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 1000 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 10⁹ 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 × 10⁸ 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 10⁹ bacteria ml⁻¹, was sprayed on the foliage of the plants (stage: 3–4 leaves) located in a square (1 m²) 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, cephalaxin 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), Friilène, 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 m² was sprayed with Mt7 at a concentration of 10⁶ 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