



Grower Summary

FV 459

**Surveillance of virus diseases
in UK Pea Crops**

2nd Interim report 2022

Project title: **Surveillance of virus diseases in UK Pea Crops**

Project number: FV459

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Location of project: York and Peterborough, plus multiple field sites

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AUTHENTICATION

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

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GROWER SUMMARY

Headline

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

Background

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recorded in the UK, but only seven (7) have ever been recorded from UK pea crops (Source: UK Virus Checklist, unpublished Fera data).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

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

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

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

Summary

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

Year 1 (2019)

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

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

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

6	Combining Pea (TBC)	TuYV	12.46	nt	nt	nt
7	Tomahawk	Negative	nt	nt	nt	nt
8	Combining Pea	PEMV1 PEMV-2	nt	40.8	86.67	nt
9	Swallow	TuYV	1.71	nt	nt	nt
10	EBBA	TuYV PEMV-2	9.71	nt	2.57	nt
11	Vidor	TuYV	60.62	nt	nt	nt
12	Amalfi	TuYV PEMV-2	16.39	0.85	20.05	nt
13	Realm	TuYV PEMV-2	32.56	0	21.8	nt
14	Ashton	TuYV PEMV-2 SbDV	93.33	nt	7.49	1.71
15	TBC	TuYV PEMV1 PEMV-2 SbDV	3.62	16.39	2.71	4.53
16	Oasis	TuYV PEMV1 PEMV-2 PEMV Sat	27.77	4.81	22.23	nt
17	Vidor/Ambassador	TuYV	21.8	nt	nt	nt
18	Kimberley	TuYV PEMV Sat	93.33	nt	nt	nt
19	Oasis	TuYV PEMV-2	86.67		28.2	nt
20	Boogie	TuYV PEMV1 PEMV-2 PEMV Sat	27.77	37.15	40.8	nt

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

Year 2 (2021)

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

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

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

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

virus-2 was identified at eleven sites with an incidence between 0.85%-66.87%. In this year's study, pea seedborne mosaic virus was identified in the site samples, it was identified at four sites with an incidence of 4.53%. Finally, pea necrotic yellow dwarf virus was identified at 1 site with an incidence of 2.64%, this represents the first finding of this virus in the UK.

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

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

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

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

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

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

Financial Benefits

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

Action Points

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