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AUTHENTICATION

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

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GROWER SUMMARY

Headline

Pea crops were surveyed using a novel approach to investigate the presence, incidence and impact of virus infections. Over two years, expected viruses such as pea enation mosaic virus-1 were present, an unexpected virus, turnip yellows virus, was present in more crops and at greater incidence. A similar suite of viruses were detected over both years, with pea necrotic yellow dwarf virus also being found to be present in year 2.

Background

Pea (*Pisum sativum)* is an important legume crop which is grown worldwide for consumption by humans and animals. Pea plants are also grown in rotation with cereals to help manage disease and improve fertility of the soil (Congdon et al., 2017, Coutts et al., 2008). Using peas, or other legumes, in rotation can reduce the need for application of pesticides and synthetic nitrogen fertilizer (Cernay et al., 2015). Peas can be infected with a number of viruses, and while Plant Virus Online lists 124 viruses which can infect pea, only 43 viruses were found to naturally infect pea (Brunt, 1996). Of the viruses with the potential to infect pea 27 have been previously recorded in the UK, but only seven (7) have ever been recorded from UK pea crops (Source: UK Virus Checklist, unpublished Fera data). However, there have been few surveys of viruses in pea crops in the United Kingdom (UK). In the late 1950's a survey was conducted covering England, the Netherlands, Sweden, and the former West Germany (Hagedorn, 1958). The UK aspect of this work covered 14 fields in England and reported the presence of 'enation mosaic' (14/14 fields affected), 'mosaic' (4/14), 'streak' (10/14), 'top yellows' (6/14) and 'stunt' (1/14). Although these reports were based purely on observed symptoms, and virus symptoms may be confused with other biotic and abiotic stresses (Latham & Jones, 2001), they give an indication of the prevalence of virus diseases in pea crops at the time. Most other pea viruses recorded in the UK have been the result of testing small numbers of samples, again as the result of diagnostic testing following symptom observation.

Recent surveys of leguminous crops in Europe have identified a new genus of virus, the genus *Nanovirus*, Family *Nanoviridae*. Viruses from this genus have been reported from legumes including clover, black medic, milk vetch, faba bean and pea. Several of these viruses have been reported to infect peas, including pea necrotic yellow dwarf virus (PNYDV), pea yellow stunt virus (PYSV), and faba bean necrotic stunt virus (FBNSV and black medic leaf roll virus (BMLRV) (Grigoras et al., 2014, Grigoras et al., 2010). Of these pea necrotic yellow dwarf virus has been shown to have spread throughout Germany and into the

Netherlands and Denmark (Gaafar et al., 2017, Gaafar et al., 2018). Prior to this project nanoviruses had not been reported from the UK.

The aim of this work is to develop a generic survey technique, which could be applied in any crop, but using pea as an initial model crop. The approach uses an integrated diagnostics approach linking screening of large bulked samples using a non-targeted approach (high-throughput sequencing; HTS) to ascertain the presence/absence of viruses, and this is supported with back testing using a targeted approach (real-time RT-PCR) to ascertain the incidence of viruses in fields which were detected in the initial screening tests. At the end of the season a sub-set of fields were sampled to assess the impact of virus infection on crops.

Summary

20 pea crops per year were sampled representing a broad geographic spread across UK pea growing regions. Samples were collected from 100m x 100m grid, with a plant sampled at random, regardless of symptom status of each plant, at each grid intersection, giving 121 sampling points. These plants were combined to make a large bulk sample. On arrival at the laboratory these samples were sub-divided to allow for two different testing approaches. One whole-crop bulk sample was prepared, and nucleic acids (RNA) extracted. This sample was then screened for the presence of viruses using High-throughput sequencing (HTS). This technique analyses all the nucleic acid present in a sample and should, detect the presence of any virus present. The other part of the sample was divided into random sub-samples, consisting of 15 individual leaves, and 15 lots of 7 leaves. RNA was extracted from these samples and stored for subsequent testing for the viruses indicated to be present in the initial screening work.

Year 1 (2019)

The combined results from 2019 testing are presented in **Table 1**.

Table 1 Results of both HTS screening and real-time RT-PCR testing showing presence and incidence of viruses from 20 pea fields in 2019. Estimate results are a calculated % mean virus content based on the number of bulk samples positive for virus, nt = Not Tested

Site	Variety	HTS result	TuYV Result Estimate	PEMV-1 Result Estimate	PEMV-2 Result Estimate	SbDV Result Estimate
1	Anubis	Negative	nt	nt	nt	nt
2	SV1022	Negative	nt	nt	nt	nt
3	Tomahawk	Negative	nt	nt	nt	nt
4	Anubis	Negative	nt	nt	nt	nt
5	Bartesa (PP)	Negative	nt	nt	nt	nt
6	Combining Pea (TBC)	TuYV	12.46	nt	nt	nt

7	Tomahawk	Negative	nt	nt	nt	nt
8	Combining Pea	PEMV1 PEMV-2	nt	40.8	86.67	nt
9	Swallow	TuYV	1.71	nt	nt	nt
10	EBBA	TuYV PEMV-2	9.71	nt	2.57	nt
11	Vidor	TuYV	60.62	nt	nt	nt
12	Amalfi	TuYV PEMV-2	16.39	0.85	20.05	nt
13	Realm	TuYV PEMV-2	32.56	0	21.8	nt
14	Ashton	TuYV PEMV-2 SbDV	93.33	nt	7.49	1.71
15	TBC	TuYV PEMV1 PEMV-2 SbDV	3.62	16.39	2.71	4.53
16	Oasis	TuYV PEMV1 PEMV-2 PEMV Sat	27.77	4.81	22.23	nt
17	Vidor/Ambassador	TuYV	21.8	nt	nt	nt
18	Kimberley	TuYV PEMV Sat	93.33	nt	nt	nt
19	Oasis	TuYV PEMV-2	86.67		28.2	nt
20	Boogie	TuYV PEMV1 PEMV-2 PEMV Sat	27.77	37.15	40.8	nt

13 of the 20 crops tested were positive for virus infections ranging in incidence from 0.85% to 93.33% estimated infection. One of the pea viruses which has been historically reported as being present in the UK, pea enation mosaic virus, was shown to be present in 5 crops, ranging from 0.85% to 30.09% virus infection. Pea enation mosaic is actually a complex infection of two viruses (PEMV1 and PEMV2), however, this was only present as a 'single' infection in one crop, at 27.44% infection. More commonly detected, and present at higher incidence, was turnip yellows virus (TuYV). This virus ranged in incidence from 1.71% to 93.33% virus and was present in 12 of the 20 crops tested. This finding represents a first report of TuYV in peas in the UK, although the virus has been reported in pea crops elsewhere in Europe. Additionally, the virus soybean dwarf virus was also detected in two of the 20 crops tested. This represents a first record of this virus in the UK. Where detected the virus was present at low incidence, and further testing for this virus will be conducted in the second and third year of the project.

Year 2 (2021)

The combined results from 2021 testing are presented in Table 2.

Table 2 Results of both HTS screening and real-time RT-PCR testing showing presence and incidence of viruses from 20 pea fields in 2021. Estimate results are a calculated % mean virus content based on the number of bulk samples positive for virus, nt = Not Tested

Site	Variety	HTS Result	TuYV Result Estimate	PEMV-1 Result Estimate	PEMV-2 Result Estimate	PSbMV Result Estimate	SbDV Result Estimate	PNYDV Result Estimate
1	Prelado	Negative	nt	nt	nt	nt	nt	nt
2	Bingo	Negative	nt	nt	nt	nt	nt	nt
3	Sakura	PSbMV	nt	nt	nt	7.22	nt	nt
4	Kaboki	TuYV, PEMV-2, PSbMV	16.91	nt	0.85	0.85	nt	nt
5	Geer	PEMV-1, PEMV-2, PEMV satRNA	nt	17.8	23.61	nt	nt	nt
6	Daytona	TuYV, PEMV-1, PEMV-2, PSbMV, PNYDV, PEMV satRNA	37.15	54.81	66.87	8.28	nt	2.64
7	Amalifi	Negative	nt	nt	nt	nt	nt	nt
8	Swallow	TuYV	5.78	nt	nt	nt	nt	nt
9	Trophy	TuYV, PEMV-2	32.56	nt	35.18	nt	nt	nt
10	Oasis	TuYV	2.71	nt	nt	nt	nt	nt
11	Romance	Negative	nt	nt	nt	nt	nt	nt
12	Dancer	TuYV, PEMV-2, PEMV satRNA	25.41	nt	6.55	nt	nt	nt
13	Vada	TuYV, PEMV-1, PEMV-2	0.85	4.53	44.91	nt	nt	nt
14	Oasis	TuYV	13.67	nt	nt	nt	nt	nt
15	Naches	TuYV, PEMV-1, PEMV-2, SbDV	8.59	44.91	22.23	nt	0.85	nt
16	Oasis	TuYV, PEMV-1, PEMV-2	11.27	3.42	2.64	nt	nt	nt
17	Fintva	TuYV, PEMV-1, PEMV-2 PSbMV, SbDV	11.27	19.61	38	1.76	3.72	nt
18	Kimberley	TuYV, PEMV-2, PEMV satRNA	60.62	nt	2.71	nt	nt	nt
19	Unknown	TuYV, PEMV-2	19.25	nt	1.71	nt	nt	nt
20	Grundy	TuYV	2.71	nt	nt	nt	nt	nt

16 of the 20 crops tested were positive for virus, the viral incidence ranged from 0.85%-66.87% estimated infection. Soybean dwarf virus which was first reported in UK peas in the first year of the study, (FV 459, (Fowkes et al., 2021)) was identified in 2 sites at a low incidence, 0.85% and 3.72%, which is similar to year 1. Turnip yellows virus which was also first detected in UK peas in the year 1 was identified at fourteen sites with incidences between 0.85%-60.62%, it was the most common virus found. Pea enation mosaic virus-1 was identified at six sites, with an incidence between 3.42%-54.81%. Pea enation mosaic virus-2

was identified at eleven sites with an incidence between 0.85%-66.87%. In this year's study, pea seedborne mosaic virus was identified in the site samples, it was identified at four sites with an incidence of 4.53%. Finally, pea necrotic yellow dwarf virus was identified at 1 site with an incidence of 2.64%, this represents the first finding of this virus in the UK.

Individual symptomatic samples were also tested. The results from these tests support the conclusions from the general field survey that TuYV is present in a greater number of crops than PEMV. In year 1 further viruses were found to be present in these symptomatic samples, including expected viruses such as pea seed-borne mosaic virus and bean yellow mosaic virus. One further novel virus-like sequence was also detected from two samples, which appears to be genetically most closely related to cucurbit aphid-borne yellows associated RNA. This sequence will be the subject of some further work in the coming year of the project to ascertain the nature of this finding. Given these samples were taken on the basis of expressing symptoms, work would need to be carried out to investigate which of these viruses, or which combination of viruses was causing the observed symptom in the plant. In year 2 no extra viruses were found in the symptomatic samples.

Further work was also conducted to investigate the potential impact of virus infection in crops. In each sampled crop an area was marked out and this was left 'untreated', compared to the rest of the field which received treatment to mitigate against aphids, the vectors of many of the viruses causing issues in pea crops. From the HTS screening work, five of these crops were identified for further study, and at the end of the growing season these were sampled and assessments of yield were made in both the treated and untreated areas of the field. From these data a statistical analysis (linear regression) was performed to estimate the impact of virus infection.

For year 1, the analysis indicates that TuYV could impact yield of a crop by an estimated 44% against an uninfected crop (CL 19%-67%), and "treatment" would reduce this effect to around 81% of the yield of an uninfected crop. Indicating that current treatment regimes are likely having a partial effect at reducing the impact of TuYV in the crop. PEMV-1 was also estimated to have a potential yield impact, but neither the impact of virus nor the effect of treatment was statistically significant.

This analysis was repeated in year 2. The analysis indicated that TuYV could impact yield of a crop by an estimated 55% against an uninfected crop (CL 33%-88%), and "treatment" would ameliorate this impact to around 88% of the yield of an uninfected crop. Differing to year 1, within year 2 PEMV-1 significantly reduced estimated yield (73%, CL 56%-94%) but "treatment" did not significantly reduce this impact (75%, CL 58%-96%). Again suggesting

that current aphicide treatments may have a greater effect at reducing the impact of TuYV infections than for PEMV-1.

It is vital to note that these effects are based on limited datasets from two years of a three-year study and will be further investigated in the next growing season.

Financial Benefits

As this is the first year of a three-year study there are no financial benefits to be reported at this stage.

Action Points

These two years of survey are showing that the viruses previously considered as the key issues in pea crops need to be considered alongside the previously overlooked virus turnip yellows virus (TuYV). The estimated yield impact data indicate that virus controls measures, even though not specifically targeted at TuYV do have some effect at ameliorating the impact of this virus.

SCIENCE SECTION

Introduction

Pea (*Pisum sativum*) is an important legume crop which is grown worldwide for consumption by humans and animals. Pea plants are also grown in rotation with cereals to help manage disease and improve fertility of the soil (Congdon et al., 2017, Coutts et al., 2008). Using peas, or other legumes, in rotation can reduce the need for application of pesticides and synthetic nitrogen fertilizer (Cernay et al., 2015). Peas can be infected with a number of viruses, and while Plant Virus Online lists 124 viruses which can infect pea, only 43 viruses were found to naturally infect pea (Brunt, 1996). Of the viruses with the potential to infect pea 27 have been previously recorded in the UK, but only seven (7) have ever been recorded from UK pea crops (Source: UK Virus Checklist, unpublished Fera data). However, there have been few surveys of viruses in pea crops in the United Kingdom (UK). In the late 1950's a survey was conducted covering England, the Netherlands, Sweden, and the former West Germany (Hagedorn, 1958). The UK aspect of this work covered 14 fields in England and reported the presence of 'enation mosaic' (14/14 fields affected), 'mosaic' (4/14), 'streak' (10/14), 'top yellows' (6/14) and 'stunt' (1/14). Although these reports were based purely on observed symptoms, and virus symptoms may be confused with other biotic and abiotic stresses (Latham & Jones, 2001), they give an indication of the prevalence of virus diseases in pea crops at the time. Most other pea viruses recorded in the UK have been the result of testing small numbers of samples, again as the result of diagnostic testing following symptom observation. The current virus health status of UK pea crops in unknown.

Recent surveys of leguminous crops in Europe have identified a new genus of virus, the genus *Nanovirus*, Family *Nanoviridae*. Viruses from this genus have been reported from legumes including clover, black medic, milk vetch, faba bean and pea. Several of these viruses have been reported to infect peas, including pea necrotic yellow dwarf virus (PNYDV), pea yellow stunt virus (PYSV), and faba bean necrotic stunt virus (FBNSV) and black medic leaf roll virus (BMLRV) (Grigoras et al., 2014, Grigoras et al., 2010). Of these pea necrotic yellow dwarf virus has been shown to have spread throughout Germany and into the Netherlands and Denmark (Gaafar et al., 2017, Gaafar et al., 2018).

Further afield, Australian researchers have had a greater focus on pea crops. This has largely focussed on mitigating the effects of pea seed-borne mosaic virus (Congdon et al., 2017, Coutts et al., 2008). However, some survey work had been carried out, which suggested that luteoviruses may be present in pea crops at a higher incidence than previously recognised (Wilson et al., 2012). Although viruses from this family, such as turnip yellows virus (TuYV)

and Bean leaf roll virus (BLRV) have been recorded from peas, the incidence of these viruses in UK and EU crops is unknown.

To date most virus surveillance work carried out on any crop follows a general formulaic approach, namely: Identify a suite of viruses likely to be present in the crop; collect samples from a number of fields based on likely symptoms; test these for the pre-selected suite of viruses using targeted diagnostics such as ELISA or PCR based methods. This approach gives limited information as it can only report on the known viruses, it leaves open questions about the identity of the causal agent of symptoms where a plant is sampled but tests negative for virus, and arguably, on this any test which is 'negative' could be considered to be wasted diagnostic resource. High-Throughput sequencing (HTS) is a technique that has been in development for plant pathology applications for around ten years. It gives a method for testing for the presence of the total genetic sequence contained in a sample, which can then be compared to known sequences to give an indication of the presence of a suspected pathogen. Thus far it has been primarily used for either screening germplasm or for single sample diagnosis where conventional diagnostics has failed to give a result, but is now being applied to landscape-scale ecology studies (Maree et al., 2018, Adams et al., 2018). However, it has not yet been applied in a plant health surveillance scenario. The aim of this project is to use an integrated approach linking HTS and conventional diagnostic methods to give a generic method for carrying out a survey for the presence of viruses in a crop, using UK pea crops as model system, where the final result is both a measure of the presence and incidence of viral pathogens. Additionally, the aim is to investigate the impact of these viral pathogens on crop production.

Materials and methods

This method has now been published by Fowkes et al., (2021).

1. *Presence and incidence of viruses in pea crops - Sampling*
    1.1. 20 pea crops were sampled for the presence of pea-infecting viruses. Sampled crops are shown in tables 3 and 4. Samples were taken c. 6 weeks prior to harvest to give a measure of viruses from seed-sources and to account for those likely to be present following early aphid migrations.
    1.2. 120 individual plants were sampled at random along a 100m x 100m grid at 10m intervals (Fox et al., 2017). Additional meta-data was also recorded including location, variety, etc.

1.3. In addition to the random sample, up to five (5) individual plants exhibiting symptoms consistent with virus infection were submitted for confirmatory testing of virus presence.

Table 3 Sites of pea crops sampled during Summer 2019

Site no.	Site	Location	Variety
1	Waterloo Farm	Ancaster	Anubis
2	Kidderminster	Low Habberly	SV1022
3	Long Sutton	Long Sutton	Tomahawk
4	Nettleham	Lincoln	Anubis
5	Woodbridge	Woodbridge	Bartesa (PP)
6	Canterbury	Adisham	Combining Pea
7	Finavon	Brechin	Tomahawk
8	Salisbury	Broughton	Combining Pea
9	Birdseye	East Riding	Swallow
10	Wooton near Ulceby	Ulceby	EBBA
11	Chatteris	Chatteris	Vidor
12	Langtoft	Langtoft	Amalfi
13	Swaby	Louth	Realm
14	Market Weighton	Market Weighton	Ashton
15	Clashbeny	Perth	TBC
16	Wainfleet	Wainfleet All Saints	Oasis
17	Stoneleigh	Stoneleigh	Vidor/Ambassador
18	Eye	Bedingfield	Kimberley
19	Langton by Spilsby	Langton	Oasis
20	Chirnside	Chirnside Borders	Boogie

Table 4 Sites of pea crops sampled during Summer 2021

Site no.	Site	Location	Variety
1	Wilsford	Ancaster	Prelado
2	Arrow	Worcester	Bingo
3	Broughton	Southampton	Sakura
4	Great Dunmaw	Great Dunmaw	Kaboki
5	Stokesby	Acle	Geer
6	Reading street	Kent	Daytona
7	Sledmere	Sledmere	Amalifi
8	Elsham	Brigg	Swallow
9	Gedney Drove End	Holbeach	Trophy

10	Spillsby	Spillsby	Oasis
11	Glamis	Perthshire	Romance
12	Reepham	Norwich	Dancer
13	Louth	Louth	Vada
14	March	Cambridge	Oasis
15	Boston	Boston	Naches
16	Beverley	Beverley	Oasis
17	Market Weighton	Market Weighton	Fintva
18	Badingham	Framlingham	Kimberley
19	Chipping Campdon	Cheltenham	Unknown
20	Coldstream	Eccles	Grundy

2. *Presence and incidence of viruses in pea crops – Laboratory testing*

2.1. On submission to the laboratory, the 120 randomly sampled plants were sub-sampled, and a composite bulked sample of all the sampled plants in each field was tested by HTS (Whole crop bulk). This initial non-target screen identified candidate pathogens for subsequent incidence testing.

2.2. Whole crop bulk samples were tested by HTS, with appropriate quality procedures, and resulting data were analysed in accordance with previously published methods (Adams et al., 2014, Fox et al., 2019, Fowkes et al., 2021).

2.2.1. Viruses inferred from HTS data were assigned provisional taxonomic placement and identified as candidate viruses for confirmation testing through mixed bulk testing (See 2.3).

2.3. Concurrently, whole crop samples were sub divided into smaller bulks for downstream confirmation testing by real-time RT-PCR. This sub-division consisted of 15 lots of 7 leaves, and a further 15 individual leaves (Mixed bulks).

2.3.1. RNA was extracted from mixed bulks using Kingfisher magnetic bead extraction in accordance with manufacturer's instructions.

2.3.2. Mixed bulks from crops shown to contain virus infection were tested for the specific candidate viruses indicated to be present in those crops by real-time RT-PCR, using existing published diagnostic assays where possible. These will be used as part validated tests and validation was at the systems level with multiple methods being used to confirm the presence of candidate viruses (Roenhorst et al., 2018).

2.3.3. The virus incidence in a sample was inferred from interpretation of bulked sample test results (see table in Appendix 1).

2.4. Individual symptomatic samples will be tested in parallel to the bulk samples detailed above to give additional intelligence on the viruses present in pea crops. These were extracted and tested by HTS as detailed above.

3. *Impact assessment of pea infecting viruses*

3.1. Using the information obtained through incidence testing detailed above, 5 crops were identified for further study. Crops were assessed at harvest to give estimates of yield loss due to High/Moderate/Low levels of virus incidence in the crop.

3.2. At all sites an area 10m x 10m was marked within the sampling area. The area remained untreated, with no aphicides applied, to allow maximum potential yield loss from virus infection to be measured. The remaining crop was treated with standard insecticide applications by the grower.

3.3. At five selected sites 16 small plots, 1m x 1m were harvested from within the 10m x 10m area, and 16 from outside the area to compare yield from the commercial crop against yield from the untreated area. Plants from those small plots were returned to PGRO and threshed using a static vining machine or combine harvester. Five (5) pea sites were harvested. Yield was measured for all 5 sites.

3.3.1. Maturity was measured for vining peas using a tenderometer machine to give a TR score, and moisture content recorded for peas that were combined.

3.3.2. Vining peas were size graded to give an additional measure of quality. Additional sub-samples of seeds were taken from all sites and assessed for symptoms of PSbMV, a virus that produces tissue scarring on the surface of the seed.

Results

In year 1., combining the results for the single sample and whole crop bulk HTS indicated the presence of six (6) viruses and a satellite RNA. Five of these were expected and are known to be common in peas in the UK as indicated by the previous literature review (AHDB FV 453). These were:

- Pea enation mosaic virus 1 (PEMV1), genus *Enamovirus*
- Pea enation mosaic virus 2 (PEMV2), genus *Umbravirus*
- Pea enation mosaic virus satellite
- Pea seed-borne mosaic virus (PSbMV), genus *Potyvirus*
- Bean yellow mosaic virus (BYMV), genus *Potyvirus*

Two viruses which had not been previously recorded in pea crops in the UK were also detected in both single symptomatic samples and from bulked field samples, and these were:

- Turnip yellows virus (TuYV), genus *Polerovirus*
- Soybean dwarf virus (SbDV), genus *Luteovirus*

Additionally, sequence fragments of a turnip yellows virus associated RNA were also detected.

Pea seed-borne virus and bean yellow mosaic virus were not detected during the randomised field survey, but only from testing individual symptomatic plants.

In year 2, testing of both the bulk field samples (BFS) and the symptomatic samples by HTS indicated the presence of six viruses and a satellite RNA. Six were found in year one, these were:

-Pea enation mosaic virus-1 (PEMV-1), genus *Enamovirus*

-Pea enation mosaic virus-2 (PEMV-2), genus *Umbravirus*

-Pea enation mosaic virus satellite RNA (PEMV satRNA)

-Pea seed-borne mosaic virus (PSbMV), genus *Potyvirus*

-Turnip yellows virus (TuYV), genus *Polerovirus*

-Soybean dwarf virus (SbDV), genus *Luteovirus*

Within this year of the project, a virus which hadn't previously been recorded in the UK was detected at one site (Kent) in the bulked field sample (BFS).

-Pea necrotic yellow dwarf virus (PNYDV), genus *Nanovirus*

No additional viruses were found by testing the individual symptomatic plants.

Presence and Incidence in Pea crops in 2019

The viruses inferred in samples from the whole crop bulk HTS analysis are listed in **Table 5**, along with the relative incidence of the three viruses which were tested for in mixed bulk samples: turnip yellows virus, soybean dwarf virus, pea enation mosaic virus-1 and pea enation mosaic virus-2. In total 13 crops out of 20 had detectable levels of virus (65%). Total virus content ranged from 1.7% up to 93.3%. Five of 20 crops contained a single virus (25%), whereas 8 crops contained multiple virus infections (40%). Generally, there was a pattern of

lower virus incidence and content earlier in the season. Five (5) crops out of 20 were found to contain PEMV-1, where detected the mean incidence of PEMV-1 was 12.59% (0.85% - 30.09%). Nine (9) crops out of 20 were found to contain PEMV-2, where detected the main incidence of PEMV-2 was 25.84% (2.57% - 86.67%). More commonly detected, and present at a higher incidence, was the virus TuYV, present in 12 of 20 crops (60%). The incidence of TuYV ranged from 1.71% - 93.33% (avg. where detected 34.3%). Soybean dwarf virus was present at low incidence in two crops with an incidence of 1.71% and 4.53%.

Table 5 Viruses inferred from whole crop bulk HTS data and mean estimated virus content (%) from the accompanying mixed bulk testing for 2019. nt=not tested

Site	Site	Location	Variety	HTS result	TuYV Result Estimate	PEMV-1 Result Estimate	PEMV-2 Result Estimate	SbDV Result Estimate
1	Waterloo Farm	Ancaster	Anubis	Negative	nt	nt	nt	nt
2	Kidderminster	Low Habberly	SV1022	Negative	nt	nt	nt	nt
3	Long Sutton	Long Sutton	Tomahawk	Negative	nt	nt	nt	nt
4	Nettleham	Lincoln	Anubis	Negative	nt	nt	nt	nt
5	Woodbridge	Woodbridge	Bartesa (PP)	Negative	nt	nt	nt	nt
6	Canterbury	Adisham	Combining Pea (TBC)	TuYV	12.46	nt	nt	nt
7	Finavon	Brechin	Tomahawk	Negative	nt	nt	nt	nt
8	Salisbury	Broughton	Combining Pea	PEMV1 PEMV2	nt	27.44	86.67	nt
9	Birdseye	East Riding	Swallow	TuYV	1.71	nt	nt	nt
10	Wooton near Ulceby	Ulceby	EBBA	TuYV	6.76	nt	2.57	nt
11	Chatteris	Chatteris	Vidor	TuYV	60.62	nt	nt	nt
12	Langtoft	Langtoft	Amalfi	TuYV PEMV2	9.7	0.85	20.05	nt
13	Swaby	Louth	Realm	TuYV PEMV2	21.8	0	21.8	nt
14	Market Weighton	Market Weighton	Ashton	TuYV PEMV2 SbDV	93.33	nt	7.49	1.71
15	Clashbeny	Perth	TBC	TuYV PEMV1 PEMV2 SbDV	2.64	0.85	2.71	4.53
16	Wainfleet	Wainfleet All Saints	Oasis	TuYV PEMV1 PEMV 2 PEMV Sat	8	3.72	22.23	nt
17	Stoneleigh	Stoneleigh	Vidor/Ambassador	TuYV	6.98	nt	nt	nt
18	Eye	Bedingfield	Kimberley	TuYV PEMV Sat	93.33	nt	nt	nt
19	Langton by Spilsby	Langton	Oasis	TuYV PEMV 2	80.01	nt	28.2	nt
20	Chirnside	Chirnside Borders	Boogie	TuYV PEMV1 PEMV 2 PEMV Sat	14.29	30.09	40.8	nt

Presence and Incidence in Pea crops in 2021

The viruses identified by HTS in the BFS are shown in Table 6 as well as the relative incidence of the six viruses identified. Of the twenty sites tested, 16 had detectable levels of virus, and of those sites, virus incidence was between 0.85%-66.87%. In five of the sites, only one virus was found and the other 11 had multiple viruses present. SbDV was identified at only two sites with incidences of 0.85% and 3.72%. PSbMV was detected at four sites with a mean incidence of 4.53% (0.85% - 8.28%). Six sites were identified as having PEMV-1, and where detected the mean incidence was 24.18% (3.42% - 54.81%). PEMV-2 was identified at eleven sites and the mean incidence was 22.30% (0.85% - 66.87%). The most common virus found was TuYV which was detected at fourteen sites with a mean incidence of 17.77% (0.85% - 60.62%). Finally, PNYDV was identified at a single site at an incidence of 2.64%.

Table 6 Sites from 2021, viruses inferred from whole crop bulk HTS data and mean estimated virus content (%) from the accompanying mixed bulk testing (%). nt=not tested

Site	Variety	HTS Result	TuYV Result Estimate	PEMV-1 Result Estimate	PEMV-2 Result Estimate	PSbMV Result Estimate	SbDV Result Estimate	PNYDV Result Estimate
1	Prelado	Negative	nt	nt	nt	nt	nt	nt
2	Bingo	Negative	nt	nt	nt	nt	nt	nt
3	Sakura	PSbMV	nt	nt	nt	7.22	nt	nt
4	Kaboki	TuYV, PEMV-2, PSbMV	16.91	nt	0.85	0.85	nt	nt
5	Geer	PEMV-1, PEMV-2, PEMV satRNA	nt	17.8	23.61	nt	nt	nt
6	Daytona	TuYV, PEMV-1, PEMV-2, PSbMV, PNYDV, PEMV satRNA	37.15	54.81	66.87	8.28	nt	2.64
7	Amalifi	Negative	nt	nt	nt	nt	nt	nt
8	Swallow	TuYV	5.78	nt	nt	nt	nt	nt
9	Trophy	TuYV, PEMV-2	32.56	nt	35.18	nt	nt	nt
10	Oasis	TuYV	2.71	nt	nt	nt	nt	nt
11	Romance	Negative	nt	nt	nt	nt	nt	nt
12	Dancer	TuYV, PEMV-2, PEMV satRNA	25.41	nt	6.55	nt	nt	nt
13	Vada	TuYV, PEMV-1, PEMV-2	0.85	4.53	44.91	nt	nt	nt
14	Oasis	TuYV	13.67	nt	nt	nt	nt	nt
15	Naches	TuYV, PEMV-1, PEMV-2, SbDV	8.59	44.91	22.23	nt	0.85	nt
16	Oasis	TuYV, PEMV-1, PEMV-2	11.27	3.42	2.64	nt	nt	nt
17	Fintva	TuYV, PEMV-1, PEMV-2 PSbMV, SbDV	11.27	19.61	38	1.76	3.72	nt
18	Kimberley	TuYV, PEMV-2, PEMV satRNA	60.62	nt	2.71	nt	nt	nt
19	Unknown	TuYV, PEMV-2	19.25	nt	1.71	nt	nt	nt
20	Grundy	TuYV	2.71	nt	nt	nt	nt	nt

Presence of virus in single symptomatic samples in 2019

The viruses detected in single symptomatic samples by HTS are presented in Table 7. TuYV was the virus most commonly detected, with PEMV-2 the second most commonly detected virus. PEMV-2 was detected in more samples than the virus PEMV-1 which is the recognised helper virus for transmission of PEMV-2. Pea seed-borne mosaic virus was only detected from samples at three sites, and bean yellow mosaic virus was detected from a single sample. Soybean dwarf virus was not detected from any of the samples submitted under this part of the study, however, small fragments of sequence of turnip yellows associated RNA were detected in two samples.

Table 7 Virus present in single symptomatic samples inferred from HTS data in 2019.

Location	Variety	PEMV-1	PEMV-2	PEMV Satellite	PSbMV	TuYV	BYMV	TuYVaRNA
Market Rasen	TBC							+
Ramsey Mereside	TBC	+	+			+		
Ramsey Mereside	TBC		+			+		
Stonea March	TBC	+	+		+	+		+
Ramsey Mereside	TBC	+	+		+	+		
Ramsey Mereside	TBC		+			+		
Deeping st.	TBC	+	+	+		+	+	
Cambridge	TBC	+	+		+	+		
Cambridge	TBC	+	+	+				
Market Weighton	Ashton		+			+		
Market Weighton	Ashton	+	+			+		
Market Weighton	Ashton	+	+			+		
Market Weighton	Ashton	+	+			+		
Market Weighton	Ashton	+	+			+		
Market Weighton	Ashton	+	+			+		
Market Weighton	Ashton	+	+			+		
Market Weighton	Ashton	+	+			+		
Wainfleet All Saints	Oasis	+	+	+		+		
Wainfleet All Saints	Oasis	+	+	+		+		
Wainfleet All Saints	Oasis	+	+	+		+		
Wainfleet All Saints	Oasis	+	+	+		+		
Wainfleet All Saints	Oasis	+	+	+		+		
Wainfleet All Saints	Oasis	+	+	+		+		
Bedingfield	Kimberley		+			+		
Bedingfield	Kimberley		+			+		
Bedingfield	Kimberley		+			+		

Langton	Oasis					+		
Langton	Oasis					+		

Presence of virus in single symptomatic samples in 2021

Symptomatic samples were collected alongside the BFS at each site, at each site between one and five symptomatic samples were taken (Table 8). For each site, the symptomatic samples were bulked together, and this was tested by HTS. Confirmation was done using real-time RT-PCR. PEMV-2 was detected in the most samples, followed by PEMV-1. PSbMV and TuYV were found in six and five samples, respectively. Neither PNYDV or SbDV were found in the symptomatic samples.

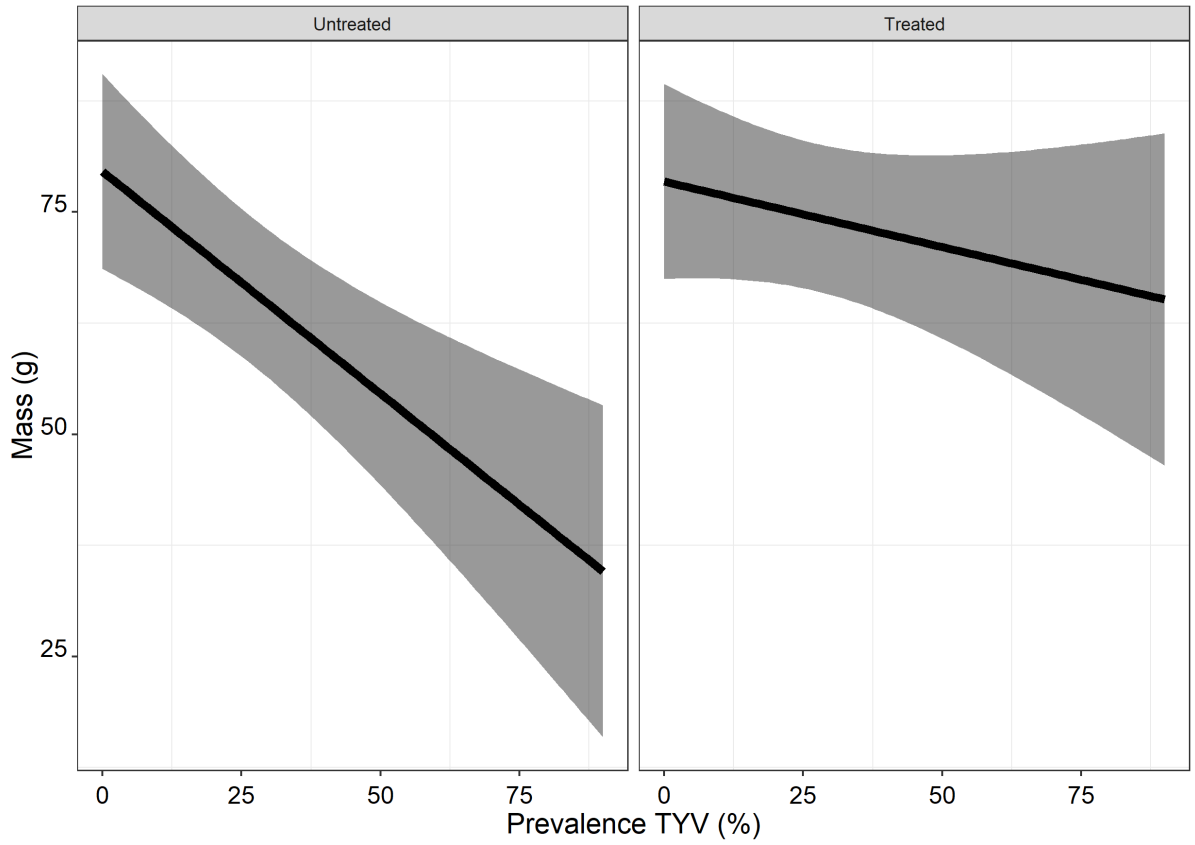
Table 8 Virus present in single symptomatic samples inferred from HTS data in 2021.

Site	HTS Result (bulked)	TuYV	PEMV1	PEMV2	PSbMV	SbDV	PNYDV
Site 1	Negative						
Site 2							
Site 2	Negative						
Site 3					-		
Site 3					+		
Site 3	PSbMV				-		
Site 4		-		-	-		
Site 4	Negative						
Site 5							
Site 5			-	-			
Site 5							
Site 5	Negative						
Site 6			+	-			
Site 6		-	+	+	-		-
Site 6			-	-			
Site 6	Negative		+	+			
Site 7							
Site 7							
Site 7	Negative						
Site 8							
Site 8		-					
Site 8	Negative						
Site 9							
Site 9		-		+			
Site 9	Negative						
Site 10		+		+			
Site 10		-		-			
Site 10	TuYV PEMV2			-			
Site 11	Negative						

Site 11							
Site 11							
Site 12		+					
Site 12		-		-			
Site 12	TuYV						
Site 13							
Site 13		-	-	-			
Site 13	Negative						
Site 14		+					
Site 14		-					
Site 14	TuYV	-					
Site 15			+	+			
Site 15		-	+	+		-	
Site 15	PEMV1 PEMV2		-	-			
Site 16							
Site 16		-	-	-			
Site 16	Negative						
Site 17							
Site 17		-	-	-	-	-	
Site 17	TuYV						
Site 18		+					
Site 18		+	-	-			
Site 18	TuYV	-					
Site 19							
Site 19		-		-			
Site 19	Negative						
Site 20	Negative	-					
Langton			+	+	+		
Langton			+	+	+		
Langton		-	+	+	+		
Langton	PEMV1, PEMV2, PSbMV,		+	+	+		
Langton	PEMV satRNA		+	+	+		

Impact of virus infection for 2019

Five crops with a range of virus content were sampled to assess the impact of virus infection both with and without treatment. These crops were at Canterbury (crop 6), Salisbury (Crop 8), Louth (Crop 13), Stoneleigh (Crop 17) and Eye (Crop 18). The unprocessed data can be found in Appendix 2. A preliminary report on the statistical analysis can be found in Appendix 3. Estimated effects are shown in Figure 3 and estimates for the effect of virus prevalence and treatments in the population, expressed as the 10-plant pea mass, were gained via a parametric bootstrap of the fitted model.



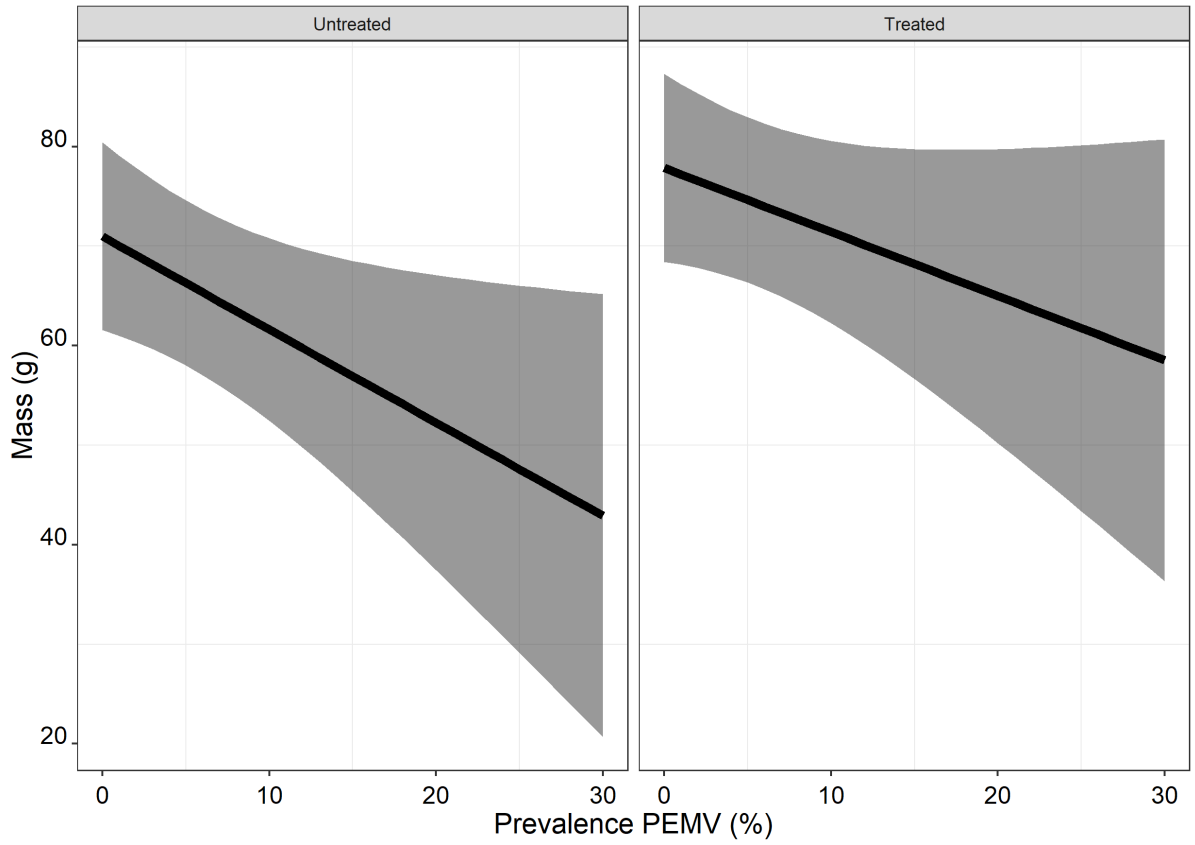


Figure 1 Estimated effects of virus prevalence of TuYV (labelled TUYV) and PEMV-1 (labelled PEMV) and treatment on productivity for 2019.

Estimates of effect size of virus infection and treatment are shown in Table 9. TuYV was found to significantly reduce productivity ($p<0.001$) in fields, and this effect appears to be ameliorated by treatment ($p>0.001$). PEMV-1 was also found to significantly reduce productivity ($p<0.011$) however treatment did not appear to ameliorate this effect ($p<0.18$).

Table 9 Estimates of effect sizes and significance of treatment and TuYV and PEMV-1 for 2019.

Value	Estimated effect	95% C.I.		Significance
10-plant pea mass in clean untreated fields	85g	73g	97g	
Effect of treatment[a]	97%	88%	106%	0.240[b]
Effect of TuYV[a]	44%	19%	67%	<0.001[c]
Effect of TuYV and treatment[a]	81%	55%	108%	<0.001[d]
Effect of PEMV[a]	70%	47%	96%	0.011[e]
Effect of PEMV and treatment[a]	76%	53%	100%	0.180[f]

[a] Expected population average 100-pea mass in this type of field expressed as a proportion of expected 10-plant pea mass in equivalent (same sites) virus free untreated fields

[b] Null hypothesis: treatment doesn't reduce the 10-plant pea mass

[c] Null hypothesis: TUYV presence doesn't reduce the 10-plant pea mass

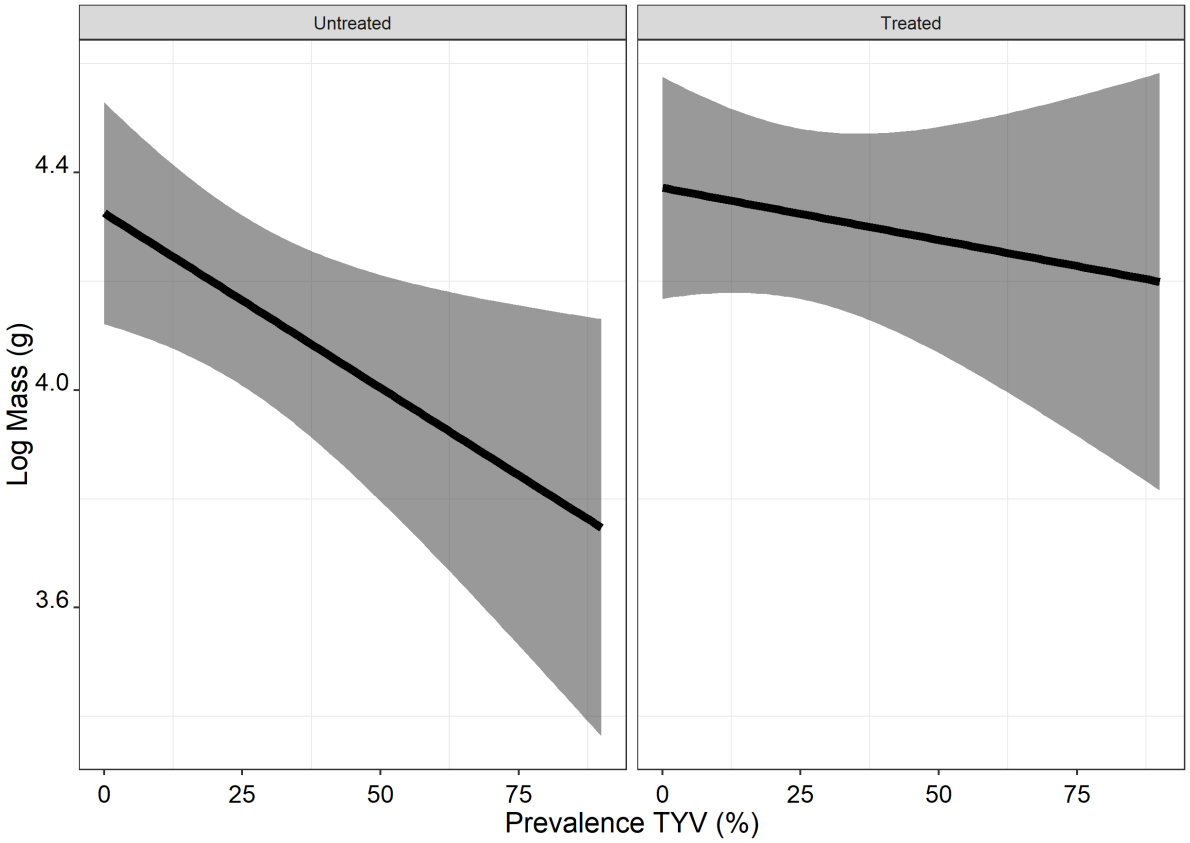
[d] Null hypothesis: treatment doesn't increase the 10-plant pea mass when TUYV is present

[e] Null hypothesis: PEMV presence doesn't reduce the 10-plant pea mass

[f] Null hypothesis: treatment doesn't increase the 10-plant pea mass when PEMV is present

Impact of virus infection for 2021

Similar to year 1, five crops with a range of virus content were sampled to assess the impact of virus infection both with and without treatment. These crops were at Southampton (crop 3), Kent (crop 6), Louth (crop 13), Beverley (crop 16) and Framlingham (crop 18). The unprocessed data can be found in Appendix 4. A preliminary report on the statistical analysis can be found in Appendix 5. Estimated effects are shown in Figure 3 and estimates for the effect of virus prevalence and treatments in the population, expressed as the 10-plant pea mass, were gained via a parametric bootstrap of the fitted model.



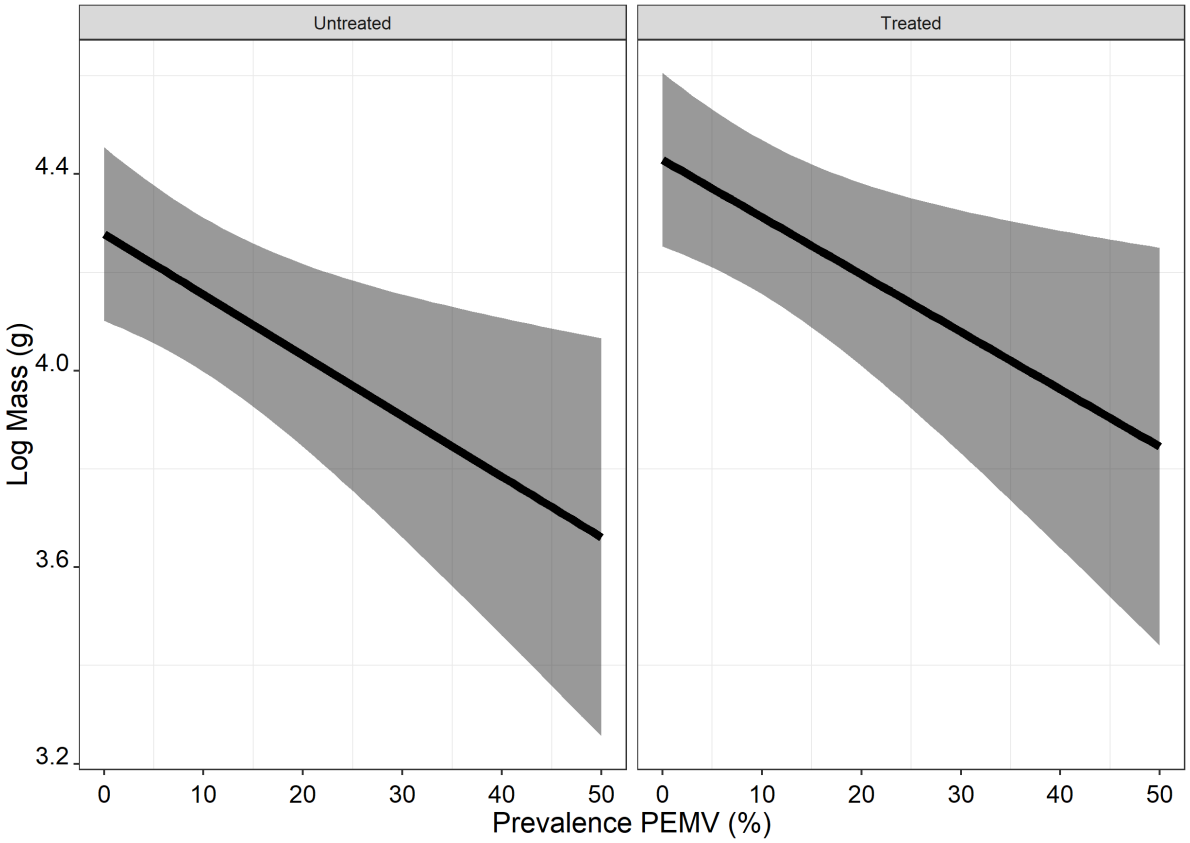


Figure 2 Estimated effects of virus prevalence of TuYV (labelled TUYV) and PEMV-1 (labelled PEMV) and treatment on productivity for 2021.

Estimates of effect size of virus infection and treatment are shown in Table 10. TuYV was found to significantly reduce productivity ($p<0.011$) in fields, and this effect appears to be ameliorated by treatment ($p>0.001$). PEMV-1 was also found to significantly reduce productivity ($p<0.006$) however treatment did not appear to ameliorate this effect ($p<0.274$).

Table 10 Estimates of effect sizes and significance of treatment and TuYV and PEMV-1 for 2021.

Value	Estimate	95% C.I.		Significance
10-plant pea mass in clean untreated fields	86g	68g	107g	
Effect of treatment[a]	103%	95%	111%	0.750[b]
Effect of TuYV[a]	55%	33%	89%	0.011[c]
Effect of TuYV and treatment[a]	88%	51%	140%	<0.001[d]
Effect of PEMV[a]	73%	56%	94%	0.006[e]
Effect of PEMV and treatment[a]	75%	58%	96%	0.274[f]

[a] Expected population average 100-pea mass in this type of field expressed as a proportion of expected 10-plant pea mass in equivalent (same sites) virus free untreated fields

[b] Null hypothesis: treatment doesn't reduce the 10-plant pea mass

[c] Null hypothesis: TYV presence doesn't reduce the 10-plant pea mass

[d] Null hypothesis: treatment doesn't increase the 10-plant pea mass when TYV is present

[e] Null hypothesis: PEMV doesn't reduce the 10-plant pea mass

[f] Null hypothesis: treatment doesn't increase the 10-plant pea mass when PEMV is present

However, it must be stressed that the results presented are based on a limited data set and further analysis of these effects will be investigated throughout the course of the project.

Discussion

The traditional approach to carrying out crop surveys would involve carrying out a literature search for potential candidate viruses that may be detected including a suite of common, unusual and those not yet present. From this list of candidate pathogens a suite of 'target' viruses would be selected and these would be tested for using a range of conventional, targeted diagnostic tests such as ELISA, PCR and real-time PCR. The generic applicability of this approach is then limited by the range of viruses 'in test' with no information given on novel or unexpected viruses not previously reported from the host. In a crop where there is limited information about the viruses likely to be encountered it is challenging to develop the suite of potential candidate viruses for testing. For example, if designing a panel of diagnostic tests for surveying pea crops, would the list of targets include the seven viruses previously recorded in

the UK on peas, the 27 viruses from the UK record which have been reported on pea elsewhere, or some of the non-UK pea viruses? Each of these decisions would incur an additional diagnostic cost. The aim of this work was to investigate the feasibility of using HTS as generic virus screen to identify candidate viruses, which could then be confirmed through downstream testing by conventional diagnostic methods.

In the first year of study using HTS revealed the presence of turnip yellows virus (TuYV) in pea crops in the UK. This virus has been previously reported to infect peas (Graichen & Rabenstein, 1996, Stevens et al., 2008), but not from the UK. It was surprising that this virus was present in more crops, and at higher incidence, than pea enation mosaic virus-1 (PEMV-1). It was also surprising that soybean dwarf virus (SbDV) was found to be present, although this was only in a limited number of crops and at a low incidence where recorded. Pea enation mosaic virus-2 (PEMV-2) was also found in nine sites at incidences up to 86% of note is that it was found at some sites without PEMV-1. The overall approach of sequencing a large bulk sample to identify pathogen candidates therefore appears to be sound, in that the pathogens identified through the sequencing work were then confirmed through follow up testing, validating the findings at the systems level (Roenhorst et al., 2018).

In the second year of study, a similar suite of viruses were found as the first year of study and at a similar number of sites as the first year. The incidences in second year were lower overall than the first year, which could be due to a number of factors including delayed growing season, aphid numbers etc. In the second year of study pea seed-borne mosaic virus (PSbMV) was found in the site samples, rather than just in the symptomatic samples, and pea necrotic yellow dwarf virus (PNYDV) was found at one site. This represents the first finding for the UK. As in the first year of study, there were sites where PEMV-2 was present without PEMV-1. After writing up the first year report, a TaqMan assay was designed for PEMV-2 allowing confirmation and determination of incidence of PEMV-2 in the sites where it was found (Fowkes et al., 2021). Traditionally, PEMV-1 and PEMV-2 are thought to exist in a synergistic relationship and only occur together (Demler et al., 1993). Within both years PEMV-2 was found without PEMV-1, the incidence of the two viruses sometimes differed at sites where they were both present.

The suite of viruses found over the two years of this study mirror what has been found by Gaafar et al. (2020). This study investigated which viruses are present in peas and surrounding weeds in pea-growing areas of Germany.

However, it is difficult to assess what may be “missed” in this process. Missed infections may arise from two sources, sampling, and analytical sensitivity. Using a sample size of 120 leaves

would give a 95% confidence of detecting approximately 3% virus incidence in the crop. This means that any finding with an incidence below this level would be detected by chance in the sampling. The consequence of this would be that setting the level of sampling would be dependent upon the surveillance/research question. For non-quarantine pathogens, likely to be present at moderate to high incidence, this low-intensity sampling is adequate, however, for emerging pathogens likely to be present at lower incidence, then higher intensity sampling should be considered. For example, a 3000-leaf sample would give an estimated 95% confidence of detecting a 0.1% infection level. Analytical sensitivity is more difficult to quantify with HTS. Recent work has suggested around 1 million sequence reads should be adequate to detect a whole viral genome (Visser *et al.*, 2016). Other research has suggested that as few as 50,000 sequence reads may be enough to detect the presence of a virus in a sample (Pecman *et al*., 2017). However, due to the nature of the extraction and sample enrichment approaches used in HTS this does not equate simply to 'number of samples' bulked in a sequencing library. The sample preparation process used at Fera involves a step to remove plant ribosomal RNA, enriching the sample for viral RNA. However, the presence of SbDV as fragments of sequence rather than whole genomes, and the possible miss of a low concentration PEMV1 in a single sample suggests that the process used here is near to the limit of detection for low levels of virus. Again, dependent upon the research question being asked (and the available budget) greater sequencing depth per sample may reveal a greater diversity of low concentration viruses.

Three of the sites tested within year 1, were tested re-tested in-depth. These sites were Market Weighton, Perth and Chirnside, these sites were chosen as they had mixed virus infections that for Market Weighton and Perth included findings of SbDV. After testing these samples in-depth, no further plant viruses were found suggesting that the initial testing was sufficient to identify plant viruses within the sample (Fowkes et al., 2021).

In year one, the testing of single symptomatic samples revealed many of the same viruses as detected through the bulk field sampling. However, pea seed-borne mosaic virus and bean yellow mosaic virus, two viruses which could have been expected to be present in crops, were detected in few individual samples, suggesting these viruses are present at low incidence of the viruses, but not a major issue in crops as a whole. In year 2, no viruses were found in the symptomatic samples that were not in site samples.

Preliminary data were also gathered on the potential impacts of infection on crops. Given the design of the surveillance aspect of the project, a 'true' controlled experiment to measure yield reduction could not be carried out. Within the project therefore, areas of crop were demarcated and 'treatment' for aphid vectors was not carried out within these areas. At the end of the season multiple plants were sampled from treated and untreated areas and the yields from

these compared. This preliminary data, from a limited number of sites across two years, suggests an ameliorating effect on yield from 'treated' areas in crops infected with TuYV. Some limited ameliorating effect was also measured in relation to the presence of PEMV-1. However, due to the limited number of sample sites these data should be considered preliminary and conclusions should not be drawn based on the current data set. However, these data have been used to develop a working hypothesis that treatment to control vectors will have an ameliorating effect on yield in relation to virus infected crops.

During year 3 of this project the assessment of presence of viruses and survey methods will be further supplemented by aerial assessment of virus incidence using remote sensing via UAV ("drone"). This work is being funded by Defra through "Future Proofing Plant Health" but should provide preliminary data to assess the use of this type of assessment at a field scale.

Conclusions

- This generic approach to surveillance work appears to be effective, and peas appear to be a successful model crop for this work
- Two viruses, turnip yellows virus and soybean dwarf virus, have been reported to infect UK pea crops for the first time as a result of these data.
- Treatment to limit virus vector aphids may have an ameliorating effect on the yield impact of pea infecting viruses
- These conclusions will be further tested in future years of the project

Knowledge and Technology Transfer

The following activities have been undertaken as KE activity:

Presentations:

- 08/02/2019 – Becky Howard presented an introduction to the project to the HMC Peas grower group and requested volunteers to provide sites
- 13/02/2019 – Becky Howard presented an introduction to the project to the Dengie Crops grower group meeting and requested volunteers to provide sites
- 08/03/2019 – Becky Howard presented an introduction to the project to the Swaythorpe Growers group meeting and requested volunteers to provide sites
- 12 and 13/06/2019 – Becky Howard presented a poster describing the project objectives at the Cereals 2019 event
- 02/07/2019 – Becky Howard presented the same poster at the PGRO Pulse Open Day at Stubton, Lincolnshire

- 10/10/2019 – Adrian Fox presented the work as part of a broader talk on new diagnostic technologies to the BCPC Workshop on pathogens, NIAB, Cambridge.
- 5/11/2019 – Adrian Fox presented the year 1 project results at the Pea and Bean growers conference, Peterborough.
- 19/11/2019 – Becky Howard presented an update of the project to Velcourt Farming managers
- 21/01/2020 – Adrian Fox presented the work as part of a broader talk on diagnostic technologies to Hutchinsons vegetable conference
- 10/11/2021 -Aimee Fowkes presented year 1 and year 2 results at the Fera Science Symposium (Using information & Data for a Sustainable Foodscape).
- 27/01/2022- Becky Howard presented year 1 and year 2 results at the PGRO/Syngenta Pulse Roadshow Webinar 2022.
- 06/06/2022- Aimee Fowkes presenting year 1 and year 2 results at the International Symposium of Plant Virus Epidemiology, Madrid 2022.

Publications:

- Pulse magazine (Winter 2019)
- Fowkes AR, Mcgreig S, Pufal H*, et al.*, 2021. Integrating High throughput Sequencing into Survey Design Reveals Turnip Yellows Virus and Soybean Dwarf Virus in Pea (Pisum Sativum) in the United Kingdom. *Viruses* **13**, 2530.
- First report of pea necrotic yellow dwarf virus in the United Kingdom (in Prep), New Disease Report currently being prepared by Aimee Fowkes and Adrian Fox.

Glossary

BYMV	Bean yellow mosaic virus
TuYVaRNA	Turnip yellows virus associated RNA
HTS	High throughput sequencing
PEMV-1	Pea enation mosaic virus-1
PEMV-2	Pea enation mosaic virus-2
PNYDV	Pea necrotic yellow dwarf virus
PSbMV	Pea seedborne mosaic virus
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase Polymerase chain reaction
SbDV	Soybean dwarf virus

TuYV Turnip yellows virus

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Appendix 1. Table for interpretation of bulk test results

Bulk size1	Bulk size2	No. positive1	No. positive2	**estimate of incidence**	lower 95% CI	upper 95% CI
1	7	0	0	0	0	2.47
1	7	1	0	0.83	0.02	3.37
1	7	2	0	1.67	0.21	4.69
1	7	3	0	2.5	0.54	6.18
1	7	4	0	3.33	0.98	7.67
1	7	5	0	p<0.001	p<0.001	p<0.001
1	7	6	0	p<0.001	p<0.001	p<0.001
1	7	7	0	p<0.001	p<0.001	p<0.001
1	7	8	0	p<0.001	p<0.001	p<0.001
1	7	9	0	p<0.001	p<0.001	p<0.001
1	7	10	0	p<0.001	p<0.001	p<0.001
1	7	11	0	p<0.001	p<0.001	p<0.001
1	7	12	0	p<0.001	p<0.001	p<0.001
1	7	13	0	p<0.001	p<0.001	p<0.001
1	7	14	0	p<0.001	p<0.001	p<0.001
1	7	15	0	p<0.001	p<0.001	p<0.001
1	7	0	1	0.85	0.02	4.66
1	7	1	1	1.71	0.21	5.16
1	7	2	1	2.57	0.54	6.25
1	7	3	1	3.42	0.98	7.68
1	7	4	1	4.28	1.5	9.18
1	7	5	1	p<0.001	p<0.001	p<0.001
1	7	6	1	p<0.001	p<0.001	p<0.001
1	7	7	1	p<0.001	p<0.001	p<0.001
1	7	8	1	p<0.001	p<0.001	p<0.001
1	7	9	1	p<0.001	p<0.001	p<0.001
1	7	10	1	p<0.001	p<0.001	p<0.001
1	7	11	1	p<0.001	p<0.001	p<0.001
1	7	12	1	p<0.001	p<0.001	p<0.001
1	7	13	1	p<0.001	p<0.001	p<0.001
1	7	14	1	p<0.001	p<0.001	p<0.001
1	7	15	1	p<0.001	p<0.001	p<0.001
1	7	0	2	1.76	0.24	6.17
1	7	1	2	2.64	0.55	6.81
1	7	2	2	3.52	0.98	7.81
1	7	3	2	4.4	1.5	9.2
1	7	4	2	5.28	2.1	10.73
1	7	5	2	6.17	2.76	12.35
1	7	6	2	p<0.001	p<0.001	p<0.001
1	7	7	2	p<0.001	p<0.001	p<0.001
1	7	8	2	p<0.001	p<0.001	p<0.001

Bulk size1	Bulk size2	No. positive1	No. positive2	**estimate of incidence**	lower 95% CI	upper 95% CI
1	7	9	2	p<0.001	p<0.001	p<0.001
1	7	10	2	p<0.001	p<0.001	p<0.001
1	7	11	2	p<0.001	p<0.001	p<0.001
1	7	12	2	p<0.001	p<0.001	p<0.001
1	7	13	2	p<0.001	p<0.001	p<0.001
1	7	14	2	p<0.001	p<0.001	p<0.001
1	7	15	2	p<0.001	p<0.001	p<0.001
1	7	0	3	2.71	0.63	7.67
1	7	1	3	3.62	1.01	8.43
1	7	2	3	4.53	1.51	9.41
1	7	3	3	5.44	2.1	10.78
1	7	4	3	6.35	2.76	12.36
1	7	5	3	7.27	3.49	14.05
1	7	6	3	p<0.001	p<0.001	p<0.001
1	7	7	3	p<0.001	p<0.001	p<0.001
1	7	8	3	p<0.001	p<0.001	p<0.001
1	7	9	3	p<0.001	p<0.001	p<0.001
1	7	10	3	p<0.001	p<0.001	p<0.001
1	7	11	3	p<0.001	p<0.001	p<0.001
1	7	12	3	p<0.001	p<0.001	p<0.001
1	7	13	3	p<0.001	p<0.001	p<0.001
1	7	14	3	p<0.001	p<0.001	p<0.001
1	7	15	3	p<0.001	p<0.001	p<0.001
1	7	0	4	3.72	1.14	9.18
1	7	1	4	4.66	1.55	10.07
1	7	2	4	5.6	2.11	11.06
1	7	3	4	6.55	2.76	12.43
1	7	4	4	7.49	3.49	14.07
1	7	5	4	8.44	4.04	15.39
1	7	6	4	9.4	4.61	16.61
1	7	7	4	p<0.001	p<0.001	p<0.001
1	7	8	4	p<0.001	p<0.001	p<0.001
1	7	9	4	p<0.001	p<0.001	p<0.001
1	7	10	4	p<0.001	p<0.001	p<0.001
1	7	11	4	p<0.001	p<0.001	p<0.001
1	7	12	4	p<0.001	p<0.001	p<0.001
1	7	13	4	p<0.001	p<0.001	p<0.001
1	7	14	4	p<0.001	p<0.001	p<0.001
1	7	15	4	p<0.001	p<0.001	p<0.001
1	7	0	5	4.81	1.74	10.73
1	7	1	5	5.78	2.19	11.76
1	7	2	5	6.76	2.79	12.8

Bulk size1	Bulk size2	No. positive1	No. positive2	**estimate of incidence**	lower 95% CI	upper 95% CI
1	7	3	5	7.74	3.5	14.18
1	7	4	5	8.72	4.3	15.89
1	7	5	5	9.71	4.97	17.38
1	7	6	5	10.7	5.33	18.09
1	7	7	5	p<0.001	p<0.001	p<0.001
1	7	8	5	p<0.001	p<0.001	p<0.001
1	7	9	5	p<0.001	p<0.001	p<0.001
1	7	10	5	p<0.001	p<0.001	p<0.001
1	7	11	5	p<0.001	p<0.001	p<0.001
1	7	12	5	p<0.001	p<0.001	p<0.001
1	7	13	5	p<0.001	p<0.001	p<0.001
1	7	14	5	p<0.001	p<0.001	p<0.001
1	7	15	5	p<0.001	p<0.001	p<0.001
1	7	0	6	5.97	2.43	12.35
1	7	1	6	6.98	2.91	13.53
1	7	2	6	8	3.55	14.64
1	7	3	6	9.02	4.31	16.06
1	7	4	6	10.05	4.97	17.43
1	7	5	6	11.09	5.61	18.75
1	7	6	6	12.13	6.38	20.3
1	7	7	6	13.18	7.18	21.98
1	7	8	6	p<0.001	p<0.001	p<0.001
1	7	9	6	p<0.001	p<0.001	p<0.001
1	7	10	6	p<0.001	p<0.001	p<0.001
1	7	11	6	p<0.001	p<0.001	p<0.001
1	7	12	6	p<0.001	p<0.001	p<0.001
1	7	13	6	p<0.001	p<0.001	p<0.001
1	7	14	6	p<0.001	p<0.001	p<0.001
1	7	15	6	p<0.001	p<0.001	p<0.001
1	7	0	7	7.22	3.2	14.05
1	7	1	7	8.28	3.71	15.39
1	7	2	7	9.35	4.39	16.61
1	7	3	7	10.42	5.21	18.08
1	7	4	7	11.5	5.99	19.6
1	7	5	7	12.59	6.72	21.08
1	7	6	7	13.69	7.55	22.75
1	7	7	7	14.8	7.99	23.66
1	7	8	7	p<0.001	p<0.001	p<0.001
1	7	9	7	p<0.001	p<0.001	p<0.001
1	7	10	7	p<0.001	p<0.001	p<0.001
1	7	11	7	p<0.001	p<0.001	p<0.001
1	7	12	7	p<0.001	p<0.001	p<0.001

Bulk size1	Bulk size2	No. positive1	No. positive2	**estimate of incidence**	lower 95% CI	upper 95% CI
1	7	13	7	p<0.001	p<0.001	p<0.001
1	7	14	7	p<0.001	p<0.001	p<0.001
1	7	15	7	p<0.001	p<0.001	p<0.001
1	7	0	8	8.59	4.05	15.86
1	7	1	8	9.7	4.61	17.37
1	7	2	8	10.83	5.33	18.73
1	7	3	8	11.96	6.2	20.3
1	7	4	8	13.1	7.1	21.98
1	7	5	8	14.26	7.56	22.81
1	7	6	8	15.43	8.46	24.64
1	7	7	8	16.6	9.39	26.56
1	7	8	8	17.79	9.91	27.65
1	7	9	8	p<0.001	p<0.001	p<0.001
1	7	10	8	p<0.001	p<0.001	p<0.001
1	7	11	8	p<0.001	p<0.001	p<0.001
1	7	12	8	p<0.001	p<0.001	p<0.001
1	7	13	8	p<0.001	p<0.001	p<0.001
1	7	14	8	p<0.001	p<0.001	p<0.001
1	7	15	8	p<0.001	p<0.001	p<0.001
1	7	0	9	10.09	5	17.84
1	7	1	9	11.27	5.62	19.52
1	7	2	9	12.46	6.38	21.05
1	7	3	9	13.67	7.31	22.75
1	7	4	9	14.89	7.99	23.92
1	7	5	9	16.12	8.88	25.61
1	7	6	9	17.38	9.89	27.65
1	7	7	9	18.64	10.49	28.84
1	7	8	9	19.93	11.31	30.51
1	7	9	9	21.23	12.28	32.52
1	7	10	9	p<0.001	p<0.001	p<0.001
1	7	11	9	p<0.001	p<0.001	p<0.001
1	7	12	9	p<0.001	p<0.001	p<0.001
1	7	13	9	p<0.001	p<0.001	p<0.001
1	7	14	9	p<0.001	p<0.001	p<0.001
1	7	15	9	p<0.001	p<0.001	p<0.001
1	7	0	10	11.76	6.03	19.96
1	7	1	10	13.02	6.74	21.85
1	7	2	10	14.29	7.58	23.66
1	7	3	10	15.59	8.46	25.29
1	7	4	10	16.91	9.39	26.94
1	7	5	10	18.25	10.33	28.66
1	7	6	10	19.61	11.27	30.51

Bulk size1	Bulk size2	No. positive1	No. positive2	**estimate of incidence**	lower 95% CI	upper 95% CI
1	7	7	10	20.99	12.27	32.52
1	7	8	10	22.4	13.33	34.75
1	7	9	10	23.83	14.5	37.23
1	7	10	10	p<0.001	p<0.001	p<0.001
1	7	11	10	p<0.001	p<0.001	p<0.001
1	7	12	10	p<0.001	p<0.001	p<0.001
1	7	13	10	p<0.001	p<0.001	p<0.001
1	7	14	10	p<0.001	p<0.001	p<0.001
1	7	15	10	p<0.001	p<0.001	p<0.001
1	7	0	11	13.64	7.18	22.29
1	7	1	11	15	8.09	24.63
1	7	2	11	16.39	8.93	26.56
1	7	3	11	17.8	9.91	28.49
1	7	4	11	19.25	10.98	30.44
1	7	5	11	20.72	12.1	32.5
1	7	6	11	22.23	13.25	34.74
1	7	7	11	23.77	14.46	37.23
1	7	8	11	25.34	14.5	37.25
1	7	9	11	26.95	15.79	40.04
1	7	10	11	28.6	17.23	43.25
1	7	11	11	p<0.001	p<0.001	p<0.001
1	7	12	11	p<0.001	p<0.001	p<0.001
1	7	13	11	p<0.001	p<0.001	p<0.001
1	7	14	11	p<0.001	p<0.001	p<0.001
1	7	15	11	p<0.001	p<0.001	p<0.001
1	7	0	12	15.8	8.77	25.51
1	7	1	12	17.3	9.57	27.62
1	7	2	12	18.84	10.49	29.9
1	7	3	12	20.41	11.56	32.22
1	7	4	12	22.03	12.79	34.63
1	7	5	12	23.69	14.13	37.19
1	7	6	12	25.41	14.5	37.64
1	7	7	12	27.17	15.79	40.2
1	7	8	12	28.99	17.23	43.31
1	7	9	12	30.88	18.46	46.18
1	7	10	12	32.82	19.94	49.53
1	7	11	12	34.83	20.81	51.57
1	7	12	12	p<0.001	p<0.001	p<0.001
1	7	13	12	p<0.001	p<0.001	p<0.001
1	7	14	12	p<0.001	p<0.001	p<0.001
1	7	15	12	p<0.001	p<0.001	p<0.001
1	7	0	13	18.36	10.41	28.83

Bulk size1	Bulk size2	No. positive1	No. positive2	**estimate of incidence**	lower 95% CI	upper 95% CI
1	7	1	13	20.05	11.31	31.14
1	7	2	13	21.8	12.32	33.83
1	7	3	13	23.61	13.51	36.73
1	7	4	13	25.49	14.61	39.07
1	7	5	13	27.44	15.84	41.4
1	7	6	13	29.48	17.25	44.08
1	7	7	13	31.6	18.86	47.4
1	7	8	13	33.83	19.94	49.73
1	7	9	13	36.16	21.6	53.34
1	7	10	13	38.6	23.54	57.63
1	7	11	13	41.16	25.86	62.62
1	7	12	13	43.85	25.86	62.62
1	7	13	13	46.65	28.63	68.04
1	7	14	13	p<0.001	p<0.001	p<0.001
1	7	15	13	p<0.001	p<0.001	p<0.001
1	7	0	14	21.52	12.28	32.64
1	7	1	14	23.5	13.34	35.3
1	7	2	14	25.58	15.19	39.99
1	7	3	14	27.77	16.27	42.99
1	7	4	14	30.09	17.53	46.16
1	7	5	14	32.56	19.03	49.52
1	7	6	14	35.18	20.81	53.27
1	7	7	14	38	22.9	57.61
1	7	8	14	41.02	25.36	62.62
1	7	9	14	44.26	25.86	63.16
1	7	10	14	47.74	28.63	68.23
1	7	11	14	51.46	31.93	73.55
1	7	12	14	55.41	35.86	78.76
1	7	13	14	59.56	35.89	78.77
1	7	14	14	63.87	40.54	83.67
1	7	15	14	68.28	46.02	88.18
1	7	0	15	25.7	15.76	40.03
1	7	1	15	28.2	17.09	43.25
1	7	2	15	30.91	18.47	47.02
1	7	3	15	33.88	20.01	51.57
1	7	4	15	37.15	21.63	56.58
1	7	5	15	40.8	23.55	62.15
1	7	6	15	44.91	26.02	68.04
1	7	7	15	49.57	28.72	73.5
1	7	8	15	54.81	31.98	78.75
1	7	9	15	60.62	35.9	83.67
1	7	10	15	66.87	40.54	88.18

Bulk size1	Bulk size2	No. positive1	No. positive2	**estimate of incidence**	lower 95% CI	upper 95% CI
1	7	11	15	73.38	46.02	92.21
1	7	12	15	80.01	52.38	95.67
1	7	13	15	86.67	59.69	98.34
1	7	14	15	93.33	68.08	99.83
1	7	15	15	100	78.2	100

Appendix 2 Pea yield data from 5 sample sites in 2019

Canterbury

Untreated		Treated	
1	50.6	21	59.9
2	83.5	22	72.2
3	67.8	23	53.3
4	71.4	24	87.9
5	65.7	25	79.3
6	66.8	26	43.7
7	51.1	27	47.2
8	82	28	62.2
9	55.2	29	53.6
10	71.6	30	83.7
11	64.6	31	77
12	48.8	32	83.3
13	62.2	33	61.4
14	88.5	34	65.3
15	70.8	35	73
16	88.4	36	83.9
17	77.3	37	55.5
18	69.6	38	81.1
19	92	39	73.7
20	58.1	40	76.2
Sum	1386		1373.4
Min	48.8		43.7
Max	92		87.9

	Untreated	Treated
Moisture	14.02	13.97
TSW	263.8	257.3

Salisbury

Untreated		treated	
1	57.4	21	62.2
2	63.1	22	57
3	55.5	23	109.3
4	63.6	24	52.9
5	68.1	25	76.8
6	41.3	26	38.1
7	46.7	27	61.1
8	75.6	28	60.6
9	45.4	29	53.4
10	48.4	30	52.7
11	63.4	31	56.9
12	50	32	57.6
13	91	33	44.1
14	53.8	34	94.8
15	51.1	35	66.6
16	69.3	36	66.4
17	67.5	37	75
18	57.7	38	50.9
19	41	39	92.6
20	57.7	40	60.4
Sum	1167.6		1289.4
Min	41		38.1
Max	91		109.3
Median	57.55		60.5

	Untreated	Treated
Moisture	11.38	11.26
TSW	353.5	366.7

Stoneleigh

Weight in grams					
Untreated	**Haulm**	**Peas**	**Treated**	**Haulm**	**Peas**
1	975	92.6	21	622	80
2	800	93.7	22	807	77
3	886	66.7	23	984	97.3
4	724	80	24	717	67.7
5	894	105.4	25	917	103.2
6	858	81.6	26	761	76.5
7	938	95.8	27	1021	73.1
8	920	98.4	28	1106	118
9	830	89.3	29	912	88.6
10	807	93.7	30	760	97.6
11	822	99	31	727	79.3
12	817	86.5	32	682	89.4
13	853	120.9	33	1013	69.9
14	839	56.1	34	769	114
15	598	77.6	35	702	88
16	656	82.5	36	743	36.3
17	655	70.1	37	822	89.3
18	797	82.3	38	658	68.6
19	858	112.4	39	805	79.9
20	693	88.2	40	818	96.5
Total	16220	1772.8	Total	16346	1690.2

					Average
	Untreated	91	94	93	92.66666667
TR	**Treated**	90	89	90	89.66666667

Eye

Note untreated area had footrot when harvested

Untreated			Treated		
	Weight of Haulm	weight of peas		Weight of Haulm	weight of peas
1	170.5	37.6	21	391.7	88.5
2	126.7	31	22	338.2	72
3	113	29.8	23	316.9	64.5
4	85.3	19.4	24	313.2	63.7
5	232.8	56.9	25	275.2	62.8
6	165.2	44.3	26	381.5	92.9
7	144.9	42.4	27	289.9	64.4
8	150.6	43.8	28	305.2	76.8
9	122.6	31	29	277.4	60.4
10	104.3	28.9	30	281.9	71.4
11	187.4	38.6	31	190.4	43.6
12	194.4	42.4	32	322.6	78.7
13	170.5	30.1	33	366.1	62
14	193.3	42.6	34	300.2	78.6
15	219.1	55.5	35	274.3	75.1
16	211.9	47.9	36	293.6	71
17	164.4	35.6	37	254	42.9
18	258.1	43.3	38	343.9	72.3
19	170.2	32.3	39	311.7	70
20	208.3	32.6	40	167.5	46.9
Sum	3393.5	766		5995.4	1358.5
Min	85.3	19.4		167.5	42.9
Max	258.1	56.9		391.7	92.9

					Average
TR	**Treated**	103	101	95	99.66666667
	Untreated	98	96	98	97.33333333

Louth

Untreated			Treated		
plot	Weight (10 plants) (kg)	Weight of peas (kg)	plot	Weight (10 plants) (kg)	Weight of peas (kg)
1	0.425	0.112	21	0.36	0.108
2	0.31	0.082	22	0.39	0.102
3	0.32	0.086	23	0.325	0.078
4	0.375	0.104	24	0.42	0.112
5	0.465	0.108	25	0.185	0.05
6	0.28	0.064	26	0.535	0.102
7	0.325	0.076	27	0.51	0.12
8	0.355	0.088	28	0.21	0.05
9	0.515	0.136	29	0.51	0.122
10	0.215	0.052	30	0.32	0.072
11	0.385	0.084	31	0.32	0.078
12	0.285	0.062	32	0.345	0.046
13	0.235	0.062	33	0.355	0.094
14	0.215	0.046	34	0.23	0.036
15	0.165	0.02	35	0.275	0.066
16	0.195	0.04	36	0.32	0.068
17	0.255	0.066	37	0.34	0.072
18	0.315	0.084	38	0.34	0.082
19	0.285	0.072	39	0.412	0.112
20	0.22	0.05	40	0.43	0.11
Sum		**1.494**			**1.68**
Min		**0.02**			**0.036**
Max		**0.136**			**0.122**

TR	**Untreated**	**Treated**
1	108	101
2	110	102
3	108	104
average	109	102

Appendix 3 The effects of TuYV, PEMV and treatment on the productivity of peas (2019)

Summary and conclusions

The aim of this analysis was to estimate the effect of the prevalence of TuYV and PEMV on the productivity of peas[1], and how treatment modifies the effect of the viruses. We used observations of productivity and virus prevalence at 5 locations. We assumed that the fields at the five locations were representative of the broader population to make inferences about the ranges within which mean effects in the population will[2] lie.

Our analysis showed that reduction in productivity is significantly correlated with estimates of the prevalence of TuYV and PEMV gained from testing samples by PCR; that treatments applied to clean fields do not reduce productivity and that treatments applied to fields with high TuYV prevalence improve productivity. Results for the effect of treatment on productivity in the presence of PEMV were inconclusive.

In more detail, we estimated the following quantities for the population across many sites:

1. The average mass of peas per 10 plants in untreated fields in which TuYV and PEMV are not detected lies[2] somewhere between 73 and 97 g
2. Treatment was not found to significantly reduce productivity in fields in which TuYV and PEMV are not detected (p=0.24); average productivity in 'clean' treated fields lies[2] somewhere between 88 and 106% of that in untreated fields.
3. TuYV was found to significantly reduce productivity (p<0.001); the average productivity in untreated fields in which TuYV is present with a prevalence of 97%[3] lies[2] somewhere between 19 and 67% of that in 'clean' fields.
4. The effect of TuYV is significantly ameliorated by treatment (p<0.001); the average productivity in treated 'TuYV fields' lies[2] somewhere between 55 and 108% of that in untreated 'clean' fields.
5. PEMV was found to significantly reduce productivity (p<0.011); the average productivity in untreated fields in which PEMV is present with a prevalence of 27%[3] lies[2] somewhere between 47 and 96% of that in 'clean' fields.
6. The effect of PEMV was not found to be significantly ameliorated by treatment (p<0.18); the average productivity in treated 'PEMV fields' lies[2] somewhere between 53 and 100% of that in untreated 'clean' fields. It is possible that the treatment does

[1] Expressed as mass of peas per 10 plants
[2] With 95% confidence
[3] The highest prevalence we observed in this study

have an ameliorating effect which is more difficult to detect because the effect of PEMV is relatively small.

These estimates rely on the five sites in this study being representative of the whole population. In addition, they apply to one season. Further observations are likely to help us reduce the size of the uncertainty that is associated with our quantitative estimates of the effects of viruses and treatments and to provide assurance that effects are consistent across seasons. Alternatively, we may find observations from further sites in subsequent seasons paint a very different picture. Either way, further observations will be very useful.

Method of analysis and results

Observations of peas per 10 plants, for treated and untreated plants, and estimated prevalence of TuYV and PEMV were provided for five sites. (Appendix 1). A linear mixed model (Equation 1) was fitted to the observations[4].

$$mass \sim Treatment * TYVn + PEMVn * Treatment + (1 \mid Location)$$

Equation 1

The prevalence of viruses and Treatment were found to have significant ($p<0.0001$) effects when compared with null models. The fitted model was:

```
Scaled residuals:
    Min      1Q  Median      3Q     Max
-3.2898 -0.5633 -0.0402  0.5486  3.4577

Random effects:
 Groups   Name        Variance Std.Dev.
 Location (Intercept)  73.49    8.572
 Residual             295.55   17.191
Number of obs: 198, groups:  Location, 5

Fixed effects:
                     Estimate Std. Error t value
(Intercept)           84.54978    6.52816  12.952
TreatmentTreated      -2.68338    3.79663  -0.707
TUYVn                  -0.49992    0.13478  -3.709
PEMVn                 -0.93596    0.42497  -2.202
TreatmentTreated:TUYVn   0.35260    0.07814   4.512
TreatmentTreated:PEMVn  0.29165    0.24775   1.177

Correlation of Fixed Effects:
            (Intr) TrtmnT TUYVn   PEMVn  TT:TUYV
TretmntTrtd -0.288
TUYVn        -0.694  0.200
PEMVn       -0.569  0.164  0.395
TrtmntT:TUYV  0.201 -0.696 -0.289 -0.114
TrtmnT:PEMV  0.163 -0.568 -0.113 -0.296  0.395
```

[4] Two unusually low observations with reasons given for why they were low were removed prior to the analysis

Appendix 4 - Pea yield data from 5 sample sites in 2021

Southampton

	Treated			Untreated	
	Total weight of plants g	Total weight of seed g		Total weight of plants g	Total weight of seed g
1	108	54.5	21	97	48.5
2	138	69.5	22	120.5	66
3	85.5	38.5	23	84	55.5
4	162.5	83.5	24	110	54.5
5	139.5	72.5	25	115	46.5
6	161	83	26	123	63.5
7	115.5	60.5	27	93	70.5
8	118.5	49	28	121.5	55.5
9	142	63	29	127	66.5
10	138.5	64	30	100.5	64
11	106.5	49.5	31	96.5	30.5
12	110.5	56	32	115	60.5
13	98	42.5	33	82	43.5
14	134.5	81	34	147.5	63.5
15	117.5	59.5	35	137	76
16	150.5	83	36	99.5	42
17	146.5	65.5	37	139.5	65
18	93	48.5	38	146.5	80.5
19	129	64	39	152	66.5
20	109	59.5	40	124.5	66.5
Sum	2504	1247		2331.5	1185.5
Mean	125.2	62.35		116.575	59.275

	Treated	**Untreated**
Moisture 1	11.1	10.7
Moisture 2	11	10.8
Moisture 3	11.1	10.7

Kent

	Treated			Untreated	
	Total weight of plants g	Total weight of seed g		Total weight of plants g	Total weight of seed g
1	85	50	21	35	15
2	107.5	50.5	22	40	15
3	82.5	37.5	23	90	45
4	78	36.5	24	110	45
5	116	58	25	125	60

6	110.5	65	26	80	40
7	107.5	53	27	30	10
8	76.5	34.5	28	105	60
9	90	43.5	29	115	60
10	114.5	56.5	30	90	55
11	50	20	31	40	15
12	75	45	32	55	35
13	93	37	33	55	25
14	90	35	34	100	40
15	121.5	61	35	115	55
16	60	30	36	70	40
17	80.5	36.5	37	55	25
18	80	30	38	75	30
19	125	55	39	90	40
20	74	34.5	40	85	40
Sum	1817	869		1560	750
Mean	90.85	43.45		78	37.5

	Treated	**Untreated**
Moisture 1	13.2	15.1
Moisture 2	13.5	15
Moisture 3	13.3	14.8
average	13.33333333	14.96666667

Louth

	Treated			**Untreated**	
	Total weight of plants g	Total weight of seed g		Total weight of plants g	Total weight of seed g
1	443.2	102.7	21	314.6	70
2	469.1	104.1	22	411.5	91.6
3	398.8	92.5	23	414.7	106.5
4	444.9	102.6	24	522.2	107.7
5	417.8	98.4	25	399.8	116.1
6	389	97	26	626.9	151.4
7	373	97.3	27	333.4	78.2
8	388.1	96.9	28	418.8	115.2
9	509.8	126	29	387	100
10	490.3	120.8	30	470.7	113.1
11	471.5	112.4	31	533.4	134.5
12	456.5	101.6	32	355	86.2
13	422.8	93.5	33	410.7	91.8

14	387.8	91	34	293.3	66.7
15	329.1	82.2	35	450.5	117.1
16	584.4	142.3	36	397.4	72.8
17	426.8	103.5	37	406	103.7
18	261.5	69.2	38	354.5	87.7
19	367.9	93.5	39	486.5	97.7
20	431.9	99.4	40	457.4	102.6
Sum	8464.2	2026.9		8444.3	2010.6
Mean	423.21	101.345		422.215	100.53

	Treated	**Untreated**
TR 1	105	104
TR 2	109	101
TR 3	107	104
Mean	107	103

Beverley

	Treated			**Untreated**		
	Total weight of plants	total weight of seed		Total weight of plants	total weight of seed	virus symptoms
1	652.8	153.1	21	388.6	95.9	
2	704.9	164.2	22	487.8	47.8	
3	557.5	163.2	23	485.9	108.2	yes
4	675.2	182.2	24	303.4	61.7	
5	635	148.1	25	376.8	73	
6	466.5	71.9	26	394.1	77.3	
7	808.5	101.3	27	532.2	138.5	
8	478.2	176.7	28	555.6	124.6	
9	431.2	95.9	29	660.9	162.3	
10	562.2	131	30	454.2	89.9	
11	722.4	169.4	31	584.9	114.7	
12	444.2	114.7	32	657.5	104.5	
13	627.5	181.8	33	427.5	91	
14	558.1	89.3	34	595.4	107.1	
15	595.4	137.4	35	897.5	193.4	
16	769.9	225.9	36	482	98.1	
17	436.7	109.5	37	389.7	61.2	
18	484.6	103.4	38	486.8	90.7	
19	603.3	167.8	39	626.8	97.4	
20	544.6	147.6	40	294.2	37.2	
Sum	11758.7	2834.4		10081.8	1974.5	

	Treated	Untreated
TR 1	107	103
TR 2	107	104
TR 3	109	102

Framlingham

	Treated			Untreated	
	Total weight of plants	total weight of seed		Total weight of plants	total weight of seed
1	297.3	80.7	21	497.9	81.5
2	372.9	71.9	22	435.6	92.4
3	325.6	89.2	23	322.4	62
4	470.5	113.8	24	268.2	63.8
5	362.7	82.1	25	406.6	83.9
6	355.5	95.5	26	280.7	59.8
7	383.5	86.7	27	438.5	84.1
8	365.8	90.1	28	244.5	41.1
9	436.8	97.5	29	277.7	55.6
10	303.3	79.9	30	412.2	90.2
11	395.5	119.4	31	397.2	105.4
12	413.3	112.1	32	383.3	107.3
13	376.4	81.4	33	431.1	76.1
14	510.3	127.9	34	455.1	78.2
15	385	90.6	35	368.7	108.5
16	412.5	106.2	36	340.9	74.6
17	349.8	76.9	37	325	94.1
18	326.3	52.9	38	545.1	156.5
19	351.9	89.1	39	378	99.9
20	452.6	105.7	40	250.3	67.5
	7647.5	1849.6		7459	1682.5

	Treated	Untreated
TR 1	105	106
TR 2	104	104
TR 3	104	106

Appendix 5 - The effects of TuYV, PEMV and treatment on the productivity of peas (2021)

Summary and conclusions

The aim of this analysis was to estimate the effect of the prevalence of viruses on the productivity of peas[5] and how treatment modifies the effect of the viruses. This was a second part of a study in which the effect of TuYV and PEMV was estimated at five sites in 2019. There were not sufficient observations to fit a model that included all of the viruses included in this addition to the study. Hence, estimates for TuYV and PYMV were updated with observations gained in 2021. We used observations of productivity and virus prevalence at 5 locations in 2019 and 5 locations in 2021. We assumed that the fields at locations were representative of the broader population to make inferences about the ranges within which mean effects in the population will[6] lie.

Our analysis showed that reduction in productivity is significantly correlated with estimates of the prevalence of TuYV and PEMV gained from testing samples by PCR; that treatments applied to clean fields do not reduce productivity and that treatments applied to fields with high TuYV prevalence improve productivity. Results for the effect of treatment on productivity in the presence of PEMV were inconclusive.

In more detail, we estimated the following quantities for the population across many sites:

1. The average mass of peas per 10 plants in untreated fields in which TuYV and PEMV are not detected lies[6] somewhere between 68 and 107 g
2. Treatment was not found to significantly reduce productivity in fields in which TuYV and PEMV are not detected ($p=0.75$); average productivity in 'clean' treated fields lies[6] somewhere between 95 and 111% of that in untreated fields.
3. TuYV was found to significantly reduce productivity ($p<0.011$); the average productivity in untreated fields in which TuYV is present with a prevalence of 97%[7] lies[2] somewhere between 33 and 89% of that in 'clean' fields.
4. The effect of TuYV is significantly ameliorated by treatment ($p<0.001$); the average productivity in treated 'TuYV fields' lies[2] somewhere between 51 and 140% of that in untreated 'clean' fields.
5. PEMV was found to significantly reduce productivity ($p<0.006$); the average productivity in untreated fields in which PEMV is present with a prevalence of 27%[7] lies[6] somewhere between 56 and 94% of that in 'clean' fields.
6. The effect of PEMV was not found to be significantly ameliorated by treatment ($p<0.27$); the average productivity in treated 'PEMV fields' lies[6] somewhere between 58 and 96% of that in untreated 'clean' fields.

[5] Expressed as mass of peas per 10 plants
[6] With 95% confidence
[7] The highest prevalence we observed in this study in 2019

These estimates rely on the sites in this study being representative of the whole population.

Method of analysis and results

Observations of peas per 10 plants, for treated and untreated plants, and estimated prevalence of TuYV and PEMV were provided for five sites. (Appendix 1). A linear mixed model (Equation 1) was fitted to the log transformed observations[8].

$$mass \sim Treatment * TuYVn + PEMVn * Treatment + (1 | Location{:}Year)$$

Equation 1

The prevalence of viruses and Treatment were found to have significant ($p<0.0001$) effects when compared with null models. The fitted model was:

```
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerMo
dLmerTest']
Formula: mass ~ Treatment * TuYVn + PEMVn * Treatment + (1 | Location:Year)
   Data: Ndata

REML criterion at convergence: 156.8

Scaled residuals:
    Min      1Q  Median      3Q     Max
-3.1750 -0.5732  0.0657  0.6104  2.8537

Random effects:
 Groups        Name        Variance Std.Dev.
 Location:Year (Intercept) 0.06183  0.2487
 Residual                  0.07104  0.2665
Number of obs: 395, groups:  Location:Year, 10

Fixed effects:
                          Estimate Std. Error         df t value Pr(>|t|)
(Intercept)             4.448e+00  1.141e-01  7.406e+00  38.997 7.63e-10 **
*
TreatmentTreated         2.834e-02  3.781e-02  3.820e+02   0.750   0.4540
TYVn                    -6.430e-03  2.760e-03  7.397e+00  -2.330   0.0507 .
PEMVn                   -1.167e-02  4.707e-03  7.429e+00  -2.480   0.0403 *
TreatmentTreated:TuYVn   4.511e-03  9.113e-04  3.820e+02   4.950 1.11e-06 *
**
TreatmentTreated:PEMVn -7.413e-06  1.568e-03  3.820e+02  -0.005   0.9962
```

[8] Two unusually low observations with reasons given for why they were low were removed prior to the analysis; three further unusually low observations were removed (See Appendix A)