

Grower Summary

Surveillance of virus diseases

in UK Pea Crops

FV 459

Final report 2022

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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previously recorded in the UK, but only seven (7) have ever been recorded in UK pea crops (Source: UK Virus Checklist, unpublished Fera data).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 (highthroughput 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	ТВС	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	Davtona	TuYV, PEMV-1, PEMV-2, PSbMV, PNYDV, PEMV satRNA	37 15	54 81	66 87	8 28	nt	2 64
7	Amalifi		nt	nt	nt	nt	nt	nt
, 8	Swallow		5 78	nt	nt	nt	nt	nt
9	Trophy	TuYV PFMV-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	ТВС	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	ТВС	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	ТВС	TuYV, PEMV-2, PEMVSatRNA (virus to investigate)	80.01	2.7 1	3.62	nt	nt	nt	nt	nt	nt
13	ТВС	TuYV, PEMV-1, PEMV-2, PSbMV, PEMV SatRNA	86.67	8.28	23.69	3.72	nt	nt	nt	nt	nt

		TuYV, PEMV-1, PEMV-2, PEMVSatRNA (virus to	10.02	22.02	40.57	nt	nt	nt	nt	2 71	nt
14	Wagtaii	investigate)	10.83	22.03	49.57					2.71	-
	Sherton?										
	(Storm			nt		nt	nt	nt	nt	nt	nt
15	seeds)	TuYV, PEMV-2, PEMV SatRNA	86.67		25.58						
		TuYV, PEMV-2, PSbMV, BYMV,									
		PEMV SatRNA, (virus to		nt			nt	nt			nt
16	Bingo	investigate)	10.42		25.49	13.02			80.01	1.76	
		TuYV, PEMV-1, PEMV-2,									
		PEMVSatRNA (virus to					nt	nt	nt		nt
17	ТВС	investigate)	15	15.59	49.57	0.85				2.71	
		TuYV, PEMV-1, PEMV-2, PSbMV,									
	Bingo or	BYMV, PEMV SatRNA (virus to					nt	nt			nt
18	Reflection	investigate)	2.71	4.53	17.38	35.18			100	4.66	
19	Darlin	TuYV	2.57	nt	nt	nt	nt	nt	nt	nt	nt
20	ТВС	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.

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Table 1 Estimates of effect sizes	and cignificance of treatment	and TuV/ and DEM//_1	for 2010 2021 and 2022
			101 ZU13, ZUZ1 anu ZUZZ.

Value	Estimate	95%	5 C.I.	Significance
10-plant pea mass in clean untreated fields Effect of treatment ^a Effect of TuYV ^a Effect of TuYV and treatment ^a Effect of PEMV-1 ^a Effect of PEMV-1 and treatment ^a	84g 106% 53% 79% 57% 61%	67g 99% 30% 45% 33% 35%	103g 114% 84% 125% 88% 96%	0.950 ^b 0.003 ^c <0.001 ^d 0.007 ^e 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

- 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.