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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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Contents

GROWER SUMMARY

Headlines

- Virus testing using serological (ELISA) methods in 2014 and 2015 suggests that several viruses are present in lettuce crops throughout the UK.
- Continued virus testing should be considered in an effort to build up a more comprehensive database of virus risk in UK lettuce crops with an emphasis on relating virus presence to symptoms, quality, yield and marketability

Background

Some common viruses such as *Lettuce mosaic virus* and *Mirafiori lettuce big vein virus* may cause characteristic and recognisable symptoms in field lettuce. However, many other viruses that infect lettuce can either be symptomless or cause a diverse range of symptoms (especially when mixed combinations of viruses occur or when varietal susceptibility varies) that can potentially be attributed to other factors. Previous AHDB-funded research found that previously unsuspected virus infections had the potential to cause both yield and quality effects. For instance 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.

In this project, the state of knowledge regarding viruses in lettuce was determined through a literature review, an appropriate list of viruses compiled for testing using commercially available ELISA kits and a virus screen performed on samples from commercial crops in July and September 2014 and 2015. ELISA screening is limited by the need to look for pre-determined viruses using specific antisera. It means that other viruses present would not be detected if present. Next Generation Sequencing, a non-targeted diagnostic technique, was also utilised in the latter stages of the project to seek additional viruses that would not be detected by the ELISA screen, due to the specificity of that method.

Summary

Over two seasons a total of 187 composite lettuce samples were screened serologically (by ELISA) for a range of viruses. In 2014, 17 viruses were assessed over two sampling periods (July and September). In 2015, lettuce samples were again screened in July and September for 12 selected viruses.

It is important to note that most samples comprised leaves from a number of plants i.e. they were composite samples. Where multiple viruses were found in a sample it should not be assumed that all viruses were present in a single lettuce plant.

The viruses selected for testing were based on the considered risk of the virus occurring in UK lettuce crops. Virus risk was based on previous findings/reports in the UK and/or its

presence in neighbouring EU Member States. The availability of appropriate commercial antisera also steered virus selection.

Eighty two samples were tested in 2014 and 105 samples tested in 2015. Screening suggested that several different viruses were present each year and a number of composite samples tested positive for multiple viruses. Across 2014 and 2015, ELISA screening suggested a total of 11 different viruses in the composite field lettuce samples received. In both years, virus incidence appeared to increase between the July and September sample dates. A number of samples tested positive for multiple viruses in both years. Many of the viruses investigated are aphid-transmitted.

Correlations between virus incidence and symptoms, variety, lettuce type and vector presence are unclear based upon the data collected over this two year project. It is not possible to conclude that viruses were necessarily responsible, in all cases, for the poor quality and low yields/increased wastage reported in 2015. Further, more detailed investigations would be required to identify any definite correlations.

Twenty one lettuce seed samples tested by ELISA in 2015 were all negative for the four viruses screened. Weed (groundsel (*Senecio vulgaris)*) samples received in June 2015 all tested negative by ELISA for the four viruses screened. Four additional weed samples were received in July 2015 and one fat hen (*Chenopodium album*) sample tested positive by ELISA for *Lettuce mosaic virus*.

Next Generation Sequencing and supporting diagnostic testing was carried out on 20 composite lettuce samples to identify any additional viruses present. Taken together, the results of the ELISA and NGS screening suggested the presence of previously unreported viruses in UK field samples. These findings should be considered as unconfirmed reports until such time that results can be verified through further analysis.

Based on the ELISA screening results, symptomatic and asymptomatic virus remains a risk in UK lettuce crops and vector control continues to be an important factor in lettuce production. Continued virus testing should be considered in an effort to build up a more comprehensive database of virus risk in UK lettuce crops with an emphasis on relating virus presence to symptoms, quality, yield and marketability.

Financial Benefits

This project aimed to carry out an initial screen of UK lettuce crops and to provide a baseline assessment of virus present in those crops. It is not clear whether the viruses found to be present are having a financial impact on yield, but it seems likely that there would be some impact on quality and therefore on marketable yield. Increased grower awareness of the presence of virus will hopefully lead to continued and improved management of virus vectors, subsequent improvements in crop quality and therefore improvements in marketability of

crops. More accurate calculations of financial benefit would require more in-depth studies of virus load and associated impact on crop quality and yield but this was outside the remit of this project.

Action Points

Basic principles of virus management should be considered by growers in order to minimise virus transmission and any potential impacts. Growers should start the season with clean seed and use tolerant and/or resistant varieties where these are available.

Crops should ideally be grown in isolation from other susceptible crops, both geographically and in time (i.e. consider crop rotations) although it is recognised that in intensive production areas this may be difficult, if not impossible, to achieve. The presence of vectors (such as aphids and nematodes) and virus reservoirs (such as weeds and other susceptible crops) should then be reduced as much as possible using integrated management practices.

SCIENCE SECTION

Introduction

Field-grown lettuce crops in the UK are susceptible to a wide range of viruses arising from a variety of sources. Viruses can potentially 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 mottling, 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 was to identify viruses that may be affecting UK lettuce based on an initial review of literature and through testing samples from commercial field lettuce crops during July and September 2014 and during July and September 2015. In addition, seed and weed (primarily groundsel) samples were tested in June 2015 to identify potential sources of viral infection.

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 1. Further details can be found in the literature review for this project (HDC FV427; Literature Review 2014).

The remit of this project was to use commercially available ELISA (enzyme-linked immunosorbant assay) kits to assess for virus presence in 40 grower-provided lettuce samples for up to twelve different viruses in each summer and autumn of 2 years. In Year 1 (2014), nine viruses known to infect UK lettuce crops were included in testing (Table 1), and additional viruses were selected based on grower consultation, symptom severity, and/or presence in neighbouring European countries either on lettuce or similar crops (Table 2). Some viruses were excluded based on the lack of availability of antisera rather than on their likelihood of being detected in UK lettuce crops. Viruses that tested negative in the July screen were not re-tested in the September screen. In Year 2 (2015), all nine viruses that tested positive in Year 1 were screened in both July and September, plus three viruses selected on the basis of either having been detected in UK lettuce previously or being common in other UK plant species. In order to identify any potential source of virus infection prior to planting, a number of seed, weed and pre-planting lettuce samples were tested in 2015.

In 2015, a small amount of additional funding was secured through AHDB to allow Fera to test a small number of samples by Next Generation Sequencing (NGS) using the Illumina MiSeq

platform. NGS is a relatively new method of RNA sequencing which allows rapid sequencing of RNA fragments in a sample (in this case lettuce), followed by alignment of retrieved sequences with the GenBank database. This method has been shown to give broad based detection of viruses present in a test sample, including those not previously known to science.

Table 1. Summary of viruses reported previously on lettuce in the United Kingdom

Virus	**Acronym**	**Transmission**	**Tested in 2014 virus screen**	**Tested in 2015 virus screen**
Arabis mosaic virus	ArMV	Nematode (e.g. *Xiphinema diversicaudatum*), seed	July only	Not tested (Negative in 2014)
Beet pseudo-yellows virus	BPYV	Whitefly (*Trialeurodes vaporariorum)*	Not tested*	Not tested*
Beet yellow stunt virus	BYSV	Aphids (e.g. *Hyperomyzus lactucae*)	September only	June, July and September
Cucumber mosaic virus	CMV	Aphids (e.g. *Myzus persicae*)	July and September	July and September
Dandelion yellow mosaic virus	DYMV	Aphids (e.g. *Myzus persicae*)	Not tested*	Not tested*
Lettuce big-vein associated virus	LBVaV	*Olpidium brassicae*	Not tested*	Not tested*
Lettuce mosaic virus	LMV	Aphids (e.g. *Myzus persicae*), seed	July and September	July and September
Lettuce necrotic yellows virus	LNYV	Aphids (e.g. *Hyperomyzus lactucae*)	Not tested*	Not tested*
Lettuce ring necrosis virus	LRNV	*Olpidium brassicae*	September only	July and September
Mirafiori lettuce big-vein virus	MiLBVV	*Olpidium brassicae*	July and September	July and September
*Tomato spotted wilt virus***	TSWV	Thrips (e.g. *Franklinella occidentalis*)	July only	Not tested (Negative in 2014)
Turnip mosaic virus	TuMV	Aphids (e.g. *Myzus persicae*)	July only	July and September
Turnip yellows virus ***	TuYV	Aphids (e.g. *Myzus persicae*)	July and September	June, July and September

* No antisera were available for these viruses

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

*** synonymous with Beet western yellows virus

Table 2. Summary of additional viruses selected for screening

Virus	Acronym	Transmission	Nearest reporting location on commercial lettuce (pre-2014)	Tested in 2014 virus screen	Tested in 2015 virus screen
Alfalfa mosaic virus	AMV	Aphids (e.g. *Myzus persicae*)	France	July and September	June, July and September
*Broad bean wilt virus I & II**	BBWV I & II	Aphids (e.g. *Myzus persicae*)	Germany / Northern Europe	July only	September only
Broad bean wilt virus I	BBWV I	Aphids (e.g. *Myzus persicae*)	Germany / Northern Europe	September only	July only
Broad bean wilt virus II	BBWV II	Aphids (e.g. *Myzus persicae*)	Germany / Northern Europe	September only	
Endive necrotic mosaic virus	ENMV	Aphids (e.g. *Myzus persicae*)	France / Germany	September only	July and September
*Impatiens necrotic spot virus***	INSV	Thrips (e.g. *Franklinella occidentalis*)	Europe	July only	Not tested (Negative in 2014)
Lettuce necrotic stunt virus	LNSV	Mechanical / soilborne. Possibly seed.	USA	July only	Not tested (Negative in 2014)
Tobacco mosaic virus	TMV	Mechanical, seed	-	July only	July and September
Tobacco rattle virus	TRV	Nematodes (e.g. *Trichodorus minor*), mechanical & seed	Denmark, Italy	July and September	June, July and September

* *Broad bean wilt viruses I & II* were tested in a combined assay in July 2014; this test was positive, and tests distinguishing between the two viruses were subsequently used in the September 2014 screen. In 2015 BBWV I tests were used in July but the wrong antisera were provided by the supplier in September and it was necessary to use the joint test for BBWV I and II instead.

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

Materials and Methods

Sampling and sample storage

Groundsel (*Senecio vulgaris*) samples were provided directly by growers from fields, or from near fields intended for lettuce planting. Lettuce seed samples were received either directly from growers or were sampled by STC staff at a commercial propagation site, with growers permission. Groundsel samples were stored at 4ºC prior to testing, whilst seed samples were germinated in order to provide fresh shoot material for testing.

Lettuce samples (which were, in the main, composite samples comprising leaves from a number of plants across a field site) were provided directly by growers and/or consultants during July and September in both 2014 and 2015. A large number of samples were received after the sampling deadline in July 2015 and additional funding was granted to allow testing of the majority of these additional samples received. Sample forms and protocols (Appendix 1) were provided to growers and although many growers provided most of the information requested, detailed information was not received for all samples. Samples were provided of many different varieties from diverse locations and sample details are provided in Appendices 2 & 3. On arrival, samples were logged in, checked for damage and stored at 3-4°C in preparation for ELISA testing.

A summary of the number of samples received, the number tested and the dates of testing can be found in Table 3.

Table 3. Details of sample numbers received and timing of testing

'Lettuce' refers to composite samples.

Season	**Number of samples received**	**Number of samples tested**	**Testing dates**
July 2014	48 lettuce	40 lettuce	9-10th July 2014
September 2014	45 lettuce	42 lettuce	23rd September 2014
June 2015	20 seed 17 groundsel	20 seed 17 groundsel	2-3rd June 2015
July 2015	36 lettuce (Round 1), of which 12 were pre-planting samples 50 lettuce (Round 2) 5 weed (Round 2) 1 seed (Round 2)	36 lettuce (Round 1) of which 12 were pre-planting samples 38 lettuce (Round 2) 4 weed (Round 2) 1 seed (Round 2)	20-21st July 2015 10-11th August 2015
September 2015	46 lettuce	43 lettuce	22-23rd September 2015

DAS ELISA testing

Samples were tested using Double Antibody Sandwich Enzyme-Linked Immunosorbant Assay (DAS-ELISA). No single company supplied reagents for all the viruses of interest in this project, so multiple sources were necessary. ELISA reagents, positive and negative controls and ready-to-use kits were purchased from Neogen (Ayr, Scotland), Loewe Biochemica (Sauerlach, Germany), DSMZ (Braunschweig, Germany), and AC Diagnostics (Fayetteville, USA).

ELISA tests 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. 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. Tissue samples (lettuce, weed or young shoot material) were then homogenised in sample buffer and added, in duplicate, to the 96-well plate along with negative, positive and blank controls. Excess sample was removed by washing. Next, conjugated antibody was added and allowed to bind to the antigen. Plates were washed four times between stages using an automated plate washer. Finally, a reactive substrate (p-nitrophenyl phosphate) was added that produced a yellow colour upon interaction with the antibody conjugate. An example 96-well plate layout is shown in Table 4.

Table 4. Example 96-well plate layout for lettuce virus screening by ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	S3	S11	S14	S19	S22	S28	S32	S35	S39	S43	Blank
B	S1	S3	S11	S14	S19	S22	S28	S32	S35	S39	S43	S47
C	S1	S4	Blank	S15	Pos	S23	S29	Blank	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	Blank	S24	S30	Pos	S37	Blank	S45	S48
G	S2	S9	S13	S18	S21	S27	S31	S34	S38	S42	S46	S48
H	Blank	S9	S13	S18	S21	S27	S31	S34	S38	S42	S46	Blank

Shaded cells provide plate co-ordinates. Pos: positive control; Neg: negative control; Blank: buffer-only control. Wells labelled with S indicate sample number (in duplicate).

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.

A number of methods can be used to determine positive thresholds, and in both 2014 and 2015 various checks were made on the data to ensure that appropriate thresholds were set.

In 2014, thresholds were set 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

When applied to 2015 data, these thresholds were deemed to result in too many potentially false positive results and so slightly more conservative thresholds, based upon the negative control (NC) values, were used, as shown below.

Possible positive (threshold for further investigation) (+)	> 2 x NC average
Strong positive (++) value	> 3 x NC average

In 2014 and initially in 2015, thresholds were set based on results from negative controls received with ELISA testing kits. There is prior evidence, however, that negative controls should be of the same plant type or species as the samples under test (Clark & Adams, 1977; Sutula *et al.*, 1986; EPPO PM7/98, 2010; EPPO PM7/125, 2015). In light of this, four lettuce samples from each sampling period that had tested negative for all viruses using kit negative controls, were selected and used as negative controls in a re-analysis of all 2015 data. This resulted in a small number of positive results becoming negative.

In initial analysis for both years, 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.

For final analyses, reporting thresholds were set to more accurately reflect the binary nature of ELISA data (positive or negative), such that only 'strong' or 'high' positive values were deemed as positive, while all other categories were classed as negative in the summaries and discussions in this document.

Next Generation Sequencing (NGS)

Twenty grower composite lettuce samples that had been tested by ELISA at STC were then submitted to Fera for sequence analysis. Samples were logged on arrival at Fera and frozen at -80^{o}C until further processing could take place. Freezing is the most robust way of preserving the viral nucleic acids in the sample for further sequence analysis and is in routine use in standard molecular protocols at Fera (A. Fox, pers. comm.).

RNA was extracted from the samples using RNeasy kit (Qiagen, UK). Ribosome depleted ScriptSeq RNA Indexed sequencing libraries (Illumina) were prepared following the manufacturer's recommended protocols. The samples were then screened using an Illumina MiSeq sequencer for the presence of both reported and unreported plant pathogens using previously published protocols (Adams *et al*., 2013). The method as used has now completed validation testing and is currently submitted to UKAS for evaluation for accreditation to ISO 17025. In addition to test samples, a negative and positive control was sequenced to confirm the system was working within acceptable parameters. The negative control was a sample of healthy tobacco plant, the positive control was a set of artificial RNAs of known size and sequence. Both negative and positive controls were sequenced as expected. Sequence data was trimmed to remove low quality sequences and viral genomes constructed (genome assembly) using appropriate software. Sequences were compared with existing sequence data on the NCBI-Genbank database (BLAST search) to identify the presence of known and unexpected pathogens.

Follow-up testing

Following the NGS work, additional PCR and sap inoculation tests were carried out at Fera in order to confirm the NGS testing results.

PCR Testing: PCR tests were carried out on the sample extracts used for NGS and also on extracts obtained from larger portions of the original samples to ensure that any viruses present had not been missed as a consequence of incomplete distribution within the plant.

Three viruses which had been previously detected in multiple samples in the ELISA screen were selected as targets for real-time PCR testing. These were *Lettuce mosaic virus* (LMV), *Cucumber mosaic virus* (CMV) and *Tobacco rattle virus* (TRV). These viruses were selected either because they are known to be viruses which infect lettuce, but are controlled in seed trade (LMV) or they are viruses which have a broad host range and are known to be already present across a range of plant species in the UK (CMV and TRV).

For two of these viruses Fera has 'off the shelf' assays for the viruses. The TRV assay (Mumford et al, 2000) is in regular use in Fera diagnostic activity for the detection of *Tobacco rattle virus* in ornamentals, potato and other field crops. The assay for CMV was developed for 'in house' use at Fera, and is currently used for seed testing and for testing soft fruits and legumes in trade (beans as seed).

The assay for *Lettuce mosaic virus* has been developed specifically for this confirmation testing. All known sequences of LMV were aligned and areas of conserved genetic sequence (similarity) were identified. Two sets of real-time PCR primers were designed to these regions

of similarity to ensure breadth of detection. The PCR primers were tested against isolates of LMV from the Fera virus isolates collection to ensure they were working effectively.

Additionally the sample extracts were tested using a conventional one-step RT-PCR assay for the presence of Potyvirus (Van der Vlugt et al, 1999). This method is used as a first screen for this common group of aphid transmitted viruses. In this scenario it has been used as a screen for the presence of *Endive necrotic mosaic virus* (ENMV), but this would also detect other potyviruses such as *Lettuce mosaic virus*.

Real-time and conventional PCR testing was carried out in accordance with Fera standard operating procedures. Samples were run with a known positive control of the target virus from the Fera virus collection. Additionally a set of negative controls are also used to ensure there has been no cross contamination of samples. All controls worked as expected, indicating that testing was carried out correctly.

Sap Inoculation: A portion of each of the frozen samples was mechanically inoculated onto a standard range of experimental test plants and lettuce. This serves two purposes: (a) This may allow the transmission of the novel virus into a long term stable host for further study and characterisation including host range and vector studies; and (b) The variety of test plants utilised have susceptibility to a wide range of plant viruses acting as an additional check on the presence or absence of plant viruses in the sample set. However, in many cases viruses are not readily transmissible or may only be transmitted efficiently by a vector. With this in mind a positive result can be taken as an indication of virus presence, but a negative result does not necessarily mean that a sample is free from virus.

The samples were macerated in buffer with the addition of a fine grit, 'celite' then dusted onto the leaves of test plants for inoculation. These plants were: *Nicotiana benthamiana*, *N. occidentalis*, *N. hesperis*, *Chenopodium quinoa* and *Lactuca sativa* (lettuce cv 'All Year Round'). With the exception of the lettuce these plants are used as standard inoculation hosts due to their range of susceptibilities to a broad range of viruses.

Fera also undertook a related piece of work investigating the effect of sample condition and choice of negative controls on ELISA results. Following the outcome of this work, negative controls used for threshold setting were changed, as described in DAS ELISA testing section above.

Beet western yellows virus / Turnip yellows virus

Beet western yellows virus (BWYV) and *Turnip yellows virus* (TuYV) are closely related but distinct species. These were previously considered to be the strains of the same virus. European virus isolates do not infect sugar beet and related species, but do infect lettuce and

brassicas. However, strains of BWYV from the USA were found to 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 species. European TuYV antisera were used in the project and, whilst BWYV terminology was used in the literature review for this project, TuYV has been used in both subsequent reports.

Results

DAS-ELISA testing

Samples from 2014 were tested using the same methodology as 2015 samples, but thresholds for determining which samples were positive and which were negative were re-defined (see Methods section for details). A summary of results from both testing years can be found in Table 5. Full results are presented in Appendices 2-7, with ELISA results presented as they were classified initially.

Note: Lettuce samples received by STC for ELISA in some cases comprised composite samples, including leaves from more than one lettuce plant. Therefore, detection of multiple viruses in a sample does not necessarily indicate presence of all the detected viruses in all of the lettuces which were sub-sampled by the grower/consultant. Rather, it is possible that different individual lettuces contributing to a sample had one, or a small number of, virus(es) present.

Table 5. Viruses assessed by DAS-ELISA in UK lettuce crops (composite samples) in 2014 and 2015 (July and September)

	2014	**2015**
Viruses testing positive	***Alfalfa mosaic virus*** *Beet yellow stunt virus* ***Broad bean wilt virus I*** *Cucumber mosaic virus* ***Endive necrotic mosaic virus*** *Lettuce mosaic virus* *Mirafiori lettuce big-vein virus* ***Tobacco rattle virus*** *Turnip yellows virus*	***Broad bean wilt virus I & II*** [ab] *Beet yellow stunt virus* *Cucumber mosaic virus* ***Endive necrotic mosaic virus*** *Lettuce mosaic virus* *Mirafiori lettuce big-vein virus* ***Tobacco mosaic virus*** [b] *Turnip mosaic virus* *Turnip yellows virus*
Viruses testing negative	*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*	***Alfalfa mosaic virus*** *Lettuce ring necrosis virus* ***Tobacco rattle virus***

Viruses highlighted in **bold** have not, to our knowledge, been reported in UK lettuce prior to this project.

[a] Tested in combination.

[b] *Tobacco mosaic virus* and *Broad bean wilt virus I & II* occur in the UK on other hosts but have not previously been reported on lettuce

2014

For detailed 2014 results, please refer to FV 427 Annual Report, January 2015. A summary is provided in

Table **6** below.

Table 6. Summary of results from lettuce crops (composite samples) in July and September 2014

		July 2014		September 2014	
Virus		**Number of positive samples (n = 40)**	**% samples testing positive**	**Number of positive samples (n = 42)**	**% samples testing positive**
AMV	**Alfalfa mosaic virus**	**4**	**10**	**1**	**2.4**
ArMV	Arabis mosaic virus	0	0	nt	nt
BBWV I & II	**Broad bean wilt viruses I & II**	**6**	**15**	nt	nt
BBWV I	**Broad bean wilt virus I**	nt	nt	**13**	**30.9**
BBWV II	Broad bean wilt virus II	nt	nt	0	0.0
BYSV	Beet yellow stunt virus	nt	nt	12	28.6
CMV	Cucumber mosaic virus	1	2.5	1	2.4
ENMV	**Endive necrotic mosaic virus**	nt	nt	**7**	**16.7**
LMV	Lettuce mosaic virus	4	10	3	7.1
LNSV	Lettuce necrotic stunt virus	0	0	nt	nt
LRNV	Lettuce ring necrosis virus	nt	nt	0	0.0
MiLBV V	Mirafiori lettuce big-vein virus	2	5	0	0
TMV	Tobacco mosaic virus	0	0	nt	nt
TRV	**Tobacco rattle virus**	**0**	**0**	**11**	**26.2**
TSWV/ INSV	Tomato spotted wilt virus / Impatiens necrotic spot virus	0	0	nt	nt
TuMV	Turnip mosaic virus	0	0	nt	nt
TuYV	Turnip yellows virus	5	12.5	16	38.1

nt = not tested
Viruses in **bold** have not, to the best of our knowledge, been reported in UK lettuce prior to this project.

Based upon the thresholds and negative control values used in 2014, nine viruses tested positive in the samples received (

Table **6**). Four of the viruses in this initial screen (*Alfalfa mosaic virus, Broad bean wilt virus I, Endive necrotic mosaic virus* and *Tobacco rattle 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 FV 427: Literature Review for further details). These records are, as yet, unconfirmed and therefore should be treated with caution.

2015

Seeds and weeds. Four viruses (AMV, BYSV, TRV and TuYV) were screened for in 17 weed (groundsel) and 20 seed samples taken early in the 2015 season. None of these seed or weed samples tested positive for any of the four viruses selected. An additional seed sample and four weed samples (one each of groundsel, nettle, cow parsley and fat hen) were received later in the season and were tested for 12 viruses alongside lettuce samples. The fat hen sample tested positive for *Lettuce mosaic virus* whilst the other three weed samples and the seed sample all tested negative for all 12 viruses.

Pre-planting samples. Twelve viruses were assessed in 12 composite lettuce samples (ex-propagation) taken prior to being planted out in the field. Two of these samples were received directly from growers; the other 10 were sampled by STC staff at a commercial propagation site, with the permission of the grower client. One sample replicate from the commercial propagation site tested positive for *Tobacco rattle virus* although this was not accepted as a positive result since the second replicate sample was found to be negative. All other samples tested negative for all 12 viruses.

July field samples: Twelve viruses were assessed in composite samples taken from 62 lettuce crops by growers and consultants in July. Results from one sample (July 15-44) have been excluded as this sample tested positive for all viruses and this was considered to be an anomalous result. Of the remaining 61 samples, six viruses tested negative in all samples, two viruses were positive in one sample each and four viruses were detected in multiple samples. Forty two samples (69%) tested negative for all viruses, 14 samples contained just one virus and five samples tested positive for multiple viruses. See Table 7 for a summary of the viruses found.

September field samples: Twelve viruses were assessed in composite samples taken from 43 lettuce crops by growers and consultants in September. Four viruses were not detected in any samples whilst eight were detected in multiple samples. A higher proportion of samples tested positive for at least one virus at this sample timing, with only 17 samples (40%) testing negative. Nine samples were found to contain a single virus and 17 samples contained two or more viruses. See Table 7 for a summary of the viruses found.

Full results for all the samples and viruses tested can be found in Appendices 2-7.

Table 7. Summary of results from lettuce crops (composite samples) in July and September 2015.

Virus		July 2015		September 2015	
		Number of positive samples (n = 61)	**% samples testing positive**	**Number of positive samples (n = 43)**	**% samples testing positive**
AMV	**Alfalfa mosaic virus**	**0**	**0**	**0**	**0**
BBWV I	**Broad bean wilt virus I**	**0**	**0**	nt	nt
BBWV I & II	**Broad bean wilt viruses I & II**	nt	nt	**8**	**18.6**
BYSV	Beet yellow stunt virus	0	0.0	9	20.9
CMV	Cucumber mosaic virus	10	16.4	3	7.0
ENMV	**Endive necrotic mosaic virus**	**1**	**1.6**	**5**	**11.6**
LMV	Lettuce mosaic virus	7	11.5	20	46.5
LRNV	Lettuce ring necrosis virus	0	0.0	0	0.0
MiLBV V	Mirafiori lettuce big-vein virus	3	4.9	4	9.3
TMV	**Tobacco mosaic virus**	**2**	**3.3**	**0**	**0.0**
TRV	**Tobacco rattle virus**	**0**	**0**	**0**	**0.0**
TuMV	Turnip mosaic virus	0	0.0	19	44.2
TuYV	Turnip yellows virus	1	1.6	7	16.3

nt = not tested
Viruses in **bold** have not, to the best of our knowledge, been reported in UK lettuce prior to this project.
Data excludes one outlier sample

Growers submitting samples were asked to provide information regarding presence of weeds (potential virus reservoirs) and insects (potential virus vectors), and were also asked whether they felt the samples submitted displayed any abnormal symptoms. Whilst drawing statistical conclusions from this data is difficult, a summary of 2015 sample details and corresponding positive virus results is shown in Table 8.

Table 8. Summary of virus findings in relation to sample characteristics (2015)

		Count	No. of samples strongly positive for any virus	% samples positive for any virus
Lettuce Type	Iceberg	61	31	50.8
	Multi-leaf	8	2	25.0
	Romaine/Cos	10	2	20.0
	Batavia	8	3	37.5
	Butterhead	3	2	66.7
Symptoms	Symptomatic	38	23	60.5
	Asymptomatic	59	22	37.3
Vectors	Aphids present	18	4	22.2
	Aphids absent (incl 'low')	26	8	30.8
	Weeds present	38	17	44.7
	Weeds absent (none, few, low)	20	10	50.0

NB. Only samples where information was provided are included in the numbers above.

Due to the very wide range of cultivars received, varietal correlation with virus incidence is difficult to assess. Five main lettuce types were received (iceberg, cos/romaine, multi-leaf, batavia and butterhead) but sample numbers received were low for all types apart from iceberg. A high proportion of iceberg type lettuce tested positive for virus, but as iceberg accounts for a large proportion of UK lettuce cropping area it is possibly more at risk from virus than other crops grown less extensively. The data indicated that over half the iceberg and butterhead lettuce samples received tested positive for at least one virus.

There was a tendency in 2015 for samples described as ‘symptomatic’ by growers / consultants to be more likely to test positive for at least one virus, compared with asymptomatic samples. This trend was not apparent in the 2014 crop.

Based upon the limited information received from growers and the results of ELISA testing, observed aphid presence does not appear to have increased the likelihood of detecting virus in 2015. However, information about aphid presence was received for <50% of samples and so the sample size is perhaps too small to achieve significant conclusions. It is also possible that low numbers of aphids were transmitting non-persistent virus in crops but were not present in high enough numbers to be readily observed. Non-colonising aphids are likely to have a strong influence on the epidemiology of virus outbreaks of e.g. *Lettuce mosaic virus* and *Cucumber mosaic virus*, but are often under-represented in crop counts as they are transitory in crops (A. Fox, Fera, pers. comm.)

Some viruses were found in more samples than others, with those that are mainly transmitted by aphids appearing to be more prevalent than those transmitted by other means. See Table 9 for further details.

Table 9: Percentage of samples testing positive for each virus in July and September 2015

Virus	% samples positive (July 2015)	% samples positive (September 2015)	Vector
AMV	0.0	0.0	Aphids
BBWV	0.0	18.6	Aphids
BYSV	0.0	20.9	Aphids
CMV	16.4	7.0	Aphids
ENMV	1.6	11.6	Aphids
LMV	11.5	46.5	Aphids
LRNV	0.0	0.0	Fungus (*Olpidium brassicae*)
MilBVV	4.9	9.3	Fungus (*Olpidium brassicae*)
TMV	3.3	0.0	Mechanical/seed
TRV	0.0	0.0	Nematodes
TuMV	0.0	44.2	Aphids
TuYV	1.6	16.3	Aphids

Next Generation Sequencing

NGS analysis suggested fewer viruses were present in the 20 samples than had been suggested during the ELISA screening.

Two samples were shown to contain *Lettuce big vein associated virus* (LBVaV) which has been previously found in the UK (Navarro et al, 2005). Although the symptoms of Lettuce big-vein disease have been shown to be caused by *Miafiori lettuce big vein virus* (MLBVV) there is evidence that infection with LBVaV on its own may not be sufficient to cause symptoms (Lot *et al*, 2002; Sasaya *et al*, 2008), However there is recent evidence that LBVaV may also be associated with a malady of necrotic rings and spots (Verbeek et al, 2012).

One sample contained a sequence of a novel member of the family *Secovirus* (a genome annotation short report giving molecular characterisation data for this virus has been prepared by Fera and a manuscript is in preparation). This family contains the genus *Torradovirus* which are whitefly and aphid transmitted viruses and the genus *Nepovirus* which are nematode transmitted viruses, as well as three known unassigned species. Although only distantly related to any previously described member of the family, the nearest genetic match for this novel virus is to *Strawberry mottle virus* (37% identity), an unassigned secovirus. At this stage it is difficult to make inferences on the biology of this virus without further study.

In addition to the viruses listed above sequence data was also obtained for a previously unknown Hypovirus and an Ourmiavirus. Although it is possible that these are novel plant viruses, the other described members of these genera are viruses of fungi. The most likely explanation for the presence of these viruses therefore is that the lettuce samples concerned had a fungal infection or contamination and these viruses were affecting these fungal infections.

Follow-up testing

Follow-up tests using PCR (carried out on the RNA extracts used for NGS) were negative for TRV, CMV and LMV. Further extracts taken from larger portions of the original twenty samples also tested negative for TRV, CMV and LMV indicating that the sampling procedure had not been a source of discrepancy in results. An additional PCR screen for Potyvirus also concluded that all twenty samples were negative for this group of viruses, which includes *Endive necrotic mosaic virus* (ENMV) as well as LMV. These additional molecular analyses based on detection of nucleic acid (RNA) of the viruses, carried out on both the original sample extractions and on re-extractions, support the results of the NGS analysis that many of the viruses identified in the ELISA screening were not present in the samples tested by Fera. It should be noted that NGS and PCR methods detect fragments of nucleic acid which are not thought to be affected by freezing.

Sap inoculation studies did not result in any plant species displaying any virus symptoms. The indicator plant species used in sap inoculation testing are from a range of common field weeds and solanaceous species which across the range of species used are known to have a broad range of detection and symptom expression. A negative result in sap inoculation does not necessarily imply that samples were free from viral infection. For instance, some viruses, such as *Lettuce big vein associated virus* and *Miafiori lettuce big vein virus*, can be challenging to work with due to the virus particles of these species being labile. Some viruses, such as Luteoviruses are only transmissible by their vector. However, many of the viruses investigated in this study such as AMV, CMV, TMV, BBWV (1 and 2), TRV, and the potyviruses ENMV and LMV are readily transmissible and present characteristic symptoms. However, degradation of essential viral structures by freezing could render a virus inactive and lead to unsuccessful sap inoculation attempts. The lack of virus detected using these methods as follow-up testing on frozen samples cannot therefore be considered conclusive.

Endive necrotic mosaic virus was detected in a number of samples tested by ELISA at STC but not detected by Fera using NGS. Due to the discrepancy in results, the supplier of the ENMV ELISA reagents agreed to carry out additional checks on the 20 samples. Samples stored at -80^{o}C were sent to DSMZ by Fera and staff at DSMZ repeated the ELISA tests on

these samples. Unfortunately, their results did not correlate with either STC ELISA tests results or Fera NGS results. Nineteen of the samples appeared to test positive for ENMV, compared with four samples identified by STC tests. DSMZ also carried out electron microscopy on six of the 20 samples but were unable to locate any virus-like particles. Further work at Fera showed that freezing of samples can result in ELISA false positives. In addition, virus degradation following freezing can result in non-detection by electron microscopy. The results from these methods as follow-up testing on frozen samples cannot therefore be considered conclusive.

Discussion

Based on two years virus testing using serological (ELISA) methods the data gathered suggests that several viruses are present in lettuce crops throughout the UK. However, at this stage, it is not possible to conclude that they are necessarily responsible, in all cases, for some of the poor quality and low yields/increased wastage reported. Further detailed work in specific targeted crops would be required to demonstrate their full economic impact on lettuce production.

The incidence of virus detection increased between early and late sampling in both years as might be expected with predominantly vector, e.g. aphid-borne viruses. Where vectors are less mobile (e.g. fungal or nematode vectors) virus levels in autumn were only slightly higher than those in summer.

The following observations were made regarding aphid levels and virus symptoms on UK lettuce crops for the duration of this study (D. Norman, Fresh Produce Consultancy, pers. comm): In 2014, aphids arrived in lettuce crops relatively early during April and May; probably as a result of a very mild winter; though heavy rain in May appeared to interrupt the aphid life-cycle and populations declined in crops thereafter. In 2015 however, whilst the winter was a bit colder there were relatively few frosts and aphids over-wintered readily and appeared in outdoor salad crops by mid-May. Migration continued and there was a steady aphid pressure in crops throughout the summer period. Whilst crop protection treatments seemed to keep levels under control, aphids were never completely eliminated from crops as fresh aphid migrants continually arrived. During late June and July virus symptoms started appearing in commercial lettuce crops and by late July symptoms of virus could be found in all the main lettuce growing areas in the country. In 2015, there was an extremely high level of virus symptoms across a range of UK crops including lettuce, celery and carrots and significant crop losses occurred in all these crops.

From the ELISA results it is evident that virus detection, whilst found in 2014, was lower than in 2015 where there was significant virus expression. In 2015, there was a positive correlation between samples being described as ‘symptomatic’ and testing positive for one or more viruses. In 2014 there was no apparent correlation between symptomatic material and the presence of virus. It is possible that where virus was present in crops in 2014, conditions were not optimal for symptom expression. This supports the hypothesis that apparently symptomless plants may harbour viruses and therefore could be a source of crop variability and yield decline at harvest, although further testing would be required before firm conclusions could be made.

During the study, previously unreported viruses in UK lettuce crops e.g. *Broad Bean Wilt Virus*, *Alfalfa Mosaic Virus*, *Tobacco Mosaic Virus*, *Endive Necrotic Mosaic Virus* and *Tobacco Rattle Virus* have potentially been detected. Prior to this study, no recent data on incidence of viruses

on UK lettuce was available. Selection of these viruses for testing was based on the knowledge that these same viruses have been found in neighbouring EU Member States on lettuce. Some are already present in the UK in other plant species and there is a recognised risk of finding them in UK lettuce crops (see Literature Review for more details). It is important however that these reports of 'new' viruses are considered as unconfirmed virus reports until such time that they can be confirmed through further analysis.

In 2015, contradictory results for virus incidence were obtained for a sub-sample of twenty composite lettuce samples tested by NGS and PCR, in comparison with previous ELISA testing results. Additional work investigated possible causes of false positives in ELISA testing but indicated that none of these were likely to have contributed significantly in this case:

- Despite use of commercially verified ELISA kits and antisera, evidence from this project has indicated that false positives can result from use of negative controls that are not of the same plant type or species as the samples under test. This has also been reported in previous research (Clark & Adams, 1977; Sutula *et al.*, 1986; EPPO PM7/98, 2010; EPPO PM7/125, 2015). In light of this, four lettuce samples from each sampling period that had tested negative for all viruses using kit negative controls, were selected and used as negative controls in a re-analysis of all 2015 data. This resulted in a small number of positive results becoming negative.
- Freezing of samples was found to be a cause of false positive results, as was sample placement on ELISA testing plates. Both of these causes can be eliminated as possible explanations for the discrepancy between the ELISA and NGS virus screen results for the following reasons: lettuce samples received from industry were not frozen prior to testing but stored at 3-4^{o}C in a refrigerator and the arrangement of replicates on ELISA plates during the screening process prevented any erroneous reporting of results.
- Variety also potentially increased false positives in the additional investigation (Little Gem produced a higher number of false positives than other cultivars) but this may have been due to placement at the edge of the ELISA testing plate. In the main virus screen, two Little Gem samples were received but neither sample tested positive for any of the viruses selected. The effect of variety on potential false positives within the ELISA screen is therefore unclear.

It was concluded that one likely cause of discrepancy in results was the presence of leaves within each sample that originated from different plants (composite samples). Sub-sampling by laboratory staff was carried out to ensure a representative portion of a sample was taken for both ELISA and NGS testing. It is possible, however, that even with a good sub-sampling technique, material from different leaves was taken and therefore that different results were obtained.

This two year survey has suggested that a range of viruses may be present in lettuce crops and their incidence and impact require further investigation. It will require more detailed analysis on a crop by crop basis, ideally using tagged plants, to track virus incidence, symptom expression and plant quality and yield characteristics.

Conclusions

- Over 2 years (2014 & 2015) 187 lettuce samples were tested for the presence of seventeen different viruses, with the following results:
    - ELISA testing suggested that each of the following nine viruses was present in at least one sampled lettuce crop in 2014: *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*.
    - Eight viruses tested negative in all samples in 2014 (*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*)
    - Lettuce samples were positive for nine of the twelve viruses selected for ELISA testing in 2015. *Broad bean wilt virus I & II, Beet yellow stunt virus, Cucumber mosaic virus, Endive necrotic mosaic virus, Lettuce mosaic virus, Mirafiori lettuce big-vein virus, Tobacco mosaic virus, Turnip mosaic virus* and *Turnip yellows virus* were all detected in more than one sample.
    - Four viruses highlighted in the ELISA screen in 2014 have not previously been reported in UK lettuce crops: *Alfalfa mosaic virus*, *Broad bean wilt virus I*, *Endive necrotic mosaic virus* and *Tobacco rattle virus*. Three viruses detected in 2015 have not previously been reported in UK lettuce: *Broad bean wilt virus I & II, Endive necrotic mosaic virus,* and *Tobacco mosaic virus*. These are as yet unconfirmed reports and should not be considered to be records of new UK lettuce viruses until these reports can be confirmed through further analysis.
- Virus incidence appeared to increase between the July and September sample dates.
- Correlations between virus incidence and symptoms, variety, lettuce type and vector presence are unclear based upon the data collected over this two year project. Further, more detailed investigations would be required to identify any definite correlations.
- Growers should continue to operate basic virus avoidance principles:
    - Use certified seed of resistant varieties where possible
    - Isolate crops to reduce transmission from one crop to another where possible
    - Reduce sources of infection such as weeds and other potential host crops
    - Reduce vectors such as aphids and nematodes

Knowledge and Technology Transfer

HDC FV 427 Literature Review 2014

HDC FV 427 Annual report 2015

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*)

Martin McPherson, UK Brassica & Leafy Salad Conference, Peterborough, 28th January 2015 (*presentation*)

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APPENDICES

Appendix 1. Lettuce sampling questionnaires

Sample Reference (STC use only)

E837 (FV 427) Lettuce Virus Screening

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

Sample supplier			
Contact email address			
Farm Address			
Sample type	Groundsel ☐	Lettuce Seed ☐	
Sample date			
For lettuce seed only:			
Seed company			
Variety			
Seed batch number			
For groundsel only:			
Field name / Reference			
Current/next crop			
Previous cropping (2014)			
Previous cropping (2013)			
Previous cropping (2012)			
Symptoms/problems in previous crop? (lettuce or otherwise)	Yes ☐	No ☐	Unknown ☐
If yes, please describe symptoms/problems			

April 2015

Sample Reference
(STC use only)

E837 (FV 427) Lettuce Virus Screening

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

Sample Supplied by (name & company)			
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) Please comment if 'No'		
Previous cropping	2014	2013	2012
Pesticides applied To current crop	Fungicides	Insecticides (incl. seed treatments)	Herbicides
Comment on presence of aphids/weeds/other virus vectors			

Appendix 2: Sampling protocols (Seed/weed, summer and autumn protocols)

E837 (FV 427) Lettuce Virus Screening

Seed and Weed Sampling 2015 –
Protocol for Growers/Consultants

Stockbridge Technology Centre
Leaders in Technology Transfer to Agriculture and Horticulture

Type of sample
Samples from batches of lettuce seed being sown in 2015 are required, along with samples of groundsel from fields (or adjacent fields) intended for lettuce cropping in 2015.

Sample Timing
Please sample and dispatch to STC during the **week commencing 11th May 2015**.

Sample Selection
Lettuce seed: Please send a small sample (approximately 1000 seeds) from any varieties that will be planted out in 2015.
Groundsel samples: Please send whole plants (or a selection of leaves from larger plants) from within and around fields intended for lettuce production in 2015. Samples from within the same field can be bulked together but keep separate from groundsel samples from field margins/surrounding areas.

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 at the following address:

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 Kirsty Wright by telephone (01757 268275) or email (Kirsty.Wright@stc-nyorks.co.uk).

Stockbridge Technology Centre Ltd., Cawood, Selby, North Yorkshire YO8 3TZ
Telephone: +44(0)1757 268275 Facsimile: +44(0)1757 268996 Website: www.stc-nyorks.co.uk
Company registered in UK No. 4175440 VAT No. 8565972 70

E837 (FV 427) Lettuce Virus Screening
Field Sampling Protocol for Growers/Consultants

Stockbridge Technology Centre
Leaders in Technology Transfer to Agriculture and Horticulture

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 second week 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.

We would also appreciate samples of young plants pre-planting, if these are available at the time of sampling. If your plants are being raised offsite by a third party and you are happy for samples to be taken directly please inform us and we can arrange to take samples directly from your propagator.

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 Kirsty Wright or Mandy Hewick by telephone (01757 268275) or email (kirsty.wright@stc-nyorks.co.uk).

K. Wright
24.06.2015

E837 (FV 427) Lettuce Virus Screening
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 second week in September 2015. **Samples must arrive at STC by Thursday 17th September 2015** in order to be included in our tests.

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 pieces of outer leaves from a number of heads (sections from 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 Kirsty Wright or Mandy Hewick by telephone (01757 268275) or email (kirsty.wright@stc-nyorks.co.uk).

K. Wright
01.09.2015

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

Sample code	Type	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. Composite 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

Appendix 4. Seed and weed sample details and ELISA results, June 2015

Sample code	Type	Previous crop lettuce?	AMV	BYSV	TRV	TuYV
Seed 15-01	Multi Leaf	N/A	-	-	-	-
Seed 15-02	Multi Leaf	N/A	-	-	-	-
Seed 15-03	Lambs Lettuce	N/A	-	-	-	-
Seed 15-04	Multi Leaf	N/A	-	-	-	-
Seed 15-05	Multi Leaf	N/A	-	-	-	-
Seed 15-06	Multi Leaf	N/A	-	-	-	-
Seed 15-07	Multi Leaf	N/A	-	-	-	-
Seed 15-08	Batavia (green)	N/A	-	-	-	-
Seed 15-09	Batavia (red)	N/A	-	-	-	-
Seed 15-10	Iceberg	N/A	-	-	-	-
Seed 15-11		N/A	-	-	-	-
Seed 15-12	Batavia	N/A	-	-	-	-
Seed 15-13	Iceberg	N/A	-	-	-	-
Seed 15-14	Little Gem	N/A	-	-	-	-
Seed 15-15	Iceberg	N/A	-	-	-	-
Seed 15-16	Romaine	N/A	-	-	-	-
Seed 15-17	Iceberg	N/A	-	-	-	-
Seed 15-18	Cos	N/A	-	-	-	-
Seed 15-19	Romaine (red)	N/A	-	-	-	-
Seed 15-20	Multi Leaf	N/A	-	-	-	-
Groundsel 15-01		Ns	-	-	-	-
Groundsel 15-02		Yes	-	-	-	-
Groundsel 15-03		Yes	-	-	-	-
Groundsel 15-04		No	-	-	-	-
Groundsel 15-05		No	-	-	-	-
Groundsel 15-06		Yes	-	-	-	-
Groundsel 15-07		Yes	-	-	-	-
Groundsel 15-08		Yes	-	-	-	-
Groundsel 15-09		Yes	-	-	-	-
Groundsel 15-10			-	-	-	-
Groundsel 15-11			-	-	-	-
Groundsel 15-12		No	-	-	-	-
Groundsel 15-13		No (Spinach)	-	-	-	-
Groundsel 15-14		No	-	-	-	-
Groundsel 15-15		No	-	-	-	-
Groundsel 15-16		Yes	-	-	-	-
Groundsel 15-17		Yes	-	-	-	-

Appendix 5. Composite Lettuce Sample Details and ELISA results, July 2015 (first round)

Sample code	Type	Sympt*	Pests and weeds**		AMV	BBWV I	BYSV	CMV	ENMV	LMV	LRNV	MiLBVV	TMV	TRV	TuMV	TuYV
			Weeds	Insects												
JULY 15-01	Iceberg	no	groundsel	aphids-	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-02	Iceberg	yes	groundsel	aphids	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-03	Iceberg	no	groundsel	aphids	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-04	Lollo Rosso	pre-plant	n/a	n/a	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-05	Multileaf	no	low	aphids	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-06	Multileaf	no	low	aphids	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-07	Batavia	yes	low	aphids	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-08	Lollo Rosso	yes	low	aphids	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-09	Butterhead	no	ns	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-10	Batavia	no	ns	aphids	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-11	Butterhead	Pre-plant	ns	aphids	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-12	Iceberg	ns	ns	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-13	Batavia	no	groundsel	aphids	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-14	Romaine/ Cos	no	groundsel	aphids	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-15	Multileaf	no	groundsel	aphids	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-16	Iceberg	no	ns	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-17	Iceberg	no	ns	ns	-	-	-	-	-	-	-	++	-	-	-	-
JULY 15-18	Iceberg	no	ns	ns	-	-	-	-	-	-	-	++	-	-	-	-
JULY 15-19	Iceberg	Yes***	ns	ns	-	-	-	-	-	-	-	++	-	-	-	-
JULY 15-20	Iceberg	pre-plant	n/a	n/a	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-21	Iceberg	pre-plant	n/a	n/a	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-22	Romaine/ Cos	pre-plant	n/a	n/a	-	-	-	-	-	-	-	-	-	++	-	-
JULY 15-23	Endive	pre-plant	n/a	n/a	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-24	Endive	pre-plant	n/a	n/a	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-25	Romaine/ Cos	pre-plant	n/a	n/a	-	-	-	-	-	-	-	-	-	+	-	-
JULY 15-26	Romaine/ Cos	pre-plant	n/a	n/a	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-27	Romaine/ Cos	pre-plant	n/a	n/a	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-28	Iceberg	pre-plant	n/a	n/a	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-29	Iceberg	pre-plant	n/a	n/a	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-30	Iceberg	no	low	no	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-31	Romaine/ Cos	no	small	no	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-32	Romaine/ Cos	ns	ns	ns	-	-	-	+	-	-	-	-	-	-	-	-
JULY 15-33	Romaine/ Cos	ns	ns	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-34	Iceberg	ns	ns	ns	-	-	-	-	+	-	-	-	-	-	-	-
JULY 15-35	Iceberg	ns	ns	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-36	Iceberg	ns	ns	ns	-	-	-	-	-	-	-	-	-	-	-	-

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

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

******* Symptom described as ‘Big Vein’. Samples 17, 18 and 19 from same location.

Appendix 6. Composite Lettuce Sample Details and ELISA results, July 2015 (second round)

Sample code	Type	Sympt*	Pests and weeds**		AMV	BBWVI	BYSV	CMV	ENMV	LMV	LRNV	MiLBVV	TMV	TRV	TuMV	TuYV
			Weeds	Insects												
JULY 15-37	Iceberg	yes	groundsel/ fat hen	ns	-	-	+	-	+	-	-	-	++	+	-	++
JULY 15-38	Iceberg	no	groundsel/ fat hen	ns	-	-	-	-	-	-	-	-	+	-	-	-
JULY 15-39		yes	groundsel/ fat hen	ns	-	-	-	-	-	-	-	-	++	-	-	-
JULY 15-40		no	groundsel/ fat hen	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-41	Iceberg	yes	ns	ns	-	-	-	-	+	-	-	-	-	-	-	-
JULY 15-42	Iceberg	no	ns	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-43	Romaine/Cos	yes			-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-44	Romaine/Cos	no			++	++	++	++	++	++	+	++	++	++	+	++
JULY 15-45	Iceberg	yes	groundsel/ fat hen	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-46	Iceberg	no	groundsel/ fat hen	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-53	Iceberg	no	ns	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-54	Iceberg	no	ns	some aphids	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-55	Iceberg	no	ns	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-56	Iceberg	no	ns	some aphids	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-57		yes	ns	ns	-	-	-	++	-	++	-	-	-	-	-	-
JULY 15-58		no	ns	ns	-	-	-	++	-	++	-	-	-	-	-	-
JULY 15-59	Iceberg	yes	ns	ns	-	-	-	++	-	-	-	-	-	-	-	-
JULY 15-60	Iceberg	no	ns	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-61	Iceberg	yes	groundsel/ fat hen	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-62	Iceberg	no	groundsel/ fat hen	ns	-	-	-	++	-	-	-	-	-	-	-	-
JULY 15-63	Iceberg	yes	fat hen/ redshank	ns	-	-	-	++	-	-	-	-	-	-	-	-
JULY 15-64	Iceberg	no	fat hen/ redshank	ns	-	-	-	++	-	-	-	-	-	-	-	-
JULY 15-65		yes	groundsel/ fat hen	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-66		no	groundsel/ fat hen	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-67	Iceberg	yes	fat hen	ns	-	-	-	++	-	++	-	-	-	-	-	-
JULY 15-68	Iceberg	no	ns	ns	-	-	-	++	-	++	-	-	-	-	-	-
JULY 15-69	Iceberg	yes	ns	ns	-	-	-	++	-	-	-	-	-	-	-	-
JULY 15-70	Iceberg	no	ns	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-71	Iceberg	yes	ns	ns	-	-	-	-	-	++	-	-	-	-	-	-
JULY 15-72	Iceberg	no	ns	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-73		yes	ns	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-74		no	ns	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-75	Iceberg	yes	ns	ns	-	-	-	-	++	-	-	-	-	-	-	-
JULY 15-76	Iceberg	no	ns	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-81	Iceberg	yes	fat hen	ns	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-82	Iceberg	no	fat hen	ns	-	-	-	++	-	-	-	-	-	-	-	-
JULY 15-85	Iceberg	no	fat hen/ redshank	ns	-	-	-	-	-	++	-	-	-	-	-	-
JULY 15-86	Iceberg	yes	fat hen/ redshank	ns	-	-	-	-	-	++	-	-	-	-	-	-
JULY 15-87	Iceberg	seed	seed	seed	-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-88	Groundsel	weed			-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-89	Nettle	weed			-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-90	Cow Parsley	weed			-	-	-	-	-	-	-	-	-	-	-	-
JULY 15-92	Fat Hen	weed			-	-	-	-	-	++	-	-	-	-	-	-

Appendix 7. Composite Lettuce Sample Details and ELISA results, September 2015

Sample code	Type	Sympt*	Pests and weeds**		AMV	BBWV (I & II)	BYSV	CMV	ENMV	LMV	LRNV	MiLBV V	TMV	TRV	TuMV	TuYV
			Weeds	Insects												
SEP 15-01	Lollo Rosso	yes	ns	ns	-	-	-	-	-	-	-	-	-	-	-	-
SEP 15-02	Batavia	yes	ns	aphids at planting	-	-	-	-	-	-	-	-	-	-	+	-
SEP 15-03	Multileaf	yes	ns	ns	-	-	-	-	++	++	-	-	-	-	++	-
SEP 15-04	Lollo Rosso	yes	ns	ns	-	++	-	-	+	++	-	-	-	-	++	++
SEP 15-05	Iceberg	yes	groundsel/ fat hen/ potatoes	ns	-	+	-	-	-	++	-	-	-	-	+	-
SEP 15-06	Cos	no	groundsel/ fat hen	ns	-	-	-	-	-	-	-	-	-	-	-	-
SEP 15-07	Iceberg	no	ns	ns	-	+	-	-	-	++	-	-	-	-	++	+
SEP 15-08	Iceberg	yes	groundsel/ fat hen	ns	+	++	++	++	++	++	+	++	-	+	++	++
SEP 15-09	Iceberg	no	groundsel/ fat hen	ns	-	-	-	-	-	-	-	-	-	-	-	-
SEP 15-11	Iceberg	no	ns	ns	-	-	-	-	-	+	-	-	-	-	++	-
SEP 15-13	Iceberg	yes	ns	ns	+	++	+	++	++	++	+	-	-	-	++	-
SEP 15-14	Iceberg	no	groundsel/ fat hen	ns	-	-	-	-	-	-	-	-	-	-	-	-
SEP 15-15	Little Gem	no	ns	ns	-	-	+	-	-	-	-	-	-	-	-	-
SEP 15-16	Little Gem	no	ns	ns	-	-	-	-	-	+	-	-	-	-	-	-
SEP 15-17	Iceberg	no	groundsel/ fat hen	ns	-	-	-	-	-	-	-	-	-	-	-	-
SEP 15-18	Iceberg	yes	ns	ns	-	+	-	-	+	++	-	-	-	-	+	-
SEP 15-20	Iceberg	no	ns	ns	-	-	+	-	-	+	-	-	-	-	++	-
SEP 15-21	Iceberg	yes	groundsel/ fat hen	ns	+	++	++	++	++	++	+	++	-	+	++	++
SEP 15-22	Multileaf	no	some	none	-	-	-	-	-	+	-	-	-	-	-	-
SEP 15-23	Iceberg	no	few	none	-	-	-	-	-	-	-	++	-	-	-	-
SEP 15-24	Iceberg	yes	few	none	-	-	++	-	-	-	-	-	-	-	-	+
SEP 15-25	Oak Leaf	no	some	none	-	+	++	-	-	++	-	-	-	+	++	+
SEP 15-26	Butterhead	yes	nettle	ns	-	-	+	-	-	+	-	-	-	-	++	+
SEP 15-27	Butterhead	no	nettle	ns	-	+	++	-	-	++	-	-	-	-	++	+
SEP 15-28	Multileaf	yes	nettle	ns	-	-	-	-	-	+	-	-	-	-	-	-
SEP 15-29	Multileaf	no	nettle	ns	-	-	-	-	-	-	-	-	-	-	-	-
SEP 15-30	Iceberg	yes	none	none	-	-	-	-	-	++	-	-	-	-	++	-
SEP 15-31	Cos	no	none	none	-	++	+	-	+	++	-	-	-	-	++	++
SEP 15-32	Iceberg	no	none	none	-	+	-	-	-	++	-	-	-	-	++	+
SEP 15-33	Iceberg	no	none	none	-	-	-	-	-	-	-	-	-	-	-	-
SEP 15-34	Iceberg	no	none	none	-	-	-	-	-	++	-	-	-	-	-	-
SEP 15-35	Multileaf	no	few	aphids early	-	+	++	-	-	++	-	-	-	-	++	-
SEP 15-36	Batavia	no	few	aphids early	-	+	++	-	-	++	-	-	-	-	++	++
SEP 15-37	Batavia	yes	few	aphids early	-	++	+	-	-	++	-	-	-	-	++	++
SEP 15-38	Cos	no	few	aphids early	-	++	++	-	-	++	-	-	+	-	++	++
SEP 15-39	Iceberg	no	some	low	-	+	-	-	++	++	-	-	-	-	+	-
SEP 15-40	Batavia	no	ns	ns	-	-	++	-	-	+	-	-	-	-	+	-
SEP 15-41	Batavia	no	ns	ns	-	-	-	-	-	-	-	-	-	-	-	-
SEP 15-42	Iceberg	yes	groundsel	ns	-	++	-	-	-	++	-	++	-	-	++	-
SEP 15-43	Romaine	yes	none	none	-	-	-	+	-	-	-	-	-	-	-	-
SEP 15-44	Romaine	yes	none	none	-	-	-	-	-	-	-	-	-	-	-	-
SEP 15-45	Iceberg	yes	none	none	-	-	-	+	+	-	-	-	-	-	-	-
SEP 15-46	Iceberg	no	none	none	-	-	-	-	-	-	-	-	-	-	-	-

Appendix 8. Next Generation Sequencing Results 2015

NGS findings showing 'Conclusive' results, where multiple sequence copies of almost complete virus genomes are obtained.

Sample	Symptom status	NGS Result (Conclusive)
SEP 15-02		nvd
SEP 15-13	symptomatic	Hypovirus (probable fungal origin)
SEP 15-16	Asymptomatic	**Lettuce big vein associated virus**
SEP 15-18	Symptomatic	nvd
SEP 15-20		nvd
SEP 15-24		nvd
SEP 15-25		nvd
SEP 15-26	symptomatic	nvd
SEP 15-28	Symptomatic	**Lettuce big vein associated virus**
SEP 15-30	Symptomatic	nvd
SEP 15-31		nvd
SEP 15-33		nvd
SEP 15-34		nvd
SEP 15-35		nvd
SEP 15-37	Symptomatic	**novel Secovirus**
SEP 15-38		nvd
SEP 15-39		nvd
SEP 15-41		nvd
SEP 15-43	Symptomatic	Ourmiavirus (probable fungal origin)
SEP 15-45	Symptomatic	nvd

nvd : No virus detected.

