulcamara	32	53	52
Water samples	294	378	405
Potato spindle tuber viroid**			
Nuclear stock testing	59	78	47
Scottish seed potatoes	54		100
Scottish tomatoes			3
Other potatoes	54	19	56
Meloidogyne chitwoodi* and *M. fallax			
Seed and ware	No survey	102	99

* 200 tuber samples, apart from nuclear stock and water sampling

** Up to 10 plants per test

Interceptions and Outbreaks

As most trade in plants and plant products destined for Scotland enters the UK *via* English ports, most plant health import inspections are done by the Plant Health and Seeds Inspectors of the Department of Environment, Food and Rural Affairs (Defra). However, quarantine organisms are intercepted in material moving to Scotland (Table 5.5).

Table 5.5 Quarantine organisms intercepted on imported material

Year	Organism	Comments
2001	*Bemisia tabaci*	5 cases on imported herbs, 5 cases on imported cut flowers, 2 cases on imported plants
	Helicoverpa zea	1 case on sugar snap peas from Guatemala
	Liriomyza trifolii	1 case on imported cut flowers
	Leptinotarsa decemlineata	1 beetle on parsley from Italy, 1 beetle on potatoes from Portugal
	Pepino mosaic virus	4 cases in tomato fruit
2002	*Bemisia tabaci*	1 case on cut flowers from Israel
	Helicoverpa armigera	1 outbreak under glass. Caterpillars found on pelargoniums imported from Canary Islands
	Leptinotarsa decemlineata	2 beetles on parsley (from Portugal and Italy), 1 found in a house
2003	*Bemisia tabaci*	8 cases on imported poinsettia plants
	Leptinotarsa decemlineata	1 beetle on parsley from Italy
	Popillia japonica	1 beetle found in an airport warehouse

In 2000, *R. solanacearum* was found in the Tay River system in Scotland for the first time. The organism was confirmed in part of the River Tay, part of the River Isla and part of the Lunan Burn (Wood *et al*., 2002). A statutory ban was put in place, prohibiting the use of irrigation water from the contaminated water courses from 2000. A SEERAD-funded eradication programme, co-ordinated by SASA, was successful in eradicating infected plants of the alternative host *Solanum dulcamara* from the Lunan Burn in 2001 and from the Lunan Burn and River Isla in 2002 and 2003. SASA continued to monitor the water in the contaminated watercourses throughout this period and no *R. solanacearum* was found in river water samples in the years 2001-2003. The irrigation ban was lifted from the Lunan Burn and the River Isla, with effect from 1 April 2004. Monitoring of the watercourse and eradication of *S. dulcamara* from the River Tay will continue in 2004.

In 2002 an outbreak of *Helicoverpa armigera* occurred in a production nursery (see section 5.5 for a summary of this outbreak and the action taken).

Research And Development

During the reporting period, SASA was involved with an EU-funded project (DIAGPRO) on production of diagnostic protocols for quarantine pests, and SASA co-ordinated the production of a diagnostic protocol for PSTVd (see section 5.3). SASA staff were involved in ring testing of protocols for *Bemisia tabaci, Clavibacter michiganensis* subsp. *sepedonicus, Impatiens necrotic spot virus* and *Tomato spotted wilt virus,* and ring tests were carried out for *Pantoea stewartii subsp. stewartii.*

A three year SEERAD flexible fund project was awarded to SASA in 2003 to assess the risks to seed potato production in Scotland from *Ralstonia solanacearum* in water courses.

Bee Diseases

Two new bee parasites were added to the European Union's list of notifiable animal diseases in August 2003. They are the small hive beetle, *Aethina tumida,* and the parasitic mite, *Tropilaelaps coleopteren.* SASA provided training to SEERAD officers and bee keepers on the biology of these organisms, their identification and the risks they pose to bees. In December 2003, the whole of Scotland was designated as a Statutory Infected Area for *Varroa destructor* (formerly *V. jacobsoni*). Throughout the reporting period SASA provided a diagnostic service to beekeepers for identification of *V. destructor* in hive debris as part of the EU "honey programme".

References

Nisbet C, Orman J, Jeffries C. 2004. Detection of *Potato mop-top virus* in microplants, glasshouse grown plants and tubers. *Proceedings of the EAPR Virology Section Meeting, Rennes, France, 13-19 June 2004,* p 60.

Wood JR, Breckenridge K, Chard JM. 2002. Survey for *Ralstonia solanacearum* in Scottish rivers. *Proceedings of the Crop Protection in Northern Britain Conference 2002,* 237-242.

5.2 *Phytophthora ramorum* (sudden oak death) (A Schlenzig and J Chard)

Phytophthora ramorum causes the death of American oaks and tanoaks in the United States (sudden oak death) and has also been found associated with ornamental plants and some tree species in Europe (Anon., 2001; Werres *et al*., 2001) (see Scientific Challenge article for symptoms and a description of the organism). The organism was found for the first time in Scotland in April 2002 and, by the end of that year, there had been 18 findings (outbreaks) of the organism. During 2002, SEERAD horticultural inspectors inspected 180 premises (365 inspections) and 121 samples were received by SASA, with 32 positive. SASA found the first infected lilac (*Syringa vulgaris*) (Beales *et al*., 2004).

Emergency legislation came into force in May 2002 (The Plant Health (*Phytophthora ramorum*) (Scotland) Order 2002)) and required certain susceptible genera imported from the USA to be from an area free from *P. ramorum* and to be accompanied by a phytosanitary certificate. In addition, Scottish producers and traders were required to notify the movement of susceptible material into and within Scotland. Emergency EC legislation was subsequently enacted (Commission Decision 2002/57/EC) and this was implemented in Scotland by The Plant Health (*Phytophthora ramorum*) (Scotland) (No. 2) Order 2002 that came into force on 1 November 2002. This required the registration of premises producing *Rhododendron* and *Viburnum* host plants and plant passports to be issued for these plants. Imports of host plants from the USA were restricted and required a phytosanitary certificate and host material from third countries required a plant passport for movement within the EC.

During 2003, SASA undertook risk assessments of outbreaks at five nurseries and extended surveillance to woodlands surrounding these sites. Inspections were made in conjunction with Forestry Commission inspectors. In the autumn of 2003 surveillance was further extended to include a survey of 51 gardens that had received host material in the last 5 years. No *P. ramorum* was found either in inspections or in the 68 samples that were analysed by SASA. However, there were a further 6 findings (outbreaks) of the organism at nurseries in Scotland during 2003 and several other *Phytophthora* spp were also found associated with plants sent in for diagnosis (Table 5.6).

Table 5.6 Other *Phytophthora* species isolated during 2002-03 associated with sampling for *P. ramorum*

Phytophthora species	Number of positive *Rhododendron* plants	Number of positive samples from other sources	Source
P. cactorum	2		
P. cinnamomi	1		
P. citrophthora		1	*Arbutus*
P. inflata?		2	*Gaultheria, Vaccinium*
P. gonapodyides		1	*Rhododendron* leaf litter
P. gonapodyides-like	2	1	Irrigation water
P. syringae	3	2	*Viburnum tinus*, gravel
Total	**8**	**7**	

Following the first finding of *P. ramorum* in a tree species in the UK (see Scientific Challenge article) in November 2003, the Forestry Commission undertook extended surveys of woodlands across Great Britain (Forestry Commission, 2004), with SASA testing the samples from Scotland; no *P. ramorum* was detected. The EC legislation was amended by Decision 2004/426/EC, requiring a plant passport for *Camellia* plants and surveys of cultivated and uncultivated plants; extending the list of known hosts of the organism; and requiring containment of the organism when it is found outside nurseries.

In 2004 SEERAD is increasing the monitoring of nurseries and continuing to survey gardens and managed landscapes in order to detect the organism and determine the extent of its spread within Great Britain. Eradication measures are being implemented in England at sites where the organism has been found in gardens in order to prevent the further spread of the organism (www.defra.gov.uk/planth/pramorum.htm).

References

Anon. (2001). *Sudden Oak Death.* http://www.camfer.cnr.berkeley.edu/oaks/

Beales PA, Schlenzig A, Inman AJ. 2004. First report of ramorum bud and leaf blight (*Phytophthora ramorum*) on *Syringa vulgaris* in the UK. *New Disease Reports* **9** (http://www.bspp.org.uk/ndr/volume.asp)

Forestry Commission 2004. *Report on the Forestry Commission Woodland Surveys 2004 to Assess the Level of Incidence of Phytophthora ramorum in British Woodlands.* Forestry Commission Plant Health Service, May 2004.

Werres S, Marwitz R, Man In'T Veld WA, De Cock AWAM, Bonants PJM, De Weerdt M, Themann K, Ilieva E, Baayen RP. 2001. *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum. Mycological Research* **105:** 1155-1165.

5.3 Development of an EU Protocol for the detection and diagnosis of *Potato spindle tuber viroid* (T James and C Jeffries)

Potato spindle tuber viroid (PSTVd) is a quarantine organism in the EC. This pest has been of particular concern in the last five years because of findings in potato in France and in tomato in Australia, England, the Netherlands and New Zealand. EU phytosanitary requirements require potato material to be free from PSTVd and there is a requirement for potato material undergoing post-entry quarantine to be tested before release. In the UK, potato nuclear stock material is also tested, demonstrating the importance placed on ensuring the health of the initial material for potato certification.

Between 2000 and 2003, SASA co-ordinated the development of an EU diagnostic protocol for PSTVd, as part of an EU SMT project (DIAGPRO) to develop diagnostic protocols for 18 quarantine organisms. Thirteen laboratories from Europe and the USA took part and the protocol was developed primarily for testing *in vitro* and glasshouse grown potato plants for the purposes of post-entry quarantine and the production of pathogen-tested nuclear stock.

After carrying out initial performance audits of the methods used by 12 laboratories in Europe and America in 2000, four (the "EU methods") were chosen for multi-laboratory validation. These were R-PAGE, DIG-probe, RT-PCR and Taqman. The performance audits involved ring tests of two sets of samples: one sample set required no extraction (to test the sensitivity of the detection method directly), and the second set was added to healthy potato leaf tissue and extracted (to check the sensitivity of the full protocol). A Russian isolate of PSTVd was used throughout the project for all evaluations.

The multi-laboratory validation of the EU methods (Jeffries and James, 2005) was undertaken by 13 laboratories during 2002 and 2003. For most laboratories, the limits of positive detection were 10-20 mg of PSTVd infective tissue for R-PAGE; 0.25-0.5 mg for DIG-probe; 0.062 mg for RT-PCR; and 0.0155 mg for TaqMan (the lowest weight of infective tissue tested). Some laboratories were able to extend the detection limit to 0.0155 mg for DIG-probe and RT-PCR.

The PSTVd diagnostic protocol, which has been approved as an EPPO diagnostic protocol (OEPP/EPPO, 2004) recommends the DIG-probe and R-PAGE as primary detection methods, with confirmation of viroid presence by any of the four validated detection methods. Specific diagnosis requires the viroid to be sequenced. Other methods may be used for primary detection, providing that they a) preferably detect all PSTVd isolates and other pospiviroids that have the potential to infect potato, and b) detect viroid in at least 1/10th of the tissue weight normally tested per plant.

References

Jeffries CJ, James C. 2005 Development of an EU protocol for the detection and diagnosis of Potato spindle tuber viroid. *OEPP/EPPO Bulletin* **25(1)** *in press.*

OEPP/EPPO. 2004. EPPO standards PM 7/33(1) Diagnostic protocols for regular pests. *Potato spindle tuber viroid. OEPP/EPPO Bulletin* **34** 257-269.

5.4 Root Rot survey in certified raspberry stocks (A Schlenzig)

Over the last 30 years the raspberry growing area in Scotland has declined dramatically from over 3,000ha at its peak in 1973 to just 570ha in 2002. One factor in this decline has been the occurrence of the root rot pathogen *Phytophthora fragariae* var. *rubi* in the soil. As a major source for contamination of raspberry plantations is infected planting material, a certification scheme was introduced to ensure that the growers receive planting material of high health standard. The certification scheme is administered and supervised by SEERAD and inspections are carried out annually to confirm that the certification requirements, which include the absence of all major raspberry diseases, are met.

In winter 2002/2003, SASA conducted a raspberry root rot survey of all raspberry stocks entered in the certification scheme at Super Elite and Elite grades, half the stocks at Standard grade and half the stocks entered for plant passporting (minimum plant health requirement). 136 samples from 57 raspberry stocks were examined and two different test methods were compared: The Duncan bait test, as recommended in the European and Mediterranean Plant Protection Organization (EPPO) guidelines, and a nested PCR method developed by SCRI and Pest Research International in Wageningen.

For the bait test, raspberry root samples are chopped into small pieces, and added to pots into which a bait plant of the highly susceptible variety "Glen Moy" is planted. After 5-6 weeks incubation in the greenhouse, if the root rot pathogen is present in the sample, the bait plant develops typical wilting and yellowing symptoms (Fig. 5.1) and oospores are observed under the microscope.

Figure 5.1 Detection of *Phytophthora fragariae* var. *rubi* by bait test 5 weeks post inoculation. Mixtures of infected/ healthy raspberry roots, from right: 300g/0g; 100g/200g; 33.3g/266.7g; 11.1g/288.9g; 3.7g/296.3g; 1.2g/298.8 g; 0g/300g

The PCR method involves a nested PCR, which targets the ITS regions of the rDNA. The first round of the PCR amplifies DNA of fungi belonging to the *Peronosporales* and the size of the expected PCR product is approximately 1310 bp. The second round amplifies specifically *Phytophthora fragariae,* with positive samples producing a band of 533 bp in size.

In general, the correlation between the results of the bait test and the PCR method was very good (Table 5.7). The bait test detected 12 samples infected with *P. fragariae* var. *rubi*, from 4 different raspberry stocks. Of these, two were entered for Standard grade and two for plant passporting. The PCR detected 10 infected samples, which were the same as in the bait test. For one stock, only 2 samples were identified as infected, rather than 3 in the bait test. Another stock was only partly contaminated, the bait test detected infection in 2 of the 4 samples and the PCR detected only one. Both tests detected the positive control.

Table 5.7 Bait test and nested PCR results for the detection of *Phytophthora fragariae* var. *rubi* in 136 samples from 57 raspberry stocks of the Scottish raspberry certification scheme

	PCR			
	samples		stocks	
Bait test	positive	negative	positive	negative
positive	10	2	4	0
negative	0	124	0	53

One problem with the PCR method is that the size of sample that can be processed in the DNA extraction is limited, representing only a small percentage (here about 1%) of a whole field sample. There is, therefore, a high probability that infected roots are absent when the sub-sample is taken for the PCR, whereas for the bait test the whole sample is tested. To increase the probability of having infected roots in the PCR sample, and therefore to increase the accuracy of the PCR, it is recommended to select apparently unhealthy roots and to take several sub-samples from each field sample.

Finding only a low number of infected raspberry propagation stocks at the lowest level of certification indicated the success of the Scottish certification scheme for raspberries. Nevertheless, a recent survey of raspberry fruiting plantations done by SCRI showed that *P. fragariae* var. *rubi* was present in some fields of most farms that were surveyed. This indicated that, at least in cases where certified planting material was used, the source of infection was probably field to field contamination through run-off water or soil movement (for example with machinery), indicating that much stricter hygiene is required on many farms.

5.5 *Helicoverpa armigera* Outbreak In Scotland (L King)

Pest Status

Commonly known as Old World Bollworm, the moth *Helicoverpa armigera* is a widespread pest throughout southern and central Europe, Asia, Africa and Australasia (Fig. 5.2). The larvae feed on a wide range of hosts, causing extensive damage to ornamental plants, flowers, and crops such as cotton, tomato, maize, tobacco, and many legume species (EPPO, 1996).

Figure 5.2 ***Helicoverpa armigera***

H. armigera is a quarantine pest listed in Annex IAII of EU Plant Health Directive (2000/29) and has been designated as an A2 pest by the European Plant Protection Organization. Consequently EU countries are required to prevent its establishment and spread. In Britain it poses a threat to glasshouse crops.

Outbreak and Control

During routine inspections of nursery premises by SEERAD Horticultural Inspectors in April 2002, *H. armigera* caterpillars were found in a glasshouse on *Pelargonium* plants imported from Kenya/Canary Islands. The plants were put under statutory notice by SEERAD inspectors and subsequent intensive inspections of all *Pelargonium* imports determined that the infestation was restricted to particular stocks. By using moth light traps, SASA's entomologists were able to determine that no adults were present initially. The absence of adults and the time interval since importation indicated that only larval and potentially pupal stages of the lifecycle were present. As a result, SEERAD imposed segregation of infested stocks, restricted movement of host plants and instituted a programme of pesticide applications as eradication measures. Monitoring for adults using the light traps continued and SEERAD notified the Plant Health and Seeds Inspectorate of Defra, in order to arrange for sister stocks in England to be traced and inspected.

Following several weeks of monitoring, one adult moth was caught, indicating that the lifecycle had been completed despite the use of chemical control. Infected stocks were therefore subsequently subject to 'controlled supervised' disposal. As a final measure, the glasshouse was cleared, fumigated and restocked with non host plant varieties.

Throughout the outbreak, SASA entomologists worked closely with SEERAD Inspectors, providing them with pest identifications, information and recommendations necessary for them to implement control measures.

Reference

EPPO. 1996. *Helicoverpa armigera. Quarantine Pests for Europe. Data sheets on quarantine pests for the European Union and European and Mediterranean Plant Protection Organisation,* 2nd Edn. CABI and EPPO, 289-294.

Secondment at the Food and Agriculture Organisation (October 2002-May 2003)

J Chard

Dr Jane Chard

Having spent seven years as a specialist adviser in agricultural microbiology at the Scottish Agricultural College, I joined SASA in January 1992 as head of the Plant Health Section. This was the year before implementation of the European Single Market for plant health and the introduction of a completely new way of approaching plant health in Europe, particularly the use of plant passports for movement of certain plants throughout the Community. Since then there have been numerous challenges, most of which requiring some action in Scotland, and including outbreaks of pests, findings of pests in new hosts and pests that are new to science.

When it was suggested that I explore options for a 3-6 month personal development project, I leapt at the opportunity. One option was a study tour of potato production in South America, but my Spanish was not good enough for that type of work. As I felt it was important for SASA to understand more about the international plant health scene, I contacted Bob Griffin, the then co-ordinator of the International Plant Protection Convention (IPPC) Secretariat to ask whether it would be possible to work with them. I received a positive response and ended up working as a visiting expert at the Food and Agriculture Organization (FAO) in Rome.

The IPPC is an international treaty that was ratified in 1952 and administered by the IPPC Secretariat (see www.ippc.int) based at FAO. The purpose of the Convention is to secure common and effective action to prevent the spread and introduction of pests of plants and plant products. It details the obligations and responsibilities of the more than 120 countries that are parties to the Convention. The Convention was amended (came into force in 1991) and further amended in1997 to take into account developments in international trade covered by the Sanitary and Phytosanitary (SPS) Agreement of the World Trade Organization (not yet in force). The 1997 Convention includes a provision for the establishment of a framework of international standards on phytosanitary measures (ISPMs).

As these standards have an impact on much of the work of SASA, particularly in relation to implementing plant health import and export requirements, one of my main aims during the secondment was to understand and participate in the standard setting process. One of the first tasks when I arrived in Rome (apart from familiarising myself with new working procedures and many phytosanitary documents) was to represent the IPPC Secretariat at a seminar on the SPS Agreement held by the Asian Productivity Organization in Tokyo, Japan. This involved making a presentation on the role of the IPPC and acting as facilitator in a workshop session.

During the seven months I was in Rome I chaired the expert working group that drafted an ISPM on pest risk analysis for regulated non-quarantine pests in February 2003. This standard has now been adopted by the Interim Commission for Phytosanitary Measures (the forum where all the parties to the Convention meet) in April 2004. Subsequently I have been involved in a second drafting group on the topic of equivalence which is under consultation during summer 2004.

My secondment provided an opportunity to meet many phytosanitary officials and to appreciate the reasons for differences in approach world wide. It was also an opportunity to live and work in a fascinating city – my walk to work past the ancient Colosseum and Forum being a particular highlight.

Working with the Sudden Oak Death Pathogen

A Schlenzig

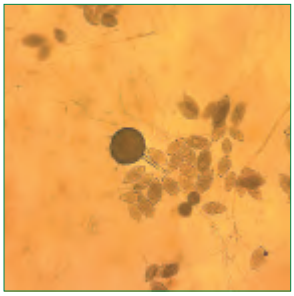
Dr Alexandra Schlenzig

After studying horticulture I did my PhD at the Technical University of Munich working on epidemiological issues of potato late blight (*Phytophthora infestans*). The subsequent post-doctoral position at the Institute of Forest Botany consisted of two projects on the detection and identification of *Phytophthora sp.* as causal agents of oak- and alder decline and this experience has proved to be unexpectedly useful for my current work at SASA. It also set up my link to Scotland since part of the work was done in collaboration with SCRI. Another postdoctoral position followed and led me to Switzerland where I worked for two years in the biotechnology group of Syngenta Crop Protection near Basel. Although I had used methods like PCR before, this job gave me a much stronger base in molecular biology and a taste of what it is like working for an industrial company.

A big part of my work in the Plant Health section of SASA is the identification and surveillance of quarantine diseases. When I started my job I was given a list of quarantine disease to watch out for with the remark "but we usually do not get them in Scotland". On the list was a new *Phytophthora* causing, since 1995, an alarming dying of oaks and tanoaks on the west coast of the USA, a phenomenon that became famous as "Sudden Oak Death" (SOD). It took some years before a British scientist from the Forestry Commission, Clive Brasier, linked the same pathogen to a disease that had already been discovered in 1993 on *Rhododendron* and later on *Viburnum* plants in nurseries in The Netherlands and Germany. In 2001 this pathogen was officially named "*Phytophthora ramorum*". After it was clear that the SOD pathogen was already present in Germany and The Netherlands, a survey of Britain's nurseries and garden centres was initiated in July 2001, and in February 2002 five infected *Viburnum* plants were found in a nursery in England.

In April 2002, 8 weeks after I had started my job at SASA, some *Rhododendron* and *Viburnum* plants (see Figure) from a nursery in Fife were brought in by the SEERAD horticulture inspectors "to check them for SOD". One of the problems in the diagnosis of quarantine diseases is that most of the time you have to identify diseases you have never seen before because they are supposed to be absent from your country. Luckily, I had some experience with *Phytophthora*s and I just tried the same method I had used before for the isolation of *Phytophthora* from alder trees. Symptom-showing parts of the plant are placed on selective agar plates with antibiotics inhibiting other pathogens. If the plant is infected by a *Phytophthora*, mycelium growth is visible by 5 days, and this was the case in one of the submitted *Viburnum*s. The species then needs to be identified by microscope, normally a task for specialists since there are around 65 *Phytophthora* sp. in existence and more are discovered every year. Characteristics are mycelium growth patterns, the presence or absence of the four possible spore types and their shapes and sizes. Fortunately, *P. ramorum* has some distinctive features, making its identification relatively easy. Its sporangia are semi-papillate and deciduous but most noticeable are the typical big and dark coloured resting spores, the so called chlamydospores (see Figure). Finding them present in my isolate, I was quite sure that I had detected the first case of *P. ramorum* in Scotland. Sending away the isolate to Clive Brasier and to the Central Science Laboratory, York, gave final confirmation. Very soon a PCR method based on the ITS regions of its nuclear ribosomal DNA became available to confirm microscopic identification. SEERAD inspectors carry out hundreds of inspections in nurseries and garden centres every year and the testing for *P. ramorum* is now routine at SASA.

Sporangia and Chlamydospore (C) of *Phytophthora ramorum*



Phytophthora ramorum* on *Viburnum tinus

Despite its name, 'Sudden Oak Death' does not affect only oaks or always cause the death of the plant. Many different tree species have been affected by the disease in the USA, such as maple, redwood, and Douglas fir. Apart from *Rhododendron* and *Viburnum*, in Scotland the pathogen has been found on lilac, and in the rest of the UK on *Camellia, Kalmia, Pieris, Leucothoe,* Yew, *Hamamelis*. For a long time it appeared that Europe would be lucky in finding the pathogen only on horticultural plants and mainly in nurseries and garden centres, but in November 2003 the disease was confirmed on a Southern red oak (*Quercus falcata*) in England and a red oak (*Q. rubra*) in The Netherlands. *P. ramorum* has been found since then on holm oak (*Q. ilex*), turkey oak (*Q. cerris*), red oak (*Q. rubra*), beech (*Fagus sylvatica*), horse chestnut (*Aesculus hippocastanum*), and sweet chestnut (*Castanea sativa*).

Phytophthora can spread through contaminated soil or water, but movement of infected plant material is the cause of spread over long distances. Therefore, after the first finding, Scotland followed England and Wales and introduced emergency legislation to prevent the further spread of the disease: the "Plant Health *Phytophthora ramorum* Order" from May 2002. The Order prohibited the import of susceptible plants, planting material, wood and bark from infested areas of the USA. Within Europe the movement of susceptible hosts was controlled, and all commercial deliveries of *Rhododendron* or *Viburnum* plants had to be notified to SEERAD for inspection. The Scottish Emergency measures were replaced by very similar EU legislation from the 1st of November 2003. All host material moving in trade within the EU now requires a valid plant passport, otherwise it will be destroyed.

We have at the moment 20 outbreak sites in Scotland all in nurseries and garden centres, apart from one private garden. Survey work has been extended to parks and woodland but, thankfully, we have not found *P. ramorum* on any trees yet. In the whole of Britain there are more than 300 known outbreak sites, most in nurseries and garden centres, but the disease has also been in found in several public parks and gardens and even on a few wild *Rhododendron*s. It seems that 2004 will be another busy year in which, possibly, the decision has to be made whether there is still a chance of eradicating this new pathogen or whether it is established in Britain and the effort has to change to managing the disease rather than eradicating it.

Scotlab

6 Diagnostic Tools

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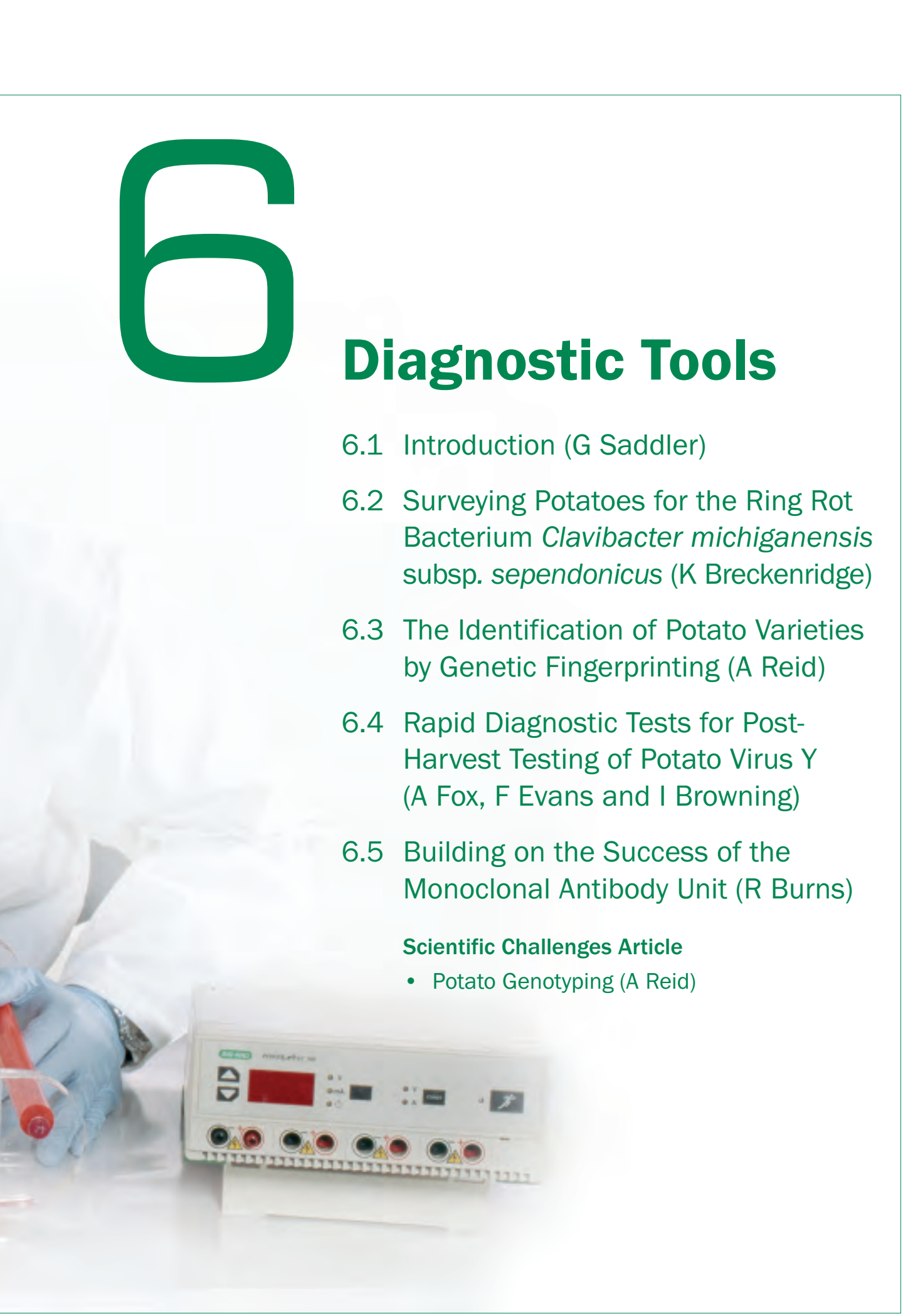
Scientific Challenges Article

- Potato Genotyping (A Reid)



Chapter 6 Diagnostic Tools

6.1 Introduction (G S Saddler)

Diagnostic tools have been employed by SASA in various aspects of its work for the last 50 years. The term 'diagnostic tool' is ill defined but, in the context of the work of the Agency, it covers two broad areas: the use of monoclonal antibodies and molecular biological methods. Both technologies are used primarily for the detection of plant pathogens but, increasingly, are being applied in the identification of GM traits. In addition, molecular biological methods are now used extensively in plant variety characterisation work.

Recent years have seen the steady migration of molecular biological methods from the research environment into more mainstream, routine usage within the work of the Agency. This trend is set against the backdrop of unprecedented developments in the wider field of molecular biology. During the period of this review the initial sequence analysis of the human genome was published (International Human Genome Sequencing Consortium, 2001), preceded slightly by the first plant genome, of *Arabidopsis thaliana* (The Arabidopsis Genome Initiative, 2000). The rice genome, the first crop plant to be studied and the staple food of two-thirds of the World's people, was published in 2002 (Yu *et al*., 2002; Goff *et al*., 2002). The first complete sequence of the bacterial plant pathogen of grape, *Xylella fastidiosa,* was produced (Simpson *et al*., 2000) and, more recently, and directly applicable to work of SASA, the sequence of the potato brown rot pathogen, *Ralstonia solanacearum* has been published (Salanoubat *et al*., 2002). The information, technology and understanding derived from these and similar genome studies are beginning to shape SASA's future work in diagnostic tools. For example, the *R. solanacearum* sequence data is being exploited as part of a major project which began late 2003, with funding from SEERAD's Flexible Fund, to develop insights into the diversity of the brown rot pathogen, detected in Scottish rivers in 2000 (Wood, 2001).

In general, though, much of the work in this area exploits developments in less high-profile branches of molecular biology. In this capacity, the Agency has spent a great deal of effort in adapting molecular biological methods so that they can be used to answer the kind of questions it asks, with the level of confidence that it requires. The remaining papers in this chapter describe some of the results of those efforts.

The majority of diagnostic test development work is conducted by a small group of scientists within SASA, dedicated to improving routine tests used throughout the Agency in pursuit of its objectives. Diagnostic tools are being used to ensure that Scotland remains free of the potato ring rot pathogen, *Clavibacter michiganensis* subsp. *sepedonicus*, and section 6.2 describes this work. Section 6.3 describes the development of a system, using genetic fingerprints, to identify potato varieties. This technology played a key role in the emergency actions of colleagues at CSL, York, and Defra in tracing the extent of a recent outbreak of ring rot, which occurred on a farm in Wales during November 2003. Section 6.4 reports on a major theme of the Agency's work, namely improved pathogen detection, showing how the relatively novel technology of real-time PCR can be used to help determine whether, and the extent to which, seed potato tubers may harbour viral infections. Finally, Section 6.6 returns to where SASA's work in diagnostic tools began within our monoclonal unit, giving details of how this technology is being applied to develop new assays for the detection of GM.

References

Goff SA. *et al.* 2002. A draft sequence of the rice genome (*Oryza sativa* L. *ssp. japonica*). *Science* **296:** 92-100.

International Human Genome Sequencing Consortium 2001. Initial sequencing and analysis of the human genome. *Nature* **409:** 860 – 921.

Salanoubat M. *et al.* 2002. Genome sequence of the plant pathogen *Ralstonia solanacearum. Nature* **415:** 497–502.

Simpson AJG. *et al.* 2000. The genome sequence of the plant pathogen *Xylella fastidiosa. Nature* **406:** 151 – 157.

The Arabidopsis Genome Initiative 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana. Nature* **408:** 796-815.

Wood JR. 2001. *Ralstonia solanacearum* in Scottish Rivers. In *Scottish Agricultural Science Agency Scientific Review 1997-2002,* 65-67.

Yu J. *et al.* 2002. A draft sequence of the rice genome (*Oryza sativa* L. *ssp. indica*). *Science* **296:** 79-92.

6.2 Surveying potatoes for the ring rot bacterium, *Clavibacter michiganensis* subsp. *sepedonicus* (K Breckenridge)

Potato ring rot is a serious bacterial disease caused by *Clavibacter michiganensis* subsp. *sepedonicus* (Cms). Foliar symptoms include interveinal discoloration leading to the development of necrotic areas that cause leaf margins to curl, and wilting can also be observed (Fig. 6.1). The primary tuber symptom is discoloration of the vascular tissue that may develop to give distinctive hollowed-out corky-brown tissue (Fig. 6.2). The disease is primarily spread by planting contaminated seed potatoes, although spread through contact with contaminated equipment may also be important. Cms has a relatively low optimum growth temperature (21-23°C) and is confined mainly to cooler potato growing regions. The climate in north and central Europe is particularly favourable for disease development and persistence. The significance of the disease as a threat to potato production has resulted in the European Community introducing legislation in the form of Council Directive 93/85/EEC for its control. The Directive requires that member states carry out surveys involving inspection and surveillance for the disease.

Figure 6.1 Foliar symptoms of ring rot of potato caused by *Clavibacter michiganensis* subsp. *sepedonicus* (Cms). Interveinal discoloration, necrotic lesions and leaf curl, can be observed leading to wilting. Figure produced with permission of Dr Solke H. De Boer, Canadian Food Inspection Agency, Charlottetown, PEI, Canada.

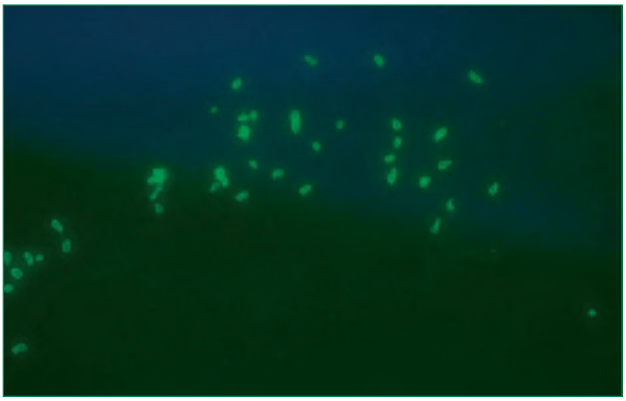
Figure 6.2 Tuber symptoms of ring rot of potato caused by *Clavibacter michiganensis* subsp. *sepedonicus* (Cms). The primary tuber symptom is discoloration of the vascular tissue that may develop to distinctive hollowed-out corky-brown tissue. Figure produced with permission of Dr Solke H. De Boer, Canadian Food Inspection Agency, Charlottetown, PEI, Canada.

Although Scotland is free of ring rot, a number of EU member states have detected the bacterium in their potato stocks. Trade in potatoes within the EU, therefore, raises the spectre of the introduction of ring rot into Scottish seed potatoes. If ring rot were to be introduced it would have a devastating affect on any affected growers: destruction of stocks, cropping restrictions etc., to say nothing of the damage it would do to the reputation of Scottish seed potatoes around the world. The recent discovery of ring rot in potatoes grown in Wales serves to highlight the need to maintain vigilance. As part of Scotland's obligations under UK and EU plant health requirements, SASA conducts annual survey of Scottish seed and ware potato stocks, as well as monitoring all imports of potatoes destined for cultivation to ensure that the country remains free from ring rot.

In 2003 the area of seed potato crops presented for classification was 11,224ha. As part of the annual ring rot survey, 562 samples were drawn from these stocks, to maintain a 1 per 20ha sampling ratio for the disease. The area of ware potatoes grown was 16,117ha, requiring a total of 81 samples for a sampling ratio of approximately 1 per 200ha. In addition to this survey work, it is important that the approximately 3 million mini-tubers produced in Scotland and classified as pre-basic seed are also free from Cms. These tubers are produced from microplants derived from cultures supplied from the UK nuclear stock collection at SASA, all of which have been officially tested for Cms. In addition, importers are encouraged to submit material for voluntary testing of stocks brought in from other parts of Europe, and all progeny of seed grown from non-Scottish seed is tested. In this way the high health status of Scottish seed potatoes is protected, thus maintaining existing markets and enabling growers to compete for new markets in Europe and elsewhere.

The application of diagnostic methods is crucial to this monitoring work. Laboratory checks are carried out for latent infection, and tubers are cut and examined for visual symptoms. The samples are prepared and tested using methods described in the Ring Rot Directive 93/85/EEC. Bacterial extracts are tested by Immunofluorescence Antibody Staining (IFAS), using a monoclonal (McAb 9A1, Agdia) antibody (Fig. 6.3). Any suspect sample extracts are inoculated into eggplants, which are checked for symptom development. After 40 days, bacterial extracts are prepared from the stem tissue and checked by IFAS testing. Additional back-up molecular tests are also carried out using real-time PCR.

Figure 6.3 Immunofluorescence Antibody Staining (IFAS) of *Clavibacter michiganensis* subsp. *sepedonicus* (Cms) positive control material using a monoclonal (McAb 9A1, Agdia) antibody

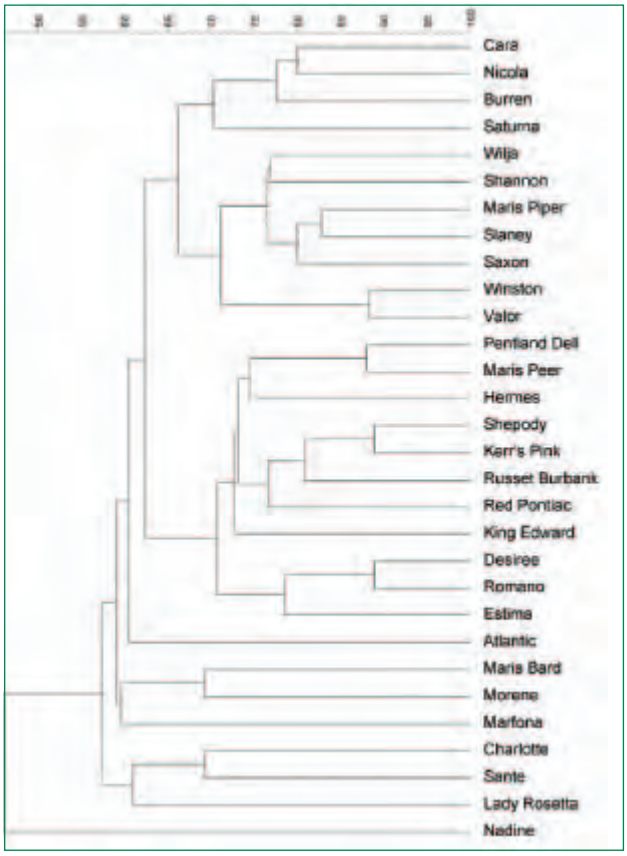


6.3 The identification of potato varieties by genetic fingerprinting (A Reid)

Genetic fingerprinting is now a commonplace and widely-recognized tool in forensic science. In the last decade the use of this technology has spread to include organisms as diverse as bacteria, fungi, animals and plants. Over the last two years SASA has developed a genetic fingerprinting method to identify potato varieties, by a technique that detects variability within microsatellite (or simple sequence repeat) markers. These are short repetitive elements, usually between 2 and 10 base pairs in length, distributed throughout the DNA of an organism. For example, the microsatellite, $(CAT)_6$, contains the sequence CAT repeated six times, the variability between cultivars arising from the number of times the element is repeated.

Twenty-eight microsatellite markers were selected from a database of over 200 generated by SCRI (Milbourne *et al*., 1998) as they were stable, easily analysed and representative of the genome. These markers were screened against 16 varieties and 12 were selected for further testing, which displayed good peak morphology (i.e. they were easy to score); the alleles were stable over a range of annealing temperatures; and they showed differences among the varieties tested. The 12 markers were then used to screen 150 varieties and the data generated stored in a BioNumerics (Applied Maths) database. By interrogating the database it was found that all the varieties could be differentiated using only 6 of the 12 markers (in fact only 4 markers are required to distinguish the 122 varieties on the current UK National List). Fig. 6.4 gives an example of the traces from 4 varieties. The 6 core markers have now been used to screen over 350 varieties, all of which can be differentiated with the exception of essentially derived varieties (EDVs); these are stable phenotypic mutants of other varieties e.g. King Edward and Red King Edward which probably differ by only a few genes. The microsatellite data can also be used to construct phylogenetic trees to show the inter-relationships and genetic distances among varieties (an example showing the top 30 varieties grown in Scotland in 2002 is shown in Fig. 6.5).

Figure 6.5 Phylogenetic tree showing the similarity between the top 30 varieties grown in Scotland in 2002. The scale is percent similarity.



The database is now in use in support of the maintenance of the potato culture collection at SASA as well as offering an identification service to industry and the public. The system has reached the stage where, from receipt of an unknown sample to identification, can be achieved in a single day. Virtually any food product containing potatoes can be analyzed and profiles have been obtained from boiled and roast potatoes, crisps, chips, instant mash, potato scones and potato based snack products.

In November 2003, the rapid identification of potato varieties became an important issue during the first recorded outbreak of the quarantine disease ring rot in the UK (See Section 6.2). This posed a serious threat to the UK seed potato industry, but rapid elimination of the disease was achieved because SASA was able to determine which of the two possible varieties involved was the source of the infection (Fig. 6.6).

Reference

Milbourne D. ***et al.*** **1998**. Isolation, characterisation and mapping of simple sequence repeat loci in potato. *Molecular and General Genetics* **259:** 233-245.

6.4 Rapid diagnostic tests for post-harvest testing of Potato Virus Y (A Fox, F Evans and I Browning)

Potato virus Y (PVY) is aphid borne, causes mild to severe leaf mosaic in potato and it can result in yield losses of 10-100%. Although PVY has the potential to cause severe damage to Scotland's seed potato stocks it can be controlled by the production of disease-free nuclear stock planting material and the operation of an effective seed classification scheme.

Figure 6.4 Microsatellite profiles yielded by four markers (black, red, green and blue peaks) from four potato varieties. The orange peaks are size standard markers. The four varieties (from top to bottom, Golden Wonder, Kerr's Pink, Arran Victory and Avondale) can all be identified by differences among the profiles generated from these four markers.

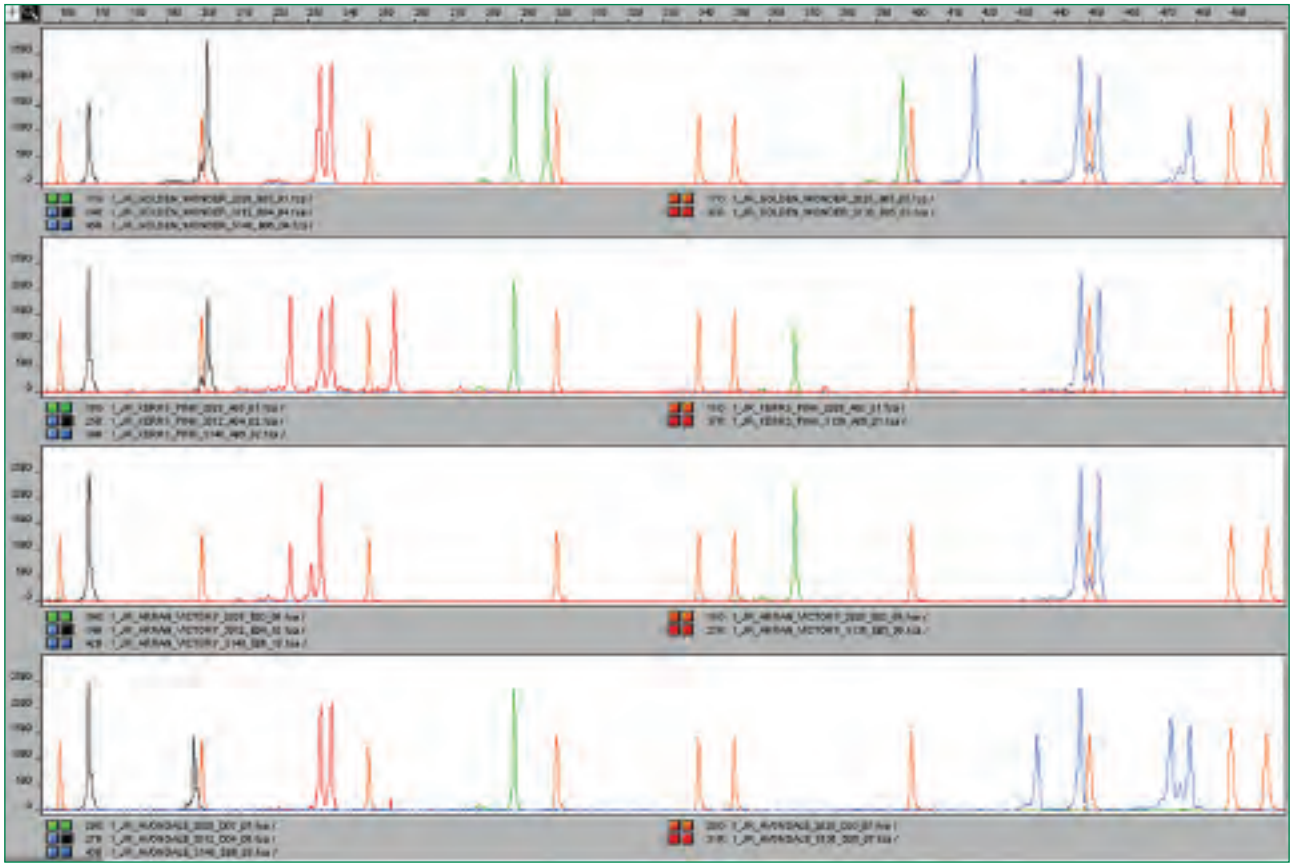
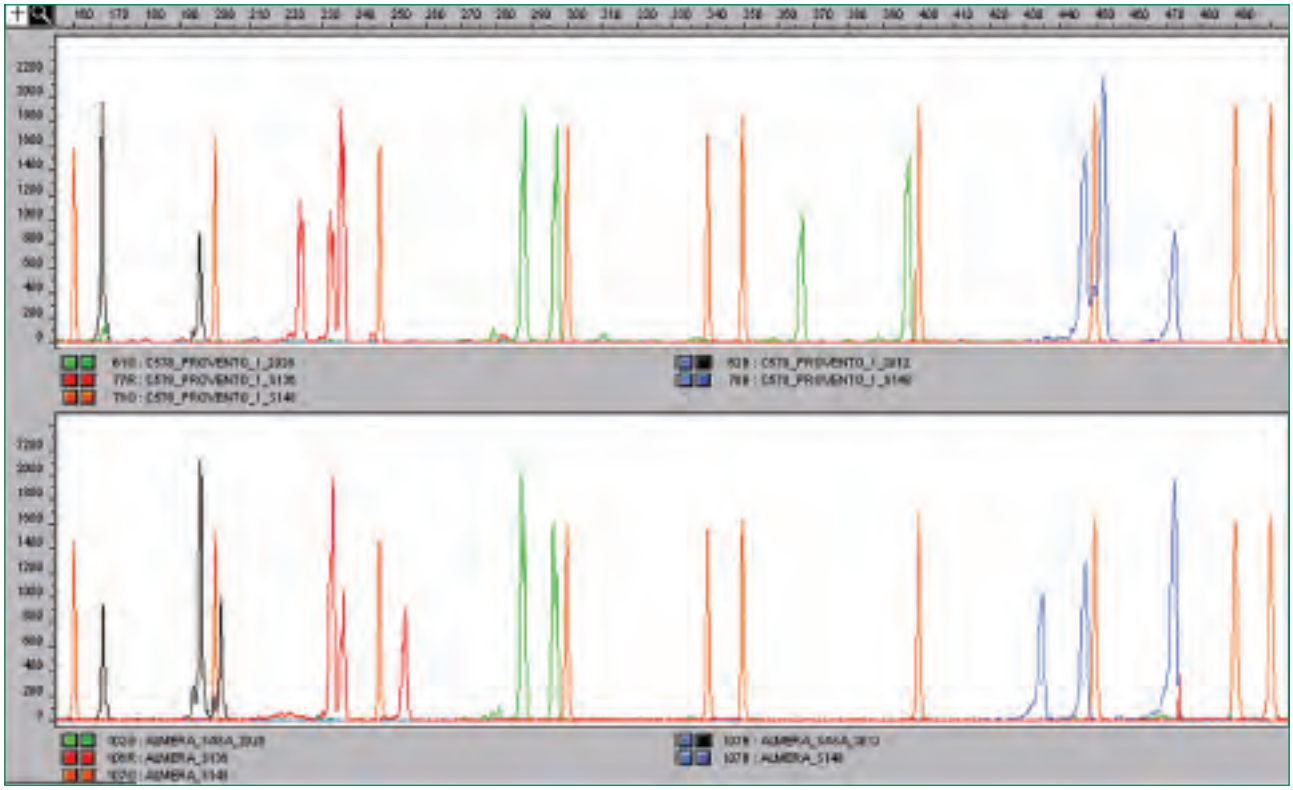


Figure 6.6 Differences in the microsatellite profiles between the two varieties at the center of the ring rot outbreak in November 2003. The use of the microsatellite typing system at SASA was crucial to narrowing the infection down to the variety Provento (top panel) and excluding the other possible variety Almera (lower panel).

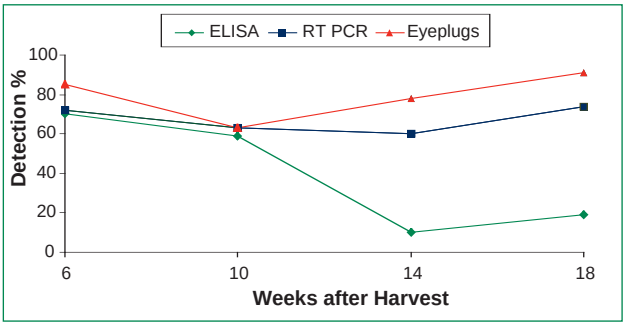


An accurate assessment of virus transmission during the growing season is essential to predict virus levels in subsequent growing crops, with the downgrading or elimination of material that exhibits high levels of infection. The detection of primary PVY infection in foliar material has been found to be unreliable and has resulted in the use of tuber testing to predict virus levels in subsequent crops. The post-harvest, 'growing-on' test has been widely adopted as giving a reliable measure of the virus content. This involves treating rose-end eyeplugs with gibberellic acid and growing in a glasshouse for five to six weeks. The resulting plantlets are tested for virus using ELISA. As the time taken for this test method means that seed producers can risk missing markets for their stocks, there is a demand for a reliable quick test that can be carried out directly on tubers. As part of a major study to identify more rapid, alternatives to the 'growing-on' test, direct tuber testing by ELISA and real-time, reverse transcriptase, polymerase chain reaction (RT-PCR) have been evaluated.

ELISA is currently the most widely used method for detection of viruses in potato. The virus is trapped on polystyrene plates coated with specific antibodies and is detected by means of a colour change following addition of a specific enzyme-labelled antibody probe. The main advantage of ELISA is the ease with which it can deal with a high volume of samples. Real-time RT-PCR offers a number of advantages over 'traditional' PCR detection methods as the analysis is a one-stop-shop with results generated on the spot. Traditional PCR methods use agarose gels for the detection of the PCR amplification product at the final phase or end-point of the reaction. This approach can be time consuming and results may not be available for a few days. There can also be problems with resolution, and throughput is generally low. Although the chemistry and technology underpinning real-time PCR are complex, its is simple to perform and is based on the detection of fluorescence emitted during the reaction. As this process can be used to determine the initial amount of target present in the reaction, it is a quantitative technique. Real time RT-PCR also eliminates post-PCR processing of PCR products, so that there is no requirement to run agarose gels to visualise results.

Results from a comparative study of the 'growing-on' test, direct ELISA and real-time RT-PCR are shown in Fig. 6.7, which permits the effectiveness of each assay over time to be assessed. They showed that real-time RT PCR provides a relatively efficient assay throughout the storage period studied, with 113 positives detected out of a possible 166. Direct ELISA was less effective over the same period as only 64 positives out of 166 were found. Although the 'growing-on' test was consistently the most accurate detection method tested, the results of this study indicate that real-time RT-PCR can be used as a rapid high throughput initial screening method for detection of PVY in tubers.

Figure 6.7 Changes in rates of detection of Potato Virus Y in tubers with time of storage after harvest for ELISA, real-time RT-PCR and the growing-on test



Additional investigations using real-time RT-PCR have shown that it is possible to bulk tuber samples before testing, so that it is possible to detect the presence of one infected tuber in a bulked-sample of 10. Based on replicated bulked samples of 10 tubers, a method has been developed to ensure that samples of seed potatoes do not contain virus at levels greater than 4% (the marketing tolerance for basic seed within the EU).

Studies on the distribution of virus in dormant and sprouting tubers are planned, with a view to improving the method using alternative sampling procedures. The new testing procedure will be implemented for PVY after the 2004 harvest. It will also be evaluated for detection of Potato Virus A and Potato Virus X.

6.5 Building on the success of the Monoclonal Antibody Unit (R Burns)

SASA has been producing antibodies for diagnostic tests since 1986. The Monoclonal Antibody Unit currently maintains and supplies a portfolio of 16 antibodies for the detection of potato viruses to a wide range of internal and external customers. The requirement to develop new assays for potato viruses, or improve the specificity of existing ones is a constant focus of the unit. For example, the last three years have seen the development of a new monoclonal antibody for the detection of Tobacco Rattle Virus (TRV), which can be used to differentiate PRN and RQ, two variants or serotypes commonly found in Scottish potatoes. In addition, antibodies capable of differentiating Potato Virus P (PVP), found in South America, from a closely related potyvirus known as Potato Rough Dwarf Virus (PRDV; Fig. 6.8) have been produced. Although these viruses produce differing symptoms in host plants there are enough similarities to suspect that they may belong the same virus species (C.Nisbet, personal communication).

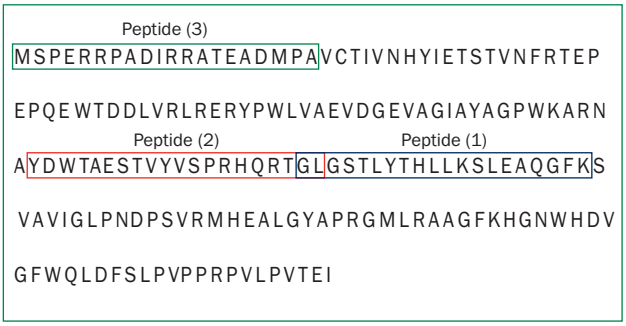
Figure 6.8 Potato plants (cv. Ditta) infected with Potato Rough Dwarf Virus (PRDV)

The current reporting period has also seen a change in emphasis away from the traditional business of potato virus detection towards developing tests in support of SASA's GM inspectorate. Although DNA-based tests such as PCR (See Section 7.3) remain the principal means of GM detection, there is a need to develop assays capable of use in the field, grain store or dockside. The use of monoclonal antibodies as part of field-based assays, particularly for pathogen detection, is long-established (e.g. Pocket Diagnostic™ as produced by the Central Science Laboratory, York). It was with a similar endpoint in mind that the unit embarked on developing antibodies for common GM traits.

Several crops including oilseed rape have been modified by genetic manipulation to include the *bar* gene derived from *Streptomyces hygroscopicus* or *pat* from *Streptomyces viridichromogenes,* which confer resistance to the herbicide glufosinate ammonium. The initial step in developing a field-based assay for the detection of *bar/pat* is the production of monoclonal antibodies capable of detecting the proteins. An iterative approach to the development of these antibodies has been adopted, attempting to tackle this problem from a number of angles. Regions of the *bar* gene product, the protein phosphinothricin-acetyl-transferase, have been investigated as potential antigens for antibody production based on their amino acid composition and location. Three candidate regions, each comprising 20 amino acids, were chosen and synthetic peptides manufactured (Fig. 6.9). These peptides were used to immunise rabbits as a source of antibodies. Although the antibodies raised in this way showed a strong affinity to the synthetic peptides in triple antibody sandwich (TAS) ELISA, they proved ineffective at detecting the native GM protein in plant material (oilseed rape cv. Archimedes).

Having met with limited success using peptides as antigens, subsequent work has concentrated on the use of the full size protein. When the full gene sequence of a protein is known, it is possible to use bacterial expression systems to manufacture the protein of interest. This is particularly useful when only small quantities (1-2 µg) are required. The protein is expressed as a fusion product, containing both the desired protein and a bacterial tag to allow secretion and subsequent purification. The protein is purified from bacterial culture medium using the bacterial tag in affinity chromatography. Using such an approach, a *pat* fusion protein was manufactured for SASA by Richard Buick of Queen's University, Belfast. The protein has been used to immunise mice but the antibodies raised again failed to recognise the native protein, *pat* in subsequent tests. Further investigation of both the synthetic protein and serum derived from the mice led to the conclusion that, although the fusion protein was identical in sequence to the native one, the protein had not folded the same way, leading to the observed immunological differences. A second fusion protein has now been made using an alternative protocol, and initial immunological assays suggest that there is good homology to native *pat*. The protein has been used to immunise mice in the first step towards the production of monoclonal antibodies for the development of an ELISA systems for detection of *pat* in crop plants and products. A fully validated field-based assay is still a few years distant, but progress made over the current reporting period would suggest that such an endpoint is an achievable target.

Figure 6.9 Amino acid sequence of phosphinothricin-acetyl-transferase, the *bar* gene product, showing areas chosen for peptide antigens



Potato Genotyping

A Reid

Alex Reid

After graduating in 1983 from the University of East Anglia in Norwich I spent four years working for the MRC in London on various aspects of mammalian development. Part of my work at the MRC included studies on sex reversal in mice where part of the Y chromosome is translocated onto the X chromosome, resulting in mice which are karyotypically female but are fully functioning males. This work later lead to the discovery of the male determining genes in humans.

In 1987 I started my Ph.D. at Imperial College on the molecular taxonomy of entomopathogenic nematodes, which are used as biological control agents for a variety of insect pests. Whilst at South Kensington I also worked on a number of projects aimed at the identification of human parasites, and taught on the molecular parasitology course. At the end of my Ph.D. I remained at Imperial but moved to the field station at Silwood Park in Ascot where I set up a molecular facility that has now become a thriving centre for molecular ecology and evolution. In 1993 I joined CABI Bioscience where my work on nematodes diversified into the molecular taxonomy of fungi, insects and invasive weeds. At the end of 2002 I achieved a long held ambition to return to Edinburgh (where I lived as a child) when I started work at SASA.

My role at East Craigs was to set up a high-throughput genotyping facility primarily for potatoes. From its inception around 18 months ago, potato genotyping has been successfully carried out on over 350 varieties including all of those on the current UK National List. All of these varieties yield distinct 'fingerprints', with the exception of a few essentially-derived varieties (phenotypic mutants of other varieties). The database is now in use to aid in the maintenance of the culture collection at SASA as well as offering an identification service to industry and the public.

In November of 2003 the rapid identification of potato varieties became an important issue following the first recorded outbreak of ring rot in the UK. Control of the disease was achieved as we were able to determine which of the two possible varieties involved was the source of the infection. The goals are now to extend the genotyping of potato varieties to cover the EU list (some 850 varieties) and to create similar fingerprinting databases for other important Scottish crops.

I am also investigating the mechanisms causing mutation in potatoes (to find the causes behind the formation of off-types). This work illustrates the 'universal' nature of studies on DNA, as the methylation of DNA is thought to be involved. Early in the development of *mammalian* embryos, cell lines become increasingly more specialized when genes not required are switched off. A fertilized egg contains all of the genes needed by the adult organism, but a brain cell does not need all of these whereas a liver cell will use different genes again. This differentiation is achieved by changes in the levels of methylation of the DNA, one of the first subjects I worked on when I was at the MRC. DNA is universal to all living organisms (bar a few viruses) which means, as a molecular biologist, it is possible to turn your hand to virtually anything whether it is the molecular taxonomy of nematodes or vegetables or the mechanisms involved for gene silencing in man or potato. However, it is always essential to have the support of colleagues who are experts on their own subjects be it parasitology or crops. One thing we are not short of at SASA is experts in fields of potatoes!

7 GM Inspectorate

7.1 Introduction (J Davey)

7.2 GM Inspection in Scotland 2001-2003 (J Davey and J Kerr)

7.3 The GM Testing Service (V Mulholland)

Scientific Challenges Article

- The GM Inspectorate and Testing Service (J Davey)

Chapter 7 GM Inspectorate

7.1 Introduction (J Davey)

SASA took over GM crop inspection and enforcement responsibilities from the Health and Safety Executive in 2000. The role of the GM Inspectorate is to ensure compliance with the GM Regulations for the deliberate release and marketing of genetically modified organisms in Scotland, and this has been achieved by inspecting GM crop research trials (Part B deliberate release sites); auditing the companies or institutes that conduct these trials; and auditing seed merchants to ensure that they are discharging their duty of care by marketing only non-GM seedlots.

Section 7.2 of this chapter describes the main activities over the past three years, giving details of the techniques and procedures that are used during site assessments, inspections and audits. It also describes some of the case-by-case investigations of specific issues relating to potential breaches of consent that have arisen during their work. Section 7.3 gives details of the diagnostic support work that is provided by a parallel team at SASA, highlighting the technology that is used and how it is applied to provide molecular evidence to confirm compliance.

Looking back over the past three years, the GM Inspectorate has found a high level of compliance to the conditions of release applied to GM trial sites. Where breaches have occurred, they have been technical in nature and these have had no implications for human health or environmental safety. Two related Inspectorate investigations, however, have highlighted that the prevention of contamination with GM material during seed production of GM varieties (i.e. adventitious contamination) is as important as it is in conventional varieties.

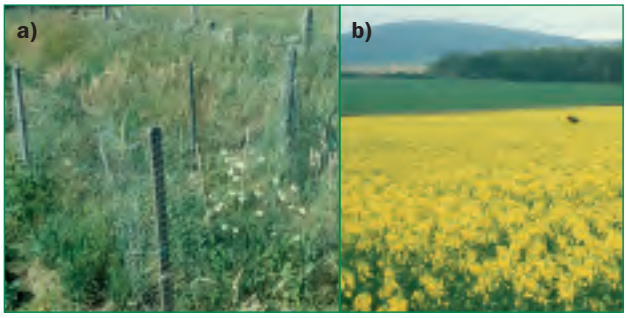
Adventitious unauthorised GM seed in seedlots sown in the spring oilseed rape Farmscale Evaluations was discovered as a result of a management audit conducted by the GM Inspectorate in Scotland in January 2002. Following this incident, the Advisory Committee on Releases to the Environment (ACRE) was asked to review the inspection and enforcement regime for GM crop research trials, and their review called for more stringent conditions under which seeds, or other propagules, are produced as part of all applications for research trials (Part B releases). In some cases, independent validation of seed purity by the GM Inspectorate will be required. These and other incidents show how the work of the Inspectorate can not only identify potential breaches of the GM Regulations but also provide evidence for improving risk assessment and monitoring procedures, which, in turn, help to optimise the GM regulatory regime.

Section 7.2 also describes the seed audit programme that the GM Inspectorate has run for the last four years. These audits determine whether companies importing or supplying non-GM seed have shown due diligence in ensuring that the seed they obtain and supply is free of unauthorised GM material. A related development has been the inclusion of thresholds for adventitious GM presence in an enhanced European regulatory regime on GM Food and Feed, and Traceability and Labelling for marketable produce. To meet these requirements, SASA is developing quantitative PCR based methods for seed and grain analysis. The European Commission is also in favour of proposing thresholds for the adventitious presence of GM seeds in non-GM varieties.

7.2 GM Inspection in Scotland 2001-2003 (J Kerr and J Davey)

The growing seasons from 2001 to 2003 were busy times for the SASA GM Inspectorate as 33 research trial sites, varying in size from 3 square metres to 15 hectares, had to be monitored regularly (Fig. 7.1). These research trials included 17 sites that were part of the GM Farmscale Evaluation Programme. The nature of the trials not only raised the profile of the work of the GM Inspectorates but also increased the workload significantly, as trial sites were larger, more numerous and more widely dispersed than the majority of research trials that had previously been grown in Scotland. At the same time there were research releases for variety trials and agronomic trials at several sites in Scotland. There was also a GM seed audit programme to verify whether the seed industry was discharging its duty of care in marketing non-GM seedlots. Investigations of potential breaches of the GM legislation, auditing of consent holders and providing scientific advice to the SEERAD GM Co-ordination Team completed the service level agreement with SEERAD. These areas of work are explained in more detail in the following sections.

Figure 7.1 Contrasting trial size used in deliberate release: (a) 3m^2, a seed burial trial and (b) 9 hectares, a farmscale evaluation trial



Pre-Planting Site Assessments

When approval is granted for GM breeding or research trials, such as the Farmscale Evaluations, not all of the proposed sites for the programme are known. Once consent holders give notice of a proposed site, the GM Inspectorate verifies whether the site description conforms to the original risk assessment. Before releasing a GMO into the environment the applicant must conduct a risk assessment to demonstrate that the GMO will not be harmful to human health or the environment. Part of the risk assessment and the associated consent includes a detailed description of the release site. Where subsequent sites are to be used, these must be equivalent to the original site, as described by the applicant. Factors such as location, size, and in particular whether the location is situated in an area where the crop is normally grown, are considered together with the proximity of each site to sites of special scientific interest (SSSIs). These details are verified using desk-based map and record studies.

Since 2002, a Geographical Information System (GIS) database and detailed environmental mapping systems have been deployed in site assessments. These systems are used to confirm the grid reference against each site, and the proposed acreage of planting is checked to ensure that it corresponds to that permitted under the terms of the consent. Each assessment is accompanied by a description of the site ecosystem. This is determined by calculating the percentage of the total area that is covered by each of several key land cover categories (e.g. arable land, woodland etc.) within 5km of each release site using the 1km square version of the 2000 Land Cover Map of Scotland. Pre-planting assessment visits were also made to some of the 2002 and 2002/3 Farmscale Evaluation sites where GIS maps were cross-referenced with information held by farmers so that the statements provided by the consent holder could be verified. In all cases the proposed sites were found to be equivalent to the original release site as described by the consent holder.

Field Inspections

During the 2001, 2002 and 2003 growing seasons, 78 inspections were conducted on the 33 trial sites, and each research trial, and previous release site that was undergoing post-harvest monitoring, was inspected at least once a year. The Inspections are carried out using a checklist of the conditions laid down in the specific consent. If a visit is made before the crop flowers, Inspectors check the plant density of the crop, whether pollen barriers, if present, are effective, the minimum distance between the GM crop and other relevant non-GM crops (Fig. 7.2) and whether there are sexually compatible weeds or wild relatives within the separation distance. At harvest, other conditions are assessed (e.g. that harvested seed is securely removed from the release site to await disposal, and that the produce of sexually-compatible crops growing within the separation distance is disposed of along with the GM crop (Fig. 7.3)). Researchers or farmers, who operate a site under the supervision of a consent holder, are also interviewed during an inspection. The trial diary or farm records are audited to verify that certain site management operations have been carried out since the previous inspection.

Figure 7.2 Operating a 50m separation distance from a GM oilseed rape research trial. The distance is applied from the edges of the outer GM plots. There were no sexually compatible crops with oilseed rape either within the distance or in neighbouring fields

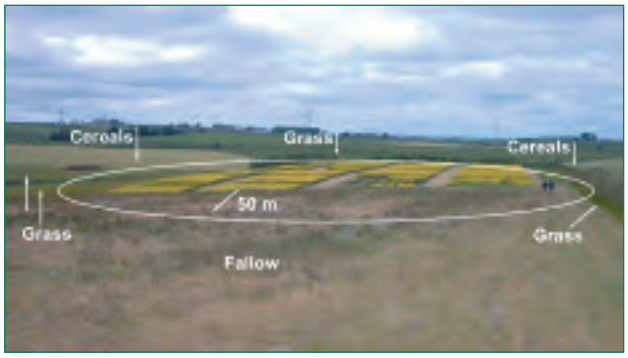


Figure 7.3 Transporting harvested produce of a GM crop for disposal

Post-harvest inspections are also conducted over the period of time that consent holders are responsible for post-harvest monitoring. The Inspectorate verifies that post-harvest monitoring is undertaken and that effective control of volunteer plants is being conducted (Fig. 7.4). The use of appropriate crops, following GM crops, are also verified in the subsequent year(s) following the official monitoring period until the site can be returned to a normal agricultural rotation. In oilseed rape the rotation gap is two years whereas, in potato, five years is routine. In this way a comprehensive assessment of all the relevant activities required in fulfilling the conditions of a consent are made.

Figure 7.4 Herbicide control of oilseed rape volunteers in a cereal crop following a GM oilseed rape trial

An example of where the combination of consent-holder monitoring and inspection has refined the post-harvest management of GM deliberate release sites arose from a consent holder recording a high incidence of potato volunteers on several sites. Persistence had continued for four years after the original release due to germination of 'true' potato seed. This led to a change in the management practice of potato sites at this research institute: sites, which would have been put into cereal crops following a GM potato release, are now kept fallow for two years so that groundkeepers and volunteers from true potato seed can be more easily observed and controlled. Where persistence has continued despite these changes, the consent holder has agreed to extend the monitoring of these sites. This and other monitoring information is reviewed by the Advisory Committee for Releases to the Environment (ACRE) when it recommends the conditions for monitoring during the assessment of new applications for the deliberate release of GM potatoes.

Contact is maintained regularly with consent holders and their site operators throughout the monitoring period over issues of compliance, including harvest arrangements, crop disposal details and volunteer control. In retrospect there was a high level of compliance in the management of Part B research trials. No issues of harm to human health or the damage to the environment were identified.

Management Audits of Consent Holders

The GM Inspectorate conducts a programme of management audits of Scottish consent holders. Where a consent holder has a pre-devolution consent that remains active throughout Great Britain or has parallel consents that apply in England and Scotland, the work is done in collaboration with the GM Inspectorate at the Central Science Laboratory (CSL) in York. Whereas inspections assess compliance at individual deliberate release sites, auditing the management procedures of consent holders at their headquarters provides an overview of their ability to organise and implement releases. Inspectors verify whether appropriate planning and operational procedures for GM research trials have been implemented and whether the conditions specified in release consents are known throughout the management chain. This is achieved by auditing records, procedures, protocols, correspondence, contractual arrangements with third parties, and interviews with staff. At each audit the consent holder is also required to provide evidence to demonstrate an adequate 'duty of care' to ensure that only those transformation events covered by the consent are released.

On the basis of information provided by consent holders, the Inspectorate found a high standard of planning, management and dissemination of information throughout management chains when conducting research trials. One widely publicised example, which illustrates the value of these audits, occurred in 2002 when unauthorised GM elements were discovered in seed supplied by Aventis CropScience Ltd (now Bayer CropScience Ltd) to SAC for release in a GM research trial. These were detected as a result of seed testing undertaken by the SAC as part of their 'duty of care' obligations. Following an extensive investigation Aventis CropScience Ltd confirmed to the Scottish Executive and Defra that related seed batches of GM oilseed rape, planted in some of the Farm Scale Evaluations, contained additional GM events.

Auditing Seed Merchants by inspection of records and correspondence to ensure non-GM seed is free from adventitious GM material

In 2000, conventional seed of oilseed rape sown in the UK was found to contain adventitious presence of GM seeds and the crops had to be destroyed before harvest. As a result, the UK GM Inspectorates were asked to implement a seed audit programme, to assist the seed industry in preventing a repetition of the 2000 incident. As Scottish seed merchants tend to be supplied by merchants or importers from England, the SASA GM Inspectorate liases closely with the CSL GM Inspectorate. This relationship has been very successful in providing reassurance to Ministers that the seed Industry has discharged its duty of care in relation to GM presence in conventional seed lots.

The SASA GM Inspectorate writes to all registered merchants, processors and packers, seeking confirmation that they do or do not trade in species at risk of containing GM adventitious presence. In the years 2000 to 2003, the species audited were oilseed rape, sugar beet, fodder beet, maize and soya. These were extended to include other brassica vegetable crops in 2004. On the basis of the responses, the Inspectorate conducts a rolling programme designed to audit each merchant at least once every four years (Fig. 7.5). During the audits, the Inspector collects the species and variety names and seed lot reference numbers of each seed lot of interest and the name of the supplying company. In addition the Inspectorate asks to see letters of assurance from the suppliers stating that seed is compliant with the guidance to seed producers and importers on prevention of adventitious GM presence. As a result of the audit programme there has not been a repetition of the 2000 incident and, through collaboration with CSL, the SASA GM Inspectorate has been able to act quickly and effectively when potential problem lots have been identified. The programme has confirmed the ability of the seed industry to take all reasonable steps to prevent the occurrence of adventitious GM presence in conventional seed.

Figure 7.5 An Inspector auditing a seed merchant's records

Contingency plan for dealing with unauthorised GM material in conventional seed

In the event of an inadvertent release of an unauthorised GMO, which has the potential to damage the environment or human health, the GM Inspectorate would provide immediate support to SEERAD. In the first instance the GM Inspectorate, along with other government departments, institutes and industry groups would provide advice in determining the scale and impact of such an incident. If it were necessary, Inspectors would use their powers to investigate further or take remedial action.

Conducting case-by-case investigations into suspected breaches of the GM legislation

The majority of Inspectorate time has been spent on investigations resulting from inspections and audits, or from incidents that have been brought to the attention of the Inspectorate. They have ranged from claims of irregularities in agricultural practice, trace deposits of GM pollen in honey taken from a hive near a Farmscale Evaluation trial, high sowing rates, and plantings of unauthorised seed. In some cases the Inspectorate concluded that breaches of the consents had not occurred. In others, where breaches were considered to have taken place, these cases were of a technical nature, and had no implications for human health or environmental safety.

These cases arose from the acknowledgement by Aventis CropScience Ltd in August 2002 that related seed batches of GM oilseed rape planted in some of the Farm Scale Evaluations, contained additional GM events. The role of the Inspectorate included screening for adventitious GM material in samples using the qualitative polymerase chain reaction (PCR) techniques described in Section 3 of this chapter. In the first case, samples of a GM variety supplied by Aventis Crop Science Ltd, for use in some of these trials, and by the CSL GM Inspectorate, secured from surplus seed from a sowing on an English site, contained additional GM sequences: *pNOS* (a promoter sequence) and *nptII* (the selectable marker for kanamycin and neomycin resistance). The consent holder later reported to SEERAD and Defra that these sequences resulted from the transgenic events Ms1, Rf1 and Rf2.

Following this incident, the GM Inspectorates were asked to investigate other varieties that were sown in these trials in 2002. The SASA GM Inspectorate took samples of harvested seed from a single site in Scotland and tests revealed a further sequence, the *CaMV 35S* promoter, in addition to *pNOS* and *nptII*. The CSL GM Inspectorate, having tested samples of the same seedlot secured from two English sites, reported a similar finding. The company was asked to investigate these findings, and on 26 June 2003, confirmed that the GM elements *nptII* (Kanamycin) and *CaMV 35S* promoter were present as a result of the following GM events: Ms1 (0.1%), Rf1 (0.1%), Rf2 (4.0%), and Topas 19-2 (1.3%). The company's test results were consistent with the findings of the GM Inspectorates and provided an explanation of the additional GM elements found in this second variety.

As a result of their investigations into these two cases, the Inspectorate were able to produce evidence that the terms of the release consents had been breached but that there was no harm either to human health or the environment. At the request of Scottish Ministers, the two related cases involving plantings of unauthorised seed in spring oilseed rape Farmscale Evaluation trials were forwarded to the Procurator Fiscal Service in October 2002 and January 2004.

The Future

In 2003, the European Union published two new GM regulations, which have established a legal basis for thresholds of GM presence in marketed food and feed produce. These allow a margin of adventitious GM presence in marketed products, provided that the GM impurities have an approval for use. The threshold was set at 0.9% (a lower threshold of 0.5% is permitted for GM events which, although they have not been given full approval, are considered by the European authorities to be safe for release i.e. have a favourable risk assessment). Any product containing GM material above the threshold level must be labelled as GM. Any GMO, which has not received an approval or favourable risk assessment remains prohibited for sale in the EU.

These new thresholds have meant that GM detection has had to move from qualitative to quantitative testing. This is a significant challenge for GM testing laboratories as quantitative assays are more complex and time consuming to design and validate than qualitative assays. Work on GM assays continues to be hampered by the lack of available standard reference material.

In view of the difficulties involved in GM testing, particularly acquiring reference material, SASA became one of Europe's designated GM testing laboratories as an inaugural member of ENGL (European Network of GM Laboratories) in December 2002. Membership of this network should assist SASA in maintaining a continually-updated suite of GM assays that are appropriate for detecting GMOs as they are approved. Maintaining the development of the GM diagnostic work and building on the Inspectorate's practical experience of enforcement to-date will be key to SASA's ability effectively to support the operation of the GM regulations in Scotland in the future.

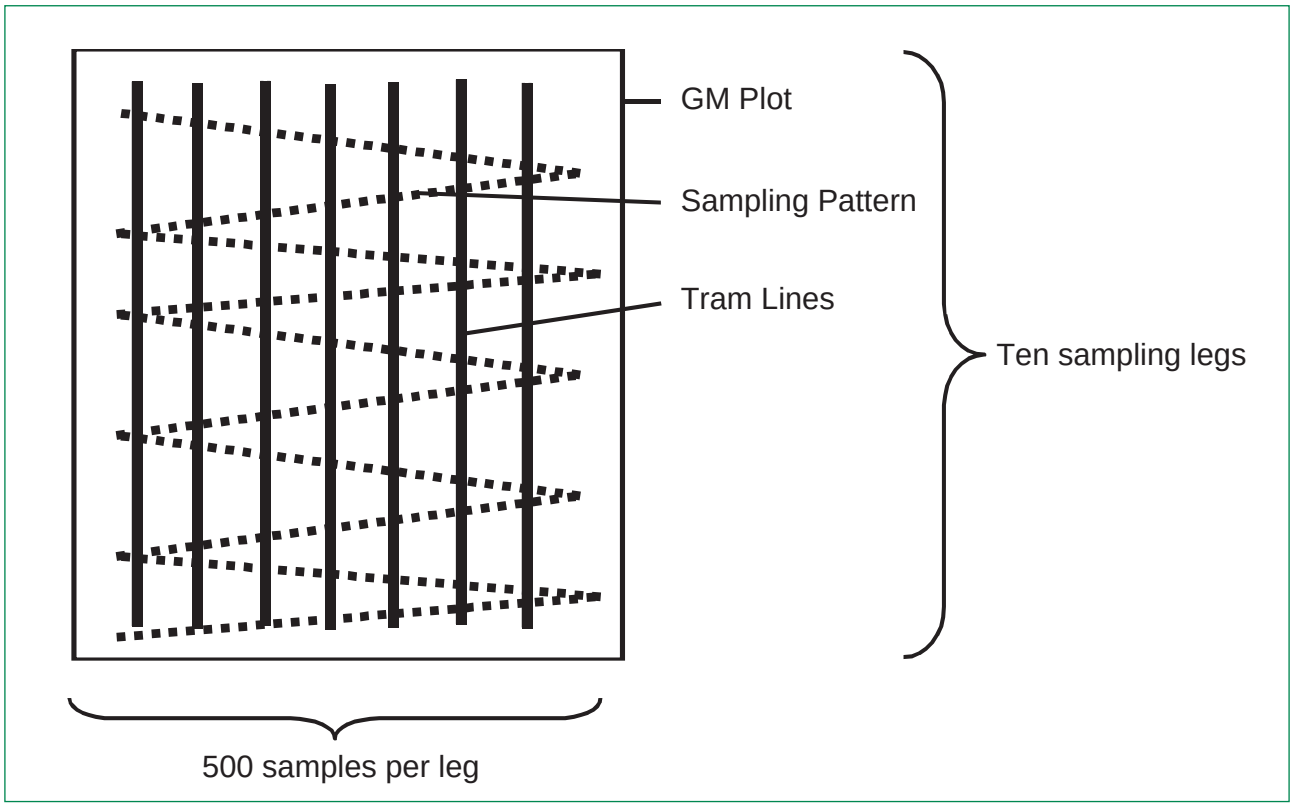
7.3 The GM Testing Service (V Mulholland)

As GM plants and their seed may look no different from untransformed, parental material, testing methods need to look for the genes engineered into the crop, or the proteins produced in the plant by the introduced DNA. To support the functions of the GM Inspectorate, DNA-based methods are used, which are highly sensitive and able to detect trace amounts of inserted DNA. The GM Inspectorate must ensure (1) that releases of GM crops have no adventitious contamination by other GM plants, (2) that release sites are managed in accordance to the release consents, including the management of volunteers and (3) that conventional seed is free from unapproved GM material. As discussed above, GM monitoring is achieved through site visits and audits. The SASA testing service identifies GM material and provides the molecular evidence needed to confirm compliance.

The polymerase chain reaction (PCR) is a molecular method that can be used to amplify regions of DNA to a level at which they can be detected. The amplification occurs between two sites, to which short lengths of synthetic DNA (primers) can bind, and these primers can be designed to bind to a desired region of DNA. A wide variety of PCR primer sets are used in a step-by-step approach to detect adventitious contamination with GM products.

An example of the type of testing undertaken at SASA is the survey of oilseed rape release sites in Scotland for adventitious contamination, by leaf disc sampling. The sample size used was 5000 leaf discs, in ten batches of 500, following the sampling plan shown (Fig. 7.6). To establish that the sensitivity and repeatability of the PCR assays used for screening the samples were sufficient, control samples were constructed consisting of one GM leaf disc added to unmodified oilseed rape leaf discs to give a dilution of 1:1000. DNA was extracted and assays were performed in a real-time PCR assay system, which allows the amplification of products to be detected during the assay (Fig. 7.7 A). The assays on the 1:1000 leaf disc sample were repeated 48 times, together with 4 positive and 4 negative controls. After PCR, the samples were subjected to melting curve analysis to indicate if the products were identical (Fig. 7.7 B – D). The samples and positive controls all gave identical profiles. These results demonstrate that the method is highly sensitive and capable of detecting a GM event at a concentration of 1:1000 (test sensitivity of 0.1%). This gives added confidence to the approach adopted in the survey where batches of 500 leaf discs were studied, so that the minimum sensitivity required for the survey was 1:500 (0.2%).

Figure 7.6 Sampling plan for leaf disc collection. An irregularly shaped field/plot will require an adaptation of this plan, but the diagonal sampling legs should be across the line of sowing.



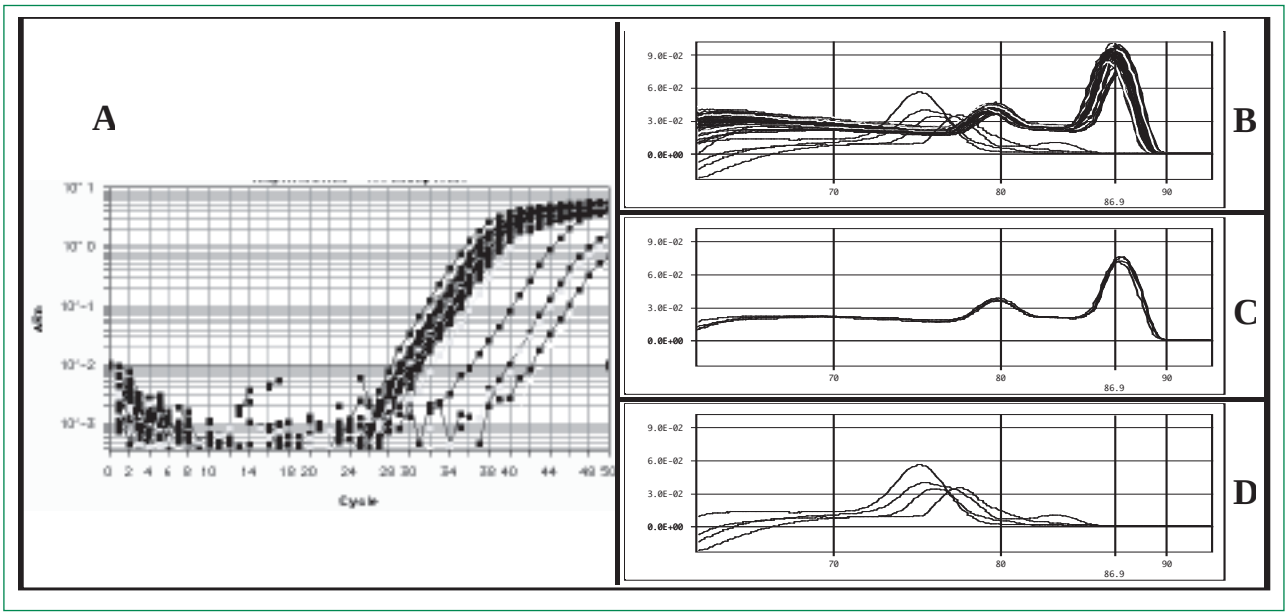
For the main survey, DNA was extracted in duplicate from each of the 10 batches of 500 leaf discs. These samples were tested to ensure that the DNA was of sufficient quality to permit PCR amplification using a positive control assay (chloroplast-specific), and all extracts were shown to have DNA suitable for further testing. The samples were then tested using the following assays; *bar*, T*nos*, P*nos*, P*nos::nptII* hybrid and LibertyLink. The test results are shown in Table 7.1 and are consistent with a crop that carries no adventitious modification.

In conclusion, a battery of PCR assays, tailored to the requirements of the GM Inspectorate, has been developed. The Agency participates in proficiency testing to ensure that it can demonstrate the testing of skills and methodologies in this technically demanding area. In the immediate future, a series of international standards will be published, which covers the entire process of testing food for the presence of GM. A selection of the methods and practices from these standards will be adopted, where appropriate, for this monitoring work.

Table 7.1 Summary of PCR data obtained from the duplicate assays performed on each of the 500-leaf disc batches. The results expected from a crop with no adventitious contamination are shown for comparison.

PCR assay	Result	Expected
Chloroplast	+	+
P*nos*	–	–
Pnos::nptII hybrid	–	–
bar	+	+
T*nos*	+	+
LibertyLink	–	–

Figure 7.7 Testing of Pnos::nptII hybrid detection assay for the leaf disc survey. (A) shows the accumulation of amplification products during the real-time PCR. (B) is the melting curve analysis for all samples tested, (C) is the analysis for the positive controls and (D) is the analysis of the negative controls.



The GM Inspectorate and Testing Service

J Davey

Dr Jonathan Davey

When I was asked to form a GM Inspectorate in April 2000, a colleague said to me: 'Why would anyone want to take on a job like that'? The answer was quite simple: 'How could I, with the right background for assessing compliance with the GMO (genetically modified organism) Regulations, possibly resist'. I had studied plant ecology for my PhD at Leeds University and had worked for twenty years in variety registration of forage and vegetable crops at SASA. During those years I had followed the advances that biotechnology had brought to plant breeding and, by 1999, was already responsible for co-ordinating the Agency's advice on genetic modification.

It was an exciting time to be taking on this challenging role. The world's largest series of experiments on the impact of genetically modified crops upon farmland biodiversity had begun across the UK. News was breaking that 5,300 hectares of conventional oilseed rape sown in the UK contained unauthorised GM impurities, and public concern over the food safety and environmental impact of GM crops was gathering momentum. Three challenges lay before me: to establish and run an inspection regime for all research trials of GM crops; to develop an audit programme to ensure conventional crop seed was free from unauthorised GM material, and to, ensure that there was a GM diagnostic service to support the work of the Inspectorate.

Initially, I formed a field inspection team of four crop specialists. They were hand picked for their knowledge of oilseed and potato crops, as genetically modified varieties of these crops were being released in Scotland at the time. Selection of staff had also to take into account the fact that unharvested seed and tubers could result in the appearance of GM plants (volunteers) in the following season. Since cereals are usually the following crop after the release of these species, as a method of controlling such GM "volunteers" before they are returned to a normal farm rotation, a cereal expert was also chosen. As the team members already conducted variety evaluation trials or certification plot checks and visited farms as part of their normal duties, they were ideally qualified to inspect GM research trials to assess whether the conditions of a release, following specified trial layouts and agricultural practice, were met. These conditions might include separation distances from other non-GM or related crops, presence and size of pollen barriers, the area and plant density of the GM release, control of volunteers/flowering plants and follow-on crops after harvest.

An inspector verifying the plant density of a GM oilseed rape crop

My colleagues also have the specialist knowledge to accompany me on visits to the offices of companies or research institutes authorised to release GMOs, and conduct audits on their managerial competence to undertake GM research trials. These inspections cover whether the correct procedures for advertising a release are followed, whether adequate training is in place, whether there are proper records for conducting and monitoring releases and whether they are exercising 'duty of care' by releasing only approved GMOs.

In the early days, I was the only full time member of the GM Inspectorate. Colleagues would join me to conduct specific inspections and then return to their regular duties. Later in 2002, John Kerr, an agricultural chemist, with expertise in pesticide survey work, joined the Inspectorate as a full time member taking over the day to day management. He also improved some of our procedures, in particular, simplifying standard operating procedures, auditing of seed merchants and conducting research on the distribution of GM material within grain commodities.

A parallel GM team at SASA provides the supporting diagnostic service: Gerry Saddler, a bacteriologist with expertise in diagnostic test development, and Vincent Mulholland, a molecular biologist with wide experience of developing and implementing PCR-based tests for a range of plant pathogens. Their forensic and diagnostic expertise underpins the field inspection and audit programmes. It is essential to be able to identify genetically modified material and determine whether GM presence is an authorised, or an unauthorised event, thereby determining whether it poses a risk to human health and the environment.

Given that the GM Inspectorate is a small group of experts working in an expanding field, it is important to discuss and share information with the wider scientific community. Central to the establishment of the Inspectorate has been membership of international groups that specialise in GM enforcement including the European Joint Enforcement Group on Deliberate Release of GMOs, which has assisted development of sampling and inspection protocols. The network also provides an early warning system of potential GM issues that could affect the UK. Similarly, Gerry Saddler and Vince Mulholland have benefited from membership of the European Network of GMO Laboratories (ENGL). The competence in detection is tested regularly by participating in GMO proficiency tests organised by the International Seed Testing Association (ISTA).

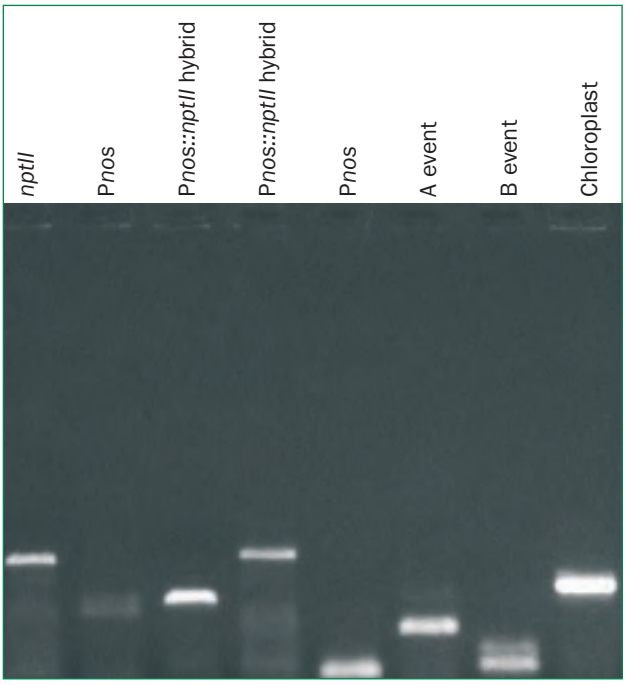
Since the GM Inspectorate took on their role, 104 inspections have been conducted on 47 release sites. There is also a regular programme of management audits of holders of consents for release in Scotland. As a consequence of these programmes, there have been five investigations of potential breaches of the GM Regulations, two of which have been forwarded to the Procurator Fiscal Service. Inspectors also operate a rolling programme of seed audits of seed merchants to check whether seed that is supplied to them is free of unauthorised GM impurities.

So the application of SASA's agroecological and molecular diagnostic expertise to the enforcement of the GM Regulations has paid dividends in protecting human health and the environment. On a personal note, it has been very rewarding to work with a successful team that has played an important contribution to the regulation of GM crops in Scotland.

Leaf disc sampling. 5,000 plants were sampled on two GM crops in the spring of 2003 to verify absence of unauthorised GM material

Oilseed Rape Diagnostic Tests: PCR amplifications of a GM oilseed rape variety

NPtII	antibiotic resistance
P nos (2 different assays)	promoter
Pnos::nptII hybrid (2 different assays)	junction sequence between P nos and NptII elements
A	GM event A (not disclosed)
B	GM event B (not disclosed)
Chloroplast	PCR control



8 Pesticides

8.1 Introduction (K Hunter and CJ Griffiths)

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Chapter 8 Pesticides

8.1 Introduction (K Hunter, CJ Griffiths)

Many pesticides are intrinsically hazardous chemicals, having biologically active properties designed to interfere with natural mechanisms. Current legislation requires the compilation of extensive data packages to show that pesticide products, when used according to the label instructions, will not give rise to unacceptable risks to users, consumers or the environment. Despite very strict controls on the sale, supply, storage and use of pesticides, there is no guarantee that all pesticides will be used according to the label and the relevant Code of Practice. Post-registration surveillance provides a means of confirming that pesticides are being used correctly and that the pre-registration risk assessments ensure the necessary levels of safety. SASA has a key role in supporting the post-registration process through the monitoring of pesticides and their metabolites in foodstuffs, investigating the impact on non-target animals and collecting usage information on agricultural and horticultural crops (see Hunter and Griffiths 2001).

Residues in Foodstuffs

SASA has been a long-standing participant in government surveillance of pesticides in the national diet since the 1960s. This work is done on behalf of, and reported to, the Pesticide Residue Committee of the UK Advisory Committee on Pesticides. In the period 2001-2003, analytical surveys were conducted on a range of foodstuff commodities including aubergine, apricot, cauliflower, cucumber, fennel, kiwi, leek, lemon, nectarine, peach and pineapple, and some of these formed the UK component of wider European Union annual survey programmes. Data on 65,564 pesticide/commodity combinations were produced, with residues being identified in 36.7% of samples tested. (The frequency of residues, calculated on the basis of pesticide/commodity combinations, was only 0.8%). Most of the residues occurred as single compounds detected at very low levels, and the relevant Maximum Residue Level (MRL) was exceeded in less than 0.5% of samples.

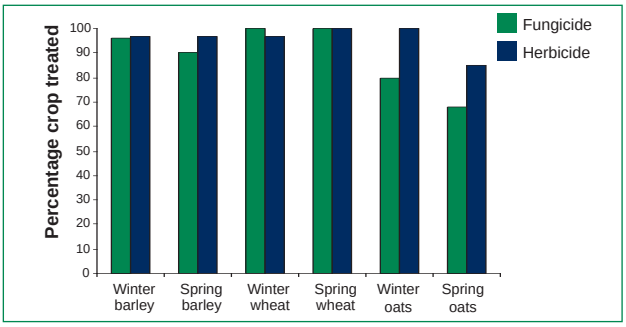
MRLs should not be exceeded for a particular pesticide and commodity combination, and are based on measurements of commodities produced following good agricultural practice. They are not safety limits, but residues in food that comply with the respective MRLs are considered toxicologically acceptable. Reporting limits for analytical surveys are usually set at a fraction of the MRL or at the limit of determination achievable using current methods.

The SASA laboratory continued to participate in relevant pesticide rounds of the FAPAS proficiency scheme to support its activities in the foodstuffs sector, achieving a consistently good performance throughout 2001-2003. SASA was also one of the best performing laboratories in the EU Proficiency Exercise 5, completed in 2003.

Agricultural and Horticultural Usage

A major increase in the use of chemicals to control weeds in agricultural crops began in the 1940s with the discovery of selective broad-leaf weed herbicides. Since then, many herbicides with different modes of action have been discovered and commercialised, providing growers with a wide spectrum of options for weed control. The benefits to growers in terms of improved yield, ease of harvest and reductions in the spread of pests and diseases have been significant and are reflected in the surveys of pesticide usage on arable crops conducted by SASA. The major cereal crops grown in Scotland are routinely treated with at least one herbicide and fungicide active ingredient (Fig. 8.1).

Figure 8.1 Percentage of Cereal Crop Areas Receiving a Herbicide or Fungicide Treatment (Arable Crop Survey 2002)



However, the widespread and continuous use of herbicides in the environment has some serious drawbacks, noted as early as 1962 by Rachel Carson in her book 'Silent Spring'. More recently attention has focused on entire ecosystems, with notable studies investigating the indirect effects of pesticides on farmland bird populations (Campbell *et al*., 1997). The decline in the populations of the grey partridge, for example, has been attributed to the use of herbicides and broad spectrum insecticides, through reduced chick survival (Potts and Aebischer, 1991).

The introduction of legislation setting the maximum levels of pesticides and their metabolites in drinking water, together with Environmental Quality Standards, has focused attention on the persistence of herbicides in the environment. The older types of herbicide widely used on cereals pose particular problems and are consistently found in surface waters at levels above the 0.1ug L^{-1} limit set by the EU Drinking Water Directive. The combination of negative environmental impacts, tighter legislation on permissible residue levels in water, and extensive data requirements, before approval to market a new product, has led to the introduction of more benign herbicides.

Post-registration surveillance in the form of pesticide usage surveys provides a feedback mechanism for the regulators and advisory committees in assessing actual and potential impacts on the environment. They also provide a useful source of information for policy makers when estimating the changes to crop management that may result from revisions of pesticide policies and legislation. Information on the changes in the usage patterns over a longer period of time

also informs Government on the success or otherwise of measures to minimise pesticide use. The section on herbicide usage on cereal crops in Scotland in this report highlights some changes that have occurred over a 20 year period as a result of innovation, improvements to environmental analysis, and changes in legislation and pesticide policies.

Poisoning Incidents

The Wildlife Incident Investigation Scheme, operated on behalf of SEERAD, investigates cases of poisoning in non-target vertebrates and honeybees in Scotland. Data generated in this way may be used to aid the U.K. regulatory process. Accumulated evidence from detailed investigation of incidents involving approved use, or misuse of a particular pesticide, can permit adjustment of the conditions of approval. A total of 430 incidents were actively investigated by SASA in the period 2001-2003, and 107 of these were confirmed as being caused by pesticide poisoning. Deliberate abuse of pesticides to poison non-target animals was the underlying cause of the majority of incidents, and relatively few were associated with approved use or unintentional misuses of pesticide formulations (Fig. 8.2). It was particularly regrettable that 3 white-tailed sea eagles were amongst the victims of abuse in the period, and that, in two of the 3 years, such illegal practices had a serious impact on the re-introduction programme for red kites in Scotland (Fig. 8.3).

Figure 8.2 Poisoning incidents, by category of involvement

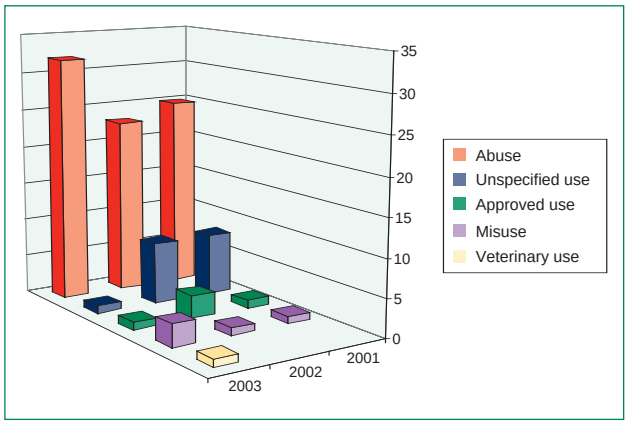
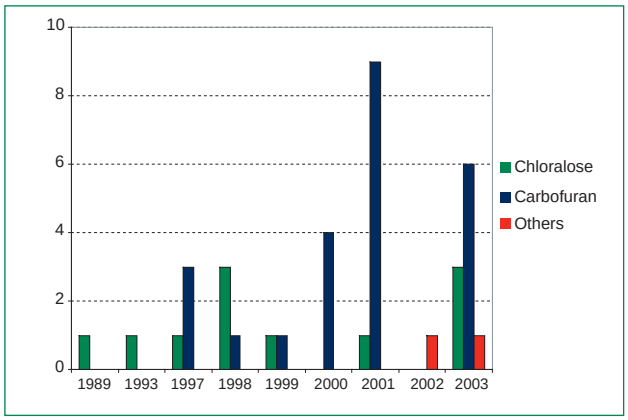


Figure 8.3 Number of red kites poisoned in Scotland



References

Campbell LH, Avery MI, Donald P, Evans AD, Green RE, Wilson JD. 1997. A Review of the Indirect Effects of Pesticides on Birds. *JNCC Report* **227.** Joint Nature Conservation Committee, Peterborough.

Hunter K, Griffiths CJ. 2001. Pesticides. In: *Scientific Review 1997-2000.* Edinburgh: Scottish Agricultural Science Agency.

Potts GR, Aebischer NJ. 1991. Modelling the population dynamics of the Grey Partridge: conservation and management. In *Bird Population Studies: Their Relevance to Conservation and Management.* Perrins CM, Lebreton J-D and Hirons GMJ eds. Oxford University Press.

8.2 Meeting the Challenges of Pesticide Residue Analysis: Exploiting and Extending the Capabilities of LC MSMS (GA Keenan)

Analytical methodologies employed in the determination of pesticide residues in foodstuffs must be capable not only of quantifying very low levels of incurred residues but also of confirming their identity and magnitude. This is essential to support enforcement of legislation, to ensure trading compliance and to carry out surveillance programmes to monitor residues in regional and national dietary components (PRC, 2002). This requirement to provide unambiguous evidence of residues is becoming increasingly challenging as reporting limits and maximum residue levels are reduced in areas where new or amended legislation, such as Commission Directive 1999/39/EC on processed cereal-based foods and baby foods for infants and young children, is introduced.

One of the most versatile and powerful analytical techniques available to analysts, to meet the challenges associated with the measurement of residues at ultra low trace levels, is undoubtedly the combination of high performance liquid chromatography with atmospheric pressure ionisation tandem mass spectrometry (API-LC-MS/MS). The value of API-LC-MSMS stems from its capability to perform selective separation on-line with sensitive, selective mass detection. For a target analyte to be detected, it must have not only the correct retention time, but also a specific combination (MRM transition) of pre-selected parent ion and daughter ion m/z values. SASA has successfully utilised this technology in its contribution to UK pesticide surveillance programmes on foodstuffs, where novel multi-residue methods have been developed, and also in the investigation of wildlife poisoning incidents (Hunter *et al*., 2004; Taylor *et al*., 2002; Taylor, 2001).

However, in less favourable cases where the target compound may exhibit poor ionisation efficiency and/or a low parent ion fragmentation probability, difficulties in achieving the relevant reporting limit may be experienced in the initial screening analyses. Also, the provision of good quality data for confirming the identity of particular analytes may be problematic. The basic multi-residue approach adopted for foodstuffs at SASA can be refined or extended in a number of ways to overcome most of these difficulties. Lack of sensitivity is a generic issue, and it has been addressed by making use of column switching as a trace enrichment technique to enhance and extend the capability of LC-MSMS methodology. The use of stable isotope-labelled internal standards has been exploited to improve analytical precision and counter problems of specific ion suppression/enhancement effects for various pesticide/matrix combinations in electrospray-LC-MSMS. The use of atmospheric pressure chemical ionisation (APCI) as an alternative ionisation mode has proved useful in overcoming difficulties experienced with certain analytes in challenging matrices when routinely applying electrospray ionisation.

Column Trapping for Analyte Pre-concentration in API-LC-MSMS

Enrichment methods are capable of high sensitivity and can also provide a degree of sample extract clean-up. Analytes can be retained and concentrated on a trapping column using a non-eluting mobile phase, while many of the sample matrix components pass to waste. After this concentration step, a switching valve is actuated allowing an eluting mobile phase from a second pump selectively to back-flush the analytes from the trapping column on to the analytical column for separation before detection (Fig. 8.4). The maximum single injection volume of many standard HPLC systems is 100 µl, but it has been found possible to overcome this limitation and accumulate analytes from multiple injections of the same sample without any apparent degradation in chromatographic performance.

Figure 8.4 Schematic of a column trapping system. Position 1 – Sample loading; Position II – Backflushing and analysis.

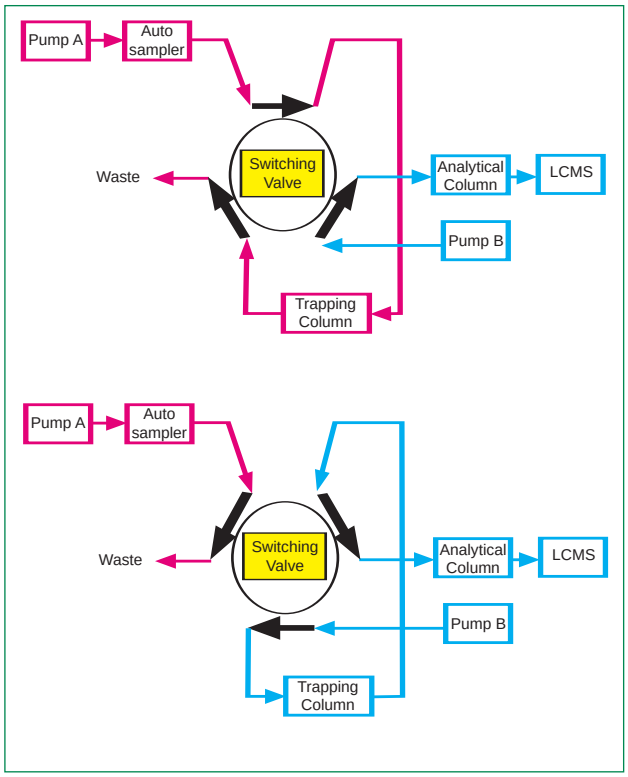
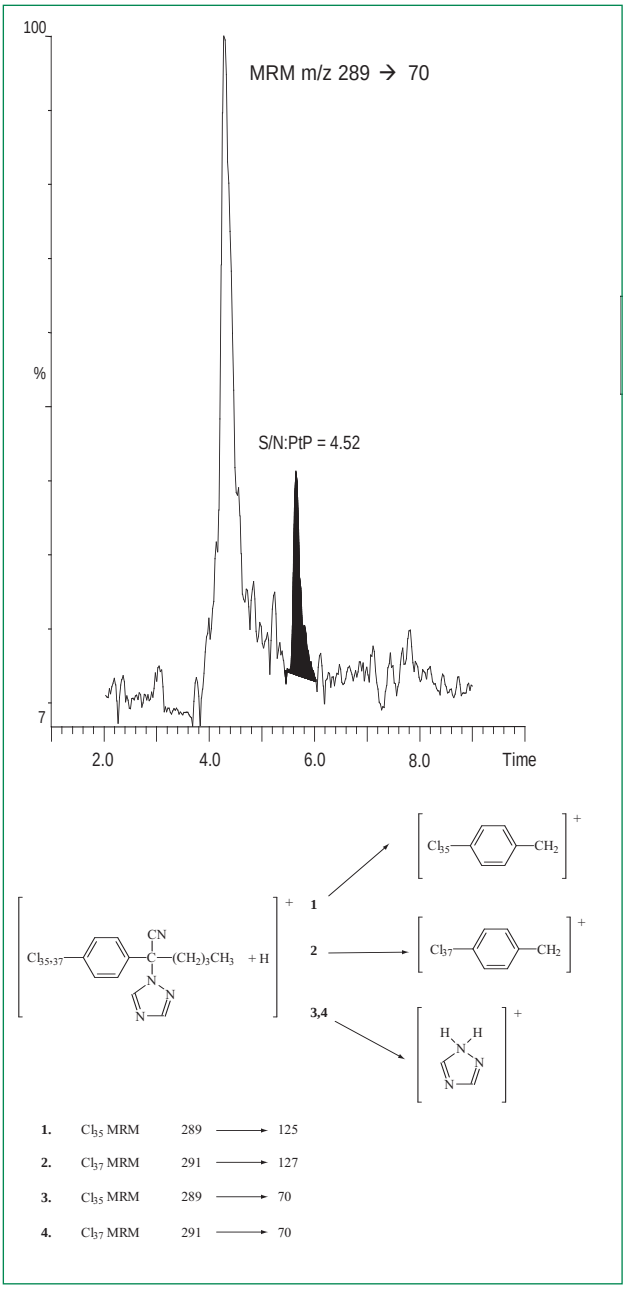


Figure 8.5 Myclobutanil in a 0.00005 µg/ml^{-1} matrix-matched standard (500 µl loading)



The potential of the method is illustrated by data for the detection and confirmation of a myclobutanil residue in apple. After optimisation of chromatographic parameters, tests with matrix-matched standards confirmed that a linear increase in response was obtained over an extended sample volume range (25 – 500 µl). Calibration curves derived for 500 µl loadings of matrix-matched standards were linear over a range 0.00005 to 0.0125 µg ml^{-1}. A marked improvement in the signal to noise ratio (S/N) was obtained when column trapping was utilised, the limit of determination (LOD) of ~ 0.0001mg kg^{-1} achievable being illustrated by analysis of a matrix-matched standard (Fig. 8.5). In addition, it was possible to use additional structurally significant MRM transitions to confirm a residue in apple puree (Fig. 8.6). Data obtained for this residue using the column trapping method compare well with those generated without trace enrichment (Table 8.1). As the magnitude of the residue was near the lower limit of the original LCMS method, analysis of residues below this level would necessitate use of the enhanced method.

Figure 8.6 Signal/noise ratios, (A) 10 µl injection of myclobutanil (0.0125 µg ml^{-1}) in apple matrix without column trapping; (B) 500 µl loading of myclobutanil (0.0125 µg ml^{-1}) in apple matrix with column trapping; (C) 500 µl loading of apple puree extract (≡ 0.5 g ml^{-1}) with column trapping. Residue ~ 0.007 mg kg^{-1}

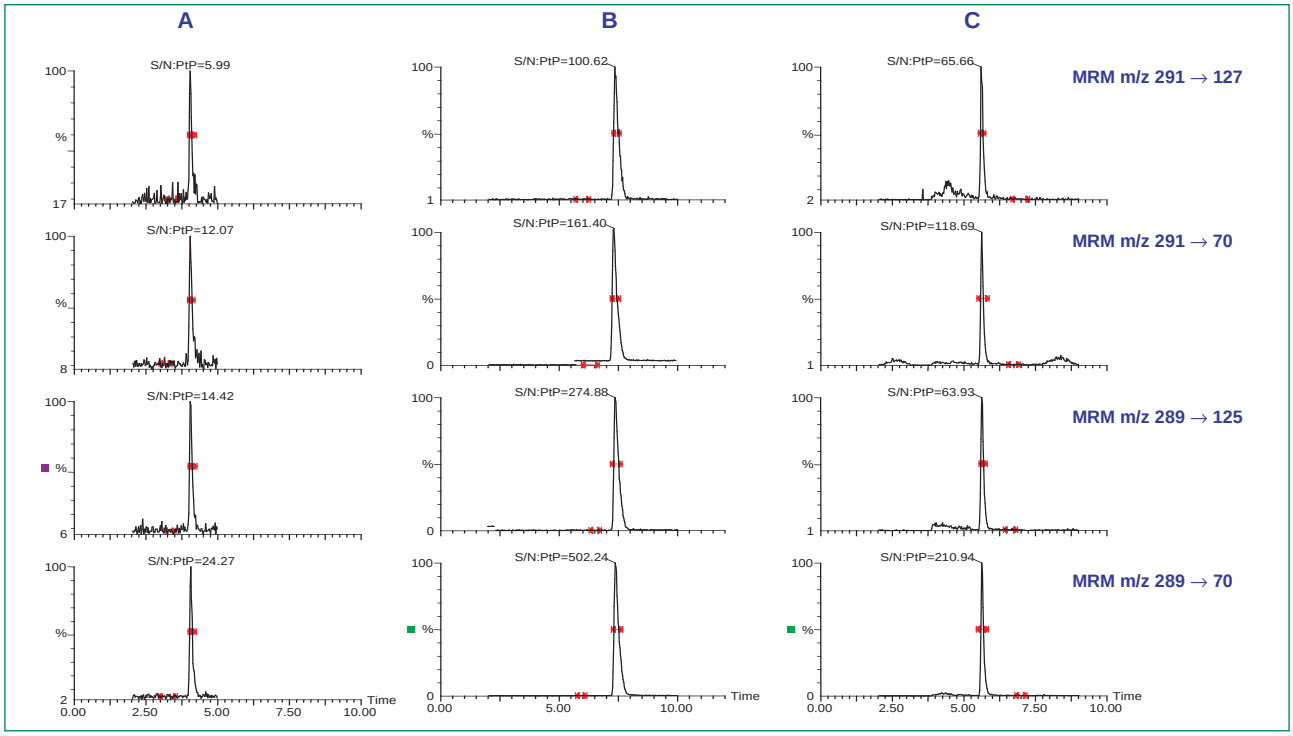
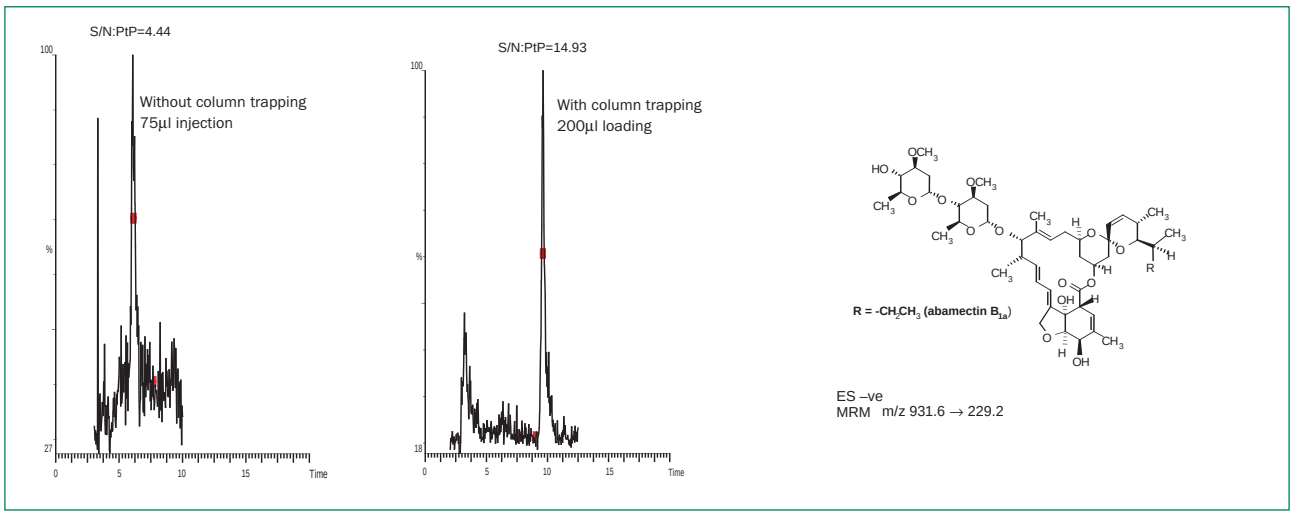


Table 8.1 Data for myclobutanil residue (mg kg^{-1}) in apple puree generated with, and without, column trapping. (* 10 µl injection, ** 500 µl sample loading)

MRM	Original LC-MSMS Method*		Column Trapping Method**	
	Extract 1	*Extract 2*	*Extract 1*	*Extract 2*
m/z 289 → 70	0.0080	0.0083	0.0065	0.0074
m/z 291 → 70	0.0076	0.0078	0.0065	0.0076
m/z 289 → 125			0.0064	0.0080
m/z 291 → 125			0.0065	0.0076

In a second example, the routine LOD obtained for abamectin, a less favourable analyte, using the original LCMS method, approached the sought reporting limit (RL) of 0.01 mg kg^{-1} in cucumber. Ideally the analytical method should be capable of achieving at least half the latter concentration, suggesting a required target LOD of 0.0025 µg ml^{-1} (≡ 0.5RL). Column trapping with a sample loading of 200 µl successfully enhanced the quality of screening data (Fig. 8.7), ensuring the target LOD was achieved and significantly increasing the reliability of the data generated by the LC-MSMS method.

Figure 8.7 Abamectin (0.0025 µg ml^{-1}) in cucumber matrix



Experience with this trace enrichment technique has demonstrated that sample amounts of crude extract equivalent to 0.25 g of matrix can be loaded without degradation in chromatographic performance; that linear calibration curves can be obtained in the range of 0.0001 – 0.025 mg kg^{-1}; that enhanced sensitivity is attained; and that the use of less intense MRM transitions can be realised for confirmation purposes.

Stable Isotope labelled Internal Standardisation

Internal standards are commonly used to improve the quality of data generated by analytical processes. Identical amounts of the internal standard are added to both calibration standards and test samples, and the responses obtained for the internal standard used to normalise the responses for the analytes sought. Using mass detection, an isotopically labelled form of a particular analyte can be employed for this purpose, as it can be discriminated from any incurred residue of the same compound by the detection system.

SASA has used deuterium (H^2) labelled carbendazim as an internal standard for the LC-MSMS analysis of pesticides in some fruit and vegetable matrices. The benefits gained from using this form of quantification are illustrated by data for lettuce. The test material from an interlaboratory analytical proficiency exercise was screened for 18 pesticides, and a residue of carbendazim tentatively identified. Subsequently, further data confirming the identification and magnitude of the residue were obtained. Scrutiny of the screening data showed that internal standardisation led to a greater degree of correlation for the calibration points used to generate the curve for carbendazim, a slight change in the slope of the line, and a decrease in the intercept value. The net effect was to increase the magnitude of residues in AQC samples by ~ 5% compared with the value that would have been obtained without internal standardisation. In the case of the lettuce test samples, the area responses obtained from the internal standard were significantly lower (~ 20%) than the mean (RSD 5.2%) obtained in matrix-matched standards and AQC samples. The lettuce matrix material used for these samples was different from that of the test samples and the matrix extract had been prepared some time earlier. This tends to point to a matrix related ion suppression effect at the elution time for carbendazim. It is not clear whether this suppression was mediated by a certain variety of lettuce only, or by fresh matrix extracts as opposed to aged extracts. Internal standardisation produced an overall positive correction of ~ 25% for the residue measurement. Similar effects were observed in the confirmation data, derived using alternative chromatographic conditions. The decrease in sensitivity that occurred throughout this sequence was corrected by internal standardisation (Fig. 8.8), the main effect again being to decrease the intercept value of the calibration line and increase residue magnitude by ~ 20%. Again an ion suppression effect reduced the area response of the internal standard by ~ 26% in the lettuce test samples. A net positive correction of ~ 45% was observed for the incurred residue of carbendazim (Table 8.2).

Figure 8.8 Calibration curves for the LC-MSMS confirmation analysis of carbendazim in lettuce using a) external standardisation, b) internal standardisation. Reporting limit (RL) units ≡ 0.05 mg kg^{-1}

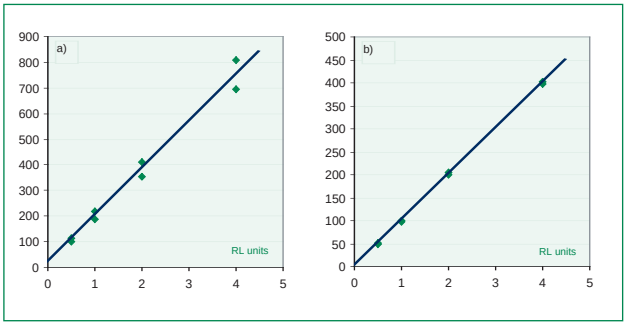


Table 8.2 Comparison of values (mg kg^{-1}) obtained for a carbendazim residue in lettuce with, and without, internal standardisation. (Assigned value of 0.053 mg kg^{-1} subsequently notified by the organising body).

	External Standardisation		Internal Standardisation	
	Screening	*Confirmation*	*Screening*	*Confirmation*
Extract 1	0.039	0.028	0.050	0.040
Extract 2	0.037	0.030	0.047	0.044

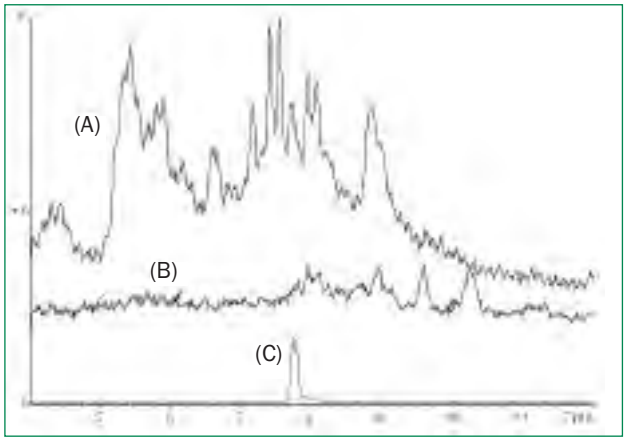
In this case, internal standardisation improved the precision of the calibration line and overcame a specific ion suppression effect on the carbendazim response observed in the test samples, but not in the matrix-matched standards. The downside in this particular analytical situation would have been the potential to overestimate residues of any of the other analytes sought, if internal standardisation using carbendazim was applied. Clearly analysts must exercise caution when interpreting such data, and relevant acceptability criteria should be promulgated in AQC protocols to deal with such situations. However, specific ion suppression effects on carbendazim and most other pesticides have not been observed in analysis of other fruits and vegetables, where internal standardisation has been successfully applied to all analytes in multi-residue suites.

APCI Determination of Thiabendazole in Cauliflower

The processes involved in API ionisation are complex and may be subject to many subtle effects. The part played by the large number of co-extracted compounds, which may be present at elevated levels in sample extracts, can be largely compensated for by the use of matrix matched standards and stable isotope internal standards. However in some particularly complex extracts, it is possible that the blank matrix material does not exactly mimic the sample extract leading to differential ion suppression or enhancement of the analyte and/or the internal standard.

In such cases, the fundamental differences between the sample introduction/ionisation modes, electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI), may offer a relatively simple opportunity to resolve the issue. Using ESI-LC-MSMS, the apparent recovery of thiabendazole from screening analyses of cauliflower extracts was consistently poor (<40%). This was probably due to ion suppression being more pronounced in the sample extract than in the matrix-matched standards. When the same analytical sequences were analysed by APCI-LC-MSMS, recoveries >70% were achieved. The suppression effect observed for thiabendazole using ESI was not exhibited for 15 other pesticides included in the same multi-residue screening analysis, whose recoveries were practically independent of ionisation mode. Full scan APCI-LC-MS traces for extracts of cauliflower and aubergine reveal the markedly more complex nature of the cauliflower matrix (Fig. 8.9). This may reflect a possible cause of the ESI ionisation phenomenon.

Figure 8.9 Full scan (m/z 80-500) positive APCI LC-MS of matrix extracts (concentration ≡ 0.5 g ml^{-1}) of cauliflower (A), abergine (B), and a thiabendazole solvent standard (C)



References

Hunter K, Taylor MJ, Sharp EA, Melton LM, Le Bouhellec S. 2004. Determination of chloralose residues in animal tissues by liquid chromatography-electrospray ionisation tandem mass spectrometry. *Journal of Chromatography B* **805:** 303-309

Pesticide Residues Committee. 2002. *Annual report of the Pesticide Residues Committee 2001.* York: Pesticide Safety Directorate.

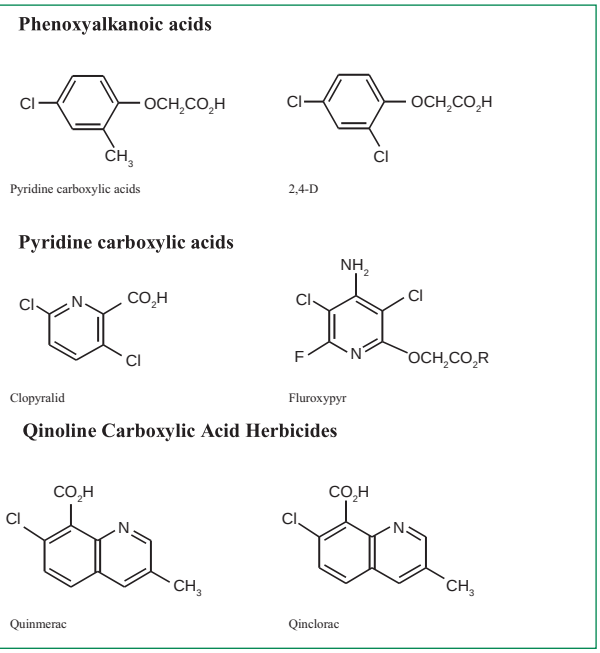
Taylor MJ. 2001. *LCMSMS: A technique for the determination of pesticide residues in the new millenium.* In: *Scientific Review 1997-2000.* Edinburgh: Scottish Agricultural Science Agency.

Taylor MJ, Hunter K, Hunter KB, Lindsay DA, Le Bouhellec S. 2002. Multi-residue method for rapid screening and confirmation of pesticides in crude extracts of fruits and vegetables using isocratic liquid chromatography with electrospray tandem mass spectrometry. *Journal of Chromatograhy A* **882:** 225-236.

8.3 Recent Changes in Herbicide Usage on Cereal Crops (CJ Griffiths)

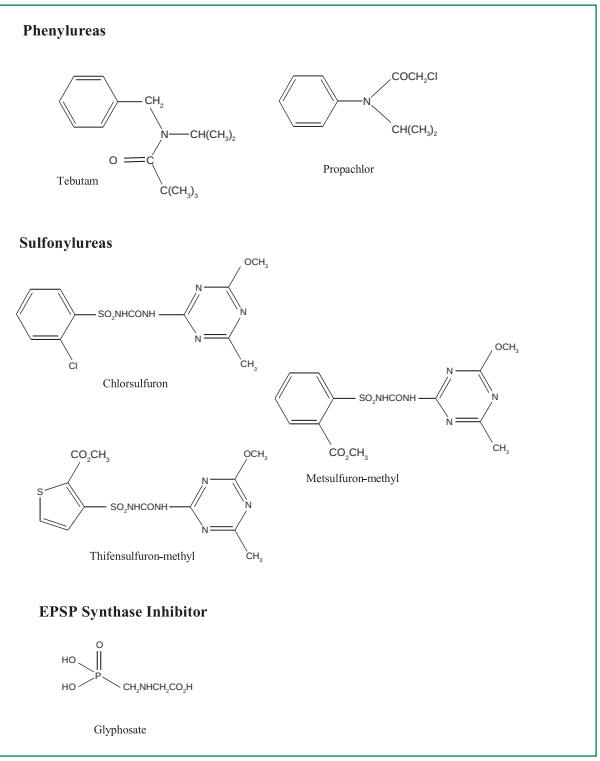
The commercialisation of synthetic chemicals to control weeds began in the 1940s when two herbicides, MCPA (Fig. 8.10) and 2,4-D (Fig. 8.10) were developed originally as candidates for use in chemical warfare. These were the first herbicides that displayed selectivity in controlling a wide range of broad-leaved weeds in cereal crops. They belong to the phenoxyacetic acid group of herbicides and to the general class of growth regulators, since they behave like the natural growth regulators, auxins, found in plants, and they interfere with the balance of regulation. Since the 1940s many other auxin-type herbicides, such as chlorpyralid and fluroxypr (Fig. 8.10), have been synthesised and approved for use on a wide range of crops. The mid-1980s saw the introduction of another group of auxin-type herbicides *viz*. the quinoline carboxylic acids, of which quinmerac and quinclorac (Fig. 8.10) are examples.

Figure 8.10 Auxin-type herbicides



Since the discovery of the auxin-type herbicides, other modes of action have been discovered, leading to the synthesis of a wide range of active ingredients that target different sites within plant cells. The next two herbicide groups discovered with different modes of action were those that affect cell division, of which the benzamides e.g. tebutam and chloracetamides e.g. propachlor (Fig. 8.11) are examples. Although the agrochemical market appears to be awash with a huge variety of herbicides, they all fall into approximately 15-20 different modes of action. The number of modes of action exploited by today's herbicides is slowly increasing as the understanding of plant physiology and biochemistry increases, spawning new groups of compounds.

Figure 8.11 Phenylurea and sulfonylurea herbicides used on cereal crops



In common with all plant protection products sold within the EU, herbicides are subject to extensive testing to ensure minimal risk to consumers and the environment. The Food and Environment Protection Act 1985 and the Control of Pesticide Regulations (1986) are the main legislative instruments that control the conditions of use for all pesticides used in the UK. The more recent EU Directives, 91/414/EEC and 98/8/EC, have created a system of European-wide registration of agrochemicals and biocides respectively, which will eventually replace the individual Member State controls on active ingredient registration. Since the mid-1970s the pre-market registration of agrochemical pesticides has been supported by a post-registration scheme of usage surveillance involving surveys to gather information on patterns of use on specific crops. A co-ordinated programme of surveys has been in place since 1992, which covers the whole of Great Britain. Northern Ireland also synchronises surveys with this programme, although separate reports are produced. Because some crops are treated more intensively and with a wide range of pesticides, they are surveyed more frequently, an example being arable crops, which are surveyed every two years. Other crops such as soft fruit, mushrooms and hardy nursery stock are surveyed every 4 years. The Pesticide Usage unit at SASA operates the programme that covers the whole of Scotland and the survey reports can be found and downloaded from the SASA web site at http://www.sasa.gov.uk/pesticide_wildlife/pesticide_usage/surveyreports.cfm

The intention of the surveys is to provide feedback to the registration authorities on the type, frequency and extent of pesticide usage, providing information for the ongoing UK and EU reviews of active ingredients. The surveys also report trends in usage over recent years, both in terms of the extent (percentage of each crop and total area treated) and the total weight of each active used. The trends data are extremely useful in identifying potential long term effects on the environment. In a review of the indirect effects of pesticides on farmland birds, conducted by the RSPB for the Joint Nature Conservation Committee (Campbell *et al*., 1997), correlation was found between the usage of pesticides and population changes of certain farmland bird species. More recently the Government policy of minimising the impact of pesticide use on the environment has been re-emphasised in the Voluntary Initiative programme agreed with the agrochemical industry and co-ordinated by the Crop Protection Association. In particular the industry has agreed to the setting of 18 key targets; for a complete description see http://www.voluntaryinitiative.org.uk/Content/default.asp. Those relating to the water environment include a 30% reduction in the number of samples containing pesticides above 0.1ppb by 2006 and the same target was set for the 9 most commonly found pesticides in untreated water. The Environment Agency's monitoring data for pesticide levels in untreated surface water will be used as the baseline for determining reductions. Trends in patterns of usage will help to inform the final analysis and confirm if the actual weights of active ingredients have fallen, treated crop areas have changed and/or if the types of active ingredients have changed over the period. Of all pesticides, the herbicide group occurs more frequently in drinking water and surface water due to the extensive areas treated and their intrinsic property of persistence. The establishment of a list of priority substances in water (Anon., 2001), made under the Water Framework Directive 200/60/EC, includes 6 herbicides, some of which are regularly found in surface water (Environment Agency, 2003).

This paper looks at trends in the usage of herbicides on cereal crops grown in Scotland over the past 20 years both in terms of crop area treated and in the changes in herbicide type. As noted previously (Griffiths, 2001), usage data can be presented in different ways depending on whether they relate to the weight of pesticide or the area treated. In the latter case it should be noted that, as a crop is often treated more than once with the same pesticide in the course of a growing season, the reported areas treated with a given pesticide have to take into account multiple applications. The reported area treated is therefore that area of a crop, which received a pesticide application, multiplied by the total number of sprays containing the same active ingredient. Furthermore, although each pesticide product has a recommended dose rate, this is rarely used in practice and the farmer will often reduce the rate by half, third, quarter or lower depending on the species of weed, density and growth stage. The survey reports take this into account when aggregating the data so that the figures given for weight of active or formulation applied to a particular crop are based on the actual information given by the farmer for each spray round.

Usage patterns also relate to crop type and area grown, although, over the years, cereal areas in Scotland have not changed appreciably (Fig. 8.12). Cereal crops in Scotland receive by far the highest proportion of agrochemicals in the arable sector, with 957.4 tonnes of active ingredients being applied to 4,702,104 hectares (see Fig. 8.13 for the distribution of pesticides on arable crops in 2000). Of the main pesticide types, herbicide treatment of cereals is second to the fungicide treated area and has remained so over recent years (see Fig. 8.14 for areas treated 1990 – 2002). Almost the whole of the cereal crop receives at least one herbicide treatment, (Fig. 8.15).

Figure 8.12 Cropping Pattern in Scotland 1982 – 2002

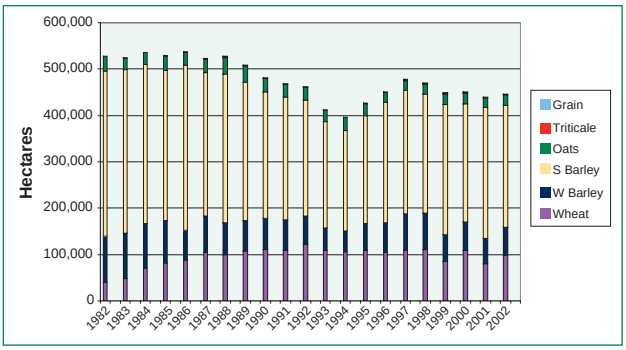


Figure 8.13 Distribution of all pesticides on main arable crops 2000

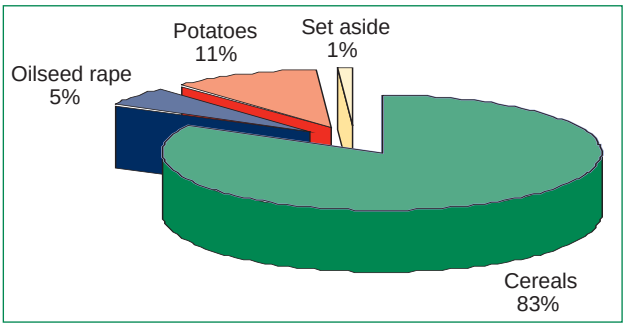


Figure 8.14 Cereal areas treated with pesticides 1990 – 2002

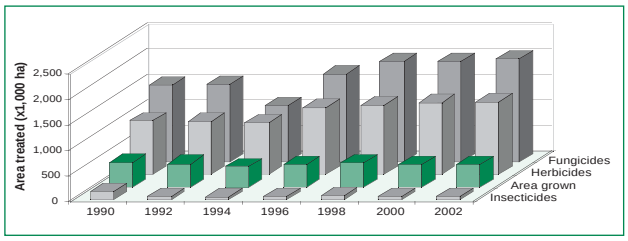
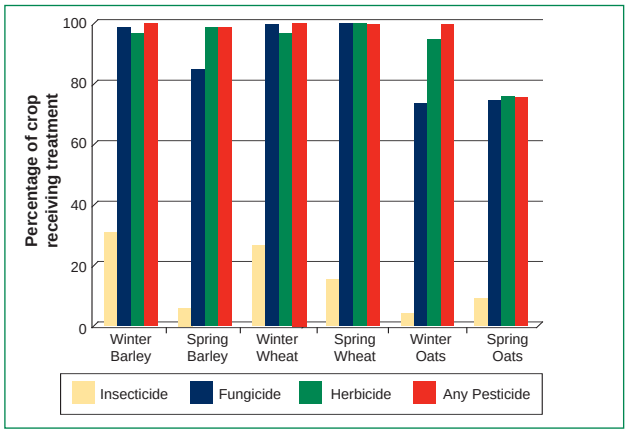
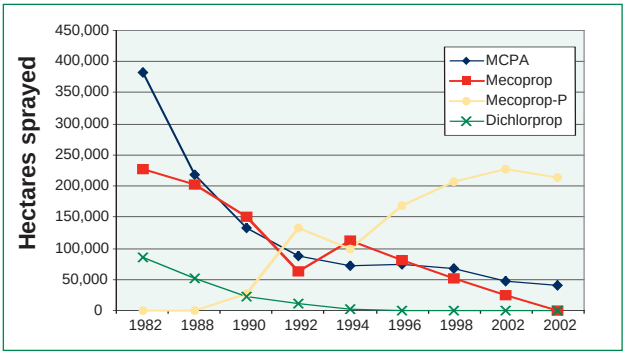


Figure 8.15 Percentage of Cereal Crop Areas Receiving Pesticides



As stated earlier, the number of modes of action used by current herbicides is relatively small and over half of the pesticides on the market today employ just three modes of action: photosynthetic inhibition, acetolactate synthase (ALS) inhibition and inhibition of protoporphyrinogen oxidase. The use of herbicides on cereal crops in Scotland over the past 20 years shows some interesting changes with respect to the types and quantities used. The top three herbicides used on cereals in 1982 were all growth regulators (Bowen and Wood, 1989) i.e. MCPA, mecoprop and dichlorprop, with a mode of action like indole acetic acid. They were used to control broad-leaved weeds in all cereals, total usage being 981 tonnes on an area of 695,000 hectares. By 1992 the usage of these three herbicides had fallen to a total of 227 tonnes on an area of 294,295 hectares. The arable survey of 2002 recorded usage of the same herbicides totalling 133.6 tonnes on 256,246 hectares. In terms of mean weight of active ingredient used per treated area of crop, the respective amounts for these survey years are 1.41, 0.77 and 0.52 kg ha^{-1}. This reduction in mean application rate, to less than half over the period, is partly due to the general trend for farmers to use less than the recommended rates. In the case of mecoprop and dichlorprop, both have chiral centres and were originally formulated as racemic mixtures containing both R and S enantiomers. However, only one enantiomer displays appreciable herbicide activity and the manufacturers later reformulated the products so that they contained only the active R form. Since the original formulations contained approximately equal amounts of the R and S forms, the maximum recommended rates for the new formulations were consequently halved, with an immediate impact on the usage pattern. Formulations containing the active ingredient mecoprop show a dramatic change over the period 1982-2002, (Fig. 8.16), with 2002 being the first year when no use of the racemic mixture was recorded. The reformulation of these products was, to some extent, a result of pressure exerted by the water industry, since monitoring of ground water over recent years has regularly detected levels above the environmental quality standard, and the EC Drinking Water Directive standard of 0.1ug L^{-1} for individual pesticide active ingredients (Environment Agency, 2003). Another important factor has been the Government's minimisation of pesticide use policy, as detailed in the 1990 White Paper 'This Common Inheritance' (Anon., 1990).

Figure 8.16 Auxin-type Herbicide Use on Cereals 1982-2002



Shortly after the discovery and commercialisation of the auxin-type herbicides in the 1940s, another group of herbicides with a mode of action affecting PS 11 (photosynthetic inhibition) was discovered. The phenylureas are one group of chemicals that operate through this mode of action, illustrated by the active ingredients linuron, chlorotoluron, monolinuron, isoproturon, methabenzthiazuron and metoxuron. All these active ingredients were recorded as being used to some extent on cereals in the arable survey of 1982 for the control of annual broad-leaved weeds and grass weeds. Total usage on cereals in 1982 amounted to 25.18 tonnes on 32,848 hectares. By 1992 usage had changed to a total of 64.49 tonnes on 85,455 hectares and to 95.62 tonnes on 138,249 hectares in 2002. Mean application rates over the period hardly changed, i.e. at 0.76kg ha^{-1} in 1982 to 0.7kg ha^{-1} in 2002. However, by 2002, only two of the original phenylurea active ingredients were in use, *viz*. linuron and isoproturon, with the latter being used to a much greater extent, being applied to 136,213 hectares compared with 2,036 hectares for linuron.

Isoproturon has been a very popular cereal herbicide for the control of annual grass weeds and broad-leaved weeds in recent years, being co-formulated with a wide range of other herbicide active ingredients such as diflufenican and pendimethalin, which extend the range of weeds controlled. However, isoproturon has moderate soil mobility and is regularly found in ground and surface waters at concentrations above the EC Drinking Water Directive limit of 0.1ug L^{-1} (Environment Agency, 2003). Stewardship schemes agreed between manufacturers, farmers and the Environment Agency, aimed to ensure best practice in the use of products containing isoproturon, were introduced in the mid-1990s for specific areas, albeit with limited success.

A major group of herbicides, which act through the inhibition of the enzyme acetolactate synthase, was introduced during the 1980s; the sulphonylureas illustrate this mode of action, having a broad spectrum of selectivity and acting at a very low application rate. Typical chemicals in this class are metsulfuron-methyl (Fig. 8.11) and thifensulfuron-methyl (Fig. 8.11), both introduced in the mid-1980s. Slight changes in the molecular structure of these ureas can result in very different physico-chemical properties, leading to large differences in stability and solubility and hence half-lives in soil.

Use of sulfonylureas on cereals first appeared in the 1988 arable survey (Snowden and Bowen, 1991) when the combined usage of chlorsulfuron (Fig. 8.11), metsulfuron-methyl (Fig. 8.11) and thifensulfuron-methyl (Fig. 8.11) accounted for 1.98 tonnes on 233,220 hectares, equivalent to an application rate of 0.0085kg ha^{-1}. This remarkably low application rate means that there is very little risk of measurable quantities being detected in surface or ground water and therefore any exceedance of the 0.1ug L^{-1} drinking water limit. Since the introduction of the sulphonylureas in the mid-1980s their number and range of use has dramatically increased. The latest arable survey covering the 2002 season recorded 5 sulphonylureas used on cereal crops, on a total of 404,378 hectares, amounting to 4.623 tonnes of active ingredients, the mean application rate being just 0.011kg $ha^{-1.}$

The sulphonylurea herbicides have been a remarkable success story as they have been approved in many countries for a very wide range of crops, particularly on those with large acreages such as wheat, maize and rice. On the downside there have been several reports of the development of resistance of some weeds to sulphonylureas, most notably in blackgrass, wild oats, chickweed and common poppy. The first confirmation of resistance in chickweed populations in Scotland occurred in the Tayside region in 2000 and has subsequently been recorded in other areas of the UK. There are several options available to farmers to avoid resistance to ASL herbicides, a simple one being to use 2 or more herbicides with different modes of action. Herbicide resistance is, of course, not only related to the sulphonylurea group. The Herbicide Resistance Action Committee, which monitors the evolution of herbicide resistant weeds, has recorded resistance to 15 different modes of action (http://www.plantprotection.org/HRAC/).

The ALS inhibitors fall into the broad class of herbicides that affect amino acid biosynthesis in plants. A widely used herbicide that disrupts the biosynthesis of the amino acids tryptophan, phenylalanine and tyrosine is the ubiquitous herbicide glyphosate (Fig. 8.11) (see Section 2.2). This organophosphorus molecule binds to the enzyme 5-enoylpyruvate shikimic acid 3-phosphate synthase (EPSP synthase), preventing the formation of amino acids that form essential intermediates for more complex material within the plant. Use of glyphosate in cereals is usually before harvest, when it aids the ripening process and controls a wide range of perennial weeds. Usage in Scotland on cereals has fluctuated over the years, both in terms of area treated and tonnes applied. In 1982 55.4 tonnes of active ingredient were applied to 36,840 hectares at an mean application rate of 1.5kg ha^{-1}, whereas in 2002 67,987 hectares were treated with 51.87 tonnes at an mean rate of 0.76kg ha^{-1}.

Glyphosate is very widely used and is, in many respects, an ideal herbicide, having low mammalian toxicity, low half-life in the soil and broad-spectrum activity. The introduction of glyphosate tolerance in genetically engineered (GM) crops has extended its use worldwide, although the commercial approval of such GM crops in the EU has yet to be resolved. Progress with respect to the approval of GM crops in the UK has been influenced by public concern over the risks for the transfer of antibiotic resistance to humans, the potential effects on biodiversity and transfer of resistance genes to other species. The recent farm scale evaluations (FSEs) have shown that, at least for fodder maize, the pesticide regime used on the GM crops had a more benign effect on the biodiversity than the conventional agronomic weed treatments (Hawes *et al*., 2003; see Chapter 7 for the work of the GM Inspectorate). Conventional maize is usually treated with the herbicide atrazine, another active ingredient that has been regularly detected in surface water above the 0.1ug L^{-1} limit. However this active ingredient has been reviewed by the EU under the 91/414/EEC Directive and will be withdrawn from use in September 2005. If GM maize were to be given commercial approval, the benefits compared with conventional practice might not be the same as those measured in the FSEs.

In conclusion, the use of herbicides on cereals shows a continuing pattern of change, in terms of diversity of the products used, their modes of action and rates of application. The main driving forces behind these changes have been the requirement for selectivity, reduced potential to contaminate surface and ground water and more recently weed resistance. Extensive legislation covers the registration and conditions of use, with continuing pressure from Government, the environment Agencies and water companies to reduce usage. The pesticide usage surveys continue to highlight these changes and *inter alia,* inform Government and the regulatory authorities during the registration and review programmes.

References

Anon. 1990. *This Common Inheritance, Britain's Environmental Strategy.* Cm 1200. London: HMSO.

Anon. 2001. European Parliament and Council Decision 2455/2001/EC. OJ L 331, 15th December 2001.

Bowen M, Wood J. 1989. *Pesticide Usage Survey Report 45, Arable Crops 1982.* Edinburgh: SOAFD.

Campbell LH, Avery MI, Donald P, Evans AD, Green RE, Wilson JD. 1997. A Review of the Indirect Effects of Pesticides on Birds. *JNCC Report* **227.** Joint Nature Conservation Committee, Peterborough.

Environment Agency 2003. *Pesticides 2002.* Wallingford.

Griffiths CJ. 2001. Quantitative and qualitative changes in pesticide usage in Scotland. *Scottish Agricultural Science Agency Review 1992-1997,* 54-56.

Hawes C, Haughton AJ, Osborne JL, Roy DB, Clark SJ, Perry JN, Rothery P, Bohan DA, Brooks DR, Champion GT, Dewar AM, Heard MS, Woiwod IP, Daniels RE, Young MW, Parish AM, Scott RJ, Firbank LG, Squire GR. 2003. Responses of plants and invertebrate trophic groups to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Philosophical TransActions of the Royal Soclety of London* **B358:** 1899-1913.

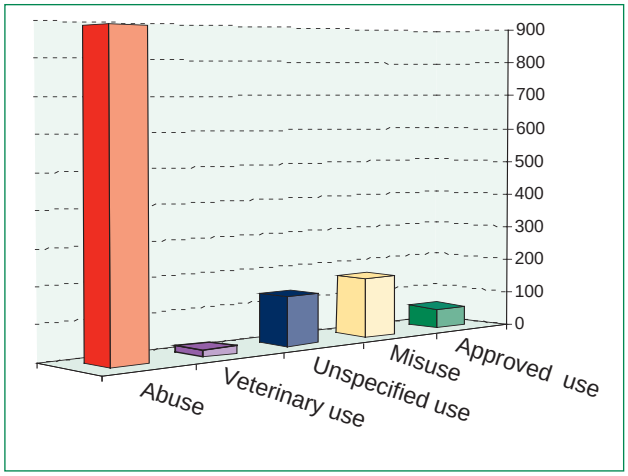
Snowden JP and Bowen HM 1991. *Pesticide Usage Survey Report 77, Arable Crops 1988.* Edinburgh: SOAFD.

8.4 Pesticide Abuse – the Long Standing Problem of Animal Poisoning (K Hunter, EA Sharp, LM Melton)

In the early part of 2004, press articles and other media coverage drew public attention to 'the worst ever' wildlife poisoning incident in the United Kingdom. A goshawk and at least 24 buzzards were poisoned in the Scottish Borders after baits prepared using a pesticide formulation had been laid. This incident demonstrated the devastating impact such practices can have on local wildlife populations. For some time concerns have been expressed about the impact of illegal poisoning on vulnerable species, especially birds of prey. The early records of the Wildlife Incident Investigation Scheme in Scotland, ironically set up to report on poisoning arising from the legitimate use of pesticides, are littered with incidents caused by the deliberate and illegal use of pesticides. Victims have included golden eagles, white-tailed sea eagles, red kites, marsh harriers, peregrine falcons, a gyr falcon, and wild mammals such as the pine marten. Companion animals have also been put at risk because of the indiscriminate nature of poisoning, although it would also appear that on occasions these animals themselves have constituted the intended target of the 'poisoners'.

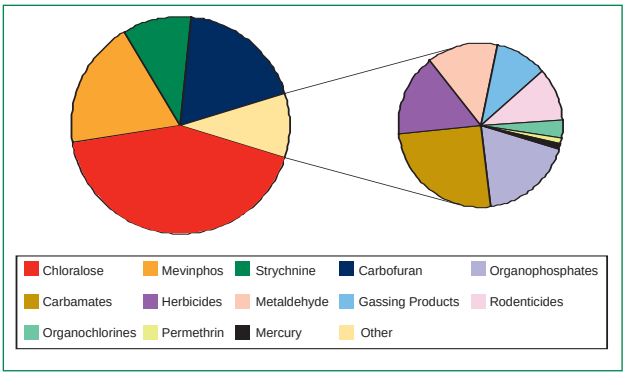
Poisoning of animals is not a new phenomenon, it was one of the methods used to control so-called 'vermin' in the 19th century, and led to the eradication of some species that are now valued as part of our natural heritage and biodiversity. In this relatively enlightened age it is difficult to understand why such an illegal practice persists, yet persist it does. Data from the WIIS in Scotland amply demonstrate the magnitude of the problem. Approximately 70% of all confirmed pesticide poisonings in the period 1972 to 2003 were attributed to the deliberate abuse of pesticides (Fig. 8.17).

Figure 8.17 Categorisation of confirmed pesticide incidents in Scotland, 1972-2003



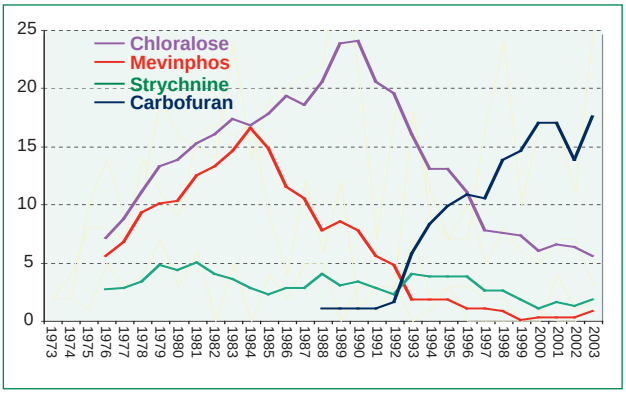
A total of 34 pesticides have been identified in wildlife casualties and/or bait materials from abuse incidents in Scotland (Fig. 8.18). It is possible that more substances may have been abused and gone unnoticed because the associated mortality was not reported.

Figure 8.18 Pesticides abused in Scotland 1972-2003 (total 923 incidents)



Four substances, carbofuran, chloralose, mevinphos, and strychnine, appear to have gained most favour as poisons of choice with perpetrators laying baits. The variation in the extent of abuse of each of these substances with time (Fig. 8.19) may well be related, in part at least, to the availability of each. The 'Phosdrin' formulations containing mevinphos were withdrawn from the market in the early 1980s. This seems to correspond to the peak in the rolling mean of the number of incidents. However, it is disappointing that incidents involving this active ingredient were still being recorded in 2003. Chloralose was without doubt the poison of choice in Scotland for many years. Its use peaked in the early 1990s, since when the annual number of incidents has declined and settled at a lower value. It has been steadily superseded by granular insecticide formulations containing carbofuran, which currently account for the vast majority of abuse incidents.

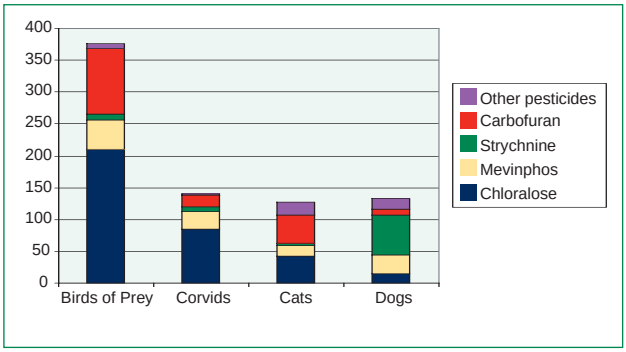
Figure 8.19 Annual number of abuse incidents (rolling mean, 4 year cycle)



Birds of prey constitute easily the most frequently poisoned sub-group of animals, making up 43% of vertebrate poisoning events, the other largest sub-groupings being corvids (16%), cats (15%), and dogs (15%). The pesticides involved with poisonings in each sub-class are illustrated in Figure 8.20. The most striking variation lies in the relative distribution for dogs where almost half of all incidents were associated with strychnine poisoning, compared with only 2-5% for the other sub-groups. Conversely, the proportion of chloralose poisoning

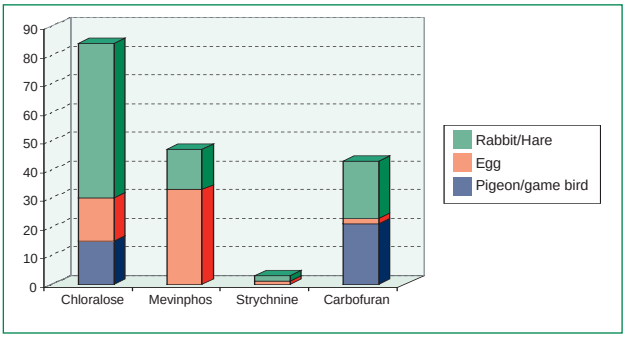
events in dogs was only ~ 10% compared with 34-60% for the other animal sub-groups. The difference in patterns may be due to a variety of factors: cats for instance are believed to be more sensitive to chloralose than dogs, but at the same time dogs are perhaps more likely to be maintained under conditions that to some extent mitigate the effects of decreased body temperature associated with chloralose intoxication. Differences in the potential food item used to prepare baits, and possible variability in repellent effect of the pesticides are other possible factors. The other noticeable feature was the greater proportion of poisoning events in cats and dogs associated with pesticides other than carbofuran, chloralose, mevinphos and strychnine. This may well be because they take place in urban situations where formulations classed as Amateur Products are subject to abuse. Such incidents are sometimes associated with disputes between neighbours, frustration arising from cats fouling seed beds in gardens or allotments, or perceived threats to pigeon lofts. The relatively high frequency of carbofuran poisoning events in cats was largely associated with activities of this type in the mid 1990s.

Figure 8.20 Pesticides abused in vertebrate poisoning events in Scotland 1972-2003



Scrutiny of data on materials clearly defined and confirmed as poisonous baits allows the majority to be categorised into three main types: those prepared from eggs, those prepared using either rabbit or hare carcases, and those prepared from pigeon or game bird carcases (Fig. 8.21). Other baits have been made up from materials such as bread, cereal seed, cooked chicken, bacon, butchered meat or sausages. Chloralose, as a powder, has been most frequently employed on animal carcases, but has also been used in egg baits to some extent. By contrast, liquid formulations containing mevinphos have predominately been used in egg baits, where, typically, a small hole has been made in the shell and the substance injected with a hypodermic syringe. This may go some way to explaining the relatively greater frequency of mevinphos poisonings in dogs as compared with cats. The same liquid formulations have also been poured onto the exposed tissues of animal carcases to form baits. The use of granular carbamate insecticide formulations, in particular those containing carbofuran, has been restricted almost exclusively to meat based baits. However, in at least one case, the remnants of a large scale bait preparation exercise have been discovered. Evidence suggested that up to a gross of egg baits had been prepared by injection, suggesting that the perpetrator had somehow produced a suitable fluid or liquid suspension from the granular material.

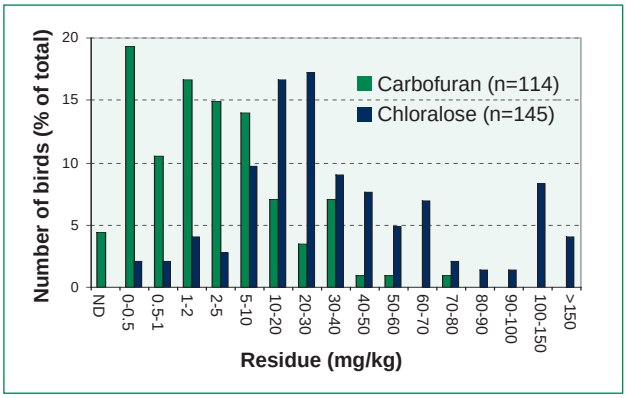
Figure 8.21 Occurrence of main bait material/pesticide combinations



To combat the perennial problem of deliberate abuse of pesticides, enforcement bodies and associated partner organisations have increasingly adopted a multi-agency approach to the investigation of individual incidents, and contributed to educational and publicity initiatives to highlight the problem. In operating the Wildlife Incident Investigation Scheme on behalf of SEERAD, SASA must strive to deliver appropriate and timely services to both SEERAD and the police. Firstly the laboratory must have appropriate procedures to document sample receipt and custody because of the potential for prosecution. The test methods employed in the analytical investigation must be robust, validated as far as practical, and be backed by relevant analytical quality control measures. The laboratory must endeavour to present high quality scientific evidence and interpretation in reports submitted to the Procurator Fiscal Service to support particular prosecutions.

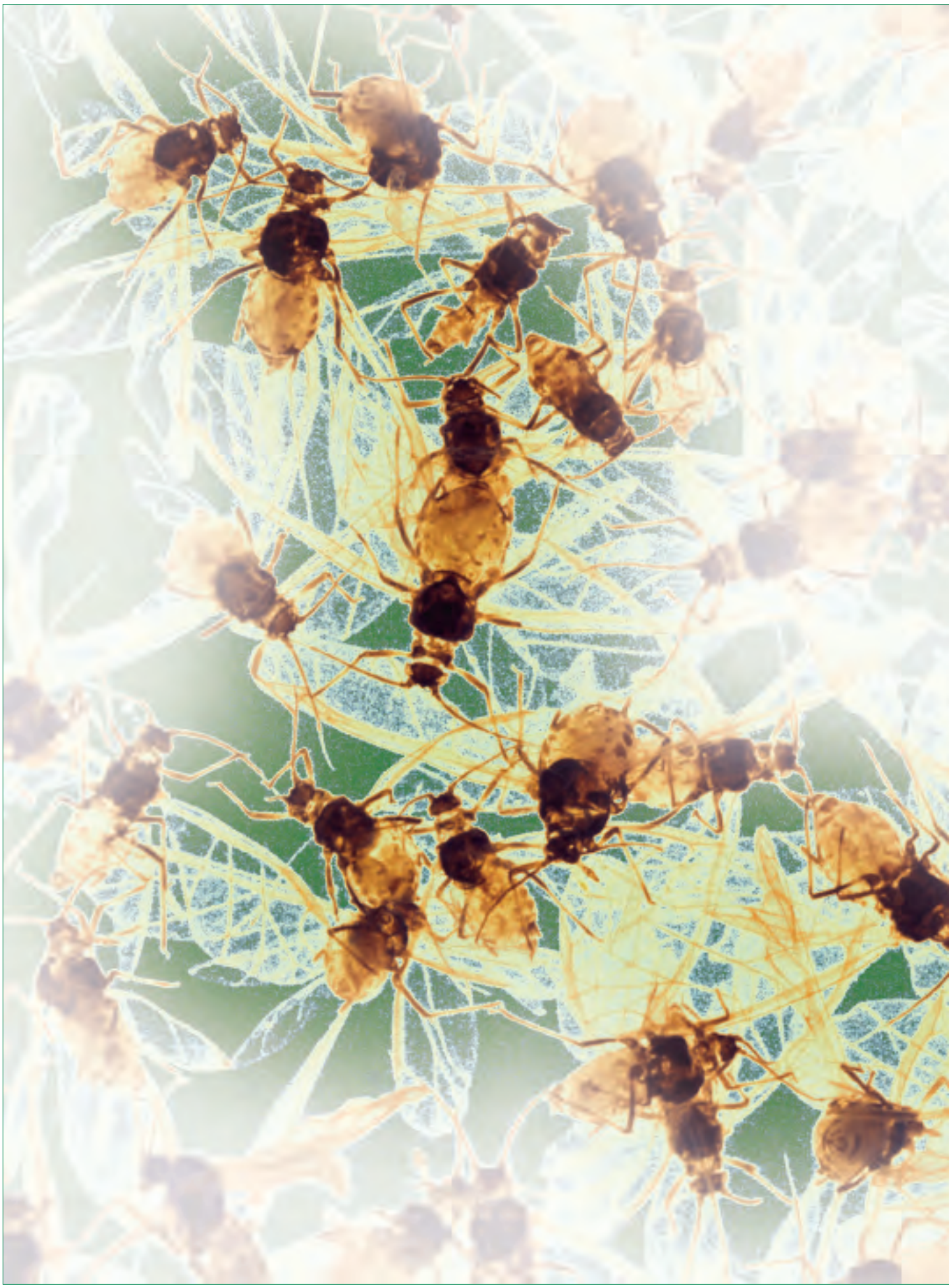
To aid interpretation, the laboratory is utilising residue data sets, gathered over a number of years, for certain toxic agents, to establish criteria indicating lethal intoxication. In the case of chloralose in birds of prey, scatter plots show no correlation between concentrations found in liver and those found in stomach content or gullet content material. However, scrutiny of the data for birds classified as being poisoned indicates that, in approximately 90% of cases, liver residues were ≥ 2 mg kg^{-1} and a residue in either the stomach content or gullet material of the same bird was ≥ 10 mg kg^{-1}. In 97% of cases at least one of these two criteria was exceeded. The frequency profile for liver residues (Fig. 8.22) indicates that their magnitude is most likely to fall in the range of 5 – 70 mg kg^{-1}. Corresponding data for poisoned corvids (n=57) demonstrates a maximum residue frequency at 5-10 mg kg^{-1} and indicates that residues are most likely to fall in the range of 2-30 mg kg^{-1}.

Figure 8.22 Liver residues in poisoned birds of prey



Carbofuran is a much faster acting and potent toxicant than chloralose. It may be more susceptible, once ingested and absorbed into the body, to enzyme mediated modification than the relatively stable chloralose. There is evidence to suggest that at least one structurally related metabolite can occur in the liver tissue of poisoned animals. Again scatter plots show no correlation between carbofuran residue concentrations in liver tissue and residue concentrations in stomach content or gullet content material from poisoned birds of prey. In 85% of cases, a residue ≥ 0.1 mg kg^{-1} was detected in liver tissue and a residue ≥ 5 mg kg^{-1} was present in digestive tract material from the same bird, and 98% of cases satisfied at least one of these criteria. The evidence for poisoning was compelling in at least three out of four cases where no residues were detected in liver tissue. The frequency profile for carbofuran (Fig. 8.22) is quite different to that for chloralose. Residues in poisoned birds are likely to fall in the range of 0 – 40 mg kg^{-1}, with the vast majority falling in the range of 0.5-10 mg kg^{-1}. Corresponding data for poisoned mammals (n= 41) show that liver residues are most likely to be in the range 0-5 mg kg^{-1}, and that no residue is likely to be detected in a much greater proportion (22%) of poisoning victims. There is some similarity here to early observations (based on limited data) that mevinphos residues were occasionally detected in avian liver tissues but rarely if ever found in the livers of poisoned mammals.

Laboratory staff preparing submissions for court purposes can enhance the value of their reports by incorporating compliance with criteria derived in this way, and by ensuring that residues measured in a particular animal are set in the context of established ranges for the relevant toxicant. Forward planning and scrutiny of data in this way will enable specialists to respond under cross examination and provide the court with good quality interpretation.



9 Wildlife/Ecology

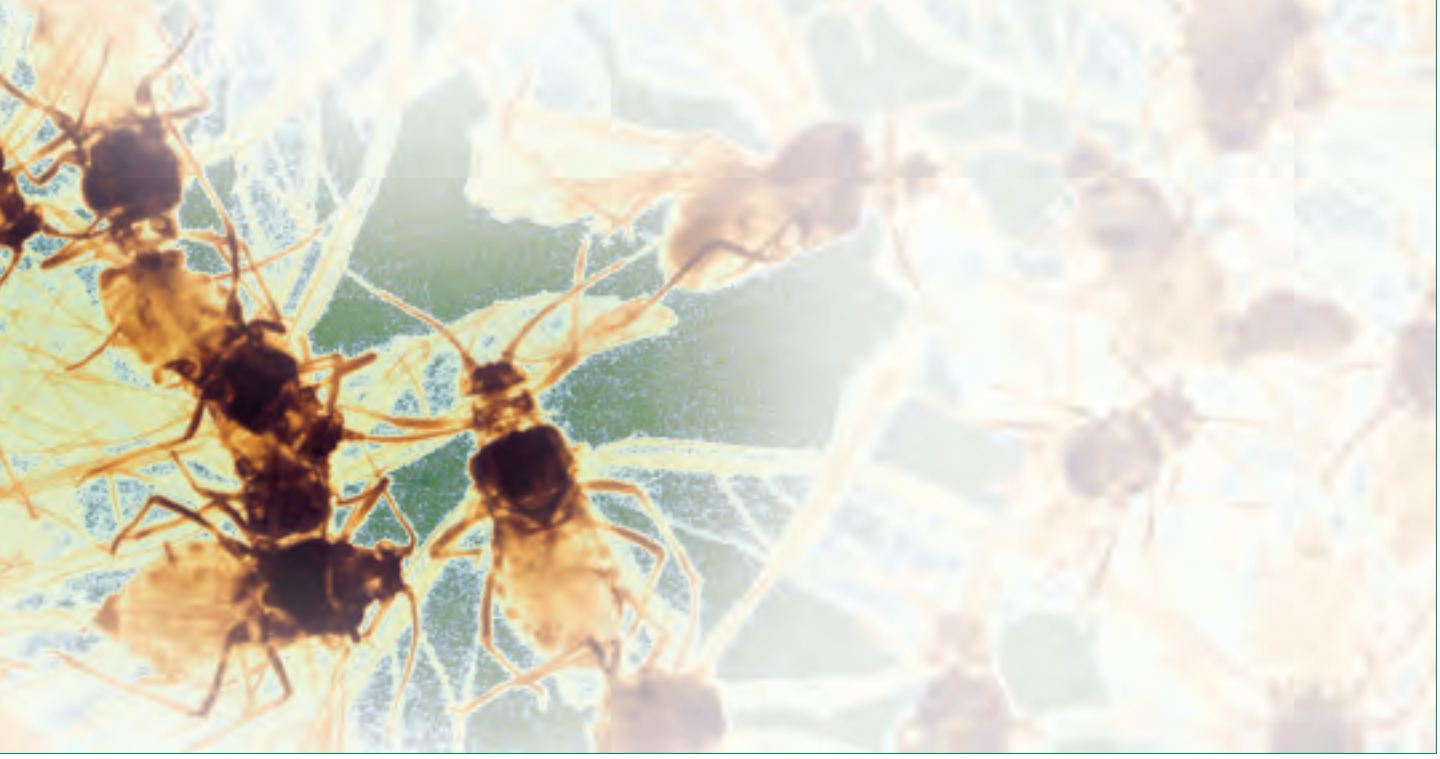
9.1 Introduction (S Campbell)

9.2 Golden Eagles and Lamb Predation (S Campbell)

9.3 The Value of Aphid Monitoring in the Detection of the Effects of Long Term Environmental Change (L King and J Pickup)

Scientific Challenges Article

- SASA's Role in the Foot and Mouth Outbreak (G Hartley)



Chapter 9 Wildlife/Ecology

9.1 Introduction (S Campbell)

The primary role of the scientists in Wildlife Management is the provision of advice to the various divisions of SEERAD on all aspects of vertebrate management, where conflict arises between wildlife species and man. That advice can be in the form of interpreting scientific literature and data, or commenting on proposed legislation changes. In addition to scientific advice, practical advice on pest control or mitigation techniques is given. SASA also receives many enquiries from outwith SEERAD relating to vertebrate pest management; these are commonly from individual farmers, pest contractors and members of the public but also include other governmental bodies and agencies, local authorities, non-governmental organisations and research bodies.

In recent years management and scientific advice has been given on issues relating to badgers, eagles, eider ducks, foxes, geese, grey squirrels, gulls, herons, rabbits, rats, sawbilled ducks and a range of other species and issues. In 2001, SASA Wildlife Staff were heavily involved in the Foot and Mouth outbreak for almost 6 months co-ordinating rodent control activities on infected premises, and G Hartley's Scientific Challenge article describes this in greater detail.

Another major component of the work for SEERAD is related to the issuing of licences to kill or take protected animal species (as permitted by the Wildlife and Countryside Act 1981). The role of SASA is to comment on the information provided by licence applicants and provide the relevant division of SEERAD with recommendations to enable them to make a well informed decision on the merits of the licence application. Often wildlife management staff will correspond with applicants to gather further background information and site visits will also be undertaken.

In recent years, Wildlife management staff have been involved in several rabbit damage cases. These arise when a landowner is suffering damage due to rabbits that originate on an adjacent landowners ground. Under the Pests Act (1954), landowners have an obligation to control rabbits on their land, and thus prevent them damaging neighbouring property. In extreme cases, when a landowner refuses to meet this obligation, a farmer may report the matter to the Agricultural Inspectorate, and if, after discussing the matter with those involved, there is still no progress then SEERAD has the authority to issue a Rabbit Control Order with which the parties involved must comply. SASA Wildlife staff may be asked to give advice on aspects of rabbit control and, in the past, have succeeded in resolving disputes by helping to mediate agreements to undertake control. As part of this work SASA staff collect data through surveys of rabbits and rabbit signs that are used to assess the severity of the problem, and later could be used as supporting evidence should SEERAD have to issue a Rabbit Control Order. To date, disputes have been resolved before it has become necessary to issue an Order.

In the past three years, research and development work has included an investigation into goose damage to reseeded grassland on Islay, and trials of electric fencing as a way of protecting pre-harvest cereal crops from goose damage on the Western Isles. An investigation of golden eagle predation of lambs on Benbecula has also been conducted (see section 9.2). Staff collaborate with colleagues from DEFRA and CSL and have worked with them on both R&D and training programmes in the last three years.

Wildlife management staff represent SEERAD on the Goose Science Advisory Committee and the Scottish working group of PAW (Partnership for Action Against Wildlife Crime), and have represented SASA at several international conferences and workshops.

9.2 Golden eagles and Lamb Predation (S Campbell)

The UK golden eagle population is almost exclusively found in Scotland, occupying remote mountain and moorland areas away from human activities. As large predators, golden eagles hunt a wide range of mammals and birds and this can on occasion bring them into conflict with sheep farming, one of the main commercial activities in such areas. Eagles are often accused of killing and taking lambs and very occasionally of taking adult sheep. There is no doubt that golden eagles can and do kill lambs, but the extent to which this occurs is a contentious issue. It may be difficult to tell if an eagle seen feeding on a fresh lamb carcass is responsible for killing the animal, or is merely feeding on a lamb that had already died.

Hill sheep in remote areas are commonly much less productive than those on lowland farms. Lambing percentages can be 70% or lower (i.e. 70 lambs per 100 ewes) compared with levels as high as 200% and above for some lowland flocks. The quality of hill and moorland grazing is normally poor, weather conditions can be adverse and management is less intensive. Previous studies of golden eagle predation of lambs in Scotland have found that it accounted for losses of up to 1 to 2% of lambs born in eagle territories, with other factors responsible for greater losses (Weir 1973; Hewson 1984 and Leitch 1986). However farmers and crofters producing lambs in eagle territories commonly suspect that the eagles may be responsible for killing many more of the lambs that disappear or are found eaten.

On the island of Benbecula in the Western Isles, crofters have, over a period of several years, made known their concerns that golden eagles have been killing and taking lambs from four remote flocks. Eagles are resident in the area and carcasses of lambs that have been eaten have been found regularly, and many lambs have gone missing. SASA were asked to investigate after several previous investigations by SEERAD inspectors had not resolved the situation. The aim was to find out what the causes of lamb mortality were and, more specifically, to what degree eagles were responsible.

Methods

Sheep flocks from four adjacent crofted sites (sites A, B, C and D) on a coastal moorland area of Benbecula were monitored between late March and mid June 2003. Ewes were gathered prior to lambing, their age was recorded and their condition was scored using a standard index based on body fat.

On sites A and B, crofters brought the ewes back to inbye land to lamb under supervision, whereas on the remaining two sites (C and D), the ewes were left to give birth without any assistance. All lambs born on sites A and B were weighed and their hindfoot measurements taken within 24h of birth; they were also given ear tags with a unique identification number so that their fate could be related to the data collected. While these procedures were being carried out, the reaction of the ewe to the handling of the lamb was noted using an established maternal behaviour index (O'Connor *et al*., 1985). The ewes from the two flocks that lambed on the hill (sites C and D) were gathered and a veterinary surgeon carried out pregnancy diagnosis (by external manipulation). Stockmarker spray was used on all four sites to give each ewe its own number to aid identification in the field. Lambs born on inbye land (sites A and B) were put out on the hill with their mothers after two to four weeks.

All flocks were monitored regularly to check for missing lambs and to locate and examine dead lambs. To assist in this task, small radio transmitters were fitted to 20 lambs selected at random from one flock before release on the hill. In late June, all flocks were gathered and a final count of lambs taken.

There was one pair of territorial non-breeding golden eagles in the study area and another two pairs approximately 7km to the north and south of the study area respectively, of which at least one was known to be breeding.

Flock Sizes, Pregnancy and Production

Lambing percentages varied between 60 and 90.5%. Although the percentage of barren ewes appears much higher on sites C and D than on the other two sites (Table 9.1), the method of pregnancy diagnosis was not completely reliable and was likely to underestimate the numbers pregnant. However, as it is not possible to quantify this error, it is not possible to confirm that pregnancy rates varied between the sites.

Mean condition scores were 2.13, 2.04, 1.90 and 1.82 for sites A, C, D and B respectively. An ordinal regression of scores on sites showed strong evidence of differences between sites, (p<0.001), and an analysis of variance also showed strong evidence of differences in mean scores between sites (p<0.001). Figure 9.1 shows that condition scores on Site A had a narrower range than other sites, with the majority of ewes scoring 2.0. According to DEFRA guidelines, a score of 2 is the ideal score for a hill sheep 8 weeks before lambing (MAFF, 2000). Although these sheep were a week or two from lambing, a score markedly lower or higher than 2 would indicate potential problems such as undernourished lambs or difficult births. Figure 9.2 shows the mean condition score on a site against the lambing percentage achieved, indicating a positive relationship.

Table 9.1 Number of ewes per flock and lamb production

Site	Ewes	Estimated pregnant	Barren ewes	Actual lambs born	Final lambs in June	Lambing %
A	106	–	7	109	96	90.5
B	69	–	6	67	42	60.8
C	89	72	17*	–	64	71.9
D	20	16	4*	–	12	60.0

* These figures are based on pregnancy diagnosis, which was not 100% accurate.

Figure 9.1 Percentage of each flock with a particular condition score

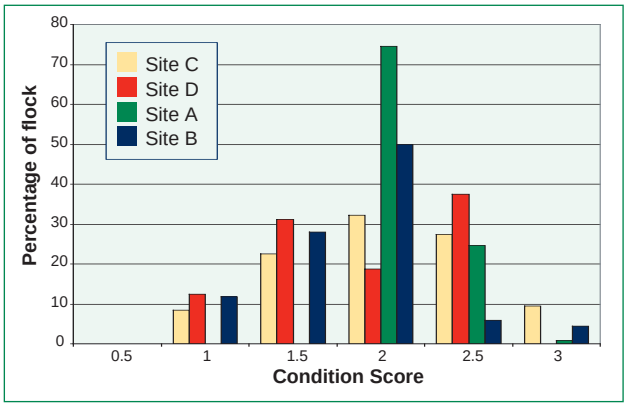
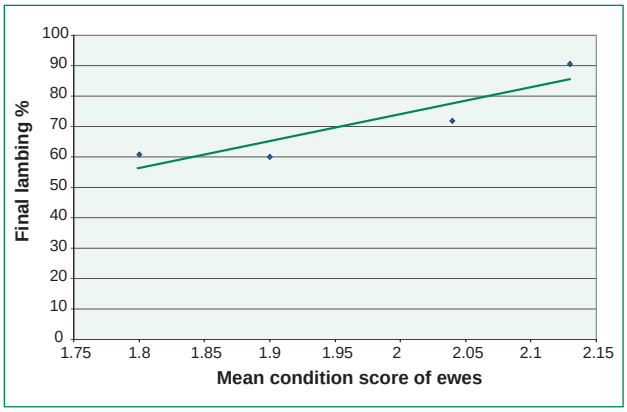


Figure 9.2 The relationship between mean condition score and lambing percentage



Effect of Mother on Survival

Relationships between characteristics of the mother and the survival of its lambs were examined separately on sites A and B. There was some evidence of an effect of the ewe's age on the subsequent survival of her lamb on site B (Chi-squared, p=0.036) but not on site A (Chi-squared, p=0.65). There was no evidence of a relationship with the maternal behaviour score, or of a relationship between the mother's condition score and the survival of its lamb, on either site. This contrasts with the indication of higher lambing production on sites with higher condition scores seen in Figure 9.2 and suggests that the higher scores and higher production may both be a result of another factor such as the type of management, rather than survival being a direct effect of higher condition score.

Lamb Measurements

Measurements of new-born lambs were collected from sites A and B only. There was no evidence that sex affected either weight or hindfoot length on either site (p=0.23 & p=0.41 respectively after taking site into account). There was evidence of a difference between the two sites in the weight of lambs at birth (p=0.037 after taking sex into account) with mean weight of 3.85 kg on site A compared with a mean of 4.11 kg on site B, the standard error for the difference being 0.12. However there was no evidence of a difference in hindfoot length between sites (p=0.73 after taking sex into account).

Unpaired t-tests comparing the measurements taken from lambs which subsequently died with those that survived on each site revealed a significant difference (p=0.008) in initial weight. Survivors on site A were heavier at birth than those that died, but there was no significant difference in hindfoot length. On site B there was weak evidence of a difference in initial weight (not significant at p=0.052), but there was a significant difference in hindfoot length (p=0.007), in both cases the survivors having larger mean values.

Causes of Death

Regular monitoring of the flocks revealed 24 dead lambs. It was possible to determine the cause of death, or at least rule out certain causes, for many of these. Other carcasses were examined for signs of damage and an assessment was made of the likelihood of eagle predation. For ten carcasses it was not possible to come to a definite conclusion. Photographs and descriptions of these carcasses, including one carcass confirmed to be an actual eagle kill as a control, were recorded electronically and later examined by four experts with experience of *post mortem* analysis of livestock and/or eagle predation investigation. A simple questionnaire was provided and the reviewer was asked to rate the likelihood of the carcass being an eagle kill. The five ratings were: Yes, Possible, Unlikely, No and Unclear, corresponding to scores of +2, +1, -1, -2 and 0 respectively. The total scores were used to determine the fate of the lambs, with a score of greater than 6 indicating a kill and a score less than -6 indicating definitely not a kill. Greater than or equal to 2 was treated as a possible kill, and less than or equal to -2 as an unlikely. Any carcass scoring between 2 and -2 was termed unclear. The assessments by the four reviewers were combined with an assessment made by SASA staff in consultation with the local veterinary surgeon, the final results being: 1 eagle kill, 2 possible eagle kills, 2 unclear, 5 unlikely and 1 definitely not an eagle kill. These figures were combined with those for other lamb carcasses where cause of death was determined, and are shown in Table 9.2. Those carcasses where cause of death could be determined are listed in Table 9.3.

For the two sites where accurate lamb data were available (A and B) the losses (as a percentage of lambs born) were calculated for each cause of death (Table 9.4).

Table 9.2 Assessment of percentage of lamb carcasses found on study sites that were killed by eagles

	Site C		Site D		Site A		Site B		All sites	
Eagle Kill?	n	%	n	%	n	%	n	%	n	%
Yes	–	–	–	–	1	50	–	–	1	4
Possible	–	–	–	–	–	–	2	22	2	8
Unknown	–	–	–	–	1	50	2	22	3	13
Unlikely	3	27	–	–	–	–	1	11	4	17
No	8	73	2	100	–	–	4*	44	15	58
Total	11	–	2	–	2	–	9*	–	24	–

* Does not include a lamb that went missing after the ewe died, because carcass not found

Table 9.3 Causes of death in carcasses found on the 'hill' which were judged definitely not to have been killed by eagles

	Site C		Site D		Site A		Site B		All sites	
Cause of death	n	%	n	%	n	%	n	%	n	%
Starvation	1	9	1	50	–	–	–	–	3	8
Birth problem/ defect	3	27	1	50	–	–	–	–	4	13
Misadventure	1	9	–	–	–	–	1	11	2	8
Illness/disease	3	27	–	–	–	–	3	33	6	25
Totals	8	–	2	–	–	–	4*	–	14	–

* Does not include a lamb that went missing after the ewe died, because carcass not found

N.B. The percentages are calculated as a percentage of the total carcasses found on each site not just those that had not been killed by eagles

Table 9.4 Causes of death in lambs from Sites A and B, including lambs lost while sheep were on inbye land before being put to hill

	Site A		Site B	
Cause of death	n	%	n	%
Birth problem	2	2	4	6
Weak lamb/illness	4	4	7	10
Misadventure	1	1	3	4
Starvation	0	0	0	0
Death of ewe	0*	0	1**	1
Eagle kill	1	1	0	0
Unknown (possible eagle kill)	0	0	2	3
Unknown, found	1	1	3	4
Unknown, missing	4	4	5	7
Totals	**13**	**12%**	**25**	**36%**

*One lamb on site B was rescued from starvation when an observer working on this project found a ewe that had become stuck in a bog

** Ewe on site B died, lamb carcass was not found but assumed to have died due to starvation

N.B. The percentages are calculated as a percentage of the total number of lambs in the flock (figures are rounded to nearest whole number

Production Comparisons

Lambing percentages for the study sites in previous years, calculated using data supplied by some of the crofters, show that production has been highly variable from year to year (Table 9.5) with 2003 being a relatively good year. Previous studies on golden eagle territories in Scotland have noted typical lambing percentages in the West of Scotland ranging from 45% to 91%, with yields of less than 70% common (Brown & Watson 1964; Lockie 1964; Hewson 1981; Leitch 1986). Data from the annual Scottish agricultural survey show that the Western Isles has consistently had the lowest lambing production among Scottish regions since 1976 (the limit of the data available). For a consecutive ten year period from 1976 to 1985, mean lambing percentage in the Western Isles was 58%, compared with 72% for both Argyll and Shetland (the regions with the next lowest production). Lambing percentages have increased since then and in the ten year period from 1994 to 2003 the Western Isles mean was 70% with Shetland and Argyll producing 79% and 80%. Means for the whole of Scotland were 91% and 99% for the two periods respectively. Thus it is apparent that lambing percentages of 70% and below are likely to be quite common on the Western Isles even today.

Table 9.5 Lambing percentage on study sites in years previous to the present study

Year	Site B	Site C	Site A	Average
1996	60.3			60.3
1997	45.5	44.7		45.1
1998	43.5	78.1		60.8
1999	66.2	61.7		63.9
2000	62.5	42.2	67.5	57.4
2001	48.3	57.3	89.1	64.9
2002	53.7	46.7	56.5	52.3
2003	60.8	71.9	90.5	74.4

Comparable data for Site D was not available

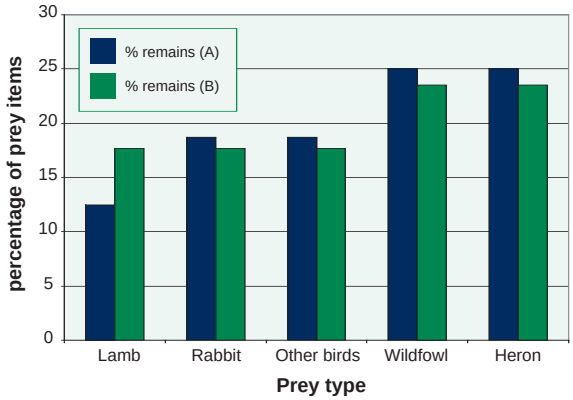
Additional information on lambing percentages was collected from some crofts on similar ground near to the study area. In 1998 the mean lambing percentage on five flocks varying in size from 30 ewes to 103 was 78% (range 75-90%). In 1999 on three of the same crofts containing 26 to 54 ewes it was also 78% (range 73-83%) and in 2000 details were available for only one flock of 119 ewes that had a lambing percentage of 59% (this flock achieved 77% in 1998).

On a single croft 7km from the study area which was in close proximity to an eyrie containing a breeding pair of eagles (no part of the croft was more than 2km from the nest and most of it was much closer), a flock of 120 ewes had lambing percentages of 67% in 2000, 68% in 2001 and 67% in 2003.

Eagle Diet

Prey remains were collected from in and around an occupied eyrie 7km to the south of the study area. Although it is unlikely that these eagles obtained much of their prey from the study area, the surrounding habitat was very similar to that of the study area, as were the available prey species. The prey remains collected were likely to be biased towards larger items of prey, since smaller items would be eaten whole or the leftovers harder to find due to their small size. Birds comprised 65% of prey items; the majority being herons or wildfowl. Rabbits comprised 18%. Several partial lamb remains were found but it was difficult to be certain how many individuals these represented. It is possible they originated from just one lamb, but it is more likely they originated from two, perhaps three animals. This would indicate that between 12 and 18% of prey items were lambs, Figure 9.3 showing the relative percentages.

Figure 9.3 Relative percentages of prey remains found in or near eyrie ('Remains A' assumes 2 lambs, 'Remains B' assumes 3 lambs)



Discussion

The body condition of ewes varied among sites, as did the consistency of condition. Although there was no evidence of a significant relationship between the condition of the mother and lamb survival there is some indication that flocks with higher mean condition had higher overall survival. This could be a result of another factor or combination of factors such as different management regimes or the quality of grazing leading both to better condition and higher survival (e.g. higher quality grazing might result in better ewe condition and also aid lamb survival).

There was some evidence that gimmers (ewes lambing for their first year) had poorer lamb survival than older ewes but this was only on one site. One way in which it is generally thought that young ewes can lose lambs more easily is by being less attentive or concerned about their lambs, but there was no evidence that the maternal behavior score (which estimates the strength of the maternal bond) influenced survival on either site.

Lightweight lambs are more likely to die if the reason they are light is lower energy reserves (e.g. less body fat). It is also conceivable that small lambs may be weaker and more susceptible to exposure and other hazards. In this study, lambs that died were significantly lighter at birth than those that survived on Site A but not on Site B. However lambs from site B exhibited significant differences in hindfoot length at birth between survivors and moralities, with survivors being significantly larger; there was no significant difference at site A for hindfoot length. Importantly, lambs on site A were significantly lighter than those on site B but survival rate was higher on site A. Overall there was inconclusive evidence that small or light lambs had a greater chance of dying; other factors related to individual sites are probably more important than size or weight in determining mortality, although if these other factors are constant, then size and birthweight may have some influence.

Overall losses from the lamb crop due to golden eagle predation were 1% on site A and 'possible' eagle kills on site B amounted to 3% of lambs born. These figures are broadly in line with previous studies in Scotland, which have cited figures of 1 to 2%. Mortality due to factors other than eagle predation accounted for 7% of lambs born on site A and 23% at site B (around seven times the number lost or possibly lost to eagles), and unknown causes accounted for 5 and 10% respectively of lambs born.

Of the total number of lambs that died, eagle predation appears to account for around 8%, the remaining 92% of lamb losses being due to factors other than predation. Birth problems (such as dystokia) and stillbirths accounted for around 15% of mortalities on sites A and B and for 27% and 50% of mortalities found on sites C and D respectively. Illness and weak lambs (this includes lambs with congenital defects) accounted for around 30% of losses on sites A and B and 27% and 0% on the sites C and D respectively. As site D had a very small sample size, a single death could have a large effect on the calculation. Exposure is often a factor in the death of lambs, increasing susceptibility to starvation and disease. During this study instances of exposure may have been lower than normal because of below average rainfall and above average temperatures during April when the majority of lambs were most vulnerable.

These data are compatible with those of other studies of lamb mortality in hill flocks, which have shown that predation generally makes a small contribution to overall losses and that factors such as birth problems, disease, congenital defects, starvation and exposure account for the majority of dead lambs. (Wiener *et al*., 1983; Henderson 1997)

Lamb production on the study sites over the past few years has been variable and there has been no consistent pattern among the sites. In 2003, production on site A was well above the mean for the Western Isles; it was close to the mean on site C and, on the remaining sites, it was below the mean. The data for 2003 show that it was a relatively productive year, yet golden eagles were present on the study area just as in previous years (although possibly not all years). This suggests that the main factor affecting production is not eagle predation since lambing percentages have varied considerably even though eagle presence is constant. The lambing percentages achieved on individual sites fall within the range of those quoted in other golden eagle studies involving hill sheep in the West and North West of Scotland where figures between 55% and 70% have commonly been reported, and production has in some cases been even lower.

From the prey remains found it is seems that lambs represented only a small proportion of the diet. Of the other species found, many were common in and around the study area and would thus be available as a source of food for the eagles that held territory there.

Conclusion

Overall production of lambs on the study area was low, but not unusually so for the type of habitat and management in this region. It is clear that causes other than eagle predation were responsible for the majority of lamb mortalities and that golden eagle predation, as a percentage of lambs born, was low, and in line with that found in previous studies. Actions take to improve lambing percentages would, therefore, seem to be best directed at improving birth success, reducing disease and lowering accident risk rather than preventing eagle predation.

References

Brown LH, Watson A. 1964. The golden eagle in relation to its food supply. *Ibis* **106:** 78-100

Henderson DC. 1997. Maximising lamb survival. *The Moredun Foundation News Sheet* **2(14)** The Moredun Foundation, Edinburgh

Hewson R. 1981. Scavenging of mammal carcasses by birds in West Scotland. *Journal of Zoology (London)* **194:** 525-537

Hewson R. 1984. Scavenging and predation upon sheep and lambs in West Scotland. *Journal of Applied Ecology* **21:** 843-868

Leitch AF. 1986. Eagle predation of lambs in the Glenelg area in 1986. *Unpublished report to the Department of Agriculture and Fisheries for Scotland.* Edinburgh

Lockie JD. 1964. The breeding density of the golden eagle and fox in relation to food supply in Wester Ross, Scotland. *The Scottish Naturalist* **71:** 67-77

MAFF. 2000. Sheep, Codes of recommendations for the welfare of livestock, MAFF Booklet **PB5162** (online at: http://www.defra.gov.uk/animalh/welfare/farmed/sheep/booklets/sheep.pdf)

O'Connor CE, Jay NP, Nicol AM, Beatson PR. 1985. Ewe maternal behaviour score and survival. *Proceedings of the New Zealand Society of Animal Production* **45:** 159-162

Wiener G, Woolliams C, MacLeod NSM. 1983. The effects of breed, breeding system and other factors on lamb mortality. 1. Causes of death and effects on the incidence of losses. *Journal of Agricultural Science (Cambridge)* **100:** 593-551.

Weir DN. 1973. A case of lamb killing by golden eagles. *Scottish Birds* **7:** 293-294

9.3 The Value of Aphid Monitoring in the Detection of the Effects of Long-term Environmental Change (L King and J Pickup)

Aphids as Agricultural Pests

In temperate regions, aphids are arguably the most important economic pests in agriculture. Because of their feeding behaviour and specialised mouthparts they are ideal vectors of plant viruses and, when present in sufficiently large numbers, they can stunt or kill plants through the direct removal of sap. Aphids have enormous reproductive potential as they have short generation times and can reproduce parthenogenetically for most of the year, giving rise to large numbers of offspring. Their success as agricultural pests has resulted in the establishment of aphid monitoring programmes and forecasting systems throughout North America and Europe as the demand increases for more effective pest management to limit the spread of viruses and protect crop quality.

UK and European Suction Trap Networks

To provide spatial data for an aphid warning service and for studies on insect population dynamics, the first aerial suction trap began operating at Rothamsted, England, in 1965 (Taylor, 1986). It has operated continuously since then, and a network of traps, standardised on the Rothamsted design, has expanded throughout Europe to include 111 traps from 19 countries, 71 of which are currently in operation (Fig. 9.4).

Figure 9.4 European traps currently in operation

A network of 16 traps currently operates across the UK in agricultural areas. SASA contributes to this by supervising the Scottish trap at Dundee, which has been in operation since 1967, as well as those at Edinburgh, Elgin and Ayr which have operated since 1969, 1970 and 1974 respectively (Fig. 9.5). Catches are collected daily throughout the main periods of aphid flight activity and SASA identifies all aphids to species. The data are incorporated into a single UK database.

Figure 9.5 12.2m Suction Trap

In Scotland, data on the abundance of key pest species is disseminated via SASA's website (www.sasa.gov.uk) on a weekly basis and is used to provide information on the potential risk of potato virus transmission throughout the growing season. Regular bulletins, incorporating all UK data, are also issued by Rothamsted Research (www.rothamsted.bbsrc.ac.uk/insect-survey/) and samples from several UK sites, including all Scottish sites, have been used to study aphicide resistance (section 4.6). Long term UK datasets have been used in the development of models for forecasting the time of arrival and migration size of pest species, and for the development of a decision support system for the control of Barley Yellow Dwarf Virus. Similar projects are carried out at local and national levels throughout Europe, but the potential of a European database to study and improve understanding of aphid abundance and populations was not explored until recently.

EXAMINE

In 2000, EXAMINE (**EX**ploitation of **A**phid **M**onitoring systems **IN** **E**urope), a project funded under the EU Concerted Action Programme and coordinated by Rothamsted Research, was initiated (Harrington *et al*., 2004). Its objective was to consolidate existing national data on aphid distribution and abundance to establish a European database, and to interrogate it against long-term data on climate, land use and atmospheric gases to investigate the effects of environmental changes on aphids as environmentally-sensitive indicators of agricultural ecosystems. This would allow predictions of the impacts of future environmental changes, which could prove valuable to agricultural scientists involved in developing aphid control measures, as well as biologists in other disciplines.

By combining data from across Europe, EXAMINE has produced the world's largest spatiotemporal standardised database for any terrestrial invertebrates. It comprises of data from 100 trap sites in 19 countries, contains 1,342 'trap years' of data and holds over ten million individual species records for 29 key species and further records for additional species.

Effects of Land Use

As part of the EXAMINE project, analyses were undertaken to determine the effects of land cover on annual abundance and phenology (time of first flight) of *Myzus persicae,* an important vector of several crop viruses. The results showed that, on a European scale, the abundance of *M. persicae* was negatively affected by increased area of coniferous plantation, whereas increases in area of deciduous and arable land had positive effects. In north west Europe, oilseed rape was associated with increases in abundance, a result which was mirrored in England and Wales, but to a lesser degree. Regarding time of first flight, areas of permanent crops and shrub cover had negative and positive effects respectively, on a European scale, although no effects were found in NW Europe. However, in all cases, the effect of land use on both phenology and abundance was significantly reduced when climatic and geographical factors such as latitude and longitude were considered.

Pollution

EXAMINE did not find, on a European scale, that air pollutants affected aphid abundance or phenology, although sufficiently detailed data on atmospheric gases were not available for all countries, making analysis difficult. The need to analyse the data more appropriately, on a finer temporal scale, has been highlighted and this may be undertaken in the future.

Climatic Effects

Using temporal climatic data supplied by the Climatic Research Unit at the University of East Anglia, the effects of temperature and rainfall on abundance and migration were investigated. For the specific case of *M. persicae,* it was found that higher temperatures in winter and spring led to earlier spring migrations and greater abundance, and that rainfall delayed the date of first flight, and limited abundance. Further analyses, using predicted higher temperatures for the future, concluded that migrations of numerous pest species are expected to occur on average 8 days earlier than at present by 2050, although this is unlikely to be accompanied by increases in annual abundance.

Implications for Future Pest Management

The EXAMINE project has illustrated that complex interactions among climate, land use and geographical location occur, to determine aphid abundance and population dynamics. While further work is planned to refine these conclusions, the current findings have considerable implications for the development of future aphid control.

If aphids migrate earlier in the season, young crops, which do not benefit from mature plant resistance, may be exposed to a higher risk of virus infection from early colonisation of aphids. The potential risk of virus infection could lead to increased insecticide use and higher incidence of insecticide resistance, a trend which has already been witnessed in the UK. In addition, changes in land use could contribute to the threat posed by pests if habitats associated with increased numbers of pest species expand. Such implications illustrate the necessity for improvements to pest forecasting, and control systems that consider integrated approaches to pest management and minimise insecticide use.

Further Applications of the UK Suction Trap Network

The usefulness of the suction trap network to other aspects of UK conservation has also been realised. SEERAD recently identified a need for improved knowledge of the impacts that future climate change may have on habitats and species distribution, and anthropogenic changes to land use, for inclusion in Biodiversity Action Plans (BAPs). Because the incorporation of contingencies for such impacts is considered integral to the future success of BAPs, in 2003, SEERAD commissioned a review of Scottish and UK species surveillance/monitoring programmes to assess their suitability for analysis against climatic data (Riley *et al*., 2003). The Rothamsted Insect Survey, which includes the UK suction trap network, was identified as a suitable candidate. This conclusion was supported by the subsequent findings of EXAMINE. Further analysis of the data on a UK and Scottish level could provide information on the impacts on aphid distribution and abundance for use at a national level.

Data collected using the suction trap network have not yet been used to look at factors influencing biodiversity of aphid species, although members of the EXAMINE consortium and scientists at the Central Science Laboratory, York, have expressed an interest in using it to monitor the arrival and spread of species across Europe and within individual countries. In this context, it should be noted that the SEERAD review recommended the expansion of the Scottish suction trap network, to cover a wider geographical area and provide information on Scottish biodiversity that would be useful on a practical level.

Conclusion

These examples show that data provided by the UK suction trap network are used to provide useful information on aphid pest species on a regular basis, while analysis *via* EXAMINE has contributed to the understanding of aphid population dynamics and abundance on a European scale. The suction trap data have practical use in the development of pest control measures and contingency planning within conservation; with additional resources, they could prove useful in monitoring biodiversity.

References

Harrington R, Verrier P, Denholm C, Hullé M, Maurice D, Bell N, Knight J, Rounsevell M, Cocu N, Barbagallo S, Basky Z, Coceano P-G, Derron J, Katis N, Luká_ová H, Marrkula I, Mohar J, Pickup J, Rolot J-L, Ruszkowska M, Schliephake E, Seco-Fernandez M-V, Sigvald R, Tsitsipis J, Ulber B. 2004. 'EXAMINE' (EXploitation of Aphid Monitoring in Europe): An EU Thematic Network for the study of global change impacts on aphids. In: JC Simon, CA Dedryver, C Rispe, M Hullé eds. *Aphids in a New Millennium* (Proceedings 6th International Aphid Symposium, Rennes, France, 3-7 September 2001) 45-49

Riley J, Kirby J, Linsley M, Gardiner G. 2003. Review of UK and Scottish surveillance & monitoring schemes for the detection of climate-induced changes in biodiversity. Report of SEERAD Flexible Fund Project JEY-001-01. Just Ecology, Gloucestershire, UK. *(Internet publication:* http://www.scotland.gov.uk/library5/environment/climatereview.pdf)

Taylor LR. 1986. Synoptic dynamics, migration and the Rothamsted Insect Survey. *Journal of Animal Ecology* **55:** 1-38

SASA's Role In The Foot & Mouth Outbreak

G Hartley

Dr Gill Hartley

I joined SASA in 1993 after a year of postdoctoral contracts, working on cetaceans and foxes, following my Ph.D. at Bristol University. However, it was the years of experience at SASA in management techniques, of wildlife, people and resources, that really provided me with the essential tools I needed for our role in the foot and mouth outbreak.

Foot and mouth disease (FMD) reached Scotland in late February 2001 and, on 13 March, I was telephoned by the Head of Veterinary Services Scotland, asking if it was possible for SASA to undertake rodent control work on infected premises on behalf of the Scottish Division of the then MAFF Animal Health Department. Rats, in particular, are considered to be a FMD transmission risk since they have been shown, under experimental conditions, to be able to retain the live virus for many days. Moreover, rat dispersal from farm steadings, potentially spreading the disease, occurs naturally in spring and summer, and would inevitably occur once cleansing and disinfection of contaminated premises began.

Several problems instantly presented themselves. Firstly, SASA had no formal agreement to undertake the work with MAFF or the Scottish Executive. Secondly, the Wildlife Management Unit had only two full-time staff and, with over 50 premises infected and rising daily, it would not have been possible for us to undertake effective control in the time necessary. Thirdly, we would need to seek agreement from the other Scottish Executive Divisions with whom we were contracted, because with the likely timescale and extent of the problem, it would not be possible for us to fulfil our normal level of service. Finally, we would have to relocate to undertake the work, since the outbreak was focused in Dumfries, some 80 miles to the SW of Edinburgh.

Given the importance of the outbreak, gaining the necessary support for our FMD role within the SE was not a problem. The staffing levels were resolved by the employment of 24 Farm Quality Assurance Scheme staff who had been made temporarily unemployed by the outbreak. The Farming and Rural Conservation Agency, which had already been undertaking rodent control on farms in England and Wales for a few weeks, kindly trained these staff in personal disinfection and in the basic essentials of rodenticide use. Administrative organisation was considerable: Health and Safety and COSHH literature had to be drawn up, along with detailed instructions for both staff and farmers. All baiting and personal disinfection equipment had to be purchased. Lists of premises and locations had to be compiled and updated twice daily, and detailed records kept of the locations of all bait points on farms, the quantity of rodenticide laid and the frequency of inspection. Nonetheless, bait laying began on 18 March, less than one week after the initial request to undertake the work, and prior to the start of cleansing and disinfection of premises.

During the following months, numerous difficulties were encountered including personnel, equipment, logistical and public relations problems. Early morning meetings provided essential briefing and feedback sessions, and allowed us to identify training and other needs. Timetabling of staff had to be continually adjusted to ensure adequate manpower resources and allow staff leave, while eliminating any risk of spread of infection. Staffing problems had to be settled, and wage problems resolved. Equipment shortages were potentially a constant source of difficulty resulting in the main store having to be relocated on no less than four separate occasions to different buildings within Dumfries. It was not until the latter weeks of the outbreak that we received our own office facilities, and before this, our 'office' and 'small equipment store' was our hotel room. The nature of the work meant that staff were laying poison baits for rodents on 'infected' and 'dangerous contact' premises, where all livestock were compulsorily slaughtered and farmers were often confined to their premises. Under these stressful circumstances, it was essential that farmers were made aware of the importance of the work, but also of the risks to non-target species, such as farm cats and dogs. All complaints or problems that arose were resolved sensitively and immediately by SASA staff. This included various PR issues that arose due to the decision not to employ local pest control workers to conduct this work, due to risks of accidental disease spread.

Rodenticide being laid at a farm

The treatments were completed in August 2001, with some 2,674 kg of rodenticide being laid. Irrespective of the difficulties encountered, the work was successfully completed, with a total of 360 premises treated effectively, and no outbreaks reported as a result of transmission by rats. This assignment demonstrated the extent of what could be achieved, with no prior experience or training for a FMD outbreak, even with limited resources, and proved to be a credit to SASA's and the Section's flexibility, organisational skills and ability to get the job done.

Appendices

1 SASA Staff Publications 2001-2003

2 List of contact names and addresses

Appendix 1 Staff Publications 2001-2003

Arnold DL, Gibbon MJ, Jackson RW, Wood JR, Brown J, Mansfield JW, Taylor JD, Vivian A. 2001. Molecular characterization of *avrPphD*, a widely-distributed gene from *Pseudomonas syringae* pv. *Phaseolicola* involved in non-host recognition by pea (*Pisum sativum*). *Physiological and Molecular Plant Pathology* **58:** 55-62.

Barnett EA, Fletcher M, Hunter K, Sharp EA. 2001. *Pesticide poisoning of animals 2000: Investigations of suspected incidents in the United Kingdom.* Environmental Panel of the Advisory Committee on Pesticides Report. London: Department for Environment, Food & Rural Affairs.

Barnett EA, Fletcher M, Hunter K, Sharp EA. 2002. *Pesticide poisoning of animals 2001: Investigations of suspected incidents in the United Kingdom.* Environmental Panel of the Advisory Committee on Pesticides Report. London: Department for Environment, Food & Rural Affairs.

Barnett EA, Fletcher M, Hunter K, Sharp EA. 2003. *Pesticide poisoning of animals 2002: Investigations of suspected incidents in the United Kingdom.* Environmental Panel of the Advisory Committee on Pesticides Report. London: Department for Environment, Food & Rural Affairs.

Braithwaite M, Leite RP, Smith JJ, Boa E, Saddler GS. 2002. First report of Citrus Canker Caused by *Xanthomonas campestris* pv. *citri* on *Citrus sinensis* in Bolivia. New Disease Reports *Plant Pathology* **51**, 382.

Brattey C, Badge JL, Burns R, Foster GD, George E, Goodfellow HA, Mulholland V, McDonald JG, Jeffries CJ. 2002. Potato latent virus: a proposed new species in the genus *Carlavirus. Plant Pathology* **51**: 495-505.

Brummer M, Arend M, Fromm J, Schlenzig A, Osswald WF. 2002. Ultrastructural changes and immuno-cytochemical localization of the elicitin Quercinin in *Quercus robur* L. roots infected with *Phytophthora quercina. Physiological and Molecular Plant Pathology* **61:** 109-120.

Campbell S, Smith A, Redpath S, Thirgood S. 2002. Nest site characteristics and nest success in red grouse *Lagopus lagopus scoticus. Wildlife Biology* **8:** 169-174.

Carnegie SF, Cameron AM, Haddon P. 2001. The effect of time of haulm destruction and harvest on the development of dry rot caused by *Fusarium solani* var. *coeruleum. Annals of Applied Biology* **139:** 209-216.

Carnegie SF, Choiseul JW, Roberts AMI. 2003. Detection of *Colletotrichum coccodes* and *Helminthospurium solani* in soils by bioassay. *Plant Pathology* **52:** 13-21.

Chard JM, Irvine SJ, Roberts AMI, Nevison IM, McGavin WJ, Jones AT. 2001. Incidence and distribution of *Raspberry bushy dwarf virus* in commercial red raspberry (*Rubus idaeus*) crops in Scotland. *Plant Disease* **85:** 985-988.

Choiseul JW, Allen L, Carnegie SF. 2001. The role of stem inoculum in the transmission of *Fusarium sulphureum* to potato tubers. *Potato Research* **44:** 165-172.

Cockerell V, 2002. Seed treatment according to need in Scotland: Barley net blotch. In: *Proceedings of Crop Protection in Northern Britain 2002,* Dundee 19-20 February, 121-126.

Cockerell V, Jacks MA, Roberts AMI, 2002. The effect of rainfall and temperature on the incidence of seed-borne *Microdochium nivale* on winter wheat in Scotland 1991-2000. In: *Proceedings of Crop Protection in Northern Britain 2002,* Dundee 19-20 February, 133-138.

Cockerell V, Thomas JE, Clark WS, 2003. Sampling wheat seed for seed-borne disease. *HGCA Topic Sheet No. 72.* London: Home-Grown Cereals Authority.

Cockerell V, Thomas JE, Clark WS, 2003. Testing winter wheat for seed-borne disease. *HGCA Topic Sheet No. 73.* London: Home-Grown Cereals Authority.

Cockerell V, Mulholland V, McEwan M, Paveley ND, Clark WS, Anthony S, Thomas JE, Bates J, Kenyon DM, Taylor EJA. 2001. Seed treatment according to need in winter wheat. In: *Seed Treatment – Challenges and Opportunities, 3rd symposium,* Wishaw, N. Warwickshire, 26-27 February 2001. (BCPC Symposium Proceedings No. 76). Farnham: British Crop Protection Council, 111-116.

Cockerell V, Mulholland V, McEwan M, Paveley ND, Clark WS, Anthony S, Thomas JE, Bates J, Kenyon DM, Taylor EJA. 2002. Control of seed and soil-borne diseases: importance of seed health. In: *HGCA R & D conference 2002: Agronomic intelligence: the basis of profitable production,* Coventry 15-16 February 2002. London: Home Grown-Cereals Authority, 4.1 – 4.11.

Cooke DEL, Young V, Birch PRJ, Toth R, Gourley F, Day JP, Carnegie SF, Duncan JM. 2003. Phenotypic and genotypic diversity of *Phytophthora infestans* populations in Scotland (1995-97). *Plant Pathology* **52:** 181-192.

David J. and Saddler GS. 2002. Systematics of the microbial kingdom(s) and fungi. In: Biological and Physiological Sciences section (theme editors Contrafatto G, Minelli A), *Encyclopedia of Life Support Systems (EOLSS),* developed under the auspices of UNESCO. Oxford, UK: Eolss Publishers. [http://www.eolss.net]

Don R. 2003. *ISTA Handbook on seedling evaluation.* 3rd edition. Zurich, Switzerland: International Seed Testing Association. ISBN 3-906549-39-9.

Don R. 2003. Moisture content proficiency test. *Seed Testing International* **125:** 25-27.

Don R. 2003. Temperature measurement and control in ISTA laboratories: guidelines for laboratories and auditors. *Seed Testing International* **125:** 28-30.

Don R, 2003. ISTA Moisture Content Proficiency Test. *Seed Testing International* **126:** 40-41.

Don R, Leist N, Steiner AM, 2001. *Proceedings of the ISTA Tetrazolium Workshop.* Zurich: International Seed Testing Association, ISBN 3-906549-33-X.

Evans HC, Holmes KA, Reid AP. 2003. Phylogeny of the frosty pod rot pathogen of cocoa. *Plant Pathology* **52:** 476-485.

Fitter AH, Hay RKM. 2002. *Environmental Physiology of Plants.* 3rd edition. London: Academic Press.

Guimarães PM, Smith JJ, Palmano S, Saddler GS. 2003. Characterisation of *Curtobacterium flaccumfaciens* pathovars by AFLP, rep-PCR and pulsed-field gel electrophoresis. *European Journal of Plant Pathology* **109:** 817-825.

Guimarães PM, Palmano S, Smith JJ, Grossi de Sá MF & Saddler GS. 2001. Development of a PCR test for the detection of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens. Antonie van Leeuwenhoek Journal of Microbiology* **80:** 1-10.

Hampton JG, Moreira de Carvalho N, Kruse M, Don R, Brodal G, Come D, Copeland LO. 2002. Quality Seed – a factor for sustainable progress. *Seed Science and Technology* **30:** 463-475.

Harrington R, Parker S, Taylor M, Adamowicz T, Cox D, Foster S, Denholm I, Dewar A, Pickup J. 2003. Trapped in 2002. *British Sugar Beet Review* **71:** 12-17.

Harrington R., Verrier P, Denholm C, Hullé M, Maurice D, Bell N, Knight J, Rounsevell M, Cocu N, Barbagallo S, Basky Z, Coceano P-G, Derron J, Katis N, Luká_ová H, Marrkula I, Mohar J, Pickup J, Rolot J-L, Ruszkowska M, Schliephake E, Seco-Fernandez M-V, Sigvald R, Tsitsipis J & Ulber B. 2004. 'EXAMINE' (EXploitation of Aphid Monitoring in Europe): An EU Thematic Network for the study of global change impacts on aphids. In: Simon JC, Dedryver CA, Rispe C, Hullé M. eds. *Aphids in a New Millennium. Proceedings 6th International Aphid Symposium,* Rennes, France, 3-7 September 2001, 45-49.

EXAMINE Consortium. 2003. EXAMINE: an EU thematic network to evaluate impacts of environmental changes on aphids at a regional scale. Proceedings IOBC/WPRS meeting 'Landscape Management for Functional Biodiversity', Bologna, Italy, May 2003. *IOBC/WPRS Bulletin (Bulletin OILB SROP)* **26:** 71-75.

Hay RKM, Gilbert RA. 2001. Variation in the harvest index of tropical maize: evaluation of recent evidence from Mexico and Malawi. *Annals of Applied Biology* **138:** 103-9.

Hoekstra R, Carnegie SF. 2001. The establishment of the European potato genetic resources database. In: van den Berg RG, van der Weerden GM, Barendse GWM, Mariani C. eds. *Solanaceae V: Advances in taxonomy and utilisation.* The Netherlands: Nijmegen University Press, 363-368.

Horgan GW, Talbot M, Davey JC. 2001. Use of statistical image analysis to discriminate carrot cultivars. *Computers and Electronics in Agriculture* **31:** 191-199.

Hunter K, Sharp EA, Melton LM. 2001. Pesticide poisoning of animals 2000: *Investigations of suspected incidents in Scotland.* Edinburgh: Scottish Agricultural Science Agency.

Hunter K, Sharp EA, Melton LM. 2002. *Pesticide poisoning of animals 2001: Investigations of suspected incidents in Scotland.* Edinburgh: Scottish Agricultural Science Agency.

Hunter K, Sharp EA, Melton LM. 2003. *Pesticide poisoning of animals 2002: Investigations of suspected incidents in Scotland.* Edinburgh: Scottish Agricultural Science Agency.

Iason GR, Manso T, Sim DA, Hartley FG. 2002. The functional response does not predict the local distribution of European rabbits (*Oryctolagus cuniculus*) on grass swards: experimental evidence. *Functional Ecology* **16:** 394-402.

Jeffries C, Chard J. 2001. International influences on potato quarantine. In: *Proceedings of the 11th Virology Section Meeting of the European Association for Potato Research,* Havlíčkův Brod-Třešt, Czech Republic, 7-13th October 2001, 49-51.

Jeffries C, James C. 2001. Development of an EU diagnostic proocol for the detection of *Potato Spindle Tuber Viroid.* In: *Proceedings of the 11th Virology Section Meeting of the European Association for Potato Research,* Havlíčkův Brod-Třešt, Czech Republic, 7-13th October 2001, 56-57.

Kerr J, Snowden JP. 2001. Arable Crops 2000. *Pesticide Usage in Scotland Report.* Edinburgh: Scottish Agricultural Science Agency.

Kerr J, 2001. Grain Stores 1998-99. *Pesticide Usage in Scotland Report.* Edinburgh: Scottish Agricultural Science Agency.

Kerr J, 2002. Rodenticides on Arable Farms 1998. *Pesticide Usage in Scotland Report.* Edinburgh: Scottish Agricultural Science Agency.

Kim SB, Goodfellow M, Kelly J, Saddler GS, Ward AC. 2002. Application of oligonucleotide probes for the detection of *Thiothrix* spp. in activated sludge plants treating paper and board mill wastes. *Water Science and Technology* **46:** 559-564.

Knapova G, Schlenzig A, Gisi U, 2002. Crosses between isolates of *Phytophthora infestans* from potato and tomato and characterisation of F1 and F2 progeny for phenotypic and molecular markers. *Plant Pathology* **51:** 698-709.

Langrell SRH, Irvine SJ. 2001. First report of *Colletotrichum acutatum* on *Kalmia. Plant Disease* **85:** 447.

Lees AK, van de Graaf P, Cullen DW, Duncan JM. 2002. The development of a quantitative real-time PCR assay for *Spongospora subterranea* f.sp. *subterranea* and its use for epidemiological studies. In: *Proceedings 5th Symposium International Working Group on Plant Viruses with Fungal Vectors,* Zürich, Switzerland, July 22-25, 2002, 31-34.

McEwan M, Mulholland V. 2002. Development of molecular diagnostic assays for seed-borne pathogens of wheat. In: *Proceedings Crop Protection in Northern Britain 2002,* Dundee 19-20 February, 127-132.

McLaren G, Don R, 2002. The effect of glyphosate treatment on the germination potential of resultant crops. In: *Proceedings of Crop Protection in Northern Britain 2002,* Dundee 19-20 February, 103-108.

McLaren G, Don R, 2002. The performance of glyphosate affected barley seed. In: *Proceedings of Crop Protection in Northern Britain 2002,* Dundee 19-20 February, 207-210.

Mracek Z, Sturhan D, Reid A. 2003. *Steinernema weiseri* n. sp (Rhabditida, Steinernematidae), a new entomopathogenic nematode from Europe. *Systematic Parasitology* **56:** 37-47.

Nechwatal J, Schlenzig A, Jung T, Cooke DEL, Duncan JM, Osswald WF. 2001. A comparison of baiting and PCR techniques for the detection of *Phytophthora quercina* and *P. citricola* in soil samples from oak stands. *Forest Pathology* **31:** 85-97.

Nisbet C, Jeffries C. 2001. The work of the UK Potato Quarantine Unit. In: *Proceedings of the 11th Virology Section Meeting of the European Association for Potato Research,* Havlíčkův Brod-Třešt, Czech Republic, 7-13th October 2001, 85-86.

Osswald WF, Jung T, Nechwatal J, Schlenzig A, Fleischmann F. 2001. Significance of *Phytophthora* and *Pythium* for oak, alder, and spruce decline. *Journal of Forest Science* **47:** 96-103.

Park KJ, Robertson PA, Campbell ST, Foster R, Russell ZM, Newborn D, Hudson PJ. 2001. The role of invertebrates in the diet, growth and survival of red grouse (*Lagopus lagopus scoticus*) chicks. *Journal of Zoology, London* **254:** 137-145.

Pickup J, King L. 2002. Aphid monitoring for virus control in Scottish seed potatoes. In: *Proceedings Crop Protection in Northern Britain 2002,* Dundee 19-20 February, 337-342.

Reid A, Manzanilla-Lopez RH, Hunt DJ. 2003. *Nacobbus aberrans* (Thorne, 1935) Thorne & Allen, 1944 (Nematoda: Pratylenchidae); a nascent species complex revealed by RFLP analysis and sequencing of the ITS-rDNA region. *Nematology* **5:** 441-451.

Reid A. 2001. Nick translation. In: Aquino de Muro M, Rapley R. eds. *Gene Probes: Principles and Protocols.* Totowa, New Jersey: Humana Press, 23-25.

Reid A. 2001. Random primed labelling. In: Aquino de Muro M, Rapley R. eds. *Gene Probes: Principles and Protocols.* Totowa, New Jersey: Humana Press, 27-28.

Reid A. 2001. Target format and hybridization conditions. In Aquino de Muro M, Rapley, R. eds. *Gene Probes: Principles and Protocols.* Totowa, New Jersey: Humana Press, 1-11.

Roberts AMI, Horgan GW, Talbot M, Davey JC. 2001. Matching photographs of plant varieties. *Plant Varieties and Seeds* **14:** 151-161.

Saddler G.S. 2002. Bacteria and plant disease. In: Waller JM, Lenné J, Waller SJ. eds. *Plant Pathologist's Pocketbook.* Third Edition. Wallingford: CABI Publishing, 94-107.

Shahina F, Anis M, Reid AP, Rowe J, Maqbool MA. 2001. *Steinernema pakistanense* sp. n. (Rhabditida: Steinernematidae) from Pakistan. *International Journal of Nematology* **11:** 124-133.

Smith JJ, Murimi K, Gouws R, Saddler GS, Trigalet A, Simons S. 2003. Biocontrol of potato bacterial wilt in Kenya. In: Serageldin I, Presley GJ. eds. *Biotechnology and sustainable development: Voices of the South and North.* Wallingford:CABI Publishing, 129-142.

Smith JJ, Saddler GS. 2001. The use of avirulent mutants of *Ralstonia solanacearum* to control bacterial wilt disease. In: Jeger MJ, Spence N. eds. *Biotic Interactions in Plant-Pathogen Associations.* Wallingford: CAB International, 159-176.

Snowden JP. 2001. Protected Crops & Mushrooms 1999. *Pesticide Usage in Scotland Report.* Edinburgh: Scottish Agricultural Science Agency.

Snowden JP. 2002. Soft Fruit 2001. *Pesticide Usage in Scotland Report.* Edinburgh: Scottish Agricultural Science Agency.

Snowden JP. 2002. Hardy Nursery Stock 2001. *Pesticide Usage in Scotland Report.* Edinburgh: Scottish Agricultural Science Agency.

Snowden JP. 2002. Crop protection in Northern Britain 2002. *Pesticide Outlook* **13:** 56-57.

Snowden JP. 2003. Outdoor Bulbs and Flowers 2001. *Pesticide Usage in Scotland Report.* Edinburgh: Scottish Agricultural Science Agency.

Snowden JP. 2003. Potato Stores 2002. *Pesticide Usage in Scotland Report.* Edinburgh: Scottish Agricultural Science Agency.

Snowden JP, Thomas LA. 2003. Arable Crops 2002. *Pesticide Usage in Scotland Report.* Edinburgh: Scottish Agricultural Science Agency.

Stock SP, Heng J, Hunt DJ, Reid AP, Shen X, Choo HY. 2001. Redescription of *Steinernema longicaudum* Shen & Wang (Nematoda: Steinernematidae); geographic distribution and phenotypic variation between allopatric populations. *Journal of Helminthology* **75**, 81-92.

Taylor MJ, Hunter K, Hunter KB, Lindsay DA, Le Bouhellec, S. 2002. Multi-residue method for rapid screening and confirmation of pesticides in crude extracts of fruits and vegetables using isocratic liquid chromatography with electrospray tandem mass spectrometry. *Journal of Chromatography* A, 982: 225-236.

Thirgood SJ, Redpath SM, Campbell S, Smith A. 2002. Do habitat characteristics influence predation on red grouse? *Journal of Applied Ecology* **39:** 217-225.

Thomas JE, Smith MJ, Thompson PS, Kenyon DM, Cockerell V, 2001. Requirements for effective seed sampling in the application of treatment according to need strategies. In: *Seed Treatment – Challenges and Opportunities, 3rd symposium,* Wishaw, N. Warwickshire, 26-27 February 2001. (BCPC Symposium Proceedings No. 76). Farnham: British Crop Protection Council, 167-172.

Thomas LA, Snowden JP. 2001. Potato Stores 1998. *Pesticide Usage in Scotland Report.* Edinburgh: Scottish Agricultural Science Agency.

Thomas LA, Snowden JP. 2002. Outdoor Bulbs and Flowers 1997. *Pesticide Usage in Scotland Report.* Edinburgh: Scottish Agricultural Science Agency.

Thomas L, Kerr J. 2002. Rodenticides on Farms Growing Fodder Crops 1997. *Pesticide Usage in Scotland Report.* Edinburgh: Scottish Agricultural Science Agency.

Thomas L, Kerr J. 2002. Rodenticides on Arable Farms 1996. *Pesticide Usage in Scotland Report.* Edinburgh: Scottish Agricultural Science Agency.

Thomas L. 2002. Rodenticides on Arable Farms 2000. *Pesticide Usage in Scotland Report.* Edinburgh: Scottish Agricultural Science Agency.

Thomas LA. 2002. Potato Stores 2000. *Pesticide Usage in Scotland Report.* Edinburgh: Scottish Agricultural Science Agency.

van de Graaf P. 2001. The fungus *Phoma clematidina* as a cause of clematis wilt. *Clematis International* 71-74.

van de Graaf P, Joseph, ME, Chartier-Hollis JM, O'Neill TM. 2002. Prepenetration stages in infection of clematis by *Phoma clematidina. Plant Pathology* **51:** 331-337.

van de Graaf P, Lees AK, Duncan JM. 2002. Effect of environmental vectors on infection of potato by *Spongospora subterranea* f.sp. *subterranea,* the vector of PMTV. In: *Proceedings 5th Symposium International Working Group on Plant Viruses with Fungal Vectors,* Zürich, Switzerland, July 22-25, 2002, 88-91.

van de Graaf P, Lees AK, Duncan JM. 2002. Effect of inoculum level and environmental conditions on powdery scab and root galling in potato. In: *Proceedings Crop Protection in Northern Britain,* Dundee, February 19-20, 2002, 281-286.

van de Graaf P, Lees AK, Cullen, DW, Duncan JM. 2003. Detection and quantification of *Spongospora subterranea* in soil, water and plant tissue samples using real-time PCR. *European Journal of Plant Pathology* **109:** 589-597.

van de Graaf P, O'Neill TM, Chartier-Hollis JM, Joseph ME. 2001. Susceptibility of clematis varieties and species to stem infection by *Phoma clematidina* as an indicator for resistance to wilt. *European Journal of Plant Pathology* **107:** 607-614.

van de Graaf P, O'Neill TM, Chartier-Hollis JM, Joseph ME. 2003. Aspects of the biology and control of benzimidazole resistant isolates of *Phoma clematidina,* cause of leaf spot and wilt in clematis. *Journal of Phytopathology* **151:** 442-450.

Wale SJ, van de Graaf P, Lees AK. 2003. *Epidemiology, autecology and control of* Spongospora subterranea, *cause of potato powdery scab.* Final report. Oxford: British Potato Council.

Young JM, Bull CT, De Boer SH, Firrao G, Gardan L, Saddler GS, Stead DE, Takikawa Y. 2001. Classification, nomenclature, and plant pathogenic bacteria – A clarification. *Phytopathology* **91:** 617-620.

Wood JR, Breckenridge K, Chard JM. 2002. Survey for *Ralstonia solanacearum* in Scottish Rivers. *Proceedings of Crop Protection in Northern Britain 2002,* Dundee 19-20 February, 237-242.

Appendix 2 List of contact names and addresses

SASA contact points from 1 October 2004.

This list gives the names and telephone numbers of those staff at SASA who will be able to provide information and advice on the subjects or services shown against their names. Telephone enquiries may be made direct to the person concerned by dialling 0131 244 (the GTN access code is 7188) followed by the 4 figure extension listed.

Staff can be contacted directly by email by using the format Firstname.Surname@sasa.gsi.gov.uk

For general enquiries, please telephone extension 8890, fax to extension 8940 or email info@sasa.gsi.gov.uk

SASA website: http://www.sasa.gov.uk

All enquiries from the media should be directed to the Scottish Executive Media & Communications Group Tel: 0131 244 2969

SASA Management Board

	Professor Gordon C Machray	8843
	Simon Cooper	8932
	Dr Kevin O'Donnell	8924
	Shelagh Quinn	8880
	Keith McLaren	4031

Pesticides, Plant Varieties and Seeds Division

Official Seed Testing Station

Head of Section and Chief Officer	Ronald Don	8891
General enquiries/seed testing and results	Caroline Cadger	8908
Requests for sample packets and price lists	Alayne Finlay	8869
Seed Health	Valerie Cockeral	8900
Seed Testing, Quality and Sampling courses	Ronald Don	8891

Cereals Section

Head of Section/General Enquiries	Gerry Hall	8856
Fees and Forms	Kate Flinn	8833
Training Courses	Robert Sunter	8815
Varietal Identity Tests	Gillian Liddle	8801
Combinable Crops VCU	Gerry Hall	8856

Herbage and Vegetable Crops Section

Head of Section	Niall Green	8853
National List/Plant Breeders' Rights tests, EC and UPOV matters, Pre-DUS and other commercial tests. Genetic resources: request for germplasm and/or information DUS Tests/	Niall Green	8853
Variety descriptions/request for seed		
	Gillian Moran	8907
	George Campbell	8962
	Tom Christie	8961
	Lesley McCarthy	8820
Certification of all non-Cereal Seed Crops/	Ken Pearson	8885
Advice to seed trade, training courses for seed Sampling/UK VCU Trials: Herbage Crops/Herbage Evaluation and Selection Trials/WPBS Trials	Russell Thomson	8899

GM Advice and Inspectorate	Dr Jonathan Davey	8837
Pesticide Usage and Wildlife Management Section		
Head of Section	Dr Chris Griffiths	8862
Pesticide Approval Information	Dr Chris Griffiths	8862
Pesticide Usage Information	Jeremy Snowden	8904
Vertebrate Pest Control: Licensing, Vertebrate R&D	Dr Gill Hartley	8804
Rabies Contingency Planning	Dr Gill Hartley	8804
Chemistry Section		
Head of Section	Dr Ken Hunter	8864
Pesticide Enquiries	Dr Ken Hunter	8864
Wildlife Incident Investigation Scheme: Vertebrates and Honey Bees	Elizabeth Sharp	8874
Pesticide Residues in Food, Gas Chromatography	David Lindsay	8855
Contact Analysis, High Performance Liquid Chromatography	Dr Ken Hunter	8864
Mass Spectrometry	Dr George Keenan	8983
Potato and Plant Health Division		
Potato Section		
Head of Section	Dr Stuart Carnegie	8858
Classification, Variety Testing (UK, DUS and Databases)	Arlene Cameron	8870
Nuclear Stock Production	Sandra Goodfellow	8852
Pathology	Dr Denise A' Hara	8931
Seed Potato Classification Scheme	Dr John Kerr	8945
Plant Health Section		
Head of Section	Dr Jane Chard	8863
General Enquiries including bee diseases	Dr Jane Chard	8863
Potato Quarantine	Dr Colin Jeffries	8868
Plant Health Licensing	Susan Irvine	8883
Potato Spindle Tuber Viroid	Tina James	0131 445 6165
Diagnostics and Molecular Biology Section		
Head of Section	Dr Gerry Saddler	8925
Molecular Biology	Vince Mulholland	8845
Bacteriology	Dr Gerry Saddler	8925
Monoclonal Antibody Production	Robert Burns	8911
Virology and Zoology Section		
Head of Section	Dr Jon Pickup	8859
Entomology (Statutory Pests)	Lorna King	8846
Virology/Virus Testing	Isla Browning	8916
Administration		
Head of Section	Shelagh Quinn	8880
Finance Manager	Anne Monk	8811
Purchasing	Graham Halliday	8849
Personnel Officer	Carol Heatlie	8805
Library	Lynda Clark	8826
Photography	Sylvia Breslin	8827
Training/Quality Assurance	Douglas Downie	8878
Support Services		
Information Technology Services	Peter Winfield	8914
Property	Keith McLaren	4031
Farms	Robert Campbell	339-8319
Gardens/Glasshouses	David MacIntyre	8810

Scottish Agricultural Science Agency
An Agency of the Scottish Executive Environment and Rural Affairs Department

82 Craigs Road, East Craigs, Edinburgh EH12 8NJ
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