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Dissemination of *Pseudomonas syringae* pv. *actinidiae* through pollen and its epiphytic life on leaves and fruits

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Summary. The role of pollen in disseminating *Pseudomonas syringae* pv. *actinidiae* (Psa) in kiwifruit orchards was investigated and the survival of the pathogen as an epiphyte on leaves and fruits was followed, from pollination time until the pre-harvest season. Pollen with natural inoculum was obtained from an infected orchard and pollen harvested without any contamination by Psa was experimentally inoculated at approximately the same contamination level. Two pollination techniques were used in glasshouse experiments: dusting and spraying. In parallel, field trials in commercial orchards were carried out: two plots were designed in two orchards, where bacterial canker was present at low incidence. From petal fall to 3 weeks before harvesting, leaf and fruit samples were taken and analysed for the presence of Psa, using two different PCR protocols and direct isolation. Results confirmed the dissemination of Psa through pollen, especially when using the aqueous suspensions. Both in glasshouse experiments and in the orchards Psa was found as an epiphyte for several weeks after pollination. Pathogen populations on leaves were, initially, 10 to 100 times less than on fruitlets. As the summer continued, the epiphytic contamination levels of fruits decreased constantly, being no more detectable from early August, whereas Psa was present at detectable levels on leaves until early October, approx. 20 days before harvesting time. Our results confirmed the role of pollen in disseminating Psa, the long epiphytic survival of the pathogen on kiwifruit leaves and the increasing unsuitability of fruits to harbour detectable, epiphytic populations of the bacterium through the summer season in commercial orchards with low disease incidence, when they are reaching the final development stage. Thus, kiwifruit surfaces do not appear to be a suitable niche for a long term survival of Psa as an epiphyte and, therefore, kiwifruits should not represent a pathway for Psa dissemination and pose a negligible risk for the introduction of the pathogen into new areas.

Key words: *Actinidia* spp., bacterial canker of kiwifruit, pathogen dispersal.

Introduction

Actinidia spp. are, with very few exceptions, dioecious plants: female plants bear fruits only when pollenated by male plants. In nature, pollination of kiwifruit plants is mainly anemophilous, since flowers are not very attractive to bees or other pollinating insects, although bee saturation pollination may be used in commercial orchards, by keeping large numbers of bee colonies nearby (Matheson, 1991). Artificial pollination of kiwifruit plants is an agricultural practice which has been extensively used by orchardists during the last 15 years, in order to obtain both greater fecundation rates of flowers and fruits of larger size, and, usually, approx. 30% increase in fruit weight (Hopping and Hacking, 1983; Gonzales *et al.*, 1998). The application of pollen in kiwifruit orchards might follow two techniques: either pollen dusting with dry pollen applicators or application of pollen in water suspensions with pumping/spraying devices. In both cases, usually, 400–500 g of pollen (250 to 750 g) are applied per ha, dusting or spraying under the canopy.

A few phytopathogenic bacteria might be asso-

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ciated with pollen, with pollen a means of pathogen dispersal in orchards. Examples are *Erwinia amylovora*, the causal agent of fireblight of pome fruits (van der Zwet and Bell, 1992) and *Xanthomonas arboricola* pv. *juglandis*, the causal agent of the bacterial canker of walnut (Garcin *et al*., 2001; Giovanardi *et al*., 2010). In the case of pollen from *Actinidia* spp., contamination by *Pseudomonas syringae* pv. *actinidiae* (Psa), the causal agent of bacterial canker of kiwifruit, was repeatedly found in samples collected from commercial orchards or from male plant orchards with visible symptoms of the disease or from fields, apparently without the disease, which later in the season reported outbreaks (Vanneste *et al*., 2011). Thus, long distance dissemination of Psa through pollen lots is possible, as reported by Biosecurity New Zealand (2010). A pest risk analysis (PRA) prepared by Australian Phytosanitary Authorities emphasized the high likelihood of Psa introduction into Australia through pollen (Biosecurity Australia, 2011). Nevertheless, the role of pollen transmission of the pathogen in orchards, and establishment of epiphytic populations on kiwifruit plants have not been demonstrated. Indeed, especially for fruits, Psa survival as an epiphyte, after artificial pollination, might pose some risks for a long distance spread of the pathogen. Thus, it is particularly important to study the fate of the pathogen as an epiphyte after artificial pollination on leaves and, especially, on fruits. This short paper outlines a single season study (May to early October 2011) on the role of pollen in disseminating Psa and its survival as an epiphyte, both under controlled conditions (glasshouse) and in commercial orchards.

Materials and methods

Plant material

Two years old *A. deliciosa* plants, cv. Hayward, were used in the glasshouse experiments. Three plots of three plants each were designed in a containment glasshouse (nine plants in total). Temperature was set at approx. 26±3°C. Plants were manually watered according to their need and they received a fertilizer complex (N, P, K) three times during the experiments. Pollen samples were provided by the Plant Protection Service of the Emilia Romagna Region and obtained from infected and non-infected orchards. Several pollen samples were accurately analysed by both PCR and direct plating, as detailed below. A total of three pollen samples were chosen for pollination. The first one had a natural load of Psa at a calculated rate of approx. 4×10^6 cfu g^{-1}; the second sample, which gave negative results for the pathogen with both the specific PCR assays (Koh and Nou, 2002; Rees-Georg *et al*., 2010) and negative direct isolation, was used as a negative control. The third pollen sample was prepared as follows: from the naturally contaminated pollen mentioned above, a pure culture of Psa was obtained by direct isolation and it was used to contaminate the Psa-free pollen sample at approximately the same inoculum rate of the naturally contaminated one. Thus, three pollen samples were used to pollinate the three kiwifruit plots: one with natural Psa contamination and one free from Psa, both used for dry pollination. The third sample, with artificial contamination, was sprayed as an aqueous pollen suspension. Additionally, two commercial kiwifruit cv. Hayward orchards, located in the Marzeno valley (eastern Emilia Romagna Region of Italy) and with low disease incidences, were chosen for the study of Psa as an epiphyte. For each orchard a five plant plot was designed, where no antibacterial treatments had been applied throughout the period of the experiments.

Pathogen isolation, identification and pollen contamination

Pollen samples were analysed according Vanneste *et al*. (2011). Direct isolation from PCR positive pollen samples was done on modified nutrient sucrose agar (NSA) (Crosse, 1959), supplemented with 80 ppm cephalexin and 200 ppm cycloheximide. Psa-like colonies were screened by hypersensitivity response (HR) on tobacco leaves, and HR positive colonies were identified with repetitive-sequence PCR (rep-PCR) using the BOXA1R primer (Versalović *et al*., 1991; Louws *et al*., 1994; Scortichini *et al*., 2002; Vanneste *et al*., 2010). Three reference strains were used as controls for plating, simplex PCR and BOX-PCR: Pan NCPPB 3739, Pan ISF-ACT1 and *Pseudomonas syringae* pv. *syringae* (Psy) strain DAFS 427, isolated from symptomatic *A. deliciosa* leaves. The Psa isolate from the naturally contaminated pollen was used to experimentally inoculate a Psa-free pollen sample.

Pollination

Three pollen samples were used to pollinate the three kiwifruit plots in the glasshouse: the naturally contaminated pollen, the Psa-free pollen experimentally contaminated with approx. 4×10^6 cfu g^{-1} and a Psa-free pollen sample; thus, each plot (three plants) was pollenated with a different pollen sample. During blossoming, the naturally contaminated and the Psa-free pollen were dusted on their plots, whereas a pollen suspension was prepared in an aqueous suspension of Psa at the concentration of approx. 4×10^6 cfu mL^{-1}. The quantity of pollen used for each plot was 1.5 g. Commercial orchards, where the disease was already present at low incidence, were not artificially pollenated.

Monitoring and sampling

The glasshouse plants were monitored every week throughout the experiment for checking possible disease outbreak and development. First samples were taken 48 h after pollination, each consisting in ten leaves and five flowers per plot. Second samples were taken 3 weeks after pollination and all other samples were taken every 3 weeks, until early October, for a total of eight samples for leaves and eight for fruitlets per plot. The commercial orchards were inspected weekly to observe possible increase in disease development in the selected plots. From the end of May to early October, samples were collected every 3 weeks (seven samplings in total). Two replicate samples, consisting in ten leaves and ten fruits each, were taken for each orchard at each sampling. All samples were analysed within 1 h from sampling, to allow maximum recovery of Psa.

Pathogen detection and identification

Leaf, flower and fruit samples were analysed according to the following procedure: the samples were put into Erlenmeyer flasks of suitable size, with 80–150 mL (according to leaf and fruit size) of sterile saline solution (0.85% NaCl), containing 0.1% of Tween 20 and then maintained at room temperature on a rotary shaker at 180 rpm for 1 h. Washing fluids were then filtered through sterile gauze into large centrifuge tubes and centrifuged at 10,000 rpm for 20 min at 6°C. Each resulting pellet was then suspended with 1.5–2.0 mL of sterile saline to obtain a final concentrate (FC). Thirty ml of the FC and its 10- and 100-fold dilutions were plated onto modified NSA and plates were incubated at 25°C and observed every 12 h for 4 days. Psa -like colonies were considered those developing on agar plates after 3–4 days, pale-whitish in colour, dull, elevated and with entire margins. For each plate, five Psa putative colonies were chosen and purified for further identification. First discrimination was performed by HR on tobacco leaves, and positive isolates were further identified by rep-PCR using the BOXA1R primer (Versalović *et al*., 1991; Louws *et al*., 1994; Scortichini *et al*., 2002; Vanneste *et al*., 2010). The above mentioned reference strains Pan NCPPB 3739 and ISF-ACT1 as well as Psy strain DAFS 427, isolated from symptomatic leaves of *A. deliciosa*, cv. Hayward, were used as controls. PCR protocols according Koh and Nou (2002) and Rees-George *et al*. (2010), the latter using both primer pairs, were applied to the FC and its dilutions, after DNA extraction using the Qiagen Dneasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) and following the manufacturer's guide.

Results and discussion

Glasshouse experiments

Results of glasshouse experiments are summarized in Table 1. Artificial pollination in the glasshouse was effective and plants produced several fruits. Plants pollenated with aqueous pollen suspensions developed approx. 20% more fruits than plants simply dusted with pollen. First leaf and flower samples were collected 48 h after pollination. Samples taken from plots pollenated with the contaminated pollen and analysed with both PCR protocols gave the specific amplicons expected, thus confirming the detection of Psa. Leaf and flower samples taken from the plot pollenated with the Psa -free pollen failed to show any of the predicted, specific bands, thus confirming the absence of the pathogen. Direct isolation from leaf and flower samples sprayed with aqueous pollen suspensions was successful in both cases and the bacteria contamination was calculated to be approx. 3×10^4 and 7×10^6 cfu mL^{-1} of the resulting concentrated washing fluids of leaves and fruits, respectively. Thus, first observation on survival of Psa showed that colonisation was more effective on flowers than on leaves, indicating that flow-

Table 1. Detection of epiphytic populations of *Pseudomonas syringae* pv. *actinidiae* (Psa) in glasshouse experiments and in commercial orchards, where the pathogen was applied in different pollen applications.

Experiments	Time of sampling after pollenation[a]							
	48 h	3 wks	6 wks	9 wks	12 wks	15 wks	18 wks	21 wks
Glasshouse (aqueous poll., leaves)	PCR[b] + DI[b] +	PCR + DI +	PCR + DI +	PCR + DI +	PCR + DI +/-	PCR + DI +	PCR + DI +/-	PCR + DI +
Glasshouse (aqueous poll., fruits)	PCR + DI +	PCR + DI +	PCR + DI +	PCR + DI -	PCR +/- DI -	PCR +/- DI -	PCR - DI -	PCR - DI -
Glasshouse (dusting, leaves)	PCR + DI -	PCR + DI -	PCR +/- DI -	PCR +/- DI -	PCR - DI -	PCR + DI -	PCR - DI -	PCR - DI -
Glasshouse (dusting, fruits)	PCR + DI +	PCR - DI -	PCR - DI -	PCR - DI -	PCR - DI -	PCR - DI -	PCR - DI -	PCR - DI -
Field plots (leaves)	NT	PCR + DI +	PCR + DI +	PCR + DI +/-	PCR + DI +/-	PCR +/- DI -	PCR + DI +/-	PCR + DI +
Field plots (fruits)	NT	PCR + DI +	PCR + DI +	PCR +/- DI +	PCR +/- DI -	PCR - DI -	PCR - DI -	PCR - DI -

[a] Times are the hours/weeks after pollination. Artificial pollination was done on May 9th, 2011; last sampling was done on October 2nd, 2011.
[b] PCR, Detection of the pathogen by PCR assays. PCR+ indicates a positive amplification (two protocols; see text). PCR +/- indicates some of the replicates gave no amplification with either protocol.
[c] DI, Detection of Psa by isolation. DI+ indicates that Psa -like colonies were detected on agar plates and further identified as Psa with HR on tobacco and BOX-PCR. DI- indicates that either no Psa -like colonies were observed on agar plates or Psa -like colonies selected from plates failed to be identified as Psa.
NT, Not tested.

ers are better niches for maintaining rather high Psa populations, approx. 100 times greater than on leaves during the first days after aqueous pollinations. From plants pollenated with the dusting applicator, direct isolation of Psa was possible from flowers, but not from leaves, although both extracts gave positive response with both PCR protocols used, as described above. Thus, dry pollination appears to be less suitable for the establishment of detectable epiphytic populations of the pathogen.

Further sampling of leaves and fruitlets (or developing fruits) from plants pollenated with aqueous pollen suspensions always resulted in PCR positive detection of Psa until early August. From the first week of August, until early October (21 weeks after pollination) PCR assays from leaf washing fluids was always positive, whereas PCR assays of fruits washing fluids gave contradictory results in August (sometimes negative and sometimes positive), being always negative in September and October. This might indicate that the epiphytic population of Psa in those fruit samples was quantitatively around the detection level of both PCR tests used during August, and that the pathogen eventually disappeared during September and early October.

Direct isolation from leaves was possible throughout the whole experimental period (May–October), although sometimes it was not possible to isolate Psa from a few samples. The calculated cfu mL^{-1} of concentrated leaf washing fluid was

barely above the detection threshold for direct isolation, being between 10^4 and 3×10^2. Direct isolation from fruitlets was constantly positive until the end of July, showing a decrease of Psa populations: last detectable contamination of Psa showed a population of 3×10^2 cfu mL^{-1} of FC. No isolation was obtained in August, September and October.

Leaves and fruitlets or developing fruits from plants pollenated with the dusting applicator always gave contradictory results during the first 11 weeks of sampling. This might indicate that, when dusting with contaminated pollen, survival of Psa as an epiphyte is more troublesome during the early development of fruits, with the populations detected being around the sensitivity threshold of the PCR assays applied. From the 12th week up to the end of the experiments (21st week) only one positive PCR sample for leaves, and no positive samples for fruits, were detected, thus indicating a possible disappearance of Psa as a fruit epiphyte. Direct isolation from dusted plants never detected the pathogen, with the exception of those samples collected 48 h after dusting pollen. The kiwifruit plants dusted with the Psa -free pollen never gave a positive response to the PCR assays applied, both from washing fluids from leaves or from fruitlets. Direct isolation always failed to detect Psa -like colonies on agar plates.

Molecular detection of Psa and its isolation on semi-selective medium showed that the establishment of epiphytic Psa populations in the glasshouse experiments was much more difficult on dusted plants than on plants pollenated with an aqueous pollen suspensions. This might be due to long periods of leaf/flower wetness, which supported the early stages of Psa colonisation. Leaf/flower wetness might have supported viability of Psa as a plant epiphyte, by possibly solubilising metabolites present on the surfaces and making them available to the pathogen for to sustain viability and growth.

During the first 3 weeks after pollination with aqueous suspensions of experimentally contaminated pollen, five leaf spots resembling typical symptoms of bacterial canker commonly visible in infected orchards were observed on a few leaves of the three treated plants. All these five spots were individually analysed and Psa was detected by both PCR assays and direct isolation on agar plates. No other symptoms developed on those plants until the end of the experiments. No spots were ever observed on plants dusted with naturally contaminated pollen and on control plants dusted with Psa -free pollen. The fact that, although Psa was always detected and isolated from plants pollenated with aqueous suspensions, but no severe disease development was observed might indicate that Psa distributed with pollen has not sufficient fitness, or the number of bacteria was too low, (4×10^6 cfu g^{-1}) to start infection processes. In other experiments, in a parallel containment glasshouse and under the same climatic conditions, the spraying of 20 1-year-old kiwifruit plants with a Psa suspensions of 8×10^7 cfu mL^{-1} resulted in the development of a severe disease (Stefani, data not published). The differences between the two experiments might be due to a behaviour already reported for *P. syringae* pvs. and related to *quorum sensing* (Monier and Lindow, 2003; Dulla *et al*., 2010). Thus, pollen has been confirmed as a source of contamination and possible dispersal of Psa, which might be able to survive for long periods on kiwifruit leaves. To date, no report has been published demonstrating the role of contaminated pollen in the spread of the disease, but researchers are already testing disinfection methods to produce and certify Psa -free) pollen lots. A high-temperature treatment has been indicated as a possible solution to kill the bacteria without affecting pollen viability. Experiments are under way in New Zealand, as reported in local newspapers (Bay of Plenty Times, on June 16, 2011; Rotorua Daily Post, on June 21, 2011), but no scientific results have yet been published.

Field experiments

Results of field experiments are summarised in Table 1. Seven surveys were done in the two commercial kiwifruit orchards for the detection of Psa as an epiphyte. Results showed consistency between the two orchards: all leaf samples collected from the end of May until early October harboured detectable populations of Psa, when tested with both PCR assays, throughout the whole sampling period. The specific PCR amplicons for Psa were detected from all fruit samples collected until early August; from mid August all fruit samples were found negative for the presence of Psa until the end of the surveys, with the exception of one single sample in one plot detected at the end of August. Direct isolation on modified NSA plates was suc-

cessful in nine leaf samples out of 14 and six fruit samples out of 14. Direct isolation from fruit samples was always positive until the 9th week after the natural occurring pollination, but were always negative for all later samples. For those leaf and fruit samples from which direct isolation of Psa was possible, a variable contamination level of washing fluids between 3×10^4 and 8×10^2 cfu mL^{-1} was estimated by plate counting, thus an average bacterial population of less than 3×10^3 Psa cells per leaf or fruit.

The semi-selective medium proved to be very good for the isolation of Psa, thus allowing the growth of the pathogen and a few other bacteria, some of them similar to Psa. The discrimination of Psa -like colonies by HR on tobacco leaves was very useful, since almost all of the HR positive colonies were identified as Psa with rep-PCR. In only one case was a HR positive colony further identified by BOX-PCR as Psy, a pathovar already detected and reported on kiwifruits in the same area where experiments were conducted (Stefani, unpublished data). Direct isolation was only possible from a number of samples yielding the Psa-specific amplicons, although from several other samples giving a PCR positive result no Psa isolations were obtained. Therefore, an enrichment step applied to the FC might be appropriate, in order to enhance sensitivity of direct isolation, as already suggested by Rees-Georg *et al*. (2010). A number of PCR protocols for Psa are available in the literature (Koh and Nou, 2002; Rees-George, 2010; Gallelli *et al*., 2011a). In the present work, the first two were assayed and they proved to be equivalent, when the original protocol of Koh and Nou (2002) was used with some modifications in order to improve its specificity (Stefani, unpublished data).

The finding of detectable populations of Psa after artificial pollination with contaminated pollen confirms the epiphytic fitness of this pathovar, as already reported several times for *P. syringae* strains, which are frequently important components of the epiphytic microflora of both cultivated and spontaneous plants (Hirano and Upper, 1990). Regarding Psa, not much information is available, although it has been reported that a low concentration of Psa cells is able to survive approx. 3 weeks on leaf surfaces before causing visible symptoms (Serizawa and Ichikawa, 1993). Our experiments showed that a few necrotic spots developed on some leaves in plants pollenated with aqueous pollen suspensions, suggesting the possibility that wet pollen applications might be involved not only in Psa dissemination, but also in the spread of the disease, although this needs further experimental confirmation, for instance on *quorum sensing*. On this point similar field experiments will be conducted, in order to have consistency with the true eco-pathological situation, as in the commercial orchards. Interestingly, the initial colonisation of the flowers by the pathogen was very effective, with a relevant number of detectable bacteria present in the first weeks of fruitlet development. Later, epiphytic populations of Psa on fruits decreased in approx. 2 months, being no longer detectable from early August until early October, i.e. 3 weeks before harvesting time in the Emilia Romagna Region. An increased fitness of Psa on flowers and young fruitlets, compared with leaf surfaces, might be due to the presence of plant exudates or waxes or metabolites from living fruit hairs: fruit hairs die during the early weeks after flowering, and becomes complete after about 50 days (Dichio *et al*. 2004). Total hair death coincides with the sharp reduction in fruit transpiration (Dichio *et al*., 2004). Ontology and morphology of kiwifruit hairs has been already described (White, 1986): hair development is initiated from epidermal cells, approx. 3 weeks after bud burst and continues to form until 107-114 days after bud burst, and after that period they desiccate. No stomata were observed on fruits (White, 1986). Then, fruitlets and young fruits are covered with living unicellular and multicellular hairs growing on amorphous layers of wax (Celano *et al*., 2009); 5 weeks after fruit set hairs begin to collapse and the epidermal cells dehydrate and suberize with an increase of the total wax from 0.33 to 2.40 mg per fruit. In parallel fruit water loss declines. Thus, the fitness of Psa as a fruit epiphyte might be excellent just after fruit set (100 times greater populations than on leaves in our study), then gradually decreases when the fruit skins suberize and fruit water loss declines. This might explain why, 12 to 13 weeks after fruit set, no detectable Psa cells were found during our experiment. These data, when referred to the kiwi fruits as a possible pathway, might indicate a negligible risk for the introduction and spread of Psa into a territory just by transfer of fruit lots as a commodity.

Different International and/or National Plant Protection Organisations have prepared or are preparing both Pest Risk Analyses (PRA) and official detection protocols for Psa. This study suggests that pollen transmission should be considered as a possible threat because of its role in pathogen dissemination. In regard to fruits, our experimental data show that fruit lots as a commodity do not represent a biosecurity threat, since Psa has almost no chance to survive as an epiphyte on fruits from orchards with low disease incidence and severity. In case of symptomless fruits collected in severely affected orchards, where several other fruits are symptomatic, Psa might survive somewhat longer as an epiphyte (Gallelli *et al*., 2011b). In this case further studies are needed to establish how long Psa is able to survive as an epiphyte after harvesting, during curing of fruits and their cold storage.

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Literature cited

Biosecurity Australia, 2011. Pest risk analysis report for *Pseudomonas syringae* pv. *actinidiae* associated with *Actinidia* (kiwifruit) propagative material. Department of Agriculture, Fisheries and Forestry, Canberra, Australia.

Biosecurity New Zealand, 2010. Psa bacteria collected in pollen in New Zealand. 20 November 2010. Ministry of Agriculture and Forestry, New Zealand.

Celano G., A. Minocci, L. Sebastiani, M. D'Auria and C. Xiloyannis, 2009. Changes in the structure of the skin of kiwifruits in relation to water loss. *Journal of Horticultural Sciences and Biotechnology* 84 (1), 41–46.

Crosse J.E., 1959. Bacterial canker of stone-fruits. IV. Investigation of a method for measuring the inoculum potential of cherry trees. *Annals of Applied Biology* 47, 395–407.

Dichio B., D. Remorini and S. Lang, 2004. Caratterizzazione e vitalità dei tricomi in frutti di actinidia (Characterization and viability of hairs in kiwifruit fruits). In: Convegno Nazionale SOI. VII Giornate scientifiche SOI, 4–6 May, 2004, Castel dell'Ovo, Napoli, Italy.

Dulla G.F.J., K.V. Krasileva and S.E. Lindow, 2010. Interference of *quorum sensing* in *Pseudomonas syringae* by bacterial epiphytes that limit iron availability. *Environmental Microbiology* 12, 1762–1774.

Gallelli A., A. L'Aurora and S. Loreti, 2011a. Gene sequence analysis for the molecular detection of *Pseudomonas syringae* pv. *actinidiae*: developing diagnostic protocols. *Journal of Plant Pathology* 93, 425–435.

Gallelli A, S. Talocci, A. L'Aurora and S. Loreti, 2011b. Detection of *Pseudomonas syringae* pv. *actinidiae*, causal agent of bacterial canker of kiwifruit, from symptomless kiwifruit organs (fruits, twigs, pollen). *Phytopathologia Mediterranea* (in press).

Garcin A., M. El-Maataoui, S. Tichadou, J.P. Prunet, T. Ginibre and C. Penet, 2001. Walnut blight, new knowledge for an old disease: summary of research (1995-2000). *Infos-Ctifl* 171, 27–30.

Giovanardi D., D. Dallai and E. Stefani, 2010. Population features of *Xanthomonas arboricola* pv. *juglandis* and epidemiology of walnut blight in Romagna (Italy). *Petria* 20, 97–98.

Gonzales M.V., M. Coque and M. Herrero, 1998. Influence of pollination systems on fruit set and fruit quality in kiwifruit (*Actinidia deliciosa*). *Annals of Applied Biology* 132, 340–355.

Hirano, S. S., and C. D. Upper. 1990. Population biology and epidemiology of *Pseudomonas syringae*. *Annual Revew of Phytopathology* 28, 155–177.

Hopping M.E and N.J.A Hacking, 1983. A comparison of pollen application methods for the artificial pollination of kiwifruits. *Acta Horticulture (ISHS)* 139, 41–50.

Koh Y.J. and I.S. Nou, 2002. DNA markers for the identification of *Pseudomonas syringae* pv. *actinidiae*. *Molecules and Cells*13, 309–314.

Louws F.J., D.W. Fulbright, C.T. Stephens and F.J. de Bruijn, 1994. Specific genomic fingerprints of phytopathogenic *Xanthomonas* and *Pseudomonas* pathovars and strains generated with repetitive sequences and PCR. *Applied and Environmental Microbiology* 60, 2286–2295.

Matheson A.G., 1991. Managing honey bee pollination of kiwifruit (*Actinidia deliciosa*) in New Zealand - A review. *Acta Horticulturae (ISHS)* 288: 213–219.

Monier, J.M. and S.E. Lindow, 2003. Differential survival of solitary and aggregated bacterial cells promotes aggregate formation on leaf surfaces. In: *Proceedings of the National Academy of Science USA* 100, 15977–15982.

Rees-George J., J.L. Vanneste, D.A. Cornish, I.P.S. Pushparajah, J. Yub, M.D. Templeton and K.R. Everett, 2010. Detection of *Pseudomonas syringae* pv. *actinidiae* using polymerase chain reaction (PCR) primers based on the 16S-23S rDNA intertranscribed spacer region and comparison with PCR primers based on other gene regions. *Plant Pathology* 59, 453–464.

Scortichini M., U. Marchesi and P. Di Prospero, 2002. Genetic relatedness among *Pseudomonas avellanae*, *P. syringae* pv. *theae* and *P. s.* pv. *actinidiae*, and their identification. *European Journal of Plant Pathology* 108, 269–278.

Serizawa S. and T. Ichikawa, 1993. Epidemiology of bacterial canker of kiwifruit. 2. The most suitable times and

environments for infection on new canes. *Annals of the Phytopathological Society of Japan* 59, 460–468.

Van der Zwet T. and R.L. Bell, 1992. Survival of *Erwinia amylovora* on apple and pear pollen. *Acta Horticulture (ISHS)* 338, 111–112.

Vanneste J.L., D. Giovanardi, J. Yu, D.A. Cornish, C. Kay, F. Spinelli and E. Stefani, 2011. Detection of *Pseudomonas syringae* pv. *actinidiae* in kiwifruit pollen samples. *New Zealand Plant Protection* 64, 246–251.

Vanneste J.L., J. Yu and D.A. Cornish, 2010. Molecular characterisations of *Pseudomonas syringae* pv. *actinidiae* strains isolated from the recent outbreak of bacterial canker on kiwifruit in Italy. *New Zealand Plant Protection* 63, 7–14.

Versalović J., T. Koeuth and J.R. Lupski, 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Research* 19, 6823–6831.

White J., 1986. Ontology and morphology of ovarian and fruit hairs in kiwifruit. *New Zealand Journal of Botany* 24, 403–414.

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