SCIENTIFIC NOTE

PEPINO MOSAIC VIRUS VACCINATION SHOULD NOT TAKE PLACE AT THE PLANT PROPAGATION SITE

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Introduction

Pepino mosaic virus (PepMV) is considered a serious pathogen in tomato production worldwide. Since it first finding in commercial tomato cultivation in The Netherlands in 1999 it has spread quickly over and outside Europe mainly due to its very infectious and persistent nature and transmission through tomato seeds and seedlings. Within European legislation it is still considered a quarantine pathogen on tomato seeds. Apart from strict hygienic measures the only control measure currently available is the so-called cross-protection (vaccination) in which a mild variant of the virus (causing only very mild symptoms) is applied to young tomato plants. The subsequent systemic infections then protects against the detrimental effects of a subsequent infection with a more aggressive variant of the virus. PepMV vaccination should be done on young tomato plants after or at planting in the greenhouse where the tomoto crop is grown, i.e. the tomato production greenhouse, to prevent unintended and unwanted spread. However, purely for practical and economic reasons there is a tendency to vaccinate young plants already at the plant propagator (nursery). Vaccination at the plant propagation (= plant nursery) site can lead to spread of aggressive mutants and recombinants, especially if more than one virus strain is used in the same crop. It is therefore strongly recommended to use *Pepino mosaic virus* vaccines exclusively at tomato production sites. Next to the risks of spreading mutants and recombinants, vaccination at the propagation site deprives other tomato growers of their freedom of choice. Tomato growers will no longer be able to choose whether they want to vaccinate their tomato crop or not, nor will they be able to choose which vaccine they want to apply. Given the highly infectious nature of PepMV, spread of the virus within the nursery will be inevitable. Technically speaking the plant propagator will be responsible for application of the virus, but it's the tomato grower who has to deal with the risks in case of mutants, recombinants or aggressive mixed PepMV infections. In addition, since most tomato plant propagators are international companies with sales in many different EU countries, vaccination at the propagation level will result in PepMV introduction in regions where PepMV is not yet prevalent. In particular for vaccines that contain other strains than de predominant Chilean (CH2) strain, such as the less prevalent Peruvian (LP) strain and the European (EU) strain, there is a substantial risk of spreading these naturally non-prevalent strains and consequently creating mixed infections throughout Europe. It is documented that mixed infections can cause more severe symptoms and thus more economic damage for the tomato industry. For these reasons, PepMV vaccination should be applied strictly and solely at the tomato grower's level.

The specific risks associated with vaccination at the plant propagation site are listed and documented further in the table below.

Risks associated with vaccination at the plant propagation site:	**Explanation:**
1) Risk of **unwanted and unintended spread** of PepMV to neighbouring crops and plants	Infection of PepMV to tomato and other Solaneceous host plants like eggplant is, especially in the first few weeks of infection, very mild in term of visible symptoms and therefore almost impossible to detect by visual inspections. PepMV is also a very infectious and stable virus and extremely readily transmitted to neighbouring plants by (human) contact, tools, through water and even birds and insects. This means that (groups of) tomato plants that have

	been inoculated with PepMV in the nursery will pose severe risks of infections to neighbouring (groups of) healthy plants in the same nursery. Previous experiences have clearly shown that even strict quarantine measures to contain PepMV within infected commercial greenhouses almost always fail. Given the infectious nature of PepMV it is not a question if spread of the virus will occur within the nursery but rather when. This means that also plants for customers who do not want their plants vaccinated will run a very high risk of becoming infected. Inoculated and infected plants cannot be distinguished from healthy plants and, unless extreme and unrealistically strict quarantine measures are taken within the nursery, tools, crates, trolleys, trucks etc. will become contaminated and contribute to the unnoticed spread of PepMV within and outside the nursery. Given the current international markets for plant propagators, this will lead to the further spread of PepMV over Europe.
2) Risk of spreading viral **mutants** to many different tomato growers from one single mutation event	PepMV is an RNA virus. Since the RNA polymerase enzymes of RNA viruses do not have proofreading activity, these viruses are prone to mutation events (Domingo and Holland, 1997). It has been shown that PepMV, which creates quasispecies populations, displays a high mutation rate during replication (Hasiów-Jaroszewska et al. 2010a, Gómez et al. 2012). A positive correlation between nucleotide diversity and severity of symptoms was observed, with more diverse populations being more aggressive than populations with lower polymorphism (Minicka et al., 2015). In a safe and sound vaccination strategy, in which all individual plants of a certain tomato production site are vaccinated at the right plant stage with a well-defined and quality-controlled virus isolate, the risk of spreading such mutants is controlled by the cross-protection effect itself: if a mutation occurs in one plant, the mutant will not be able to spread because the adjacent plants are already protected. However, if such a mutation occurs at the plant propagation level and it spreads to non-vaccinated plant batches, it can lead to huge damage in the non-vaccinated (and thus non-protected) plants. The damage will only become visual after several weeks, so the plants will already be delivered to – in the worst case – many different tomato growers throughout Europe.
3) Risk of spreading viral **recombinants** to many different tomato growers from one single recombination event	PepMV vaccination is based on cross-protection, in which viable but mild virus isolates are used to protect the plants from the adverse effects of more aggressive variants of the virus. When viruses multiply, recombination events can occur. When using a vaccine consisting of only one strain, there is no risk for recombination to occur. However, recombination events in mixed infection with two strains of PepMV have been shown to occur (Pagan et al., 2006;

	Hanssen et al., 2008; Hasiów et al., 2010b). When two or more virus strains are applied at the propagation level and an aggressive recombinant would arise from such a recombination event, this recombinant could be established (within the viral quasispecies cloud; Hasiów et al., 2010a) and spread throughout the entire propagation site (not limited to one location because of common employees, tools, trucks, …). It might take several weeks before the symptoms of such an aggressive recombinant isolate start to develop in the plant, so by the time the damage becomes visible the plants have most likely already been shipped to tomato growers throughout Europe. By contrast, if a recombinant is formed at the tomato grower level, the spread is limited and the damage can be contained.
4) Risk of **spreading non-prevalent strains** to regions with low infection pressure	Given the reasons listed above it is not possible for a plant propagator to produce both vaccinated and virus-free tomato plants at the same production site. PepMV is very infectious and easily mechanically transmittable. Therefore avoiding cross-contamination in a commercial young plant production environment is not feasible. It is known that plant propagators (nurseries) vaccinating with mild isolates of PepMV spread the vaccine also to non-vaccinated plant batches. Even if the propagator uses two different locations (one for vaccination and the second one to grow virus-free plants), practical experience teaches us that cross-contamination will also occur in the virus-free greenhouses. Thus, plant batches with mild virus strains from vaccines will be spread all over Europe, also to regions with no or little PepMV infection pressure. In particular for vaccines that contain other strains than de predominant Chilean (CH2) strain, such as the less prevalent Peruvian (LP) and European (EU) strains, there is a substantial risk of spreading these non-prevalent strains and consequently creating mixed infections throughout Europe. It is well documented that mixed infections cause more severe symptoms and thus more economic damage for the tomato industry (Hanssen et al., 2008; Hanssen et al., 2009; Mayne & O'Neill, 2017).
5) Risk of **cross-contamination** between different vaccines	It is not possible for a plant propagator to produce at the same time different plant batches, each of them vaccinated with different PepMV vaccines. As indicated above, PepMV is highly infectious and easily mechanically transmittable. Therefore, if vaccines are applied at the plant propagator site, cross-contamination between the different vaccines will occur during the incubation period of the mild virus, when plants are vaccinated but not yet fully protected. Also in this case, cross-contamination may occur, even if the propagator uses two different locations (see also point 1 and 4).

Overall conclusion:

Vaccination to protect tomato plants against PepMV damage can only be done in a safe and sustainable way at the tomato growers' site and not at the plant propagator site, because vaccination at the plant propagation site will result in:

- Increased risk for the **spread of strains that are not naturally occurring** in certain regions, thus leading to **mixed infections** which can cause more damage
- Increased risk for the **spread of recombinants**
- Increased risk for the **spread of mutants**
- **Unacceptable risks** for tomato growers who do not want their crops to be vaccinated

For these reasons we strongly advise against vaccination with weak variants of PepMV at plant propagator sites (nurseries).

References:

- Domingo E, Holland JJ (1997), RNA Virus mutations and fitness for survival. Annu. Rev. Microbiol. 51: 151–78.
- Gómez P, Sempere RN, Aranda MA, Elena SF (2012), Phylodynamics of *Pepino mosaic virus* in Spain. Eur. J. Plant Pathol. 134:445-449.
- Hanssen IM, Paeleman A, Wittemans L, Goen K, Lievens B, Bragard C, Vanachter ACRC, Thomma BPHJ (2008). Genetic characterization of *Pepino mosaic virus* isolates from Belgian greenhouse tomatoes reveals genetic recombination. Eur. J. Plant Pathol. 121: 131-46.
- Hanssen IM, Paeleman A, Vandewoestijne E, van Bergen L, Bragard C, Lievens B, Vanachter ACRC, Thomma BPHJ (2009). *Pepino mosaic virus* isolates and differential symptomatology in tomato. Plant Pathol. 58: 450-460.
- Hasiów-Jaroszewska B, Jackowiak P, Borodynko N, Figlerowicz M, Pospieszny H (2010a). Quasispecies nature of Pepino mosaic virus and its evolutionary dynamics. Virus Genes 41(2): 260–267.
- Hasiów-Jaroszewska B, Kuzniar A, Peters S, Leunissen JAM, Pospieszny H (2010b). Evidence for RNA recombination between distinct isolates of *Pepino mosaic virus*. Acta Bioch. Pol. Vol. 57, 3: 385-388
- Mayne S, O'Neill T (2017). *Pepino mosaic virus* of tomato - new results on strains, symptoms and persistence. AHDB Agriculture and Horticulture Development Board, Factsheet 25/16.
- Minicka J, Rymelska N, Elena SF, Czerwoniec A, Hasiów-Jaroszewska B (2015). Molecular evolution of Pepino mosaic virus during long-term passaging in different hosts and its impact on virus virulence. Annals of Applied Biology 166: 389–401.
- Pagán I, Cordoba-Selles MC, Martinez-Priego L, Fraile A, Malpica JM, Jorda C (2006). Genetic structure of the population of *Pepino mosaic virus* infecting tomato crops in Spain. Phytopathol. 96: 274–279.

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