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Summary of practical research:

Effect of USAF™ ultrasonic pressure waves in combination with H2O2 on ToBRFV virus in irrigation water

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By: Ron Peters

Commissioned by:
Luijkx Ultrasound B.V. and Ultramins B.V.

Involved parties:

Mr. Ron Peters of Experimental greenhouse Ron Peters, contractor.
Mr Kees Luijkx from Luijkx ultrasound bv, producer USAF™ ultrasonic equipment
Mr. Robert van Hoo, USAF™ Ultrasonic Distributor
Mr. Rien Rodenburg, supervisor
NAK Horticulture laboratory

Background information:

Experimental greenhouse Ron Peters was approached by Kees Luijkx of Luijkx Ultrasound B.V. and by Robert de Hoo of Ultramins B.V. to evaluate the effect of USAF™ ultrasonic transmitters on ToBRFV virus in irrigation water. The claim is that USAF™ can inactivate all virus present in the water within 24 hours in combination with a regular non-stabilized hydrogen peroxide.

The research focused on ToBRFV alone as it is an urgent problem in tomato farms and one of the most difficult pathogens to control. This is because ToBRFV belongs to the so-called Tobamo viruses. These viruses are part of the non-enveloped viruses, the most difficult type of virus to fight. It is known from scientific reports that enveloped viruses, fungi and bacteria are easier to combat with cavitating ultrasonic transmitters.

ToBRFV in water

It may be assumed that ToBRFV can be transferred to tomato plants via water. Mr. Ron Peters of Experimental greenhouse Ron Peters has established in several studies prior to this study that ToBRFV is absorbed by plants via the irrigation water.
These findings are confirmed by several scientific reports, in which viruses have been investigated whether they can be spread via water, such as the study by Dorst 1988 et al (Surface water as source in the spread of cucumber green mottle mosaic virus) and Paludan 1985 et al. (Spread of viruses by agricultural nutrient solutions in soilless cultures)

Research question:

Are ToBRFV viruses in irrigation water still contagious after a treatment of USAF™ ultrasonics in combination with H2O2.

Operation combination USAF™ ultrasonic and H2O2.

When treated with only an oxidizing agent, the H2O2 must first dissolve (oxidize) the membrane of the pathogens. This can take some time and most of the added H2O2. Once the membrane has been made permeable, a small amount of H2O2 can destroy the inner DNA, permanently destroying the germs.

When combined with USAF™ ultrasonics, the membrane is first broken within a fraction of a second. The USAF™ cavitating ultrasonic transmitters produce micro gas bubbles that, when they implode, create a 2000 bar pressure wave. The membranes of pathogens cannot withstand such high pressures and will rupture immediately.
If the membrane is ruptured, the way is cleared for the H2O2 present in the water to destroy the inner DNA.

This technique works on all pathogens because there are no known pathogens that can withstand the pressures produced by cavitating USAF™. Existing fungi and bacteria will therefore also be destroyed.

Goal:
It can be assumed that viruses do not only occur in the drain water, but also in the basin water. Rapid and effective treatment of the entire water flow is especially important with ToBRFV, because of the exclusion of risks. Only treating drain water does not eliminate the risk.

PCR test
A test on live plants was chosen because it is not sufficient to analyze the virus water with a PCR test. This is because if pieces of DNA are present, a positive result will still come from a PCR test. The PCR test does not give a live or dead result. Alive or dead can only be determined by means of contamination of plants. In this case through the roots.

Composition ToBRFV mixture:

Water:
There are 2 vessels (barrels) filled with 100 liters of tap water.
The following additives have been added to the barrels.

Addition ToBRFV:

15 kilograms of ToBRFV contaminated tomatoes and tomato leaves were smashed with a small amount of tap water with a mixer. The plants used all had the appearance of a ToBRFV infection.
The ground substance is filtered by means of a cloth filter to remove the bigger particles from the mixture.
The aqueous part of the filtering, with high dose ToBRFV, was added 50/50 to vessel 1 and vessel 2.

Container 1 and 2 with tomatoes plants got water from barrel 1.
Container 3 and 4 with tomatoes plants got water from barrel 2
Container 5 with tomatoes plants got only fresh tap water.

A test strip showed the presence of ToBRFV in the water.

Addition H2O2:
Vessel 1:
40 ppm of un-stabilized hydrogen peroxide is added (35%).

Vessel 2:
20 ppm un-stabilized hydrogen peroxide (35%) has been added
(Used hydrogen peroxide authorization number 13279N)

Addition USAF™:
An USAF™ ultrasonic transmitter is installed in both vessel 1 and vessel 2. The USAF™ ultrasonic transmitter is turned on for a 24-hour period.
24 hours has been chosen, because in practice the day stock silo will be treated. The day stock silo can also be treated for 24 hours or a significant part thereof.
Also included in the consideration of opting for 24 hours is that the solution used for these tests is many times more heavily contaminated with ToBRFV, compared to a normal situation at a greenhouse. In a normal situation at a greenhouse, it will be easier to destroy the germs present.

Vessels:
After the additions, 2 vessels were available:
Vessel 1: 100 liters of tap water with 40 ppm H2O2, treated with USAF™ 24 hours
Vessel 2: 100 liters of tap water with 20 ppm H2O2, treated with USAF™ 24 hours

The vessels are equipped with a tap to drain water before pouring. The first liters are not used to prevent tapping water from the tap that contains no or less virus, or too much sediment. Before draining, the mixture is stirred.

Setup:

The greenhouses at Ron Peters are fully equipped for conducting tests with ToBRFV.
Experimental greenhouse Ron Peters is the only company in the Benelux with NVWA (governmental inspection) approval to work with ToBRFV. The test rooms are completely separated from each other for insects, birds and other animals. The ventilation windows are fitted with insect screens and every test rooms is separated with an insect lock to a central corridor. The floors are made from concrete.
Entering the test room is only permitted by one and the same person and only for necessary work. In this case, all activities, such as planting, casting and taking leaf samples, were carried out by Mr. Ron Peters.
The plants are placed in the middle of a table and far enough apart to prevent mechanical contamination through contact with each other.

The plants were planted in disinfected plastic containers with new rock wool. 3 plants in each container. The tomato plants were about 4 weeks old and un-inoculated.

Performance:

At the start of the test, all plants were dried out for several days in order to create stress in the plants, so that uptake of the ToBRFV virus would take place more quickly.

Plant container 1 and 2 received water from vessel 1
(tap water + 40 ppm H2O2 + USAF™ 24 h)
Plant container 3 and 4 received water from vessel 2
(tap water + 20 ppm H2O2 + USAF™ 24 h)
Plant container 5 only received clean tap water. This was to demonstrate that the plants were free of ToBRFV.
For further watering, all plant containers received clean tap water.

Result analysis:

The test report of NAK horticulture laboratory is enclosed with this report.
Translation: Niet aangetoond = not found
Aangetoond = found

The test report of NAK horticulture (number: INS-21-21914) shows the following:

Plant container 1 and 2
Container 1 and container 2 are ToBRFV free in both channels (tests), this was with the treatment of 40 ppm H2O2 and USAF™ ultrasonication.

Plant container 3 and 4
At container 3 and 4 there is a contradiction in the results. At channel 1, the Ct values (Cycle threshold, the number of times the sample has to be doubled to detect the virus) are lower than 32. This means that ToBRFV has been detected. The values are so close to the limit value of 32, that this result could also be a false positive
In channel 2, the Ct value is higher than 32 for both samples, so no ToBRFV has been detected. Positive and negative result for the same sample is not possible.
A third determination (channel) to obtain a definitive result has not been carried out.

To the extent to which ToBRFV has been demonstrated in these tests, it is assumed that these low values, even if they are not a false positive, cannot cause recontamination.

With regard to the Ct value of 32, you can also remark that in France, for example, a limit value of 30 is used. With these results, this would mean that all samples were free of ToBRFV.

Plant container 5
The same explanation can be done for container 5 as for container 3 and 4. Plant container 5 has only been given clean tap water, so in principle it cannot contain ToBRFV

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Conclusion:

Treatment of contaminated irrigation water with ToBRFV, by means of USAF™ ultrasonics in combination with 40 ppm H2O2 will completely inactivate ToBRFV viruses present in the irrigation water.

Treatment of irrigation water by means of USAF™ ultrasonics in combination with 20 ppm H2O2 gives varying results.
Because the positive results are so close to the limit values, it is also possible that these are false positive results and all the samples were still free of ToBRFV.

Ron Peters.
Director of Experimental Greenhouse Ron Peters.