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Epiphytic life is the main characteristic of the life cycle of *Pseudomonas syringae* pv. *pisi*, pea bacterial blight agent

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Abstract

Pseudomonas syringae pv. *pisi*, pea bacterial blight agent, is seed-transmitted. Some aspects of its life cycle and its biology were investigated. The colonization of pea plants obtained from naturally infected seeds was studied in natural conditions while high populations of bacteria developed on plants showing no symptoms. Two streptomycin-resistant mutants were used to study the epiphytic life of the pathogen. Populations were monitored in different host-parasite compatibilities. When race 2 or race 6 of the pathogen was surface-inoculated on susceptible cultivars, a decrease of population size was observed during the following one to three days but was followed by an increase to levels 1 000 times greater than the initial number detected, without symptoms for most of the plants. When race 2 was surface-inoculated on resistant genotypes or race 6 on non-host plants, bacteria did not multiply but population levels slightly decreased. *Pseudomonas syringae* pv. *pisi* shows a resident phase and its development is race-specific. Weeds collected in naturally contaminated pea fields, diseased or not, often harboured the pathogen but with levels smaller than those observed on peas. Pea crop debris and volunteers kept high levels of bacteria for at least eight months after the harvest of a diseased crop. As long as two pea crops are not grown one after the other in the same field, it is unlikely that debris and volunteers will act as an important inoculum source. The development of this pathogen during the growing season is considered as an important parameter to take into account for controlling the disease through seed health testing.

Introduction

Pea bacterial blight, caused by *Pseudomonas syringae* pv. *pisi* (*P. syringae* pv. *pisi*), has been widespread in Europe since 1985. The pathogen is seed-transmitted [Skoric, 1927] and the increase of international seed exchanges simultaneously with the increase of growing areas has contributed to the spread of the disease. A gene-for-gene relationship between pathogen races and pea cultivars has been established [Taylor et al., 1989] on the basis of seven races, the sixth being pathogenic for all cultivars. Epiphytic survival of *P. syringae* pv. *pisi* was reported in a preliminary study [Samson et al., 1988] where it was found that this pathogen was frequently isolated from asymptomatic plants. The existence of a permanent pathogenic microflora on the leaf surface was first noted for *P. syringae* pv. *morsprunorum* [Crosse, 1959, 1963]. It was concluded that the epiphytic populations could be the main source of inoculum for the leaf scars in autumn.

The research reported here is a study of populations of *P. syringae* pv. *pisi*, in order to define the biology and life cycle of the pathogen. We demonstrate that the development of bacteria is race-specific and that population levels associated with asymptomatic plants are related to the host genotype. The survival of *P. syringae* pv. *pisi* on weeds, crop debris and volunteers is also examined to determine if the disease can be transmitted by routes other than seeds.

Materials and methods

Bacterial isolates for surface inoculation

Two spontaneous mutants of *P. syringae* pv. *pisi* races 2 and 6 (Mt7 and VISm2 respectively) resistant to 250 μg ml^{-1} streptomycin, were obtained by conventional selective plating methods (respectively from strains Si544–69 and Si114A-6). The biochemical and serological characters of Mt7 and VISm2 as well as pathogenicity did not differ from those of parental wild-types. Population dynamics of the wild-type strain and the mutant strain were compared on cultivar Belinda for both races. Plants in glasshouse were sprayed with an inoculum of about 10^9 bacteria ml^{-1} of either strain or race. Bacterial population recovered from plants was determined by blending the above-ground part of eight individual plants taken every two days during two weeks, using an Ultraturrax homogenizer. It was found that the two mutants showed the same dynamic as its wild-type and thus the same ability to colonize a susceptible host. Therefore, mutants could be used for the following studies.

Inocula were prepared by rinsing the bacteria grown for 24 h on King's medium B into sterile water. Cell concentration was adjusted to about 5×10^8 bacteria ml^{-1} by optical density measurement at 450 nm with a spectrophotometer. Inoculum concentration was determined by dilution plating and viable cell count.

Comparison between washing and blending methods to recover P. syringae *pv.* pisi *from asymptomatic plants*

In a field sown with cultivar Belinda, a suspension of strain Mt7, concentrated at 10^8 bacteria ml^{-1}, was sprayed on the foliage of the plants (stage: 3–4 leaves) located in a square (1 m^2) in the center of the field. Two weeks later, eight plants were taken in concentric areas around the focus, between 0 and 2 m, 2 and 4 m, and 4 and 6 m, from the focus. The above-ground parts of the plants were cut and placed in 10 ml of sterile tap water in large test tubes, and shaken for two hours with a rotative shaker. The number of bacteria present in the washing water was determined by dilution plating and viable cell count on borate-levan agar medium (borate 1.5 g l^{-1}, sucrose 50 g l^{-1}, agar 15 g l^{-1}, cephalexin 40 ml l^{-1}), a medium commonly used for *P. syringae* pv. *pisi* isolation [Grondeau et al., 1992b] added with 100 μg ml^{-1} streptomycin and after four day incubation at 25 °C. Plants were then blended in the washing water, using an Ultraturrax homogenizer. Bacterial numbers in the blending water were determined as previously.

Monitoring P. syringae *pv.* pisi *after surface inoculation in semi-controlled conditions*

Pea (*Pisum sativum*) cultivars used were: Kelvedon Wonder, Belinda (susceptible to races 2 and 6), Frilène, Monitor and Lincoln (resistant to race 2 and susceptible to race 6). The non-host leguminous plants used were bean (*Phaseolus vulgaris*) cultivar Michelet, white clover (*Trifolium repens*) cultivar Aran, red clover (*T. pratens*) cultivar Lossam, bird's-foot trefoil (*Lotus corniculatus*) cultivar Oden, alfalfa (*Medicago sativa*) cultivar Kara. A non-host, non-leguminous plant, *Chenopodium* sp. cultivar Quinoa was also used. Plants were grown in a glasshouse. They were inoculated when 13–23 days old (at development stages varying from two to four expanded leaves) by gently spraying upper leaf surfaces until they were wet. Spraying was stopped before inoculum droplets were large enough to run off the leaf surface. After leaf drying, plant samples were taken to determine bacterial populations on day zero. The remaining plants were maintained in a glasshouse or in a climatic growth chamber. Samples of eight healthy plants were regularly taken, and the above-ground part of those plants was individually analysed.

Monitoring P. syringae *pv.* pisi *after surface inoculation in field experiments*

To study the spread of *P. syringae* pv. *pisi* from an inoculum source in relation to host genotype, field trials were conducted at Brain (Maine-et-Loire, France) at the FNAMS (Fédération Nationale des Agriculteurs Multiplicateurs de Semences) station in winter-sown pea in 1992. Two pea cultivars, Belinda (susceptible to race 2) and Monitor (resistant to race 2), were sown in four plots per cultivar. Plots measured 5 by 5 m and were separated by 3 m of uncropped land. In the middle of each plot, 1 m^2 was sprayed with Mt7 at a concentration of 10^8 cells ml^{-1} when plants had five expanded leaves (30 March 1992). This area constituted the source of primary inoculum (focus) for secondary spread.

The population dynamic of the inoculated pathogen was then monitored on pea foliage, first in the focus by sampling one plant per focus, and secondly around the focus on the four diagonals of each plot at distances

of 0.5–1–1.5–2 and 2.5 m from the focus (one plant was taken on each diagonal for each distance, 160 plants were then sampled per date). Individual plants were brought back to the laboratory to determine the number of streptomycin-resistant bacteria carried by each sampled plant. There were 10 sampling dates after inoculation.

Determination of bacterial populations after surface inoculation

The above-ground part of asymptomatic plants, sampled from glasshouse or field, was individually blended for one minute in a sterile water volume adjusted to plant size, with an Ultraturrax apparatus when small, and ground with a blender when more developed. Extracts were allowed to settle before being diluted. Several drops of 100 μl each were then laid on borate-levan agar medium containing 100 μg ml^{-1} streptomycin. Plates were incubated in the dark at 25 °C and the number of white pearly convex colonies counted after two and four days of incubation. Populations were expressed as the decimal logarithm of the number of bacteria (or colony forming unit) per plant, except for the experiment with non-host plants where they were expressed per gram of fresh weight because of considerable differences in plant size between species. Samples were therefore weighted before being blended.

Appearance of disease

Sampled plants were observed for the presence of water-soaked lesions.

Statistical analysis

The mean numbers of bacteria per plant or per gram of fresh weight were compared between genotypes at one date and during time, by unidimensional variance analysis (ANOVA) and multidimensional variance analysis (MANOVA), using the statistical procedure STATITCF on computer (Manufacturer: Technical Institute of Cereals and Forages, Paris; works with MS-DOS, PC). ANOVA was performed to compare genotypes on one sampling date with the test of Newman-Keuls, and MANOVA gave information about the interaction between genotypes and time, to see if the differences observed were due to genotypes only, or if they were not constant during time, and so if time interfered. Errors α and β were 5%.

Monitoring natural populations of P. syringae *pv.* pisi *on asymptomatic pea plants in field conditions*

A naturally infected seed lot was sown in April 1989 in a 8 by 2 m plot at Brion (Maine-et-Loire, France). The plot was divided into 64 squares (0.25 m^2 each) and one plant was sampled in each square on three different dates: 18/05, 07/06 and 03/07. All plants were observed carefully for disease.

Monitoring natural populations of P. syringae *pv.* pisi *on weeds, pea debris and volunteers in field conditions*

Natural populations of *P. syringae* pv. *pisi* were determined on weeds collected in two naturally infected pea crops. The first crop was sown with Belinda at Brion (Maine-et-Loire, France). The crop did not show any visible symptoms on the day when weed samples were taken. The second crop was sown during the autumn with cultivar Belinda at Villefranche-de-Lauragais (Haute-Garonne, France). This crop displayed disease symptoms when weed samples were collected.

Weeds, as well as pea debris and pea volunteers, were also analysed for the presence of *P. syringae* pv. *pisi* after the harvest of a pea crop which had been naturally diseased by pea blight. This field was sown with cultivar Belinda during the autumn at Le Plessis (Maine-et-Loire, France). Weed, debris and volunteer samples were randomly taken in these fields. Results were expressed as the number of bacteria per gram of fresh weight because of the heterogeneity of plant material.

Identification of natural populations of P. syringae *pv.* pisi *on pea plants, weeds, pea debris and volunteers in field conditions*

The aerial part of plants was ground in a Waring blender and the extract was diluted and plated on the semi-selective borate-levan agar medium. After 3–4 days incubation at 25 °C, white, raised and pearly colonies were counted and sub-cultured. Isolates were identified according to a scheme [Grondeau et al., 1992a] based on fluorescence test, oxidase and arginine dihydrolase activities, tobacco hypersensitivity, aesculin hydrolysis, Ouchterlony reaction and, if necessary, inoculation to Kelvedon Wonder, a susceptible pea cultivar.

Table 1. Numbers of *P. syringae* pv. *pisi* (log), strain Mt7, detected by washing and then blending 12 asymptomatic plants (Pea cultivar Belinda, sown on 22/11/89 at Le Plessis, sampled on 12/02/90)

Plant number	1	2	3	4	5	6	7	8	9	10	11	12
Washing	4.00	1.40	5.80	3.50	5.60	6.60	0.55	1.40	1.60	0.55	1.90	0.85
Blending	5.60	1.90	6.10	3.80	6.10	6.50	1.70	0.55	0.55	2.30	1.10	1.40

Detection limit: 13 bacteria/plant.
According to Wilcoxon's test, the two methods are not significantly different (p = 1%).

Results

Location of P. syringae *pv.* pisi *on/in asymptomatic plants*

For 12 plants out of 24 analysed, strain Mt7 could be detected neither by washing nor by blending method. Results for the positive plants are given in Table 1. No significant difference appeared between the two methods. This result tends to demonstrate that populations isolated from asymptomatic but contaminated pea plants were mostly external and could be detached from leaf surface by shaking in water. If a fraction of this population was already internal, the amount of internal bacteria could not be detected by the method used here (blending in washing water, dilution-plating) and therefore, must be under the detection limit.

Development of P. syringae *pv.* pisi *on asymptomatic plants grown from naturally infected seeds*

The contamination of the pea crop from naturally infected seed by natural populations of *P. syringae* pv. *pisi* at Brion is shown by Figure 1. No symptom was seen throughout the growing season. However, 16% of the plants were contaminated by the pathogen five weeks after sowing. Then, 28 and 45% of contaminated plants were found two (07/06) and three (03/07) months after sowing respectively. The last sampling date was characterized by high numbers of bacteria isolated from contaminated plants. This experiment demonstrated the ability of *P. syringae* pv. *pisi* to colonize a pea crop without inducing any symptoms.

Populations of P. syringae *pv.* pisi *race 2 in relation to host genotype (susceptible or resistant) and non-host plants*

The two cultivars tested were Belinda (susceptible) and Monitor (resistant). A suspension of Mt7 (race 2, 5×10^8 bacteria ml^{-1}) was sprayed on Belinda and

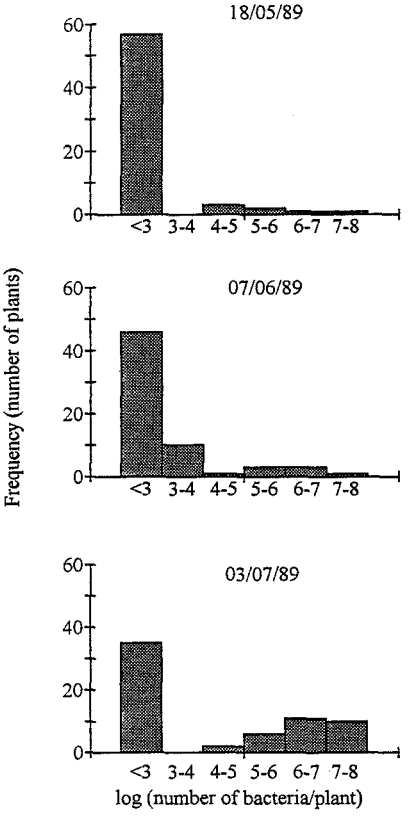

Figure 1. Distribution into 6 classes of the contamination of 64 pea plants in a field naturally contaminated (but not diseased) by *P. syringae* pv. *pisi* (Pea cultivar Belinda sown on 06/04/89 at Brion; detection limit: 10^3 bacteria/plant).

Monitor plants. A decrease in the number of bacteria was first observed for the two genotypes (Figure 2A). Then, from the third day after inoculation, strain Mt7 multiplied on Belinda to reach about 10^8 bacteria per plant and then remained constant. Growth on Monitor was slight and populations oscillated between 10^5 and 10^6 bacteria per plant. All the sampled plants looked healthy. Population levels on Belinda were significantly higher than levels on Monitor from the third to the 16th day after inoculation. Interaction between

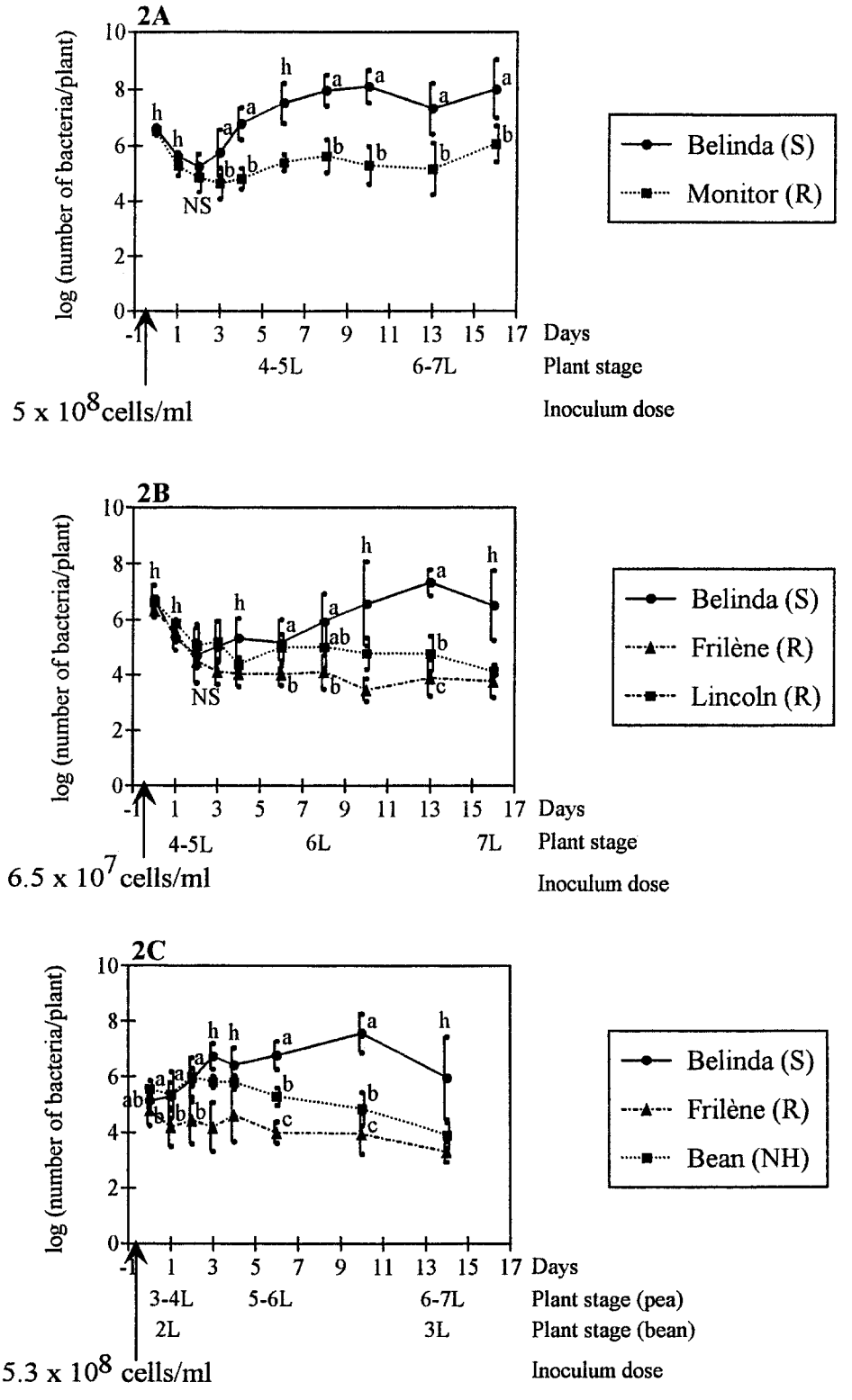

Figure 2. Development of *P. syringae* pv. *pisi* race 2 (strain Mt7) on susceptible (S) and resistant (R) pea cultivars and on non-host plants (NH) after inoculum spray on day 0 and in semi-controlled conditions (a, b, c: groups significantly different at 5% according to Newman-Keuls's test – h: heterogeneous mean squares – L: leaves).

time and cultivar was found by MANOVA. Thus, two days were necessary after inoculum spray for differences between genotypes to become significant. Water-soaked lesions appeared on day 10 on plants remaining in the glasshouse for Belinda only.

This experiment repeated with the other resistant cultivars (Frilène and Lincoln) and with a non-host plant (bean), showed a greater multiplication associated with the susceptible cultivar (Belinda), whereas populations fell progressively on Frilène, Lincoln or bean (Figures 2B and 2C). Symptoms were observed only on Belinda, about one week after the last sampling date, and for less than 10% of the remaining plants. Those plants showed one or two small blight spots.

For the field experiment at Brain, strain Mt7 was sprayed on the foci and at the concentration of 2.2 $\times$ 10^8 bacteria ml^{-1}. A few hours after the inoculation, population levels in foci were significantly higher on Belinda than on Monitor (Figure 3). The number of bacteria increased on Belinda to reach 10^7–10^8 bacteria per plant whereas populations on Monitor levelled off around 10^3–10^4 or very slightly increased. Differences between bacterial numbers on the two cultivars were always significant. This experiment took place during a period of continuous rain. Cultivar Monitor remained visually healthy, whereas 75% of the plants of cultivar Belinda were blighted on day 21 in the focus of infection.

Populations of P. syringae *pv.* pisi *race 6 in relation to host genotype (susceptible) and non-host plants in semi-controlled conditions*

Figure 4A shows bacterial numbers on Belinda, Frilène and Monitor after spraying a suspension of race 6 (3 $\times$ 10^9 bacteria ml^{-1}). A decrease of the total number of bacteria per plant was observed until day two with no significant difference between cultivars on days zero and one. From day two, numbers of bacteria increased on all cultivars. The rate of increase was more rapid on Frilène for which populations were significantly higher than those of Monitor and Belinda on day three. From day six, the numbers of cells per plant levelled off or slightly decreased. There were no significant differences between cultivars.

Race 6 colonization was compared on host and non-host plants (Figure 4B). For all sampling dates, heterogeneous mean squares were found in spite of the change of plant units (grams instead of whole plants). This may be due to wide variation in foliage of the different plant species. Moreover, because the sampling method was labour intensive, species were sampled over two days, but the two days were considered as one sampling date in the statistical analysis. Inoculum sprayed was about 6 $\times$ 10^8 bacteria ml^{-1}. One day after inoculation, about 5 $\times$ 10^5 bacteria g^{-1} of fresh weight were detected on pea and other leguminous plants whereas only about 10^4 were recovered from *Chenopodium* leaves. Subsequently, increase in race 6 counts occurred only on pea plants to level off between 10^7 and 10^8 bacteria g^{-1}. Race 6 numbers on legumes were similar and tended to slowly decrease, the populations remaining below 10^6 bacteria g^{-1}. The numbers of race 6 bacteria on *Chenopodium* leaves were the lowest since they stayed around 5 $\times$ 10^4 bacteria g^{-1}.

Whereas race 6 multiplied on its host, it could only survive on non-host plants, during the time of the experiment, with no increase of population size.

Spread of P. syringae *pv.* pisi *from a focus, on susceptible and resistant pea cultivars in field conditions*

In the field experiment conducted at Brain, no diseased plants were observed outside the foci of infection for both cultivars. Despite this, bacteria were detected on Belinda and the number of plants contaminated with the mutant strain sprayed at time 0 in the focus, increased with time (Figure 5) showing a spread of the pathogen from the inoculum source. For Belinda, bacteria were detected 2.5 m from foci only seven days after inoculation. For Monitor, they were detected at this distance 27 days after inoculation. Therefore, a resistant genotype slows the spread of the pathogen and delays it but does not entirely prevent it. Without inducing symptoms, *P. syringae* pv. *pisi* is able to spread but the speed of this spread is reduced when the cultivar is resistant.

Survival of natural populations of P. syringae *pv.* pisi *on non-host plants, pea volunteers and debris, in field conditions*

Survival on weeds in a contaminated but not diseased pea crop (Brion)

Seven species were analysed: *Polygonum persicaria, Polygonum aviculare, Solanum nigrum, Convolvulus arvensis, Senecio vulgaris, Equisetum arvense, Chenopodium album.* The detection limit was 400 bacteria g^{-1} of fresh weight. *P. syringae* pv. *pisi* populations

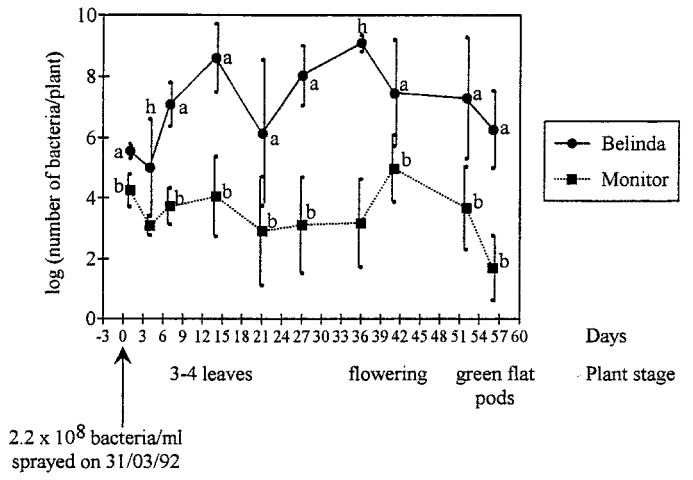

Figure 3. Development of *P. syringae* pv. *pisi* race 2 (strain Mt7) on susceptible (Belinda) and resistant (Monitor) pea cultivars, in foci, after inoculum spray on day 0, in field conditions (a, b: groups significantly different at 5% according to Newman-Keuls's test – h: heterogeneous mean squares; pea sown on 28/02/92 at Brain).

were detected on *Equisetum arvense* (2.0×10^6 bacteria g^{-1} of fresh weight), *Polygonum persicaria* (3.8×10^6 bacteria g^{-1} of fresh weight) and *Polygonum aviculare* (8.5×10^5 bacteria g^{-1} of fresh weight). On the day when samples were taken, the pathogen had not contaminated all the pea plants (only 32%, with populations up to 10^8 bacteria per plant). This crop was grown as spring pea and cultivation took place in dry conditions. However, three contaminated weed samples could be found out of 12 analysed.

Survival on weeds in a diseased pea crop (Villefranche-de-Lauragais)

Nearly all the weeds analysed were contaminated with numbers of the pathogen ranging from 10^2 to 2×10^5 bacteria g^{-1} of fresh weight (Table 2). However, these numbers were less than those detected on peas (10^6–10^7).

Survival on weeds, pea volunteers and pea debris after harvest (Le Plessis)

The pathogen was detected on half of the weed species sampled two months (end of August) after the harvest (Table 3). Five months after pea harvest (end of November), less weeds were available in the field, but half of them still carried the pathogenic organism. Finally, eight months after the harvest of the pea crop (mid-February), *P. syringae* pv. *pisi* was not detected in any of the 10 weed samples analysed.

Pea volunteers found on each sampling date at different growth stages always carried high populations of *P. syringae* pv. *pisi*. But they became rare as time passed and were all found in the same area on the last sampling date. The field was ploughed between the second and the third visit.

The number of viable bacteria g^{-1} of fresh weight slightly decreased in pea debris. In mid-February, about eight months after the harvest, debris still carried about 10^6 bacteria g^{-1} of fresh weight.

Table 2. Populations of *P. syringae* pv. *pisi* detected on the weeds present in a naturally diseased pea field (Belinda sown on 04/11/88 at Villefranche-de-Lauragais, sampled on 29/05/88)

Samples	Fresh weight (g)	Number of bacteria g^{-1} of fresh weight
Pea	30	2.9×10^7
Pea	73	1.1×10^6
Avena elatior	185.4	1.7×10^5
Reseda lutea	23	1.0×10^5
Galium aparine	49	9.5×10^4
Reseda lutea	33	5.4×10^4
Avena elatior	225	1.5×10^4
Lepidium campestre	28.5	1.3×10^4
Fumaria officinalis	159.5	9.7×10^3
Lepidium campestre	44	3.7×10^3
Fumaria officinalis	102	2.0×10^3
Polygonum convolvulus	43	1.5×10^3
Anagalys coerulea	10	4.5×10^2
Galium aparine	41	1.1×10^2
Polygonum convolvulus	4.3	<

<: less than the detection limit (23 bacteria g^{-1} of fresh weight).

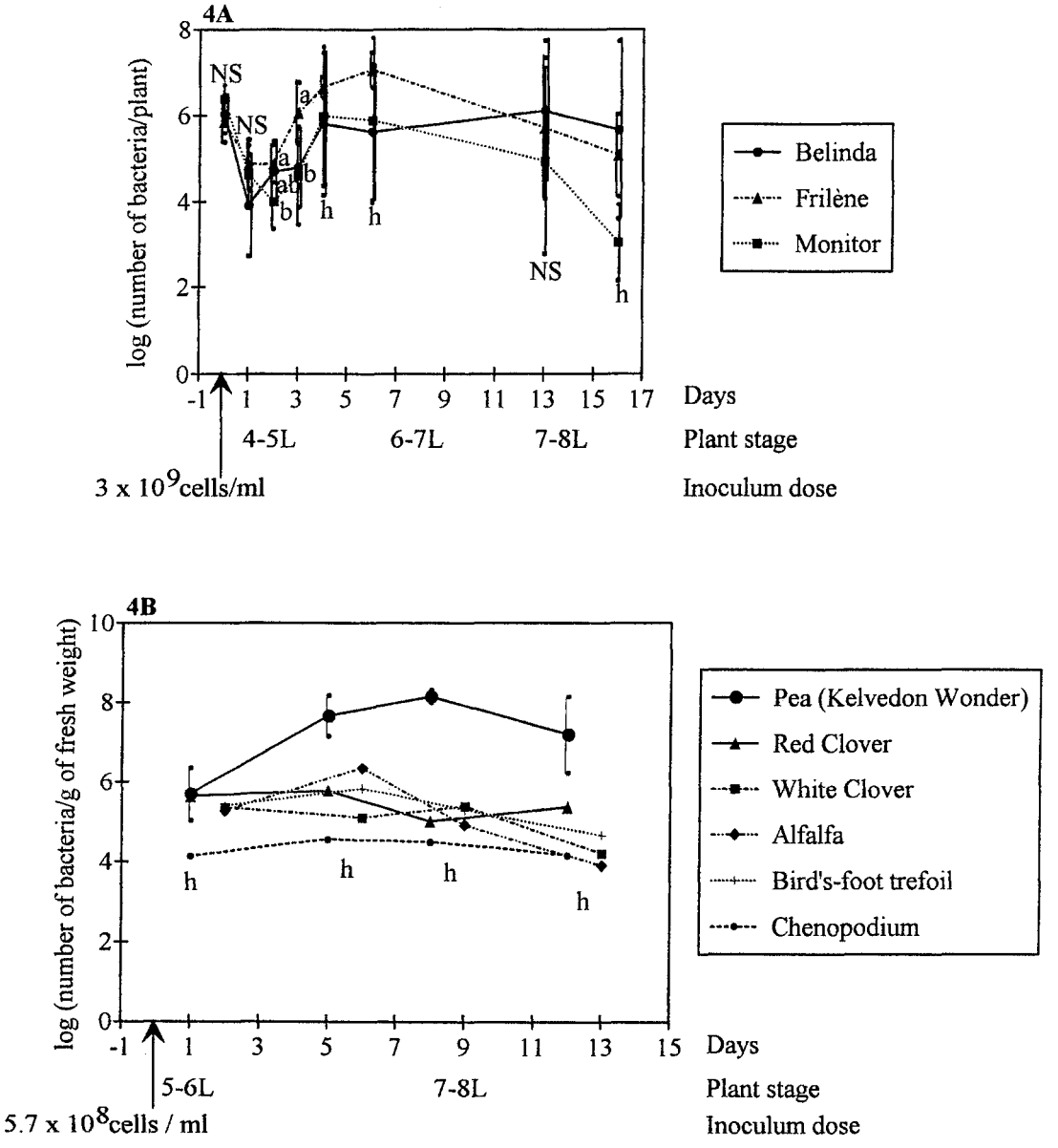

Figure 4. Development of *P. syringae* pv. *pisi* race 6 (strain VISm2) on susceptible pea cultivars (4A) and on non-host plants (4B) after inoculum spray on day 0 and in semi-controlled conditions (a, b: groups significantly different at 5% according to Newman-Keuls's test – NS: difference non significant – h: heterogeneous mean squares – L: leaves).

Discussion

The present study shows that *P. syringae* pv. *pisi* is able to multiply and to survive on pea foliage, mostly in the absence of symptoms. This ability to colonize peas and to spread to neighbouring plants changes the perception of this seed-transmitted disease. From a contaminated seed, bacteria can multiply with no (or cryptic) symptoms on the seedling, and achieves high levels of populations on all the plants in a field. Field inspection would declare a crop free from contamination, whereas the bacterium is present on nearly all plants. Furthermore, if climatic conditions necessary for bacterial penetration in plant tissues occur (most frequently frost, hail, or any kind of injury), disease can occur in the whole field within one or two weeks.

A microbial epiphyte is cultured from the plant surface, and an epiphyte resident multiplies on the

Table 3. Populations of *P. syringae* pv. *pisi* detected on pea volunteers, pea debris and weeds after the harvest (26/06/90) of a naturally diseased pea crop (Pea cultivar Belinda sown on 22/11/89 at Le Plessis)

Samples	Number of bacteria g^{-1} of fresh weight [Fresh weight in g] on:					
	31/08/90		29/11/90		18/02/91	
Pea volunteers	1.1×10^6	[33.5]	1.7×10^5	[50]	6.3×10^6	22.3
Pea debris	3.2×10^7	[21.5]	1.7×10^6	[69]	1.3×10^6	[133.7]
Digitaria sanguinalis	1.1×10^4	[35]	–	–	–	–
Setaria verticillata	3×10^3	[23]	–	–	<	[25.9]*
Convolvulus arvensis	8.4×10^2	[15.5]	–	–	–	–
Symphytum officinale	3.3×10^2	[107]	<	[80]	–	–
	2×10^2	[112]	–	–	–	–
	2×10^2	[105]	–	–	–	–
Chenopodium album	45	[20]	–	–	–	–
Symphytum officinale	<	[115]	–	–	–	–
	<	[184]	–	–	–	–
Medicago lupulina	<	[105]*	–	–	–	–
Lathyrus pratensis	<	[5.5]*	–	–	–	–
Melilotus officinalis	<	[0.3]*	–	–	–	–
Lotus corniculatus	<	[43.5]*	–	–	–	–
Carduus tenuiflorus	<	[115]	<	[51]	<	[4.7]
Cynodon dactylon	–	–	1.9×10^4	[35]	<	[26.9]*
Mercurialis annua	–	–	1.7×10^3	[61]	<	[1.2]
Sinapis arvensis	–	–	74.5	[46]	<	[12.7]*
Daucus carota	–	–	<	[80]	<	[1.1]
Zea mays	<	[44]	<	[55]	<	[117.5]
Solanum tuberosum	<	–	<	[120]	–	–
Helianthus annuus	<	[81]	–	–	–	–
Artemisia vulgaris	–	–	–	–	<	[41]
Primula officinalis	–	–	–	–	<	[45.1]*
Senecio vulgaris	–	–	–	–	<	[10.9]
Veronica hederaefolia	–	–	–	–	<	[1.2]

–: no plant to be sampled; <: less than the detection limit (200 bacteria g^{-1} of fresh weight).
*: plants sampled in the field border.

surface of healthy shoots [Leben, 1965]. Pathovars of *P. syringae* have already been reported to have a resident phase on their hosts: pv. *morsprunorum* [Crosse, 1963] and *phaseolicola* [Leben et al., 1970]. When an inoculum was sprayed on pea leaves in glasshouse, a multiplication period of three to six days was observed only on susceptible genotypes. This period was followed by a plateau around 10^7–10^8 bacteria per plant. After the decrease period, bacteria on resistant cultivars did not seem to multiply, and population levels remained below 10^6 bacteria per plant. However, even if these population levels were weaker than those detected on susceptible genotypes, the bacterium could persist on resistant plants and act as an inoculum in the field. Observations that population dynamics of bacteria were constrained by host genotype were reported by Crosse [1963] who found higher epiphytic populations of *Pseudomonas syringae* pv. *morsprunorum* on a susceptible cherry cultivar than on a resistant one. Mew and Kennedy [1971] showed that *Pseudomonas syringae* pv. *glycinea* multiplied on susceptible hosts but not on resistant ones. Stadt and Saettler [1981] noted that the multiplication of *Pseudomonas syringae* pv. *phaseolicola* was more rapid on susceptible genotypes than on resistant ones. Our results from glasshouse or growth chamber are in accord with these findings with the additional observation that resistant hosts were similar to the non-host species tested in that they harboured the pathogen on their foliage surface without allowing it to multiply. Resistant genotypes seemed to tolerate contamination with the pathogen since population decreases were

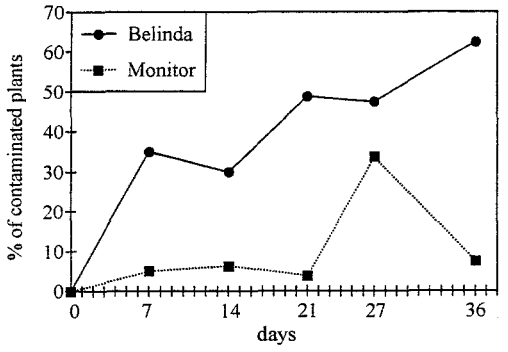

Figure 5. Spread of *P. syringae* pv. *pisi* race 2 (strain Mt7) on susceptible (Belinda) and resistant (Monitor) pea cultivars from foci sprayed on day 0, and in field conditions (Pea sown on 28/02/92 at Brain; detection limit: 35 bacteria/plant).

slow after inoculation. Bacteria could maintain themselves to levels greater than or equal to 10^4 cells g^{-1} of fresh weight when an inoculum of 6×10^8 bacteria ml^{-1} was sprayed on leaves. That survival of incompatible bacteria was illustrated in field experiments by recovery of the bacterium on Monitor outside the focus of infection.

Pseudomonas syringae has been reported on non-host plants for pv. *syringae* [Ercolani et al., 1974; Lindemann et al., 1984] and pv. *tomato* [Schneider and Grogan, 1977] for example. Our results with *P. syringae* pv. *pisi* race 6 (chosen because it is pathogenic for all present commercial pea cultivars) sprayed on non-host plants, both leguminous and non-leguminous, tend to indicate that race 6 was unable to multiply on the non-host plants tested. However the studies of natural populations of *P. syringae* pv. *pisi* on weeds that grew in a pea crop showed that non-host plants can be contaminated by the pathogen. The role of contaminated weeds acting as a source of inoculum for the next pea crop was investigated. Results demonstrated that bacteria could survive on weeds at least five months after the harvest, in the climatic conditions of the South-West of France. But weeds tend to be destroyed by winter conditions (frost) or by cropping patterns (ploughing). Since pea crop is never sown in two successive years in the same land, it is unlikely that weeds represent a major source of primary inoculum. In our experiment, *P. syringae* pv. *pisi* was able to survive at least seven months on pea debris and volunteers. But this survival on diseased plant debris is likely to stop with their decomposition [Young et al., 1969]. This is the reason why a two year rotation of pea crops should be retained. Additionally, it was recently demonstrated that a strain of *Pseudomonas syringae* introduced on a bean crop, was not necessarily detected on the bean crop grown the next season [Hirano and Upper, 1993].

Bacterial diseases have been usually compared to fungal diseases, in which inoculum was produced entirely in lesions, whereas epiphytic populations were unknown or unconsidered [Hirano and Upper, 1990]. *P. syringae* pv. *pisi* is able to undertake an active multiplication phase on apparently healthy plants and to spread over neighbouring plants without lesion formation. If we are allowed to use the words used to describe fungal epidemics, we should conclude that the latent period of pea bacterial blight is shorter than the incubation period, since no diseased tissues are required for the pea plant to be contagious. The approach of the epidemiology of bacterial diseases having an epiphytic phase has to be rethought taking this fact into account, as it was already pointed out for *P. syringae* pv. *morsprunorum* and sour cherry trees [Latorre and Jones, 1979].

The multiplication of *P. syringae* pv. *pisi* is restricted to compatible hosts. Do bacteria develop only on external sites of the leaf surface or do they multiply in sub-stomatal cavities? When asymptomatic plants are considered, bacteria can easily be recovered by washing leaf surfaces [Crosse, 1959]. In our study, no difference was seen between washing and blending methods. A greater significant number of bacteria per asymptomatic plant could not be detected by blending the plants after their washing. Observed by scanning electron microscopy, trichomes and microscopic fissures of the cuticular layer of pear leaves appeared to be important niches for *P. syringae* pv. *syringae* allowing entry into host tissue [Mansvelt and Hattingh, 1987]. *P. syringae* pv. *syringae* enters apple leaves through stomata where it can multiply profusely. Masses of these cells would then be extruded through stomata and epiphytic populations could be replenished by this way [Hattingh et al., 1989]. The real problem is not to know where bacteria are, but that they have already invaded the host, strictly on plant surfaces or deeper in sub-stomatal cavities, and that their presence is invisible. Therefore, any woundings or climatic events may trigger a general disease outbreak.

Survival can occur on resistant peas or non-host plants. Despite the observation that populations on resistant peas are far less than those observed on susceptible peas, a spread can occur but is reduced compared to a susceptible genotype. If some studies have been conducted by scanning electron microscopy to determine where, what are called 'epiphytic

bacteria' are truly located on their compatible host, none is known about the behaviour of natural populations detected on a resistant host or on non-host plants. Do bacteria invade the same niches as on the susceptible host?

Such a significant development of epiphytic pathogenic bacteria on compatible pea cultivars raises the question of seed health testing efficiency to control the disease. What should be the detection threshold of bacteria in seed lots low enough to prevent the epiphytic spread of the pathogen to the whole crop? Are the same genes as pathogenicity involved in the race specificity of the epiphytic development? Investigations are in progress to try to answer some of these questions.

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