Tomato Brown Rugose Fruit Virus (TOBRFV) Webinar: Q&A

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Host: AUSVEG
Link to recording:

Q1: You mentioned a grower who was able to eradicate ToBRFV after years of infection. What practices enable growers to eradicate ToBRFV after years of infection?

Answer: That eradication case involved three years of attempts. In the first two years, eradication was not successful because they missed cleaning registration terminals that workers used. The third year, they implemented comprehensive measures including:

- Gave all staff a week off
- Thoroughly cleaned all staff accommodation
- Used disinfection beyond research-tested levels
- "Disinfected everything to within an inch of its life"

The key was being thorough and diligent about cleanup, ensuring no contamination sources were overlooked.

Q2: When testing with three-piece PCR tests, is that 3,000 seeds total or multiples of 1,000 per test?

Answer: We test three pooled samples of 1,000 seeds each (so 3,000 total). If we get an initial positive on our screening assay (single PCR), we re-extract from the homogeneous samples - we don't use the same extract. We test on multiple different PCRs before declaring a positive result.

Q3: How would ToBRFV management apply to open field tomato production and capsicum?

Answer: Open field production has advantages over glasshouse systems; Because plants aren't handled at the same intensive level as glasshouse tomatoes the opportunities for rapid virus spread are much fewer. It seems to be less of an issue generally due to reduced handling.

However, there are some concerns about the virus in field tomato seed supply chains entering Europe. The lack of handling makes it less problematic, but genetic resistance would likely be the most practical management option for field production.

Q4: What's the value of field ELISA test kits for routine sampling in seedling nurseries?

Answer: Field ELISA kits are useful for:

- Confirming symptomatic plants
- Inspector field testing when regulatory importance is limited

- Getting immediate results on-site

Limitations:

- You must pick the right part of the plant to sample
- Need adequate time for testing
- Not ideal for testing very early in virus/disease establishment
- Not suitable for asymptomatic screening

Q5: Comment on shortened growing seasons as a management strategy

Answer: (Comment from participant with experience in Israel, Spain, Italy) Many growers have shifted from one long cycle to two shorter cycles to avoid severe late-season damage.

Adrian confirmed similar feedback from colleagues in Israel, particularly in plastic tunnel systems - moving to two crops per season rather than one long season is becoming the preferred approach.

Q6: How is virus management going in Spain's mid to low-tech production houses?

Answer: Limited information is available. Suspected that growers are likely moving to shorter growing cycles similar to Israel, but no actual data is available.

Q7: What if seed testing is negative but seedlings from the same seed test positive?

Answer: First consideration is defining what "negative" means - this depends on the detection method used. Different labs may use different cut-offs or conventional PCR versus real-time PCR, affecting sensitivity.

If seed has been through a 20,000 seed test on real-time PCR and tested negative, the seed is as free from virus as can be assured. In this case, I would look at other contamination sources but would want the full genome of the virus before drawing conclusions.

Q8: Is anything known about diesel's effect on virus survival/inactivation?

Answer: No research has been done on diesel. We've conducted disinfection work using standard disinfection products. Interestingly, we wanted to test skimmed milk powder (known to remove viral proteins from cutting tools), but growers asked us not to because they would have to market fruit as "not for lactose intolerance."

Q9: With continuous growing across a site, is compartment-by-compartment eradication possible?

Answer: If compartments are directly linked, this is incredibly challenging. We have sites with five standalone glasshouse compartments that successfully limited outbreaks to single compartments. However, sites with compartments separated only by sliding doors ended up with both sides infected, partly due to bumblebee pollination moving between compartments.

Q10: How has understanding transmission pathways reshaped policy on eradication?

Answer: Our advice, in the UK, from the beginning was to maintain eradication between seasons. We (UK) leave the timing of eradication to growers as a commercial decision, as long as eradication action is taken before the next crop. One grower who had recurrent infection now advocates for eradicating as soon as virus presence is found.

Note: Fruit movement policy in the UK was influenced by neighbouring countries considering fruit low-risk, preventing discriminatory action against domestic growers.

Q11: If fruit movement is allowed but imported fruit is a contamination source, doesn't this help spread the virus?

Answer: While I do think fruit is a contamination source, I consider it a lower risk pathway than direct contamination into production areas. Risk management strategies include:

- Not bringing fruit onto production sites
- Limiting worker fruit access to only on-site grown fruit
- Regular swab testing protocols
- Testing staff hands, lockers, coffee machines, and growing areas
- Using positive results for worker re-education and training

Q12: If comparing ToBRFV to bacterial canker eradication in Australia, is eradication of TOBRFV more difficult?

Answer: Yes, TOBRFV is more difficult to eradicate than bacterial canker.

I believe genetic resistance developed by the seed industry is our "exit ramp" from this virus. Current options include:

- Hypersensitive resistance: somewhat fragile but provides good control
- Intermediate resistance: limits spread but still allows virus replication

We need to understand how to best deploy different resistance types while maintaining biosecurity measures. All biosecurity measures effective against bacterial canker also work against TOBRFV.

Q13: Comments on field ELISA dipstick tests

Answer: These are useful tools that we provide to inspectors. When they see suspected positive fruit, inspectors can do dipstick tests on-site rather than sending samples to the lab for confirmation of limited regulatory importance. However, you need to sample the right plant parts and allow adequate time for results.

Key Management Recommendations Discussed:

Routine Biosecurity Measures:

- Hand washing (limited effect on TOBRFV but works against other pathogens)
- Disposable gloves and protective clothing
- Boil washing of uniforms
- Zonal working to prevent staff movement between areas
- Equipment cleaning and disinfection
- Row-specific cutting knives rather than workers carrying tools
- Restricted access to production areas
- Meeting seed representatives off-site
- Staff training on disease recognition
- Limiting movement between facilities
- Prohibiting outside fruit on-site
- Limiting non-essential items (jewellery, watches, mobile phones)
- Using zip-seal bags for electronics if they must enter glasshouses

Testing Protocols:

- Regular swab testing of staff hands, lockers, equipment, and growing areas
- Multiple PCR confirmation for positive screening results
- Comprehensive seed testing protocols