



Actinidia pollen is a pathway for the dissemination of *Pseudomonas syringae* pv. *actinidiae* and for the spread of the bacterial canker of kiwifruit

E. Stefani, R. Tontou, and D. Giovanardi

Department of Life Sciences, via Amendola 2, Pad. Besta, 42122, Reggio Emilia, Italy.

E-mail: emilio.stefani@unimore.it



Fig. 1 & 2 – Symptoms of bacterial canker on trunk and leaf spots caused by *Psa* on *Actinidia chinensis*, cv. Hort16A

INTRODUCTION

The bacterial canker of kiwifruit is the most destructive disease of cultivated *Actinidia* spp. (Fig. 1 & 2). The causal agent is the Gram negative bacterium *Pseudomonas syringae* pv. *actinidiae* (*Psa*). Both *Actinidia deliciosa* (the green fleshed kiwifruit) and *Actinidia chinensis* (the yellow fleshed one) are susceptible to the disease.

Artificial pollination is widely applied as agricultural practice in kiwifruit orchards, either by dusting or by spraying, in order to obtain fruits of bigger size and better quality. Moreover, the pollen of *Actinidiae* spp. has been proven to be a good substrate for *Psa* and a pathway for its dissemination. The aim of this research has been to study if the dispersal of *Psa* through pollen is followed by the establishment of the pathogen in the orchard and the disease outbreak.

MATERIALS & METHODS

Naturally contaminated pollen was collected from an orchard with symptomatic plants: contamination was calculated to be approx. 6×10^4 cfu/ml. In addition, an experimental orchard with two-year-old actinidia plants was planted and four theses, each one with four replicates, were designed in it: in the first two theses non contaminated pollen was applied either by dusting or spraying, in the third one naturally contaminated pollen was dusted and in the fourth same contaminated pollen was sprayed in a water suspension. Each thesis was represented by 16 plants. Then, after artificial pollination, sampling of flowers, fruits and leaves was done every two weeks until middle October for two consecutive seasons. The presence of epiphytic *Psa* was monitored, and evaluated throughout the whole period. Moreover, for every monitoring time, the possible development of typical symptoms was carefully checked in plants. Detection of *Psa* was done both with PCR and direct isolation. *Psa* putative colonies were always subject to identification.

RESULTS

In the first experimental season (2012), *Psa* was detected already 24 h post pollination, which confirms that the pathogen survives in the orchard after the pollination (Table 1). Additionally, a higher number of *Psa* colonies grew on selective media in case of pollination by spraying than by dusting and the same occurred after isolation from flowers in comparison to leaves. Analysis of fruits washings allowed detection of *Psa* until early August in the spraying treatment, whereas *Psa* was detected until early July in the dusting treatment. *Psa* was detected on leaves from May to August in the case of spraying and sometimes in October too. In the case of dusting it was detected until early July instead. During winter, sampling *Psa* didn't result in any positive detection/isolation. During the second sampling season (2013) *Psa* was detected and isolated again from both wet and dust pollinated plots during June-July. Typical necrotic leaf spots developed in June/July on four plants: from those lesions *Psa* was isolated and identified. Genotyping of *Psa* isolates confirmed its identity as the same strain present in pollen.

| | Time of sampling after pollination | | | | | | | |
|-----------------------|------------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| | 24 h | 2 wks | 4 wks | 6 wks | 8 wks | 12 wks | 16 wks | 21 wks |
| | 2012/2013 | 2012/2013 | 2012/2013 | 2012/2013 | 2012/2013 | 2012/2013 | 2012/2013 | 2012/2013 |
| Wet poll., (Flowers) | +/- | NT/NT | | | | | | |
| Wet poll., (Fruits) | NT/NT | +/- | +/- | +/- | +/- | -/- | -/- | -/- |
| Wet poll., (Leaves) | +/+ | +/+ | +/+ | +/+* | -/- | +/+* | +/- | +/- |
| Dust poll., (Flowers) | +/- | NT/NT | | | | | | |
| Dust poll., (Fruits) | NT/NT | +/- | +/- | -/- | -/- | -/- | -/- | -/- |
| Dust poll., (Leaves) | +/- | +/- | -/- | +/+ | +/+* | -/- | -/- | -/- |

Table 1 – Detection of epiphytic populations of *Psa* in field experiments after pollination (wet and dry) with naturally contaminated pollen done in 2012. In 2013 pollination was natural. Results shown in table summarise detection of *Psa* in two following seasons, 2012 and 2013. The epiphytic presence of *Psa* in 2013 was erratic and only on leaves. An asterisk indicates that **symptoms associated with presence of *Psa* were detected** on plants three plants during the season 2013. *Psa* isolated from those symptoms was genetically identified as the same present in pollen used for pollination
2012/2013 = *Psa* detection in 2012/*psa* detection in 2013

DISCUSSION & CONCLUSIONS

Psa was detected in the experimental field 24h post artificial pollination, which confirms that it survives after pollination. From plants pollinated by dusting of pollen, 100 times less colonies were isolated in comparison with plants pollinated by spraying, which may be due to the fact that wet conditions favour the establishment and growth of bacteria. Additionally, flowers showed constantly a higher number of bacteria colonies than leaves, which has already been described in similar experiments. Analysis of fruits and leaves during the first experimental season led to detection of epiphytic *Psa* populations until early August in the spraying treatments and until early July in the dusting treatments. During wintertime *Psa* could not be detected, but during the second experimental season *Psa* was detected and isolated from both sprayed and dusted plots. This is an evidence that small *Psa* populations survived during winter on the plants. Typical symptoms of the disease (necrotic leaf spots) were observed the second year, samples taken and indeed *Psa* was isolated and identified. The genetic profiles of the isolates were compared with the profile of the inoculum of the previous year and they were found to be identical, confirming that they derived from the applied pollen. Isolation and detection of *Psa* from the experimental orchard indicates that establishment of *Psa* populations in an orchard from contaminated pollen is possible. The occurrence of typical disease symptoms the year following pollination indicates that the pollen can carry a bacterial load adequate to establish a new disease niche. Therefore, pollen might be considered a pathway for *Psa* dissemination and disease spread into new areas: results justify the listing of pollen as a pathway for *Psa* introduction and spread into new areas, as precautionary mentioned in pest risk analyses (PRAs) prepared in the EU, EPPO Region, Australia, New Zealand.

ACKNOWLEDGEMENTS

This research was financed in part by Regione Emilia Romagna / CRPV, under the Project: “Ricerche sul *Psa* del Kiwi”, L.R. 28/1998.