| | |
|---|---|
| **Project title:** | Outdoor lettuce: screening crops for presence of virus |
| **Project number:** | FV 427 |
| **Project leader:** | Dr G. M. McPherson MBPR (Hort.)<br>Stockbridge Technology Centre |
| **Report:** | Annual Report, January 2015 |
| **Previous report:** | Literature Review, June 2014 |
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| **Location of project:** | Stockbridge Technology Centre |
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| **Date project commenced:** | 01 January 2014 |
| **Date project completed (or expected completion date):** | 31 January 2016 |

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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 studies 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.

## *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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## *CONTENTS*

## *GROWER SUMMARY*

### Headline

Four of the nine different viruses that were detected in lettuce tested in July and September 2014 had not been previously reported in lettuce in the UK. Virus detection was not always linked to symptom expression and a higher virus incidence was detected in samples tested in September than in July.

### Background

Well-known viruses such as *Lettuce mosaic virus* and *Mirafiori lettuce big vein virus* usually cause characteristic and recognisable symptoms in field lettuce. However, many other viruses that infect lettuce can either by symptomless or cause diverse range of symptoms that can potentially be attributed to other factors. Previous HDC-funded research found that hitherto unsuspected virus activity caused crop problems. For example, project FV 384 linked long-term decline in asparagus crops to virus presence. Similarly, FV 365, which looked at *Turnip yellows virus* in brassicas, found that a high percentage of plants were infected and, while plants exhibited minimal symptoms, yield and shelf life were affected. A survey of viruses in UK lettuce has not been performed for a considerable time and this research effectively establishes a baseline for further study. In this project, the state of knowledge regarding viruses in lettuce was determined through a literature review, and an initial screen of viruses was performed on samples from commercial crops in July and September 2014. It is important to note that, while this was a fairly comprehensive screen, virus detection was not exhaustive as we were constrained by the availability of antisera reagents for ELISA (serological) detection.

### Summary

Seventeen viruses were assessed over two sampling periods during this study. Forty samples were tested in July and forty-two in September. Of these, lettuce tested positive for nine viruses and negative for eight viruses (Table 1). Four of these viruses (*Broad bean wilt virus I, Endive necrotic mosaic virus*, *Tobacco rattle virus*, and *Alfalfa mosaic virus*) have not previously been reported in UK lettuce crops, although some are known to be present in the UK on other crop or weed species (see *Literature review* for further details). There was a general increase in the number of samples testing positive for viruses between the July sampling period and the September sampling period, which was perhaps to be expected, particularly for aphid or similar vectors. No apparent correlation was seen between virus incidence and samples declared by growers as symptomatic. Virus incidence also appeared

 

not to be linked to specific lettuce cultivars, but may have been linked to geographical location. Definitive conclusions regarding such correlations cannot yet be drawn. Further testing in year 2 of this project may clarify any links between virus incidence and variety, geography, and/or apparent symptoms.

**Table 1.** Viruses assessed in UK lettuce in 2014 (July and September)

| | |
|---|---|
| Viruses testing positive in UK lettuce samples | **Alfalfa mosaic virus** |
| | **Broad bean wilt virus I** |
| | Beet yellow stunt virus |
| | Cucumber mosaic virus |
| | **Endive necrotic mosaic virus** |
| | Mirafiori lettuce big-vein virus |
| | **Tobacco rattle virus** |
| | Turnip yellows virus |
| | Lettuce mosaic virus |
| Viruses testing negative in UK lettuce samples | Arabis mosaic virus |
| | Broad bean wilt virus II |
| | Impatiens necrotic spot virus |
| | Lettuce necrotic stunt virus |
| | Lettuce ring necrosis virus |
| | Tobacco mosaic virus |
| | Tomato spotted wilt virus |
| | Turnip mosaic virus |

Viruses highlighted in **bold** tested positive and have not, to our knowledge, been reported in UK lettuce previously.

## Financial Benefits

It is not yet clear whether the viruses found in the UK lettuce samples in this project have an impact on yield or product quality, and therefore financial benefits cannot yet be assessed.

## Action Points

At this stage, it is not clear exactly what strategies growers should use, beyond the usual vector (e.g., aphid) control measures already employed, to mitigate against virus infection. It may become clearer as the project progresses whether viruses lead to yield/quality losses and how control might be addressed both practically and economically. This will depend to some extent on how the individual viruses carry over between seasons, e.g., by seeds, transplants, and weeds etc. The majority of the viruses found in this study are known to be aphid-transmitted, some can be seed-borne, and many are also found in a variety of weeds. Identification of virus reservoirs may therefore of importance when considering control measures.

## *SCIENCE SECTION*

### Introduction

Field-grown lettuce crops in the UK are prone to a wide range of viruses arising from a variety of sources. Viruses can be introduced to field crops via infected seed and young plants, weed hosts, or via insect, fungal, nematode or other potential vectors. Dispersal from initial crop infection sites or from alternate hosts (e.g., weed species) can occur through mechanical field operations or via insect or other vectors. A range of visible symptoms may be observed in infected plants, including stunting, twisting, chlorosis, discolouration, and necrosis. However, it is unclear whether UK lettuce crops may also be harbouring asymptomatic viruses that nevertheless lead to reductions in quality or yield. The aim of this project is to identify viruses that may be affecting UK lettuce through an initial review of literature and through testing samples from commercial field lettuce crops during July and September 2014.

The literature search for this project revealed that approximately 61 viruses are known to have the capacity to infect lettuce by either natural or artificial means. Of these, 34 have been reported to occur naturally on lettuce crops worldwide. Thirteen of these viruses have previously been reported in the UK or are assumed to be present due to their known associations with other viruses. These viruses are summarised in Table 2. Further details can be found in the literature review for this project (HDC FV 427; Literature Review 2014).

The remit of this project was to use double antibody sandwich (DAS) enzyme linked immune-sorbent assay (ELISA) testing to assess for virus presence in 40 grower-provided lettuce samples for up to 12 different viruses in each of summer and autumn. ELISA antisera were not available for *Beet pseudo-yellows virus, Lettuce big-vein associated virus, Lettuce necrotic yellows virus,* or *Dandelion yellow mosaic virus.* These viruses were therefore excluded from testing, but may still be present in UK lettuce crops. Nine viruses known to infect UK lettuce crops were included in testing (Table 2), and additional viruses were selected based on grower consultation, symptom severity, and/or presence in European lettuce (Table 3). Viruses that tested negative in the July screen were not re-tested in the September screen.

 

**Table 2.** Summary of viruses reported previously on lettuce in the United Kingdom

| Virus | Acronym | Transmission | Tested in 2014 virus screen |
|---|---|---|---|
| Arabis mosaic virus | ArMV | Nematode (e.g. *Xiphinema diversicaudatum*), seed | July only |
| Beet pseudo-yellows virus | BPYV | Whitefly (*Trialeurodes vaporariorum)* | Not tested* |
| Beet yellow stunt virus | BYSV | Aphids (e.g. *Hyperomyzus lactucae*) | September only |
| Cucumber mosaic virus | CMV | Aphids (e.g. *Myzus persicae*) | July and September |
| Dandelion yellow mosaic virus | DYMV | Aphids (e.g. *Myzus persicae*) | Not tested* |
| Lettuce big-vein associated virus | LBVaV | *Olpidium brassicae* | Not tested* |
| Lettuce mosaic virus | LMV | Aphids (e.g. *Myzus persicae*), seed | July and September |
| Lettuce necrotic yellows virus | LNYV | Aphids (e.g. *Hyperomyzus lactucae*) | Not tested* |
| Lettuce ring necrosis virus | LRNV | *Olpidium brassicae* | September only |
| Mirafiori lettuce big-vein virus | MiLBVV | *Olpidium brassicae* | July and September |
| Tomato spotted wilt virus** | TSWV | Thrips (e.g. *Franklinella occidentalis*) | July only |
| Turnip mosaic virus | TuMV | Aphids (e.g. *Myzus persicae*) | July only |
| Turnip yellows virus<br>(syn. Beet western yellows virus) | TuYV | Aphids (e.g. *Myzus persicae*) | July and September |

* No antisera were available for these viruses

** Tested in a combined assay with *Impatiens necrotic spot virus*

**Table 3.** Summary of additional viruses screened in July and September

| Virus | Acronym | Transmission | Nearest prior reporting location on commercial lettuce | Tested in virus screen |
|---|---|---|---|---|
| Alfalfa mosaic virus | AMV | Aphids (e.g. *Myzus persicae*) | Europe | July and September |
| Broad bean wilt virus I & II* | BBWV I & II | Aphids (e.g. *Myzus persicae*) | Europe | July only |
| Broad bean wilt virus I | BBWV I | Aphids (e.g. *Myzus persicae*) | Europe | September only |
| Broad bean wilt virus II | BBWV II | Aphids (e.g. *Myzus persicae*) | Europe | September only |
| Endive necrotic mosaic virus | ENMV | Aphids (e.g. *Myzus persicae*) | Europe | September only |
| Impatiens necrotic spot virus** | INSV | Thrips (e.g. *Franklinella occidentalis*) | Europe | July only |
| Lettuce necrotic stunt virus | LNSV | Mechanical / soilborne. Possibly seed. | USA | July only |
| Tobacco mosaic virus | TMV | Mechanical, seed | - | July only |
| Tobacco rattle virus | TRV | Nematodes (e.g. *Trichodorus minor*) | Europe | July and September |

* Broad bead wilt viruses I & II were tested in a combined assay in July; this test was positive, and tests distinguishing between the two viruses were subsequently used in the September screen

** Tested in a combined assay with *Tomato spotted wilt virus*

## Materials and methods

### Sampling and sample storage

Asymptomatic and symptomatic lettuces were provided directly by growers in late June/early July (sampling dates: 30.06.14 – 07.07.14) and September (sampling dates 08.09.14 – 17.09.14). Sample forms and protocols (Appendix 1) were provided, but detailed information was not received for all samples. Samples were provided from several varieties in diverse locations. Sample details are provided in Appendices 2 & 3. On arrival, representative leaves were photographed, damaged tissues were removed, and samples were stored at 4°C in preparation for ELISA testing. Summer ELISA testing was conducted on 9–10$^{th}$ July 2014. Autumn testing was performed on 23$^{rd}$ September 2014. Sample degradation was minimal in summer and very few samples were discarded. Several of the autumn samples had already degraded upon receipt and did not store well. Samples were stored for as short a time as possible, but the late arrival of some samples necessitated storage for up to two weeks prior to testing. Ideally, storage times will be reduced further in subsequent testing; however, in general, samples that arrived in good condition stored well and were suitable for testing. This suggests that sample damage prior to arrival was more detrimental to sample quality than was storage duration at STC. Forty-eight samples were received in July and forty-five in September and the majority of these were tested.

### DAS ELISA testing

DAS ELISA kits were used in the July screen and antisera-only reagent sets were used for the September screen. ELISAs were conducted in 96-well plates. In high throughput reagent kits the primary antibody is adsorbed to the plate wells during manufacture, whereas in antisera-only reagent sets, antibody must be adsorbed to wells by the user as part of the ELISA protocol. High-throughput ELISA reagents were purchased from Neogen (Ayr, Scotland) and Loewe Biochemica (Sauerlach, Germany). Antisera were purchased from Loewe Biochemica, DSMZ (Braunschweig, Germany), and AC Diagnostics (Fayetteville, USA). Reactions were performed according to the manufacturers' protocols. Briefly, antibody for the virus of interest was coated onto individual wells of a 96-well plate. Lettuce samples were then homogenised in sample buffer and added to the 96-well plate. Excess sample was removed by washing. Next, conjugated antibody was added and allowed to bind to the antigen. Finally, a reactive substrate (p-nitrophenyl phosphate) was added that produced a yellow colour upon interaction with the antibody conjugate. Plates were washed four times between stages using an automated plate washer. Forty lettuce samples were tested in duplicate in July and forty-two samples were tested in September. The remaining wells of

 

each plate were used for negative (virus-free plant tissue or buffer) and positive controls. In July, positive controls were used as provided by the manufacturer. In September, a positive control dilution series was included in order to provide additional information regarding the virus detection limits with each antiserum. General 96-well plate layouts are shown in Figure 1.

**July**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | buffer | S3 | S11 | S14 | S19 | S22 | S28 | S32 | S35 | S39 | S43 | buffer |
| **B** | S1 | S3 | S11 | S14 | S19 | S22 | S28 | S32 | S35 | S39 | S43 | S47 |
| **C** | S1 | S4 | buffer | S15 | pos. | S23 | S29 | buffer | S36 | neg. | S44 | S47 |
| **D** | pos. | S4 | S12 | S15 | S20 | S23 | S29 | S33 | S36 | S40 | S44 | neg. |
| **E** | neg. | S8 | S12 | S17 | S20 | S24 | S30 | S33 | S37 | S40 | S45 | pos. |
| **F** | S2 | S8 | neg. | S17 | buffer | S24 | S30 | pos. | S37 | buffer | S45 | S48 |
| **G** | S2 | S9 | S13 | S18 | S21 | S27 | S31 | S34 | S38 | S42 | S46 | S48 |
| **H** | buffer | S9 | S13 | S18 | S21 | S27 | S31 | S34 | S38 | S42 | S46 | buffer |

**September**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | pos. A | S5 | S10 | S14 | S18 | S22 | S26 | S29 | S33 | S37 | S40 | neg. |
| **B** | S1 | S5 | S10 | S14 | S18 | S22 | S26 | S29 | S33 | S37 | S40 | S13 |
| **C** | S1 | S6 | neg. | S15 | buffer | S23 | pos. C | S30 | S34 | buffer | S41 | S13 |
| **D** | S2 | S6 | S11 | S15 | S19 | S23 | S27 | S30 | S34 | S38 | S41 | S44 |
| **E** | S2 | S7 | S11 | S16 | S19 | S24 | S27 | S31 | S35 | S38 | S42 | S44 |
| **F** | S4 | S7 | buffer | S16 | pos. B | S24 | buffer | S31 | S35 | neg. | S42 | S45 |
| **G** | S4 | S9 | S12 | S17 | S21 | S25 | S28 | S32 | S36 | S39 | S43 | S45 |
| **H** | neg. | S9 | S12 | S17 | S21 | S25 | S28 | S32 | S36 | S39 | S43 | pos. D |

**Figure 1:** ELISA 96-well plate layout for lettuce virus screening

Shaded cells provide plate co-ordinates. Pos., positive control; neg., negative control; water, water control. Wells labelled with S indicate sample number. In July, positive controls were at the manufacturer's recommended dilution. In September, positive control A was at the recommended dilution. Positive controls B, C, and D were further diluted 1/25, 1/625, and 1/15625, respectively.

## Data analysis

ELISA test plates were scanned at 405 nm using a colorimetric plate reader. As colour development can vary between assays, plates were scanned 1 hour and 2 hours after substrate addition and were also scanned after an overnight incubation.

The criteria for determining a positive signal were based upon the average and standard deviations (SD) of the negative controls (NC), as follows.

| | |
|---|---|
| Low positive (+) value | > NC average + 2SD of NC |
| Medium positive (++) value | > NC average + 3SD of NC |
| High positive (+++) value | > NC average + 4SD of NC |

Both sample duplicates were required to reach the low positive (+) threshold for a sample to be considered positive. Where positive determination differed between samples, the lowest positive value was used For example, if one duplicate was '+' and the other duplicate was '++', then the sample would be considered as '+'. Where results differed between scan timings, the scan that provided the best discrimination between negative and positive controls was used.

### *Beet western yellows virus / Turnip yellows virus*

*Beet western yellows virus* (BWYV) and *Turnip yellows virus* (TuYV) are closely related, if not identical (at least in some parts of the world). European virus isolates do not infect sugar beet and related species, but do infect lettuce and brassicas. However, some isolates of BWYV from the USA do infect sugar beet as well as lettuce and brassicas. As a result, European isolates were reclassified as TuYV to provide a distinction between the two infective agents. At present, BWYV and TuYV continue to be classified in the USA as different viruses. European TuYV antisera were used in the project and, whilst BWYV terminology was used in the literature review for this project, TuYV will be used henceforth.

 

## Results

Forty-eight lettuce samples were received for the July virus screen and forty-five for the September screen. Of these, 40 and 42 samples were tested, respectively. While the majority of samples were described as asymptomatic by growers, some samples were noted as displaying symptoms such as chlorosis, spotting, and distortion. Representative images of symptomatic leaves are shown in Figure 2.

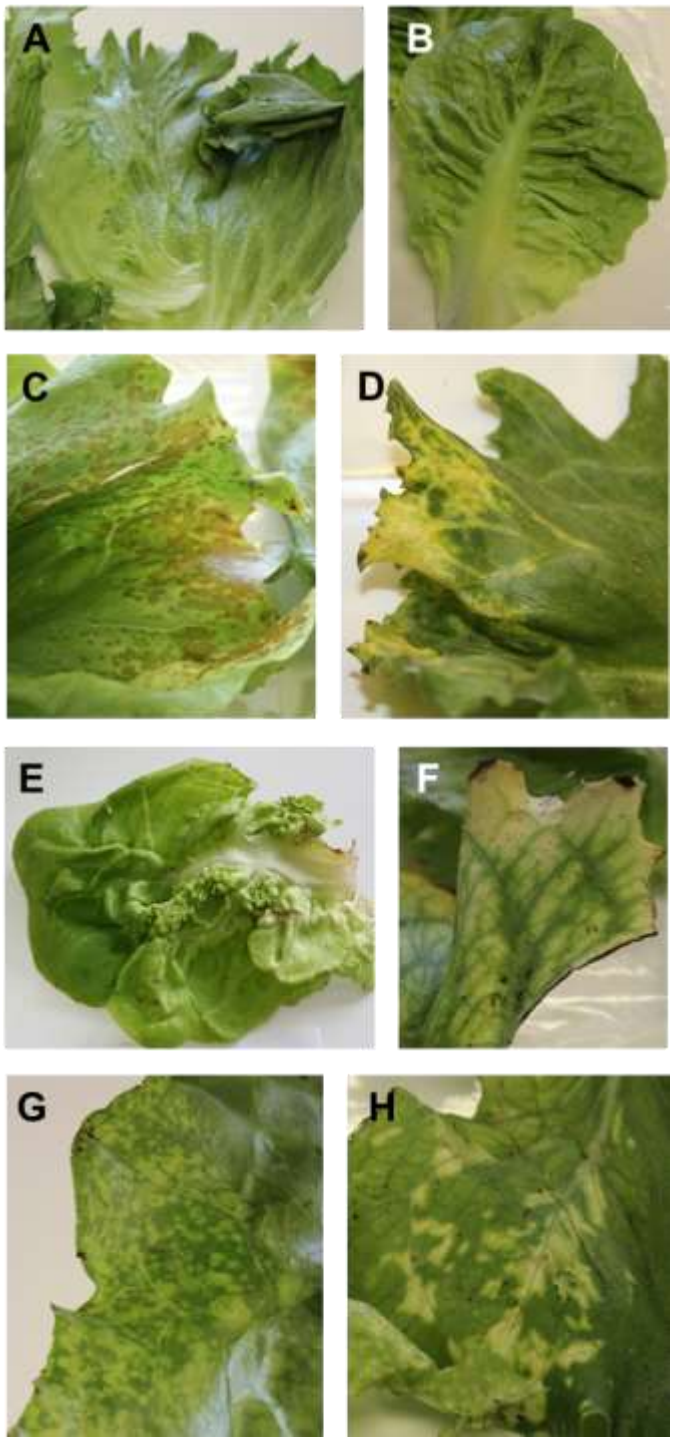


**Figure 2.** Examples of symptomatic lettuce samples

A-B, asymptomatic; C, brown spotting; D, veinal chlorosis; E, distortion; F-H, variable chlorotic symptoms on a single sample.

A: JUN-30
B: SEP-23
C: JUN-32
D: SEP-45
E: SEP-44
F-H: JUN29

 

Fourteen viruses were assessed in the July screen. Based on the threshold criteria for a positive response, six of the viruses were found in at least one sample and six were not found in any of the forty samples tested (Table 4). In addition, 26 samples tested positive for one or both of *Broad bean wilt viruses I* and *II.* However, as these two viruses were tested in combination in the July screen, it was not possible to discern which virus (BBWVI, BBWVII, or both) was present.

Viruses that tested negative in the July screen were not tested in the September screen. Additional viruses were selected for the second screen and *Broad bean wilt viruses I* and *II* were tested individually. Of the eleven viruses assessed in the September screen, nine tested positive in at least one sample and two were not found in any of the forty-two samples tested (Table 5). No samples tested positive for *Broad bean wilt virus II*, suggesting that July samples were probably all affected by *Broad bean wilt virus I.*

In total, seventeen different viruses were screened and samples tested positive for nine of these. Most significantly, four viruses were found that had not previously been reported in UK lettuce. Full results for all the samples and viruses tested are in Appendices 2 & 3.

## Discussion

### Virus incidence increased between July and September

Virus incidence was higher in September than in July, as might be expected from ongoing virus transmission throughout the growing season (Tables 4 & 5, Appendices 2 & 3). In July, 80% of samples were positive for at least one virus. Most were affected by one or two viruses (78%), with only two samples testing positive for three viruses and one for four different viruses. By contrast, 90% of samples were positive for at least one virus in September and 60% of samples tested positive for three or more viruses. This indicates that there was a general increase in virus prevalence between July and September. The majority of the viruses examined in this study are aphid transmitted and the increase in aphid pests over the season may explain the increase in virus incidence between the two testing dates. However, incidence of AMV, CMV, LMV, and MiLBVV was at approximately the same levels in July and September. AMV and LMV have the potential to be transmitted by seed, and, although seed transmission was not directly assessed in the 2014 screen, it is therefore possible that this was a major route of infection for these viruses. CMV and MiLBVV were found infrequently and this may disguise any general increase in prevalence. It should be noted that the positive control for the MiLBVV test was detected only at the highest concentration of the dilution series, indicating that this assay had limited sensitivity

 

compared to the other tests used in this study. Therefore, the MiLBVV incidence in this study may be an underestimation.

**Table 4.** Summary of virus screening results, July 2014

| Acronym | Virus | Number of positive samples (n = 40) | Percentage of samples testing positive |
|---|---|---|---|
| **AMV** | **Alfalfa mosaic virus** | **8** | **20** |
| ArMV | Arabis mosaic virus | 0 | 0 |
| **BBWV I & II** | **Broad bean wilt viruses I & II** | **26** | **65** |
| CMV | Cucumber mosaic virus | 1 | 2.5 |
| LMV | Lettuce mosaic virus | 7 | 17.5 |
| LNSV | Lettuce necrotic stunt virus | 0 | 0 |
| MiLBVV | Mirafiori lettuce big-vein virus | 2 | 5 |
| TMV | Tobacco mosaic virus | 0 | 0 |
| **TRV** | **Tobacco rattle virus** | **3** | **7.5** |
| TSWV/ INSV | Tomato spotted wilt virus / Impatiens necrotic spot virus | 0 | 0 |
| TuMV | Turnip mosaic virus | 0 | 0 |
| TuYV | Turnip yellows virus | 10 | 25 |

Viruses in **bold** have not, to the best of our knowledge, been reported in UK lettuce previously.

**Table 5.** Summary of virus screening results, September 2014

| Acronym | Virus | Number of positive | Percentage of |
|---|---|---|---|

 

| | | samples (n = 42) | samples testing positive |
|---|---|---|---|
| **AMV** | **Alfalfa mosaic virus** | **6** | **14.3** |
| **BBWV I** | **Broad bean wilt virus I** | **33** | **78.6** |
| BBWV II | Broad bean wilt virus II | 0 | 0.0 |
| BYSV | Beet yellow stunt virus | 20 | 47.6 |
| CMV | Cucumber mosaic virus | 2 | 4.8 |
| **ENMV** | **Endive necrotic mosaic virus** | **13** | **31.0** |
| LMV | Lettuce mosaic virus | 3 | 7.1 |
| LRNV | Lettuce ring necrosis virus | 0 | 0.0 |
| MiLBVV | Mirafiori lettuce big-vein virus | 6 | 14.3 |
| **TRV** | **Tobacco rattle virus** | **20** | **47.6** |
| TuYV | Turnip yellows virus | 21 | 50.0 |

Viruses in **bold** have not, to the best of our knowledge, been reported in UK lettuce previously.

## Virus incidence does not appear to correlate with declared symptoms or lettuce variety

Fourteen of the forty samples tested in July and fifteen of the forty-two samples tested in September were denoted ‘symptomatic’ by the grower/consultant. However, there was no clear correlation between this and virus presence. For example, half of the samples described as ‘symptomatic’ in July did not test positive for any viruses (Appendices 2 & 3). Similarly, several samples that tested positive for five or more viruses in September were described as ‘not symptomatic’. There was also no clear association between any particular virus and a ‘symptomatic’ description: all viruses were found in both ‘symptomatic’ and ‘non-symptomatic’ samples. Similarly, no clear correlation was apparent between specific lettuce cultivars and virus incidence. It should be noted that determining the cause of unusual growth patterns can be challenging as such patterns may be due to a range of additional factors such as herbicide damage, variations in water availability, and soil type. Ultimately, specific inoculation studies may be needed to correlate symptoms with particular viruses or virus complexes.

The designation of ‘symptomatic’ is highly subjective and was determined by the individuals taking the samples. It is therefore possible that independent assessment of leaf symptoms upon receipt of samples might reveal a clearer association between symptom and virus. Images of whole heads prior to sample removal might also facilitate symptom classification, and photographs will be requested from growers in subsequent screening rounds.

 

A number of samples provided in July and September were 'paired', that is, symptomatic and asymptomatic samples were provided from the same sampling location and by the same individual (Samples 27–36 in July and 1–21 in September, Appendices 2 & 3). While there appeared to be little difference between the numbers of positive tests in the asymptomatic and symptomatic samples (7 vs. 8 positive tests in July and 30 vs. 38 positive tests in September), more of the positive tests in the symptomatic samples were strong (+++) positives (1 vs. 3 positive tests in July and 12 vs. 21 positive tests in September). These numbers are indicative of a possible association between overall viral load and symptom expression that should be explored further.

### Incidence of some viruses may be linked to geographical location

Lettuce samples were received from diverse sites ranging from Kent to southern Scotland. Insufficient samples tested positive for virus in July to allow observations regarding a possible correlation with geographical distribution. In September, most of the viruses that were found in >10 samples were found at all the sample locations. However, perhaps not surprisingly, overall virus incidence was higher in areas with a long history of lettuce cultivation and high intensity of lettuce production. Notably, samples from several regions were free of *Endive necrotic mosaic virus.* Conversely, *Tobacco rattle virus* was found primarily in samples from a single geographic area. However, it must be noted that many of the samples from the regions in question came from one or two farms, and it is possible that site-to-site variation rather than geographical location could account for differences in virus incidence.

## Conclusions

- Overall, lettuce samples were tested for the presence of seventeen different viruses, with the following results:
    - Nine viruses tested positive in at least one lettuce sample (*Alfalfa mosaic virus, Broad bean wilt virus II, Beet yellow stunt virus, Cucumber mosaic virus, Endive necrotic mosaic virus, Mirafiori lettuce big-vein virus, Tobacco rattle virus, Turnip yellows virus, Lettuce mosaic virus*)
    - Eight viruses tested negative in all samples (*Arabis mosaic virus, Broad bean wilt virus II, Impatiens necrotic spot virus, Lettuce necrotic stunt virus, Lettuce ring necrosis virus, Tobacco mosaic virus, Tomato spotted wilt virus, Turnip mosaic virus*)
    - Four of the nine positive viruses are new records, and to our knowledge have not previously been reported in the literature as being found in UK commercial lettuce

 

(*Alfalfa mosaic virus, Broad bean wilt virus I, Endive necrotic mosaic virus,* and *Tobacco rattle virus*)

- Virus incidence increased between the July and September sample dates.
- There was no clear correlation between virus incidence and symptom expression and/or lettuce cultivar. There is a slight indication that virus incidence may correlate with certain geographical locations (particularly those areas with a long history of lettuce growing) but, given the relatively small number of samples examined, this is not conclusive.

This initial screen showed that hitherto unsuspected viruses are present in UK outdoor lettuce, in some cases at high frequency. It remains to be determined whether a) virus presence in UK field lettuce is associated with marketable yield loss and/or post-harvest disorders and b) additional viruses, particularly those found in European lettuce, are also present in the UK. Additional yield data are being sought from growers to address the first of these questions, but an alternative research strategy may be required to obtain a definitive answer. A second round of virus screening will be undertaken during 2015 to determine whether a similar range of viruses is found on repeated investigation.

## Knowledge and Technology Transfer

HDC FV427 Literature Review 2014

Claire Burns, HDC Leafy Salads Roadshow, Huntapac Farms, Lancashire, 6th Nov 2014 (*presentation*)

Claire Burns, HDC Leafy Salads Roadshow, Chichester College, Brinsbury, 12th Nov 2014 (*presentation*)

Martin McPherson, HDC Leafy Salads Roadshow, Farm Energy Centre, Stoneleigh, 19th Nov 2014 (*presentation*)

 

## Appendices

### **Appendix 1.** Lettuce sampling questionnaire and protocol

**E837 (FV 427) Lettuce Virus Screening**

**Sample Details (please complete one form for each sample sent)**

| | | | |
|---|---|---|---|
| Sample Supplied by (name) | | | |
| Contact email address | | | |
| Sample date | | | |
| Farm Address | | | |
| Field Name/Reference | | | |
| Lettuce Type & Variety | | | |
| Planting Date (or approximate age of crop) | | | |
| Growth Stage | | | |
| Any symptoms in crop? | Yes/No (delete as appropriate) | | |
| Describe any symptoms (where applicable) | | | |
| Is crop performing as well as expected? | Yes/No (delete as appropriate)<br>Please comment if 'No' | | |
| Previous cropping | 2013 | 2012 | 2011 |
| | | | |
| Pesticides applied To current crop | Fungicides | Insecticides (including seed treatments) | Herbicides |
| Comment on presence of aphids/weeds/other virus vectors | | | |

 

**Field Sampling Protocol for Growers/Consultants**

**Type of sample**
Samples of any outdoor lettuce type are required from crops across the UK.

**Sample Timing**
Please sample and dispatch to STC during the first two weeks in July (first sampling period) and again during the second week in September (second sampling period).
Where symptomatic plants are found at other times, these may be sent in and will be included when we carry out the main batches of testing. However, the closer to the main sampling periods these are sent, the more accurate the results will be.

**Sample Selection**
Where heads are showing symptoms of any description, please sample separately and make a note on the sample record sheet of the specific symptoms seen. These may be typical virus symptoms (such as mottling, chlorosis, stunting etc) or other symptoms not attributable to any specific cause (perhaps small plants, unexplained leaf discoloration, vein discoloration or tip burn).

Where the crop is apparently healthy (asymptomatic), please sample from across the field, taking a representative sample as you would a soil sample (eg. in a 'W' across the field).

Keep symptomatic and asymptomatic samples from the same field separate. Please also keep varieties separate.

It is not necessary to sample whole heads, instead sample outer leaves from a number of heads (15-20 leaves from separate heads across the field would be ideal). Place leaves in a sealed plastic bag labelled with the field name, date sampled and symptom where appropriate.

**Sample Hygiene**
Wear fresh disposable gloves for each sample location (i.e. no need to change gloves between leaves going into the same bag).

**Sample Dispatch**
Dispatch samples to Stockbridge Technology Centre (address below) as soon as possible, refrigerating them until you are able to send them, try to avoid samples spending the weekend in the post.

Lettuce Virus Screen
c/o Plant Clinic
Stockbridge Technology Centre
Stockbridge House
Cawood
Selby
YO8 3TZ

If you require additional materials for sampling (gloves, bags, address labels etc) please contact Mandy Hewick or Claire Burns by telephone (01757 268275) or email (Claire.burns@stc-nyorks.co.uk).

 

## **Appendix 2.** Lettuce Sample Details and ELISA Results, July 2014

| Sample code | Variety | Symptoms* | Pests and weeds** | | Problems at harvest*** | MiLBVV | CMV | LMV | TRV | AMV | TuYV | BBWV I&II |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Insects | Weeds | | | | | | | | |
| JUN14-01 | Iceberg | yes | Aphids | ns | ns | - | - | +++ | - | - | - | +++ |
| JUN14-02 | Romaine | yes | Aphids | ns | ns | - | - | - | - | - | - | +++ |
| JUN14-03 | Iceberg | yes | Aphids | ns | ns | - | - | +++ | - | - | - | +++ |
| JUN14-04 | Iceberg | no | None | ns | yes | - | - | - | - | - | + | +++ |
| JUN14-05 | Iceberg | no | None | ns | yes | | | | | | | |
| JUN14-06 | Iceberg | no | None | ns | yes | | | | | | | |
| JUN14-07 | Iceberg | no | None | ns | yes | | | | | | | |
| JUN14-08 | Iceberg | no | None | ns | no | +++ | - | - | - | +++ | - | - |
| JUN14-09 | Iceberg | no | None | Few weeds | yes | - | - | +++ | - | +/- | - | +++ |
| JUN14-10 | Iceberg | no | None | Few weeds | yes | | | | | | | |
| JUN14-11 | Little Gem | no | None | Few weeds | no | - | - | - | - | - | - | ++ |
| JUN14-12 | Little Gem | no | None | Few weeds | no | - | - | - | ++ | - | - | ++ |
| JUN14-13 | Romaine | no | None | Few weeds | yes | - | - | - | - | - | + | +++ |
| JUN14-14 | Romaine | no | ns | Some weeds | yes | - | - | - | - | - | - | ++ |
| JUN14-15 | Romaine | no | None | Few weeds | yes | - | - | - | - | - | - | - |
| JUN14-16 | Romaine | no | None | Few weeds | no | | | | | | | |
| JUN14-17 | Other | yes | Aphids | ns | no | - | - | - | - | - | - | - |
| JUN14-18 | Other | yes | Aphids | ns | no | - | - | - | - | - | - | ++ |
| JUN14-19 | Other | no | Aphids | ns | no | - | - | ++ | - | - | - | + |
| JUN14-20 | Other | yes | Aphids | ns | no | - | - | +++ | ++ | - | +++ | + |
| JUN14-21 | Romaine | no | ns | ns | no | - | - | - | - | - | - | ++ |
| JUN14-22 | Other | no | ns | ns | no | - | - | ++ | - | - | - | ++ |
| JUN14-23 | Other | no | Minimal | Minimal | no | - | - | - | + | - | ++ | - |
| JUN14-24 | Romaine | no | Aphids | None | no | - | - | - | - | - | - | ++ |
| JUN14-25 | Romaine | no | Aphids | None | no | | | | | | | |
| JUN14-26 | Romaine | no | Aphids | None | no | | | | | | | |
| JUN14-27 | Iceberg | no | ns | ns | no | - | - | ++ | - | ++ | +++ | - |
| JUN14-28 | Iceberg | yes | ns | ns | no | - | - | - | - | +++ | +++ | - |
| JUN14-29 | Iceberg | yes | ns | ns | yes | - | - | - | - | - | - | ++ |
| JUN14-30 | Iceberg | no | ns | ns | yes | - | - | - | - | ++ | - | ++ |
| JUN14-31 | Iceberg | no | ns | ns | no | - | - | - | - | +/- | - | - |
| JUN14-32 | Iceberg | yes | ns | ns | no | - | - | - | - | - | +++ | +/- |
| JUN14-33 | Iceberg | yes | ns | ns | no | - | - | - | - | - | + | ++ |
| JUN14-34 | Iceberg | no | ns | ns | no | - | - | - | - | ++ | - | +/- |
| JUN14-35 | Iceberg | yes | ns | ns | yes | - | - | - | - | - | ++ | ++ |
| JUN14-36 | Iceberg | no | ns | ns | yes | - | - | - | - | - | - | ++ |
| JUN14-37 | Romaine | no | ns | ns | no | - | - | - | - | - | - | + |
| JUN14-38 | Romaine | no | ns | ns | no | - | - | - | - | - | - | ++ |
| JUN14-39 | Romaine | no | None | None | no | - | - | - | - | - | - | + |
| JUN14-40 | Iceberg | no | ns | Groundsel | no | +++ | - | - | - | +++ | - | - |
| JUN14-41 | Iceberg | no | None | None | no | | | | | | | |
| JUN14-42 | Little Gem | no | None | None | no | - | - | - | - | + | +++ | ++ |
| JUN14-43 | Little Gem | no | ns | Groundsel | no | - | - | - | - | - | - | ++ |
| JUN14-44 | Iceberg | no | ns | ns | no | - | - | - | - | - | - | - |
| JUN14-45 | Iceberg | yes | ns | ns | no | - | +++ | - | - | - | - | - |
| JUN14-46 | Iceberg | yes | ns | ns | no | - | - | - | - | - | - | - |
| JUN14-47 | Iceberg | no | Thrips | ns | no | - | - | - | - | +++ | - | + |
| JUN14-48 | Little Gem | yes | ns | Groundsel | no | - | - | - | - | - | - | - |

Sample not tested is denoted by

*Symptomatic/asymptomatic rating on sample information form from grower.

** Where no details were provided by growers, “ns“ (not stated) is used.

***Problems at harvest defined as <75% yield (where yields known) or symptoms noted at harvest, e.g. breakdown, tip burn, twisting.

For ELISA testing, strength of positive response is denoted by '+' numbers, where +++ indicates the strongest positive signal. Samples with both + and - (e.g., ++/-) indicate that one of the two replicates tested negative and one tested positive.

All samples tested negative for TMV, TuMV, ArMV, LNSV, and TSWV/INSV.

 

## **Appendix 3.** Lettuce Sample Details and ELISA results, September 2014

| Sample code | Variety | Sympt* | Pests and weeds** | | Probs at harv*** | TuYV | TRV | ENMV | BYSV | BBWV I | CMV | AMV | LMV | MiLBVV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Insects | Weeds | | | | | | | | | | |
| SEP14-01 | Little Gem | no | ns | ns | yes | +++ | + | - | + | + | - | - | +++/- | - |
| SEP14-02 | Little Gem | no | ns | ns | yes | +++ | +++/- | - | + | ++ | - | - | - | - |
| SEP14-03 | Little Gem | no | ns | ns | yes | | | | | | | | | |
| SEP14-04 | Romaine | no | ns | ns | no | - | - | + | +++ | + | - | - | - | - |
| SEP14-05 | Romaine | no | ns | ns | no | + | + | +++ | +++ | - | +++ | - | +++ | - |
| SEP14-06 | Iceberg | no | ns | ns | yes | +++ | - | - | - | +++ | - | - | - | - |
| SEP14-07 | Romaine | no | ns | ns | yes | ++ | - | - | - | + | - | - | - | - |
| SEP14-08 | Iceberg | no | ns | ns | yes | | | | | | | | | |
| SEP14-09 | Iceberg | no | ns | ns | yes | - | - | - | ++ | ++ | - | - | +++/- | - |
| SEP14-10 | Romaine | yes | ns | ns | yes | +++ | +++ | +++ | +++ | +++ | + | +/- | +++/- | + |
| SEP14-11 | Little Gem | no | ns | ns | yes | +++ | +++ | - | ++ | +++ | - | ++ | - | +/- |
| SEP14-12 | Iceberg | no | ns | ns | no | - | + | - | - | + | - | - | - | - |
| SEP14-13 | Romaine | no | ns | ns | yes | - | - | - | + | +/- | - | - | - | - |
| SEP14-14 | Romaine | yes | ns | ns | yes | - | - | +++ | ++ | + | - | - | - | - |
| SEP14-15 | Iceberg | yes | ns | ns | yes | +++ | + | + | - | ++ | - | - | - | - |
| SEP14-16 | Romaine | yes | ns | ns | yes | +++ | - | +++ | +++ | + | - | - | - | - |
| SEP14-17 | Iceberg | yes | ns | ns | no | ++/- | +++ | +++ | +++ | +++ | - | + | - | - |
| SEP14-18 | Romaine | yes | ns | ns | yes | + | - | + | ++ | +++ | - | - | +++/- | - |
| SEP14-19 | Iceberg | yes | ns | ns | yes | +++ | ++ | +++ | +++ | +++ | - | + | +++ | + |
| SEP14-20 | Little Gem | yes | ns | ns | yes | | | | | | | | | |
| SEP14-21 | Little Gem | yes | ns | ns | yes | - | - | ++ | +++ | + | - | - | - | - |
| SEP14-22 | Other | no | ns | ns | no | +++ | - | - | - | - | - | - | - | - |
| SEP14-23 | Little Gem | no | ns | ns | no | +++ | +++ | - | - | + | - | +/- | - | - |
| SEP14-24 | Little Gem | yes | ns | ns | no | - | +++ | - | ++ | + | - | - | - | - |
| SEP14-25 | Other | yes | ns | ns | no | - | +++ | - | - | +++ | - | - | - | + |
| SEP14-26 | Other | ns | ns | ns | no | +++ | ++ | - | +/- | +++ | - | - | - | - |
| SEP14-27 | Romaine | ns | ns | ns | no | +++ | +++ | - | +++ | +++ | - | +/- | - | +/- |
| SEP14-28 | Romaine | ns | ns | ns | no | +++ | +++ | - | +++ | +++ | - | ++ | - | + |
| SEP14-29 | Other | no | None | Groundsel Fat hen | no | +++ | + | - | - | + | - | - | +++/- | - |
| SEP14-30 | Other | no | None | ns | no | +++ | +++ | - | - | ++ | - | - | - | - |
| SEP14-31 | Other | no | None | Groundsel Fat hen | no | + | - | +++ | +++ | + | - | - | - | - |
| SEP14-32 | Other | no | Aphids | None | no | - | - | + | ++/- | - | - | - | - | - |
| SEP14-33 | Other | no | Aphids | None | no | - | - | + | +++ | - | - | - | - | - |
| SEP14-34 | Other | no | Aphids | None | no | - | +++ | - | - | +++ | - | - | +++ | - |
| SEP14-35 | Little Gem | yes | ns | Groundsel Fat hen | no | ++/- | - | - | - | + | - | - | ++/- | - |
| SEP14-36 | Romaine | no | None | None | yes | - | +/- | - | - | ++/- | - | - | - | - |
| SEP14-37 | Iceberg | no | ns | Groundsel | no | +++/- | - | +++/- | +/- | ++ | - | - | - | - |
| SEP14-38 | Iceberg | yes | None | None | no | + | - | - | - | +++ | - | - | - | - |
| SEP14-39 | Iceberg | no | Aphids | Some weeds | no | - | +++ | - | - | ++ | - | - | - | - |
| SEP14-40 | Iceberg | yes | ns | Some weeds | yes | - | - | - | - | +/- | - | - | - | - |
| SEP14-41 | Iceberg | no | ns | ns | yes | - | ++ | - | - | ++ | - | ++ | - | - |
| SEP14-42 | Romaine | no | ns | ns | no | - | - | - | - | +/- | - | - | - | - |
| SEP14-43 | Little Gem | no | ns | ns | no | - | - | - | - | - | - | - | - | - |
| SEP14-44 | Little Gem | yes | ns | ns | yes | - | - | - | ++/- | + | - | - | +++/- | - |
| SEP14-45 | Iceberg | yes | ns | ns | yes | +++ | ++ | - | +++ | +++ | - | +++ | +++/- | - |

Sample not tested is denoted by

*Symptomatic/asymptomatic rating on sample information form from grower.

**Where no details were provided by growers, “ns“ (not stated) is used.

**Problems at harvest defined as <75% yield (where yields known) or symptoms noted at harvest, e.g. breakdown, tip burn, twisting.

For ELISA testing, strength of positive response is denoted by '+' numbers, where +++ indicates the strongest positive signal. Samples with both + and - (e.g., ++/-) indicate that one of the two replicates tested negative and one tested positive.

All samples tested negative for BBWV II and LRNV

 

 