

# BACTERIAL BLIGHT (*Xanthomonas campestris*) OF SUNFLOWER (*Helianthus annuus*), A NEW DISEASE<sup>1</sup>

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## 1. INTRODUCTION

Sunflower has its center of origin in America having been introduced in Europe and in other continents during the Sixteenth Century. The economical importance of the crop in Brazil has been increasing in the last few years, mainly in the western states (16). Minas Gerais State, located at the southeastern part of Brazil, has no extensive plantations of sunflower and the crop is grown in small areas either for ornamental purposes or for seeds to feed birds and other animals. Recently, farmers and extensionists have brought sunflower whole plants and/or plant organs to the Department of Plant Pathology of the Universidade Federal de Viçosa for diagnosis of what apparently was a new disease breaking through the region.

After the bacterial etiology for the disease was determined, it was found that there was no register of a pathovar of *Xanthomonas campestris* described in sunflower neither in Brazil (2, 3, 17, 20, 21, 22, 23, 24) nor abroad (4, 5, 13, 27, 28, 30,). In Brazil, particularly, only a soft rot due to *Erwinia carotovora* subsp. *carotovora* (19), a wilt caused by *Pseudomonas solanacearum* (19), and a bacterial blight induced by *Pseudomonas cichorii* (18) have been described.

This paper is the first report of a new bacterial disease occurring on sunflower in the southeastern region of Brazil due to a xanthomonad. It also

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proposes a new pathovar name for the etiological agent, namely *Xanthomonas campestris* pv. *silvia*.

## 2. MATERIAL AND METHODS

**Symptomatology:** Sunflower whole plants or plant organs were collected in small crop areas located in different regions of Viçosa, Minas Gerais, Brazil by farmers and extensionists and brought to the Department of Plant Pathology of the Universidade Federal de Viçosa for diagnosis and control recommendation.

Typical symptoms (Figure 1) consisted of necrotic lesions, dark to black, usually surrounded by chlorotic haloes, predominantly in leaves but



FIGURE 1 – Typical symptoms of the bacterial blight of sunflower under field conditions.

also present in stems, flowers and petioles. It was possible to distinguish two basic symptomatological patterns in leaves: wedge-shaped, single, large lesions starting at leaf borders and clusters of tiny and punctiform lesions everywhere in the leaf blade. Both patterns probably indicate distinct ways of secondary spreading. In spite of the fact that the disease seems to be severe and symptoms are drastic, field visits at different periods of the

culture cycle indicated that there was no plant death as a consequence of the disease unless rust (*Puccinia helianthii*) - endemic in the region - occurred simultaneously. It was also observed that heavily infected plants had a tendency to produce smaller flowers with high proportion of empty seeds even though it is impossible to conclude whether it might be due to the bacteriosis itself, to the rust or due to a synergism of both bringing about a general plant weakening.

**Prognosis:** The bacterial etiology of the disease was investigated by using the drop diffusate test (11, 25). Small fragments were excised from lesions and mounted in water with cover slip and observed under light microscope with the lowest magnification.

**Isolation:** Infected organs with typical symptoms were thoroughly washed with soap and water (25). From borders of lesions square fragments (0.5 cm) were removed and immersed in ethanol (20 seconds) and then transferred to 2% NaClO (2 minutes). After that, surface sterilized fragments were washed three times in autoclaved distilled water, aseptically ground in a drop of sterile water and allowed to rest for 10 minutes. Past that length of time, the macerate was streaked onto the surface of solid culture medium 523 (9) and plates incubated at 28°C/48 hours (26).

**Pathogenicity tests:** For the hypersensitivity test, 24 hour old cultures in solid slants were used to prepare a bacterial cell suspension in saline (0.85% NaCl) whose concentration was calibrated to  $OD_{550} = 0.10$  prior to infiltration in test plant leaves such as tobacco, coffee, passion-fruit, tomato and bean (14). The same bacterial suspension was used for inoculating sunflower seedlings previously kept in moist chamber for 24 hours by techniques such as spraying, leaf injection and sectioning of leaf blade with a scissors immersed in inoculum suspension. Inoculated plants returned to moist chamber for an additional 24 hour period.

**Cell morphology and cultural characteristics:** Cell morphology and arrangement was observed by using the Gram staining and Indian ink negative staining (7). Presence of endospores was checked by using the method described by GUNSALUS and STANIER (8). Colony morphology (size color, elevation and edges) were also investigated by using the standard culture medium (9).

**Genus determination:** Since isolates under investigation were clearly non-fastidious provided they grew easily and quickly in the standard non-complex culture media, such as medium 523 of KADO and HESKETT (9), GYCA, NYGA and PDA (26), identification tests were directed to the classical non-fastidious genera. Xanthomonadines production was investigated according to LELLIOTT and STEAD (14), asparagine utilization as postulated by STARR (29), fluorescent pigment in King's B medium (10), relations with free oxygen by the anaerobic jar technique (1), the technique described by SCHAAD (26) for the Gram stain, and endospore staining according to GERHARDT (7) after GUNSALUS and STANIER (8).

**Species determination:** Biochemical, biological, cultural and staining procedures were performed according to information obtained from FAHY and PERSLEY (6), SCHAAD (26), LELLIOTT and STEAD (14), ROMEIRO (22) and KRIEG and HOLT (13).

### 3. RESULTS

Careful examination of infected plant parts always followed by the drop exsudate test allowed us to distinguish bacterium induced lesions with no doubt from those with a different etiology.

After 48 hour isolation rendered colonies yellow in color, small (2-4mm in diameter), elevated, brilliant, with regular borders, smooth and translucent. Isolates were obtained from different isolation plates, transferred individually to fresh slants, preserved in 15 % glycerin at -80°C and incorporated to the culture collection of the Institution. In culture medium 523 (9) growth was fast enough to be discarded the possibility of fastidious growth behavior and without detectable production of any water-soluble pigments able to bring about culture medium darkening.

As shown in Table 1, the hypersensitive response was only observed in bean leaves, as necrotic, dry, localized lesions in infiltrated areas within 18-24 hours (11, 12), being negative for the other test plants. On the other hand, 48 hours after inoculation of the original host, susceptibility symptoms started becoming evident in sunflower plants, despite the inoculation technique chosen. Host inoculation brought about reproduction of original symptoms of the disease in sunflower leaves, earlier (48 hours) by leaf infiltration and later by sectioning of leaf border with sterile scissors dipped into inoculum suspension (72-96 hours). Host inoculation by spraying took more than 7 days for symptoms to show up.

Under the light microscope, cells of the pathogen were seen as straight, single, regular, non-pleomorphic rods, with conspicuous capsule.

Being able to grow easily in standard routine culture media, it was assumed that isolates could be positioned within the group of classical, non-fastidious genera of plant pathogenic bacteria (22). Production of xanthomonadines (Figure 2) leaves no doubt that plant pathogenic isolates obtained from infected sunflower plants belong to the genus *Xanthomonas*.

In spite of that, corroborative biochemical, physiological and staining tests were carried out anyway, as seen in Table 2, to check other basic characteristics of the genus *Xanthomonas* such as Gram staining, relations with free oxygen, and so forth.

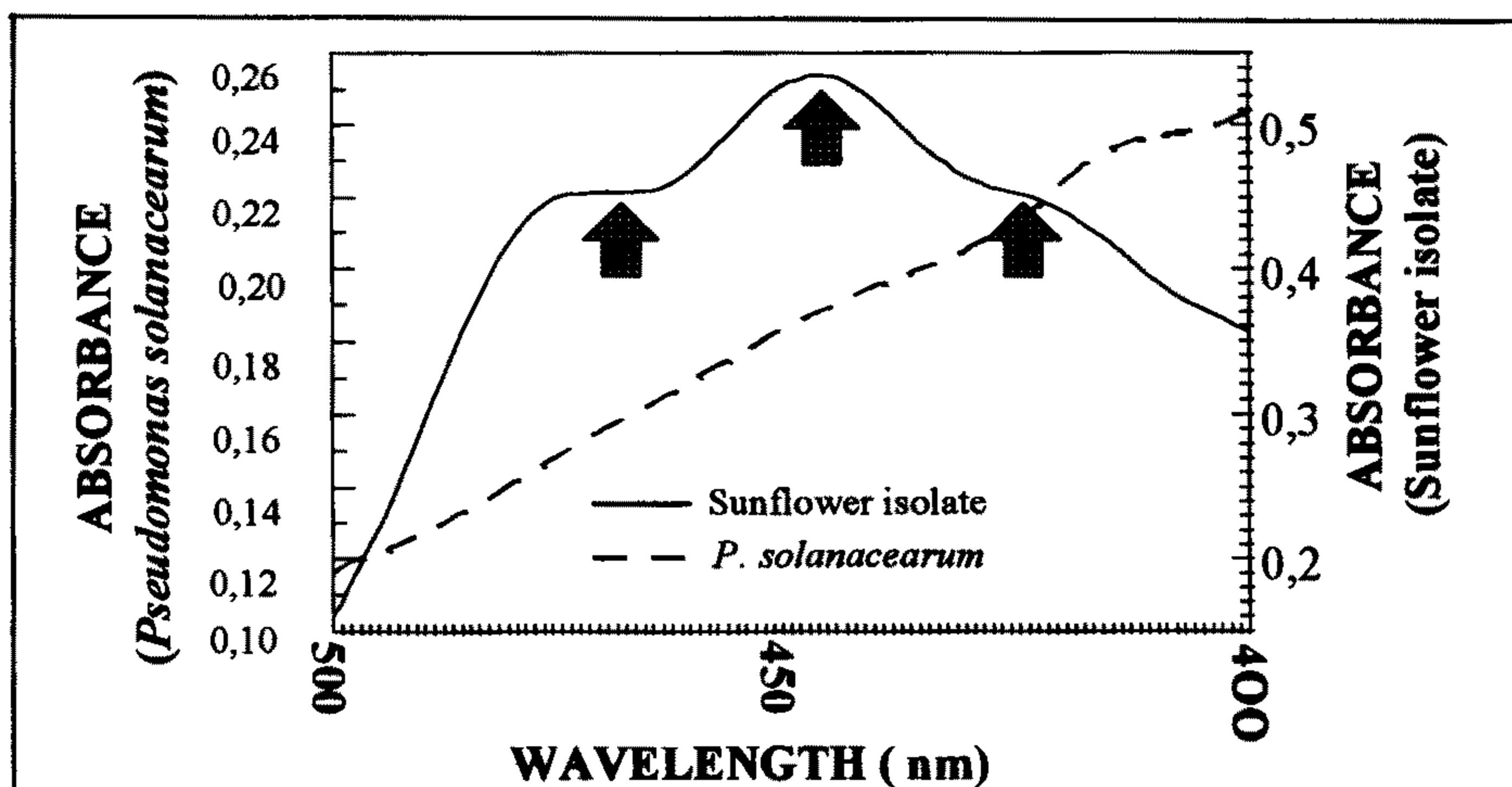


FIGURE 2 - Xanthomonadines production by one of sunflower isolates (—) compared to the lack of production of the same pigments by an isolates of *Pseudomonas solanacearum* (---) used as a negative control.

TABLE 1 - Pathogenicity tests: Results of HR and original host inoculations performed with isolates obtained from infected sunflower plants

Test-Plant	Type of Association	Observed Symptoms	
		HR (24 hours or less)	Susceptibility (More than 48 hours)
Tobacco (*)	Incompatible	-	-
Tomato (*)	Incompatible	-	-
Passion-fruit	Incompatible	-	-
Coffee	Incompatible	-	-
Bean	Incompatible	+	-
Sunflower	Compatible	-	+

(\*) = Some yellowing in infiltrated leaf areas after 48 hours

Additional tests were performed to verify in which species within the xanthomonads isolates under investigation could be positioned. Results in Table 3 clearly indicate they fit better in the species *Xanthomonas campestris*.

**TABLE 2 - Biochemical, biological, physiological and staining characteristics of sunflower isolates, at genus level, when compared to the expected ones (6, 12, 13, 14, 24, 26) for the classical genera of non-fastidious plant pathogenic bacteria**

INVESTIGATED CHARACTERISTICS	Genus <i>Agrobacterium</i>	Corineform Group	Genus <i>Erwinia</i>	Genus <i>Pseudomonas</i>	Genus <i>Xanthomonas</i>	SUNFLOWER ISOLATES
Rod-shape	+	+	+	+	+	+
Growth in routine media	+	+	+	+	+	+
Endospores	-	-	-	-	-	-
Growth factors required	-	-	-	-	+	+
Gram staining	-	+	-	-	-	-
Asparagine utilization	+	+	+	+	-	-
Xanthomonadines	-	-	-	-	+	+
Facultative aerobes	-	-	+	-	-	-
Tumor induction	+	-	-	-	-	-

#### 4. CONCLUSIONS

It seems there is no doubt that the disease observed in sunflower plantations at Viçosa, Minas Gerais, Brazil here described has a bacterial etiology. Bacterial streams were always observed in lesions supposed to have a bacterial etiology and a bacterium was consistently isolated from such lesions. Isolates of the bacterium always brought about the original symptoms in a perfect reproduction of Koch's postulates.

The lack of fastidious growth lead us to disconsider other plant pathogenic prokaryotes like mycoplasmas, rickettsias and so on. The absence of endospores eliminates the possibility of unusual plant bacterial pathogens such as species of *Bacillus* and/or *Clostridium* in the same way that unobserved pseudomycelium-like structures rules out possibilities like *Streptomyces* sp. and related organisms. As a consequence, we cannot put too much restriction to position unknown isolates among the classical genera of plant pathogenic bacteria, namely those comprising species of either *Agrobacterium*, Corineform Group, *Erwinia*, *Pseudomonas* or *Xanthomonas*.

Merely by having detected the production of xanthomonadines (Figure 2) we can assume with almost 100% of probability that isolates obtained

TABLE 3 - Biochemical, physiological, staining characteristics of sunflower isolates when compared with some standard ones (13) for the recognized plant pathogenic species of the genus *Xanthomonas*

DIFFERENTIAL CHARACTERISTIC	SPECIES OF <i>Xanthomonas</i> <sup>(1)</sup>						
	I	II	III	IV	V	VI	VII
Xanthomonadines	+	+	+	+	-	-	+
Gelatin	D	+	D	-	-	-	+
Hydrolysis Esculin	+	-	+	+	-	-	+
Starch	D	+	-	+	-	-	-
Milk proteolysis	+	-	-	-	-	Slow	+
Urease production	-	-	-	-	+	-	-
NaCl tolerance (%)	2,0-5,0	0,5-1,0	0,5	1,0	1,0	0,4-0,6	5
Arabinose	+	-	-	-	+	-	+
Glucose	+	+	+	+	-	+	+
Sucrose	+	+	+	+	-	+	+
Acid production	+	+	+	-	-	+	+
from Mannose	+	+	+	-	-	+	+
Galactose	+	-	D	-	+	+	+
Threalose	+	-	-	+	-	+	+
Melobiose	+	-	-	-	-	-	+

(1) *Xanthomonas* species: I = *X. campestris*, II = *X. fragariae*, III = *X. albilineans*, IV = *X. axonopodis*, V = *X. ampelina*, VI = *X. populi*, VII = Sunflower isolates

from infected sunflower plants were true xanthomonads. Data showed in Table 2 not only confirm this assumption but also rules out the probability of being a new or a non described genus other than *Xanthomonas*. Moreover, results presented in Table 3 clearly indicate that sunflower isolates can undoubtedly be positioned within the species *Xanthomonas campestris*.

This is the first description of a bacterial disease in sunflower induced by a species of *Xanthomonas* in Brazil (2, 3, 15, 17, 19, 20, 21, 22, 24) or abroad (4, 5, 27)

Additionally, specific references about pathovars of *Xanthomonas campestris* (13, 30) give no description of a pathovar that specifically infects sunflower. Therefore, it is quite possible that the isolates here described are a new pathovar and we propose to name this pathovar as "silvia" after the first author's only daughter's name.

Finally, it is proposed that *Xanthomonas campestris* pv. *silvia* would be the name for the pathogen causing the new bacterial disease here described as "bacterial blight of sunflower"

## 5. SUMMARY

In Viçosa, Minas Gerais, Brazil, a disease in sunflower (*Helianthus annuus*) was observed, whose symptoms show up as necrotic lesions, always surrounded by chlorotic haloes, predominately located at the leaf margin and, less frequently, dispersed in the leaf limb. The same lesion patterns were also visualized in stem and flower parts. Lesion fragments mounted in water and observed under the lowest microscope magnification showed copious bacterial streams indicating a bacterial etiology for the disease. From lesions a bacterium was isolated that produces yellow, shining, regular edges, elevated colonies in standard culture media, able to induce HR in bean leaves and to reproduce original symptoms if inoculated in the host. Isolates are straight, regular, rod-shaped cells, Gram-negative, unable to use asparagine a sole Carbon and/or Nitrogen source, strict aerobes, xanthomonadines producers, are not tumorigenic and can be positioned in the genus *Xanthomonas*. Biological, biochemical and staining tests lead to the identification of the pathogen as *Xanthomonas campestris*. Its is not known the way by which the pathogen was introduced in the region but it might be through botanical seeds. Losses to the pathogen are very severe in spite of that fact that plant death was not observed or suppression of flower production. A proposition for creation of a new pathovar "silvia", being, as a consequence, the name of the pathogen *Xanthomonas campestris* pv. *silvia* has been made.



## 7. RESUMO

### QUEIMA BACTERIANA (*Xanthomonas campestris*) DO GIRASSOL (*Helianthus annuus*), UMA NOVA DOENÇA

Em Viçosa, Minas Gerais, Brasil, foi observada uma enfermidade em girassol (*Helianthus annuus*) cujos sintomas se manifestam como lesões necróticas, sempre circundadas por halos cloróticos, predominantemente localizadas nas margens das folhas e, menos freqüentemente, dispersas no limbo foliar. O mesmo padrão sintomatológico era observado em caule e verticilos florais.

Fragmentos de lesões montados em água e observados ao menor aumento do microscópio exibiram abundante exsudação, indicando uma etiologia bacteriana para a enfermidade. A partir de lesões, foi isolada uma bactéria que produz colônias amarelas, elevadas, brilhantes, de bordos regulares em meios padrão de cultivo, capaz de induzir HR em folhas de feijoeiro e de reproduzir os sintomas originais se inoculada no hospedeiro.

O isolamento produz células com forma de bastonete retos e regulares, é Gram-negativo, não utiliza asparagina como única fonte de Carbono e Nitrogênio, é aeróbico estrito, produz xantomadinas e pode ser posicionado no gênero *Xanthomonas*. Testes biológicos, bioquímicos e tintoriais permitiram a identificação do patógeno como *Xanthomonas campestris*. Desconhece-se a forma como o patógeno foi introduzido na região mas poderia ter sido via sementes. Perdas decorrentes da incidência do patógeno são bastante severas ainda que não se tenha observado morte de plantas e, ou, supressão da produção de flores. Propões-se a criação de um novo patovar - "silvia" - sugerindo-se pois, como nome do patógeno *Xanthomonas campestris* pv. *silvia*

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