

BIOLOGICAL AND EPIDEMIOLOGICAL ASPECTS OF *XANTHOMONAS ARBORICOLA* PV. *PRUNION* PEACH IN ITALY

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SUMMARY

Survival in the field of *Xanthomonas arboricola* pv. *pruni* was studied using two mutants marked by resistance to 200 µg ml⁻¹ of rifampicin (Rif^r) and rifampicin-chloramphenicol (Rif^r-Chlor^r). The mutants were used to contaminate branches (July 1995) and buds/leaf scars (October 1995) of peach trees cv. 'Elegant Lady' in two peach orchards in the district of Verona. A suspension of the Rif^r-Chlor^r mutant was also used for vacuum infiltration (September 1995) of leaves on detached shoots of the same cultivar. Survival was assessed on the basis of periodic reisolation on appropriate recovery media from bud/leaf scar samples (October 1995-March 1996) and debris of infected leaves kept on the soil (January-April 1996). The pathogen was found in bud/leaf scar samples and in infected leaf debris up to 6-7 months after contamination. The Rif^r mutant was only reisolated (frequency 13%) from leaf tissue samples with typical spots collected in one of the two experimental peach orchards in the summer of 1996. The mutants were never found associated with asymptomatic leaf or flower samples (summer-autumn 1995/spring-autumn 1996). *X. arboricola* pv. *pruni* apparently survives in buds/leaf scars and in infected leaf debris on the soil, providing potential sources of inoculum for primary infections even in the absence of spring ('black tips') and summer cankers, never observed during the experiments.

RIASSUNTO

ASPETTI BIOLOGICI ED EPIDEMIOLOGICI DI *XANTHOMONAS ARBORICOLA* PV. *PRUNION* SU PESCO IN ITALIA. È stata studiata la sopravvivenza in campo di *Xanthomonas arboricola* pv. *pruni* mediante l'uso di due mutanti marcati per resistenza a rifampicina (Rif^r) ed a rifampicina-cloramfenicolo (Rif^r-Chlor^r) alle dosi di 200

µg ml⁻¹ per entrambi. I due mutanti sono stati rispettivamente usati per contaminare branche (luglio 1995) e gemme/cicatrici fogliari (ottobre 1995) di alberi di pesco cv. "Elegant Lady" in due pescheti del veronese. Una sospensione del mutante Rif^r-Chlor^r è stata altresì usata per infiltrare sotto vuoto (settembre 1995) foglie su germogli distaccati della medesima cultivar. La sopravvivenza di *X. arboricola* pv. *pruni* è stata valutata in base ai risultati di reisolamenti periodici su appropriati substrati di crescita da campioni di gemme/cicatrici fogliari (ottobre 1995-marzo 1996) e di residui di foglie infette mantenute sul terreno (gennaio-aprile 1996). Nelle gemme/cicatrici fogliari e nei residui di foglie infette il patogeno è stato trovato fino a 6-7 mesi dopo la contaminazione. Solo il reisolamento del mutante Rif^r (frequenza 13%) ha avuto successo da campioni di tessuto fogliare con tipiche maculature prelevati da uno dei due pescheti sperimentali durante l'estate 1996. In nessun caso, i mutanti sono stati trovati associati a campioni di foglie e fiori asintomatici (estate-autunno 1995/primavera-autunno 1996). Si discutono le modalità di sopravvivenza di *X. arboricola* pv. *pruni*. L'evidenza sperimentale indica che sia sopravvissuto nelle gemme/cicatrici fogliari e nei residui di foglie infette sul terreno, potenziali sorgenti di inoculo per infezioni primarie in assenza di cancri primaverili ("cime annerite") ed estivi, mai osservati durante il periodo della sperimentazione.

Key words: *Xanthomonas arboricola* pv. *pruni*, antibiotic resistant mutants, epidemiology, *Prunus persica*.

INTRODUCTION

Xanthomonas arboricola pv. *pruni* (Vauterin et al., 1995; Young et al., 1996), causal agent of bacterial spot of peaches [*Prunus persica* (L.) Batsch] and other stone fruits, is a harmful organism subject to phytosanitary legislation in the EU (EEC Directive no. 92/103) and in Italy (M.D., 31/01/1996, Annex II, Part A, Section II, point 8). In Europe and in the countries of the Mediterranean basin the pathogen is locally established in Austria, Bulgaria, Italy, Lebanon, Netherlands, Romania

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and the ex-USSR. It is found but not established in Cyprus and Switzerland (EPPO/CABI, 1992). In France, it has recently been reported (Anonymous, 1997). Severe epidemics of bacterial spot associated with heavy losses have occurred in Italy (Po Valley, north-eastern areas) on plum (*P. salicina*) and peach (Bazzi and Mazzucchi, 1980; Stefani et al., 1989; Saccardi and Goio, 1990). The disease is a serious threat for the most susceptible cultivars.

Epidemiology is linked to host susceptibility (Bazzi et al., 1990; Simeone, 1991) and to several environmental factors modulating epiphytic survival, penetration and spread of *X. arboricola* pv. *pruni* (Anderson, 1953; Foster and Petersen, 1954; Feliciano and Daines, 1970; Gasperini et al., 1984; Du Plessis, 1986, 1987, 1990; Shepard and Zher, 1994). In the fruit-growing areas of the Po Valley, summer cankers on plum trees provide important overwintering sites and sources of inoculum for two to three years on peaches. Severe outbreaks of the disease commonly occur even in the absence of the canker phase. In France, the appearance of cankers on the branches is quite rare (Bernadette et al., 1997). We still know little about several aspects of the disease cycle. The purpose of this study was to document the overwintering of *X. arboricola* pv. *pruni* in infected fallen leaves, terminal/axillary buds and leaf scars of peach trees.

MATERIALS AND METHODS

Bacterial concentration. Aqueous bacterial suspensions were calibrated at $A_{660} = 0.1$ and $A_{660} = 0.3$ and colony forming units (cfu) were determined from plate counts.

Antibiotics. Rifampicin (Sigma, R-3501) and chloramphenicol (Serva, 16785) were used.

Parental wild-type. *X. arboricola* pv. *pruni* VR 69, originally isolated from infected peach leaves (Regional Phytosanitary Service, Verona, Italy) was used. It was routinely grown on plates of SP-agar (Hayward, 1960) and YDC-agar (Stolp and Starr, 1964) plates for 48 h at 27°C. The strain was maintained freeze-dried and/or at -80°C in 15% glycerol-SP broth.

Resistant mutants. The parental wild-type was marked for rifampicin and for rifampicin/chloramphenicol resistance. Resistant colonies were first plated on SP-agar plus 200 µg ml⁻¹ of rifampicin. The Rif^r mutants were then selected for chloramphenicol resistance (Chlor^r) with increasing doses up to 200 µg ml⁻¹

(Miller, 1972; Bazzi et al., 1984, 1989). In vitro stability of Rif^r and Rif^r-Chlor^r mutants was tested following three transfers in SP-broth for 48 h at 27°C on a rotary shaker (Bazzi et al., 1984). The two mutants were routinely grown on SP-agar containing 200 µg ml⁻¹ each of both antibiotics.

Recovery media. SP-agar plus 200 µg ml⁻¹ of rifampicin and actidione (Fluka, 10700) and SP-agar plus 200 µg ml⁻¹ of rifampicin, chloramphenicol and actidione were used.

Pathogenicity tests. Bacteria were grown on SP-agar for 48 h at 27°C. Drops (20 µl) of aqueous bacterial suspensions (1.3×10^8 cells ml⁻¹), were used to inoculate 5 holes per peach fruitlet cv. 'Elegant Lady', using a sterile needle and a cross pattern (Zaccardelli et al., 1992). Two fruitlets were used for each strain. After a week in a moist chamber at 27°C with a 14 h photoperiod, the fruitlets were examined for the presence of water-soaked spots around the holes in the pulp, and reisolation was attempted.

In greenhouse inoculations, bacterial suspensions at a similar concentration were thoroughly sprayed on 2 year-old peach trees cv. 'Elegant Lady', using an electric hand sprayer. Before and after inoculation, each tree was kept covered with a large polyethylene bag (moist chamber) for 24 h, to induce water congestion. When the first angular water-soaked areas appeared on the lower surface of the leaves, the trees were transferred to the open air.

In separate tests, bacterial suspensions (1.3×10^7 cells ml⁻¹), were locally infiltrated in leaves on detached peach shoots using a disposable syringe without a needle (Randawa and Civerolo, 1985). The shoots were kept with their basal ends in beakers containing tap water for a week at 27°C, and subsequently examined for symptom expression.

Sterile distilled water and the parental wild-type strain VR 69 were used as controls in all the pathogenicity tests.

Hypersensitive reaction. Small volumes of bacterial suspensions (1.3×10^8 cells ml⁻¹) were locally infiltrated in parenchymal tissue of green bean pods (4-6 infiltrations per pod) and kept in a moist chamber for 72 h at 27°C. Sterile distilled water and the parental wild-type strain VR 69 of *X. arboricola* pv. *pruni* were used as controls.

Contamination of peach branches in the field. In two peach orchards (cv. 'Elegant Lady') located in the district of Verona (experimental orchards), two branch-

es of the central peach tree out of five arranged in a cross pattern, were contaminated (July 31st, 1995) with the Rif^r mutant. The mutant was grown in nutrient broth A without salts (nutrient broth 8.0 g, casamino acids 5.0 g, yeast extract 1.0 g, per litre; pH 7.0) (Stolp and Starr, 1964) for 48 h at 27°C on a rotary shaker and then centrifuged. A dense suspension of the final pellet in PBS was kept in a portable refrigerator for 2 h, and in the field resuspended in demineralized water to a concentration of about 10⁸ cells ml⁻¹. For inoculation, each branch was covered with a large polyethylene bag in the late afternoon and then thoroughly sprayed with the suspension. The bag was removed the next morning (Bazzi et al., 1990). A preliminary experiment was done in the laboratory to check the viability of a similar dense suspension (maintained as described above) and to determine the volume of water required to obtain a suitable inoculum concentration.

Contamination of peach buds/leaf scars in the field.

Two series of contaminations were made in each peach orchard, on 4th and 24th October 1995 respectively. In the first series, the buds and leaf scars, on the basal and medial halves of 50 shoots randomly chosen on 4-5 peach trees, were contaminated with the Rif^r-Chlor^r mutant. The inoculum contained approximately 9.0 x 10⁸ cells ml⁻¹. The leaf petioles were broken off at the base just before contamination; a small soft paint brush was dipped in the bacterial suspension and the buds and leaf scars were touched with its tip (Gasparini et al., 1984).

In the second series of contaminations, the buds and leaf scars in the apical halves of 50 different shoots were contaminated with the mutant using the same technique.

Peach leaf infiltration. A suspension of the Rif^r-Chlor^r mutant (8.6 x 10⁸ cells ml⁻¹) was infiltrated under vacuum into the intercellular spaces of leaves. Twenty bunches of 5 healthy peach shoots cv. 'Elegant Lady' collected in September 1995, were separately placed upside-down in a vacuum cylinder containing the bacterial suspension for 2 min at 100 kPa negative pressure. After returning to atmospheric pressure, the leaves were detached and placed on plastic grids in trays that were closed in polyethylene bags (humid chamber) for 8 days at 27°C. When the first symptoms appeared, the leaves were distributed in several containers of 1 cm mesh plastic netting and subsequently placed on the soil in a peach orchard (district of Ozzano Emilia). Some containers with naturally infected leaves, collected from the Verona area, were placed under other trees in the same orchard.

Reisolation from asymptomatic leaves and flowers.

At 7-10 day intervals, asymptomatic leaves were collected from contaminated branches and twigs in the two experimental peach orchards and from surrounding branches on the five trees arranged in a cross pattern. Attempts were made to reisolate the mutant Rif^r, in the summers of 1995-96 and Rif^r-Chlor^r in 1996. Four samples, each consisting of 12-15 leaves (5 g) were collected at each interval. Each leaf was cut into 3-5 parts and washed in 100 ml sterile PBS-Tween 20 (0.2%) for 1 h at 27°C on rotary shaker. The washing liquid was filtered through sterile gauze, centrifuged for 15 min at 10,000 *g* and the final pellet resuspended in 1 ml of PBS. One aliquot of 0.5 ml was plated on the recovery media and one transferred to 200 ml of recovery SP-broth. After incubation in a rotary shaker for 4-6 days at 27°C, the suspensions were centrifuged and the pellets were resuspended in PBS and used for plate counting on recovery media. In March 1996, four samples, consisting each of 30 open asymptomatic flowers, were collected from each of the two experimental orchards. They were collected from branches contaminated with the Rif^r-Chlor^r mutant and surrounding branches. The mutant was reisolated following the same method used for reisolation from asymptomatic leaves. Attempts were also made, using the same procedure, to isolate on SP-agar the wild-type *X. arboricola* pv. *pruni* strains from asymptomatic leaf and flower samples collected from naturally infected trees.

Reisolation from buds and leaf scars. Three samples were collected: the first (T¹), in the last 10 days of October 1995, twenty days after experimental contamination with Rif^r-Chlor^r mutant; the other two (T² and T³) in mid January and mid March 1996 to assess winter survival. At each interval, four samples each consisting of 30 buds and leaf scars were excised with a sterile scalpel from the basal and medial halves of about ten twigs previously contaminated with the mutant. Each sample was placed in double bag containing 10 ml of ice-cold sterile PBS and blended in a Stomacher (Lab-Blender-80) for 3 min. The resulting suspensions were filtered through gauze and centrifuged at 480 *g* for 5 min. The supernatant was refiltered and centrifuged at 10,000 *g* for 10 min. The final pellets were resuspended in 1.0 ml of PBS and used for plate counting after 5-6 days incubation at 27°C on recovery media. The same technique was used to reisolate and count the colonies of the mutant from 8 replications, each consisting of 10 leaf scars, sampled in the two peach orchards, taken from the apical halves of twigs 20 days after the experimental contamination.

Reisolation from leaves on the soil. In the period January-April 1996, leaf samples were taken at monthly intervals from plastic netting containers kept on the soil at the base of the trees during the winter. The 1 cm pores of the containers ensured direct contact between the leaves and the soil. Samples of leaf tissue (3-4 fragments generally showing bacterial spots) were ground in a mortar and the suspensions streaked on plates of recovery medium for the Rif^r-Chlor^r mutant and incubated at 27°C. At the same time attempts were made to isolate wild-type *X. arboricola* pv. *pruni* strains on SP-agar from naturally infected leaf samples kept on the soil during the winter.

Reisolation from leaf spots. Leaf samples with typical bacterial spot symptoms were gathered at random in the two experimental peach orchards during the summer 1996. Tissue fragments from 3-4 lesions were ground in a mortar and the suspensions streaked on SP-agar and the mutant recovery media. The plates were incubated at 27°C. The colonies (280) isolated on SP-agar from symptomatic leaves, collected from branches contaminated with the Rif^r mutant, were streaked as small spots (about 8 mm in diameter) on recovery medium to assess the growth of antibiotic resistant cells (reisolation frequency). The isolation frequency for the wild strains resistant to rifampicin was assessed on single colonies (518) isolated in the summer 1996 from naturally infected leaves of cv. 'Elegant Lady' collected in three different peach orchards in the district of Verona, using the procedure as described for mutant reisolation.

The cultural conditions of the trees and the infected materials used for isolations were similar to those of the experimental orchards.

Identification of mutants and their reisolates. This was based on antibiotic resistance, on the morphology on recovery media and on YDC-agar, pathogenicity tests, hypersensitive reaction on green bean pods and the gel electrophoretic pattern obtained with one-dimensional SDS-PAGE of the total soluble proteins of the bacterial cell envelope (Stefani et al., 1994). The parental wild-type was used as a control.

RESULTS

Antibiotic resistant mutants and recovery media. The parental wild-type was sensitive to rifampicin and chloramphenicol but spontaneous resistant mutants appeared on the plates with concentration gradients of the

antibiotics. In stability tests on 15 mutants no reversion to rifampicin sensitivity was observed. All mutants were similar morphologically to the wild strain VR 69 on SP-agar and YDC-agar; they induced HR on green bean pods within 48 h but showed slightly less virulent than the control strains on peach fruitlets. They were successfully reisolated on recovery media. One of these stable mutants (Rif^r) was chosen, that caused lesions on peach fruitlets with an average diameter of 3.50 mm as compared with those of 4.85 mm caused by the wild-type strain. This Rif^r mutant was then selected for chloramphenicol resistance following the same procedure. Following the in vitro test, six colonies out of fifteen showed stable resistance to both antibiotics (200 µg ml⁻¹); only four, morphologically indistinguishable from the parental wild-type, induced a similar HR, although in the pathogenicity test they appeared less virulent. One of these isolates, Rif^r-Chlor^r, was chosen; it caused lesions with an average diameter of 3 mm on peach fruitlets. The SP-medium containing rifampicin-chloramphenicol eliminated wild *X. arboricola* pv. *pruni* strains; however, in some cases, the growth of unidentified bacteria was observed and, despite the addition of actidione (200 µg ml⁻¹), growth of *Acremonium*, *Penicillium* and *Rhizopus* spp. occurred especially during summer.

Detection of epiphytic mutants on leaves and flowers. Reisolation attempts in summer-autumn 1995 and spring-autumn 1996, to detect epiphytic mutant populations on asymptomatic leaf and flower samples were not successful. Moreover, the isolations on SP-agar from asymptomatic leaf and flower samples collected from other peach trees not contaminated with the mutants did not reveal any wild-type *X. arboricola* pv. *pruni* strains. There was abundant growth of unidentified bacterial flora on non-recovery media.

Reisolation from buds and leaf scars. Twenty days after contamination of wounds, following the detachment of leaves in the apical third of the twigs, the mutant was found in the apical leaf scars and the numbers of bacteria reisolated per sample varied from 1.0×10 to 1.0×10^2 (Table 1). In the period October 1995-March 1996, the Rif^r-Chlor^r mutant was successfully reisolated from bud and leaf scar samples. The quantitative re-isolation technique on recovery medium revealed progressively decreasing mutant populations from the last 10 days in October ($4.5-7.5 \times 10^2$ bacteria per sample) to mid March ($1.02-3.12 \times 10^2$ bacteria per sample) (Table 2). In spring 1996, no spring cankers (black tips) were observed.

Table 1. Rif^r-Chlor^r populations of *X. arboricola* pv. *pruni* detected in samples each consisting of 10 apical leaf scars collected in the last 10 days of October 1995 from peach trees cv. 'Elegant Lady' in two experimental orchards.

	Samples	No. bacteria/sample
Peach orchard 1	1	8.3 x 10
	2	8.1 x 10
	3	1.1 x 10
	4	1.4 x 10
Peach orchard 2	1	2.5 x 10
	2	6.1 x 10
	3	1.5 x 10
	4	1.0 x 10 ²

Table 2. Rif^r-Chlor^r populations of *X. arboricola* pv. *pruni* detected in buds and leaf scar samples of peach cv. 'Elegant Lady' collected in the Verona district. Numbers of bacteria per sample are expressed as the mean of four replicates each consisting of 30 buds and leaf scars.

Sampling Dates	Peach orchard 1			Peach orchard 2		
	Max	Min	Average	Max	Min	Average
T ₁ 10/24/1995	12 x 10 ³	2 x 10 ³	7.5 x 10 ³	8 x 10 ⁵	1.5 x 10 ⁵	4.5 x 10 ⁵
T ₂ 1/15/1996	3 x 10 ³	5 x 10 ²	1.57 x 10 ³	1.7 x 10 ³	4 x 10 ²	8.93 x 10 ²
T ₃ 3/15/1996	6.5 x 10 ²	1.2 x 10 ²	3.12 x 10 ²	2.6 x 10 ²	3.4 x 10	1.02 x 10 ²

Survival in leaves on the soil. Almost completely disintegrated debris were all that remained in the plastic netting containers after the first three months; however, there were still leaf parts with spots suitable for mutant reisolations. The mutant was found in leaf samples up to 7 months after contamination. Attempts to isolate, on SP-agar, wild-type strains of the pathogen from naturally infected leaves, detached and kept on the soil during the same period, were unsuccessful. On the other hand, growth of a large number of unidentified bacteria was observed on SP-agar.

Reisolation from leaf spots. Attempts to reisolate the Rif^r-Chlor^r mutant used to contaminate buds and leaf scars (October 1995), from symptomatic leaves (July-September 1996), were unsuccessful. In the same period, the Rif^r mutant, sprayed on peach tree branches in the experimental fields (end of July 1995), was successfully reisolated on SP-agar from leaf spot samples from one of the two experimental orchards. After transfer to recovery medium, the reisolation frequency of colonies Rif^r was 13%. The isolation frequency of wild-type rifampicin resistant strains from naturally infected orchards was only 3%.

Mutant phenotypes. The phenotypic characteristics of mutants were compared with those of the parental wild-type. After 72 h at 27°C on SP-agar and YDC-agar, mutants colonies (1-1.5 mm in diameter) were typically pale yellow to yellow, round, mucoid, domed and lucent, and induced HR on green bean pods within 48 h. Protein profiles from the mutants, their reisolates

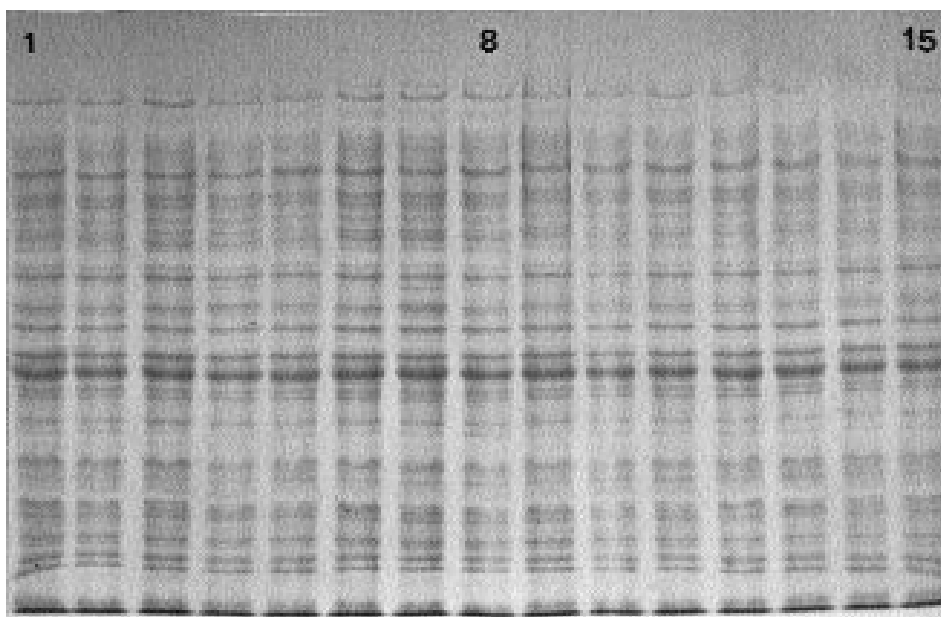


Fig.1. Electrophoretic total protein profiles of Rif^r (lane 10) and Rif^r-Chlor^r (lane 7) mutants and their reisolates from symptomatic plant material (lanes 1-6 and 11-15) compared with the wild-type strain VR 69 of *X. arboricola* pv. *pruni* (lane 9).

and the wild-type strain VR 69 revealed a very similar general pattern (Fig.1). All mutant characteristics were indistinguishable from those of the parental wild-type with the exception of the pathogenicity test on peach fruitlets, where they were less virulent, on the basis of the mean diameter of the lesions.

DISCUSSION

The wild-type strain VR 69 of *X. arboricola* pv. *pruni*, although sensitive to the action of rifampicin and chloramphenicol (Lech and Brent, 1987), produced mutant colonies resistant to both antibiotics with concentrations of up to 200 µg ml⁻¹. These two antibiotics were chosen because in previous experiments no mutant colonies resistant to other antibiotics (kanamycin, ampicillin and tetracycline) were obtained. No reversion to rifampicin and chloramphenicol sensitivity was observed. The mutants were phenotypically indistinguishable from the parental wild-type, they induced HR on green bean pods, but their virulence on peach fruitlets was lower. A decrease in virulence can be induced by experimental mutations (Kearney and Staskawicz, 1990; Swarup et al., 1992) and reduced growth in planta may be a consequence.

Attempts to reisolate the Rif^r-Chlor^r mutant and wild-type *X. arboricola* pv. *pruni* strains from asymptomatic flowers and leaves were unsuccessful. The abundant growth of microbial flora on the SP-agar plates might have inhibited the growth of the pathogen on this medium.

The epiphytic persistence of *X. arboricola* pv. *pruni* on leaves (especially in the summer after rainy periods) and asymptomatic peach and plum flowers, described by Shepard and Zehr (1994) in the eastern United States, were not confirmed here.

The unsuccessful attempts to reisolate the mutants Rif^r and Rif^r-Chlor^r on recovery media from asymptomatic flowers and leaves during the summer-autumn 1995 and spring-autumn 1996 might be due to various factors: unfavourable environmental conditions for multiplication and onset of infection cycles, presence of pesticide residues (for example some dithiocarbamates), poor competitiveness towards other micro-organisms and reduced adaptation to the epiphytic habitat.

In the experimental orchards, the bacterium was only able to persist in the buds and leaf scars. On plum, *X. arboricola* pv. *pruni* overwinters in leaf scars, which act as efficient sources of primary inoculum with early contamination of the wounds (Gasperini et al., 1984). The Rif^r-Chlor^r mutant was successfully reisolated from buds and leaf scars, between October and March in de-

creasing amounts. Therefore, in the absence of a true resident epiphytic phase (Leben, 1965, 1971; Hirano and Hupper, 1983), buds and leaf scars can represent important sites for the survival and spread of *X. arboricola* pv. *pruni*.

The successful reisolation of the Rif^r-Chlor mutant from debris of inoculated peach leaves, kept on the soil at the base of trees from October 1995 to April 1996, shows that *X. arboricola* pv. *pruni* was able to overwinter and survive in infected leaves on the ground for at least 7 months.

Attempts to reisolate the Rif^r mutant were only successful (13%) from symptomatic leaf samples collected from one of the two experimental orchards, while only 3% of the wild-type rifampicin resistant strains of *X. arboricola* pv. *pruni* were reisolated from natural spots on samples of different origins. The use of recovery media was quite effective for reisolations from buds, leaf scars and leaf debris during winter. Conversely, SP-agar was found to be more appropriate for reisolation from leaf spots, since the recovery media slowed down the growth of the mutants, favouring competition by other micro-organisms, in particular fungi, very abundantly present on leaves during summer. The recovery media were however essential to assess the reisolation frequency of the Rif^r mutant grown on SP-agar.

The results obtained in this study establish that *X. arboricola* pv. *pruni* survives during winter in infected fallen leaves on the ground and in buds and leaf scars without causing spring cankers (black tips). The latter are described in the disease cycle on peach from North America, South Africa and New Zealand, as the primary sources of inoculum from which bacteria escape in the form of cryptoexudate, causing the first leaf infections. However, severe attacks of bacterial spot may occur in peach orchards where cankers are rarely observed (Shepard and Zehr, 1994). During the two years of the experiments, no spring and summer cankers were observed. The only symptoms resembling spring cankers were a very few necrotic twig tips, but attempts to isolate the pathogen from these were unsuccessful. Despite the absence of cankers, quite severe outbreaks of bacterial spot were recorded especially in the summer of 1995. Our data confirm previous observations in the Veneto (Saccardi and Goio, 1990) and Friuli (Stefani et al., 1989) regions: that buds, leaf scars and fallen leaves may be important sources of inoculum for spring infections even in the absence of black tips.

To conclude, in the light of these results, the biological cycle of *X. arboricola* pv. *pruni* on peach in the Po Valley appears to differ from that on plum, where cankers act as important sources of inoculum for early infections (Bazzi and Mazzucchi, 1980).

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