WP 2: PepMV trials

Deliverables

2.1. Completed glasshouse trials over two seasons on four different locations

2.2. Assessment of impact of PepMV on infected tomato crops

2.3. Econometric model for assessing economic impact

2.4. Cost benefit analysis of PepMV control measures

WP 2 deals with field and glasshouse trials to assess the damage and economic impact of PepMV on late infected tomato crops.
Four partner institutes from Hungary, Netherlands, Spain and UK (Table 1) set up replicate greenhouse trials over two full seasons to assess the economic impact of *Pepino mosaic virus* (PepMV) in late-infected tomato crops. Previous research from the UK has suggested that PepMV has a significant effect on fruit quality and has no overall effect on fruit yield (Spence et al. 2006), while other EU countries reported either effects on yield or a limited impact of PepMV infection on both fruit yield and quality. It is unclear whether the observed effects on yield and quality apply to tomato crops grown in all EU-member states. Replicate trials were carried out in four major tomato producing areas to assess the possible damage of PepMV under different environmental and cropping conditions to provide an EU-wide perspective. In these greenhouse trials, we determined the effects of the mild PepMV isolate *1066* and the aggressive PepMV isolate *PCH-06/104* on yield and quality on tomato plants. These isolates cover the impact ranging from a mild PepMV infection to a worse case scenario. The isolate *1066* belongs to the European tomato strain, while the isolate *PCH-06/104* belongs to the Chile2 strain of PepMV. Trial data were combined with results from a cost-benefit analysis to allow an assessment of the possible overall economic impact of PepMV in each EU-member state.

Table 1. Participants in the PepMV greenhouse trials

Partner	Institute	Location	Country
2	Food & Environment Research Agency (Fera)	York	UK
4	Universidad Politénica de Valencia	Valencia	Spain
5	Csongrád Megyei Mezőgazdasági SZakigazgatási Hivatal (CsM-MgSzH)	Hódmezővásárhely	Hungary
7	Wageningen UR Glastuinbouw (WUR)	Bleiswijk	The Netherlands

Materials and methods

Replicate greenhouse trials were carried out during two growing seasons. Crops were infected with the mild PepMV isolate *1066* and with the aggressive isolate *PCH-06/104* in the first and second growing season, respectively. These trials were conducted following a standardized set-up, while tomato plants were grown according to the local commercial practice of each participating partner country. Conformity between sites was ensured by using standardized protocols to manage the trials and to collect the data. Inter-site variation was minimized by holding a workshop prior to the start of the first trial, which was held before the project kick-off meeting in the Netherlands (May 2007). An interim meeting was held in Hungary to review the progress made during the Hungarian trials in 2007, when the other trials had not yet started. This opportunity was used to make refinements to the initial protocols. A second interim meeting was held in York (UK) between the first and second growing season to discuss results from the first season and to evaluate the protocols.

Eventually, two different protocols were drafted by the workpackage leader (Partner 2) and circulated amongst the other partners involved in WP2. Revisions were made based on comments received and the final versions were circulated. The two protocols produced were: **1. Trials Monitoring Protocol,** which provided guidance on the monitoring of the virus infection, virus-related symptoms and fruit-set. **2. Quality Assessment Protocol,** which is based on Directive 790/2000 EC "Marketing Standard for tomatoes", and provides guidance on how to assess the post-harvest crop yield and quality of the trials. This protocol defines criteria such as fruit size and quality. Advice from both industry and Horticulture Marketing Inspectors proved to be essential when drafting this protocol. Horticulture Marketing Inspectors are required to enforce Directive 790/2000 and hence have an excellent understanding of the boundaries between different fruit classes; a critical and highly subjective area.

Common trial design

The tomato cultivar Cedrico was cultivated in all trials in both growing seasons. Tomato plants were planted and grown according to the local commercial practice. Two treatments were assessed during both growing seasons, namely a PepMV infected treatment and a healthy control treatment. Both treatments were represented by four replicate plots that consisted of 20 tomato plants each. All Cedrico plants in the infected plots were mechanically inoculated with the *1066* isolate (first season) or the *PCH-06/104* isolate (second season). Partner 7 (the Netherlands) had grown tomato plants infected with these isolates and subsequently distributed infected leaves from these plants to the other partner institutes. They inoculated several cv. Cedrico plants in order to have a fresh source of inoculum available on site. Trial plants were subjected to a late inoculation. Prior to the inoculation, all trial plants were sampled by removing the terminal leaflet from the highest fully-developed leaf. Leaf material was tested by ELISA to ensure the absence of PepMV. Virus inoculum was prepared by grinding a few leaves of the infector plants in inoculation buffer (the Netherlands, Hungary and UK: PBS, pH 7.4; Spain: 0,01M PBS, pH 7.2 + 0.2% (p/w) DIECA + 0.2% (p/w) sodium bisulphite). Inoculations were conducted by rubbing sap extracts plus carborundum or Celite on the upper fully developed leaves when the plants were in the 5th truss-stage. Fera (UK) isolated RNA from leaves from infected tomato plants and subsequently confirmed that the PepMV present in the trial belonged to the European tomato strain (first season) and the Chile2 strain (second season).
Prior to the inoculation, sanitation methods were put in place to minimise the risk of early PepMV infection and of other pathogen infections. Once inoculations were done, all work in the compartment/area with healthy plants was carried out prior to the work in the compartment/area with infected plants to avoid the unwanted spread of PepMV. Both compartments/areas had their own stock of equipment, overalls, gloves, shoe covers and caps. Disinfection mats were placed in front of the entrance of both compartments/areas and were wetted regularly with disinfectant. The same instructed personal performed all labor throughout both growing seasons and no uninvited entry was allowed. All harvest from the trial was disposed in a safe way. As the same facilities were used during both growing seasons, all compartments and tunnels were thoroughly disinfected in between the two seasons. The watering and irrigation systems were also disinfected. All tools were either disinfected or replaced by new tools.

Site-specific trial design

Because the tomato growing seasons are not synchronic throughout the EU, tomato plants were sown, planted and inoculated at different times of the year to ensure that crop management was in line with the local commercial practice (Table 2). The type of greenhouse used at commercial facilities varies between the different partner countries *(Fig. 2)*. As such, the trial lay out was designed to fit the local type of facilities. As an example the UK trial design is shown below*(Fig. 3)*.

Figure 1. *Local green- and glasshouse facilities in the (a.) UK, (b.), Spain, (c.) Hungary and (d.) the Netherlands as used during the PEPEIRA trials.*

Table 2. Site-specific trial management in each participating EU-country.

Country	Growing season	Sowing	Transplantation	Inoculation	First harvest	Final harvest
UK	2007-2008	14 March 2008 [1]	15 May 2008	18 June 2008	9 July 2008	17 September 2008
	2008-2009	14 January 2009	5 March 2009	9 April 2009	20 May 2009	26 August 2009
Spain	2007-2008	21 August 2007	1 October 2007	20 November 2007	6 February 2008	28 May 2008
	2008-2009	22 September 2008	10 November 2008 [2]	18 February 2009 [3]	15 April 2009	1 July 2009
Hungary	2007	13 March 2007	20 April 2007	18/19 June 2007	4 July 2007	4 October 2007
	2008	15 March 2008	28 April 2008	20 June 2008	27 June 2008	24 September 2008
Netherlands	2007-2008	14 November 2007	8 January 2008	3 March 2008	9 April 2008	17 September 2008 [4]
	2008-2009	21 November 2008	6 January 2009	11 March 2009	14 April 2009	27 October 2008

[1] Seed was originally sown late November 2007. However, just after planting on 11 January, Defra's Plant Health Division decided that the glasshouse facilities at STC needed to be upgraded to ensure that the virus could be contained and destroyed on site. This overruled the earlier approval of the trail set-up by the Plant Health Division and delayed the start of the trial by approximately 3 months.

[2] In the second Spanish growing season, sowing and transplantation dates were adapted to avoid problems with whitefly-transmitted viruses.

[3] The inoculation date in the second Spanish growing season was also changed relative to the first season, because the cooler weather conditions of that winter slowed down plant development considerably

[4] In 2009, yields decreased steadily towards the end of the season after the 19th week of harvesting. In 2008, production levels also decreased after the 19th week, but subsequently dropped sharply after the 23rd week and the trial was therefore stopped earlier in 2008.

UK-specific trial design

Plants in the UK were propagated and grown at Stockbridge Technology Centre (STC), North Yorkshire *(Fig. 2a)*. The experimental layout is shown in *Fig. 3*. The glasshouse was split by treatment. The infected treatment consisted of four replicated plots on one side of the glasshouse, while the non-inoculated (healthy) plots were on the other side. Each experimental plot consisted of two rows, each row containing ten plants. Bell pepper is considered to be a non-host of PepMV and was planted between each plot to avoid the spread of PepMV by plant-to-plant contact.
Between sowing and planting, 200 plants were tested for the presence of PepMV by ELISA. On 13 May 2008 and 3 February 2009, 180 tomato plants (at the 5th true leaf stage) were sampled by removing the terminal leaflet from the highest fully-developed leaf. Similarly, the uppermost fully developed leaf of 20 bell pepper plants (barrier crop) was sampled and tested. Tomato seedlings were transplanted into single rock wool blocks that were subsequently placed on rock wool slabs. Plants were trained to a crop wire at circa 4 m height. In both growing seasons, the healthy control treatment and infected treatment were located in two separate areas of the same glasshouse compartment. Both areas were identical in size and had a cropping area of 90 m^2. Crop density was 2.2 tomato plants per m^2. Sanitation methods were put in place to minimise the risk of transmitting PepMV, and other pathogens. For example, entry of bumble bees and larger sized pests that may spread PepMV was avoided by screening the glasshouse vents. Plant pollination was done by hand.

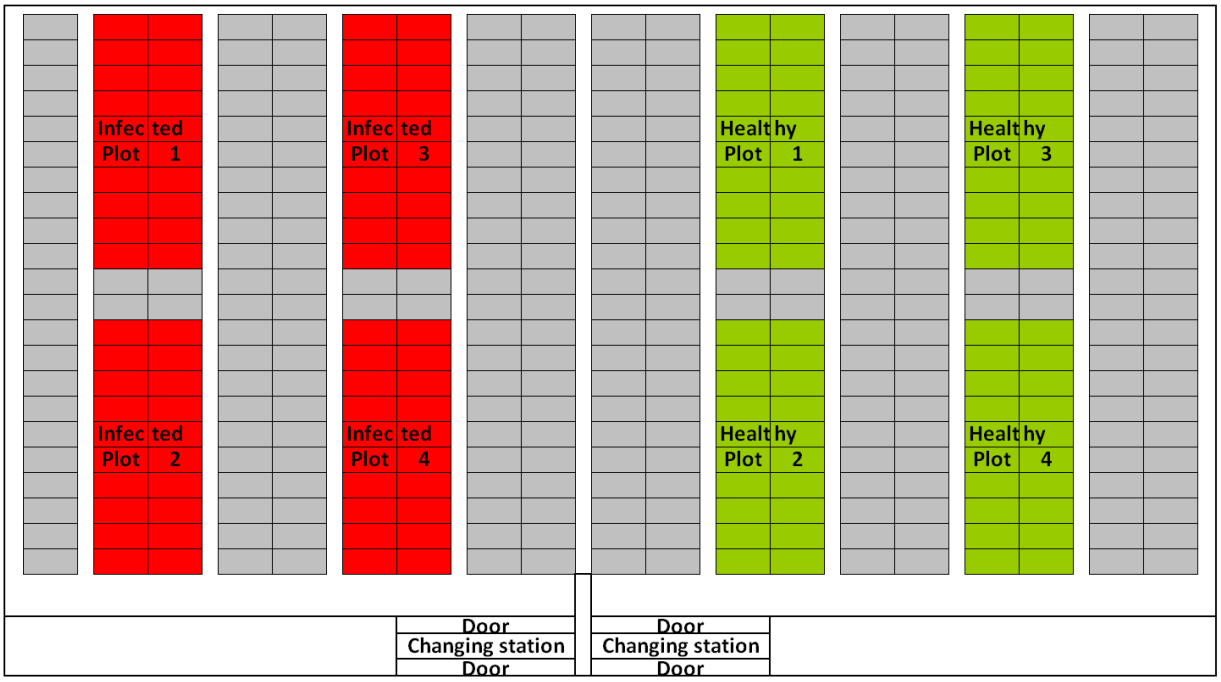

Figure 3. *Schematic map of the UK trial set-up. A double door entry system was present for both glasshouse sections. Each square represents a single plant of the tomato cv. Cedrico (healthy = green squares; PepMV-infected = red squares) or of the sweet pepper barrier crop (grey squares). Tomato stems were rotated clockwise within the replicate plots.*

DAS-ELISA and PepMV concentration

Double sandwich enzyme-linked immunosorbant assays (DAS-ELISA) was used to test whether all tomato plants were free of PepMV prior to the inoculation. During the trials, the healthy tomato plants were tested by DAS-ELISA to confirm the absence of PepMV on a weekly basis.
Every two (Hungary) or four weeks (other trial sites), four pooled samples of infected Cedrico plants were taken to determine the PepMV concentration. The terminal leaflet of the upper fully developed leaf was collected from three plants per plot and 10 ml of PBS-Tween buffer (pH 7.4) was added per gram of leaf tissue to allow grinding. Anti-PepMV rabbit polyclonal antiserum (Prime Diagnostics, The Netherlands) was diluted to 1:1,000 (v/v) and used according to the

appended specifications. Reactions were measured at 405 nm with an ELISA reader (Bio-Tek Elx808 or Multiskan RC). Samples were considered positive if absorbance levels exceeded background levels by a factor three. Viral load is either reported as (relative) absorbance values (Spain, UK, and Hungary) or as virus concentration (the Netherlands). To quantify viral concentration, a dilution series of known amounts of the *1066* isolate was assayed on each plate to establish a standard curve.

Flowering and fruit-set

Flowering and fruit-set were monitored on at least three plants per replicate plot, adding up to a total of 12 plants per treatment. Monitoring started on the first truss above the inoculated leaf and continued for the next five to seven trusses (the Netherlands: truss 5 to 11; UK: truss 5 to 11; Hungary: truss 5 to 9; Spain: truss 4 to 9). Flowering and fruit-set data were collected weekly.

Leaf symptom development

Symptoms were monitored weekly with a standardized scoring form on which plant heads and foliage were rated for the presence of virus-related symptoms. Rated symptoms as described by the monitoring protocol were leaf bubbling, mosaic and nettle heads (in plants heads), and yellow spots and scorching (foliage). The protocol allowed for additional symptoms to be monitored as well. Additionally observed and rated symptoms were leaf deformation (the Netherlands, UK) and necrosis on the petals (Spain, Hungary). All symptoms were scored on a 0 (no symptoms) to 4 (very severe) scale. Eight plants (Spain) or three plants (other sites) were visually assessed per replicate plot.

Shelf-life

Samples of healthy and PepMV-infected fruits were subjected to a shelf-life test at three times during both growing seasons (early, mid- en late-season) according to the prescribed trial protocol.

Harvesting

Tomato fruits were harvested weekly. Fruits were sorted into size classes, graded, counted and weighted on the day of harvest. Fruit quality assessments were based on Directive 790/2000 EC "Marketing standard for tomatoes". Fruits were assigned to the size categories <35 mm, 37-47 mm, 47-57 mm, 57-67 mm or >67 mm. All fruits were also graded as either Class I, Class II or waste, based on the presence of marbling, fruit discoloration, uneven ripening or other deviations, such as lack of firmness, cracks, blemishes, and blossom-end rot (Table 3; *Fig. 4)*. Fruits that were smaller than 35 mm were always graded as waste.

Table 3. *Definition of classes as described in the quality assessment protocol.*

Class	Characteristics
Class I	Even colour (although does allow a slight defect in colouring) Firm No cracks or blemishes
Class II	Reasonably firm Small defects in colouring, e.g. mottling, uneven red colour Healed cracks less than 3 cm in length Minor blemishes
Waste (out grades)	Irregular colour severe uneven ripening, ‘greenbacks’ Over ripe (soft/rotting) Major defects e.g. bad bruising, blossom-end rot Open wounds/cuts Fruit smaller than 35 mm

Figure 4 *Examples of fruits with colour defects. Fruits on the top left are still considered to be class I despite of the small colour defects. Fruits on the top right are downgraded to class II. The bottom row illustrates the difference between a class II (left and right) and a waste class fruit (middle).*

Statistical analysis

An analysis of variance for repeated measures was done on fruit yield and quality data from season 2 (2008 for the Hungarian trial; 2009 for other countries). All analyses were carried out using Genstat release 10.2 (VSN International Ltd, UK). The first year trial data (2007 for the Hungarian trial; 2008 for other countries) were analysed using a REML linear mixed model for repeated measures approach because the data sets were unbalanced.

Results

DAS-ELISA and PepMV concentration

At all trial sites, tomato plants were free of PepMV at the start of the trials as determined by DAS-ELISA. At none of the trial sites, PepMV was detected in the healthy control plants at anytime. In those plants that were inoculated with either *1066* or *PCH-06/104*, PepMV could be detected by DAS-ELISA on the first day that the plants were tested. The first DAS-ELISA test took place 2-4 wpi in Hungary, UK and Spain. In the Dutch trials, PepMV was already detected in all inoculated plants at nine dpi.

The general pattern which arises from the tests on the PepMV concentration is that this concentration fluctuates somewhat throughout the season *(Figure 5)*. It should be kept in mind that the growing seasons at the different sites are not synchronic, which may explain differences in these fluctuations. Local weather conditions may also affect the PepMV concentration. One notable exception to the moderate fluctuations is a sharp drop at the end of August in the Hungarian trial on *1066 (Fig. 5c)*, when temperatures in the greenhouse were very high. A second exception is the Dutch trial, in which the concentration of PepMV in the *PCH-06/104*-infected plants was initially much higher than the PepMV concentration in the *1066*-infected plants *(Figure 5a)*, but then experienced a sharp drop to end up on a similar level as *1066* in June.

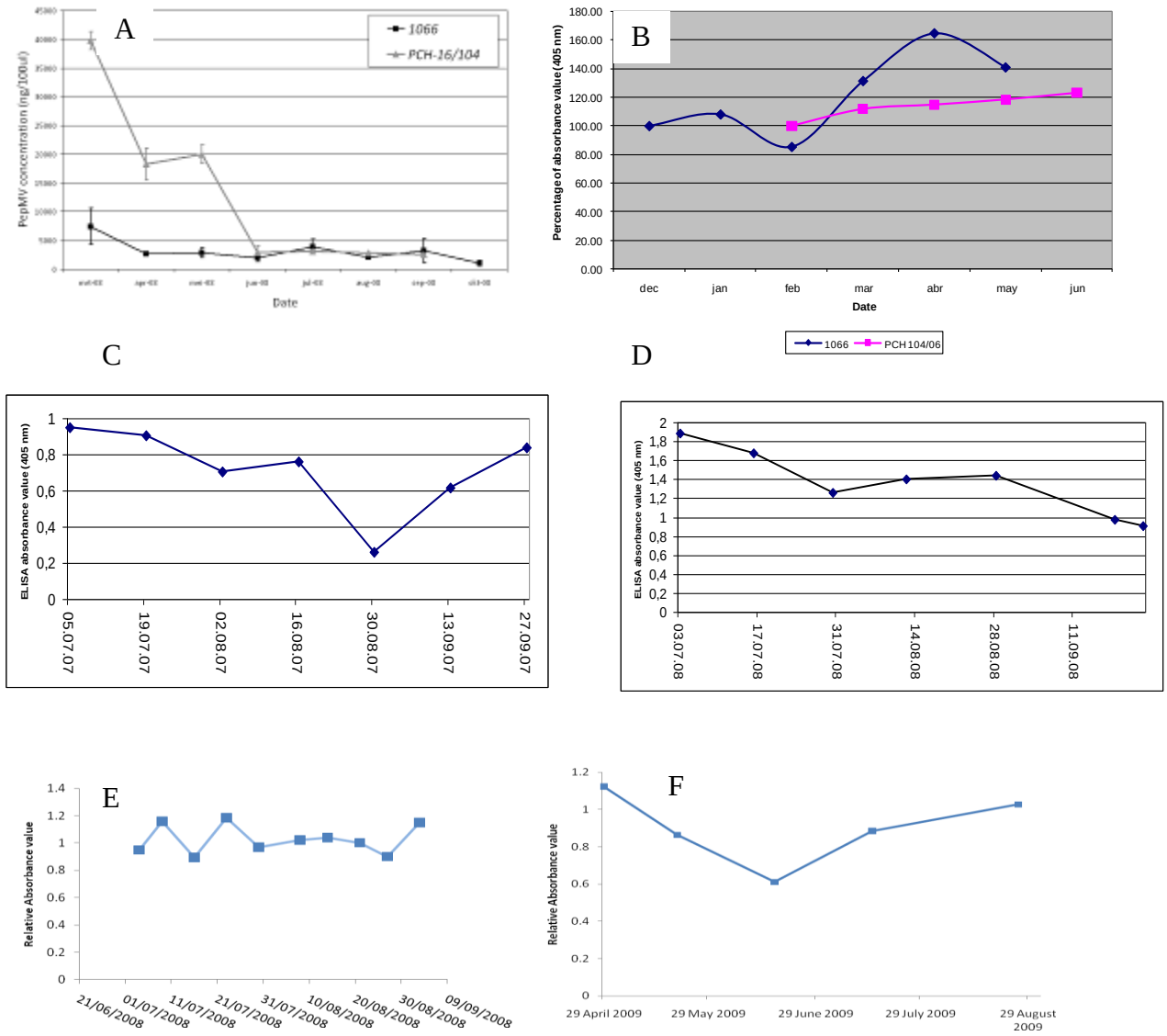

Figure 5. *PepMV concentration throughout the growing season as measured by DAS-ELISA. (a.) PepMV concentration (± s.e.) in the Dutch trials. (b.) Relative absorbance values in the Spanish trial. Absorbance values at the first time point were set to 100%. (c.) Absorbance values of 1066-infected plants in the Hungarian trial. (d) Absorbance values of PCH-06/104-infected plants in the Hungarian trial. (e). Adjusted absorbance values of 1066-infected plants (relative to healthy control plants) in the UK trial. (f). Adjusted absorbance values of PCH-06/104-infected plants (relative to healthy control plants) in the UK trial.*

Flowering and fruit-set

1066 and *PCH-06/104* had no effect on fruit set. A small tendency towards fewer flowers was observed after inoculation with both *1066* and *PCH-06/104.*

Symptom development

The mild PepMV isolate *1066* and the aggressive isolate *PCH-06/104* differed clearly from each other with regard to symptom development on leaves and sepals *(Fig. 6)*. One should, however, keep in mind that both isolates were assessed in different growing seasons and the crops were consequently affected by different weather conditions. This may have influenced the symptom expression to some extent. Therefore, healthy tomato plants were assessed as well to provide a baseline figure.

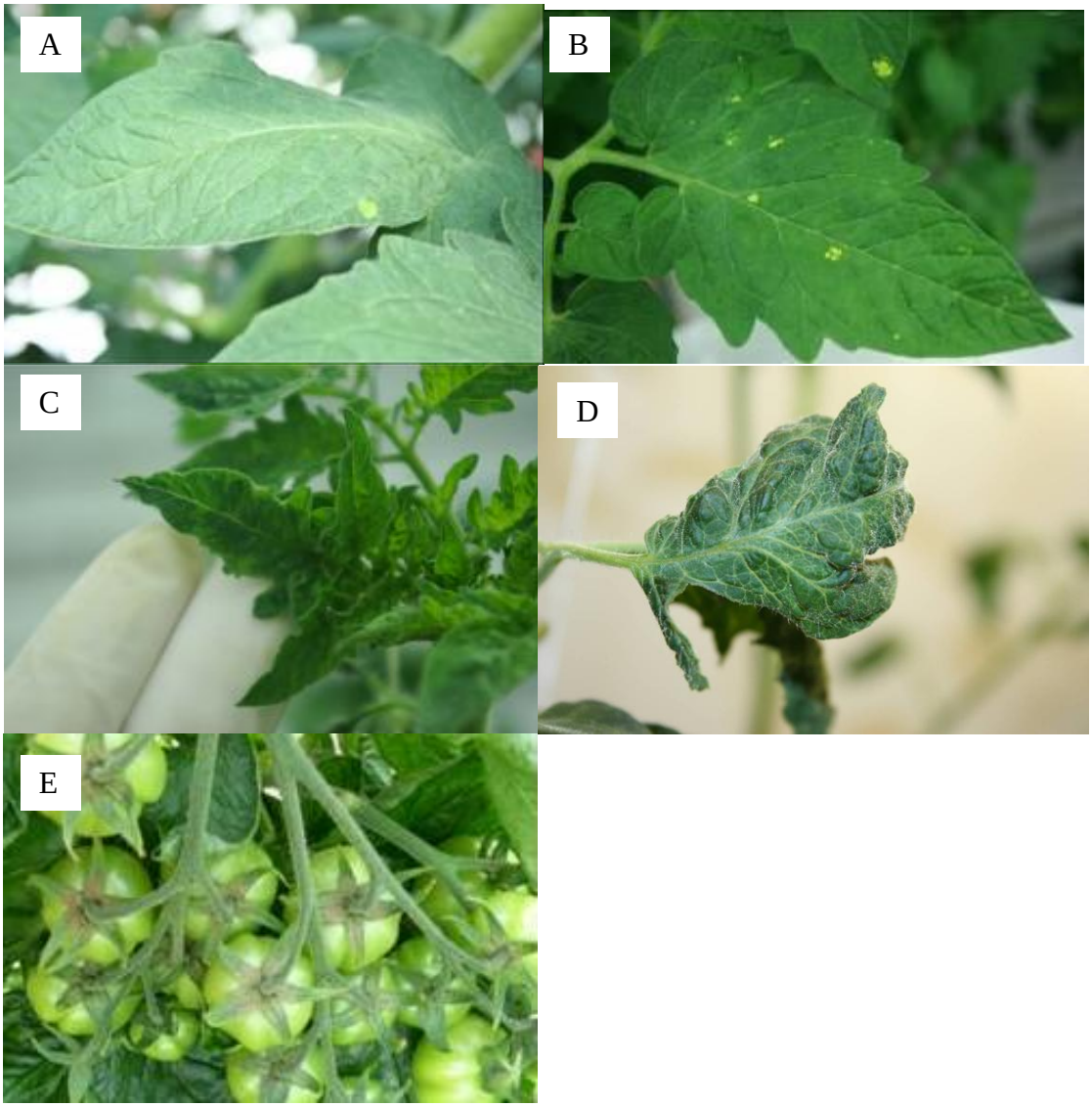

Figure 6. *Both (a. Hungary) 1066 and (b. the Netherlands) PCH-06/104 caused the typical PepMV symptom of yellow spots. PCH-06/104 also caused (c. the Netherlands) leaf bubbling and nettle heads, (d. Hungary) leaf deformation, and (e. Spain) necrosis on the sepals of the developed fruits below the 5th truss.*

Table 4. *PepMV-related symptoms observed in the greenhouse trials. The table displays the mean symptom score.*

Treatment Trial, symptom	Healthy (1st season)	*1066* (1st season)	Healthy (2nd season)	*PCH-06/104* (2nd season)
Dutch trial	*(n=348)*	*(n=348)*	*(n=336)*	*(n=336)*
Plant heads/foliage				
Leaf bubbling	0.08	0.22	0.11	0.90
Nettle heads	0.01	0.08	0.01	0.66
Discoloration/Mosaic	0.22	0.89	0.31	1.39
Yellow spots	0.00	0.23	0.00	0.90
Leaf deformation	0.00	0.01	0.00	0.24
Spanish trial	*(n=544)*	*(n=544)*	*(n=384)*	*(n=384)*
Leaves				
Leaf bubbling	0.0	0.1	0.1	0.1
Nettle Heads	0.0	0.0	0.2	0.2
Mosaic	0.0	0.0	0.0	0.2
Yellow spots	0.0	0.0	0.01	0.2
Yellowing [1]	0.8	0.8	1.6	1.3
Purple appearance[1]	0.9	0.9	1.2	0.9
Necrotic reddening [1]	1.0	0.8	1.6	1.0
Petals				
Mosaic/necrotic spots	0.0	0.4	0.0	0.9
Hungarian trial	*(n=168)*	*(n=168)*	*(n=168)*	*(n=168)*
Plant heads/foliage				
Leaf bubbling	0.0	0.0	0.0	0.4
Yellow spots	0.0	0.3	0.0	0.2
Scorching	0.0	0.0	0.0	1.0
Petals				
Necrosis	0.0	0.0	0.0	0.9
UK trial	*(n=132)*	*(n=132)*	*(n=168)*	*(n=168)*
Plant heads/foliage				
Leaf bubbling	0.04	0.05	0.05	1.23
Nettle heads	0.00	0.00	0.00	0.01
Discoloration/Mosaic	0.01	0.06	0.03	0.38
Yellow spots	0.00	0.00	0.00	0.02
Leaf deformation	0.00	0.00	0.00	0.02

[1] These symptoms are probably caused by viruses other than PepMV

Effect of PepMV on yield

When the yield data for all four participating countries are analysed together, a number of factors need to be considered. First, the location of the trial had the largest influence on yield, whereby southern sites tended to produce less yield than northern sites. Second, sampling date had a large influence on yield . In general, yield increased shortly after sampling began, and then decreased. This was true for all countries except Spain, where yield tended to increase over the season. As there was no interaction between site and treatment in both the 2007/08 (P=0.919) and 2009 (P=0.181) seasons, overall conclusions can be made about the effects of PepMV isolate *1066* and *PCH-06/104* on tomato yield across all sites. With this in mind, PepMV isolate *1066* had no measurable effect on total yield of Class I, Class II and waste together. However, isolate PCH-06/104 reduced the total yield of Class I, Class II and waste together by 4.24%.

Despite each trial site showing similar effects in terms of the impact of PepMV on yield, it is worth considering the results from each country separately. In each of the individual trials carried out in the first year of the project (i.e. in Hungary, Spain, the Netherlands and UK) isolate *1066* had no significant effect on total yield at any location. Also, in all countries, the number of harvested fruits was not affected by the virus. *PCH-06/104* had no effect on total yield (g/plant/week) at any of the four individual trial locations except Spain where yield was reduced by 10%. However, there was a tendency towards lower total yield in the *PCH-06/104*-infected plants in the Hungarian and UK trials compared to healthy plants.

Figure 7. Marbling (upper left) and uneven ripening (upper right) caused by the *PCH-06/104* isolate in the Hungarian trial. Uneven ripening (bottom left and right) also occurred on the first four trusses of plants infected with the isolate *PCH-06/104* in the Dutch trial.

Effect of PepMV on fruit quality

Clear differences could be observed between the PepMV isolates *1066* and *PCH-06/104* with regard to the occurrence of fruit symptoms. PepMV isolate *1066* had very little effect on fruit quality in the trials. Typical PepMV-related symptoms (i.e. fruit marbling and uneven ripening, Figure 7) were observed at all four trial sites, but their occurrence in infected plots did not differ statistically with fruit symptoms in uninfected plots..

PCH-06/104 had a large effect on fruit quality. In Hungary, the first fruit symptoms (.i.e. marbling) appeared shortly after inoculation (Figure 7) and were observed throughout the whole trial. Necrosis was observed on the petals of some fruits. In the Dutch trial, especially the first four trusses displayed clear PepMV related symptoms (uneven ripening, Figure 7). These trusses were already developing at the time of the inoculation. Similarly, in the UK trial, marbling on fruit was seen within four weeks after inoculation in plants infected with *PCH-06/104*.

Overall, isolate *PCH-06/104* reduced the yield of Class I fruit in the first seven weeks of harvests. This represents a drop in yield of Class I fruit of 23%. Consequently, there was a 60% increase in Class II fruit in the same period. In weeks 8 to 14, there was no effect of virus on fruit quality.

In the 2007/08 (1066) trials, Class I yields were higher in the beginning of the season than later in the season. Isolate *1066* did not affect the yield of Class I, Class II or waste compared with non-infected control plants.

Table 5a. Effect of PepMV isolate *1066* on yield of tomato (g/plant/week) over a 14 week harvest period. Values in brackets are changes in yield within each class category as a result of PepMV infection (% yield compared with healthy control plots). A minus value represents a yield reduction due to virus infection. * and ** represent significant yield effects at $P<0.05$ and $P<0.01$ respectively.

	Class I			Class II			Waste		
Country	Healthy	Inoculated		Healthy	Inoculated		Healthy	Inoculated	
Hungary	165.2	144.7	(-12.4%)	59.4	60.4	(1.6%)	47.2	69.2	(46.4%)**
Netherlands	661.4	642.9	(-2.8%)	57.2	61.9	(8.2%)	2.1	3.1	(47.1%)
Spain	17.1	25.9	(50.8%)	134.1	144.2	(7.5%)	215.3	204.4	(-5.1%)
UK	580.6	567.7	(-2.2%)	55.2	90.7	(64.6%)*	4.3	3.8	(-10.7%)
Country Mean	**266.6**	**256.1**	**(-4.0%)**	83.2	96.0	**(15.4%)**	88.5	91.3	**(3.2%)**

Table 5b. Effect of PepMV isolate PCH-06/104 on yield of tomato (g/plant/week) over a 14-week harvest period[2]. Values in brackets are changes in yield within each class category as a result of PepMV infection (% yield compared with healthy control plots). A minus value represents a yield reduction due to virus infection. *, ** and *** represent significant yield effects at P<0.05, P<0.01, and P<0.001 respectively.

	Class I			Class II			Waste		
Country	Healthy	Inoculated		Healthy	Inoculated		Healthy	Inoculated	
Hungary	218.0	153.7	(-29.5%)**	84.7	118.3	(39.7%)**	48.6	62	(27.6%)
Netherlands	642.7	558.3	(-13.1%)***	87.6	167.1	(90.7%)***	21.4	34.4	(60.7%)
Spain	63.5	48.7	(-23.3%)	161.6	123.8	(-23.4%)**	280.0	277.2	(-1.0%)
UK	593.6	530.3	(-10.4%)**	60.1	93.9	(56.2%)**	37.5	37.8	(0.8%)
Country Mean	**376.2**	**320.4**	**(-14.8%)*** **	**98.3**	**123.9**	**(26.0%)*** **	**96.9**	**102.8**	**(6.1%)**

[2] The means presented in the analysis of variance are calculated after estimating the values for the weeks, treatments, countries and replicate blocks when data were missing. As a result of this, the predicted means will therefore not match the observed means exactly for the treatments for each country where missing values were present (and in the case of the Netherlands, data were collected beyond 14 weeks).

Conclusions

Replicated semi commercial-scale trials were carried out over two consecutive seasons in four major tomato producing areas throughout Europe (i.e. Hungary, the Netherlands, Spain and UK) to assess the economic effect of PepMV under different environmental and cropping conditions. A mild isolate, 1066, had no effect on total fruit yield or yield of Class I, Class II or waste fruit over a 14 week production. *PCH-06/104*, selected as an aggressive PepMV isolate, reduced the yield of Class I fruit by around 14% over a 15-week period ($P<0.001$). This isolate also reduced total fruit yield by just over 4% ($P=0.057$). The effect of isolate *PCH-06/104* on Class I and total yield reduction tended to be greater during the earlier part of the season.

There were differences in fruit yields between countries. In general, the trials in the southern European sites produced lower yield that those in the northern countries. Despite these differences, the effects of the aggressive isolate, *PCH-06/104*, on Class I fruit yield was consistent: yields were reduced by between 10 and 29%. The effects of *PCH-06/104* on total fruit yield was less consistent: yields were reduced by between 4 and 11% in three out of four countries. In one country, the Netherlands, total yield was unaffected by *PCH-06/104*. All results are summarized in table 6.

Table 6. Summary of he effects of the PepMV isolates *1066* and *PCH-06/104* on tomato plants grown under conditions similar to the commercial practice in the UK, Hungary, Spain and the Netherlands.

Isolate	***1066* (mild isolate)**				***PCH-06/104* (aggressive isolate)**			
Country / **Effect on:**	**UK**	**Hungary**	**Spain**	**Netherlands**	**UK**	**Hungary**	**Spain**	**Netherlands**
Fruit set	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*
Symptoms in plant heads and foliage	Mild symptoms: Yellow spots	Mild symptoms: Yellow spots	Mild symptoms: One plant with yellow spots, necrosis on petals	Mild symptoms: Yellow spots and discoloration	leaf bubbling, mild mosaic	leaf bubbling, scorch, yellow spots	Mild symptoms: yellow spots, necrosis on petals	Clear symptoms shortly after inoculation, mild symptoms later on
Total yield - weight	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	10% reduction	*No effect*
Total yield - No. of fruits	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*
Fruit size distribution	*No effect*	*No effect*	Slightly larger fruits	No effect	*Smaller fruits early season*	smaller fruits	No general effect on size	*No effect*
Fruit quality (% downgrading from Class I to Class II)	*No effect*	*No effect*	*No effect*	*No effect*	10%	29%	23%	13%
Fruit symptoms	*No effect*	Uneven ripening observed, but not PepMV related	*No effect*	Uneven ripening observed, but not PepMV related	Marbling and uneven ripening	Marbling and uneven ripening observed	*No effect*	Increased occurrence of uneven ripening
Shelf-life	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	Small negative effect early in the season