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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

The ability to detect tomato brown rugose fruit virus (ToBRFV) from leaves is influenced by the growth stage at which the plant is infected, however, by sampling different plant parts (upper leaves, fruit, sepals) we can maximise the chance of detecting the virus.

Background

Tomato brown rugose fruit virus is a rapidly emerging virus of significant economic and regulatory importance. It emerged in 2014 in Jordan and has since entered production systems and spread to most tomato growing regions in the world, including now being reported affecting tomatoes and peppers across most of Europe, The Americas and Asia. As part of the ongoing efforts to mitigate against the risk of ToBRFV in the UK, both plant health regulatory authorities and growers are routinely requesting testing for the virus from propagation plants (plants for planting), production crops and from import/packhouse fruit. It is therefore crucial to understand how the results of laboratory tests relate to infection status of plants to allow accurate interpretation and reporting of test results.

Summary

Trials were conducted to investigate the development of infection of ToBRFV. These trials attempted to mimic growing conditions in UK crops, and were set up in a mock hydroponic set up, under quarantine conditions at Fera in York, UK (see figure 1 (a) and (b)). To keep the trials relevant to the UK industry the cultivars Roterno and Piccolo were used, with four plants of each variety included in each “treatment”. In each case plants were brought into the glasshouse.

Four treatments were investigated namely:

- Winter crop (initiated - 04/11/2020)
    - Glasshouse 1: Early inoculation on entry to glasshouse – 04/11/2020
    - Glasshouse 2: Late inoculation after 9 weeks in glasshouse – 06/01/2021
- Spring crop (Initiated – 21/04/2021)
    - Glasshouse 3: Early inoculation on entry to glasshouse – 21/04/2021
    - Glasshouse 4: Late inoculation after 9 weeks in glasshouse – 16/06/2021

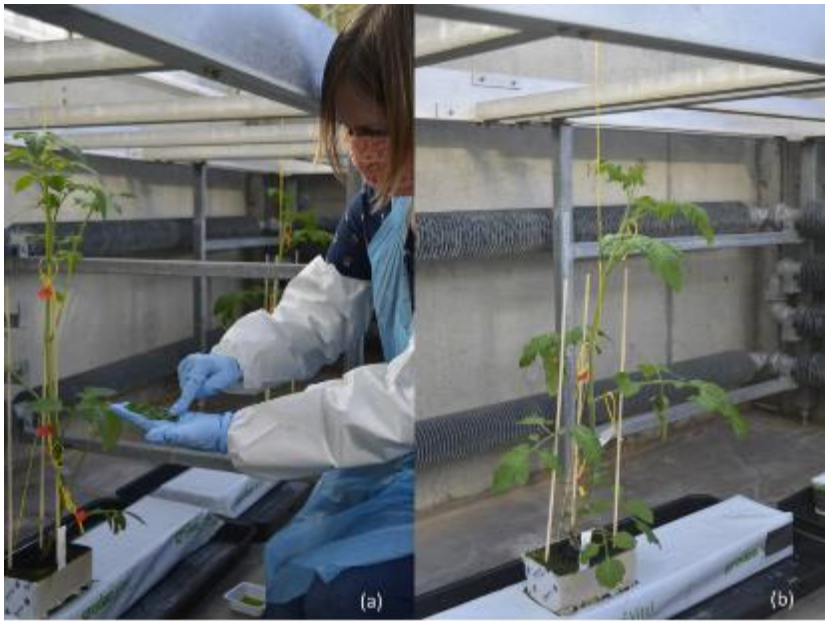

Figure 1. (a) Inoculation of tomato plants showing specific biosecurity measures and mock-hydroponic set up. (b) Inoculated plant showing nylon twin with white label denoting inoculation point.

Initial trials (Winter crop/early infection) ran for 140 days (20 weeks), based on the results from these initial trials, and due to the deterioration in the late infected plants, subsequent treatments ran for 126 days (18 weeks), with additional sampling points included early in the trial to give greater resolution to the initial stages of infection. Following inoculation, plants were sampled on the following schedule: day 2, 5, 7, 9, 12, weekly for weeks 2 through 12 and fortnightly for weeks 14, 16, 18 and 20. Samples were taken of leaves from the upper, middle and lower parts of the plant. When present, samples were also taken of sepals and ripe fruit. Additionally, symptoms were recorded, and a photographic record kept throughout the trial.

In total over 1600 plant samples were tested for the presence of ToBRFV. Samples were tested following standard Fera testing procedures to replicate the routine testing carried out by the laboratory in accordance with UK, EU and EPPO requirements. Briefly, nucleic acid was extracted from samples and tested using real-time RT-PCR, with results expressed as cycle threshold (Ct) values, where the lower the Ct value is indicative of a greater titre of virus (i.e the reaction has detected the presence of virus earlier due to high titre). Due to many laboratories applying a Ct “cut off”, for further analysis where result interpretation was required an arbitrary Ct-value <31Ct was applied. A Ct value of 40 would be considered no virus detected. This reflects the current approach in the laboratory to determine a positive result from an “inconclusive” or “negative” result.

There were slight differences in the speed at which virus was detectable from different plant parts observed between winter and spring crops. However, the most marked difference in the pattern of infection development in different plant parts was observed between early and late infection points, consequently showing a different response dependent upon the physiological age of the plant at time of infection.

In early infected plants (Circa 8 weeks old) detection from leaves of early infected plants looks to be predictable with the virus detected from leaves at the top of the plant approximately 2 weeks after inoculation with middle and lower leaves becoming infected approximately 2 and 4 weeks later respectively. An example of this is shown in figure 2 below.

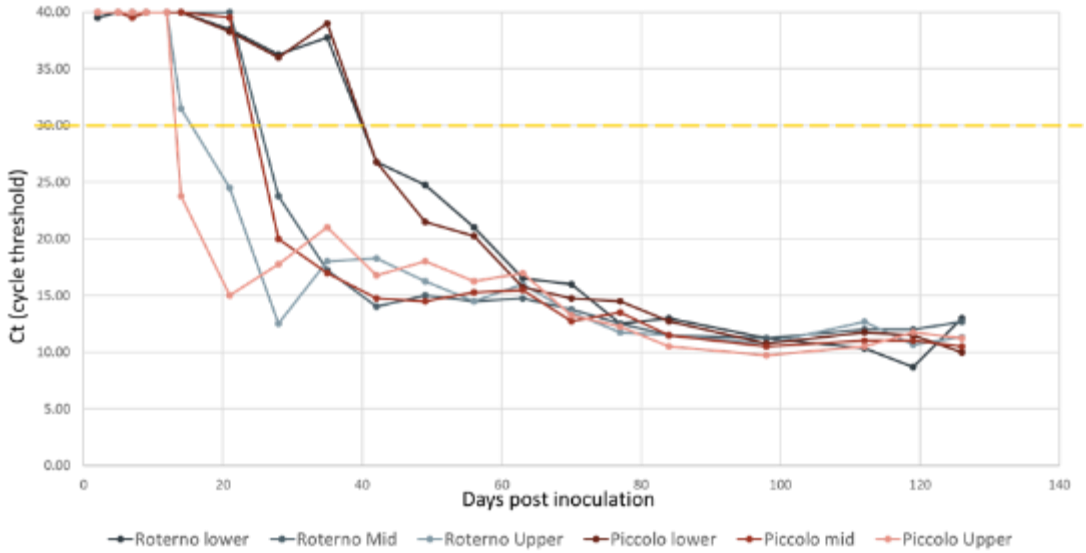

Figure 2. Example data for early inoculation results showing Cycle Threshold (Ct) results for Winter crop/Early inoculation, showing development of infection from leaf detection. (Lower Ct equates to a higher titre of virus in a sample).

When mature plants were inoculated the plants appeared to be less susceptible to infection, with fewer plants becoming infected, in this case seven out of 16 plants inoculated in the late treatments compared to 15 from 16 plants in the early treatments. The development of infection in different plant parts took much longer than early infections and was erratic, with some leaves of plants testing negative when leaves from other sites on the same plants were consistently testing positive (see figure 3 for example data). The earliest leaf detection from late inoculation treatments was in upper leaves after 28 days and 49 days in spring and winter crops respectively.

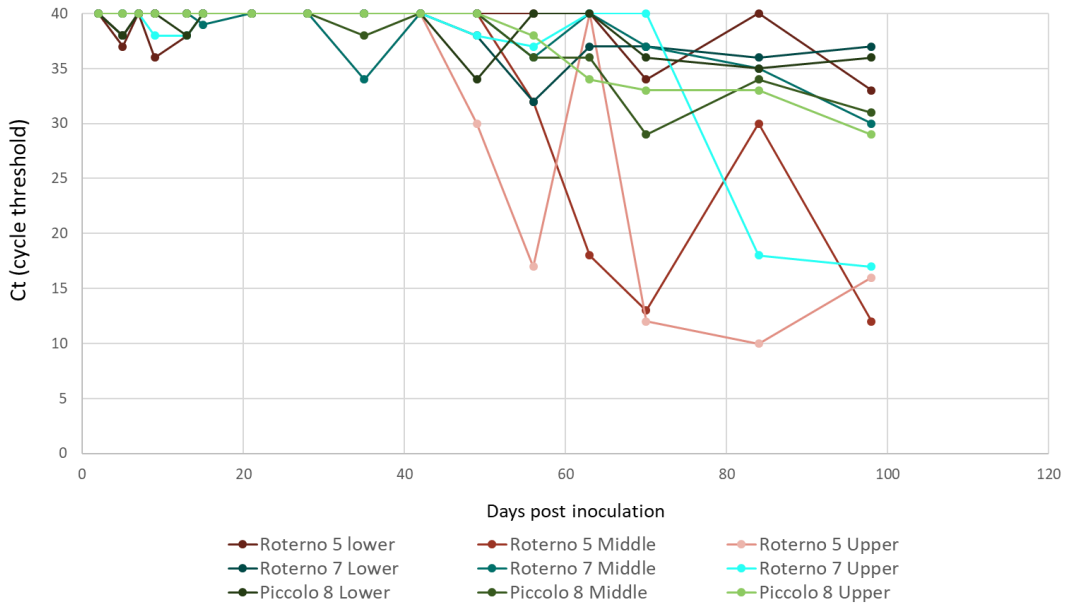

Figure 3. Example data for late inoculation results showing Cycle Threshold (Ct) results for Winter crop/Late inoculation, showing development of infection from leaf detection. (Lower Ct equates to a higher titre of virus in a sample).

Table 1. Days post inoculation of the first detection of ToBRFV from different plant parts (Leaf, sepal and fruit) and sampling sites for each treatment regardless of variety.

Infection time	Crop	Sample site	Leaf	Sepal	Fruit
Early	Spring	Lower	13	56	56
Early	Spring	Middle	28	63	63
Early	Spring	Upper	13	70	126
Early	Winter	Lower	28	77	77
Early	Winter	Middle	28	77	77
Early	Winter	Upper	14	77	112
Late	Spring	Lower	36	14	21
Late	Spring	Middle	2[a]	21	14
Late	Spring	Upper	28	21	21
Late	Winter	Lower	98	14	35
Late	Winter	Middle	63	35	35
Late	Winter	Upper	49	35	Inf

(a) individual plant result on the borderline of positive/inconclusive, virus was not detected again in this plant until 36 dpi.

Additionally, a comparison of detection from different plant parts and matrices was also carried out (see table 1). In early infected treatments (young plants) upper leaves were consistently found to be the sample site with most reliable detection. Sepals (Calyx) and ripe

fruit being found to be positive several weeks later. However, this is a reflection that these were the earliest sample points where sepals and ripe fruit were available for testing, and these were found to be positive at the first sample point.

In mature plants (late inoculation), sepals and fruit were found to be positive earlier than leaf samples. In most cases this was between one and three weeks earlier, however, in one case (Winter, late inoculation, lower plant) the sepals were positive for infection nearly 12 weeks earlier than leaf samples from the corresponding region on the sampled plants (see table 1).

Although this trial was limited in scope by the need to carry out the work under strict quarantine conditions, the similarity to previously published work, most notably a report from 1934, give cross validation to the reported findings.

Financial Benefits

Although these data do not correspond directly to financial benefits for individual growers, early detection of the virus, and retaining a high health status from this damaging pathogen ensures growers can continue to operate free from plant health restrictions. In the event of an outbreak, early detection can be instrumental in preventing further spread of the virus to other parts of a grower premises and help to inform the grower about the best course of action to limit further crop damage.

Action Points

When sampling plants for ToBRFV infection:

- Before sepals and fruit are present on the plant ensure samples are taken from the top of the plant/growing tips.
- Once sepals and fruit are present a sample of sepals and/or fruit should be taken in addition to leaf samples from the top of the plant/growing tips.

SCIENCE SECTION

Introduction

Tomato brown rugose fruit virus is a member of the genus *Tobamovirus*. The virus is rapidly emerging as a transboundary pathogen. Following an initial outbreak of tomato brown rugose fruit virus (ToBRFV) in Jordan in 2014/15 (Salem et al., 2016) the virus was subsequently also reported from Israel (Luria et al., 2017). The virus has rapidly emerged as a risk to commercial tomato and pepper production because it is able to overcome resistance genes, such as the *Tm-2*^{2} genes which provide resistance in tomato to several tobamoviruses (EPPO, 2020), therefore due to the risk to tomato crops the virus is under eradication in many countries where it has been detected (EPPO, 2021a). Infected crops suffer from reduced yield and fruit appearance and quality is affected resulting in loss of marketable yield (EPPO, 2020).

ToBRFV, like other tobamoviruses, is mechanically transmitted, It has also been demonstrated to be seedborne and may be spread by bumblebees (Levitzky et al., 2019, Davino et al., 2020, Salem et al., 2021). Once the virus has infected a plant in a susceptible crop it can spread through normal working practices and bumblebee pollination and under experimental conditions has been shown to infect a whole crop within a single cropping cycle (Panno et al., 2020). As with other robust contact transmitted pathogens where resistance strategies are not available, control of the virus relies on prophylactic biosecurity measures e.g. testing of seed and application of hygiene best practice measures (EFSA, 2011). The virus is robust, can remain infectious for at least 6 months in dried sap, and is resistant to many disinfectants on both seed and a range of glasshouse surfaces (Davino et al., 2020, Samarah et al., 2021, Skelton & Fox, 2021, Chanda et al., 2021a). To support surveillance inspections of plants and seeds it is important to be able to reliably detect the virus. Multiple diagnostic assays have been developed including serological assays using ELISA and molecular tests such as RT-PCR, RT-qPCR, LAMP and CRISPR (Alkowni et al., 2019, Alon et al., 2021, Bernabé-Orts et al., 2021, Chanda et al., 2021b, Fidan et al., 2021, Levitzky et al., 2019, Yan et al., 2021). Some of the molecular tests have been further validated for regulatory use and are recommended in international standards and for regulatory diagnostic activity (EU, 2020, EPPO, 2021b).

Whilst a great deal of research has been focused on developing and validating detection methods, less research focus has been given to the relative influence of sampling on diagnostic outcome, namely how much to sample, of which host tissues (matrices) to maximise the potential for detection. Recommended sample sizes for regulatory inspections are laid out within International standards on phytosanitary management (ISPM), standard 31

"Methodologies for Sampling of Consignments" (IPPC, 2008), with consideration being given to the thresholds of detection afforded by given number of samples from a consignment. Therefore, two key aspects which should be considered when sampling are the likely distribution of the pest/pathogen and also the diagnostic efficacy of the test being used. This latter aspect is often addressed through the generation of validation data, however, the expression of the target pathogen in the plant, and consequently the choice of sampled tissue, will also have major influence on the outcome of the test. However, little is known about the in-plant distribution of ToBRFV with respect to time after infection and plant age. In 1934, Samuel tracked the movement of tobacco mosaic virus (TMV) through tomato plants using an approach of sectioning up infected plants and testing them using bioassay. This was repeated in young and mature plants. In young plants, it was found that the virus could first be detected in the roots, before moving to the top of the plant, and eventually infecting every branch. Whereas, in mature plants, the virus could first be detected in the roots and then the top of plants, but the plant was never fully systemically infected and detection was erratic (Samuel, 1934). It is recognised that systemic infection of plants does not always occur and this asymmetric infection is noted more in viruses that move inefficiently. Further, even when systemic infection is achieved, the virus accumulates to different levels within the plants, the highest virus concentration being found in symptomatic leaves/stem (Hull, 2014a). Given that viruses tend to be unevenly distributed through plants, the choice of where to sample for a diagnostic test is crucial (Hull, 2014b).

Materials and methods

Virus isolates and inoculation:

The glasshouse trials were set up with the same basic format across the four treatments. To avoid cross contamination between trials each treatment was sited in a different glasshouse, but under identical conditions. These four treatments were:

- Winter crop (initiated - 04/11/2020)
    - Glasshouse 1: Early inoculation on entry to glasshouse – 04/11/2020
    - Glasshouse 2: Late inoculation after 9 weeks in glasshouse – 06/01/2021
- Spring crop (Initiated – 21/04/2021)
    - Glasshouse 3: Early inoculation on entry to glasshouse – 21/04/2021
    - Glasshouse 4: Late inoculation after 9 weeks in glasshouse – 16/06/2021

In each case, plants were approximately 8 weeks old when brought into glasshouse. In each treatment four plants of each of the two varieties were grown in a mock-hydroponic set up,

with each plant being of alternating variety. Additionally, two healthy control plants, one of each of variety were included in a separated mock-hydroponic set up. Plants were grown in insect proof glass house cubicles under an appropriate plant health quarantine licence. The set up was sterilised to mitigate against inadvertent contamination with ToBRFV following procedures from PE033/a. In all treatments the photo period was 16h light/8h dark with temperature maintained at 22^{o}C (day) 18^{o}C (night).

Plants were inoculated with a commercially available ToBRFV isolate (DSMZ, PV-1236) at a 1:1000 dilution. One leaf of each plant, approximately 1/3 of the way up the plant (approximately 0.5m from the base of the plant), was mechanically inoculated with the diluted isolate and celite following standard Fera procedures (see figure 1 a and b). Due to the persistence of the virus, to avoid inadvertent sampling from the inoculated leaf, inoculated leaves were marked by tying a piece of nylon twine with a white label around the petiole of the whole compound leaf ensuring sampled leaves were infected via systemic infection.

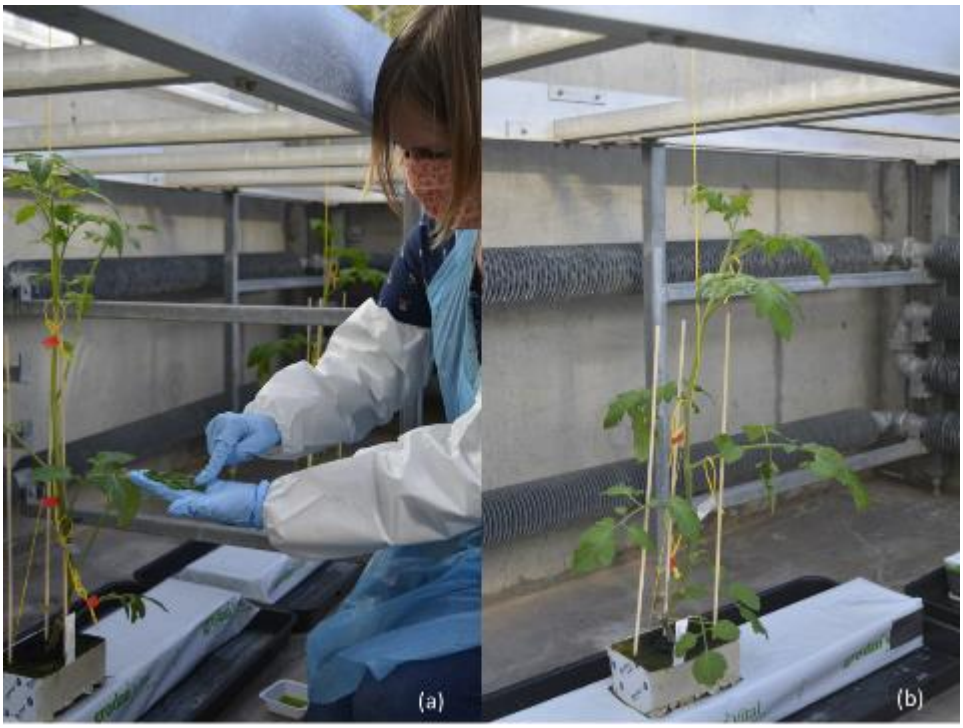

Figure 1. (a) Inoculation of tomato plants showing specific biosecurity measures and mock-hydroponic set up. (b) Inoculated plant showing nylon twine with white label denoting inoculation point.

Sample collection and symptoms:

Leaf: Symptoms were recorded and leaf samples were taken across 16-20 time points, Day 2, 5, 7, 9, 12, weekly for weeks 2 through 12 and fortnightly for weeks 14, 16, 18 and 20.

Sample collection was discontinued when plants were no longer fit for testing, generally when leaves were dried and necrotic. At each time point leaf samples were taken from the top, middle and bottom of each plant with gloves being changed between each sample. It is key to note that the same leaf was not sampled each time but leaves from the same region on the plant.

Sepals (calyx): Samples of sepals were taken from plants as they developed and recorded at the specific time point and plant location.

Fruit: tomato fruit were taken from plants in all four glasshouses as they were ripe. In winter crop treatments these were taken at two time points, but in spring crop treatments these were picked throughout the growth period with time point and sample location being recorded.

Additionally, In the spring replicates, side shoots were also collected when present at the time points. A piece of stem representing the top, middle and bottom of the ten plants were taken at the last time point of the spring late infection. In the Spring crop, early infection, additional plants were inoculated and destructively sampled for root material soon after infection. Roots were sampled at the end of the trial in line with the stem sampling above.

In each case, samples were stored at -80 °C until tested.

Total RNA extraction and ToBRFV screening:

The samples were ground using a HOMEX 6 (Bioreba) then RNA was extracted by magnetic bead extraction using Invimag Virus DNA/RNA mini-kit (Invitek GmbH). The RNA extracts were stored at -20 °C.

Real-time RT-PCR was performed using iTaq universal probes one-step reaction mix (Bio-Rad) containing 1 µl of total RNA extract. All samples were initially tested for cytochrome oxidase (COX) (Weller et al., 2000) as an internal control, then run in duplicate wells for ToBRFV (Menzel & Winter, Unpublished). All testing for ToBRFV was carried out on a QuantStudio 6 Flex Real-time PCR System according to the following the manufacturer's instructions.

The average cycle threshold (CT) value between the two wells was recorded for each sample and it was compared chronologically for leaves, and where applicable sepals, fruits, side shoots and roots.

Results

Over the time course of the four treatments over 1600 real-time PCR tests were carried out, therefore full results from the trial are presented in the appendices. Leaf sampling results are

included in Appendix 1, Sepals and Fruit are in Appendix 2. In each case these are presented as real-time RT-PCR Ct values without any form of interpretation as to “positive/negative”.

Interpretation of positive and negative results is made with reference to real-time RT-PCR cycle threshold values (Ct-values). Real-time RT-PCR, also known as RT-qPCR, expresses a result via the generation of fluorescence during a reaction in the presence of the target pathogen. This reaction is regulated by a number of heating and cooling cycles, and the number of these cycles taken before fluorescence reaches a detectable level is known as the Cycle Threshold value, or Ct value. The reaction runs for 40 thermal cycles (40Ct), however due to the high sensitivity of the test there is a difference between a result having some level of detectable fluorescence and that test result being interpreted as positive for infection. Within this section where possible an interpretation of “positive/negative” is made with reference to the Fera standard reporting procedures for ToBRFV detection in plant matrices where a Ct >31 is considered to be an inconclusive result, therefore for any further analysis below assumes a “positive” result to be <31Ct unless otherwise stated.

Although there were some minor differences between cropping time (Winter and Spring), there appeared to be minimal differences between varietal response and the most marked difference in detection was between early and late infection. A more detailed analysis of the probability of detecting a positive result from the different treatments is given below under the section “Analysis of leaf detection results”.

Overview of leaf detection

In early infections (Figure 2) the virus appears to be detectable at levels considered to be “positive” from leaves at the top of the plant approximately 14 days post inoculation, with detection in the middle of the plant two weeks later and detection in the lower leaves a further two weeks later. This relationship was broadly similar between varieties tested. Titre of virus. assessed crudely via Ct value, appears to increase more slowly in upper and middle leaves from Systemic infection throughout the plant appeared to plateau from 60 through to around 80 days post-inoculation. Plants inoculated early in the growth cycle were highly susceptible to infection, with 15 of the 16 plants inoculated over the two early treatments becoming readily infected. The other plant (Spring crop, late infection, Roterno, Plant 4) had some leaf detection results which were consistently of a Ct level to be considered positive, but a different pattern of detection was observed with the first “positive” in the middle of the plant after 28 days and the next leaf in the lower plant at 63 days, possibly indicating that this plant had become infected later in the growth cycle and not during the initial trial inoculation (See Appendix 1).

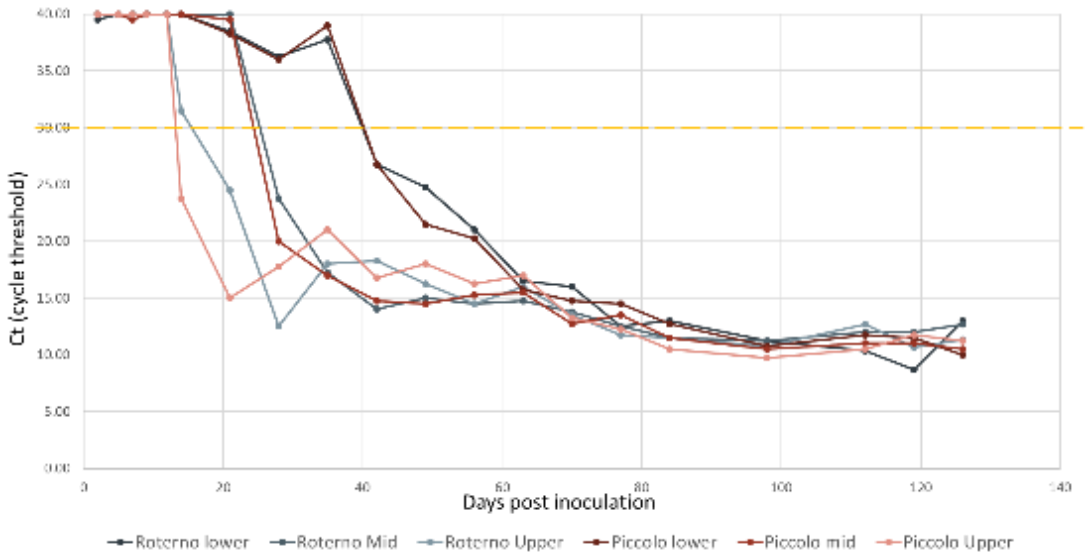

Figure 2. Example data for early inoculation results showing Cycle Threshold (Ct) results for Winter crop/Early inoculation, showing development of infection from leaf detection. (Lower Ct equates to a higher titre of virus in a sample).

In late inoculation trials the movement and consequent detection of the virus throughout the plant appeared to be highly erratic. The results shown in figure 3 highlight this issue, the results from three plants where results from some sample sections were consistently positive. In one case (Winter crop, late infection Roterno, Plant 5) the pattern of infection was similar to that observed in early infections, although the earliest infected leaf in that plant was detected at 49 days post inoculation, and the lower leaves positive 7 weeks after that (98 days post inoculation). In another case (Spring crop, Roterno, Plant 7) leaf detection was consistently strong in the top of the plant at every sample date from 28 days post inoculation (Ct 7-19), with the rest of the plant testing at levels which would be considered inconsistent or negative (40Ct) for a further 4 weeks. In most cases plants were exhibiting detectable levels of virus from very early in the trial, as early as day 2 post inoculation, however, these results were erratic and inconsistent throughout the trial and rarely at levels which would be considered to be clearly “positive”.

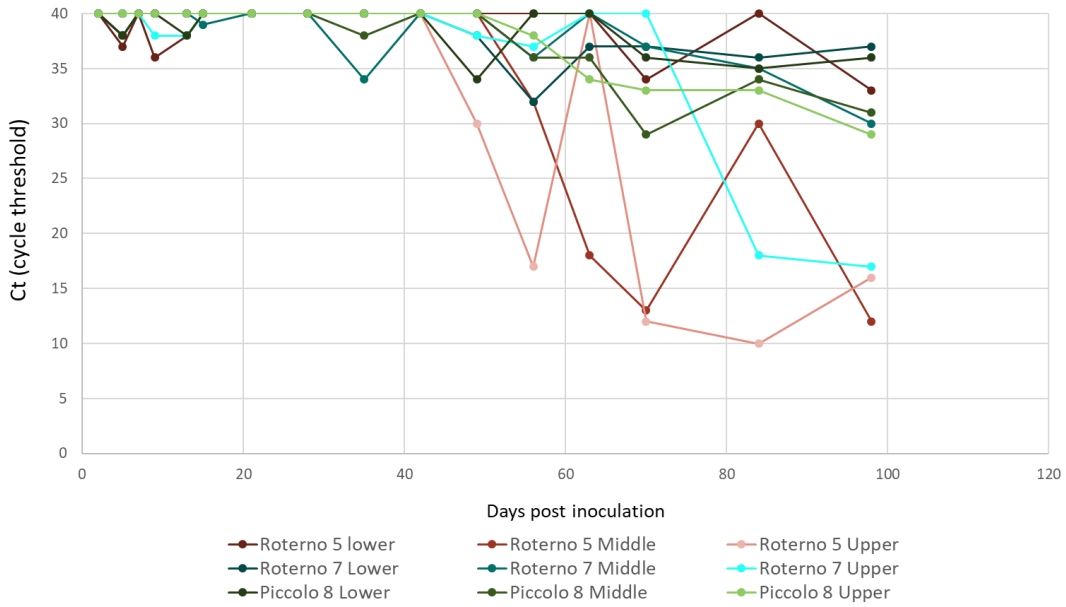

Figure 3. Example data for late inoculation results showing Cycle Threshold (Ct) results for Winter crop/Late inoculation, showing development of infection from leaf detection. (Lower Ct equates to a higher titre of virus in a sample).

Consequently, this erratic distribution and inconsistency of virus titre had a confounding impact on the interpretation of results for the determination positive and negative plants in the overall trial. Overall, fewer plants in the late inoculation treatments became infected, with 7 out of 16 plants having multiple “positive” results over the Ct <31 threshold.

Sepal and fruit timing compared to leaf

Results from the sepal and fruit testing are presented in table 1. In early crops sepals and fruit were not present for several weeks after inoculation. Consequently, virus is consistently detected earlier in the leaf samples than sepals and fruit, and generally in the upper leaves. The apparent delay in detection from sepals and fruit in these early inoculated plants is a consequence of timing of fruit development and ripening rather than virus movement. As soon as sepals and ripe fruit were available for testing virus was consistently detected from these plant parts.

In late inoculated treatments the sepals and fruit were generally found to be positive earlier than leaf samples. In one exception to this pattern, a Spring crop/late inoculated plant (Plant 2, Piccolo) was detected with a borderline positive result (Ct 31) in a leaf from the middle of the plant 2 days post inoculation, 12 days earlier than detection from fruit, and 19 days earlier than detection from sepals in the earliest virus detections in that treatment. However, no

further virus was detected in leaves from this plant until 36 days post inoculation, almost 5 weeks later, when the leaves were detected with strong virus levels in the upper leaf sample (Ct 10) and moderate virus levels (Ct 28) in the middle leaf sample. By comparison in the specific plant the sepals and fruit were both strongly positive at 21 days post inoculation (Ct 13 and 12 respectively).

Table 1. Days post inoculation of the first detection of ToBRFV from different plant parts (Leaf, sepal and fruit) and sampling sites for each treatment regardless of variety, na= no date at which samples were detected with virus at Ct<31

Infection time	Crop	Sample site	Leaf	Sepal	Fruit
Early	Spring	Lower	13	56	56
Early	Spring	Middle	28	63	63
Early	Spring	Upper	13	70	126
Early	Winter	Lower	28	77	77
Early	Winter	Middle	28	77	77
Early	Winter	Upper	14	77	112
Late	Spring	Lower	36	14	21
Late	Spring	Middle	2[a]	21	14
Late	Spring	Upper	28	21	21
Late	Winter	Lower	98	14	35
Late	Winter	Middle	63	35	35
Late	Winter	Upper	49	35	na

a) individual plant result on the borderline of positive/inconclusive, virus was not detected again in this plant until 36 dpi.

Analysis of leaf detection results

For the purposes of this analysis leaf results were assessed as either positive or negative, Therefore, samples which would be interpreted as “inconclusive” were treated as negative results, i.e samples which produced a Ct of 31 or lower were scored as positive; other samples were scored as negative.

Detecting plants infected with virus by testing leaves

A binomial generalised linear mixed model was fitted the to the positive and negative results to provide an estimate of the probability that a leaf would give a positive result under the conditions examined in this study. The model was based on the assumption that there was an underlying probability for each glasshouse; that the probability increased linearly (on the logit scale) over time-since-inoculation with a gradient that depended on each combination

of: the age of plant at inoculation (Early, Late), the height of the leaf (Lower, Middle, Upper) and the crop (Spring, Winter). In addition, the probability was assumed to vary at random to some degree between plants.

The gradients of the model are shown in Figure 4.

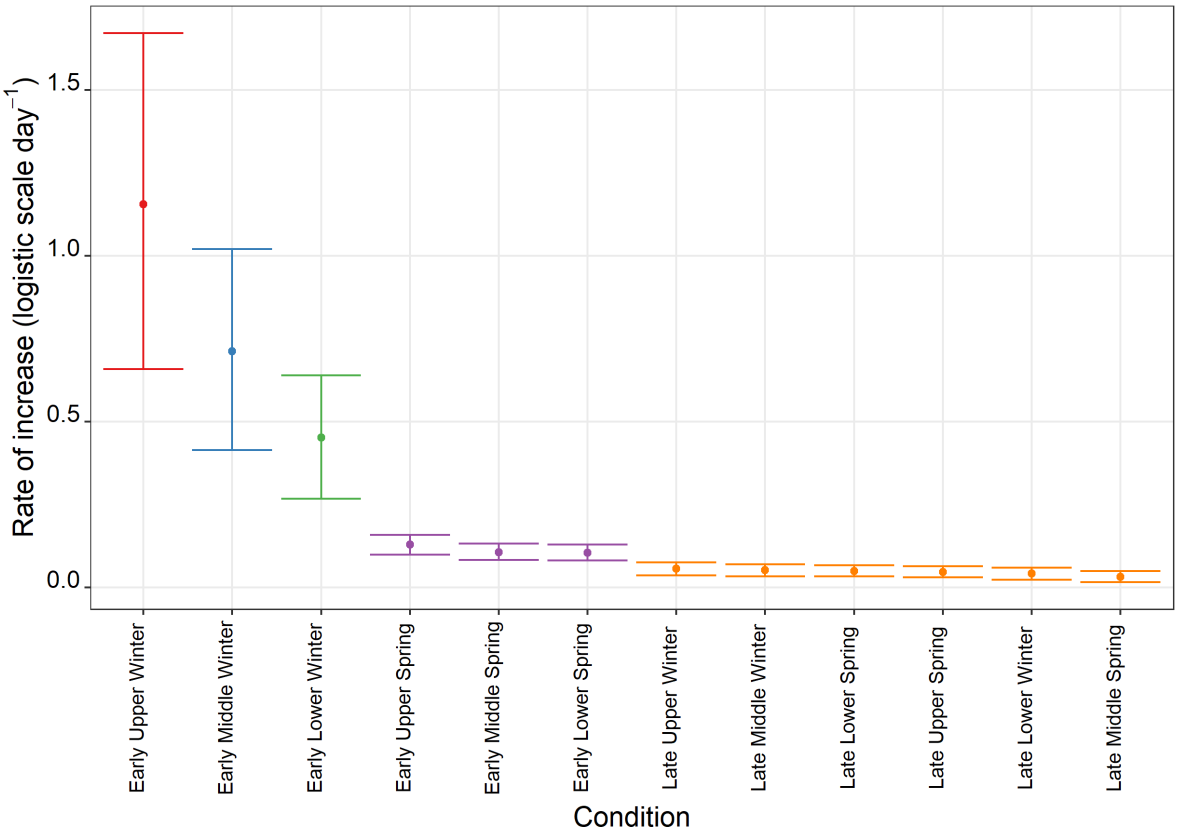

Figure 4: Rate of increase in probability that leaves will give a positive test result (conditions with different colours have a significantly different rate)

The significance of the difference between pairs of gradients was tested by sampling from the multivariate normal distribution describing each of the parameters and correcting for multiple comparisons. Based on this assessment and saying that rates are different for "p<0.05", the rate at which the probability increases is highest for upper leaves taken from winter crop plants infected early. The next highest rate is for middle leaves from the early-infected winter crop plants and the third highest rate is for lower leaves from those plants. The rates for leaves taken from early infected spring crop plants were lower than for early infected winter crop plants but higher than all late-infected plants. Significant differences between rates for different leaf-heights were not detected for the early-infected spring crop plants. Late-infected plants had a lower rate of increase in the probability that a leaf will give a positive response.

No difference in the rate of increase associated with leaf-height or crop was detected in leaves taken from late-infected plants.

Figure 5 shows the observed proportions of leaves giving a positive response and the estimated probability that a leaf will give a positive response under each of the conditions studied derived from the fitted model.

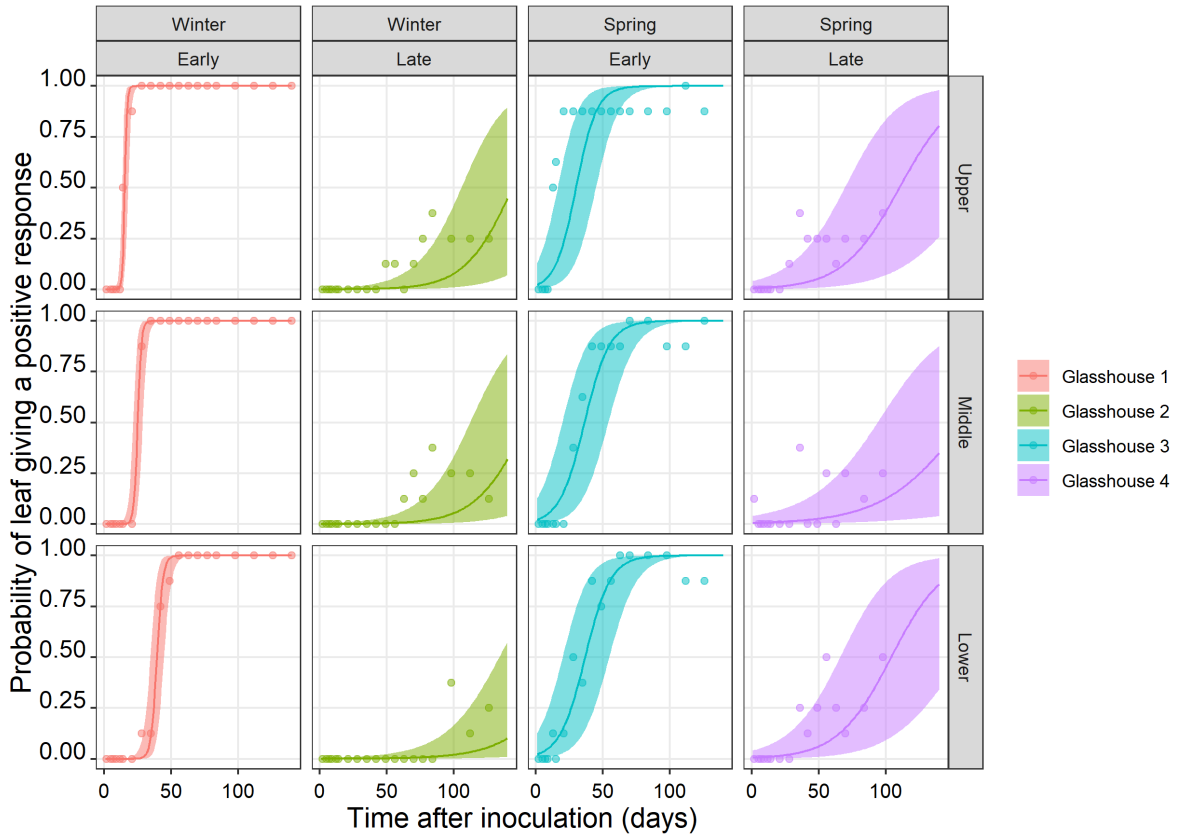

Figure 5: Observed proportions of positive leaves and estimated probability of leaf giving a positive result with 95% confidence interval

The estimated probabilities of a positive response (shown in Figure 5) were used to estimate the time after infection until 10, 50 and 90% of leaves will give a positive response. The central estimate with a range taken from the 95% confidence interval derived from the fitted model is given in Table 2. Early infection leads to a high proportion of positive leaves much faster than late infection, and a high proportion of positive leaves are found in winter crop more quickly than in the spring crop. Within crops that are infected early, a large proportion of high leaves are positive more quickly than middle and low leaves. We didn't detect the same patterns in plants which were infected later in growth but because the proportion of positive leaves was so much lower this is absence of evidence rather than evidence that a similar relationship is not present in late-infected plants.

Table 2: Estimated time after inoculation by which 10, 50 and 90% of leaves will give a positive PCR result

Infection time	Crop	Leaf height	Time for 10% positive (days)			Time for 50% positive (days)			Time for 90% positive (days)		
			Estimate	95% confidence interval		**Estimate**	95% confidence interval		**Estimate**	95% confidence interval	
Early	Winter	Upper	**14**	12	16	**16**	14	18	**18**	16	21
Early	Winter	Middle	**22**	18	26	**26**	22	29	**29**	25	33
Early	Winter	Lower	**35**	29	40	**40**	35	45	**45**	40	52
Early	Spring	Upper	**14**	1	27	**30**	17	45	**47**	33	65
Early	Spring	Middle	**16**	1	33	**37**	20	55	**57**	40	78
Early	Spring	Lower	**16**	1	33	**37**	21	55	**58**	41	79
Late	Winter	Upper	**105**	68	>140	**>140**	106	>140	**>140**	>140	>140
Late	Winter	Middle	**113**	72	>140	**>140**	113	>140	**>140**	>140	>140
Late	Winter	Lower	**140**	87	>140	**>140**	134	>140	**>140**	>140	>140
Late	Spring	Lower	**61**	22	103	**105**	67	>140	**>140**	106	>140
Late	Spring	Middle	**93**	34	>140	**>140**	94	>140	**>140**	>140	>140
Late	Spring	Upper	**64**	24	110	**111**	70	>140	**>140**	111	>140

These estimates can be used to inform the interpretation of test results for the presence of the virus: in particular, to interpret results where no virus is detected. Where no virus is detected in “n” randomly selected samples then an approximate upper limit (with 95% confidence) for the prevalence of infection in the population from which samples were taken is given by 3/(n.p) where p is the probability that an infected sample will give a positive result. Hence, if 200 randomly selected leaves from different plants are tested and found to be negative (e.g. 20 pools of 10 leaves each all found to be negative), and assuming the probability of detection is 100% for leaves from a plant that was infected a sufficiently long time ago, we can say that the prevalence of plants that were infected "a long time ago" is less than approximately 1.5% with 95% confidence. However, Table 2 shows that in some scenarios there is much less information, and much less assurance, provided by negative test results. For example, the 90% of leaves taken from the top of early-infected winter crop plants provide a positive result after 18 days (95% confidence interval 16 to 21 days). Hence if plants are young enough (in this case infected at no more than 8 weeks) and samples are leaves taken from the tops of plants then a finding of no positive results from 200 leaves tells us:

- that the prevalence of plants infected "a long time ago" is less than 1.5%;
- that the prevalence of plants infected at least **21 days** ago is no more than 1.7%;
- but that the test results tell us little about the potential presence of plants that may have been infected more recently than **16 days** ago

If young winter crop plants are tested but 200 leaves from locations other than the top of the plant are tested then a finding of no positive results is less informative about recent infection:

- the prevalence of plants infected "a long time ago" is less than 1.5%;
- the prevalence of plants infected at least **52 days** ago is no more than 1.7%;
- but the test results tell us little about the potential presence of plants that may have been infected more recently than **40 days** ago

Test results from older plants which may have been infected later (in this case 17 weeks old) are much less informative about plants which may have been infected recently. If 200 leaves are tested and provide only negative results:

- we still assume this means that the prevalence of plants infected "a long time ago" is less than 1.5%;
- *we think* that the prevalence of plants infected about **100 days ago** is less than **2%**;
- but we are not confident about that estimate. Infected plants may not be detectable even after 140 days.

Symptom expression

Observations on symptom development were made throughout the time course of the experiment and these are included in Appendix 3. The range of symptoms was scored on the basis of standard virology symptom descriptions. Below are some of the extensive range of photographs which were taken to illustrate different symptoms (Figure 6-12) including leaf yellowing (chlorosis), purple spotting, necrosis and fruit symptoms including mottle and splitting.

Despite attempts to mimic glasshouse conditions as closely as possible in a trial set up, the symptom development observed here may not be typical of those within a real outbreak scenario. Likely as a consequence of stress of sub-optimal growing conditions combined with virus infection all plants developed excessive levels of purpling followed by necrosis within weeks of inoculation.

Figure 6. Leaf showing bubbling, distortion and thinning, winter crop, early inoculation (28 dpi)

Figure 7. Leaves showing bubbling, leaf distortion and chlorosis, Winter crop, early inoculation (28dpi)

Figure 8. Leaves showing early signs of leaf chlorosis, Winter crop, early inoculation (28dpi)

Figure 9. Leaf showing severe chlorosis and necrosis, Winter crop, early inoculation (63 dpi)

Figure 10. Leaves showing purple spotting and necrosis, Winter crop, early inoculation (76dpi)

Figure 11. Fruit showing mottle. Spring crop, early inoculation (45dpi)

Figure 12. Fruit splitting, Winter crop, early inoculation (63 dpi)

Discussion

Since tomato brown rugose fruit virus was first described in 2014, it has now been reported from 31 countries in three continents. The virus infects tomato and pepper crops and causes severe impact through loss of yield and effects on fruit quality (EPPO, 2021a). The virus is mechanically transmitted and because the virus movement protein can overcome the *TM-22* resistance mechanism which protects against other tobamoviruses in commercial tomato crops (Hak & Spiegelman, 2021), once a crop is infected it can spread rapidly within an affected crop reaching 100% incidence (Panno et al., 2020). In the absence of genetic resistance, currently the only effective measures to control the introduction and onward spread of the virus are through a suite of biosecurity measure combining both regulatory mitigation measures, e.g. import seed testing and surveillance of production sites (EPPO, 2021b), and industry measures such as hygiene, staff vigilance, and cultural control measures similar to those recommended for limiting the spread of other contact transmissible tomato pathogens such as pospiviroids (EFSA, 2011). Therefore, it is critical to understand the reliability of detecting the virus with reference to infection dynamics within the plant to be able to better devise sampling strategies to maximise the chance of early detection of the virus. Currently, laboratories across Europe implement diagnostic “cut offs”, i.e. a decision threshold dependent up validation data supporting a positive/negative inference. Within Fera validation data of both assays currently in use in the laboratory, in a dilution series from infected leaf detection of ToBRFV could still be achieved reliably at 38Ct ($1x10^6$), however, it is not known how this dilution rate reflects “real” infection in a plant. For this reason, it is also crucial to understand how detection of virus relates to the development of the virus within the plant, and how this is affected by plant growth stage, cropping season and variety.

As early as 1934 the movement of viruses had been studied in tomato using the closely related tobacco mosaic virus (Samuel, 1934). This study, carried out decades before serological or molecular diagnostic tools were available, utilised biological testing (Sap inoculation to test plants) for confirmation of virus entering leaf tissue and different plant parts. This study, utilising the variety “Dwarf Champion”, indicated that the virus was detectable in the inoculated leaf approximately 3 days post inoculation (dpi); from the roots at 4 dpi; from the top of the plant at 5 dpi; and with fully systemic infection at 25 dpi. The results from both early inoculation treatments showed a similar pattern of development, despite minor differences between the two cropping times, where the virus is detectable in the upper plant first and then spreads back down through the plant.

On face value, comparing the results from the early inoculation in this study with those from Samuel (1934) suggests that initially there is a delay of approximately 8 days between the virus being present in the upper leaf tissue and it being detectable by real-time RT-PCR, but

detection of systemic infection through the whole plant was comparable. However, this may not be as clear as this initial comparison would suggest. Within the Samuel (1934) study plant material was excised at the stated sample date it was then incubated in a test tube with wet cotton wool and supplemental lighting for 7-10 days to bio-amplify the virus in the sample prior to detection, suggesting the corresponding days of detection are comparable across the two approaches. An additional complicating factor is that since the 1934 study commercial tomato plants now have the *TM-22* resistance gene. Whilst ToBRFV can overcome this genetic resistance, a recent study suggests that the gene will still attenuate movement of the virus, effectively slowing down the development of infection (Hak & Spiegelman, 2021). In work currently being conducted as part of AHDB PE035 studies on irrigation water and comparing to detection from the top of the plant suggest that for cv. “Moneymaker” the virus can be detected from young plants between 3 to 5 dpi (data not presented). Although these plants are approximately four weeks younger than the early inoculation plants from PE034, this suggests the real-time RT-PCR can detect the virus very early in the infection cycle. Additionally, the resistance status of the commercial varieties used in the PE034 is contributing to delaying the development of infection within the plant.

In another similarity to the Samuel (1934) study, infection in older plants showed a more erratic distribution. As noted by the 1934 study, “…the presence of a developing fruit truss a few nodes above the insertion may sometimes exert a pull...”, and this appears to be supported by the results from this study. In the late infection treatments, the sepals and fruit were consistently clearly determined as virus positive before the virus could be reliably detected from leaf tissue. Similarly, to the pattern of virus infection, this is accounted for by the active transport system in the plant being directed to the developing plant parts, i.e. the growing tips in young plants and the fruit in mature plants. This pattern of infection development, and consequent detection, indicates that sampling regimes should account for the presence of fruit trusses to maximise the chance of early discovery of the virus in an infected crop.

It is also key to note that more mature plants may not be as susceptible to infection as young plants, with a much lower proportion of plants developing infection. The phenomenon has been noted in other crops, mainly in relation to insect transmitted viruses of potato and cereal crops (Lindblad & Sigvald, 2004, Sigvald, 1985, Gibson, 1991). Further work on a larger scale is needed to confirm if this phenomenon is at play in this tomato-tobamovirus pathosystem but may indicate that the risk of virus infections in crops diminishes with the age of the crop. The applicability of these data to “real world” situations should be caveated with caution given the low numbers of plants tested and the “mock” conditions which could not accurately replicate a commercial glasshouse, the likely high levels of inoculum compared to a real

outbreak, and the obvious stress plants were under once inoculated within this trial. Additionally, there are “unknowns” regarding infection dynamics of plants infected at time points not covered within this trial. However, this is the first study to indicate the within plant spread of ToBRFV with respect to the chance of detecting the virus and gives a strong indication that sampling regimes should be altered to account for the results presented here.

Out with the scope of this trial, and those conducted under PE033/a, there are still serious knowledge gaps concerning the detection, survival and disinfection of ToBRFV within organic and other crops in soil-based growing systems. There are additional knowledge gaps on sustainable methods for disposal of infected planting material (e.g. composting). Both of these knowledge gaps require further research both in the UK and globally, and also ongoing knowledge sharing and co-operation through collaborative efforts such as the UK ToBRFV working group, to ensure the UK remains as biosecure as possible to protect growers from ToBRFV and other plant pathogens.

Conclusions

The data presented here indicate that early infected plants are likely to be more susceptible to infection, but pathogen spread is relatively predictable. In mature plants the virus movement within the plant is more erratic, but likely to be detected from fruit and sepals earlier than from leaves. Therefore, the main conclusion from this work is to recommend a two-tier sampling regime to account for this difference to maximise the chance of detection from a plant of unknown infection status at any given point in the growth cycle. Briefly these are:

- In crops prior to the development of fruit trusses, sampling should focus on leaves from the top of the plant
- In crops following fruit setting, a sampling regime should take leaves from the tops of plants, however, an additional sample of sepals and/or fruit should also be taken

Knowledge and Technology Transfer

The outcomes of this work have been presented at the following events:

- Regular updates to AHDB/TGA ToBRFV Steering Group (Monthly progress reporting)
- Presentation at TGA tomato growers conference 2021 (23rd September 2021, online)
- Presentation at Fera online Seminar, with invitation extended to Defra and APHA, EPPO virology panel members and other European stakeholders. Attended by approximately 50 Fera internal attendees, and 35 external attendees. (4 November 2021)

- Publication in preparation with an aim of submitting to EPPO Bulletin before end of 2021.

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Appendix 1

Leaf detection, Winter crops early infection (Glasshouse 1), late infection (Glasshouse 2), Ct values from each sampling point.

Sample set Leaf `	04/11/2020		Plant 1 (roterno)			Plant 2 (picollo)			Plant 3 (roterno)			Plant 4 (picollo)			Plant 5 (roterno)			Plant 6 (picollo)			Plant 7 (roterno)			Plant 8 (picollo)		
Glasshouse	Date	Day/week	Days post inoculation (dpi)	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper
Glasshouse 1	06/11/2020	Day 2	2	40	40	40	40	40	40	40	40	40	40	40	40	38	40	40	40	40	40	40	40	40	40	40	40
Glasshouse 1	09/11/2020	Day 5	5	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Glasshouse 1	11/11/2020	Day 7	7	40	40	40	40	40	40	40	40	40	40	38	40	40	40	40	40	40	40	40	40	40	40	40	40
Glasshouse 1	13/11/2020	Day 9	9	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Glasshouse 1	16/11/2020	Day 12	12	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Glasshouse 1	18/11/2020	Day 14	14	40	40	40	40	40	40	40	40	35	40	40	11	40	40	33	40	40	25	40	40	18	40	40	19
Glasshouse 1	25/11/2020	Week 3	21	40	40	24	40	40	13	34	40	40	39	39	17	40	40	18	40	39	14	40	40	16	34	40	16
Glasshouse 1	02/12/2020	Week 4	28	30	17	13	39	27	13	40	39	12	38	16	20	40	21	11	35	19	21	35	18	14	32	18	17
Glasshouse 1	09/12/2020	Week 5	35	40	14	22	40	19	23	40	23	15	40	19	19	40	18	18	40	18	22	31	14	17	36	12	20
Glasshouse 1	16/12/2020	Week 6	42	26	15	17	32	16	18	33	14	18	29	14	16	30	14	19	22	13	16	18	13	19	24	16	17
Glasshouse 1	23/12/2020	Week 7	49	21	14	16	31	14	16	34	14	16	21	15	20	25	16	16	16	14	16	19	16	17	18	15	20
Glasshouse 1	30/12/2020	Week 8	56	20	14	14	30	15	17	27	14	16	16	15	16	20	15	14	14	14	16	17	15	14	21	17	16
Glasshouse 1	06/01/2021	Week 9	63	18	16	17	16	15	20	18	14	15	16	15	16	14	15	16	16	15	16	16	14	16	15	17	16
Glasshouse 1	13/01/2021	Week 10	70	16	16	15	17	14	17	23	15	15	12	11	12	16	12	12	13	12	12	9	12	12	17	14	12
Glasshouse 1	20/01/2021	Week 11	77	11	12	10	14	13	11	13	12	13	13	14	15	14	14	13	18	15	11	12	12	11	13	12	12
Glasshouse 1	27/01/2021	Week 12	84	11	11	11	12	12	11	17	11	13	13	11	11	12	12	11	11	12	10	12	12	11	15	11	10
Glasshouse 1	10/02/2021	Week 14	98	12	11	12	11	9	10	13	13	10	11	11	9	9	11	11	11	12	9	11	10	10	10	10	11
Glasshouse 1	24/02/2021	Week 16	112	N/A*	N/A	N/A	10	11	11	11	11	10	14	12	10	11	14	11	12	11	11	9	11	17	11	10	10
Glasshouse 1	10/03/2021	Week 18	126	N/A	N/A	N/A	11	11	11	9	13	11	13	10	11	9	11	11	11	11	16	8	12	10	11	12	9
Glasshouse 1	24/03/2021	Week 20	140	N/A	N/A	N/A	11	12	11	8	11	12	11	10	10	19	13	12	9	11	12	12	14	10	9	9	12
		06/01/2021		Plant 1 (roterno)			Plant 2 (picollo)			Plant 3 (roterno)			Plant 4 (picollo)			Plant 5 (roterno)			Plant 6 (picollo)			Plant 7 (roterno)			Plant 8 (picollo)		
Glasshouse 2	08/01/2021	Day 2	2	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Glasshouse 2	11/01/2021	Day 5	5	40	40	34	40	37	40	40	38	40	40	33	38	37	38	40	40	38	40	38	40	40	38	40	40
Glasshouse 2	13/01/2021	Day 7	7	40	40	40	40	37	40	40	40	33	38	38	40	40	40	40	40	38	40	40	40	40	40	40	40
Glasshouse 2	15/01/2021	Day 9	9	40	40	40	40	40	40	40	40	40	40	40	40	36	40	40	34	40	40	40	40	38	40	40	40
Glasshouse 2	18/01/2021	Day 12	12	40	40	40	40	40	40	40	40	40	40	40	38	38	40	40	36	38	40	40	40	38	38	40	40
Glasshouse 2	20/01/2021	Day 14	14	40	38	40	40	40	40	40	38	37	38	37	40	40	40	40	37	40	38	40	39	40	40	40	40
Glasshouse 2	27/01/2021	Week 3	21	40	40	40	38	38	38	40	40	40	40	40	37	40	40	40	38	39	40	40	40	40	40	40	40
Glasshouse 2	03/02/2021	Week 4	28	37	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Glasshouse 2	10/02/2021	Week 5	35	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	38	40	40	34	40	40	38	40
Glasshouse 2	17/02/2021	Week 6	42	40	40	40	40	40	40	40	40	40	40	40	38	40	40	40	40	40	40	40	40	40	40	40	40
Glasshouse 2	24/02/2021	Week 7	49	39	40	40	40	40	40	38	37	40	40	40	40	40	40	30	38	40	40	38	40	38	34	40	40
Glasshouse 2	03/03/2021	Week 8	56	40	40	40	40	40	40	40	40	40	40	40	40	40	32	17	38	40	40	32	36	37	40	36	38
Glasshouse 2	10/03/2021	Week 9	63	38	40	40	40	40	40	40	40	40	40	40	40	40	18	40	36	38	40	37	40	40	40	36	34
Glasshouse 2	17/03/2021	Week 10	70	36	40	40	37	40	40	40	40	40	40	40	40	34	13	12	40	40	37	37	37	40	36	29	33
Glasshouse 2	24/03/2021	Week 11	77	40	40	40	40	40	40	36	40	40	40	40	40	40	30	10	38	40	40	36	35	18	35	34	33
Glasshouse 2	31/03/2021	Week 12	84	40	40	40	37	40	40	40	40	40	40	40	38	33	12	16	32	40	40	37	30	17	36	31	29
Glasshouse 2	14/04/2021	Week 14	98	40	40	40	29	40	40	33	38	40	37	40	40	30	35	35	40	40	40	36	25	29	29	26	31
Glasshouse 2	28/04/2021	Week 16	112	40	37	40	40	37	38	34	40	40	40	37	40	31	40	10	40	40	40	33	29	33	32	17	12
Glasshouse 2	12/05/2021	Week 18	126	40	40	40	35	40	38	34	40	40	40	40	40	16	39	22	37	40	38	17	40	40	33	9.5	28
Glasshouse 2	26/05/2021	Week 20	140																								

Leaf detection, Spring crop early infection (Glasshouse 3) and late infection (Glasshouse 4). Ct values from each sampling point.

				Plant 1 (picollo)			Plant 2 (roterno)			Plant 3 (picollo)			Plant 4 (roterno)			Plant 5 (picollo)			Plant 6 (rotemo)			Plant 7 (picollo)			Plant 8 (roterno)		
		21/04/2021																									
Glasshouse 3	23/04/2021	Day 2	2	36	40	40	40	36	40	40	40	40	36	40	40	40	40	38	40	40	40	40	40	40	37	37	40
Glasshouse 3	26/04/2021	Day 5	5	40	40	40	40	40	40	40	37	40	40	40	40	40	38	40	40	40	40	40	38	40	38	40	36
Glasshouse 3	28/04/2021	Day 7	7	40	40	40	40	40	40	40	40	40	40	36	40	40	40	40	40	40	40	37	35	40	40	40	40
Glasshouse 3	30/04/2021	Day 9	9	40	40	40	36	36	40	40	37	40	40	36	40	35	40	40	40	40	40	40	37	40	40	40	40
Glasshouse 3	04/05/2021	Day 13	13	40	36	40	40	38	40	32	36	40	40	40	38	38	40	21	40	40	17	40	37	30	29	40	20
Glasshouse 3	06/05/2021	Day 15	15	40	33	40	40	37	28	40	40	40	40	34	35	40	40	14	34	34	17	35	40	16	40	36	20
Glasshouse 3	12/05/2021	Week 3	21	40	35	15	40	40	11	40	40	13	40	40	40	30	40	14	40	37	10	40	40	10	38	40	10
Glasshouse 3	19/05/2021	Week 4	28	37	38	10	28	35	11	29	40	12	40	30	34	28	10	14	34	34	13	26	35	12	33	29	12
Glasshouse 3	26/05/2021	Week 5	35	34	37	13	35	8	13	26	11	12	37	35	40	23	10	11	21	14	16	33	24	11	34	40	19
Glasshouse 3	02/06/2021	Week 6	42	25	11	12	31	13	16	10	7	10	36	36	35	11	9	10	16	14	14	16	13	11	30	14	12
Glasshouse 3	09/06/2021	Week 7	49	27	12	17	27	12	13	17	11	14	40	38	40	11	13	13	32	11	12	16	13	16	31	10	15
Glasshouse 3	16/06/2021	Week 8	56	22	14	15	20	11	13	24	13	14	40	33	40	11	15	17	12	11	17	23	10	12	12	11	11
Glasshouse 3	23/06/2021	Week 9	63	17	12	17	19	11	11	13	15	10	31	37	38	11	10	11	11	10	15	12	12	10	11	10	11
Glasshouse 3	30/06/2021	Week 10	70	12	12	10	14	13	13	13	12	10	20	30	36	12	10	11	10	12	12	12	9	10	10	12	13
Glasshouse 3	14/07/2021	Week 12	84	12	10	11	12	9	12	10	12	12	28	28	34	10	12	11	12	11	11	9	10	10	7	11	15
Glasshouse 3	28/07/2021	Week 14	98	12	10	10	29	12	11	13	12	13	26	35	33	11	8	10	10	12	11	10	9	10	10	11	10
Glasshouse 3	11/08/2021	Week 16	112	12	10	9	14	10	12	16	13	12	32	34	30	12	14	12	14	13	15	12	12	11	13	15	19
Glasshouse 3	25/08/2021	Week 18	126	13	12	10	23	12	12	12	14	15	35	31	32	10	12	12	13	12	12	12	11	10	12	11	20
				Plant 1 (roterno)			Plant 2 (picollo)			Plant 3 (rotemo)			Plant 4 (picollo)			Plant 5 (roterno)			Plant 6 (picollo)			Plant 7 (roterno)			Plant 8 (picollo)		
		16/06/2021																									
Glasshouse 4	18/06/2021	Day 2	2	40	32	40	40	31	39	40	40	40	40	34	37	40	40	40	40	40	40	40	40	40	40	40	40
Glasshouse 4	21/06/2021	Day 5	5	40	40	40	40	40	40	40	40	40	40	40	40	40	40	38	40	40	40	40	37	40	38	40	40
Glasshouse 4	23/06/2021	Day 7	7	40	40	40	40	40	40	37	40	37	40	40	40	38	40	40	40	40	40	40	40	40	40	40	38
Glasshouse 4	25/06/2021	Day 9	9	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Glasshouse 4	28/06/2021	Day 12	12	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Glasshouse 4	30/06/2021	Day 14	14	40	40	40	40	40	40	40	40	40	38	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Glasshouse 4	07/07/2021	Week 3	21	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Glasshouse 4	14/07/2021	Week 4	28	40	40	40	34	40	35	40	40	40	40	40	40	40	40	40	40	40	40	36	40	12	40	40	40
Glasshouse 4	22/07/2021	Week 5	36	33	40	37	36	28	10	35	40	40	38	36	36	27	27	32	24	25	23	36	32	7	40	40	40
Glasshouse 4	28/07/2021	Week 6	42	40	40	40	31	35	11	35	40	40	38	40	40	40	40	40	40	40	40	40	40	8	37	38	38
Glasshouse 4	04/08/2021	Week 7	49	40	39	40	21	35	36	40	36	40	37	38	34	34	40	19	18	40	40	40	40	10	34	34	37
Glasshouse 4	11/08/2021	Week 8	56	27	35	32	26	26	30	36	40	38	33	35	35	33	40	38	28	37	35	27	30	19	32	38	40
Glasshouse 4	18/08/2021	Week 9	63	32	33	36	35	32	37	40	40	40	37	40	40	30	34	40	40	40	40	37	35	9	27	40	40
Glasshouse 4	25/08/2021	Week 10	70	35	40	34	34	19	28	40	40	40	40	40	40	34	36	40	40	40	40	30	29	13	37	40	40
Glasshouse 4	08/09/2021	Week 12	84	34	39	36	14	25	13	33	34	40	35	34	40	37	40	40	32	40	36	32	33	11	24	37	34
Glasshouse 4	22/09/2021	Week 14	98	37	35	37	14	12	10	40	40	40	40	40	36	32	40	31	31	40	40	28	29	11	31	40	32

Appendix 2

Results of Sepals

Ct value of sepals from Winter crops early infection (Glasshouse 1), late infection (Glasshouse 2), Spring crop early infection (Glasshouse 3) and late infection (Glasshouse 4), indicating sample point.

Sample set Sepal			Plant 1 (roterno)			Plant 2 (picollo)			Plant 3 (roterno)			Plant 4 (picollo)			Plant 5 (roterno)			Plant 6 (picollo)			Plant 7 (roterno)			Plant 8 (picollo)		
Glasshouse		Day/week	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper
Glasshouse 1	20/01/2021	Week 11	11	13	13	11	14	16	11	11	14	11	12	16	15	13	14	10	14	17	14	13	16	11	16	16
Glasshouse 1	10/02/2021	Week 14	12	15	13	15	15	12	14	15	15	14	13	14	12	14	17	13	12	14	12	13	15	16	16	N/A
Glasshouse 2	20/01/2021	Day 14	40	39	N/A	40	40	40	40	40	40	27	37	38	40	40	38	40	40	40	40	40	40	40	37	40
Glasshouse 2	10/02/2021	Week 5	40	40	38	29	40	40	40	40	40	40	40	40	37	40	21	40	40	40	40	27	24	13	15	16
Glasshouse 2	28/04/2021	Week 16	40	40	40	40	40	38	40	40	36	40	40	37	18	40	13	39	40	40	40	40	11	9	9	10
			Plant 1 (picollo)			Plant 2 (roterno)			Plant 3 (picollo)			Plant 4 (roterno)			Plant 5 (picollo)			Plant 6 (roterno)			Plant 7 (picollo)			Plant 8 (roterno)		
		Day 2																								
Glasshouse 3	02/06/2021	Week 6																								
Glasshouse 3		Week 8	10																							
Glasshouse 3	23/06/2021	Week 9	10	11		14			13	14		34	38		12	12		14			14	12		13		
Glasshouse 3		Week 10	12	11	17	11	13	20	15	12	15	31	34	37	13	13	18	12	12	16	12	12	17	14	15	22
Glasshouse 3	14/07/2021	Week 12	12	13	15	13	12	13	15	14	18	29	35	37	15	15	16	11	10	14	14	13	11	12	13	16
Glasshouse 3	25/08/2021	Week 18	15	12	15	12	11	13	16	11		27	33	4	13	14	13	11	12	11	15	15	12	11	11	12
			Plant 1 (roterno)			Plant 2 (picollo)			Plant 3 (roterno)			Plant 4 (picollo)			Plant 5 (roterno)			Plant 6 (picollo)			Plant 7 (roterno)			Plant 8 (picollo)		
Glasshouse 4		Day 0		40		40			40			40														
Glasshouse 4		Day 7				40							40					40	40							
Glasshouse 4	30/06/2021	Day 14	40	40	40	28	35	40	40	40	40	40	40	40	40	40	40	40	40	38	40	40	40	40	40	40
Glasshouse 4		Week 3	40	40	40	13	14	14	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Glasshouse 4	14/07/2021	Week 4	40	40	40	15	12	13	40	39	40	40	40	40	40	40	40		40	40	40	17	16	40	40	40
Glasshouse 4	28/07/2021	Week 6	40	40	40	40	11	13	40	40	40	40	40	40	40	40	40		40	40	16	13	14	40	40	40
Glasshouse 4		Week 8			37		16			39	40			40			40			40			14			38
Glasshouse 4	18/08/2021	Week 9	35	39	40		10	11	40	40	40	40	40	40	40	40	40	40	40	40	15	13	16	39	40	40
Glasshouse 4		Week 10															40					17	14	40		
Glasshouse 4		Week 11															40					16	15			
Glasshouse 4	08/09/2021	Week 12	40	38	40		13	11	40	40	40	40	39	40	36	39	40	40	40	40	12	12	12	40	40	40
Glasshouse 4	20/10/2021	Week 18																								

Results of Fruit

Ct value of ripe fruit from Winter crops early infection (Glasshouse 1), late infection (Glasshouse 2), Spring crop early infection (Glasshouse 3) and late infection (Glasshouse 4), indicating sample point.

Sample set Fruit			Plant 1 (roterno)			Plant 2 (picollo)			Plant 3 (roterno)			Plant 4 (picollo)			Plant 5 (roterno)			Plant 6 (picollo)			Plant 7 (roterno)			Plant 8 (picollo)		
Glasshouse		Day/week	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper
Glasshouse 1	20/01/2021	Week 11	16	15	N/A	13	19	N/A	14	22	N/A	16	13	N/A	19	15	N/A	16	14	N/A	17	15	N/A	17	16	N/A
Glasshouse 1	10/02/2021	Week 14	21	17	16	14	14	15	18	17	N/A	14	14	14	18	15	N/A	14	13	N/A	14	16	N/A	14	16	N/A
Glasshouse 2	20/01/2021	Day 14	N/A	40	N/A	40	40	N/A	36	N/A	N/A	38	38	N/A	38	49	N/A	37	40	40	40	40	N/A	40	34	N/A
Glasshouse 2	10/02/2021	Week 5	33	37	N/A	40	30	N/A	33	36	N/A	40	36	N/A	27	27	N/A	38	34	N/A	38	14	N/A	13	12	N/A
			Picollo and roterno inverted for GH3!																							
			Plant 1 (picollo)			Plant 2 (roterno)			Plant 3 (picollo)			Plant 4 (roterno)			Plant 5 (picollo)			Plant 6 (roterno)			Plant 7 (picollo)			Plant 8 (roterno)		
Glasshouse 3	02/06/2021	Week 6																								
Glasshouse 3	16/06/2021	Week 8	13												17											
Glasshouse 3	23/06/2021	Week 9	14	15					16	20		40	40		13	12		16			17	13		14		
Glasshouse 3		Week 10	15	14		14	15			15		37	40		14	15		15	16		17	14			13	
Glasshouse 3		Week 11	15			15	14		13	13		36			14	14		14	15		13	13		15	14	
Glasshouse 3	14/07/2021	Week 12	15	15		16	15		13	15			40			14					14	14		15	16	
Glasshouse 3		Week 17												34						13	15		14		16	14
Glasshouse 3	25/08/2021	Week 18																					14			
			Plant 1 (roterno)			Plant 2 (picollo)			Plant 3 (roterno)			Plant 4 (picollo)			Plant 5 (roterno)			Plant 6 (picollo)			Plant 7 (roterno)			Plant 8 (picollo)		
Glasshouse 4	16/06/2021	Day 0																								
Glasshouse 4	30/06/2021	Day 14	40				31			40		36	37		40			40	40		40	40		40		
Glasshouse 4		Week 3					12	12	40				40		40	40		40	40		22	40				
Glasshouse 4	14/07/2021	Week 4		40			12	12	40	40			40			40			35		13	12			40	40
Glasshouse 4	28/07/2021	Week 6		40			12	12		39	40		37	40		40	40		40	38	12		11		38	39
Glasshouse 4		Week 8			37		16			39	40			40			40			40			14			38
Glasshouse 4	18/08/2021	Week 9																								
Glasshouse 4		Week 10															40					13	14	40		
Glasshouse 4		Week 11															40					16	15			
Glasshouse 4	08/09/2021	Week 12										40						39								
Glasshouse 4		Week 14									40	40						40						40		
Glasshouse 4	20/10/2021	Week 18																								

Appendix 3

Symptoms recorded from tomato plants throughout the trial.

Key to symptom recording:

B - bubbling of leaves
N - narrowing of leaves
D - distortion of the leaves
Da - dark coloured patches on the leaves
Bs - black speckling of leaves
Y - yellowing of leaves
R- ragged shaped leaves
NF- necrotic patches on fruit
I- interveinal yellowing (patches)
YS -yellow spots on leaves
IP- interveinal purple on leaves
Ne- necrosis of leaves
P- purple speckling
Ye - yellow speckling
Nt - necrosis on edge/tips of leaves
Nb - Necrosis of all leaves and branch up to stem
Yt - yellowing on edges/tips of leaves or near necrotic patches
Ns - Brown/Necrosis on stem

Winter/Early			Plant 1 (roterno)			Plant 2 (picollo)			Plant 3 (roterno)			Plant 4 (picollo)			Plant 5 (roterno)			Plant 6 (picollo)			Plant 7 (roterno)			Plant 8 (picollo)		
Glasshouse		Day/week	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper
Glasshouse 1	06/11/2020	Day 2																								
Glasshouse 1	09/11/2020	Day 5																								
Glasshouse 1	11/11/2020	Day 7																								
Glasshouse 1	13/11/2020	Day 9																								
Glasshouse 1	16/11/2020	Day 12																								
Glasshouse 1	18/11/2020	Day 14																								
Glasshouse 1	25/11/2020	Week 3																								
Glasshouse 1	02/12/2020	Week 4			Slight N,B,D,Da			N,B,D,Da						Severe N,B,D,Da			Slight N,B,D,Da			Severe N,B,D,Da			N,B,D,Da			Severe N,B,D,Da
Glasshouse 1	09/12/2020	Week 5			Slight N,B,D,Da			N,B,D,Da						Severe N,B,D,Da			Slight N,B,D,Da, Bs, Y			Severe N,B,D,Da, Bs, Y			N,B,D,Da, Bs, Y			Severe N,B,D,Da
Glasshouse 1	16/12/2020	Week 6		N, R	N,B,D,Da		N,B,D,Da	N,B,D,Da		NF	N,B,D,Da		Severe N,B,D,Da	Severe N,B,D,Da		Bl	Slight N,B,D,Da, Bs, Y		Severe N,B,D,Da, Bs, I,	Severe N,B,D,Da, Bs, Y	Bs, Y	N,B,D,Da, Bs, Y	N,B,D,Da, Bs, Y		N,B,D,Da	Severe N,B,D,Da
Glasshouse 1	23/12/2020	Week 7		N, R, Y	N,B,D,Da		N,B,D,Da	N,B,D,Da		Y, BS, NF	N,B,D,Da		Severe N,B,D,Da	Severe N,B,D,Da		Bs	Slight N,B,D,Da, Bs, Y		Severe N,B,D,Da, Bs, I,	Severe N,B,D,Da, Bs, Y	Bs, Y	N,B,D,Da, Bs, Y, NF	N,B,D,Da, Bs, Y		N,B,D,Da	Severe N,B,D,Da
Glasshouse 1	30/12/2020	Week 8	I	N, R,Y	N,B,D,Da		N,B,D,Da	N,B,D,Da	I (at edges of leaves)	Y, BS, NF	N,B,D,Da	Few Ye	Severe N,B,D,Da	Severe N,B,D,Da		Ye	Slight N,B,D,Da, Bs, Y	I, IP	Severe N,B,D,Da, Bs, I,	Severe N,B,D,Da, Bs, Y	Ne- especially leaf tips, I	N,B,D,Da, Bs, Y, NF, I	N,B,D,Da, Bs, Y		N,B,D,Da	Severe N,B,D,Da
Glasshouse 1	06/01/2021	Week 9	I	N, R,Y, NF (x2)	N,B,D,Da		N,B,D,Da	N,B,D,Da	I (at edges of leaves)	Y, BS, NF (x2)	N,B,D,Da	Few Ye	Severe N,B,D,Da	Severe N,B,D,Da		Ye	Slight N,B,D,Da, Bs, Y, NF(x1)	I, IP	Severe N,B,D,Da, Bs, I,	Severe N,B,D,Da, Bs, Y	Ne- leaf tips, I	N,B,D,Da, Bs, Y, NF (x2), I	N,B,D,Da, Bs, Y		N,B,D,Da	Severe N,B,D,Da
Glasshouse 1	13/01/2021	Week 10	I, Ne	N, R,Y, NF (x2)	N,B,D,Da		N,B,D,Da, P	N,B,D,Da	Ne at tips, P	Ne at tips, Y, BS, NF (x2)	N,B,D,Da	Ye, P	Severe N,B,D,Da	Severe N,B,D,Da		Ye, P, NE of leaf tips	Slight N,B,D,Da, Bs, Y, NF(x1)	severe I, IP	Severe N,B,D,Da, Bs, I,	Severe N,B,D,Da, Bs, Y	Ne- leaf tips, I	N,B,D,Da, Bs, Y, NF (x2), I, Ne of leaf tips	N,B,D,Da, Bs, Y	P	N,B,D,Da	Severe N,B,D,Da
Glasshouse 1	27/01/2021	Week 12																								
Glasshouse 1	10/02/2021	Week 14																								
Glasshouse 1	24/02/2021	Week 16				Y, P, Nt,	N, B, D, Nt, Ne	N, B, D, Nt, Ne	Ye, Nt, P	Nt, Ne, Yt,	N,B,D,Da, Nt	Y, P, Nt,	P, Ye, Nt, Ne	N, B, D, Da	Y, Nt(v. few)	Nb, Y	B, D, Da, Y, Nt	P, Y, Bs	Nt, Y, Bs,	N, D, Da, Y Bs	Ne, Nb(few), Y	Y, bs, Nb(some)	Nt, D, Bs, Ye	P, Y, Nt	Ne, Nt, D, Da, P	D, N, Nt, Ne, p
Glasshouse 1	10/03/2021	Week 18				Y, P, Bs, Nt, Nb	Y, Bs, Ne, Nb	N, D, Nt, Ne	Y, Bs, Nt	Ne, Nb, Y	N,B,D,Da, Nt	Nb, P, IP, Y, Nt	P, Ye, Ne, Nb	N, D, Ye, Bs	Y, Nt Bs	Ne, Nb	Y, Bs, Nt	P, Y, Bs, IP, Nt	Nb, Ne, Y P,	N, D, Da, Y Bs	Y, Bs, Nt, Ne	Nb, Ye	Y, Bs, Nt	P, IP, Y, Nt	Nb, P, Y, Ne	D, N, Nt, p
Glasshouse 1	24/03/2021	Week 20				Y, P, IP, Nt, Ne, Nb	Ye, Nt, Ne, Nb	D, y, Nt	Y, Bs, Nt, Ne, Nb	Nb (all)	Nb (all)	Y, P, Ne, Nb	P, Ne, Nt, Nb	Nt, P	Ye, Bs, Nt, Ne	Nb (all)	Y, Bs, Nt, Ne	Y, P, Nt, Ne, Bs	Nb (all)	Y, Bs, Nt, Ne, N, D	Ye, Bs, Nt, Ne, Nb	Nb, Ye	Ye, Bs, Ne	P, Ip, Y, Nt, Ne	Ne, Nb, Ns	Bs, Nt, Ye, N
Winter/Late																										
Glasshouse 2	08/01/2021	Day 2																								
Glasshouse 2	11/01/2021	Day 5																								
Glasshouse 2	13/01/2021	Day 7	P												P, Ye											
Glasshouse 2	15/01/2021	Day 9																								
Glasshouse 2	18/01/2021	Day 12																								
Glasshouse 2	20/01/2021	Day 14																								
Glasshouse 2	27/01/2021	Week 3																								
Glasshouse 2	03/02/2021	Week 4																								
Glasshouse 2	10/02/2021	Week 5																								
Glasshouse 2	17/02/2021	Week 6	Ye, Bs, Nt	Ye, Bs, Nt, Nb (on few)	D, Ye, Nt, Ne		P (small amount)		Ye, Nt	Nt, Nb (few)	D, Nt	Y, P	P (Less then L)	P(small amout), D, N	Ye, Nt (not bad)	Nt, Ne, Nb, D	D, Nt, Ne, N	Y, P,	P (less then lower)	D (small amount). P (less then lower)	Y, Bs (on eddge)	P, Yt, Nt	D, N, Bs, Nt	Y, P	P(less then L)	
Glasshouse 2	24/02/2021	Week 7	Ye, Bs, Nt	Ye, Bs, Nt, Nb (on few)	D, Ye, Nt, Ne	P (small amount)	P (small amount)		Ye, Nt	Ne, Nb(few)	D, Nt, Ne	Y, P	P (Less then L)	P(small amout), D, N	Ye, Nt (not bad)	Nt, Ne, Nb, D	D, Nt, Ne, N	Y, P,	P (less then lower)	D (small amount). P (less then lower)	Y, Bs (on edge) Nt	Yt, Bs, Ne, Nb (not many)	D, N, Bs, Nt, Ne (some	Y, P	P(less then L)	
Glasshouse 2	03/03/2021	Week 8	Ye, Bs, Yt Nt	Ye, Bs, Nt, Nb (on few)	D, Ye, Nt, Ne	P	P		Yt Nt	Ne, Nb,Ye	Nt Ne Nb	Y, P, IP	P	P, N	Ye, Nt (not bad)	Nt, Ne, Nb, Ye	Nt, Ne, N	Y, P, IP	P	D, P, Da	Y, Bs, Ne (few)	Yt, Bs, Ne, Nb	N, Yt, Bs, Ne, Nb	Y, P, IP	P	P
Glasshouse 2	10/03/2021	Week 9	Yt, Bs, Nt, Ne	Ye, Bs, Ne, Nb	Nb, Ye, Bs, D	P	P		Yt, Bs, Nt	Ne, Nb, Y	Ne, Nb,	Y, P, IP	P	P,	Y, Bs, Nt (not bad)	Ne, Nb, Nt, Ye	Ne, Nb, Nt, Ye	Y, P, IP	P	D, P, NT	Yt, Bs, Ne	Yt, Bs, Ne, Nb	N, Yt, Bs, Ne, Nb	Y, P, IP	P	p
Glasshouse 2	17/03/2021	Week 10	Yt, Bs, Nt, Ne	Yt, Bs, Ne, Nb	Yt, Bs, Ne, Nb	p	p		Yt, Nt, Bs	Ne, Nb, ye	Ne, Nb, ye	Y, P, IP	P, Ip	P	Yt, Nt, Bs,	Ne, Nb, (mostly Nb) Yt, Nt	Ne, Nb, (mostly Nb) Yt, Nt	Y, P, IP	Y, P, IP	Y, P, Nt	Yt, Ne, Nt, BS	Yt, Ne, Nt, Bs, Nb (mostly Nb)	Yt, Bs, Ne (nearly all)	y, P, IP	P, Ip	p
Glasshouse 2	31/03/2021	Week 12	Yt, Bs, ne	Ne, Yt, Bs, Nb	Yt, Bs, Ne, Nb	P, Ip	P,	P	Yt, Bs, Nt	Yt, Bs, Nt, Ne, Nb	Yt, Bs, Nt, Ne, Nb	Y, P, Ip	p, Ip	p, IP	Bs, Yt, Ne, Nt	Mostly Nb, Yt, Ne	mostly Nb, y	Y, P, IP	Y, P, IP	Y, P, Ip,Nt	Yt, Bs, Nt, Ne	Yt, nt, ne, Nb	Yt, nt, ne, Nb	Y, P, IP	P, IP	P
Glasshouse 2	14/04/2021	Week 14	Yt, Bs, Nt, Ne	Yt, Bs, Ne, Nb	Yt, Bs, Ne, Nb	Y, P, IP	P. IP	P. IP	Yt, Bs, Nt, Ne, Nb	Ye, Nt, Ne, Nb	Ye, Nt, Ne, Nb	Y, P, IP	p, ip	p, ip	Yt, Nt, Bs, Ne, Nt	P, Nb	p, Nb	Y, P, IP	Y, P, IP	Y, P, IP, Nt	Yt, Bs, Nt, Ne	Nt, Yt, Bs, Ne, Nb	Ne, Nb	Y, P, IP	P, Ip	P
Glasshouse 2	28/04/2021	Week 16	Ye, Bs, Nt, Ne,	Ye, Bs, Nt, Ne, Nb	Ye, Bs, Nt, Ne, Nb	Y, P, IP	P, Ip	P, IP	Yt, Bs, Nt, Ne	Ye (small amount), Ne, Nb	Ye (small amount), Ne, Nb	Y, P, Ip	P, IP	P, IP	Yt, Bs, Nt, Ne	P, NB	Nb	severe P	severe P	severe P	Y, P, Nb	Ne, Nb, yt, P	Ne, Nb, P	severe P	severe P	severe P
Glasshouse 2	12/05/2021	Week 18	p, Nb	p, Nb	p, Nb	severe P	severe P	severe P	I, Nb	I, Nb	I, Nb	severe P	severe P	severe P	severe P, Nb	severe P, Nb	severe P, Nb	severe P	severe P	severe P	severe P, Nb	severe P, Nb	severe P, Nb	severe P	severe P	severe P

Spring/Early			Plant 1 (Picollo)			Plant 2 (Roterno)			Plant 3 (Picollo)			Plant 4 (Roterno)			Plant 5 (Picollo)			Plant 6 (Roterno)			Plant 7 (Picollo)			Plant 8 (Roterno)		
			Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper
Glasshouse 3	23/04/2021	Day 2																								
Glasshouse 3	26/04/2021	Day 5																								
Glasshouse 3	28/04/2021	Day 7																								
Glasshouse 3	30/04/2021	Day 9																								
Glasshouse 3	04/05/2021	Day 13																								
Glasshouse 3	06/05/2021	Day 15																								
Glasshouse 3	12/05/2021	Week 3																								
Glasshouse 3	19/05/2021	Week 4	P		D	P, I	P, I, slight M	B	P				P	Slight B			B,N,D	P	Ys, P	N, D		P	B, N	P	P, Yt	
Glasshouse 3	26/05/2021	Week 5	Mild P		D, B	I	I	Mild B	P		B, D				IP		B, D	P	P		P		B, D	P, I		
Glasshouse 3	02/06/2021	Week 6	P		D, B	P, slight Yt	Yt, Nt	N	IP	P	B, D, N	IP	P		P	B	B, D	P, Y	Y, Yt, Nt	D	P	P, B	N, B, D	P, Yt	Yt, Nt	
Glasshouse 3	09/06/2021	Week 7	Bs		B, D	Yt, Nt, Bs, P	Yt, Nt	N, D	P, Bs	P, B, N, D	B, D, N	Yt, Bs	P		P Bs	B, N, D	B, N, D	P, bs, Yt, Nt, Ne (few)	Yt, bs, Nt,	D	P, Bs	B, N,	N, B, D	P, Yt, Bs	Yt, Nt, D	D
Glasshouse 3	16/06/2021	Week 8	P, Nt- 1 leaf	N,D,P	B, N,D	Ne,P,Nt	D,N,Ne, Nt	D,N	IP-on innoc branch, P, Nt-on innoc branch	B,N,D,P	B,N,D	IP-innoc branch, Ne,P,Yt	Ne,P,Yt	N,D	P,Yt	P,Yt,B,N, D	B,N,D	IP-at innnoc branch and more, Ne,P,Nt	N,D,Ne,P ,Nt	N,D	IP,P	B,N,D,P	B,N,D	P,Ne	D,Ne	N,D
Glasshouse 3	23/06/2021	Week 9	P, Ne - 1 leaf	B,N,D, Nt	B,N,D, Nt	Ne,P,Nt	Ne, Nt		P, Nt	B,N,D, Nt	B,N,D	IP, Nt	Nt		P, Nt	B,N,D, Nt	B,N,D	IP, Nt	Nt		IP, P, Nt	B,N,D, Nt	B,N,D	Nt, Ne	Nt	
Glasshouse 3	30/06/2021	Week 10	P,IP,Nt	P,Nt,Yt,B,N ,D	P,B,N,D	P,Ne(1leaf),Nt,Yt	Ne, Nt,Yt		P,IP,Nt	P,Nt,Yt,B, N,D	P,B,N,D	P,Ne,Nt, Yt	Ne, Nt,Yt		Nt,P	B,Nt, Ne(1 leaf),P		IP,Ne	Ne	Nt	IP,Y,P	Y,P		Nb	Y(yellow branch), Y	
Glasshouse 3	07/07/2021	Week 11 (fruit sampling)	NF															Uneven ripening on tomato+ NF								
Glasshouse 3	14/07/2021	Week 12	IP,P,I,Nt, Ne	Yt Y,Bs,Ne,Nt, split tom	N,D,Nt,Yt ,Ne	P,Ne,Nt, Yt,Y	Ne,Nt,Yt	Mottling (light and dark green)	IP,P,Ne, Nt,Yt,Split tom	I,Nt,Ne,Y t, split tom	P,Ne,Nt, N,D	IP,P,Nt,N e,	Ne,Nt,P, yellow branch	Nt,D, mottle	Yt,IP,P,N e,Nt,Y,Yt	Ne,Nt,Y, necrotic branches	Yt,D	IP,P,Ne, Nt,Y,Yt	Nt,Ne,Y, necrotic branches	Nt,mottl w	IP,Nt,Ne, I	Nt,Ne,Y, Yellow branches , split tom	N,D,Nt	Ne,Nt,IP, P,(Leaf upside down)	Ne,Yello w end of branch, Ne end of branch	Nt,D,mot tle
Glasshouse 3	28/07/2021	Week 14	IP, Ne, Nt, P, Nb	Ne,Nt,Nb,Y ,D,P,Yt, split toms	N,D,Yt,N e,Nt	Ne,Nb,Y, P,IP, upside down leaf	Nb,Ne,Nt ,Mottling	Mottle,N t	Ne,Nt,P,I P,Y,Nb,sp lit toms	Ne,Nt,P, Y,Yellowi ng branch, split toms	Nt,Ne,D, N	Ne,IP,Nb	Ne,Nb,Nt ,mottle	Mottle,N t,D	Ne,Nb,Y, Nt, split toms	Ne,Nb,Nt , split toms	D,Nt,Y	Ne,Nt,IP	Ne,Nb	Nt,Ne,m ottle	Ne,Nt,Nb , split toms	Ne,Nt,Nb , split toms	Y,Nt,N,D, split tom	Ne,Nb,Nt ,IP,mottl e	Ne,Nb,Ye llow branch	Ne,Nt,m ottle
Glasshouse 3	11/08/2021	Week 16																								
Glasshouse 3	25/08/2021	Week 18	IP,Ne,Nt, Nb	Nb,Ne,N	IP,N,Nt,D	P(speckled tips), Ne,Nb	Ne,Nb	Ne,Nt,Y	Ne,Nb	IP,Ne,Nt	Ne,D	Ne,Nb	Ne,Nb	Ne	IP,Ne,Nt, Nb	Ne,Nb	N,D,Ne,IP,Nt	Ne,Nb	Ne,Nb	Ne	Ne,Nb	Ne,Nb,IP	IP,N,D,Y, Nt,Ne, split toms	Ne,Nb	Ne,Nb	Ne

Spring/Late			Plant 1 (roterno)			Plant 2 (picollo)			Plant 3 (roterno)			Plant 4 (picollo)			Plant 5 (roterno)			Plant 6 (picollo)			Plant 7 (roterno)			Plant 8 (picollo)		
Glasshouse 4	18/06/2021	Day 2	Innoculated leaf on all plants inc. healthy shrivelled Ne																							
Glasshouse 4	21/06/2021	Day 5	P, Nt	Yt- 2 leaves, 1 on innoculated branch and 1 on innoculated leaf Nt- and high middle				Nt, D (upper leaves dry and curling but not very very top)	Yt	Nt, Yt		P, Nt		N	Nt-Lower-middle			P, Nt			P	Nt (tips dry on all apart from very top and very bottom), P, IP (dense purple from tips on some)			P-lower middle	
Glasshouse 4	23/06/2021	Day 7	IP, Nt	Nt		IP			IP, Nt	Nt, Yt		IP			IP,Nt	Nt		IP			IP,Nt	Nt		IP		
Glasshouse 4	25/06/2021	Day 9	IP, Nt	P, Nt	N,D	P, Nt		N,D, Yt (golden rust colour on side at edge of leaf more than tip)	P, Nt, Yt	P, Nt, Yt	N,D	P, Nt	P	B	P,Nt,Yt-slight	P	B	P,Nt, Yt-slight	P	N,D	IP,Nt,Yt,I (purple veins yellow leaf)	Nt,Yt	N,D	IP,Nt-slight	IP	N,D
Glasshouse 4	28/06/2021	Day 12	P, Nt, Yt	P, Nt, Yt	Nt	P	P	D, B, Yt (golden rust colour on many top leaves)	P, Nt, Yt	P, Nt, Yt	Nt	P, Nt	P	N. D	Nt, Ne-slight	Nt, Ne-slight	Nt	P, Nt	P, Nt	N, D	IP,Y	Y, Nt, IP, P		P, Nt	P	D
Glasshouse 4	30/06/2021	Day 14	Nt,P, Yt	Nt	Nt, N	IP,P	P	B,D,N, Yt (golden rust colour on many top leaves), Purple edges, Nt	Ne,Y,IP,P	Yt,Nt,Ne	N	IP,Y, P, Nt	P		IP,P,Nt	Nt,Y,P	Nt	P,Nt,Yt	P,Nt,Purpling edges	N,D	IP,P,Y,Ne	Nt,P	Yt,Nt,B(slight)	Nt,P	Nt	Yt,D,Nt (between golden and necrotic)
Glasshouse 4	07/07/2021	Week 3	IP,P,Y,Nt, Ne,uneven ripening on tomato	P,Nt,Ne,Yt, Y,Uneven ripening on tomato	Nt,Yt,N,Ne	IP,P,Nt	P,D,Ne (on innoc branch), Nt	P,Nt,D,N, Ne,Yt	IP,Y,P,Yt, Nt,Ne	Yt,Nt,Ne, P,Y	Nt,Yt,N	IP,P,Nt	Nt,P,B	D,N,Darkening and flattening of whole very upper leaves	Nt,Ne,IP, P, NF	Nt,Yt,Ne	Yt,D,Ne	P,IP,Nt	P,Ne,Nt	D,N,Darkening and flattening of whole very upper leaves	IP,Y,Ne,Nt,Yt	Ne,Nt,Yt	N,Nt	IP,P,Nt	P,Nt	D,N,Nt, Y,B, Ne(Necrotic branch),
Glasshouse 4	14/07/2021	Week 4	Ne,IP,Yt	Y,Ne,Yt,Nt, Purple patches	Ne, N, Dark & flat uppermost	IP,P	P,Nt - innoculated branch, Ne at innocc branch, toms split	N,D,Ne,Nt,curling	IP,Y,Ne,Nt	Some very small and distorted green tomatoes	D,N,Nt	IP,P	P	N	Nt,IP,Ne, Yt	Nt,N,YtSome very small and distorted green toms(pinched in star shape)	D,Y,Nt	IP,P,Nt	Nt,P, tom split	N,D	Uneven ripening-green/yellow repening patches on a few toms	Ne,Nt,Yt, Y	Dark&Flat,Nt	IP,P	P,Nt	Ne,Nt,P, Dark &flat uppermost leaves

Glasshouse 4 cont.

Glasshouse 4	28/07/2021	Week 6	IP,P,Yt,Nt,N	Nt,Ne,P	D,Nt,Thick,flat leaves	Nt,P,IP	Nt,P,IP, few split toms	Nt,N,D no new leaves, Tomato split	Ne,Nt,P, upside down leaves	Ne,Nt,P	Nt,Yt	IP,Y,P,Ne,Nt	Nt,Yt,Y,P	Nt,Dark flat leaves	IP,P,Nt,Ne branch	Ne,Ne end of branch, yellow branch, necrotic branch end	Ne,Nt, tomato growing on its side (but this was also the plant that was bent at the top which may have caused this?)	IP,P,Y,Ne,Nt,Ne branch	Y,Yt, purple leaves, Ne-on innoculation branch, few split toms	N,D,Ne,Dark flat leaces, necrotic patches on toms	Ne,Ne branches but ones that had toms are ok.	Ne, Ne branches-ones that held toms ok.	Ne,Y branch, Nt, N,D,Uneven ripening, distorted toms - small and flattened	IP,P,Y,Nt	P, Purple leaves, Yt, Y patches, toms split	N,D,Ne,Nt,Y,toms split, uneven ripening
Glasshouse 4	04/08/2021	Week 7	Ne,Nt,Y,IP,P,Yellow branches	Nt,Ne,I,mottle,P,upside down leaf, yellow branches	Nt,N,D	Nt,IP	Nt,IP	N,D,Nt,purple tips,YS/Yt	Ne,Ne branches, Y,I, uneven ripening	Ne,Nb,Nt,YS,Purple patches, uneven ripening	N,D,Nt	IP,Y,Nt,Yt,Nb	Yt,Nt,Y,IP	Dark flat leaves, D,Nt	IP,Y,Ne,Nt,Nb	Ne, Yellow branch	Yt,Nt,Ne,D	IP,P,Nt,Yt-slight	Nt,Yt,IP,YS	N,D,flat leaves, Nt,Yt	Nb	Ne,Nb	D,N,Ne,Nt,Uneven ripening	IP,Y,Nt,Yt	Nt,Yt,IP, Ne, YS	N,D,Y,Ne,Nt
Glasshouse 4	11/08/2021	Week 8	Small?		One pumpkin shape tom	tom very small. Necrotic patch on 1 tom				Tomato patchy (All?)	Toms 2/4 mis-shapen, 1/4 rugose, Uneven ripening, 1 tom pumpkin shape			Split tom									Uneven ripening, small, distorted-pumpkin shape	1/2 fruit split (LMU unknown)		
Glasshouse 4	18/08/2021	Week 9	IP,Ne,Nb	Ne,Nb	Nt,Ne	IP,Nt,Nb	Nt,IP	IP,D,N,YS	Ne,Nb	Ne,Nb	N,Ne,Nb-slight,Yellowing at end of branch	Y,IP,Nt	IP,Y,Ne,Nt	D,Nt,flat uppermost leaves	Ne,Nb	Ne,Nb	Ne,Uneven ripening on 1 tom	IP,Y,Nt	Y,IP,Ne,Nt	N,D,IP,Nt	Ne,Nb	Ne,Nb	Ne,Nb,IP,Y	IP,Y,Ne,Nt	IP,Y,Ne,Nt	IP,Y,Nt,N,D,(Nb uppermost)
Glasshouse 4	25/08/2021	Week 10	Ne,Nb	Ne,Nb	Nt,Ne|Side shoot-D,N,Curling|	Ne,Nt,IP	Ne,Nt,IP	N,D,Y,B,IP,Nt,Ne	Ne,Nb	Ne,Nb	Ne,Nb	IP,Ne,Nb	Nt,IP,Ne,Y	IP,Nt, some dark flat, some curling	IP,Ne,Nb	Ne,Nb	Ne,Nb	IP,Nt,Ne,Y	Nt,Ne,IP,Nb	D,Nt,P,IP	Ne,Nb	Ne,Nb, uneven ripening	Ne,Nt	IP,Nt,Ne,Y	IP,Nt,Ne	D,Y,N,P,IP,Nt,Ne
Glasshouse 4	08/09/2021	Week 12	Ne,Nb	Ne,Nb	Ne	IP,Ne,Nt,Nb	Ne,Nt,IP	Ne,Y,D,N	Ne,Nb	Ne,Nb	Ne,Nb	IP,Ne,Nt,Nb	IP,Ne,Nt,Nb	IP,Nt	Ne,Nb	Ne,Nb	Ne	Ne,Nb,IP,Nt	Ne,Nt,Nb,IP	N,D,Nt	Ne,Nb	Ne,Nb	Ne	IP,Ne,Nb,Nt	IP,Ne,Nt	N,Nb,Ne,Nt
Glasshouse 4	22/09/2021	Week 14	Ne,Nb	Ne,Nb	Ne|site-shoot mottled	Ne,Nb,Nt,IP	Ne,Nt,IP	IP,Ne,Nt,Nb	Ne,Nb	Ne,Nb	Ne,Nb, 1 tom with uneven ripening and uniform tiny yellow speckles	IP,Ne,Nb,Nt	IP,Ne,Nt	IP,Ne,Nt	Ne,Nb	Ne,Nb	Ne, 2 toms growing elongated and tear drop shape,	IP,Nb,Nt,Ne	Ne,Nb	IP,Nt,Ne	Nb,Ne	Nb,Ne	Nb,Ne	IP,Nb,Ne,Nt	IP,Ne,Nt	Nt,Ne,IP
Glasshouse 4	06/10/2021	Week 16	All leaves look the same except any side shoots have grown more																							