USE OF THERMAL FOGGING FOR DISINFECTION IN GREENHOUSES. WHAT ABOUT ANIMAL HOUSES ?

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Introduction

Disinfection treatment by thermal fogging has been considered since a long time as a very efficient method for hygiene maintenance. This method garantees good results in many domains as agriculture, food industry... Thermal fogging is used for the disinfection of greenhouses, shelters or grain silos, particularly against dust mites, aphids or other caterpillars since the 1980's. This method was also developped against bacteria and fungi, leading to new homologations for these uses. Until now, no official protocol for testing the virucidal activity of disinfectant applied by thermal fogging exists. This leads to the impossibility to homologate a disinfectant as virucide applied through thermal fogging.

BBV laboratory at Saint-Pol de Léon (29) has developed protocols to test disinfectants applied by thermal fogging against vegetable viruses. Since there is no chemical treatment to control viruses once plants are infected, hygiene (on tools, hands and structures) is a key aspect for an effective control. Prophylaxis and disinfectant treatments are of primary importance. In accordance with the current requirements of approval for sale for this category of usage in virucidy, as defined by the French Ministry of Agriculture, BBV develops protocols to test disinfectants. The objective is to officially approve efficient products, allowing their use by producers. During these studies, we valid protocols for testing efficiency of products to disinfect structures by fogging.

The most important virus for tomato producers is Pepino mosaic virus (PepMV). The virus was recently introduced in Europe (1999). In 2003, in Brittany, 55 ha were contaminated. In spite of bad conditions for virus development, this mechanically transmitted virus induced significant damage in tomato production. Losses of 5 % yield and 25 % quality were observed for early contamination. Because of its virulence, PepMV is classified as a quarantine pathogen on seeds in Europe (EC decision 2004 / 200). Thus, we work on another virus, as similar as possible to the PepMV: the Potato Virus X (PVX), this choice being made according to the French Ministry of Agriculture.

Materiel & Methods

Plants of *Chenopodium album* subsp. *amaranticolor* were used as an indicator plant. Plants were sowed in peat pot and transferred 3 weeks later into plastic jars (5 x 5 x 6 cm). The compost used was a mixture of fair peat, black, sandcoloured peat of fine structure. Plants were grown up to the 5-6 node stage.

- tomato leaves infected with PVX were diluted 1:5 in 30 ° F water hardness (4) sterile water and ground up using a mortar and pestle.
- The macerate was filtered on sterile stamen and presented a dilution end point superior or equal to 10^{-4}.
- 1 ml or 2.5 ml of plant sap was placed on glass Petri dish (85 x 65 mm). Dishes are then placed in a greenhouse in which a thermal fogging is applied with the Nebul'Ops (a commercial product) by means of a Thermal Fog Application System Igeba TF35. Nebul's Ops is an acid and oxydative disinfectant made of peracetic acid and of hydrogen peroxyd.
- The thermal fogging of Nebul'Ops was done on the plant sap at the concentration of 1.5 ml/m^3 with water at 1 ml/m^3 as vehicule and 2 ml of Vector to make visible the fog, and at the concentration of 2 ml/m_3 with Forneb at 5 % as vehicule. Durations of contact were 2 and 4 hours.

For each condition, the upper faces of 20 leaves (2 hours of contact) or 8 leaves (4 hours of contact) of *Chenopodium album* subsp. *amaranticolor* were mechanically inoculated by gently rubbing with a sterile compress soaked with 30 ° F sterile water dipped into the plant sap / tested product. The plants were then placed into a greenhouse compartment (12 hours light periods) and monitored for symptom development.

As a phytotoxicity control, the thermal fogging was performed at the final test concentration on 1 ml or 2.5 ml healthy plant sap on glass Petri dish. In negative control plants, sterile 30 ° F water was used instead of tested product on healthy plants sap, in the same conditions. Positive control plants consist in 1 or 2.5 ml of infected plants sap on glass Petri dish put in contact with 30 ° F sterile water during 2 and 4 hours of contact.

The estimations of the symptoms were visual and performed 15 days after inoculation. The notation of the symptoms was done according to a six points scale (0, 1, 2, 3, 4, 5) based on the number of necrotic spots by leaf. The score 0 corresponded to leaves without visible symptoms, score 1 for leaves exhibiting less than 5 necrotic spots and the score 5 to completely infected leaves. Leaves inoculated by objects were independently scored. Then, an average score by object was calculated, which determines the efficiency of the products.

Results

Phytotoxicity control

Leaves inoculated by healthy plant sap / 1.5 or 2 ml/m^3 Nebul'Ops mixture do not show any phytotoxicity. These concentrations do not thus require the use of a neutralising agent.

Control plants

The negative control plants are always free of disease, showing the absence of accidental contaminations.

In positive control plants, a strong infection, near level 5 on our notation scale, is always detected, showing that experimental conditions are relevant to evaluate the antiviral effect of products.

Test of Product

Leaves inoculated with 1 ml of infected plant sap / 1.5 ml/m^3 Nebul'Ops mixture show between 0 and 4 necrotic spots for 2 hours of contact and none for 4 hours of contact. Leaves inoculated with 2.5 ml of infected plant sap / 1.5 ml/m^3 Nebul'Ops mixture show between 0 and 12 necrotic spots for 2 hours of contact and none for 4

hours of contact. Leaves inoculated with 1 or 2.5 ml of infected plant sap / 2 ml/m^3 Nebul'Ops mixture do not show any necrotic spots for 2 or 4 hours of contact.
The desinfectant proved its efficiency to eliminate the PVX virus.
However it was found essential to properly remove the organic matter from all the surfaces before disinfection.

Discussion

From greenhouses to animal houses ?

To our best knowledge no similar protocol yet exists for testing the efficiency of disinfectants applied by thermal fogging in animal houses. In this respect, the present work could serve as first step in order to build up such protocols.
When disinfecting greenhouses and animal houses similar issues need to be addressed. Hygiene is nothing new, but it is the corner stone to good health (1) (2) (3).
The major goal of desinfection is to eliminate the specific pathogens. In both cases the latter are mostly viruses, bacteria (and fungi for greenhouses). The resistance of those pathogens to the disinfectants varies a lot depending on their physical and biological characteristics. In animals, both enveloped and non enveloped viruses can be found. Non enveloped viruses like Porcine Parvovirus and Porcine circoviruses are considered as among the most resistant within this group of pathogens. Additionnally, as well DNA as RNA viruses can be found in both animals and plants. Regarding bacteria, a broad spectrum of pathogens is involved. In animal production like in plant production, bacteria that can sporulate can be found despite those of interest do not belong to the same species.
Although there can be a huge number of equipments, the type of material used is for part similar in greenhouses and animal buildings. Concrete, metallic and plastic material can be found in both cases. The point is of course of paramount importance in respect to decontamination easiness and to corrosion.
In any case a thorough cleaning of the buildings is a prerequisite. Then, in agreement with the vet. / agronomist, the choice of a disinfectant that is independently tested and which has been officially approved is necessary.
A broad-spectrum product is often wise ie showing a biocidal activity against viral, bacterial, spores and fungal organisms. Another common issue is to avoid environmental pollution and the products as well as the application process should not show significant health and safety concerns.
Another aspect needs careful consideration. From a medical standpoint, there must not be confusion between a disinfectant and an antiseptic. The former has to be applied on inert surfaces, on non-living material, whereas the second is a treatment that can be applied onto the skin or the mucosa of humans or animals. Disinfection of livestock buildings is started after the buildings have been totally depopulated, the slurry removed and after the place has been thoroughly cleaned. One of the problems encountered with thermal fogging in animal houses and in greenhouses might be the difficulty to have air tight rooms avoiding any escape to the neighbouring rooms. Another issue which needs to be properly addressed is the method to be used to assess decontamination efficiency and eventually make official decisions on approval. Specific equipment should be adopted. In addition the difficulty of a proper residual contamination measurement still remains. In plants, bio-assays can be used when testing. In animals, for welfare reasons, such assays to reveal a residual contamination after disinfection can hardly be accepted.
An official method aiming at testing virucidal, bactericidal and fungicidal efficiency of disinfectants applied through thermal fogging is being finalized by a scientific working group (4)
Further efforts should be directed to studies about standardized protocols to evaluate disinfectant efficiency applied through thermal fogging in animal houses, in view of official approval .

References

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