Project title	Asparagus – Screening UK crops for virus infection
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Project leader:	Dr G M McPherson
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Location of project:	Stockbridge Technology Centre Ltd
Project coordinator:	Mr Peter Knight (PK Consultancy)
Date project commenced:	September 2010
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Key words:	Asparagus, *Asparagus officinali*s, virus, *Asparagus virus I, Asparagus virus II, Asparagus virus III, Tobacco streak virus, Tomato back ring virus, Strawberry latent ringspot virus, Arabis mosaic virus, Cucumber mosaic virus,* ELISA, sap inoculation, indicator plants.

The results and conclusions in this report are based on an investigation conducted over a 4-month period during one season. The conditions under which the study was carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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Science Director

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CONTENTS

GROWER SUMMARY

Headline

Results of a short-term study suggest that *Asparagus virus 1* (AV-I) and *Cucumber mosaic virus* (CMV) are present in the majority of UK asparagus. Growers should note however that the presence of these viruses within a crop is not the sole factor responsible for 'asparagus decline'.

Background

Commercial asparagus crops once planted can remain in production for up to 10-15 years if well managed. However, some UK producers have reported a loss in yield and quality in mature crops in recent years. A potential contributing factor of this yield reduction, generally referred to as *asparagus decline*, is the presence of viruses within a crop.

The 3 most important viruses of asparagus are considered to be AV-I, *Asparagus virus II* (AV-II) and *Tobacco streak virus* (TSV). In addition, *Cucumber mosaic virus* (CMV), *Arabis mosaic virus* (ArMV), *Strawberry latent ringspot virus* (SLRV) and *Tomato black ring virus* (TBRV) are also known to infect asparagus. *Asparagus virus III* (AV-III) has also been reported on asparagus, but to-date has only been detected in Japan. It is also known that elsewhere crops can be infected with more than one virus and that a combination of viruses can reduce quality and yield significantly (Bandte *et al.*, 2008).

Following an HDC funded literature search on asparagus viruses carried out in 1998 (FV 213) a recommendation was made for a survey of asparagus crops of different ages over 3 successive seasons to determine the presence, distribution and identity of viruses that are potentially responsible for the asparagus yield decline seen in the UK. Up to this point there was no evidence of any formal or detailed studies into virus infection in UK asparagus

The aim of this study was to carry out an initial survey of UK asparagus crops during September and October 2010 to assess the presence of virus infection.

Method

A total of 40 fern samples (2 per field – one from the edge and one from the centre of the crop) were collected from 20 geographically dispersed UK crops from 6 to 8 September 2010. Of samples collected, 85% were cv. Gijnlim and the remaining 15% of were cv. Backlim. All asparagus crowns used to establish the crops were imported from the

Netherlands. The youngest crop sampled was planted in 2008, with the oldest having been planted in 2000.

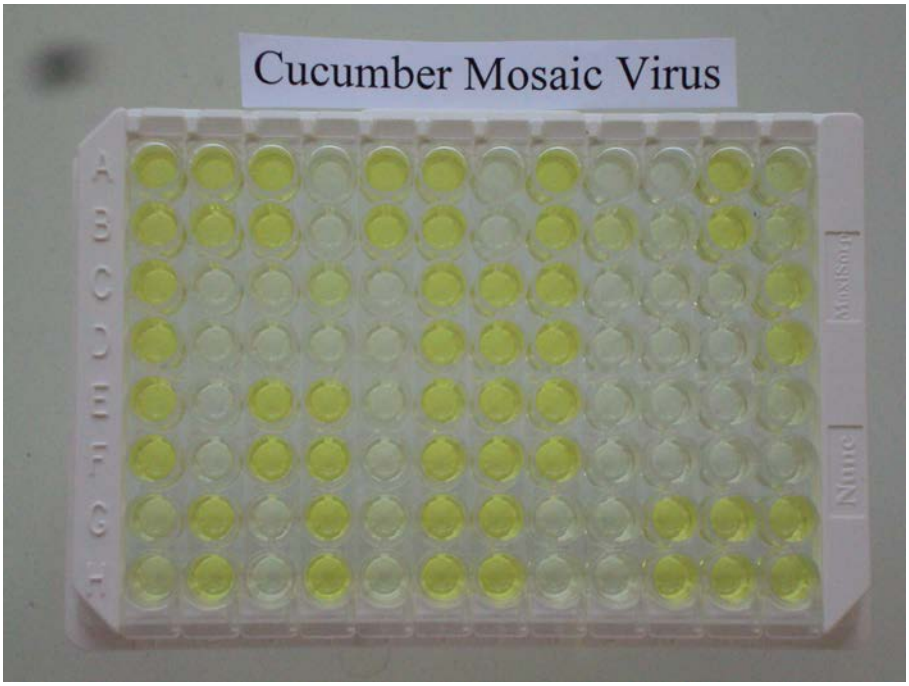
An ELISA technique and a general Potyvirus assay was used to detect and quantify viruses present within samples. Indicator plants were then inoculated with sap from infected samples to confirm the identities of viruses present. The full methodology carried out to sample the crops; detect, quantify and identify the viruses present is given in the science section of the full report.

Results

1) Virus Testing Results

The most commonly detected viruses were AV-I and CMV. When either of the viruses was detected they were present at high concentrations. Only small numbers of crops were found to be infected with AV-II, ArMV, TSV and SLRV. No *Tomato black ring virus* was detected in any crop. No symptom development consistent with AVIII was observed in inoculated indicator plants.

Plate1: Example of ELISA plate test; yellow colouration indicates a positive

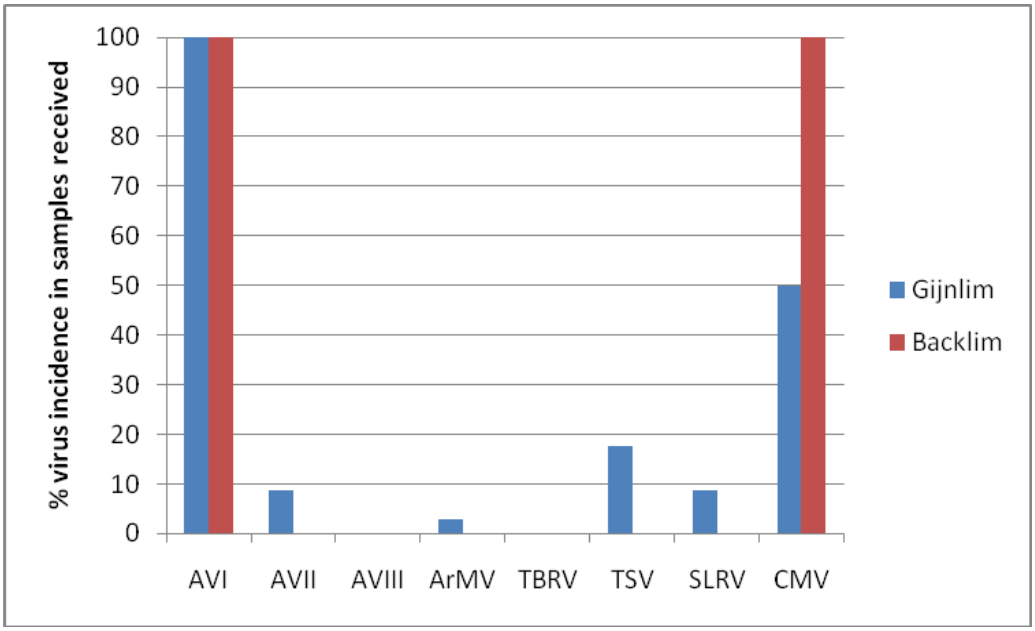


Similar levels of AV-I and CMV were detected in samples from field edges and centres. AV-II and ArMV were only detected in a few samples collected from the edge of the crops, whilst TSV and SLRV was only detected in small numbers of samples collected from crop centres.

The significance of these findings is not clear due to the relatively low rate of detection of virus in the sampled material.

Although fewer samples of cv. Backlim were received, the detail in Figure 1 shows that all Backlim crops were infected with both AV-I and CMV. No other viruses were detected in this cultivar. All cv. Gijnlim crops sampled were infected with AV-I, with 50% being infected with CMV. Four other viruses affected small percentages of Gijnlim (Figure 1).

Figure 1. The frequency of occurrence of viruses in the two cultivars sample



The table below is a summary of the incidence of viruses relative to the age of the crowns

Virus	Incidence
AV-I	Detected in all samples in each represented age of crop
AV-II	Only detected in 50% of the 2002 planted crops and approx 17% of the 2005 planted crowns.
ArMV	Detected only in the crops planted in 2002
TSV	Not detected in crops planted prior to 2003, but has been detected at varying levels in all planting years since then with the exception of the crops sampled from 2005.
SLRV	Detected at low to moderate levels in the samples from crops planted in 2003, 2006 and 2008.
CMV	All of the sampled crops which were planted in 2000 and 2002 were infected. The virus was also detected in sampled crops from 2003, 2005 and 2006 – but at a reduced incidence.

In addition the majority of the samples tested were found to contain more than one virus; all of the Backlim had AV-I and CMV, as mentioned previously. About 44% of the Gijnlim samples were infected with two viruses, 17% contained three viruses, and almost 3% were infected with four of the viruses tested for.

2) Sap inoculation tests

The sap inoculated plants were held in the glasshouse for 4 weeks and monitored regularly for the development of symptoms consistent with virus infection e.g. local lesions on inoculated leaves of mottling, mosaic symptoms or any other symptoms which might be consistent with the presence of AV-III. No symptoms suggestive of infection were observed on any of the plants.

Conclusions

The results from this initial short-term study suggest that AV-I and CMV are present in the majority of UK crops. The other four viruses detected, AV-II, ArMV, SLRV and TSV, were found in a smaller number of crops, and often at lower virus titres (concentrations). It is likely that many growers will be concerned by the findings reported, however the results should be considered in conjunction with the reported crop performance data, collected as part of the survey (see Table 1 in the full report). Crop performance at the time of sampling was described as 'good', 'moderate' or 'poor' by the growers. Previous knowledge and research suggests a possible correlation between the number of viruses present (i.e. multiple infections) and the susceptibility of the crop to other pathogens, e.g. fungal infection such as *Fusarium, Phytophthora* and *Stemphylium* spp., and the general decline of the crop resulting in poor fern growth and reduced yield.

The results found in this study, albeit on only 40 samples from 20 crops, do not show a correlation in this respect. In the study some crops carrying only one virus infection were reported to be performing only moderately or poorly, whilst other crops where up to four viruses were detected were reported to be performing well. Of course it also has to be taken into account that the various crops were all of different ages and this would also have an impact on their performance. We must therefore conclude that the presence of virus in the crop is not the only factor implicated in the reduction in crop performance.

Action Points

There is no action that growers can take to eradicate viral infection from these crops although some measures may be possible to prevent secondary spread, depending on the individual viruses.

Growers must purchase and plant virus free propagative material and should pay close attention to the presence of aphids in crops with a view to making regular insecticide applications to limit vector spread through the crop.

Improving hygiene standards can limit mechanical infection between crowns, although this is likely to be the hardest area to control, particularly during harvest operations. However, the additional cost of these actions must be off-set against the overall benefit to the crop in terms of increased yield and crop longevity.

SCIENCE SECTION

Introduction

Commercial asparagus crops once planted can remain in production for up to 10-15 years if well managed and maintained. However, some UK asparagus producers have reported a decline in yield and quality of their crops in recent years. A potential contributing factor of this yield reduction, generally referred to as asparagus decline, is the presence of virus within the crops.

Following an HDC funded literature search on asparagus viruses carried out in 1998 (FV 213) a recommendation was made for a survey of asparagus crops of different ages over 3 successive seasons to determine the presence, distribution and identity of viruses that are potentially responsible for the asparagus yield decline that has occurred in the UK. As far as is understood there have been no formal or detailed virus studies carried out in the UK previously. The aim of this study was to carry out a preliminary survey of UK asparagus crops during September to October 2010 to check for the presence of virus infection.

There are several viruses known to affect asparagus, although, unlike in many other crops, virus symptoms tend not to be exhibited e.g. there is no distortion or foliar mottling visible. However, it is reported elsewhere that infected crops may be weakened leaving crowns susceptible to other pathogens e.g. *Fusarium*, *Stemphylium* and *Phytophthora* spp. (Evans & Stephens 1989).

The 3 most important viruses of asparagus are considered to be *Asparagus virus I* (AV-I), *Asparagus virus II* (AV-II) and *Tobacco streak virus* (TSV). In addition, *Cucumber mosaic virus* (CMV), *Arabis mosaic virus* (ArMV), *Strawberry latent ringspot virus* (SLRV) and *Tomato black ring virus* (TBRV) are also known to infect asparagus. *Asparagus virus III* (AV-III) has also been reported on asparagus, but to-date has only been detected in Japan. It is also known that elsewhere crops can be infected with more than one virus and that a combination of viruses can reduce quality and yield significantly (Bandte *et al.*, 2008).

Details of viruses previously reported on Asparagus crops worldwide

Virus	Family	Transmission		
		Insect vector	Mechanical	Seed/Pollen -borne
Asparagus virus I	Potyvirus	✓ (aphid)	✓	X
Asparagus virus II	Ilarvirus	X	✓	✓
Asparagus virus III	Potexvirus	X	✓	X
Cucumber mosaic virus	Bromovirus	✓ (aphid)	✓	✓
Arabis mosaic virus	Nepovirus	✓ (nematode)	X	✓
Tobacco streak virus	Ilarvirus	✓ (thrips)	✓	✓
Tomato black ring virus	Nepovirus	✓ (nematode)	X	✓
Strawberry latent ringspot virus	Nepovirus	✓ (nematode)	✓	✓

This study employed two techniques for detecting viruses in a total of 40 collected fern samples. Initial investigation determined that a serological or an Enzyme-Linked Immunosorbent Assay (ELISA) was available for the viruses listed above with the exception of AV-III. A general Potyvirus assay was used to test for AV-1.

Sap inoculation tests were carried out on a range of indicator species to capture additional information specifically with regard to AV-III which could not be detected by ELISA techniques.

Materials & Methods

Sample collection

A total of 40 fern samples were collected in accordance with the sampling protocol (Appendix 1) from 6 to 8 September 2010. Twenty individual crops were offered by 5 different growers who participated in the investigation. Each grower provided 4 crops for sampling. Two samples per crop were taken, one from the edge of the crop and one from the centre. All crops chosen were over 2 years old. Sampled crops were given a unique identifying code and data regarding variety, planting year, soil type and pesticides applied were collated. Latex gloves were worn during sampling and changed between samples to avoid cross-contamination. Samples were held in cold storage and dispatched to STC in bulk for ELISA testing using 96-well plates.

ELISA testing

Samples were tested using ELISA reagents supplied by Agdia-Biofords and Neogene Europe. Protocols for the work and reagents varied slightly from one virus test to another. However, a generalised method is detailed below.

Materials: Sample bags with mesh liner
Extraction buffer
96-well ELISA plates
Positive & Negative control material from a suitable host
Phosphate Buffered Saline + Tween (Wash buffer)
Antibody reagent
Enzyme conjugate
PNP Buffer
PNP substrate
Micropipette + sterile tips
Dynex Opsys MR plate reader
Tecan Columbus plate washer

Method

1. Field samples were uniquely numbered from 1 - 40. Sub-samples of fern were collected aseptically and placed in the ELISA sample bags.
2. Extraction buffer was added and the sample was macerated.
3. Sap from the test samples along with the positive and negative controls were pipetted into duplicate wells in a pre-coated ELISA plated in a designated sample layout.
4. The test plates were incubated for the required time.
5. Sample plates were washed and the antibody reagent was added prior to a further incubation period.
6. The test plates were washed and coated with the conjugate reagent and incubated.
7. The test plates were washed and the PNP substrate was added prior to final incubation.
8. The absorbance of the test plates was read using an Opsys plate reader at 405nm wavelength.
9. The absorbencies were interpreted using the following threshold values:

Values	**Threshold equation**	**Interpretation**
Negative	Under 2 x the mean value of negative control wells	negative response
+ Positive	2 x the mean value of negative control wells	positive response
++ Positive	3 x the mean value of negative control wells	strongly positive response
+++ Positive	4 x the mean value of negative control wells	very strongly positive response.

This interpretation provided a quantification of the virus titre within samples. The background absorbance values provided from the blank (buffer only) wells was subtracted from the remaining well values prior to reading.

Sap inoculation

Materials: Sap from samples
Celite (diatomaceous earth)
Cotton buds
Indicator plants. Species used:
Cucumis sativa (cucumber)
Gomphrena globosa (globe amaranth)
Nicotiana glutinosa
Nicotiana tabaccum (tobacco)
Petunia hybrid (petunia)

Method

1. The indicator plants were sown and potted into 9cm pots prior to inoculation.
2. A suitable leaf was chosen and abraded (worn down through rubbing) gently with Celite using a cotton bud.
3. The test sample sap was applied to the abraded area using a fresh cotton bud.
4. The inoculated plants were labelled and maintained in a glasshouse for 4 weeks to allow time for symptom development to occur.

Results

Sample collection information

Samples were collected from 20 crops, with 2 samples taken per crop.

15% of samples collected were cv. Backlim whilst the remaining 85% were cv. Gijnlim

All asparagus crowns used originally to establish the crops had been imported from the Netherlands.

The youngest crop sampled was planted in 2008, with the oldest having been planted in 2000.

Virus Testing Results

The results shown in the tables and charts which follow indicate that the most commonly detected viruses were AV-I and CMV. When either of the viruses was detected they were present at high concentrations. Only small numbers of crops were found to be infected with AV-II, ArMV, TSV and SLRV. No *Tomato black ring virus* was detected in any crop. We did not observe symptom development consistent with AV-III in the sap inoculated indicator plants.

Plate 1: Example of ELISA plate test; yellow colouration indicates a positive response.

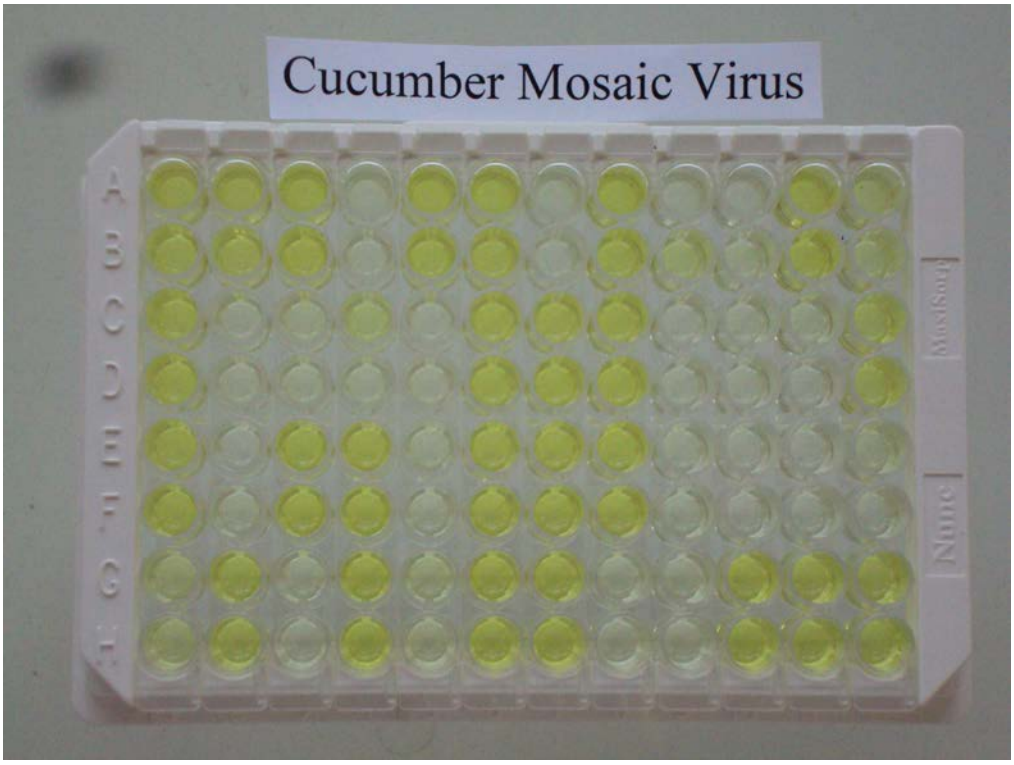


Table 1. Virus testing results

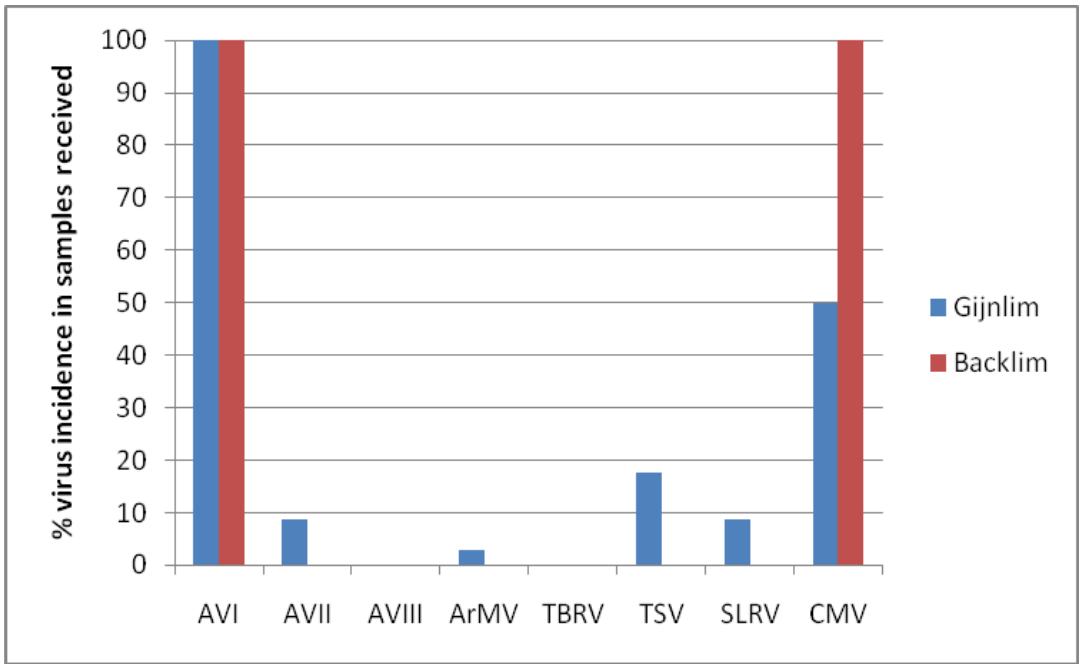
Crop No.	Results from field edge								Crop No.	Results from field centre								Year of planting
	AVI	AVII	AVIII (SAP)	ArMV	TBRV	TSV	SLRV	CMV		AVI	AVII	AVIII (SAP)	ArMV	TBRV	TSV	SLRV	CMV	
1	+++	-	-	-	-	-	-	+++	1	+++	-	-	-	-	-	-	+++	2006
2	+++	-	-	-	-	-	-	-	2	+++	-	-	-	-	-	-	-	2005
3	+++	-	-	-	-	-	-	+++	3	+++	-	-	-	-	-	-	+++	2006
4	+++	-	-	-	-	-	-	+++	4	+++	-	-	-	-	-	-	+++	2003
5	+++	-	-	-	-	-	-	-	5	+++	-	-	-	-	-	-	-	2004
6	+++	-	-	-	-	-	-	+++	6	+++	-	-	-	-	-	-	-	2005
7	+++	+++	-	-	-	-	-	+++	7	+++	-	-	-	-	-	-	+++	2000
8	+++	-	-	-	-	-	-	-	8	+++	-	-	-	-	-	-	-	2004
9	+++	++	-	-	-	-	-	+++	9	+++	-	-	-	-	-	-	+++	2005
10	+++	-	-	-	-	-	-	+++	10	+++	-	-	-	-	-	-	+++	2006
11	+++	-	-	-	-	-	-	+++	11	+++	-	-	-	-	-	-	+++	2006
12	+++	-	-	-	-	-	-	+++	12	+++	-	-	-	-	+++	-	+++	2006
13	+++	+++	-	+	-	-	-	+++	13	+++	-	-	-	-	-	-	+++	2002
14	+++	-	-	-	-	-	-	-	14	+++	-	-	-	-	+++	-	-	2004
15	+++	-	-	-	-	-	-	-	15	++	-	-	-	-	+++	-	-	2006
16	+++	-	-	-	-	-	-	-	16	+++	-	-	-	-	++	-	-	2007
17	+++	-	-	-	-	-	-	+++	17	+++	-	-	-	-	+++	+	-	2003
18	+++	-	-	-	-	-	-	-	18	+++	-	-	-	-	+++ ?	+?	-	2008
19	+++	-	-	-	-	-	-	+++	19	+++	-	-	-	-	-	+	+++	2006
20	+++	-	-	-	-	-	-	-	20	+++	-	-	-	-	-	-	+++	2006

Key	
-	negative response
+	positive result
++	strongly positive result
+++	very strongly positive result

The data in Table 1 indicate that similar levels of AV-I and *Cucumber mosaic virus* were detected in samples from the edge and centre of the crops sampled. AV-II and *Arabis mosaic virus* were only detected in a few samples collected from the edge of the crops, whilst *Tobacco streak virus* and *Strawberry latent ringspot virus* was only detected in small numbers of samples collected from the central area of crops. The significance of these findings is not clear due to the relatively low rate of detection of virus in the sampled material.

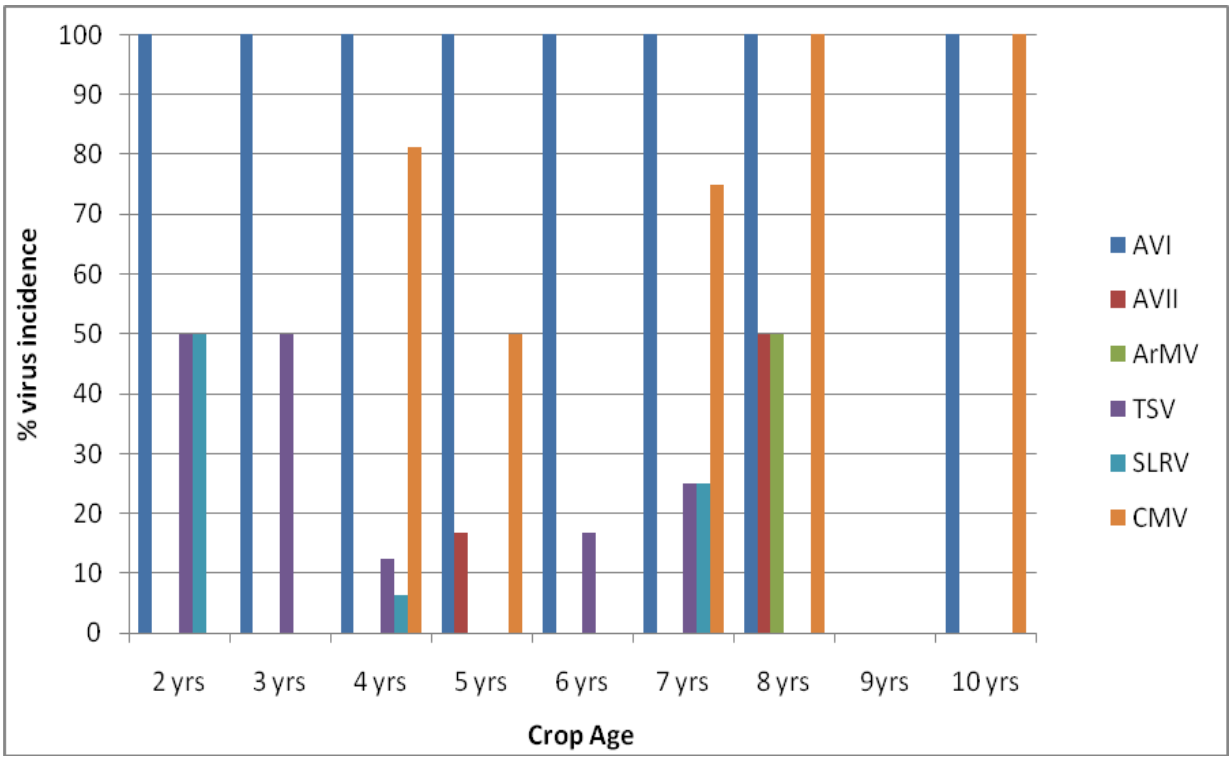
Although fewer samples of cv. Backlim were received, the detail in Figure 1 shows that all Backlim crops were infected with both AV-I and CMV. No other viruses were detected in this cultivar. All cv. Gijnlim crops sampled were infected with AV-I, with 50% being infected with CMV. Four other viruses affected small percentages of Gijnlim (Figure 1).

Figure 1. Showing the frequency of occurrence of viruses in the two cultivars sampled



Samples were collected from crops with crowns of various ages, the oldest having been planted 10 years ago and the youngest 2 years ago. No samples were collected from crops planted in 2001.

Figure 2. Incidence of virus in crops relative to the age of crowns

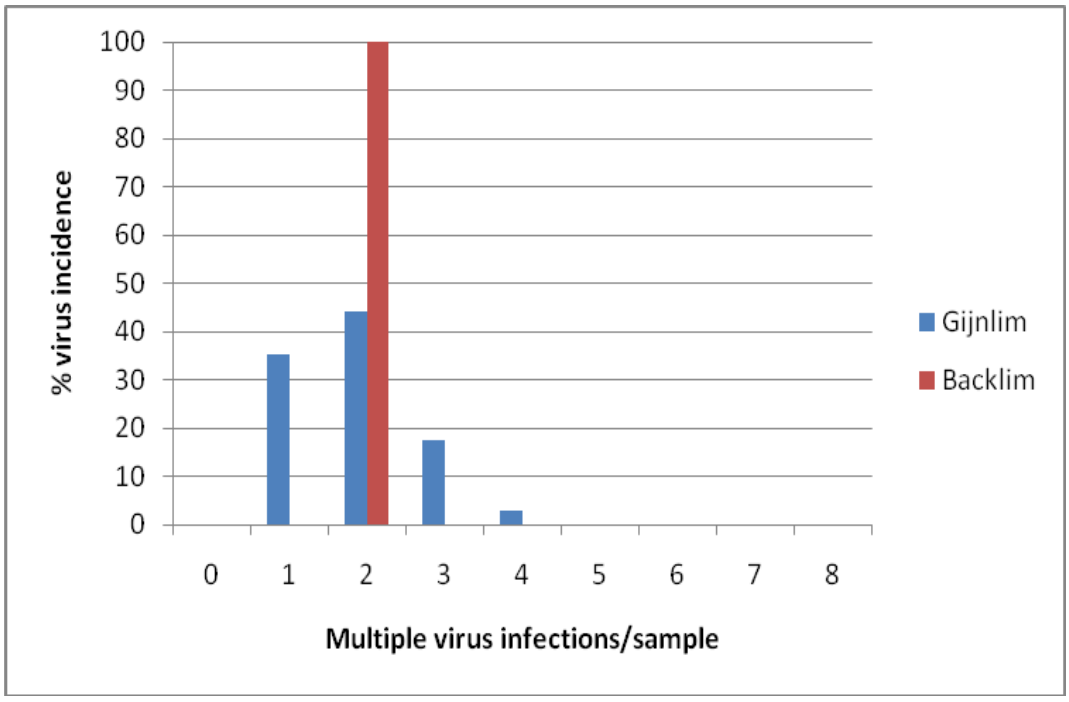


Summary of data on crop age (from Figure 2)

Virus	Incidence
AV-I	Detected in all samples in each represented age of crop
AV-II	Only detected in 50% of the 2002 planted crops and approx 17% of the 2005 planted crowns.
ArMV	Detected only in the crops planted in 2002
TSV	Not detected in crops planted prior to 2003, but has been detected at varying levels in all planting years since then with the exception of the crops sampled from 2005.
SLRV	Detected at low – moderate levels in the samples from crops planted in 2003, 2006 and 2008.
CMV	All of the sampled crops which were planted in 2000 and 2002 were infected. The virus was also detected in sampled crops from 2003, 2005 and 2006 – but at a reduced incidence.

The majority of the samples tested were found to contain more than one virus (Figure 3). All of the Backlim samples tested were infected with two viruses, AV-I and CMV. Approximately 44% of the Gijnlim samples were infected with two viruses, whilst 33% contained only AV-I. Approximately 17% contained three viruses, and almost 3% were infected with four of the viruses tested for.

Figure 3. Incidence of multiple virus infections by cultivar



Sap Inoculation Tests

The sap inoculated plants were held in the glasshouse for 4 weeks and monitored regularly for the development of symptoms consistent with virus infection e.g. local lesions on inoculated leaves of mottling, mosaic symptoms or any other symptoms which might be consistent with the presence of AV-III. No symptoms suggestive of infection with AV-III were observed on any of the plants.

Conclusions

The virus tests were carried out on 20 geographically dispersed UK crops (2 samples per crop) collected from growers situated in the principal growing areas for this crop. No virus testing on a large scale had previously been carried out on UK asparagus crops and the aim of this study was to assess the levels of virus infections present in crops and to consider the impact of these findings on crop performance, e.g. yield, fern quality, disease susceptibility.

The results from this initial short-term study suggest that AV-I and CMV are present in the majority of UK crops. The other four viruses detected (AV-II, ArMV, SLRSV and TSV) were found in a smaller number crops, and often at lower virus titres (concentrations). It is likely that many growers will be concerned by the findings reported, however the results should be considered in conjunction with the reported crop performance data, collected as part of the survey (see final column in Table 1). Crop performance at the time of sampling was described as 'good', 'moderate' or 'poor' by the growers. Previous knowledge and research suggests a possible correlation between the number of viruses present (i.e. multiple infections) and the susceptibility of the crop to other pathogens, e.g. fungal infection such as *Fusarium, Phytophthora* and *Stemphylium* spp*.,* and the general decline of the crop resulting in poor fern growth and reduced yield. The results found in this study, albeit on only 40 samples from 20 crops, do not show a correlation in this respect. In the study some crops carrying only one virus infection were reported to be performing only moderately or poorly, whilst other crops where up to four viruses were detected were reported to be performing well. Of course it also has to be taken into account that the various crops were all of different ages and this would also have an impact on their performance. We must therefore conclude that the presence of virus in the crop is not the only factor implicated in the reduction in crop performance. Other factors are likely to include presence of other pathogens, soil structure, quality, fertility and crop vigour.

The presence of virus in the asparagus crops tested is now becoming clear. There is no action that growers can take to eradicate infection from these crops, although some measures may be possible to prevent secondary spread, depending on the individual viruses. We know that the majority of the viruses tested for, with the exception of AV-II and AV-III, can be transmitted by aphid, thrips or nematode vectors, that all can be transmitted mechanically and also that several can be present in seed or pollen. It is possible to take precautions to purchase and plant virus free propagative material and to pay close attention to the presence of aphids in crops with a view to making regular insecticide applications to limit vector spread through the crop. Improving hygiene standards can limit mechanical infection between crowns, although this is likely to be the hardest area to control, particularly during harvest operations. However, the additional cost of these actions must be offset against the overall benefit to the crop in terms of increased yield and also in crop longevity.

An important and possibly significant result is the very low incidence of AV-II in the crops sampled. Much of the reported literature suggests that the presence of AV-II, whether singly or in multi-virus infections can have the biggest impact on reduction of both yield and crop

life. In our tests this virus was only found in 7.5% of the samples which is potentially good news for UK growers.

It is important that this first round of testing is considered as providing baseline data on virus incidence in UK asparagus. Greater value will be gained by carrying out repeat testing on the same crops, and by increasing the sampling regime.

Data may be able to be gathered on varietal susceptibility, crop age, performance and a link to yield data for sampled crops may also provide a method of quantifying the effect of virus if sampling were repeated over a number of seasons.

Recommendations for further work

- Similar testing should be repeated on the same crops in future seasons to measure whether the incidence of individual viruses is increasing.
- If additional work is undertaken the sample number should be increased.
- If possible virus testing on other cultivars should be undertaken to investigate the potential for natural virus resistance.
- Linking future virus results with yield data will provide a quantifiable measure of the potential impact on virus in crops if regular virus testing is undertaken.
- Consider the possible need for further insecticide approvals to control aphids.

Acknowledgements

STC would like to thank the project co-ordinator Peter Knight for his guidance and assistance with this project. Thanks also to the growers who participated in this study by providing samples and crop details.

References

Bandte M, Grubits E, Schmidt C, Weber D, Uwihs F & Buettner C. (2008) Occurrence of viruses in asparagus (*Asparagus officinalis* L) in North Germany. In *1st Symposium on Horticulture in Europe, Vienna 2008*, International Society for Horticultural Science. p192.

Evans T.A, and Stephens C.T. (1989) Increased susceptibility to Fusarium Crown and Root Rot in Virus Infected Asparagus. *The American Phytopathological Society*.(Vol 79, No. 3).