



**PROJECT REPORT No. 4**

**EXTRACTION AND  
IDENTIFICATION OF WEED  
SEEDS AND PLANT-PARASITIC  
NEMATODES FROM SOIL  
SAMPLES COLLECTED FROM  
98 CEREAL FIELDS  
THROUGHOUT SCOTLAND**

**1989**

**FREE**

Report to the Home-Grown Cereals Authority

Extraction and identification of weed seeds and plant-parasitic  
nematodes from soil samples collected from 98 cereal fields throughout  
Scotland (Ref. 62(b)/87)

H.M. Lawson and B. Boag

Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA.

Report No. 1

Final report on a 6-month project commencing 1st May 1987

Abstract: Soil samples, from 98 farms randomly selected from the cereal acreage statistics compiled by the Department of Agriculture and Fisheries for Scotland, were analysed at SCRI for weed seeds and migratory plant-parasitic nematodes. In a related project the East of Scotland College of Agriculture analysed a second set of samples for cereal cyst nematodes. Seeds of 41 weed species were identified and counted, the commonest being annual meadow-grass (Poa annua), rough meadow-grass (Poa trivialis) and common chickweed (Stellaria media), which occurred in samples from 90%, 62% and 60% of fields respectively. More than 20 plant-parasitic nematode species were recovered, the major species being one of the needle nematodes (Longidorus elongatus), the stunt nematode (Tylenchorhynchus dubius) and a root lesion nematode (Pratylenchus crenatus), which occurred in samples from 60%, 59% and 52% of fields respectively. Cereal cyst nematode (Heterodera avenae) was found in soil samples from 69% of the fields.

The data from this project will be matched with information also collected on farm type, location, cropping practices and soil physical and chemical characteristics for each of the 98 fields, with the objectives of predicting which types of cereal field are likely to be at risk from particular weed associations and nematode species and of devising cost-effective control measures.

i) Objectives

The objectives of the major research programmes to which this 6-month project was linked are to compile data-bases of information which will permit the prediction of weed and nematode control requirements for individual crops, fields and rotations. This project involved the extraction, identification and counting of weed seeds and nematodes from soil samples taken from 98 cereal fields throughout Scotland, as a first step in compiling such a data-base for cereal crops.

ii) Introduction

There is a lack of comprehensive information on the weed seed and nematode populations associated with cereal cropping in Scotland which could be used for prediction and selection of cost-effective control strategies, both in individual crops and in rotations. Surveys of emerged

weeds in, for example, spring cereal crops (Carnegie, 1974; Scragg, 1974) are of value for that crop in that season, but give no indication of what might have happened in a winter cereal crop or with different soil and weather conditions. It is also difficult to use such data to monitor changes over a period of years. Similarly, although a range of plant-parasitic nematodes is known to attack and damage cereal crops in England and Wales, only cereal cyst nematode has been associated with damage to cereals in Scotland (Anon, 1986). There is little published data on the incidence and pathogenicity of other nematode species, although two needle nematodes, *Longidorus elongatus* and *L. leptocephalus*, are reported to be widely distributed in Scotland (Taylor & Brown, 1976).

A previous survey conducted by SCRI into the weed seed and plant-parasitic nematode populations associated with 100 randomly-chosen swede fields in Scotland recorded 89 weed species (Smoktunowicz, 1986) and 28 nematode species (B. Boag, unpublished results). Data from that survey were also used to investigate associations between different weed and nematode species and their geographical distribution, the previous cropping of the field, soil characteristics and farm type. Results indicated that all these factors did in fact influence the prevalence and incidence of certain weed and nematode species. This information is being used in an attempt to predict likely associations of species in various field situations. The soil-sampling survey carried out by the East of Scotland College of Agriculture in 1987, primarily to assess cereal cyst nematode populations, offered a unique opportunity to SCRI to collect data on cereal weed seedbanks and plant-parasitic nematode populations comparable to that in the swede survey and capable of similar types of interpretive analysis.

#### Materials and methods

A stratified sample was drawn from a list of cereal-producing farms throughout Scotland (based on the DAFS June returns for 1986) to give a total of 100 farms. Two of the farms selected were on islands and were not visited, the survey being restricted to the 98 farms on the Scottish mainland. Each farm was visited between January and April 1987 and one field, in which cereals were grown regularly, was selected per farm. Soil samples were taken with a prod corer for the cereal cyst nematodes and with a trowel for the migratory nematodes and weed seeds. The bulk sample taken by trowel from each field comprised at least 20 sub-samples collected systematically across the field. The farm visits and soil sampling and the extraction of cereal cyst nematodes were carried out with financial provision by DAFS. The analysis of the soil samples at SCRI for weed seeds and free-living nematodes was the part of the programme funded by the present H-GCA grant.

At SCRI individual soil samples were thoroughly mixed and a 200 g sub-sample for nematode extraction was removed and placed in a refrigerator at 5°C; a 1 litre sub-sample to be used for weed seed determination was frozen at c. -20°C and a third sub-sample stored for use in soil physical and chemical analysis.

For analysis of weed seed content soil samples were removed from deep-freeze storage and allowed to thaw. Each sample was pressed through a funnel into a bucket/bowl and then stirred with a trowel to ensure thorough mixing. Three 200 ml volumes of soil were then removed and weighed. The

three sub-samples were analysed separately. The soil was washed, with mechanical agitation through a series of three sieves, using a Fritsch Analyssette. Seeds were extracted from the contents of each sieve by means of flotation in water or calcium chloride, using the technique described by Roberts & Ricketts (1979). In both the water and calcium chloride fractions floating seeds which were firm and resistant to gentle pressure were assumed to be viable and therefore removed. These seeds were then counted and identified by reference to standard samples of weed seeds and to publications on weed seed identification by Hanf (1983); Musil (1963) and Martin & Barkley (1961). Species about which there was any doubt were sent to the Official Seed Testing Station, DAFS, East Craigs, Edinburgh for verification.

Migratory plant-parasitic nematodes were extracted from the soil sample using a modified sieving and decanting technique (Boag, 1974). The 200 g of soil were added to approx 600 ml water in a plastic bucket and allowed to stand for  $\frac{1}{2}$  hr. The soil was agitated into a suspension and decanted through a rough 2 mm sieve to remove stones and roots. The suspended material was then passed through 251  $\mu$ m and 125  $\mu$ m aperture sieves, combined and placed on 90  $\mu$ m nylon mesh supports, and put into water in Baermann funnels. The residues from a further two sieves 75 and 53  $\mu$ m in aperture were similarly combined and suspended on paper tissue filters on supports in another Baermann funnel. After approximately 15 hours the nematodes which had wriggled through the supports and fallen to the bottom of the funnels were drawn off and counted using a low powered stereo-microscope. To determine the species present a sub-sample of approx 50 nematodes was examined in greater detail using a higher-powered research microscope. Specimens were counted and identified after being heat-killed at 60°C and fixed in triethanolamine-alcohol-formalin.

## Results

The first month of the 6-month period was devoted to training the research assistant appointed to the project in the extraction and identification techniques outlined above. Thereafter approximately one month was spent on the nematode extraction, three months on weed seed extraction and the remaining time on data entry and tabulation.

Forty one species of weeds were extracted and identified from the soil samples. The twenty most common species are shown in Table 1. Poa annua (annual meadow-grass) and P. trivialis (rough meadow-grass) were recorded from 90% and 62% of the fields respectively, while Stellaria media (common chickweed) was recorded from 60% of fields. Other frequently-recorded weed species included common spurrey (Spergula arvensis), rushes (Juncus spp.), fat-hen (Chenopodium album), knotgrass (Polygonum aviculare), smooth meadow-grass (Poa pratensis) and redshank (Polygonum persicaria). In general there was a positive relationship between the percentage of fields in which a species was recorded and the number of seeds recovered per litre of soil.

More than 20 species of plant-parasitic nematode species were recorded, the 13 most prevalent being listed in Table 2. Longidorus elongatus was found in 60% of the fields, while Tylenchorhynchus dubius and Pratylenchus crenatus were both found in over 50% of the fields. The spiral nematodes Rotylenchus goodeyi, Helicotylenchus psuedorobustus and H. digonicus were



recorded from around 30% of fields while other spiral nematodes H.

vulgaris, H. canadensis, H. varicaudatus and R. fallorobustus were found in considerably fewer fields. The stubby root nematode (Trichodorus primitivus) and needle nematodes L. leptocephalus and L. goodeyi were also found in low numbers in relatively few fields. The percentage of fields in which individual nematode species were found in the survey did not relate particularly well to the size of their populations, due to the differences in their biology and ecology.

For comparison and completeness, the results of the cereal cyst nematode counts (courtesy ESCA) are summarised in Table 3, 69% of the samples having had either dead or live cysts and 38% having had live cysts present.

### Discussion

The limited results given in this report reflect the nature of the project, which was primarily one of data collection rather than of analysis and interpretation. These further stages await the analysis of the physical and chemical characteristics of the soil samples and their inclusion, together with information on the farms and their rotations, into the data-base. The temporary research assistant carried out her somewhat tedious and repetitive, but highly skilled, duties in a competent manner. Her abilities have since been recognised by her appointment to a full-time laboratory post at SCRI.

Some preliminary conclusions can be derived from the data presented in Tables 1-3. The numbers of weed seeds recovered per litre of soil were considerably lower for most species than those recorded in a previous survey of weed seeds in Scottish swede fields (Smoktunowicz, 1986) and may reflect more efficient weed control management in predominantly cereal rotations compared to the more livestock-oriented rotations which include the swede crop.

A major difference between the two surveys in the range of species recorded was the inclusion of many more species usually associated with grassland in the swede list as compared to the cereal list. Nevertheless the principal species recorded in the cereal survey included most of these reported in other surveys of weed seed or seedling populations in arable rotations in Scotland (Anon, 1977; Carnegie, 1974; Lawson & Boag, 1984; Scragg, 1974; Warwick, 1984). The high incidence of seeds of rushes (Juncus spp.) in cereal fields was surprising, although rushes were also a major feature of the swede survey (54% of fields). Williams (1984) found that rushes were the main constituents of the seed-bank below a long-term pasture, but were not represented in the sward. They are not normally recorded as weeds in either cereals or swede crops in Scotland, but may survive in the seedbank for many years and pose a potential problem when a return to grassland occurs, particularly on poorly drained soils. The incidence and high numbers of seeds of the various Poa species is worth noting as are the complete absence of wild-oat (Avena fatua), black-grass (Alopecurus myosuroides) and bromes (Bromus species). Broad-leaved species also absent included cleavers (Galium aparine), poppies (Papaver spp.), groundsel (Senecio vulgaris) and orache (Atriplex patula). In this context, the failure of a species to be recorded means that its population fell below the threshold of one seed per 600 ml of soil per field rather

than that it was not present at all. Nevertheless, the absence of even one seed of a species from approximately 300 subsamples of soil suggests a low general incidence of that species or a very local and patchy distribution within a few fields. Warwick (1984) found no seeds of wild-oat in a survey of seeds from soils of Scottish potato fields. Evidence from regular surveys by the North of Scotland College of Agriculture (E. Scragg, personal communication) suggest that populations of this weed species in Scotland are on the decline. Black-grass has so far been found only very occasionally in cereal crops in the East of Scotland College area, while seeds of brome grasses, which are increasing in importance as weeds (D.H.K. Davies, personal communication) might not have been included in the soil samples because sampling was intentionally carried out well away from field margins. More detailed assessment of the weed species which were recorded in the survey will be possible once farm type, rotational practices and soil characteristics have been included in the data-base.

The numbers of plant-parasitic species recovered per litre of soil and their population size were also generally lower than those recorded from the similar survey of nematodes associated with the swede crop (B. Boag, unpublished results). This may reflect differences in soil type or more probably the more intensive arable rotation in which cereals are grown. The majority of the species recorded in the swede survey were also recorded from the present cereal survey. Of the main plant-parasitic species found, L. elongatus, L. leptocephalus, T. dubius and Pratylenchus spp. have all been shown to decrease the yield of cereals in England (Corbett, 1972; Brown & Sykes, 1975; Empson & Gair, 1982) and nematode numbers from the present survey would suggest that yields of cereals could be depressed by populations of these species present in some fields in Scotland. The absence from the soil samples of Meloidogyne nassi, which is widespread in England and Wales and can do considerable damage to cereal crops (Franklin, 1965; York, 1980), would suggest that although it has been reported once from Scotland (Stephan & Trudgill, 1982) it is not a common species. Seven of the 14 spiral nematodes were found in this survey. Although no one species was found in large numbers, collectively they constituted a large proportion of the plant-parasitic nematode fauna. Further analyses of the results and the association of different species with cropping history and farm and soil type may help to identify farms which are at particular risk from plant-parasitic nematodes.

The cereal cyst nematode (Heterodera avenae) was the most widespread of the species recorded in the survey, confirming its position as the major nematode pest species in Scotland (Anon, 1986).

As well as carrying out an in-depth examination into the factors affecting the incidence and distribution of weed and nematode species in cereal crops in Scotland, we also intend to investigate possible relationships between the nematode and the weed populations. This could have potential implications for control strategies for both groups of species.

The investment by the Home-Grown Cereals Authority in this small project has therefore provided databases of information which can be used to increase greatly our understanding of the potential weed and nematode problems associated with cereal crops in Scotland and to contribute to better-targeted and more cost-effective control.

#### Acknowledgements

Thanks are due to Dr Simon Bowen of the East of Scotland College of Agriculture for carrying out most of the field sampling and supplying the cereal cyst nematode data, and to the H-GCA for the grant which funded the project reported above.

Table 1

Weed seeds extracted from soil samples collected from 98 cereal fields

Species		% fields in which recorded	Total no. recovered from 98 fields (1 litre soil/field)
<u>Poa annua</u>	(Annual meadow grass)	90	1847
<u>Poa trivialis</u>	(Rough meadow-grass)	62	1105
<u>Stellaria media</u>	(Common chickweed)	60	628
<u>Spergula arvensis</u>	(Common spurrey)	41	568
<u>Juncus</u> spp	(Rushes)	40	758
<u>Chenopodium album</u>	(Fat-hen)	34	460
<u>Polygonum aviculare</u>	(Knotgrass)	34	270
<u>Poa pratensis</u>	(Smooth meadow-grass)	30	215
<u>Polygonum persicaria</u>	(Redshank)	30	152
<u>Viola</u> spp	(Pansies)	22	202
<u>Matricaria</u> spp	(Mayweeds)	19	313
<u>Ranunculus</u> spp	(Buttercups)	19	98
<u>Veronica</u> spp	(Speedwells)	19	40
<u>Capsella bursa-pastoris</u>	(Shepherd's purse)	18	103
<u>Galeopsis tetrahit</u>	(Common hempnettle)	18	52
<u>Trifolium repens</u>	(White clover)	18	52
<u>Fumaria officinalis</u>	(Common fumitory)	16	57
<u>Trifolium dubium</u>	(Lesser trefoil)	16	37
<u>Polygonum hydropiper</u>	(Water pepper)	10	48
<u>Phleum pratense</u>	(Timothy)	9	23
Others			509
Grand total			7537



Table 2

Plant-parasitic nematodes extracted from soil samples collected from  
98 cereal fields

Species	% fields in which recorded	Max no. recovered from 98 fields (1 litre soil/field)
<u>Longidorus</u> spp. (needle nematodes)		
<u>L. elongatus</u>	60	1800
<u>L. leptocephalus</u>	11	375
<u>L. goodeyi</u>	2	15
<u>Helicotylenchus</u> spp. (spiral nematodes)		
<u>H. pseudorobustus</u>	28	1225
<u>H. digonicus</u>	28	575
<u>H. vulgaris</u>	12	1250
<u>H. canadensis</u>	10	1250
<u>H. varicaudatus</u>	6	475
<u>Tylenchorhynchus dubius</u> (stunt nematode)	59	1500
<u>Pratylenchus crenatus</u> (root lesion nematode)	52	2750
<u>Rotylenchus</u> spp. (spiral nematodes)		
<u>R. goodeyi</u>	31	800
<u>R. fallorobustus</u>	13	400
<u>Trichodorus primitivus</u> (stubby root nematode)	12	1025
Others		3531
Grand total		16971

Table 3

Cereal cyst nematodes extracted from soil samples collected from  
98 cereal fields (ESCA data)

Species	No. fields with live and/or dead cysts	%	No. fields with live cysts only	%
<u>Heterodera avenae</u>	68	69	37	38

## References

- ANONYMOUS (1977). Turnip and swede survey. Research investigations and field trials 1975-76. North of Scotland College of Agriculture, Aberdeen. pp. 200-205 and 232-233.
- ANONYMOUS (1986). Cereal Pests. Annual Report of the Edinburgh School of Agriculture for 1985. p. 55.
- BOAG, B. (1974). Nematodes associated with forest and woodland trees in Scotland. Annals of Applied Biology 77, 41-50.
- BOAG, B. (1978). Spiral and stunt nematodes. AAB Workshop, Rothamsted Experimental Station, pp. 1-30.
- BROWN, E.G. and SYKES, G.B. (1975). Studies on the relationship between density of Longidorus elongatus and yield of barley and potatoes. Plant Pathology 24, 221-223.
- CARNEGIE, H.M. (1974). A survey of dicotyledonous weeds in spring-sown cereals in north-east Scotland. British Crop Protection Council Monograph No. 10, 106-114.
- CORBETT, D.C.M. (1972). The effect of Pratylenchus fallax on wheat, barley and sugar beet roots. Nematologica 18, 303-308.
- EMPSON, D.W. and GAIR, R. (1982). Nematodes (Eelworms) In: Cereal Pests, Ministry of Agriculture Fisheries and Food Reference Book 186 London HMSO. 116 pp.
- FRANKLIN, M.T. (1965). A root-knot nematode, Meloidogyne nassi n.sp., on field crops in England and Wales. Nematologica 11, 79-86.
- HANF, M. (1983). The arable weeds of Europe - with their seeds. BASF Aktiengesellschaft, Ludwigshafen am Rhein, West Germany.
- LAWSON, H.M. and BOAG, B. (1984). A review of weed control practices in swede crops in Scotland in 1982. Proceedings Crop Protection in Northern Britain 1984 177-182.
- MARTIN, A.C. and BARKLEY, W.D. (1961). Seed identification manual. University of California Press, Berkeley, California.
- MUSIL, A.F. (1963). Identification of crop and weed seeds. Agriculture Handbook No. 219. United States Department of Agriculture, Washington D.C.
- ROBERTS, H.A. and RICKETTS, M.A. (1979). Quantitative relationships between the weed flora after cultivation and the seed population in the soil. Weed Research, 19, 269-275.
- SCRAGG, E.G. (1974). Regional weed problems - dicotyledonous weeds in tillage crops. British Crop Protection Council Monograph No. 10, 19-27.

- SMOKTUNOWICZ, N.T. (1986). A study of weed seed populations in 100 Scottish swede fields. Internal Report Scottish Crop Research Institute, Dundee, 28 pp.
- STEPHAN, Z.A. and TRUDGILL, D.L. (1982). Root-knot nematodes (Meloidogyne spp.) In: Scottish Crop Research Institute Annual Report for 1981 p. 123.
- TAYLOR, C.E. and BROWN, D.J.F. (1976). The geographical distribution of Xiphinema and Longidorus nematodes in the British Isles and Ireland. Annals of Applied Biology 84, 383-402.
- WARWICK, M. (1984). Buried seeds in arable soils in Scotland. Weed Research 24, 261-268.
- WILLIAMS, E.D. (1984). Changes during 3 years in the seedbank beneath a long-term pasture as influenced by defoliation and fertiliser regime. Journal of Applied Ecology 21, 603-615.
- YORK, P.A. (1980). Relationship between cereal root-knot nematode Meloidogyne nassi and growth and grain yield of spring barley. Nematologica 26, 220-229.

**HGCA**

**Caledonia House, 223 Pentonville Road**

**London N1 9HY**

**Tel: 020 7520 3920 Fax: 020 7520 3931**

E-mail: [publications@hgca.com](mailto:publications@hgca.com)

Website: [www.hgca.com](http://www.hgca.com)

For price, including postage and packing within the UK, see title page