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What drives the endophytic stage of *Pseudomonas syringae* pv. *actinidiae* in symptomless kiwifruit plants?

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Executive summary

What drives the endophytic stage of *Pseudomonas syringae* pv. *actinidiae* in symptomless kiwifruit plants?

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Whether we wish to eradicate a virus affecting people or reduce the economic impact of a plant pathogen, it is crucial to determine as accurately as possible which individuals in the population may carry the pathogen without showing any symptoms. Those symptomless carriers could indeed later express symptoms and help spread the pathogen. The overall goals of this project was to (i) verify our initial assumption that *Pseudomonas syringae* pv. *actinidiae* (Psa) can survive in symptomless kiwifruit plants and to identify the tissues (stem or leaf) where this phenomenon happens; (ii) identify the genes or the metabolites responsible or required for Psa to stay alive in symptomless plants; and (iii) find the triggers that would signal the end of the latency phase, i.e. the triggers that will lead to the plant expressing symptoms.

This report presents the results obtained since September 2019 and the general conclusions that can be drawn from the work carried out since the inception of this project.

The main results obtained in the last 12 months are that keeping potted plants of *Actinidia chinensis* var *chinensis* 'Hort16A' and 'Zesy002' (commonly known as Gold3) or A*. chinensis* var *deliciosa* 'Hayward' and 'Zes007' (commonly known as Green11) under flooding-like conditions did not always result in the suppression of symptom expression. The concentration of inoculum and potentially the rate of growth of the plant were more influential in determining whether an inoculated plant would become a symptomless carrier. Psa was able to survive and multiply in symptomless potted plants of *Actinidia arguta*, a species known to be resistant to Psa. This result suggests that plants of resistant cultivars or species may be symptomless carriers and pose a risk to spread the disease.

Analysis of RNASeq data, indicates that in the first 48 hours post-inoculation, bacterial gene expression is similar in plants expressing symptoms and in symptomless plants. In contrast, a large number of kiwifruit genes were differentially expressed in plants inoculated with a low concentration of Psa (plants that would become symptomless carriers) versus plants inoculated with a high concentration of Psa (plants that would express symptoms). In particular, a number of auxin responsive proteins were highly overexpressed within 3 hours of inoculation.

The conclusions from the overall project are that:

- In all the cultivars tested, 'Hayward', Gold3, 'Hort16A', Green11 and in *A. arguta,* leaf infection and stem inoculation can lead to Psa multiplying endophytically without the plant expressing symptoms.
- The bacterial load (number of bacteria per unit of plant tissues) in symptomless tissues can be as high as that in tissues showing symptoms.

- The bacteria were able to travel at least short distances in symptomless plants.
- The physiological state of the bacteria influence its ability to infect, but once the bacteria is in the plant it seems that it is the plant that determines whether the interaction will lead to symptom development.
- The physiological state of the plant and its rate of growth seem to be factors controlling symptom expression.
- Results obtained 2 years in a row suggest that following leaf infection some cultivars, e.g. 'Hayward' are able to kill the pathogen while others cannot. This ability to kill the pathogen could be an important characteristic of plants that are not systemically colonised.

The first contracted objective, which aimed to determine which plants and where in symptomless plants Psa was multiplying, has been fully met. Metabolites and genes that were differentially expressed in symptomless plants have been identified; this was the goal of the second objective. However, further analysis is needed to fully understand the mechanisms supporting pathogen multiplication in a symptomless plant. The third objective aimed at identifying environmental triggers that could break latency in kiwifruit. Some of those triggers have been identified, e.g. inoculum concentration and water stress; however, other factors that interfere with plant growth might also been involved.

Practically, the results of this project mean that late summer and autumn infections are most likely to allow Psa in the tissues without any visible symptoms. The usefulness of late season application of protectants might have been underestimated.

Some cultivars or some species considered as resistant because they do not show symptoms can be symptomless carriers (e.g. *A. arguta*) and therefore could still play a role in the distribution of Psa.

Actively growing plants seemed more likely to express symptoms; this could explain the relatively high occurrence of symptoms expression in early spring. It would be useful to determine whether the rapid growth of spring time is enough to break the latency-associated late season infection or with the colonisation of the scion tissues from infected rootstocks. In which case, the lack of widespread symptoms in Gold3 orchards could indicate that Psa was not present in those plants even though they were grafted on infected rootstocks.

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1 Introduction

Whether we wish to eradicate a virus affecting people or reduce the economic impact of a plant pathogen, it is crucial to determine as accurately as possible which individuals in the population carry the pathogen. Testing every individual (people or plants) is not always feasible and therefore we need to understand where and how the pathogen survives in the population and we need to identify individuals that carry or may carry the pathogen but do not show any symptoms (symptomless carriers).

The time between infection and symptom development is known as the latency phase, during that phase infected individuals are symptomless carriers. For plant pathogenic bacteria, the length of this phase can vary based on various factors including environmental factors such as temperature. It is extremely difficult to identify plants that are in a latency phase; since to determine whether a symptomless plant is infected usually requires destructive sampling of the plant tissue. We know when a plant expresses symptoms that it was infected and that earlier it was a symptomless carrier but that is when the latency phase is over. In addition, it is rare to know when the plant was first infected, and therefore how long the latency period was. Yet understanding why an infected plant does not express symptoms and what are the triggers to "break" the latency phase, are important pieces of information that are needed to put in place strategies to prevent or manage outbreaks.

The situation described above applies to *Pseudomonas syringae* pv. *actinidiae* (Psa) causing bacterial canker on *Actinidia* spp. Until symptoms develop on a kiwifruit plant we do not know if the plant is infected, and if it is infected when it will express symptoms or whether it will ever express symptoms. Detecting Psa in a symptomless plant requires destroying the tissues and therefore it is not possible to determine whether the tissue tested was ever going to display symptoms. This makes working on symptomless carriers challenging. Yet there are reasons to suspect that many kiwifruit plants in the Bay of Plenty could be symptomless carriers. A large number of *Actinidia chinensis* var. *chinensis* 'Zesy002' (commonly known as Gold3) were grafted on rootstocks infected by Psa. We know that Psa can cross the graft union and therefore we cannot rule out that Psa has not infected the Gold3 scions even though those plants have not shown signs of infection. In addition, plants grafted on non-infected rootstocks but growing in an environment where Psa is present could also become infected without showing symptoms.

Since we cannot determine which symptomless vine in an orchard is infected, and we do not know how to cure potentially infected vines from Psa, knowing the triggers that end the latency phase of Psa is the best option to prevent large-scale disease. Knowledge of the triggers that end the latency phase would allow us to put in place a strategy to prevent those triggers taking place. Such a strategy would prevent vines potentially infected by Psa to express symptoms that include shoot die back, loss of leaders and/or vine death.

This project had three overall major objectives. The first objective was to verify our initial assumption that Psa can survive in symptomless kiwifruit plants and to identify the tissues (stem or leaf) where this phenomenon happens. The second objective was to identify the genes or the metabolites responsible or required for Psa to stay in a latent phase. The last objective aimed to find the triggers that would signal the end of the latency phase, i.e. the triggers that will lead to the plant expressing symptoms.

Previously, in the first parts of this project, we showed that stem inoculation of *A. chinensis* var. *deliciosa* 'Hayward' or *A. chinensis* var. *chinensis* 'Hort16A', using low concentration of inoculum, led

to the plants being symptomless carriers (Vanneste et al. 2019a; Vanneste et al. 2019b). We also showed that keeping plants of 'Hort16A' in flooded-like conditions seemed to increase the likelihood of the plants being symptomless. The bacterial populations in some of those symptomless plants was as high as that found in plants expressing symptoms (Vanneste et al. 2019b). In addition, we showed that leaf infection of *A. chinensis* var *deliciosa* 'Zes007' (commonly known as Green11), *A. chinensis* var. *chinensis* 'Russell', 'Hayward' or 'Hort16A' also resulted in Psa surviving as an endophyte in symptomless plants (Vanneste et al. 2019a; Vanneste et al. 2019b). The expression of PR1, a gene linked to Psa resistance in kiwifruit plants, was overexpressed in the early phase of the interaction (3 hours post-inoculation) in Gold3 plants inoculated with low concentration of Psa. However, over time this difference disappeared (Vanneste et al. 2019b). Finally, we showed that there were some significant differences in the metabolites present in plants inoculated with high or low concentrations of inoculum. Some of those compounds were identified as belonging to classes linked with disease resistance such as terpene, lipid, isoflavonoid or flavonoid (Vanneste et al. 2019a).

The goals of this last part of the project were to confirm the impact of inoculum concentration and flooding conditions on symptoms expression in different cultivars of kiwifruit, and to analyse the RNASeq data in order to identify the plant and the bacterial genes where expression is linked with a plant being a symptomless carrier. This could help us understand whether latency is determined by the plant or the pathogen and might offer clues on the triggers that end the latency period.

2 Materials and methods

Experiments on potted plants were carried out as described previously (Vanneste et al. 2019a; Vanneste et al. 2019b); they are briefly described below to allow this report to be read as a stand-alone document.

2.1 Plant material

The five cultivars of kiwifruit used in this study: Gold 3, 'Hort16A', 'Hayward', Green11, and *Actinidia arguta* 'Hortgem Tahi' were obtained from Multiflora Laboratories Limited (Auckland, New Zealand) as tissue culture plants. Individual plantlets were planted into 1.0-L pots containing a 50:50 ratio of potting mix and perlite. Potting mix was sourced from Daltons Limited (coated extend 3-month starter 1 kg/m^3; Dolomite 2 kg/m^3; Gypsum Coarse 2 kg/m^3; Lime – Ag Grade 2 kg/m^3; Microplus 0.5 kg/m^3; Osmocote® Exact 8/9 Standard Start 5 kg/m^3; Permawet™ 0.75 kg/m^3). The plants were placed in the glasshouse at 16–24°C, with a day length of approximately 16 hours achieved using high pressure sodium lamps when necessary. Plantlets were watered once daily and fertilised weekly with Thrive® (Yates NZ Ltd) at the recommended rate. For a number of reasons, including the lock-down period following the COVID-19 New Zealand outbreak, some plants of 'Hayward' and Gold3 were cut back to one or two buds and left to regrow in the glasshouse before being used in an experiment. When such plants were used in an experiment, all the plants in that experiment had been cut back.

2.2 Bacterial inoculum

Two strains of Psa biovar 3, were used in this study. Psa 10627 was isolated from leaf spots on kiwifruit leaves from a Te Puke commercial orchard (Vanneste et al. 2013) and SR123 a naturally occurring streptomycin-resistant isolate. The strain SR123 was used to determine the epiphytic and endophytic Psa population. Psa was grown on agar plates of King's B medium with or without streptomycin (100 µg/ml) (King et al. 1954) at 28°C for 48 hours before being resuspended in sterile water to a final concentration of c.1 x 10^4 colony forming units (CFU) per mL (low inoculum) or c. 1 x 10^8 cfu/ml (high inoculum).

2.3 Inoculation

Stem inoculation was done by stabbing the stem of young potted plants with a toothpick at a single point between the two youngest fully developed leaves. A 2-µl drop of bacterial suspension was then immediately pipetted into the wound, 2 µl of sterile water was pipetted in the wound of the control plants. All plants were then placed into high-humidity tents (relative humidity >90%) for 3 weeks. The temperature inside the tents varied depending on the time of the year the experiment was carried out but we aimed to maintain the temperature as close to 21°C as possible. Disease assessment was carried out weekly for 3–4 weeks following inoculation and was recorded as the length of stem being necrosed. Plants that were not stabbed (not wounded) and not inoculated, and plants that were stabbed but not inoculated (wounded but not inoculated) were used as negative controls.

2.4 Bacterial isolation

Except for the first two experiments on Gold3, in which several stem sections of 2-cm each were analysed per plant, endophytic populations of Psa were usually determined by cutting one 2-cm section around the inoculation point (1 cm above and 1 cm below). Sections were weighed, then surface sterilised using the same protocol as leaf discs, except for the sodium hypochlorite concentration was increased to 5%. Sterile stem sections were macerated in plastic bags by crushing with a hammer in 1 ml of sterile water. Of this, 100 µl was removed, serially diluted and three 100 µl drops of each dilution were plated onto King's B medium supplemented with streptomycin (100 µg/ml).

2.5 Experimental set up

Eleven experiments on potted plants were carried out after September 2019 when the last interim report was submitted (Vanneste et al. 2019a) (Table 1). These experiments can be divided in two groups. Those where the goal was to determine if the pathogen could survive in symptomless kiwifruit plants, inoculated with low concentrations of Psa (c. 10^4 cfu/ml) and kept in flooded conditions (their pots standing in water with the water level being just few centimetres below soil level), and, those where the goal was to determine the conditions which lead to symptoms development. This phenomenon about flooded plants was first described for 'Hort16A' (Vanneste et al. 2019b). Unless stated otherwise, the plants were kept in flooded conditions for the duration of the experiment. The other experiments aimed to determine whether Psa symptoms expression was linked to the rate of growth of the plant when plants were inoculated with low or high concentrations of Psa inoculum.

When the bacterial suspension used to inoculate the plants was c. 10^4 cfu/ml the inoculum was considered 'low concentration inoculum'. When the concentration of Psa was c. 10^8 cfu/ml the inoculum was considered 'high concentration inoculum'.

The lockdown associated with the incursion of COVID-19 in New Zealand offered the opportunity of undertaking more plant experiments using plants made available by other teams who could not use them because of the lockdown. This is the case for Experiments 4, 6 and 10. Although for those experiments we were not always able to determine the presence of live cells of Psa in symptomless plants, the results are still very informative and they are presented in this report.

Table 1. Experiments carried out since September 2019 with results presented in this report.

Experiment Number	Host plant	Cultivar	Criteria measured
1	*Actinidia chinensis* var. *chinensis*	'Zesy002'	Length of stem necrosis and Psa endophytic population
2	*A. chinensis* var. *chinensis*	'Zesy002'	Length of stem necrosis and Psa endophytic population
3	*A. chinensis* var. *chinensis*	'Zesy002'	Length of stem necrosis
4	*A. chinensis* var. *chinensis*	'Zesy002'	Length of stem necrosis
5	*A. chinensis* var. *chinensis*	'Zesy002'	Length of stem necrosis and Psa endophytic population
6	*A. chinensis* var. *chinensis*	'Hort16A'	Length of stem necrosis
7	*A. chinensis* var. *deliciosa*	'Zes007'	Length of stem necrosis
8	*A. arguta*	'HortGem Tahi'	Length of stem necrosis and Psa endophytic population
9	*A. chinensis* var. *deliciosa*	'Hayward'	Length of stem necrosis and Psa endophytic population
10	*A. chinensis* var. *deliciosa*	'Hayward'	Length of stem necrosis
11	*A. chinensis* var. *chinensis*	'Zesy002'	Length of stem necrosis

A. chinensis var. *chinensis* 'Zesy002' is commonly known as Gold3, and *A. chinensis* var. *chinensis* 'Zes007' is commonly known as Green11.

2.6 Origin of the RNASeq data

The experimental design used for generating the RNASeq data has been described earlier (Vanneste et al. 2019a); the information is summarised below to allow this report to be read as a stand-alone document.

The experiment was set up to compare gene expression of young potted Gold3 plants that were not wounded and not inoculated, with that of plants wounded but not inoculated and plants wounded and inoculated. Stem tissues were sampled before inoculation (six plants) and six plants per treatment were sampled 3, 24 and 48 hours after inoculation, except for the not wounded and not inoculated treatment for which only four plants were sampled. This gave us a total of 72 samples, which was the optimum number for RNASeq analysis. In addition, 10 to 12 plants of each treatment were kept in the glasshouse to monitor symptom development and determine endophytic and epiphytic populations of Psa in stems.

Plants used in this experiment were approximately 50–60 cm in height with 6–8 fully developed leaves. They were stem-inoculated using two concentrations of the pathogen: high concentration (1×10^8 cfu/ml) or low concentration (1×10^4 cfu/ml). Sampling involved cutting a 4-cm section of stem, 2 cm above and 2 cm below the inoculation point, at each time point. Stem sections were immediately frozen in liquid nitrogen and stored at –80°C until the RNA was extracted.

2.7 RNASeq data analysis

From each sample, total RNA was isolated from about 100 mg of kiwifruit tissue ground by mortar and pestle in liquid nitrogen using the Spectrum Plant Total RNA Kit (Sigma-Aldrich). Quality control of the RNA was carried out as described in Appendix 1, the RNA was then sequenced by Novogene using an Illumina MiSeq™V2 platform. The raw reads received from Novogene were subjected to a quality control step and then analysed using the bioinformatics programmes described in Appendix 1. The fold change gene expression for the plant genes and the bacterial genes was estimated using DESeq2 (Love et al. 2014) with a front end developed by A. Saei. The results are presented as volcano plots showing for each of the genes analysed the log2-fold change of gene expression and its associated Log 10 *p* adjusted value.

3 Results

3.1 Impact of inoculum concentration and flooding on symptoms expression in different cultivars of kiwifruit

3.1.1 Impact of inoculum concentration and flooding on symptoms expression on Gold3 plants

The first two experiments on Gold3 were carried out to determine whether Psa could survive and travel in symptomless plants after stem inoculation with low concentrations of Psa. In the first experiment, 3 weeks post-inoculation, only one of the six plants inoculated with a low concentration of Psa (4.3×10^4 cfu/ml) showed symptoms (a stem necrosis of 10 mm), but all the plants inoculated with a high concentration of Psa (4.3×10^8 cfu/ml) had a stem necrosis ranging from 6 to 30 mm.

The stem of four of the six plants inoculated with a low concentration of Psa and of four of the six plants inoculated with a high concentration of Psa were cut in sections of 2 cm each with one of the sections centred on the inoculation point (Figure 1). Psa was isolated from the section containing the inoculation point for the eight plants analysed. In two of the plants inoculated with a low concentration of Psa the pathogen was isolated in sections of the stem neighbouring the inoculation point. The plant in which Psa was found 10 cm away from the inoculation point was the only plant inoculated with a low concentration of Psa that showed symptoms (Figure 2).

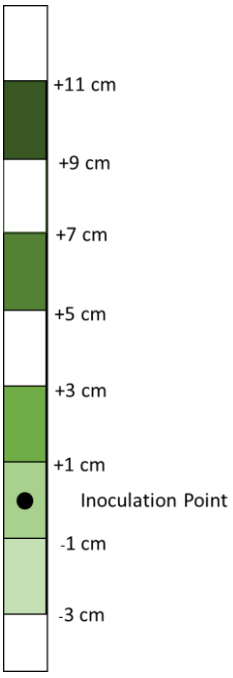

Figure 1. Diagram representing the stem sections that were analysed for presence of *Pseudomonas syringae* pv. *actinidiae* (Psa) in plants of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) plants inoculated with different concentrations of Psa. The numbers represent the distance in centimetres from the inoculation point. The segments in different shades of green are the stem segments that were analysed for Psa population. The segments in white were not analysed.

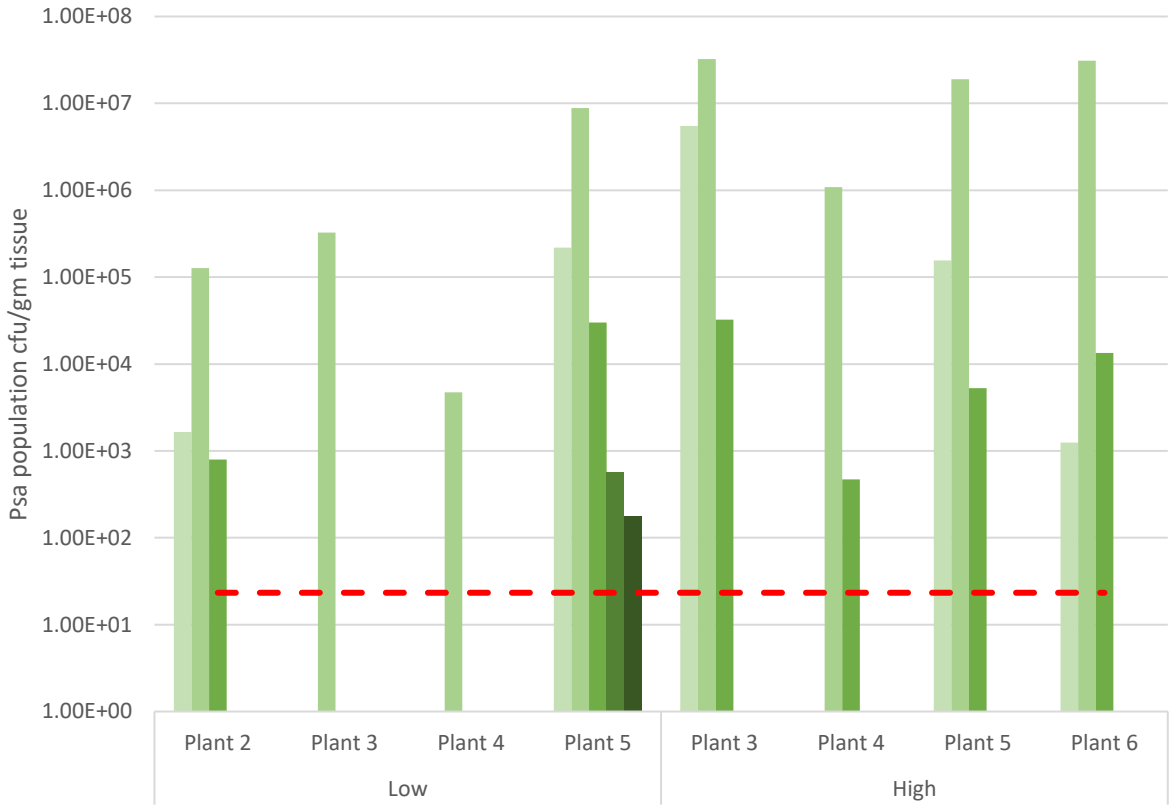

Figure 2. Endophytic population of *Pseudomonas syringae* pv. *actinidiae* (Psa) in cfu/g of tissue isolated from stem sections of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) plants inoculated with low concentration (4.3 x 10^4 cfu/ml) or high concentration (4.3 x 10^8 cfu/ml) of Psa. The colour of the bars represents the section of the stem from which Psa was isolated (Figure 1). The four plants inoculated with high concentration of Psa and Plant 5 inoculated with low concentration of Psa showed stem necrosis. The three other plants inoculated with low concentration of Psa were symptomless. The red dotted line represents the limit of detection of Psa by dilution plating on agar plates of King's B medium (23 cfu/gm of tissue).

This experiment was repeated using 3.7 x 10^4 cfu/ml as low concentration of Psa and 3.7 x 10^8 cfu/ml as a high concentration of Psa. Four weeks post-inoculation, only two of the five plants inoculated with high concentration of Psa showed visible stem necrosis (8 and 13 mm). None of the plants inoculated with low concentrations of Psa showed any symptoms. Psa was isolated from the five plants analysed, two inoculated with low concentration and three inoculated with high concentration of Psa (Figure 3).

These results indicate that Psa is not only able to survive and but also to travel at least short distances in symptomless plants of Gold3.

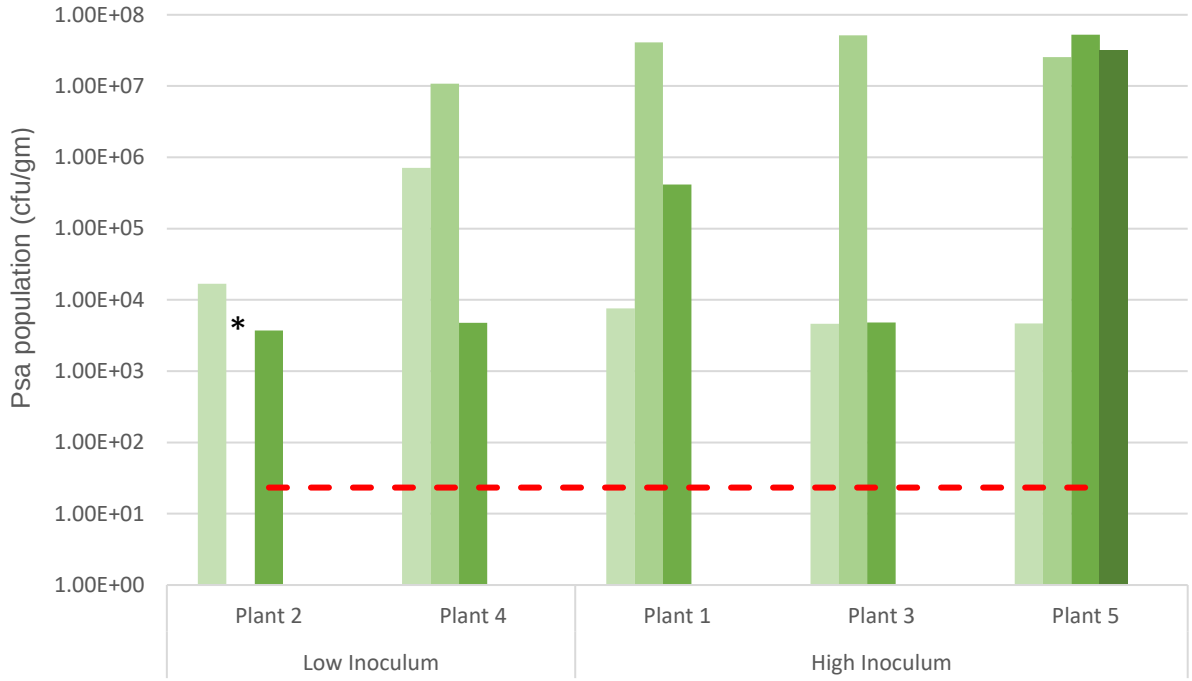

Figure 3. Endophytic population of *Pseudomonas syringae* pv. *actinidiae* (Psa) in cfu/g of tissue isolated from stem sections of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) plants inoculated with low concentration of 3.7 x 10^4 cfu/ml or high concentration (3.7 x 10^8 cfu/ml) of Psa. The colour of the bars represents the section of the stem from which Psa was isolated (Figure 1). Only Plant 3 and Plant 5 inoculated with high concentration of Psa showed symptoms of stem necrosis. All the other plants were symptomless. The asterisk indicates a missing data point. The red dotted line represents the limit of detection of Psa by dilution plating on agar plates of King's B medium (23 cfu/gm of tissue).

Experiments 3, 4 and 5 were carried out to determine whether keeping the plants under flooding-like conditions after stem inoculation with a low concentration of inoculum increased the likelihood of having symptomless carriers, as a previous experiment with 'Hort16A' suggested (Vanneste et al. 2019b).

In Experiment 3, the six plants inoculated with the high concentration of Psa (3.3 x 10^8 cfu/ml) and kept under normal conditions and the six plants kept under flooding-like conditions showed stem necrosis. Of the six plants inoculated with low concentration of Psa (3.3 x 10^4 cfu/ml) and kept under normal conditions only one plant showed symptoms. Similarly, of the six plants kept under flooding-like conditions, only one showed symptoms (Figure 4).

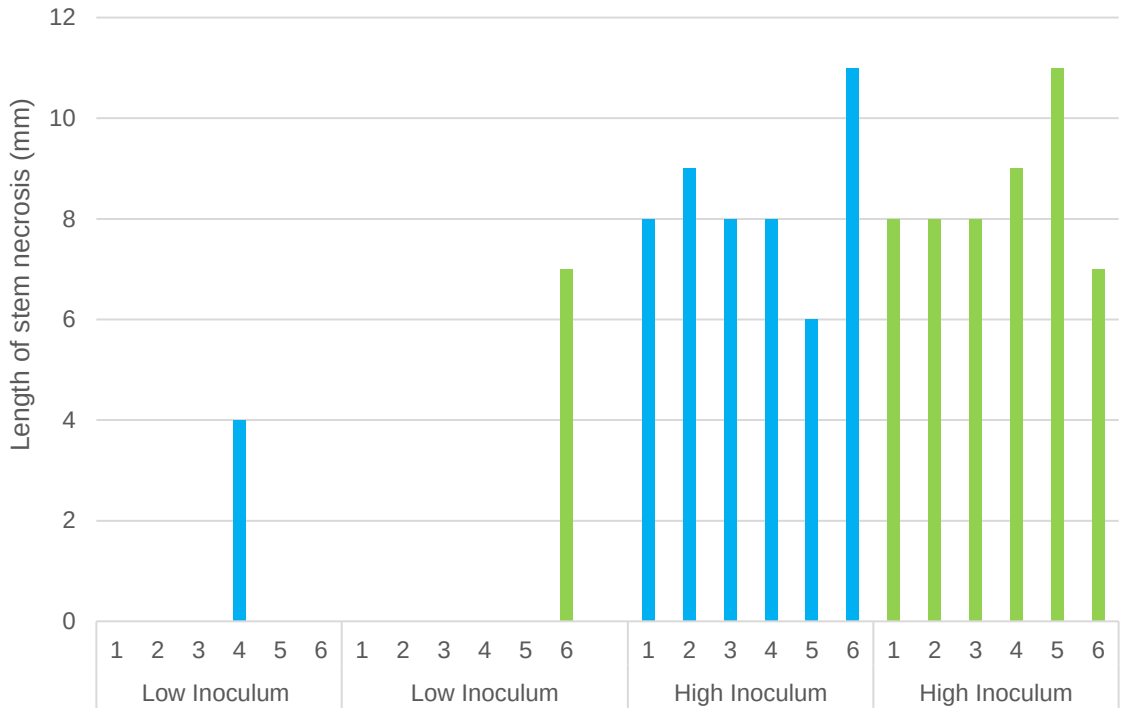

Figure 4. Length of stem necrosis of young potted kiwifruit plants of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) inoculated with a low concentration (3.3 x 10^4 cfu/ml) or a high concentration (3.3 x 10^8 cfu/ml) of *Pseudomonas syringae* pv. *actinidiae*. The blue bars represent length of stem necrosis of plants kept under flooding-like conditions (water level constantly kept a few centimetres below soil level) and the green bars represent length of stem necrosis of plants kept under normal conditions.

Experiment 4 was carried out on approximately 4-month-old plants. All plants inoculated with high concentration of Psa (2.7 x 10^8 cfu/ml) showed stem necrosis of similar length, regardless of whether they were kept in flooding-like conditions or not. Two of the four plants inoculated with low concentration of Psa (2.7 x 10^4 cfu/ml) and kept under flooding conditions and two of those that were not kept under flooding conditions showed stem necrosis (data not shown).

In Experiment 5, none of the 12 plants inoculated with low concentration of Psa (1 x 10^4 cfu/ml) showed symptoms. In contrast, the four plants inoculated with high concentration of Psa showed stem necrosis. There was no difference in the endophytic populations of Psa in plants inoculated with a low concentration of Psa and kept under flooding-like conditions with those kept under normal conditions (Figure 5).

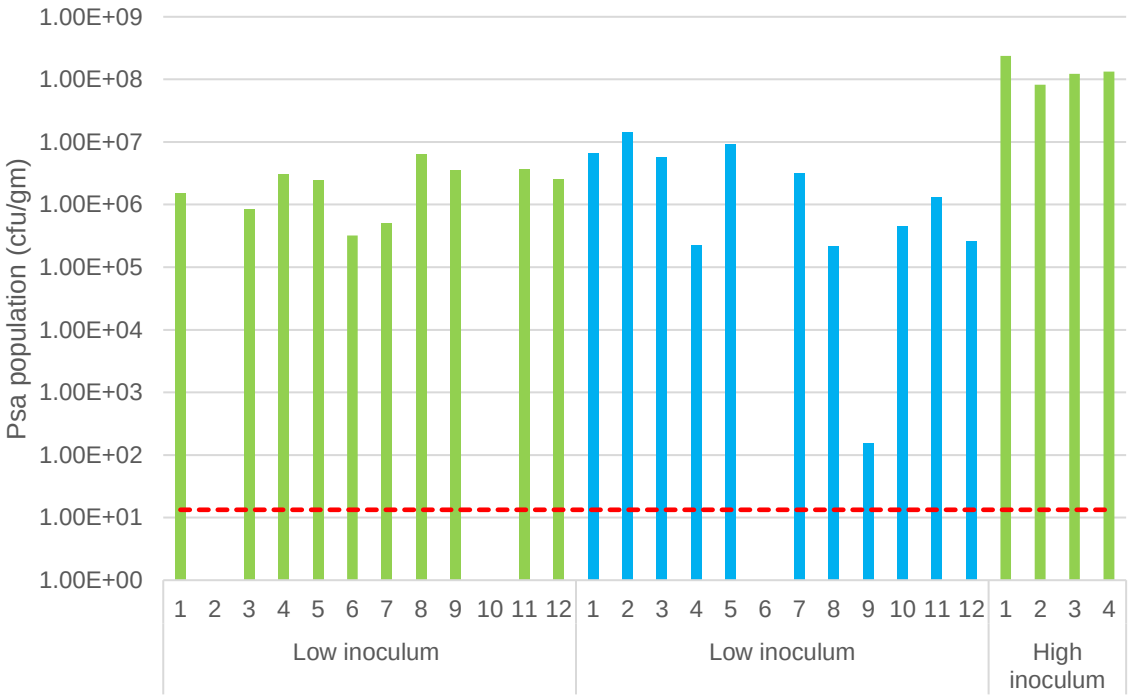

Figure 5. Endophytic populations of *Pseudomonas syringae* pv. *actinidiae* (Psa) in cfu/g of tissue isolated from stem sections of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) plants inoculated with low concentration (1.0 x 10^4 cfu/ml) or high concentration (1.0 x 10^8 cfu/ml) of Psa. The blue bars represent bacterial populations from plants kept under flooding-like conditions (water level constantly kept a few centimetres below soil level) and the green bars represent bacterial populations from plants kept under normal conditions. The red dotted line represents the limit of detection of Psa by dilution plating on agar plates of King's B medium (13 cfu/gm of tissue).

Keeping the plants under flooding-like conditions did not alter the number of plants that expressed symptoms or the average length of stem necrosis in any of the three experiments. The inoculum concentration was the major factor leading to symptoms expression, the higher the inoculum the higher the probability that the plant would express symptoms.

3.1.2 Impact of inoculum concentration and flooding on symptoms expression on 'Hort16A', Green11 and *A. arguta* plants

These experiments were carried out to determine whether the ability of Psa to survive in symptomless tissues after stem inoculation was limited to a few cultivars or was a more general phenomenon of the kiwifruit/Psa interaction.

Experiment 6 was conducted on 3-month-old 'Hort16A' plants that were inoculated with a bacterial suspension containing 2.0 x 10^4 cfu of Psa/ml (low inoculum) or 2.0 x 10^8 cfu/ml (high inoculum) (Figure 6).

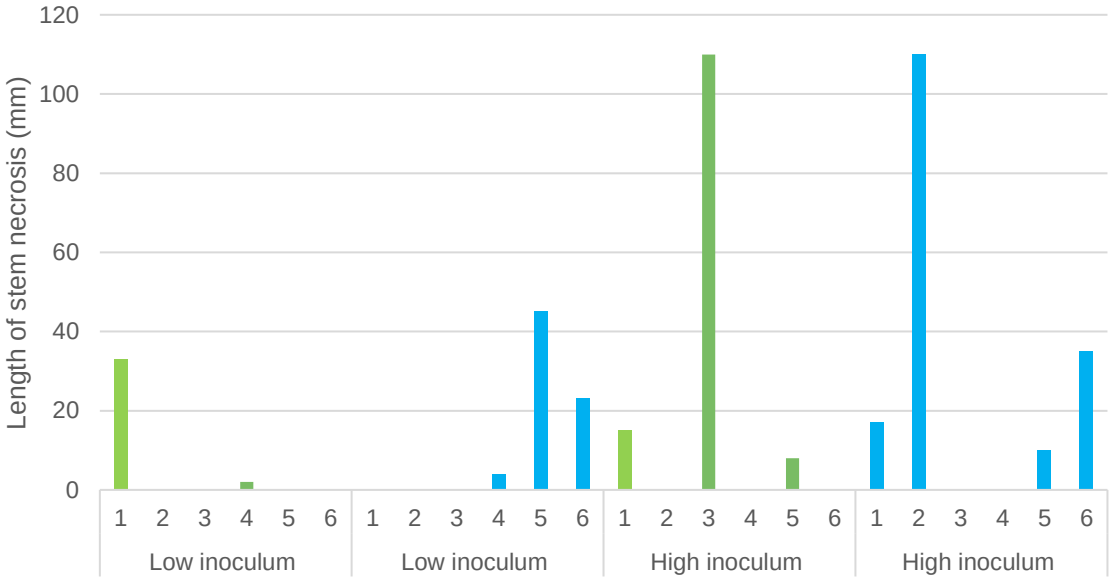

Figure 6. Length of stem necrosis of young potted kiwifruit plants of *Actinidia chinensis* var. *chinensis* 'Hort16A' inoculated with a low concentration (2.0 x 10^4 cfu/ml) or a high concentration (2.0 x 10^8 cfu/ml) of *Pseudomonas syringae* pv. *actinidiae*. The blue bars represent length of stem necrosis of plants kept under flooding-like conditions (water level constantly kept a few centimetres below soil level) and the green bars represent length of stem necrosis of plants kept under normal conditions.

Keeping the plants under flooding-like conditions had only a minor effect on the number of plants showing symptoms. The experiments carried out on Green11 (Experiment 7) and *A. arguta* (Experiment 8) led to a similar conclusion (Figures 7 and 8).

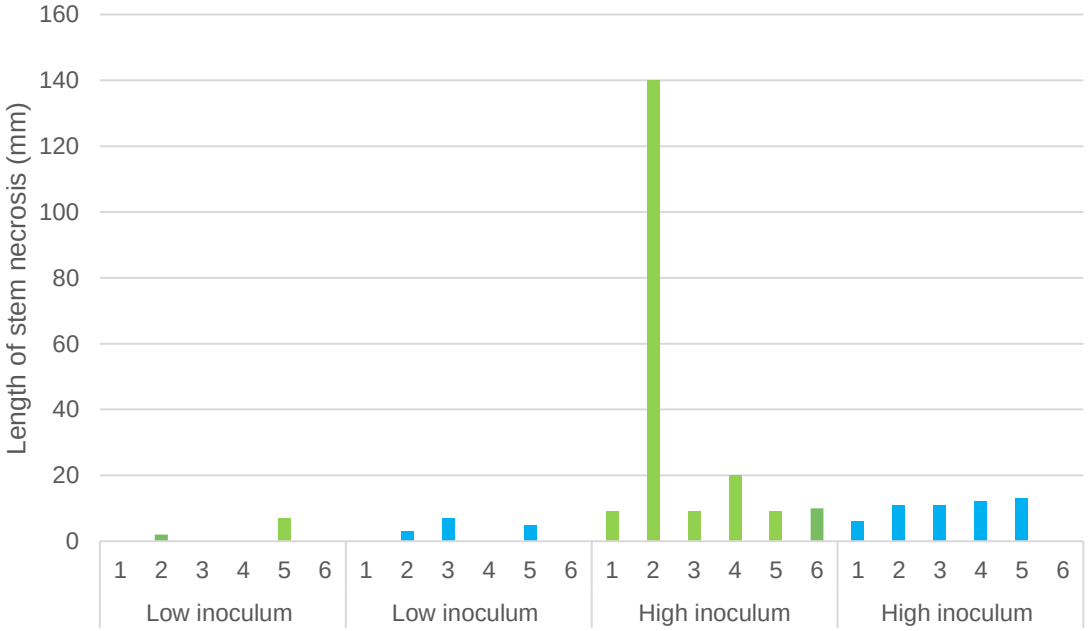

Figure 7. Length of stem necrosis of young potted kiwifruit plants of *Actinidia chinensis* var. *deliciosa* 'Zes007' (Green11) inoculated with a low concentration (2.0 x 10^4 cfu/ml) or a high concentration (2.0 x10^8 cfu/ml) of *Pseudomonas syringae* pv. *actinidiae*. The blue bars represent length of stem necrosis of plants kept under flooding-like conditions (water level constantly kept a few centimetres below soil level) and the green bars represent length of stem necrosis of plants kept under normal conditions.

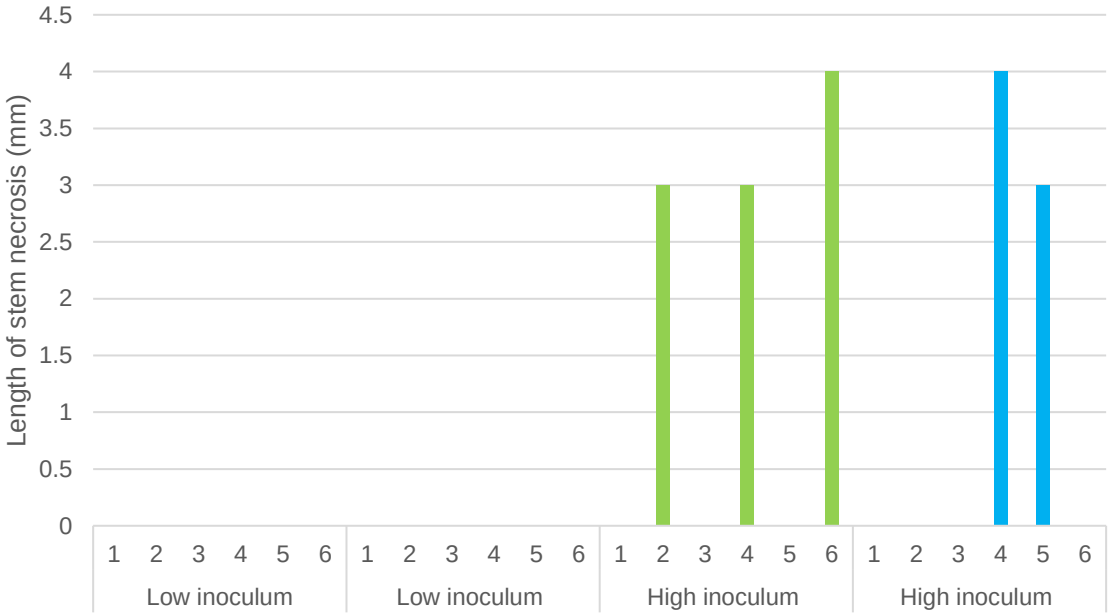

Figure 8. Length of stem necrosis of young potted kiwifruit plants of *Actinidia arguta* 'HortGem Tahi' inoculated with a low concentration (2.0 x 10^4 cfu/ml) or a high concentration (2.0 x 10^8 cfu/ml) of *Pseudomonas syringae* pv. *actinidiae*. The blue bars represent length of stem necrosis of plants kept under flooding-like conditions (water level constantly kept a few centimetres below soil level) and the green bars represent length of stem necrosis of plants kept under normal conditions.

In these three experiments, maintaining the plants under flooding-like conditions did not influence the outcome of the infection (symptomatic or symptomless). This is true for 'Hort16A' for which we previously showed that keeping the plants under flooding-like conditions reduced the likelihood of symptoms expression. This indicates that there are other factors at play and that flooding has an effect only under some, yet unknown, circumstances.

Not all plants of *A. arguta* showed symptoms even when inoculated with a high concentration of Psa, which is consistent with *A. arguta* being considered resistant to this bacterium. However, in all but one plant inoculated with a low concentration of Psa (2.0 x 10^4 cfu/ml), the pathogen could be recovered 36 days post-inoculation (Figure 9). The lack of symptoms following stem inoculation was therefore not because of the inability of Psa to survive and multiply in those tissues. Under the conditions of this experiment, *A. arguta* can be a symptomless carrier.

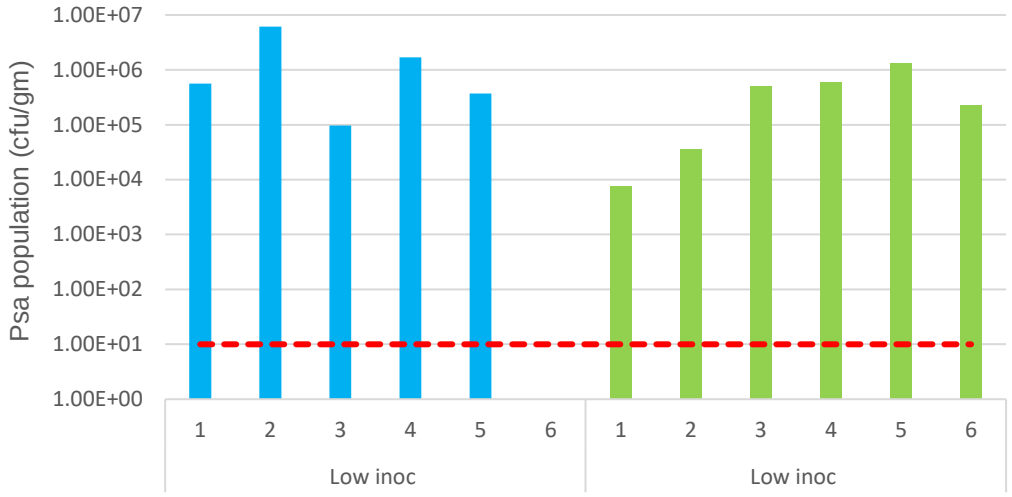

Figure 9. Endophytic populations of *Pseudomonas syringae* pv. *actinidiae* (Psa) in cfu/g of tissue isolated from stem sections of *Actinidia arguta* 'HortGem Tahi' plants inoculated with low concentration of (2.0 x 10^4 cfu/ml) of Psa. None of those plants expressed symptoms at the time of the analysis (36 days post-inoculation). The blue bars represent bacterial populations from plants kept under flooding-like conditions (water level constantly kept a few centimetres below soil level) and the green bars represent bacterial populations from plants kept under normal conditions. The red dotted line represents the limit of detection of Psa by dilution plating on agar plates of King's B medium (10 cfu/gm of tissue).

3.1.3 Impact of inoculum concentration and flooding on symptoms expression on 'Hayward' plants

A similar experiment to that described above was conducted on 'Hayward' plants to determine the impact of inoculum concentration and flooding on symptoms expression. Four of the six plants inoculated with a low concentration of Psa (1.6 x 10^4 cfu/ml) showed stem necrosis, while none of the six plants inoculated with the same bacterial suspension but kept under flooding conditions showed any symptoms 5 weeks post-inoculation (Figure 10). Endophytic populations of Psa in those symptomless plants was similar whether the plants had been kept under flooding-like conditions or not (Figure 11). These results are similar to those obtained previously with 'Hort16A' (Vanneste et al. 2019b) but in contrast to those reported above. The impact of keeping plants under flooding-like conditions on symptoms expression might have more to do with the status of the plant at the time of inoculation rather than the cultivar being inoculated.

In that same experiment, five of the six plants inoculated with a high concentration of Psa showed symptoms whether they were kept under flooding-like conditions or not. But the only two plants showing a stem necrosis longer than 25 mm were plants kept under flooding-like conditions (Figure 10).

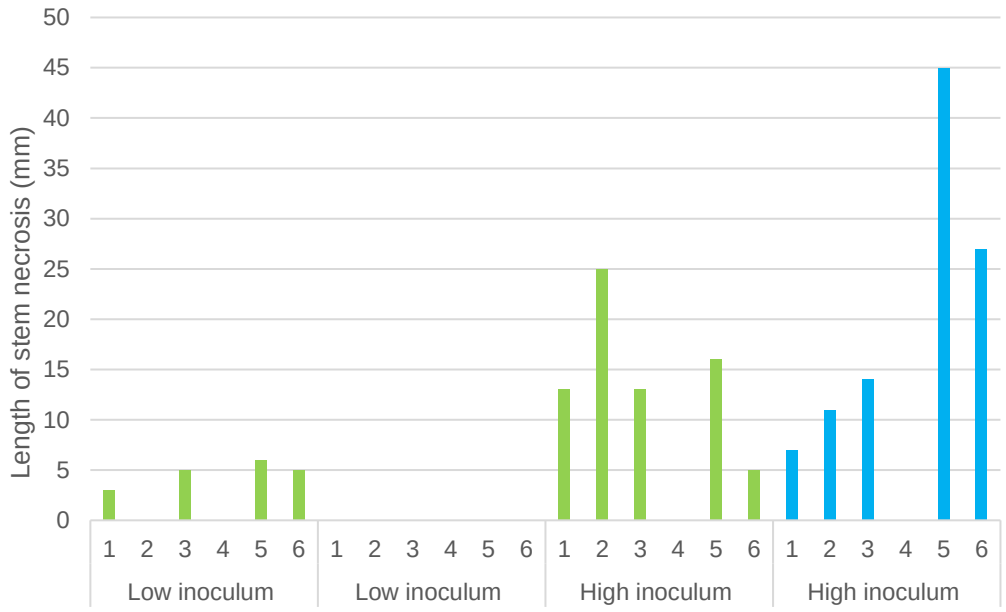

Figure 10. Length of stem necrosis of young potted kiwifruit plants of *Actinidia chinensis* var. *deliciosa* 'Hayward' inoculated with a low concentration (1.6 x 10^4 cfu/ml) or a high concentration (1.6 x 10^8 cfu/ml) of *Pseudomonas syringae* pv. *actinidiae* 5 weeks post-inoculation. The blue bars represent length of stem necrosis of plants kept under flooding-like conditions (water level constantly kept a few centimetres below soil level) and the green bars represent length of stem necrosis of plants kept under normal conditions.

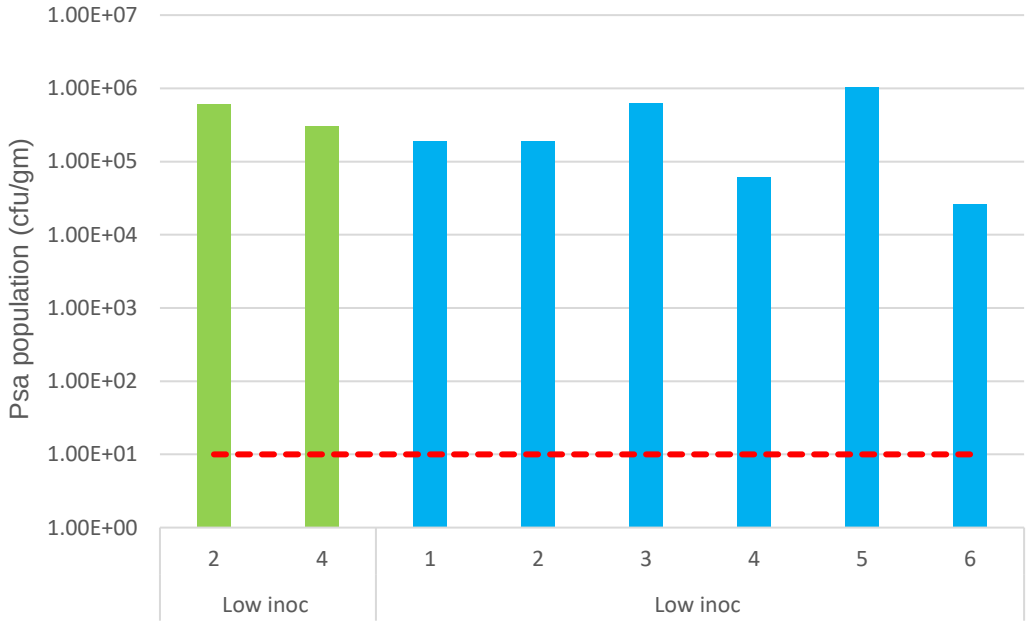

Figure 11. Endophytic populations of *Pseudomonas syringae* pv. *actinidiae* (Psa) in cfu/g of tissue isolated from stem sections of *Actinidia chinensis* var. *deliciosa* 'Hayward' plants inoculated with low concentration of (1.6 x 10^4 cfu/ml) of Psa. None of those plants expressed symptoms at the time of the analysis (5 weeks post-inoculation). The blue bars represent bacterial populations from plants kept under flooding-like conditions (water level constantly kept a few centimetres below soil level) and the green bars represent bacterial populations from plants kept under normal conditions. The red dotted line represents the limit of detection of Psa by dilution plating on agar plates of King's B medium (10 cfu/gm of tissue).

To determine whether the duration of the flooding had an influence on its impact, three lots of five "Hayward' plants each were inoculated with a suspension of Psa containing 5.6 x 10^8 cfu/ml. One lot was kept under normal conditions, one lot was kept under flooding-like conditions for 48 h only and the last lot was kept under flooding conditions for the duration of the experiment. All the plants showed symptoms but only plants that were kept under flooding conditions had a stem necrosis longer than 15 mm (Figure 12). The duration of the flooding conditions did not seem to have any effect on the length of the stem necrosis, suggesting that the outcome of the inoculation was dictated early in the kiwifruit/Psa interaction.

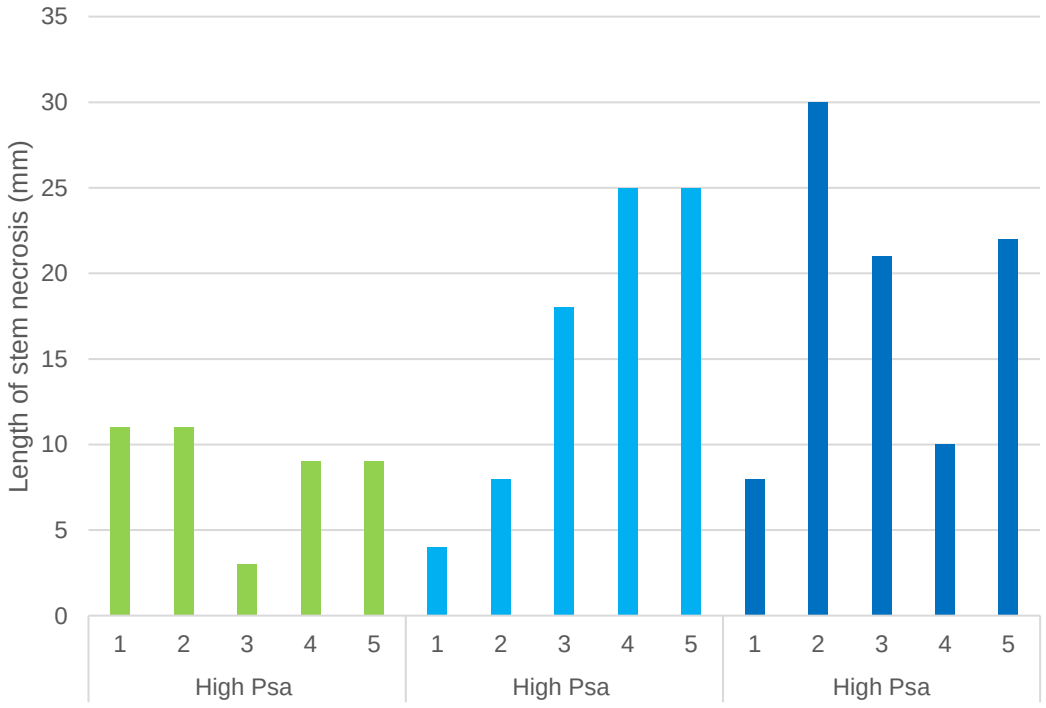

Figure 12. Length of stem necrosis of young potted kiwifruit plants of *Actinidia chinensis* var. ***deliciosa*** 'Hayward' inoculated with a high concentration (5.6 x 10^8 cfu/ml) of *Pseudomonas syringae* pv. *actinidiae*. The bars represent length of stem necrosis of plants, green bars represent plants kept under normal conditions, light blue bars represent plants in flooded (water level constantly kept a few centimetres below soil level) conditions for 48 h and dark blue bars plants kept under flooding-like conditions for 13 d.

In those two experiments carried out on 'Hayward', it appears the stress caused by the flooding-like conditions, which prevented symptoms expression at low inoculum, increased symptoms expression at high inoculum.

3.2 Influence of the rate of growth on symptoms expression

The effect of flooding on the ability of kiwifruit plants to express symptoms following inoculation with low concentrations of Psa was not consistent. At times, flooding suppressed symptom expression, while at other times it did not have any effect. One hypothesis is that flooding affects plant growth. If the plants were not growing actively, flooding had a minor effect, while on fast-growing plants it had a

much larger effect resulting in a suppression of symptoms expression. If this hypothesis is correct then plants that are not in an active phase of growth would be less likely to express symptoms than fast-growing plants.

To confirm this hypothesis, two groups of Gold3 plants were inoculated with a low (5 x 10^3 cfu/ml) or a high (5 x 10^7 cfu/ml) concentration of Psa. The development of the terminal bud of the plants in one of the groups had stopped to develop, while the plants of the second group had their terminal bud growing actively (Figure 13).

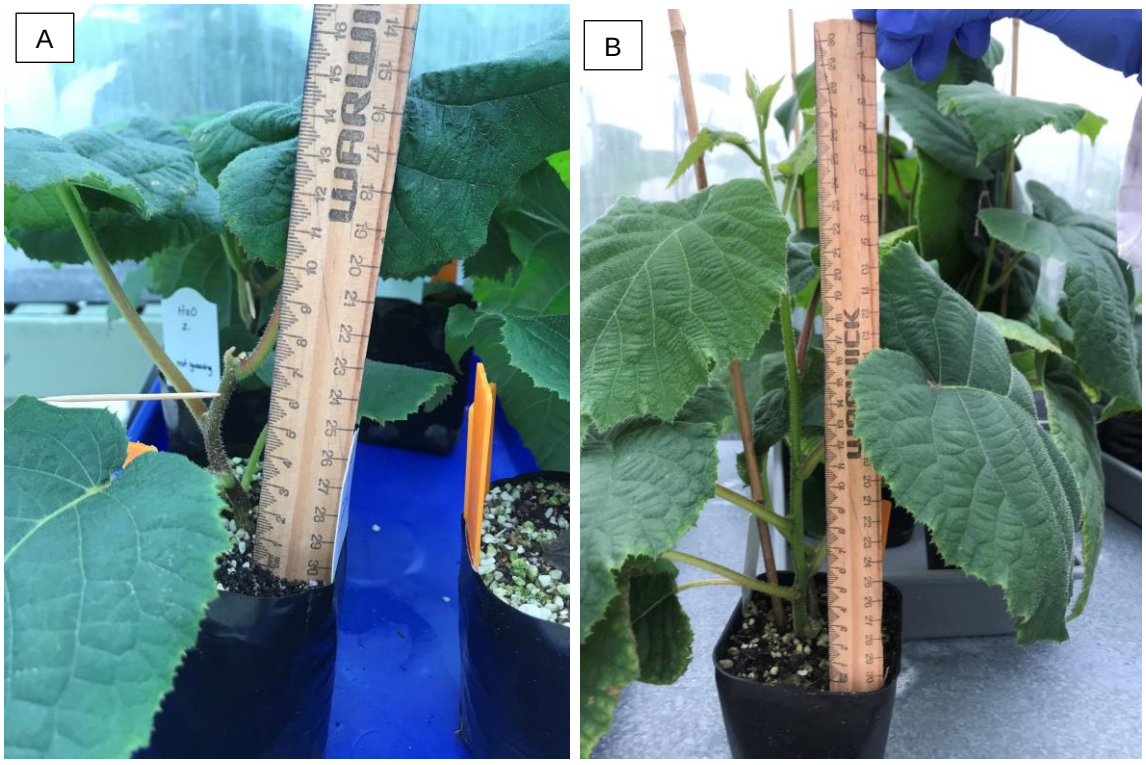

Figure 13. Panel A: plants of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) in which terminal bud had stopped to develop resulting in a slow-growing plant. Panel B: Gold3 plants with an active terminal bud resulting in a fast-growing plant.

The group of plants where terminal bud stopped developing and were inoculated with a low concentration of Psa did not show any symptoms 3 weeks post-inoculation, while those inoculated with a high concentration of Psa showed very limited stem necrosis. In contrast, three of the five plants with an active terminal bud inoculated with a low concentration of Psa showed symptoms and all the plants inoculated with a high concentration of Psa showed symptoms (Figure 14). Of the plants inoculated with high concentrations of Psa, four of the five slow-growing plants showed symptoms but the necrosis was less than 10-mm long. While the fast-growing plants were all infected and all except one plant had stem necrosis longer than 10 mm.

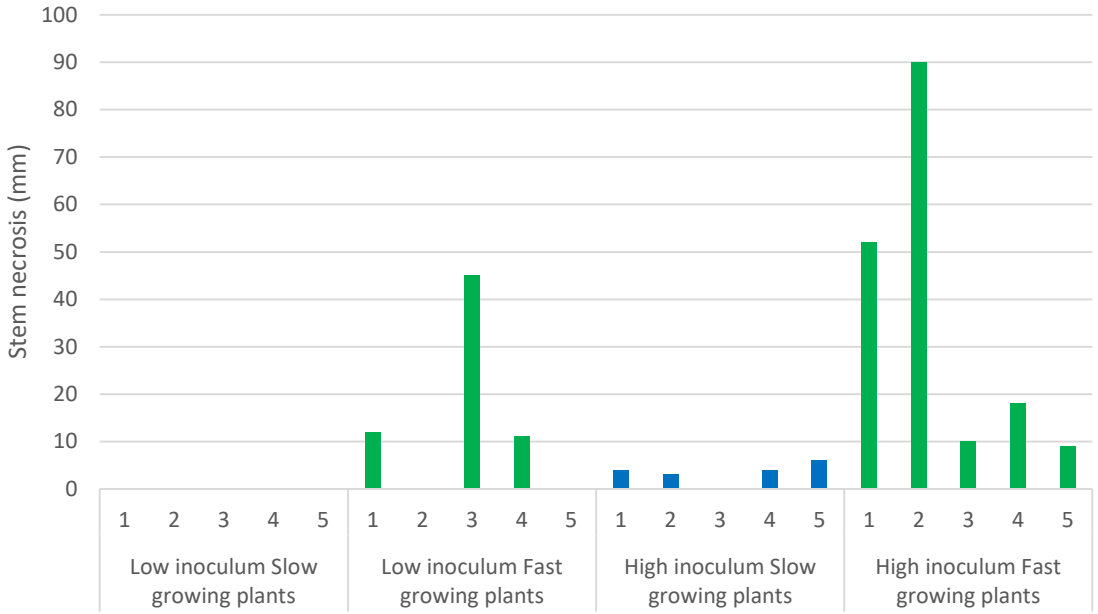

Figure 14. Length of stem necrosis of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) plants inoculated with low concentration of *Pseudomonas syringae* pv. *actinidiae* (5 x 10^3 cfu/ml) or high concentration of Psa (5 x 10^7 cfu/ml), 3 weeks post-inoculation. The blue bars represent length of stem necrosis of plants in which terminal bud stopped developing and the green bars represent length of stem necrosis of plants in which terminal bud was still developing.

Fast-growing plants expressed symptoms more readily than slow-growing plants, and the length of stem necrosis was consistently longer in fast-growing plants. These results suggest that the rate of growth of the plant affects its ability to express symptoms. A corollary of this result is that any factor that interferes with the rate of growth also affects symptom expression.

3.3 Differential gene expression in symptomless carriers

The RNASeq experiment was carried out on Gold3 plants and was designed to allow the identification of the plant and of the bacterial genes that might be responsible for infected plants to stay symptomless. The idea was to compare plant and bacterial gene expression in infected symptomless plants with that in symptomatic ones. Because we were interested in the early phases of the plant pathogen interaction, the samples were taken at times (3, 24 and 48 hours post-inoculation) when we did not know which plants were going to be symptomatic. So only some of the plants inoculated with water, a low concentration of Psa or a high concentration of Psa were used for RNA extraction. The other plants were kept in a glasshouse for symptoms expression. None of the 12 plants inoculated with low concentration of Psa showed any symptoms 3 weeks post-inoculation. However, live endophytic Psa populations were recovered at the point of inoculation from 10 of those 12 plants. Endophytic populations varied from 3.2 x 10^5 cfu/g of stem tissue to 6.4 x 10^6 cfu/g of stem tissue. In contrast, the 12 plants inoculated with high concentration of Psa showed stem necrosis 3 weeks post-inoculation; in those plants, the endophytic populations varied from 8.2 x 10^7 cfu/g to 2.4 x 10^8 cfu/g.

Based on these results, we considered that all the plants inoculated with low concentrations of Psa were symptomless carriers and the plants inoculated with high concentration of Psa were symptomatic plants.

A comparison of the level of expression of the bacterial genes mapped to the Psa genome in symptomatic plants versus symptomless plants (high inoculum versus low inoculum) revealed that only one gene was differentially expressed using a cut-off of a 2-fold change with an adjusted *p*-value of 0.01. This gene was 2-fold overexpressed in the plants showing symptoms. This overexpression was observed only at the 48-hour time point. The function of this gene is not known but it is unlikely that it is linked with virulence, as it is only one gene and overexpressed only 48 hours post-inoculation. Usually a large number of genes are involved in virulence and they are expressed much earlier during the plant/pathogen interaction. Therefore, this result indicates that the bacteria were expressing all the other genes at the same rate whether they were inoculated at a low or a high concentration and suggests that it is not bacterial gene expression that dictates whether a plant will express symptoms after infection with Psa.

Because plant gene expression in the nil treated plants evolved over the course of this experiment (data not shown), only samples taken at the same time point were compared. The number of genes differentially expressed between plants subjected to different treatments was quite large. Table 2 shows the number of genes where change in expression was at least 10-fold different, while Table 3 shows the number of genes where change in expression was at least 2-fold different.

Comparing gene expression of water-treated plants with plants that were nil treated showed that a large number of genes are involved in the wound response. This was especially true 3 hours post-treatment. The number of genes differentially expressed after inoculation with a high or low concentration of Psa was actually lower than that involved in the wound response. Several genes were overexpressed at 3 and 24 hours post-treatment in plants inoculated with a low concentration of Psa compared with the plants inoculated with a high concentration of Psa. While at 48 hours post-treatment most of the genes differentially expressed between those two groups of plants were down-regulated (Tables 2 and 3).

This indicates that the plant recognised presence of low concentration of Psa and reacted to it rapidly and in a different manner than when it was inoculated with a high concentration of Psa. The genes differentially expressed in plants inoculated with low concentrations of Psa might be responsible for the lack of symptoms expression, i.e. for latency in kiwifruit plants inoculated with Psa.

As found earlier by qPCR (Vanneste et al. 2019b), 3 hours post-inoculation, the expression of PR1 was increased in plants inoculated with low concentration of Psa. However, many other genes are differentially expressed at that time point and PR1 might not be responsible or not the only gene responsible for suppression of symptoms expression.

Volcano plots corresponding to the different comparisons presented in Tables 2 and 3 and the list of the genes differentially expressed by least 10-fold between plants inoculated with low concentrations of Psa and those inoculated with high concentration of Psa are presented in Appendix 2. It is important to note that a large proportion of the genes overexpressed in plants inoculated with low concentration of Psa in the early phase of the interaction are linked with hormonal balance. This is especially true 3 hours post-inoculation when 7 of the 18 genes overexpressed by at least 10-fold are auxin-responsive proteins.

Table 2. Number of genes differentially expressed (down-regulated or up-regulated) in plants of *Actinidia chinensis* var. *chinenis* 'Zesy002' (Gold3) inoculated with low concentration (1.0 x 10^4 cfu/ml) or high concentration (1.0 x 10^8 cfu/ml) of *Pseudomonas syringae* pv. *actinidiae*.

Comparison	Time point	Number of genes up-regulated	Number of genes down-regulated
Water-treated plants vs nil treatment	3	206	23
	24	75	6
	48	12	2
Plants inoculated with low concentration of Psa vs water-treated plants	3	5	1
	24	22	0
	48	0	5
Plants inoculated with high concentration of Psa vs water-treated plants	3	5	4
	24	17	0
	48	64	2
Plants inoculated with low concentration of Psa vs plants inoculated with high concentration of Psa	3	18	0
	24	11	0
	48	0	33

The threshold was set at 10-fold change with a *p*-value of 0.01.

Table 3. Number of genes differentially expressed (down-regulated or up-regulated) in plants of *Actinidia chinensis* var. *chinenis* 'Zesy002' (Gold3) inoculated with low concentration (1.0 x 10^4 cfu/ml) or high concentration (1.0 x 10^8 cfu/ml) of *Pseudomonas syringae* pv. *actinidiae*.

Comparison	Time point	Number of genes up-regulated	Number of genes down-regulated
Water-treated plants vs nil treatment	3	1351	598
	24	311	91
	48	108	88
Plants inoculated with low concentration of Psa vs water-treated plants	3	37	50
	24	86	6
	48	20	51
Plants inoculated with high concentration of Psa vs water-treated plants	3	23	58
	24	36	22
	48	245	24
Plants inoculated with low concentration of Psa vs plants inoculated with high concentration of Psa	3	387	53
	24	40	1
	48	3	380

The threshold was set at 2-fold change with a *p*-value of 0.01.

4 Discussion

Effective disease management relies on knowing where the pathogen resides, including and especially where it resides when it does not cause disease. Therefore, understanding the circumstances that allow Psa to multiply in symptomless plants is an important element of the epidemiology of this pathogen, which could lead to better disease control.

The goal of this project was to better understand the risks posed by the presence of symptomless kiwifruit plants carrying endophytic Psa populations. This project comprised three different but related objectives. The first objective was to confirm the existence and characterise the conditions leading to kiwifruit plants being symptomless carriers. The second objective was to find markers linked with the presence of live Psa in a symptomless plant, and the third objective aimed to identify triggers that lead to infected plants expressing symptoms.

We previously reported progress made on these three objectives (Vanneste et al. 2019a; Vanneste et al. 2019b). We showed that Psa was able to survive and multiply in the leaves or stems of *A. chinensis* var. *chinensis* or *A. chinensis* var. *deliciosa* of different cultivars without the plants showing any symptoms, and that this situation was facilitated when plants were inoculated with low concentration of Psa and/or when they were kept in flooding-like conditions. In addition, several metabolites, belonging to the terpene, flavonoid or isoflavonoid classes, were identified as being present in higher concentrations in symptomless carriers. Several compounds from those classes have been shown in different plant models to provide defence against biotic and abiotic stresses (Zhu et al. 2013; Liu et al. 2017; Lu et al. 2017).

The goals of this last part of the project were first to confirm the impact of inoculum concentration and flooding on symptoms expression in different cultivars of kiwifruit, and to analyse the RNASeq data in order to identify what plant and bacterial genes are linked with lack of symptoms expression in Psa-infected plants.

The influence of flooding on symptom expression seemed to be independent of the *Actinidia* species or of the cultivar used for the experiment. Flooding modified symptoms expression in both ‘Hort16A’ and ‘Hayward’ but not consistently. Keeping the plants under flooding-like conditions did not always prevent symptom expression even when the plants were inoculated with low concentrations of Psa. When flooding affected symptom expression, it reduced or prevented symptom expression in plants inoculated with low concentration of inoculum but increased symptom expression in plants inoculated with high concentration of Psa.

This suggests that in areas prone to regular flooding or areas where soils are waterlogged, high inoculum of Psa might lead to increased symptoms expression. This also suggests that frost fighting with irrigation might not only help the pathogen to spread and colonise the canopy but might also lead to increased symptoms expression in areas of high inoculum and prone to waterlogging.

Using high concentration of inoculum (c. 10^8 cfu/ml) generally led to infected plants expressing symptoms, regardless of the environmental conditions. However, those very high concentrations of Psa might not reflect the conditions of infection in the field.

One of the hypotheses initially put forward to explain why a bacterial pathogen can survive in a plant without causing symptoms is that the pathogen was not able to express some of its genes necessary for virulence. We previously showed that growing Psa in the presence of kiwifruit leaf extracts influenced its ability to infect kiwifruit leaves (Vanneste et al. 2019b). This is consistent with earlier

findings that Psa express genes linked with virulence following recognition of the favourable environment (high concentration of Psa, presence of other bacteria and presence of a host plant) (Cellini et al. 2019). However, the RNASeq experiments indicate that once in the plant the bacterial genes expression was similar whether the plant was going to express symptoms or become a symptomless carrier. It might be that by stem inoculating the plants, as was done for the plants used for the RNASeq analysis, the bacteria did not need to express their virulence genes and they only expressed genes necessary for their survival, i.e. those necessary for nutrient acquisition. These results indicate that virulence genes need to be expressed only during the very early phase of the plant/pathogen interaction and Psa has the same nutritional requirement in infected kiwifruit plants whether these plants will express symptoms or not.

There seems to be a number of conditions that result in lack of symptom expression and plants being symptomless carriers. Keeping the plants under a flooding-like situation is one of those conditions; however, flooding might not affect directly the ability of a plant to express symptoms but might rather interfere with its rate of growth, which might be directly linked with symptoms expression. In addition, we cannot rule out that the flooding conditions also resulted in a major modification of the root microbiome, which in turn might have led to differential gene expression in the plant.

A large number of kiwifruit genes were overexpressed in the early stages of the kiwifruit/Psa interaction in plants which will not express symptoms. It might be that the low initial inoculum of Psa cannot suppress a resistance mechanism in kiwifruit plants. Interestingly, some of the genes overexpressed by at least 10-fold in plants that will not express symptoms are transcription factors and auxin-responsive proteins (Appendix 2).

The hormonal balance in the plant, including auxin concentration, might play an important role in symptom expression. Auxin is a phytohormone synthesised from the shikimate pathway, the same pathway that leads to synthesis of salicylic acid. It has been suggested that increased auxin concentration reduces concentration of salicylic acid (Koo et al. 2020), which has been shown to reduced disease incidence caused by Psa (Cellini et al. 2014). Furthermore, it was recently demonstrated that auxin down-regulated the expression of virulence genes in *P. syringae* pv. *tomato*, a plant pathogen closely related to Psa, and interfered with expression of genes involved in resistance to this pathogen (Djami-Tchatchou et al. 2020). In addition, we recently showed that suppression of apical dominance lessened the ontogenic resistance (resistance linked to age) to Psa (unpublished data). Suppression of apical dominance was achieved by removing the apical meristem, which results in a reduction of auxin in the tissues. These results would then strengthen the hypothesis presented earlier that cell multiplication and cell elongation, which are linked with a rapid growth rate, are key to symptoms expression and possibly to the length of latency phase in kiwifruit.

Moreover, the metabolomics analysis conducted last year identified a gibberellin, another plant hormone linked with cell elongation, as one of the most significant reoccurring mass spectrophotometric features in plants inoculated with low concentrations of Psa (Vanneste et al. 2019a). From those experiments it would seem that it is the hormonal balance in the plant that determines or drives symptom expression in kiwifruit infected by Psa.

In two independent experiments involving leaf inoculation of ‘Hayward’, live Psa could not always be recovered from symptomatic tissues. In contrast, Psa was easily recovered from ‘Hort16A’ necrotic leaf spots. It would be useful to determine whether this is an indication that ‘Hayward’ is able to kill Psa after infection and whether there is a link between the ability of a cultivar to kill Psa post-infection and the ability of Psa to move systemically in plants of that cultivar. The main reason why the economic consequences of Psa in ‘Hort16A’ is much higher than that in ‘Hayward’ is because in

‘Hort16A’ Psa can move systemically and kill large portions of a kiwifruit vine, while in ‘Hayward’ symptoms are mostly limited to leaf spots (Vanneste 2017). The inability of Psa to move from leaf spots to the vascular system could be linked with the ability of ‘Hayward’ plants to kill Psa after infection. Identifying the compound(s) that kill Psa in ‘Hayward’ could provide some useful selection characteristics in future breeding programmes.

It is generally accepted that the inability of a plant pathogenic bacteria to multiply following infection (i.e. the pathogen invading the plant tissues) indicates that the plant is not a host plant or that it is resistant to the pathogen. On the other hand, multiplication of the pathogen in the plant tissues is believed to lead to symptoms development and is a sign of susceptibility. In this project we showed that in the kiwifruit/Psa pathosystem, this was not always the case and that Psa could multiply in a susceptible cultivar without leading to symptoms development. When this phase is relatively short it is called latency phase. However, if this situation lasts for a long period of time or if the symptoms never develop fully, it could be called tolerance. In which case, this study showed that under some specific conditions, every cultivar could show some level of tolerance to Psa.

5 Conclusions and practical consequences

This project has uncovered some interesting and useful features about the Psa/kiwifruit interaction:

- In all the cultivars tested, 'Hayward', Gold3, 'Hort16A', Green11 and in *A. arguta*, leaf infection and stem inoculation can both lead to Psa multiplying endophytically without causing symptoms.
- The Psa bacterial load (number of bacteria per unit of plant tissues) in symptomless tissues can be as high as that in tissues showing symptoms.
- The Psa bacteria were able to travel at least short distances in symptomless plants.
- The physiological state of the bacteria influence its ability to infect but once the bacteria is in the plant it seems that it is the plant that determines whether the interaction will lead to symptoms development.
- The physiological state of the plant and its rate of growth seems to be one of the factors involved in symptoms expression.

Practically, this means that when Psa infects plants in late summer and autumn, these infections may not result in any visible symptoms. This in turn means that the usefulness of late season application of protectants might have been underestimated.

Some cultivars or some species considered as resistant because they do not show symptoms can be symptomless carriers (e.g. *A. arguta*) and therefore could still play a role in the spread and distribution of Psa.

Actively growing plants seemed more likely to express symptoms; this could explain the relatively high occurrence of symptoms expression in early spring. It would be useful to determine whether the rapid growth of spring time is enough to break the latency associated late season infection or with the colonisation of the scion tissues from infected rootstocks. If the early grafting of Gold3 on Psa-infected rootstocks led to those vines being symptomless carriers and if the rapid growth experienced in spring would be enough to break Psa latency, then we could expect those symptomless carriers to express symptoms during spring. The lack of widespread symptoms in Gold3 orchards these last few years could indicate that either rapid spring growth is not enough to break latency or that Psa was actually not present in those plants.

6 Acknowledgements

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Appendices

Appendix 1: RNA preparation and analysis of the sequence data

Total RNA was prepared from about 100 mg of kiwifruit tissue ground by mortar and pestle in liquid nitrogen. RNA was isolated using the Spectrum Plant Total RNA Kit (Sigma-Aldrich) following the supplier's recommendations. To remove any genomic DNA that might have been extracted, RNA was DNase treated using DNase 1 (Quanta BioSciences) according to the manufacturer's instructions. DNAse was then removed using AMPure® XP beads (Beckman Counter, USA). Sample purity and RNA concentrations were determined using a Nanophotometer (Implen). RNA integrity was then checked on a BioAnalyser (Agilent). All RNA samples have a RIN number of at least 8.0. A sample of 6 µg of RNA was then sent to Massey Genome Service at Massey University, Palmerston North for sequencing with Illumina MiSeq™ V2.

A total of 3,576,321,504 raw reads were obtained from Novogene who did the sequencing on behalf of Massey Genome Service. After discarding the sequences that did not meet the quality standard recommended by Yan et al. (2013), we had 3,519,371,022 clean reads. The sequence effective rate varied between 96.6 to 99.3% and sequencing errors were less than 0.03%. The average number of clean reads per sample was 54,144,169. This depth of sequencing should allow us to analyse bacterial gene expressions. The majority of the reads belong to the plant genome and are being mapped on the *Actinidia chinensis* var. *chinensis* genome using BioStar to achieve counts per sample. The counts will go through the Deseq programme to detect which genes are differentially expressed in the different samples.

A total of 3,576,321,504 raw reads were generated by Novogene and downloaded as FastQ files. Because of some problems during the sequencing run, the complete dataset is made of the initial raw dataset plus three top-up runs done by Novogene. As a result of this sequencing strategy used, and because all four datasets were needed for the analysis, we had to make sure that there was no lane effects that could have led to an unknown bias being added. We circumvented this problem by keeping the dataset separate during the analysis.

The Quality Control (QC) was performed using FastQC/0.11.7 (Andrews, 2010) and the results collated using multiqc/1.7 (Ewels et al. 2016). Contaminant adapter sequences and 12pb from the 5' end were removed using fastp/0.20.0c (Chen et al. 2018). The rRNA content of the datasets was determined using fastq_screen/v0.11.1 (Wingett and Andrews 2018). No significant rRNA content was observed in the processed reads. The GC content of the reads was bimodal because our samples contained plant and bacterial RNA sequences.

Read alignment to the Kiwifruit Genome (CK51) and to the Psa genome and plasmid sequences (CP011972.2_psa.fasta CP011973.1_psa.fasta) was performed using STAR/2.6.1d (Dobin et al. 2013). Analysis of the fastp output on the processed reads indicated that a significant portion of the library insert sizes were small and would result in overlapping paired end reads. This would lead to a low alignment rate when using STAR. As a result, the setting "--peOverlapNbasesMin" was set to 5pb in the STAR command. Reads counts were then extracted from the STAR alignment data to both the Kiwifruit and Psa genomes. Separate read count files were generated for each of the four raw read datasets. The fold change gene expression for the plant genes and the bacterial genes was estimated using DESeq2 (Love et al. 2014) with a front end developed by A. Saei.

Appendix 2: Gene expression analysis

Table A1. List of the genes differentially expressed in plants of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) inoculated with low concentration of *Pseudomonas syringae* pv. *actinidiae* (Psa) vs plants inoculated with high level of Psa 3 hours post-inoculation. Only genes showing a 10-fold change with an adjusted *p*-value of 0.01 are listed. Under those criteria there were no down-regulated genes.

Gene id	Potential gene function	Log-fold change	Gene expression	Adjusted *p*-value
Acc02354.1	Transcription factor like protein	3.76	Up	2.45E-06
Acc03558.1	Unknown	4.5	Up	1.87E-10
Acc04112.1	Transcription factor like	3.39	Up	3.64E-08
Acc05203.1	Auxin-responsive protein	4.6	Up	3.85E-05
Acc07165.1	Auxin-responsive protein	3.36	Up	0.001366
Acc07169.1	Auxin-responsive protein	4.33	Up	5.68E-07
Acc07221.1	Auxin-responsive protein	3.9	Up	0.000115
Acc07652.1	MADS-box protein	5.07	Up	0.000664
Acc08274.1	WRKY transcription factor	3.43	Up	0.000709
Acc09117.1	putative protein	3.9	Up	3.14E-07
Acc16246.1	Auxin-responsive protein	4.42	Up	0.000408
Acc16267.1	Auxin-responsive protein	5.25	Up	1.18E-10
Acc16269.1	Auxin-responsive protein	3.43	Up	1.15E-08
Acc16444.1	Scarecrow-like protein	3.47	Up	1.46E-08
Acc17327.1	Legume lectin domain protein	3.68	Up	6.96E-05
Acc17735.1	B3 domain-containing protein	3.57	Up	0.000402
Acc32402.1	Unknown	4.08	Up	0.000154
Acc33023.1	Protein NRT1/ PTR FAMILY 2.13 like	3.82	Up	3.08E-05

Table A2. List of the genes differentially expressed in plants of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) inoculated with low concentration of *Pseudomonas syringae* pv. *actinidiae* (Psa) vs plants inoculated with high level of Psa 24 hours post-inoculation. Only genes showing a 10-fold change with an adjusted *p*-value of 0.01 are listed. Under those criteria there were no down-regulated genes.

Gene id	Potential gene function	Log-fold change	Gene expression	Adjusted *p*-value
Acc01741.1	BAHD acyltransferase like	5.2	Up	0.000301
Acc04970.1	Ethylene-responsive transcription factor	4.82	Up	0.001888
Acc08011.1	NAC domain-containing protein	4.71	Up	0.00499
Acc15506.1	Apical endosomal glycoprotein	4.08	Up	0.007687
Acc20680.1	Pathogenesis-related genes transcriptional activator like	4.84	Up	0.006594
Acc20765.1	Dammarenediol II synthase like	3.52	Up	0.006193
Acc22643.1	Abscisic acid 8'-hydroxylase like	3.94	Up	0.001888
Acc24981.1	Unknown	3.8	Up	6.23E-05
Acc25505.1	Late embryogenesis abundant protein like	4.06	Up	0.004907
Acc27677.1	Caffeoylshikimate esterase like	4.06	Up	0.004569
Acc28692.1	Abscisic acid 8'-hydroxylase like	5.51	Up	0.002063

Table A3. List of the genes differentially expressed in plants of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) inoculated with low concentration of *Pseudomonas syringae* pv. *actinidiae* (Psa) vs plants inoculated with high level of Psa 48 hours post-inoculation. Only genes showing a 10-fold change with an adjusted *p*-value of 0.01 are listed. Under those criteria there were no up-regulated genes.

Gene id	Potential gene function	Log–fold change	Gene expression	Adjusted *p*-value
Acc03403.1	Late embryogenesis abundant protein	–3.5	Down	0.000958
Acc03855.1	Calcium-binding protein CML31	–3.98	Down	0.001255
Acc04162.1	Glutathione S-transferase	–3.94	Down	3.14E-13
Acc06953.1	Ethylene-responsive transcription factor	–3.6	Down	0.004958
Acc07390.1	Ethylene-responsive transcription factor	–4.26	Down	5.98E-05
Acc09101.1	BON1-associated protein	–4.03	Down	0.005206
Acc09102.1	BON1-associated protein	–3.5	Down	0.006063
Acc13543.1	Glutathione S-transferase like	–5.02	Down	6.29E-08
Acc13544.1	Glutathione S-transferase like	–6.02	Down	9.46E-13
Acc14580.1	BON1-associated protein	–3.46	Down	0.000644
Acc15719.1	Dehydration-responsive element-binding protein like	–4.93	Down	0.001398
Acc15720.1	Dehydration-responsive element-binding protein like	–4.27	Down	0.000253
Acc15722.1	Dehydration-responsive element-binding protein like	–3.68	Down	0.004123
Acc16608.1	Xyloglucan endotransglucosylase/hydrolase protein	–3.4	Down	0.004564
Acc18955.1	Fusaric acid cluster transcription factor	–3.94	Down	0.000152
Acc18956.1	Fusaric acid cluster transcription factor	–3.39	Down	3.24E-06
Acc18958.1	Protein mms22 like	–3.6	Down	2.14E-05
Acc18962.1	Tyrosine aminotransferase like	–4.46	Down	7.91E-11
Acc19196.1	Glutathione S-transferase like	–4	Down	2.55E-14
Acc19197.1	Glutathione S-transferase like	–5.21	Down	4.55E-15
Acc19199.1	Glutathione S-transferase like	–5.35	Down	3.59E-23
Acc19994.1	Salutaridine reductase like	–5.22	Down	8.61E-10
Acc21125.1	Ethylene-responsive transcription factor	–4.05	Down	0.000583
Acc22761.1	Transcription factor bHLH92 like	–3.61	Down	0.000145
Acc25889.1	Zinc finger protein	–3.37	Down	0.000582
Acc26543.1	UDP-glycosyltransferase like	–3.34	Down	0.001174
Acc26727.1	Polyhomeotic-like protein	–3.38	Down	0.000129
Acc28692.1	Abscisic acid 8'-hydroxylase like	–3.46	Down	0.002379
Acc30541.1	Calcium-binding protein	–3.52	Down	0.009154
Acc31802.1	ABC transporter G family member 31 like	–4.34	Down	8.15E-11
Acc32410.1	E3 ubiquitin-protein like	–3.59	Down	0.000859
Acc33669.1	Phospholipase like	–3.59	Down	0.000391
Acc33775.1	Ethylene-responsive transcription factor	–4.37	Down	0.000974

Representing in a graph differential gene expression of the plant genome is relatively complex because of the sheer number of genes being compared. Often the results are presented as a scatterplot that shows the fold change (log2 Ratio) on the x-axis plotted against the 'Absolute Confidence' (-log10 adjusted p-value) on the y-axis. These plots are commonly called 'Volcano Plots'. Each dot on a volcano plot represents one gene, and the "outliers" on such graphs represent the most highly differentially expressed genes. The threshold used for the plots presented below is set at 10-fold changes with an adjusted p-value of 0.01.

For all the plots presented below, the red dots show genes that are significantly up-regulated and the green dots show genes significantly down-regulated.

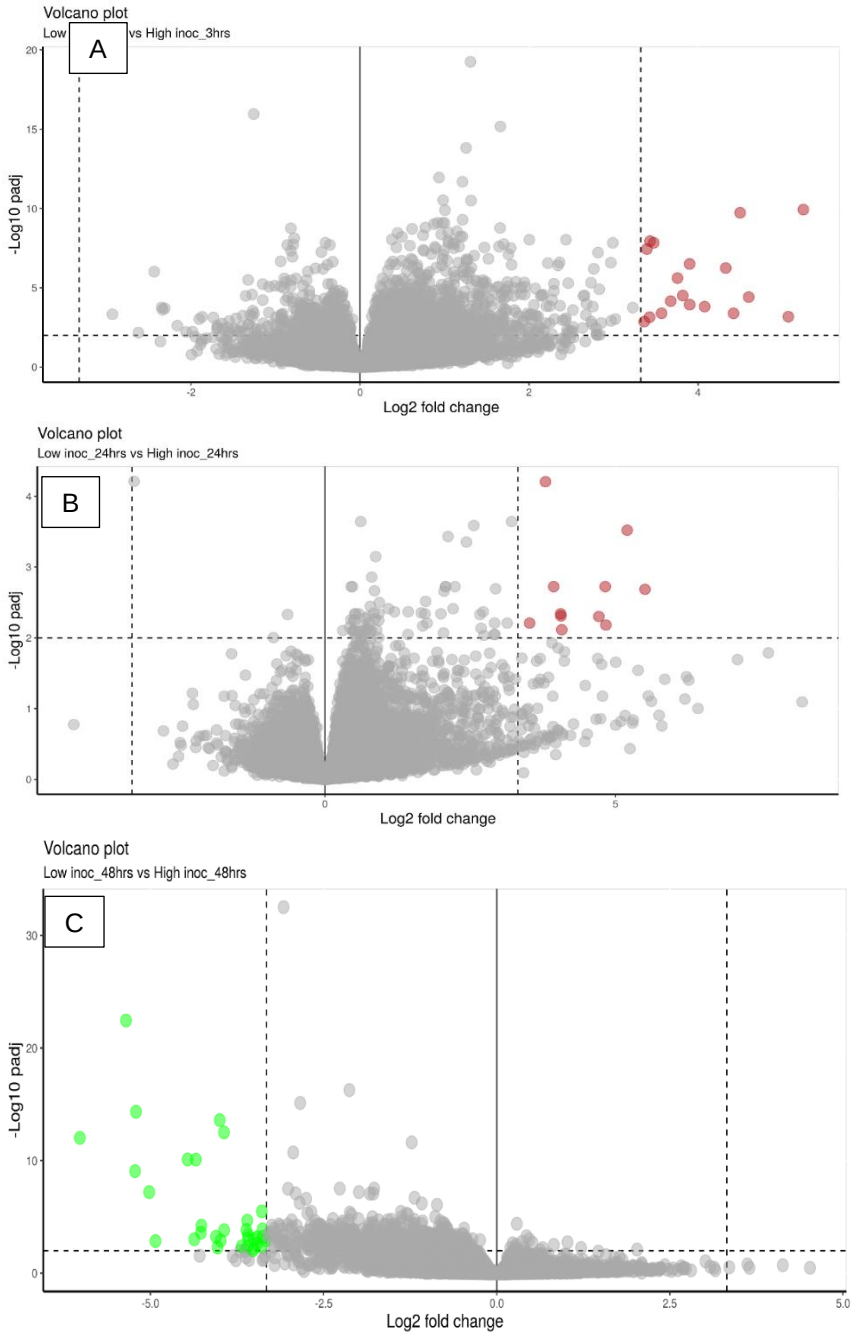

Figure A1. Volcano plot representing gene expression in plants of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) inoculated with low concentration of *Pseudomonas syringae* pv. *actinidiae* (Psa) vs plants inoculated with high level of Psa. Time points of (A) 3 hours, (B) 24 hours and (C) 48 hours post-inoculation are presented.

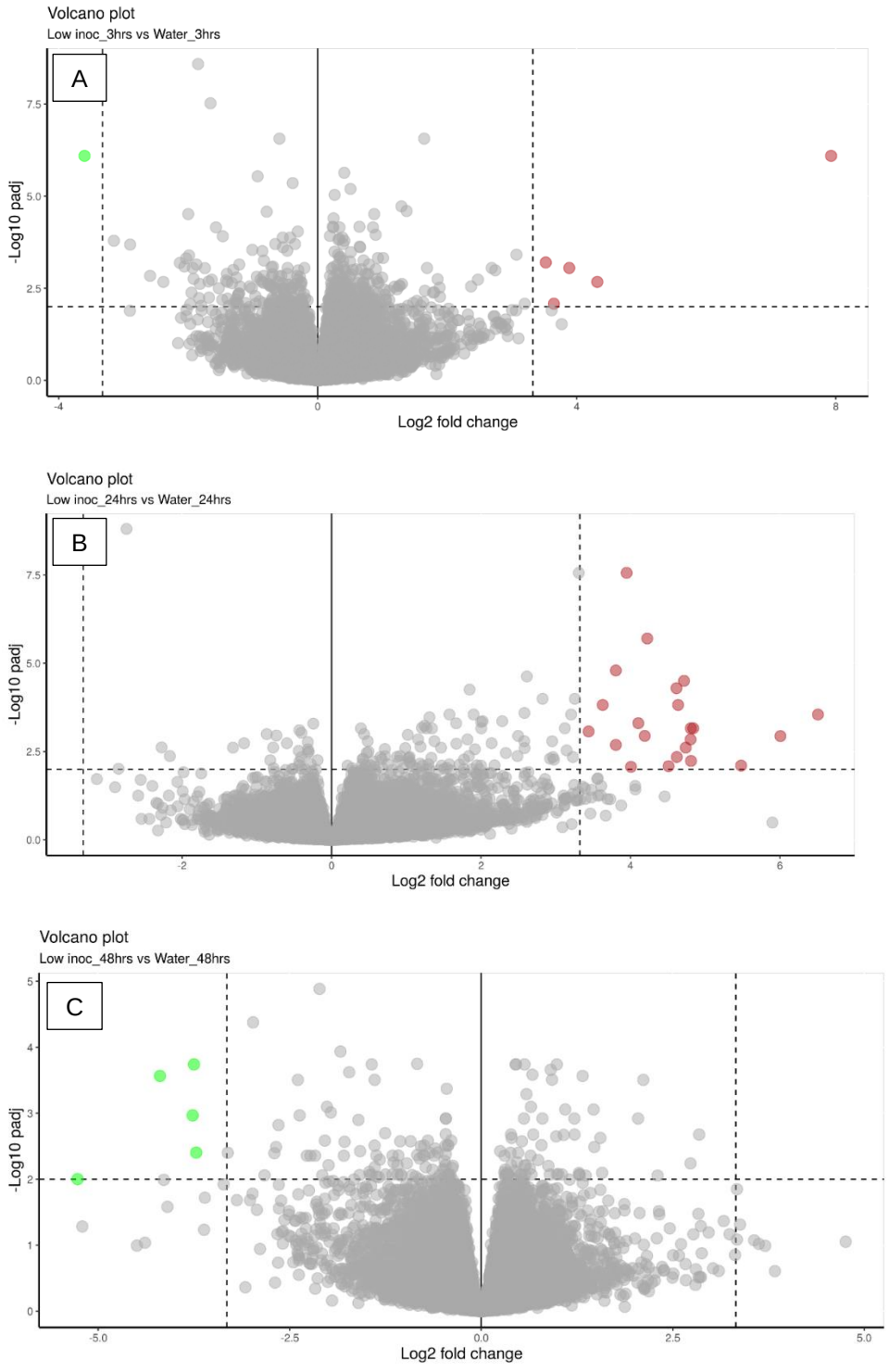

Figure A2. Volcano plot representing gene expression in plants of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) inoculated with low concentration of *Pseudomonas syringae* pv. *actinidiae* (Psa) vs water-treated plants. Time points of (A) 3 hours, (B) 24 hours and (C) 48 hours post-inoculation are presented.

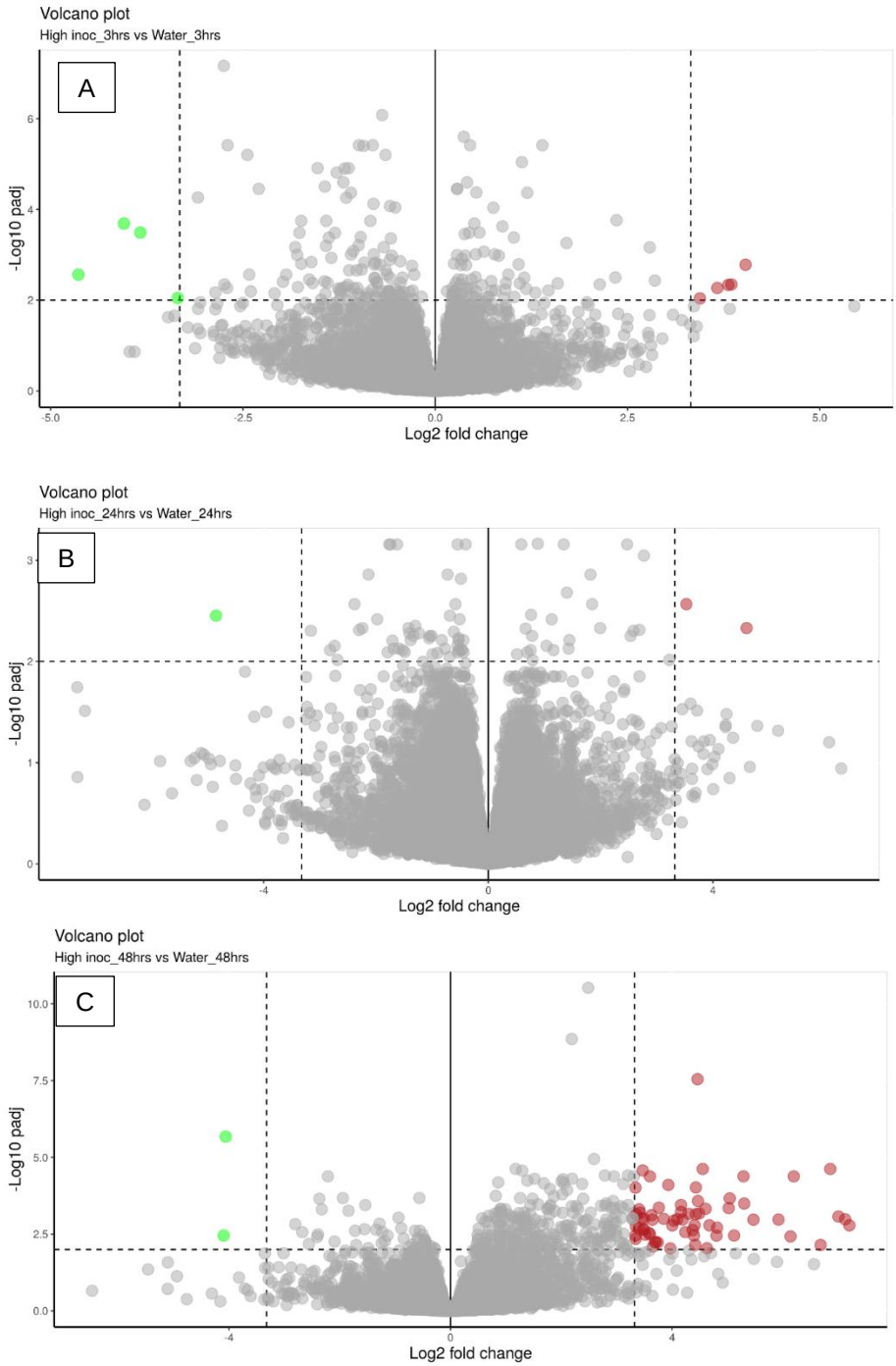

Figure A3. Volcano plot representing gene expression in plants of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) inoculated with high concentration of *Pseudomonas syringae* pv. *actinidiae* (Psa) vs water-treated plants. Time points of (A) 3 hours, (B) 24 hours and (C) 48 hours post-inoculation are presented.

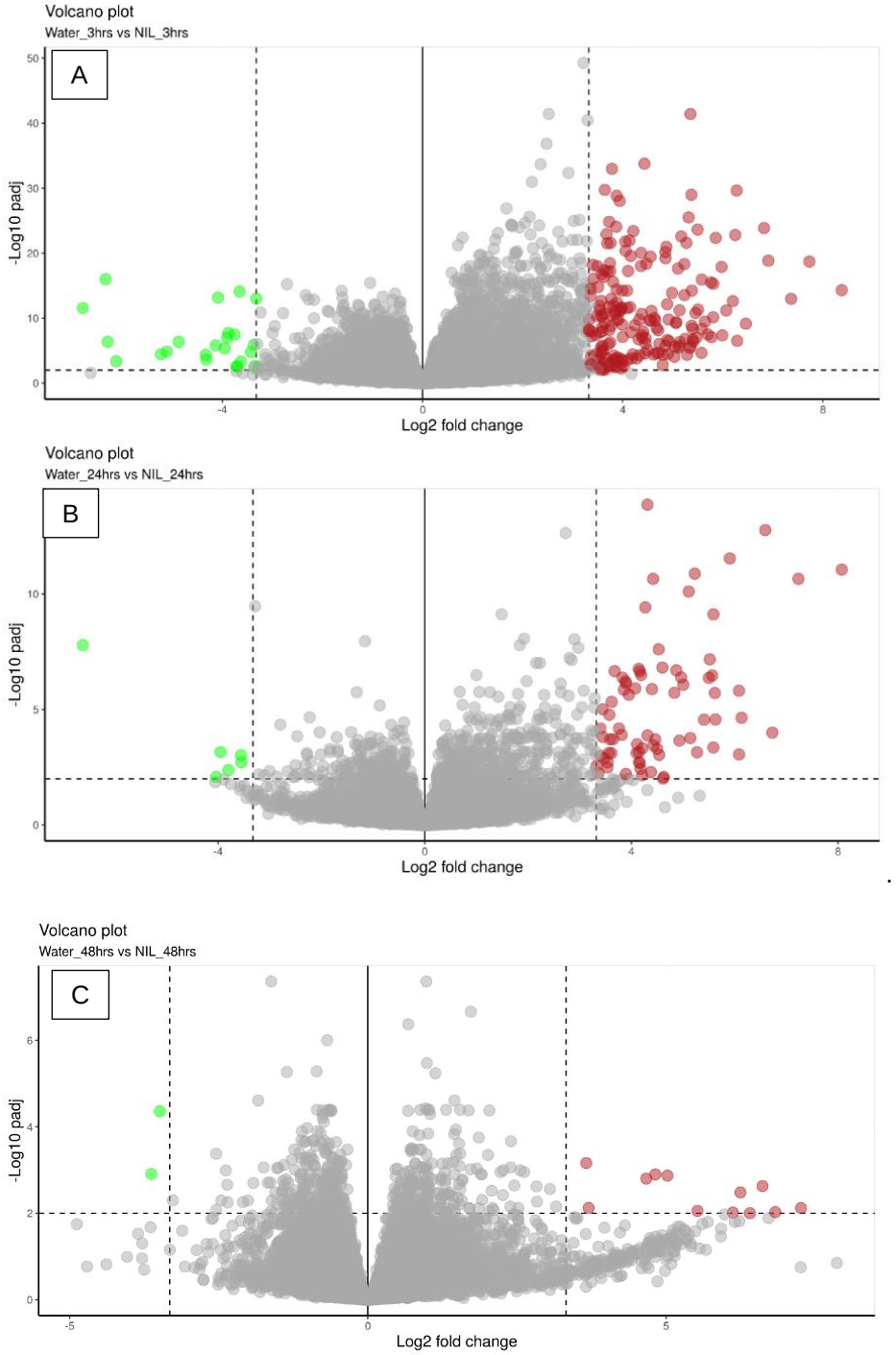

Figure A4. Volcano plot representing gene expression in water treated plants of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) vs nil-treated plants. Time points of (A) 3 hours, (B) 24 hours and (C) 48 hours post-inoculation are presented.

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