Recherche Agronomique (1998), 3, 1-8
INRAA

# The ecological significance of toxin production by *Pseudomonas syringae pv. tabaci* in soil and rhizosphere.

[1]HARZALLAH, D, and [1]LAROUS, L.

[1]Department of Microbiology, Institute of Biology
UFA, Setif 19000 Algeria.

***Abstract*** *: Pseudomonas Syringae pv. tabaci is the causal agent of the wildfire disease of tobacco (Nicotiana spp.). It produces the clhorosis-inducing tabtoxin. The main aim of this research was to identify the role of tabtoxin production in the ecology and survival of Pseudomonas Syringae pv. tabaci, in natural environments. Experimental approach of this problem involved a comparison of the growth in soil and in the rhizosphere of wild-type strain of pv. tabaci [Tox$^+$] with mutant that did not produce tabtoxin [Tox$^-$]. These strains are suitably marked with antibiotic resistance. The Tox$^+$ strain had no significant advantage over the Tox$^-$ strain in sterile soil, unsterile soil and in the rhizosphere.*

***Key Words*** *: Pseudomonas syringae pv. tabaci, Soil, Rhizosphere, Tabtoxin.*

**الملخص** : تعتبر البكتيريا *Pseudomanas syringae pv. tabaci* لعامل المسبب لمرض اللفحة البرية الذي يصاب به نبات التبغ *(Nicotinia spp.)* تفرز هذه البكتيريا سم التابتوكسين المحدث للشحوب اليخضوري. تضمنت هذه الدراسة مقارنة نمو عزلات من هذه البكتيريا مفرزة و غير مفرزة للسم في التربة المعقمة و غير المعقمة و في المنطقة الجدرية، حيث استعملت عزلات بكتيرية مكتسبة لصفة المقاومة للمضادات الحيوية. سمحت النتائج بإيضاح أن العزلة المفرزة للسم ليس لها مزايا معنوية في المعيشة عن العزلة غير المفرزة للسم سواء في التربة المعقمة أو التربة غير المعقمة أو المنطقة الجدرية.

**الكلمات الدالة** : *Pseudomanas syringae pv. tabaci*، التربة، المنطقة الجذرية ،التابتوكسين.

## INTRODUCTION

Bacteria constitute a significant portion of microbial life in the soil. Among these, certain are detrimental to the growth of plants and are broadly classified as bacterial plant pathogens. Phytopathogens are introduced mainly into the soil by infected crop plants as a leakage from diseased plants, by decomposing infected tissues, by altrenate hosts and in some cases even by insects.

Since Valleau et al. (1944) demonstrated that virulent *Pseudomonas syringae pv. tabaci* and Pseudomonas angulata are present in the soil and apparently persist there, increasing attention has been paid to the pathogen as a saprophyte in soils, in the rhizosphere of the host as well as other plants. Although *P. tabaci*, the causative agent of wildfire disease in tobacco, was regarded as a leaf pathogen, it bas been found associated with the roots of various crops, pasture and weed plants in addition to those of its natural host, tobacco, (Valleau et al., 1944).

The role of this patogen in the causation of a leaf spot disease (Turner, 1986; Lucas, 1975) and the presence of this pathovar in the rhizosphere bas been known for some time, but it is not understood how plant growth and development may be affected by the presence of this pathogen in the soil or in the rhizosphere, or by production of tabtoxin in this area . In this work we report our experiments into a study of the ecological significance of tabtoxin production by *P. tabaci* growing in the rhizosphere, in sterilized and in unsterilized soil.

## MATERIAL AND METHODS

### Inoculation of the soil :

Two *Pseudomonas syringae pv. tabaci* Streptomycin ($Strep^R$) or rifampicin ($Rif^R$) resistant mutants were used in an investigation of the effect of toxigenicity on growth and survival in soil. Each mutant was grown for 18 hr at 30°C in nutrient broth in shake culture. Culture was then centrifuged (1000 g, 10min, 4°C). The pellet was washed and resuspended in saline (0.85%) to give a suspension of A550 nm = 0.2. One ml of the suspension was added to sterile McCartney bottles containing 10g of sterilized soil or unsterilized soil (sieved with 2 mm sieve) which were then shaken for approximately two minutes. These soil samples were incubated at 28°C during the whole experiment.

### Sampling :

Bacteria were harvested by adding 10ml saline to each bottle. Bottles were shaken and left on the bench for 30 min to allow the bottle contents to settle. Dilutions were made with saline from 1ml removed from each bottle, a volume (o.1 ml) of appropriate dilution was spread with sterile glass rod on nutrient agar containing streptomycin or rifampicin (25 $\mu g\ ml^{-1}$), as required.

**Inocutation of the rhizosphere :**

Two *pv. tabaci* mutants were used to inoculate the rhizosphere area of several 4 to 6 week old plants. One ml of the Tox$^+$ cells suspension (1,o x $10^8$ cells $ml^{-1}$) was injected into the root area The same procedure was used for Tox$^-$ mutant (for which a 1 ml suppension contained 1,4 x $10^8$ cells). All inoculated plants were kept in greenhouse at 20°C.

**Sampling :**

Viable counts in rhizosphere and in soil surrounding the roots were determined starting from the 7th day of inoculation. The root system was separated from loeasly adhering soil. Two grans of roots were ground with 10 ml saline in a sterite mortar and five grams of the removed soil were suspended in 10ml saline.

Five plants from three different families (Solanaceae, Leguminosae and graminae) were used in the above experiment. Thereafter, only tobacco the usual host of *pv. tabaci*, was used for studing the growth and survival of the two bacterial strains in the root area.

**RESULTS :**

Populations of the two strains (Tox$^+$ and Tox$^-$) increased similarly in sterilized soil during the first five days after inoculation. Thereafter, the growth curves diclined, but at day 20 the number of both strains remained similar to, or above, the number counted at the time of inoculation (Figure 1 and 2). In unsterilized soil, however, populations of all strains started decreasing from the time of inoculation.

A considerable decrease was noticed in the first week and at day 20, the number of the Tox$^+$ and the Tox$^-$ strains was under $10^4$ cells/ml. (Figure 1 and 2).

The effect of rhizosphere and soil surrounding the roots of six plants (*Pisum sativum, Nicotiana tabacum, Hordeum vulgare, Vicia faba and Lycopersicum esculentum*) on the populations of *pv. tabaci* strains (Tox$^+$ and Tox$^-$ ) was studied.

The populations of the test bacteria collected from the rhizosphere of these plants varied according to the plant species. Number of the Tox$^+$ strain was higher in the rhizosphere than in the soil surrounding the roots. There was no consistent difference between the number of the Tox$^-$ strain in the rhizosphere and in soil surrounding the roots.

In general Tox$^-$ strain survived much better than the Tox$^+$ strain in both regions. In all samples, the inoculated bacteria represented only a very small proportion of the total (aerobic) bacterial flora (Table I). The time-course, of the populations changes of *pv. tabacci* Tox$^+$ and Tox$^-$ strains in the rhizosphere (Figure 3) and in soil surrounding the roots of tobacco plant (Figure 4), showed that the population of Tox$^-$ strain gave a small initial increase followed by a gradual decrease towards the end of experiment. The Tox$^+$ started its decrease from the time of inoculation.

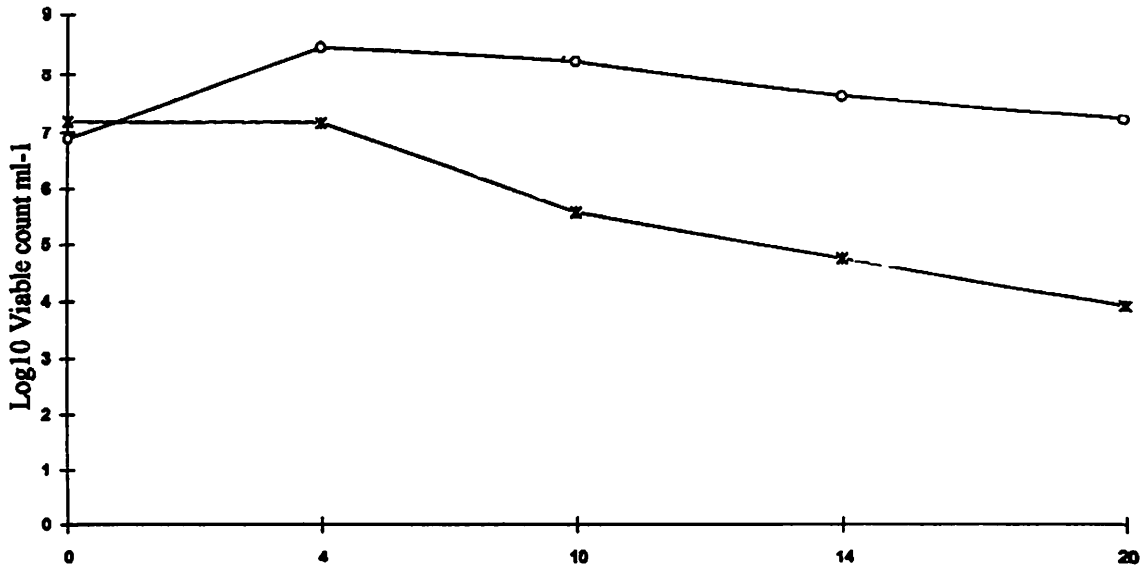


**Fig.1** : Grouth of *Pseudomanas syringae pv. tabaci* $Tox^{+}$ in sterlized soil an in unsterlized soil.

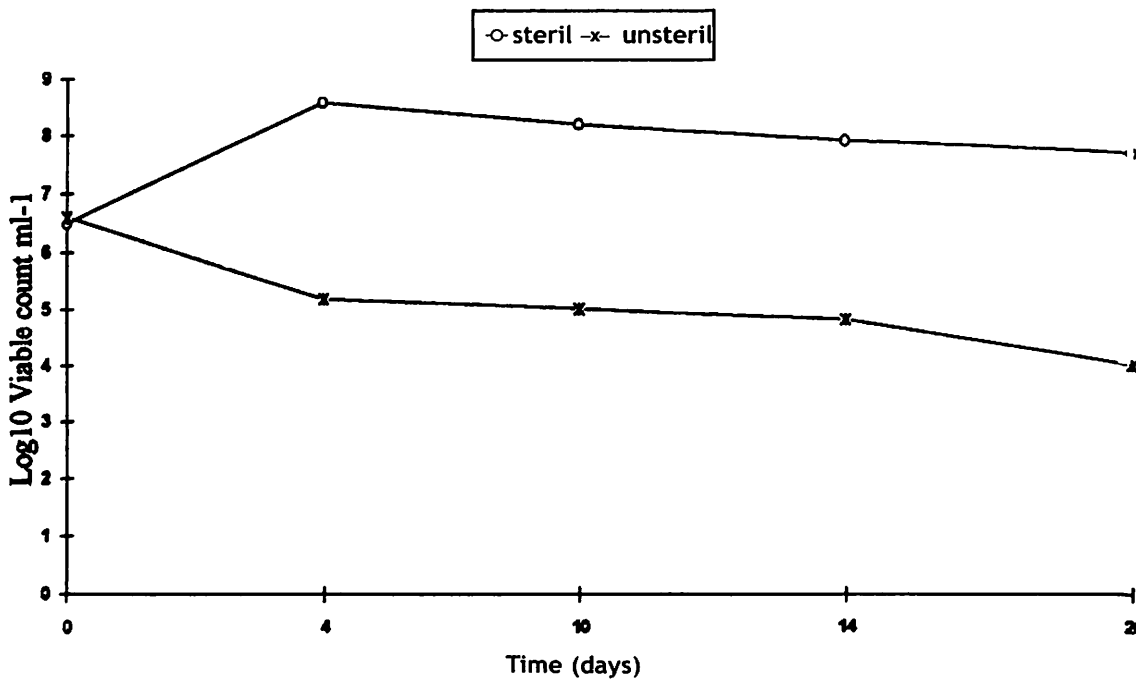


**Fig.2** : Grouth of *Pseudomanas syringae pv. tabaci* $Tox^{-}$ in sterlized soil an in unsterlized soil.

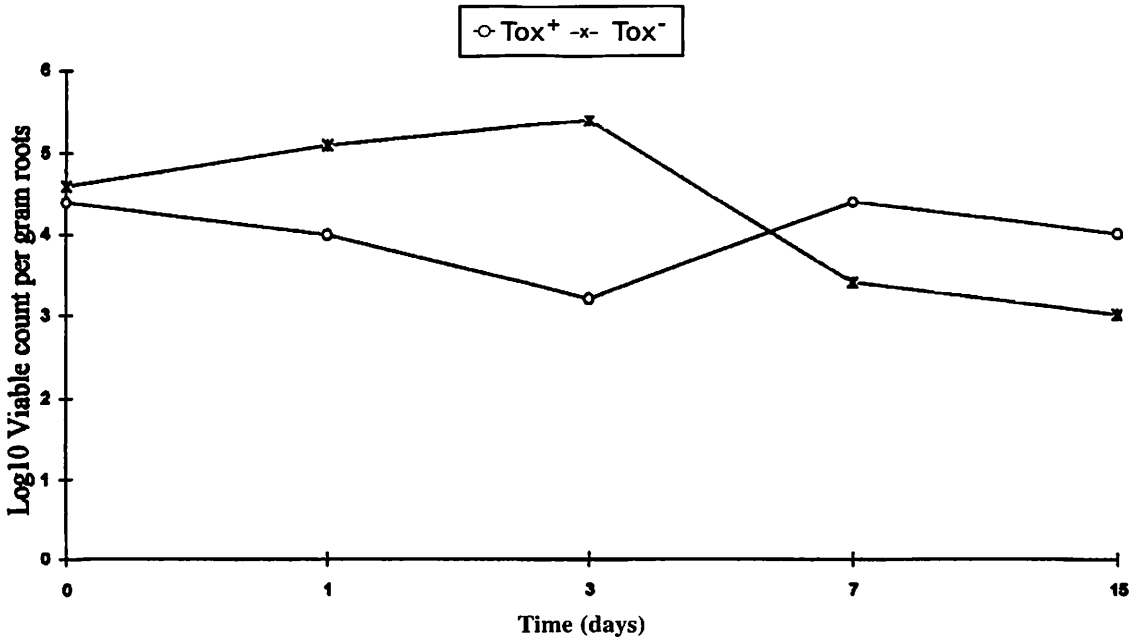


**Fig.3** : Time course of the population changes of pv. *tabaci* $Tox^+$ and $Tox^-$ in the rhizosphere area of tobacco.

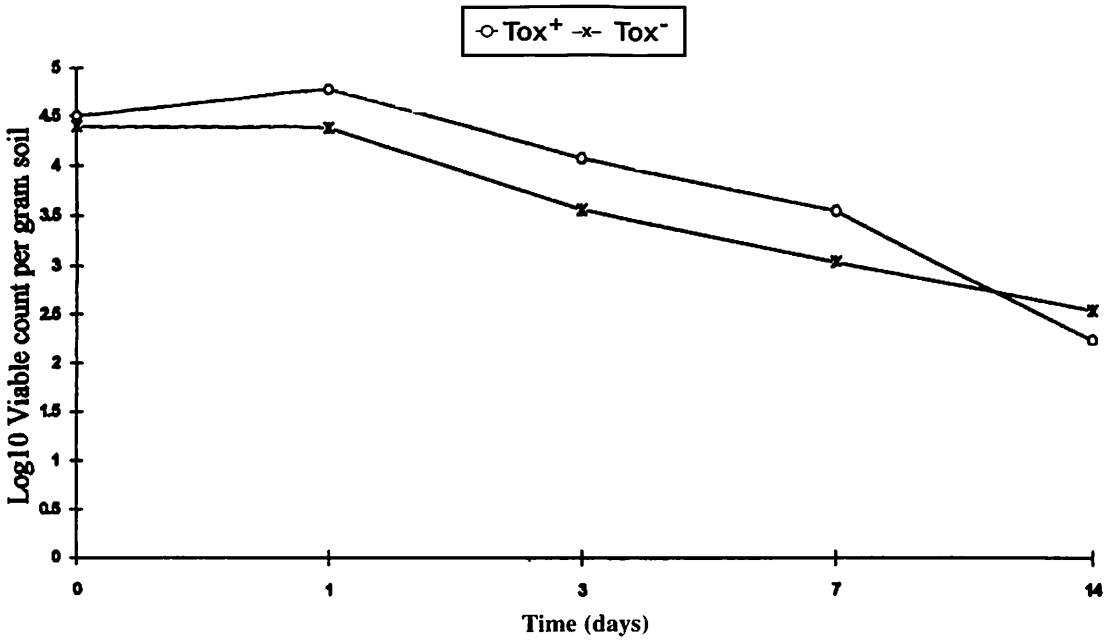


**Fig.4** : Time course of the population changes of pv. *tabaci* $Tox^+$ and $Tox^-$ in the soil surrounding the roots of tobacco.

Both strains survived better the rhizosphere than in soil surrounding the roots of tabacco plant. At day 15, number of $Tox^+$ was ten fold higher than those of the $Tox^-$, in the rhizosphere.

The total bacterial number was constant in the rhizosphere and in soil surrounding the roots. Which was readly similar when the tobacco rhizosphere area inoculated either with $Tox^+$ or with the $Tox^-$ strain (Table I).

**Table I** : Log Number of cells/ml of *Pseudomonas syringae pv. tabaci* Tox+ and Tox- strains isolated from the rhizosphere of several plants.

| **Plant species** | **Inoculation area** | **Inoculation with $Tox^+$** | | **Inoculation with $Tox^-$** | |
|---|---|---|---|---|---|
| | | **Total bacteria** | **$Tox^+$** | **Total bacteria** | **$Tox^-$** |
| *Pisum sativum* | Rhizosphere | 7.40 | 6.75 | 7.60 | 4.00 |
| | Surr. soil | 6.88 | 6.77 | 7.04 | 3.67 |
| *Nicotiana tabacum* | Rhizosphere | 8.87 | 4.46 | 7.43 | 3.90 |
| | Surr. soil | 7.44 | 4.73 | 7.61 | 3.78 |
| *Hordeum vulagre* | Rhizosphere | 7.13 | 5.23 | 6.47 | 4.08 |
| | Surr. soil | 6.35 | 4.95 | 6.18 | 3.45 |
| *Vicia faba* | Rhizosphere | 7.29 | 4.84 | 6.69 | 4.47 |
| | Surr. soil | 6.27 | 5.98 | 6.46 | 4.10 |
| *Lycopersicum esculentum* | Rhizosphere | 7.29 | 4.72 | 3.37 | 4.13 |
| | Surr. soil | 6.10 | 4.07 | 6.26 | 3.95 |
| Mean ± S.D. | Rhizosphere | 7.55 | 9.2 | 6.91 | 4.32 |
| | | ± 0.56 | ± 0.81 | ± 0.50 | ± 0.45 |
| | *Surr. soil | 6.60 | 5.3 | 6.71 | 3.79 |
| | | ± 0.49 | ± 0.95 | ± 0.54 | ± 0.22 |

*Surr. soil : Soil surrounding the roots.

## DISCUSSION :

Results of the survival of all *pv. tabaci* strains ($Tox^+$ and $Tox^-$ ) in unsterilized soil show that the populations declined continuously during the experimental period of up to 20 days. However, this decline is eliminated or subtantially less for populations introduced into sterilized soil. The inability of these strains to survive in unsterilized soil could be due either to the presence of inhibitors or to the absence of nutrients required for microbial growth. Brown (1973) reported that untreated soil contained a factor, thereafter called bacteriostasis, which is of biological origin and was thought to involve protozoa, and it was also probably associated with organic matter in the soil and was removed by prolonged drying or heating. It is known that soil flora and soil protozoa partially inhibit survival of bacterial pathogens. In the field, additional factors such as, fluctuating moisture levels and temperatures could also account for the inhibition of growth and survival of plant pathogenic bacteria.

From previous work (Bosshard-heer and Vogelanger, 1977), it was reported that *Pseudomonas tomate* survived in sterilized soil for long periods of time, whereas in unsterilized soil it could be isolated only after one month. The same conclusion can be drawn. *pv. tabaci* strains survived well in sterelized soil compared with their survival in unsterilized soil. Stimulatory effects of sterilized soil are explained on the basis of physical and chemical changes resulting in an increase in readily available nutrients for soil microflora, the killing of predators such as amoebae and flagellates which are present in a large numbers in unsterilized soil and removing the effect of bacteriostasis in general.

It bas been found, generally, in this work that there was better survival of *pv. tabaci* in the rhizosphere then in normal soil, this might be because the rhizosphere is a rich environment and that *Pseudomonas sp*, are rhizosphere organisms (Cross, 1968).

Lynch (1982) found that plants release about 20% of their total photosynthate through the roots, and this provides much substrate for microbial biomass in the rhizosphere.

There was, on the whole; not much difference in growth and survival between the two strains ($Tox^+$ and $Tox^-$) inoculated into either the rhizosphere area or the soil.

Clearly, virulent strain of *pv. tabaci* grow and survive in the rhizosphere better than the Tox- strain. This may be due to the ability of the Tox+ strain to produce tabtoxin which may have a harmful effect upon some other residents of the soil and rhizosphere. For instance, Singh (1945) reported that plant pathogenic bacteria are eaten by flagellates and amobae whilst others are not, and the plant pathogenic bacteria were inedible because they produced a flourescent pigment, or exotoxin.

It is apparent from this work that tabtoxin production plays, at best, only a minor role in the survival of *pv. tabaci* in the rhizosphere and in the soil. Although tabtoxin does process antibiotic activity, the populations of toxin-producing strain decline in the soil and in the rhizosphere in much the same way as Tox$^-$ strain.

## References :


**Brown M. E. (1973).** Soil bacteriostasis limitation in growth of soil rhizosophere bacteria. Canadian Journal of Microbiology, 19 : 195-199.

**Boosshard-Heer, E. and Vogelsanger, J. (1977).** Uberlensfahig keit von Pseudomonas tomato (Okabe) Alstatt in verscheidenen Boden Phytopathologische Zeitschritt, 19 : 193-200.

**Crosse, J. E. (1968).** Plant pathogenic bacteria of soil. In: Ecology of soil bacteria (Ed. by T. R, Gray and D. Parkinson) pp. 552-527. University of Toronto Press. Toronto.

**Lucas, G. B. (1975).** Diseases of tobacco. Biological Consulting Associates Raleigh, North Carolina pp. 397-409.

**Lynch, J. M. (1982).** Limits of microbial growth in soil. Journal of General Microbiology. 128 : 405-410.

**Singh, B. N. (1945).** The selectivity of bacteria food by soil amoebae, and the toxic effects of bacterial pigments and other products on soil protozoa. British Journal of Experimental Pathology, 26 : 316-325.

**Turner, J. G. (1986).** Activities of ribulose-5- bisphosphate and glutamate synthetase in isolated mesophyll cells exposed to tabtoxin Physiological and Molecular Plant Pathology, 29 : 59-68.

**Valleau, W. D. Johnson, E. M and Diachim, S. (1944).** Root infection of crop plants and Weeds by tobacco leaf-spot bacteria. Phytopathology, 34 : 163-174.