Project title:	**Surveillance of virus diseases in UK Pea Crops**
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Project leader:	Dr Adrian Fox, Fera Science Ltd
Report:	Final Report, December 2022.
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Key staff:	Dr Becky Howard, PGRO Shona Duffy, PGRO Aimee Fowkes, Fera Science Ltd
Location of project:	York and Peterborough, plus multiple field sites
Industry Representative:	Philip Langley, G's produce
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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, prevalence 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 prevalence. Five viruses were found repeatedly through the three years (turnip yellows virus, pea enation mosaic virus-1, pea enation mosaic virus-2, pea seed-borne mosaic virus and soybean dwarf virus), additional viruses such as pea necrotic yellow dwarf virus were found only in one year.

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. 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 naturally, 27 have been previously recorded in the UK, but only seven (7) have ever been recorded in UK pea crops (Source: UK Virus Checklist, unpublished Fera data).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). A survey was conducted covering England, the Netherlands, Sweden, and West Germany in the late 1950s (Hagedorn, 1958). The UK part of this work consisted of 14 fields in England; it 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 the diagnostic of testing small numbers of samples, again 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). Nanoviruses had not been reported from the UK prior to this project.

The aim of this work was to develop a generic survey technique, which could be applied in any crop, 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 prevalence of viruses in fields which were detected in the initial screening tests. At the end of the season a sub-set of the fields were sampled to assess the health of crops.

As a result of this project the viruses of concern in UK pea crops are now known to be:

Pea enation mosaic virus-1 (genus: *Enamovirus*, PEMV-1) was known to be in UK peas prior to this work. PEMV-1 has a mutualistic relationship with pea enation mosaic virus-2 (genus: *Umbravirus*, PEMV-2), and had previously been thought to only occur together. These viruses are only known to infect leguminous species and cause mottling, stunting and enation symptoms. As a virus complex, PEMV-1 and PEMV-2 can be persistently transmitted by aphids, persistent transmission is characterised by long acquisition times which means chemical control is possible. Pea varieties are available which are resistant to PEMV-1.

Pea seed-borne mosaic virus (genus: *Potyvirus*, PSbMV) was also known to be present in the UK. This virus is restricted to leguminous hosts and causes mosaic and distortion on the plant and necrotic rings on pea seed. This virus is non-persistently transmitted by aphids, characterised by short acquisition times so chemical control is more difficult. As this virus is seed-borne the main pathway for control is the use of clean seed.

Turnip yellows virus (genus: *Polerovirus*, TuYV) is known to be present in the UK and causes high yield loss in oil seed rape. This was the most prevalent virus found within this study. It has also been reported on peas in Germany and Australia but this project is the first report of it in UK peas. TuYV has a wide host range, including brassicas and legumes. Stunting and yellowing have been previously associated with TuYV infection, however a specific yield loss study on TuYV by Nancarrow *et al*., (2022) did not observe symptoms associated with TuYV infection. Despite lack of symptoms, up to 45% yield loss were reported in this study. TuYV is persistently transmitted by aphids, it is not known to be transmitted by seeds or mechanically.

Pea necrotic yellow dwarf virus (genus: *Nanovirus*, PNYDV) has been reported in Austria, Denmark, Germany and the Netherlands and this project is the first report of it within the UK. PNYDV is only known to infect leguminous species and can cause stunting and yellowing, it

is persistently transmitted by aphids. It is not known to be transmitted by seeds or mechanically. This virus has potential to cause high yield loss, especially when the virus infects young plants.

Soybean dwarf virus (genus: *Luteovirus,* SbDV) was not known to be present in the UK prior to this project. This virus is restricted to leguminous species and can cause mild yellowing in peas. It is persistently transmitted by aphids. In each year of the project SbDV was found at a couple of sites at low incidence.

Bean yellow mosaic virus (genus: *Potyvirus*, BYMV) was known to be in the UK prior to this project. It is restricted to legumes and is non-persistently transmitted by aphids. BYMV can cause a mild mottle and vein chlorosis symptoms. Within the third year of the project it was found at high incidence later in the season, but only found once between years one and two.

Bean leafroll virus (genus: *Luteovirus*, BLRV) was also known to be in the UK prior to this project. It is restricted to legumes, is persistently transmitted by aphids and can cause yellowing and stunting symptoms.

Additionally, two viruses belonging to the genus *Cytorhabdovirus* were identified for the first time in the UK and in peas. Cabbage cytorhabdovirus-1 (CCyV1) and trifolium virus A (TpVA) have previously been identified in a cabbage from Greece and clover in the Czech Republic, respectively. At this time no symptoms are reported to be associated with either virus. There are also no vectors reported for these viruses, however species of this genus are known to be vectored by aphids, planthoppers, leafhoppers and whiteflies. They are also known to be mechanically transmitted.

Summary

20 pea crops per year were identified for sampling 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. This technique analyses all the nucleic acid present in a sample and should, in theory, detect the presence of any virus present in the sample. 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 prevalence of viruses from 20 pea fields in 2019. Estimates are calculated mean proportion of infected plants based on the number of bulk samples positive for virus, nt = Not Tested

Site	Variety	HTS result	TuYV Estimate (%)	PEMV-1 Estimate (%)	PEMV-2 Estimate (%)	SbDV 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 PEMVSatRNA	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 PEMVSatRNA	27.77	37.15	40.8	nt

13 of the 20 crops tested were positive for virus infections ranging in prevalence from 0.85% to 93.33% estimated infection. Pea enation mosaic virus has been historically reported in the UK, however it is not a single virus. 'Pea enation mosaic virus' is a complex of two different species of viruses, pea enation mosaic virus-1 (PEMV-1), genus *Enamovirus* and pea enation mosaic virus-2 (PEMV-2), genus *Umbravirus*. Pea enation mosaic virus satellite RNA may also be present but is not required for infection. PEMV-1 was present in 5 crops, ranging from 0.85% and 40.08%. PEMV-2 was identified in 9 crops, ranging from 2.57% and 86.67%. More commonly detected, and present at higher prevalence, was turnip yellows virus. This virus ranged in prevalence from 1.71% to 93.33% virus and was present in 13 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 was 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 prevalence of viruses from 20 pea fields in 2021. Estimates are calculated mean proportion of infected plants based on the number of bulk samples positive for virus, nt = Not Tested

Site	Variety	HTS Result	TuYV Estimate (%)	PEMV-1 Estimate (%)	PEMV-2 Estimate (%)	PSbMV Estimate (%)	SbDV Estimate (%)	PNYDV 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 prevalence ranged from 0.85%-66.87% of plants infected. 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 prevalence: 0.85% and 3.72% of plants, 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 prevalences between 0.85% and 60.62% of plants; it was the most common virus found. Pea

enation mosaic virus-1 was identified at six sites, with prevalences between 3.42% and 54.81% of plants. Pea enation mosaic virus-2 was identified at eleven sites with prevalences between 0.85% and 66.87% of plants. In this year's study, pea seedborne mosaic virus was identified in the site samples, it was identified at four sites with a prevalence of 4.53% of plants. Finally, pea necrotic yellow dwarf virus was identified at 1 site with a prevalence of 2.64% of plants, this represents the first finding of this virus in the UK.

Table 3 Results of both HTS screening and real-time RT-PCR testing showing presence and prevalence of viruses from 20 pea fields in 2022. Estimates are a calculated mean proportion of infected plants based on the number of bulk samples positive for virus, nt = not tested

Site	Variety		TuYV Estimate (%)	PEMV-1 Estimate (%)	PEMV-2 Estimate (%)	PSbMV Estimate (%)	SbDV Estimate (%)	BLRV Estimate (%)	BYMV Estimate (%)	CCyV-1 Estimate (%)	TpVA Estimate (%)
1	Boston	TuYV	4.66	nt	nt	nt	nt	nt	nt	nt	nt
2	Kabuki	TuYV	33.88	nt	nt	nt	nt	nt	nt	nt	nt
3	Bingo	TuYV, PEMV-1, PEMV-2, PSbMV, BYMV, BLRV, PEMV SatRNA (virus to investigate)	66.87	2.64	6.76	20.41	nt	0.85	4.81	28.99	nt
4	TBC	TuYV, PEMV-1, PEMV-2, PSbMV, PEMV SatRNA (virus to investigate)	38	31.6	54.81	44.91	nt	nt	nt	nt	0.85
5	Selune	TuYV, PEMV-1, PEMV-2, SbDV, BLRV, PEMV SatRNA	14.89	5.78	29.48		18.64	13.67	nt	nt	nt
6	Kactus	TuYV, PEMV-1, PEMV-2 PSbMV, PEMVSatRNA	44.91	0	9.35	59.56	nt	nt	nt	nt	nt
7	Ida	TuYV, PEMV-1, PEMV-2, SbDV (virus to investigate)	86.67	4.53	18.25	nt	0.85	nt	nt	3.72	nt
8	Celebration	TuYV, PEMV-1, PEMV-2	36.16	1.76	2.71	nt	nt	nt	nt	nt	nt
9	TBC	TuYV, PEMV-1, PEMV-2, BYMV, PEMVSatRNA	47.74	86.67	93.33	0	0		1.76		nt
10	2	TuYV	1.76	nt	nt	nt	nt	nt	nt	nt	nt
11	Amalfi	TuYV, PEMV-1, PEMV-2, PEMVSatRNA (virus to investigate)	54.81	2.71	2.71	nt	nt	nt	nt	nt	nt
12	TBC	TuYV, PEMV-2, PEMVSatRNA (virus to investigate)	80.01		3.62	nt	nt	nt	nt	nt	nt
13	TBC	TuYV, PEMV-1, PEMV-2, PSbMV, PEMV SatRNA	86.67	8.28	23.69	3.72	nt	nt	nt	nt	nt

14	Wagtail	TuYV, PEMV-1, PEMV-2, PEMVSatRNA (virus to investigate)	10.83	22.03	49.57	nt	nt	nt	nt	2.71	nt
15	Sherton? (Storm seeds)	TuYV, PEMV-2, PEMV SatRNA	86.67	nt	25.58	nt	nt	nt	nt	nt	nt
16	Bingo	TuYV, PEMV-2, PSbMV, BYMV, PEMV SatRNA, (virus to investigate)	10.42	nt	25.49	13.02	nt	nt	80.01	1.76	nt
17	TBC	TuYV, PEMV-1, PEMV-2, PEMVSatRNA (virus to investigate)	15	15.59	49.57	0.85	nt	nt	nt	2.71	nt
18	Bingo or Reflection	TuYV, PEMV-1, PEMV-2, PSbMV, BYMV, PEMV SatRNA (virus to investigate)	2.71	4.53	17.38	35.18	nt	nt	100	4.66	nt
19	Darlin	TuYV	2.57	nt	nt	nt	nt	nt	nt	nt	nt
20	TBC	TuYV	23.5	nt	nt	nt	nt	nt	nt	nt	nt

20 of the 20 crops tested were positive for virus in year three (**Table 3**). The virus prevalence ranged from an estimated 0.85% to 100% of plants infected. Turnip yellows virus was first detected in UK peas within the first year of this project. Within the final year of the project it was detected at all 20 sites with prevalences between 1.76% to 86.67% of plants infected. As in previous years, it was the most common virus found. Pea enation mosaic virus-1 was identified at eleven sites with prevalence between 1.76% to 86.67% of plants infected. Pea enation mosaic virus-2 was identified at fifteen sites with prevalence between 2.71% and 54.81% of plants infected. Soybean dwarf virus, which was first identified within the first year of the project, was identified at 2 sites with prevalences at 0.85% and 18.64% of plants infected. Pea seed-borne mosaic virus was identified at seven sites with prevalences between 0.85% and 59.56% of plants infected. Bean yellow mosaic virus was identified in the site samples. This virus was found within symptomatic samples in the first year but was not seen in site samples in either the first or second year. It was found at four sites with prevalences between 1.76 and 100% of plants infected. Bean leafroll virus is known to be present in the UK but had not been identified in this project in the first and second year. In the final year of the project it was found at prevalences of 0.85% and 13.67% of plants infected. Within this year, a further two viruses were identified for the first time in the UK; these were cabbage cytorhabdovirus-1 and trifolium pratense virus A. Cabbage cytorhabdovirus-1 was identified at six sites with prevalences between 1.76% and 28.99% of plants infected. While trifolium pratense virus A was identified at one site at a prevalence of 0.85%.

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-1 and PEMV-2. 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. Additionally turnip yellows virus associated RNA was identified in two of the samples. Given these samples were taken on the basis of expressed 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 both years 2 and 3 no viruses were found in the symptomatic samples that were not found in the site samples.

Further work was also conducted to investigate the impact of virus infection in crops, specifically on the mean mass of peas per plant, and its interaction with treatments against aphids. Aphids are a vector of many of the viruses that cause issues in pea crops. Marked areas were left untreated against aphids in each sampled crop. A sub-set of the sites were identified for further study, using the HTS screening work to give a range of virus prevalences. Plants were sampled from untreated and treated areas at the end of the growing season to

provide estimates of yield. From these data a statistical analysis (linear mixed modelling) was performed to estimate the impact of virus infection on yield (**Figure 1)**.

Estimates of effect size of virus infection and treatment are shown in **Table 4**. Estimates based on all three years results are qualitatively consistent with estimates made in the previous two years with respect to the significance of effects. TuYV was found to significantly reduce productivity (p=0.003) in fields, and this effect appears to be ameliorated by treatment (p<0.001). PEMV-1 was also found to significantly reduce productivity (p=0.007) however treatment did not appear to ameliorate this effect (p=0.153).

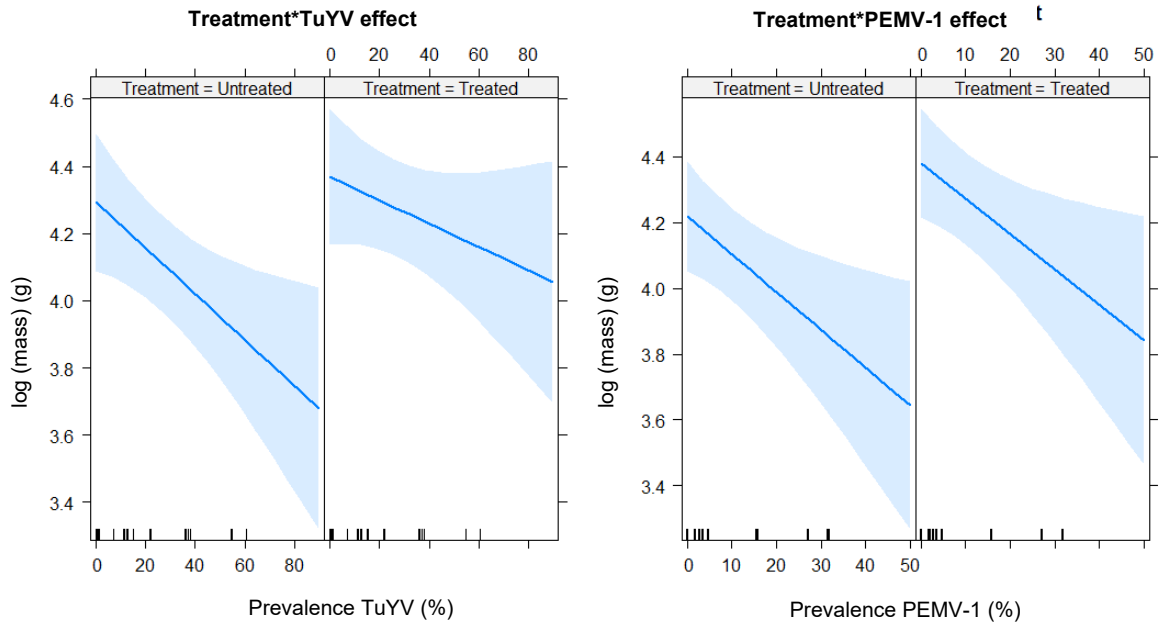

Figure 1 Estimated effects of virus prevalence of TuYV (labelled TUYV) and PEMV-1 (labelled PEMV) and treatment on productivity for 2019, 2021 and 2022.

Table 4 Estimates of effect sizes and significance of treatment and TuYV and PEMV-1 for 2019, 2021 and 2022.

Value	Estimate	95% C.I.		Significance
10-plant pea mass in clean untreated fields	84g	67g	103g	
Effect of treatment[a]	106%	99%	114%	0.950[b]
Effect of TuYV[a]	53%	30%	84%	0.003[c]
Effect of TuYV and treatment[a]	79%	45%	125%	<0.001[d]
Effect of PEMV-1[a]	57%	33%	88%	0.007[e]
Effect of PEMV-1 and treatment[a]	61%	35%	96%	0.153[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-1 doesn't reduce the 10-plant pea mass

[f] Null hypothesis: treatment doesn't increase the 10-plant pea mass when PEMV-1 is present

Financial Benefits

A model was used to predict the yield loss associated with the presence of virus. Through this model, it is predicted that yield loss is reduced when chemical control is applied.

Action Points

A similar suite of viruses have been identified over all three years of the project, this is a mix of viruses previously known to be present in the UK (PEMV-1, PEMV-2 and PSbMV) and viruses which were identified within this project (TuYV and SbDV). The estimated yield impact data indicate that virus control measures, even though not specifically targeted at TuYV do have some effect at ameliorating the impact of this virus.

- Virus appears to be widespread and impacting on yield of crops, therefore action should be taken to monitor and control and entry and spread of virus in crops.
- Data from this study indicates that although symptomless TuYV impacts on yield however current treatments appear to ameliorate some of this impact. Therefore, growers should continue with current treatment regimes against vector insects for virus management.
- Indications from FV460 looking at control of viruses in carrot crops indicate early season control may have greater impact on maintaining yield than season long control. The applicability of these results for pea crops should be investigated.
- The current resistance status of pea varieties in the UK is not known and work is recommended to screen varieties for TuYV to inform future breeding programmes.
- The epidemiology of TuYV in pea crops is not currently known and work is recommended to understand the infection dynamics of this virus at landscape scale or across rotations.
- The surveillance method developed here has yet to be applied to other crops. Future work could investigate viruses within other legume crops or leguminous cover crops.

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 not known.

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 was unknown prior to this extensive survey.

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, until now, been applied in a plant health surveillance scenario. The aim of this project was 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 was 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 from all three years are shown in **Table 5, Table 6 and Table 7**. 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 5 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 6 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

Table 7 Sites of pea crops sampled during Summer 2022

Site no.	Site	Location	Variety
1	Leasingham	Leasingham	Boston
2	Great Dunnow	Berners Roding	Kabuki
3	Alcester	Dunnington	Bingo
4	Broughton	Stockbridge	TBC
5	Sutton Norfolk	Sutton	Selune
6	Lenham Kent	Lenham	Kactus
7	Fockerby	Goole	Ida
8	Kilham	North Yorkshire	Celebration
9	Long sutton	Long sutton	TBC
10	Kirriemuir	Kirriemuir	2
11	Spilsby	Spilsby	Amalfi
12	Aylsham	Norfolk	TBC
13	Thorney	Thorney	TBC
14	North Cave	North Cave	Wagtail
15	South Willingham	South Willingham	Sherton? (Storm seeds)
16	Bedingfield	Bedingfield	Bingo
17	Barmstone	Lisset	TBC
18	Snowshill	Moreton-in-Marsh	Bingo or Reflection
19	Drummy	Crief	Darlin
20	Borders Growers	Chirnside	TBC

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 were 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 were 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 in 2019 and 2021 and 4 in 2022. Yield was measured for all 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 seed-borne mosaic virus (PSbMV), genus *Potyvirus*
- Bean yellow mosaic virus (BYMV), genus *Potyvirus*

And the satellite RNA identified was:

- Pea enation mosaic virus satellite RNA (PEMVSatRNA)

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 turnip yellows virus associated RNA (TuYVaRNA) were also detected.

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

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

In year 3, testing of both the bulk filed samples (BFS) and the symptomatic samples by HTS indicated the presence of viruses and a satellite RNA. Six were previously found in years one and two, 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*

-Bean yellow mosaic virus (BYMV), genus *Potyvirus*

One virus had not previously been identified in this study, but is known to be present in the UK was:

-Bean leafroll virus (BLRV), genus *Luteovirus*

Two viruses found this year that had not been previously reported in the UK were:

-Cabbage cytorhabdovirus-1 (CCyV-1), genus *Cytorhabdovirus*

-Trifolium pratense virus A (TpVA), genus *Cytorhabdovirus*

No additional viruses were identified in the symptomatic samples.

Presence and Incidence in Pea crops in 2019

The viruses inferred in samples from the whole crop bulk HTS analysis are listed in **Table 8** , 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 14 crops out of 20 had detectable levels of virus (70%). 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 13 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 8 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 Estimate (%)	PEMV-1 Estimate (%)	PEMV-2 Estimate (%)	SbDV 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 9** 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 9 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 Estimate (%)	PEMV-1 Estimate (%)	PEMV-2 Estimate (%)	PSbMV Estimate (%)	SbDV Estimate (%)	PNYDV 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

The viruses identified in the site samples within 2022 can be found in **Table 10**, alongside the relative incidence of the nine viruses identified. All twenty sites had detectable levels of virus (100%), and the virus incidence ranged from 0.85% up to 100 %.

Five sites contained a single virus (25%), with the remaining fifteen sites having multiple viruses (75%). TuYV was identified at all twenty sites with a mean incidence of 37.44% (1.76% - 86.67%). PEMV-1 was identified at eleven sites with an average incidence of 15.51% (2.64% - 86.67%), with a further identification at site 6 which couldn't be confirmed by real-time RT-PCR. PEMV-2 was identified at fifteen sites with a mean incidence of 27.49% (2.71% - 93.33%). PSbMV was identified at seven sites with an average incidence of 25.38% (0.85% - 59.56%). Additionally, SbDV was identified at two sites with incidences of 0.85% and 18.64%. BLRV was identified at two sites with an average incidence of 7.26% (0.85-13.67). BYMV was identified at four sites with an average incidence 46.65% (1.76%-100%). CCyV-1 was identified at eight sites with a mean incidence of 5.9% (0.85%-28.99%). TpVA was identified at one site with an incidence of 0.85%.

Table 10 Sites from 2022, viruses inferred from whole crop bulk HTS data and mean estimated virus content from the accompanying mixed bulk testing (%). nt=not tested

Site	Vairety	HTS Result	TuYV Estimate (%)	PEMV-1 Estimate (%)	PEMV-2 Estimate (%)	PSbMV Estimate (%)	SbDV Estimate (%)	BLRV Estimate (%)	BYMV Estimate (%)	CCyV-1 Estimate (%)	TpVA Estimate (%)
1	Boston	TuYV	4.66	nt	nt	nt	nt	nt	nt	nt	nt
2	Kabuki	TuYV	33.88	nt	nt	nt	nt	nt	nt	nt	nt
3	Bingo	TuYV, PEMV-1, PEMV-2, PSbMV, BYMV, BLRV, PEMV SatRNA (virus to investigate)	66.87	2.64	6.76	20.41	nt	0.85	4.81	28.99	nt
4	TBC	TuYV, PEMV-1, PEMV-2, PSbMV, PEMV SatRNA (virus to investigate)	38	31.6	54.81	44.91	nt	nt	nt	nt	0.85
5	Selune	TuYV, PEMV-1, PEMV-2, SbDV, BLRV, PEMV SatRNA	14.89	5.78	29.48	nt	18.64	13.67	nt	nt	nt
6	Kactus	TuYV, PEMV-1, PEMV-2 PSbMV, PEMVSatRNA	44.91	0	9.35	59.56	nt	nt	nt	nt	nt
7	Ida	TuYV, PEMV-1, PEMV-2, SbDV (virus to investigate)	86.67	4.53	18.25	nt	0.85	nt	nt	3.72	nt
8	Celebration	TuYV, PEMV-1, PEMV-2	36.16	1.76	2.71	nt	nt	nt	nt	nt	nt
9	TBC	TuYV, PEMV-1, PEMV-2, BYMV, PEMVSatRNA	47.74	86.67	93.33	0	0	nt	1.76	nt	nt
10	2	TuYV	1.76	nt	nt	nt	nt	nt	nt	nt	nt
11	Amalfi	TuYV, PEMV-1, PEMV-2, PEMVSatRNA (virus to investigate)	54.81	2.71	2.71	nt	nt	nt	nt	0.85	nt
12	TBC	TuYV, PEMV-2, PEMVSatRNA (virus to investigate)	80.01	nt	3.62	nt	nt	nt	nt	1.76	nt
13	TBC	TuYV, PEMV-1, PEMV-2, PSbMV, PEMV SatRNA	86.67	8.28	23.69	3.72	nt	nt	nt	nt	nt
14	Wagtail	TuYV, PEMV-1, PEMV-2, PEMVSatRNA (virus to investigate)	10.83	22.03	49.57	nt	nt	nt	nt	2.71	nt
15	Sherton? (Storm seeds)	TuYV, PEMV-2, PEMV SatRNA	86.67	nt	25.58	nt	nt	nt	nt	nt	nt

16	Bingo	TuYV, PEMV-2, PSbMV, BYMV, PEMV SatRNA, (virus to investigate)	10.42	nt	25.49	13.02	nt	nt	80.01	1.76	nt
17	TBC	TuYV, PEMV-1, PEMV-2, PEMVSatRNA (virus to investigate)	15	15.59	49.57	0.85	nt	nt	nt	2.71	nt
18	Bingo or Reflection	TuYV, PEMV-1, PEMV-2, PSbMV, BYMV, PEMV SatRNA (virus to investigate)	2.71	4.53	17.38	35.18	nt	nt	100	4.66	nt
19	Darlin	TuYV	2.57	nt	nt	nt	nt	nt	nt	nt	nt
20	TBC	TuYV	23.5	nt	nt	nt	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 11**. 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 11 Virus present in single symptomatic samples inferred from HTS data in 2019. Pos= positive, Neg= negative, nt=not tested.

Location	Variety	HTS Result (bulked)	TuYV	PEMV-1	PEMV-2	PSbMV
Market Rasen	TBC	TuYVaRNA	Pos	Pos	Pos	nt
Ramsey Mereside	TBC	TuYV, PEMV-1, PEMV-2	Pos	Pos	Pos	nt
Ramsey Mereside	TBC	TuYV, PEMV-2	Pos	Pos	Pos	nt
Stonea March	TBC	TuYV PEMV-1, PEMV-2, PSbMV TuYVaRNA	Pos	Pos	Pos	Pos
Ramsey Mereside	TBC	TuYV, PEMV-1, PEMV-2, PSbMV	Pos	Pos	Pos	Pos
Ramsey Mereside	TBC	TuYV, PEMV-2	Pos	Pos	Pos	nt
Deeping st.	TBC	TuYV, PEMV-1, PEMV-2 PEMV SatRNA, BYMV	Pos	Pos	Pos	nt
Cambridge	TBC	TuYV, PEMV-1 PEMV-2, PSbMV	Pos	Pos	Pos	Pos
Cambridge	TBC	PEMV-1, PEMV-2, PEMV SatRNA	Neg	Pos	Pos	nt
Market Weighton	Ashton	TuYV, PEMV-1, PEMV-2	Pos	Pos	Pos	nt
Market Weighton	Ashton		Pos	Neg	Neg	nt
Market Weighton	Ashton		Pos	Pos	Pos	nt
Market Weighton	Ashton		Pos	Neg	Pos	nt
Market Weighton	Ashton		Pos	Neg	Pos	nt
Market Weighton	Ashton		Pos	Neg	Neg	nt
Market Weighton	Ashton		Pos	Pos	Neg	nt
Market Weighton	Ashton		Pos	Neg	Neg	nt
Wainfleet All Saints	Oasis	TuYV, PEMV-1 PEMV-2, PEMV SatRNA	Pos	Neg	Pos	nt
Wainfleet All Saints	Oasis		Pos	Pos	Pos	nt
Wainfleet All Saints	Oasis		Pos	Pos	Pos	nt
Wainfleet All Saints	Oasis		Pos	Pos	Pos	nt
Wainfleet All Saints	Oasis		Pos	Pos	Pos	nt

Wainfleet All Saints	Oasis		Pos	Pos	Pos	nt
Bedingfield	Kimberley	TuYV, PEMV-2	Pos	Neg	Neg	nt
Bedingfield	Kimberley		Pos	Neg	Neg	nt
Bedingfield	Kimberley		Pos	Neg	Pos	nt
Langton	Oasis	TuYV	Pos	Neg	Neg	nt
Langton	Oasis		Pos	Neg	Pos	nt

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 12**). 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 nor SbDV were found in the symptomatic samples.

Table 12 Virus present in single symptomatic samples inferred from HTS data in 2021. Pos= positive, Neg= negative, nt=not tested.

Site	HTS Result (bulked)	TuYV	PEMV-1	PEMV-2	PSbMV	SbDV	PNYDV
Site 1	Negative	nt	nt	nt	nt	nt	nt
Site 2	Negative	nt	nt	nt	nt	nt	nt
Site 2		nt	nt	nt	nt	nt	nt
Site 3	PSbMV	nt	nt	nt	Neg	nt	nt
Site 3		nt	nt	nt	Pos	nt	nt
Site 3		nt	nt	nt	Neg	nt	nt
Site 4	Negative	Neg	nt	Neg	Neg	nt	nt
Site 4		Neg	nt	Neg	Neg	nt	nt
Site 5	Negative	nt	Neg	Neg	nt	nt	nt
Site 5		nt	Neg	Neg	nt	nt	nt
Site 5		nt	Neg	Neg	nt	nt	nt
Site 5		nt	Neg	Neg	nt	nt	nt

Site 6	Negative	Neg	Pos	Neg	Neg	nt	Neg
Site 6		Neg	Pos	Pos	Neg	nt	Neg
Site 6		Neg	Neg	Neg	Neg	nt	Neg
Site 6		Neg	Pos	Pos	Neg	nt	Neg
Site 7	Negative	nt	nt	nt	nt	nt	nt
Site 7		nt	nt	nt	nt	nt	nt
Site 7		nt	nt	nt	nt	nt	nt
Site 8	Negative	Neg	nt	nt	nt	nt	nt
Site 8		Neg	nt	nt	nt	nt	nt
Site 8		Neg	nt	nt	nt	nt	nt
Site 9	Negative	Neg	nt	Pos	nt	nt	nt
Site 9		Neg	nt	Pos	nt	nt	nt
Site 9		Neg	nt	Pos	nt	nt	nt
Site 1nt	TuYV PEMV2	Pos	nt	Pos	nt	nt	nt
Site 1nt		Neg	nt	Neg	nt	nt	nt
Site 1nt		Neg	nt	Neg	nt	nt	nt
Site 11	Negative	nt	nt	nt	nt	nt	nt
Site 11		nt	nt	nt	nt	nt	nt
Site 11		nt	nt	nt	nt	nt	nt
Site 12	TuYV	Pos	nt	Neg	nt	nt	nt
Site 12		Neg	nt	Neg	nt	nt	nt
Site 12		Pos	nt	Neg	nt	nt	nt
Site 13	Negative	Neg	Neg	Neg	nt	nt	nt
Site 13		Neg	Neg	Neg	nt	nt	nt
Site 13		Neg	Neg	Neg	nt	nt	nt
Site 14	TuYV	Pos	nt	nt	nt	nt	nt

Site 14		Neg	nt	nt	nt	nt	nt
Site 14		Neg	nt	nt	nt	nt	nt
Site 15	PEMV1 PEMV2	Neg	Pos	Pos	nt	Neg	nt
Site 15		Neg	Pos	Pos	nt	Neg	nt
Site 15		Neg	Neg	Neg	nt	Neg	nt
Site 16	Negative	Neg	Neg	Neg	nt	nt	nt
Site 16		Neg	Neg	Neg	nt	nt	nt
Site 16		Neg	Neg	Neg	nt	nt	nt
Site 17	TuYV	Neg	Neg	Neg	Neg	Neg	nt
Site 17		Neg	Neg	Neg	Neg	Neg	nt
Site 17		Neg	Neg	Neg	Neg	Neg	nt
Site 18	TuYV	Pos	Neg	Neg	nt	nt	nt
Site 18		Pos	Neg	Neg	nt	nt	nt
Site 18		Neg	Neg	Neg	nt	nt	nt
Site 19	Negative	Neg	nt	Neg	nt	nt	nt
Site 19		Neg	nt	Neg	nt	nt	nt
Site 19		Neg	nt	Neg	nt	nt	nt
Site 2nt	Negative	Neg	nt	nt	nt	nt	nt
Langton	PEMV1, PEMV2, PSbMV, PEMV satRNA	Neg	Pos	Pos	Pos	nt	nt
Langton		Neg	Pos	Pos	Pos	nt	nt
Langton		Neg	Pos	Pos	Pos	nt	nt
Langton		Neg	Pos	Pos	Pos	nt	nt
Langton		Neg	Pos	Pos	Pos	nt	nt

Presence of virus in single symptomatic samples in 2022

Symptomatic samples were collected alongside the BFS at each site, at each site between one and five symptomatic samples were taken (**Table 13**). For each site, the symptomatic

samples were bulked together, and this was tested by HTS. Confirmation was done using real-time RT-PCR. TuYV was the most common virus in the symptomatic samples, followed by PEMV-2, PSbMV and PEMV-1. BYMV and CCyV-1 were found in a couple of samples. While being identified by HTS, BLRV and SbDV was not confirmed in the symptomatic samples by TaqMan.

Table 13 Virus present in single symptomatic samples inferred from HTS data in 2022. Pos= positive, Neg= negative, nt=not tested.

Site	HTS Result (bulked)	TuYV	PEMV-1	PEMV-2	PSbMV	SbDV	BLRV	BYMV	CCyV1	TpVA
Site 1	Negative	Neg	nt	nt	nt	nt	nt	nt	nt	nt
Site 1		Neg	nt	nt	nt	nt	nt	nt	nt	nt
Site 1		Neg	nt	nt	nt	nt	nt	nt	nt	nt
Site 2	Negative	Pos	nt	nt	nt	nt	nt	nt	nt	nt
Site 2		Neg	nt	nt	nt	nt	nt	nt	nt	nt
Site 2		Pos	nt	nt	nt	nt	nt	nt	nt	nt
Site 3	TuYV, PEMV-1, PEMV-2, PSbMV, PEMV SatRNA	Pos	Pos	Pos	Pos	nt	Neg	Neg	Neg	nt
Site 3		Pos	Neg	Pos	Neg	nt	Neg	Neg	Neg	nt
Site 3		Neg	Pos	Pos	Neg	nt	Neg	Neg	Neg	nt
Site 4	PEMV-1, PEMV-2, PSbMV, Insect virus	Pos	Neg	Pos	Neg	nt	nt	nt	nt	Neg
Site 4		Neg	Pos	Pos	Pos	nt	nt	nt	nt	Neg
Site 4		Neg	Pos	Pos	Pos	nt	nt	nt	nt	Neg
Site 5	PEMV-2, BLRV	Neg	Neg	Pos	nt	Neg	Neg	nt	nt	nt
Site 5		Pos	Neg	Pos	nt	Neg	Neg	nt	nt	nt
Site 5		Neg	Neg	Neg	nt	Neg	Neg	nt	nt	nt
Site 6	TuYV	Neg	Neg	Neg	Neg	nt	nt	nt	nt	nt
Site 6		Neg	Neg	Neg	Neg	nt	nt	nt	nt	nt
Site 6		Neg	Neg	Neg	Pos	nt	nt	nt	nt	nt
Site 7	TuYV	Pos	Neg	Neg	nt	Neg	nt	nt	Neg	nt
Site 7		Pos	Neg	Neg	nt	Neg	nt	nt	Neg	nt
Site 7		Pos	Neg	Neg	nt	Neg	nt	nt	Neg	nt
Site 8	PEMV-1, PEMV-2, PEMV SatRNA	Neg	Pos	Pos	nt	nt	nt	nt	nt	nt
Site 8		Pos	Neg	Neg	nt	nt	nt	nt	nt	nt
Site 8		Neg	Neg	Neg	nt	nt	nt	nt	nt	nt
Site 9	TuYV, PEMV-1, PEMV-2, PSbMV, SbDV, PEMV SatRNA	Pos	Pos	Pos	Pos	Neg	nt	Neg	nt	nt
Site 9		Pos	Pos	Pos	Pos	Neg	nt	Neg	nt	nt

Site 9		Pos	Pos	Pos	Neg	Neg	nt	Neg	nt	nt
Site 11	TuYV	Pos	Neg	Neg	nt	nt	nt	nt	Neg	nt
Site 11		Pos	Neg	Neg	nt	nt	nt	nt	Neg	nt
Site 11		Pos	Neg	Neg	nt	nt	nt	nt	Neg	nt
Site 12	TuYV, PEMV-2	Pos	nt	Neg	nt	nt	nt	nt	Neg	nt
Site 12		Pos	nt	Neg	nt	nt	nt	nt	Neg	nt
Site 12		Pos	nt	Pos	nt	nt	nt	nt	Pos	nt
Site 13	TuYV, PEMV-2	Pos	Neg	Pos	Pos	nt	nt	nt	nt	nt
Site 13		Pos	Neg	Pos	Neg	nt	nt	nt	nt	nt
Site 13		Pos	Neg	Neg	Neg	nt	nt	nt	nt	nt
Site 14	TuYV, PEMV-1, PEMV-2	Pos	Pos	Pos	nt	nt	nt	nt	Neg	nt
Site 14		Neg	Pos	Pos	nt	nt	nt	nt	Neg	nt
Site 14		Neg	Neg	Pos	nt	nt	nt	nt	Neg	nt
Site 15	TuYV, PEMV-2, WAV-1	Pos	nt	Neg	nt	nt	nt	nt	nt	nt
Site 15		Pos	nt	Neg	nt	nt	nt	nt	nt	nt
Site 15		Pos	nt	Pos	nt	nt	nt	nt	nt	nt
Site 16	PEMV-2, BYMV, PEMVSatRNA (virus to investigate)	Neg	nt	Neg	Neg	nt	nt	nt	Neg	nt
Site 16		Pos	nt	Pos	Neg	nt	nt	nt	Pos	nt
Site 16		Pos	nt	Neg	Neg	nt	nt	nt	Neg	nt
Site 17	TuYV, PEMV-2, PSbMV (pea mitovirus and aphis citricidus bunyavirus)	Neg	Neg	Neg	Pos	nt	nt	nt	Neg	nt
Site 17		Pos	Neg	Pos	Neg	nt	nt	nt	Neg	nt
Site 17		Neg	Neg	Pos	Neg	nt	nt	nt	Neg	nt
Site 18	PSbMV, BYMV	Neg	Neg	Neg	Pos	nt	nt	Pos	Neg	nt
Site 18		Neg	Neg	Neg	Pos	nt	nt	Pos	Neg	nt
Site 18		Pos	Neg	Pos	Pos	nt	nt	Neg	Neg	nt
Site 19	Negative	Pos	nt	nt	nt	nt	nt	nt	nt	nt
Site 19		Neg	nt	nt	nt	nt	nt	nt	nt	nt
Site 19		Neg	nt	nt	nt	nt	nt	nt	nt	nt

Site 20	TuYV	Neg	nt	nt	nt	nt	nt	nt	nt	nt
Site 20		Pos	nt	nt	nt	nt	nt	nt	nt	nt
Site 20		Neg	nt	nt	nt	nt	nt	nt	nt	nt

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 4** 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.

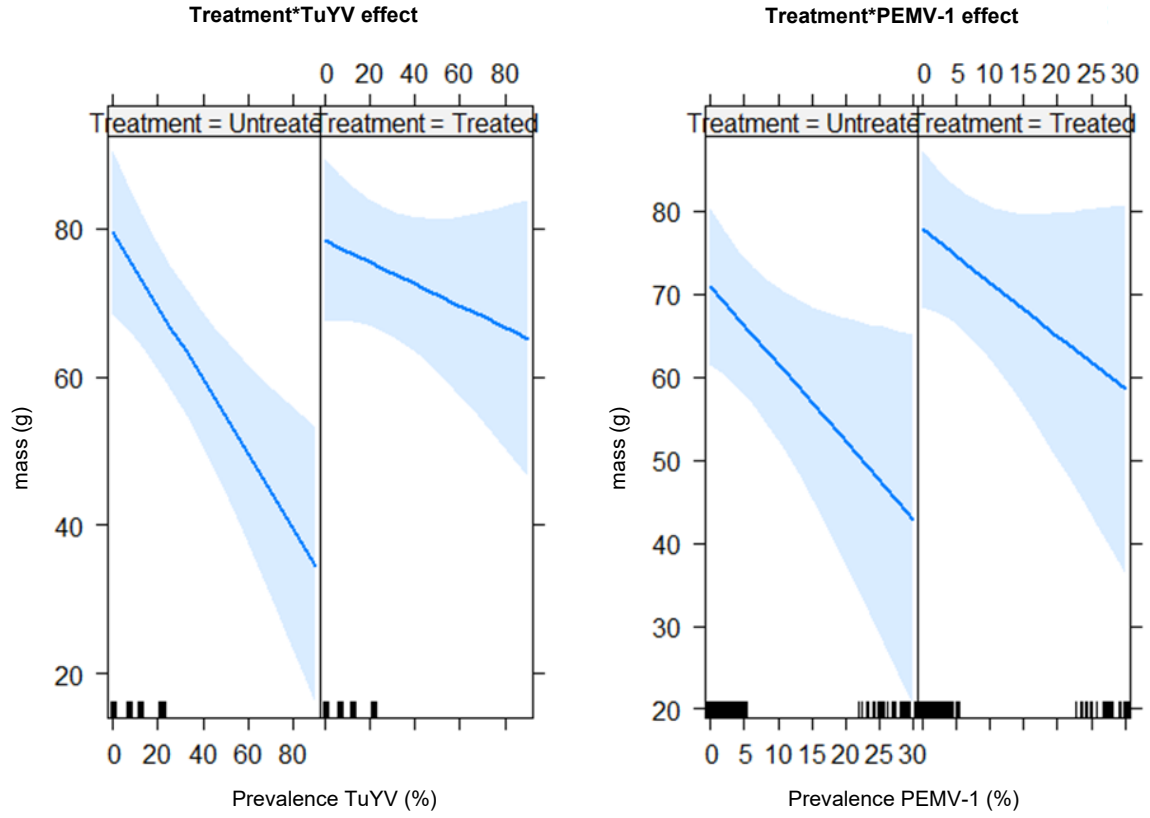

Figure 2 Estimated effects of virus prevalence of TuYV and PEMV-1 and treatment on productivity for 2019.

Estimates of effect size of virus infection and treatment are shown in **Table 14**. 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 14 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-1[a]	70%	47%	96%	0.011[e]
Effect of PEMV-1 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 increases the 10-plant pea mass

[c] Null hypothesis: TuYV presence does not reduce the 10-plant pea mass

[d] Null hypothesis: treatment does not increase the 10-plant pea mass compared to no treatment when TuYV is present

[e] Null hypothesis: PEMV-1 presence does not reduce the 10-plant pea mass

[f] Null hypothesis: treatment does not increase the 10-plant pea mass compared to no treatment when PEMV-1 is present

Impact of virus infection for 2019 and 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 which used observations gained in 2019 and 2021 can be found in Appendix 5. Estimated effects are shown in **Figure 5** 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.

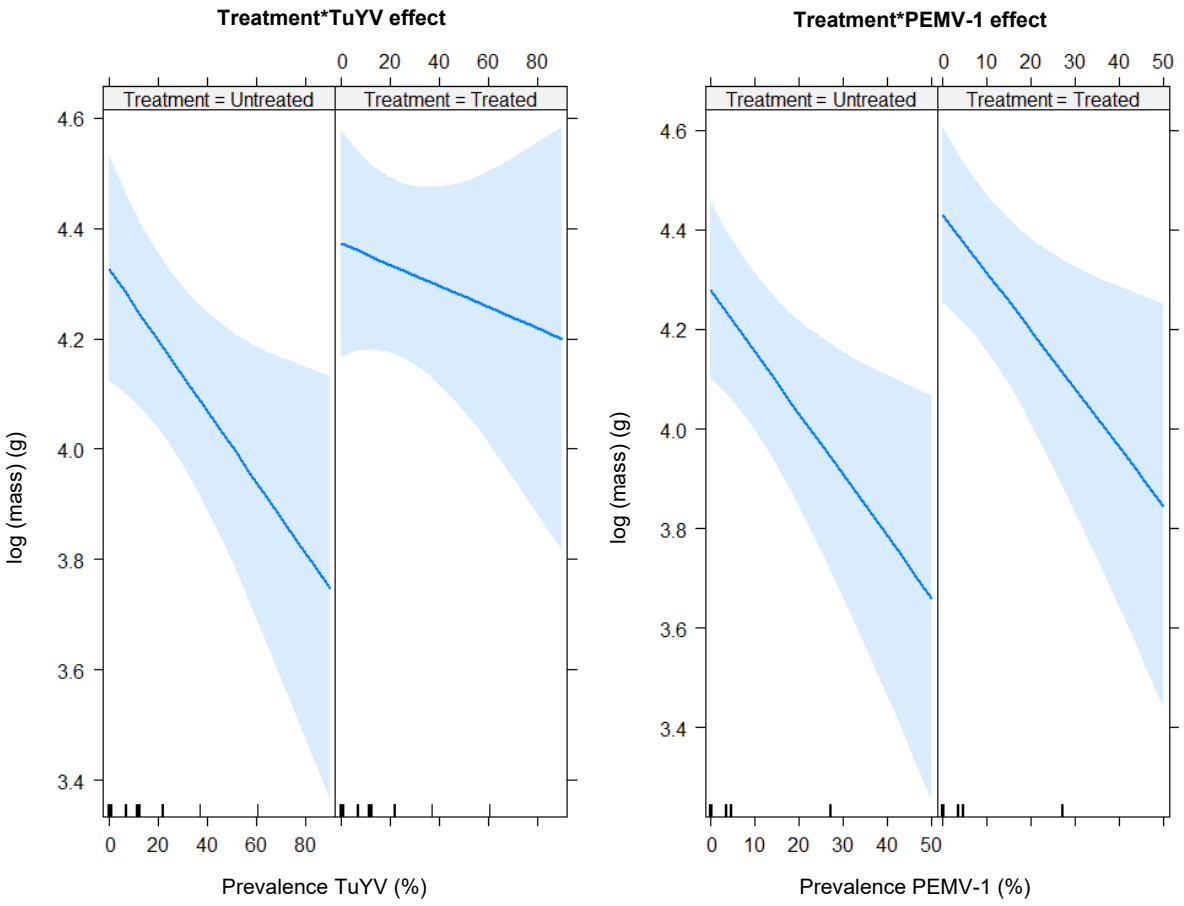

Figure 3 Estimated effects of virus prevalence of TuYV and PEMV-1 and treatment on productivity for 2019 and 2021.

Estimates of effect size of virus infection and treatment are shown in **Table 15**. 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 15 Estimates of effect sizes and significance of treatment and TuYV and PEMV-1 for 2019 and 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-1[a]	73%	56%	94%	0.006[e]
Effect of PEMV-1 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: 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-1 doesn't reduce the 10-plant pea mass

[f] Null hypothesis: treatment doesn't increase the 10-plant pea mass when PEMV-1 is present

Impact of virus infection for 2019, 2021 and 2022

Within year 3, four crops with a range of virus content were sampled to assess the impact of virus infection both with and without treatment. Due to early aphid flights and aphicide applications 2023, it was difficult to find crops that had not been treated therefore only peas from four sites were harvested. A report on the statistical analysis which used observations gained in 2019, 2021 and 2022 can be found in Appendix 6. Estimated effects are shown in **Figure 6** 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.

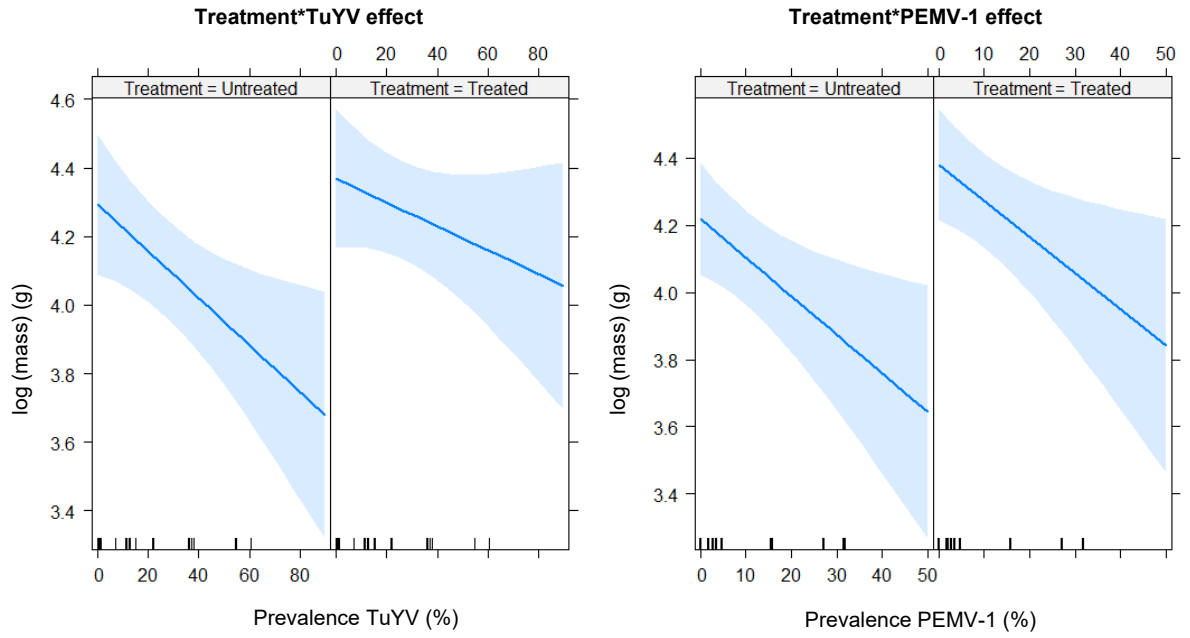

Figure 4 Estimated effects of virus prevalence of TuYV and PEMV-1 and treatment on productivity for 2019, 2021 and 2022.

Estimates of effect size of virus infection and treatment are shown in **Table 16**. Estimates based on all three years results are qualitatively consistent with estimates made in the previous two years with respect to the significance of effects. TuYV was found to significantly reduce productivity (p=0.003) in fields, and this effect appears to be ameliorated by treatment (p<0.001). PEMV-1 was also found to significantly reduce productivity (p=0.007) however treatment did not appear to ameliorate this effect (p=0.153).

Table 16 Estimates of effect sizes and significance of treatment and TuYV and PEMV-1 for 2019, 2021 and 2022.

Value	Estimate	95% C.I.		Significance
10-plant pea mass in clean untreated fields	84g	67g	103g	
Effect of treatment[a]	106%	99%	114%	0.950[b]
Effect of TuYV[a]	53%	30%	84%	0.003[c]
Effect of TuYV and treatment[a]	79%	45%	125%	<0.001[d]
Effect of PEMV-1[a]	57%	33%	88%	0.007[e]
Effect of PEMV-1 and treatment[a]	61%	35%	96%	0.153[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-1 doesn't reduce the 10-plant pea mass

[f] Null hypothesis: treatment doesn't increase the 10-plant pea mass when PEMV-1 is present

UAV disease monitoring in pea fields 2022

Additionally, to the AHDB-project presented here, the GIS team at Fera flew drones with Multispectral sensors over eight of the twenty sites. Image processing was done with Pix4D and then investigations into spectral information indicative of disease presence was undertaken in eCognition Developer.

The drones captured 5 bands: RGB bands, Near Infra-Red and Red Edge Bands. Various combinations of these bands were used to generate different indices to visualize crop health in the various fields. The indices with most potential for indicating stress were determined to be the Simple Ratio Index (ρNIR/ρRed). After compiling the indices, the area of interest (AOI) of the fields were used to limit the study area and then soil/bare ground was removed with a combination of NDSI, Chlorophyll Green and NDVI.

After removal, a filter using the SR ratio was used to determine whether areas of the crop field were facing high stress. In **Figure 7**, **Figure 8**, **Figure 9** and **Figure 10** examples from the outputs of the imaging are presented. The green areas indicate healthy crops and is displayed in red in the false colour NVDI, whereas brown patches coinciding with darker areas in the false colour NVDI indicate plants under stress. The imaging data from both site 13 (**Figure 7**) and site 16 (**Figure 8**) indicate that the plants are experiencing stress within these fields.

Sites 13 and 15 have similar viruses present and comparable incidences of TuYV and PEMV-2 (shown in **Table 17,** the full results for year three can be found in **Table 10**). However, where signs of stress can be identified in Site 13 there are no indications in Site 15. Site 16 also

shows symptoms of stress at the site, this site has a lower incidence of TuYV than the other sites included here but has a high incidence of BYMV.

Table 17 Average incidence of viruses for four sites from year 3

Site	TuYV Average	PEMV-1 Average	PEMV-2 Average	PSbMV Average	BYMV Average	CCyV-1 Average
11	54.81	2.71	2.71	nt	nt	0.85
13	86.67	8.28	23.69	3.72	nt	nt
15	86.67	nt	25.58	nt	nt	nt
16	10.42	nt	25.49	13.02	80.01	1.76

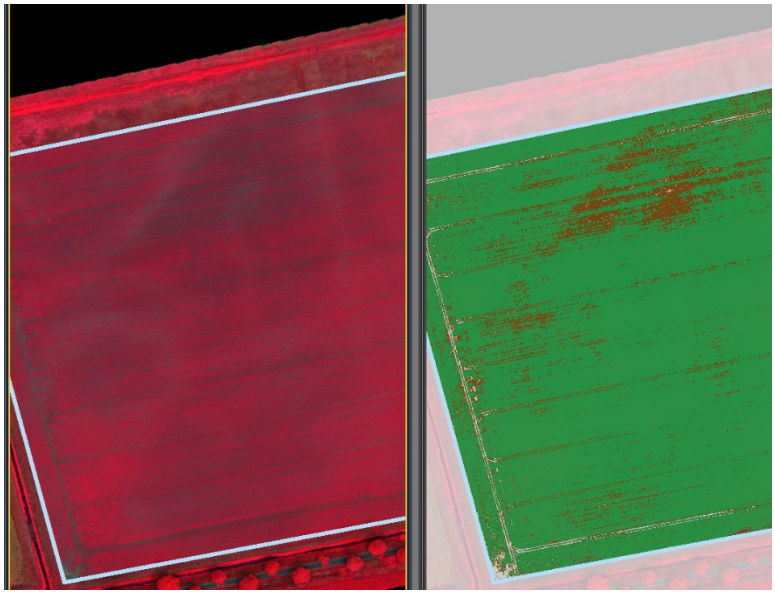

Figure 5 UAV imaging of site 13

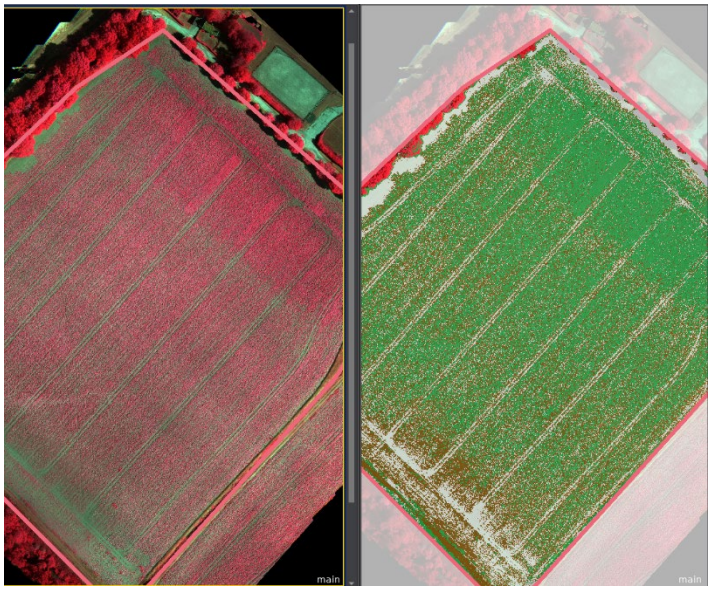

Figure 6 UAV imaging of site 16

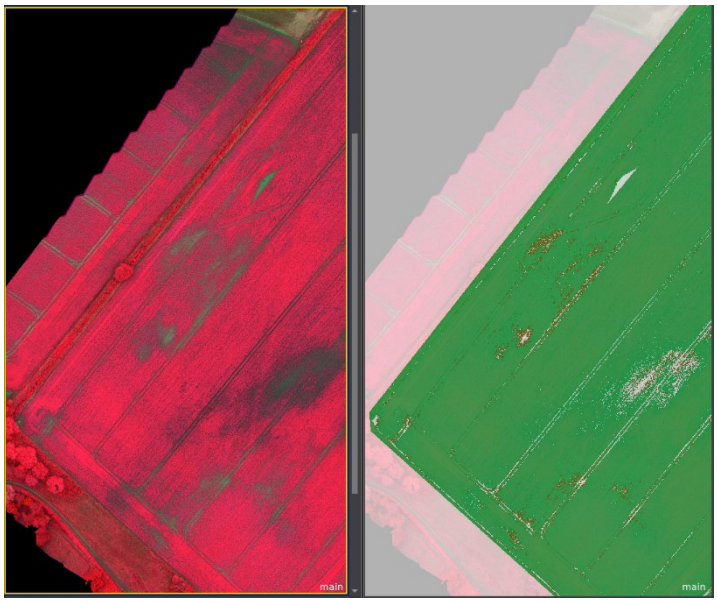

Figure 7 UAV imaging of site 11

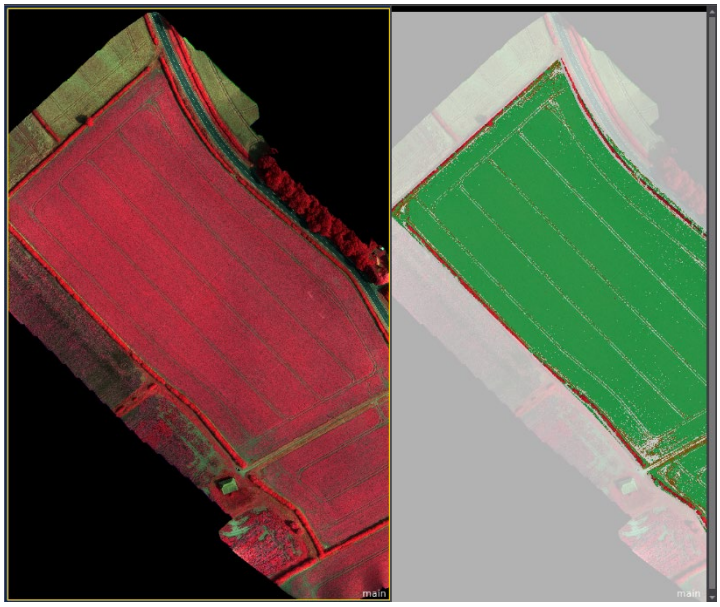

Figure 8 UAV imaging of site 15

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 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 was 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).

Within the third year, TuYV, PEMV-1, PEMV-2, PSbMV and SbDV were all present in the site samples. As in previous years, TuYV was the most common virus followed by PEMV-2 and PEMV-1. Additionally, in the third year bean leafroll virus (BLRV), bean yellow mosaic virus (BYMV), cabbage cytorhabdovirus virus (CCyV1) and trifolium pratense virus A (TpVA) were found in the site samples. BYMV was seen in a symptomatic sample in the first year. BLRV, CCyV1 and TpVA were not seen in previous years, and this provides the first report of CCyV1 and TpVA in the UK. TuYV was found at all twenty sites, fifteen sites had mixed virus infections, and virus incidence was high across the viruses found. This is suspected to be due to exceptional weather conditions, mild winter/warm spring promoting weed and aphid populations followed by a hot summer, which negatively affected the weed populations meaning aphids migrated to nearby crops. This could explain both the higher virus incidences and the new virus findings for the third year.

PEMV-1 (genus *Enamovirus*) and PEMV-2 (genus *Umbravirus*) are two different viral species that have a synergistic relationship. PEMV-1 produces a coat protein which enables aphid transmission while PEMV-2 allows for movement within the plant. These viruses have been thought to only occur together, so the finding of these viruses separately was unexpected (Demler et al., 1993).

Gaafar et al., (2020) performed a study investigating which viruses are present in peas and surrounding weeds in pea-growing areas of Germany. The study involved sequencing of smaller bulks than the work reported here. Within this work a total of thirty-five viruses were

identified, of which twenty-five were either new records for Germany or novel viruses. Like the work reported here TuYV, PEMV-1, PEMV-2, PNYDV and PSbMV were the most common viruses. SbDV, BYMV and BLRV were identified at some sites, but findings were more sporadic. PNYDV is one the most common viruses in Germany, whereas within the work reported here PNYDV was only identified at one site, further survey work should test for this virus to better understand its UK distribution.

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 quality 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, throughout the three years of the project there have been multiple instances of identifying viruses at low incidences by the presence of sequence fragments rather than whole genomes, e.g. SbDV, PNYDV and TpVA. In particular, within the symptomatic samples there have been cases where the sample tests positive by real-time RT-PCR for a virus that was not identified by HTS. Often this is associated with high Ct values from the real-time RT-PCR testing (>Ct 30), which suggests the virus is at a low level and at the limit of detection for HTS. 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 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). Therefore, no in-depth testing was performed on site samples in year 2 or 3 of the project.

Following the initial finding of PNYDV at one site in year 2, further work was done to characterise this isolate, as it is the first finding in the UK. Unlike the other viruses identified within this project, PNYDV has a DNA genome. The HTS method used within this project is optimised for detection of RNA viruses, that combined with the low viral incidence led to fragments of the virus being identified. Therefore, further work was done to obtain a whole genome, this work also helped to understand sequencing of virus DNA targets and was funded by Defra through 'Future Proofing Plant Health' (Submitted for publication).

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 symptomatic samples, suggesting these viruses are present a 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. Within third year, no extra plant viruses were found within the symptomatic samples that were not found in the site samples. Broadly, findings in the symptomatic samples correlated with findings at the site. All together this suggests that testing the site as a bulk doesn't miss viruses, as additional viruses were not commonly found in the symptomatic samples. Within the first year, the extra viruses were found in symptomatic samples not associated with the twenty sites.

Over the three years, there have consistently been findings of TuYV, PEMV-1, PEMV-2, PSbMV and SbDV. Additionally, extra viruses have been identified, suggesting they are present but at low incidence and may only be an issue in certain years e.g., high incidence of BYMV within year 3.

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 three 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.

The potential yield impact presented here is preliminary but across the three years a yield loss of 40%-50% is associated with the presence of TuYV, where no treatment is used. A yield loss study in Australia reports yield losses on field pea of up to 45% in early TuYV infection. These yield losses match those predicted from this model, however specific yield loss studies are required to show whether a similar result would be seen in UK crops. Nancarrow et al., (2022) report that the yield losses in peas from TuYV was due to factors such as fewer grains, reduction in 1,000-grain weight and reduced number of pods. During the study the plants were assessed for symptoms using methods such as visual examination, weight of dried leaves and measurements of chlorophyll content. From these assessments, there were significant reductions of 8% and 18% in plant height and whole plant weight, respectively, in one of the assessments performed. Within this project, TuYV was found to be widespread and present at high incidence in UK peas but was not reported prior to this project, and high virus prevalence levels were not expected from site visits when samples were collected as no typical virus symptoms were identified. Lack of typical virus symptoms and more subtle symptoms, such as number of pods, may have meant that this virus has remain unnoticed until this point. An AHDB project running alongside this project was investigating the aphids associated with virus incidence and in the final year of the project investigated spray treatments (FV 460). Within this project, it was found that earlier sprays were associated with a limiting impact on yield loss.

During year 3 of this project the assessment of presence of viruses and survey methods was further supplemented by aerial assessment of virus incidence using remote sensing via UAV (“drone”). This work was funded by Defra through “Future Proofing Plant Health”. From this project, results from four of the sites are presented here, and of these only two fields showed signs of stress. Of the three sites with high incidence of TuYV (>50%) only one site was identified to show symptoms of stress. Site 16 was shown to have stressed plants in the field, it had a low TuYV incidence (10%) but a high incidence of BYMV (80%). Nancarrow et al., (2022) did not identify typical viral symptoms on peas infected with TuYV, which could explain that even where the TuYV incidence is high at the site, there are no signs of stress. Within Site 16, the high incidence of BYMV could have caused the stress identified at the sites, but the symptoms of BYMV on pea can be variable including bright mosaic, necrosis or symptomless (Trębicki, 2022). The analysis presented here is still being developed, however, the analysis may not be as informative where viruses do not present typical virus symptoms limiting the use of it in viral assessments.

In conclusion, it has been shown that high throughput sequencing can be used in a generic surveillance workflow, and further work could investigate the use of this method on other crops. Through this survey, there is now a better understanding of which viruses are present in UK peas, which can be used to better understand how to manage them. Future work is required to determine if the yield loss model presented here mirrors what happens in the field, and how this compares to the results from the yield loss work in Australia. There are questions such as can resistance be bred into peas for TuYV? How does TuYV affect the physical properties of the pea (e.g. protein content) as well as the yield loss? What are the origins of TuYV in the field? Is it spreading from oil seed rape or weeds?

Conclusions

- This generic approach to surveillance work appears to be effective, and peas appear to be a successful model crop for this work.
- During this project turnip yellows virus, soybean dwarf virus, pea necrotic yellow dwarf virus, cabbage cytorhabdovirus-1 and trifolium pratense virus A 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.

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 presented year 1 and year 2 results at the International Symposium of Plant Virus Epidemiology, Madrid 2022.
- 08/11/2022- Aimee Fowkes presented year 1-3 results at the Fera Science Symposium (Biosecurity)
- 09/11/2022- Aimee Fowkes presented year 1-3 results at the Pea and Bean Growers Conference
- 25-26/01/2023- Aimee Fowkes and Adrian Fox presented year 1-3 results at the PGRO/Syngenta Pulse Roadshow.
- End of January- Aimee Fowkes presented year 1-3 results at the PGRO/Syngenta Pulse Roadshow Webinar 2023.
- 2nd February 2023 – Becky Howard presented year 1 to 3 results to Crop Advisors agronomy group meeting
- 6th February 2023 – Becky Howard presented year 1 to 3 results to Agrovista agronomists meeting
- 10th February 2023 – Becky Howard presented year 1 to 3 results to the HMC Peas Grower Group meeting.
- 16th February 2023 – Becky Howard presented year 1 to 3 results to Velcourt grower group meeting.
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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.
- Winter 2023- Aimee Fowkes wrote an article 'Virus surveillance in peas' for PGRO's publication 'The Vegetable Magazine'.

Glossary

BYMV	Bean yellow mosaic virus
BLRV	Bean leafroll virus
CCyV1	Cabbage cytorhabdovirus-1
DNA	Deoxyribonucleic acid
TuYVaRNA	Turnip yellows virus associated RNA
HTS	High throughput sequencing
PEMV-1	Pea enation mosaic virus-1
PEMV-2	Pea enation mosaic virus-2
PEMVSatRNA	Pea enation mosaic virus satellite RNA
PNYDV	Pea necrotic yellow dwarf virus
PSbMV	Pea seed-borne mosaic virus
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
SbDV	Soybean dwarf virus
TuYV	Turnip yellows virus
TpVA	Trifolium pratense virus A

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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-1 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-1 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-1 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-1 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-1 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-1 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-1 was found to significantly reduce productivity ($p<0.011$); the average productivity in untreated fields in which PEMV-1 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-1 was not found to be significantly ameliorated by treatment ($p<0.18$); the average productivity in treated 'PEMV-1 fields' lies[2] somewhere between 53 and 100% of that in untreated 'clean' fields. It is possible that the

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

treatment does have an ameliorating effect which is more difficult to detect because the effect of PEMV-1 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-1 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-1 and treatment on the productivity of peas (2019 and 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-1 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 PEMV-1 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-1 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-1 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-1 are not detected lies[2] somewhere between 68 and 107 g
2. Treatment was not found to significantly reduce productivity in fields in which TuYV and PEMV-1 are not detected (p=0.75); average productivity in 'clean' treated fields lies[2] 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-1 was found to significantly reduce productivity (p<0.006); the average productivity in untreated fields in which PEMV-1 is present with a prevalence of 27%[3] lies[2] somewhere between 56 and 94% of that in 'clean' fields.
6. The effect of PEMV-1 was not found to be significantly ameliorated by treatment (p<0.27); the average productivity in treated 'PEMV-1 fields' lies[2] 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].

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

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 * TYVn + 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:TYVn    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)

Appendix 6 - The effects of TuYV, PEMV-1 and treatment on the productivity of peas (2019, 2021 and 2022)

Summary and conclusions

The aim of this analysis was to estimate the effect of the prevalence of viruses on the productivity of peas[9] and how treatment modifies the effect of the viruses. This was a third part of a study in which the effect of TuYV and PEMV-1 was previously estimated at five sites in 2019 and across 2019 and 2021. Hence, estimates for TuYV and PYMV were updated with observations gained in 2022. We used observations of productivity and virus prevalence at five locations in each of 2019, 2021 and four locations in 2022. 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[10] lie.

Our analysis showed that reduction in productivity is significantly correlated with estimates of the prevalence of TuYV and PEMV-1 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-1 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-1 are not detected lies[2] somewhere between 67 and 103 g
2. Treatment was not found to significantly reduce productivity in fields in which TuYV and PEMV-1 are not detected ($p=0.95$); average productivity in 'clean' treated fields lies[2] somewhere between 99 and 114% of that in untreated fields.
3. TuYV was found to significantly reduce productivity ($p<0.003$); the average productivity in untreated fields in which TuYV is present with a prevalence of 97%[11] lies[2] somewhere between 30 and 84% 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 45 and 125% of that in untreated 'clean' fields.
5. PEMV-1 was found to significantly reduce productivity ($p<0.007$); the average productivity in untreated fields in which PEMV-1 is present with a prevalence of 27%[3] lies[2] somewhere between 33 and 88% of that in 'clean' fields.

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

6. The effect of PEMV-1 was not found to be significantly ameliorated by treatment ($p<0.15$); the average productivity in treated 'PEMV-1 fields' lies[2] somewhere between 35 and 96% of that in untreated 'clean' fields.

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-1 were provided. A linear mixed model (Equation 1) was fitted to the log transformed observations of yield[12].

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

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 ['lmerM
odLmerTest']
Formula: mass ~ Treatment * TYVn + PEMVn * Treatment + (1 | Location:Year)
   Data: Ndata

REML criterion at convergence: 237.9

Scaled residuals:
    Min      1Q  Median      3Q     Max
-4.5165 -0.5567  0.0728  0.6414  2.7211

Random effects:
 Groups        Name        Variance Std.Dev.
 Location:Year (Intercept) 0.06773  0.2603
 Residual                  0.07548  0.2747
Number of obs: 558, groups:  Location:Year, 14

Fixed effects:
                          Estimate Std. Error         df t value Pr(>|t|)
(Intercept)             4.409e+00  1.141e-01  1.161e+01  38.654 1.25e-13 *
**
TreatmentTreated        6.819e-02  3.715e-02  5.410e+02   1.835 0.066995 .
TYVn                   -6.834e-03  2.707e-03  1.161e+01  -2.525 0.027249 *
PEMVn                  -1.149e-02  4.467e-03  1.161e+01  -2.572 0.024993 *
TreatmentTreated:TYVn   3.361e-03  8.807e-04  5.410e+02   3.817 0.000151 *
**
TreatmentTreated:PEMVn  7.404e-04  1.453e-03  5.410e+02   0.510 0.610552
```

[12] 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)

