Masteroppgave 2021 60 stp
Fakultet for biovitenskap

Pepinomosaikkvirus – overlevelse under kompostlignende forhold

Pepino Mosaic Virus Survival Under Compost-like Conditions

Endre Mogstad Ananiassen
Plantevitenskap, Plantevern

Forord

Denne oppgaven er utført som det avsluttende arbeidet i Masterstudiet i Plantevitenskap, retning Plantevern ved Norges miljø- og naturvitenskapelige universitet. Arbeidet er utført i samarbeid med Norsk institutt for bioøkonomi.

Oppgavens tema ble valgt etter forslag fra min hovedveileder Dag-Ragnar Blystad, og ble utarbeidet i større detalj i fellesskap med hovedveileder, og tilleggsveileder Zhibo Hamborg.

Det valgte temaet var av interesse fordi det gav en mulighet til å bidra med ny kunnskap som kunne være av praktisk nytte, og fordi det gav anledning til å lære mer om plantvirus, en ganske særegen og spennende gruppe plantepatogener. Kompost som en potensielt nyttig ressurs, og pepinomosaikkvirus som et plantepatogen av økonomisk betydning gjorde at det føltes meningsfullt å forsøke å bidra til å kartlegge virusets overlevelsespotensiale igjennom komposteringsprosessen.

Jeg vil takke alle som på noe vis har bidratt til denne oppgaven, da spesielt mine to dyktige og tålmodige veiledere Dag-Ragnar og Zhibo for all den gode hjelpen, Ketil Stoknes og hans medarbeidere ved Lindum AS som bidro med faglige innspill og forsøksmateriale, og Sissel Haugslien for hennes uvurderlige hjelp med den praktiske utførelsen.

Ballstad, 31.08.2021

Endre Mogstad Ananiassen

Table of Contents

Forord ....

1 Sammendrag 1

2 Abstract 1

3 Introduction 2

3.1 Plant pathogens in compost 2

3.2 Pepino mosaic virus 2

3.3 Plant virus diagnostics 3

3.4 Goal of study 5

4 Materials and methods 6

4.1 Materials 6

4.2 Methods 7

4.2.1 Plant growth conditions 7

4.2.2 Sap inoculation 7

4.2.3 Preparation of infected leaf material 8

4.2.4 Small-scale compost experiments 10

4.2.5 Temperature control 11

4.2.6 Temperature measurement 11

4.2.7 DAS- ELISA 11

4.2.8 Experimental setup 12

4.2.9 Results registration 13

5 Results 15

5.1 Main result 15

5.2 Experiment 1 16

5.3 Experiment 2 17

5.4 Experiment 3 18

5.5 Experiment 4 18

5.6 Test plant symptoms 19

5.7 ELISA testing 21

6 Discussion 22

6.1 Methods 22

6.2 Effect of compost 23

6.3 Temperature 23

7 Conclusion 25

8 Bibliography 26

9 Appendix 29

9.1 Compost water mass fraction 29
9.2 Sampling times 30

Table of Figures

Figure 4.1 6
Figure 4.2 8
Figure 4.3 8
Figure 4.4 9
Figure 4.5 10
Figure 4.6 12
Figure 4.7 14
Figure 5.1 15
Figure 5.2 16
Figure 5.3 17
Figure 5.4 18
Figure 5.5 19
Figure 5.6 20
Figure 5.7 21
Figure 9.1 29

Table of Tables

Table 5.1 16
Table 9.1 30

1 Sammendrag

Bruken av kompostert plantemateriale som en komponent i vekstmedier og til jordforbedring kan medføre et potensiale for spredning av plantepatogener. Kunnskap om patogeners respons på ulike komposteringsforhold er nødvendig for å kunne sikre at disse ikke overlever komposteringsprosessen. For mange patogener er temperaturforholdene i en kompostprosess ansett som en av hovedfaktorene for utryddelsestid. Det har blitt publisert relativt få artikler som omhandler utryddelse av plantevirus igjennom kompostering, og disse beskriver responsen til et fåtall arter. Pepinomosaikkvirus (PepMV) er av stor økonomisk betydning i tomatkulturer, men det er lite tilgjengelig informasjon om artens respons på ulike komposteringsforhold. Denne studien undersøkte ulike komposttemperaturers effekt på utryddelsestid for PepMV, samt effekten av kompost på utryddelsestid. Bladmateriale infisert med PepMV ble utsatt for kompost i et småskala system med konstant temperatur, samt en tørr, konstant temperaturbehandling. Dette bladmaterialet ble så brukt til å saftinokulere testplanter. Smitte var fortsatt tilstede etter 65 dager ved 20°C. PepMV var utryddet etter 3-5 dager ved 40°C, 1-3 dager ved 60°C, og 6-9.5 timer ved 80°C. Sammenligning mellom resultater fra kompostbehandling og tørr behandling viste at kompost reduserte utryddelsestiden betraktelig ved samme temperatur.

2 Abstract

The distribution and use of compost based on plant matter as a component in growth media and for soil improvement is a potential pathway for the spread of plant pathogens. Knowledge about pathogen response to different compost conditions is necessary for proper management of a composting operation to ensure conditions are sufficient for eradication. Compost temperature is for many plant pathogens considered an important factor influencing eradication time. Only a limited number of papers have been published on plant virus eradication during composting, describing the response of a relatively small number of virus species. For pepino mosaic virus (PepMV), a pathogen of considerable economic importance in tomato crops, little information is available about its survival under different compost conditions. This study investigated PepMV eradication times under different composting temperatures and the effect of compost on PepMV eradication time. Infected leaf material was subjected to constant temperature compost conditions in a laboratory-scale system, as well as constant temperature dry treatments, then used for sap inoculation of indicator plants. PepMV was still detected in compost after 65 days at 20°C and eradicated at 40°C, 60°C, and 80°C in 3-5 days, 1-3 days, and 6-9.5 hours, respectively. Comparison between eradication times in compost treatments and dry treatments showed that compost reduced eradication time considerably at the same temperature.

3 Introduction

3.1 Plant pathogens in compost

Viral plant pathogens' ability to survive through composting has been explored only to a limited degree (Noble & Roberts, 2004; Wichuk et al., 2011). Composting can be useful for disposing of crop plant residue, as a way of recovering a portion of the invested nutrients, and producing decomposed organic material for soil improvement or as a component for growth media mixes. Understanding the response of different virus species to different composting conditions when dealing with infected or potentially infected plant matter is important for commercial or "in-house" compost producers to be able to reasonably guarantee a non-infectivity of the product to susceptible plant species. Only a small number of virus species' survivability has been studied, using different methods of composting or laboratory scale "simulated" composting, and then with variation in feedstock and treatment (Wichuk et al., 2011).

Due to convenience and the possibility of control over variables such as temperature, water content and availability of oxygen over time, several publications on virus survivability have been done using "laboratory scale" or "bench scale" composting systems (Noble & Roberts, 2004; Wichuk et al., 2011). The setup described in one publication studying the eradication of *Plasmodiophora brassicae* (Fayolle et al., 2006) used 2 litre flasks, containing material to be composted, partially immersed in a temperature regulated water bath, with a system in place for actively controlling air flow rates to the flask interior. In Noble et al. (2011) a similar system was used where the material used in the flasks was mature green waste compost, to limit the microbial activity.

Compost typically has an initial mesophilic phase of increasing microbial activity and temperature. Temperatures can reach well into the thermophilic regions, above 70°C is not uncommon, and remain at a steady high temperature for several days before it starts dropping off again. The trailing mesophilic phase, also referred to as the curing phase, is characterised by a slow, steady decline in temperature as microbial activity declines (Herrmann & Shann, 1997). The transition through the different phases are also associated with changing communities of species of compost microorganisms (Herrmann & Shann, 1997). As many plant pathogens are reported as sensitive to temperature (Noble & Roberts, 2004; Wichuk et al., 2011), the temperature in and the duration of the thermophilic phase is considered particularly important for successful eradication of some species of plant pathogens.

3.2 Pepino mosaic virus

Pepino mosaic virus (PepMV) was described in the scientific literature for the first time in 1980 (Jones et al., 1980) after being discovered in a crop of pepino plants (*Solanum muricatum*) in Peru in the first half of the 1970s. It has since gained notoriety as a menace in commercial tomato (*Solanum lycopersicum*) crops in EU member states, and infections have been reported in Africa, Asia, and on both North and South American continents (Werkman & Sansford, 2010).

PepMV is a monopartite, positive sense, single strand (ss)RNA virus in the genus *Potexvirus*, in the plant virus family *Alphaflexiviridae*. The virion is a filamentous, hollow cylinder-like structure with a diameter of ~13nm and an average length of ~508nm (Adams et al., 2004; Jones et al., 1980). The genome consists of approximately 6400 bases (Bibi et al., 2017). All *Potexvirus* species have 5 open reading frames (ORFs), from the 5'-end, expressing the

replicase complex proteins, the triple gene block proteins (TGB 1-3) and the coat protein subunit (Adams et al., 2004; Kreuze et al., 2020).

The virus causes systemic infections in several species in the family *Solanaceae* across different genera, including *Datura*, *Nicotiana*, *Physalis*, and *Solanum* (Jones et al., 1980). In tomato, expression of symptoms has been shown to vary depending on local climate, tomato cultivar and virus strain (Blystad et al., 2015), and as of 2015 there were five main strains of PepMV. When systemic symptoms develop in the vegetative parts of the plant, they often include chlorosis in leaves, leaf mosaic, and leaf bubbling (Blystad et al., 2015). In fruit, symptoms often present as being unevenly ripe with a marbled or striped appearance, which is of economic importance for a producer due to reduction in or complete loss of commercial value (Spence et al., 2006).

Mechanical transmission is considered to be the most important route for PepMV transmission between individuals. This can be direct contact between plants resulting in rubbing and sap transmission, or indirectly, for instance via intermediate deposition of virus-rich sap on clothing, greenhouse surfaces and on tools used for pruning or harvesting. PepMV is very contagious in tomato, and contaminated surfaces remains infective for a relatively long time, up to several weeks depending on conditions (Mayne & O'Neill, 2017; Vlugt, 2009). While research experiments in greenhouse show that bumblebees (Shipp et al., 2008) and *Olpidium virulentus* (Alfaro-Fernández et al., 2010) can play a role in PepMV infection of healthy plants, vector transmission is not considered important. No other vector organisms have been reported for PepMV (Vlugt, 2009). PepMV has been reported to be present in or on the seed coat of, in one study at least 25% (Córdoba-Sellés et al., 2007), of tomato seeds from infected, symptomatic fruit. The same study found ~2% of seedlings from positive seeds to result in positive DAS-ELISA tests, indicative of infection and demonstrating a potential for pathogen dispersal via commercial seed lots. Indeed, the initial isolation and characterisation of the Ch2 strain was done from material collected from a Chilean commercial tomato seed lot (Ling, 2007).

Previous investigations of PepMV eradication in relation compost seems limited to an unpublished result by Mumford (Mikkelsen et al., 2006) and Mayne and O'Neill (2017), both on chipped tomato plants infected with the virus. In both cases, the information on PepMV behaviour in compost is rather limited. Other relevant works, as temperature is held to be an important factor in eradication of pathogens, may include work done on heat treatment of tomato seeds (Ling, 2010).

3.3 Plant virus diagnostics

Compared to many plant pathogens, working with plant viruses can be challenging because of their minuscule size and inability to reproduce outside a host cell. Because of physical limitations of light microscopy, meaningful direct observation and characterisation of the individual virion's morphology requires high-energy methods like electron microscopy, or other very high-resolution methods. Electron microscopy alone does not guarantee precise identification of a virus since many species have similar dimensions, however it is possible in combination with species-specific marker methods.

Nucleic acids tendency to hybridise with a complementary strand has given rise to a wide variety of diagnostics methods. Knowledge of virus genome sequences allows for production of shorter strands complementary to specific and unique regions, which can be exploited for purposes of identifying a specific clade (Wilson, 2014). A relatively simple approach to this is the attachment of detectable labels, for instance radioactive isotopes or fluorescent dyes, to the (probe) short complementary strands to determine if a plant virus is present (Wilson,

2014). A group of nucleic acid amplification techniques are based on the same annealing principle, but instead of binding a probe marker a set of DNA polymerase primers is bound to specific target sequences in the genome. The polymerase is present in the same solution as the primers together with the necessary nucleotides to exponentially multiply a target sequence in the virus genome, resulting in a potentially very specific and sensitive method for detection of virus nucleic acids when used in combination with some visualisation method (Wilson, 2014). Of particular importance is the polymerase chain reaction (PCR) methods which uses a forward and a reverse primer together with a thermostable DNA polymerase to produce copies of the virus DNA sequence and its complementary strand (Wilson, 2014). The reaction is kept going by low-high thermo cycling to allow primers to anneal and initiate polymerisation, then denature the resulting double stranded products for the next round of primer annealing (Wilson, 2014). PCR-based methods have become widely used in research and diagnostics (Wilson, 2014). “Next-generation” DNA sequencing methods, like for instance Illumina, have the advantage of not requiring any previously known target genome sequence, and can be used to characterise previously unknown viruses, as well as to recognise already known genomes (Wilson, 2014). These methods are rather elaborate, but are typically based on complementary binding to anchored probes followed by a nucleotide-by-nucleotide DNA polymerisation and read of a fragmented but complete virus genome (Wilson, 2014). The sequencing methods are typically more expensive per assay (Wilson, 2014). For the all DNA-based methods, working with RNA viruses requires an initial step of RNA isolation and reverse-transcription to be applicable (Wilson, 2014).

Serological test methods are similar in principle to the use of nucleic acid probes to detect complementary target sequences, in that they employ specific animal antibodies to bind exposed target protein sequences and are typically used with a similar label (Wilson, 2014). One important method is enzyme-linked immunosorbent assay (ELISA). It is based on immunoglobulins (Ig) harvested from animals that bind to specific polypeptide sequences in virus proteins (Wilson, 2014). Production of Ig is induced in the animal by injection of plant virus particles or a selection of virus proteins, stimulating an immune response. Ig can then be harvested from the animal’s blood (Wilson, 2014). When conjugated with an enzyme that catalyses an indicator reaction, typically resulting in a colour change, the Ig-enzyme can be used to indicate the presence of plant virus proteins. (Wilson, 2014) One commonly used approach to this is the “double antibody sandwich” (DAS)- ELISA, where a surface coated with virus specific Ig anchors target virus protein, and virus particles where the relevant protein is attached and exposed, to the surface. Any non-anchored particles are removed by rinsing and enzyme-linked Ig is introduced and binds to any anchored proteins, resulting in an Ig-protein-Ig sandwich. Rinsing removes unanchored Ig-enzyme, an enzyme substrate is added, and the resulting reaction indicates the target protein is present (Wilson, 2014). Igs can be used as mixtures with different binding sites (polyclonal), purified directly from blood plasma, or with a single binding site on a target protein, typically produced by cell culture (monoclonal) (Wilson, 2014). Compared to the nucleic acid-based methods, ELISA tests are often cheaper to perform per test, and typically require less sophisticated equipment (Wilson, 2014).

Bioassay methods are useful and, in many cases, necessary in the study of plant viruses because of their inability to reproduce independently. Bioassay is defined as “the use of living cells or organisms to make quantitative and/or qualitative measurements of the amounts or activity of substances” (Allaby, 2012). For detection purposes when working with plant viruses this typically means introduction of the pathogen into well-characterised indicator plants and observing symptom development (Legrand, 2015). While serological and molecular methods can be extremely sensitive detection tools, being based on recognition of limited protein or nucleic acid sequences means they do not discern between virion

fragments and complete, functional virus particles (Wilson, 2014). As successful development of systemic symptoms requires production of new virus particles, bioassay provides information about virus viability as well as presence (Wilson, 2014).

Because of the difference in host range and disease expression different viral plant pathogens can have in different plant species, varieties or forms, plants from different taxonomic groups with known reactions to specific pathogens can be quite useful as a tool for investigating suspected virus infections in crop plants. Detection of a pathogen in a host is achieved simply by successful transmission to and development of symptoms in a test plant. Further information towards the identity of a pathogen is acquired through which test plants are susceptible to infection, the disease progression and symptoms in the different plants, and in some cases what transmission methods are necessary for successful transmission in the first place. While for many virus species crude sap transmission is sufficient, some may require specific vector organisms, transmission through grafting or "dodder (genus *Cuscuta*) bridges".

Commonly used indicator plants include species from families *Amaranthaceae, Chenopodiaceae, Fabaceae,* and *Solanaceae,* including several species from genus *Nicotiana. Nicotiana occidentalis* 37B and *Nicotiana benthamiana* are two useful test plants for working with PepMV. Both are susceptible to EU-tom, Ch2, and US1 strains, easily infected through sap inoculation, and develop obvious systemic symptoms (Blystad et al., 2015). Additionally, neither plant showed any difference when tested under different climatic conditions (Blystad et al., 2015). Systemic symptoms in 37B are very well characterised for Ch2 and include vein clearing, chlorosis, mosaic and necrosis (Blystad et al., 2015). Similarly, *N. benthamiana* develops leaf mottling (Fakhro et al., 2011). *N. benthamiana* has also been reported to have a considerably higher virus concentration in its leaves compared to five other *Nicotiana* test plant species (Fakhro et al., 2011).

3.4 Goal of study

Information about PepMV survival in plant tissues in compost is very limited. By subjecting symptomatic *N. benthamiana* leaves infected with a PepMV isolate to constant temperature, dry treatments and laboratory-scale compost simulation treatments, then testing their infectivity by sap inoculation of suitable indicator plants, this study seeks to determine (1) eradication times of PepMV in infected leaf material under different composting temperatures, (2) the importance of temperature compared to compost conditions for eradication, and (3) the importance of compost maturity.

4 Materials and methods

4.1 Materials

A PepMV isolate, TomA2001-1 (Blystad et al., 2015), strain Ch2, was used in this study. *N. benthamiana* plants were infected with the isolate by sap inoculation for virus multiplication and production of symptomatic leaf material for use in the experiments. *N. occidentalis* 37B plants were used as indicators for virus survival testing. Both species were grown from seeds, and the seeds and the virus isolate were made available from NIBIO's collection.

Compost used in the experiments was provided by Lindum AS, a company that, amongst other things, run a commercial composting operation based on garden waste in Drammen, Norway. The compost was produced in large scale outdoor windrows from garden waste. Compost in two different stages of development was used for comparison: a mature compost and a still active compost.

The mature compost had spent approximately 12 months in windrows before being packaged into perforated plastic bags and stored before use. pH was measured to be in the range of 6.8-7.1, both before and after end of treatment. Water mass fraction at the start of and throughout treatment is shown in Figure 9.1. While the range and distribution of the size of the compost particles was not measured, the mature compost was visually evaluated to be made up of finer material than the active compost, with the largest particles present notably smaller as well (Figure 4.1).

Figure 4.1
Mature compost(left) and active compost(right) displayed to illustrate difference in particle size and aggregation.

The active compost was collected from a recently established windrow. Material was collected from different temperature layers in the windrow, at about 40°C, 50°C and 60°C and stored in plastic bags during transport and preparation, which took close to 3 hours in

room temperature. The drop in temperature was not measured, though by touch was determined to be, relatively, much higher than room temperature just before transfer to drying cabinets. Compost pH was within 6.7-7.0 before and after treatment. Figure 9.1 shows the active compost water mass fraction.

4.2 Methods

4.2.1 Plant growth conditions

Plants used in this study were raised and kept in a greenhouse with an actively regulated climate. Lighting consisted of natural light and a mix of high-pressure sodium and metal halide lamps. Air humidity was controlled by misting and ventilation, and temperature by radiator pipes circulating hot water. Two different compartments were used, a smaller nursery compartment (KNVH0701) and a large main compartment (KNVH0707) to which all plants were moved after sap inoculation, either as part of experiments or production of infected plant material for use in experiments. The nursery compartment had a photoperiod of 16 hours and was set to maintain at least 150umol/s PAR using a mix of high-pressure sodium (HPS), mercury-vapour lamps and sunlight. Day-time air temperature was set to be above 22°C with a relative humidity set to be above 65%, and 22°C and 65% during the night. The main compartment had a 16-hour photoperiod and was set to maintain at least 150umol/s PAR, with a day- and night-time air temperature and relative humidity setting of 20°C, 20°C, 65% and 65%, respectively.

All plants used were raised from seeds in the nursery compartment following a 10+10+5 day program. The seeds were sown in a small 9 cm x 9cm pot and the seedlings transplanted to a 26cm x 56cm x 7cm tray, approximately 5cm apart, after 10 days. The plants were transplanted again after 10 days to 9x9cm plastic pots individually and were considered sufficiently recovered and developed for inoculation after another 5 days. All test plants remained in the nursery compartment after the 25-day development program was complete until inoculated, up to 14 days later, though more typically only up to 7 days.

A sphagnum-based growing medium, composed of 10% fine sand, 80% and 10% sphagnum moss at von Post decay state H1-H4 and H4-H6 respectively, was used in all stages of plant development. Perlite was mixed in before use. Watering in the nursery compartment was done once per day by hand from the top with a nutrient solution. The same was done in the main compartment, but in addition a manually adjusted drip-watering system was used.

To prevent cross-contamination when reusing trays, they were submerged in a bath of potassium hydroxide and detergent for no less than three days before being washed with a water jet to remove any remaining debris.

4.2.2 Sap inoculation

To inoculate plants for virus propagation and production of test material, and for determining infectivity of test material after treatment, a typical approach to sap inoculation was used. Infected leaf material was ground with mortar and pestle (Figure 4.2) in a 0.03M phosphate buffer solution to form a deeply coloured suspension. Teabags containing treated leaf material were left to soak in buffer for a few minutes to make grinding easier since most of the material had a substantially reduced water content. The resulting suspension was applied to test plants dusted with silicon carbide powder (carborundum), which acted as an abrasive. Using two side-by-side ‘Q-tip’-style cotton swabs, the suspension-soaked Q-tips were smeared over the adaxial surface of the leaves of the test plants, covering as much of the leaf area as possible. About 5-15 minutes after application, the plants were rinsed in tap water and moved to the greenhouse main compartment (Figure 5.7).

Figure 4.2
Partially decayed leaf material after grinding.

The buffer solution was prepared in 1-liter batches using 3.136 grams of Na_2HPO_4 x $2H_2O$ and 1.684 grams of KH_2PO_4. After the salts had dissolved, the pH was measured by pH-meter and the solution was stored in a capped glass container in a refrigerator until used.

To sanitize mortars and pestles between uses a Duromatic Polaris pressure cooker with a pressure valve set to 1.2 bar, which should correspond to a steam temperature of ~123°C, was used. It was kept at max pressure for a minimum of 15 minutes, following local laboratory convention, before being allowed to cool down in a passive manner.

4.2.3 Preparation of infected leaf material

Infected leaf material was used to study the effect of the different treatments. Leaves were acquired from *Nicotiana benthamiana* grown in greenhouse following the 25-day development program. After 25 days the plants were infected by sap inoculation method described above, using symptomatic leaves of *Nicotiana occidentalis* var. *P1* kept in storage at -80°C since March 2020, and moved to the main chamber. After at least 14 days, to allow for a systemic distribution and infection, symptomatic leaves would be harvested from the

Figure 4.3
Shows *N. benthamiana* leaf preparation (left) by removal of most of the first order vein. The leaf is then be inserted into a tea bag (right) and is ready for either compost or temperature-only treatment. This leaf was about 1 gram after vein removal.

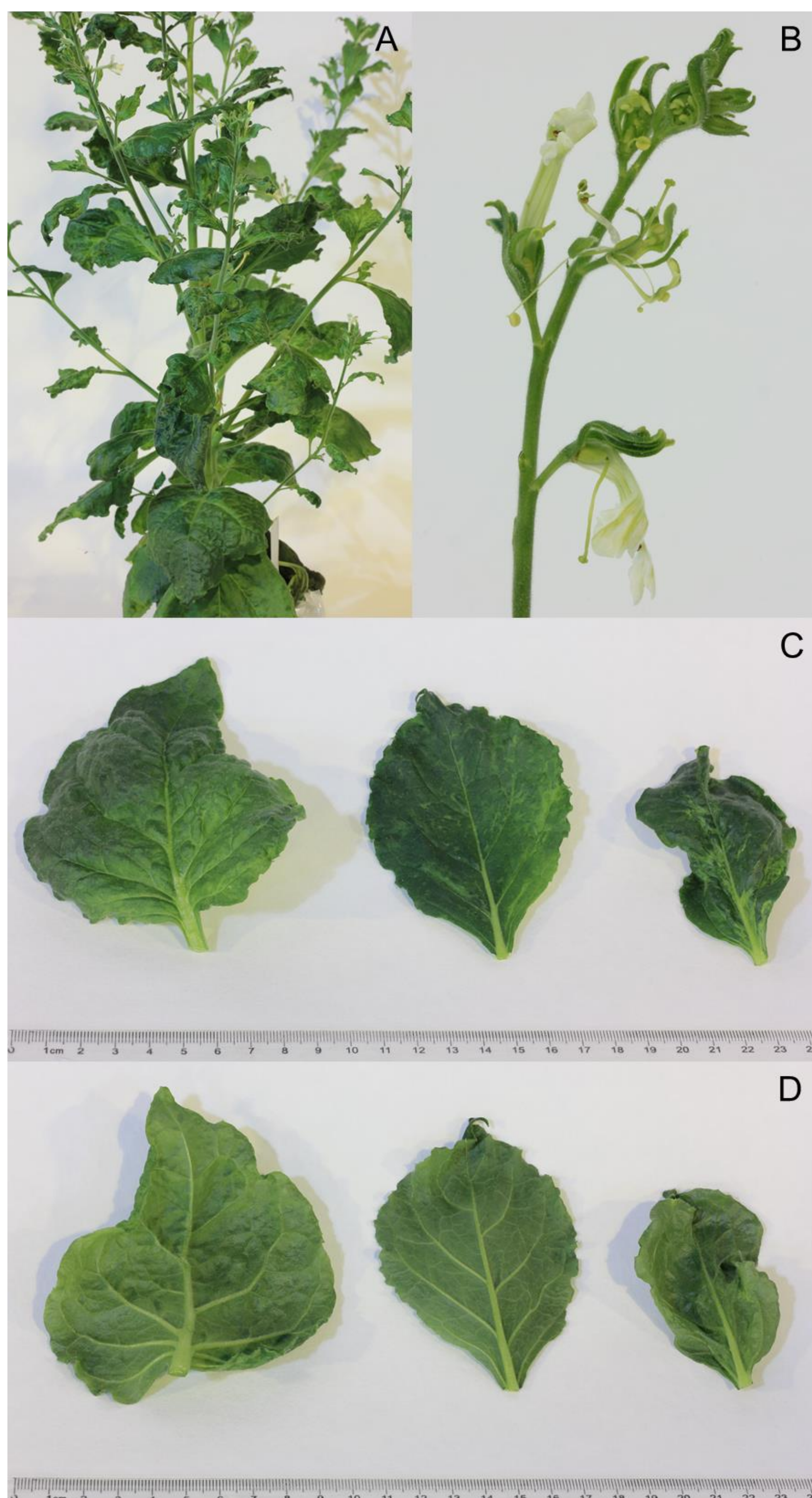

Figure 4.4

Figure A shows *N. benthamiana* approximately 6 weeks after infection with PepMV TomA2001-1. In figure B, flowers on the same individual as A, with pistil and stamen only partially covered by perianth during development which is severely deformed. C and D shows change in leaf morphology after infection with PepMV. Leaf early after inoculation and systemic spread (left), (middle) in 'intermediate' and later stages, and (right) only in later stages.

plants. Leaves harvested were selected based on leaf weight, signs of senescence and to some degree visual evaluation of other unspecified criteria. The intention was to reduce the variation in leaf development and the effect this likely has on the breakdown rate under compost-like conditions. Leaf weight was measured after the petiole and approximately 2/3

of the first order vein was removed using a scalpel. The weight was in the 0.9-3.2 grams interval. Leaf material in a single piece with a target weight of 1 gram was cut from the harvested leaves, allowing deviation from the target weight within a 0.85-1.15 grams interval. The leaf material was then wrapped around the index finger with the abaxial surface facing outwards in a way that would minimise the number of layers of the resulting fold, typically to 2-3 layers, then inserted into a 55mm by 70mm teabag, distributed by CDON AB. The material used in the teabags were not specified from the distributor, but the fact that they were mostly, if not completely unaffected by the different compost treatments made it seem unlikely to be cellulose fibres. By visual inspection they appeared as non-woven plastic polymer fibres, likely some variant of “Ethylene-Propylene Side by Side”. The teabags served to contain the leaf material throughout treatment, even when severely decayed, allowing for material exposure to compost liquids and surrounding atmosphere, and ease of retrieval at end of treatment.

4.2.4 Small-scale compost experiments

For the composting part of the experiments large outdoor plant containers in polyethylene were used as reaction vessels (Figure 4.5). These containers had a shape close to that of an inverted circular conical frustum, with a top and bottom diameter of 40 cm and 30 cm respectively. The height was 35 cm. The container bottom surface was not perforated, and the outer surface was in a light grey colour.

Figure 4.5
Compost “reaction vessel”, with perforated cover(left), and plastic insert(right).

The container interior during experiments could be divided into three layers. From the top, a relatively large volume of air, the middle layer a volume occupied by compost, and the bottom layer a comparatively small air volume. A perforated plastic insert was used, sitting between the container bottom and the layer of compost, forming an air volume of about 4 litres, with no direct gas exchange with the container exterior, or the air volume above the compost layer in the container. To reduce the rate of evaporation from the compost during experiments the opening of the container was covered with a double layer of aluminium foil that was folded down around the outer edge and held in place using a piece of string tied around the circumference. Depending on the specific experimental setup, the foil would be perforated to increase the availability of oxygen in the container interior, though with an increased rate of water loss, or remain unperforated.

The compost itself was used as provided with no sieving or other forms of intentional particle size selection performed, and was distributed in an even-depth, uncompacted layer in the container. The same weight of compost was used in each container within each experiment series to maintain a similar water content and oxygen consumption over time.

4.2.5 Temperature control

To maintain close to constant temperature conditions during treatments the main greenhouse compartment and three drying cabinets with adjustable temperature were used to contain the experiments. Each of the three cabinets were initially adjusted to an internal temperature setting, by glass thermometers in a capped flask filled with water, that would remain unchanged throughout the entire study. They were adjusted towards one of three target temperatures, 40°C, 60°C and 80°C. The drying cabinets were all different models and were kept in locations with different and variable external temperatures. All three cabinets were controlled by an internal thermostat. The 80°C cabinet was indoors close to room temperature, while the 60°C cabinet was in a garage kept above freezing. The 40°C cabinet was in a garage with temperature conditions closer to outdoor conditions.

4.2.6 Temperature measurement

All temperature measurements were done using a laboratory style partial immersion glass thermometer. The thermometer was an Amarell Precision "green spirit" 76mm partial immersion thermometer with an unknown working fluid. The thermometer was used to take temperature readings directly in compost and indirectly in the cabinets or greenhouse compartment. Taking readings in compost was done by inserting the thermometer in the centre of the compost volume and letting the fluid column settle while the compost container's top cover was on. To measure the temperature in the drying cabinets an Erlenmeyer flask or glass beaker filled with water was kept in the cabinet. Under the assumption that the flask or beaker with contents would reach a state of thermodynamic equilibrium with the cabinet interior between measurements, the water acted as a thermal reservoir with relatively high specific heat capacity, resistant to temperature change on a short time scale, from which the temperature could be measured. The measured temperature was taken to be the same as the cabinet's interior previous to opening the door. The flask or beaker was capped with aluminium foil, also during measurement, to reduce the effect of evaporation on the liquid's temperature and was placed on a shelf a good distance from the cabinet walls and any compost container present. The same method was employed in the greenhouse compartment, but here the flask was placed in the same plastic box as the tea bags undergoing "dry" treatment, which covered by a V-shaped roof fashioned from aluminium foil to reduce the effect of the compartment's variable irradiance throughout the day.

4.2.7 DAS- ELISA

Leaf material was collected from the majority of the test plants visually evaluated as negative and sporadically from plants evaluated as positive for PepMV. Tissue (0.5g) acquired from leaves not sap inoculated directly, was placed in BIOREBA extraction bags and stored at -80°C before use. Samples were prepared by adding 4.5 ml extraction buffer and homogenising leaf material using a homogeniser. A commercially available BIOREBA test kit for PepMV with polyclonal coating and conjugate antibodies was used with a *p*-nitrophenyl phosphate disodium hexahydrate chromogenic substrate. The test was carried out by hand in 96 well microtiter plates, with 2 blank, negative and positive wells as control and reference. The wells were coated with antibodies diluted in a coating buffer (1:200), pipetting the solution into the wells by hand, and incubating the plates in a fridge overnight in a plastic bag. The buffer solution was removed with a washing buffer and 100 µl of homogenised leaf was pipetted into each well and was again left to incubate in the fridge overnight. The wells were washed, and 100 µl enzyme-linked antibodies in a conjugate buffer (1:200) was pipetted into the wells and incubated overnight. After the wells were washed, the substrate solution was added and the plates placed in a dark drawer for 30 minutes before being read at 405 nm.

4.2.8 Experimental setup

A small-scale compost simulation was set up using plant containers with compost, tea bags containing symptomatic leaf material and temperature-controlled cabinets or greenhouse compartments, prepared as described in the paragraphs above. A container would be placed in one of the four constant temperature environments for approximately 24 hours to allow the compost to reach something close to an equilibrium temperature for the system. It was then brought to a workbench in a temperate room and tea bags were then buried in the compost to a depth of about 7cm, the bags lying down in the horizontal plane. When the tea bags were buried the time was noted as start of treatment. The temperature of the compost and the weight of the container was measured before it was then returned to the controlled environment. This entire process would take about 5-10 minutes. At end of treatment for the different tea bags the container would be brought out to the workbench, compost temperature and weight was measured, and tea bags randomly collected. This would be done as quickly as possible to minimise the change in temperature in the compost. The container would again be returned to the controlled environment. The collected tea bags were used to inoculate test plants, as described above, to determine when the loss of infectivity of symptomatic leaf material occurs under the different treatments.

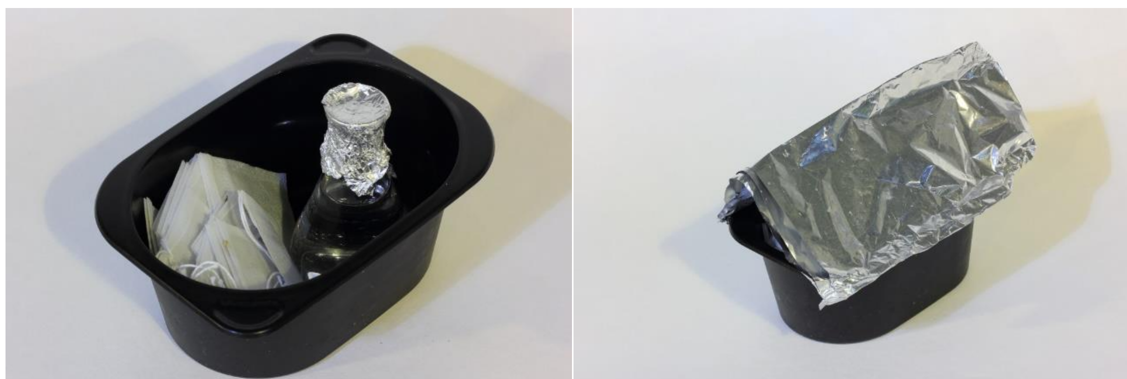

Figure 4.6
Plastic container with infected leaf material for use in experiment 4 (temperature-only treatment). An aluminium “roof” (right) was used to minimise the effect of the variable irradiance in the greenhouse compartment for the 20°C treatment. Contains Erlenmeyer flask with water for temperature measurement.

The study was divided into four separate experiments as shown in Figure 4.7.

Experiment 1 served as a pilot experiment for testing out the study’s methodology, and produce some initial, lower resolution results as an indicator for what time intervals to expect eradication to occur under the four different temperature treatments. The experiment was done at all four temperatures (20, 40, 60, and 80°C) with 10 kg of mature compost. The aluminium lid was not perforated for this experiment. Single tea bags were sampled at random from each temperature treatment as shown in Table 9.1 and three test plants per tea bag were used for bioassay.

In experiment 2, which was done to investigate effect of temperature in mature compost, compost from the same batch as experiment 1 was used and resolution was increased by increasing the number of tea bags per sample collection to three, while keeping the number of test plants inoculated per tea bag at three, nine plants in total. Selection of experiment run times for each temperature was decided based on results from experiment 1. This experiment was done using 6 kg mature compost and perforated lids on the containers. Three tea bags were sampled at random from each of the four temperature treatments

following a schedule as seen in Table 9.1. Three test plants per tea bag was used for bioassay.

Experiment 3 was done using compost freshly collected at three different temperatures in a more recently established, active windrow reaching internal temperatures above 60°C. This was done to determine if development stage of the compost had any effect on eradication time of PepMV. Compost collected at temperatures of 40°C and 60°C was used in treatments with the corresponding temperature. Additionally, compost collected at a temperature of 50°C was used in a parallel 60°C treatment. As in experiment 2, 6 kg of compost and a perforated lid was used. Three tea bags were collected according to the schedule shown in Table 9.1, and three test plants per tea bag was used for bioassay.

Experiment 4 was done to determine the effect of temperature on eradication time. Tea bags with infected leaf material were simply placed in open plastic boxes under dry conditions for the duration. Initial "pilot" experiments are here included under the same experiment. At end of treatment the tea bags were used to inoculate test plants to test infectivity. The sampling schedule for each temperature is shown in Table 9.1.

4.2.9 Results registration

Test plants inoculated from treated leaf material were visually inspected for symptoms of PepMV after 14 days and evaluated as either positive, if symptoms were present, or negative.

As a control for the visual evaluation, material from test plants was collected and tested by DAS-ELISA. The results from the test were registered as positive or negative, read values evaluated by comparison with positive and negative control wells.

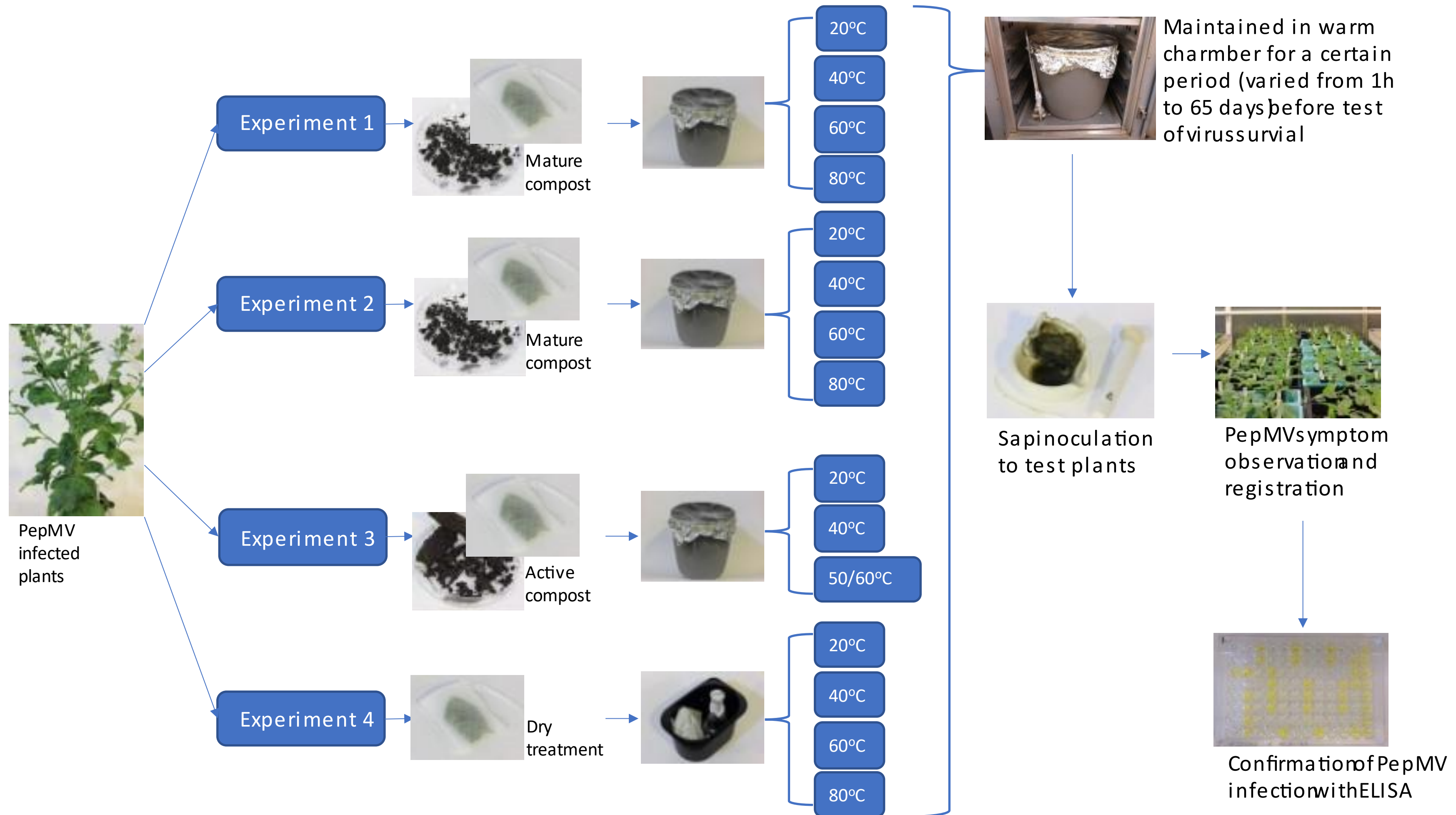

Figure 4.7
Experiment flow diagram. The study was divided into four experiments, subdivided into separate temperature treatments.

5 Results

5.1 Main result

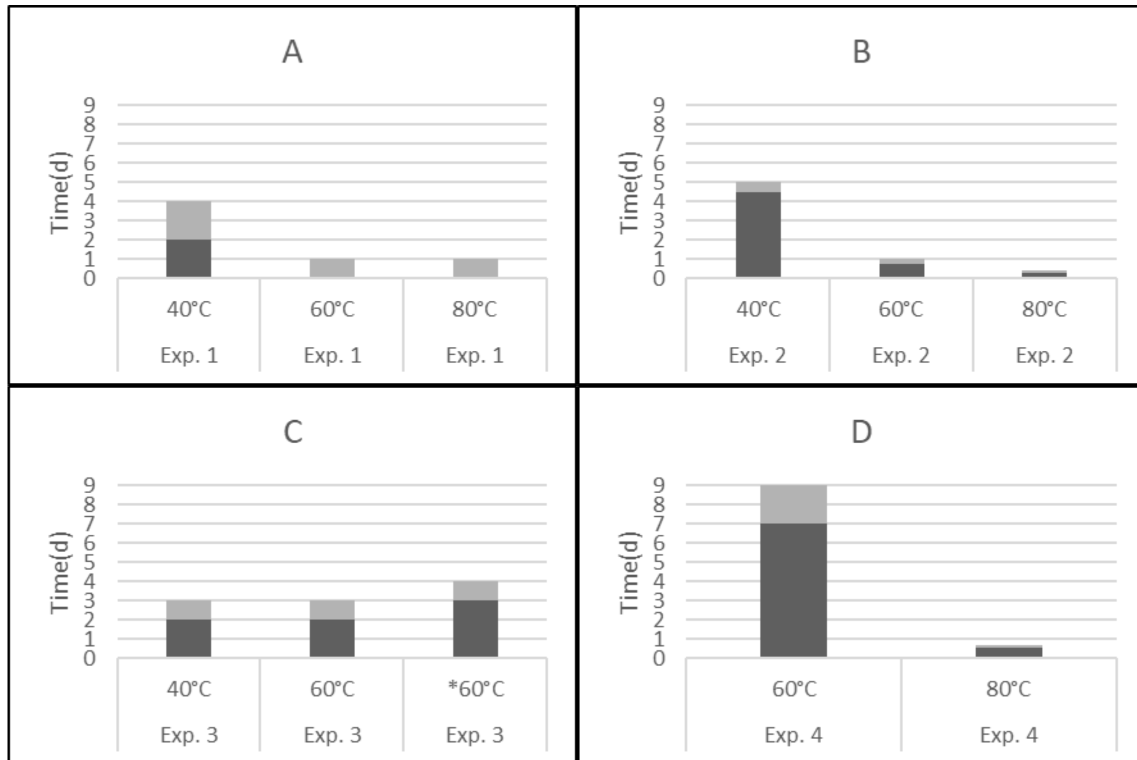

Figure 5.1
PepMV eradication times at different temperature treatments in experiment 1 (A), 2 (B), 3(C), 4 (D). The dark bar denotes the period within which the leaf material was still confirmed infectious, until the last infection occurred. The light bar denotes the period between the last positive and first negative result. * denotes “50/60” treatment.

The eradication time of PepMV under the different treatments is shown in Figure 5.1 and Table 5.1. Virus eradication times were found to be consistently higher in experiment 4 (dry treatment) where comparable, and for experiment 1 (pilot with mature compost), 2 (mature compost), and 4 (dry) virus eradication time decreased with increasing temperature. A more detailed description of the results is given below.

Table 5.1
Shows individual treatment series within each experiment. 'Time range' shows the sampling intervals, 'eradicated' the interval between the last positive and first negative result, 'average temperature' the measured average treatment temperature start-end. Bold text highlights series where the pathogen is eradicted. All temperatures are given in °C. * denotes "50/60" treatment.

Experiment	Cabinet temperature	Time range	Average temperature	Eradicated
Exp. 1	20	0-21d	16.8	>21d
Exp. 1	**40**	**0-21d**	**39.3**	**2-4d**
Exp. 1	**60**	**0-12d**	**63.6**	**<1d**
Exp. 1	**80**	**0-12d**	**79**	**<1d**
Exp. 2	20	0-21d	16	>65d
Exp. 2	**40**	**0-5d**	**40.8**	**4.5-5d**
Exp. 2	**60**	**0-24h**	**63**	**18-24h**
Exp. 2	**80**	**0-24h**	**72.3**	**6-9.5h**
Exp. 3	**40**	**0-10d**	**45.3**	**2-3d**
Exp. 3	**60**	**0-6d**	**56.9**	**2-3d**
Exp. 3	***60**	**0-6d**	**56**	**3-4d**
Exp. 4	20	0-65d	15	>65d
Exp. 4	40	0-65d	44	>65d
Exp. 4	**60**	**0-12d**	**64**	**<9d**
Exp. 4	**80**	**0-24h**	**77.8**	**13.25-16.75h**

5.2 Experiment 1

All test plants at 20°C developed symptoms (Figure 5.2) except for the final day where one of three plants did not. At 40°C, one of three plants developed symptoms at day 2, and from day 4 non were infected. After 24 hours at the 60°C and 80°C treatments no test plants developed symptoms. Eradication time decreased from 40°C to 60°C with 3 days.

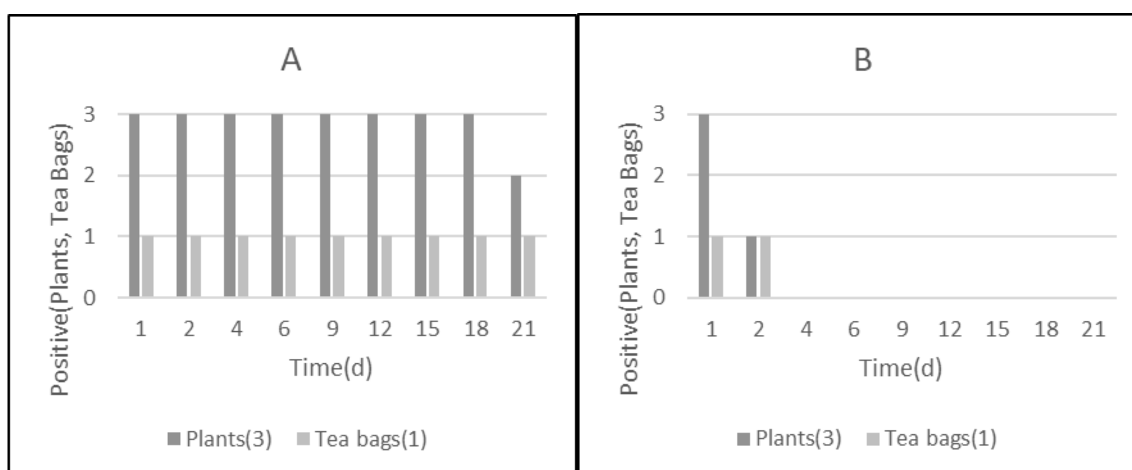

Figure 5.2
Experiment 1, results from temperature treatments 20°C (A) and 40°C (B). The dark bar shows number of positive test plants, the light-grey bar the number of positive tea bags.

5.3 Experiment 2

Figure 5.3 A show test plants still developed symptoms after 65 days of the 20°C treatment with some variation in the number of positive test plants. Only 2 out of 3 tea bags were positive on day 51, 57 and 65. The lowest number of positive plants was 2 and occurred on day 51.

At 40°C all test plants were positive up to and including day 3 (Figure 5.3 B), and then started trailing off until day 5, when no plants developed symptoms. A total number of 6 tea bags were tested with 18 test plants 4 days treatment time, with a result of 11/18 test plants and 5/6 tea bags positive, as the treatment was done in two partially overlapping series.

For the 60°C treatment, the first negative plants occurred after 18 hours (Figure 5.3 C) with 4/9 plants and 1/3 tea bags negative. At the next sampling time, 24 hours, all plants were negative.

The number of positive test plants started to drop after 4 hours at 80°C (Figure 5.3 D) and was no longer detected after 9.5 hours. With two overlapping series, at 6h 2/18 plants and 1/6 tea bags were positive, none of them in the 6-24h series.

Eradication time decreased with temperature with an unknown amount from 20°C down to 5 days at 40°C, with a further reduction of 4 days when increased to 60°C, and with another 14.5 hours from 60°C to 80°C.

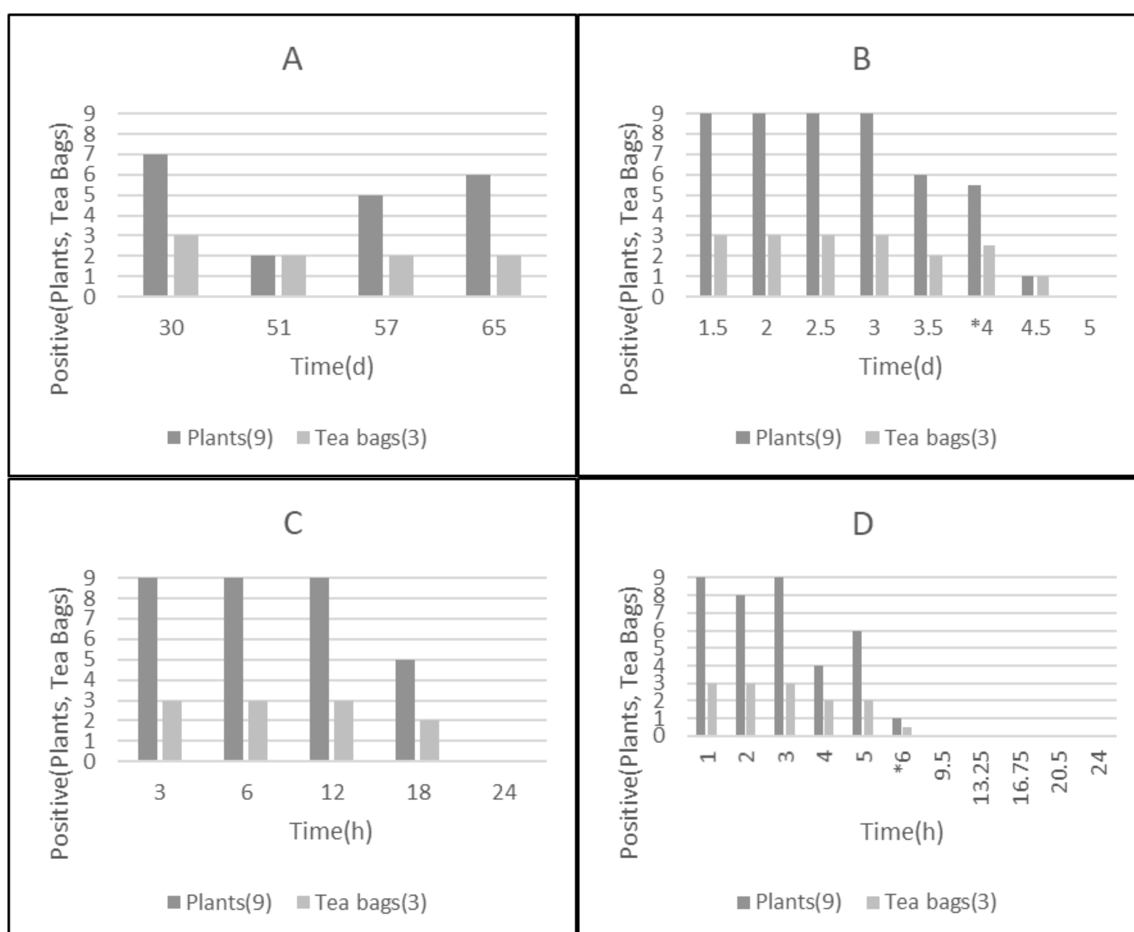

Figure 5.3
Results from experiment 2 from temperature treatments 20°C (A), 40°C (B), 60°C (C), and 80°C (D). The dark bar shows number of positive test plants, the light-grey bar the number of positive tea bags. * denoted average of overlapping series.

5.4 Experiment 3

For all three temperature treatments, the first negative test plants occurred on day 2 (Figure 5.4). While no further infection occurred at 40°C and 60°C (A, B) from day 3, for the "50/60" treatment, denoted (C), after all tea bags had been negative at day 2, the infection re-emerged on day 3 with 1/3 plants positive for all three tea bags. From day 4 all test plants were negative for 50/60°C.

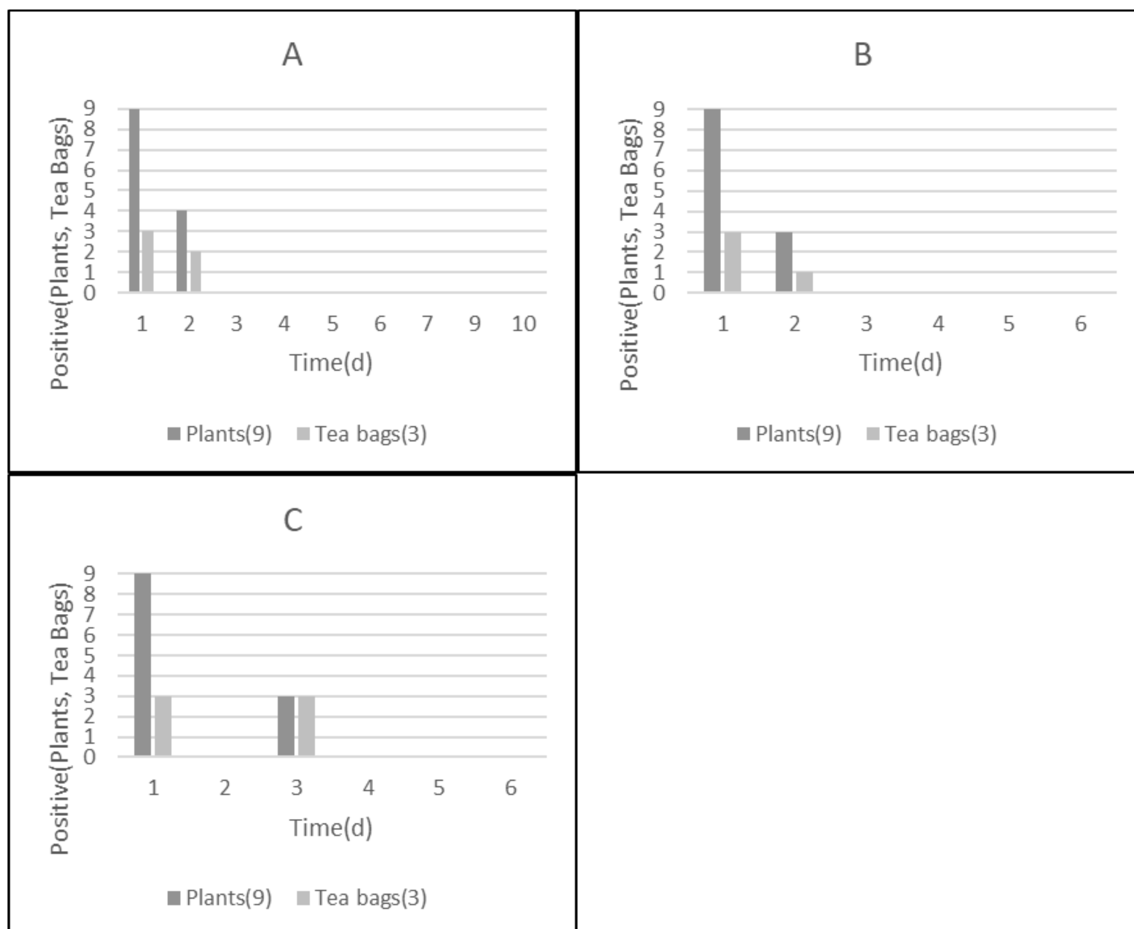

Figure 5.4
Results from experiment 3. The dark bar shows number of positive test plants and the light-grey bar the number of positive tea bags for treatments 40°C (A), 50°C (B), and "50/60°C" (C).

5.5 Experiment 4

The 20°C and 40°C treatments had no visible effect over 65 days, with only a single test plant not developing symptoms (Figure 5.5 A, B).

At 60°C considerable variability in the number of positive test plants was seen from day 2 until day 7 (Figure 5.5 C), which was also the last day inoculation resulted in any symptomatic plants.

No effect was seen in the 80°C treatment (Figure 5.5 D) before 6 hours. At 6 hours 13/18 test plants and 6/6 tea bags were positive. At 9.5 hours and 13.25 hours ~half the test plants and all tea bags were positive. From 16.75 hours, no further infections occurred.

From 40°C to 60°C the eradication time was reduced with an unknown amount to 9 days. Increasing the temperature to 80°C reduced the eradication time further with ~8.3 days.

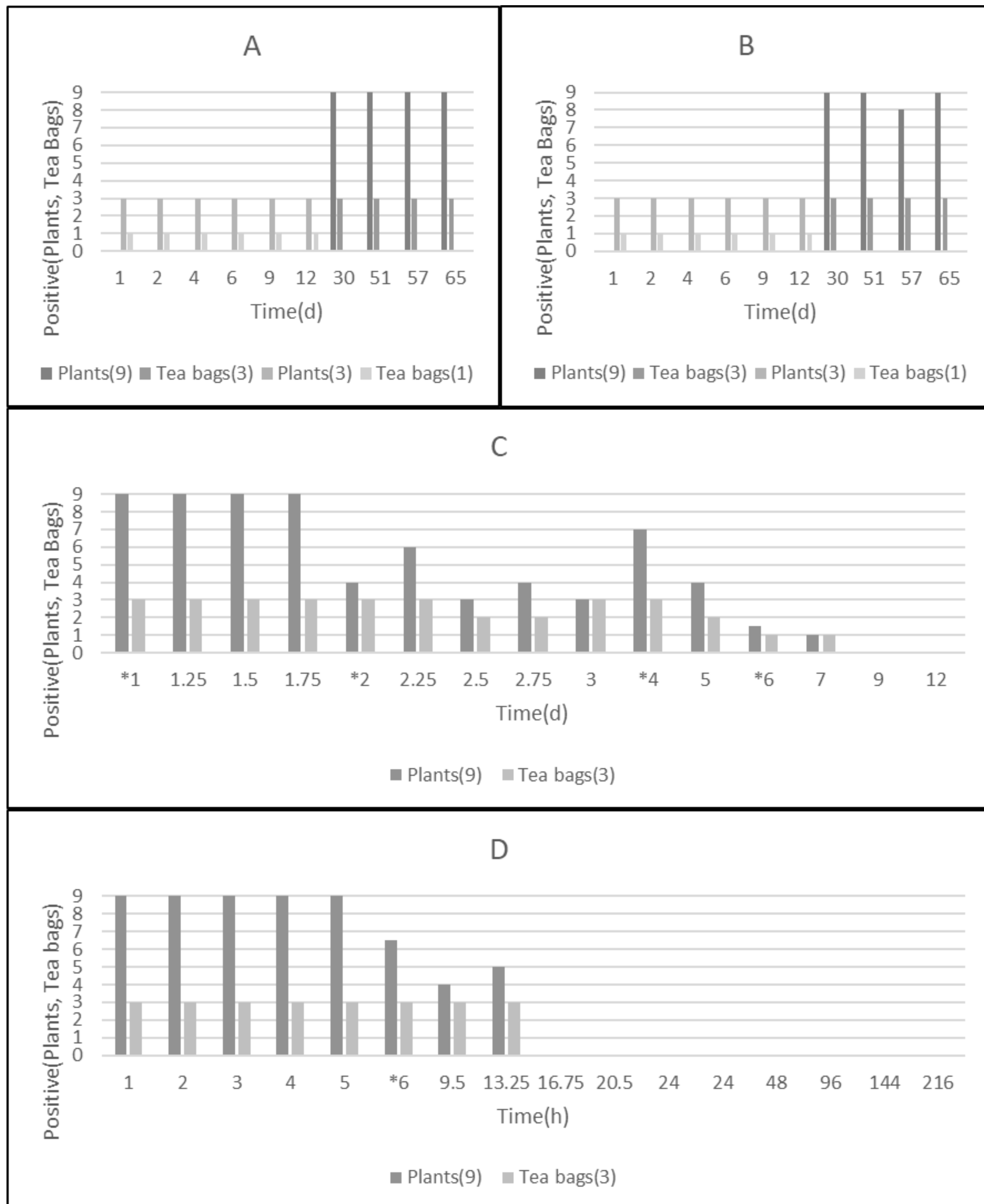

Figure 5.5
Result from experiment 4, with 20°C in A, 40°C in B, 60°C in C, and 80°C at D. Lightly coloured pairs of bars indicate series where only one tea bag was collected per sample time. Within bar pairs the darker bar shows number of positive test plants and the light bar the number of positive tea bags. * denotes average values.

5.6 Test plant symptoms

Infected *N. occidentalis* 37B typically started showing systemic symptoms within a week after inoculation. The initial symptom in all plants was a very unambiguous vein clearing at one or

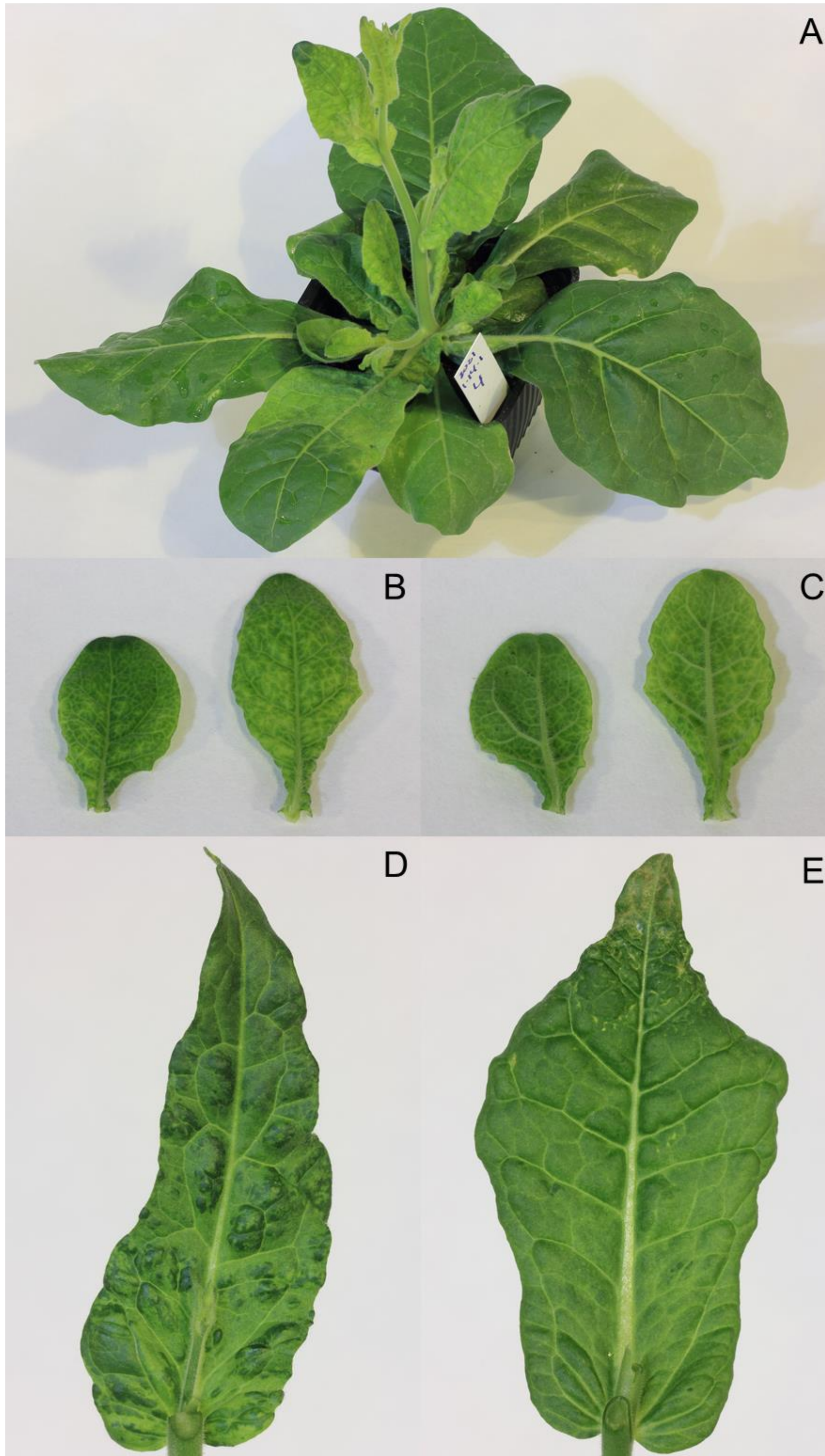

Figure 5.6
A shows 37B ~2 weeks after infection with PepMV TomA2001-1. Systemic symptoms typically initially showed as vein clearing close to the leaf base and progressed towards the leaf tip. Figure B and C show younger leaves with typical symptoms. D shows mottling, chlorosis and bubbling on a stem leaf, more typical of later stage symptoms. Figure E shows a bit more uncommon presentation, with initial symptoms in the leaf tip being mottling and necrosis.

Figure 5.7
Test plants in greenhouse main compartment after inoculation.

a few younger leaves, starting close to the base of the leaf (Figure 5.6 A-C). The vein clearing was often first visible only on one side of the primary vein, with a later distribution more symmetrical around the vein as it progressed towards the leaf tip, covering the entire

leaf area. Leaf bubbling in combination with chlorosis (Figure 5.6 D) would typically occur after the initial vein clearing symptoms had presented.

A sporadic but specific deviation from systemic symptoms as described above (Figure 5.6 A-D) occurred. Figure 5.6 E shows a leaf with no obvious symptoms from the leaf base to close to the leaf tip, which has small scattered chlorotic spots and some necrosis. No attempt was made on mapping the rate of occurrence of this particular symptom pattern.

While it was not measured in any rigorous fashion, indicator plants not showing any systemic symptoms, and therefore evaluated as not infected, appeared to typically be of a more uniform height and taller than infected plants.

Due to what appeared to be a reliable occurrence of systemic symptoms, and because of frequently severe damage to the leaf surface from the mechanical sap inoculation, no systematic observation of local lesions was done.

5.7 ELISA testing

Out of the 501 plants tested for PepMV by DAS-ELISA four results deviated from the visual evaluation of the plants. Three were positive where the test plants showed no symptoms, all from different tea bags. One of the three tea bags did not result in symptoms in any of the test plants it was used to inoculate and this was confirmed by testing.

One plant was visually evaluated to be symptomatic where the test gave a negative result. This individual was inoculated from a tea bag that did not result in infection in the two other plants, which was confirmed by testing of both individuals. The other two parallel treatment tea bags ("50/60, 72h) both resulted in one symptomatic and two asymptomatic plants, with the asymptomatic confirmed by testing.

For the 122 symptomatic and the 379 asymptomatic plants tested ~99.2% in both categories, were in agreement with the visual evaluation of the test plants.

6 Discussion

6.1 Methods

Visual evaluation of *N. occidentalis* 37B indicator plants for systemic symptoms 14 days after inoculation with PepMV strain Ch2 is a reliable method for determining infectivity of test material. Out of the 501 indicator plants tested for presence for PepMV with ELISA, only 4 (~99.2%) deviated from the visual evaluation.

When familiar with the visual appearance of 37B plants, there was no sense of ambiguity when evaluating symptoms. The results of the ELISA testing confirms this, as comparison with positive and negative control wells left no doubt about the result of each individual test well. The rate of latent infection appears to be very low, at ~0.8% or less, based on the three apparent false negatives. As a large portion of the ELISA testing was carried out by hand by an untrained operator, it is not unlikely that some or all of these are as a result of well contamination, possibly making the actual occurrence of visual false negatives lower.

The laboratory-scale approach to constant temperature simulated compost conditions, while undoubtably primitive compared to the more elaborate and well controlled systems used in other publications (Fayolle et al., 2006; Noble et al., 2011), was a useful setup for testing. Little equipment was needed, and it was easily available and affordable. With preparation and preheating of the container with compost done within a relatively short amount of time, it allowed for testing on short notice with little pre-planning or work. The setup was used to successfully show a difference in eradication time between dry temperature only treatments of infected leaf material, a mature compost medium, and an active compost medium, likely in large part due to microbial action. However, how similar the treatment conditions in the containers are compared to any kind of commonly used, actual compost system remains an open question. It is assumed that using mature compost as an environment and a source of inoculum of compost-relevant microorganisms for any introduced pathogen infected plant material will result in a fairly pessimistic estimate of what eradication times will be in a newly established compost of comparable temperature. This is because of the already partially depleted resources available for microorganisms in the mature compost, which likely limits population growth rates.

The characterisation of the composts used in testing, as well as the conditions in the containers while the experiments were running leaves much to be desired. Nothing is known about the oxygen concentration and consumption in the compost containers during treatment. Neither was the C/N ratio determined, though this is probably within some typical range for “common” garden waste compost in southern Norway. pH in the compost was measured at the start and end of the treatments and was found to be in the 6.8-7.1 range, which is reasonably close to the pH of the sap inoculation buffer, and therefore assumed to be of little effect where PepMV eradication is concerned. While the water mass fraction was measured at each sampling time, the matric potential was not determined. All these factors are likely to have at least some influence on the microbial activity during treatment, and a more detailed knowledge about them would probably make it easier to compare the results under these conditions to other publications on compost-related eradication.

While cost, the need for work hours and availability of equipment limited this study to some extent, another unforeseen and important factor was the COVID-19 pandemic. The outbreak limited access to lab facilities, caused some delays, and prevented some planned work from getting done. Still, the main parts of the study were completed.

6.2 Effect of compost

The effect of compost on eradication of PepMV from leaf material was found to be considerable. When comparing dry treatment (exp. 4) to the results compost from treatments in experiments 2 and 3 (Table 5.1), the least difference in eradication time was a factor ~2.8 times higher. The study also indicates that the maturity of the compost when the infected leaf material is introduced can have a big effect on the temperature response in eradication time. While the eradication time in the active compost at 40°C was 0.6 times that measured in mature compost, at 60°C it was higher by a factor of 3 (Table 5.1).

The decrease in eradication times from dry (Figure 5.1 D) to compost treatments (Figure 5.1 A, B, C) was expected. While there are no previous publications comparing survival of PepMV under compost-like conditions with comparable dry temperature treatments, other works have compared dry temperature treatments with moist or wet temperature treatments. Ling (2010) reported a difference in eradication time for PepMV in or on the seed coat of tomato seeds as 1 hour when submerged in a 55°C water bath and 48 hours at 72°C of dry heat, using a similar approach to bioassay. Assuming no significant dilution effects in the hot water bath gave rise to the difference, this seems to demonstrate the importance of the presence of water in PepMV eradication during temperature treatment. The leaf material in dry treatments at temperatures of 40°C or higher was dehydrated relatively quickly, while material buried in compost likely maintained a water content similar to that of the surrounding compost. This highlights a limitation of this study. The lack of a water x temperature only experiment, analogous to the dry experiment, makes it difficult to evaluate how important the effect of microbial action is for eradication time under the different temperature treatments in compost. Still, a notable effect of microbial action at 40°C and 60°C can reasonably be inferred from the comparison between the active and mature compost experiments, as the initial water mass fraction (Figure 9.1) in the active compost was similar for both temperatures and higher than in the mature compost while still yielding such differing results (Figure 5.1 B, C).

The higher eradication time at 60°C (Figure 5.1 B, C) in the still active compost was surprising. Kerins et al. (2018) speculates that "… fresh material for composting is, at any given time x temperature combination, likely to provide a more deleterious environment for a pathogen than the same time x temperature with mature compost." While the result does not seem to fit with this thinking, it is unclear whether this is an effect inherent to the particular compost conditions, or if it arises as a result of flawed methodology, for example from some stage in handling before introduction of the infected plant material, or if it is related to some change in environmental conditions, like availability of oxygen, having a severe negative effect on an already established large microbial population. Given the progressively decreasing eradication times with increasing temperature in experiment 1, 2, and 4, some explanation along the lines of the latter examples seems likely.

6.3 Temperature

PepMV in leaf material was found to be very stable at 20°C, and at 40°C when dry. The virus was still detected at day 65, and positive tea bags resulted in infection in all inoculated test plants in both dry and mature compost treatments (Figure 5.3 A, B, & Figure 5.5 A, B). Temperatures above 40°C were effective at eradicating the virus from leaf material exposed to mature compost. At 40°C no infection occurred after 5 days (Figure 5.1 B), a 20°C increase in temperature reduced the eradication time to 1/5 of that, and a further increase by 20°C to 80°C reduced the time needed to 1/4 again, with eradication occurring within 6

hours. Dry temperature eradication occurred within 9 days at 60°C and 16 hours 45 minutes at 80°C, ~13 times faster (Figure 5.1 D).

The stability at 20°C is comparable to previously reported results (Jones et al., 1980) where PepMV remained infective in sap and desiccated leaves for at least 3 and 6 months, respectively. Since the leaves in the 20°C dry treatment in this study never dried out due to the relatively high surrounding air humidity, the treatment is likely more accurately compared to the sap than the desiccated leaf material.

The higher eradication time in experiment 2 compared to experiment 1 at 40°C (Table 5.1 B, A) likely reflects the higher resolution in experiment 2 from 3 times the number of tea bags and test plants per sampling time.

The results from the active compost experiment, where the eradication time was the same at 40°C as at 60°C (Figure 5.1 C), one would expect a reduction simply on account of the increased temperature, and especially when considering the results of the mature compost and dry treatments. This is likely some effect of the chosen method to simulate compost conditions that becomes noticeable in this particular treatment, as discussed above, and should probably be disregarded.

Mikkelsen et al. (2006) cited a result on eradication of PepMV in shredded infected tomato plants. The virus was no longer detected after 5 days in a windrow at a minimum temperature of 60°C. No more details about the methods used were available, including the detection method. This result and the results from this study at 60°C were all within the same order of magnitude (Figure 5.1 A-D). With no further composting results for PepMV or any species in Alphaflexiviridae found, this was the only somewhat comparable result available.

This study's use of leaf material only, with the first-order vein removed, done under the assumption that variability in eradication time imposed by variable vein thickness with regards to non-temperature effects, where penetration depth into the tissue might be a factor, would be reduced, makes it dangerous to interpret the results of this study as directly translatable to an equivalent-temperature compost or a comparable litter situation. First-order veins, petioles, and pieces of, or intact, stem with their closer to cylindrical structures with greater thickness would possibly take longer to have all their tissue colonised and exposed to microbial enzymatic action. This together with the interpretation of the difference in active compost as a result of microbial action means eradication times for PepMV could be longer for thicker and possibly more resilient tissues. Consequently, eradication time likely varies with what plant species' infected residue is being composted. As possibly the most relevant PepMV host, composting of tomato plant residue under different conditions should be studied in greater detail.

Compared to other plant viruses' reported eradication times at different temperatures in compost, most of which were in the 40-80°C range (Noble & Roberts, 2004), PepMV would seem to be a comparatively unstable virus in the same range. Most have been reported to survive more than 3 days around to 60°C and some several days at temperatures around 70°C, though only a very limited number of species have been studied (Noble & Roberts, 2004; Wichuk et al., 2011). However, with the variation in detection methods, and in many cases limited information about compost temperature over time, comparisons should be done only tentatively. Some of the reported bioassay results used centrifugation of sap at ~100,000g to increase test sensitivity (Avgelis & Manios, 1989; Avgelis & Manios, 1992), likely well beyond what would be detectable with sap inoculation alone. For virus species more sensitive to microbial action, as discussed above for PepMV, particle size of the infected material to be composted is a possible source for variation in eradication time. This

also varies between the different publications, with some using leaf material only, as cited in Wichuk et al. (2011), while others used all parts of the plant, milled to particles in the centimeter (Avgelis & Manios, 1992) or millimeter (Ghaly et al., 2006) range.

7 Conclusion

The eradication time of PepMV in infected leaves in compost is more than 65 days at 20°C. At 40°C and 60°C it is in the range of 3-5 days and 1-3 days, respectively, depending on compost maturity. Eradication time at 80°C is between 6-9.5 hours.

In the 40-80°C temperature range, the compost environment reduces eradication times of PepMV considerably.

Maturity of compost used in laboratory-scale systems simulating compost conditions can have a considerable effect on PepMV eradication time.

8 Bibliography

Adams, M. J., Antoniw, J. F., Bar-Joseph, M., Brunt, A. A., Candresse, T., Foster, G. D., Martelli, G. P., Milne, R. G., Zavriev, S. K. & Fauquet, C. M. (2004). The new plant virus family Flexiviridae and assessment of molecular criteria for species demarcation. *Arch Virol*, 149 (5): 1045-60. doi: 10.1007/s00705-004-0304-0.

Alfaro-Fernández, A., del Carmen Córdoba-Sellés, M., Herrera-Vásquez, J. Á., Cebrián, M. d. C. & Jordá, C. (2010). Transmission of Pepino mosaic virus by the Fungal Vector Olpidium virulentus. *Journal of Phytopathology*, 158 (4): 217-226. doi: 10.1111/j.1439-0434.2009.01605.x.

Allaby, M. (2012). *A dictionary of plant sciences*: Oxford University Press.

Avgelis, A. D. & Manios, V. I. (1989). Elimination of tomato mosaic virus by composting tomato residues. *Netherlands Journal of Plant Pathology*, 95 (3): 167-170. doi: 10.1007/BF01999972.

Avgelis, A. D. & Manios, V. I. (1992). *ELIMINATION OF CUCUMBER GREEN MOTTLE MOSAIC TOBAMOVIRUS BY COMPOSTING INFECTED CUCUMBER RESIDUES*: International Society for Horticultural Science (ISHS), Leuven, Belgium.

Bibi, I., Djelouah, K., Remah, A. & Afechtal, M. (2017). Pepino Mosaic Virus: a serious threat to tomato crops worldwide. *Revue Marocaine des Sciences Agronomiques et Vétérinaires*

Blystad, D.-R., van der Vlugt, R., Alfaro-Fernández, A., del Carmen Córdoba, M., Bese, G., Hristova, D., Pospieszny, H., Mehle, N., Ravnikar, M., Tomassoli, L., et al. (2015). Host range and symptomatology of Pepino mosaic virus strains occurring in Europe. *European Journal of Plant Pathology*, 143 (1): 43-56. doi: 10.1007/s10658-015-0664-1.

Córdoba-Sellés, M. d. C., García-Rández, A., Alfaro-Fernández, A. & Jordá-Gutiérrez, C. (2007). Seed Transmission of Pepino mosaic virus and Efficacy of Tomato Seed Disinfection Treatments. *Plant Disease*, 91 (10): 1250-1254. doi: 10.1094/PDIS-91-10-1250.

Fakhro, A., von Bargen, S., Bandte, M., Büttner, C., Franken, P. & Schwarz, D. (2011). Susceptibility of different plant species and tomato cultivars to two isolates of Pepino mosaic virus. *European Journal of Plant Pathology*, 129 (4): 579-590. doi: 10.1007/s10658-010-9722-x.

Fayolle, L., Noble, R., Coventry, E., Aime, S. & Alabouvette, C. (2006). Eradication of Plasmodiophora brassicae during composting of wastes. *Plant Pathology*, 55 (4): 553-558. doi: 10.1111/j.1365-3059.2006.01399.x.

Ghaly, A. E., Alkoaik, F., Snow, A. & Singh, R. (2006). Effective Thermophilic Composting of Crop Residues for Inactivation of Tobacco Mosaic Virus. *American Journal of Biochemistry and Biotechnology*, 2 (3): 111-118. doi: 10.3844/ajbbsp.2006.111.118.

Herrmann, R. F. & Shann, J. F. (1997). Microbial Community Changes During the Composting of Municipal Solid Waste. *Microbial Ecology*, 33 (1): 78-85. doi: 10.1007/s002489900010.

Jones, R. A. C., Koenig, R. & Lesemann, D. E. (1980). Pepino mosaic virus, a new potexvirus from pepino (Solanum muricatum). *Annals of Applied Biology*, 94 (1): 61-68. doi: https://doi.org/10.1111/j.1744-7348.1980.tb03896.x.

Kerins, G., Blackburn, J., Nixon, T., Daly, M., Conyers, C., Pietravalle, S., Noble, R. & Henry, C. M. (2018). Composting to sanitize plant-based waste infected with organisms of plant health importance. *Plant Pathology*, 67 (2): 411-417. doi: 10.1111/ppa.12729.

Kreuze, J. F., Vaira, A. M., Menzel, W., Candresse, T., Zavriev, S. K., Hammond, J., Hyun Ryu, K. & Report Consortium, I. (2020). ICTV Virus Taxonomy Profile: Alphaflexiviridae. *J Gen Virol*, 101 (7): 699-700. doi: 10.1099/jgv.0.001436.

Legrand, P. (2015). Biological assays for plant viruses and other graft-transmissible pathogens diagnoses: a review. *EPPO Bulletin*, 45 (2): 240-251. doi: 10.1111/epp.12222.

Ling, K.-S. (2010). Effectiveness of Chemo- and Thermotherapeutic Treatments on Pepino mosaic virus in Tomato Seed. *Plant Disease*, 94 (3): 325-328. doi: 10.1094/pdis-94-3-0325.

Ling, K. S. (2007). Molecular characterization of two Pepino mosaic virus variants from imported tomato seed reveals high levels of sequence identity between Chilean and US isolates. *Virus Genes*, 34 (1): 1-8. doi: 10.1007/s11262-006-0003-x.

Mayne, S. & O'Neill, T. (2017). Pepino mosaic virus of tomato – new results on strains, symptoms and persistence. *Agricultural and Horticulture Development Board*, Factsheet 25/16.

Mikkelsen, L., Elphinstone, J. & Jensen, D. (2006). Literature review on detection and eradication of plant pathogens in sludge, soils and treated biowaste.

Noble, R. & Roberts, S. J. (2004). Eradication of plant pathogens and nematodes during composting: a review. *Plant Pathology*, 53 (5): 548-568. doi: https://doi.org/10.1111/j.0032-0862.2004.01059.x.

Noble, R., Dobrovin-Pennington, A., Pietravalle, S., Weekes, R. & Henry, C. M. (2011). Indicator organisms for assessing sanitization during composting of plant wastes. *Waste Manag*, 31 (8): 1711-9. doi: 10.1016/j.wasman.2011.04.007.

Shipp, L., Buitenhuis, R., Stobbs, L., Wang, K., Kim, W.-S. & Ferguson, G. (2008). Vectoring Pepino mosaic virus by bumble-bees in tomato greenhouses. *Annals of Applied Biology*, 153: 149-155. doi: 10.1111/j.1744-7348.2008.00245.x.

Spence, N. J., Basham, J., Mumford, R. A., Hayman, G., Edmondson, R. & Jones, D. R. (2006). Effect of Pepino mosaic virus on the yield and quality of glasshouse-grown tomatoes in the UK. *Plant Pathology*, 55 (5): 595-606. doi: 10.1111/j.1365-3059.2006.01406.x.

Vlugt, R. (2009). Pepino mosaic virus. *Hellenic Plant Protection Journal*, 2: 47-56.

Werkman, A. W. & Sansford, C. E. (2010). Pest Risk Analysis for Pepino Mosaic Virus for the EU. *EU Sixth Framework Project Project PEPEIRA.*, Deliverable Report 4.3.

Wichuk, K. M., Tewari, J. P. & McCartney, D. (2011). Plant Pathogen Eradication During Composting: A Literature Review. *Compost Science & Utilization*, 19 (4): 244-266. doi: 10.1080/1065657x.2011.10737008.

Wilson, C. R. (2014). *Applied plant virology / Calum R. Wilson, Tasmanian Institute of Agriculture, University of Tasmania, Australia*. Modular texts. Wallingford, Oxfordshire: CABI.

9 Appendix

9.1 Compost water mass fraction

Figure 9.1
Water mass fraction of compost throughout treatment.

9.2 Sampling times

Table 9.1
Shows points of sample collection in treatment time within each experiment temperature. Time in hours is shown in italics.

	Temp.	Series	Time range	Sampling times									
Exp 1	20, 40	1	1-21d	1	2	4	6	9	12	15	18	21	
Exp 1	60, 80	1	1-12d	1	2	4	6	9	12				
Exp 2	20	1	30-65d	30	51	57	65						
Exp 2	40	1	1.5-4d	1.5	2	2.5	3	3.5	4				
Exp 2	40	2	4-5d	4	4.5	5							
Exp 2	*60*	*1*	*3-24h*	*3*	*6*	*12*	*18*	*24*					
Exp 2	*80*	*1*	*1-6h*	*1*	*2*	*3*	*4*	*5*	*6*				
Exp 2	*80*	*2*	*6-24h*	*6*	*9.5*	*13.3*	*16.8*	*20.5*	*24*				
Exp 3	40	1	1-10d	1	2	3	4	5	6	7	8	9	10
Exp 3	60	1	1-6d	1	2	3	4	5	6				
Exp 3	50/60	1	1-6d	1	2	3	4	5	6				
Exp 4	20	1	1-21d	1	2	4	6	9	12				
Exp 4	20	2	30-65d	30	51	57	65						
Exp 4	40	1	1-21d	1	2	4	6	9	12				
Exp 4	40	2	30-65d	30	51	57	65						
Exp 4	60	1	1-12d	1	2	4	6	9	12				
Exp 4	60	2	1-2d	1	1.25	1.5	1.75	2					
Exp 4	60	3	2-3d	2	2.25	2.5	2.75	3					
Exp 4	60	4	4-9d	4	5	6	7	9					
Exp 4	80	1	1-9d	1	2	4	6	9					
Exp 4	*80*	*2*	*1-6h*	*1*	*2*	*3*	*4*	*5*	*6*				
Exp 4	*80*	*3*	*6-24h*	*6*	*9.5*	*13.3*	*16.8*	*20.5*	*24*				

Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway