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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were 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.

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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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GROWER SUMMARY

Headline

Second year trials have confirmed the presence of *Asparagus virus I* (AV-I) and *CMV* as the main viruses in UK asparagus crops. Older crops had more viruses than younger crops but virus presence did not appear to reduce yield nor affect crop performance.

Background

Commercial asparagus crops can remain in production for 10-15 years. However, UK growers have reported a noticeable decline in the yield and quality of their crops in recent years. This project was proposed by the Asparagus Growers' Association to investigate possible causes for this observed decline. A brief review of the literature relating to a phenomenon known as 'asparagus decline' (AD) was carried out in 2010 which identified this asymptomatic disorder e.g. no obvious foliar symptoms, as a possible cause of yield loss. An earlier HDC study (FV 151) investigated the use of salt application over a 4 year period following reports from elsewhere that soil applied salt was effective in reducing asparagus decline and increasing yields. The study reported a consistent trend towards benefits to the asparagus crops from a split dose salt application at the 0.5 to 1.0 t/ha rate. However, the differences observed were not always statistically significant ($p=0.05$). The report concluded that, as a long-term project, treatment differences could be expected to develop over a prolonged number of years. It is understood that whilst salt is still used by a few growers its popularity has declined in recent years due to difficulties associated with the availability of suitably formulated products.

An initial study was carried out in autumn 2010 to investigate the incidence and frequency of a range of 7 viruses in collected fern samples from UK crops (FV 384). The results showed a very high incidence of AV- I and CMV in the crops tested, with a number of other viruses being detected at lower frequencies. Multiple virus infections were common. Growers were asked to rate the performance of each crop as 'poor', 'moderate' or 'good' though the virus incidence results were found not to correlate well with recorded crop performance. Growers were left unsure as to what the consequences or impacts of the virus results were.

A second study was commissioned in 2011 to review the literature pertaining to 'asparagus decline' in greater depth, carry out additional virus testing and to try and determine the possible consequences of the virus infections via analysis of yield data from the crops sampled.

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Summary

Literature Review

A thorough search of the scientific literature relating to 'asparagus decline' (AD) was carried out. The potential causes of AD were identified by a number of researchers as overuse of herbicides, overharvesting, production of allelopathic compounds by asparagus roots, fungal diseases of roots, crowns and foliage along with infection by viruses (Shelton & Lacy, 1980; Schofield, 1991).

Many researchers have demonstrated that young asparagus crops inoculated with multiple viruses show reduced vigour and yield. Knaflewski (2008) described the situation where viruses and fungi form a 'disease complex' and lead to AD. He also reported a correlation between crop age and virus incidence and confirmed that all asparagus viruses could be mechanically transmitted during harvest, stating that this is likely to be to main mechanism of virus infection. Pawlowski & Fiedorow (2002) also confirmed that virus could be spread via natural root grafting in the soil. Asparagus virus 2 (AV-II) alone can be transmitted by seed, work done by Falloon (1986), Bertacchini (1990) and Fiedorow (2002) detected varying levels of seed-borne infection ranging from 0-25%, although very much higher levels had been reported during the 80s.

Much work has been done investigating the role of *Fusarium* and *Phytophthora* infections in AD. It has been shown that virus infected plants are more susceptible to fungal infections by *Rhizoctonia solani*, *Fusarium oxysporum* and *Phytophthora* spp. and that this can be linked to seedling survival as well as crop yield and vigour.

Crop establishment problems following replanting of new crops into land previously affected by AD was also discussed by Grogan & Kimble (1959); Conroy (1975); Young (1984), and Huiskamp & Kanters (1989). This was initially attributed to high levels of *Fusarium* inoculum in the soil, but later work by Young &, Chou (1985) and, Hartung & Putnam (1986) suggested that asparagus tissues and plant residues present in the sites contained toxic substances that inhibited young asparagus plants.

There are no available methods to remove virus from infected crops, therefore prevention or limitation of infection are the only means available to growers. The use of virus-free seed or propagation material is the first step. Careful harvesting, crop monitoring and good geographical spread between plantings can also help.

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Virus testing results for 2011

A total of 74 samples, collected from 37 crops were collected and tested for 4 viruses (AV-I, AV-II, CMV and TSV) in 2011. Samples were collected from sites spread between Somerset in the South and Angus in the North. AV-I was found to be present in **all** crops tested and should therefore be considered virtually ubiquitous in UK crops. CMV was found in 63% of samples, whilst AV-II was only detected in 27% and TSV in 7% of samples.

No firm correlations between cultivar susceptibility or location was observed. There was however a strong indication that crop age appeared to be significant, with crops between 5 and 9 years old being found to be most heavily infected with multiple viruses.

Re-testing of 10 crops that were originally tested in 2010, showed a worrying 43% increase in the presence of virus in these crops. However, perhaps surprising given information in the scientific literature, there did not appear to be a correlation between virus incidence and reported crop performance or yield. It is important to note though that yield data was only available on approximately half the tested crops, and generally only as total yield with little data provided regarding the weight of Class 1 and 2 spears. It is possible that more detailed yield data may have shown a significant reduction in crop quality in infected crops, particularly those with multiple infections.

Financial Benefits

No immediate financial benefits will result directly from this work. However, it is hoped that increased knowledge and understanding of the current science and the state of virus infections in UK crops should help growers to make more informed decisions regarding asparagus production in the future and thus mitigate any potential loss of marketable yield or crop quality from such virus infection.

Action Points

- Source new planting material from the most reputable and certified propagators to ensure virus-free healthy material.

- Consider new crop locations e.g. planting in land with a previous history of AD, or close to old or failing crops should be avoided if possible as this can lead to poor establishment and rapid infection with viruses and/or fungal pathogens.

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- Keep good yield data records to permit individual monitoring of crops for any signs of failure.

- Consider additional surveying of UK crops on a regular basis to ensure there is a good understanding of the current state of virus infection in asparagus crops.

- Maintain good soil structure and try to reduce the risk of infection with fungal pathogens by the use of suitable fungicides and limit all stress-causing factors in crops as these can increase the risk of AD in crops.

- Insecticides currently in use by growers are used primarily for the control of asparagus beetle. It is worth considering though that as some viruses may be introduced and spread by vectors e.g. aphids or thrips then insecticide application may help slow down virus transmission in crops. They are unlikely to prevent initial infection though as some viruses are transmitted in a non-persistent manner (that is, they acquire and transmit the virus after a very short feeding time) and effective control with chemical application is much less effective in these situations. However, growers may perhaps need to consider seeking SOLAs for insecticides specifically for aphid control.

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Literature Review

Asparagus production

Although enjoyed by the Romans and perhaps originally introduced to Britain at this time, asparagus production in the UK is not thought to have begun commercially until around 1540. Production in the UK is almost entirely of green asparagus, with low production levels of white and purple cultivars. Asparagus is also produced across Europe, Asia, Africa and North and South America, Australia and New Zealand. In 2005 China was the world's leading producer of asparagus producing almost 6 million tonnes, whilst Peru produced approximately 200 thousand tonnes and the US 90 thousand tonnes.

DEFRA report that the area of land under asparagus production in the UK in 2009/10 was approximately 1,580 hectares, with a production value in 2009 of approximately £14.5M. The majority of asparagus planting undertaken currently uses crowns produced in the Netherlands, although there is a renewed interest in using UK produced crown material.

Asparagus crowns are produced from seed either in modules or in open field situations. Once planted, crowns generally begin to produce a marketable crop when crowns are 2-3 years old. Some literature suggests that asparagus plantations should remain economically viable for up to 20 years, although a general productive period of 10-20 years is more accepted. In temperate climates, asparagus crops reach peak production after 5-8 years and slowly weaken thereafter so that the commercial life is c. 10-20 years (Johnston *et al.* 1979, Falloon & Tate, 1986; Bussell & Ellison, 1987). However, in crops around the world a disorder termed 'asparagus decline' has been reported which is reducing the economic viability of crops to 5-8 years in many instances.

Asparagus decline

AD was defined by Grogan and Kimble (1959) as "a slow decline in the productivity of old asparagus plantings … to the point where the plantings become unprofitable to maintain". Since this early work was undertaken much research into the causes, interactions, impact and possible control strategies has been undertaken by scientists around the world.

The symptoms of AD are described as

- Reduced number of fern stalks and thinner stems.
- Spears may shrivel or wilt at any stage of growth.
- Ferns are usually yellowed and stunted with reddish brown vascular discolouration and rusty flecks or lesions on the external layers of the lower portion of fern stalks.

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- Reddish colouration may extend into crowns from fern sockets or from discoloured fleshy roots.
- Feeder roots may be absent or shrivelled and where present, show discoloured reddish areas at the junction with fleshy storage roots.
- Some of the fleshy roots show vascular discolouration and many collapse completely. Soft rot bacteria or secondary organisms are usually associated with such a collapse.

Factors which are thought to cause stress in asparagus plants and to contribute to decline include tillage, overuse of herbicides, overharvesting, production of allelopathic compounds by asparagus roots, fungal diseases of roots, crowns and foliage, as well as infection by viruses (Shelton & Lacy, 1980; Schofield, 1991).

Impacts of virus infection

Over the last 50 years there has been much research carried out which investigated the presence and impact of virus in asparagus. (*Asparagus officinalis* L.). Much of the work has been carried out in North America and Europe; both areas of high asparagus production. The first report of the presence of virus being linked to a lack of vigour in plants was made by Weissenfels & Schmelzer (1976).

Asparagus is host to several viruses. AV-I, AV-II, Asparagus virus 3 (AV-III), Cucumber mosaic virus (CMV), Tobacco Streak Virus (TSV), Arabis Mosaic virus (AMV) and Strawberry Latent Ring-spot Virus (SLRV). Distribution of the different viruses hosted by asparagus varies geographically. It has been shown that crowns may be infected with more than one virus. Often crops infected with virus show little or no obvious symptoms as noted by Knaflewski *et al* (2008).

In a study carried out by Yang (1979) in Washington USA, young plantlets were cloned from virus-free, AV-I infected, AV-II infected and AV-I + AV-II infected plants. The 4 groups of cloned plants were grown on for 6-8 months in a glasshouse, where no differences in growth were observed, before being planted in separate field sites and monitored for 16 months. Following this time the results shown in Table 2 were observed.

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Table 2. Effects of asparagus virus 1 and Asparagus virus 2 on asparagus plant survival and vigour after 16 months in the field (Yang, 1979)

Virus infection	No. of plants	No. of survivors	Height (cm)	No. of stems	Fresh weight (g)	Dry weight (g)
None	30	28	119.4	7.6	216.0	103.5
AV-I	35	31	112.5	5.0	157.5	72.0
AV-II	30	26	116.8	6.0	184.5	94.5
AV-I + AV-II	28	16	94.7	4.6	81.0	31.5

(from Yang, 1979)

Yang noted that AV-I appeared to be slightly more debilitating than AV-II when single infections occurred. Also those plants infected with both viruses showed much greater growth retardation effects than single virus infections.

Studies by Jaspers & Falloon in 1999 showed that the incidence of AV-II in a trial crop in New Zealand reduced marketable yield significantly ($P<0.05$), whilst yield from non-AV-II infected plants increased year-on-year. The number of spears from infected plants which were ≤8mm diameter was significantly greater each year (by 109%, 88%, 220% 499% and 216% in 1992-96 respectively) from AV-II infected plants than from AV-II free plants. They also noted that there was a reduction in fern number and size. Robb (1984) showed that plants with smaller fern stand accumulate less photosynthate, resulting in reduced carbohydrate reserves and reduced bud development in the following season. Cycles of reducing productivity develop, in which size and number of buds, spears and fern stalks decrease annually and this may be the a mechanism caused by the presence of AV-II.

Knaflewski *et al* (2008) carried out research at the University of Poznan in Poland and reported the results of his research and a summary of work undertaken by other researchers worldwide. He stated that “very often viruses form a complex together with fungi and they are undoubtedly one of the most important biotic factors of asparagus decline”. He records that, as virus is stored in the crown material, once infected a plant remains infected for its whole life and becomes the source of infection for other plants. In his research they found a total of 11 viruses (the 8 most frequently detected viruses were investigated in the previous (2010) study) capable of infecting asparagus, with the most common being CMV, AV-II and AV-II in Europe, and AV-I being the most commonly detected virus in the USA. Hartung *et al*, (1985) reported a correlation between crop age and virus incidence, increasing from 35% in 0-2yr old crops to 69% in crops 16-29 years old (AV2 in Michigan USA).

Blaszczak and Weber (1979) found that it was not possible to prove that infection of asparagus by fungi increased the chance of virus infection. They stated that roots infected

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with fungi often limits virus transmission because most fungi secrete antiviral compounds. Also, necrotic and rotted tissues of plants cannot be infected by viruses.

Knaflewski (2008) states that whilst visible symptoms of virus are often difficult to see, plants are sometimes smaller, produce lower yields and are less vigorous. Yield reductions can range from 8 -30%, but where there is infection with a complex of 2 viruses, reduction by 70% has been observed. However, it is difficult to distinguish the effect of viruses from the effect of fungal infections and abiotic factors except in controlled experiments. The presence of virus in plants can also affect the quality of spears. Jaspers (1996) found, on average, in a two-year experiment, 100% more bent and 78% more thin spears harvested from plants infected with AV-II. The percentage of spears with open heads was also higher from the infected plants. Further work by Fiedorow (2001) and Jaspers (1996) showed that the longer the period that the plants are infected for, the greater the yield reduction. In the 1st year of harvest yield was reduced by 19% and in the 2nd year by 31%.

Virus Transmission

According to Knaflewski (2008), all viruses occurring in asparagus can be transmitted mechanically during harvest by inoculation with the sap of infected plants. This is probably the main mechanism of virus infection.

Studies carried out by Kegler *et al* (1991), Gasiorowska (1998) and Pawlowski (2002) demonstrated that 1/3 of the plants harvested with an infested knife became infected. Pawlowski & Fiedorow (2002) also found that virus can be spread by natural root grafting. Transmission of AV-II through roots in pot experiments did occur in 75% of the cases between 12-22 months.

Only AV-II is known to be transmitted by asparagus seed. This varies from 0-25% infection in some seed batches (Falloon *et al* 1986; Bertacchini *et al* 1990; Fiedorow *et al*, 2000). AV-II is seed-borne because it can be carried in pollen by bees during feeding and pollination. Jaspers & Falloon (1999) carried out studies in New Zealand to investigate other methods of transmission by which AV2 (and other viruses) could be spread in a crop. They planted seedlings of the cultivar UC157 in a field in 1990. Two AV-II infected plants were sown in each row, with 8 virus-free plants on either side (24 rows). Spears were harvested between 1992 and 1995. At the start of each row the cutting knife was disinfected by soaking for 1 minute in a 3% w/v solution of trisodium phosphate. Each row was cut in one direction only over the life of the trial. One block of 12 rows was cut in the direction of the prevailing wind

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and the other block was cut against the prevailing wind. This created 4 treatments whereby the effect of transmission by knife could be separated from other factors e.g. windblown pollen. They found that the incidence of AV-II infection in plants increased annually, but was significantly greater ($P<0.5$) in areas cut after the initial AV-II infector plants, than in areas cut before the infector plants. By 1995 AV-II incidence was 96.5% in areas cut after source plants compared to 26% in areas cut before. They found that spread caused by wind was less significant.

More recent studies (Anon.) which carried out testing on seed stock plants in the USA for AV-II found that the incidence of infection had reduced dramatically from previously reported levels in the 80s (100%) to only 25% in 2005. This is thought to be due to raising seed plants using tissue culturing of meristems and also because harvesting in Michigan is carried out by snapping rather than cutting.

AV-I and CMV can be transmitted in a non-persistent[1] way by aphids and AV-II and TSV also by pollen. CMV infection can come from other plants, e.g. cucumber, tomato, other vegetable crops and numerous weed species. TSV can occur on clover, bean, soybean and strawberry, among others and is transmitted mostly by thrips. Work by Fiedorow & Pawlowski (2002) has also shown that AV-II can be transmitted by spores of asparagus rust (*Puccinia asparagi*).

AV-III can be transmitted only mechanically with the sap of infected plants (Fujisawa, 1986; Brunt *et al*, 1996) and is still only reported in Japan.

Impacts of Virus & *Fusarium* infections and other fungal pathogens of asparagus

In all situations where AD is reported, isolations have confirmed that *Fusarium* infections are associated with the disorder (Grogan & Kimble 1959, Conroy 1975; Johnston *et al.,* 1979; Fantino, 1990). Work done by a number of researchers indicated that *Fusarium oxysporum* f. sp. *asparagi* and *F. moniliforme* are often considered to be part of the complex. *F. oxysporum* is a wilt pathogen that has been isolated from vascular root tissue, cortical root lesions, crown and stem lesions, and stem vascular tissue. *F. moniliforme* is less commonly isolated from roots but has been frequently isolated from stem and crown tissue where it often causes a more invasive, dry, brown rot than *F. oxysporum* (Schofield, 1991).

[1] Non-persistent transmission involves the virus particles being carried on the stylet tip of the vector. The virus is then transmitted to the next plant visited by the vector. The virus particles do not enter the gut of the vector and cannot replicate within the vector. The vector only carries the viable virus (viruliferous) for a short period of time if further feeding on a suitable host does not occur.

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Work carried out by Evans & Stephens (1989) showed that AV-II or AV-I + AV-II infected plants were more easily infected *by Fusarium oxysporum* f. sp. *asparagi*. Pawlowski (2002) found the same to be true of *Rhizoctonia solani*. Plants infected with AV-I and AV-II had an 85% higher infection rate than virus-free plants. Joint fungal/viral infections were found to impact greatly on seedling survival rates (Pawlowski 2002 & 2004). Evans & Stephens (1989) and Pawlowski (2002 & 2004) postulated that virus infected roots produce more monosaccharides and other exudates and that this might attract fungi, allow easier germination of fungal spores and also that asparagus roots were less resistant to infection. Other mechanisms proposed for the increased fungal root rot induced by virus infection are that the inherent susceptibility of root tissue is increased by virus infection, and that increased leakage of nutrients from roots of virus-infected plants increases the inoculum potential of the fungi in the rhizosphere (Beute, 1970). In general it is considered that *F. oxysporum* f. sp. *asparagi* plays a more significant role in AD than *F. moniliforme*. Evans & Stevens (1989) conducted a number of studies using seed and seedlings inoculated with combinations of AV-I, AV-II, and the two *Fusarium* species. They found that seedlings which were inoculated with AV-II and *F.oxysporum* f. sp. *asparagi* became significantly more diseased than AV-II infected seedlings inoculated with *F. moniliforme*. Seedlings infected with either virus and *F. oxysorum* f. sp. *asparagi* became more diseased than virus-free seedlings and also that seedlings doubly infected with both AV-I & AV-II were more diseased when inoculated with *F.oxysporum* f. sp. *asparagi* than seedlings with a single virus infection. They also carried out work on the exudates produced by virus infected seedlings. They found that seedlings infected with AV-II and grown in a liquid culture released 3 times more electrolytes in a 24-hour period than virus-free seedlings. When tested they found the seedlings released more glucose (3x), total carbohydrates (1.7x) and amino-acids (8x) than virus free plants. When aliquots of the root exudates from AV2 infected and healthy seedlings were added to water agar, significantly greater numbers of microconidia of *F.oxysporum* f. sp. *asparagi* and *F. moniliforme* germinated on the media supplemented with the exudate from the virus infected seedlings. They also found that in virus infected seedlings, the ability of the roots to produce lignin was significantly reduced. Lignin production in plants provides a method of ‘walling-off’ areas of damage or infection, so reducing the spread of pathogens. Reducing the plant’s ability to do this would result in more severe levels of infection of plant tissues.

An additional problem associated with AD is that of replanting problems on land where decline symptoms have been prevalent. Reports from Asia, Australia, North America and Europe all indicate that replanting land with asparagus leads to establishment failure and reduced plant vigour; and where re-establishment is achieved, the economic life of the

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stand is shorter than with fields established on land that has not previously been in asparagus production (Grogan & Kimble 1959; Conroy, 1975; Young, 1984; Huiskamp & Kanters 1989). Initially researchers attributed the replanting problems to the presence of *Fusarium* inoculum in the soil, as many of the symptoms seen in failing plants were similar to those observed in AD and crown and stem rots caused by *Fusarium* (Grogan & Kimble 1989). Yang (1982) carried out work investigating the effect of soil fumigation on replant disease i.e. reducing *Fusarium* inoculum in the soil. He found that there were some initial benefits, but that the effect was short-lived and the problem persisted. Later work has shown that asparagus tissue and asparagus plant residues contain a toxic substance that is inhibitory to asparagus plants (Young & Chou 1985; Hartung & Putnam 1986; Hartung *et al* 1989).

The root pathogen *Phytophthora megasperma* var. *sojae* has also been noted as a cause of death of asparagus plants, particularly in wet conditions, although it is not often linked directly to AD. It is, however, linked to problems during replanting when young seedlings can be particularly susceptible to infection by oospores in the soil (Falloon & Fraser, 1991).

Other fungal pathogens of asparagus have been reported as being associated with AD. Cooperman *et al* (1986) identified *Cercospora asparagi* (*Cercospora* blight), which can cause the development of lesions at the base of the fern which progress upward in warm and humid conditions. Blighted ferns turn yellow to brown and eventually die prematurely. Purple spot (caused by *Pleospora herbarum*) also contributed to AD by damaging ferns during the growing season, causing defoliation. The destruction of photosynthetically active fern tissue reduces the potential photosynthate translocated to the crown and storage roots (Elmer *et al*; 1996).

Prevention of virus infections in asparagus

Once infected with one or more viruses no treatments are available to remove the infection. Therefore prevention of infection is the only way that growers can control virus levels in crops. Clearly, the first step is to ensure that virus-free seed or crown material is used for new plantings. Seed producers should take steps to inhibit infection of seed crops, e.g. by using plants raised by meristem tissue culture rather than using already infected seed, and also by protecting seed-raising crops from potential insect vectors, by careful harvesting, and also by good geographical spread between older and new plantings.

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Commercial growers should monitor crops for potential virus-carrying aphid and thrips vectors, although this is unlikely to stop insect transmission completely, it may reduce rate of spread through the crop, and a reduction in the incidence of virus reduces rate of spread by insects as there is a lower probability of them collecting virus-infected sap from one plant and transferring it to another. Growers should plant new crops as far as possible from older, possibly already virus-laden crops and should consider adopting less transmissive harvesting techniques, such as snap harvesting or using knife dips between plants, although these option may have impacts on rate of harvest. Work by Fiedorow & Pawlowski (2002) (Table 3) showed that very simple knife cleaning treatments could have a very significant effect on the transmission of AV2 in indicator plants (*Chenopodium murale*).

Table 3. Effect of knife treatment during spear cutting on the infection by Asparagus virus 2

Treatments	Number of local spots on the leaves of *Chenopodium murale* in replicates.				Spot number per leaf	
	I	II	III	IV	Mean	(%)
Not cleaned knife	12	17	14	15	14.5	100
Water cleaned knife	11	14	10	12	11.8	81.4
Milk soaked knife	0	0	0	0	0.0	0.0
Wiped knife	0	0	0	0	0.0	0.0

Source: Fiedorow & Pawlowski (2002)

Reducing AD

Virus infection makes plants more susceptible to attack by *Fusarium.* Reducing *Fusarium* in soils may be a method of reducing AD. Soil fumigation can help, but is not a long-term solution, giving only temporary effects in crops. Reducing or eliminating virus from seed and from cloned propagating material may help by reducing the susceptibility of plants to later *Fusarium* infections (Schofield, 1991). Studies have also shown that the use of various sodium chloride treatments of soils can be effective in reducing the infection rate of *Fusarium* in asparagus (Elmer, 1992), resulting in increased fresh weight of spears and increased fern growth. The practice of salting asparagus beds which was probably used from before 1860 to around the 1940's to control weeds and boost yields (Rudolph, 1927) appears to be a practice unique to the US and was discontinued after herbicides were developed in the 1940s. About this time the number of reports of *Fusarium* crown and root rots in asparagus began to increase (Cohen, 1941; Graham, 1955 & Grogan & Kimble, 1959).

The use of biological control of *Fusarium* spp. by inoculation with a non-pathogenic race of *Fusarium* has been investigated by Damicone & Manning (1982). Work carried out in Taiwan with soil amendments has shown that a mixture consisting of rice husks, bagasse, oyster shell, urea, potassium, nitrate, calcium, superphosphate, and mineral ash

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suppressed natural populations of *F. oxysporum* and reduced both the incidence and severity of the disease (Sun & Huang, 1985; Tu *et al* 1990). Scher & Baker (1980) also found a species of *Pseudomonas* that suppressed *Fusarium* infections in soils with high inoculum levels.

Further research into the development of *Fusarium* resistant cultivars of asparagus could also be an economically sensible approach to reducing AD.

Fusarium infection can also be reduced by the use of treated seed or fungicide applications to crowns and ferns.

Literature cited – see reference section at the end of the report for more detail.

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SCIENCE SECTION

Introduction

Commercial asparagus (Asparagus officinalis L.) crops can remain in production for up to 10 -15 years if well managed and maintained, although many growers budget on a plantation life of 10yrs with 8 - 9 harvests during that period. However, some UK asparagus producers have reported a decline in yield and quality of their crops in recent years. A potential contributing factor for this yield reduction, generally referred to as asparagus decline (AD), is the presence of virus infection in crops.

Following an HDC-funded literature review (FV 213) on asparagus viruses in 1998, a recommendation was made for a survey of asparagus crops of different ages over 3 successive seasons to determine the presence, distribution and the identity of any viruses potentially involved in asparagus decline in the UK. STC were commissioned by HDC in 2010 to carry out a survey of asparagus crops by testing fern samples for a number of viruses late in the season. The findings from the preliminary survey in 2010 provided growers with some indication of the incidence of virus in their crops; however it was felt that an additional year of testing together with an increased number of sampled crops would add further value and help draw some conclusions regarding distribution and spread of 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 in affected crops. 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). Further detail regarding the link between virus infections and AD are explored in the literature review included in this report.

Table 1: Details of viruses previously reported on Asparagus crops worldwide

Virus	Family	Transmission		
		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)	✓	✓

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

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Virus (CMV), Arabis Mosaic Virus (AMV), 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 crops can be infected with more than one virus and that mixed virus infections can reduce quality and yield significantly (Bandte et al. 2008).

More detailed testing for a total of 8 viruses was carried out in 2010, the results from those tests suggested that only 4 viruses were present in UK crops to any extent and this allowed us to focus on these viruses during the survey carried out in 2011. We used a serological test - Enzyme-Linked Immunosorbent Assay (ELISA) to test the 2011 samples for AV-I, AV-II, CMV and TSV.

Materials and Methods

Literature Review

A small-scale review of the existing literature on the phenomenon called *Asparagus Decline* was carried out as a component of the year 2 project.

Virus detection: Sample collection

A total of 74 fern samples were collected during the period 24th August – 10th September 2011. A sampling protocol (Appendix 1) was produced to standardise this process. Ten of the crops which had been sampled in 2010 were re-sampled in 2011. To increase the geographical spread of the sampling an additional 8 growers provided material for the survey this year. Samples were collected from a regionally diverse area ranging from Somerset to Angus in Scotland.

At each of the new sites for 2011, growers identified between 2 and 4 crops for sampling. Two samples/crop were collected, one from the edge of the crop and one from the centre. Some of the crops chosen had been newly planted in 2011, whilst others were older – the oldest crop sampled having been planted in 1993. Sampled crops were given a unique identifying code so that testing could be carried out 'blind' and reporting could be anonymous within the group. Additional crop information (e.g. variety, planting year, soil type, pesticides applied) was also collated. Latex gloves were worn for the collection of samples and changed between samples to avoid any potential for cross-contamination.

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Care was also taken to minimise any risk of virus or other pathogen transfer between sites. The collected samples were dispatched to STC for virus testing using ELISA methodology.

Virus detection: ELISA testing

Samples were tested using ELISA reagents supplied by Agdia-Biofords. Protocols for the work and reagents varied slightly from one 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 1-74. Sub-samples of the 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 in to 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 the last incubation.
8. The absorbance of the test plates was read using the Opsys plate reader at 405nm wavelength.
9. The absorbencies were interpreted using the following threshold values:

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Values	Threshold equation	Interpretation
Negative	Minus 2 x the mean of the Standard deviation (SD) of the negative control wells	Indicates a negative response
+ Positive	2 x the mean of the SD of the negative control wells	Indicates a positive response
++ Positive	3 x the mean of the SD of the negative control wells	Indicates a strong positive response
+++ Positive	4 x the mean of the SD of the negative control wells	Indicates a very strong positive response.

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

Results

Sample collection information

Samples were collected from 37 crops, with 2 samples per crop giving 74 samples in total.

78% were cultivar Gijnlim, 11% of samples collected were cultivar Backlim, approximately, 5% of the samples were cv. Millennium, and 3% each of Fileas and Jersey Giant were sampled.

The majority of the crops had been established following the importation of asparagus crowns from Holland or France; however, just over 22% of the crops sampled had been raised from UK produced plugs.

The youngest crop sampled was planted in 2011, with the oldest having been planted in 1993.

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 virus titres (concentrations).

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Plate1: Example of ELISA plate test – yellow colouration denotes a positive response.

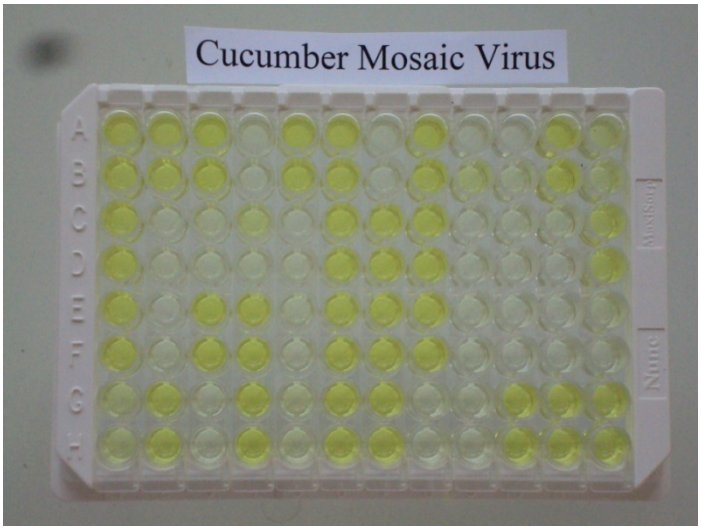

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Table 4. Virus testing results for samples collected during autumn 2011

Crop No.	Results from Field Edge				Crop No.	Results from Centre of Field				Yr of planting
	AV1	CMV	AV1I	TSV		AV1	CMV	AV1I	TSV	
1					1					2006
2				-	2			-	-	2006
3		-	-	-	3			-	-	2006
4				-	4			-	-	2008
5					5				-	2002
6				-	6				-	2003
7			-	-	7			-	-	2006
8		-	-	-	8				-	2005
9				-	9			-	-	2000
10				-	10				-	2004
11				-	11			-	-	2001
12			-	-	12			-	-	2010
13		-		-	13		-	-	-	2010
14		-	-	-	14	-	-	-	-	2011
15		-		-	15		-	-	-	2010
16			-	-	16				-	2003
17				-	17				-	1997
18			-	-	18			-	-	2010
19		-		-	19	-	-	-	-	2011
20		-	-	-	20		-	-	-	2010
21			-	-	21		-	-	-	2010
22		-	-	-	22		-	-	-	2007
23		-	-	-	23		-		-	2008
24		-	-	-	24		-	-	-	2008
25		-	-	-	25		-	-	-	2011
26			-	-	26			-		2004
27			-	-	27			-		2003
28		-	-	-	28		-	-	-	2008
29		-	-	-	29		-	-	-	2007
30		-	-	-	30		-	-	-	2009
31			-	-	31			-	-	2005
32			-	-	32			-	-	2010
33			-	-	33			-	-	2002
34			-	-	34			-	-	2003
35			-	-	35			-	-	2001
36			-	-	36			-	-	2001
37			-	-	37			-	-	1993

- Indicates a negative response
+ indicates a positive result
++ indicates a strongly positive result

The data in Table 4 indicates that the most predominant virus detected was AV-I, this was followed by CMV and AV-II whilst only a few samples (6.75%) were infected with Tobacco Streak Virus. The distribution of infection between the different cultivars is shown in Chart 1 below.

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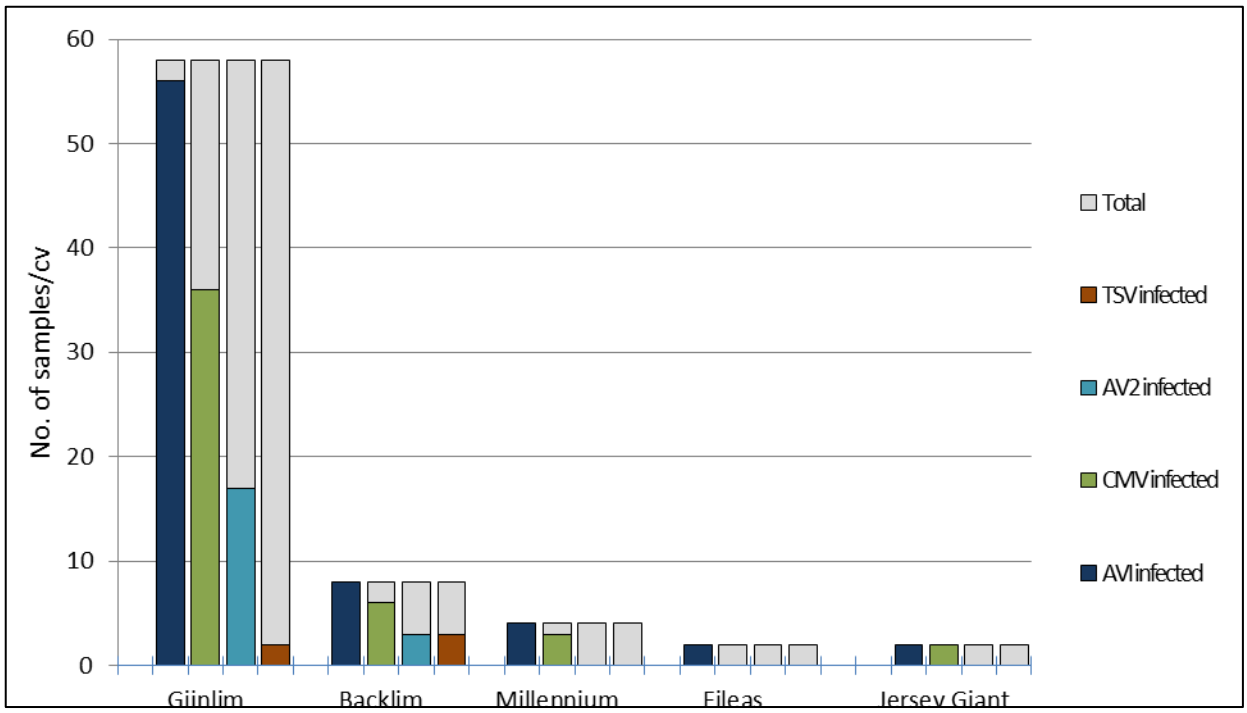

Chart 1. Showing the distribution of viruses between the two cultivars sampled

As was observed during the 2010 sampling, the vast majority of the UK crops tested were cv. Gijnlim, followed by Backlim, Millennium, Fileas and Jersey Giant (the latter 3 accounting for only 1 or 2 crops per cultivar). Both Gijnlim and Backlim show similar virus incidence patterns, with all 4 viruses represented to in similar proportions. Only AV-I and CMV were detected in the 3 less well represented cultivars. It is not clear whether this is significant i.e. whether these cultivars are more resistant to those viruses, or whether the lower detection rate of these viruses is the cause of them not being found here i.e. fewer samples of these cultivars were tested.

Crop Age

Samples were collected from crops with crowns of various ages, the oldest having been planted 18 years ago and the youngest in 2011 (Chart 2 & Table 5).

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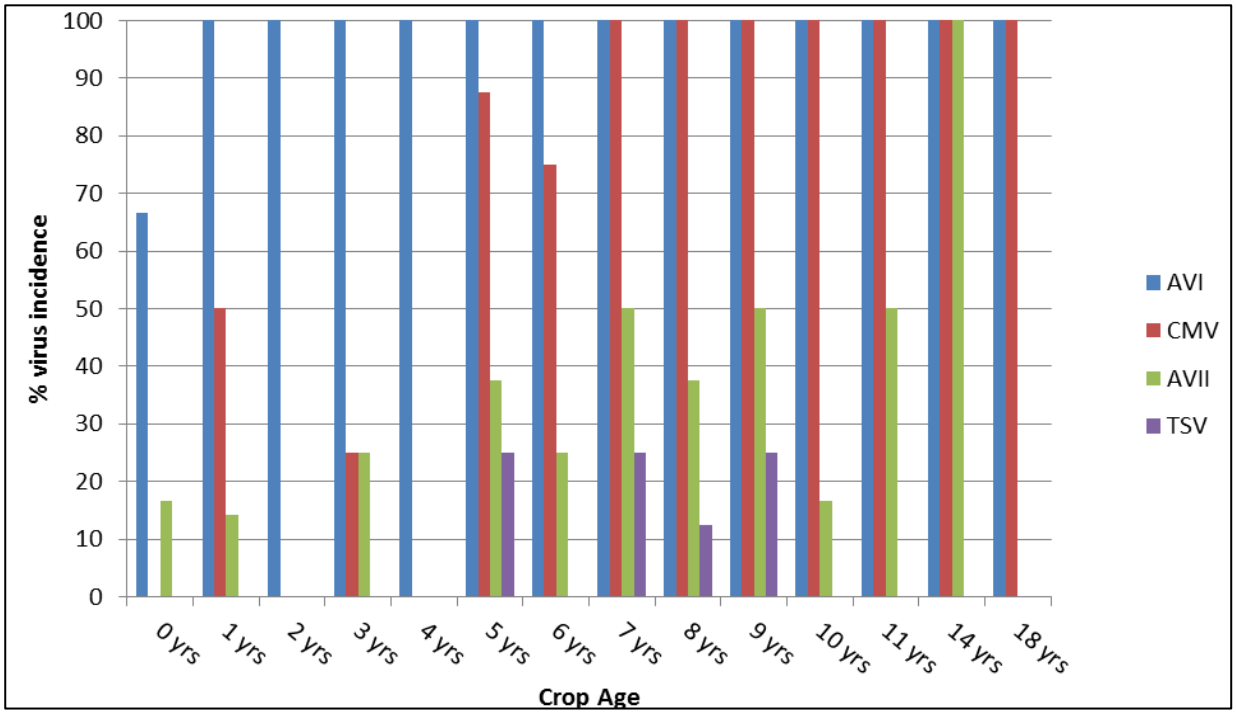

Chart 2. Showing the incidence of virus in crops relative to the year of planting

No samples were received from crops which were 12, 13, 15, 16 or 17yrs old. Results based on 2 samples/crop.

Table 5. Summary of data on crop age

Virus	Incidence
AV-I	Detected in all samples in each represented age of crop, there was a slightly lower incidence of the virus in newly planted crops.
CMV	Detected in approx. 78% of the crops sampled, there was a slightly greater incidence in crops older than 5yrs.
AV-II	Detected in 27% of the crops sampled. Again, there was a slightly higher incidence of infection in older crops.
TSV	Was only detected in approximately 7% of crops sampled. Affected crops were all approximately 5-9yrs old.

Multiple virus infections

The incidence of multiple virus infections in samples varied across cultivars (Chart 3). Two Gijnlim samples were completely free of virus during this year's tests. Neither of these had been tested during 2010 and both were from newly planted crops. Of the two samples/crop tested, interestingly the samples from the edge of these newly planted crops were infected with AV-II (and AV-II in one instance), whilst the samples from the middle of the crop were virus-free. The remaining Gijnlim samples contained between 1 and 4 viruses, with just less than half the samples being infected with 2 viruses – this result was similar to that seen during testing carried out in 2010. Backlim samples contained between 1 and 4 viruses, again, approximately half the samples were infected with 2 viruses. Fern samples from the cv. Millennium contained either just 1 or 2 viruses (no AV-II or TSV was detected in these

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samples). The samples of cv. Fileas were only infected with AV-I, whilst the Jersey Giant samples were infected with AV-I and CMV. However, it should be remembered that only very low numbers of samples of the cultivars Millennium, Fileas and Jersey Giant were received for testing and therefore the result may not be entirely representative.

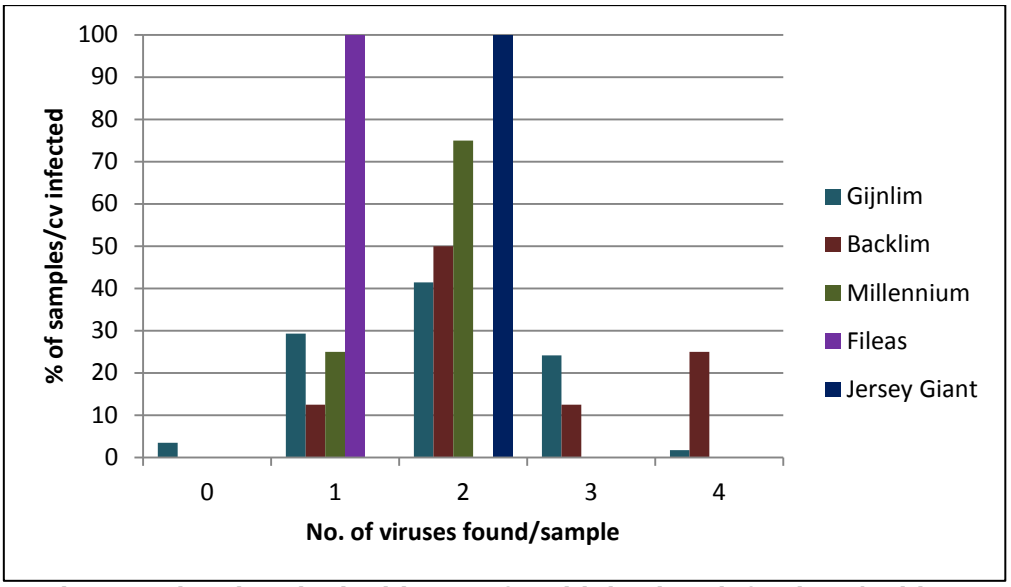

Chart 3. Showing the incidence of multiple virus infections/cultivar

Source of Crowns

The majority of the crops tested began life as crowns imported either from France or the Netherlands, whilst 21% of the crops tested in 2011 originated either from crowns or cell propagation in the UK. A comparison of the number of viruses found to be present in each instance is shown in Chart 4.

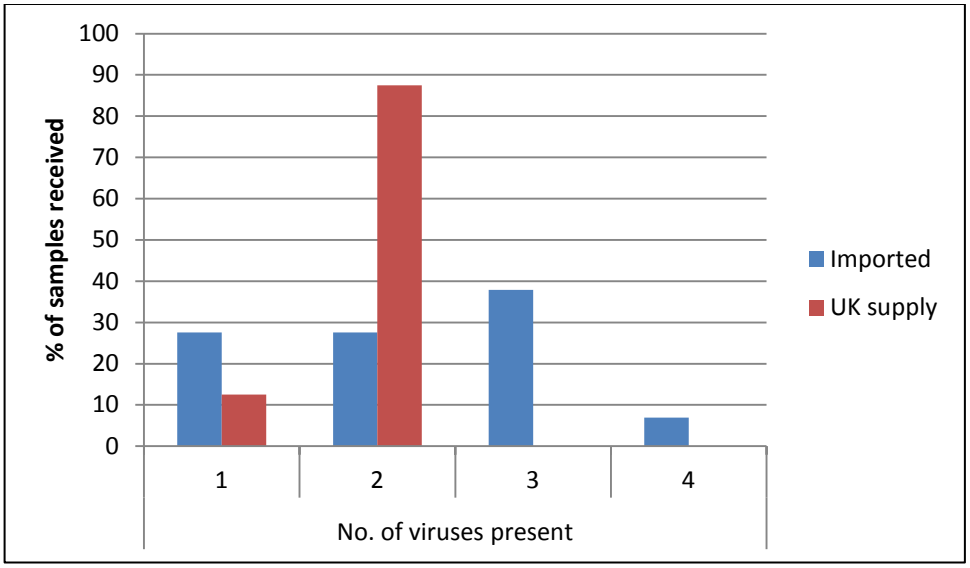

Chart 4. A comparison of virus incidence and crop origin

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The incidence of virus infection in the imported crowns shows that many of the crops contained up to 3 viruses and some had 4. The material collected from the UK-propagated crops contained only 1 or 2 viruses, which may suggest a possible reduction in virus levels from using UK-propagated material. However, although the UK propagated samples were from a good age range of crops, care should be taken when interpreting the results as the majority of the UK-sourced material was found in Yorkshire and therefore a geographical influence on virus presence cannot be ruled out.

Geographical distribution

Sampling in 2011 was aimed at obtaining a broad geographical distribution of crops, and material was obtained from sites from as far apart as Somerset and Angus for virus testing.

As in 2010, AV-I was found in all areas sampled. CMV was detected over a relatively widespread area in 2011, although was less frequently detected in samples received from Lincolnshire, Staffordshire, Somerset and Scotland. It was found consistently in samples from Yorkshire, Kent, Suffolk, Hereford and Worcester. AV-II was *not* detected in samples collected from Scotland, Kent or Yorkshire, and at only low frequency in samples from Staffordshire and Somerset. AV-II was detected more frequently in samples from Norfolk, Suffolk, Hereford, Evesham and Lincolnshire. TSV was detected only in samples from Norfolk, Suffolk and Somerset.

Comparison of the results from 2010 and 2011 samples

Twenty of the samples tested in 2011 were collected from crops sampled initially in 2010. A comparison of the virus infections detected in each year is shown in Table 6.

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Table 6. Comparison of positive virus findings in repeated samples – 2010 and 2011

Sample No. (2011)	Cultivar	Yr of Planting	AV-I Detected 2010	AV-I Detected 2011	CMV Detected 2010	CMV Detected 2011	AV-II Detected 2010	AV-II Detected 2011	TSV Detected 2010	TSV Detected 2011
1 Edge 1 Middle	Backlim	2006								
2 Edge 2 Middle	Gijnlim	2006								
3 Edge 3 Middle	Gijnlim	2006								
4 Edge 4 Middle	Gijnlim	2008								
5 Edge 5 Middle	Gijnlim	2002								
6 Edge 6 Middle	Gijnlim	2003								
7 Edge 7 Middle	Gijnlim	2006								
8 Edge 8 Middle	Gijnlim	2005								
9 Edge 9 Middle	Gijnlim	2000								
10 Edge 10 Middle	Gijnlim	2004								

The results show that there was a 43% increase in the number of positive virus detections from 2010 to 2011. These were mostly accounted for by a large increase in the detection of CMV and AV-II from one year to the next. There were two samples which had tested positive in 2010 for TSV where the virus was not detected in 2011, although 3 new positive results were found in different crops in 2011. The low incidence of this virus in the crops tested would suggest that this may well correlate with sample selection e.g. not all plants in the crop would be likely to be infected. In general the results would suggest that the incidence of virus infections in most crops is increasing year-on-year.

Yield data & crop performance

Growers were able to supply yield data for a number of the crops which were tested in 2011 (Table 7). In the majority of instances information was only available for the total yield of the crop in tonnes/hectare. Data was requested on the amount of Class 1 and 2 spears produced which would have provided some quantifiable data regarding crop quality. This was only available for 2-3 crops; since no conclusions could be drawn from such limited

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data it is omitted here. Several growers were able to provide yield data extending back to 2007. In recently planted crops (2010 and 2011) only short harvests or no harvests had taken place as yet and therefore no data was available.

Where full data sets are represented fluctuations in yield/annum are observed in individual crops, however there does not appear to be any overall trend towards year on year reductions in yield, which might be expected based on the virus testing results and the reported knowledge that virus infections, and multiple infections in particular, can lead to reductions in yield. It is likely that year to year yield fluctuations reflect changing environmental conditions which may have had an impact on the levels of other yield-impacting factors such as root diseases.

Growers were asked to subjectively rate the performance of the sampled crops in terms of vigour and yield. The results were surprising in that the performance of many of the crops which were found to be infected with three or four viruses were rated by growers as ‘good’ or ‘moderate’, whilst several of the crops rated as poor were only found to be infected with one or two viruses. The results (Table 6) which show a comparison of the reported crop performance for the repeated crops suggest that growers were generally not observing a decline in crop performance between 2010 and 2011 – rather, several of them rated crop performance more highly in 2011. No correlation between yield and crop performance was observed. This would suggest that growers are not noticing a decline in crop performance or yield in heavily infected crops.

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Table 7. Total yield (t/ha) for crops tested during 2011

Crop No.	Yr planted	Reported crop performance		total yield (t/Ha)				
		2010	2011	2007	2008	2009	2010	2011
1	2006	Poor	Moderate	0.00	2.16	2.64	1.39	1.76
2	2006	Good	Mod/Good	0.00	2.29	2.53	1.31	1.34
3	2006	Good	Good	0.00	0.00	2.60	2.88	2.29
4	2008	Not rated	Good	0.00	0.00	1.73	2.18	2.17
5	2002	Poor	Good	0.00	4.63	5.73	3.61	4.25
6	2003	Moderate	Good	8.91	6.74	6.49	5.11	6.09
7	2006	Moderate	Moderate	No data supplied				
8	2005	Poor	Poor	No data supplied				
9	2000	Good	Moderate	5.10	3.49	3.99	2.38	2.56
10	2004	Good	Good	5.61	3.47	3.00	2.33	4.34
11	2001		Moderate	3.46	1.33	1.65	1.68	1.48
12	2010		Not cropped	0.00	0.00	0.00	0.00	1.23
13	2010		Not cropped	Not harvested yet				
14	2011		Not cropped	Not harvested yet				
15	2010		Moderate	Only 1 short harvest				
16	2003		Good	No data supplied				
17	1997		Good	No data supplied				
18	2010		Moderate	Only 1 short harvest				
19	2011		Not cropped	No data supplied				
20	2010		Not cropped	Not harvested yet				
21	2010		Not cropped	Not harvested yet				
22	2007		Good	0	0	0	8.24	9.37
23	2008		Moderate	0	0	0	0.19	0.56
24	2008		Good	0	0	0	0.38	1.13
25	2011		Not cropped	Not harvested yet				
26	2004		Good	2.8	3.3	3.2	3.8	4.2
27	2003		Moderate	0	0	3.5	2.9	3.4
28	2008		Moderate	0	0	0	0	1.6
29	2007		Poor	Not recorded				
30	2009		Moderate	Not recorded				
31	2005		Poor	Not recorded				
32	2010		Not cropped					
33	2002		Good					
34	2003		Poor					
35	2001		Poor	Not recorded				
36	2001		Poor					
37	1993		Poor					

Pesticide use

Details of pesticide applications were requested from all participating growers on the 'Crop Details' form completed at the time of sample collection.

The most widely used insecticide was Toppel 10 (cypermethrin) which was used by 40% of growers primarily for asparagus beetle control in crops. A few growers also used Calypso (thiacloprid) for the same purpose. Signum (boscalid + pyraclostrobin) was the most

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commonly used fungicide, used by approximately 57% of growers alongside other products containing strobilurins e.g. Amistar, Amistar Top and Olympus. There was no clear correlation between the number or frequency of pesticide application and the number of viruses found in crops. Indeed, some of those crops which had received very few pesticide applications only contained 1 virus, whilst others that had received multiple applications were found to be infected with 3-4 viruses.

Discussion & Conclusions

The findings from the virus testing work carried out in 2011 are interesting but remain unclear in many ways; however some conclusions can be drawn.

Virus testing was carried out on a more geographically diverse group of UK crops in the 2011 survey than in 2010, and it focused on the 4 predominant viruses identified in the 2010 tests – AV-I, AV-II, CMV and TSV. A total of 37 crops were sampled (2 samples/crop) collected from growers situated in locations from Somerset to Angus. Ten of the crops sampled (20 samples) were from sites which had been tested during the 2010 survey and this has enabled a comparison of virus infection frequency and also of virus incidence to crop yield. The aim of this study was to determine the levels of virus infections present in a greater number of crops over a larger geographical area. It was envisaged that this would provide a robust set of results and allow us to better consider the impact of these findings on crop performance e.g. yield, harvest quality and disease susceptibility.

The majority of the samples received in 2011 were of cv. Gijnlim. Samples from three other cultivars were received but in very low numbers. A determination of any potential cultivar resistance is therefore impossible from these data. The recorded results (Chart 1) indicate that both Gijnlim and Backlim samples appear to be similarly susceptible to all 4 of the viruses, the lack of detection of both AV-II and TSV in the cvs. Millennium, Fileas and Jersey Giant offer no evidence of resistance to these viruses because of low sample numbers. A more complex study involving the collection of a known number of samples from each cultivar, from a variety of locations and at a variety of crop ages would be required before stronger conclusions could be drawn.

The clearest correlation observed was that between crop age and virus incidence; infection was lower in younger crops older ones, with highest infection rates observed between 5 -9 years. This is unsurprising since each of the viruses can be transmitted mechanically within the crop and older crops will have been harvested more frequently resulting in a higher

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likelihood of transmission. However, the fact that AV-I virus particularly was detected in crops newly planted during 2011 is interesting and may indicate that the crowns were infected at the time of planting, i.e. were propagated from infected material. This may not be the case, however; studies in Germany (Bandte *et al* 2008), showed that freshly planted, virus-free crops became infected with AV-I within 7 months. It should also be considered whether the new, potentially virus-free crops were planted close to crops already carrying viruses, and infection transmitted mechanically or by insect vectors between the crops. This hypothesis is supported by the detection of AV-I in the samples collected from the edges of the newly planted crops 14 and 19 where samples from the middle of these crops were virus-free.

Other possibly useful observations regard geographical distribution of viruses across the UK. AV-I is present in all of the locations tested over the two years. CMV was detected in just over half the samples tested in 2011, but no geographical pattern was observed. AV-II appeared to have been present more frequently in samples from Lincolnshire southwards, but was not present in Somerset. TSV was detected in fewer locations, and no distribution pattern is seen.

The re-testing of 10 of the crops first tested in 2010 was useful and provided some information which may concern growers. The results (Table 6) show a 43% increase in the frequency of positive detections for the 4 viruses tested for in 2011. In both years 100% of the samples tested were infected with AV-I. A 50% increase in the number of crops infected with CMV was observed, whilst an additional 45% of samples were found to be infected with AV-II. The results for TSV incidence were rather mixed with no virus being detected in the 2 crops found to be infected in 2010, but new detections in 3 crops in 2011. This is likely to be linked to the low frequency of detection of this virus in such a small sample group. These results suggest that there is a year-on-year increase in the incidence of virus in these crops, and this could well be representative of crops across the UK. This is perhaps unsurprising and can be linked to additional harvesting and insect movement & migration during the additional year which are both potential routes for virus transmission.

Details regarding the application of both insecticides and fungicides are of some interest with regard to virus infection in asparagus crops. Insecticide applications may possibly influence the presence and persistence of virus vectors e.g. aphids in the crop. The findings of the literature review for this project also identified a clear link between the presence of virus and the crops susceptibility to infection by fungal root pathogens e.g. Fusarium and

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Phytophthora spp. and the link to AD. However, no correlation between virus incidence and the use of pesticides was observed during this study. There was no suggestion that the use of insecticides and fungicides in the crop helped to reduce the amount of virus present and this may be linked to the use of insecticides primarily for the control of asparagus beetle in crops rather than aphid control. It is possible that the fungicides were having a positive effect on root health and that this was proving beneficial in reducing the potential damage and yield reductions which might otherwise be expected following virus infection. A possible but unproven connection between reduced virus presence and the origin of propagation material was also observed.

Of most significance, and also surprise given the impacts of viral infection in the reviewed literature is the fact that both yield data and growers' perception of crop performance did not show the expected relationship with the incidence of viral infection - growers often recorded 'moderate' or 'good' performance from crops which were carrying multiple infections, whilst the converse situation was also represented. However, this is a very subjective classification and crops rated as 'poor' may be considered so in relation to other adjacent crops or may have shown 'poor' yields for several years i.e. any better performance was prior to 2007 when this data set was initiated. In such cases as these the crop performance, if not related to virus presence may potentially be linked to other factors e.g. soil type and structure, crop quality and vigour, moisture levels, aspect or crop age, which have either singularly or collectively reduced plant population.

Yield data was available for 43% of the crops, mostly only as tonnes/ha of total crop. Some fluctuations year-on-year were observed but with no overall negative trends in heavily virus-infected crops, and variation was more likely to be linked to weather patterns in individual years and the resulting effects on crop production. Unfortunately, very few growers were able to provide data showing harvest quality details e.g. the weight of Class 1/2 spears which may have been useful as a reduction in crop quality might have been observable, and this would certainly equate to a reduction in the economic viability of certain crops.

The presence of virus in the asparagus crops tested is now clear. There is no action that growers can take to eradicate infection from these crops though some measures may help 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 vectors (aphid, thrips or nematodes), that all can be transmitted mechanically and also that several can be present in pollen. It is possible to take precautions by planting virus free

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crowns, to pay attention to the presence of aphid in crops and to consider applying regular insecticide applications to limit the spread of aphids, and thereby potentially virus, and also to introduce hygiene methods to limit mechanical infection between crowns – although this is likely to be the hardest area to control 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 relatively low incidence of AV-II in the crops sampled, particularly when the data is compared to AV-II incidence in other asparagus producing areas of the world. Much of the reported literature suggests that the presence of AV-II either singly or in multiple infections can have the biggest impact on yield reduction and crop life reduction. In the testing carried out in 2011 this virus was only found in 27% of the samples which is potentially good news for UK growers. However, this figure has increased dramatically from the previous testing carried out in 2010 when AV-II was only found to be present in 7.5% of the samples tested and this suggests that virus has spread to a much higher percentage of plants in these crops, thus increasing the probability of collecting a sample from an infected plant. It appears that the incidence of this virus may be increasing in UK crops and this could be a cause for concern.

This second season of testing has provided more useful data on the incidence and frequency of virus infections within UK asparagus crops, and has allowed us to build up a better picture of the impacts, or lack of them, in crops. As always, the results and data provided and reported provide only a snapshot of the results for a number of crops in each year. As with the data provided in 2010 the additional data collected should be considered as baseline data, forming a starting point to which later results can be compared.

The origin of this study was based on grower observations of premature crop decline and loss of yield, and yet few of the crop performance results gathered as part of this project actually support this and this is a little intriguing.

Recommendations for further work

- Further testing on these crops in future seasons may provide a useful assessment of viral progress.

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- Virus testing on larger sample numbers of other cultivars could be undertaken to assess genetic potential for virus resistance.
- Virus testing of alternative host crop species may be determine if there is a natural reservoir of the virus in the vicinity of the main asparagus production areas.
- 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. As such, growers should endeavour to keep good records of crop yield and quality.
- Future emphasis should be directed to research which seeks to minimise infection during propagation and in the early years post-planting to delay the onset of epidemic development of any particular virus pathogen.
- Growers may wish to consider the implementation of aphid monitoring systems and also the development of control strategies, including insecticide use, for reducing infestations in ferns.
- It would also be useful to have Lateral Flow Device (LFD) ELISA tests available for on-site use against the predominant asparagus viruses so that growers and their consultants could keep a regular track of infection progress in specific crops or areas.

Acknowledgements

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Knowledge and Technology Transfer

Full results from the virus testing were provided to each of the participating growers as an anonymous list. Each grower was provided with the sample numbers for their own crops so that they could see the specific virus incidence in their own crops and compare this with crops in general without specific knowledge about other individual crops to ensure complete confidentiality. Information from the project has been effectively communicated to the industry and should assist in improving overall crop health and hopefully aid long-term crop and economic performance.

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APPENDIX 1

SAMPLING PROTOCOL

FV 384a – Further virus testing in UK asparagus

Field Sampling Protocol

2 samples from each field/site are required.

The first of these should be from the edge of the field, roughly 3 or 4 rows into the crop. The second of these should be from the middle of the field (or at least one third of the way into large fields). The samples at each of these locations will be taken from a single row.

Wear fresh disposable gloves for each sample location (ie. One pair for the 'field edge' sample, one pair for the 'centre of field' sample).

In the selected row, start by pinching off a 4-6 inch sample from the end of a low fern branch. Avoid sampling material that is more than 1-2mm in diameter. Move 10-20m down the row and take a sample from the middle level of the fern. A further 10-20m down the row, take a sample from high up the fern. Continue working down the row and up and down the plant levels until approximately 15 samples have been taken.

Bulk these 15 samples into one plastic bag and secure the bag closed.

Please ensure that the bag is labelled with the farm and field reference, and also with the in-field location (edge or middle).

Dispatch samples to Stockbridge Technology Centre (address below) as soon as possible, refrigerating them until you are able to send them.

Stockbridge Technology Centre
Stockbridge House
Cawood
Selby
YO8 3TZ

15th August 2011

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