Grower Summary

Understanding disease development of tomato brown rugose fruit virus (ToBRFV)

PE 034

Annual report 2021

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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

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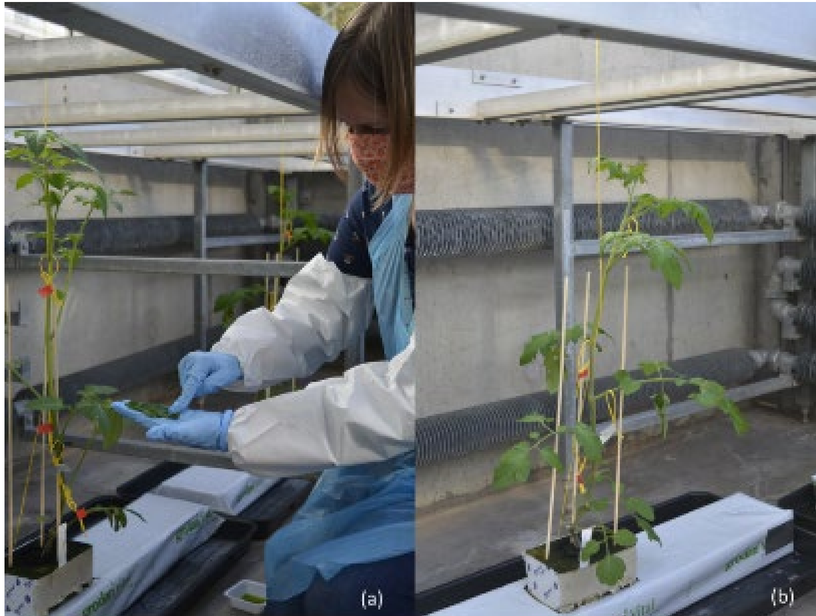

Figure 1. (a) Inoculation of tomato plants showing specific biosecurity measures and mock-hydroponic set up. (b) Inoculated plant showing nylon twin with white label denoting inoculation point.

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.

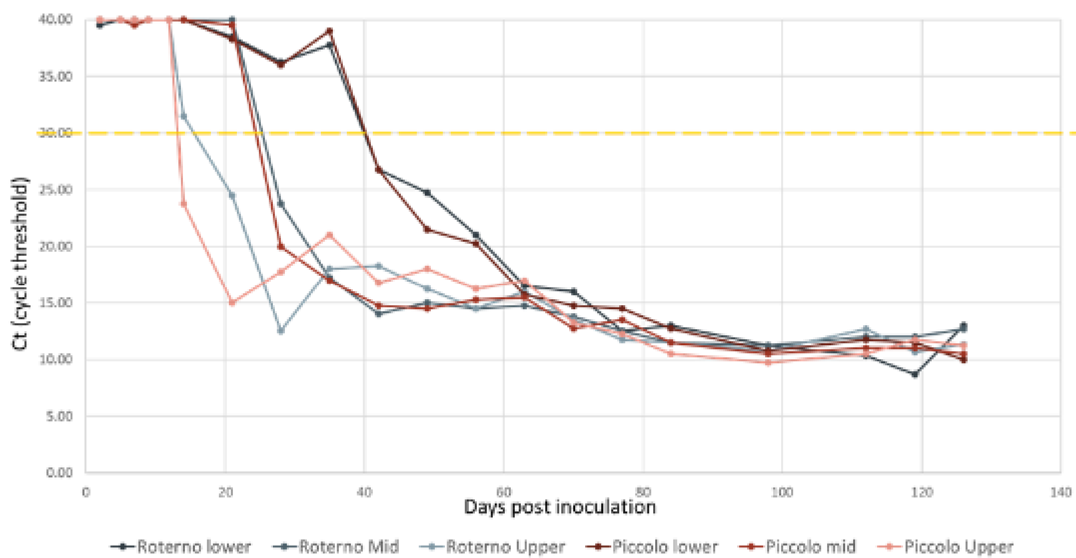

Figure 2. Example data for early inoculation results showing Cycle Threshold (Ct) results for Winter crop/Early inoculation, showing development of infection from leaf detection. (Lower Ct equates to a higher titre of virus in a sample).

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.

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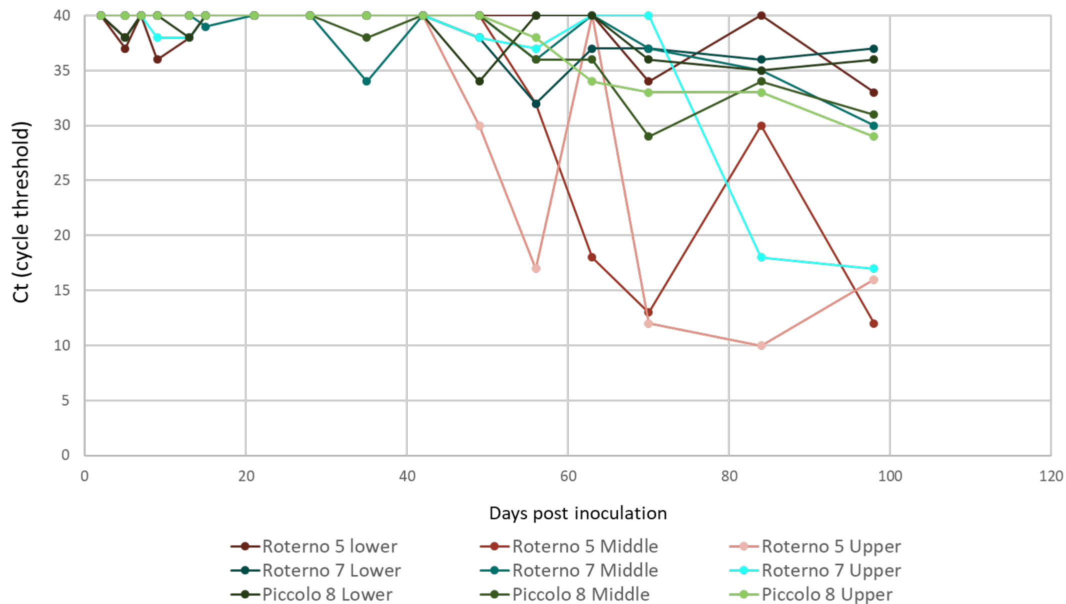

Figure 3. Example data for late inoculation results showing Cycle Threshold (Ct) results for Winter crop/Late inoculation, showing development of infection from leaf detection. (Lower Ct equates to a higher titre of virus in a sample).

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

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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.

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