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EVALUATION OF THE EPIDEMIC POTENTIAL OF A NEW OAT VIRUS: ITS DETECTION, VECTORING AND ECOLOGY

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EVALUATION OF THE EPIDEMIC POTENTIAL OF A NEW OAT VIRUS: ITS DETECTION, VECTORING AND ECOLOGY

by

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CONTENTS

Summary

The detection, ecology and epidemic potential of an oat virus, first detected in the UK in 1986 and now named oat chlorotic stunt virus (OCSV), was studied. A simple virus purification method was used and an antiserum with good specificity in sensitive serological tests prepared. Virus isolates from Aberystwyth and Brecon (in South Wales) were serologically identical but no relationships with any other virus were detected. OCSV was detected in roots and root exudates, chlorotic and necrotic parts only of symptom-bearing leaves, all floral parts including the glumes and lemmas (the husk), and in dried soil about twelve months after virus-infected plants had died.

In two field trials conducted on a virus-contaminated site during 1992/93 and 1993/94, 0.1% and 0% of plants showed systemic symptoms, but root infections were detected in some 26% and 13%, respectively. In the latter trial, equal numbers of infected plants were detected in oats grown from seed saved from the previous crop as in those raised from virus-free, commercially supplied, seed. Plants with shoot or root only infections were detected from early spring to summer, but there was no evidence of secondary spread from OCSV-infected to virus-free plants. Virus appeared to have no effect on grain yield in total, although about 88% of plants with systemic symptoms died before harvest in 1993.

The virus was not mechanically transmitted but virus-infected seedlings which showed the characteristic leaf symptoms of OCSV, were produced following embryo-wound inoculation using a razor blade. The maximum infection rate was 27%. In this way, OCSV was transmitted to four *Avena* spp., and to four of six winter barley, five of seven winter wheat and one of three triticale cultivars. Systemic leaf symptoms were only produced by *Avena* spp. Roots of a common grass, *Poa annua*, appeared to be a host of the virus as well.

Virus was detected in oat seedlings grown in association with virus-infected oat. OCSV was not transmitted by the soil-borne fungus *Olpidium brassicae* and was not detected within resting bodies of the soil-borne fungus *Polymyxa graminis*. Severely affected plants which survived produced sterile seed, but viable seed was obtained from both plants and tillers with very slight or no systemic symptoms, and from those with root infections only. Under laboratory conditions, root infections were detected in seedlings raised from seed originating from field-infected or embryo-wound inoculated plants. No seedling infections were detected in plants grown from seed from which the husk had been removed.

The results suggest that OCSV is a root infecting virus of the Gramineae, which may have changed in recent years to become more suited to infecting *Avena* spp. Developments of the embryo-wound inoculation technique may make it suitable for application to the mass testing of seedlings in a breeding programme. However, there is currently no evidence for genetic variation in virus susceptibility, particularly of root infections, between species or cultivars.

OCSV was actively secreted from infected roots into soil where it is stable for many months, and passive mechanical infection subsequently occurred. These properties are consistent with OCSV having affinities with carmo- or tombusviruses, though some viruses from these groups are transmitted by *Olpidium* spp. Although seedling infections through virus-infected glumes and lemmas under field conditions was not proven, the dissemination of virus in this way may be possible. OCSV was not detected, however, in a survey conducted during 1994, of oat cv. Aintree crops grown at nine

NIAB field trial sites, although it was present at IGER, Aberystwyth (the farm at Brecon was not included). Since oat may not be a unique host, the expense of screening seed crops for virus infection of the husk may not be worthwhile.

II Introduction

Oats (*Avena sativa*) offer an attractive alternative for cereal growers because of increasing appreciation of the high nutritional quality of the grain both for animal and human consumption.

Winter-sown oats are often infected by two viruses singly or in combination, both of which are transmitted by the fungus *Polymyxa graminis*. These are: oat mosaic baymovirus (OMV; Barnett, 1991) a flexuous, filamentous rod about 700 nm long (Hebert and Panizo, 1975), and oat golden stripe furovirus (OGSV; Brunt and Shikata, 1987), a rigid rod-shaped virus with lengths of 150 and 300 nm (Adams, Jones and Swaby, 1988). The objectives of the winter oat breeding programme at Aberystwyth include the production of new cultivars with resistance or tolerance to these viruses.

An apparently new and potentially devastating virus disease of oats was observed in nurseries at Aberystwyth in 1986 (Catherall and Valentine, 1987). Affected plants were severely stunted exhibiting leaf spiralling and conspicuous leaf symptoms of elongated bright golden yellow to white chlorotic streaks and eye-spots, often becoming necrotic with age. Numerous isometric virus particles of about 35 nm diameter were detected in leaf sap by electron microscopy (Thomas, 1988), but no serological reaction was obtained with the virus using antisera to viruses known to infect Gramineae in the UK (Thomas, unpublished). More recently, the nucleotide sequence and genome organisation of the virus has been determined and the name oat chlorotic stunt virus proposed (OCSV; Boonham, Henry and Wood, 1995).

The mode of OCSV transmission has not been determined. In 1986 infected plants were confined to an area at the foot of a gentle slope where drainage water accumulated (Catherall and Valentine, 1987). This indicates that the virus is soil-borne or transmitted by zoosporic fungi such as *P. graminis* or *Olpidium* sp. However, OCSV re-occurred at Aberystwyth in 1987 and in 1990 in winter oats grown in adjacent fields; and from 1991 in a field about 1 km distant from these, and separated by an arterial road. Coincidentally, winter oats with identical symptoms and containing a virus with isometric particles of about the same diameter were found for the first time in 1991 on a farm some 70 km distant from Aberystwyth in Brecon, South Wales by the Agricultural Development and Advisory Service (Bradshaw and Wright, personal communication). A replicated varietal trial established on this farm, in which plants with systemic symptoms were recorded, suggested that cultivars may vary in their susceptibility to OCSV and that good levels of resistance probably occur within existing UK varieties (Thomas *et al.*, 1992).

The work reported here was undertaken to evaluate the epidemic potential of OCSV through an understanding of its ecology and mode of transmission. To achieve this, propagation of the virus in oats after mechanical inoculation was established, the virus was purified and a polyclonal antiserum to OCSV produced, which allowed sensitive and reliable detection of virus in infected plants. For most experiments, oat cv Aintree was used as this variety was one of two which appeared to be more susceptible to infection by OCSV (Thomas *et al.*, 1992).

III Propagation of virus, production of specific antiserum and virus detection

Objectives

Studies of the occurrence and movement of virus within and to other crops, require sensitive serological detection methods. To achieve this, the initial phase of the work necessarily concerned virus purification and the production of an antiserum, which could be used to evaluate detection methods available to this laboratory.

Experimental

To semi-purify virus as a source of inoculum for embryo-wounding experiments, fresh or frozen leaves were homogenised in tris-phosphate buffer pH 8.0 containing thioglycolic acid (2 ml/g) and clarified with 1:1 n-butanol/chloroform, followed by one cycle of differential centrifugation. The virus-containing pellets were re-suspended in 0.05 M tris-HCl pH 7.4 buffer and clarified by low-speed centrifugation. For antiserum production, virus was further purified by a second cycle of differential centrifugation followed by sucrose gradient centrifugation. OCSV-containing fractions were pooled, the virus was recovered by centrifugation and re-dissolved in 1 ml of buffer with an estimated concentration determined by UV spectrophotometry of 3.4 mg/ml.

An antiserum to OCSV from Aberystwyth was prepared in rabbit; 340 µg of purified virus in 0.5 ml of buffer was emulsified with adjuvant and injected subcutaneously on three occasions three weeks apart and finally seven weeks later. Serum was collected after a further three weeks and on four other occasions two weeks apart.

The antiserum was used to evaluate serological relationships using the Ouchterlony agar double-diffusion test. Thereafter more sensitive methods were used to detect OCSV in plant and other extracts. These were: the enzyme-linked immunosorbent assay (ELISA; Clark and Adams, 1977); a dot immuno-binding assay (DIBA) developed from the method of Hawkes, Niday and Gordon (1982); and immune-specific electron microscopy (ISEM) developed from Derrick (1973).

To maximise the number of samples which could be analysed, a gift of the antiserum was made to the MAFF, Central Science Laboratory (CSL), at Harpenden where automated ELISA was performed. A number of tests were subsequently purchased from CSL at a price which reflected this initial collaboration. This procedure had an advantage in that the analysis of some aspects of several experiments was performed independently at both CSL and IGER.

DIBA employed a polyvinylidene membrane (Immobilon-P, Millipore (UK) Ltd); sample discs were cut out and reacted in microtitre plates containing 250 µl of substrate. After allowing for colour development, the reaction was stopped by removal of the discs. The concentration of p-nitrophenol produced in both the DIBA and ELISA tests was estimated spectrophotometrically at a wavelength of 405 nm (A_{405}).

On occasions, in order to improve the reliability of virus detection in DIBA tests, plant tissue homogenised in 0.05 M tris-HCl buffer pH 7.4 (2 ml/g) and clarified with 1:1 n-butanol/ chloroform was mixed with one quarter volume of 30% polyethylene glycol 6000 (PEG) in 0.6 M NaCl. The precipitate was collected by centrifugation and redissolved in 1 ml of buffer.

Observations

In gel diffusion tests, OCSV from Aberystwyth produced a single confluent line of precipitation. Serum produced from the first and last bleeding had end-point titres of 1:128 whilst that from all other bleedings was 1:256. Co-precipitation with the homologous virus without spur formation also occurred with purified preparations of virus prepared from leaves of diseased plants found at Brecon. The antiserum reacted to the same end-point titre with virus prepared from diseased tissue grown at Aberystwyth and Brecon indicating that both isolates were serologically identical.

In ELISA and DIBA, little reaction was produced when samples were prepared from non-infected (healthy) oats or other graminaceous plants (ELISA: 200 samples, range A_{405}0.068 - 0.112; DIBA: 69 samples, range A_{405}0.008 -0.345). In contrast, those samples prepared from leaf or root from known infected plants always produced a significant reaction in about 30 minutes (ELISA: 40 samples, range A_{405}2.6 - 3.04; DIBA: 69 samples, range A_{405}0.110 - 2.8). Samples prepared from infected and healthy plants were included as controls in each assay. Assays of plants for which infection was to be determined, were considered positive when reactions exceeded twice (ELISA) or three times (DIBA, which was slightly more variable than ELISA) those of non-infected plants.

The antiserum to OCSV failed to react in DIBA with eight spherical viruses from three virus groups which infect members of the Gramineae (Table 1). However, it reacted equally with samples prepared from chlorotic leaves from plants naturally infected at Aberystwyth or Brecon (sap dilution end-point 1:10k). Furthermore, positive reactions were only obtained when samples were prepared from chlorotic or necrotic tissue. Those prepared from dark green or apparently unaffected tissue were apparently free of virus. Tests to detect virus in floral parts showed that virus was present in the pistil, glumes and lemmas combined (A_{405}1.4 and 1.59 respectively) and to a lesser extent anthers (A_{405}0.62); compared with the mean for healthy controls (A_{405}0.042) Virus was readily detected in roots treated with 10% SDS (sodium dodecyl sulphate) which is known to disrupt virus on the root surface (14 samples: range, A_{405}0.927 - 1.7) confirming an earlier observation that significant quantities of virus were released when infected plants were stood in water (Thomas, unpublished).

Virions (virus particles) were detected in sap extracts from obviously infected material in the transmission electron microscope (TEM) after negative staining with 2% $^{w}/_{v}$ methylamine tungstate (Milne, 1984). However ISEM, which is much more sensitive, was generally used to ensure virus detection in the TEM. Virus was readily trapped in large numbers on TEM grids coated with antiserum to OCSV and exposed to sap extracted from newly chlorotic tissue (100-1000 particles visible on the small TEM viewing screen). Virus was less numerous when grids were exposed to sap from old or dead tissue, or from poorly infected plants (1-100 particles visible). Using suitable dilutions of sap extracts from chlorotic tissue, few virions were bound to TEM grids coated with dilutions of antiserum to clover yellow vein potyvirus or with buffer alone. Consequently, ISEM was often used to confirm or reject infection when DIBA tests produced results close to the exclusion value.

Table 1. *Spherical plant viruses infecting Gramineae but unreactive in DIBA tests to antiserum to oat chlorotic stunt virus.*

Virus Group	Virus
Cocksfoot mild mosaic virus group	cocksfoot mild mosaic virus festuca mottle virus holcus transitory mottle virus phleum mottle virus phleum mottle virus
Luteoviruses	barley yellow dwarf virus
Sobemoviruses	cocksfoot mottle virus cynosorus mottle virus

Conclusions

The virus was easily purified and an antiserum with good specificity prepared. No serological difference was detected between the virus isolates from Aberystwyth and Brecon, and no relationship established between OCSV and other viruses of the Gramineae. Virus was detected within tissues by three sensitive serological methods, DIBA, ELISA and ISEM and they were used to monitor virus infections within crops and to evaluate the epidemic potential of OCSV.

IV Mechanical infection of oats by oat chlorotic stunt virus OCSV

Objectives

The ability to manipulate OCSV infection is important both for virus-resistance breeding, and to evaluate the host range, since alternative hosts may provide sources of virus within the environment from which oats are infected.

In previous tests, all conventional methods to infect oats, other Gramineae and some key dicotyledonous plants with OCSV by mechanical inoculation failed (Thomas, unpublished). However, infections were achieved using an embryo wounding technique (Zang, Zitter and Lulkin, 1991; Thomas, unpublished). The number of plants infected was variable, with a maximum of 25% showing systemic symptoms.

To initiate a glasshouse-based virus-resistance breeding programme, many plants must be reliably infected at each inoculation, and so attempts were made to improve the embryo-wound method.

Experimental and Observations

For embryo-wound inoculations, two cuts were made using a fresh razor blade through a drop of purified virus (c. 3 µl) into the embryo of seed previously soaked for approximately 4 h in water to aid removal of the glumes and lemmas (the husk). After allowing the virus preparation to be absorbed into the embryo, the inoculated seed was germinated on water-soaked compost (Levington, Fisons plc) in a seed tray in a glasshouse. When seedlings had developed approximately 4 to 6 cm of first leaf growth, they were transplanted individually into compartmented seed trays filled with Levington compost in a glasshouse. They were grown until growth stage 16 (Zadoks, Chang and Konzak, 1974), leaves assessed visually for systemic infection, and on occasions the roots tested by DIBA for non-systemic infections. In some experiments, seeds were germinated in an illuminated growth cabinet providing 63.1 to 77.0 µmol m^{-2} s^{-1} depending on position, at various temperatures.

Other methods used to wound the embryo and introduce virus, were: injection with a syringe using a fine hypodermic needle (10 µl of virus preparation into the centre of the embryo, at the interface of the plumule and radicle); injection with fine glass probes controlled by a micromanipulator to pierce the embryo 12 times in a defined pattern; or stabbing in a similar way through a 3 µl drop of inoculum using a hand-held glass probe. To increase cell division following wounding, in some tests using the standard razor cut technique, seeds were soaked in gibberellic acid (GA; 20 µg l^{-1}) prior to removal of the husk, and GA was included in the virus inoculum at 40 µg l^{-1}.

Inoculations using a razor blade to cut embryos, often caused significant seedling malformation and reduced their survival. Passing a fine glass needle controlled by a micromanipulator through the inoculum into the embryo, however, inflicted very little damage when seeds were germinated and grown on in a growth cabinet at 20 °C. Nevertheless, only 2 of 60 seedlings developed systemic symptoms (c. 4%), though 2 of 14 (c. 14%) root samples of non-systemically infected plants tested by DIBA were infected also (A_{405}0.799 - 1.133). Although virus-infected plants were obtained, the method was not useful because it was time consuming and induced only a low rate of infection compared to the normal method.

In further tests, the micromanipulation method was compared to injection using a hypodermic syringe (40 seeds for each treatment). Seedlings were raised in a growth cabinet at 20 °C or 10 °C for the light (14 h) and dark periods respectively. Both techniques produced similar results with systemic symptoms induced in c. 15% of the seedlings (6 and 7 of 40 seedlings, respectively).

In a separate test using the same virus preparation as an inoculum, embryos (100 per treatment) were inoculated by either syringe injection or the standard razor cut method. Of the surviving seedlings, systemic leaf symptoms were produced by 11 of 96 (11.5%) injected seeds, and by 19 of 96 (20%) seeds cut by razor blade. Virus was not detected by DIBA in 20 root samples of apparently healthy plants from each treatment (mean A_{405}0.489; healthy control, A_{405}0.426)

A single virus preparation was employed throughout in controlled temperature experiments in which 80 seeds per test of oat cv. Aintree were inoculated using the standard razor blade method. A range of temperatures from 10 °C and 5 °C for 12 h light and dark periods respectively, to a continuous 30 °C were used (Table 2). Seeds wounded with buffer only (20) were raised in each experiment to determine whether

temperature influenced seedling survival. Systemic leaf symptoms were recorded from test seedlings about one month later, when the roots of 20 apparently healthy plants and a control seedling were tested by DIBA.

Roots from a very few apparently healthy virus-inoculated plants contained virus (2%, 3 of 150 plants tested in all experiments, mean A_{405}1.128) but most of the infected plants expressed systemic leaf symptoms. Thus, recording plants with systemic symptoms was an adequate way of evaluating the experiments. The percentage of plants with systemic symptoms increased as the mean daily temperature was raised from 7.5 °C to 15 °C, but then decreased when the mean daily temperature was increased further to a maximum of 30 °C. Hence, the optimum temperature at which maximum infection was produced (c. 26%) appeared to be continuous 15 °C (Table 2). The survival of embryo-wounded seed was apparently not affected by the temperatures used in these tests.

Table 2. *Effect of temperature on the induction of systemic symptoms in oat cv. Aintree by OCSV after embryo-wound inoculation.*

Temperature °C			No. of	Plants with symptoms		Plants with root infections	
Light	Dark	*Mean*	tests	Plants inoculated*	*%*	No. plants tested by DIBA**	*%*
10	5	*7.5*	1	3/60	*5.0*	0/13***	*0*
10	10	*10*	1	6/64	*9.4*	2/37	*5.4*
15	10	*12.5*	1	9/63	*14.3*	0/20	*0*
20	5	*12.5*	1	9/63	*14.3*	0/20	*0*
15	15	*15*	2	36/140	*25.7*	0/20	*0*
20	15	*17.5*	1	9/63	*14.3*	1/20	*5.0*
20	20	*20*	1	8/52	*15.4*	0/20	*0*
30	30	*30*	1	0/60	*0*	not tested	

*Number of inoculated plants surviving.

**Roots from plants without systemic symptoms were tested by DIBA.

***Number of plants with A_{405} values exceeding A_{405} healthy control roots by a factor of 3 or more/ number of plants tested.

Tests with gibberellic acid were inconclusive. In two experiments, 1 of 25 (4%) and 4 of 25 seedlings (16%) treated with gibberellic acid showed systemic symptoms whilst, in both cases, none of 25 seedlings imbibed with only water developed symptoms. It was subsequently shown by negative staining that the virus inoculum used in this experiment contained mainly defective particles which were readily penetrated by methylamine tungstate. Since root-only infections were not assessed by DIBA or ISEM, further tests are necessary to verify any benefit from using gibberellic acid.

Conclusions

These results showed that oats could be infected by the embryo-wound technique in which the standard, razor blade technique was most effective. The method generally produced infected plants with systemic leaf symptoms. Although incubation temperature was influential, the rate of infection (maximum 27% at 15 °C) could not be improved. Moreover, the total number of plants showing symptoms was still inadequate to readily evaluate different oat germplasm for susceptibility to OCSV and this remains a difficulty for a virus-resistance breeding programme.

V Assessment of disease occurrence within crops and impact on yield

Objectives

A site at IGER within which OCSV-infected plants had been detected in previous years, was selected to study natural infections of winter oat by OCSV in 1993 and 1994. Regular crop screening for the location and appearance of diseased plants was designed to provide data on the numbers of plants affected, the distribution and movement of OCSV within the crop and the effect of virus on yield.

Experimental and Observations

For each crop, seed of oat cv. Aintree was sown in October of the previous year. The plots were divided into blocks 4 m × 5 m (approximately 8460 plants each) and inspected regularly for infected plants; those showing symptoms were marked in the field with a cane and on a representative map (Appendix 1).

In 1993, seed was sown at a density of 423 m^{-2}. Infected plants were first observed on 23/3/93, following some days of bright sunny weather and cool evenings, and virus was confirmed by negative staining in the TEM. Diseased plants were often stunted, appeared to have a slightly darker green colour and areas of prominent leaf chlorosis. Such plants became easier to identify as the healthy plants grew at a much faster rate. Most diseased plants were first observed in April and May, after which no more new diseased plants were recorded (Table 3). Altogether, 789 plants (0.093% of the crop) were infected (Appendix 1 and Table 3), but 695 of these plants (88%) died before grain filling. Thus, diseased plants appeared over a relatively short period suggesting that all plants were infected at about the same time. However, it was not clear whether certain weather conditions were required to stimulate virus multiplication within leaves and to induce the appearance of disease symptoms, so that infection may have occurred progressively during the winter.

Preliminary tests (Section III) indicated that virus was only detected in parts of leaves bearing symptoms. To determine whether apparently healthy plants growing adjacent to OCSV-infected plants may become infected without exhibiting symptoms, 48 leaf samples were taken, 12 each from plants around six diseased plants within blocks with the highest infection rate (A5, B4, B5, F5, G7 & G8). Three plants were taken either side of the infected plant in the same row and three plants were taken from the adjacent rows (Appendix 2).

Table 3. *First detection of plants exhibiting leaf symptoms of OCSV in oat cv. Aintree grown during 1992/93.*

Screening date	Number of plants first detected on each date in blocks:									*Total*
	A	B	C	D	E	F	G	H	I	
20/2/93*	0	0	0	0	0	0	0	0	0	*0*
17/3/93	0	0	0	0	0	0	0	0	0	*0*
25/3/93	2	2	1	0	0	3	3	1	0	*12*
6/4/93	44	84	28	11	30	66	124	64	61	*512*
10/5/93	42	49	43	33	10	19	24	19	26	*265*
15/6/93	0	0	0	0	0	0	0	0	0	*0*
28/6/93	0	0	0	0	0	0	0	0	0	*0*
20/7/93	0	0	0	0	0	0	0	0	0	*0*
10/8/93	0	0	0	0	0	0	0	0	0	*0*
Total	*88*	*135*	*72*	*44*	*40*	*88*	*151*	*84*	*87*	*789*

*Each screening lasted approximately 4 days ending on the date indicated.

In separate tests to evaluate the incidence of root infections, roots were assayed from 122 apparently healthy plants, each harvested in a symmetrical pattern (Appendix 3) from around a healthy and diseased plant (as determined by ELISA). The roots were thoroughly washed with a jet of water to remove soil and any loose roots which might have originated from virus-infected plants. The root and leaf samples from both sets of plants were frozen and later tested by ELISA.

None of the leaf samples contained virus (A_{405} healthy controls, mean 0.071; test samples, mean 0.073), indicating an absence of latent infections within leaves. Virus infection of the roots was detected in 22% of the plants sampled around the infected plant (A_{405} healthy controls, mean 0.074; infected controls, mean 3.396; 27 diseased plants, mean, 0.483; range, 0.148 - 3.613); and in 29.5% of the plants from around the non-infected plant (A_{405} 36 diseased plants, mean, 0.643; range, 0.0175 -3.504). However, virus infection appeared to be at random and was not clustered around the infected plant (Appendix 4).

Clearly, a significant number of field-grown plants contain virus in the roots but do not express systemic symptoms. It further appears that, under field conditions systemically infected plants always exhibit characteristic leaf symptoms.

The apparently random distribution of plants with root infections suggests that secondary infection from diseased to adjacent healthy plants was not common. This was despite the likely presence in the soil of virus inoculum released from infected plants. If infection normally occurs through root infection from contaminated soil it would appear that conditions during spring are not suitable. This may be because of environmental conditions, the age of the plants, or the absence of a vector.

To assess the effect of OCSV on individual plants, 50 infected plants, selected using random numbers as map coordinates, were located and tagged in the field. Matched pair analysis using adjacent apparently healthy plants was used to minimise environmental

variation. Seed heads were collected where possible from the infected plant (34 of 50 had died when they were harvested on 10/8/93), and the associated apparently healthy plant, stored in individual paper bags, allowed to dry at room temperature (about a month), threshed and the weight of seed recorded (Table 4). Grain yield from infected plants was significantly reduced (t = 8.78; mean grain weight 0.213 g and 3.2 g, respectively for infected and healthy plants, 16 plants each).

Table 4. *Grain yield of 16 OCSV-infected and adjacent apparently healthy plants of oat cv. Aintree grown during 1992/93.*

Matched pair number	Grain yield (g)	
	Apparently healthy plant	OCSV infected plant
1	4.714	0.339
2	4.500	0.173
3	4.273	0.078
4	4.200	0.234
5	4.140	0.191
6	3.850	0.040
7	3.640	0.030
8	3.350	0.885
9	3.090	0.231
10	2.696	0.440
11	2.630	0.100
12	2.380	0.154
13	2.357	0.104
14	2.271	0.224
15	0.222	0.120
16	0.906	0.058
mean	3.200	0.213

No effect of OCSV on crop productivity was detected when grain yield of areas with high (blocks B5, G7 and G8) and low (D7, E3 and E6; Appendix 1) infection were compared (Table 5; mean weight of seed from blocks with high and low infection 7.34 and 7.09 kg, respectfully; t = 0.618).

Table 5. *Effect of OCSV infection on grain yield (kg) of blocks of oat cv. Aintree* grown during 1992/93 with high or low incidence of plants with systemic symptoms.*

Blocks with low virus incidence				Blocks with high virus incidence			
Code	number infected**	(%)	Yield	Code	number infected**	(%)	Yield
D7	1	(0.01)	6.88	B5	25	(0.29)	8.07
E3	2	(0.02)	7.07	G7	37	(0.43)	6.77
E6	2	(0.02)	7.31	G8	30	(0.35)	7.18
mean	1.67	(0.02)	7.09	mean	30.67	(0.36)	7.34

*4 m × 5m blocks containing approximately 8460 plants (see Appendix 1).

**Plants with systemic leaf symptoms only were recorded and field position marked during 1993.

For 1994, seed from two sources was sown; that harvested from the previous crop and new seed from a commercial supplier, each to one half of the site. Our intention was to compare the performance of a crop raised from seed saved from a known infected crop to that raised from seed of apparently healthy status. The crops had an estimated plant density using the straight stick method (Anon, 1987) of 433 plants m^{-2}. Infection was monitored monthly from February to July; systemic leaf symptoms were assessed by visual screening, and washed roots from 12-45 plants selected at random, individually tested by DIBA. Calculations following the incidence of virus in the 1992/93 field trial had indicated that roots from a minimum of eight plants were required for testing to detect a single infected plant (J.F. Potter, personal communication).

Throughout the life of the crop there was no clear difference between the plants raised from the self-saved or new seed and grain yield was similar (466.2 kg from plants of self-saved seed; and 458.4 kg from plants of new seed). No systemically infected plants were observed, but root infections were detected in both halves of the crop (c. 13% of plants grown from self-saved or new seed). Although the chronological sequence of virus detection differed (Table 6) it was not significant ($t = 0.5651$) and it may only reflect variation resulting from small sampling size. As the level of infection in both halves of the crop was similar, the origin of the seed appeared to be immaterial to the occurrence of virus. Presumably, the environmental conditions required to induce systemic infections were not reproduced during the winter of 1993/4, though we were unable to identify any major differences between the two preceding winters.

Table 6. *Detection of OCSV by DIBA in two crops of oat cv. Aintree grown during 1993/94 sown with seed saved from an OCSV infected crop at IGER in 1993 and with seed of a commercial supplier.*

Sample date (1994)	Number of roots with OCSV / Number tested			
	IGER seed	(%)	Commercial seed	(%)
9/2/94	6/12	50	1/12	8.3
8/3/94	5/40	12.5	1/40	2.5
11/4/94	4/20	20	0/20	0
31/5/94	0/20	0	2/20	10
27/6/94	5/20	25	5/20	25
12/7/94	0/45	0	12/45	26
Total	20/157	12.7%	21/157	13.4%

Conclusions

These observations indicated that a site contaminated by OCSV is likely to result in root infections of some plants every year, but the appearance of plants expressing systemic symptoms was erratic. Leaves of plants without symptoms did not contain virus. Plants with systemic symptoms were recorded over a few months, which suggested that infection had occurred earlier perhaps over a similar length of time. The conditions required to induce systemic symptoms were not identified. The number of plants with root infections only varied between the field trials in 1992/93 and 1993/94. In the field, there was no secondary spread from infected plants to healthy ones. The studies confirm previous work (Thomas, unpublished) that for an individual plant, systemic invasion by OCSV is either lethal or has a severe effect on productivity. Nevertheless, on the evidence of the block yield for the 1992/93 field trial, where there was a mortality rate of c. 88% for all plants in the crop expressing symptoms, yield loss remained insignificant.

VI Survey of winter oat crops for disease

Objectives

To estimate the occurrence of OCSV in the UK, a survey of winter oat cv. Aintree sown in Autumn 1993 was conducted.

Experimental

Plants were collected in July 1994 from nine trial sites of the National Institute of Agricultural Botany (NIAB) and from the trial site at IGER. Staff at NIAB sites preferentially selected samples of stunted or sickly looking plants where possible, whilst at IGER random numbers were used as co-ordinates to locate plant samples. The NIAB and IGER samples were assembled at IGER where the root and stem bases were

thoroughly washed with a high pressure spray, then individually packaged, labelled and sent to CSL, Harpenden for ELISA testing.

Observations

Only samples from the IGER site contained virus-infected plants (Table 7, A_{405} healthy controls, mean 0.068; infected controls, mean 2.883; infected plants, range 0.198 - 2.769).

Table 7. *Detection of OCSV by ELISA in roots of oat cv. Aintree grown during 1993/94.*

Location	Number of samples	Number of plants infected by OCSV
Aberystwyth*	90	12
Cambridge	23	0
Devon	25	0
Edinburgh, plot 10	27	0
Edinburgh, plot 31	32	0
Harper Adams	30	0
Morley	27	0
Nottingham	25	0
Winchester	25	0
Wye	32	0

*All sites were at NIAB field stations except for the crop grown at Aberystwyth which was at IGER.

Conclusions

These results suggest that OCSV does not occur widely. At the time of sampling many diseased plants with systemic symptoms might have already died. However, it has been shown previously that many more, apparently healthy plants contain virus in the roots. Indeed, such plants were detected in the samples from IGER which had been selected at random. Thus to our knowledge, the virus has only been detected at two sites in Wales and, we believe, from one farm in Shropshire (CSL, personal communication).

VII Alternative hosts

Objectives

Determining the origin of any apparently new virus is often difficult or impossible. For OCSV, two possibilities might be considered. The virus may have entered the UK as a latent infection of exotic germplasm acquired for breeding purposes. Alternatively, OCSV may have existed as a latent infection of oat and other plants, particularly the Gramineae, and became manifest when either oat genotypes became more suitable for virus multiplication or the prevailing cultural and/or environmental conditions changed.

As OCSV was not mechanically transmitted under laboratory conditions except latterly by the embryo-wound method, the host range of the virus was unknown. Seeds of weed species commonly occurring in cereal crops, which may also act as alternative hosts for OCSV, were too small to embryo-wound inoculate. Therefore, a number of these were grown under field conditions, sampled and then tested by DIBA. Mixed sowings of winter cereals with oat, and embryo-wound inoculation were undertaken to establish whether other cereals might express symptoms of OCSV.

Experimental and Observations

In 1993, five weed species (4 dicotyledons and 1 monocotyledon) were commonly found in the oat crop and samples were taken at random from close proximity to virus-infected plants. Whole plants (three per species) were collected, their root systems thoroughly washed to remove soil and other unattached roots, blended in buffer and a "virus" protein fraction purified by differential centrifugation. Extracts were examined by electron microscopy and by DIBA.

Of the five species tested (Table 8) only extracts from the roots of the monocot, *Poa annua*, appeared to contain virus-like particles and additionally induced an A_{405} value more than 3 times greater than that from roots of uninfected oats or other weed species (*P. annua*, mean 0.201; OCSV-infected oat, mean 1.144; healthy oat, mean 0.057).

Thus, *P. annua* may be an alternate host of OCSV. However, it was possible that the test sample contained infected material, for example fine root pieces from infected oat plants. This seems unlikely since the other samples which were apparently virus-free were taken from the same area in a similar manner. Nevertheless, no species-specific healthy controls were available, and the absorption value obtained for *P. annua* may have reflected an unusual species-specific response to the antibodies.

To confirm the susceptibility of *P. annua* to OCSV, seed was sown into four 130 mm pots containing a) embryo-wound inoculated OCSV-infected oat cvs Solva or Aintree grown in Levington compost; b) soil from the OCSV contaminated field at Brecon; and c) an uninfected oat cv. Aintree also in Levington compost. Seedlings were grown for about three months to establish a sward, during which time the oats were kept vegetative by regular trimming. Ten samples of an unknown number of seedlings were removed from each pot in a radial pattern, 1 g of root tissue removed, washed and then surface sterilised by immersion in 10% SDS for four hours. The roots were re-washed, an extract prepared and then clarified with 1:1 butanol/chloroform before testing by DIBA and ISEM.

Table 8. *Detection of OCSV in roots of weed species harvested from the 1993 IGER winter oat field trial.*

Plant		DIBA value A_{405}	ISEM Result
Oat cv Aintree : uninfected		0.057	-*
Oat cv Aintree : OCSV infected		1.144	+++
Scarlet pimpernel	(*Anagallis arvensis*)	0	-
Meadow cranesbill	(*Geranium pratense*)	0.079	-
Annual meadowgrass	(*Poa annua*)	0.201	+/-
Knotgrass	(*Polygonum aviculare*)	0.03	-
Field pansy	(*Viola arvensis*)	0.127	-

*Number of virus particles per field of view in the transmission electron microscope:
-, none; +/-, <1; +, 1-10; ++, 10-100; +++, 100-1000.

In DIBA, only 1 of 10 samples prepared from seedlings grown in the pot with an OCSV-infected oat cv. Aintree apparently contained virus (Table 9; A_{405}, 0.296) but some others produced A_{405} values just less than 3x those of the healthy control (A_{405}, 0.087), and these were further tested by ISEM. After both tests, OCSV was considered to be present in 5 of 40 samples (Table 9).

As it was possible that virus trapped by TEM grids coated with antiserum to OCSV was from an infection by another virus, the samples were retested by ISEM using antisera to viruses known to infect grasses. These were antisera to cocksfoot mild mosaic virus (cocksfoot mild mosaic, festuca, holcus and phleum mottle serotypes) and cocksfoot mottle virus. Only those grids coated with antiserum to OCSV were successful in trapping virus.

In further studies, the trial site at IGER was sown in October 1994 with four replicates each of several winter oat cultivars (cvs Aintree, Chamois, Gerald, Harpoon, Kynon, Peniarth and Solva), winter barley (cvs Halcyon, Maris Otter and Pastoral), winter wheat (cvs Mercia and Riband), and two grasses, *Lolium perenne* (perennial ryegrass; PRG) and *Poa annua*.

Plants with systemic symptoms were observed in all cultivars of oat but none were noted during March to July 1995 in any other cereal. To test for root infections of *P. annua* or PRG by OCSV, c. 20 individual plants each from three replicate plots were sampled on 10/7/1995 and mixed, the roots (c. 25 g fresh weight) removed and thoroughly washed, and then surface sterilised in 10% SDS as before. Extracts were prepared in buffer, clarified with 1:1 n-butanol/chloroform and concentrated into 1 ml after precipitation with PEG (see Section III). Since no homologous root preparations from known healthy plants were available, comparable preparations from glasshouse grown uninfected oat cv. Aintree roots were used in DIBA.

Table 9. *Detection of OCSV in roots of* Poa annua *grown in soil from an OCSV-infected crop at Brecon and in pots in association with embryo-wound inoculated oat.*

Treatment: (plants grown with or in)	No. samples with OCSV / No. of plants tested		DIBA
	by DIBA	*by* ISEM*	A_{405} ratio of plants tested by ISEM
OCSV- infected oat cv. Solva	0/10	2/3	(2.9) (2.87) (2.65) **
OCSV- infected oat cv. Aintree	1/10	2/2	3.4 (2.67)
Uninfected oat cv. Aintree	0/10	-	-
Brecon soil	0/10	-	-

*ISEM of samples with DIBA A_{405} ratio just less than x3 that of uninfected *P. annua* root extract.

**An A_{405} value of at least x3 that of uninfected root extract was considered positive; A_{405} value of uninfected control root extract 0.087. Values in parenthesis give DIBA ratio of the samples with OCSV detected by ISEM only.

OCSV was apparently detected in the roots of both PRG (1 of 3 preparations) and *P. annua* (2 of 3 preparations) by DIBA (Table 10), although contamination of the samples by decaying infected oat roots from the oat crop of the previous year was still possible.

To establish whether other cereals were hosts of OCSV, purified preparations of virus were embryo-wound inoculated to seeds of winter wheat (*Triticum aestivum*), winter barley (*Hordeum vulgare*), triticale (*Triticum aestivum* × *Secale cereale*), sweet corn (*Zea mays*) and oat cv. Aintree. Wounded seed was germinated in Levington compost in a growth cabinet at 15 °C and 12 h photoperiod. Seedlings were transplanted individually in John Innes No 3 compost in a compartmented seed tray and maintained in the glasshouse until at least 6 expanded leaves had developed. Any systemic symptoms were noted; roots were tested by DIBA and when apparently positive or nearly so, by ISEM of simple extracts.

Table 10. *Detection of OCSV by DIBA in root extracts of* Lolium perenne *and* Poa annua *grown in an OCSV contaminated site at IGER during 1994/95.*[*]

Replicate	DIBA Value (A_{405})	
	Lolium perenne	*Poa annua*
1	0.153	0.216
2	0.192	0.348[**]
3	0.672[**]	0.599[**]

[*]Seeds were sown into the OCSV-contaminated field at IGER and grown during 1994/95. Roots from three separate batches of 20 plants were harvested and a concentrated protein extract prepared.

[**]Signifies values of at least x3 A_{405} obtained from an equivalent root preparation made from glasshouse grown oat cv. Aintree (A_{405} = 0.098).

OCSV induced systemic symptoms in 3 of 4 *Avena* spp., and root infections in all, but since most plants with systemic symptoms were induced in *Avena sativa*, this appeared to be the most susceptible species. Root infections were also detected in cultivars of winter barley (4 of 6 cvs), winter wheat (5 of 7 cvs) and triticale (3 of 3 cvs; Table 11). Virus was detected more easily by DIBA in *Avena* spp. than in other cereal roots which would appear to suggest that virus multiplied poorly in the latter. However, viral purifications were not made to determine absolute virus concentrations by other methods. As all *Avena* spp. were induced to produce systemic symptoms, it would appear possible that oat cultivars inherited the potential to support OCSV early in the development of cultivated *Avena sativa*.

Conclusions

Root infections of the embryo-wound inoculated cereals was clear evidence that OCSV might be maintained in the soil by release from the roots of infected plants in such crops. Nevertheless, when these cereals were grown in mixed culture with oat, only oat showed systemic symptoms of OCSV. This suggests that, at the moment, only oat crops are at risk from this virus. Although there were reservations about the results of experiments using grasses, the repeated positive tests obtained with *P. annua* suggest that it is most likely that this common grass is an alternative host as well. Thus, it may be extremely difficult to eradicate the virus from soils by simple crop rotation.

Table 11. *Detection of OCSV in roots of cereals following embryo-wound inoculation.*

Cereal	No. roots infected / No. plants tested			DIBA ratio of positive test plant to uninfected controls (range)
	DIBA	(%)*	ISEM	
Avena species				(0.117)**
A. byzantina	2/8	(0)	nt	3.5 and 4.15
A. fatua	0/19	(9.5)	nt	-
A. sativa cv. Aintree	0/40	(25.7)	nt	-
A. sterilis	0/10	(9)	nt	-
total	41/193 = *21.2%***			
Triticale cultivars				(0.149)
Lasko	0/26		1/4	-
Purdy	0/20		3/3	-
Trick	1/30		1/1	5
total	5/76 = *6.6%*			
Winter barley cultivars				(0.212)
Athene	3/40		0/2	3.3 to 7
Pipkin	2/13		nt	3.2 and 5
Puffin	0/40		nt	-
Sprite	0/17		0/1	-
Surprise	2/23		0/8	3.0 and 3.27
Willow	23/40		5/11	3.3 to 7
total	30/173 = *17%*			
Winter wheat cultivars				(0.181)
Admiral	1/38		5/5	4
Beaver	6/40		6/6	3.0 to 4.8
Cadenza	2/13		0/2	3.0 and 4
Haven	0/38		0/8	-
Mercia	9/44		nt	4 to 7
Prophet	0/40		nt	-
Rialto	3/6		nt	.25 to 4.04
total	25/219 = *11.4%*			
Zea mays				(0.709)
Maris jade	0/12		nt	-
total	0/12 = *0%*			

*Percentage of plants with systemic symptoms as shown in parenthesis.

**Mean A_{405} value for roots of each cereal inoculated with buffer only.

***Total number of plants infected as determined by DIBA, ISEM and where applicable by observation of systemic symptoms/ Total number of plants grown.

nt = not tested.

VIII Studies on the mode of virus transmission

Objectives

An understanding of the mode of transmission and means of the annual reappearance of OCSV in oat crops is necessary to estimate the epidemic potential of the virus. Many plant viruses are vectored by insects (principally aphids), some nematodes or certain fungi. Others have no vector, and many passively infect plants by physical (mechanical) means, for example through crop cultivation, or through some interaction of root growth within contaminated soil.

In previous studies not detailed here, no evidence was found for transmission by a common aphid of oat (*Rhopalosiphum padi*; Thomas, unpublished) nor by populations of nematodes in soil from a field with many diseased plants, and in which oat cv. Solva was subsequently grown (Cook and Thomas, unpublished). In addition, virus was not detected by DIBA in nematodes including *Xiphinema* sp., extracted from soil attached to roots of field grown OCSV-infected oat (Thomas, unpublished). Thus further experimentation was designed to evaluate possible fungal transmission, seed transmission, or passive soil-borne infection.

Indeed, the original outbreak of OCSV was not suggestive of an aerial vector or of transmission by nematodes. However, it was considered that virus incidence might be associated with water-logged soil (Catherall and Valentine 1987), which is typical of virus transmission by the soil-borne fungi *Polymyxa graminis*, *Olpidium brassicae* and *O. radicale*.

Attempts were made to verify fungal transmission either by correlating virus infection with fungal infections; by serological detection of viral protein in thin sections of *P. graminis* zoosporangia (since culture of *P. graminis* was not possible); or by establishing a viruliferous culture of *Olpidium* sp. isolated from oat.

Experimental and Observations

Fungal transmission. In an attempt to correlate fungal and virus infection, a number of plants were selected at random in June from the 1992/93 IGER field trial. Following DIBA tests to ascertain virus infection, 20 infected and 20 healthy plants were chosen, and their roots thoroughly washed to remove all traces of soil. To aid identification in the light microscope, fungal chitin was stained with trypan blue after hot 10% KOH treatment.

All samples contained *P. graminis*, and *Olpidium* resting spores were present in 17 of 20 OCSV-infected and 15 of 20 healthy plants. The identity of the *Olpidium* sp. was not authenticated but it resembled *O. brassicae*. These tests showed that both fungi were common throughout the plot. If either fungus were a virus vector it is necessary to assume that only part of the fungal population was viruliferous. The availability of the virus might be the limiting factor, though non-vector clones of these fungi occur.

Plant viruses transmitted by *P. graminis* appear to be carried within the fungal zoospore (Adams, 1988). Therefore, an attempt was made to detect virus within the *P. graminis* resting spore bodies (cystosori) found in roots.

Cystosori were located in fresh root pieces c. 1-2 mm long from healthy and OCSV systemically infected field-grown oat by light microscopy. They were fixed, low-

temperature resin embedded and ultrathin sections of the cystosori were cut and probed for viral coat protein by immunogold labelling (Gordon, Thomas and James, 1995), in which rabbit anti-OCSV antiserum was bound to sections containing whole virus or free virus coat protein. Rabbit antibody was in turn detected by exposing sections to a gold-labelled goat anti-rabbit antiserum which is revealed in the electron microscope by its electron density. To ensure specificity of the labelling, control sections were exposed to antiserum to clover yellow vein potyvirus, which does not occur in oat. In addition, control sections were exposed to OCSV antiserum in which the antibodies to OCSV and any that were made at the same time to plant proteins, were incubated with acetone precipitated oat proteins (Fasseas, Roberts and Murant, 1989). These proteins were prepared from healthy and OCSV-infected oats; 5, 10 and 20 mg of the preparations were mixed at room temperature with 200 µl of antibody diluted 1:100, left for 4 h, and clarified by low-speed centrifugation.

No specific labelling of the *P. graminis* cystosori was evident in any of the tests which suggest that this fungus is not a vector of OCSV. However, this is not a sensitive technique as only those protein molecules whose antigenic sites is exposed at the surface of the section can be labelled. It is possible, therefore, that virus or virus coat protein may have been present in undetectable amounts.

Infection of plants by viruses for which *Olpidium* spp. are a vector is initiated when the *Olpidium* zoospore encysts on the root. Virus is bound to the zoospore membrane and some virions are taken inside the cyst when the flagellum is withdrawn (Temmink, Campbell and Smith, 1970). *O. brassicae* is relatively easily cultured and attempts were made to infect oat with the fungus and OCSV.

A preliminary experiment was performed using disposable universal containers (Sterilin) pierced to make drainage holes in the base. Six plants of oat cv. Solva, germinated on moist tissue until the plumule was just emerging, were sown individually in sand immediately over a piece of cover slip upon which roots about 10 mm long from an OCSV-infected plant containing *O. brassicae* resting spores were positioned (Tomlinson and Thomas, 1986). The tubes were immersed to the base of the screw thread, daily for 8 h in a half-strength hydroponic solution (Maxicrop Garden Products Ltd, UK) and left to drain for 16 h. The seedlings were maintained at 15 °C and 16 h light in a growth room for c. two months.

None of the plants exhibited any symptoms of virus infection in the leaves, but at this time root-only infections had not been discovered so no roots were tested. Examination of the roots by light microscopy revealed extensive infection and multiplication of *O. brassicae*.

In subsequent experiments, a recirculating weak hydroponic solution was used which bathed nine culture tubes simultaneously. The tubes were suspended through a plate positioned over a lower, approximately 2 l tank, which was filled by a siphon from a 3 l header tank. The header tank was refilled from the lower tank by a continuously running peristaltic pump, which exposed the tubes and allowed them to drain. At the completion of the siphon cycle, the tubes were immersed in hydroponic solution to about 5 mm below the screw thread. The tubes were fully exposed for c. 45 minutes and there was c. 2 h from the start of one siphon to the next. The hydroponic solution was changed weekly to maintain the pH and nutrient balance. The culture apparatus was sterilised with a 1% solution of Virkon (Antex International, from Anachem Ltd, UK) for 72 h between experiments.

A two stage test was established. Initially, culture tubes were prepared as before but with root pieces from DIBA tested, virus-free field-grown oat cv. Aintree containing *O. brassicae* resting spores as a fungal source, and embryo-wound inoculated seedlings as soon as systemic symptoms were detected. After about two months, the plants were harvested and a proportion of the root stained with trypan blue. The remainder of the infested roots from plants with *O. brassicae* (7 of 9) were refrigerated.

In the subsequent procedure, three tubes each were established using oat cv. Aintree seedlings raised from seed of a commercial supplier and three different root treatments: cover slips without inoculum; root pieces from *O. brassicae* free, systemically-infected embryo-wound inoculated plants; and root pieces from the OCSV-infected plants which had been infested with *O. brassicae* in stage one. They tubes were positioned on the lower tank plate at random.

After two months the oats were harvested and portions of the roots from each plant were either tested for OCSV using DIBA or stained with trypan blue to reveal *O. brassicae*. All plants were infected by *O. brassicae*, but none contained OCSV.

Since all the seedlings became infected by *O. brassicae,* the experiments demonstrated that *O. brassicae* was successfully cultured from the *Olpidium* and virus-infected root pieces, through zoospores released into the recirculating nutrient solution. In addition, all seedlings remained virus-free, thereby showing that *O. brassicae* was not a vector of OCSV. Had some of the plants become infected by virus we would not have known if the virus had been acquired from virions released into the nutrient solution or through infection by *O. brassicae*. Nevertheless, under the conditions of the test it would appear that passive virus infection was ineffective, though no attempt was made to detect virus in the recirculating nutrient solution.

Seed transmission. Initially, seed transmission appeared unlikely because plants with severe systemic symptoms died before flowering (e.g. see Section V). On occasions, however, such plants produced tillers in the spring in which virus symptoms were less damaging, and small amounts of very poor, but sterile seed was produced (Thomas, unpublished).

Nevertheless, little was known about the quality of seed produced by embryo-wound inoculated plants showing systemic symptoms, or by field-grown plants in which virus is detected in the roots only. To study these, two experiments were conducted. In experiment one, 40 seeds each of oat cv. Aintree from three sources (from a seed merchant, the 1993 IGER field trial, and embryo-wound inoculated plants with systemic symptoms) were grown in seed trays in fresh sterile Levington compost. In experiment two, since virus was detected in the living glumes and lemmas of spikelets from virus-infected plants, comparisons were made using whole (4 sowings) or naked (de-husked; 2 sowings) seed from embryo-wound inoculated plants grown under the optimum conditions for virus propagation as determined in Section IV. The husks (the dried glummes and lemmas surrounding the seeds) were removed after soaking the seed for 4 h in distilled water.

When seedlings had developed more than six expanded leaves they were harvested, and the soil thoroughly washed from the roots, which were surface sterilised in 10% w/v SDS. After thorough rinsing root extracts clarified by 1:1 n-butanol/chloroform were tested by DIBA and ISEM.

No systemic infection was apparent in any of the plants from either test. However, in experiment one, virus was detected in 13 of 40 roots (32.5%) of seedlings grown from seed collected from the embryo-wound inoculated plants, and in 9 of 40 roots (22.5%) of seedlings grown from seed harvested from the IGER field-grown plants (Table 12). No root infections were detected in seedlings raised from commercially supplied seed.

Table 12. *Detection of OCSV by DIBA in roots of oat cv Aintree raised from a) self-saved seed harvested in 1993 from the IGER field trial, b) seed harvested from systemically infected embryo-wound OCSV-inoculated plants, and c) seed from a commercial supplier.*

Seed source	No. infected plants* / No. plants tested		DIBA value (A_{405})	
	DIBA	ISEM**	infected	ratio of infected plants to uninfected controls
IGER field trial	9/40	7/9	0.46 to 1.05	3.5 to 8.0
Embryo-wound inoculated	13/40	8/13	0.26 to 0.431	3.1 to 5.1
Commercial supplier	0/40	0/10***	not applicable	

* Those plants with an A_{405} x3 that of uninfected oat root extracts.

** Plants apparently infected with OCSV by DIBA were tested by ISEM only.

*** 10 samples were selected at random for ISEM.

In experiment two, virus was detected in roots of 5 of 77 (6.5%) whole seed, but in none of 40 naked seed (Table 13). These results suggest that the husk was the source of virus by which seedlings became infected after germination in these glasshouse experiments.

To further establish that the husk was the source of virus infection in young seedlings, tests were made directly on the seed. A protein preparation made from 100 whole seeds saved from the 1993 IGER field trial was compared with that prepared from 100 whole seeds supplied by a seed merchant. Seeds were homogenised in buffer, clarified with 1:1 n-butanol/chloroform, and the proteins concentrated by precipitation with PEG (Section III). Virus was not detected in either seed preparation by DIBA (healthy leaf extracts were used for comparison). It was expected that the husk of some of the seed from the IGER trial would have been viruliferous, however perhaps by chance, insufficient contaminated seed was present for virus detection. On the other hand, virus may not have been released from the dried husk, or it may have irreversibly bound to material that precipitated after clarification with the organic solvents and was removed from the extract before PEG precipitation.

Table 13. *Detection of OCSV by DIBA in roots of whole or naked * oat cv. Aintree raised from self-saved seed harvested in 1993 from the IGER field trial.*

Sowing	Number of plants infected/ number tested		DIBA
	Naked seed	Whole seed	ratio infected plants to uninfected controls
1	nt	1/22**	4.9
2	nt	2/22	3.8 & 5.9
3	0/20	1/13	3.04
4	0/20	1/20	3.16
total	0/40	5/77 (6.5%)	

*Seed from which the husk (glumes and lemmas) was removed before sowing.

**Those plants with an A_{405} value of at least x3 that of uninfected oat root extracts.

nt = not tested.

Thus, the virus is not seed transmitted in the sense that virus is carried within the embryonic tissue of the seed, but it is clear that it might be disseminated by whole seed in which the husk acts as a source of virus infection. This would suggest that in those plants with root infections only, and those with tillers exhibiting slight systemic symptoms, virus is transported through the vascular tissue to the bracts of developing flowers. Alternatively, seed from field-grown plants might be contaminated with resting spores of a possible still unidentified, soil-borne fungal vector by rain splash. However, the embryo-wound inoculated plants were grown in the glasshouse in fresh compost, and this mechanism seems unlikely. Therefore, it is most probable, given the affinity of OCSV to the carmo- or tombusviruses (Boonham, Henry and Wood, 1995), that virus released from the degenerating glumes and lemmas initiated infection in the emerging roots. The rate of root infection in plants grown from whole seed in these tests, however, was apparently greater in the first experiment than the level of infection found in seedlings grown from the same seed in the 1993/94 field trial. In addition, root infection of plants raised in this trial from seed supplied by a commercial supplier, for which we have no evidence of virus contamination, also apparently became infected by OCSV in similar numbers to those oats grown from the self-saved seed. This may suggest that under field conditions, virus-infected husk does not in fact, initiate seedling infections. Furthermore, it would appear that the husk-induced glasshouse infections were a reflection of the conditions employed, perhaps for example, the use of very wet Levington peat compost.

Soil transmission. A further experiment was based on observations (Thomas, unpublished) that, when stood in water, OCSV-infected plants released virions in sufficient quantity to enable a purified preparation of virus to be made. To determine if virus is stable under soil conditions, virus purifications were made from three sources of soil; from the sites in Brecon and IGER, both of which had maintained naturally infected plants; and John Innes compost, which had maintained an embryo-wound inoculated plant. The OCSV-infected plants were re-potted in their original soil in 180 mm pots and transferred to the glasshouse and grown on for a year or more. The soils were allowed to dry for up to 12 months after the diseased plants had died.

All the soil from each pot was soaked in tris/phosphate buffer pH 8 for c. 72 hours, filtered through glasshouse aphid-proof nylon mesh to remove plant debris and subjected to differential centrifugation after treatment with n-butanol. All protein pellets were resuspended in 1 ml of tris/HCl buffer. Virus was detected in all soil preparations when tested by DIBA and ISEM (Table 14).

Table 14. *Detection of OCSV by DIBA and ISEM in extracts prepared from dry soils which had grown an OCSV infected plant.*

Soil origin	Virus detected by:	
	DIBA (A_{405})	ISEM
Brecon field site	0.494*	+**
IGER field site	0.49	+
John Innes no. 3	0.59	+
None (healthy oat root)	0.039	-
None (OCSV infected oat)	>3.0	+++

* An A_{405} value of at least x3 that of the uninfected root extract was considered positive.

** Number of virus particles per field of view in the transmission electron microscope:

-, none; +/-, < 1; +, 1 to 10; ++, 10 - 100; +++, 100 - 1000.

It seems most likely that new infections of oat by OCSV occur when seed germinates in soil into which virus has been released either by an infected plant, which may or may not be present, or possibly from its own virus-infected glumes and lemmas. To test the first option, a mature OCSV-infected plant was repotted after washing away as much of the original soil as possible, in a 180 mm pot using John Innes No 3 compost. Ten oat cv. Aintree virus-free seeds from the seed merchant were sown radially about 5 cm from the centre of the pot. Once the seedlings were established (about one month) the infected plant was cut to soil level to reduce competition and to stimulate root decay. Control seedlings were grown in the same compost in parallel. Washed seedling roots were tested for OCSV infection by DIBA after a further two months.

Three seedlings had A_{405} values more than three times those of roots from control oats, and thus appeared to be infected by OCSV (A_{405} healthy roots, 0.057; roots from OCSV-infected plant, 1.389; seedling roots, range, 0.176 to 0.823). However, although roots were thoroughly washed with a jet of water before sampling, it was possible that the positive results from the seedling samples were obtained because small pieces of root from the virus-infected source plant contaminated the samples. Nevertheless, the experiment may indicate that OCSV-infected plants might act as a direct source of inoculum when seeds are germinated in close proximity.

Conclusions

Transmission by a fungal vector was not proved. The evidence against transmission by *Olpidium* sp. was clear, but that against *Polymyxa graminis* was less certain. On the other hand, the results support the concept of passive mechanical infection of roots as these grow or move within virus-contaminated soil. Virus is released in large quantities from infected roots and survived in dry soil for at least a year, although no attempt was made to determine its infectivity. In contemporary experiments, an oat was infected in the roots when grown alongside an infected plant. Oat root infections occurred under laboratory conditions, when seed saved from virus-infected plants was sown, because the husk contained virus. This means of virus dissemination remains a possibility, though there was no evidence to suggest that it occurred under field conditions. Thus, seedlings may be exposed to inoculum within the soil throughout their development from water imbibition, seed coat rupture, to root and radical emergence. When a crop is sown, all seeds will go through this temporal sequence simultaneously and it may explain field trial results which suggested that virus-infection occurred very early in crop development without secondary infection of mature plants.

IX Conclusions

The results described in this report and in associated work, demonstrate that OCSV has characteristics which are typical of the carmo- and tombusvirus groups. Profuse multiplication of virus particles, which are about 35 nm in diameter, occurred in chlorotic oat leaf tissue (Thomas, 1988). The number of crop plants exhibiting systemic symptoms was apparently never great and such plants were not detected in some seasons. Nevertheless, a significant number of plants with root infections were detectable on sites in which the virus had occurred on more than one year. The virus was readily exuded from roots and remained stable in soil for at least 12 months. No aerial or soil-borne vector was proven, thus indicating that OCSV may be transferred by soil and water movement. These observations are consistent with the premise advanced by Catherall and Valentine (1987) that the pattern of the original occurrence was associated with water drainage.

From molecular studies, Boonham, Henry and Wood (1995) also considered that OCSV had affinities with both the carmo- and tombusvirus groups. Members of these groups frequently occur in high concentrations in diseased tissue, have no aerial vectors, are very stable and virus is often released abundantly from the roots of infected plants in which they preferentially multiply (Koenig, 1988). Re-infection of healthy plants may occur by passage of virus through roots, though plant to plant spread by mechanical contact above ground or in the soil occurs. Wide host ranges are common, and dissemination occurs *via* drainage water, streams and rivers during which virus is protected by absorption to inorganic and organic matter such as clays and plant debris

(Koenig, 1988). In some instances, virus is transmitted by a soil-borne fungus, *Olpidium brassicae* or *O. radicale*.

Although in a single pot experiment, three oat seedlings grown in association with a virus-infected plant appeared to become infected, such transmission was not achieved in previous experiments (Thomas, unpublished) and the virus remained extremely difficult to manipulate. Nevertheless, this work demonstrated that infected plants can be produced by an embryo-wound inoculation technique. The method was relatively inefficient and successful infection may have been related to the temperature at which the wounded seeds germinated and grew. Diseased plants invariably expressed systemic symptoms indicating that the cultural conditions used were not analogous to those experienced by an emerging winter oat crop. Nor do we know why only some individuals from an apparently genetically uniform cultivar should express systemic symptoms in some years.

As embryo-wound inoculation was the only reliable method available, a limited host range study, essentially of other Gramineae, was attempted. Root infections of winter barley, winter wheat and triticale were achieved; and circumstantial evidence from field and pot experiments indicated that the common grass, *Poa annua*, was a host. The concentration of virus in the roots of these plants as revealed by DIBA, suggested that virus did not multiply as well in the roots of these species as in the roots of *Avena* spp. However, more substantial experimentation is needed to verify this. Although three other *Avena* spp. were artificially infected with OCSV, *Avena sativa* appeared to be the most susceptible to the virus. Nevertheless, it is probable that OCSV is not new to the UK, but is a hitherto unknown root-infecting virus of the Gramineae which perhaps has mutated to become more aggressive to oat.

Oats in which OCSV became systemic normally died before seed production. Some of these plants, however, managed to produce tillers in which the systemic symptoms were poorly expressed or there were apparently no symptoms at all, from which much reduced numbers of viable and inviable seed was obtained. On all of these categories of plants, therefore, virus infection was devastating. For those oats with only root infections, OCSV appeared to have no effect at all on grain yield. Indeed, since so few plants exhibited leaf symptoms, the yields of both crops produced on the field-trial site were similar to those produced elsewhere at IGER, and consistent with what would be expected from a commercial crop (Valentine, personal communication). Unless environmental or cultural circumstances change leading to significantly more plants being stimulated to multiply virus systemically, OCSV poses no threat to oat production.

On the evidence of the small survey of oat crops, the virus does not appear to be widespread. There is clear evidence that OCSV infection occurs through roots from virus released into the soil in which it remains viable for a considerable time. Dissemination within IGER may thus have occurred from movement of contaminated water or soil. These studies also suggest an alternative mechanism. Under laboratory conditions using very wet peat compost, root infections were detected in seedlings raised from seed collected from embryo-wounded plants with systemic symptoms, and from seed saved from the 1992/93 IGER field trial. The compost was new and it was extremely unlikely that it could have been supplied contaminated by OCSV. Furthermore, newly supplied commercial seed remained uninfected as did seedlings raised from the IGER seed in which the husk had been removed prior to sowing. Virus was present in all floral parts of spikelets, including the glumes and lemmas, produced by plants with systemic symptoms. Thus, it seems probable that OCSV also occurred in the spikelets of oats with root infections only. Presumably, at the time of flowering,

virus is transported from the roots through the vascular system of the culm to the flowers, without invading leaf tissue. Indeed, virus has been detected in ultrathin sections of the vascular tissue of infected leaves (Thomas, 1988). Seed produced by flowers on severely systemically infected plants may contain so much virus that the embryo is inviable. On the other hand, since removal of the husk from the 1992/93 self-saved seed prevented seedling infection, it may be that, in plants with root infections only, virus invaded the glumes and lemmas at grain filling and the embryo itself was resistant to invasion. When the self-saved 1992/93 seed was sown in the field in 1993 together with commercial seed which was shown to be virus free, similar levels of root infection were detected in 1994 in both crops. There was, therefore, no evidence that field infections occurred through virus-contaminated husk. The possibility of transmission from husk to seedling is being investigated in further experiments, in which seed collected from oat cultivars used in the 1994/95 IGER field trial, were sown in boxes outdoors in October 1995 with sterile John Innes compost. Tests on roots later in 1996 will ascertain whether seedlings may become infected from contaminated husks under conditions approaching natural field conditions.

Although the virus appears to pose no serious threat to oat production, virus-resistance breeding may still be desirable. A replicated variety trial established at Brecon, in which plants with systemic symptoms were recorded (Thomas *et al.*, 1992), suggested that susceptibility to OCSV infection may have been associated mainly with non-UK lines and segregating breeding material containing parents of diverse origin. These results indicated that good levels of resistance may occur within UK varieties. However, our field trials using one of these more susceptible cultivars (Aintree), demonstrated that the production of systemic symptoms was unreliable. In addition, as many more plants contained root infections only, the induction of systemic symptoms in some may not reflect the true susceptibility of a cultivar. Embryo-wound inoculations invariably induced systemic symptoms in those seedlings which became infected, but at present, testing genotypes by this method appears too laborious and time consuming. A re-appraisal of the gibberellic acid pre-treatment might be worthwhile in this respect. If seedling infection through the roots reflects genetic susceptibility to the virus, another approach might be possible. This would utilise the occurrence, under laboratory conditions, of root infections of seedlings raised from seed collected from embryo-wound inoculated plants with systemic symptoms. In this way, only a small number of seeds of each cultivar would need to be embryo-wound inoculated at one time. However, since all the *Avena* spp. tested were liable to infection by OCSV, some evidence that genotypes differ in their susceptibility to root and shoot infections is required before routine laboratory resistance screening is initiated.

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XI References

Adams, M.J. (1988). Evidence for virus transmission by plasmodiophorid vectors. In: Developments in applied biology II. Viruses with fungal vectors pp 203-211. Eds J.I. Cooper and M.J.C. Asher. Wellesbourne, Association of Applied Biologists.

Adams, M.J., Jones, P. and Swaby, A.G. (1988). Purification and some properties of oat golden stripe virus. Annals of Applied Biology **112**, 285-290.

Anon (1987). In: Cereal Crop Manual. Drilling, pp 37-44. Eds C.D. Farman, P.J. Warry, M.R. Saul, P.V. Biscoe and D. Jansen. Kenilworth, National Agricultural Centre.

Barnett, O.W. (1991). Potyviridae, a proposed family of plant viruses. Archives of Virology **118**, 139-141.

Boonham, N., Henry, C.M. and Wood, K.R. (1995) The nucleotide sequence and proposed genome organization of oat chlorotic stunt virus, a new soil-borne virus of cereals. Journal of General Virology **76**, 2025-2034.

Brunt, A.A. and Shikata, E. (1987). Fungus-transmitted and similar labile rod-shaped viruses. In: The plant virus. Vol. 2. The rod-shaped viruses, pp 305-335. Eds M.H.V. Van Regenmortel and H. Fraenkel-Conrat. New York & London, Plenum Press.

Catherall, P.L. and Valentine, J. (1987). Oat virology. In: Report of the Welsh Plant Breeding Station for 1986, Aberystwyth, UK, pp 132-134.

Clark, M.F. and Adams, A.N. (1977). Characterisation of the microplate method of ELISA for the detection of plant viruses. Journal of General Virology **34**, 475-484.

Derrick, K.S. (1973). Quantitative assay for viruses using serologically specific electron microscopy. Virology **56**, 652-653.

Fasseas, C., Roberts, I.M. and Murant, A.F. (1989). Immunogold localization of parsnip yellow fleck virus particle antigen in thin sections of plant cells. Journal of General Virology **70**, 2741-2749.

Gordon, A.J., Thomas, B.J. and James, C.L. (1995). The location of sucrose synthase in root nodules of white clover. New Phytologist **130**, 523-530.

Hawkes, R., Niday, E. and Gordon, J. (1982). A dot-immunobinding assay for monoclonal and other antibodies. Analytical Biochemistry **119**, 142-147.

Hebert, T.T. and Panizo, C.H. (1975). Oat mosaic virus. Commonwealth Mycological Institute/ Association of Applied Biologists. Descriptions of Plant Viruses No. 145, 4 pp.

Koenig, R. (1988). Detection in surface waters of plant viruses with known and unknown natural hosts. In: Developments in Applied Biology II. Viruses with fungal vectors pp 305-313. Eds J.I. Cooper and M.J.C. Asher. Wellesbourne, Association of Applied Biologists.

Milne, R.G. (1984). Electron microscopy for the identification of plant viruses in *in vitro* preparations. In: Methods in Virology **7**, pp 87-120. Eds K. Maramorosch and H. Koprowski. London, Academic Press.

Temmink, J.H.M., Campbell, R.N. and Smith, P.R. (1970). Specificity and site of *in vitro* acquisition of tobacco necrosis virus by zoospores of *Olpidium brassicae*. Journal of General Virology **9**, 201-213.

Thomas, B.J. (1988). Oat virology. In: Report of the Institute for Grassland and Animal Production for 1987, volume 2 The Welsh Plant Breeding Station, Aberystwyth, UK, p 15.

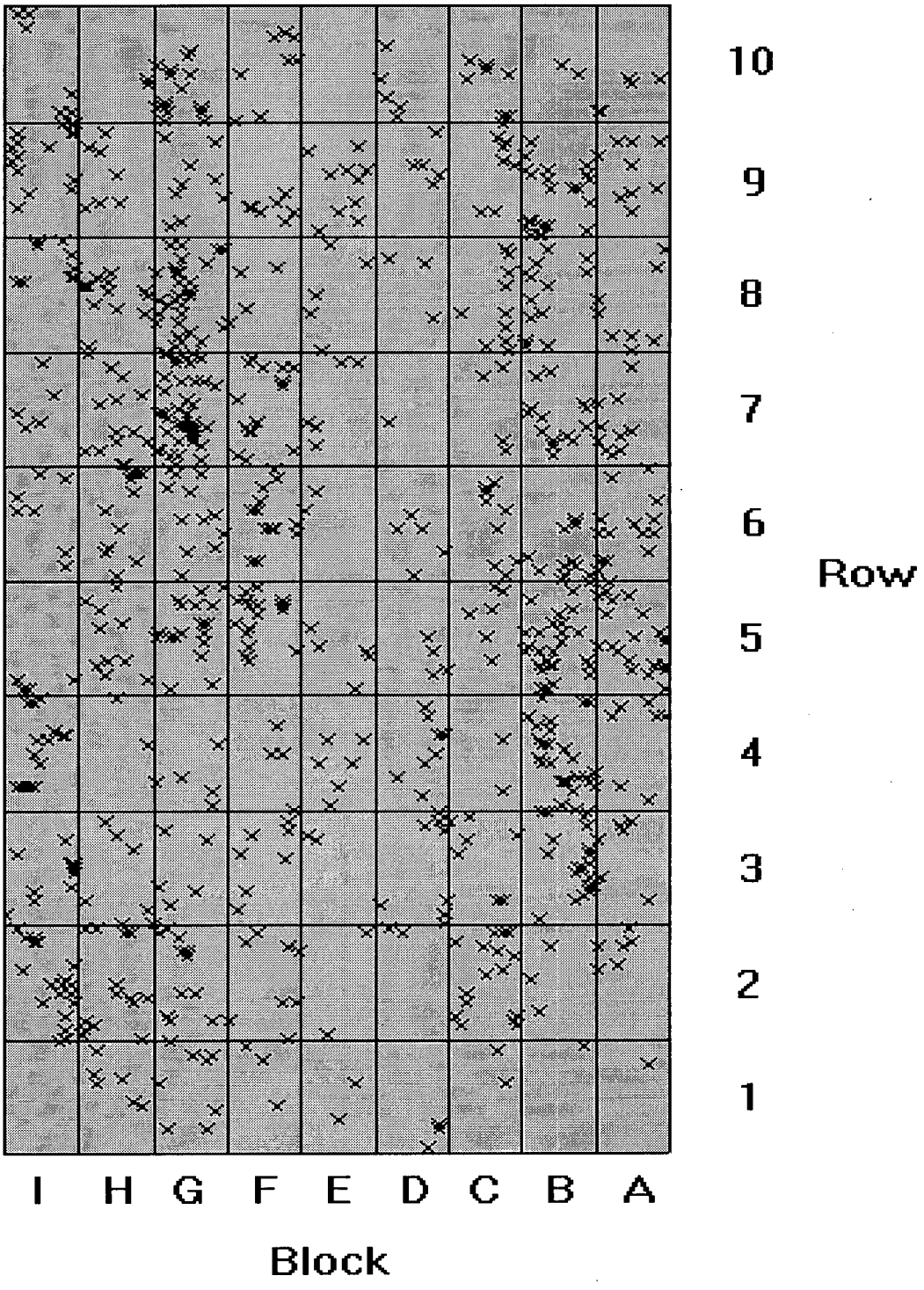
Thomas, B.J., Wright, D.M., Valentine, J., Bradshaw, N. and Middleton, B.T. (1992). A virus attacking winter oats in the UK In: Proceedings of the Fourth International Oat Conference, Adelaide, South Australia, 20 October 1992, Vol. III. General Sessions, p 66. Eds Andrew R. Barr, Robyn J. Mclean, John D. Oates, Glen Roberts, John Rose, Ken Saint and Suzanne Tasker.

Tomlinson, J.A. and Thomas, B.J. (1986). Studies on melon necrotic spot virus disease of cucumber and the control of the fungus vector (*Olpidium radicale*). Annals of Applied Biology **108**, 71-80.

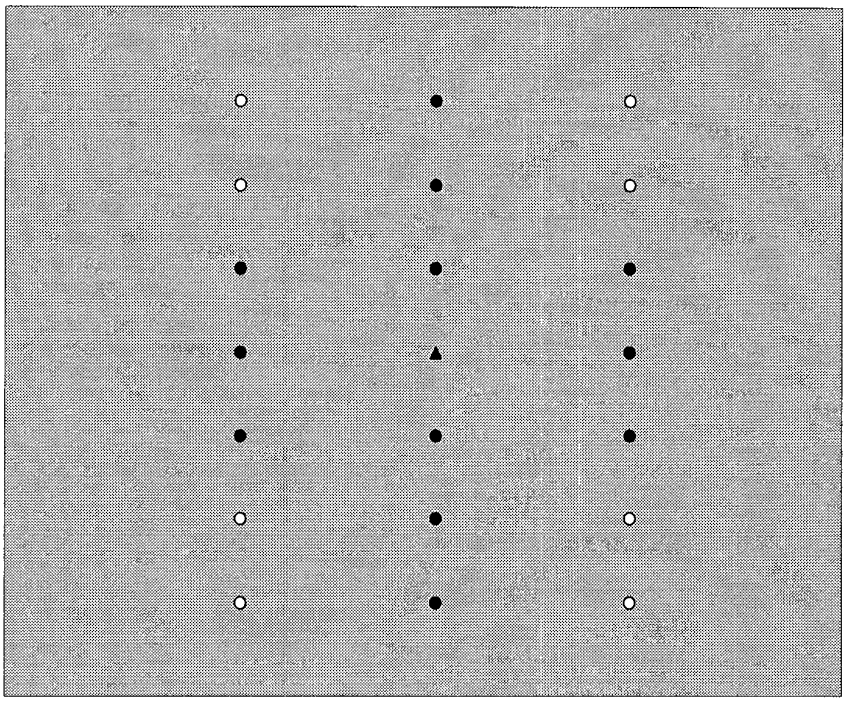
Zadoks, J.C., Chang, T.T. and Konzak, C.F. (1974). A decimal code for the growth stages of cereals. Weed Research **14**, 415-421.

Zang, L., Zitter, T.A. and Lulkin, E.J. (1991). Artificial inoculation of maize whiteline mosaic virus into corn and wheat. Phytopathology **81**, 397-400.

Appendix 1. *Location of oat cv. Aintree plants grown during 1992/93 expressing systemic symptoms of OCSV.*

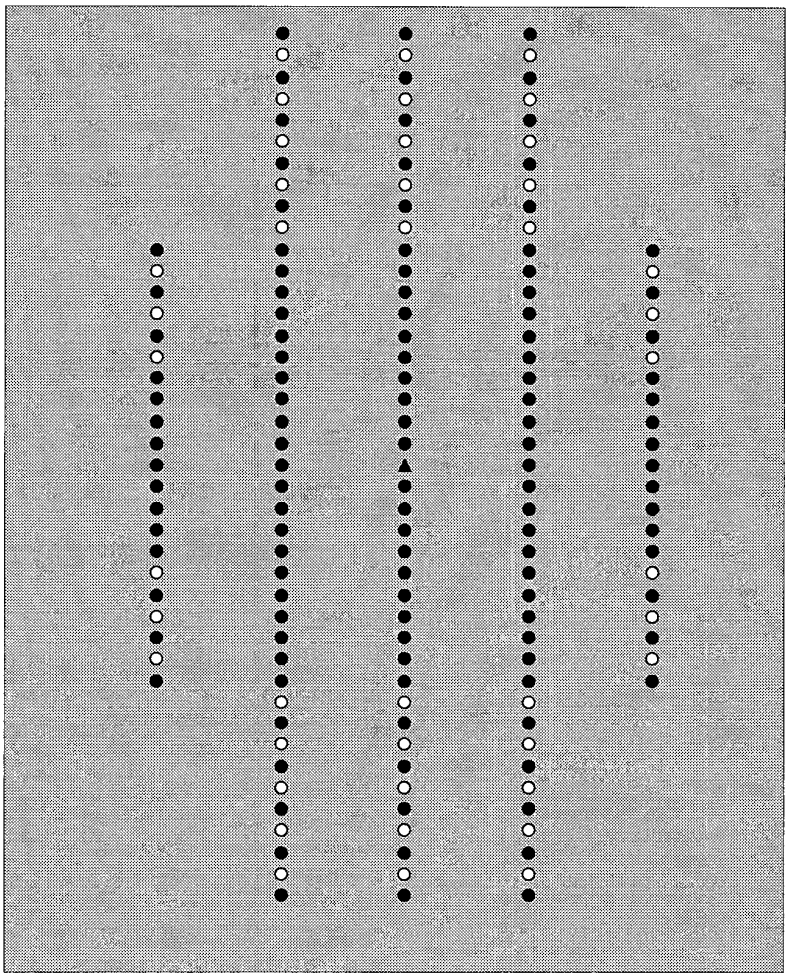


Appendix 2. *Distribution of 12 plants around an OCSV-infected oat cv. Aintree grown at IGER during 1992/93 selected for leaf testing by ELISA.*



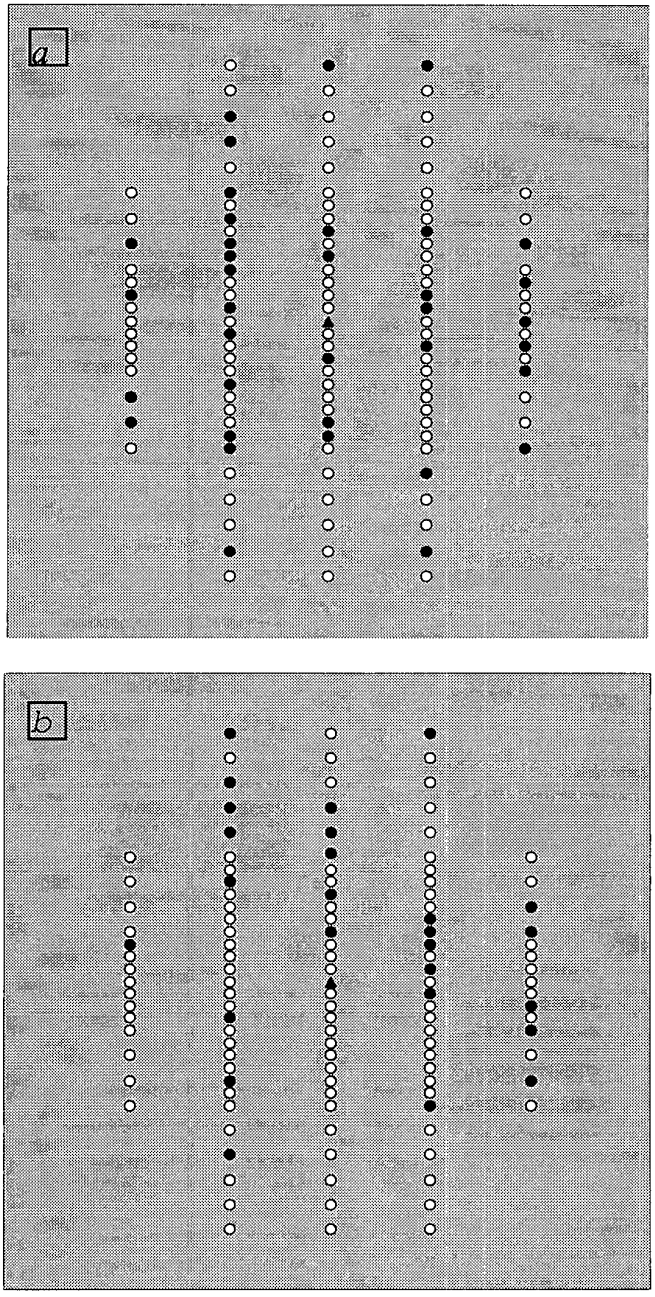
Open circle = not tested.
Closed circle = selected for ELISA testing of leaves.
Triangle = plant with systemic symptoms of OCSV.

Appendix 3. *Distribution of 122 plants grown at IGER during 1992/93 around an apparently healthy or OCSV-infected oat cv. Aintree selected for root testing by ELISA.*



Open circle = not tested.
Closed circle = selected for ELISA testing of roots.
Triangle = central oat; either healthy or one with systemic symptoms of OCSV.

Appendix 4. *Distribution of plants with OCSV-infected roots located by ELISA growing around a) an healthy or b) a systemically infected oat cv. Aintree grown at IGER during 1992/93.*



Open circle = uninfected roots.
Closed circle = OCSV root infections.
Triangle = central healthy or OCSV infected plant.